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ANNONA MURICATA FRUIT MEDIATED BIOSYNTHESIS, PHYSICOCHEMICAL CHARACTERIZATION OF MAGNETITE (Fe_3O_4) NANOPARTICLES AND ASSESSMENT OF ITS IN VITRO ANTIDIABETIC ACTIVITY

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ABSTRACT

The biological preparation of magnetite (Fe_3O_4) nanoparticles (NPs) plays a vital role in the nanotechnology field. In the current study, we report here the preparation and physicochemical characterization of Fe_3O_4 nanoparticles by using *Annona muricata* fruit source, which acted as reducing material in the nanoparticle preparation. UV-Visible spectra of magnetite nanoparticles showed a peak at 340 nm due to the SPR. The Fourier transform infrared spectra indicate a very intense absorption band at 657cm^{-1}, which proved the existence of magnetite in the prepared material. The crystal size of magnetite NPs was calculated using an X-ray diffractometer and it was estimated to be 23nm; the prepared nanoparticles are cubic in structure. The surface morphology, crystalline purity and magnetic property were examined by SEM-EDX and VSM. The synthesized nanoparticles were examined for their antidiabetic strength by α-Amy inhibitory activity. Acarbose was used as a pharmacological inhibitor. The inhibitory potential of prepared nanoparticles against alpha-amylase proves their therapeutic role.

Keywords: Acarbose, *Annona muricata*, Alpha amylase, Magnetite, VSM

INTRODUCTION

Magnetite (Fe_3O_4) phase, Iron (III) oxide, hematite (α-Fe_2O_3) phase and maghemite (γ-Fe_2O_3) phase are a few iron oxide phases in nature, in various forms. Among all these forms Fe_3O_4 is the well-known iron oxide (IO) which is used in varied fields. Fe_3O_4 is the chemical formula for magnetite, which is also considered as a mineral. The tetrahedral-octahedral layer at the inverse spinel arrangement is observed in the crystal structure of the magnetite. This arrangement leads to an inference that the Fe^{2+} ion of Fe_3O_4 occupies half the octahedral site because of the higher stabilization energy of the ferrous crystal. On another side, Fe^{3+} ion of Fe_3O_4 occupies another octahedral site and all tetrahedral sites.[1] Superparamagnetic nanoparticles magnetized until their magnetic saturation, while an external magnetic force field is applied. The magnetic force field is eliminated; while the magnetic interaction is not shown by Superparamagnetic nanoparticles.[2] It is rather surprising to note that Fe_3O_4 NPs exhibit the behavior of superparamagnetic nanoparticles. Apart from that, Magnetite nanoparticles are biodegradable, biocompatible and potentially non-toxic to human.[3-4] These characteristic features show great potential in biomedical applications.

The bioactive compounds found in biological material can act as a reducing agent, which can stabilize the nanomaterials during the preparation process. The biological compounds present in the plant control

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the size as well as the shape of the nanomaterials in respective applications. During the nanoparticle preparation, whereas the required materials are only metal salt and then a green substrate. While receiving the properties required for different applications, a few parameters like concentration, pH and temperature of reaction altered respectively. The non-toxic and biocompatible, special surface coated Magnetite NPs could be easily used in biomedical fields. It also helps to deliver drugs, in particular areas at ease. The toxicity of green synthesized Fe_3O_4 NPs is minimized; thereby it acts as a safer biomedical application to human beings.[5] Besides, Magnetite nanoparticles can conjugate with proteins or enzymes that can be targeted to cancer cells with the help of magnetic external force field, while the magnetic force field will be altered by heating for magnetic hyperthermia treatment.[6]

Fruit peels are very thick to be consumed by human beings and they are used as natural fertilizers. During recent days, scientists often used natural resources to synthesize nanoparticles. There are a couple of studies in synthesizing Fe_3O_4 NPs by using fruit extracts are *Passiflora tripartite*, *Averrhoa carambola*, *Couroupita guianensis* and *Ananas cosmos*.[7-10] The citric *acid* present in lemon juice was used for controlling the size and surface capping purpose.[11]A close study of these characteristic features of Fe_3O_4 NPs proves that it will be a great use in its application in future biomedicine.

The current work deals with the structural characterization of Fe_3O_4 material synthesized using *Annona muricata* fruit extract and it also aims to detect the inhibitory potential of Fe_3O_4 NPs against alpha amylase (α-Amy) enzyme.

