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Evaluation of Anti Oxidant and Anti inflammatory Activity of Banana Peels

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Abstract
In the preset study, synthesis iron oxide nanoparticles (Fe3O4-NPs) were synthesized using a rapid, single step and completely green biosynthetic method by reduction of ferric chloride solution with banan peel water extract containing sulphated polysaccharides as amain factor which acts as reducing agent and efficient stabilizer. The structural and properties of the Fe3O4-NPs were investigated by X-ray diffraction, FTIR Fourier transforminfrared spectroscopy and Ultra violate UV. The diameter of iron nanoparticles was predominantly found within the range 15-35 nm. we evaluated the anti-inflammatory effect of Fe3O4-NPs by in vitro method by using membrane stabilization test and protein denaturation test. Membrane stabilization test was done by using human red blood cells (HRBCs). Protein denaturation test was done by using bovine serum albumin (BSA). The results revealed that (Fe3O4-NPs) was capable of rendering membrane stabilization by inhibiting the hypotonically-induced hemolysis of HRBCs in dose-dependentmanner).
Keywords: Nanoparticles, Fe3O4-NPs, X-ray, HRBCs, BSA.

Introduction

Nanotechnology implies the creation and utilization of materials, devices and systems through the control of matter on the nanometer-length scale i.e. at the level of atoms, molecules and supramolecular structures (1-3).A nanoparticle can be defined as a microscopic particle that has at least one dimension less than 100 nanometers in size (3). Unlike bulk materials, they have unique optical, thermal, electrical, chemical, and physical properties (2) and, hence, they find a variety of applications in the areas of medicine, chemistry, environment, energy, agriculture, information, and , communication, heavy industry and consumer goods (3). Conventional nanoparticle synthesis methods like attrition and pyrolysis have draw backs such as defective surface formation, low production rate, high cost of manufacturing, and large energy requirement (4). Chemical synthesis methods (e.g., chemical reduction, sol gel technique, etc.) involve the usage of toxic chemicals, formation of hazardous byproducts, and contamination from precursor chemicals (4). Plant extracts reduce the metal ions in a shorter time as compared tomicrobes.Depending upon planttype and concentration of phytochemicals, nanoparticlesare synthesized within a few minutes or hours, whereas microorganism-based methods require a longer time (5). The current work describes a green and rapid method using Banana peel, plant extract solution for the biosynthesis of iron oxide nanoparticles in ambient conditions, without any additive protecting nanoparticles from aggregating, template shaping nanoparticles or accelerants. The current simple synthetic green method using rapid precursors of Banana peel extract provides high-yield nanosized materials with good optical properties, and the method can be used to prepare nanocrystalline oxides of other interesting materials. The Fe3O4-NPs were prepared using ferric chloride as iron precursor and Banana peel extract as reducing agent and stabilizer.

Materials and Methods

Banana peel extract (BPE) preparation:

Banana peels were washed and boiled in distilled water for 30 min at 90°C. The peels (100 g) were crushed in 100 ml distilled water and the extract was filtered through a cheese cloth to remove insoluble fractions and macromolecules. This filtrate was treated with equal volume of chilled acetone and the resultant precipitate was centrifuged at 1000 rpm for 5 min. This precipitate was resuspended in distilled water and stored in refrigerator 4 °C for further studies. This extract was used as reducing as well as stabilizing agent.

Preparation of Fe_3O_4 Nanoparticles:

Iron oxide nanoparticles (Fe_3O_4-NPs) were prepared by adding 0.1 M FeCl3 solution to the (BPE) in a 1:1 volume ratio. Fe_3O_4-NPs were immediately obtained with the reduction process. The mixture was stirred for 20 min and then allowed to stand at room temperature for another 30 min.The obtained colloidal suspensions were then centrifuged and washed several times with ethanol an then dried at 40 °C under vacuum to obtain the Fe_3O_4-NPs.

