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Original Article

PHOTOCATALYTIC DEGRADATION OF METHYLENE BLUE USING IRON OXIDE NANOPARTICLES SYNTHESIZED USING *ANNONA MURICATA* LEAF EXTRACT

BEENA JOSE[1*], FEMY THOMAS[1]

Department of Chemistry, Vimala College (Autonomous), Thrissur, Kerala 680009
Email: drbeenajose@gmail.com

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ABSTRACT

Objective: The objective of the present study is the synthesis of iron oxide nanoparticles using *Annona muricata* aqueous leaf extract, characterization of the synthesized nanoparticles and evaluation of the antibacterial, photocatalytic activity and cytotoxicity.

Methods: The iron oxide nanoparticle was synthesized using *Annona muricata* aqueous leaf extract and the crystal structure of the iron oxide nanoparticle was determined by UV-Visible spectroscopy, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR). The *in vitro* cytotoxicity of iron oxide nanoparticles was evaluated using Dalton's lymphoma ascites cells and the antibacterial assay was conducted using agar well diffusion method.

Results: The UV-Visible spectrum of iron oxide nanoparticle showed a maximum absorption peak at 265 nm. This is the XRD pattern of iron oxide nanoparticles exhibited a distinct peak at 26.029 (2θ), accounting for crystal plane (211). SEM images revealed that the synthesized iron oxide nanoparticles were aggregated as irregular sphere shapes with rough surfaces. TEM image reveals the size of the synthesized iron oxide nanoparticles are spherical in shape with an average size of 20 nm. Green synthesized iron oxide nanoparticles using *Annona muricata* leaf extract effectively degraded methylene blue dye.

Conclusion: This study showed that the synthesized iron oxide nanoparticles using *Annona muricata* aqueous leaf extract exhibited pronounced antibacterial, anticancer and photocatatytic activity and can be used in the textile industry for the purification of water contaminated with carcinogenic textile dyes. It can also be used as an external antiseptic in the prevention and treatment of bacterial infections.

Keywords: *Annona muricata*, Green synthesis, Characterization, Iron oxide nanoparticle Photocatalytic degradation, Methylene blue, Antibacterial activity, Cytotoxicity

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INTRODUCTION

The biological synthesis of the nanoparticle is a challenging idea which is well known as green synthesis. Biosynthesis of nanoparticles could be a substitute to traditional chemical methods for the production of metallic nanomaterials in a clean, non-toxic and ecologically sound manner. Green synthesis of nanoparticles is cost-effective, easily available, eco-friendly, non-toxic, large scale production can be done easily and acts as reducing and capping agent when compared to the chemical method [1]. Plant-mediated biological synthesis of nanoparticles has gained importance only in recent years [2] and plant extracts reduce the metal ions in a shorter time as compared to microbes [3]. The synthesized nanoparticles can be used for the purification of water contaminated with carcinogenic textile dyes due to its photocatalytic activity.

The need for the purification of the wastewater is of great concern to today's world and the water gets polluted by various ways. The water containing organic pollutants such as dyes coming out from textile industries affects the biological cycle, mainly photosynthesis in aquatic plants and also makes unfit for human use and also for the marine animals. Many studies have shown that dyes such as methylene blue and crystal violet are carcinogenic and mutagenic [4]. Thus, environmental contamination by these toxic chemicals has emerged as a serious global problem. In this study, we have studied the photodegradation of the methylene blue by iron oxide nanoparticle synthesized using *Annona muricata* leaf extract.

Annona muricata leaves extract was chosen for this study because of many pharmacological effects including anti-cancer, anti-inflammatory, anti-diabetic and antioxidant activities it possess [5-7]. The phytochemical screening of *Annona muricata* confirmed the presence of f1avonoids, terpenoids, reducing sugar, anthraquinone, tannins and cardiac glycosides. Phytoconstituents in the leaves of *A. muricata* L. contain an alkaloidal principle named 6-Hydroxyundulatine and other alkaloids [8, 9].

