



## Utilizing *Camellia Sinensis* Extract for the Biosynthesis of Iron Oxide Nanoparticles and Assessing their Antibacterial Effectiveness

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**ABSTRACT:** In this study, an extract from green tea leaves (*Camellia sinensis*) was prepared using 5 grams of leaves in 100 ml of distilled water, then the mixture was heated to 80 °C using a magnetic stirrer to obtain the extract for half an hour. After cooling, iron oxide particles were synthesized by adding 0.01 molar iron chloride to 100 ml of distilled water and using a magnetic stirrer for half an hour. The extract was mixed in ratios of 1:1, 1:2, and 2:3 with iron chloride, and the mixture immediately turned black. The results showed that the light absorption of iron oxide particles decreased with increasing ratios, resulting in a shift towards longer wavelengths. The indirect band gap increased from 3.2 eV at a 1:1 ratio to 3.3 eV at a 2:3 ratio, which may be attributed to the confinement effect. X-ray diffraction (XRD) patterns show the amorphous nature of the particles, and crystal sizes were estimated using the Scherrer formula. In terms of surface properties, particle aggregation was observed in the 1:1 and 2:3 ratios, while the 1:2 ratio had a more uniform distribution. The results showed that iron oxide particles have antibacterial activity against *Staphylococcus aureus*, with a highest inhibition zone at a ratio of 3:2. This indicates their potential in managing infections caused by these organisms.

**KEYWORDS:** Inhibition of bacterial, iron oxide, green method, tea extract, Gram-positive *S. aureus*, Optical characteristics, Structural characteristics.

**Cite the Article:** Najeeb, I. R., AL Mutleb Hamood, S.A., gabal, B.C., Hussein, N.N. (2026). Utilizing *Camellia Sinensis* Extract for the Biosynthesis of Iron Oxide Nanoparticles and Assessing their Antibacterial Effectiveness. *Contemporary Research Analysis Journal*, 3(5), 319–326. <https://doi.org/10.55677/CRAJ/06-2026-Vol03105>

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Publication Date: May 13, 2026

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### 1. INTRODUCTION

One of the most important technologies of the twenty-first century is nanotechnology. One important advancement in the science of nanotechnology is the creation of new nanomaterials with a variety of uses. Nanoparticles and nanomaterials are produced using both top-down and bottom-up approaches [1,12]. By using a range of mechanical processes to divide the bulk material into nanoscale size, the top-down process produces metal nanoparticles. Metal nanoparticles are produced bottom-up from atoms or molecules to molecular structures of nanoscale size using a range of chemical or biological processes [3,4]. Numerous disciplines, including optics, mechanics, biotechnology, engineering, remediation, microbiology, environmental medicine, and electronics, can make use of these nanoparticles. They range in size from 1 to 100 nm and have a large surface area to volume ratio. [5]. It is interesting to note that because nanoparticles are non-toxic, non-immunogenic, and can be altered to target certain uses, they have a wide range of applications [6]. Bio-nanotechnology techniques have emerged as the main focus of numerous research disciplines in recent years in an effort to create safe nanomaterials with a variety of applications, particularly in a pharmaceutical and biomedical industries [7–9]. As a result, biological methods have been developed where green synthesis offers greater benefits than traditional techniques. According to numerous studies, metal nanoparticles produced using green methods are simple, quick, affordable, nontoxic, environmentally benign, and easily scalable for large-scale synthesis [10–14]. Because iron oxide nanoparticles may interact with a variety of bacterial compounds and prevent the growth of microorganisms, they offer a viable approach to reducing pathogenicity and resistance to antimicrobial drugs [15]. The most stable of the many iron oxide polymorphs is hematite ( $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>), which has strong chemical stability, biocompatibility, tuneable optical and magnetic properties, and anti-corrosive properties that make it

## Utilizing Camellia Sinensis Extract for the Biosynthesis of Iron Oxide Nanoparticles and Assessing their Antibacterial Effectiveness

affordable and appropriate for a variety of technological applications. Novel nanotechnologies for gas sensors, water splitting, water purification, anticorrosive agents, high-density magnetic storage medium, catalysts, and solar energy conversion were made possible by hematite's advantages [16-18]. In addition, because of their great chemical stability, low toxicity, and biocompatibility,  $\alpha$ - $\text{Fe}_2\text{O}_3$  nanoparticles are better suited for biomedical applications [19]. Aim of the work biosynthesis of Iron Oxide nanoparticles using Camellia sinensis Extract and evaluation of their antibacterial efficiency.

