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Synthesis of Green ZnO/Fe₃O₄ Nanocomposite by Microplasma Jet and Anti-Bacterial Agent

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Abstract:

There has been an increase in demand for nanocomposite, which has resulted in large-scale manufacturers employing high-energy processes and harmful solvents. Because of this, the need for environmentally benign "green" synthesis processes has grown. Other methods for making nanocomposite include using plants and plant products, bacteria, fungi, yeast, and algae. Green synthesis has minimal toxicity and is safe for human health and the environment compared to other processes, making it the ideal option for creating nanocomposite materials. This work reveals an environmentally friendly synthesis method for magnetic nanocomposites. In particular, they were using an aqueous extract of Artemisia to obtain ZnO/Fe₃O₄ using cold plasma technology. The magnetic nanocomposite was prepared with different concentrations (0.01, 0.02, and 0.03) of M and (2:8) of the aqueous extract. The structural properties were studied using X-ray diffraction, where the crystal size ranged from 30 to 40 nm, while the surface morphology was studied through the field emission scanning electron microscope, and it was found that the shape of the particles is semi-spherical and within a particle size range of 30 to 60 nm. "Green" magnetic nanocomposites showed low toxicity and high biocompatibility, allowing their application in biomedicine, where magnetic nanocomposites were employed as anti-agents for *E. coli* and *S. aureus* using the agar diffusion method. Its high effect on bacterial inhibition was noted when the concentration was increased, as the diameter of inhibition ranged (11-22) mm for *E. coli* and (15-24) mm for *Staphylococcus aureus*.

Keywords: Green, Artemisia, ZnO: Fe₃O₄, Nano composite, Cold plasma, *E.Coli*, *S.aureus*

توليف المترابكات النانوية الخضراء ZnO/Fe₃O₄ باستخدام تقنية نفاث البلازما الدقيقة وتطبيقها
كعوامل مضادة للميكروبات

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الخلاصة:

زيادة الطلب على تحضير المتراكبات النانوية أدى الى تصنيعها بشكل واسع النطاق وباستعمال طاقات عالية ومذيبات ضارة. مما أدى الى ازدياد الحاجة إلى عمليات تحضير "خضراء" صديقة للبيئة، عن طريق استعمال طرق سهلة وغير مكلفة بمستخلصات نباتية. يحتوي التوليف الأخضر على حد أدنى من السمية، وهو آمن لصحة الإنسان والبيئة مقارنة بالعمليات الأخرى، مما يجعله خيارًا مثاليًا لتخليق المتراكبات النانوية. تكشف هذه الدراسة طريقة توليف المتراكبات النانوية المغناطيسية على وجه الخصوص باستعمال مستخلص مائي لنبات الشيح (*Artemisia*) للحصول على ZnO / Fe_3O_4 باستعمال تقنية البلازما الباردة. تم تحضير ZnO / Fe_3O_4 المغناطيسية بتركيز مختلفة (0.01 و 0.02 و 0.03) مولاري من المستخلص المائي بنسبة (8:2). تمت دراسة الخواص التركيبية باستعمال حيود الأشعة السينية، حيث تراوح الحجم البلوري بين (30-40) نانومتر، بينما تمت دراسة الشكل السطحي من خلال المجهر الإلكتروني الماسح ووجد أن شكل الجسيمات شبه كروية ومتداخلة حيث تراوح حجم الجسيمات (30-60) نانومتر. أظهرت المتراكبات النانوية المغناطيسية "الخضراء" سمية منخفضة وتوافقًا حيويًا عاليًا، مما يسمح بتطبيقها في الطب الحيوي، حيث تم استعمال المتراكبات النانوية المغناطيسية كعوامل مضادة للبكتريا الإشريكية القولونية والعصبية الذهبية باستعمال طريقة الانتشار (اجار)، وقد لوحظ تأثيرها العالي على تثبيط البكتريا عند زيادة التركيز حيث تراوح قطر التثبيط بين (11-22) ملم للإشريكية القولونية و (15-24) ملم للمكورات العنقودية الذهبية.