Green synthesized nanoparticles are cheap and economical and green synthesis is an eco-friendly method for the production of well-characterized metallic nanoparticles using plants. In this work, the iron nanoparticles were synthesized by reduction of ferric chloride solution with *Annona muricata* aqueous leaf extract, which acts as a reducing agent. Size, shape, functional groups and morphology of synthesized nanoparticle were determined with Fourier Transform Infrared spectroscopy (FTIR), UV-Vis spectrometry, Scanning electron microscopry (SEM), Transmission electron microscopy (TEM). The anti-antimicrobial efficacy of the synthesized nanoparticle was investigated via the agar well diffusion method. The UV-Visible spectrum of synthesized iron nanoparticle revealed absorbance at 265 nm, which confirmed the formation of iron oxide nanoparticles.

From the FTIR spectroscopic analysis of the different functional groups present in the *Annona muricata* leaf extract was identified. It showed the ability of this plant to act as reducing agents and stabilizers of an iron nanoparticle. The FTIR analysis of iron nanoparticles showed bands corresponding to O-H, C=C, C=O, C-H, and Fe-O bond such as γ-Fe_2O_3 bonds.

The present study is a subject of great interest and in the present study the synthesis and characterization of iron oxide nanoparticle from *Annona muricata* leaves were reported for the first time. The iron oxide nanoparticles synthesized using *Annona muricata* leaf extract showed significant catalytic activity in the photo degradation of methylene blue and hence it can be used as a promising candidate for the purification of waste water contaminated with dyes from textile industries.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Annona muricata* were collected from Munumuri village of Thrissur district, Kerala during December 2017 and authenticated by Dr. Sheeja Tharakan, Department of Botany, Vimala

College, Thrissur and the voucher specimen no VMA-SGB-005 was kept in the herbarium of the department for future reference.

Chemical reagent

Ferrous chloride, ferric chloride, sodium hydroxide,, Mueller Hinton agar, DMSO, methylene blue, ciprofloxacin, potassium bromide.

Preparation of *Annona muricata* aqueous leaf extract

Fresh leaves of *Annona muricata* were thoroughly washed with tap water and then by distilled water to remove all the dust and unwanted visible particle. Then leaves dried in room temperature and cut into small pieces. About 50g of these leaves were weighed and transferred into a 250 ml beaker containing 150 ml distilled water and boiled for 20 min. The extract was then filtered using Whatmann No.41 filter paper to remove particulate matter and to get a clear solution. The solution was then concentrated, which was then refrigerated (4 °C) for further study.

Preparation of iron oxide nanoparticles

Fifty ml of 0.1M $FeCl_2$ and 100 ml of 0.1M $FeCl_3$ were mixed in a conical flask. It was heated to 80 °C and stirred using a magnetic stir for about 10 min. (fig. 1). Then 50 ml of plant extract was added to the mixture and stirred for another 5 min at 80 °C; then, the yellow-colored solution gets changed to black color (fig. 2).

Fig. 1: Mixture of $FeCl_2$ and $FeCl_3$

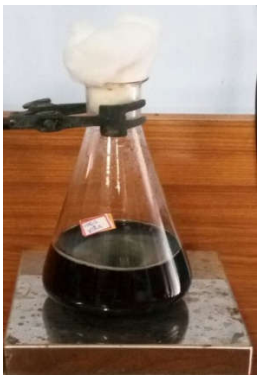

Fig. 2: Mixture of $FeCl_2$ and $FeCl_3$ and plant extract

Then 20 ml of 0.1M NaOH solution was added with a rate of 3 ml per min. It was again stirred for 5 min and the solution was cooled and the froth was removed. It was decanted and the plant residue was removed. The decanted solution was the centrifuged and residue obtained was washed several times using distilled water. The nanoparticles obtained are dried at 40 °C under vaccum to obtain the Iron oxide nanoparticles [10] and characterized by UV-Visible, FTIR spectroscopy, SEM, TEM and XRD analyses. The evaluation of *in vitro* cytotoxicity and antibacterial activity was also conducted.

