

Title

Author

Abstract

Cobalt ferrite nanoparticles (CoFe_2O_4) have gathered substantial academic and industrial interest due to their diverse applications across biological systems, photocatalytic degradation of dyes, environmental remediation, medicine, energy storage devices, and various industrial sectors. Recognizing the ecological and environmental associated with conventional chemical and physical methodologies for synthesising cobalt ferrite nanoparticles, contemporary research has increasingly focused on alternative approaches. Biological synthesis protocols have emerged promising, offering cost-effectiveness, non-toxicity, biocompatibility, and environmental sustainability (Dichayal et al., 2024). This review critically examines the established protocols for the biogenic synthesis of cobalt ferrite nanoparticles utilizing various plant-derived components. The underlying mechanism of this green synthesis route, which involves utilising naturally occurring reducing and capping agents present in plant extracts to facilitate the formation of nanoparticles with tailored properties, is thoroughly discussed. Furthermore, the review evaluates the array of characterization techniques employed to investigate synthesized nanoparticles' structural, morphological, and magnetic characteristics. A comprehensive overview of their diverse applications is also presented. Finally, the review identifies extant challenges and potential opportunities for future research and development within this domain, underscoring the imperative for optimising synthesis parameters and exploring novel, environmentally benign precursors and stabilizing agents. In summary, the green synthesis of cobalt ferrite nanoparticles represents a sustainable and ecologically responsible strategy for producing advanced materials for many technological applications. This review provides a comprehensive synthesis of the current advancements in the eco-friendly synthesis of cobalt ferrite nanoparticles, encompassing their characterization methodologies and potential applications (Dichayal et al., 2024).

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I. Introduction

Nanoparticles are materials that range between 1-100 nanometres in size. It is impossible to see nanoparticles with the naked human eye. Nanoparticles could have various properties physical and chemical, according to their various counterpart materials. Nanoparticles could made of only a few hundred atoms. It is rare to range to 100 nm; otherwise, it always ranges below it. NPs could change their size with respect to the atomic scale. This is due to the area of the surface; volume changes with the increasing ratio because the material atoms could dominate the other materials' performance. This could possess the unexpected properties of NPs like optical, physical, and chemical, as they are small enough to confine their electrons and produce quantum effects. Extracts of many plant species, several acids and the salts of different metals (e.g., Cu, Au, Ag, Pt, Se and Fe) have been active in the green synthesis of nano-sized materials. Because no bacteria or toxic chemical contaminants are present, plant materials are more favourable when used for NP fabrication compared to methods involving microbes or deleterious chemicals (H. Singh et al., 2023).

Plumbago zeylanica (also known as *Chitrak* or *Ceylon leadwort*) and *Nyctanthes arbor-tristis* (also known as *Parijat* or *Night-flowering jasmine*) extracts and using them to synthesize nanoparticles with iron nitrate ($\text{Fe}(\text{NO}_3)_3$) and cobalt nitrate ($\text{Co}(\text{NO}_3)_2$) can result in the formation of bimetallic nanoparticles (e.g., iron-cobalt nanoparticles) with unique properties. The phytochemicals in these plant extracts act as reducing and stabilizing agents during the synthesis process.

The cobalt ferrite nanomaterials have attracted amplified attention due to their potential applications as high-density magnetic recording media, catalysts, and microwave absorbers. They are used as carriers for drug delivery, as substrates in various diseases like cancer treatment methods, biosensors and also in magnetic resonance imaging. The goal of today is to develop synthesis strategies for nanomaterials that are eco-friendly, simple, and clean to "reduce or eradicate the use of the peers of hazardous substances in the design, production and application of chemical products". The ideal strategy of synthesis is the combination of the methods of soft chemistry and green chemistry (Gingas et al., 2016). The vivid applications of NPs or nano ferrites in the ferrofluids, magnets, storage devices, targeted drug delivery and more attracted more attention to their way of synthesizing these nanomaterials. CoFe_2O_4 is a well-known high-magnetic solid material with high coercivity and moderate magnetization. These materials possess great physical and chemical stability, which makes these NPs

suitable for magnetic recording applications such as audio and video with higher-density digital recording disks etc. (Houshiar et al., 2014).

