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Research paper

Ocimum Tenuiflorum-mediated Biosynthesis of Silver Iron (Ag/Fe) Bimetallic Nanoparticles and Evaluation of their Antiglycation and Biocompatibility Activities

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ABSTRACT

Nano-biotechnology based revolution, which rests on the foundation laid by the green synthesis of nanoparticles (NPs), has given us hope to make significant developments in the area of biomedical science. In this study, we synthesized Ag/Fe Bimetallic Nanoparticles from the *Ocimum Tenuiflorum* plant extract. The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of the fresh leaves of plant extract were determined to find out their antioxidant potential. UV-Visible spectrophotometry of green synthesized Ag/Fe Bimetallic NPs presented a distinctive band of surface Plasmon resonance within the range of 380-420 nm. The synthesized NPs were characterized using Fourier Transform Infrared spectroscopy and X-Ray Diffraction analysis techniques. Synthesized NPs were checked for their biological activities such as anti-glycation and biocompatibility activities. FTIR showed the presence of phenols, aliphatic and aromatic hydrocarbons and flavonoid compounds that were used in bioreduction and capping of Ag/Fe NPs. XRD was conducted to determine the particle size and crystalline nature of both types of NPs. The Ag/Fe bimetallic nanoparticles had spherical shape and a particle size of 13 nm. In case of antiglycation and biocompatibility activities results showed their high potential in clinically multi-drug resistant strains.

KEYWORDS: Nanobiotechnology, Ag/Fe Bimetallic nanoparticles, Ocimum Tenuiflorum Plant, characterization, antiglycation, biocompatibility activities.

INTRODUCTION

Nanotechnology and nano-sciences are emerging as newest and wide field since 1980. Nanoparticles are very small in size i.e.one billionth or 10-9 because of their small size and their specific properties like optical, magnetic, catalytic, and electrical properties they're behaving as building blocks for complexed nanostructures (1,2). In 20th Century, Richard Feynman introduced the fundamental idea of present-day nanotechnology. These days, we are noticing an incredible development within the area which is especially attributed to the way nanotechnology has born to hope for treating infectious diseases that were tagged incurable before. Based on classification nanomaterials are distributed based on their dimensions such as volume to surface ratio, morphology (e.g., flatness, spatial position, and aspect ratio) in hybrid nanoparticles. Nanotechnology involves at least one-dimensional particles which can be less than 100 nm while particle can be zero dimensions in reference to quantum dots (2,3).

For the green synthesis of Ag and Fe Bimetallic nanoparticles plant extract of any medicinal plant is used. In this study, Plant extract of *Ocimum tenuiflorum* was used for the synthesis of Ag and Fe Bimetallic NPs.

These two have not been reported from this plant to the best of our knowledge. *Ocimum tenuiflorum*, Tulsi, from the family *Lamiaceae* has been described as the "Queen of plants" and the "mother medicine of nature" due to its perceived medicinal qualities (4,5). Traditionally, Tulsi is used in different forms; aqueous extracts from the leaves (fresh or dried as powder) are used in herbal teas or mixed with other herbs or honey to enhance the medicinal value. Traditional uses of Tulsi aqueous extracts include the treatment of different types of poisoning, stomach-ache, common colds, headaches, malaria, inflammation, and heart disease. Oils extracted from the leaves and inflorescence of Tulsi have been claimed to have numerous useful properties, including as expectorants, analgesics, anti-emetics, and antipyretics; stress reducers and inflammation relievers; and as anti-asthmatic, hypoglycemic, hepatoprotective, hypotensive, hypolipidemic, and immunomodulatory agents (6,7). Plant extracts are known to have biologically active compounds that can work as capping agents that can reduce the ions to for NPs. The plant extract has a lot to do with the nature of synthesized NPs because it affects morphology (7,8).

During the synthesis, if the working conditions are changed, then the morphology of NPs can also change. It has been reported that UV radiations can significantly alter the shape of these particles. With the emergence of antibiotic resistance of microbes towards antibiotics, scientists and pharmaceutical companies have started investing their efforts to look for other ways to get rid of these. AgNPs have proved to be the most beneficial in this regard and hence they are being used a lot (9,10). In this study, we aim to synthesize, characterize and check different biological activities of Ag and Fe Bimetallic NPs from *Ocimum tenuiflorum* plant. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of the plant extract was checked. The physio-chemical properties will be characterized by using various techniques such as FTIR (Fourier-transformed infrared spectroscopy), and XRD (X-ray diffraction). Furthermore, biological activities of both types of NPs such antiglycation and biocompatibility activities were also evaluated in this project.