### 2. MATERIALS AND METHODS

Green tea (*Camellia sinensis*) leaf extract was made by heating 5 grams of leaves in 100 milliliters of distilled water to 80 degrees Celsius for 30 minutes with a magnetic stirrer. After cooling, 0.01 molar iron chloride was added to 100 ml of distilled water, and the mixture was stirred with a magnetic stirrer for 30 minutes to create iron oxide particles. When iron chloride was added to the extract in 1:1, 2:1, and 3:2 ratios, the mixture instantly became black. The hue shift happened right away. The supernatant layer was disposed of once the solution was decanted. To get rid of any contaminants, the particles were cleaned twice more and then decanted once more. They were dried for an hour at 60°C in an electric oven. The produced  $\text{Fe}_2\text{O}_3$  nanoparticles' antibacterial activity against Gram-positive *S. aureus* bacteria was assessed using Mueller-Hinton agar medium and the conventional zone of inhibition method. Mueller-Hinton broth was used to cultivate the bacterial strain for 16–20 hours at 37°C.

### 3. RESULTS AND DISCUSSION

#### 3.1 Optical characteristics

##### 3.1a. The optical absorption

The optical absorption spectra are displayed in Figure 1. of composite  $\text{Fe}_2\text{O}_3$  nanoparticles at different material ratios. The variation in absorption may result from the variation in the concentration of nanoparticles within the solution. As the absorption decreased with increasing material ratios, the edges of the optical absorption shifted toward longer wavelengths (red shift) this behaviour close to obtained by [20].

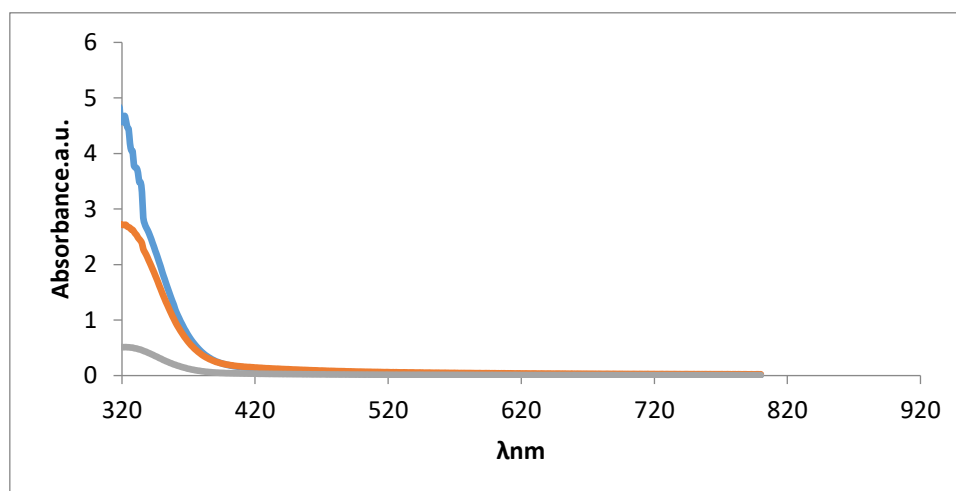


Figure 1: optical absorption spectra of composite  $\text{Fe}_2\text{O}_3$  nanoparticles at different material ratios

##### 3. 1.b Optical bandgap

The indirect optical bandgap of the  $\text{Fe}_2\text{O}_3$  NPs shown in Figure 2. was increased from 3.2 in 1:1, 3.25 in 1:2 eV to be 3.3 eV in 2:3. this behaviour close to obtained by [20] which might be attributed to the confinement effect [20]. A bandgap of an examined samples can be evaluated by Tauc's relation as following [21]:

$$\alpha h\nu = C (h\nu - E_g)^x \dots\dots\dots (1)$$

## Utilizing Camellia Sinensis Extract for the Biosynthesis of Iron Oxide Nanoparticles and Assessing their Antibacterial Effectiveness