1. Introduction

Antibacterial compounds are used in various sectors, including packaging, construction, food, textiles, medicine, and water disinfection. Toxicity, high temperature, and pressure sensitivity are among the organic compounds' drawbacks [1]. A growing number of researchers are focusing on using inorganic metal oxides as a source of antibacterial activity since they are non-toxic, stable in harsh environments, and include mineral components [2]. The antibacterial activities of metal and metal oxide nanoparticles have been extensively documented in the scientific literature [3]. Therefore, due to their unique physical, chemical, and biological features, metal and metal oxide nanoparticles have drawn much interest. The size of these nanomaterials may be adjusted [4]. Tailor-made nanomaterials with the necessary characteristics have also been frequently reported. Developing antibacterial nanoparticles is difficult owing to their stability, cost-effectiveness, and great efficacy [5, 6]. Antibacterial nanomaterials, such as nanoparticles, nanowires, and nanotubes, have a high surface area-to-volume ratio, making them ideal for use in these applications. However, accumulation during preparation is the limiting factor [7, 8].

The dispersion factor of metal oxide nanostructures is important for in vivo and in vitro studies. Among the powdered metal oxides, ZnO is a semiconductor with catalytic, optical, electrical, and significant growth inhibition capabilities [9, 10]. Exposing these semiconductors to UV radiation is necessary to activate them [11]. Because of its high catalytic activity and low toxicity, ZnO is an ideal photocatalytic material among these semiconductors [12]. There are a lot of active sites on ZnO nanoparticles since they have a large surface area. The semiconductor ZnO is an n-type semiconductor. Approximately 3.37 eV [13] is the energy of the broadband gap. ZnO's broadband gap allowed it to be excited in the UV spectrum below 400 nm. Recombination of holes and electrons is another limitation of ZnO [13]. If this problem persists, ZnO may be modified to fix it [14]. This change made it possible to get higher absorption in visible light. The recombination between electrons and holes is also reduced. Many approaches have been developed to circumvent ZnO's limitations, such as doping, surface modification using metal nanoparticles, and heterostructure advancement [14]. For example, the separation

rate of electron-hole pairs may be accelerated by coupling ZnO with p-type materials such as Fe₃O₄ [15]. Consequently, the Fe₃O₄/ZnO nanocomposite's photocatalytic performance is more active because of the holes-to-electrons separation in the p-n junction. Photocatalysts may be isolated and reused from wastewater using an external magnet because of their current magnetic characteristics [16]. To find out how to create nanoparticles, some options include expanding supercritical fluids rapidly, electrospray crystallization, or hot plasmas [17].

All three approaches, however, do not allow for the industrial-scale production of low-cost organic crystals with a nanometer size. While electrospray crystallization may be scaled up, the rapid growth of supercritical solutions is time-consuming and expensive. Nanoparticles may be produced using thermal plasmas, which are both effective and cost-effective [18]. On the other hand, thermal plasmas are not suitable for organic substances due to their high temperature. The breakdown of organic substances is prevented by using cold plasmas, which operate at temperatures near the ambient temperature. The problem is that cold plasmas are generally operated in a vacuum, which makes the process more complex and costly, especially at larger scales [19, 20]. When reasonably high production rates are needed, atmospheric pressure plasma systems are an attractive option for decreasing equipment and processing costs. The dielectric barrier discharge, a cold plasma that may be employed at atmospheric pressure, was used in this work as a particular form of plasma. Synthesis of organic compounds with scalability and low operating costs is possible using atmospheric-pressure cold plasma [21, 22].

2. Experimental setup :

2.1 Synthesis of green ZnO/Fe₃O₄ nanocomposite by cold plasma:

ZnO: Fe₃O₄ nanoparticles were prepared with molar ratios (0.01, 0.02, and 0.03) M from zinc nitrate Zn(NO₃)₂.6H₂O and iron nitrate Fe(NO₃)₃.9H₂O using artemisia extract. Both zinc and iron nitrate were prepared in the same way, and the following method was used to know the weight per molar:

1. Take (0.3, 0.6, 0.9 gm) for zinc nitrate and (0.24, 0.48, 0.72 gm) for iron nitrate, weighed for each of (0.01, 0.02, 0.03) M.
2. Put in a glass beaker containing 100 ml of distilled water and stir with a magnetic stirrer for 10–15 minutes.
3. Take 5 ml of ZnO and 5 ml of Fe₃O₄ (0.01, 0.02, and 0.03) M, and the compound was placed in a 10 ml small beaker.
4. Exposed to the plasma jet system for 10 minutes to obtain the nanomaterial for each of the three concentrations.

