



Original Research Article

Green Synthesis, Characterizations and Pharmacological Studies of Ag/Fe Bimetallic Nanoparticles Mediated by Rose Petal Extract

Article History:

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Abstracts: Synthesis of bimetallic nanoparticles has employed many field of applications such as medicinal, industrial, electrical, fertilizer etc, these bimetallic nanoparticles therefore need to be produced via a cost-effective and eco-friendly route. Here, green synthesis of Ag/Fe hybrid nanoparticles obtained from aqueous petal extract of rose was reported. The hybrid nanoparticles formation was noticed by a colour change from yellowish milky to brown and further studied using UV-Vis, FT-IR, FL spectrophotometers. The maximum absorption wavelength, λ_{max} as determined by the UV-Visible Spectrophotometer was found to be 420 nm. The FT-IR showed the formation and stabilization of the bimetallic nanoparticles. The morphological were studied by XRD, DLS, SEM Analysis, the electrical properties were studied by cyclic voltogram, the biological studies of antioxidant, anti-diabetic and anti-inflammatory activities were screened.

Keywords: Green Synthesis, Ag/Fe, Rose petal extract, Antioxidant, Antidiabetic, Anti-Inflammatory

INTRODUCTION

Nanotechnology is the branch of science which deals with very small structures of size 0.1 to 100 nm [1]. Now a day's nanotechnology and nanomaterials entice great attention because of their numerous applications [2]. Synthesis of nanoparticles can be achieved with the aid of bottom-up technique which includes constructing up of the atoms or molecules. Top-down technique includes making tinier and tinier structure via etching from the bulk material [3]. Nanoparticles have distinct physical properties like large surface area, shape, aggregation and size, state of size distribution, surface morphology, crystallinity, solubility and defect in structure [4]. The photocatalytic activity and stability of nanoparticles can be influenced by their surface chemistry and zeta

potential [5]. Physical and chemical properties exhibited by nanoparticles are unique when compared to the bulk materials[6]. Nanoparticles are also used in various fields like medicine, manufacturing materials, environmental, electronics[7] etc .Various metal nanoparticles such as gold, silver, platinum, palladium, copper, iron, nickel and cobalt can be produced by means of chemical, physical and biological methods[8]. Due to some poisonous chemical substances absorbed on the surface of metals more dangerous results have been related with chemical synthetic methods [9]. Biological methods are environmentally safe and cost effective in comparison with chemical and physical strategies for the synthesis of nanometals. Nanoparticles synthesized through the use of vegetation or plant extracts [10], microorganisms

[11,12], fungus[13] and enzymes[14] are recognized as ecofriendly and vital branch of nanotechnology. Bimetallic nanoparticles consists of two one of a kind metals have remarkable properties when compared to monometallic nanoparticles from both technological and scientific point of view[15,16]. Biosynthesized bimetallic nanoparticles are used in several contemporary fields viz. imaging, labeling, luminescence tagging, drug delivery and biomedical field because of their superior properties.[17].Biosynthesized bimetallic nanoparticles additionally have been found to possess extraordinary catalytic activities[18].

The combination of two metals in a single nano-object is as interesting as it is challenging. Indeed, bimetallic nanoparticles have been widely studied during recent years because of the multiple possibilities which can arise from the interaction between the two components [19]. Alongside, in the fields of energy harvesting and catalysis, it is of paramount importance to shift from noble metals to first and second row transition metals [20]. The aim of this research is therefore to study the impact of Ag doped iron nanomaterials on the and optical, spectral, structural morphological and biological characteristics of Ag/Fe bimetallic nanoparticles.

MATERIAL AND METHODS

2.1 Collection of materials

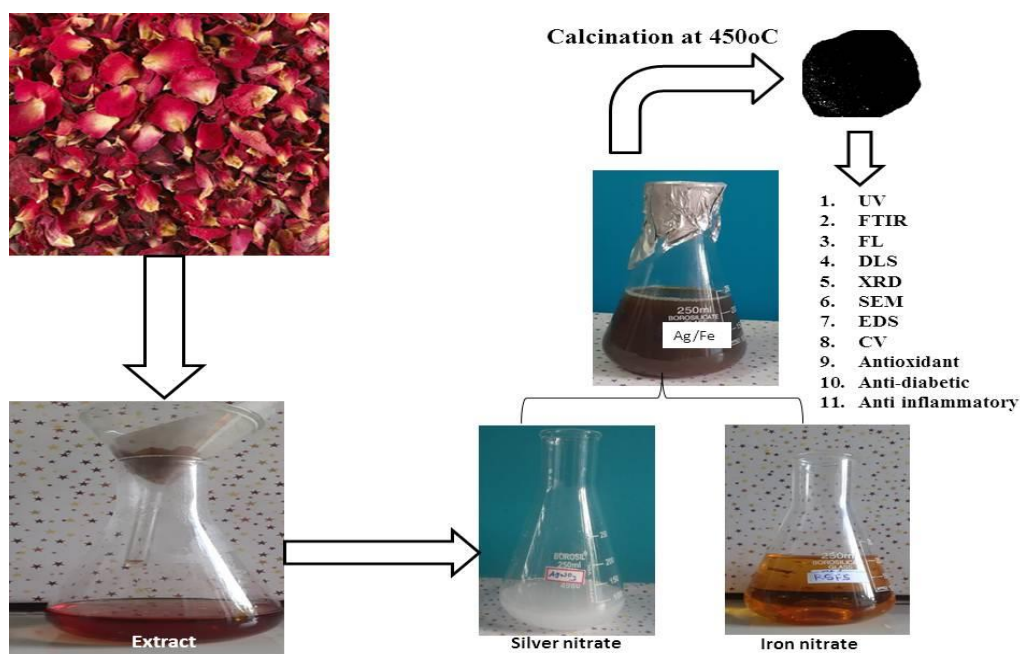
Flower wastes collected from Sri Narayanaperumal temple, Thiruvanthipuram, Cuddalore district, Tamilnadu, India. The collected wastes were separated by hand picking method. The roses were separated from the waste collections and then washed three times in running tap water to remove dust particles attached within and three times in double distilled water. Then the washed roses were dried at room temperature for five to six days and ground well until turn into powder using a pestle and mortar. This powder was collected in airtight glass tube used for preparation of extract.