EXPERIMENTAL

Materials

The chemicals required were purchased from the following sources: Iron chloride ($FeCl_3.6H_2O$), Iron sulphate ($FeSO_4.7H_2O$), Sterile distilled water (SDW) and Ammonium Hydroxide (NH_4OH) from Sigma-Aldrich with 99% purity.

Plant Description

Annona muricata is known to be soursop (or) Graviola. The Graviola plant is about 15-83 cm in diameter and 5-10 m height. The leaves of *Annona muricata* are used to treat colds, colds as well as flu disease. Soursop fruits are not only known as food, but juice is used as a galactogogues to treat heart disease, liver diseases and diarrhea. The fruit of *Annona muricata* is also found to be used in the treatment of cancer and diabetes patient.[12]

Preparation of Plant Extract

Indian medicinal fruit, *Annona muricata* was selected from Trivandrum, Kerala, based on herbal character, availability and its economical affordability. Fresh fruits were cleaned thoroughly with sterile distilled water (SDW) to clear all heavy biomaterials.

About 15g of the fruit were transferred into 400ml beaker containing 150ml of the SDW and then heated for about 30 minutes. The *Annona muricata* fruit extract was filtered through Whatman 11µm filter paper to suspend heavy biomaterials. Finally, the fruit extract was kept at 4^0c for the magnetite nanoparticles preparation.[13]

Phytochemical Screening

Fresh and healthy fruits were selected for phytochemical tests. The phytochemical screening of *Annona muricata* was carried out by the standard method that previously described.[14]

Preparation of Magnetite Nanoparticles

Iron chloride ($FeCl_3.6H_20$) and Iron sulphate ($FeSO_4$. $6H_2O$) were taken in 2:1 ratio and dissolved in SDW. This metal mixture solution was boiled and maintained at 30-40^{0}C under a mild stirring using a magnetic stirrer for 20 minutes. After 10 minutes, *Annona muricata* fruit extract was added slowly into the solution. After one hour, 1N NH_4OH was added drop by drop into the mixture solution for uniform precipitation of Fe_3O_4NPs. The P^H level of the mixed solution was reached in10 and then the solution was left undisturbed and allowed to settle down at room temperature. The black-colored nanoparticles get deposited at the bottom side of the conical flask. The deposited nanoparticles were repeatedly washed with sterile distilled water. This solution was subjected to centrifugation at 12,000 RPM for 15 minutes. A pellet containing the nanoparticles was dried in the dryer hot oven machine at 70°C for 20 hours.[15] The sample is again calcined using a muffle furnace at 400°c for 4 hours. The biosynthesized nanoparticle is subjected to characterization and application (Fig.-1).