The nanocomposite was prepared using cold plasma technology at a voltage of 15 kV, a frequency of 23 Hz, and a flow rate of 3 l/min of argon gas using Artemisia extract at a ratio of 2:8 at (0.01, 0.02, and 0.03 M) as illustrated in Fig. 1.

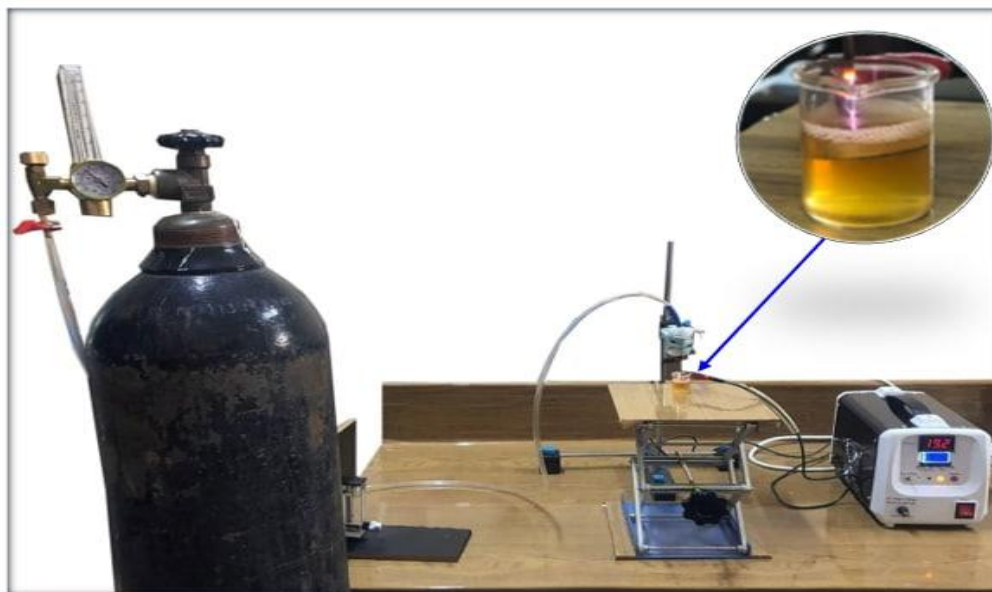


Figure 1: Prepared of Nanocomposite by cold plasma

Study of the effect of Nanocomposite on pathogenic bacteria:

The inhibition activity of the bacteria was tested after choosing the best results on two bacteria types, *Aureus staphylococcus* and *Escherichia coli*, where bacterial isolation and the good diffusion method were used. (Neutrian agar) media and left for 30 minutes at room temperature. After which, a drill is made in the culture medium using a cork drill measuring 6 mm with 3 holes in each plate. And then, we named and marked each hole. This drilling is the prepared solution concentration (0.01, 0.02, and 0.03 M). Without wormwood and with wormwood, 75 microliters of pre-prepared ZnO:Fe₃O₄ particle solution were placed in each hole. The dishes were left at room temperature for a period and then incubated at 37 °C for a period of 18–24 hours, after which the inhibition area around the holes was measured in millimeters.