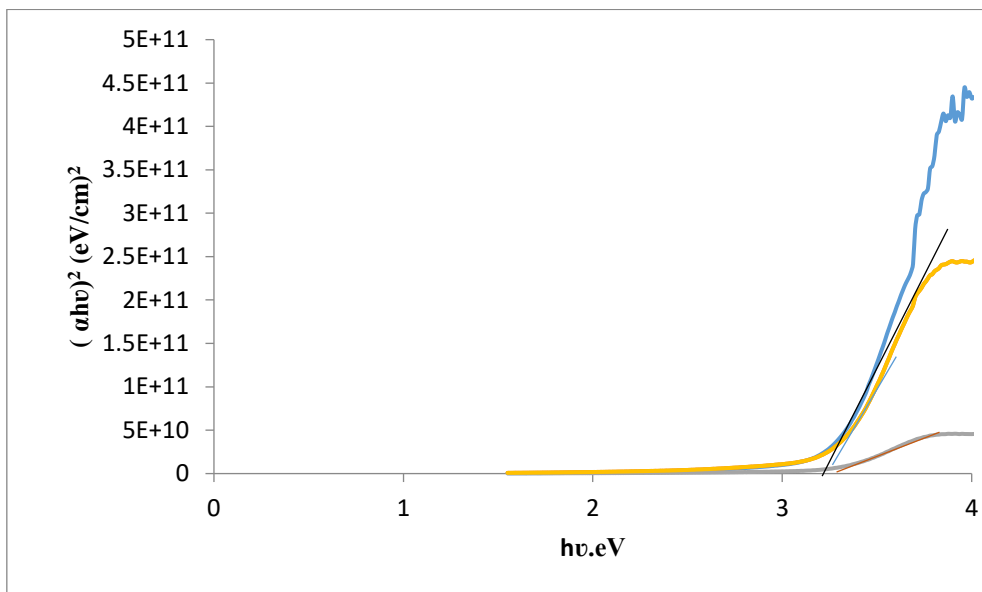


Figure 2: optical bandgap of composite Fe<sub>2</sub>O<sub>3</sub> nanoparticles at different material ratios

### 3.2 Structural characteristics

Figure (3a,3b,3c). The XRD pattern of the sample shows an amorphous nature, characterized by the absence of sharp peaks. This pattern is explained by the tiny size of the nanoparticles formed by the green method and their coating with organic compounds extracted from green tea, which inhibits long-term crystalline arrangement. The crystallite size has been evaluated using Scherrer's formula based on information obtained from the XRD patterns [22,23].

$$D = (K \lambda) / (\beta \cos \theta) \dots\dots\dots (2)$$

The size of the crystals varies according to the proportions of the manufactured samples, as shown in Table 1.

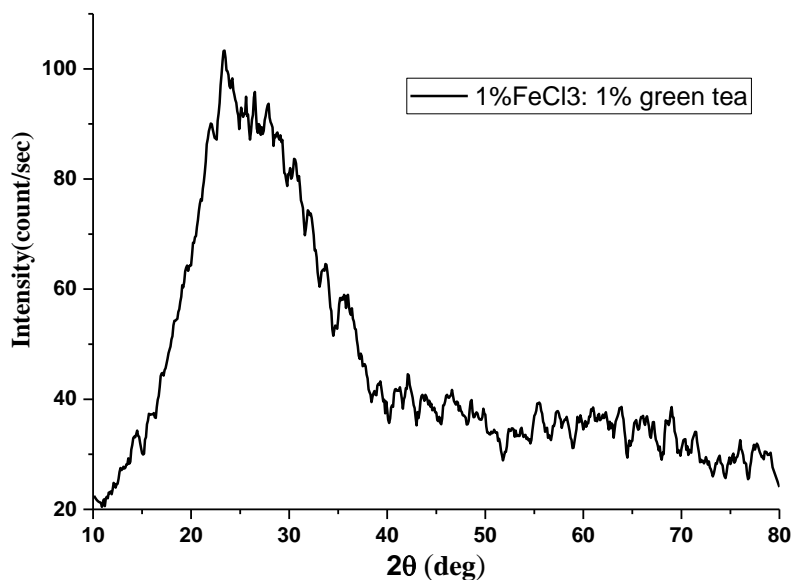


Figure 3a: X-ray diffractograms of the synthesized Fe<sub>2</sub>O<sub>3</sub> nanoparticles in 1:1

## Utilizing Camellia Sinensis Extract for the Biosynthesis of Iron Oxide Nanoparticles and Assessing their Antibacterial Effectiveness

