

Green Synthesis of Zinc Ferrite Nanoparticles from *Nyctanthes arbor-tristis*: Unveiling Larvicidal Potential, Protein Binding Affinity and Photocatalytic Activities

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Abstract

An ecofriendly method was used to synthesize the novel zinc ferrite nanoparticles (ZnFe_2O_4 NPs) utilizing leaf extract of *Nyctanthes arbor-tristis* by hydrothermal method. The synthesized ZnFe_2O_4 NPs were characterized by different pivotal techniques. X-ray diffraction confirms the arrangement of the fcc crystal structure of nanoparticles and some organic substance that was encapsulated on the zinc ferrite. According to SEM analysis, the resulting nanoparticles are agglomerated and spherical in shape. The ZnFe_2O_4 nanoparticles are in their pure form, and all of their elemental compositions are shown by the EDAX spectrum. FTIR results reveal that the produced nanoparticles contained distinctive functional groups. Fluorescence spectroscopic technique was used to examine the binding affinities between bovine serum albumin (BSA) and ZnFe_2O_4 nanoparticles in terms of protein binding, stability, and conformation. The interaction between BSA and ZnFe_2O_4 NPs was examined using steady-state and time-resolved fluorescence measurements, and it was evident that there was static quenching at play. The ability of ZnFe_2O_4 nanoparticles to kill larvae of *Culex quinquefasciatus* (*C. quinquefasciatus*) was evaluated. The synthesized NPs demonstrated a noteworthy toxic effect against 4th instar larvae of *C. quinquefasciatus* with LC_{50} values of 43.529 $\mu\text{g/mL}$ and LC_{90} values of 276.867 $\mu\text{g/mL}$ respectively. This study explains the toxicity of green synthesized ZnFe_2O_4 NPs upon the mosquito larvae proving it to be a good and effective larvicide. Furthermore, the ZnFe_2O_4 NPs were utilized for dye degradation of methylene blue effectively under visible light treatment and achieved 99.5% degradation.

1. Introduction

In recent years, the advancement of nanotechnology have revealed potential uses for nanoparticles (NPs) in a broad range of biological domains, including diagnostics, drug delivery, biomolecular sensing, multiscale imaging, and others (Šimšíková et al. 2013; Huang and Lau 2016). Pharmaceutical and medical diagnostics have made use of several nanoparticles with innovative and distinctive physicochemical characteristics. Because of their enormous surface to mass ratio and unique chemical and physical properties, NPs can interact with a variety of biomolecules in such a process, which makes them useful for a wide range of biological processes (Verma et al. 2013). In terms of the research issue on nanoparticles, the chemical method is hazardous to the environment, and the physical synthesis is a top-down method requiring a significant amount of energy and space (Ouda 2014). Among these, the most convenient method for the synthesis with lesser toxicity towards environment is through biological/green approach. Biomaterials are used to synthesis nanoparticles through reduction process and later act as a stabilizer to increase the stability of the NPs (Singh et al. 2015). Plant materials are a significant source of secondary metabolites with redox potential, including flavonoids, terpenoids, and phenolics. So, plants and their parts are the best materials for synthesizing nanoparticles (Sun et al. 2014). Moreover, biologically synthesized nanoparticles are utilized in various emerging applications, such as antibacterial, dye degradation, organic reaction and fields for controlling mosquitoes. Plant-assisted nanoparticles effectively suppress pre-adult vector mosquitoes in their native environments by causing egg death and preventing oviposition (Benelli et al. 2016).

Mosquitoes are the most important insect, which can carry a number of infectious diseases, including malaria, dengue, filariasis, yellow fever and tularemia. Mosquito-borne diseases are widespread in over 100 countries and account for about two million annual deaths, with at least one million of those deaths occurring in children. *Aedes aegypti* is one of the important vector mosquitos, carrying numerous arboviruses that cause diseases in humans like dengue fever, chikungunya, and zika that annually impact a large number of people (Li et al. 2020). Synthetic chemical insecticides have been used to control these mosquitoes for over 50 years (Becker et al. 2010). These chemical pesticides can harm both humans and insects in addition to being hazardous to the environment. Recently, many vector control nanomaterials are developed via chemical synthesis, and it became a challenge towards environmental safety. Therefore, researchers are focused on synthesizing nanoparticles through the green approach. Generally, plants and plant materials contain numerous phytochemicals that act as a reducing as well as capping agents.

Protein is a biomacromolecule that could control several functions in living organisms in its native 3D structure. The large surface and mass ratio of the NPs enables them to bind and interact with different biomolecules, for example proteins, nucleic acids, and other metabolites (Sousa et al. 2021). Interaction between protein and NPs forms a complex that could extensively modulate the surface properties of the NPs making them an unique biological entity. In unison, the NPs may also induce some conformational changes in the structure of the proteins thereby leading to several biological side effects. Therefore, research on the interactions between proteins and NPs is mandatory to gain a basic understanding of how these NPs affect the structure and function of proteins (Randika Perera et al. 2019). The literature contains a large number of research about the study between proteins and NPs, including silver (AL-Thabaiti et al. 2017), metal oxide Click or tap here to enter text., fluorescent polymeric NPs (Welsch et al. 2013) and magnetic iron oxide NPs(Paul et al. 2012) etc. Water contamination by dyes has been a major global concern that is fundamental to all future planning and policy discussions. Since methylene blue (MB) is one of the most often used industrial dyes, it is frequently utilized in research to evaluate the effectiveness of recently discovered adsorbent materials. The adsorption, swelling, and water-holding capabilities of green synthesized metal nanoparticles can be used to remove MB from wastewater in an efficient manner.

To the best of our knowledge and the literature review, zinc ferrite nanoparticles (ZnFe_2O_4 NPs) have not been synthesized using *Nyctanthes arbor-tristis* (**Figure S1**). Herein, we have synthesized the ZnFe_2O_4 NPs through greener way using *Nyctanthes arbor-tristis* by hydrothermal method. Synthesized ZnFe_2O_4 NPs were analyzed using different spectroscopic methods. Additionally, a variety of spectroscopic techniques, including steady state absorption, fluorescence and time resolved spectroscopy, were used to study the *in-vitro* interaction between ZnFe_2O_4 NPs and BSA. Through these experiments, the quenching process, binding characteristics, and effects of zinc ferrite on BSA conformation, stability, and activity during interaction with ZnFe_2O_4 NPs were ascertained. Moreover, the ZnFe_2O_4 NPs were utilized for larvicidal activity against mosquito larvae and photocatalytic activity against methylene blue.

2. Experimental Section

2.1. Material

A taxonomic identification of the *Nyctanthes arbor-tristis* leaf was performed by Dr. G. Subramani of the Department of Botany at Arignar Anna Govt. Arts College in Namakkal, Tamil Nadu, India, using plant samples collected from the area. Sigma-Aldrich chemical company, India supplied the hydrated iron nitrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$) (97%) and the hexahydrate zinc nitrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) (96%). Methylene blue(dye) was purchased from Fischer Scientific.