3. Results and Discussion:

3.1 X-ray Diffraction

ZnO: Fe₃O₄+Artemisia Nanoparticles of the crystal structure were examined using the x-ray diffraction spectra obtained with (0.01, 0.02, and 0.03 M) molarity concentrations. The crystal size was estimated using Eq. (1) (Table 1). With the aid of the whole width at half-maximum peak, the average dimension of a crystal, denoted by the symbol D, may be quickly determined from the X-ray range of the full width at half-maximum (FWHM). It can be determined by applying Scherrer's formula [10]. After subtracting the instrumental broadening, the FWHM in radians is the corresponding diffraction angle. The primary peaks of ZnO and Fe₃O₄ are in Figure 2. Parallel faces in a cubic crystal structure display the X-ray diffraction analysis's findings using examples from the study of ZnO:Fe₃O₄. In Figure 2a, the concentration (0.01)M shows (022), (100), (311), and (102), corresponding to the angles (30.3889°, 31.8659°, 36.39°, and 43.35°, respectively. In figure 2b, the concentration (0.02)M shows (010), (100), (311), and (311) corresponding to the angles (21.33°, 31.68°, and 35.37°, respectively, and in figure 2c, the concentration (0.03)M reveals the matching values for (022), (100), (311), and (102). It exhibits a high degree of crystallinity, and by increasing the concentration, the crystal size is altered from 34 nm to 41 nm.

$$2d\sin\theta = n\lambda \quad \dots\dots (1)$$

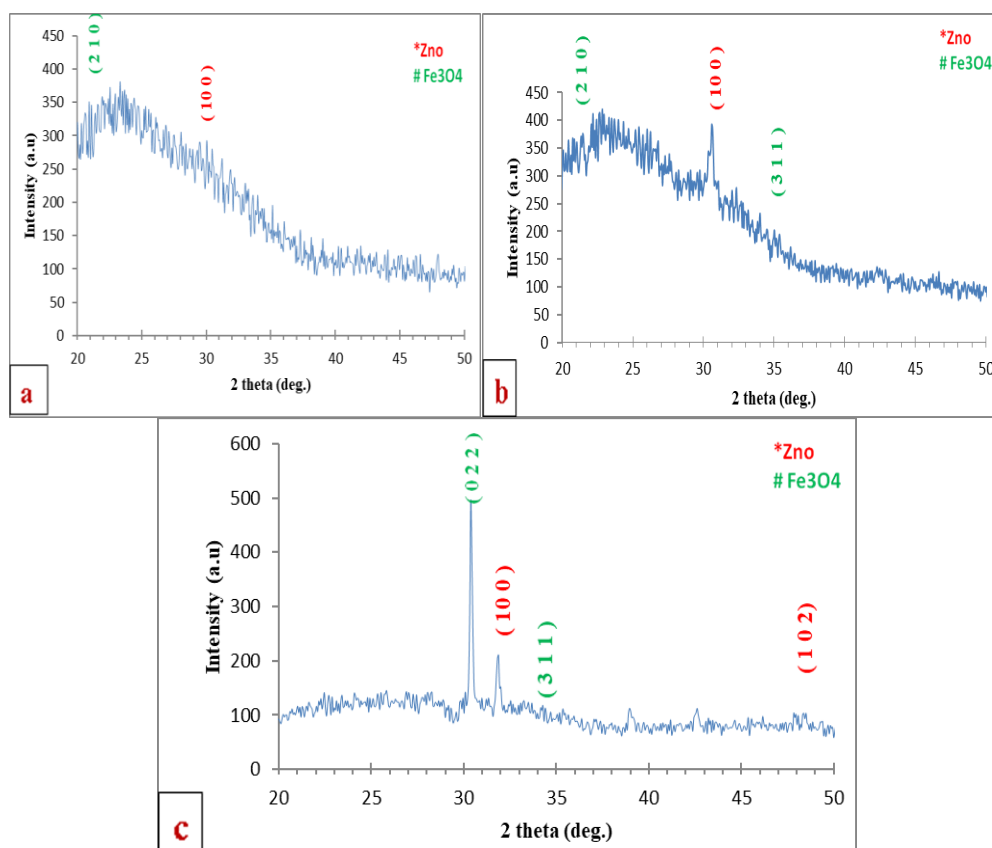


Figure 2: (a, b, and c) X-ray of (ZnO: Fe₃O₄+ Artemisia) nanocomposite at different concentrations

Table 1: X-ray of ZnO: Fe₃O₄+ Artemisia Nanocomposite at Different Concentrations