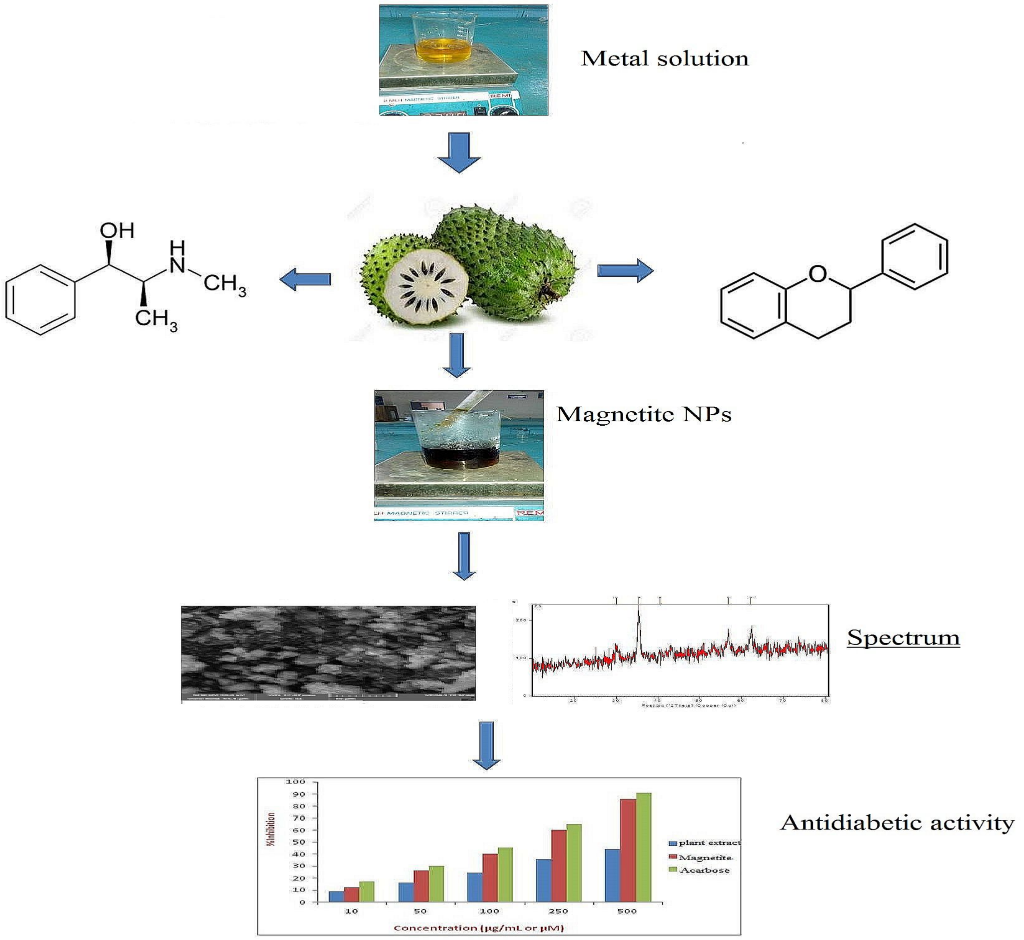

Fig.-1: Schematic Diagram of Biosynthesized Fe_3O_4 NPs

Pancreatic Alpha Amylase Method

The α-Amy inhibition activity of synthesized Fe_3O_4 NPs was performed; according to our former publication.[16] Acarbose is a standard diabetic medicine used to treat diabetes patients.

Characterization

UV-Vis Spectral Analysis

The optical spectral studies were performed using (Beckman-Model No.DU-50, Fullerton) spectrophotometer.

FTIR Spectral Analysis

FTIR (Fourier Transform Infrared) spectra of *Annona muricata* extract and magnetite nanoparticles were obtained by Perkin Elmer Spectrum Express version 10,300 using KBr pellet method.

XRD Spectral Analysis

Crystallographic data about the synthesized Fe_3O_4 nanoparticles was examined from X-ray diffractometer (PANalytical, Philips PW 1830) in the range of 20°- 70° with 2°/min scanning rate.

SEM-EDX Analysis
The morphological character (SEM) and elemental analysis (EDX) were done using TESCANS 9000 instrument.

VSM Study
The room temperature magnetization of magnetite nanoparticles was measured using Vibrating Scanning Magnetometer (Cryogenic, UK).

Antidiabetic Activity
The antidiabetic activity was tested by the pancreatic alpha amylase model.

RESULTS AND DISCUSSION

Phytochemical Analysis
Data reveals that this *Annona muricata* fruit gives positive results for anthraquinone, terpenoids, flavonoids, alkaloids, reducing sugar, phenols and carbohydrates. The presence of phlobatannins and cardiac glycosides could not be recorded (Table-1). The chemical constituents present in *Annona muricata* fruit extract are anthraquinone, terpenoids, flavonoids, alkaloids, reducing sugar, phenols and carbohydrates, they can act as reducer, stabilizer and a chelating agent in the nanoparticle preparation. The above bioactive compounds present in the fruit extract can change the size, shape and morphology character of the prepared nanoparticles. These phytochemicals can generate nanoparticles with high stability and dispersity.

Table-1: Phytochemical Analysis of Annona muricata Fruit Extract

Phytochemical Test	Aqueous Fruit Extract
Anthraquinone	+
Saponins	+
Terpenoids	+
Flavonoids	+
Alkaloids	+
Reducing sugar	+
Phenols	+
Carbohydrates	+
Phlobatannins	_
Cardiac glycosides	-

UV-Vis Spectral Analysis
The UV-Visible spectral pattern is used to investigate the optical properties of the nanoparticles. The visible changes from colorless to dark black evidenced the formation of magnetite nanoparticles. The UV-Visible spectrum of fruit extract and Fe_3O_4 NPs are shown in Fig.-2. The optical absorption band obtained at 340 nm for the black color magnetite nanoparticles synthesized from iron chloride, iron sulphate with the molar ratio 2:1.[17]

