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Chemical Preparation of Iron Oxide Nanoparticles Using Plants Extracts in Antibacterial Application

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Abstract: In of this studying the green synthesis of Iron Oxide nanoparticles (Fe_2O_3 NPs) with Celery stalks and green tea leaves extract were used. The fresh suspension of plant extracts ware green- brown in color. However, after acting of $FeNO_3$ within 20min, the suspension showed the change in color and turned dark brown after 4 hours of incubation at room temperature. Formation of Iron oxide nanoparticles was confirmed using X-ray is spectral analysis and showed the characteristic Bragg peaks of (111) to green tea extract and (111) to celery extract, plant of the face center cubic (FCC) Iron Oxide nanoparticles. The scanning electron microscope (SEM) Iron oxide nanoparticles see small particles and rode. The synthesized Fe_2O_3 NPs colloidal solution has shown better antibacterial activity against both Gram-positive and Gram-negative bacterial strains. The diameters of the inhibition zones of Fe_2O_3 NPs against the bacterial strains were, S. aureus (27 mm) p. aerugino (29mm) with camellia sinensis extract and S. aureus (22 mm) p. aerugino (25mm) with Apium graveolens exiract at 50 µg/ml concentration.

Keywords: Synthesis, Iron Oxide Nanoparticles, Camellia Sinensis Leaves (Green Tea), Apium Graveolens (Celery), Extract, Antibacterial Activity

1. Introduction

Nanomaterial's molecules in a way that is very necessary and common green synthesis is create environmentally friendly nanomaterial with high specifications Since the plant extract contains various secondary metabolites, it acts as the reducing and stabilizing agent for the bioreduction reaction to synthesize the optimum nanoparticles properties [1]. This approach has been actively used in recent years as an alternative, efficient, inexpensive, and environmentally safe method for producing nanoparticles with specific properties [2]. Chemical methods involve the reduction of chemicals phytochemicals [3], electrochemical procedures [4] microemulsion, chemical precipitation, chemical vapor condensation, pulse electrode position [5]. A typical procedure involves growing nanoparticles in a liquid medium containing various reactants, in particular reducing agents, such as sodium borohydride or potassium bitartrate [6] or methyl polyethylene glycol [7] or hydrazine [8]. A nanometer (nm) is a billionth of a meter, 10-9. The bacterial membrane contains sulfur-containing proteins, and the Iron oxide nanoparticles interact with these proteins in the cell as well as with the phosphorus-containing compounds like DNA. The nanoparticles release Iron oxide in the bacterial cells enhancing their bactericidal activity [9]. The present review focuses on the synthesis of Fe_2O_3 NPs with particular emphasis on biological synthesis using plant extracts and most commonly proposed mechanisms regarding the antibacterial properties of nanoparticles [10].

2. Material and Methods

2.1. Chemical and Reagents

In this study, has been used Iron nitrate hexhydrate ($Fe(NO_3)_2.6H_2O$), (99%), (Reagent World, USA, purity 99.99%), Sodium hydroxide, Celery stalks and green tea leaves buds were collected from the local market. Distill water (DW) was used as a solvent.

2.2. Preparation of Green Reducing and Stabilizing Agent

The Celery stalks (Apium graveolens) and green tea leaves (camellia sinensis) were collected from the local market, (Baghdad). The plants have been catted into small pieces and washed with distil water to remove impurities Apium graveolens (30g) and 200 mL (DW) were homogenized at 80°C in continuous stirring, in cooled down and filtered. The filtrate (brown color) was collected and used for the synthesis of Iron oxide nanoparticles, the same procedure was don for green tea leaves.

2.3. Synthesis of Iron-Oxide Nanoparticles

To synthesis the Iron oxide nanoparticles, freshly extract (30 mL) was added to 0.02 M solution of Iron nitrate hexahydrate, heated at 75°C till precipitates appeared and then temperature reduced to 60°C and kept the solution at for 60 min. The mixture was kept overnight at room temperature and then centrifuged at 14000 rpm for 10 min. The precipitates were washed this with ultrapure water and absolute ethanol to remove unreacted particles and impurities. The obtained precipitates were dried in an furnace at 500°C for 2h, grinded and subjected to characterization, Synthesis of Iron oxide nanoparticles using plant extracts and mixing it with Iron nitrate and turning it from pink to gray forming the Iron oxide. The process to synthesis of Fe_2O_3 NPs with plants is show in figure 1.

