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Biogenic Synthesis and Optimization of Metallic Silver and Gold Nanoparticles from *Musa pardisiaca* Flower Extract with Their Potential Antibacterial and Antioxidant Study



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ABSTRACT

Phytobiological synthesis of metallic nanoparticles is gaining lot of importance due to its more reliable and economic route over other conventional methods. Both physical and chemical methods are not feasible and eco-friendly due to the use of expensive and hazardous chemicals. To overcome the drawback of these methods, biogenic synthesis of metallic nanoparticles came into existence which is one-step synthesis, inexpensive and eco-friendly method. This research article focuses on *Musa pardisiaca* flower extract mediated synthesis of metallic silver and gold nanoparticles and its characterization. The aqueous extract of act as both *M. pardisiaca* reducing as well as a stabilizing agent for the synthesis of metallic silver nanoparticles and gold nanoparticles. Synthesized metallic silver and gold nanoparticles was then further characterized by UV-Vis spectroscopy, Particle size analyser, Zeta sizer, FESEM (Field Emissions Scanning Electron Microscopy), TEM (Transmission Electronic Microscopy) for their Surface Plasmon Resonance (SPR), size, stability and morphology respectively and further evaluated for antimicrobial and antioxidant activity.



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INTRODUCTION

Nanotechnology is a branch of technology which deals with the synthesis, characterization and application of materials in the nanoscale range of 1 – 100 nm¹. The term metallic nanoparticles are used to describe nanosized structures in which at least one of its phases has one or more dimensions (length, width or thickness) in nanometre size range. Metallic nanoparticles exhibit large surface area to volume ratio and their modified remarkable properties compared to the bulk counterparts²⁻³. Synthesis of metallic nanoparticles exhibiting biological activities is one of the prime features of nanotechnology⁴. Conventional methods including physical and chemical synthesis of metallic nanoparticles has several disadvantages including use of hazardous chemicals as reducing and/or capping substances, low material conversion, high energy consumption, difficulty in purification and unsafe chemicals⁵⁻⁷. Hepatotoxicity or renal toxicity has also been reported for metallic nanoparticles when administered through oral, inhalation or subcutaneous route synthesized from chemical approach due to the use of hazardous and unsafe chemicals⁸⁻⁹. Due to several disadvantages associated with chemical method, there is a need for an approach to design eco-friendly, inexpensive and biocompatible route for synthesis of metallic nanoparticles in order to meet its growing demand in diverse sectors¹⁰. Green synthesis approach is considered to be one of the most feasible and superior process in comparison to other conventional methods¹¹. Plant has the ability to synthesize metallic nanoparticles which can be attributed to phyto mediated reduction of metals which depends on diverse nature of chemical constituents which enables the conversion of metal ions to metallic nanoparticles (12). Plant extract act as both reducing as well as stabilizing agent for metallic nanoparticles. In this research article, we are going to focus on *M. paradisiaca* flower extract mediated synthesis of metallic silver and gold nanoparticles and its potential antibacterial and antioxidant application in nanomedicine¹³. *M. paradisiaca* is the largest herbaceous flowering plant belonging to the family Musaceae commonly called as ‘Banana’^{14,15}. *M. paradisiaca* consists of several bioactive molecules including alkaloids, flavonoids, tannins, and phenolic compounds. Phenolic compounds and other easily oxidizable phytochemicals can reduce metallic ions to metallic nanoparticles¹⁶.

MATERIALS AND METHODS

Banana flowers procured from vendor of local market (Mumbai) were authenticated for its confirmation with species (*M. paradisiaca* belonging to family Musaceae) at St. Xavier College (CSMT, Mumbai). Silver nitrate, Chloroauric acid (25%), Ascorbic acid and 2,2-

diphenylpicrylhydrazyl (DPPH) was procured from Vishal Chem, Mumbai. Nutrient agar was procured from HIMEDIA, Mumbai.

Synthesis of metallic silver and gold nanoparticles from *M. paradisiaca* flower extract

The synthesis of metallic silver and gold nanoparticles from *Musa paradisiaca* flower extract has been reported for their ability to reduce metal ions to metallic nanoparticles.