Characterization Methods and Instruments:

FT-IR spectra of the Fe_3O_4-NPs were recorded in the range 500–4000 nm by (Shimadzu, Tokyo, Japan). The crystalline structure and phase purity of the Fe_3O_4-NPs produced were identified by X-ray diffraction

measurement (Shimadzu, Tokyo, Japan).

UV-Vis Spectra Analysis:
The reduction of pure Fe^{+3} ions to Fe° was monitored by measuring the UV-Vis spectrum by sampling of aliquots (0.3 ml) of Fe Nanoparticle solution diluting the sample in 3 ml distilled water. UV-Vis spectral analysis was done by using UV-Vis, at the range of 100 -450 nm and observed the absorption peaks at 240-440 nm regions due to the excitation of surface Plasmon vibrations in the FeNPs solution, which are identical to the characteristics UV-visible spectrum of metallic Iron and it was recorded.

DPPH radical scavenging assay:
To the methanolic solution of DPPH (1mM) an equal volume of the nano particle extract dissolved in water was added at various concentrations from 2 to 1000 µg/ml in a final volume of 1.0 ml. An equal amount of alcohol was added to the control. After 20 min, absorbance was recorded at 517 nm. Experiment was performed in triplicate (6).

ABTS radical scavenging assay:
To the reaction mixture containing 0.3 ml of ABTS radical, 1.7 ml phosphate buffer and 0.5 ml nano particle was added at various concentrations from 2 to 500 µg/ml. Blank was carried out without drug. Absorbance was recorded at 734 nm. Experiment was performed in triplicate (7).

Iron chelating activity assay:
The reaction mixture containing 1 ml O-Phenanthroline, 2 ml Ferric chloride, and 2 ml extract at various concentrations ranging from 2 to 1000 µg/ml in a final volume of 5 ml was incubated for 10 minutes at ambient temperature. The absorbance at 510 nm was recorded. Ascorbic acid was added instead of nano particle and absorbance obtained was taken as equivalent to 100% reduction of all ferric ions. Blank was carried out without drug. Experiment was performed in triplicate (8).

Lipid peroxidation assay:
The mixture (Egg phosphatidylcholine in 5 ml saline) was sonicated to get a homogeneous suspension of liposome. Lipid peroxidation was initiated by adding 0.05 mM ascorbic acid to a mixture containing liposome (0.1 ml). The pink chromogen was extracted with a constant volume of n-butanol and absorbance of the upper organic layer was measured at 532 nm (9).

Determination of anti-inflammatory activity by membrane stabilization activity:
The membrane stabilizing activity of nanoparticle was assessed by the method developed byShinde et al.(10) and designed and employed by Sikder et al.,(12,13) and Md Reyad-Ul-Foerdous et al with a slight modification, ie., mice RBCs were used in their studies where as healthy human volunteer's RBCs (HRBCs) were used in our study.

HRBCs preparation:
The venous blood was collected from the anticubital vein by using asyringe that contained EDTA as anticoagulant. After centrifuging, the RBCS were separated and was washed three times in isotonic buffered saline at (154 mM Nacl) in 10 mM sodiumphosphate buffer at a pH 7.4 through centrifugation for 10 min at 3000 rpm.The test sample consisting of 0.5 ml of RBC suspension from the HRBC stock and mixing itwith 5 ml of hypotonic solution (5500, 1000 µg/mL was added in test sample tubes and diclofenac sodium (200 µg/mL) was added to the standard tube. The control sample tube containing 0.5 ml of HRBCs was mixedwith only hypotonic buffered saline. The tubes with these mixtures were incubated for 10 minat 56ºC and cooled under running tap water for 20 min. Then the tubes were centrifuged for10 min at 3000 rpm and the supernatant was collected to measure the absorbance at 540 nm.The membrane stabilization was calculated by using the formula.% inhibition of hemolysis = (OD1– OD2/OD1) X 100Where, OD1 = optical density of hypotonic-buffered saline solution alone (control)OD2 = Optical density of test sample in hypotonic solution.