Usually, fabricated nanoparticles have a metallic nature and show an effect commonly known as SPR “Surface Plasmon Resonance”, which plays a vital role in the quantum mechanical effects of light in UV-visible regions, leading to unique optronics or optoelectronic properties. Obviously, any type of change in the shape or size of a nanoparticle is reflected in its inter-particle interactions and absorption properties. Due to their exceptional properties, nanoparticles are widely utilized in various biomedical applications (H. Singh et al., 2023). The use of plant extracts from leaves, seeds, roots, or flowers in the biosynthetic method of NPs manufacturing has already been reported. Plant extracts have a variety of secondary metabolites such as flavonoids, polyphenols, alkaloids, sugars, polysaccharides, amino acids, and vitamins. The primary objective of this study was to synthesize CoFe_2O_4 NPs using a sustainable and environmentally friendly green chemistry technique that uses *Nyctanthes arbor-tristis* leaf extract and *Plumbago zeylanica* root extract as a fuel and reducing agent (Ansari et al., 2023).

This work is an attempt to propose a novel one-step green synthesis of CoFe_2O_4 -NPs with colloidal stability using chitosan, a biopolymer, as a reducing and capping agent. The synthesis of CoFe_2O_4 -NPs using chitosan as both a reducing and capping agent has been reported (Muthukrishnan, 2015). Ferrites with distinctive physical characteristics have been the focus of many studies in the pharmaceutical and industrial sectors. Within these nanomaterials, cobalt-ferrite nanoparticles (CoFe_2O_4 NPs) exhibited interesting properties such as strong overloaded magnetization without favoured ferromagnetic mechanism and good penetration (Ansari et al., 2023).

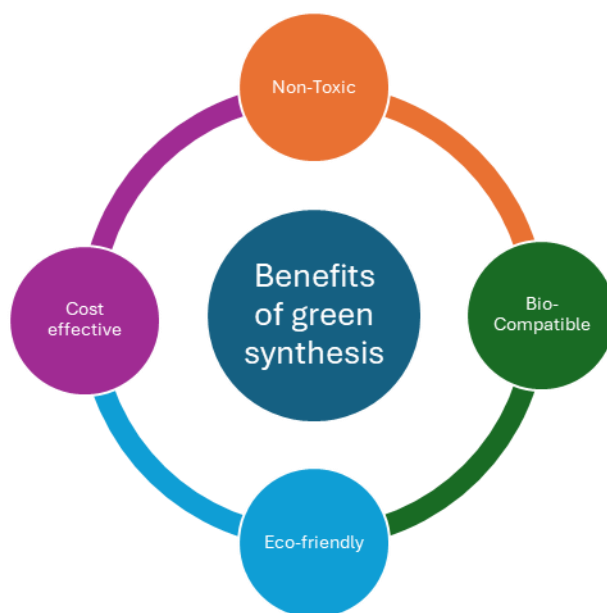


Fig. 1 Benefits of Green Synthesis

Phytochemical Contributions

- *Plumbago zeylanica* contains bioactive compounds like plumbagin, flavonoids, and phenols, which have antioxidant, antimicrobial, and anti-inflammatory properties.
- *Nyctanthes arbor-tristis* contains iridoid glycosides, flavonoids, and alkaloids, exhibiting antioxidant, antimicrobial, and anti-inflammatory effects.

Nanoparticle Synthesis

- The phytochemicals will reduce the iron nitrate and cobalt nitrate in the plant extracts to form iron-cobalt nanoparticles (Fe-Co NPs).
- The plant extracts act as green reducing agents, replacing toxic chemical reductants, stabilising the nanoparticles, and preventing aggregation.

II. Review Of Literature

This review highlights that pectin-based silver (Pe-AgNPs) and gold nanoparticles (Pe-AuNPs) demonstrate promising antibacterial and anticancer/drug delivery properties, respectively. Both types showed biocompatibility and low toxicity, confirming the potential of Ag and Au as effective metals for pectin-mediated synthesis (Devasvaran & Lim, 2021).

This review explores the green synthesis of cobalt ferrite nanoparticles (CoFe_2O_4) using various plant-based methods as eco-friendly, cost-effective, and biocompatible alternatives to conventional chemical techniques. It highlights the mechanism of biogenic synthesis, involving natural reducing and capping agents, and details the characterization methods used to study structural, morphological, and magnetic properties. The paper also outlines the wide range of applications, including in biomedicine, photocatalysis, environmental cleanup, and energy, while identifying key challenges and future opportunities for optimizing synthesis and exploring novel natural resources (Dichayal et al., 2024).

III. Objectives

1. Synthesis of Cobalt Ferrite nanoparticles by green approach
2. Fabrication of Cobalt Ferrite nanoparticles with Chitosan and its Physico-chemical and biological characterization
3. To study the anti-inflammatory and antioxidant properties.