FTIR Spectral Analysis
The FTIR spectral pattern was performed to study the bioactive functional groups found in aqueous *Annona muricata* fruit extract and Fe_3O_4 NPs. The FTIR spectrum of aqueous fruit extract exhibited characteristic stretching frequencies in 3405.2937,2058,1637,1408,1255,1059,920,866,631cm^{-1}(Fig.-3).The FTIR spectral data of synthesized nanoparticles represent that the absorption band observed at 3444cm^{-1} corresponding to –OH, -NH stretching vibration of carboxylic acids, alcohols and phenols. The peak observed at 2072cm^{-1} indicated the C-H stretches of the CH_3 group. The peak assigned at 1634cm^{-1} is due to the C=C stretches of aromatic rings.[18] The band indicated at 1385 cm^{-1} correspond to the C-H bending vibration of methyl groups. The absorption peak at 1014cm^{-1} can be attributed to the C-O-C stretches in *Annona muricata* fruit extract. The FTIR spectral data strongly suggested the presence of reducing sugars, alkaloids, carbohydrates, flavonoids, and polyphenols apart from other phytochemicals.

Also, a strong peak observed at 657cm^{-1} was indicated the formation of magnetite nanoparticles, which is due to the characteristic stretching vibration of Fe-O.[19]

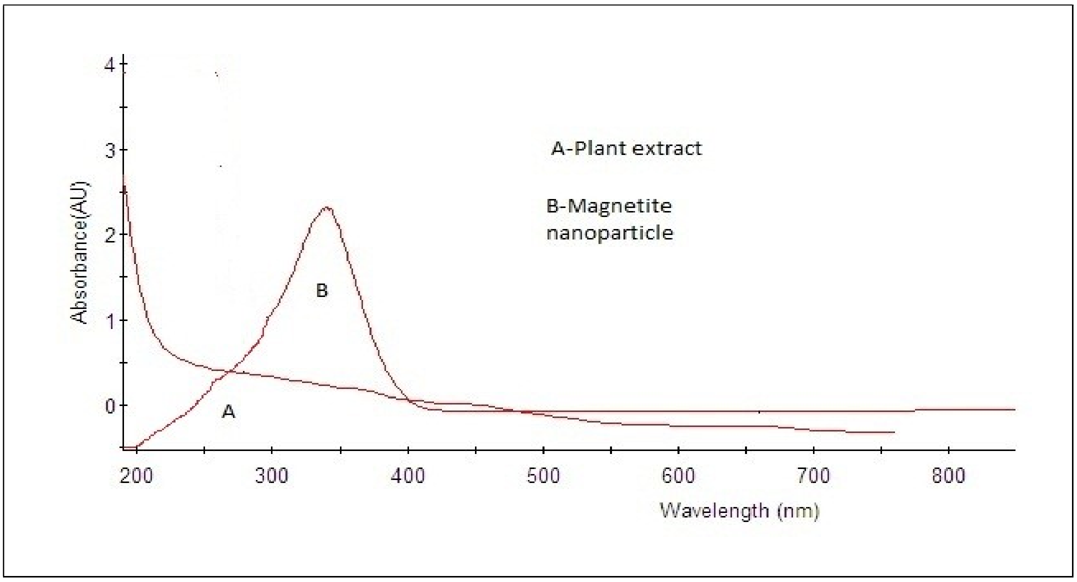

Fig.-2: UV-Vis Spectral Data of Plant Extract (A) and Magnetite Nanoparticles (B)