Co. M	2θ (Deg.)	FWHM (Deg.)	C.S (nm)	Av. C.S nm	Phase	hkl	card No.
0.01+Artemisia	21.7559	0.1771	45.79539	34.54326	Fe ₃ O ₄	210	96-900-6195
	31.89	0.3542	23.29114		ZnO	100	96-230-0113
0.02+Artemisia	21.33	0.1771	45.79526	38.71136	Fe ₃ O ₄	210	96-900-6195
	31.68	0.1771	46.74165		ZnO	100	96-230-0113
	35.37	0.3542	23.59719		Fe ₃ O ₄	311	96-900-6195
0.03+Artemisia	30.3889	0.2952	46.61612	41.17930	Fe ₃ O ₄	220	96-900-6195
	31.8659	0.3542	23.38155		ZnO	100	96-230-0113
	36.39	0.1771	46.34205		ZnO	101	96-230-0113
	43.35	0.1771	48.37749		Fe ₃ O ₄	400	96-900-6195

3.2 Field emission-scanning electron microscopy (FE-SEM):

The surface morphologies of ZnO: Fe₃O₄ +Artemisia with different concentrations in particular have been investigated using Field Emission Scanning Electron Microscopy (FE-SEM). As shown in Fig. 3, the FE-SEM image indicates that the ZnO: Fe₃O₄ +Artemisia nanoparticles had a diameter of approximately 10–100 nm, which proves the nanosize of the prepared samples with different concentrations (0.01, 0.02, 0.03) M.

