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# Fabrication of nanoparticles using *Annona squamosa* leaf and assessment of its effect on liver (Hep G2) cancer cell line

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**Abstract.** *Annona squamosa* is a fruit bearing plant possesses potent bioactive compounds in all its part. In this present investigation iron oxide nanoparticle was synthesized from hydroethanol extract of *Annona squamosa* leaves at 60<sup>0</sup> c temperature. Production of iron oxide nanoparticles in extraction is detected by UV–V spectrophotometer, Scanning electron microscopy was employed to analyse the structure of nanoparticles. Fourier transform infrared spectroscopy (FT-IR) analysis were performed, in order to determine the functional groups on *Annona squamosa* leaves extract. The synthesized Fe<sub>3</sub>O<sub>4</sub> NPs shows potential cytotoxicity against liver carcinoma cell line (HepG2), and there is no toxicity on the normal liver cell line. Our reports confirmed that the *Annona squamosa* leaf is a very good eco-friendly and nontoxic bioreductant for the synthesis of Iron oxide nanoparticle and opens up further opportunities for fabrication of drugs towards cancer therapy.

## 1. Introduction

From our ancient history, our ancestors used various plant as a drugs for many diseases, many of our traditional plants has healing properties. Cancer treatments do not have potent medicine as the currently available drugs are causing side effects in some instances. The main obstacle in treating cancer is delivering of drug to a specific carcinoma cell. Thus besides from plants, effort has also been made to discover natural products from iron oxide nanoparticles to provide clinic drugs directly for the synthetic. More specifically for drug delivery purposes, the use of nanoparticles is attracting, increasing attention due to their unique capabilities and their negligible side effects not only in cancer therapy but also in the treatment of other ailments. Among all types of nanoparticles, biocompatible superparamagnetic iron oxide nanoparticles (SPIONs) with proper surface architecture and conjugated targeting ligands/proteins have attracted a great deal of attention for drug delivery applications [1]. *Annona squamosa* plant, a angiosperm fruit bearing plant which exhibit medicinal property in all their parts such as leaves of *Annona squamosa* have strong antibacterial activity [2], Hypoglycemic [3] and Anticancer activity [4]. In the present study, we report the use of *Annona squamosa* leaf in the biosynthesis of iron oxide nanoparticle and its cytotoxic effect on liver (HepG2) cancer cell lines.

## 2. Materials and methods

### 2.1. Chemicals

The chemicals were purchased from Sigma, USA. All other chemicals used are of analytical grade.



## 2.2. Collection of sample and authentication

Fresh leaves of *Annona squamosa* plant was collected locally during the month of November to January and authenticated by the Taxonomist Prof. P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai, India. (PARC/2009/456).

## 2.3. Preparation of leaf extract

Extract were prepared by cold maceration method using 30:70 hydro ethanol and air dried *Annona squamosa* leaf powder. The concentrated crude extracts were lyophilized into paste (5 and 15 g respectively) and were taken for further investigation.

## 2.4. Synthesis of iron oxide nanoparticles

Iron oxide nanoparticles were synthesized by a modified co-precipitation method [5]. Ferric chloride ( $\text{FeCl}_3$ , 0.074 g) and ferrous chloride ( $\text{FeCl}_2$ , 0.190 g) at a ratio of 2:1 were dissolved in 20 ml deionized water, which was then stirred with 1:1, 1:2, 1:3, 1:4 and 1:5 ratios of plant extract and heated to 60°C. The nanoparticles were then removed from solution by magnetic separation.

## 2.5. Determination of synthesized iron oxide nano particles

**2.5.1 UV-Visible spectroscopy:** The Surface Plasmon Resonances (SPR) of synthesized iron oxide nanoparticles have been studied by UV-Visible double-beam bio-spectrophotometer ELICO-BL-198 using the software SPECTRAL TREATS VERSION 2.37.4 REL-1.

**2.5.2. SEM analysis (scanning electron microscopy):** SEM was used to verify uniformity of nanoparticle shape and size as previously described. The fabricated nanoparticles were dropped onto black carbon tape with a double-side. After that, they were vacuum-coated with a platinum mixture for 45 s and morphologically analyzed with a FE-SEM (CamScan Apollo 300 Field-Emission SEM, UK) at 20 kV.