Antibacterial assay

For *in vitro* screening, Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria were selected and the antibacterial activity of iron oxide nanoparticle was studied using the agar well diffusion method. Ciprofloxacin was used as the reference standard. DMSO was used as a negative control. The iron oxide nanoparticles synthesized using *Annona muricata* leaf extract was prepared in 10 mg/ml concentration and 10, 20 and 40 µl was tested against Gram-positive *Staphylococcus aureus* (MTCC 2825) and Gram-negative *Escherichia coli* (MTCC 40) cultures. The microorganisms used for this antibacterial activity evaluation where obtained from Microbial Type Cultute Collection and gene bank (IMTECH, Chandigrah, India).

***In vitro* cytotoxicity**

The iron nanoparticles prepared from *Annona muricata* leaf extract were studied for the short term *in vitro* cytotoxicity using Dalton's lymphoma ascites cells by Trypan Blue exclusion method. Viable cell suspension (1×10^6 cells in 0.1 ml) was added to tubes containing various concentrations of the iron oxide nanoparticles and the volume was made upto 1 ml using phosphate-buffered saline. Control tube contained only cell suspension and the assay mixture was incubated for 3 h at 37 °C and the cell suspension was mixed with 0.1 ml of 1% Trypan Blue and loaded on a hemocytometer. Dead cells were stained blue by Trypan Blue, while live cells did not take up the dye and the number of stained and unstained cells was counted separately [11].

Characterization of the nanoparticles

The crystal structures of the Iron nanoparticles was determined by X-Ray diffraction analysis using X-Ray Diffraction Unit (XRD) Pan Alytical, X-Pert pro, Netherlands operating at 40kV with 2 sec time interval at room temperature. The morphology of the prepared nanoparticles was determined by Scanning Electron Microscopy (JEOL Model/JSM-6390LV). The sample was analysed by Tranmission Electron Microscopy (TEM) to determine the size and morphology of the particles. TEM analysis was done using a JEOL/JSM-2100F. The prepared nanoparticles were subjected to FTIR spectroscopic measurements to identify the possible biomolecules present in the plant extract responsible for the reduction and capping of the nanoparticles. FTIR spectrum was recorded using FTIR spectrometer (Model RXI, Make Perkin Elemer) in the range of 4000-400 cm^{-1} using KBr pellet method. The surface Plasmon resonances of synthesized nano particles were studied by UV-Visible double beam spectrometer (Varian, Cary 5000) in the range of 175–800 nm.

RESULTS AND DISCUSSION

Characterization of iron oxide nanoparticles from aqueous leaf extract of *Annona muricata*

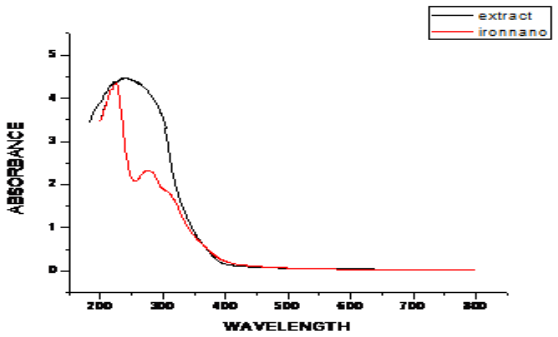

Fig. 3: UV-visible spectrum of (a)-*A. Muricata* leaf extract,(b)-iron nanoparticles

UV-Visible analysis is one of the most important characterization methods to study nanoparticles. The surface plasmon resonances

(SPR) of iron oxide nanoparticles synthesized from the aqueous leaf extract of *Annona muricata* have been studied by UV-Visible double beam spectrometer. The absorption of visible radiations due to the excitation of SPR, imparts various colours to nanoparticles. As the nanoparticles size changes, colour of the solution also changes. So UV-Vis absorption spectrum is quite sensitive to the formation of nanoparticles and both the nanoparticles and leaf extract were subjected to UV-Visible study (fig. 3).