2.2 Preparation of flower extract

5 grams of rose powdered with 50 mL of double-distilled water (DDW) taken in the 250 mL round bottomed flask, water condenser fitted and fix the running tap water then heated for 20 min at 80°C. Then the extract was filtered with Whatman 1 μ filter paper. The filtrate was used to the further green synthesis of process.

2.3 Synthesis of Ag/Fe bimetallic nanoparticles

Silver nitrate and Iron nitrate solution of 0.1m M were prepared separate conical flask. Equal volume (100 ml of 0.1M AgNO₃ +100 ml of 0.1M Fe(NO₃)₂) of the above solution taken in 500ml conical flask, drop wise 10 ml of rose flower petal extract has been added and color changes were observed after 10 min. By adding 1M NaOH solution, the pH is checked and adjusted to 12



**Figure:1 Scheme of green synthesis of Ag/Fe bimetallic nanoparticles by
petal extract**

Rose flower

RESULT AND DISCUSSION

3.1 UV Spectral analysis

UV-visible spectroscopy is one of the most widely used techniques for structural characterization of Ag/Fe bimetallic nanoparticles. **Figure:2a** shows the UV-Vis spectra of obtained Ag/Fe bimetallic nanoparticles mediated by Extract of pink roses flower petal. SPR band was broad, indicating poly-dispersed nanoparticles. SPR band around 386.30 nm broadened and slightly moved to the long wavelength region indicating the presence and formation of

Ag/Fe bimetallic nanoparticles. The optical absorption spectra of metal NPs were dominated by SPR, which shift to longer wavelengths with increasing particle size. The position and shape of plasmon absorption of Ag/Fe bimetallic particles were strongly dependent on the particle size, dielectric medium and surface-adsorbed species. The surface Plasmon absorption of Ag/Fe bimetallic nanoparticles has the short wavelength band in the visible region around 386.30 nm due to the transverse electronic oscillation of bimetallic nanoparticles.

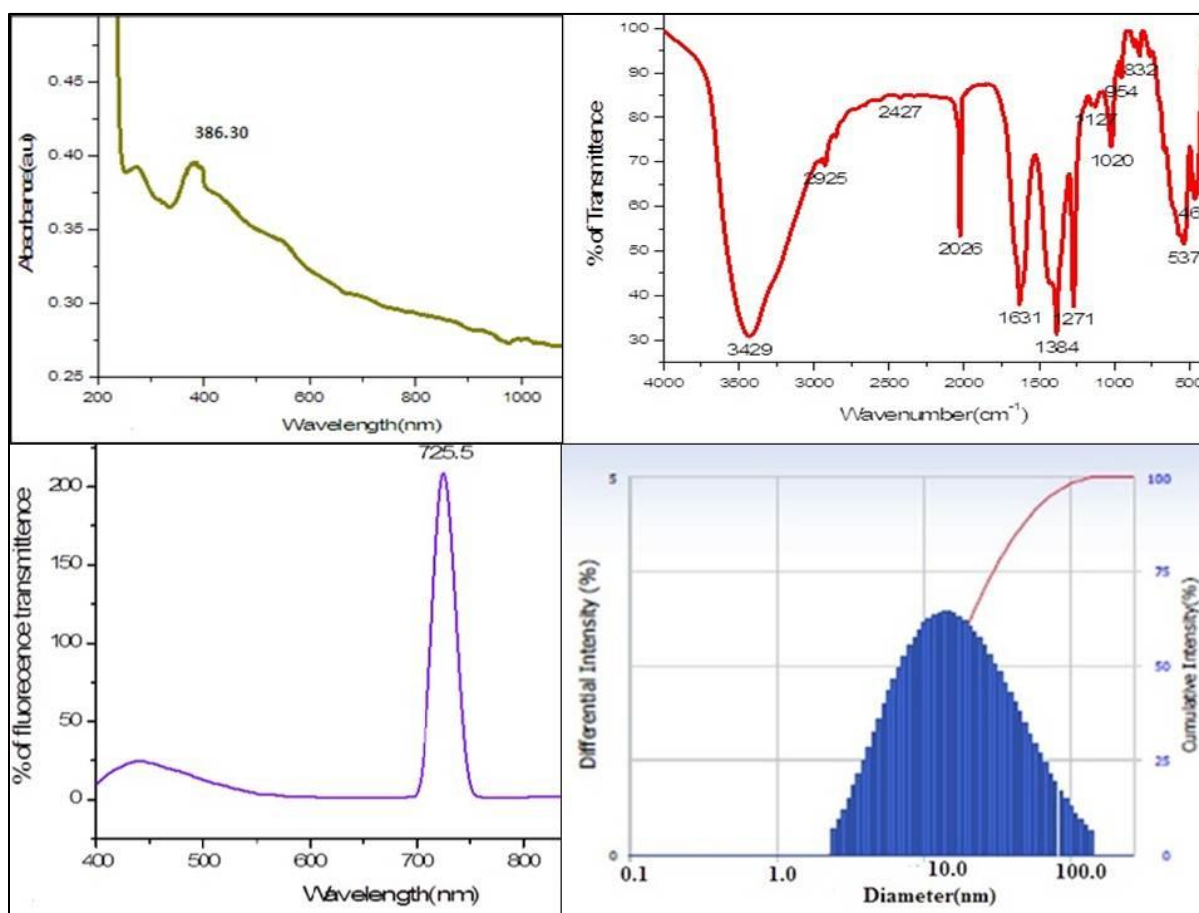


Figure:2 UV Spectrum of Ag/Fe bimetallic nanoparticles by pink roses flower petal Extract