Protein denaturation method:
The ability of nao particle to inhibit protein denaturation was studied by the method of Shravan kumar et al.,(14) to evaluate the anti-inflammation activity of aoparticle.

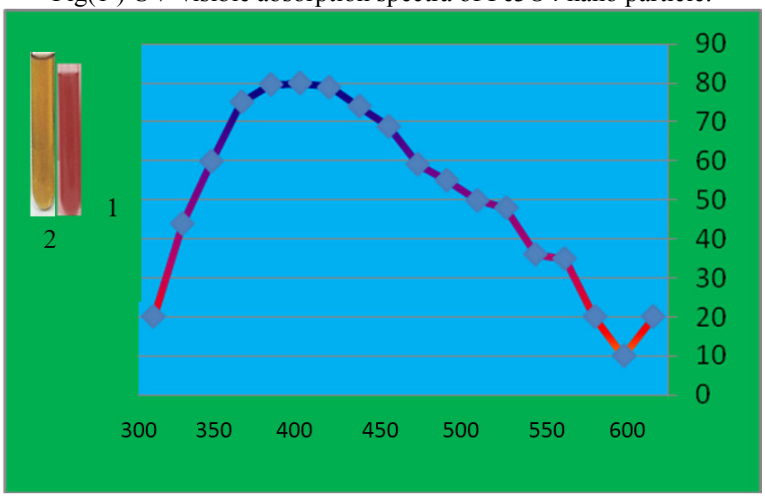
Procedure:
The tubes were taken as for control, product control, standard tube (with Diclofenac sodium at 200 µg/ml) and test (with *nanoparticle.*The reagents were added in the following order, 0.45 ml of 5% aqueous solution of

Bovine serum albumin (BSA), 0.050 ml of sample in different concentrations, as 50,100,200,300,400,500,1000 µg/ml. In control distilled water was used instead of sample.In product control, all reagents were added except BSA. Then, the pH of the solution in all the tubes was adjusted to 6.3 using 1N Hcl. The tubes with the samples were incubated at 37 ºC for 20 mins and then heated at 54 ºC for 3 mins in water bath. All the tubes were later cooled in running tap water and then 2.5 ml of phosphate buffer solution in each tube. A turbidity was produced in the tubes, which was measured spectrophotometrically at 600 nm. Control represents 100 % protein denaturation. The results were compared to the standard diclofenac sodium.
Percentage inhibition = 100 - OD of test – OD of control / OD control X 100Antiinflammatory action of noparticle HRBC membrane stabilization.

Statistical analysis:
All results are expressed as mean ± S.E.M. Linear regression analysis (Origin 6.0 version) was used to calculate the IC_{50} values.

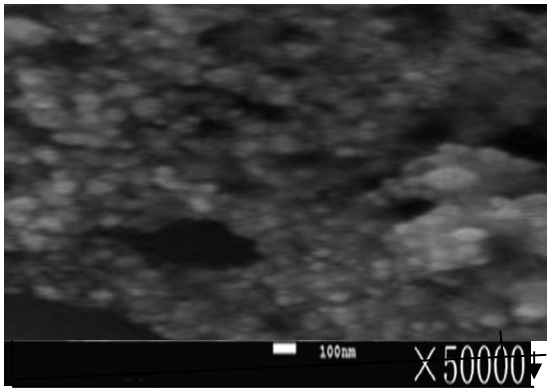
Results and Discussion:
Ultraviolet-visible spectroscopy (UV-Vis) refers to absorption spectroscopy in the UV-Visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared) ranges. The absorption in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. The UV Visible spectrum of Fe3O4-NPs in the aqueous banana extract is shown in Figure (1). The two absorption peaks at wavelengths of absorption peaks at 350-450 nm indicate the formation of iron nanoparticles.

Fig(1) UV-visible absorption spectra of Fe3O4 nano particle.