In the UV-Visible spectrum the leaf extract showed an absorption maximum (λ_{max}) peak around 245 nm and iron oxide nanoparticle shows a absorption maximum (λ_{max}) peak around 265 nm indicating the formation of iron oxide nanoparticle. The characteristic absorption peak occurs at wavelength in the range of 200 to 300 nm indicated the formation of iron oxide nanoparticles [12]. FTIR analysis was performed in the order to determine the functional group and their role of synthesis of iron nanoparticle.

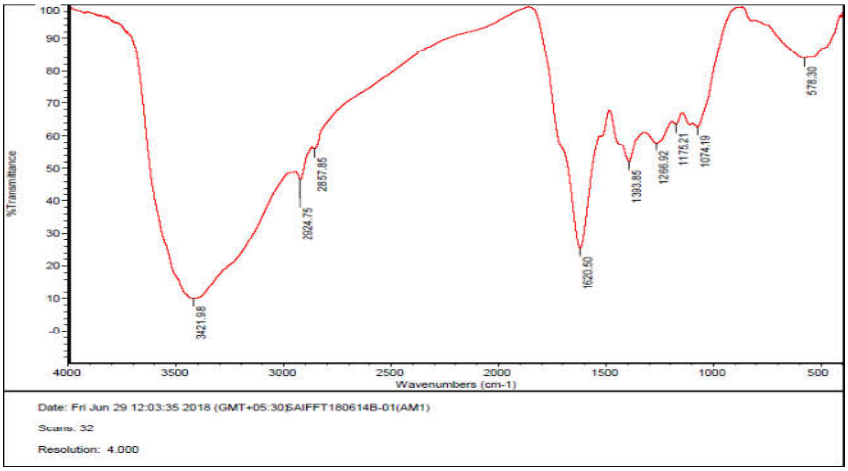

Fig. 4(a): FTIR spectrum of *Annona muricata* leaf extract

Annona muricata leaf extract showed peaks at 2924.75 cm^{-1}, 2857.85 cm^{-1}, 1620.50 cm^{-1}, 1393.85 cm^{-1}, 1266.92 cm^{-1}, 1175.21 cm^{-1}, 1074.19 cm^{-1} and 578.30 cm^{-1} in the FTIR spectra were due to the–CH2–and–CO groups present in the *Annona muricata* leaf extract. A broad peak at 3421.98 cm^{-1} was observed, which may be assigned to–OH, hydroxyl groups present in *Annona muricata* extracts (fig. 4a). The presence of phenolic compounds in *Annona muricata* extracts is responsible for the formation and stabilization of synthesized iron oxide nanoparticles [13].

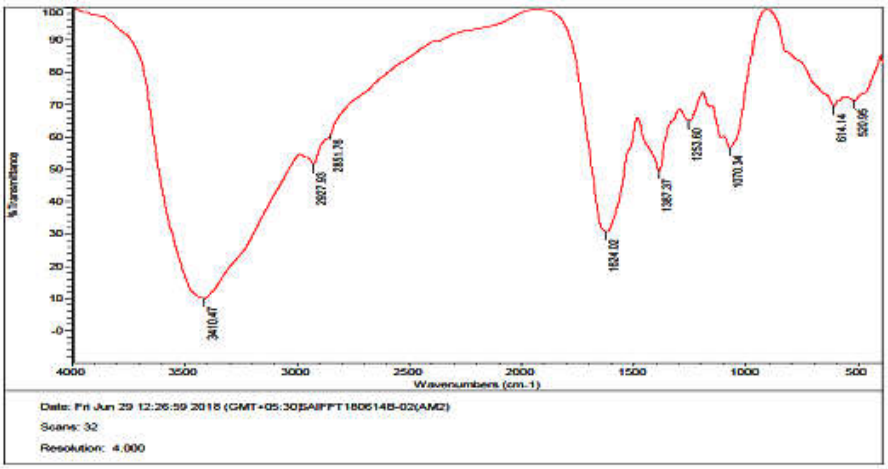

Fig. 4(b): FTIR spectrum of *Annona muricata* iron oxide nanoparticle

The FTIR spectrum of iron oxide nanoparticles shows bands at 3410.47 cm^{-1} and 1624.02 cm^{-1}corresponding to O–H stretching and bending bands. The frequencies at low wavenumbers 614.14 cm^{-1} come from vibrations of Fe–O bonds of iron oxide. The band at 614.14 cm^{-1} refers to Fe-O stretches of maghemite (γ-Fe_2O_3) [14]. From this result it has been concluded that the soluble biomolecule group present in the *Annona muricata* leaves extract acted as capping agents preventing the aggregation of iron oxide nanoparticle in the solution (fig. 4b).