Preparation of *M. paradisiaca* flower extract

M. paradisiaca flowers were thoroughly washed with distilled water to remove surface impurities. Banana flowers were crushed and a defined amount of distilled water was added and kept for heating on magnetic stirrer (REMI MS-500) which was maintained at 80°C for 30 minutes. The extract obtained was filtered through muslin cloth and then subjected to centrifugation (REMI C24B41) which was carried out at 10000 rpm for 15 mins for the debris to settle. The supernatant obtained was then filtered through Whatman no. 1 filter paper to eliminate impurities. The supernatant was stored in refrigerator at 4°C for further synthesis of metallic silver and gold nanoparticles. This extract was used as reducing as well as stabilizing agent¹⁷⁻¹⁹.

Preparation of metal salt solution

Preparation of silver nitrate solution (1mM)

1mM solution of silver nitrate was prepared by dissolving 0.017 gm of silver nitrate in 100 ml distilled water.

Preparation of chloroauric acid solution (1mM)

1mM solution of chloroauric acid was prepared by dissolving 0.034 gm of 25% chloroauric acid in 100 ml distilled water.

Synthesis of metallic silver and gold nanoparticles from *M. paradisiaca* flower extract

30 ml of *M. paradisiaca* flower extract was added to 50 ml of 1 mM of metal salt solution at room temperature. The reaction mixture was allowed to incubate at room temperature for 24 to 48 hrs. in the dark place to avoid the photoactivation of metal salts under static conditions. The completion of process for silver nanoparticles was indicated by colour change from clear white to yellowish brown whereas for gold nanoparticles from clear white to purple colour which was due to the reduction of M^+ ions into M^0 indicating the formation of metallic nanoparticles. The synthesized metallic nanoparticles were separated by centrifugation

(REMI, C24B41) at 12000 rpm for 30 minutes. The metallic nanoparticles were then dispersed in distilled water and used for further characterization and evaluation²⁰⁻²³. The effect of reaction time was studied by incubating the reaction mixtures with optimum concentration at 24, 48, 72 and 96 hrs. The effect of higher temperature on synthesis of metallic nanoparticles was studied by incubating the reaction mixtures at 40, 60, 80 and 100°C. The effect of pH on synthesis of metallic nanoparticles was carried out by using pH meter (TOSCHON INDUSTRIES PVT. LTD, EQ-601) and studied by adjusting the pH of the reaction mixtures with 0.1 N NaOH and 0.1 N HCl. The effect of varying concentration of silver nitrate and chloroauric acid (1, 1.5, 2mM) on synthesis of silver nanoparticles and gold nanoparticles was studied respectively. The effect of volume of extract added (1, 2, 3, 4 ml) to the reaction mixtures on the synthesis of metallic nanoparticles was studied²⁴.

Characterization of metallic silver nanoparticles

UV-Visible spectroscopy

The absorption spectrum of metallic nanoparticles was scanned in between 200 to 800 nm using a UV-Vis spectrophotometer (SHIMADZU, UV-1800)²⁴.

Field Emission Scanning Electron Microscopy (FEGSEM) and Transmission Electron Microscopy

The shape and size of metallic nanoparticles were determined by FEGSEM. For FEGSEM, a drop of aqueous metallic nanoparticles dispersion sample was loaded on a copper grid and allowed to dry in room temperature. After complete drying, the micrographs were recorded using FEGSEM (JEOL JSM-7600). In similar fashion, the TEM analysis was carried out. For a selected area, the electron diffraction pattern was also recorded through TEM (JEOL JSM)²⁴.

Particle Size Analysis (PSA)

The Z-average size and Polydispersity Index (PDI) of metallic nanoparticles was determined by Dynamic Light Scattering (DLS) technique (Horiba, Malvern Nano sizer)¹⁷.

Zeta potential

Zeta potential analysis is carried out to measure the stability of metallic nanoparticles and explains the agglomeration phenomenon of nanoparticles (Horiba, Malvern Nano sizer)¹⁷.

