BIOSYNTHESIS AND CHARACTERIZATION OF SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES FROM THE LEAF EXTRACT OF *HEMIGRAPHIS COLORATA* (BLUME) HALLIER F. AND ITS CYTOTOXICITY STUDY

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Abstract:

Iron oxide nanoparticles are gaining importance for their uses in environmental remediation technologies and in medicine. In the present investigation, iron nanoparticles were synthesized and characterized by green route using leaf extracts of *Hemigraphis colorata* (Blume) Hallier F. The reductant present in the plant extracts act as reducing and stabilizing agent for synthesizing iron oxide nanoparticles. FeNps were characterized by UV-Visible spectroscopy, FTIR Analysis, XRD, SEM, TEM and SAED Analysis. UV-Vis spectroscopy analysis was done in the range of 200-400nm and the maximum absorption was observed at 215nm regions for the formation of FeNPs characteristic of iron nanoparticle. The FTIR spectrum shows prominent bands at 3448.87, 3411.26, 2884.67, 2813.30, 1628.95, 1268.25, 1050.29 and 1426.42 cm^{-1} The SEM micrographs showed that the synthesized iron oxide nanoparticle has irregular sphere shapes and TEM reveals the size of the synthesized FeNPs to be less than 10 nm that can act as super paramagnet. XRD analysis determines angles that correspond to crystal planes of 100, 311 and 511 of crystalline iron oxide nanoparticle. The average crystallite size was calculating using Debye-Scherrer equation which gives 9.01nm for 311 peak. The cytotoxic study of synthesized FeNPs from *Hemigraphis colorata* has short term in vitro anticancer activity against DLA cell lines. FeNPs synthesized from *Hemigraphis colorata* showed 44% of cell death in 200µl concentration against Dalton's lymphoma ascites cells bearing mice. These eco-friendly, cost effective stable nanoparticles synthesized from leaf extracts of *Hemigraphis colorata* can therefore be used as an economic and valuable alternative for the large-scale production of iron oxide nanoparticles.

Keywords: FeNps, UV-Vis spectroscopy, SEM, TEM, SAED, XRD, FTIR, Cytotoxic

1. Introduction:

Nanotechnology research has emerged rapidly during the past year in a broad range of product domains. It provides opportunities for the development of materials including those for medical applications, where conventional techniques may reach their limits (Le V N *et al.*, 2014). Nanotechnology has a wide range of applications mainly in the field of biomedical, drug delivery system and ultra-sensitive disease detection (Panyam J *et al.*, 2013). Plant-mediated biological synthesis of nanoparticles has gained importance only in the recent years (Gardia J L *et al*., 2002). Synthesis of nanoparticles using plants provides more biocompatible nanoparticles than chemical synthesis, whereas chemical synthesis may lead to the presence of some toxic chemical species on the surface of nanoparticles that may have undesirable effects in biomedical application (Ahmad N *et al.*, 2011). Iron nanoparticles have attracted intensive research interest because of their important applications in cancer therapy, drug delivery, magnetic resonance imaging (MRI) and wastewater treatment (Vicky M *et al.*, 2010). Nanoparticles are attractive for cancer applications as they can be engineered to have multifunctional serving simultaneously as imaging contrast agents, therapeutic agents, drug delivery vehicles (Vasquez K M *et al.*, 1998). Some researchers have reported that small-sized nanoparticles are more readily able to enter the cancer cells and interact with components inside the cancer cells, hence those nanoparticles may act as a good system for drug delivery and often new perspectives for diagnostic and targeted therapeutic approaches for cancers (Bigger I *et al.*,2002).

2. Materials and Methods

2.1. Collection of plant materials:

Fresh leaves of *Hemigraphis colorata* (Blume) Hallier f. were collected from ATIC medicinal plants, Mannuthy and the taxonomic identification was made by Taxonomist Anto P V, Assistant Professor, St. Thomas College, Thrissur.

2.2. Preparation of plant extract:

The leaves of *Hemigraphis colorata* (Blume) Hallier f. were washed thoroughly and dried using hot air oven at 60^0C. 2.5g of fine powder was weighed and boiled with 100ml of deionized water at 80^0C for 15 minutes using water bath. The plant extract was filtered and collected separately.

2.3. Synthesis of iron oxide nanoparticle from plant extract:

Take 10ml of leaf extracts of *Hemigraphis colorata* in a beaker. Add 90 ml of aqueous solution of 1mM ferric chloride solution into it. If iron nanoparticle is present, there will be a color change from yellowish brown to

brownish or greenish black after certain time period of incubation at room temperature. The solution containing FeNPs were separated and concentrated by repeated washing and centrifugation at 16,000 rpm for 20 minutes. The final suspension was dried in hot air oven and the characterizations of obtained nanoparticles were carried out.

