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Synthesis of Iron Nanoparticles from Banana Peel Extract and Its Genotoxic Effect on Human Blood Leukocyte Culture

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ABSTRACT In the present study, zero-valent iron particles are synthesised in nano-dimension from a banana peel extract under atmospheric conditions. Further characterisations of the nanoparticles are carried out to determine the particles' size, shape, and features using UV-visible spectrophotometry (UV-V) spectroscopy and atomic force microscopy (A.F.M.). The genotoxicity of these particles is checked for its future use in nano-biotechnology. Thus, the current work is a biological approach for synthesising iron nanoparticles, which is environmentally friendly and easy to carry out. The researchers also have used the biological approach to synthesise the same, as it is environment-friendly and easy to carry out. Iron nanoparticles are produced by their reduction from Fe (II) or Fe (III) in an aqueous medium with the help of ferric chloride. Later their aggregation in the medium is reduced by adding chitosan and polyvinyl chloride (P.V.A.) as stabilisers.

INTRODUCTION

Nanotechnology is the manipulation of matter at the atomic and molecular levels. The nanoparticles are in nano dimensions and differ from each other in their synthesis, properties, and applications (Khan and Hossain 2022). Though nanotechnology has recently advanced, its idea was given long back in 1959 by R. Feynman in his talk "*There is plenty of room at the bottom*" (Feynman 1992). These particles can be produced in various physical, biological, and chemical processes (Belloni et al. 1998). The bottom-up approach of the physical method is gaining much interest as it is the convergence of mechanics and chemistry. However, this needs a lot of technological research to make atomically precise nanoparticles. Using harmful chemicals and dangerous by-products, conventional chemical synthesis of nanoparticles leads to several issues. As a result, scientists' focus has switched to the green process, which facilitates the creation of safe, non-toxic, eco-friendly practices. In light of this, green-synthesised nanoparticles have seen a remarkable expansion in development and application over the past decade (Harmansah et al. 2022).

The use of nanotechnology in the biological field is an important application. Nevertheless, this requires a lot of research and testing as its effect on the human body is highly debatable. So, nano-biotechnology emphasises more on those substances which make up the human body to subside their toxic effects. An average human body contains almost ten gms of iron. However, in its usual form, iron is highly reactive in the presence of oxygen (Solomon et al. 2021). Iron nanoparticles are inert and can be easily used for many biological applications, such as drug delivery (Hemben et al. 2021; Machado et al. 2013).

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Banana, one of the most important tropical fruits in the world market, belongs to the *Musaceae* family. For thousands of years, the banana has been a highly cultivated fruit. India is the largest producer, producing 39 thousand tons (Singh et al. 2011). 18-33 percent of the whole fruit is peeled and is a waste product. The banana peel contains much more insoluble and soluble fibre than their flesh, besides being a rich source of iron and potassium. Banana peels were analysed for minerals and nutritional content. It was found to be rich in the content of iron, potassium, calcium, sodium and manganese and rich in protein, crude lipids, carbohydrates, and crude fibre (Anhwange et al. 2009). In another work, banana peel was rich in dietary fibre, essential amino acids, polyunsaturated fatty acids, and minerals like iron and potassium (Emaga et al. 2007). Medicinal benefits of banana peel have also been reported, including relief from pain, swelling, itching, bruising, wrinkles, and sunburn (Edwards 1999). However, these peels are not used for other purposes despite so many benefits. They are dumped as solid wastes at a considerable expense. It is thus essential and significant to find applications for these peels as they might contribute to real environmental problems (Zhang et al. 2005). Therefore, more studies should be conducted on the nutritional compositions and health benefits of banana peel and its extracts. The current work exploits the peel's potential value and possible utilisation.

Objective

The current work is a biological strategy for producing iron nanoparticles, which is environmentally friendly and easy to carry out.

MATERIAL AND METHODS

Reagents and Chemicals

From Sigma Aldrich, 0.001 M Ferric chloride was obtained. Throughout the experiment, freshly prepared triple distilled water was used.

Collection of the Extracts

The raw banana was collected from the local region and carefully removed its peels. They were washed and cleaned correctly with triple distilled water, and with the help of water-absorbent paper, they were dried out. This was followed by cutting the peels with an ethanol-sterilised knife, and then they were crushed with the help of a mortar and pestle. This was then dispensed in 10 ml of sterile distilled water, followed by heating at 70-80°C for 3-4 minutes. Now the extract was filtered with Whatman's filter paper number 1. The filtrate was collected and stored in a clean and dried conical flask by the standard sterilised method.