IV. Materials Required

The iron ($\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$) and the cobalt ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) nitrates were reagents. *Nyctanthes arbor-tristis* (night flowering jasmine) dried leaves powder and *Plumbago zeylanica* (Chitrak) dried roots powder, chitosan, blood, Eppendorf, scissors, Falcon tubes, muffle furnace, BOD incubator, centrifuge.

V. Methodology

Preparation of the Plant Extract

Nyctanthes arbor-tristis leaves extract

The *Nyctanthes arbor-tristis* also known as night jasmine, aqueous extract was prepared according to the following protocol: 10 g *Nyctanthes arbor-tristis* leaves powder were mixed with 100 mL of distilled water and heated up to 80 °C for 30 min under magnetic stirring. After cooling the centrifuge, it is in Remi-centrifuge for 15 mins, with 4000 rpm.

Plumbago zeylanica roots extract

The *Plumbago zeylanica* also known as Chitrak, aqueous extract was prepared according to the following protocol: 10 g *Plumbago zeylanica* leaves powder were mixed with 100 mL of distilled water and heated up to 80 °C for 30 min under magnetic stirring. After cooling the centrifuge, it is in Remi-centrifuge for 15 mins, with 4000 rpm.

Synthesis of CoFe_2O_4 nanoparticles

The metal salts, cobalt nitrate hexahydrate and ferric nitrate nonahydrate, are the precursors of the synthesis of nanoparticles by the green synthesis method. These metal salts were added slowly into the aqueous solution of plant extract one by one with the (2:1) and stirred for 15 mins over the magnetic stirrer and maintained the pH 10 by adding slowly NH_4OH 25%. During this time dark brown or blackish precipitation was formed. The obtained suspension was continued stirred at ~80 °C for 3 hours. After this time, keep it at normal room temperature for cooling; after cooling, dry it in hot air over the oven for 2 days. After the dried suspension, crush it with the help of mortar and pastel and make powder of that dried suspension.



Fig: Green synthesis of Cobalt Ferrite nanoparticles

Synthesis of Cobalt Ferrite Nanocomposites

Cobalt ferrite nanocomposites were synthesized by the Green approach. Then we have to fabricate the cobalt ferrite nanoparticles with chitosan. The *Nyctanthes arbor-tristis* and *Plumbago zeylanica* synthesized CoFe_2O_4 nanoparticles were mixed with chitosan in an equal ratio of 1:1 in the alumina Crucible and kept in the muffle furnace for 1 hour at 120°C . After one hour, the metal nanoparticles were coated with the polymer, formed a nanocomposite. Then we have stored it for further characterization studies.

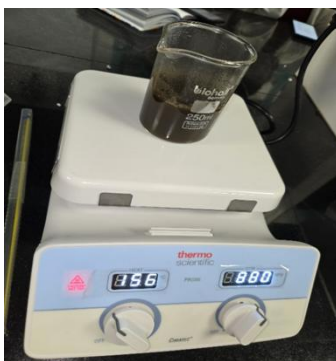


Fig - Synthesis of Cobalt ferrite nanocomposites

VI. Results And Discussion

UV- Visible Spectroscopy

UV-Vis spectroscopy (UV-Vis) is another relatively facile and low-cost characterization method often used to study nanoscale materials. It measures the intensity of light reflected from a sample and compares it to the intensity reflected from a reference material. NPs have optical properties sensitive to size, shape, concentration, agglomeration state and refractive index near the NP surface. UV-Vis spectroscopy is an important tool for identifying, characterizing, and investigating these materials and evaluating the stability of NP colloidal solutions.