Figure 1. *Synthesis of Iron oxide nanoparticles using plant extracts Camellia and Apium with Iron nitrate.*

3. Results and Discussion

3.1. X-Ray Analysis

Reveal XRD of Iron oxide nanoparticles using green tea and celery extracts. Such as Bragg diffraction values are (002, 200, 140, 110, 111, 006, 130, 023, 132, 004, 214, 200) of Iron oxide nanoparticles with camellia sinensis extract. XRD pattern in Figure 3 show the Iron oxide nanoparticles formed with crystalline in nature with a mixed phase structure (Rhombohedral) in peaks (002, 111, 220, 020, 200, 100, 220, 411, 322, 004, 315, 424, 505, 312) celery extract, XRD pattern indicates that the Iron oxide nanoparticles formed are crystalline in nature with a mixed phase structure (Orthorhombic). The average crystallite size of the Iron oxide nanoparticles was calculated, using Debye-Scherrer equation):

$$D = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

Where: D is the particle size (nm), k is a constant equal to 0.94, λ is the wavelength of X-ray radiation (1.541A), b is the full-width at half maximum (FWHM) of the peak (in radians) and 2 theta is the Bragg angle (in degrees). The average crystallite size was found to be in the range of (23-43 nm) from Camellia sinensis extract and (20-99 nm) from Apium graveolens extract.

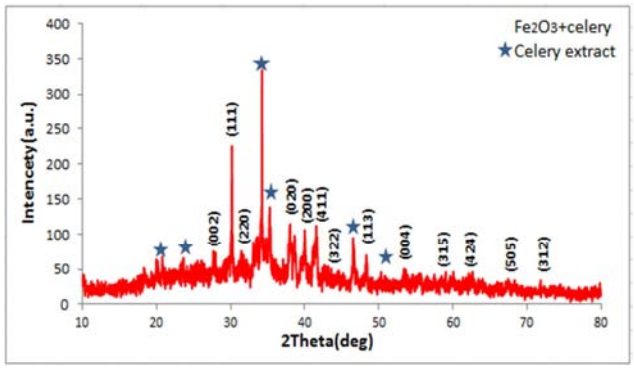

Figure 2. *XRD pattern of Iron oxide nanoparticles using Camellia sinensis extract.*

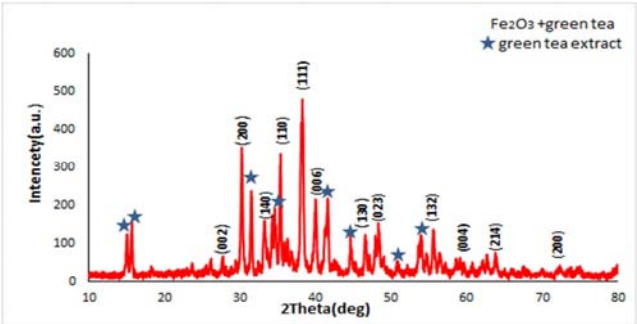

Figure 3. *XRD pattern of Iron oxide nanoparticles using Apium graveolens extract.*

3.2. FE-SEM Analysis

The FE-SEM images of Iron Oxide nanoparticles are shown in Figure 4. The morphology of the nanoparticles indicates irregular, cubic and hexagonal shapes of various sizes that are agglomerated. Further observations with higher magnifications reveal these images possess smooth surfaces. At much higher magnification the images are seen as large particles which can be attributed to aggregation or clustering of smaller particles.