MATERIAL AND METHODS

Preparation of Reagents:

1. Prepared 1000 ug/ml standard solution of Gallic Acid by dissolving 10 mg of GA in 10ml of DW. Dilutions of standard solution were done with water to make 50-1000 µg/ml concentration solutions.
2. Prepared 20% sodium carbonate solution by adding 20g Na_2CO_3 to 80ml of DW, mixed thoroughly and made final volume up to 100ml by adding water.
3. Prepared 100µg/ml Quercetin standard by dissolving 1mg quercetin 10ml of DW. Dilutions of standard solution were done with water to make 25-200µg/ml concentration solutions.
4. Prepared 10% (w/v) Aluminum Chloride solution by dissolving 2.5g $AlCl_3$ in 25ml of methanol with the help of heat stirring and made final volume 100ml by adding methanol.
5. Prepared 1M KAC solution by dissolving 2.45g in Potassium Acetate in 25ml of DW.

Selection of Medicinal Plant for Synthesis of Nanoparticles:

Ocimum Tenuiflorum **(**Tulsi in Urdu/Hindi**)** was selected for the synthesis of Silver/Iron Bimetallic Nanoparticles**.** Fresh leaves were collected from premises of Kinnaird College for Women, Lahore.

Preparation of Aqueous Leaf Extract of Medicinal Plant:

Firstly, the *ocimum tenuiflorum* (tulsi) leaves were collected from premises of Kinnaird College for Women, Lahore. 10g of dry plant

leaves were weighed; afterwards, leaf extract was prepared after washing plant leaves thoroughly under running tap water followed by using distilled water. Leaves were left for drying on room temperature for 30 mins. 400 ml of distilled water was added in 10g of leaves and boiled till volume reduced to 100 ml, then it was grounded finely by using pestle and mortar. Plant extract was filtered by using Whatman's filter paper. The prepared plant extract was stored at 4°C for further use.

Phytochemical Analysis of Plant Extract

Determination of Total Phenolic Content (TPC)

For the determination of Total Phenolic Content, of the plant extract *ocimum tenuiflorum*, Kamtekar *et al.* (38) instructions for the Folin- Ciocalteu's method were followed. To begin with, 6 different concentrations of the standard Gallic acid in test tubes were taken, subsequently 1 ml plant extract was added in each test tube. The concentrations made were 50, 125, 250, 500, 750, 1000 µg mL^{-1}, to which 0.5 ml Folin-Ciocalteu's reagent was added, along with 5 ml distilled water. The mixtures were shaken well before letting them incubate at room temperature for 5 mins.

After incubation, the total volume in each test tube was increased to 5 ml with the help of distilled water and 1.5 ml of 20% sodium carbonate solution. Color change was observed as the mixture turned deep blue, at this point, the solutions were left to incubate for 2 hrs. at room temperature. Afterwards, spectrophotometer was used to measure the absorbance of all the solutions at 750 nm. The whole process was done in triplicates. Using the absorbance values, calibration curve was plotted of the phenolic content of the leaf extract with the help of Gallic Acid standard. The concentrations were assigned to the x-axis and the absorbance to the y-axis. The TPC values were stated in mg/g of Gallic acid equivalents (mg GAE/g).

Determination of Total Flavonoid Content (TFC)

For the determination of the Total Flavonoid Content (TFC) of the plant extract *ocimum tenuiflorum*. Aryal *et al.* (11) instructions for the Aluminum chloride colorimetric method were followed. 1 ml of quercetin standard (25-200 µg mL^{-1}) or 1 ml of *ocimum tenuiflorum* leaf extract was added to a test tube. Afterwards, 5.6 ml of distilled water, 0.2 mL 10 % (w/v) aluminum chloride solution as well as 0.2 ml 1M potassium acetate was added into the same test tube. The solution was incubated for 30 mins at room temperature after mixing all the solutions well. Spectrophotometer was used to measure the TFC absorbance at 415 nm. The whole process was done in triplicates. Using the absorbance values, calibration curve was plotted of the flavonoid content of the leaf extract with the help of Quercetin standard. The concentrations were assigned to the x-axis and the absorbance to the y-axis. The TFC values were stated in mg/g of quercetin equivalent (mg QE/g) of leaf extract.

Free Radical Scavenging Activity (FRSA)

For the determination of the antioxidant activity of the plant extract (*Ocimum Tenuiflorum*), Kamtekar *et al.* (12) instructions for the DPPH FRSA (Free Radical Scavenging Activity) assay were followed. The whole experiment was done in triplicates. 4.5 ml of DPPH (2, 2-Diphenyl-1-picrylhydrazyl) (3.2 mg/100 mL methanol) is taken in a test tube, to which 0.5 ml of the *O.tenuiflorum* plant extract was added, followed by the incubation of the mixture for 1 hr. at room temperature. Spectrophotometer was used to measure the absorbance at 517 nm. Percentage (%) of discoloration of DPPH was taken as the unit to measure the FRSA with the help of the equation mentioned next;

$$\textbf{FRSA (\%) = 100 x (1-A}_c\textbf{/A}_s\textbf{)}$$

Where A_C represent the absorbance of *Ocimum Tenuiflorum* plant extract and DPPH, and A_S represents the absorbance of standard DPPH solution.

Reagents' Formation

For the formation of bimetallic NPs, the stock solution of Ag nitrate and Fe nitrate were formed. 0.04 M stock solution of silver nitrate was formed for the production of silver nanoparticles. For this 0.1 grams of salt in 12.5ml distilled water was mixed at room temperature. 0.03 M stock solution of Fe Nitrate was formed for the production of iron nanoparticles. For this 0.1 grams of iron nitrate was added to 12.5ml of distilled water at room temperature.