Zero Valent Iron Nanoparticles

The precursors and the reducing agent were mixed in 1:1 proportion in a sterile clean flask while synthesising iron nanoparticles. 5 ml of filtered banana peel extract and 5 ml of freshly prepared 0.001 M aqueous $FeCl_3$ solution were mixed at 50-60°C with constant stirring. The nanoparticle synthesis obtained a change in colour from light green to black within a particular time. The iron nanoparticles prepared were stabilised by adding 1 percent P.V.A. and 1 percent chitosan.

UV-Vis Spectra Analysis

By measuring the UV-Vis spectrum by sampling aliquots (0.3 ml) of iron nanoparticle solution and diluting the sample in 3 ml distilled water, the reduction of Fe^{+3} ions to Fe nanoparticles was monitored. UV-Vis spectrophotometer at 200-600 nm, UV- Vis spectral analysis. Absorbance peaks were observed at 216- 268 nm due to the absorbance of light by Π- electrons to higher anti-bonding molecular orbital (surface Plasmon resonance), which are identical to the characteristics of the UV-visible spectrum of metallic iron, and it was recorded. The sample and distilled water (blank) were subjected to UV-Vis Spectrophotometer every 15 minutes thrice to check the reaction rate. Then the same aliquot and one freshly prepared sample and stabilisers (P.V.A.) were again subjected to the same after 15 days to review the stability of the nanoparticles.

Atomic Force Microscopy (A.F.M)

This analytical instrument helps in determining the size and shape of the nanoparticles. 2-3 drops of the sample were poured on pre-cleaned

glass slides and dried in the hot-air oven for 5 minutes at 50°C. The dried sample was immediately subjected to A.F.M. The Cantilever of A.F.M. deflects when it encounters the particle, and the detector measures the strength and deflection distance to give the dimension of the nanoparticles.

pH Analysis

With the help of a digital pH metre, the pH was determined. The pH of the reduced solution with the synthesis of nanoparticles was found to be 2.16.

Leukocyte Culture Method

Chromosome preparations were obtained following the modified method of Moorhead et al. (1960) and Hungerford (1965) from P.H.A.- stimulated peripheral blood lymphocytes. 2 ml of the venous blood sample was collected in a sterile heparinised syringe, and 0.5 ml of the blood was inoculated into the vials that contained 5ml of RPMI 1640 medium containing 1 ml of F.B. serum and 0.2 ml of P.H.A. under aseptic conditions. After this, the culture vials were placed at 37°C. Periodically the cultures were shaken, and carbon dioxide was released once every day.

Various sets of experiments were conducted as listed below:

- Treatment with 20 µg/ml of peel extract
- Treatment with 40 µg/ml of peel extract
- Treatment with 60 µg/ml of peel extract
- Treatment with 80 µg/ml of peel extract

The cultures were treated with different extract concentrations at the 71st hour of incubation for precisely 1 hour. The cultures were then thoroughly washed by centrifuging the content at 1000 rpm for 10 minutes after 1 hour of incubation. The supernatant was discarded carefully, after which 6 ml of RPMI 1640 medium was added to the pellet and mixed well with the help of a Pasteur pipette.

Culture Harvest

After washing, the dividing cells were arrested by adding two drops of 0.001 percent colchicine solution to each culture vial. The cultures were further incubated for 20 minutes at 37°C. The content of the vials was later transferred to 15 ml centrifuge tubes and centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded, after which the cells were resuspended in a small amount of solution left behind by gently tapping the cell button. 6 ml of prewarmed (37°C) hypotonic solution (0.075 M KCl) was added to all the tubes, and the contents were mixed gently. It was then incubated for 5 minutes at 37°C and centrifuged for 6 minutes. The supernatant was carefully removed, and the cells were then fixed with 6 ml of filtered Carnoy's Fixative (3:1 methanol: acetic acid). The tubes were incubated at room temperature for 2 hours, and one change of fixative was given before slide preparation.