2.4. Characterization of iron oxide nanoparticles

The formation of nanoparticle was confirmed and monitored with the help of various analytical methods. The preliminary characterization of iron oxide nanoparticles was carried out using UV- visible spectroscopy based on optical properties. The reduction of iron oxide was monitored by recording the absorption spectra at the range of 400 to 500 nm (Shimadazu, Japan) X-ray diffraction pattern was obtained by using lyophilized powders of iron oxide nanoparticles Phillips PW- 1710 automated diffractometer using a Cu Kα radiation in the 2θ range of 10° to 70º operated at a voltage of 40kV and a current of 35mA. XRD provides information about the grain size of the iron oxide nanoparticles which can be determined using Debye Sherrer's equation.

$D = K\lambda / \beta \cos\theta$

The Fourier transform infrared spectroscopy (FT-IR) spectroscopic analysis were performed using Shimadazu (Japan) FT-IR spectrophotometer with KBr pellet (1:100 ratio) in the wave number region of 4000 to 500cm^{-1}. Scanning electron microscope (SEM) analysis were carried out using SEM (JEOL-MODEL 639O) at an accelerating voltage of 20KV and Transmission electron microscope (TEM) analysis was done using TEM (JEOL 2100F model) to determine the size and morphology of nanoparticles examined.

2.5. Cytotoxicity assay:

The cytotoxicity of synthesized FeNPs was studied for short term in vitro cytotoxicity using Dalton's lymphoma ascites cells (DLA). This tumour cells were aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by Tryphan blue exclusion method. Viable cell suspension (1×10^6 cells in 0.1 ml) was added to tubes containing various concentrations of the FeNPs solution made by adding deionized water and the volume was made up to 1 ml using phosphate buffered saline (PBS). Control tube contained only cell suspension. These assay mixture were incubated for 3 hours at 37^0C. Further cell suspension was mixed with 0.1 ml of 1% Tryphan blue and kept for 2-3 minutes and loaded on a Haemocytometer. The numbers of stained and unstained cells were counted separately.

3. RESULT

3.1 Synthesis of FeNPs:

The green synthesis of iron oxide nanoparticle was carried out by adding 1mM $FeCl_3$ solution into the aqueous leaf extract of *Hemigraphis colorata* results a color change from yellowish-brown to brownish black. This color change is due to the redution of $FeCl_3$ solution to Fe^{3+}ionswhich indicate the formation of iron nanoparticle. The micro-centrifuged and air dried samples of iron nanoparticle were used for the characterization of FeNPs.

3.2 Characterization of FeNPs:

3.2.1 UV-Visible spectral analysis:

The synthesis of FeNPs from leaf extracts of *Hemigraphis colorata* were analyzed by using UV-Vis spectroscopy ranging from 200 nm-400 nm wave length as given in graph 1. In UV-Vis spectrum, the surface plasma resonance (SPR) peak was observed at 215nm, indicating the reduction of Fe^{3+} ions which further confirms the formation of iron nanoparticles.

3.2.2 FTIR Analysis:

FTIR Analysis was carried out to determining the functional groups of synthesized iron nanoparticles. The observed intense bands were compared with standard values to identify the functional groups. The FTIR spectrum shows prominent absorption peak at 3963.89, 3776.78, 3448.87, 3411.26, 2884.57, 2813.230, 1628.95, 1426.42, 1268.25, 1050.29, 605.67cm^{-1} as shown in graph 2. The peaks at 3448.87 and 3411.26$cm^{-1}$was assigned for N-H strech present in the FeNPs, the peaks at 1183.38cm^{-1} was assigned for C-N strecth and peaks at 1059.29cm^{-1} corresponds to C-O streching in the FeNPs; which confirms amide linkage of proteins.

3.2.3 XRD Analysis:

The XRD study of the sample indicates the formation of iron nanoparticles. The observed peaks were compared with the standard powder diffraction card of (JCPDS File No. 87-0720), and the results are in agreement with the standard XRD pattern of FeNPs as shown in table no.1. The average crystallite size was calculating using Debye-Scherrer equation which gives 9.01nm for 311 peak.

