Electronic Supplementary Information (ESI)

Green Synthesis of Iron Oxide Nanoparticles using *Psidium guajava* L. Leaves Extract and its Application Towards Degradation of Organic Dyes and Anti-microbial Activities

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1.1 Chemicals, reagents and test organisms

Iron (III) chloride hexahydrate (Merck), Iron (II) chloride tetrahydrate (Merck), sodium hydroxide (Fisher Scientific), methylene blue, methyl orange, H_2O_2 (30.0%, Merck 64271), DMSO (50%), distilled water, and Whatman filter paper 1 like chemicals, solvents and materials were used. The model bacterial pathogens *Shigella sonnei, Staphylococcus aureus,* and *Escherichia coli*, along with fungal pathogens *Candida tropicalis* and *Candida albicans* were collected from Research Institute for Bioscience and Biotechnology (RIBB), Chyasal Road, Lalitpur, Nepal.

1.2 Characterization of iron oxide nanoparticles

The formation of IONPs was confirmed by analyzing the absorption band recorded with the help of the SPECORD 200 PLUS, analytikjena (An Endress+Hauser Company) UV-Visible spectrophotometer. Scanning range was maintained in the range of 250 – 600 nm at a medium scanning rate and resolution of 1 nm. Distilled water was used as a blank reference for the baseline correction of the spectrophotometer during the experiment. All the samples were loaded into a 2 mL quartz cuvette with a 1 cm path length.

The presence of secondary metabolites in the *Psidium guajava* extract and IONPs were investigated empirically using FTIR (Nicolet iS50 FT-IR), with spectra scanned in the range of 4000–400 cm^1.

XPS measurements were carried out on Nexsa XPS system (ThermoFisher Scientific UK). The experimental condition was adjusted to, base pressure: 2.0×10^{-7} Torr, beam size: 400 μm, pass energy: 50 eV, dwell time: 50 ms, and step: 0.1 eV/step. All samples C_{1s}, N_{1s}, O_{1s}, and Fe_{2p} signals were acquired using Al Kα monochromatized X-ray radiation (hv = 1486.6 eV).

For determining crystallinity of the IONPs, a routine powder X-ray diffraction pattern was recorded using the Rigaku D/MAX-2500/pc diffractometer with monochromatic Cu Kα radiation of wavelength 1.540598 Å 2θ scanning between 5° and 80°. The crystallite size of powdered IONPs was determined via Scherrer's equation [7].

$$D = \frac{k\lambda}{\beta\cos\theta}$$

where,

D = crystalline grain size,

k = dimensionless shape factor, with a value close to unity,

λ = wavelength of x-ray radiation

θ = Bragg's angle (half of the 2θ value of chosen peak), (in radians), and

β = full width at half maximum, FWHM (in radians)

Field emission scanning electron microscopy (FE-SEM) was used to study the morphology of nanoparticles running at a voltage of 10 kV.

High resolution transmission electron microscopy (HR-TEM) was used to visualize the size and morphology of nanoparticles.

To determine the stability of the colloidal nanostructure system, the zeta potential of the IONPs was measured using a HORIBA Scientific SZ-100V2. IONP samples were adjusted to 2, 4, 6, 8, 10, and 12 pH with NaOH. Subsequently, 1 mL of the pH-adjusted sample was injected into a flow cell. All samples were measured at a fixed temperature and angle of 25°C and 17°, respectively.

2.1 XPS analysis of biosynthesized IONPs

Table S1

Name	Peak (BE)	FWHM (eV)	Area CPS (eV)	Atomic %
C 1s	284.36	1.53	106140.1	29.33
N 1s	406.85	1.21	14026.07	2.51
O 1s	530.46	3.34	538697.3	61.54
Fe 2p	709.49	3.66	247298.2	6.62

BE and FWHM indicates binding energy and full width at half maxima respectively

2.2 Calculation of average crystallite size biosynthesized IONPs using XRD

Table S2

No.	2 θ in degree	FWHM in degree	h	k	l	θ in radian	FWHM in radian	Size (nm)	Average size (nm)
1	31.68845	0.16236	1	1	1	0.277274	0.002841	49.00254	21.75
2	32.56763	0.454608	2	2	0	0.284967	0.007956	17.46207	
3	35.15226	0.64944	3	1	1	0.307582	0.011365	12.13935	
4	45.46933	0.454608	4	0	0	0.397857	0.007956	16.77468	
5	56.4963	0.32472	4	2	2	0.494343	0.005683	22.4245	