Slide Preparation

The remaining cell button was suspended in a small quantity of freshly prepared fixative. A test slide was prepared by gently placing a drop or two of the cell suspensions on a previously cleaned glass slide and then dried immediately on a hot plate (40°C). The prepared slides were examined for cell density, and metaphase spreads under a light microscope. Other slides were prepared after suitable modifications.

Staining Procedure

The slides were stained for 4 minutes in 4 percent Giemsa solution and washed in distilled water for 1 minute. Later the slides were air-dried.

Microscopic Analysis

Well-spread and stained cells were scored under the light microscope's oil immersion objective (100X lens) .

RESULTS

Here, the researchers use UV-spectroscopy, X-ray diffraction, Fourier transform infrared spectroscopy, and AFM to characterise the iron nanoparticle. UV-Spectroscopy helps recognise and characterise nanoparticles since various ocular properties are sensitive to the nanoparticle surface's shape, concentration, size, refractive index, and aggregation state. Synthesised iron nanoparticles interact with light at a particular wavelength, and the unique optical properties of these

materials lay the groundwork for plasmonics (Skoog et al. 2007).

Particles can be seen and studied as individuals in three dimensions. The ability to see biological samples in buffers that maintain their native structure for long durations complements E.M. Among the many biomedical uses of A.F.M. is the evaluation of nanoparticle and carbon nanotube toxicity to individual cells. Nanoparticle size and aggregation can be analysed with this method (Addala et al. 2013).

Qualitative Analysis

This study uses banana peel extracts to synthesise iron (Fe) nanoparticles. Peel extract was mixed with $FeCl_3$ solution in a 1:1 ratio to reduce Fe^{3+} ions to Fe^0 nanoparticles. An immediate colour change from light green to black was followed along with a change in pH after reducing the solution. Ferric Chloride exhibits bright yellowish colour when mixed with water. When mixing Ferric Chloride solution with the plant extract, the immediate colour change and a reduction in pH indicated the formation of iron nanoparticles. In this work, the pH change was observed from high to low and presented in Figure 1.

U.V. Visible Spectroscopy

The U.V. visible spectroscopy of the synthesised nanoparticles was in the range of 570-575 nm. The observation of suitable surface plasmon resonance (S.P.R.) with high band intensities and

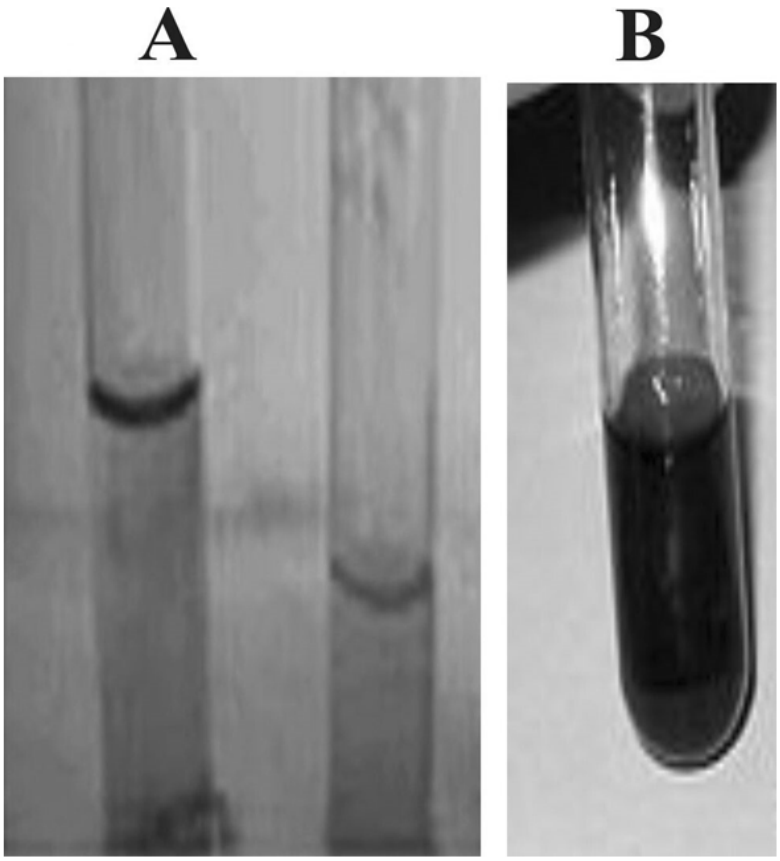


Fig.1. Shows the sample's colour change after adding the $FeCl_3$ solution

peaks under the visible spectrum proved the synthesis of iron nanoparticles from banana peel extract. Graphs are presented in Figure 2.