2.2. Preparation of Plant Extracts

Nyctanthes arbor-tristis leaves were washed with water and dried in the shade at ambient temperature for 5 to 6 days (27–37 °C). The dried leaf (1000 g) was mechanically ground into a powder using a commercial mortar-pestle. It was then extracted for 6 hours in a Soxhlet apparatus (boiling point range 60–80°C) with 1000 mL of methanolic solvent. Further, the extracts were filtered through Whatman filter paper. Then, the filtered solution was concentrated under vacuum pressure at 45°C and the extract was kept in the refrigerator at 4°C until further use. The schematic representation of plant extraction steps were depicted in scheme 1.

2.3. Collection of Larvae:

The *C. quinquefasciatus* mosquito larvae were collected from National Centre for Disease Control (NCDC), Mettupalayam, Tamil Nadu, India. The collected larvae were maintained at room temperature ($28 \pm 2^\circ\text{C}$) with 65–80% relative humidity and all the larvae were kept under a dark/light of 12:12 h (photoperiod). Methoprene (1.0, 2.5, and 5.0 g/ml) was given to *C. quinquefasciatus* larvae and larval growth reaches the fourth instar stage for a period of seven days. Further, the larvae were used for the bioassays analysis with various concentrations of ZnFe_2O_4 NPs.

2.4. Phytochemical analysis of plant extract

2.4.1. GC-MS analysis of methanol extracts

Thermo GC-Trace ultra-version 5.0 gas chromatography interfaced to Thermo MS DSQ mass spectrometer apparatus was used to perform GC-MS analysis of *Nyctanthes arbor-tristis* leaf methanol extract under the following conditions: DB5-MS Standard nonpolar capillary column (30 x 22.5 mm x 22.5 µm) and 99.99% pure helium gas were used as the carrier gas at a steady flow rate of 1 ml/min. The oven temperature was maintained at 70°C and was set to rise to 260°C at a rate of 6°C per minute. The mass range was 45–450 m/z. In 36 minutes, the entire running time was over. After gas chromatography, the chromatogram was examined in mass spectrometry to determine the mass of each fraction. Retention time and mass spectrometry were exploited to recognize the bioactive components by comparing the mass spectra of unknown peaks with those kept in the GC-MS library. Gas

chromatography-mass spectroscopy (GC-MS) was used to find out the phytoconstituents present in the extract which could have been a reason for the exhibited larvicidal activity.

2.5. Green Synthesis of Zinc-ferrite nanoparticles

Zinc-ferrite Nanoparticles were synthesized (Scheme 2) by the reported method (Din et al. 2020) with trivial modifications. A 1mM concentration of $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ solution was freshly prepared in aqueous medium under stirring condition. Further, 90ml of both metal solutions and 10ml of plant extract were merged as one under stirring conditions (30 min). The mixture of solutions was transferred to the autoclave and maintained at 120°C (90 min). Then, the semi-solid brown product was filtered and washed with water followed by ethanol. Finally, the nanoparticles were dried in the oven (60°C for 5 h) and then the impurities were removed by annealing of nanoparticles for about 1 h at 400°C . The systematic synthesis method is displayed in the schematic diagram (Scheme 2).

2.6. Experimental Section

The absorption spectra of Zinc-ferrite Nanoparticles were recorded using a SPECORD 200 PLUS UV–Visible spectrophotometer. Agilent Technologies' Cary Eclipse fluorescence spectrometer was used for the protein quenching analysis. The functional groups were examined by using JASCO FT-IR ATR 6300 spectrometer in the spectral range of $4000 - 400 \text{ cm}^{-1}$. To carry out the EDAX analysis, a drop of extract with reduced zinc nanoparticles were taken and analysis was performed on Hitachi S-3400 N SEM instrument equipped with the thermo EDA Thermochments. X-ray diffraction spectrometer (Rigaku Miniflex) with monochromatic Cu K α radiation ($\lambda_{\text{Cu}} = 1.54 \text{ \AA}$) was used to check the phase purity and crystalline nature of nanoparticles.

2.7. Larvicidal activity

Larvicidal activity was performed with varying concentrations (25, 50, 75, 100 $\mu\text{g/mL}$) of synthesized NPs. Briefly, the NPs were dissolved in purified water and extract supernatants were dissolved in distilled water for the preparation of various dilutions required according to the concentrations mentioned above. This activity was performed using *C. quinquefasciatus* in their third instar. The control was sterile deionized water. A castor leaf with a diameter of 8.5 cm was immersed in various ZnFe_2O_4 concentrations, plant supernatant, and 2 mM zinc oxide. The assay was carried out in triplicate for 24h. Mortality was observed. LC_{50} and LC_{90} values were determined using the values obtained from the mortality.

2.8. Bovine serum albumin binding studies

1% concentration of protein solution was prepared in Phosphate buffer (pH 7.4) and the BSA concentration was measured by applying $\epsilon_{280} = 44,300 \text{ M}^{-1} \text{ cm}^{-1}$ (Anjomshoa et al. 2014). Moreover, the nanoparticles stock solution (1mg/mL) was made with water. The quenching measurement were

monitored in the range between 290 to 500 nm with excitation wavelength of BSA at 285 nm ($\lambda_{\text{emi}} = 347\text{nm}$) and fixed the slit width of excitation (5nm) and emission (5nm).

2.9. Photocatalytic study

The dye degradation by photocatalytic manner was performed by green synthesis of zinc ferrite nanoparticles under visible light irradiation for methylene blue. The degradation studies were carried out with photoreactor (HEBER annular). The nanoparticles were added into quartz tube and further added to the MB dye. Further, the mixture was irradiated with 250W mercury lamp and samples were collected every 15 minutes to calculate the percentage of degradation of dye. The samples were monitored the absorbance by UV-Visible absorbance spectroscopy at 650nm of MB.

$$\% \text{ of decolorization} = [C_0 - C_t / C_0] \times 100$$

Herein C_0 and C_t are the initial dye absorbance and time taken for the dye degradation of absorbance.