Table no.1. X-ray diffraction data of FeNPs

Hkl	(100)	(311)	(400)	(422)	(511)	(440)
2θ (Degree)	31.84	35.46	43.07	53.44	56.93	62.54
D (A^0)	2.80	2.52	2.09	1.71	1.61	1.48

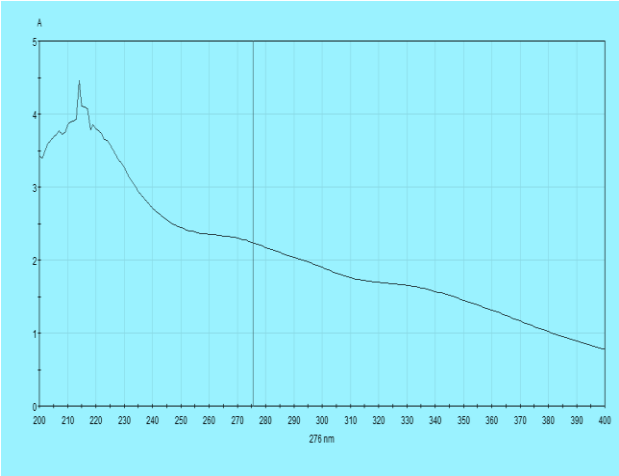
3.2.4 SEM Analysis:

SEM technique was employed to characterize the size, shape and morphology of iron oxide nanoparticles. SEM images were obtained for the iron nanoparticles of leaf extracts of Hemigraphis colorata as shown in fig.1. The SEM micrograph revealed that the synthesized iron nanoparticles were aggregated as irregular sphere shapes and ranges from 100-200 nm with inter particle distance. The larger size of particle is due to capping agent binding the Fe nanoparticle as determined using FTIR.

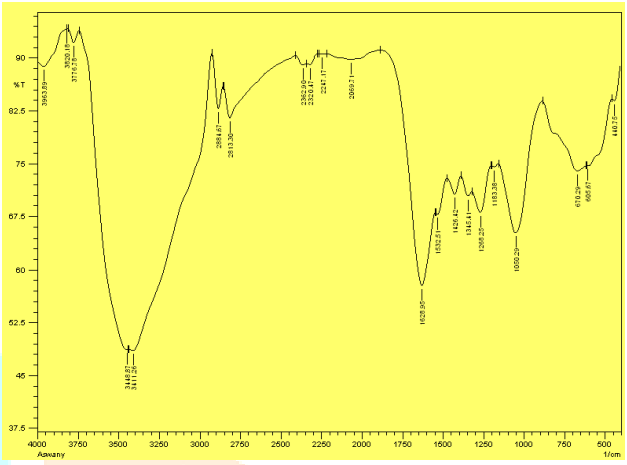
3.2.5 TEM and SAED pattern of FeNPs:

Transmission electron microscopy (TEM) provided further insight into the morphology and size. The irregular sphere nature of the FeNPs from aqueous extracts of *Hemigraphis colorata* was visualized by TEM analysis with average diameter ranging from 5-9nm. Selected area electron diffraction (SAED) spots that corresponds to the different crystallographic planes of face centered cubic structure (FCC) of elemental iron. Since the nanoparticle size is <10nm, they behave as superparamagnetic FeNPs. This behaviour can be attributed to their size.

Graph 1. UV-Vis Spectra analysis of FeNPs of leaf extracts of *Hemigraphis colorata*



Graph. 2 FTIR Spectra of FeNPs of leaf extracts of *Hemigraphis colorata*



Graph. 3 Bragg reflection angle of FeNPs using XRD

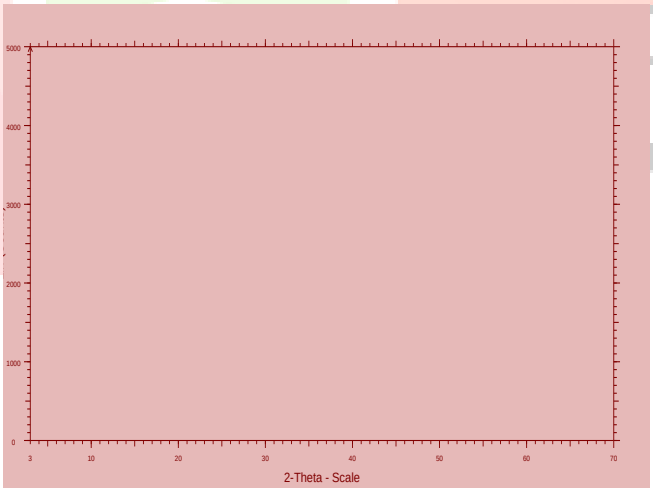


Fig. no. 1. SEM Micrograph of FeNPs of the leaf extracts of *Hemigraphis colorata*