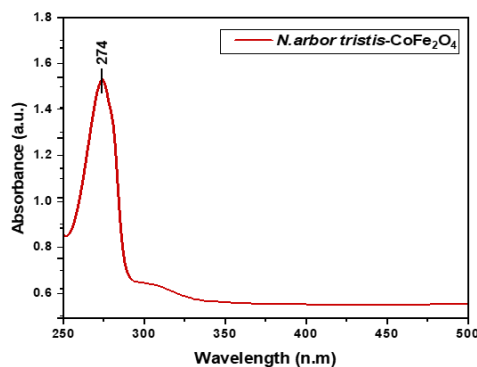


Fig.2: Visible UV-spectrum analysis of *Nyctanthes arbor-tristis*

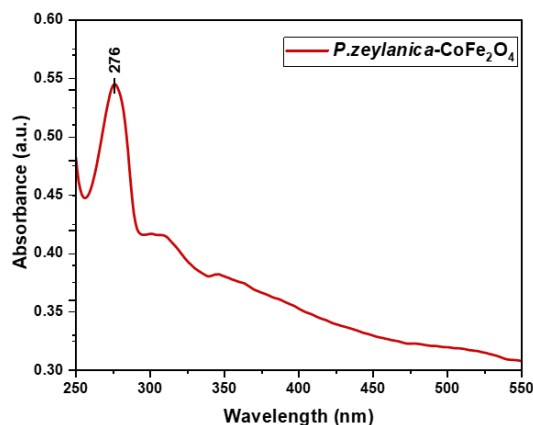


Fig.3: Visible UV-spectrum analysis of *Plumbago zeylanica*

FT-IR analysis

The FTIR analysis displayed the functional groups and phytochemicals present in the synthesized CoFe_2O_4 NPs and help in determining the role of plant extract which reduce the metal salts into the metal nanoparticles. The FTIR analysis recorded at $4000\text{--}500\text{ cm}^{-1}$ are shown in **Table 1**. The intense and broad peak observed at $3849.74\text{--}3645.46\text{ cm}^{-1}$ corresponded to the OH stretching vibration. The two weak bands at 3375.74 cm^{-1} and 3106.01 cm^{-1} attributed to the OH stretching and deformation. Peak at 2355.10 cm^{-1} shows vibration of CO_2 . Peaks at $2158.00\text{--}1975.26\text{ cm}^{-1}$ represented maybe the presence of metal ligands on the surface of nanoparticles. Peaks at 1632.12 cm^{-1} and 1520.40 cm^{-1} assigned to COO^- carboxylic group with symmetric stretch. Peaks at 1055.97 cm^{-1} represented C-O alkoxy group with stretching vibrations in the nanoparticles. The emergence of peaks at 837.32 cm^{-1} assigned for the C-O stretching and metal oxide presence. Emergence of peak at 787.84 cm^{-1} was due to CO-O and Fe-O vibration in octahedral position. The current findings are supported by previous reports with the FTIR spectrum of CoFe_2O_4 NPs synthesized from *Plumbago zeylanica* root extracts.