The crystallinity of the iron nanoparticle was examined by XRD (fig. 5) and the distinct peak was found at 26.029 (2θ), accounting for crystal plane (211). The graph was compared and found to be equal to the card JCPDS no. 39-1346 of maghemite (γ-Fe_2O_3). The average crystal size was found to be 37.04 nm by Scherrer equation. The crystal system is cubic in nature. Lattice parameter for the iron oxide nanoparticle equal to 8.3787 is close to standard lattice parameter of γ-Fe_2O_3 [15].

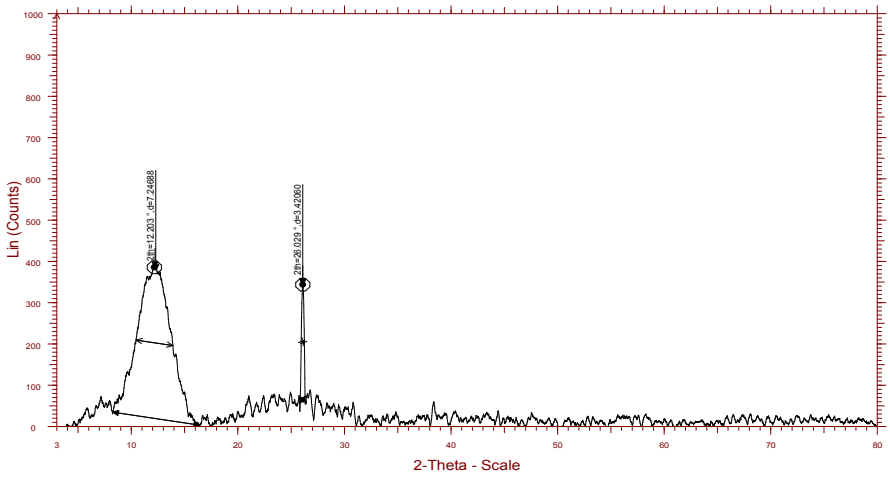

Fig. 5: XRD pattern of γ-Fe_2O_3 synthesized from *Annona muricata* leaf extract

Scanning electron microscope (SEM) was employed to analyze the morphology of iron oxide nanoparticle (fig. 6). SEM was taken with accelerating voltage of 20kV and the image was observed at 5000X magnification. SEM images revealed that the synthesized iron oxide nanoparticles were aggregated as irregular sphere shapes with rough surfaces. The morphology of the nanoparticles mostly appeared to be a porous and spongy [16].

TEM images revealed that the iron oxide nanoparticles were spherical in shape. The image shows agglomerates of small grains and some dispersed nanoparticles, confirming the results obtained by SEM. The iron oxide nanoparticle was analyzed by TEM to determine the size and morphology of the particle. The TEM image supports the crystalline structure of iron oxide nanoparticles and the lighter regions are mainly on the surface of the particle and darker region concentrated at the center of the particle. TEM image shows the size distribution and shape of nanoparticles based on the transmittance of electron beam through an ultra-thin specimen. It is clear from the TEM image that the size of nanoparticle is almost uniform and all particles are in spherical in shape and the average size of particle is found to be 20 nm [17].

SEM and TEM images of synthesized iron oxide nanoparticle from *Annona muricata* leaf extract is shown in fig. 6 and 7.