Synthesis of Ag/Fe Bimetallic Nanoparticles:

Protocol mentioned in the literature (13), was followed for the generation of silver iron bimetallic nanoparticles. For silver, the precursor salt i.e., silver nitrate was utilized and for iron i.e., iron nitrate was utilized. It can be formed by adding 12.5 ml of 0.04 M AgNO3 and 12.5 ml of 0.03 M of Fe (NO3)3 in a falcon tube. Then mixed both mixtures in a single falcon tube, and heated until reaching 60^C. you can use hot plate for heating it, placed the thermometer in it. When this temperature was reached, 1 ml of leaf extract of therapeutic plant (ocimum tenuiflorum) was added to the mixture, kept at a constant stir and heated at 80^C to 100 for 35 mins. The formation of the nanoparticles was observed and confirmed by the colour change of mixture, by monitoring through spectrophotometer.

The reaction was cooled at room temperature, centrifuged at 14,000 rpm for 5 min. Containing Ag/Fe Bimetallic NPs, darkish brown pellets were achieved. With distilled water pellets were washed after discarding the supernatant. Before centrifuge at 14000rpm for 5 mins, with distilled water Eppendorf having pellets were filled and dissolved with the help of vortex mixer. Pellets were washed carefully to eliminate impurities from the manufactured NPs. The supernatant was discharged. This step (washing) was repeated three times. At room temperature NPs were dried. Pellets having black powder colour were obtained for the purpose of characterization after all the moisture was evaporated.

Optimization of Protocol for synthesis of NPs: For Silver & Iron Bimetallic Nanoparticles:

On the basis of parameters such as absorbance, model peak in ultraviolet – vis spectrophotometer, time of colour variation, stability, decrease the time of silver ions to silver NPs, the ratio of plant extract with a salt solution was selected. The quantity of Stock sol of salt was changed in every ratio while the quantity of leaf extract of the therapeutic plant remained standard i.e., 1:10, 1:15, 1:20, and 1:25 were carried out. After dissolving stock sol with leaf extract of the therapeutic plant, the time of colour variation was observed. After 16 to 20 mins the quickest colour variation was seen in 1:20 and 1:25. On the other hand, even after 6 hours, no colour variation was observed in 1:10 and 1:15. All the solutions were checked in ultraviolet-vis spectrophotometer immediately after colour variation. Absorption peaks of all the solutions were checked in the range between 300-800 nm wavelength.

For 1:10, 1:15, & 1:20 no peaks were observed at all. The peaks were better at ratio 1:25, the reaction mixture showed quicker change in colour for synthesis (indication of faster synthesis of nanoparticles. Absorbance peaks were checked from time to time.

Among these, 1:25 was best. The change in colour was very quick, stable adsorption peak were observed at 24 hours. That's means the nanoparticles formed in the solution were stable even after 1 day. Considering all these factors, 1:25 using plant extract was selected for the synthesis of silver iron bimetallic nanoparticles.

Characterization of Ag/Fe Bimetallic NPs: UV-Visible Spectroscopy

UV-Visible Spectroscopy was performed using a Specord 200 plus spectrophotometer, throughout the process of Ag/FeNPs synthesis. Absorbance was measured within the range of 350-800 nm with the interval of 25 mins.

Characterization of Ag/Fe Bimetallic NPs: Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR)

Attenuated Total Reflection-Fourier-transform Infrared (ATR-FTIR) spectra of Ag/Fe bimetallic nanoparticles were recorded. A Burcker V70 Interferometer (in reflective mode, under a de-hydrated airflow, and gold crystal containing ATR accessory) was used to attain the reflectance spectra of the sample Ag/Fe NPs. The wave number present in the mid IR range was measured (400 – 4000 cm^{-1}). The readings were mostly seen on the 64 scans, with the resolution set at 4 cm^{-1}. IR readings provided in this study are the mean of three replicates along with their standard deviations.

Characterization of Ag/Fe Bimetallic NPs: Powder X-Ray Diffraction

XRD was performed to inspect the crystalline nature of the Ag/Fe bimetallic nanoparticles. X-ray diffractometer (Shimadzu-Model, XRD6000, Nettetal, Germany was used in its scanning mode to measure all types of Ag/FeNPs placed on the XRD grid. The diffractometer with its Cu/kα radiation for the 2θ angles in 20°-80° range, 30 mA current at 40 kV voltage. The Debye-Scherrer equation mentioned below was used to calculate the size of all *O.tenuiflorum* Ag/Fe NPs.

$$(\boldsymbol{D = k\lambda})/(\boldsymbol{\beta \cos \theta})$$

where k = shape factor (0.94); λ = X-ray wavelength (λ = 1.5418 Å); β = full width at half maximum (FWHM) in radians; and θ = Bragg's angle.