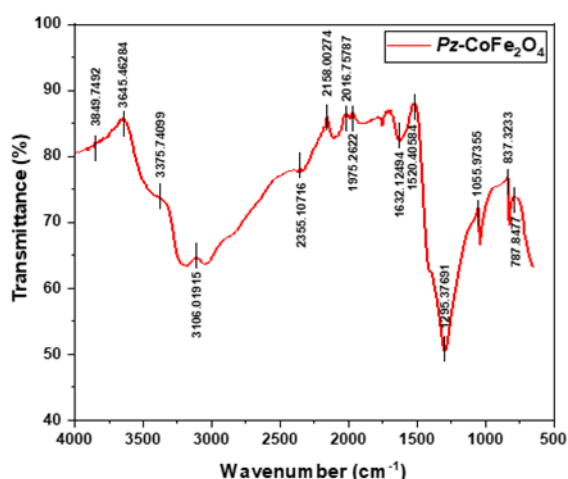


Fig.4- FT-IR analysis of *Plumbago zeylanica*

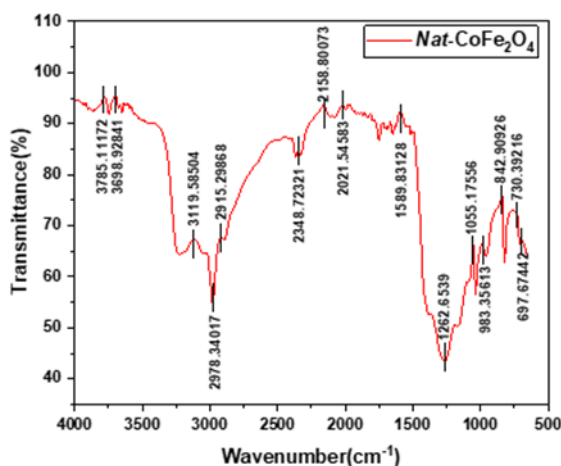


Fig.5- FT-IR analysis of *Nyctanthes arbor-tristis*

The FTIR analysis displayed the functional groups and phytochemicals present in the synthesized CoFe_2O_4 NPs and helped in determining the role of plant extract, which reduces the metal salts into the metal nanoparticles. The FTIR analysis recorded at $4000\text{--}500\text{ cm}^{-1}$ is shown in **Table 2**. The intense, broad peak observed at $3785\text{--}3698\text{ cm}^{-1}$ corresponded to the OH stretching vibration. The two weak bands at 3119 cm^{-1} and $2915\text{--}2978\text{ cm}^{-1}$ attributed to the OH stretching and deformation. The peak at 2348 cm^{-1} shows the vibration of CO_2 . Peaks at $2158\text{--}2021\text{ cm}^{-1}$ may represent metal ligands on the surface of nanoparticles. Peaks at 1589 cm^{-1} were assigned to the COO^- carboxylic group with symmetric stretch. Peaks at 1262 cm^{-1} represented the C-O alkoxy group with stretching vibrations in the nanoparticles. The emergence of peaks at 1055 cm^{-1} was assigned for the C-O stretching and metal oxide presence. The emergence of the peak at 787.84 cm^{-1} was due to CO-O and Fe-O vibration in the octahedral position. The findings are supported by previous reports with the FTIR spectrum of CoFe_2O_4 NPs synthesized from *Plumbago zeylanica* root extracts.

Table 1.- FT-IR peaks analysis of *Nyctanthes arbor-tristis*

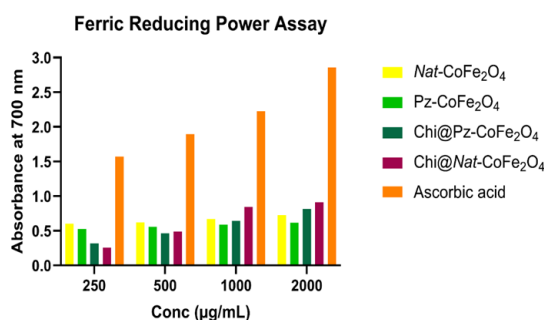
S. no.	Wavenumber cm^{-1}	Bonds present	Phytochemicals
1.	$3785\text{--}3698\text{ cm}^{-1}$	O-H stretching, Free hydroxyl groups	Phenols, Flavonoids, Alcohols
2.	3119 cm^{-1}	N-H stretching, Possible amine (-NH)	Alkaloids, Proteins, Amides
3.	$2915\text{--}2978\text{ cm}^{-1}$	C-H stretching	Lipids, Terpenoids
4.	2348 cm^{-1}	Adsorbed atmospheric CO_2	Carbonyl-containing metabolites
5.	$2158\text{--}2021\text{ cm}^{-1}$	$\text{C}\equiv\text{C}$, $\text{C}\equiv\text{N}$, Alkynes or cyanides	Alkaloids, Cyanogenic Glycosides
6.	1589 cm^{-1}	C=O stretching	Flavonoids, Tannins, Carboxylic Acids
7.	1262 cm^{-1}	C-O, metal-oxygen interactions	Carbohydrates, Glycoside
8.	1055 cm^{-1}	C-O-C stretching	Sugars, Cellulose, Polysaccharides
9.	983 cm^{-1}	Fe-O Stretching	Cobalt Ferrite Nanoparticles Formation
10.	$842\text{--}697\text{ cm}^{-1}$	Co-O and Fe-O, spinel ferrite structures (CoFe_2O_4)	Cobalt Ferrite Nanoparticles Formation

Table 2.- FT-IR peaks analysis of *Plumbago Zeylanica*

S. no.	Wavenumber cm^{-1}	Bonds present	Phytochemicals
1.	3849-3645 cm^{-1}	O-H stretching	Phenols, Flavonoids, Alcohols
2.	3375-3106 cm^{-1}	Conformation of O-H stretching and deformation	Alkaloids, Proteins, Amides, Terpenoids, Lipids
3.	2355 cm^{-1}	Vibrations of CO_2	Carboxylic Acids, Esters
4.	2158-1975 cm^{-1}	Maybe metal ligands are found	Alkaloids, Cyanogenic Compounds
5.	1632-1520 cm^{-1}	COO^- carboxylic group, Symmetric stretch	Flavonoids, Quinones, Tannins
6.	1055 cm^{-1}	C-O alkoxy group with stretching vibrations	Carbohydrates, Glycosides
7.	837 cm^{-1}	C-O stretching, metal oxide	Cobalt Ferrite Nanoparticles Formation
8.	787 cm^{-1}	CO-O and Fe-O in octahedral position	Cobalt Ferrite Nanoparticles Formation

Ferric Reducing Power Assay (FRAP)