**2.5.3. Fourier transform infrared spectrophotometer (FTIR):** Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The Liquid extract of plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a scan range from 400 to 4000 $\text{cm}^{-1}$  with resolution of 4  $\text{cm}^{-1}$ .

## 2.6. Antiproliferation assay

**2.6.1 Cell lines and culture:** Human hepatocellular carcinoma cell lines, HepG2 cells (American Type Culture Collection [ATCC] CRL 8024) were obtained from National center for cell science, Pune, India was maintained in monolayer culture at 37 °C and 5%  $\text{CO}_2$  in DMEM supplemented with 10% FBS, 100 U/ml of penicillin, 50  $\mu\text{g}/\text{ml}$  of streptomycin.

**2.6.2. Cytotoxicity assay:** The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately  $1.2 \times 10^4$  cells/well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated with different concentrations of the iron oxide nanoparticles synthesized from leaf extract of *Annona squamosa* (25, 50 and 100  $\mu\text{g}$ ) for 24 hours. After the incubation, medium was discarded and 100  $\mu\text{l}$  fresh medium was added with 10  $\mu\text{l}$  of MTT (5mg/ml)[14]. After 4 hours, the medium was discarded and 100  $\mu\text{l}$  of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a microtitre plate reader. Cyclophosphamide was used as a positive control. Cell survival was calculated using standard formula.

## 3. Result and discussion

In the present study, we report on rapid biosynthesis of Iron oxide nanoparticle using *A. squamosa* leaf extract. The plant mediated iron oxide nanoparticles was prepared at room temperature.

### 3.1. Synthesis of iron oxide nanoparticles

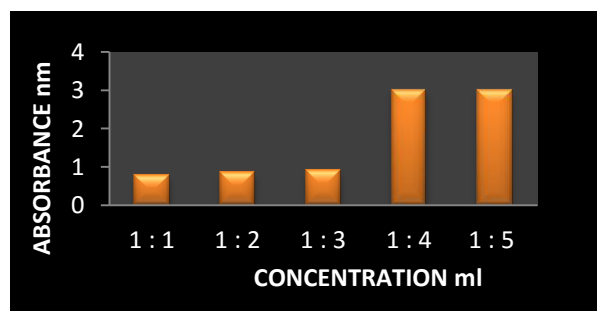
The addition of Ferric chloride solution to the *Annona squamosa* leaves extract the reduction reaction takes place of  $\text{Fe}^{3+}$  to  $\text{Fe}_3\text{O}_4$ . A possible reaction beyond the formation of iron oxide nanoparticles is Ferric chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) and *Annona squamosa* leaf extract are involved in the reaction of aqueous phase medium. Figure 1 represents the formation of iron oxide complex by dark brown colour appearance. Initially, the  $\text{C}=\text{O}$  of aldehyde group in *Annona squamosa* leaf extract chelated with  $\text{Fe}^{3+}$  ions to form ferric protein chains  $\text{HO}^- \dots \text{Fe}^{3+} \dots$  bonds and as result in the formation of suspended ferric hydroxide  $\text{Fe}(\text{OH})_3$ . Subsequently on slow evaporation, ferric hydroxide in a core is dehydrated ( $-\text{H}_2\text{O}$ ) to form a black colored magnetite ( $\text{Fe}_3\text{O}_4$ ) nanoparticle as a crystals. The protein chain in *Annona squamosa* leaf extract covered on  $\text{Fe}_3\text{O}_4$  surface through chelation of  $\text{COO}^- \dots \text{Fe}^{3+}$ .