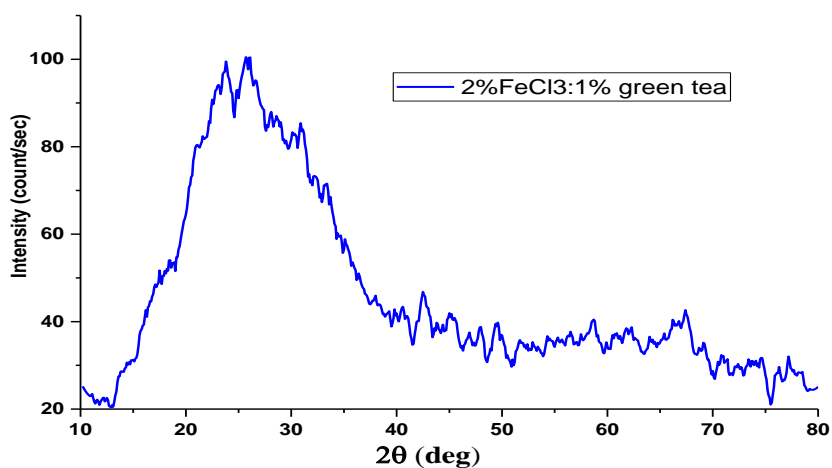


Figure 3b: X-ray diffractograms of the synthesized Fe<sub>2</sub>O<sub>3</sub> nanoparticles in 2:1

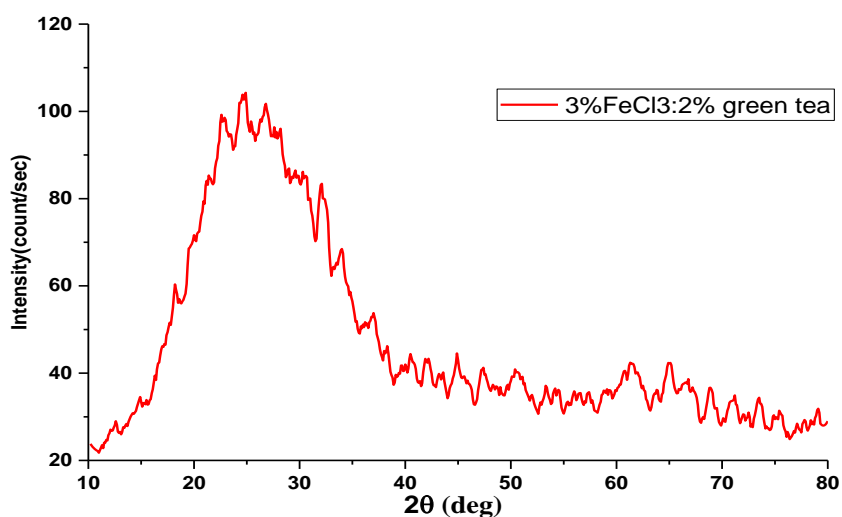


Figure 3c: X-ray diffractograms of the synthesized Fe<sub>2</sub>O<sub>3</sub> nanoparticles in 3:2

Table 1: X-ray diffractograms of the synthesized Fe<sub>2</sub>O<sub>3</sub> nanoparticles

2 $\theta$	I%	FWHM	D nm	hkl
22.129	94.4	0.195	42.2	012
23.657	100	0.534	15.4	104
28.272	91.6	0.433	19.2	110
43.033	42.1	0.202	42.8	113
43.894	47.7	0.163	53.2	202
55.345	41.1	0.323	28.4	024
66.269	43.9	0.175	55.4	116
77.483	35.5	0.187	55.9	300
23.931	100	0.182	44.6	012
42.104	45.5	0.34	24.9	113
49.800	37.5	0.57	15.3	024
51.750	42	0.159	55.3	116
32.24	100	0.252	32.8	110
33.719	83	0.214	38.7	104

## Utilizing Camellia Sinensis Extract for the Biosynthesis of Iron Oxide Nanoparticles and Assessing their Antibacterial Effectiveness

### 3.3 FESEM of iron oxide nanoparticles

Figure (4a,4b,4c) illustrates the surface morphology of iron oxide nanoparticles synthesized using the green method at different ratios, with the aqueous solution then deposited onto a glass slide by distillation. Figure 4a,4c demonstrates that the iron oxide nanoparticles prepared at 1:1 and 3:2 ratios aggregate into larger crystals, and the surface of the samples appears free of any microscopic holes or cracks. In contrast figure 4b, the 1:2 ratio results in a more homogeneous distribution and smaller particle size, this behaviour close to obtained by [20].