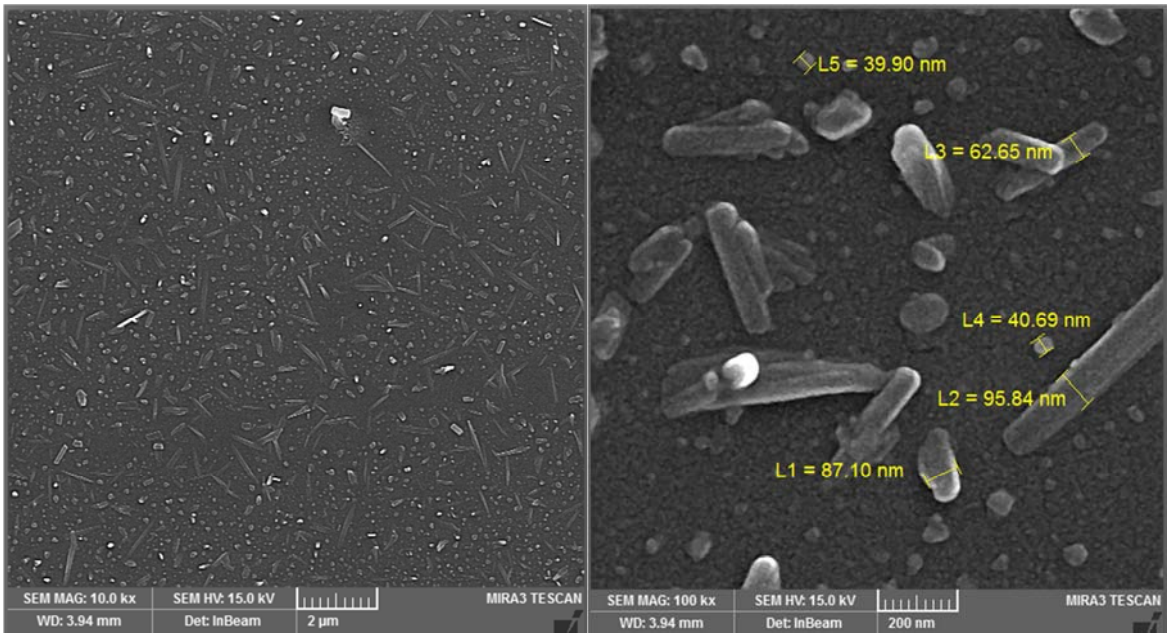

Figure 4. *FE-SEM of Iron oxide nanoparticles preparing using Camellia sinensis extract.*

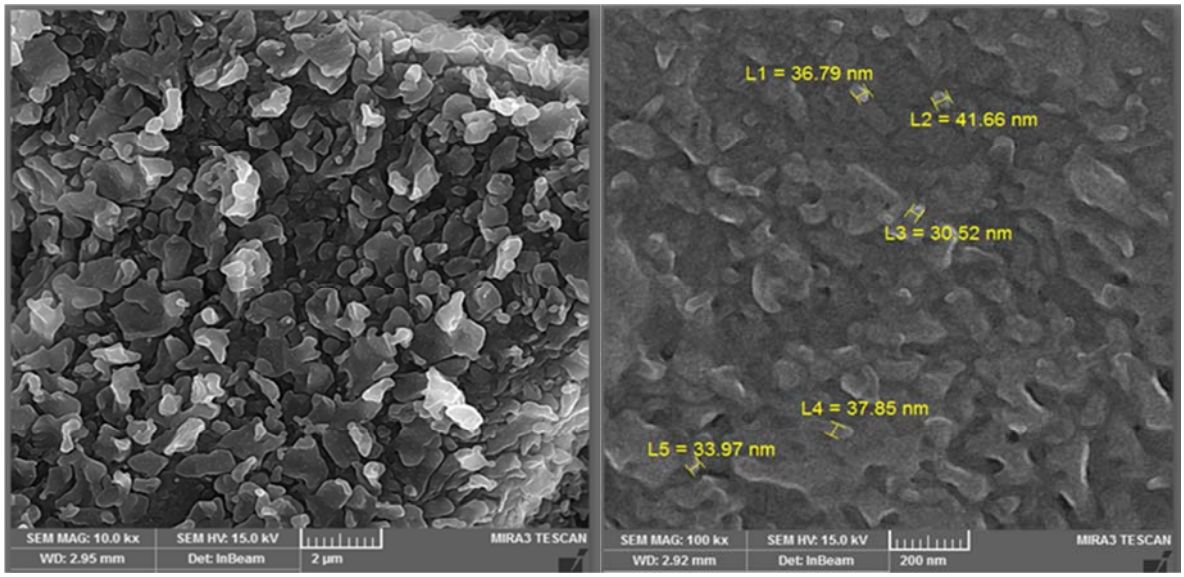

Figure 5. *FE-SEM of Iron oxide nanoparticles preparing using Apium graveolens extract.*