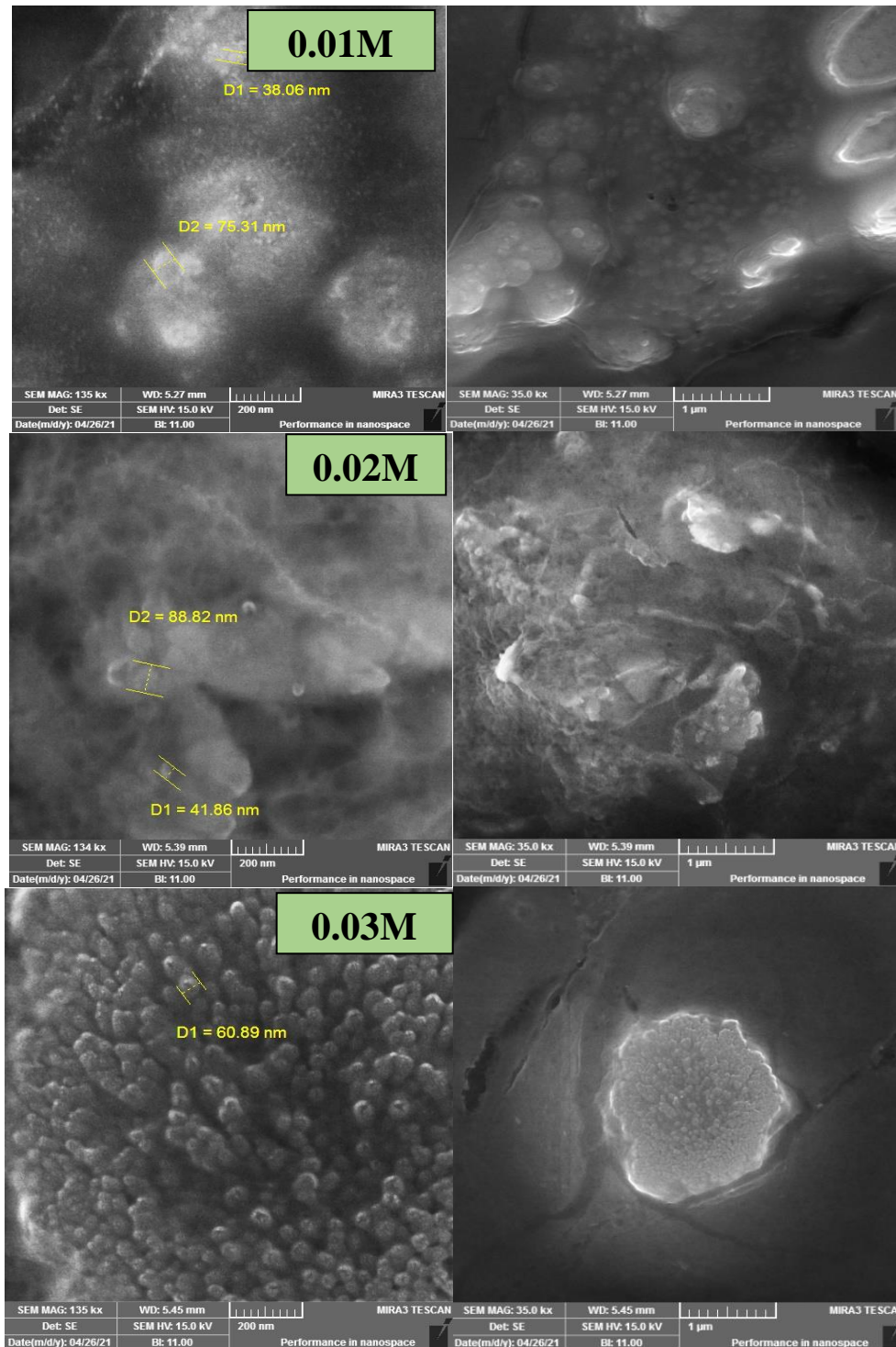


Figure 3: FE-SEM images for (ZnO: Fe₃O₄+Artemisia) nanocomposite at different concentrations (0.01, 0.02, 0.03) M

3.3 Optical properties

In semiconductor physics, the optical energy gap is one of the most often used expressions in the essential constants of light scattering and refraction. How useful semiconductors are for optical and electrical applications depends on the value of this constant. Figure 4 displays the final spectrum achieved on ZnO: Fe₃O₄+ Artemisia. All the spectrum information that has been gathered revealed a substantial cut-off at wavelengths 420, 433, and 435 nm, where the absorbance value is lowest. Assuming a direct energy gap value for the ZnO: Fe₃O₄+ Artemisia

nanocomposite at various concentrations of (0.01, 0.02, and 0.03) M, it is determined in Fig. 4. It can be seen that when concentrations grow, the EG will close, resulting in a reduction in particle size. This is consistent with XRD studies showing that grain size reduces as concentrations rise. To calculate the energy gap, researchers looked at how absorption changed as one moved closer to the basic absorption in the spectrum. Eq. (2) [18] is employed in Table 2.

$$E_g = hc / \lambda \quad \dots\dots (2)$$

With the rise in concentrations, energy gap values were reduced. The number of photon collisions with the substance increased with concentration, which explains the observed drop. As a result, the material will absorb more photons, and the energy gap will narrow. This narrowing of the energy gap will cause the distribution of atoms within the material to be regulated and the crystal phases to change.

By decreasing the energy gap, the grain size will shrink. Large particle size (at higher concentrations) might result in additional energy states and a diminished confinement effect, which generates a small bandgap, explaining why this is happening.

Table 2: The values of E_g of (ZnO: Fe₃O₄+Artemisia)

Concentrations (M)	$\lambda =$ Cut off wavelength (nm)	E_g (eV)
0.01	417	2.97
0.02	430	2.88
0.03	440	2.81