Antibacterial activity of synthesized metallic nanoparticles

Agar well diffusion assay was carried out to study the bactericidal activity of plant mediated synthesis of metallic nanoparticles against the panel of selected pathogens²⁵. The tested microorganisms were uniformly swabbed on nutrient agar medium using sterile inoculum loop. Four wells of 6mm diameter were made using sterile well borer. 100 microliter of metallic nanoparticles solution with varying concentrations (25, 50, 75 and 100 µg/ml) was poured into the corresponding well. Banana flower extract was used as a control sample to access the antibacterial activity. The plates were then incubated at 37°C for 24 h. The bactericidal activity of metallic nanoparticles was evaluated in terms of inhibition zone²⁴.

Antioxidant activity of synthesized metallic nanoparticles

The antioxidant activity of nanoparticles was characterized using DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) assay. A stock solution of DPPH was prepared in methanol. 0.5 ml of metallic nanoparticles solution of 20, 40, 60, 80 and 100 µg/ml was added to 3 ml of ethanol and 0.3 ml of DPPH radical solution. The mixture was vigorously shaken and allowed to stand at room temperature for 30 min. After incubation of sample for 30 mins, the sample colour changes from deep violet to light yellow which indicates that the sample gets reduced. Then the absorbance was measured at 517 nm by using a UV-Visible spectrophotometer (SHIMADZU UV-1800). Antioxidant activity was measured by calculating the % inhibition by following formula (a)^{26,27}:

$$\text{Percentage inhibition} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

RESULTS AND DISCUSSIONS

Visual observation and UV – Visible Spectroscopy

The formation of metallic nanoparticles was monitored with colour change and UV-Visible spectroscopy. The colour of the reaction mixture started changing from pale white to reddish brown as shown in figure no: 1a and pale white to purple colour as shown in figure no: 1b which indicates the formation of silver and gold nanoparticles respectively. This was due to the reduction of metal ions into metallic nanoparticles via the active chemical constituents present in the banana flower extract²⁸. The excitation of SPR is attributed by colour of the reaction mixture. The unique optical properties exhibited by the noble metals is due to the property of Surface Plasmon Resonance (SPR)²⁹. As shown in figure no: 2 and 3, a well-defined and characteristic SPR band for metallic silver nanoparticles is obtained in the range

of 400 – 500 nm whereas for gold nanoparticles SPR band is obtained in the range of 500 – 600 nm. Silver nitrate and chloroauric acid solution used as a control neither developed reddish brown colour and purple colour respectively nor did they display the characteristic SPR band which indicates that the metal salts did not reduced under the used conditions²⁴.

Effect of metal salts concentration

Pale yellow to reddish-brown and purple colour were observed at varying concentration of silver nitrate and chloroauric acid (1, 1.5 and 2mM) respectively. With increasing the concentration of metal salts, the SPR peak of metallic nanoparticles become distinct. The maximum peak intensity was obtained at concentration of 2mM as shown in figure no: 2a and 3a. A variation in the metal salt concentration and biological material is known to have influence on synthesis of metallic nanoparticles³⁰.

Effect of *M. paradisiaca* flower extract

The SPR peaks was found to be proportional as the volume of extract (ml) increased as shown in figure no: 2b and 3b. As the concentration of the biological material involved in metallic nanoparticle synthesis increases, higher contents of the chemical constituents of the biological material are involved in metal reductive nanoparticle synthesis²⁴.

Effect of pH

The pH of aqueous silver nanoparticles dispersion was carried out using pH meter. pH has an influence on metallic nanoparticles synthesis as the colour of the reaction mixture and the intensity of the SPR peaks were proportionally more intense. No colour changes and a white precipitate was observed at pH value 1 – 3. Varying shades of reddish-brown and purple colour was observed at pH values of 5 – 9 for silver and gold nanoparticles respectively as shown in figure no: 2c and 3c³¹. When the reaction mixture was conducted at acidic pH (1 – 3), neither colour change nor the characteristic SPR peaks were observed. A variety of biomolecules present in biological material are known to be involved in biological nanoparticles synthesis, such biomolecules are likely to be inactivated under extremely acidic conditions. The variation in the colour intensity over the range of pH could be postulated to a variation in the dissociation constants (pka) of the functional groups on the biomass that are involved³⁰.