3.2 FTIR study of Ag/Fe bimetallic nanoparticles:

The FTIR spectrum of Ag/Fe bimetallic nanoparticles obtained in this study. FTIR measurement was carried out to identify the possible biomolecules responsible for capping and efficient stabilization of Ag/Fe bimetallic nanoparticles synthesized using pink roses

flower petal extract. FT-IR spectrum have shown in **Figure:2b**. In the FTIR spectrum of pink roses flower petal extract capped Ag/Fe bimetallic nanoparticles, the spectrum showed absorptions at 3429 cm^{-1} due to -OH group of polyphenolic compounds, the frequency of 2925 cm^{-1} have shown

in –NH group of amine and amide compounds, Aromatic C=C have shown in 2427 cm^{-1} , 2026 cm^{-1} have indicates alkyne region, Carbonyl group contain acetamide group have shown in 1631 cm^{-1} , the strong peak of 1384.30 cm^{-1} have reveals that –O-H bending vibration for Hydroxyl group contain phyto-constituent and silver & iron hydroxide, this hydroxide peaks getting only to using sodium hydroxide for maintain pH ~ 10 , The vibration peaks of 1271 cm^{-1} indicates amine bending vibrations, 1020 cm^{-1} have shown C-O bending vibrations, The vibration peak of 537 and 446 cm^{-1} have been indicates the Ag/Fe bimetallic nanoparticles formations. The existence of phenolic compounds and proteins was assured by the functional vibrations bands group as demonstrated in the FTIR spectrum. All these confirm that water-soluble phytochemicals present in the leaf extract of pink roses flower petal Extract have the ability to perform dual functions of reduction and stabilization of the Ag/Fe bimetallic nanoparticles.

3.3 Fluorescence spectra

The fluorescence emission spectrum has been shown an obvious peak at 725.5 nm in Ag/Fe bimetallic nanoparticles sample, shows no trend in neither the Stern-Volmer quenching plot nor the direct fluorescence method (**Figure:2c**). Overall, even in the presence of Ag/Fe bimetallic nanoparticles.

3.1 Dynamic Light Scattering Analysis

DLS is often interpreted to as a scattering of quasi-elastic light. It fulfills the role of size distribution and agglomeration of selective NPs(**Figure:2d**). This process is quite sensitive, rapid and it can calculate the mean size of a particle on both macro and nano scale. The speed of the DLS technique is based on particle size. Small particles in suspension undergo random thermal motion known as Brownian motion. This random motion is modeled by the Stokes-Einstein equation. Below the equation is given in the form most often used for particle size analysis. The DLS analysis of green synthesized chromium

nanoparticle to shown the average size of the nanoparticle is 52.35 nm . This result also shown in some of the nanoparticle having more than 100 nm , this is due to growth of the nanoparticle and aggregation of the two is more nanoparticles.

3.2 SEM Analysis of Ag/Fe Bimetallic nanoparticles by pink rose petals extract

The scanning electron microscope uses a beam of high-energy electrons to produce a variety of signals at the surface of specimens used. The signals show information about the sample including chemical composition, and crystalline structure, external morphology (texture) and orientation of materials which make up the sample. SEM analysis is normally considered to be non-destructive because the x-rays generated do not lead to loss of volume of the sample, so it becomes possible to repeatedly analyse the same materials. A scanning electron microscope is a kind of electron microscope which images a sample by scanning it using a high-energy electron beam. The electrons then interact with the atoms making up the sample, thus producing signals which reveal information about the sample's composition, surface topography and other properties such as electrical conductivity.

The SEM analysis was used to determine the structure of the reaction products that were formed. Thin films of the sample were prepared on a gold coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min . SEM image has showed individual zinc particles as well as a number of aggregates. SEM analysis is done to visualize shape and size of nanoparticle. The SEM image of synthesized silver/iron bimetallic nanoparticles are shown in **Fig.3**. From the SEM images were seen in different magnification ranges like $1\mu\text{m}$ – $2\mu\text{m}$ which clearly demonstrated the presence of spherical and mountain rock shaped nanoparticles.