A.F.M. Analysis

The A.F.M. analysis of the sample showed the size and shape of the iron nanoparticles synthesised. The shape of the nanoparticles formed was spherical, with the advantage of a high surface area to load the drug. The size of the nanoparticles formed was 60.16 nm in length and 42.54 nm in width. Figure 3 shows the 2D image of the A.F.M. analysis along with the graph. The graph depicts the attractive and repulsive regimes acting on the sample. The force is in the attractive regime as the cantilever is pulled towards the sample. Thereby the crests are formed, and as the cantilever moves away from the sample, the force is in the repulsive regime, thereby creating the troughs of the graph. Figure 4 shows a 3D image of the nanoparticle where the size and height of the particle can be seen clearly.

Genotoxic Effects

The prepared metaphase chromosomes were analysed in the four cytogenetic variables (frequency of chromosomal and chromatid breaks). But no significant difference was observed between normal and treated samples (Table 1). The percentage of chromosome break, that of dicentric, the percentage of chromatid break, and the percentage of deletion were the same in both normal and treated samples, as shown in Figure 5.

DISCUSSION

In the present study, the iron nanoparticle was characterised with the help of various spectroscopic and microscopic techniques like UV-Spectroscopy, X-Ray Diffraction, Fourier Transform Infra-Red Spectroscopy, and Atomic Force Microscopy. Nanoparticles have some ocular properties sensitive to shape, concentration, size, refractive index, and agglomeration state of the nanoparticle surface, making UV-Spectroscopy a valuable tool for identifying and characterising these materials. The iron nanoparticle synthesised interacts with a specific wavelength of light, and the unique optical properties of these materials provide the foundation for the field of plasmonics (Skoog et al. 2007).

Extraction of biologically friendly nanoparticles such as iron will produce cost-effective nanoparticle production, which can be used in various applications. This research article sums up the search for an iron nanoparticle that is ecologically friendly for use in environmental sustainability. It was noted that the synthesis rate of nanoparticles from other biological materials, such as bacteria, takes up more time for synthesis than compared synthesis from plants (Dhillon et al. 2012). The genotoxic effect of other nanoparticles extracted from different biological materials was reported (Maurer-Jones et al. 2013). The genotoxicity for iron nanoparticles extracted from banana peel was found insignificant when tested in the human leukocyte culture. The toxic effects of metals like iron have been related to several neurological and other problems in humans (Kannabiran 2016; Vellingiri et al. 2022). In this study, iron nanoparticles were synthesised utilising banana peel extract, a household waste found almost anywhere, making it more economical.

Individual particles can be resolved and offer visualisation and analysis in three dimensions. It complements E.M. by allowing the visualisation of biological samples in buffers that preserve their native structure over extended periods. A.F.M.

Table 1: Chromosome aberration of control and treated with banana peel extract

Lab code	*Concentration*	*The number of cells scored*	*Chromatid aberration*		*Chromosome aberration*	*Total number of aberrations*	
			Breaks	*Deletion*	*Breaks*	*No.*	*%*
P1	Control	100	-	1	1	2	2
	Total	100	0	1	1	2	2
P3	20 µl (Extracted)	50	-	1	-	1	2
P4	40 µl (Extracted)	50	-	-	-	-	-
P5	60 µl (Extracted)	50	1	-	-	1	2
P6	80 µl (Extracted)	50	2	-	2	4	8
	Total	200	3	1	2	6	3