Effect of temperature

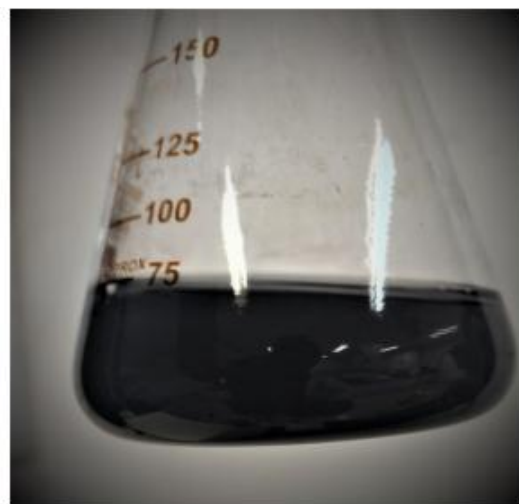
The temperature also has an influence on biological metallic nanoparticles synthesis. The reaction mixtures incubated at temperature 25°C and 40°C showed pale yellowish and pale purplish colour for silver and gold nanoparticles respectively and the intensity of the SPR peaks was not so pronounced. At higher reaction temperatures (60, 80 and 100°C) dark reddish-brown and purple colour was observed for silver and gold nanoparticles respectively and the intensity of the SPR peaks was found to be more intense and pronounced. The colour change took 1 hr to develop at room temperature whereas at higher reaction temperatures the reduction process was found to be faster and colour was developed within 30 minutes. The highest intensity of the SPR peak was found to be at 100°C as shown in figure no: 2d and 3d. As the reaction temperature increases, UV spectra shows sharp and narrow peaks at lower wavelength range which indicates the formation of smaller size metallic nanoparticles whereas at lower temperature, UV spectra shows SPR peak at higher wavelength region which indicates the formation of larger size metallic nanoparticles. It is clear that the reactants are consumed rapidly as the temperature of the reaction mixture increases leading to the formation of smaller size metallic nanoparticles³². Thus, it can be stated that the size of the metallic nanoparticles synthesis was decreased with an increase in temperature of the reaction mixtures.

Effect of incubation time

As the incubation time of the reaction mixture increases, the intensity of colour also goes on increasing which means that the reduction process was slow initially and gradually increases as the incubation time of the reaction mixture increases. The peaks were found to be more intense and pronounced after longer incubation time up to 96 hrs as shown in figure no: 2e and 3e. The maximum reduction of metal ions was obtained after 96 hrs. The increase in absorbance along with colour intensity could be postulated to an increase in the number of metallic nanoparticles with incubation time³³. The rapid generation of the metallic nanoparticles was owing to the excellent reducing potential of the active components of *M. paradisiaca* flower extract and their polymeric stabilization within a narrow size spectrum²⁴.

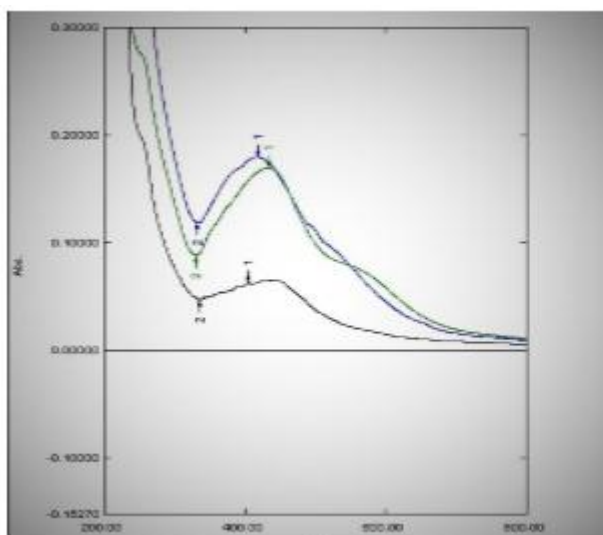


(a)

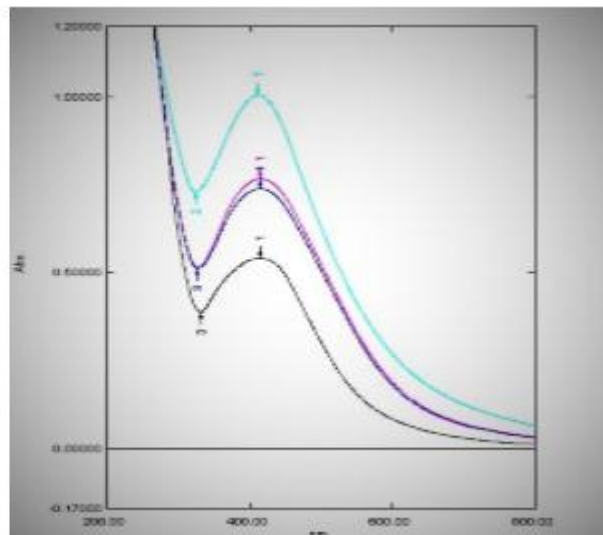


(b)