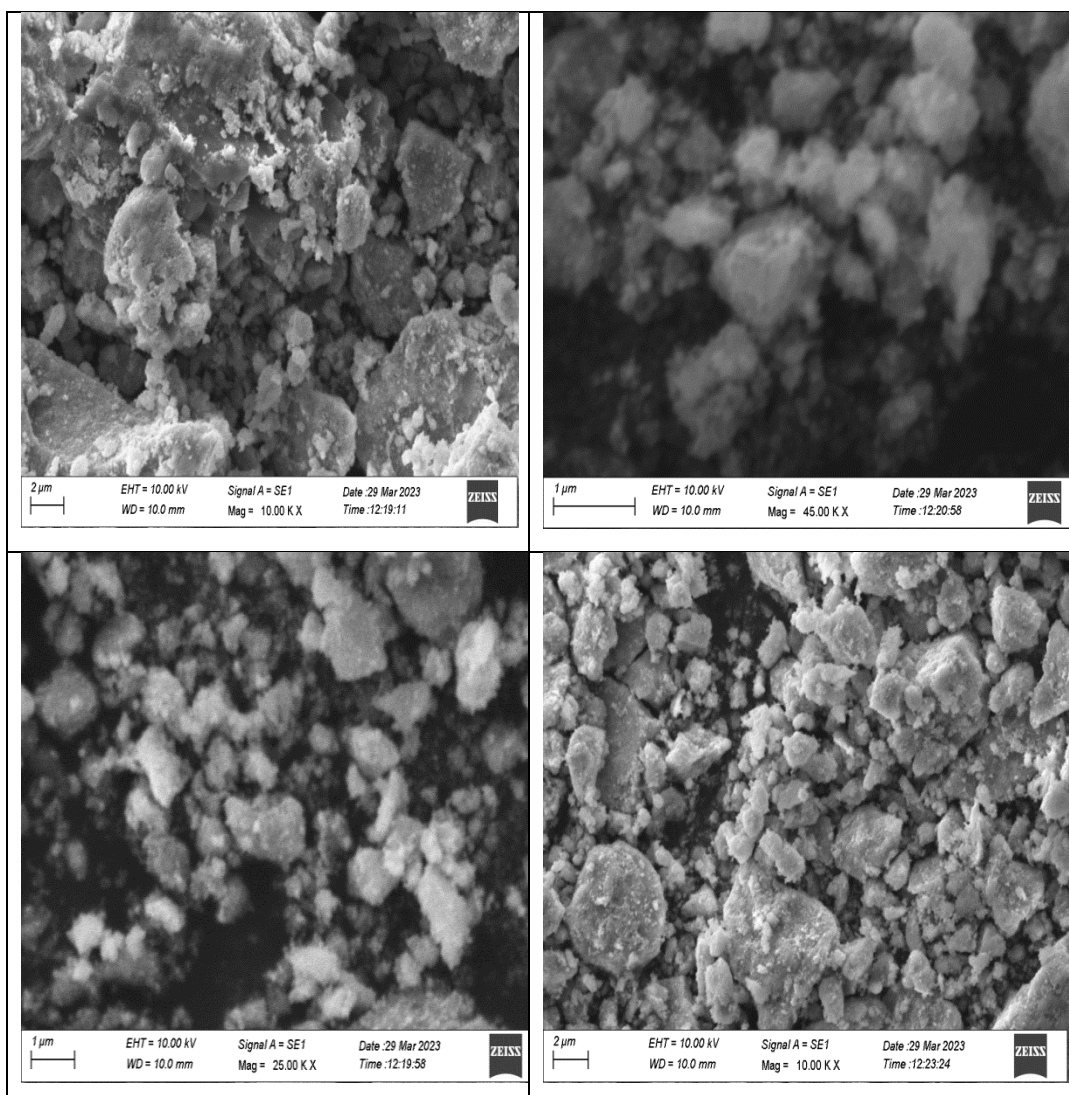


Figure:3 SEM Images of Ag/Fe bimetallic nanoparticles mediated by pink rose petals nanoparticles

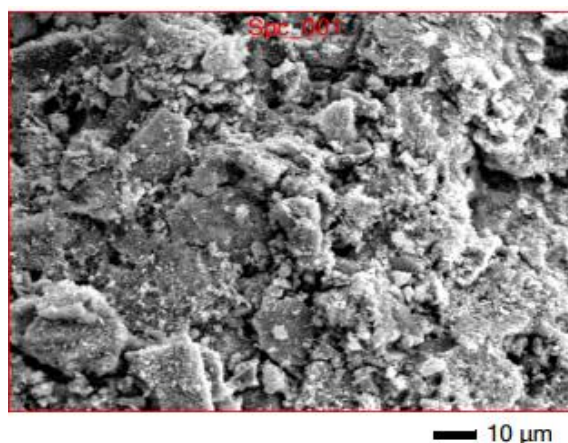


Figure:4 EDS Spectrum of Ag/Fe bimetallic nanoparticles mediated by pink rose petals nanoparticles

The elemental information of biosynthesized Ag/Fe bimetallic nanoparticles mediated by pink rose petals

nanoparticles was analysed by EDX . As shown in the **Figure 4**, evident that particle size 10µm. The

intensive peak signals of Ag, Fe and O were observed in the spectrum **Figure:5**, indicating the composition of Ag/Fe and O. Besides, the spectrum also exhibits the existence of O and W, which could due to the

biomolecules on the surface of the biosynthesized Ag/Fe bimetallic nanoparticles mediated by pink rose petals nanoparticles. Percentage of the element have shown in Table:1

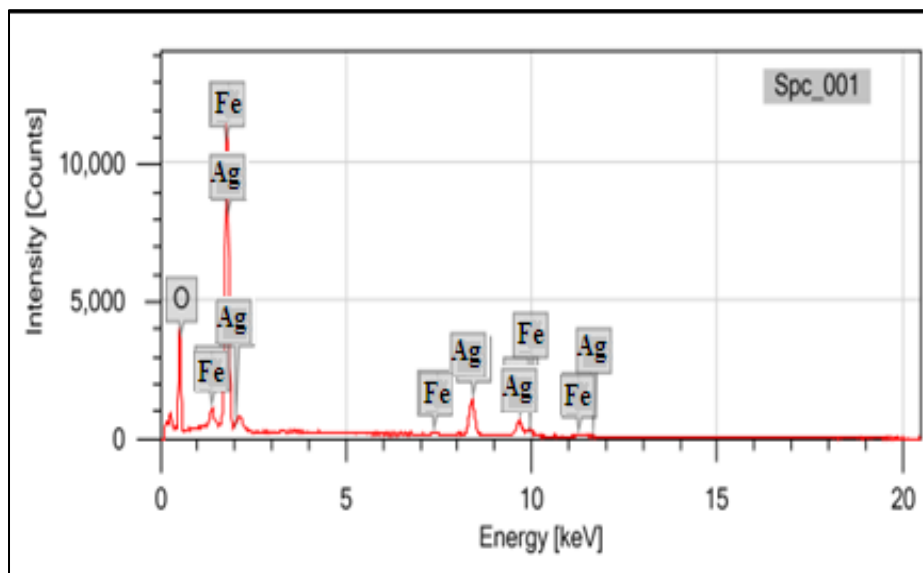


Figure:5: Intensity peak signal of Ag/Fe bimetallic nanoparticles mediated by pink rose petals nanoparticles