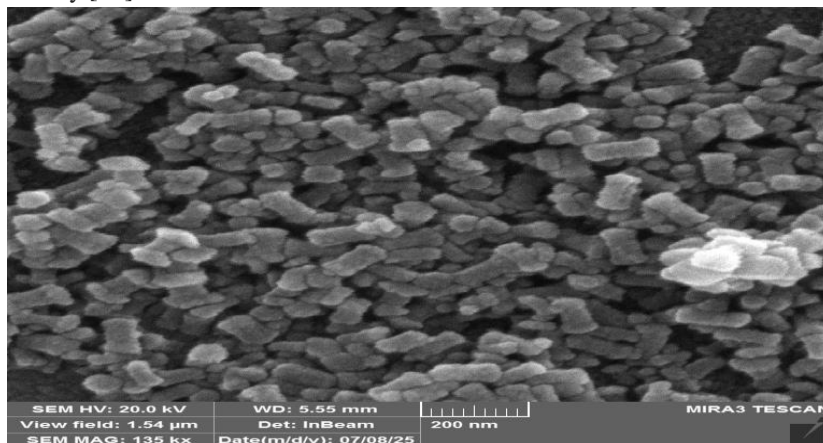


Figure 4a: Surface morphology of iron oxide nanoparticles in 1:1.

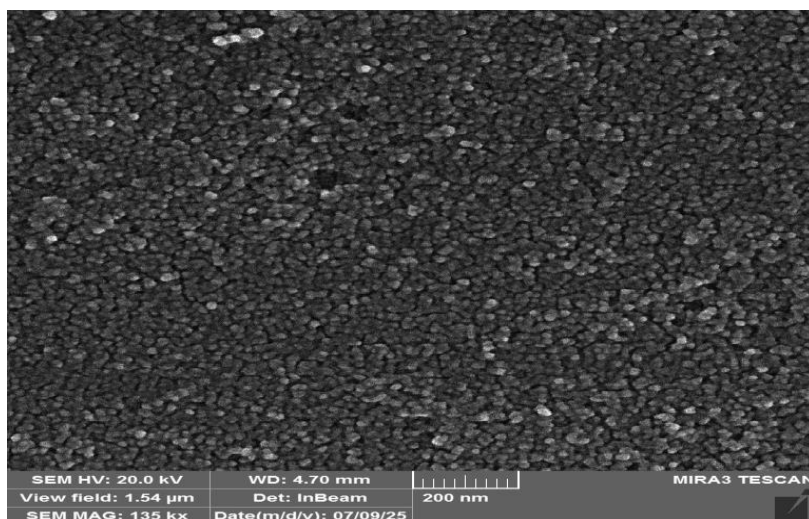


Figure 4 b: Surface morphology of iron oxide nanoparticles in 2:1.

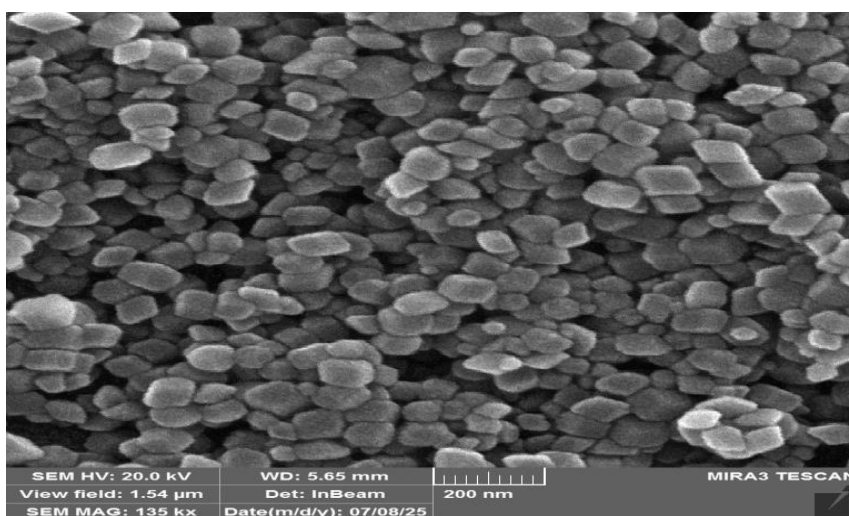


Figure 4c: Surface morphology of iron oxide nanoparticles in 3:2.

## Utilizing *Camellia Sinensis* Extract for the Biosynthesis of Iron Oxide Nanoparticles and Assessing their Antibacterial Effectiveness

### 3.4 The iron oxide nanoparticles' EDS spectrum

Strong oxygen (O) peaks in the EDS spectrum of iron oxide nanoparticles made with green tea extract verify that oxygen (O) is the main element present (Figure 5a,5b,5c). Furthermore, oxidation-induced iron oxide production is suggested by oxygen (O) peaks. O (60.30 wt%) and Fe (39.70 wt%) are shown by EDS quantification.