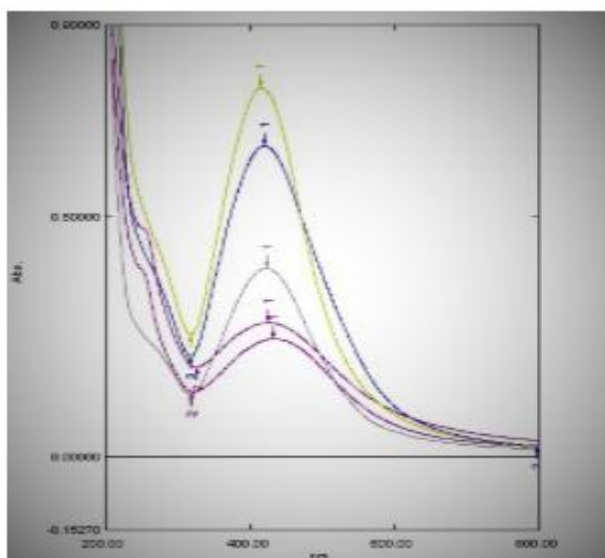
Figure No. 1: Colour change (a) reddish brown colour indicating the formation of silver nanoparticles (b) purple colour indicating the formation of gold nanoparticles



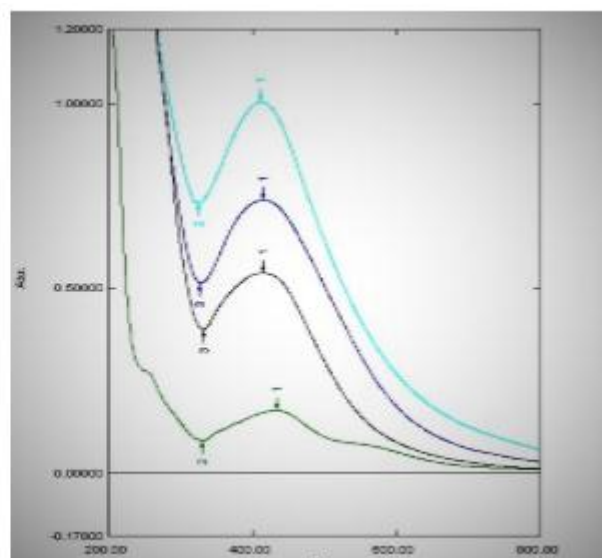
(a)



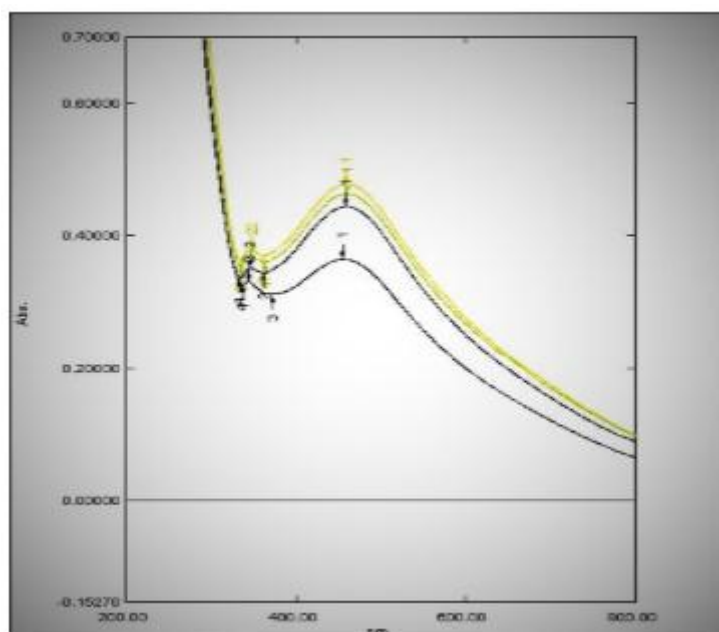
(b)