To determine the functional groups on banana peel extract and predict their role in the synthesis of Iron nanoparticles,FTIR analysis was performed. The band intensities in different regions of the spectrum for Iron NPs(before and after reaction with Iron Sulfate, respectively)were analyzed and are shown in Figure(2). There was a shift in the following peaks: 3,346–3,336, 2,920–2,918, 2,355–2,351, 1,643–1,641, 771–769, and 426–422 cm-1. The broad and intense absorption peak at around 3,394 cm21 corresponds to the O–H stretching vibrations of phenols and carboxylic acids. The shift from3,394 to 3,388 cm-1 may indicate the involvement of O–H functional group in the synthesis of nanoparticles. The peak located at around 2,355 cm21 was attributed to the N–H stretching or the C = O stretching vibrations. The peak shift from 2,355 to 2,351 cm implicated that these groupsmay be involved in the process of nanoparticle synthesis. The peak located at 1,641 cm21 could be assigned to the C = O stretching in carboxyl or C = N bending in theamide group. A shift in this peak (from 1,641 to1,643 cm_{21}) indicated the possible involvement of carboxylor amino groups of the CBPE powder in nanoparticle synthesis The peak at 771 and 760 cm21 corresponds to C–H stretching of aromatic compounds. The formation of Fe3O4 is characterized by two absorption bands at 535 and 307 cm−1 which correspond to the Fe–O bond in magnetite (15) From the FTIR result, the soluble elements present in BS extract could have acted as capping agents preventing the aggregation of nanoparticles in solution,and thus playing a relevant role in their extracellular synthesis and shaping (16).

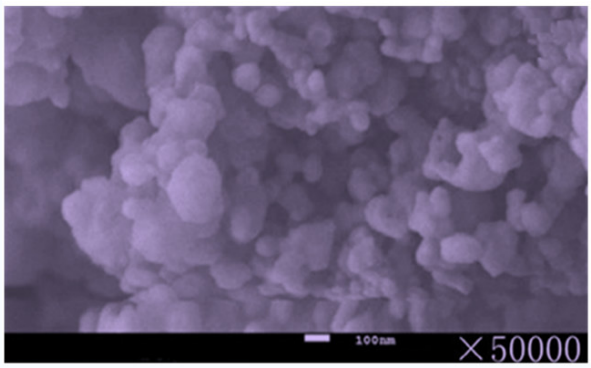
SEM Images of Iron:
SEM analysis results of iron nanoparticles were clearly distinguishable at different enlargements. Iron nanoprticles in the Banana peel extract were found to be polydispersed (figure) and measured in size from 15 to 34 nm.



scavenging activity in various concentrations of DPPH used for testing radical scavenging activity by the compound. When the stable DPPH radical accepts an electron from the antioxidant compound, the violet color of the DPPH radical was reduced to yellow colored diphenyl picrylhydrazine radical which was measured colorimetrically. Substances which areable to perform this reaction can be considered as antioxidants and therefore radical scavengers (17).

Several concentrations ranging from 2–1,000 µg/ml of nano particle were tested for their antioxidant activity in different in vitro models. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration in all the models. The percentage scavenging and IC_{50} values were calculated for all models given in Table (1). The anti oxidative mechanism of antioxidants can result from metal chelation, free radical scavenging (hdrogen-donatation free radical quenching, or co-operative effects of these properties (12). The ability of test sample to donate hydrogen was checked using stable free radical DPPH is formed from the scavenger the reaction is monitered by the decrease of the absorbance at 518 nm (13).