3. Results and Discussion

3.1. Phytochemical activity

Studies in biological systems have shown that plant secondary metabolites have medicinal properties (Kalaivani 2013). After successful extraction of *N. arbor tristis* plant extract and performed a preliminary phytochemical analysis of plant extract to determine the existence of phytochemicals including proteins, alkaloids, amino acids, glycosides, anthraquinones, saponins, carbohydrates, flavonoids, tannins, and phenolic compounds. Terpenes and steroids were absent in the *N. arbor tristis* methanolic extract, as summarized in Table 1. It has been observed that the bioactive elements of the *N. arbor tristis* plant, such as phenolics, alkaloids, flavonoids, protein, and amino acids, have biological effects in the prevention and treatment of numerous human illnesses (El-Beshbishy and Bahashwan 2012). Tannins are widely known for having modest quantities of antibacterial, antioxidant, and astringent properties (Vergara-Jimenez et al. 2017). Moreover, phenolic and flavonoid compounds have also been found to exhibit hepatoprotective, anti-inflammatory, antiallergenic, antioxidant, and anticarcinogenic properties, due to their capacity to scavenge and chelate free radicals as well as their methods of action (Cai et al. 2006).

Table 1
Preliminary phytochemical analysis of the methanol extracts of
N. arbor tristis

Phytochemical Constituents	Test/Reagents	Result
Alkaloids	Mayer's test	-
	Wager's test	+
	Dragendroff's test	+
Proteins and amino acids	Millions test	-
	Ninhydrin test	+
	picric acid	+
Anthraquinone glycoside	Bontrager's test	+
	Coumarin	+
	Terpenes	-
Flavonoids	Sodium hydroxide test	+
Phenolic compounds	Tannins	+
Carbohydrates	Molisch's test	+
	Fehling's test	+
	Anthrone test	-
Phytosterol	Saponins	+
	Coumarin	+
	Steroid	-
(+) = Presence; (-) = Absence		

3.2. Identification of bioactive compounds of *N. arbor tristis* by GC-MS Analysis:

Bioactive components of methanol extracts were associated by GC-MS analysis (Fig. 1). The GC-MS unknown spectrum of these plant extracts were assessed with the known spectrum stored in Turbomass GC-MS library using NIST version-2005. The phytochemical compounds are identified and further it was confirmed based on the peak area, molecular formula, and retention time. GC-MS Investigation of *N. arbor tristis* suggested the presence of 50 bioactive compounds that could contribute to the medicinal quality of the plant and these compounds are mentioned in **Table S1**. From these results, it could be

determined that "*N. arbor tristis*" contains various bio-active compounds. Therefore, it is recommended as a plant of phytopharmaceutical importance.

3.1. Structural analysis of ZnFe_2O_4 nanoparticles

The plant extract contains some reducing compounds like phenolic, protein, carbohydrate, vitamin, and fat. These compounds serve as a reducing agent; therefore, the nanoparticles were synthesized using the extract of *N. arbor tristis* along with the zinc and ferrite metal as precursors. Further, ZnFe_2O_4 NPs were characterized using pivotal techniques such as FTIR, XRD, HR SEM and absorption studies. Figure 2a displays the FTIR spectrum of green synthesized ZnFe_2O_4 nanoparticles. The metal-oxygen complex was identified by peaks below 1000 cm^{-1} , according to the spectrum, which was noted within the region of $4000\text{--}400\text{ cm}^{-1}$ (Kombaiah et al. 2016). The prepared ZnFe_2O_4 nanoparticles shows the stretching vibration of Zn-O in tetrahedral sites and stretching vibration of Fe-O in octahedral sites vibrational bands were observed in the range of $400\text{--}600\text{ cm}^{-1}$. The ZnFe_2O_4 nanoparticles shows the tetrahedral and octahedral vibrational bands were observed to be between 400 and 600 cm^{-1} . FTIR analysis of the produced ZnFe_2O_4 nanoparticles reveals peaks at 3254 , 1638 , 1422 , 1064 , 756 , 595 , and 493 cm^{-1} . These observed peaks could be due to the different functional groups present in the prepared NPs. The broad peak at 3254 cm^{-1} was assigned to hydroxyl groups (Jagajjanani Rao and Paria 2013). The small peak at 1638 cm^{-1} was recognized as $\text{--C}=\text{C--}$ group due to the amino acids and flavonoids. The peak at around 1422 cm^{-1} and 1064 cm^{-1} due to presence of the methylene group and C--O--C group (Zhao et al. 2016; Gowri). The aforementioned findings make it clearly evident that the prepared ZnFe_2O_4 nanoparticles contain different functional groups.

X-ray diffraction pattern analysis was used to confirm the synthesized ZnFe_2O_4 NPs from *N. arbor tristis*. The results showed clear diffraction peaks in the XRD spectrum of 2θ values between 10° and 80° (Fig. 2b). Bragg's reflections demonstrated a face-centered cubic (fcc) crystal structure correlated with the peaks, and this structure matched to the (111, 200, 220, 311, 440, 511) plane. From these peaks are confirmed that the formation of nanoparticles in single phase face-centered cubic and there is no secondary phase was observed. The surface morphology of prepared NPs was examined by SEM analysis and the image was presented in Fig. 3c. From the image clearly enlightened the formation of nanoparticles shows the aggregation with homogeneous nanoparticle distribution and spherical shape. Moreover, the formed nanoparticles are pure form, and it was confirmed by EDAX analysis (Fig. 2d). The agglomeration of nanoparticles are formed during the synthesis of nanoparticles due to its high energy created from the annealing method (Madhukara Naik et al. 2019; Govindan et al. 2020). From the EDAX analysis, the chemical compositions such as Zn, Fe, and O are present in prepared nanoparticles were examined. From these results, the produced ZnFe_2O_4 nanoparticles cannot contain any impurities, as seen in Fig. 2d.

The spectrophotometer testing is one of the most widely used simple and sensitive techniques for the examination of nanoparticles (**Figure S2**). The production of zinc ferrite nanoparticles from leaf extract

of *N. arbor tristis* was analyzed ranging from 200 to 800nm. This showed a peak at 380 nm. As the extract was mixed with zinc ferrite, it started to change color from watery to brown owing to the reduction of zinc ions which implies the formation of zinc ferrite nanoparticles. From the above analysis, the prepared nanoparticles were surrounded by different functional groups, and it shows more accessible surface area for dye degradation. Furthermore, the green synthesis of nanoparticles may lead to the application in the field of biological.

3.3. Protein binding studies

Recently, various nanoparticles were used in different kind of emerging biological fields such as diagnostics, drug delivery, biosensing and imaging process. Proteins are important macromolecules in cells and maintain a key role in regular cell processes. One of the main components of plasma protein is BSA and it is needed for metabolism and transportation of numerous endogenous as well as exogenous substances. Moreover, BSA has tryptophan residues that make a maximum emission peak at 347 nm. In the study of binding interactions between protein molecules and ligands (such as dyes, medications, nanoparticles, etc.), fluorescence spectroscopy is receiving more interest because of its greater selectivity, sensitivity, speed, and convenience of usage at very low concentration in physiological circumstances (Momeni et al. 2017).