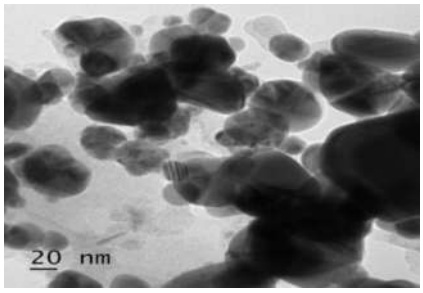

Fig. 6: SEM image

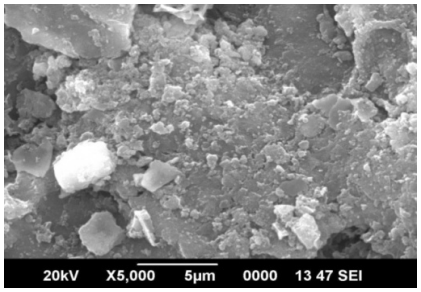

Fig. 7: TEM image

***In vitro* cytotoxicity of iron oxide nanoparticles**

The iron oxide nanoparticle in various concentrations was applied to tumor-bearing mice and the percentage of cytotoxicity was calculated (table 1).

Table 1: Percentage cell death due to *Annona muricata* leaf extract and iron oxide nanoparticle

	Percentage of cell death (%)				
	Concentration (µg)				
	200	100	50	20	10
1. *A. muricata* leaf extract	23.42±0.22	18.91±0.12	15.62±0.16	7.14±0.24	5.10±0.14
2. Iron oxide nanoparticles	83.56±0.20	78.43±0.16	74.50±0.18	58.86±0.12	37.2±0.10

(n=3, mean±SD)

From the cytotoxicity analysis, it was found that the iron oxide nanoparticle synthesized using *Annona muricata* leaf extract exhibited more ability to destroy tumorous cells than the *Annona muricata* leaf extract. The percentage of cytotoxicity increases when drug concentration increases and vice versa. At 100µg, the iron oxide nanoparticles synthesized using *Annona muricata* leaf extract have 83.56% cell death of Daltons Lymphoma Ascites cells. It was found that the iron nanoparticle can induce a cytotoxic effect on DLA cells in a dose-dependent manner, inhibiting tumor progression and thereby effective controlling disease progression without toxicity to normal cells [18]. Iron oxide nanoparticles can serve as anti-tumor agents by decreasing progressive development of tumor cells.

Antibacterial activity of iron oxide nanoparticles

Antibacterial activity of the synthesized iron oxide nanoparticle was studied against *Staphylococcus aureus* and *Escherichia coli* using agar

well diffusion method. Wells of standard size were cut and the extracts were added to each well. The petri dishes were incubated for 24 h at 37 °C and the diameter of zone inhibition is the measure of antibacterial activity [19]. The treatments were repeated thrice, and the mean was taken. The results of zone inhibition in mm are reported in table 2.

Table 2: Inhibition zones formed by iron oxide nanoparticles synthesized using *Annona muricata* aqueous leaf extract

Microorganisms	Diameter of inhibition zones (mm)			
	Iron oxide nanoparticles (µg) *Ciprofloxacin* (µg)			
	10	20	40	10
1. *Staphylococcus aureus*	-	-	12+0.20	20+0.18
2. *Escherichia coli*	12+0.16	14+0.22	18+0.12	20+0.14

(n=3, mean±SD)

The result showed that iron oxide nanoparticles at a concentration of 40µg exhibited significant activity against *Staphylococcus aureus* and *Escherichia coli* and was quite comparable to the standard antibiotic Ciprofloxacin (10µg) screened under similar conditions. The mechanism of the bactericidal activity of iron oxide nanoparticles are due to the attachment of the iron oxide nanoparticles to the cell wall. Iron oxide nanoparticles disturb the permeability of the membrane by penetrating to the cell membrane and causing intracellular ATP leakage and cell death [20].

Photocatalytic degradation of methylene blue

Photo catalytic activity of the synthesized iron oxide nanoparticle was evaluated by the decolorization of methylene blue dye in aqueous solution. The experiment was carried out in the presence of visible light irradiation without any catalyst. 10 mg of methylene blue dye was added to 1000 ml of double-distilled water used as a stock solution and 10 mg of iron oxide nanoparticles was added to 100 ml of methylene blue dye solution. A control was also maintained without the addition of iron oxide nanoparticles.