Table:1, Percentage of atoms in Ag/Fe bimetallic nanoparticles

Element	Line	Mass %	Atom %
O	K	20.32±0.14	74.56 ±0.51
Ag	L	36.35± 0.23	13.12± 0.26
Fe	M	33.33 ± 0.39	12.34 ± 0.12
Total		100.00	100.00
Spc_001		Fitting ration 0.0852	

3.1 XRD Analysis

The XRD analysis was done to deduce the crystalline nature with the lattice properties of biosynthesized Ag–Fe nanoparticles from pink rose petals extract, as shown in **Figure:6**. The XRD diffraction peaks were obtained, and the particle size was calculated by using the

Debye–Scherrer equation as:

$$D = K/lb \cos q \dots\dots\dots(2)$$

where D represents the average crystallite size, K is the Scherrer constant (0.94), *l* is the wavelength of X-ray sources (0.15406 nm), *b* is the full width at half maximum in radians (FWHM) and *q* is the diffraction angle in radians. By utilizing each term's numerical values in Equation (1), the average particle size of biosynthesized Ag–Fe nanoparticles from pink rose petals aqueous extract was 15.65 nm.

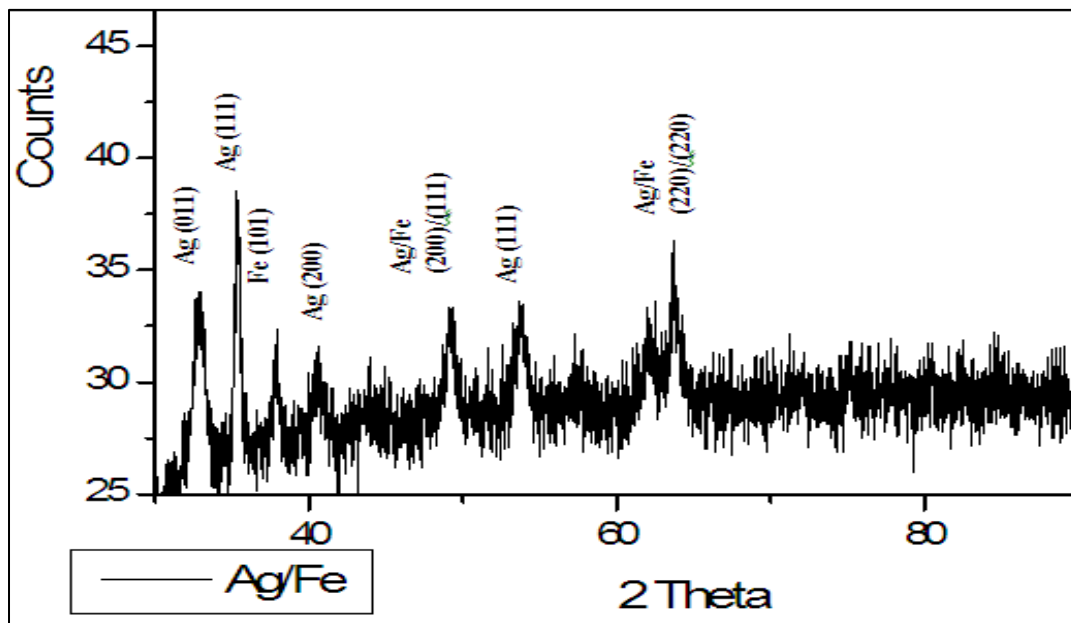


Figure:6 XRD spectrum of Ag/Fe bimetallic nanoparticles by pink rose petal extract

Besides, the crystalline nature of Ag–Fe nanoparticles was deduced from 2θ values of major diffraction peaks at 38.01, 44.21, 64.7 and 77.49° with lattice planes (111), (200), (220) and (311) respectively, as is crystal clear in Figure 10. The results of XRD patterns show a face-centered cubic (fcc) phase for Ag particles, according to the JCPDS number 00-004-0783. However, Fe shows diffraction peaks at 2θ values 44.21 and 64.7° with lattice planes (110) and (220), and the absence of other peaks in the XRD

pattern reflects that the Fe nanoparticles are protected from oxidation and are possibly reduced onto the surface of Ag in Ag–Fe nanoparticles biosynthesized from pink rose petals extract.

3.2 Cyclic voltammetry study

The shape of the cyclic voltammetry curves (Figure:7) is an ideal rectangular shape observed at 10 mV/s. Further increasing the scan rate the observed pattern of the CV curve is altered and it confirms the pseudo capacitive nature of the material.

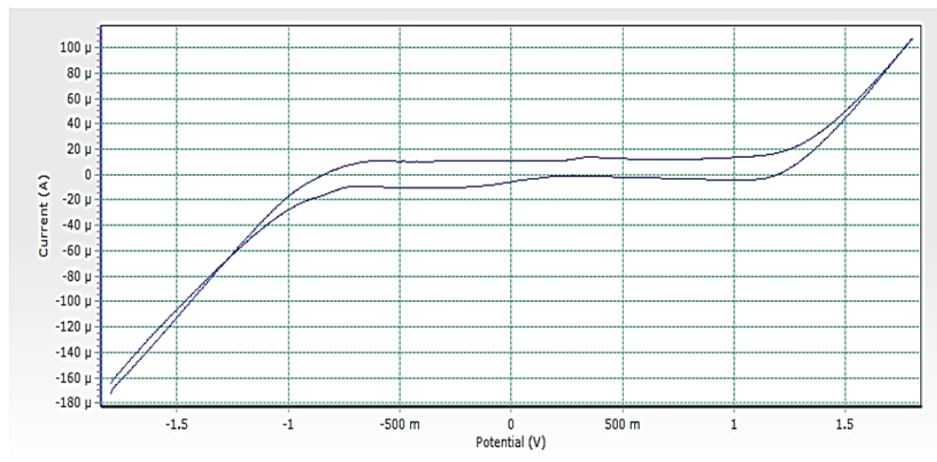


Figure:7 cyclic voltammetry study of Ag/Fe bimetallic nanoparticles by pink rose petal extract