Biological activities of Ag/Fe Bimetallic NPs: Antiglycation Activity of Ag/Fe Bimetallic NPs

For *in vitro* cell free Antiglycation activity, two assay Vesperlysine and Pentosidine like AGEs were performed, mentioned in detail below.

Vesperlysine and Pentosidine like AGEs Activity

Advanced End Products (AGEs) are formed due to the presence of Oxidative stress. So, the antioxidant capacity also links with antiglycation activity in terms of inhibiting AGEs formation. The inhibitory percentage of AGEs formation of Ag/Fe Bimetallic NPs was measured by following Shah *et al.* (14) protocol. Each sample of Ag/Fe Bimetallic NPs was mixed with 0.5 M glucose solution prepared in phosphate buffer, 20 mg/mL BSA solution was prepared in 0.1 M phosphate buffer with the pH maintained at pH 7.4, and 1mM phosphate buffer having 0.02 % sodium azide. The whole mixture was incubated in darkness for 5 days at 37 °C. The AGEs produced were recorded at 330 nm excitation wavelength along with 410 nm emission wavelength. The anti-AGEs formation was specified as percentage (%) inhibition relative to the control.

Evaluation of Biocompatible Properties: Hemolysis Assay:

Biocompatibility was measured by using hemolysis assay to check compatibility of Ag/Fe Bimetallic NPs with human RBCs. To investigate, fresh RBCs was taken from a donor. Erythrocytes were isolated by using EDTA tube and centrifuged at 14,000 rpm for 5 min, supernatant was superfluous and pallet were washed thrice with Phosphate buffer saline (PBS). 200µL pallet was added to 4800µL PBS. Different concentration of prepared erythrocytes was added to tested samples and incubate them for 1 hr. at 37°C. after incubation centrifugation was performed at 2500 rpm for 10 mins. Hemoglobin release was use in 96 well plate containing treated supernatant and observed at 530 nm. 0.5% Triton and DMSO were used as positive and negative controls respectively. Percentage of hemolysis was determined by using following formula:

% Hemolysis = sample absorbance - negative control absorbance/ positive control absorbance – negative control absorbance × 100

Brine Shrimp Assay:

Another assay was used to check biocompatibility i.e., Brine Shrimp Lethality assay which is used to assess the preliminary cytotoxicity test of bioactive chemicals. It works by killing of an organism i.e., brine shrimp. Several groups work on this assay but first proposed by Micheal et al (15) This assay is used to evaluate toxicity of heavy metals, medicines, pesticides and plant extract (16) DMSO is used as a solvent in brine shrimp assay which is safe for working beside this methanol and Tween 20 was also suggested. As Tween 20 is used to dissolve oil, water soluble functional groups and alkyl chains (17). This assay utilizes and explains the potential of laboratory cultured larvae (nauplii) about 22 mm long and large enough to observe under high throughput and have potential to hatch without any special requirements (18). Researchers observe the survival rate of nauplii with different concentration of plant extract and take reading of their motion after every 30 sec. Often, nauplii (larvae) were observed with diverse concentration of plant for 24 hours. Motile nauplii was calculated and exhibit the effectiveness of Ag/Fe Bimetallic NPs. Formula that used to confirm their survival is (19);

% Death = No. of dead nauplii/No. of dead nauplii + No. of live nauplii × 100

RESULTS

Phytochemical Analysis of Plant Extract: Determination of Total Phenolic Content (TPC)

Gallic acid was used as a standard for TPC and a graph was plotted for different concentrations of gallic acid against the absorbance. From this graph, this equation was derived $y = 0.0002x + 0.2558$, $R^2 = 0.9879$ and value of x was calculated by using the absorbance of leaf extract. Then by using this value in the following formula, TPC expressed in Gallic acid equivalents (GAE) per gram DW was calculated.

TPC (GAE) = $C \times V\ (ml)$ **/ mg**

Where, C= concentration of extract obtained from standard curve, V= volume of extract and m= mass of extract.

Total phenolic content of the *Ocimum tenuiflorum* leaf extract was found out to be 23.02 ± 0.08 mg GAE/g DW.

Determination of Total Flavonoid Content (TFC)

Quercetin was used as a standard for TFC and a graph was plotted for different

concentrations of quercetin against the absorbance. From this graph, this equation was derived y = 0.0057x + 0.0127, R2 = 0.9973 and value of x was calculated by using the absorbance of leaf extract. Then by putting this value in the following formula, TFC expressed in Quercetin equivalents (QE) per gram DW was calculated.

$$\textbf{TFC (QE) = C} \times \textbf{V (ml) / mg}$$

Where, C= concentration of extract obtained from standard curve, V= volume of extract and m= mass of extract.

The total flavonoid content of *ocimum tenuiflorum* leaf extract was found out to be 2.02 ± 0.04 mg QE/g DW.