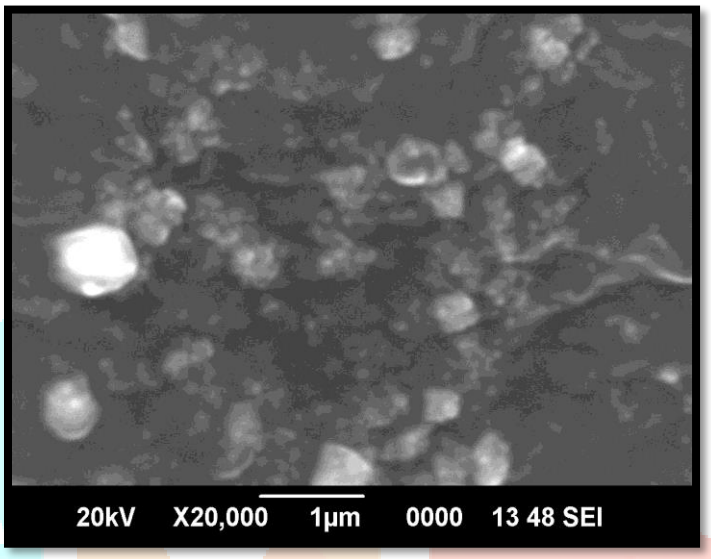
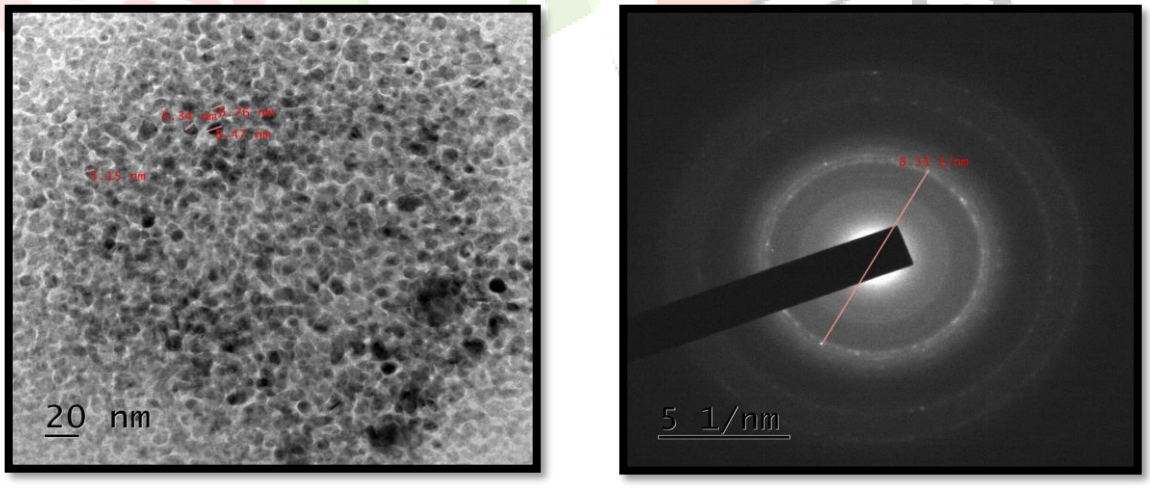


Fig no. 2. TEM Micrograph and SAED pattern of FeNPs of the leaf extracts of *Hemigraphis colorata*



(a) TEM micrograph **(b) SAED pattern**

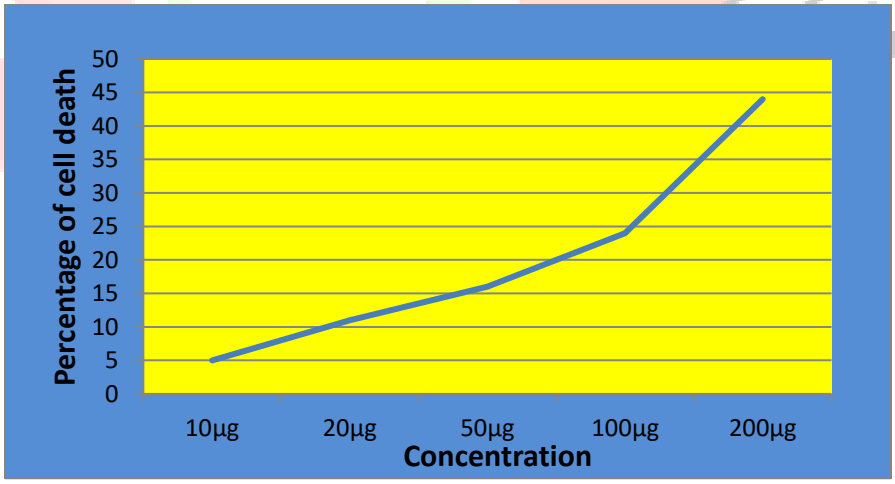
3.3 Cytotoxicity assay:

The short term invitro cytotoxicity were studied for synthesized FeNPs using Dalton's Lymphoma Ascites (DLA) cells and evaluated by Tryphan blue dye exclusion method using Haemocytometer. The table no.2 represents the cytotoxicity of the DLA with FeNPs at different concentrations ranging from 10µg/ml to 200µg/ml.