The binding mechanism of the ligand and protein can be easily and conveniently explored using the fluorescence quenching technique. The maximum emission intensity of BSA was diminished with addition of ligand, it is due to presence of some molecular interaction processes such as excited-state reactions, molecular rearrangement, electron/energy transfer, static/dynamic quenching (Naveenraj and Anandan 2013; Millan et al. 2016). In this work, BSA concentration (3 μ M) was kept constant and addition of various concentrations of ZnFe₂O₄ NPs (1 μ g/mL to 5 μ g/mL). The emission intensity of BSA was gradually diminished (Fig. 3a), but there was no discernible wavelength shift in the emission maxima. This result showed that there was an interaction between them, and this interaction had no effect on the polarity of the microenvironment surrounding the Tryptophan residue (Chaves et al. 2017). Moreover, there is no significant alteration found in the absorption spectrum measurement between them (**Figure S3**).

The mechanism of quenching is followed static/dynamic quenching was evaluated using Stern-Volmer equations 1&2 (J. R. Lakowicz 1999).

$$I_0/I = 1 + K_{SV} [Q] \quad (1)$$

$$K_{SV} = k_q \times \tau_0 \quad (2)$$

where I_0 and I represents the emission intensities of BSA in the absence and presence of quencher. The K_{SV} , k_q and τ_0 denotes the quenching constant, quenching rate constant and lifetime of BSA (6 ns). $[Q]$ represent the quencher concentration. Figure 3c shows the Stern-Volmer plot and obtained the linear S-V plot indicates that the quenching is follow dynamic. However, static type also shows linear plot

sometimes. The K_{sv} and k_q were determined using equations 1 and 2 and the values are 1.34×10^6 g/mL and 2.23×10^{14} (g/mL.s)⁻¹. Generally, the higher value of dynamic k_q in the water is 2.0×10^{13} (g/mL.s)⁻¹ (Ponce et al. 2013; Kosuru et al. 2024). From these results, the measured k_q was notably higher than the reported one. This result clearly showed the quenching mechanism follow static quenching rather than dynamic (Douadi et al. 2020). Further, it was confirmed with the help of lifetime measurement analysis (Fig. 3b). The lifetime value of BSA is significantly varied with the addition of NPs in dynamic quenching, whereas the values remain constant for static quenching (Kathiravan and Renganathan 2008; Kathiravan et al. 2009a; Asha Jhonsi et al. 2009; Kathiravan et al. 2009b; Ugin Inba Raj 2020). Thus, these findings proved that the suggested fluorescence quenching mechanism was static, rather than dynamic.

The binding constant(K_b) and number of binding site (n) were computed from the double-logarithmic Eq. (3),

$$\log (I_0 - I) / I = \log K_b + n \log [Q] \quad (3)$$

From the Fig. 3d shows the linear plot and calculated the values of K_b and n. The calculated values are 6.70×10^5 (g/mL)⁻¹ and 1 for K_b and n, respectively. The strong binding relationship between BSA and ZnFe₂O₄ NPs was shown by the increased binding constant value. However, the binding site value of 1 was identified, implying that BSA binds to NPs as 1:1 ratio.

3.4. Larvicidal activity

Larval mosquito control, especially in infested areas, has become heavily reliant on a limited selection of target-specific materials. In this work, larvicidal activity was achieved for aqueous, methanol extract, and synthesized ZnFe₂O₄ NPs against *C. quinquefasciatus* mosquito larvae. The synthesized ZnFe₂O₄ NPs unveiled potent larvicidal activity against *C. quinquefasciatus* mosquito larvae. Table 2 display the larval mortality results and Fig. 4 shows the fourth instars exposed to altering the concentrations of synthesized NPs and plant extracts ranging from 25 µg/mL- 100 µg/mL at 24h. The mortality was assessed. The maximum mortality was observed, LC₉₀ values and LC₅₀ was also determined (Fig. 5). This study revealed that ZnFe₂O₄ NPs were more potent with a LC₅₀ 43.529 µg/mL when compared with methanolic extract (LC₅₀ 52.023 µg/mL) and aqueous plant extract (LC₅₀ 104.088 µg/mL) against the larvae of *C. quinquefasciatus*. Herein, the prepared nanoparticles from *N. arbor tristis* plant extract shows the potential toxic effect against *C. quinquefasciatus* mosquito larvae.

Table 2
Larval mortality of secondary metabolites against *C. quinquefasciatus* larvae.

S.No	Treatments	LC ₅₀ (LCL-UCL) (µg/mL)	LC ₉₀ (LCL-UCL) (µg/mL)	χ ² (df = 10)
1.	Plant extract	104.088 (76.3-231.6)	862.462 (325.1-22298.1)	6.542
2.	Methanolic extract	52.023 (39.3–66.6)	327.483 (181.6-1492.8)	5.469
3.	ZnFe ₂ O ₄ NPs	43.529 (30.5–67.3)	276.867 (160.2-1127.5)	1.409
LC ₅₀ -Lethal concentration for killing 50% of the exposure larvae; LC ₉₀ - Lethal concentration for killing 90% of the exposure larvae; LCL- Lower Confidence limit, UCL-Upper Confidence limit ; χ ² -Chi square; df-Degree of freedom. No mortality was observed in the control.				

3.5. Photocatalytic activity for decolorization of Methylene blue (MB)

The photocatalytic decolorization of methylene blue was examined by utilizing ZnFe₂O₄ NPs as catalyst using visible light source. 10 mg of MB in 100 mL of water and an addition of 50 mg of NPs was taken into the quartz tube. The irradiated samples were collected in different time intervals and the absorption was measured by absorption spectroscopy. Initially, the methylene blue dye absorption observed at 650 nm and after the absorbance of MB decreases drastically with increase the irradiation time. The corresponding absorption spectral profiles were depicted in Fig. 6. Finally, we achieved the complete decolorized the MB dye solution (Fig. 7) and gave 99.5% of degradation in 90 minutes. The efficiency of dye degradation of prepared ZnFe₂O₄ NPs is mainly related to nature of crystallites, unique electron transfer and surface morphology of NPs. The dye degradation process may follow pseudo first order kinetics and the equation is given by,

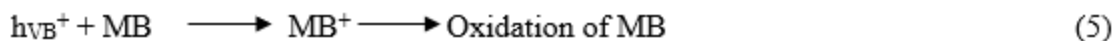
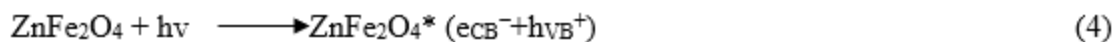
$$C_t = C_0 e^{-Kt} \text{ or } \ln (C_0/C_t) = Kt$$

Here, C₀ and C_t were absorbance of initial and different time intervals of dye solution, time (t) in minutes and K is represented as rate constant (min⁻¹). From the above equation was utilized to compute the rate constant (K) is 3.2 x 10⁻² min⁻¹.