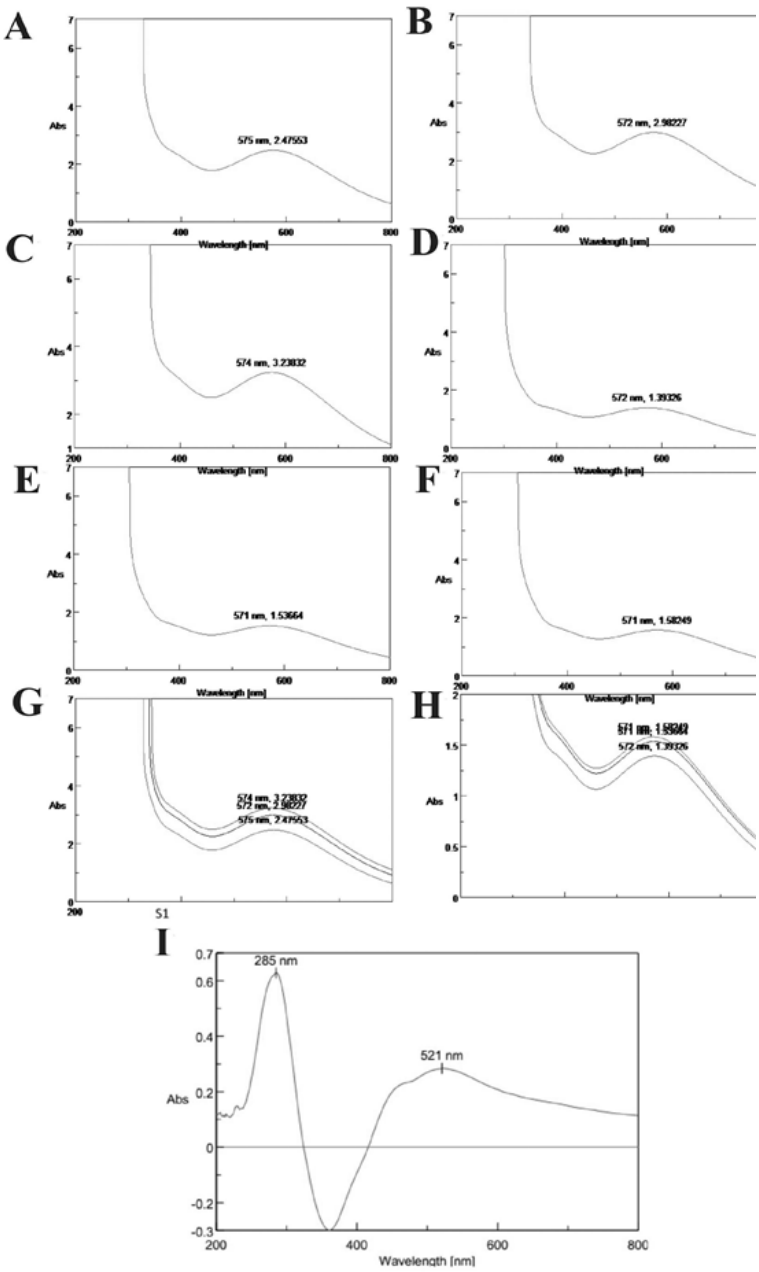
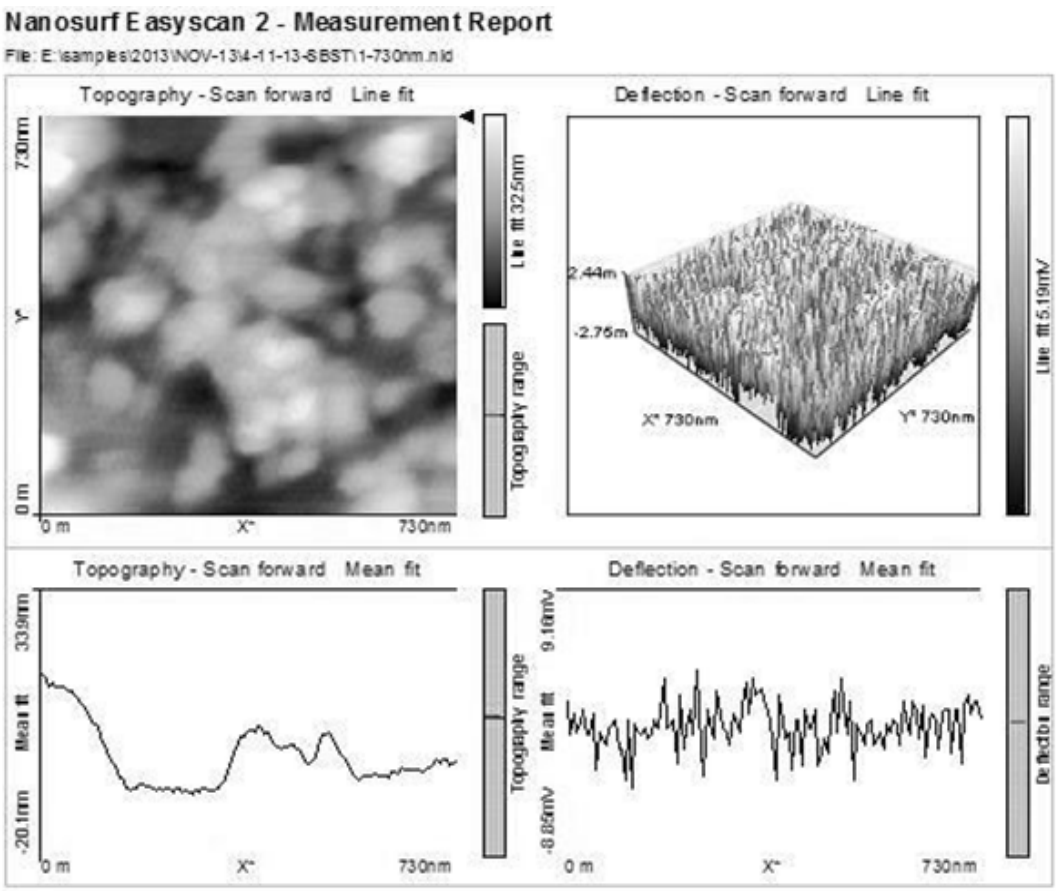