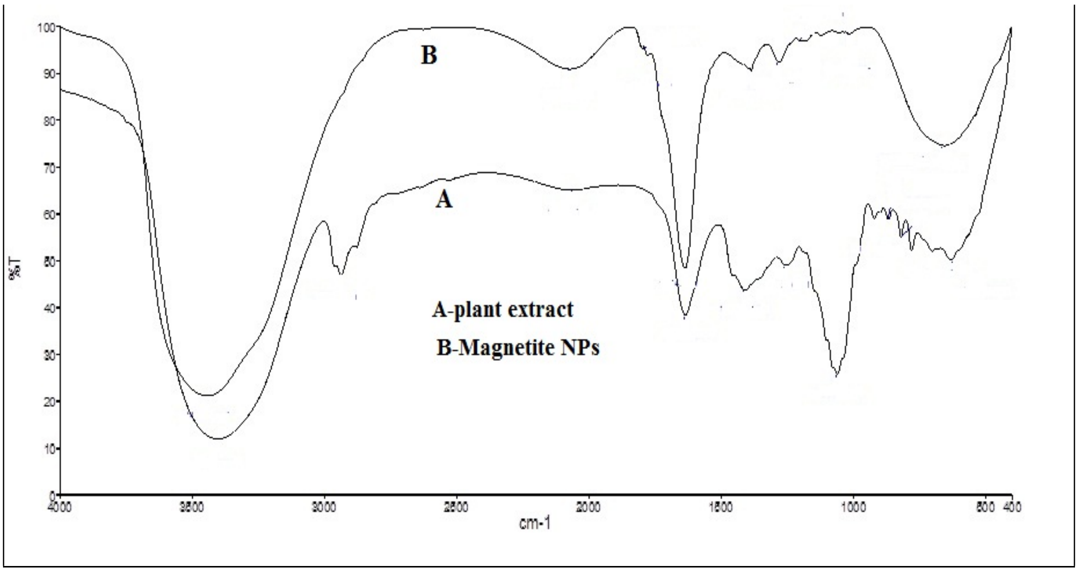

Fig.-3: FTIR Spectral Data of Plant Extract (A) and Magnetite NPs (B).

XRD Spectral Pattern Analysis

X-ray diffractometer is used to identify the crystallographic structure of the prepared material. The XRD obtained for the magnetite material using *Annona muricata* fruit extract is shown in fig.-4.The crystallite size of the magnetite material can be evaluated using Debye-Scherrer relation. The following relation is $D = 0.9\lambda / \beta \cos\theta$.

The estimated crystallite size of Fe_3O_4NPs was found to be 23nm. The XRD Spectrum shows the crystallographic nature of the nanoparticles. The reflection peaks observed at 2 theta values of 30.0857, 35.3845, 42.4107, 56,8172 and 62.2730 which could be indicated to (220), (311), (400), (511) and (440) planes of crystal Fe_3O_4NPs respectively. The results show the cubic structure of magnetite nanoparticles and observed reflection peaks are well-matched with JCPDS file no (89-0950).

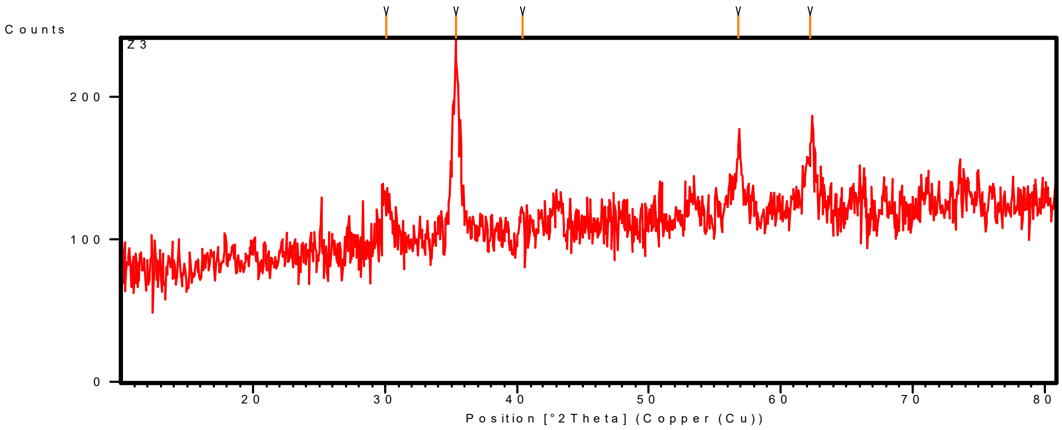

Fig.-4: XRD Spectral Pattern of Fe_3O_4 NPs (Magnetite Nanoparticles)

SEM-EDX Analysis

The synthesized magnetite nanoparticles were analyzed by SEM- EDX to evaluate the surface morphology as well as atomic percentages. The SEM images of Fe_3O_4NPs using *Annona muricata* fruit extract are depicted in Fig.-5. SEM micrograph revealed that the prepared sample is spherical.[20] The intense peak obtained from the EDX spectra (Fig.-6) are Fe and O. Therefore EDX spectra confirm that the synthesized Fe_3O_4NPs are pure without forming any impurity peaks. The atomic percentages obtained from EDX spectra were 56.99 (Fe) and 43.01 (O).