**Figure 1.** Photograph of visible color change of *Annona squamosa* leaf extract.

### 3.2. UV-Visible spectroscopy

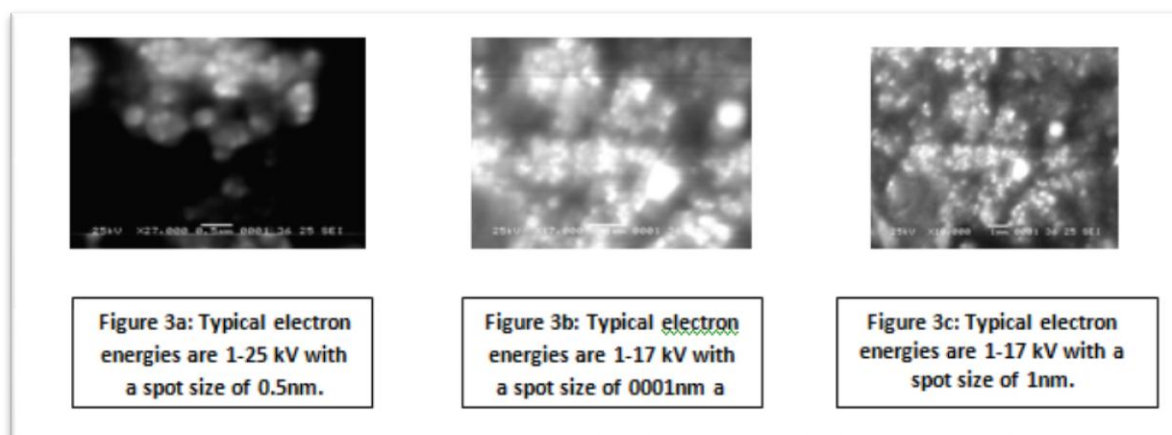
After the addition the *Annona squamosa* leaf extract into the aqueous solution of  $\text{FeCl}_3$ , the solution was filled in glass cuvette of path length 10mm and UV-Vis spectral analysis has been done in the range of 300 to 700 nm. DI water was used as blank. Figure 2 represents the absorbance obtained by UV-spectroscopy on various concentration gradient of sample.



**Figure 2.** Absorbance obtained by UV-spectroscopy on various concentration of sample.

### 3.3. SEM analysis (scanning electron microscopy)

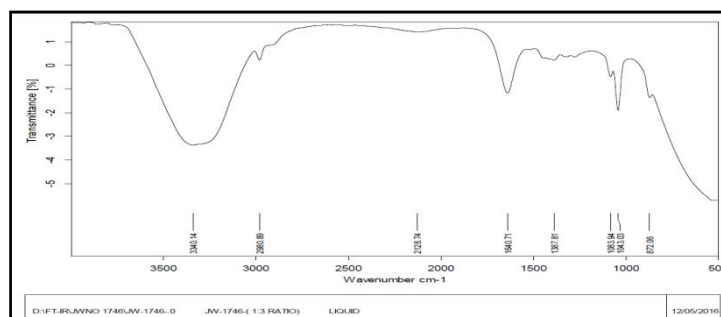
The surface morphology of the iron oxide and silica particles has been studied by scanning electron microscopy method. As shown in Figure 3a-3c represents the SEM images of  $\text{Fe}_2\text{O}_3$  nanoparticles grafted with *Annona squamosa* with small spot by SEM analysis. Figure 3a-3c suggests the aggregation of the particles and small grains are present at the surface. A scanning electron microscopy was employed to analyse the structure of nanoparticles that were formed. Figure.3a shows the low magnification 25k x 27,000 SEM image of  $\text{Fe}_3\text{O}_4$  powder; it can be seen that the particles are agglomerated. The size distribution and morphology is irregular. The particles are plate like structure with coarsened grains, whereas the magnification at 25k x17,000 in Figure3b, the  $\text{Fe}_3\text{O}_4$  nanoparticles showed uniformly distributed small spherical shaped particles. The magnification at 25kx 10,000 (Figure3c) under the same conditions, which showed that large number of homogeneous nano capsule like morphology of iron oxide nanoparticles.