3.3. UV-Visible Spectroscopy

The formation of Iron nanoparticles was first confirmed based on a change in color of the reaction mixture at room temperature from light brown to dark brown within 15 min. This was followed by UV-vies spectroscopy which is frequently used to characterize synthesized metal nanoparticles. Figure 6 shows the UV–vies absorption spectrum of the synthesized Iron nanoparticles. The maximum absorption peaks are (220 nm) for camellia sinensi and (225 nm) for Apium graveolens, The energy band gaps are (2.9 eV) of camellia sinensi extract and (4.01 eV) of Apium graveolens extract as a result of quantum confinement and small molecules as shown in Figure 7.

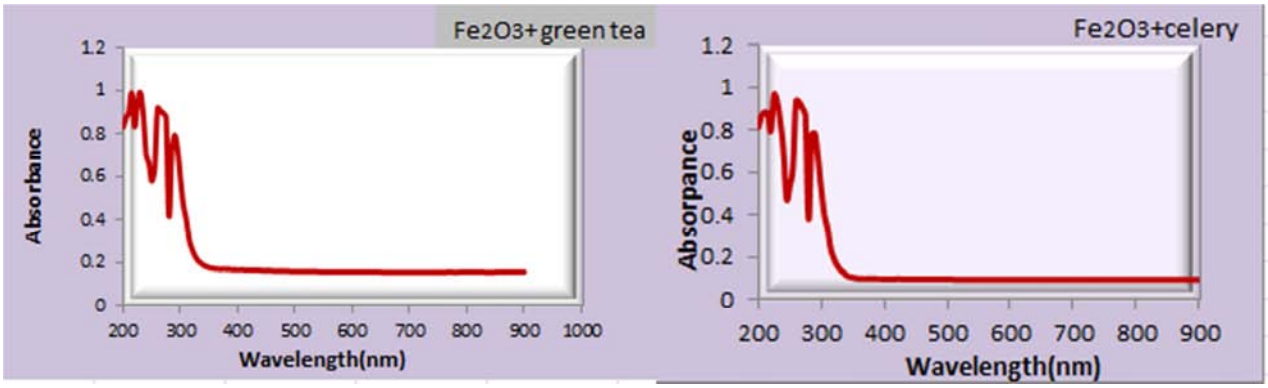

Figure 6. *UV-Vis absorption spectra of Iron oxide NPs with Camellia sinensis extract and Apium graveolens extract.*

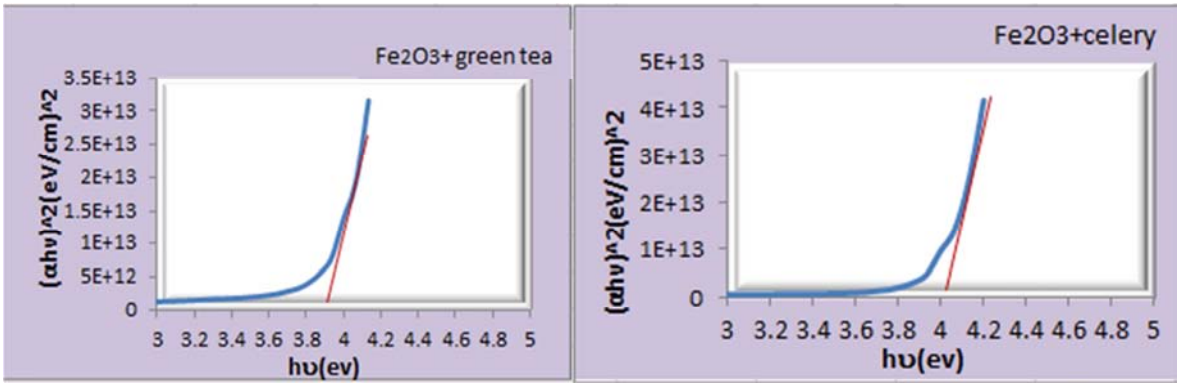

Figure 7. *The energy band gap of Iron oxide Nanoparticles with Camellia sinensis extract and Apium graveolens extract.*