DPPH + antioxidant(purpul color) ---------- DPPH-H+ antioxidant(yellow color)



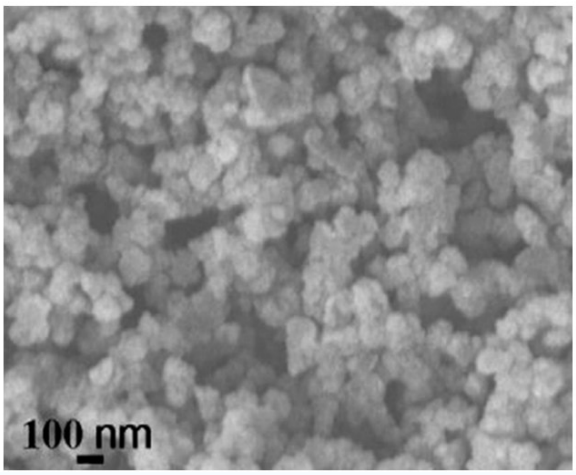


Table (1) : Comparison of IC_{50} values of Iron nanoparticle in compartion with standard.

No	Test	IC_{50} *µg* / ml
1	DPPH radical scavenging activity	32.21
2	ABTS radical scavenging activity	22.64
3	Iron chelating method	47.6
4	Lipid peroxidation method	29.4

Membrane stabilization effect:

Nanoparticles effectively inhibited the membrane lysis of HRBCs which was expressed as the percentage inhibition (%inhibition) of hemolysis. It was dose-dependent: in lower concentration (25µg/ml), the %inhibition value was (26.80%) in compare with increased concentrations (50, 100, 150, 200, 300, 400, and 500 µg/mL), as shown (10.98%, 19.5%, 24.04%, 37.81%, 44.57%,76.30%.65.71, 70.52, 81.54) this was in comparison with the

anti-inflammatory effect of standard drug viz. Diclofenac sodium (200 µg/ml, 83.61%) (Table 1). stabilization of lysosomal membrane is important to prevent the inflammatory processes. We have chosen the hypotonically induced erythrocyte lysis to prove the membrane stabilizing effect because HRBC membrane is considered to be similar to the lysosomal membrane(18).

Protein denaturation was effectively inhibited by nano particle in dose-dependent manner (50, 100, 150, 200, 300, 400, and 500 µg/mL), the inhibition value (10.98%, 19.5%, 24.04%, 37,81%, 44.57%, 65.71%, 70.52%, 81.54%, 83.61). When compared to diclofenac sodium (200 µg/mL, 88.64%), nanoparticle was almost equally effective in inhibiting the protein denaturation in higher concentration (Table 2). Protein denaturation is a pathological process by which the proteins lose their configuration and become functionless (19). This usually happens when the proteins are exposed to external stress like heat, strong acid or base. These external forces make the organic or inorganic solvents in the proteins to lose their tertiary and secondary structure and make them lose their functional capacity(20).

Table 1. Effect of *PD* Extract on membrane stabilization activity.

Extract/ Drug	Concentration	% inhibition of hemolysis
Nano particl	25 µg/mL	10.98
	50 µg/mL	19.5
	100 µg/mL	24.05
	150 µg/mL	37.81
	200 µg/mL	44.57
	300 µg/mL	65.71
	400 µg/mL	70.52
	500 µg/mL	81.54
Diclofenac Sodium	200 µg/mL	83.61

Protein denaturation study

Extract/ Drug	Concentration	% inhibition of hemolysis
Nano particle	25 µg/mL	15.98
	50 µg/mL	22.67
	100 µg/mL	34.01
	150 µg/mL	47.63
	200 µg/mL	50.66
	300 µg/mL	64.21
	400 µg/mL	71.60
	500 µg/mL	84.97
Diclofenac Sodium	200 µg/mL	88.64

Conclusion

Nanotechnology is the development of reliable and ecofriendly processes for synthesis of metal oxide nanoparticles. Fe3O4-NPs with an 15-34nm were synthesized by bio reduction of ferric chloride solution with a green method using Banana peel aqueous extract contain sulphated polysaccharides as the reducing agent and efficient stabilizer. The involvement of these groups in biosynthesis is revealed by FTIR analysis. The characteristics of the obtained Fe3O4-NPs were studied using FTIR, XRD, UV-visible. As-synthesized nanoparticles have been successfully implemented in the fields of medicine and environmental remediation.

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