The reaction suspension was well mixed by stirring for 30 min to make the equilibrium of the working solution. The dispersion was then put under the sunlight and 10 ml of the solution was withdrawn at 30 min time interval to evaluate the photocatalytic degradation of the dye. The solution was centrifuged for 1 min and filtered to remove the iron oxide catalyst particles before measuring the absorbance. That was characterized by UV-Visible spectroscopy [21].

Photocatalytic degradation of methylene blue occurs in the presence of iron oxide nanoparticle of *Annona muricata* leaf extract, which act as catalyst and decrease the absorption peak of methylene blue (665 nm). With the increase in reaction time, the intensity of the peak gradually decreases and after 4 hour of the reaction the peak at 665 nm nearly vanishes and it indicates that the total methylene blue present in the solution has been degraded (fig. 8).

Discolorization of the dye increased as contact time also increases and the UV spectrum has shown a continuous decrease in absorption. This indicates that methylene blue can be removed by iron oxide nanoparticles synthesized using *Annona muricata* extracts.

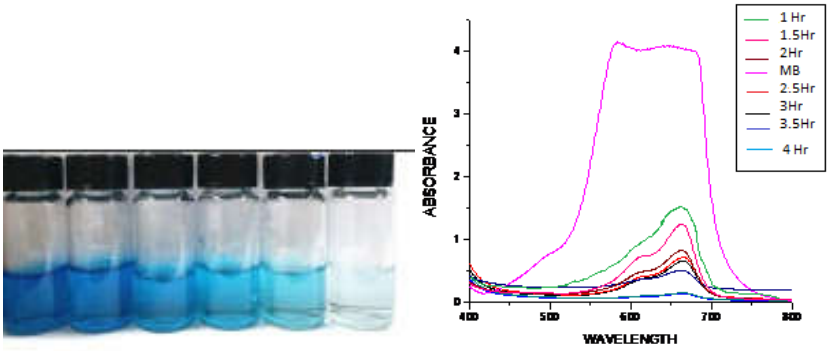

Fig. 8: The absorption spectra of an aqueous solution of methylene blue treated with 10 mg of synthesized iron oxide nanoparticles using *A. muricata* at different time intervals

CONCLUSION

In the present study, iron oxide nanoparticles were synthesized using *Annona muricata* leaf extract and are characterized by FTIR, XRD, UV-Visible SEM and TEM techniques. The secondary metabolites such as phenols, flavonoids, tannins, steroids, coumarin, saponins and terpenoids present in the leaf extract played a dual role of both reducing the metal salt and stabilizing the resultant nanoparticles. Synthesized nanoparticles are quite stable without any chemicals as capping and stabilizing agent because of biomolecules present in the extract act as capping agent.

The *in vitro* cytotoxicity analysis showed that the ability of the synthesized iron oxide nanoparticle to destroy the tumor cell is greater than the *Annona muricata* leaf extract. The green synthesized iron oxide nanoparticles showed good antibacterial activity against the tested pathogens. The present study highlights the potential application of iron oxide nanoparticles in biomedical industries.

The synthesized iron oxide nanoparticles also exhibited pronounced photocatalytic activity in the degradation of methylene blue. The textile industries produce effluents with high levels of toxic and recalcitrant compounds, such as dyes, which generate disastrous effects on the environment and the human being. The synthesized iron nanoparticles are very effective in the purification of water contaminated with textile dyes and can be efficiently placed as a great tool to promote sustainable development to the present and to the future.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally in the research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