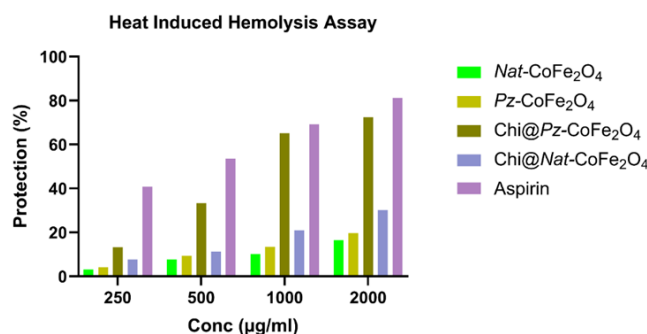
The method was followed to perform the FRAP assay. In brief, FRAP reagent comprised of 2.5 mL of 2,4,6-tripyridyl-s-triazine (10mM) was prepared using 60 mM of HCl, 2.5 mL of 20 mM FeCl_3 solution, and 30 mL of 300 mM acetate buffer (pH 3.8). A 200 μL FRAP reagent was incubated with various concentrations of *Nat* and *Pz*. CoFe_2O_4 NPs and composites (80–200 $\mu\text{g/mL}$) along with Ascorbic acid (standard, 200–1800 μM) in a black chamber for 30 min to obtain blue colour, then the OD was taken at 594 nm. Using the calibration curve of standard Ascorbic acid, the values were converted into FRAP units (equivalent amount of Ascorbic acid), and the graph was plotted against the concentration of the compounds.

Fig 6: - FRAP of *NAT/Pz* CoFe_2O_4 nanoparticles and composites

Heat-Induced Haemolysis Assay

The method was carried out briefly, 0.10 mL of 10% RBC suspension was pre-incubated with dose-dependent concentrations of *NAT* and *Pz*. CoFe_2O_4 NPs and composites (200 $\mu\text{g/mL}$), and to the RBC suspension, 3.0 mL of buffer was added and incubated the mixture in a water bath at 30 °C for 30 min. After that, test tubes were cooled at room temperature and were spun at 3000 rpm for 10 min. The haemoglobin content released into the supernatant was measured using a spectrophotometer at 540 nm. The suspension without test sample was taken as a control, and Aspirin was used as a standard drug. The percentage of haemolysis inhibition was calculated by using the below formula.

$$\text{Percentage inhibition of haemolysis} = 1 - \frac{\text{OD of Sample}}{\text{OD of control}} \times 100$$

Fig 7: - Heat-Induced Haemolysis Assay of *NAT* and *Pz*. Nanoparticles and composites

Hydroxyl Radical Scavenging Assay

Scavenging hydroxyl radical is an important antioxidant activity because of the very high reactivity of the OH radical, enabling it to react with a wide range of molecules found in living cells, such as sugars, amino acids, lipids, and nucleotides. It has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods (Sowndhararajan & Kang, 2013). The CoFe_2O_4 nanoparticles have the ability to neutralize the hydroxyl radicals by reducing them. It was found that the CoFe_2O_4 nanoparticles exhibited 46.46 % inhibition at concentrations of 1000 and 2000 $\mu\text{g}/\text{mL}$. The HRSA of CoFe_2O_4 NPs absorbance measured at 420 nm, while decreasing absorbance, the inhibition activity also increased it leading to increasing antioxidant activity as prepared NPs (Kumar et al., 2019).

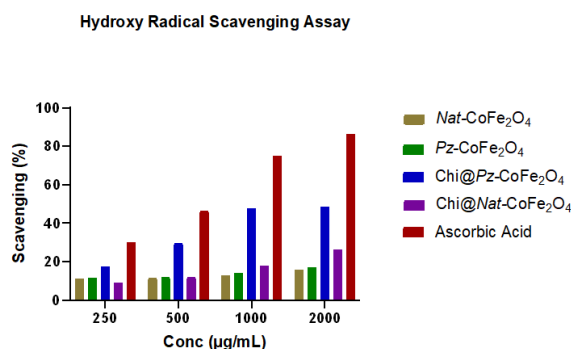


Fig 8: - Hydroxyl Radical Scavenging Assay of CoFe_2O_4

Oxidative Induced Hemolysis Assay

The method followed and oxidative stress was induced in RBCs using 10 mmol/L CoFe_2O_4 . Different concentrations of NAT and Pz. CoFe_2O_4 NPs and composites (250–2000 $\mu\text{g}/\text{mL}$) were incubated with 1 mL of 2% RBCs and incubated for 30 min at 37 °C. The RBCs without CoFe_2O_4 were taken as a positive control, whereas only CoFe_2O_4 -treated with RBCs (without sample) were used as a reference control. Approximately 2 mg/mL concentration of reaction mixture was taken from each test tube and performed for further activities such as lipid peroxidation, protein carbonyl content, total thiols, enzyme activities like superoxide dismutase and catalase activities.