The specific capacitance (SC) values of manganese ferrite electrode can be estimated by using the formula $C_s = Q / m\Delta V$

Where, C_s is the specific capacitance, Q the anodic and cathodic charges on each scanning, m is the mass

of the electrode material (mg) and ΔV is the scan rate (mVs^{-1}). Electrochemical measurements were

performed in 0.2 tetra butyl ammonium perchlorate with a standard three electrode configuration consisting of a sample (working electrode), an Ag/AgCl (reference electrode) and a high platinum wire (counter electrode). The scan rate increased in the range from 10 mV/s to 100mV/s and its corresponding specific capacitance values depicted in Fig.15 Further, the specific capacitance values of 290.4 F/g observed in the scan rate of 10 mV/s for the sample annealed at 700°C. The reason for high specific capacitance at low scan rate is observed in the present study suggested that the ionic diffusion takes place both inner and outer surfaces. The higher specific capacitance values observed in the present study confirm the good crystallinity of the Ag/Fe bimetallic nanoparticles.

3.4 Antioxidant Activity

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Diets high in vegetables and fruits, which are good sources of antioxidants, have been found to be healthy; however, research has not shown antioxidant supplements to be beneficial in preventing diseases.

Total antioxidant activity

Total antioxidant activities of samples were observed. About 3 ml of antioxidant reagent (0.6 M H₂SO₄, 28 mM Na₃PO₄ and 4 mM ammonium molybdate) were added to the test samples with various concentrations. The test mixture to accomplish proper diffusion with phosphomolybdenum reagent was incubated at 95°C for 90 min in a water bath. The total antioxidant activity of extracts and vitamin C standard drug were measured and determined their absorbance at 695 nm using a spectrophotometer. The total antioxidant activities were calculated using the given formula.

$$TOA = [(A_t - A_c) / A_t] \times 100.$$

Table:2 Antioxidant Activity of Ag/Fe Bimetallic nanoparticles by pink rose petals extract

S.No	Concentration (µg/mL)	Vitamin C (standard)	Ag/Fe
1	50	31.37255	5.405405
2	100	51.38889	12.5
3	200	84.30493	20.45455
4	250	92.23947	30
5	500	97.77919	35.18519

The antioxidant activity of Ag/Fe bimetallic nanoparticles have shown in moderate activity against phosphor molybdatinum reagent. Vitamic C act as standard as the present investigation, Table 2 have shown in antioxidant activity of Ag/Fe bimetallic nanoparticles, Figure:8 and:9 has shown in antioxidant activity Ag/Fe bimetallic nanoparticles and standard Vitamin C, Figure:10 have shown in Cluster column chart of anti-oxidant activity of Ag/Fe Bimetallic nanoparticles mediated by pink rose petals extracts.



Figure:8 Total Antioxidant activity of Ag/Fe Bimetallic nanoparticles by Pink rose petal extract



Figure:9 Total Antioxidant activity of Standard Vitamin C

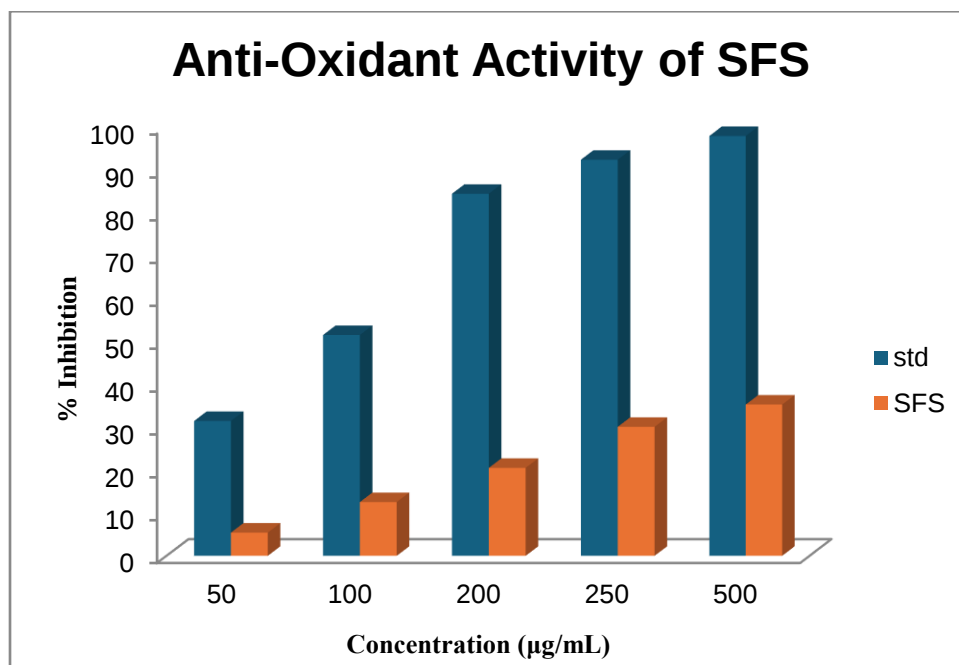


Figure:10 Cluster column chart of Total Antioxidant activity of Ag/Fe Bimetallic nanoparticles by Pink rose petal extract and Standard Vitamin C

3.4 Anti-diabetic Activity α -amylase inhibition technique

The antidiabetic activity of the samples was performed using α -amylase inhibition method. Briefly, Amylase (0.2%) was incubated with and without samples (in 1.5 mL) and standard for 10 min at 25°C. This experiment was performed in 0.2M

phosphate buffer (pH 6.9). After pre incubation, the 1% starch solution (0.5mL) was added and the reaction mixture was incubated for 30 min at 25°C. In order to stop the enzymatic reaction, DNSA reagent (0.5 mL) was added as the colour reagent and then incubated in a boiling water bath for 90 min.