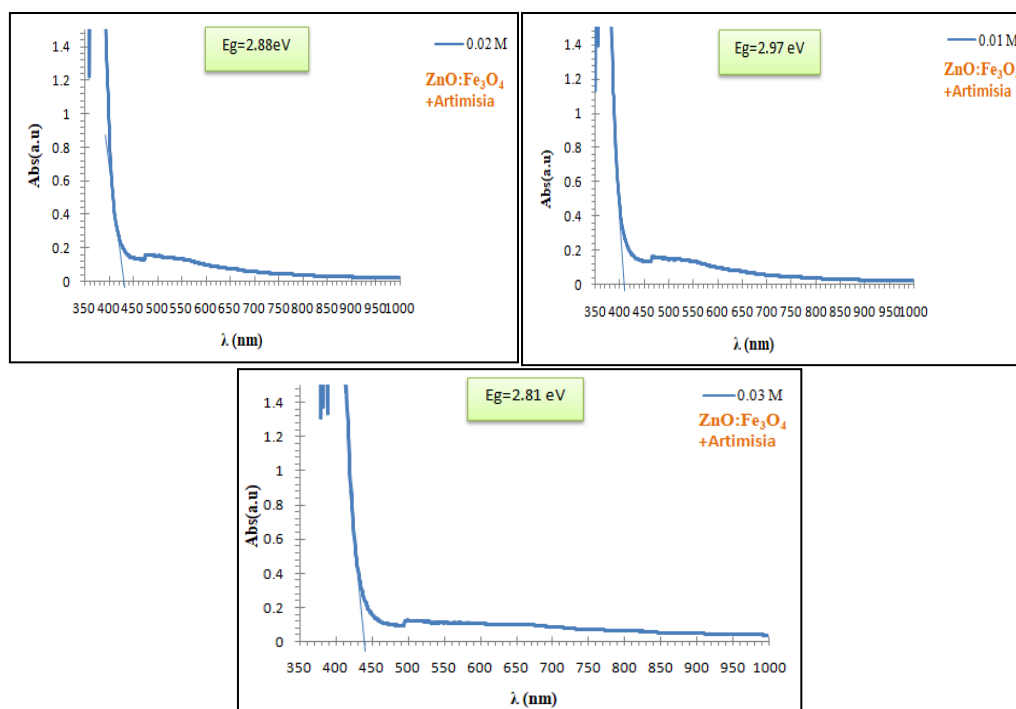


Figure 4: E_g for (ZnO: Fe₃O₄+Artemisia) at different concentrations

3.4 Inhibiting activity of bacteria:

This study tested the antibacterial efficacy of a synthesized (ZnO:Fe₃O₄) nanocomposite to resist two strains of *E. coli* and *Staphylococcus aureus*. Nanoparticles of ZnO:Fe₃O₄ prepared by treating Artemisia leaf extract with ZnO:Fe₃O₄ nitrate were found to be antibacterial against

Gram-positive and Gram-negative bacteria when evaluated by the agar diffusion method. This study shows that nanoparticles made from plant extract successfully prevent the development of bacterial strains or isolates employed, with the findings of research on inhibitory activity varying with bacterial type and concentration demonstrating the usefulness of nanoparticles. ZnO:Fe₃O₄ nanoparticles, on the other hand, were very successful at inhibiting bacteria of both gram-positive and gram-negative varieties.

Also in Fig. 5, observed were ZnO:Fe₃O₄ nanoparticles made from Artemisia extract (green method). Inhibiting bacterium activity was observed to decrease in both positive and negative ways. This indicates that the herb made ZnO:Fe₃O₄ nanoparticles less toxic. In addition, the function of the bacterium *Staphylococcus aureus* in blocking it was demonstrated. Table 3 illustrates the findings of ZnO: Fe₃O₄-induced bacterial inhibition particles were made with a 1 ml molar concentration of zinc and iron nitrate and a 2:8 mixing ratio, where the ZnO:Fe₃O₄ proportion is 8 and the Artemisia extract percentage is 2. The inhibitory zone diameter was 11 mm at a concentration of 0.01 M in a solution of ZnO:Fe₃O₄ nanoparticles made with Artemisia extract. At a concentration of 0.02 M, the diameter of the zone was 15 mm, and at a concentration of 0.03 M, it was 20 mm (22 mm). Previously, table 2 showed that at concentrations of 0.01M and 0.02M, coli-form bacteria were inhibited to the same degree (15 and 17 mm, respectively), whereas at concentrations of 0.03M and 0.04M, the most significant suppression was achieved (25 mm and 30 mm, respectively) (24 mm).

Because bacteria have negative charges and nanometallic oxides have positive charges, there is an electrical attraction between the surface of the bacteria and the nanoparticles. Additionally, nanoparticles produce ions that interact with the group of carrier proteins. Nutrients leak out of the membrane of a bacterial cell and cause the membrane's permeability to decrease, killing the cell [23]. Since it blocks nanoparticles from entering the cell wall, this multilayer is thicker and more solid than peptidoglycan. The cell walls of gram-negative and gram-positive bacteria are structurally distinct because of different lipopolysaccharides and lipoproteins in the former and higher lipid content in the latter, respectively [24]. It was found from the above that the inhibition activity of *E.coli* was higher in all conditions than that of *staph.* bacteria.