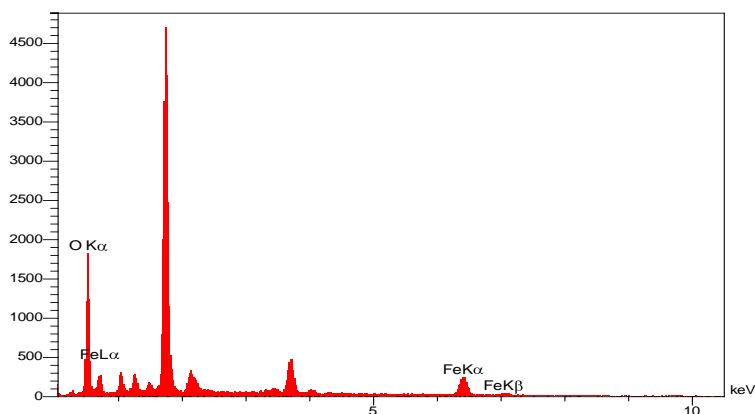


Figure 5a: the EDS spectrum of iron oxide nanoparticles in 1:1.

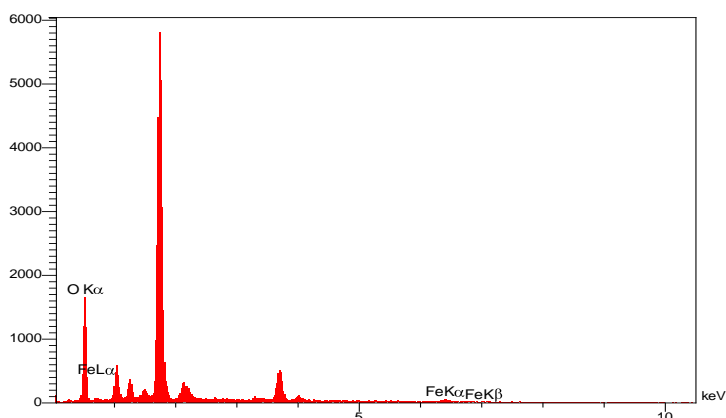


Figure 5b: the EDS spectrum of iron oxide nanoparticles in 2:1.

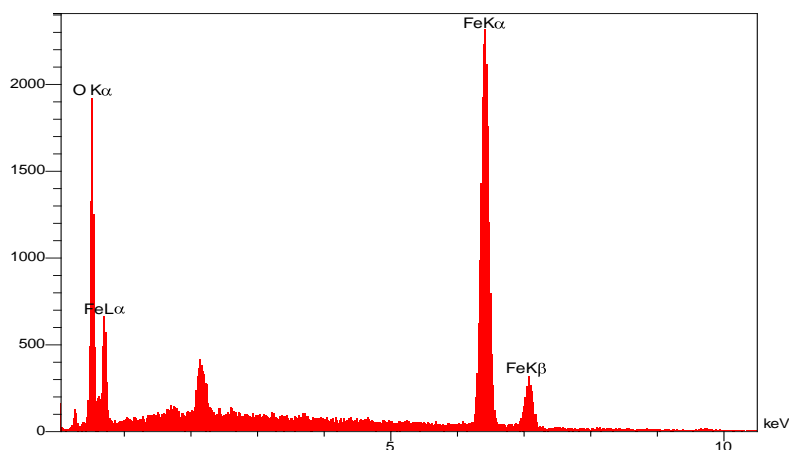


Figure 5c: the EDS spectrum of iron oxide nanoparticles in 3:2.

## 4. ANTIBACTERIAL CHARACTERISTICS

A plate of the Gram-positive bacterial strain *Staphylococcus aureus* is displayed in Figure 6, along with the organism's zone of inhibition at different iron oxide nanoparticle (IONP) concentrations. Agar diffusion method used to measure an inhibition zones in millimeters. The measured diameters varied overall. The most sensitive Gram-positive bacterium was *S. aureus*, which showed greater activity for iron oxide nanoparticles made with green tea extract at a 3:2 ratio than for the other ratios. Additionally, it was discovered that the concentration and the inhibition zone were closely proportionate.