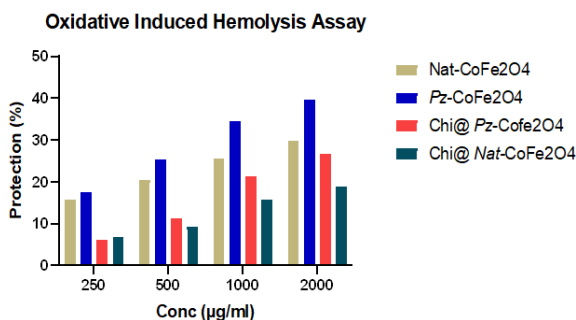


Fig 9: - Hydroxyl Radical Scavenging Assay of CoFe_2O_4

Hemocompatibility Assay

The hemolytic activity of CoFe_2O_4 ferrites against human erythrocyte membranes was investigated in an aqueous solution of PBS. It was a stabilizing agent to prevent the coagulation of whole blood samples collected from healthy volunteers. Blood: anticoagulant ratio was adjusted as 8:2. PBS was used to dilute the anticoagulated whole blood samples. RBCs were separated from plasma by centrifugation at 4000 rpm for 10 min. Precipitated RBCs were diluted up to 50 mL by adding PBS. 0.5 mg/mL and 2.0 mg/mL concentrations of CoFe_2O_4 suspensions were prepared and mixed with 2% of RBC stock solution. Distilled water and PBS were used in positive and negative control tests. In a positive control test, DW causes complete (100%) hemolysis of all erythrocytes; a negative control test corresponds to 0% hemolysis. (Yalcin et al., 2021b)

RBCs were incubated in the presence of varying nanoparticle concentrations in the BOD incubator at 37 °C for 3 h. Each test was performed thrice. At the end of the incubation period, the samples were centrifuged at 4000 rpm for 10 min. The absorbance (ABS) value of the supernatant was used to quantify the degree of haemoglobin release into the medium following cell lysis. (Yalcin et al., 2021b)

Hemolysis values were calculated using the Abs value at 540 nm using the equation given below

$$\text{Haemolysis ratio} = \frac{(OD(\text{test}) - OD(\text{negative control}))}{(OD(\text{positive control}) - OD(\text{negative control}))} \times 100\%.$$

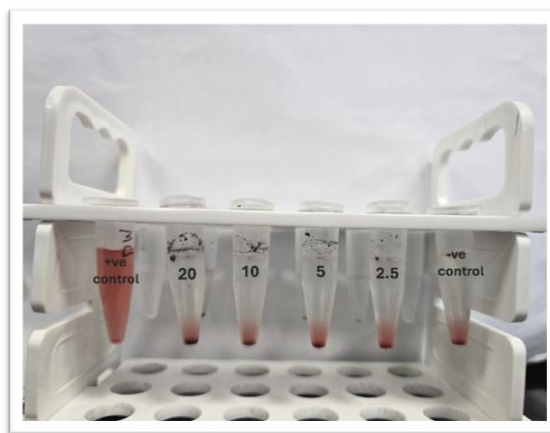


Fig 10: - Haemolysis Assay of CoFe_2O_4

Protease Inhibitory Assay

The method was used to perform a protease inhibitory assay with some alterations. Briefly, 500 μL of aspirin was pre-incubated with different concentrations of *NAT* and *Pz*. CoFe_2O_4 NPs (250–2000 $\mu\text{g/mL}$) and incubated for 5 min at room temperature. About 1 mL of 20 mM Tris–HCl buffer of pH 7.4 was added and further incubated for 10 min at room temperature. Then, adding about 500 μL of 0.8% (w/v) casein, the mixture was incubated for about 60 min at room temperature. After the incubation, about 1.25 mL of TCA was added to precipitate the reaction mixture, the test tubes were centrifuged at 4000 rpm for 5 min, and about 500 μL of supernatant was collected from each test tubes and added with 2.5 mL of Na_2CO_3 ; after that, 500 μL of FC reagents (1:2 dilutions) was added. The developed colour intensity was read at 660 nm by a spectrophotometer. About 10 μL of 5 mM PMSF was used as a standard protease inhibitor. The blank was monitored by Tris–HCl buffer, and the reaction mixture without a test sample was taken as a reference control. The percentage inhibition of protein denaturation was measured by using the following formula;

Table 3: - Representation of Protein Denaturation by the *Nat* and *Pz* CoFe_2O_4 NPs and NCs.