2.2 Degradation of methylene blue by biosynthesized IONPs

Table S3

Time (min)	Maximum Absorbance (A)	Degradation %
0	4.1122	0
5	3.5123	14.58829823
15	2.8094	31.68133846
25	2.481	39.66733136
35	2.381	42.09911969
45	2.22191	45.96785176
55	1.85382	54.91902145
65	1.26956	69.12698799
75	0.88405	78.50177521
85	0.816	80.15660717
95	0.736	82.10203784

2.3 Degradation of methyl orange by biosynthesized IONPs

Table S4

Time (min)	Maximum Absorbance (A)	Degradation %
0	5.6859	0
5	4.6373	18.44211119
25	4.6373	18.44211119
45	4.7977	15.6210978
65	4.0446	28.86614256
85	4.06	28.59529714
105	3.6184	36.36187763
125	3.5184	38.12061415
145	3.2184	43.39682372
165	3.0184	46.91429677

185	2.8184	50.43176982
205	2.6184	53.94924286

2.4 Anti-bacterial activity of biosynthesized IONPs

Table S5

Bacteria	Diameter of zone of inhibition (ZOI) in mm			
	Neomycin (PC) (20 µL)	IONPs (20 µL)	Plant extract (PE) (20 µL)	50% DMSO(NC) (20 µL)
S. sonnei	30	13	12	-
S. aureus	23	13	11	-
E. coli	18	-	11	-

2.5 Anti-fungal activity of biosynthesized IONPs

Table S6

Fungi	Diameter of zone of inhibition (ZOI) in mm			
	Clotrimazole (PC) (20 µL)	IONPs (20 µL)	Plant extract (PE) (20 µL)	50% DMSO(NC) (20 µL)
C. tropicalis	25	15	11	-
C. albicans	22	13	7	-

Figure S1. Visible color change illustrating the formation of IONPs

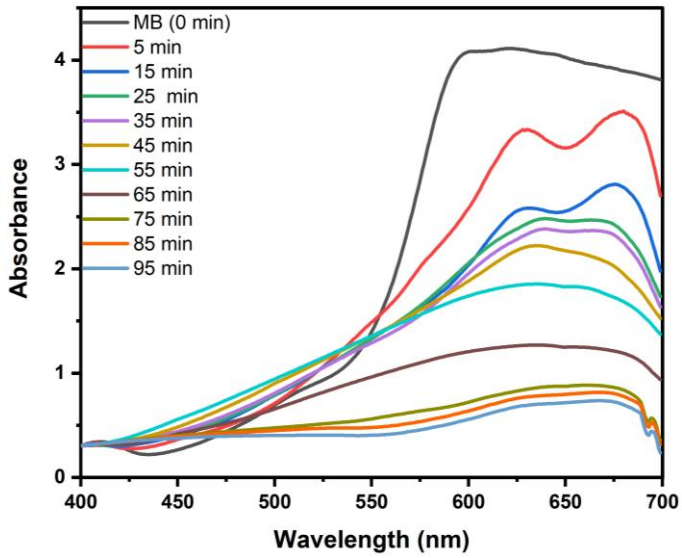

Figure S2. UV-Vis absorption of methylene blue at a different time in presence of IONPs

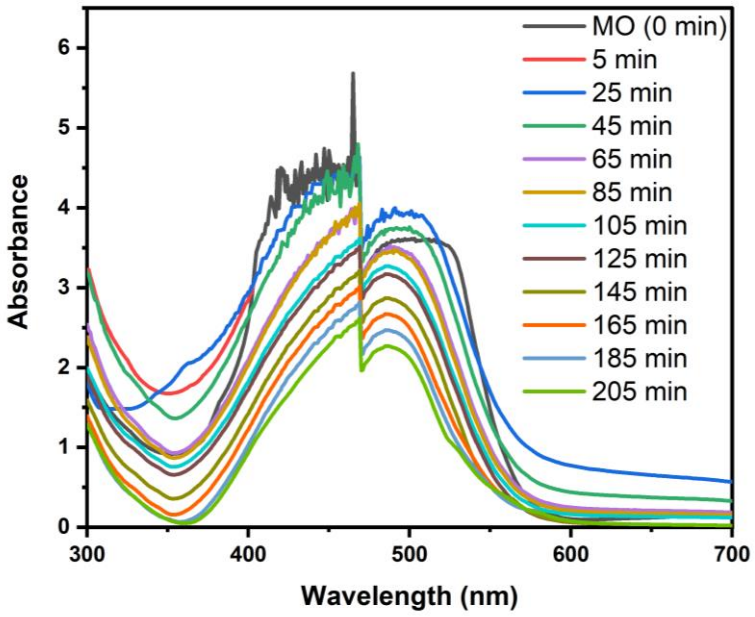

Figure S3. UV-Vis absorption of methyl orange at a different time in presence of IONPs