## Utilizing *Camellia Sinensis* Extract for the Biosynthesis of Iron Oxide Nanoparticles and Assessing their Antibacterial Effectiveness

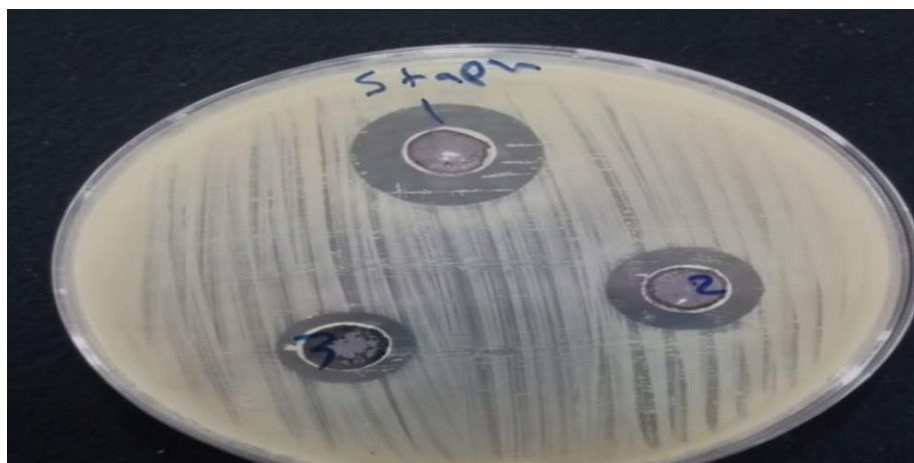


Figure 6: zone of inhibition of the strain at varying concentrations of iron oxide nanoparticles

### CONCLUSION

Green tea leaves were employed to produce iron oxide nanoparticles. is an economical and ecologically good way to create nanoparticles without harming the environment. *S. aureus* was prevented from growing by these iron oxide nanoparticles. Consequently, It makes sense to suggest that iron oxide nanoparticles could be used to treat diseases and infections caused by these organisms. A highest differentiation of  $\text{Fe}_2\text{O}_3$  nanoparticles with green tea was best prepared chemically in a straightforward and understandable manner, and the best suppression of *Staphylococcus aureus* bacteria was obtained when green tea extract was present at a ratio of 3:2.

### REFERENCES

1. H. Barabadi, M. Najafi, H. Samadian, A. Azarnezhad, H. Vahidi, M.A. Mahjoub, M. Koohiyan, A. Ahmadi, A systematic review of the genotoxicity and antigenotoxicity of biologically synthesized metallic nanomaterials: are green nanoparticles safe enough for clinical marketing? *Med.* 55 (2019) 1e16,
2. A.K. Mittal, Y. Chisti, U.C. Banerjee, Synthesis of metallic NPs using plant extracts, *Biotechnol Adv.* 31 (2013) 346e356,.
3. Alsultan, H. K. A. A. K., & Guda, M. A. (2026, March). Green synthesis and characterization of zirconium oxide nanoparticles using *Matricaria Chamomilla L.* plant extracts for antibacterial application. In *AIP Conference Proceedings* (Vol. 3396, No. 1, p. 080001). AIP Publishing LLC. <https://doi.org/10.1063/5.0318554>
4. H.D. Yu, M.D. Regulacio, E. Ye, M.Y. Han, Chemical routes to top-down nanofabrication, *Chem Soc Rev.* 42 (2013) 6006e6018.
5. I. Khan, K. Saeed, I. Khan, Nanoparticles: properties, applications and toxicities, *Arab J Chem.* 12 (2019) 908e931.
6. L. Aakash, B.S. Vinduja, T. Arunkumar, T. Sivakumar, D. Gajalakshmi, A systematic review on green synthesis of nanoparticles and its medical applications, *J Xidian Univ.* 14 (2020) 6069e6076,.
7. Alshebly, E. A. J., & Guda, M. A. (2026, March). Green synthesis of silver oxide nanoparticles and their effect on inhibition growth of bacteria. In *AIP Conference Proceedings* (Vol. 3396, No. 1, p. 030008). AIP Publishing LLC. <https://doi.org/10.1063/5.0318556>
8. S. Ahmad, S. Munir, N. Zeb, A. Ullah, B. Khan, J. Ali, M. Bilal, M. Omer, M. Alamzeb, S.M. Salman, S. Ali, Green nanotechnology: a review on green synthesis of silver nanoparticles -An ecofriendly approach, *Int J Nanomed.* 14 (2019) 5087e5107,.
9. S. Bayda, M. Adeel, T. Tuccinardi, M. Cordani, F. Rizzolio, The history of nanoscience and nanotechnology: from chemical-physical applications to nanomedicine, *Molecules.* 25 (2020) 1e15.
10. P. Khandel, R.K. Yadaw, D.K. Soni, L. Kanwar, S.K. Shahi, Biogenesis of Metal Nanoparticles and their pharmacological applications: present status and application prospects, *J Nanostructure Chem.* 8 (2018) 217e254,.
11. H. Bahrulolum, S. Nooraei, N. Javanshir, H. Tarrahimofrad, V.S. Mirbagheri, A.J. Easton, G. Ahmadian, Green synthesis of metal nanoparticles using microorganisms and their application in the agrifood sector, *J Nanobiotechnol.* 19 (2021) 1e26,.
12. S. Patil, R. Chandrasekaran, Biogenic nanoparticles: a comprehensive perspective in synthesis, characterization, application and its challenges, *J Genet Eng Biotechnol.* 18 (2020),.
13. J. Singh, T. Dutta, K.H. Kim, M. Rawat, P. Samddar, P. Kumar, Green synthesis of metals and their oxide nanoparticles: applications for environmental remediation, *J Nanobiotechnol.* 16 (2018) 1e24,.