Fig. 2. UV-Vis spectroscopy result: Iron nanoparticle at 570-575 nm. f1 – Pure peel extract at different time intervals, from 0 minutes, 15 minutes, and 30 minutes from extraction. f2 – Peel extract with stabilisers P.V.A. and chitosan at different time intervals, from 0 minutes, 15 minutes, and 30 minutes from extraction (A: f1-0 minutes; B: f1-15 minutes; C:f1-30 minutes; D: f2-0 minutes; E: f2-15 minutes; F: f2-30 minutes; G: Comparison between A, B, and C; H: Comparison between F, E, and D; I: f1-15 days later)



Parameter

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Time/Line	= 0.6 s	I-Gain	= 1000	Controller S/N	= 023-06-154
Points	= 128	D-Gain	= 0	– Module –	
Lines	= 128	Tip voltage	= 0nV	Controller Board	= 2
X-Slope	= 0 °	Feedback mode	= Free running	AFM Basic Module	= 2
Y-Slope	= 0 °	Feedback algo.	= Adaptive PID	AFM Dynamic Module	= 0
Rotation	= 0 °	Error range	= 20 V	AFM Extension Module	= 0
X-Pos	= 1.7µm	– Global –		Video Module	= 1
Y-Pos	= -1.3µm	Measurement environment	= Air	Signal Module S	= 0
Z-Plane	= 0µm	Operating mode	= Static Force	Signal Module A	= 0
Overscan	= 5 %	Cantilever type	= CONTR	USB Module	= 2
Const.Height-Mode	= Disabled	Head type	= EZ2-AFM	Nanosurf Report	= 0
Date	= 04-11-2013	Scan head	= 10-06-176.hed	Scripting Interface	= 0
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Fig. 3. A.F.M. image showing the shape and size of the iron nanoparticle synthesised in 2 dimensions