Figure:11. Anti-diabetic activity of standard α -amylase

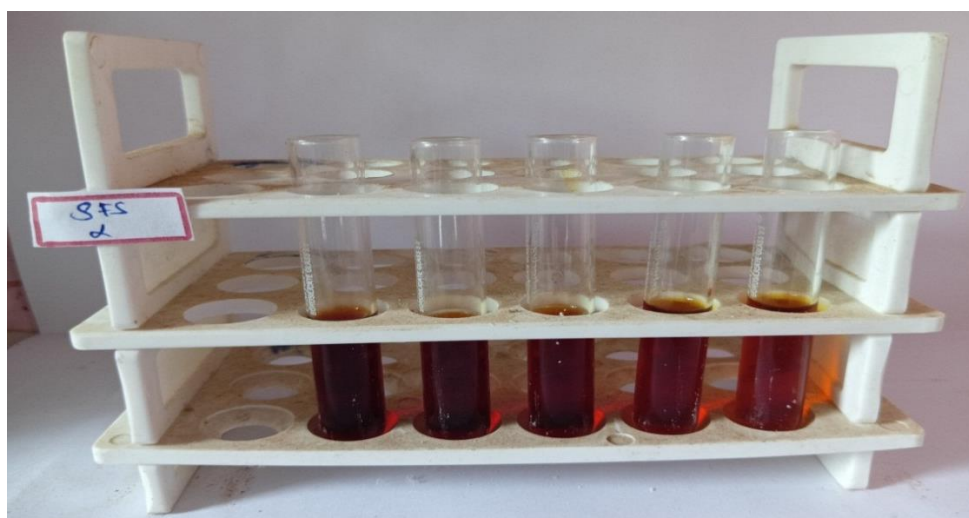


Figure:12. Anti-diabetic activity of Ag/Fe bimetallic nanoparticles by pink rose petal extract

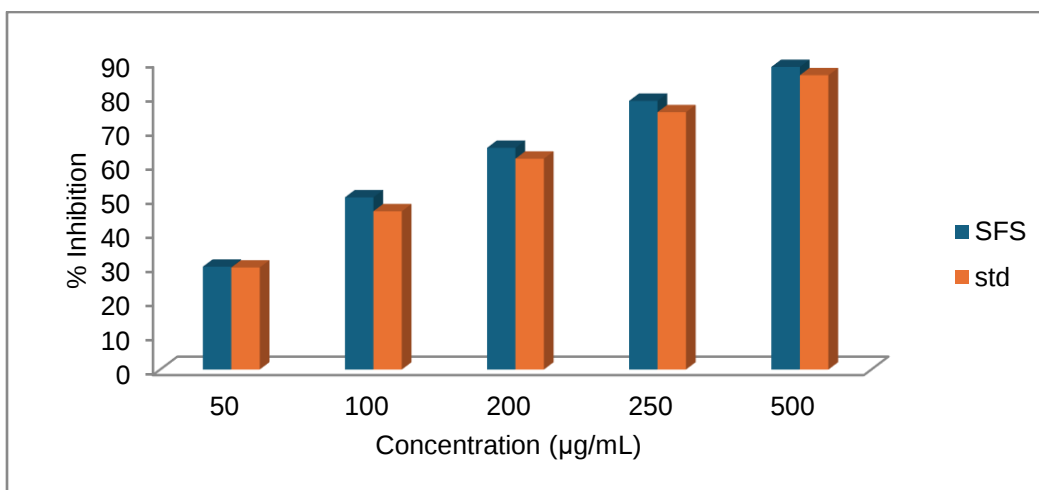


Figure:13. The cluster column chart of Anti-diabetic activity of Ag/Fe bimetallic nanoparticles by pink rose petal extract

After cooling down to the room temperature, 0.5 mL of samples was diluted to 2.5mL of distilled water and the absorbance measured at 540 nm using a UV-Visible spectrophotometer. The measured absorbance was compared with that of the control experiment. The percentage inhibition was calculated from the given formula.

$$\% \text{ of Inhibition} = 100 \times [Ac - At / Ac]$$

At: Absorbance of test

Ac: Absorbance of control

Table:3 Anti-diabetic activity of Ag/Fe bimetallic nanoparticles by pink rose petal extract

S.No	Ag/Fe	Standard(α -amylase)
50	30.16477	29.91128
100	50.4436	46.38783
200	64.89227	61.7237
250	78.58048	75.28517
500	88.46641	86.0583

Table 3 have shown in Anti-diabetic activity of Ag/Fe bimetallic nanoparticles, Figure:11 and:12 has shown in Anti-diabetic activity Ag/Fe bimetallic nanoparticles and standard α -amylase, Figure:13 have shown in Cluster column chart of Anti-diabetic activity of Ag/Fe Bimetallic nanoparticles mediated by pink rose petals extracts.