3.6. Mechanism for decolorization of MB

The common pathway states that various radicals and charged species are formed when metal nanoparticles are photoexcited in an aqueous solution during the photocatalytic destruction of pollutants (Kusmierek 2020). The ZnFe₂O₄ NPs photocatalyst originates the photodegradation of MB dye

under light irradiation, which leads to excitement of the electrons from valence bond (VB) to conduction bond (CB). The same number of holes are simultaneously created in the VB, where they react with the OH^- or water surface to produce $\bullet\text{OH}$ (hydroxyl radical). A sequence of processes is involved in the suggested mechanism of dye degradation (Scheme 3), as exhibited in equations 4–7.



Concurrently, superoxide radicals ($\bullet\text{O}^{2-}$) are produced by reduction in the reaction between the electrons in the CB on the surface of the nanoparticles and the dissolved oxygen molecules. This radical participates in the oxidation process and prevents e-/h+ couples from recombining, maintaining ZnFe_2O_4 electron neutrality. Hydroperoxyl radicals are created when $\bullet\text{O}^{2-}$ is protonated, resulting in hydrogen peroxide (H_2O_2), which is subsequently dissociated to yield $\bullet\text{OH}$ radicals. The strong oxidizing agent ZnFe_2O_4 nanoparticles attack organic molecules by producing reactive species on their surface. When these reactive species attack organic molecules, they become mineralized and produce carbon dioxide and water molecules (equations 8,9). The most reactive species from the process for the photodegradation efficiency of the MB dyes are the hydroxyl ($\bullet\text{OH}$) and superoxide radicals ($\bullet\text{O}^{2-}$). When compared to the published ferrites, the synthesized ZnFe_2O_4 nanoparticles show good photocatalytic activity (Harish et al. 2013).

4. Conclusion

In conclusion, the leaf crude methanol extract of *Nyctanthes arbor-tristis* has been used to prepare the ZnFe_2O_4 nanoparticles and the extract acts as a reducing as well as stabilizing agent. The synthesized NPs were thoroughly characterized by UV-Visible, FTIR, XRD and SEM-EDX techniques. Additionally, the intrinsic BSA fluorescence was quenched by the ZnFe_2O_4 nanoparticles, and static quenching predominated over dynamic quenching as the main mode of quenching. Lifetime analysis verified the static quenching mechanism. The results here open up the possibility of further investigations of the efficacy of the larvicidal properties of natural product extracts. This procedure provides a simple, energy-efficient, low-cost, and environmentally benign way to synthesize ZnFe_2O_4 nanoparticles. Significant photocatalytic activity for Methylene blue dyes is demonstrated by the synthesized ZnFe_2O_4 nanoparticles. This approach may be helpful in the large-scale synthesis of related metallic nanomaterials since such environmentally benign nanoparticles can be effectively employed in wastewater treatment, antibacterial, wound-healing, and targeted drug delivery systems.

Declarations

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Data availability: All data generated and analyzed during this study are available from the corresponding author on reasonable request.

Ethical approval : Not applicable.

Consent to participate : Not applicable.

Consent to publish : Not applicable.

Author contribution:

Duraisamy Kavitha – Data curation, Writing- Original draft, **Venkatesan Srinivasan** – Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Supervision, Writing - review & editing, **Sivaji Ilakkia** - Writing- Original draft, **Kosuru Rekha Yamini** – Methodology, Data curation, Writing – original draft, **Prithika Palaniyappan** –Data curation, Writing – original draft, **Sureshkumar Muthusamy** – Writing – original draft, **Dhakshinamurthy Divya**– Software.

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Competing interests :The authors declare no competing interests.

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Schemes

Schemes 1-3 are available in the Supplementary Files section.

Figures

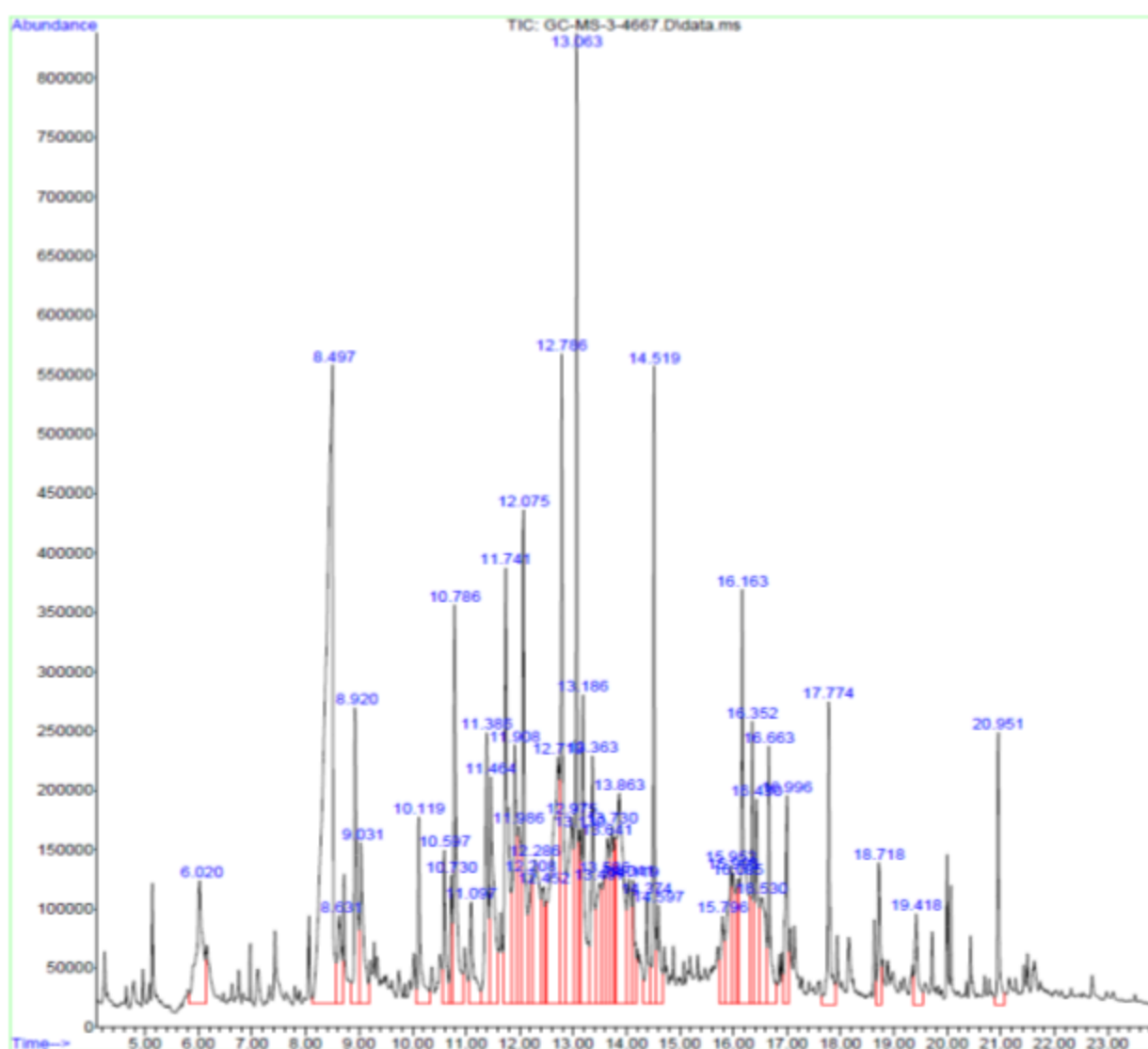


Figure 1

GC-MS chromatogram of methanolic extract of *N. arbor tristis*.