Table 3: Inhibition zone of *S.aureus* and *E.coli* bacteria at 0.01, 0.02, and 0.03 M for ZnO: Fe₃O₄+Artemizinin

Concentration%	Types of bacteria	Inhibition zone with Artemisia mm
0.01	S. aureus	11
0.02		15
0.03		22
0.01	E.coli	15
0.02		17
0.03		24

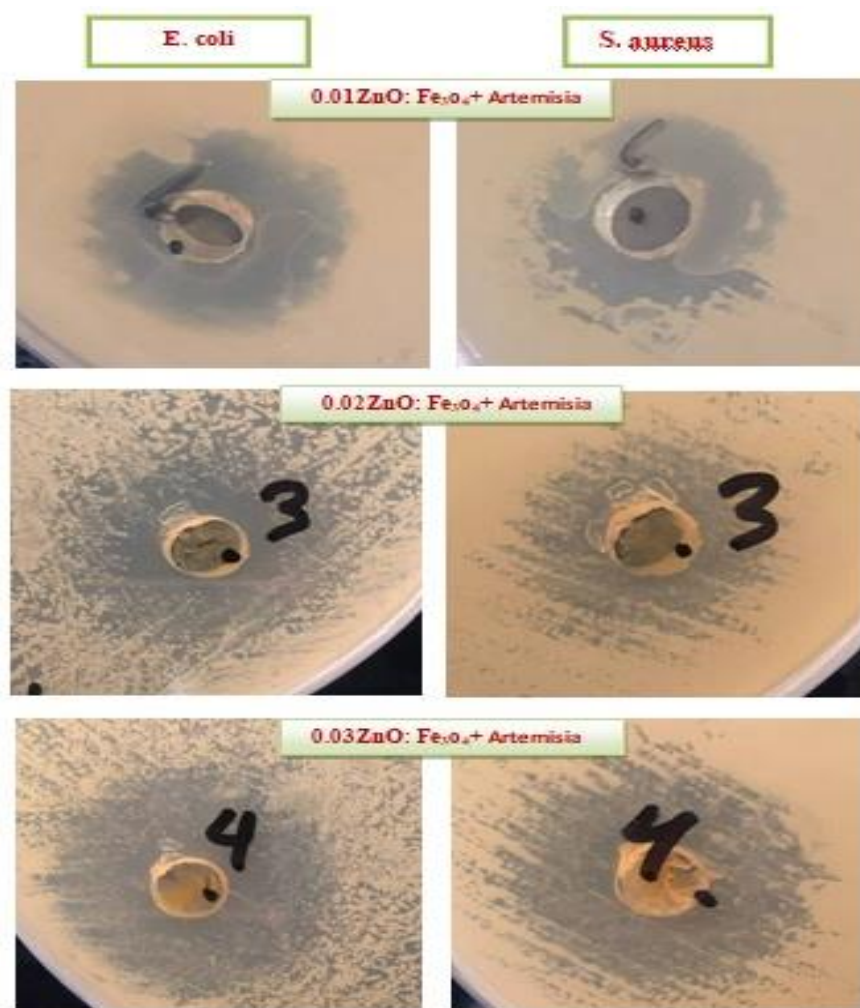


Figure 5: Inhibition zone of Staph bacteria and E.coli bacteria at different concentrations of ZnO: Fe₃O₄+Artemisia

5. Conclusion:

The synthesis of green ZnO/Fe₃O₄ nanocomposites was achieved by microplasma jet technology. The nanocomposites were characterized by field emission scanning electron microscopy and XRD techniques, where the crystal size ranged from 30 to 40 nm. The surface morphology was studied through the field emission scanning electron microscope, and it was found that the shape of the particles is semi-spherical and within a particle size range of 30–60 nm. Antimicrobial studies have further evaluated the developed nanocomposites. The nanocomposites showed more efficacy for *S. aureus* than for the second type of *E. coli* due to the thickness of the bacteria's cell membrane.

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