3.4 Anti-Inflammatory Activity BSA denaturation technique

The synthesized compound and standard diclofenac sodium were screened for anti-inflammatory activity by using the inhibition of albumin denaturation technique with minor modification. The standard drug and compound were dissolved in minimum

quantity of Dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, PH 7.4). The final concentration of DMF in all solution was less than 2.5%. Test Solution (2.5 mL) containing different concentrations of the drug was mixed with 1 mL of 1 mM Bovine serum albumin solution in phosphate buffer and incubated at 37 °C in an incubator for 10 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm. Percentage of Inhibition of denaturation was calculated from control where no drug was added. The percentage inhibition of denaturation was calculated by using the following formula.

$$\% \text{ of Inhibition} = 100 \times [Ac - At / Ac]$$

At: Absorbance of test

Ac: Absorbance of control

Table:4 Inhibition of Anti-inflammatory activity of Ag/Fe bimetallic nanoparticles mediated by pink rose petals nanoparticles

Concentration (μ g/mL)	Ag/Fe	Standard (diclofenac sodium)
50	24.46483	29.96942
100	35.57594	40.1631
200	43.32314	48.82773
250	56.67686	62.58919
500	78.89908	83.3843

The anti-inflammatory activity of green synthesised Ag/Fe bimetallic nanoparticles mediated by pink rose petal extract have shown comparable activity compared with standard diclofenac sodium.



Figure:14 Anti-inflammatory activity of standard diclofenac sodium

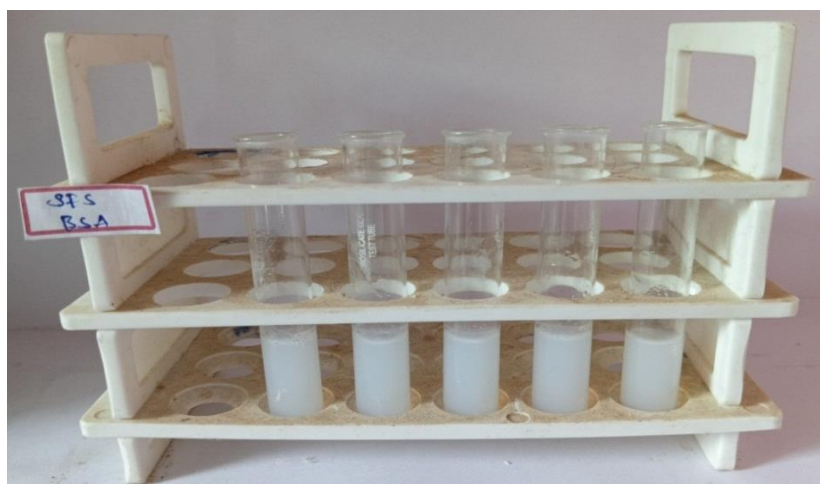


Figure:15 Anti-inflammatory activity of Ag/Fe bimetallic nanoparticles mediated by pink rose petals nanoparticles

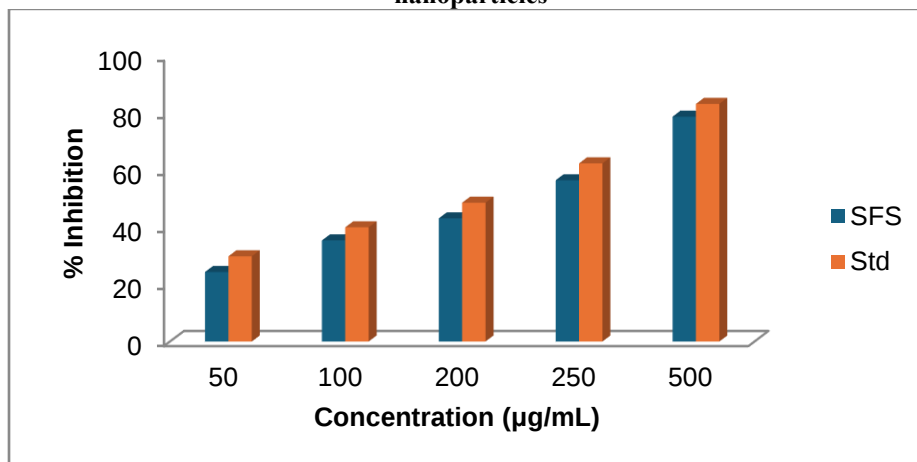


Figure:16 Cluster column chart of Anti-inflammatory activity of Ag/Fe bimetallic nanoparticles mediated by pink rose petals nanoparticles

Figure.14 and 15 have shown anti-inflammatory activity of standard diclofenac sodium and green synthesised Ag/Fe bimetallic nanoparticles mediated by pink rose petal extract. Figure.16 have shown in Cluster column chart of anti-inflammatory activity of Ag/Fe bimetallic nanoparticles mediated by pink rose petals nanoparticles. Table.4 has shown Inhibition of Anti-inflammatory activity of Ag/Fe bimetallic nanoparticles mediated by pink rose petals nanoparticles.

CONCLUSION

In summary, the Ag/Fe bimetallic nanoparticles were successfully synthesized by green method through pink rose petal extract. This is a green method of nanoparticles synthesis that does not introduce harmful substances into the environment and ensures cost effectiveness. The absorption maximum of the cobalt nanoparticles of Ag/Fe bimetallic are 386.30 nm, FTIR spectroscopy showed by 537 cm^{-1} and 463 cm^{-1} have evident that Ag/Fe bimetallic nanoparticles, FL spectroscopy have been showed by 725 nm, DLS have showed average size is 21.2 nm. XRD analysis gives particle size was calculated to be in the range of 28–42 nm. SEM analysis of Ag/Fe bimetallic nanoparticles to report 2 μm and 1 μm , and also purity of the cobalt nanoparticles conformed by EDS spectrum, Super capacitor performance application also studied by cyclic voltammetry of cobalt nanoparticles. Antioxidant and anti diabetic activity have showed significant activity compared to standard drug but anti-inflammatory activity have shown in the very high and effective activity.

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