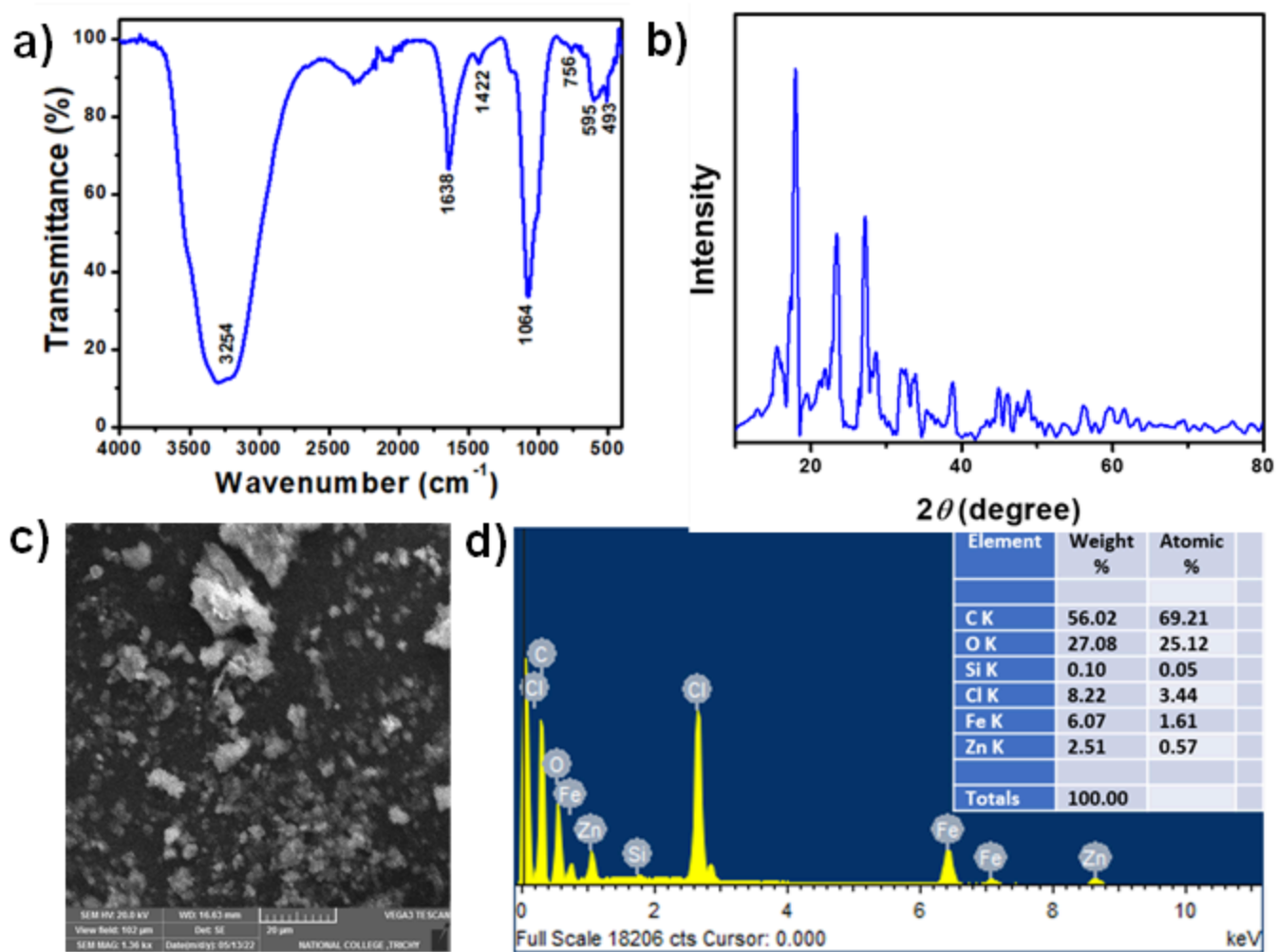


Figure 2

a) FT-IR spectrum, b) XRD spectrum, c) HR-SEM image and d) EDAX data of ZnFe_2O_4 NPs.