Table no.2 Showing percentage of cell death recorded by using Haemocytometer.

Sl.No	Concentration(µg/ml)	Percentage of cell death
1	200	44
2	100	24
3	50	16
4	20	11
5	10	5

Graph no.4. showing the percentage of cell death when treated with FeNps.



4. Discussion

The super paramagnetic iron nanoparticles were synthesized from aqueous leaf extract of *Hemigraphis colorata* (Blume) Hallier f. UV-Vis spectroscopy analysis was done in the range of 200-400nm and the maximum absorption due to the excitation of surface Plasmon vibrations was observed at 215nm regions for the

formation of FeNPs characteristic of iron nanoparticle as reported earlier. The FTIR measurements were carried out to identify the possible biomolecules for the reduction of Fe ions and capping of reduced iron nanoparticles. The FTIR spectrum shows prominent bands at 3448.87, 3411.26, 2884.67, 2813.30, 1628.95, 1268.25, 1050.29 and 1426.42 cm^{-1}. SEM and TEM reveals the size of the synthesized FeNPs to be lesser (<10 nm), which behave as super paramagnet. In the paramagnetic state, the individual atomic magnetic moments are randomly oriented, and the substance has a zero net magnetic moment if there is no magnetic field. SAED analysis helped to visualize the crystal planes of synthesized iron nanoparticles. The average size, the crystalline nature of the particles and quality of compounds were determined by X-Ray Diffraction Spectrum showing different diffraction peaks at 31.84^0, 35.46^0, and 56.93°. XRD analysis was done in order to determine the actual size of FeNPs which was corresponding to crystal planes of 100, 311 and 511 of crystalline FeNPs. The average crystallite size was calculating using Debye-Scherrer equation which gives 9.01nm for 311 peak. The cytotoxic study of synthesized FeNPs from *Hemigraphis colorata* has short term in vitro cytotoxicity activity against DLA cell lines. FeNPs synthesized from *Hemigraphis colorata* showed 44% of cell death in 200µl concentration against Dalton's lymphoma ascites cells bearing mice.

5. Conclusion

From this study; we conclude that the synthesized nanoparticles have super paramagnetic property due to their size less than 10 nm. The thermal fluctuations can also change the direction of magnetization of the entire crystal. This behaviour of super paramagnets is preferred in biomedicine as they are biocompatible and potentially non-toxic to humans. Thus, chemotherapeutics agents encapsulated with super paramagnetic FeNPs is used in targeted drug delivery system and hence can be used to treat soild tumours. The invitro cytotoxicity study of nanoparticles showed remarkable percentage of cancerous cell death.

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6. References

- Ahmad N, Sharma S, Sigh V N, Shamsi S F, Fathima A and Mehta B R (2011). Biosynthesis of silver nanoparticle from *Desmodium triflorum*: A Noval approach towards weed utilization, *Journal of Biotechnology and Research*, Vol. 1(8), P. 54-66.
- Bigger I, Dubernet C and Couvreur P (2002). Nanoparticles in cancer therapy and diagnosis, *Advanced Drug Delivery Reviews*, Vol. 4(5), P. 631-651.

- Gardia J L, Gomez E, Peralta-Videa J R, Parsons J G, Troiani H and Jose-Yacaman M (2003). Alfalfa sprouts: a natural source for the synthesis of silver nanoparticle, *Langmuir*, Vol. 19(4), P. 1357-1361.
- Le V N, Rui Y, Gui X, Liu S and Han Y (2014). Uptake, transport, distribution and bioeffects of SiO2 nanoparticles in Bt. transgenic cotton, *Journal of Nanotechnology*, Vol. 12(1), P. 50-55.
- Panyam J and LabhaSetwar V (2013). Biodegradable nanoparticles for drug and gene delivery to cells and tissue, *Advance Drug Delivery Reviews*. Vol. 55(3), P. 329-347.
- Vasquez K M and Wilson J H (1998). Triplex-directed modification of gene and gene activity, *Trends in Biochemical Sciences*, Vol. 33(1), P. 4-9.
- Vicky M., Rodeny S., Ajay S., Hardhik M (2010). Intoduction to metallic nanoparticles, *Journal of Pharmaceutical and Bioallied Science*. Vol. 2(4), P. 282-289.