## Utilizing *Camellia Sinensis* Extract for the Biosynthesis of Iron Oxide Nanoparticles and Assessing their Antibacterial Effectiveness

14. P. Velusamy, G.V. Kumar, V. Jeyanthi, J. Das, R. Pachaiappan, Bio-inspired green nanoparticles: synthesis, mechanism, and antibacterial application, *Toxicol Res.* 32 (2016) 95e102.
15. Zúñiga-Miranda J, Guerra J, Mueller A, Mayorga-Ramos A, Carrera-Pacheco SE, Barba-Ostria C, Heredia-Moya J, Guamán LP, Iron Oxide nanoparticles: Green Synthesis and their antimicrobial activity. *Nanomaterials* (2023),13(22):2919. 10.3390/nano13222919 10.3390/nano13222919.
16. Alabassi MM, Guda MA, Muhammed MA. The removal efficiency of natural nano-coagulant produced from *Phragmites communis*, *Schanginia aegyptiaca* and *Portulaca oleracea* in wastewater treatment. *Int J Aquat Biol.* 2022;10(2):181-6.
17. Husayn DM, Guda MA. Response of some wild plants in antioxidant enzymes by zinc oxide nanoparticles. *AIP Conf Proc.* 2023;2787(1):90049. doi:10.1063/5.0148199.
18. Guda MA. Retraction: Response of antioxidant in some plants to iron oxide nanoparticles. *AIP Conf Proc.* 2023;2977(1):40144. doi:10.1063/5.0182305.
19. S. Naz, M. Islam, S. Tabassum, N.F. Fernandes, E.J.C. Blanco, M. Zia, Green synthesis of hematite ( $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>) nanoparticles using *Rhus punjabensis* extract and their biomedical prospect in pathogenic diseases and cancer, *J. Mol. Struct.* 1185 (2019) 1–7
20. Wisam Jafer Aziz, Aliyaa Abd Urabe, Chemical Preparation of Iron Oxide Nanoparticles Using Plants Extracts in Antibacterial Application, *International Journal of Bioorganic Chemistry*, 2019; 4(1): 1-6.
21. Guda MA, Younus AS, Altamimi AJT, Ayoub AF, Al Regawi SM, Al-Edhari AHM, et al. Effect of irrigation with magnetized water on some vegetative and flowering parameters of wheat (*Triticum aestivum* L.) cultivar IP A 99. *ARN J Eng Appl Sci.* 2019;14(2):5466-70. doi:10.36478/JEASCI.2019.5466.5470.
22. Nesma H. Ibrahim, Gharib M. Taha, Noura Sh. A. Hagaggi and Marwa A. Moghazy, Green synthesis of silver nanoparticles and its environmental sensor ability to some heavy metals, *BMC Chemistry*, (2024) 18:7, pp.1-34.
23. Inass Abdulah Zgair, Adel H. Omran Alkhayatta, Asmahana Asaad Muhmood, Shaymaa K. Hussain, Investigation of structure, optical and photoluminescence characteristics of Li doped CuO nanostructure thin films synthesized by SILAR method, *Optik - International Journal for Light and Electron Optics* 191 (2019) 48–54

. 24