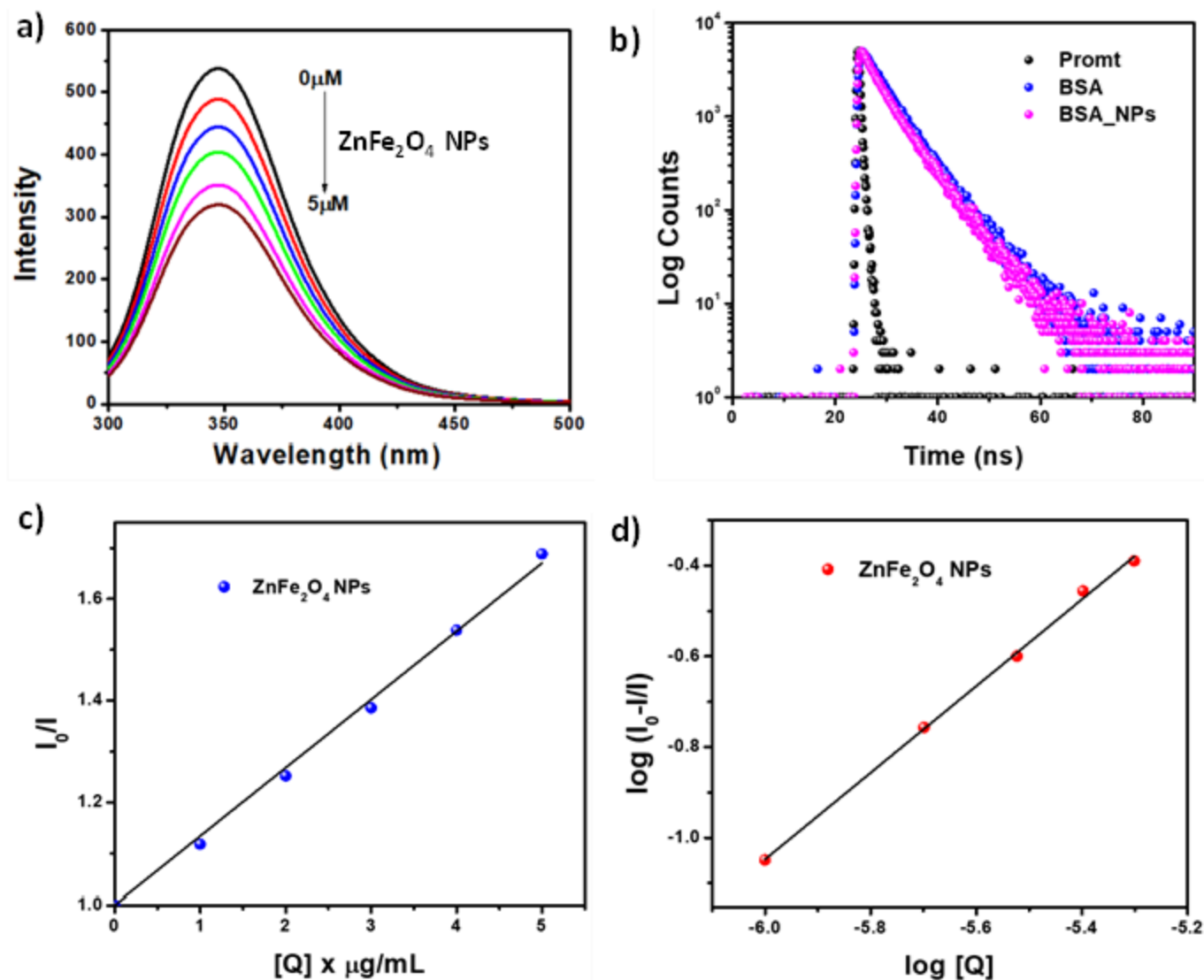


Figure 3

(a) Emission spectra of BSA with NPs, (b) Lifetime decay, (c) Stern-Volmer plot and (d) double-logarithmic plot of the fluorescence quenching of BSA with NPs.

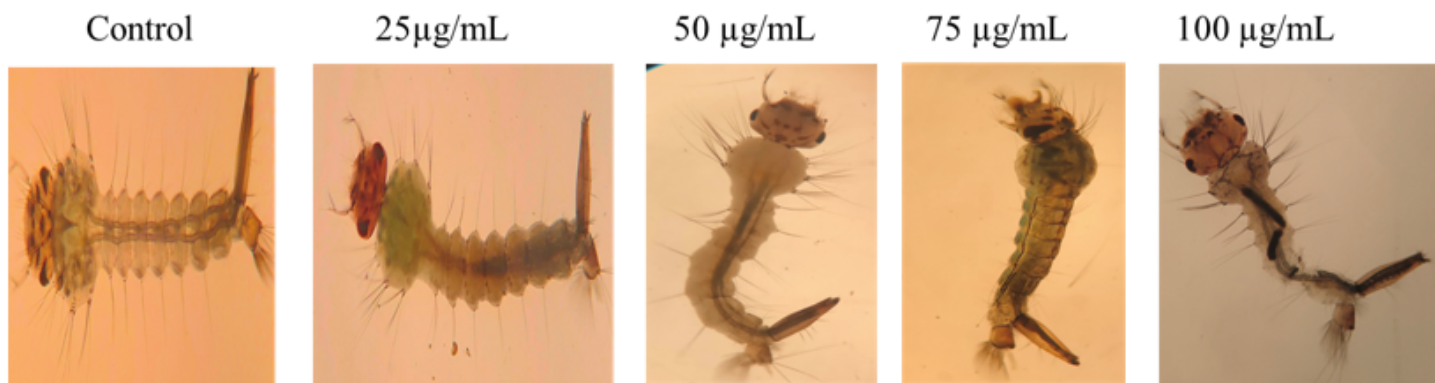


Figure 4

Various concentration of lethal concentration for killing of the exposure larvae.

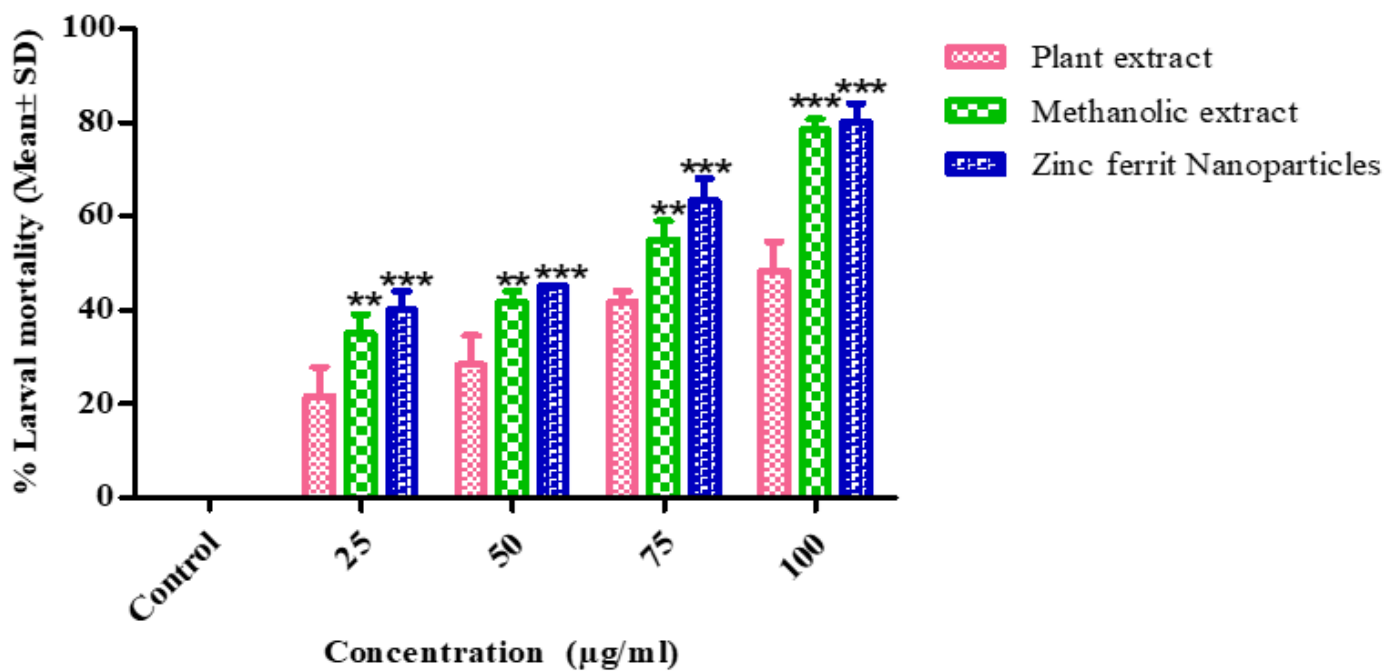


Figure 5

Larvicidal activity of ZnFe_2O_4 nanoparticles.