1. Rai M, Yadav A, Gade A. CRC 675-current trends in phytosynthesis of metal nanoparticles. Crit Rev Biotechnol 2002;28:277-84.
2. Sravanthi M, Munikunar M, Ravichandra M, Hemalatha KP. Green synthesis and characterization of iron oxide nanoparticles in *Wrightia tinctoria* leaf extract and their antibacterial studies. Int J Curr Res Acad 2016;4:30-44.
3. Bharani M, Thirunethiran K, Varalakshmi B, Gayathri G, Lakshmi P. Synthesis and characterization of silver nanoparticles from *Wrightia tinctoria*. Int J Appl Biol Pharm Tech 2012;3:58-63.
4. Bruno Lellis C, Zani Favarp PJ, Alencar PJ, Cesar P. Effects of textile dyes on health and the environment and bioremediation potential of living organisms. Biotechnol Res Innov 2019;3:275-90.
5. Misas C, Hernandez N, Abraham A. Contribution to the biological evaluation of cuban plants. Cuban J Trop Med 1979;31:29-35.
6. Baskar R, Rajeswari V, Kumar TS. *In vitro* antioxidant studies in leaves of Annona species. Indian J Exp Biol 2007;45:480-5.
7. Sousa OV, Vieira GD, Pinho JJ, Yamamoto CH. Antinociceptive and anti-inflammatory activities of the ethanol extract of Annona muricata L. leaves in animal models. Int J Mol Sci 2010;11:2067-78.
8. Vimala JR, Leema RA, Raja S. A study on the photochemical analysis and corrosion inhibition on mild steel by *Annona muricata* leaves extract in 1N hyhrochloric acid. Chem Sin 2012;3:582-8.
9. Patel S, Patel JK. A review on a miracle fruits of *Annona muricata*. J Pharmacogn Phytochem 2016;5:137-48.
10. Amrutha S, Sridhar S. Green synthesis of magnetic iron oxide nanoparticle using leaves of *Glycosmis maurintiana* and their antibacterial activity against human pathogens. J Innov Pharm Biol Sci 2018;5:22-6.
11. Phull AR, Abbas QA, Raza H, Kim SJ, Zia M. Antioxidant, cytotoxic and antimicrobial activities of green synthesized silver nanoparticles from the crude extract of *Bergenia ciliate*. Future J Pharm Sci 2016;6:320-44.
12. Brajesh K, Smita K, Cumbal L, Debut A. Biogenic synthesis of iron oxide nanoparticles for 2-arylbenzimidazole fabrication. J Saudi Chem Soc 2014;18:364-9.
13. Pattanayak M, Nayak PL. Ecofriendly green synthesis of iron nanoparticles from various plants and spices extract. Int J Plant Anim Environ Sci 2013;3:68-78.
14. Kim T, Grady AN, Karl J, Justin S. Synthesis, characterization, and alignment of magnetic carbon nanotubes tethered with maghemite nanoparticles. J Phys Chem 2010;114:6944–51.
15. Tzitzios VK, Bakandritsos A, Georgakilas V, Basina G, Boukos N, Bourlinos AB, *et al*. Large-scale synthesis, size control, and anisotropic growth of-Fe_2O_3 nanoparticles: organosols and hydrosols. J Nanosci Nanotechnol 2007;7:1–5.
16. Mihir H, Siddhivinayak B, Rakesh KM. Plant mediated green synthesis of iron nanoparticle. J Nanopart 2014;10:1-9.
17. Amutha S, Sridhar S. Green synthesis of maganetic iron oxide nanoparticle using leaves of Glycosmis Mauritiana and their bacterial activity against human pathogen. J Innov Pharm Biol Sci 2018;5:22-6.
18. Nurul AH, Hock IC, Muggundha RR, Wai HT. Cytotoxicity of plant-mediated synthesis of metallic nanoparticles: a systematic review. Int J Mol Sci 2018;19:1725-48.
19. Deepa RH, Monnanda SN. Antibacterial activity of lianoid and arboreal species of schefflera from sothern India. Asian J Pharm Clin Res 2020;13:46-50.
20. Sheena V, Sunny K, Soumya J. Antimicrobial activity of carbon nanoparticles isolated from natural sources against pathogenic gram-negative and gram-positive bacteria. J Nanosci 2013;10:1-5.
21. Vanaja M, Paulkumar K, Baburaja M, Rajeshkumar S, Gnanajobitha G, Malarkodi C, *et al*. Degradation of methylene blue using biologically synthesized silver nanoparticles. Bioinorg Chem Appl 2014;10:1-8.