Free Radical Scavenging Activity (FRSA)

When a substance like an antioxidant is added to DPPH assay, its nitrogen atom receives a hydrogen atom from the antioxidant and it changes into its reduced form. As a result, the violet color disappears and absorption is also decreased at 517 nm which shows that the added substance had strong antioxidant capacity. Absorbance of the leaf extract in DPPH assay was measured at 517 nm using a spectrophotometer after 1 hour incubation and percentage inhibition was calculated. The dark purple color of DPPH totally disappeared in the presence of leaf extract and its absorption was also decreased due to which there was an increase in percentage inhibition.

$$\textbf{Percentage Inhibition} = \frac{\textbf{Absorbance of blank} - \textbf{Absorbance of sample}}{\textbf{Absorbance of blank}} \times 100$$

Absorbance of blank

FRSA of leaf extract of *ocimum tenuiflorum* was found out to be 87% ± 0.03 %.

Characterization of Ag/Fe Bimetallic NPs: UV-Vis Spectroscopy Analysis of Ag/Fe NPs

Fe/Ag Bimetallic nanoparticles exhibit maximum Surface Plasmon Resonance (SPR) peak in the range of 350-425 nm wavelength. The figure 1 shows the absorption spectrum of Ag/Fe Bimetallic NPs. The results showed absorption spectrum peak at 408 nm wavelength with the absorbance of 4.4973 for Ag/Fe Bimetallic NPs. The UV-Vis spectrum showed sharp absorption peak confirming the presence of NPs.

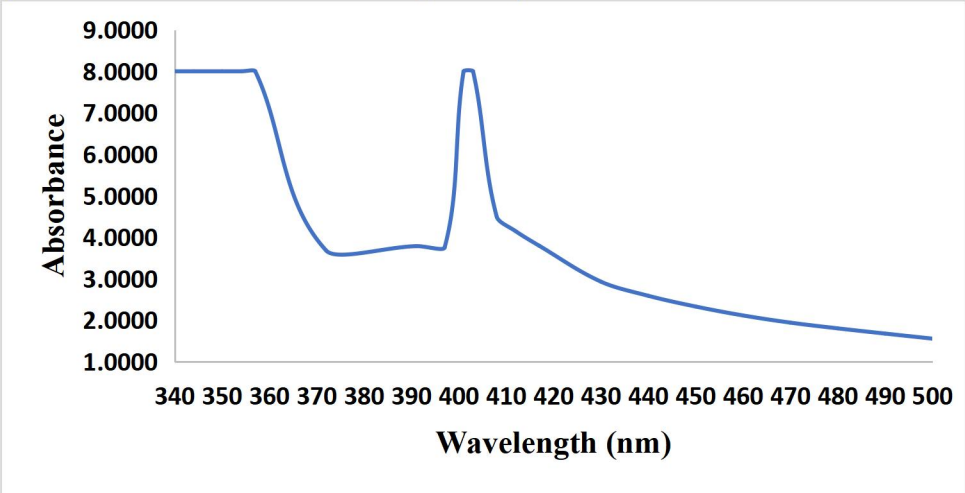

Figure 1: Absorption spectrum of Ag/Fe Bimetallic nanoparticles showing a sharp peak at 402 nm.

Fourier Transform Infrared Spectroscopy:

FTIR was performed on both leaf extract of *Ocimum Tenuiflorum* and Ag/Fe Bimetallic NPs. Data was analyzed by plotting a graph between wavelength and transmittance. Different peaks were observed in the range of 500-4500 cm^{-1} which showed the presence of various functional groups in both plant extract and Ag/Fe Bimetallic nanoparticles. For the leaf extract of *Ocimum Tenuiflorum*, different peaks at 530.42 cm^{-1}, 565.14 cm^{-1}, 1479.4 cm^{-1}, 1624.0 cm^{-1}, 2328.08 cm^{-1} 3350.35 cm^{-1} and 3811.34 cm^{-1} were observed (Figure 2). For Ag/Fe Bimetallic nanoparticles, different peaks at 532.35 cm^{-1}, 590.21 cm^{-1}, 1624.06 cm^{-1}, 2347.36 cm^{-1}, 3711.04 cm^{-1} and 3888.4 cm^{-1} were observed (Figure 3).

These peaks showed the presence of corresponding bonds of C-O, O-H (oxygen groups), C=C– stretch (alkenes), –C≡C– stretch (alkynes), C=N stretch (nitriles), C–N stretch (aromatic amines), and N-H (1˚, 2˚ amines, amides) which correspond to phenolic, alkaloids and amide group.