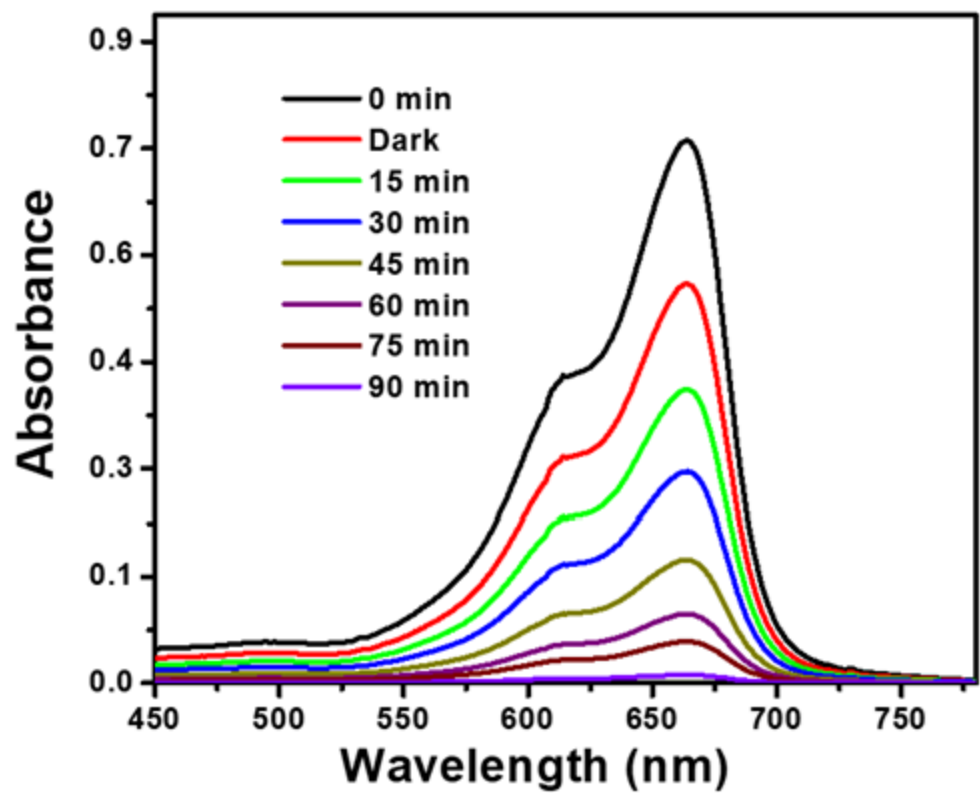


Figure 6

Absorption spectra for the decolorization of methylene blue dye in presence of ZnFe_2O_4 .

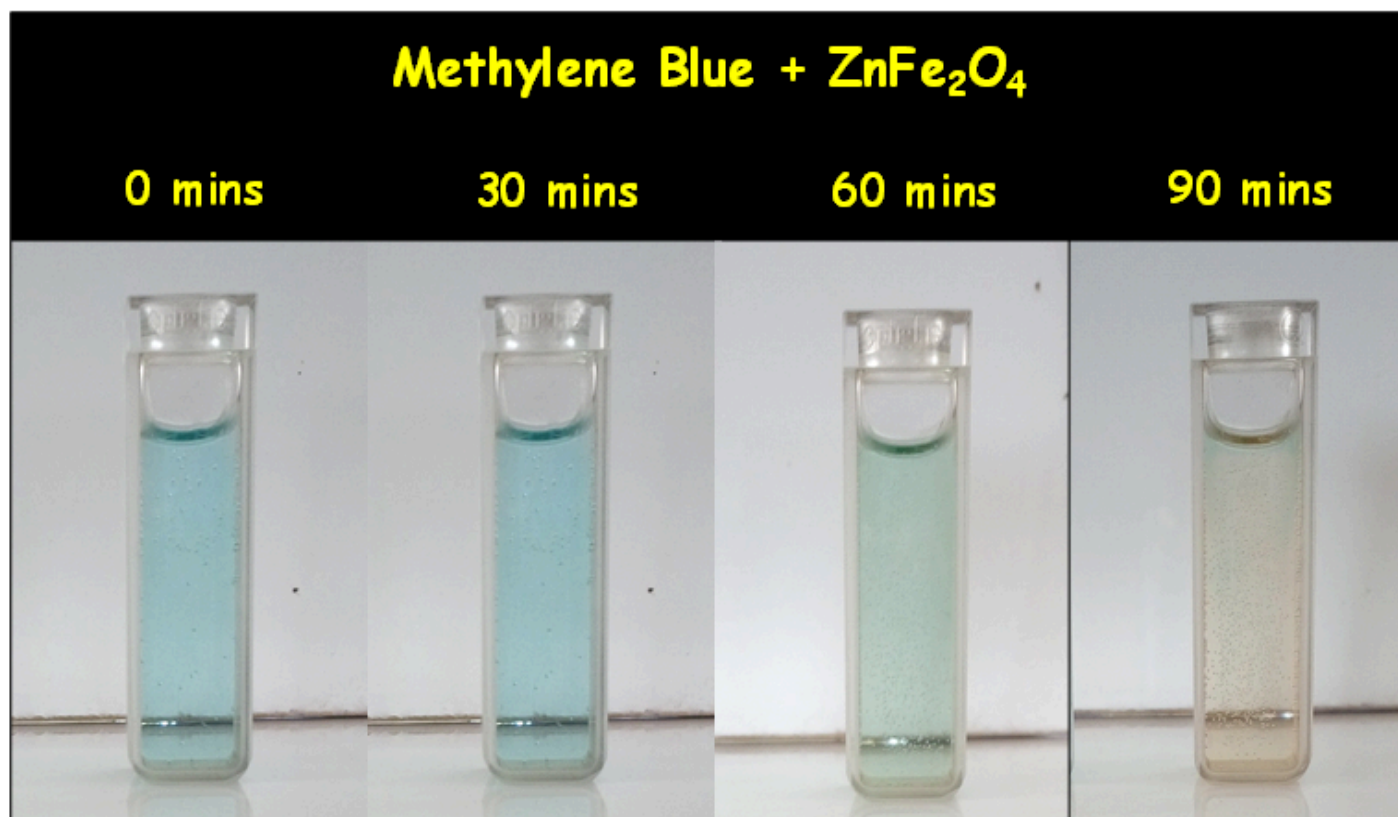


Figure 7

Images shows the color changes of the MB solution by adding ZnFe_2O_4 NPs.

Supplementary Files

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