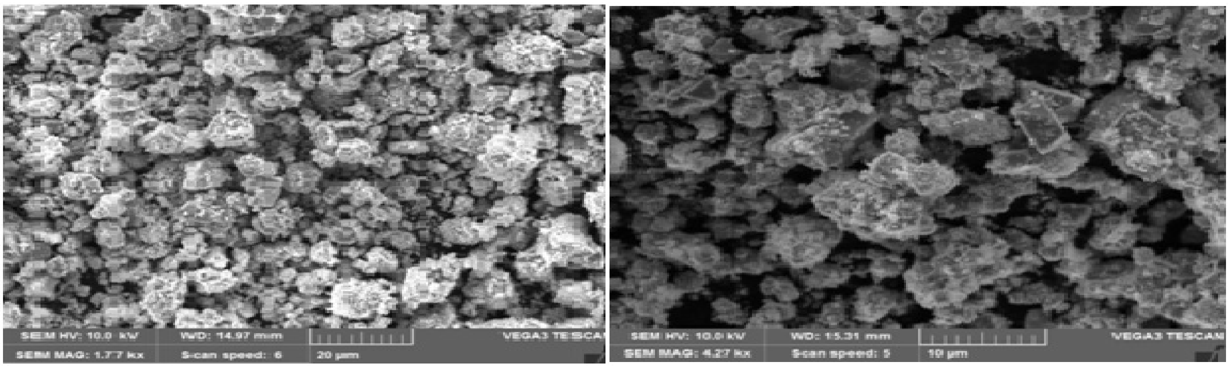

Fig.-5: SEM Micrographs of Biosynthesized Fe_3O_4NPs

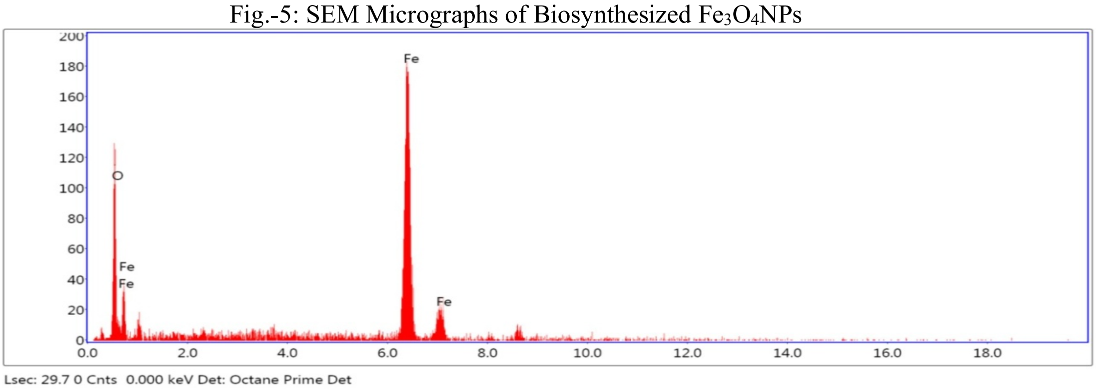

Fig.-6: EDX Spectrum of Fe_3O_4NPs

VSM Study

The magnetic behavior of synthesized Fe_3O_4 NPs was evaluated at 300K (RT) by a VSM with an applied field of +6k to -6k Oe. The magnetic saturation curve of the synthesized Fe_3O_4NPs is presented in Fig.-7. It is clear that the sigmoidal curve passes through the origin and shows no hysteresis loop, indicating that the prepared material is superparamagnetic. The specific magnetic saturation calculated value was found to be 44.2emu/g for Fe_3O_4NPs.