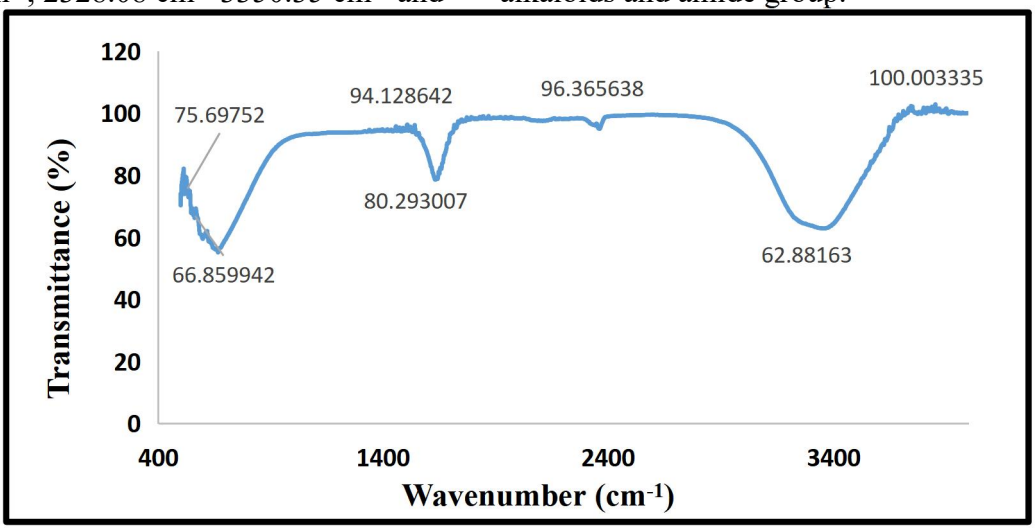

Figure 2: FTIR analysis of leaf extract of *O. tenuiflorum.*

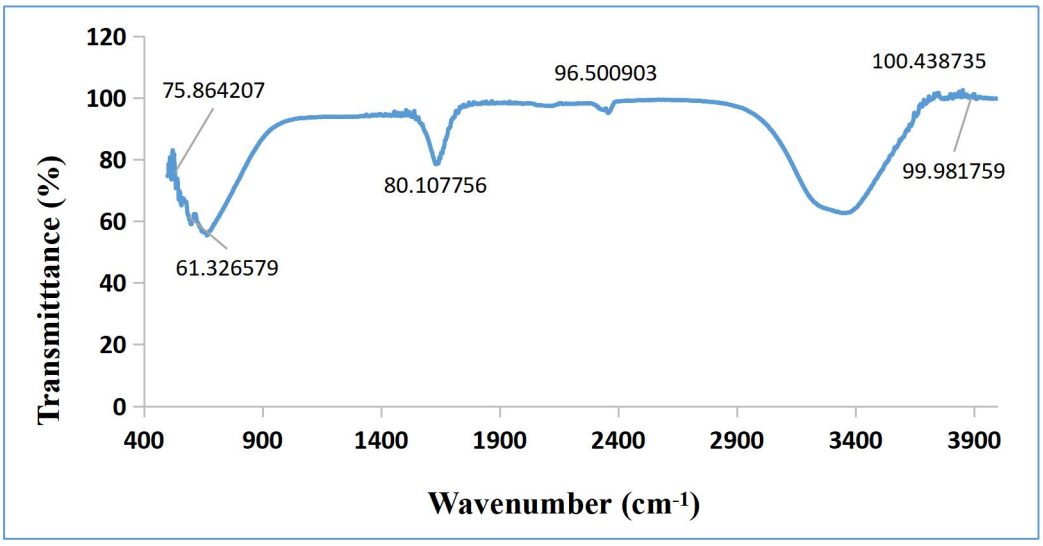

Figure 3: FTIR Analysis of Ag/Fe Bimetallic NPs.

X-Ray Diffraction (XRD)

To confirm the crystalline nature of Ag/Fe nanoparticles, XRD analysis was carried out. The diffraction peaks for Ag/FeNPs with 2θ were at 26.92, 32.28, 49.68, 56.6 suggesting a good crystalline nature of synthesized *O.tenuiflorum* Ag/FeNPs. These peaks confirmed the hexagonal structure of green synthesized *O.tenuiflorum* Ag/FeNPs. Different peaks were observed including low, medium and high intensity peaks. Three intense peaks confirmed that Ag/Fe nanoparticles are crystalline in nature. The peaks at the range of 20-85° were at positions 26.9, 32.2, 49.6, 56.6, 62.8, 68.0 and 68.2 degrees as mentioned in the figure 4. The size of the particles was found to be 13 nm using Scherrer equation.

Biological Activities of Ag/Fe Bimetallic NPs: Anti-Glycation Activity

The anti-Glycation activity was expressed in % inhibition. It is basically the % of anti-AGEs formation which is expressed in % inhibition. Oxidative stress leads to formation of advanced glycation end products. To check the anti-Glycation activity of the bimetallic NPs, Vesperlysine-like AGEs and Pentosidine like AGEs tests were carried out. Fe NPs showed less Vesperlysine-like AGEs inhibition than AgNPs. Similarly, Iron also showed less Pentosidine-like AGEs inhibition than AgNPs. Overall, Ag/Fe bimetallic NPs were shown to be better as antiglycation agents.