3.4. Antibacterial Susceptibility Assay

The inhibition zone of Iron oxide nanoparticles biofabricated from the Apium graveolens and camellia sinensis extracts against two pathogens is shown in Figure 8, Table 1. And Both each of Gram-negative (p. aerugino) and Gram-positive (S. aureus) bacteria organisms were used in this study. Human pathogens capable of causing diseases ranging from skin infections, pneumonia, sepsis, toxic shock syndrome, urinary tract infections, vomiting, anaemia, kidney infections, osteomyelitis, septicemia, lung infection to wound infections. The surfaces of the Iron oxide nanoparticles might have interacted directly with the bacterial outer membrane, causing the membrane to rupture thereby killing the organism. So, the antibacterial activity exhibited by the Iron nanoparticles here is attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes. Result was used as a positive control in the experiment. The minimum inhibitory concentration (MIC) of the Iron oxide nanoparticles, Note that the presence of plant extracts increase the effectiveness of material nanoparticles as green tea extract increase the proportion of negative bacteria killing of 20 mm with celery extract and 18 mm without the extract to 31 mm with leaves extract and the presence of plant extracts increase the effectiveness of oxide nanoparticles as green tea extract increase the proportion of positive bacteria killing of 18 mm with celery extract and 17 mm without the extract to 25 mm with leaves extract.

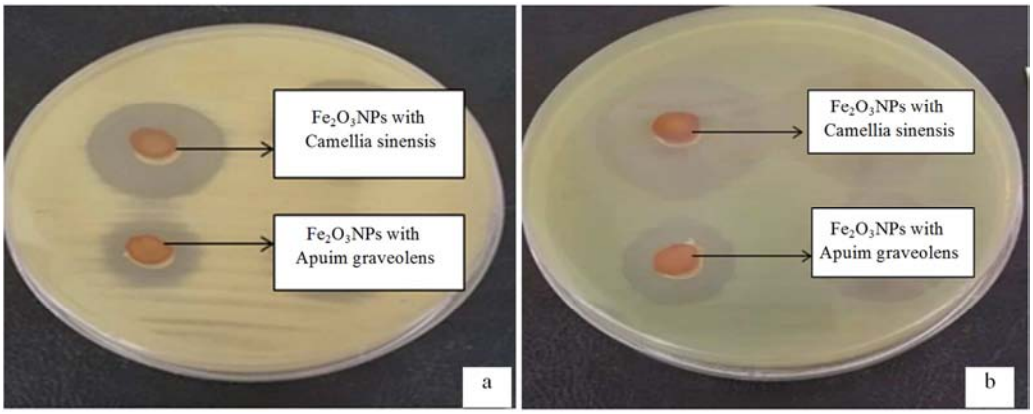

Figure 8. *Rate of inhibition for bacteria Pseudomonas a eruginosa a) S. aureus b) P. aeruginosa for Iron oxide NPs of plant extracts.*

Table 1. *Zone Inhibition (mm) of Iron oxide NPs against pathogens.*

Type of material nanoparticles used	Zone of inhibition (mm) at 200µg/ml concentration S. aureus	Zone of inhibition (mm) at 200µg/ml concentration p. aerugino	Percentage of Inhibition (%) S. aureus	Percentage of Inhibition (%) P. aerugino
Fe_2O_3 NPs pure	17	18	18.8	20
Fe_2O_3 with Apium graveolens	18	20	20	22.2
Fe_2O_3 with Camellia sinensi leaves	25	31	27.8	34.4

4. Conclusions

Iron oxide nanoparticles were synthesized using camellia sinensi leaves and Apium graveolens extracts as a green method of nanoparticles synthesis that does not introduce harmful substances into the environment and ensures cost effectiveness. The particle size has been calculated to be in the range (20–99 nm). These Iron oxide nanoparticles inhibited the growth of S. aureus, p. aerugino. Therefore, it is pertinent to conclude that the Iron oxide nanoparticles could be used in the treatment of diseases and infections caused by these organisms. The best results were obtained from chemical preparation simple and clear differentiation peake of Fe_2O_3 NPs with green tea and best to inhibition the existence of green tea extract was (25-31mm) of S. aureus and P. aerugino bacteria respectively.

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