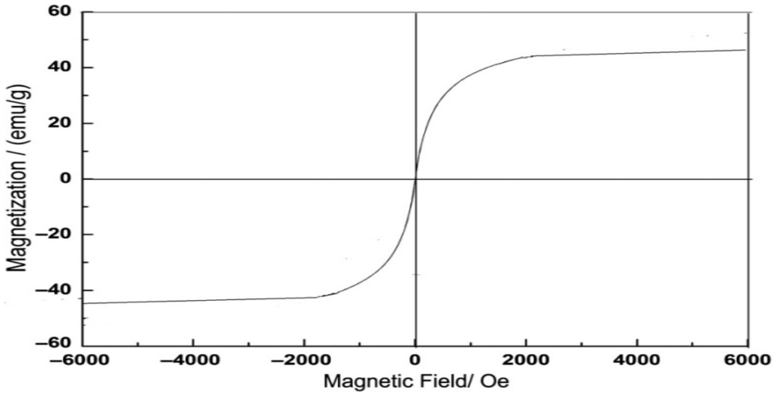

Fig.-7: VSM Spectrum of Biosynthesized Fe_3O_4NPs

Antidiabetic Activity

The synthesized magnetite nanoparticles were tested by using an in vitro alpha amylase inhibition model. The inhibitory activity of biosynthesized magnetite nanoparticles in combination with the commercially used antidiabetic drug, Acarbose was examined. Acarbose, a synthetic pharmacological inhibitor delays the digestion of carbohydrates and inhibits the action of pancreatic amylase for the breakdown of oligosaccharides and disaccharides into monosaccharides suitable for starch absorption. The inhibition of the digestive enzyme (α-Amy) is used for the treatment of non-insulin diabetes.[21] The results indicate that the alpha amylase enzyme was significantly inhibited by various concentrations of magnetite nanoparticles (Table-2).

Comparison of α-Amy inhibition of Plant extract, Acarbose and Fe_3O_4 nanoparticles were shown in Fig.-8. The percentage inhibition of magnetite nanoparticles at various concentrations 10, 50,100,250,500 were found to be 12.37, 26.72, 40.11, 60.17, and 89.15 respectively. The result suggests that biosynthesized Fe_3O_4 nanoparticles using *Annona muricata* exhibit a good level of inhibition under in vitro conditions.

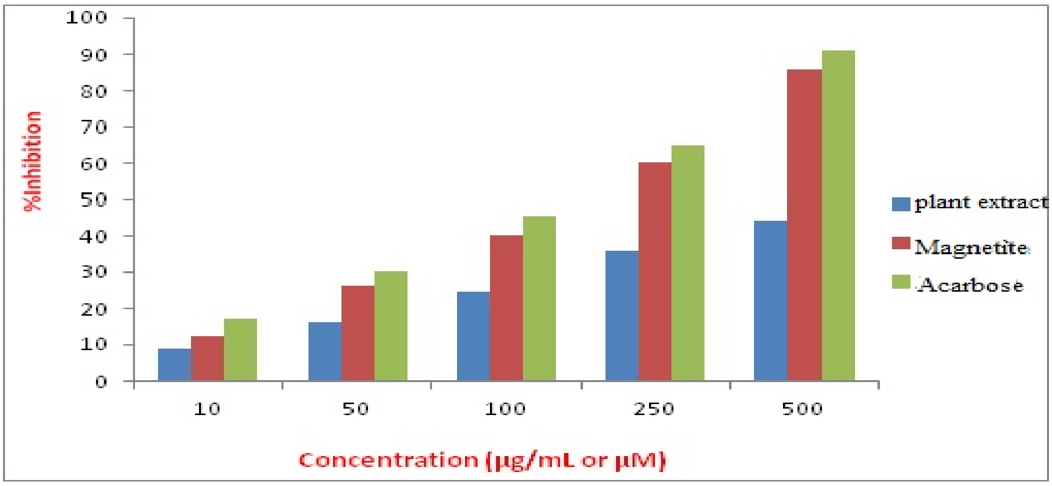

Fig.-8: Comparison of α-Amy inhibition of Plant Extract, Acarbose and Magnetite NPs

Table-2: Antidiabetic Activity of Biosynthesized Fe_3O_4NPs

Concentration (µg/mL)	Standard (Acarbose)	Plant Extract	Fe_3O_4
10	17.21	8.71	12.37
50	29.98	16.28	26.72
100	45.43	24.87	40.11
250	65.33	35.98	60.17
500	91.11	44.44	89.15

CONCLUSION

In conclusion, nanoparticles synthesized through a biological route using *Annona muricata* fruit extract are safe, cheaper and Eco friendly. The organic phytochemicals present in the *Annona muricata* fruit extract acts as reducing material during the nanoparticle synthesis. The synthesized nanoparticles were successfully characterized and confirmed by physicochemical techniques. From the antidiabetic results, we conclude that the biosynthesized Fe_3O_4NPs were found to show remarkable potential against the alpha amylase enzyme and it was found to be appropriate nanomedicine in diabetes management. However, biosynthesized Fe_3O_4NPs have high therapeutic efficiency in type 2 diabetes mellitus and in various biomedical applications.

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