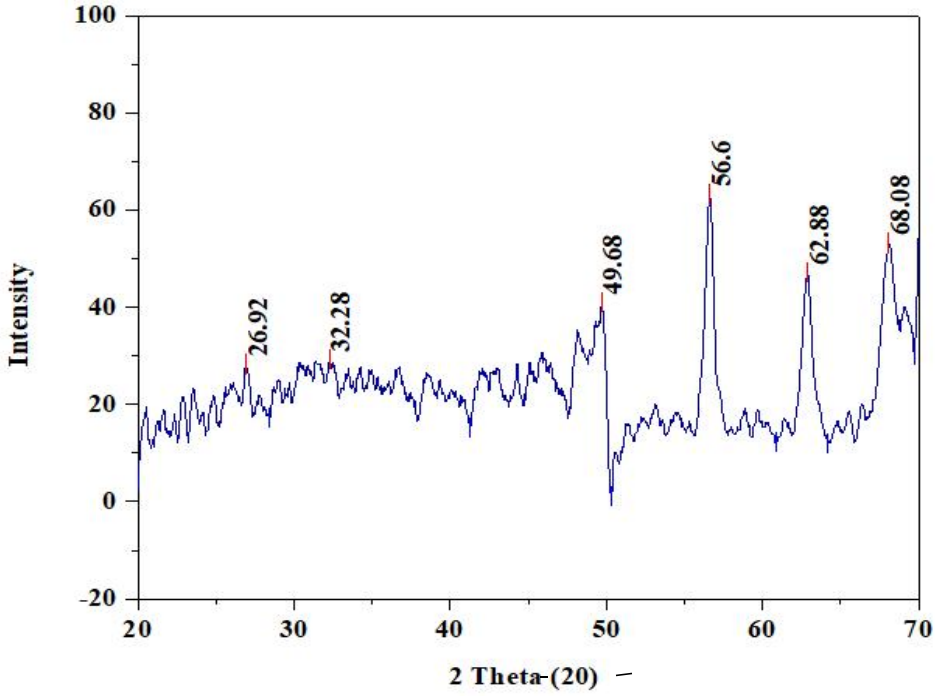

Figure 4: XRD pattern of Ag/Fe Bimetallic NPs

Table 1: Vesperlysine like AGES (% inhibition) of Ag/Fe Bimetallic NPs

Sample Type	Vesperlysine-like AGEs (% inhibition) Mean ± SD	Pentosidine-like AGEs (% inhibition) Mean ± SD
Ag/Fe Bimetallic NPs	41.5 ± 1.1	45.5 ± 1.3

Evaluation of Biocompatible of Ag/Fe Nanoparticles

Hemolytic assay was performed and observed their biocompatibility through Ag/Fe Bimetallic NPs with human RBCs. Hemolytic activity was done in three different concentrations and it reveals average activity potential of 2.40% + 0.4 which comes in the range of 2-5 % expressing that they are slightly hemolytic in nature. Similarly brine shrimp method has also provide information of effectivity of *Ocimum Tenuiflorum* mediated Ag/Fe bimetallic NPs which was measured by three different concentrations under LC50 in µg/ml, results shows that LC50 value of brine shrimp lethality assay is 14.32 µg/ml + 1.86 mobility of nubile which exhibit the range of 10-30 µg/ml, this range comes under category of less toxic for cells.

DISCUSSION

For the past few years, nanotechnology has shown massive steps to revolutionize the world by making fundamental improvements to the biomedical area. Like we have discussed above, nanotechnology has hard to summarize applications because of their diverse nature. They have attracted a lot of attention lately because of their uses in catalysis, electronics and much more (20). A wide number of metallic and bimetallic NPs have been manufactured but AgNPs and FeNPs hold a distinct position because they are useful for a very wide range of purposes. Their Antifungal, Antibacterial, Anticancer, Antidiabetic, Antiglycation, Antiviral and many more such activities have earned these two the prominence (). We have also discussed above that biological synthesis is a better option than chemical and physical methods for a number of reasons. This is a safe and cheap method of manufacture and makes use of several parts of the plants or trees and other biological entities.

Total phenolic and flavonoid content is determined in order to check if the plant extract contains enough capping agents to synthesize NPs. These secondary metabolites reduce the metal salts and stabilize the synthesis of NPs. Among secondary metabolites, phenolic compounds are the most important for this purpose as they are excellent redox agents (21,22). The total phenolic content of our plant extract was found to be 23.02 ± 0.08 mg GAE/g DW. Rababa et al (23) , also found similar values of TPC content (24.09 ± 0.09 mg GAE/g DW) in the medicinal plant *T. foenumgraecum*. The total flavonoid content of *ocimum tenuiflorum* plant extract was found to be 2.02 ± 0.04 mg QE/g DW. Similar results have been reported by Valentin with a TFC value of 3.02 ± 0.08 mg QE/g DW (24). Hence, TFC content of our plant extract is in accordance with other studies. TPC and TFC values of our plant extract shows that it has enough phytochemicals for the synthesis of NPs.

Several research studies have been conducted that reported synthesis of AgNPs and FeNPs through biological means (25,26). For AgNPs Leaf Extract of *T. Ciliata* was mixed with AgNO3 salt and this solution was prepared in 12 different ratios from 1:1 to 1:20 and this method was adopted from the research study reported by Alwan et al. (27) The ratios were observed for a period of 24-48 hours after

which 1:20 using diluted plant extract was selected because it was found to be the most stable one and took least time to synthesize too.