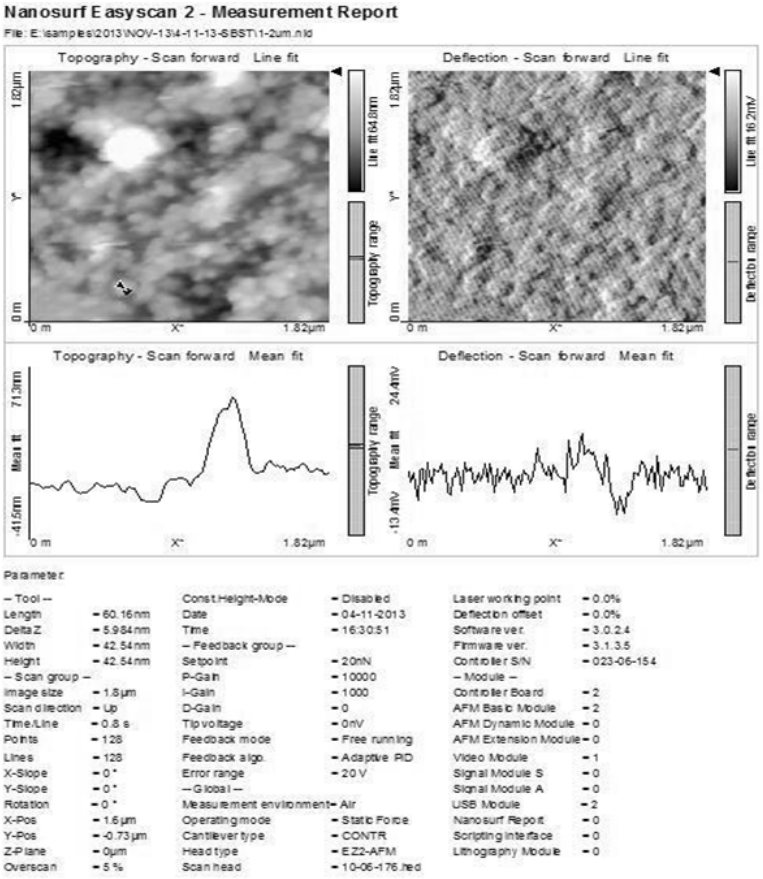

Fig. 4. A.F.M. image showing the shape and size of the iron nanoparticle synthesised in 3 dimensions

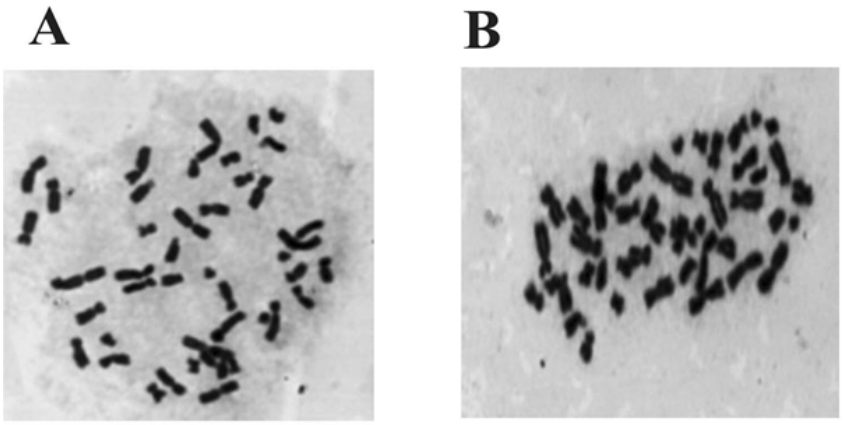

Fig. 5. Images of metaphase chromosomes treated with banana peel extract

has been used in various biomedical applications, including the testing of cellular toxicity of nanoparticles and carbon nanotubes. It can be used to analyse the reproducible measurements of nanoparticle size and aggregation (Addala et al. 2013).

CONCLUSION

From the present study, it has been confirmed that the banana peel extract can produce iron nanoparticles that show good stability in the solution. Under the UV-Visible wavelength, nanoparticles show quite good surface plasmon resonance behaviour. A.F.M. analysis has offered visualisation of the nanoparticles in three dimensions, presenting a base to analyse the nanoparticle size and aggregation measurement. Ferric Chloride, which has been used as a reducing agent along with banana peel extract, has shown a significant colour change and a concerning change in the pH of the solution. The success of such a rapid time scale for synthesising metallic nanoparticles is an alternative to chemical synthesis protocols and low-cost reductants for synthesising iron nanoparticles. Further, the genotoxic effect of the banana peel extract on human leukocyte culture was checked, which showed no variation from normal control cells. Thus, it can be concluded that there should not be any genotoxicity of the extracted iron nanoparticles on human blood cells.

RECOMMENDATIONS

The nutritional contents and health advantages of banana peel and its derivatives require additional research. This study exploits the peel's potential worth and utility.

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CONFLICTS OF INTEREST

The authors declare that this study does not have conflicts of interest.

AUTHO R CONTRIBUTIONS

Conceptualisation: S.M., K.B., J.D., N.S.; resources and data curation: S.M., K.B., J.D., N.S., K.R., S.K., A.G.M., R.M., U.R.W.; writing and original draft preparation: A.S., S.M., K.B., J.D., N.S., K.R.; writing, review and editing: K.R., A.G.M., U.R.W.; visualisation: A.V.G., K.R.; supervision: A.V.G., K.R.; project administration: A.V.G., K.R.

All authors have read and agreed to the published version of the manuscript.

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