**Figure 3.** The SEM images of  $\text{Fe}_2\text{O}_3$  nanoparticles grafted with *Annona squamosa*.

### 3.4. Fourier transform infrared spectrophotometer (FTIR)

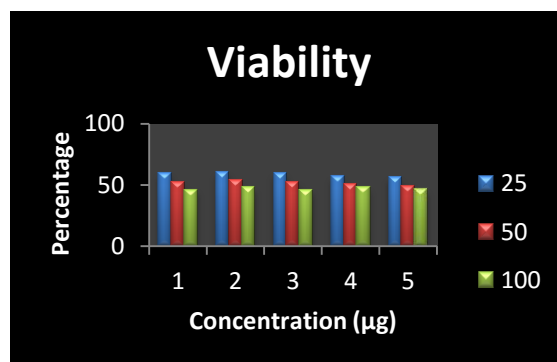
FTIR analysis was performed, in order to determine the functional groups on *Annona squamosa* leaves extract and predict their role in the synthesis of iron oxide nanoparticles. Figure 4 shows the FT-IR spectrum of *Annona squamosa* leaf extract. The strong absorption peak at  $3340\text{ cm}^{-1}$  is assigned to O-H stretching of alcohol and phenolic compounds or stretching of the -NH band of amino group. The presence of peak at  $2980\text{ cm}^{-1}$  are assigned to aliphatic C-H stretching in methyl and methylene groups. The peak at  $1640\text{ cm}^{-1}$  is due to stretching vibration of CO groups in the ketones, aldehydes and carboxylic acids. The peak belonging to  $1387\text{ cm}^{-1}$  is due to COO stretching vibration. The peak at  $1640\text{ cm}^{-1}$  is attributed to the presence of carboxylate ions ( $\text{COO}^-$ ), which is responsible for the formation of iron oxide nanoparticles. The peaks at  $1083$  and  $1043\text{ cm}^{-1}$  indicate the presence of CO groups. This peak was absent in plant extract which indicate the formation of iron oxide nanoparticles. The absorbance band at  $872\text{ cm}^{-1}$  might be assigned to the existence of some amount of oxidised iron oxide on the surface. FT-IR analysis confirmed that the bio reduction of ferric chloride into iron oxide nanoparticles is due to the reduction by capping material of *Annona squamosa* leaf extract.



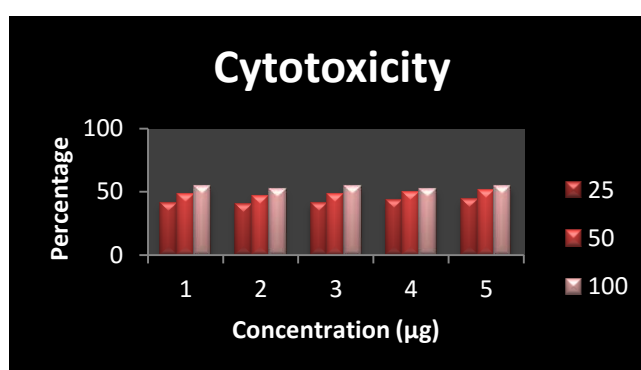
**Figure 4.** Representation of wave number  $\text{cm}^{-1}$  obtained by FT-IR analysis.

### 3.5. Anti proliferative assay- MTT against Hep G2 cells

The synthesized iron oxide nanoparticle obtained was subjected to MTT assay against Hep G2 cell line. Observation of morphological changes in cells indicated that  $\text{Fe}_3\text{O}_4$  NPs inhibited proliferation of the various cancer cell lines in a dose-dependent and time-dependent manner. No toxicity was seen in the normal liver cell line. Figure 5 represent the percentage of cell viability of HepG2 Cell line after treated with synthesized  $\text{Fe}_3\text{O}_4$  NPs and figure 6 represent the percentage of cytotoxicity exhibited by synthesized  $\text{Fe}_3\text{O}_4$  NPs on HepG2 Cell line.



**Figure 5.** Percentage of cell viability by synthesized  $\text{Fe}_3\text{O}_4$  NPs on HepG2 Cell line.



**Figure 6.** Percentage of cell cytotoxicity by synthesized  $\text{Fe}_3\text{O}_4$  NPs on Hep G2 Cells.

The cytotoxicity of the iron oxide nanoparticle was studied against the Hep G2 cell line by MTT assay. The cytotoxicity effect of synthesized iron oxide nanoparticle from the ratio 1:1 to 1:5 was studied at different concentrations 25 µg, 50 µg, and 100 µg against Hep G2 cell line. Nanoparticles synthesized from this ratio also had significantly higher yield. The cytotoxic activity of the nanoparticles synthesized from various ratios had almost the cytotoxicity which indicates that the activity arises from compounds of extract rather than ferrous sulphate.

#### 4. Conclusion

This study shows that the *Annona squamosa* iron oxide nanoparticle exhibits cytotoxic activity at very less dose. The synthesized iron oxide nanoparticles showed promising anticancer activity against human liver cancer cell line. From the study, it can be concluded that the iron oxide nanoparticles synthesized using *Annona squamosa* possess high anticancer activity against cell lines which further suggest the potential therapeutic use of these nanoparticles.

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