The FTIR results of our plant extract, Ag and AgFe NPs indicated the presence of a number of functional groups. These results mainly reveal the occurrence of carbonyl compounds, saturated and unsaturated hydrocarbons, aromatic compounds, amines, amides, flavonoids and polyphenolic compounds. These compounds are the capping agents that are responsible for synthesis of stable NPs. The absorbance peaks of both NPs were very similar, however the plant extract showed comparatively a greater number of peaks. The peaks of plant extract were also sharper and denser compared to NPs. This may suggest that plant extract originally had a greater number of compounds but in NPs, only the compounds used for capping were used up so there are fewer compounds in NPs. Similar results of the presence of flavonoids and phenolic compounds were reported by Mandal D et all, in their research report. Another report by Yedurkur et al (28); discussed the presence of C-H group in AgFeONPs. Other than that, presence of alkenes and alcoholic groups in FeNPs have also been reported. Sharp peaks determining the presence of methoxy,nitryl, carbonyl and alkanes in AgNPs have been reported by Gardea-Torresdey. Hence, our results are clearly in accordance with the previous findings. Another experiment was performed by scientists for the observation of peaks for silver and iron. Data was taken for the 2θ range of 30 to 80 degrees with a step of 0.0202 degree. The diffractogram has been compared with the standard powder diffraction card of JCPDS, silver file No. 04-0783. Four peaks at 2θ values of 38.2901, 44.5583, 64.8185, and 77.4383 degree in the experimental diffractogram have been identified to be due to silver metal, iron metal and corresponding to (hkl) values - (111), (200), (220) and (311) planes of silver. The XRD study has thus confirmed that the resultant particles in the prepared sample are silver iron nanoparticles having spherical structure. There are four more peaks in the diffractogram at 32.35, 46.38, 54.03 and 57.66 degrees. XRD for Fe and AgNPs was done and the particle size was calculated along with the determination of shape. The presence of intense peaks at (101), (115), (176), (200) ,(223), (244), (245) planes indicate the FCC structure of AgNPs according to JCPDS. The average particle size was found to be 120.03455 nm. The presence of intense peaks at (100), (150) and (220) spherical or truncated triangular planes indicate the highly crystalline nature and single phase of the synthesized AgFeNPs. The hexagonal crystal geometry according to JCPSD card no. 01-007-2551. The average particle size was found to be 110.6754 nm. Our results were in accordance with those reported by Ankamwar B et al (29). After characterization of the synthesized NPs, we moved towards analyzing their potentials for various biological activities, including antidiabetic and antiglycation. Today, diabetes has evolved as a disease that is showing a rapid and worldwide increase largely due to the unhealthy lifestyles adopted, ageing and increasing urbanization. In a period of last few decades, the disease has affected 368 million people. It is being speculated that by the time we reach 2035, we will have a total of 592 million people affected by diabetes. Two enzymatic assays were used and each one had promising results for both Fe and AgNPs. Ag showed 41.6% better inhibition relative to control for α-Glucosidase and 54.93% for α Amylase. Fe worked better than control but was not as effective as AgNPs. The results show that Fe and AgNPs are effective against the disease but AgNPs do better overall. Our results for both the NPs are in harmony with previous reports Antiglycation is another biological

activity that was carried out on two of the NPs. Glycation is a process that generates glycated products. In this study, to check the antiglycation potential, 2 assays were used; Vesperlysine and Pentosidine AGES like. The results show that out of the two, AgNPs showed a greater inhibition activity of 45.5% than FeNPs that inhibited Vesperlysine like AGES activity by 41.5%. Our results match to the previous studies. American Society of Cancer works on the collection of cancer statistics in the United States. They have estimated that in the year 2021, 1,898,160 new cases have been reported and more than 600,000 deaths are projected to occur. The increasing number of cases has made it mandatory for researchers to look for solutions. Ag and Fe made from *Ocimum Tenuiflorum* were tested for their antiglycation activity and biocompatibility activities.

CONCLUSION

In this study, Silver Iron bimetallic (Ag/Fe) NPs were successfully synthesized from the leaf extracts of *ocimum tenuiflorum* (tulsi) by using the green synthesis approach. The present study clearly shows that Ag/Fe NPs are potential replacement for other NPs that are manufactured through conventional ways. Ag/Fe NPs showed positive results for all the biological activities including Antiglycation and Biocompatibility. XRD showed that the Ag/Fe NPs had hexagonal nature and their size was 13 nm. FTIR analysis showed the involvement of phytochemicals like phenols, amine and carbonyls. Biocompatibility activity using hemolysis assay and brine shrimp larvae were also determined that represents *ocimum tenuiflorum* Ag/FeNPs were proved to be better anti-oxidant agents. Although, Ag/FeNPs seem to be a better option. The synthesized NPs are ecofriendly and nontoxic and these two properties together with the biological behaviors and characteristic properties of these two make them potential candidates for their use to fight off several diseases. Our study supports the idea that green synthesized NPs are a better replacement for conventional synthesis methods but we cannot ignore the associated risks.

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