	Nat NPs	Pz NPs	Pz Composite	Nat Composite	Std. (Aspirin)
Conc. ($\mu\text{g/ml}$)	Percentage %	Percentage %	Percentage %	Percentage %	Percentage %
250 $\mu\text{g/ml}$	22.3	2.3	16.92	6.9	36.22
500 $\mu\text{g/ml}$	31.53	6.92	29.23	15.38	62.49
1000 $\mu\text{g/ml}$	43.07	12.3	43.07	23.84	81.86
2000 $\mu\text{g/ml}$	47.69	20.76	59.23	41.53	91.62

Protein Denaturation Inhibition Assay

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as a strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. Most biological proteins lose their biological function when denatured. Denaturation of tissue proteins is one of the well-documented causes of inflammation. The decrease in absorbance of the test sample with respect to control indicated protein stabilisation, i.e., inhibition of protein (albumin) denaturation or anti-denaturation effect by the test extract and the reference drug Aspirin (Mirke et al., 2020).

Table 4: Representation of Protein Denaturation by the *Nat* and *Pz* CoFe_2O_4 NPs and NCs.

	Nat CoFe_2O_4	Pz CoFe_2O_4	Pz Composite	Nat Composite	Std. (Aspirin)
Conc. ($\mu\text{g/ml}$)	Percentage %	Percentage %	Percentage %	Percentage %	Percentage %
250 $\mu\text{g/ml}$	9.41	28.03	18.82	12.25	42.96
500 $\mu\text{g/ml}$	12.45	32.05	27.84	21.27	60.81
1000 $\mu\text{g/ml}$	14.8	37.35	34.9	35.98	76.15
2000 $\mu\text{g/ml}$	22.15	42.45	40.39	50.09	98.66

VII. Conclusion

CoFe₂O₄ nanoparticles were synthesized via an eco-friendly green method. Their structural, magnetic, and photocatalytic characteristics were examined, considering the influence of phytochemical constituents and their associated biological functionalities (Yalcin et al., 2021b). Plant-mediated green synthesis of nanoparticles marks a significant advancement in nanotechnology and eco-friendly approaches. Research in this area emphasizes this environmentally friendly method's benefits and wide-ranging applications. Using plant extracts as natural reducing and stabilizing agents offers several advantages over conventional chemical approaches, such as lower cost, scalability, and eliminating toxic byproducts—positioning this method as a sustainable and safer alternative. Renewable, plant-based resources enable controlled nanoparticle synthesis, yielding particles with improved size uniformity and stability—key features that enhance their suitability for a wide range of applications. Plant-derived, bio-inspired nanoparticles exhibit notable pharmacological traits, including biocompatibility and nanoscale dimensions, making them particularly promising for biomedical applications, such as targeted drug delivery and disease management. Beyond healthcare, these nanoparticles also show potential in agriculture, bioremediation, and various industrial processes. Green synthesis using plant extracts as natural reducing agents offers a cost-effective and environmentally sustainable alternative to conventional methods while also imparting valuable biological functionalities. This review aims to serve as a valuable reference for researchers and scientists in the field, encouraging further innovation and advancement in the expanding nanoscience and green chemistry domains. (Singh et al., 2023)

VIII. Future Scope And Further Enhancement Of The Project

While the eco-friendly production of *Nat/Pz-CoFe₂O₄* and *Nat/Pz-CoFe₂O₄@Chi* shows great promise, several obstacles obstruct its widespread adoption. These include difficulties in sourcing appropriate natural materials (due to complex extraction, seasonal or geographical limitations, and variability in their composition), establishing optimal synthesis parameters, and ensuring consistent product quality and quantity (often resulting in low purity and yield). These limitations delay the scalability and reproducibility of the process for large-scale industrial applications, making production dependent on the time and location of resource availability. Future research should focus on identifying readily available and cost-effective natural resources, developing synthesis conditions suitable for large-scale production, improving nanoparticle yield and quality (in terms of purity and size control), and utilizing energy-efficient technologies. Addressing these challenges will unlock the broad prospects and significant developmental potential of Green-Synthesized cobalt ferrite nanoparticles (Tamboli et al., 2023).

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