(c)

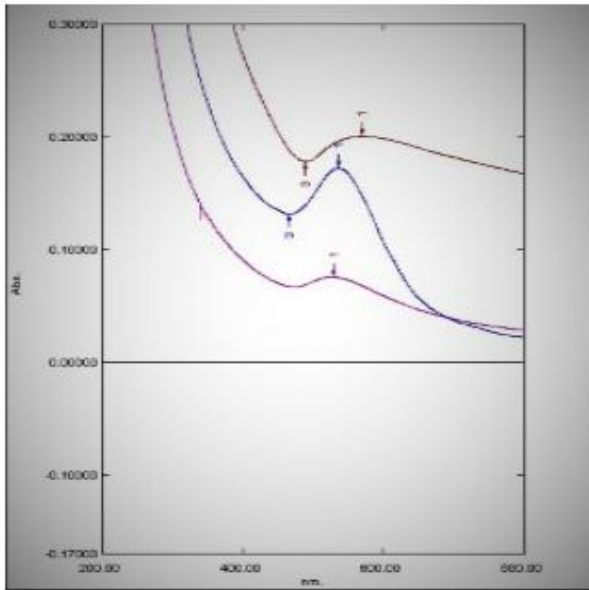


(d)

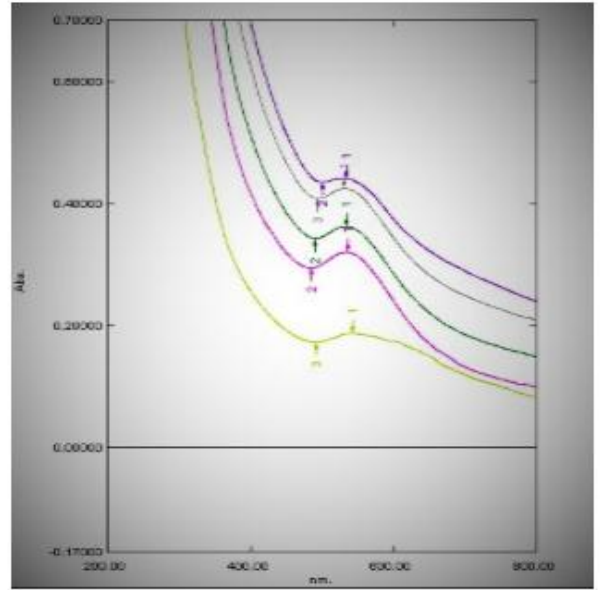


(e)

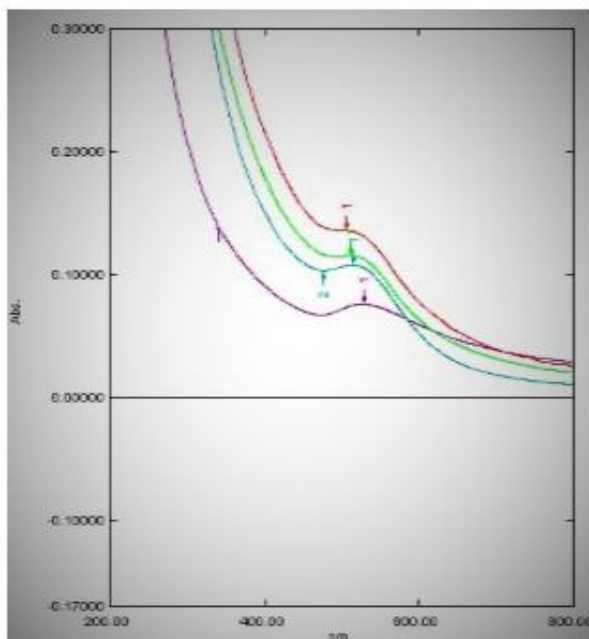
Figure No. 2: UV-Visible spectra of biosynthesized silver nanoparticles, (a) at different concentration of AgNO_3 (b) at varying volume of *M. paradisiaca* flower extract (c) at different pH conditions (d) at different temperature conditions (e) at different incubation time.



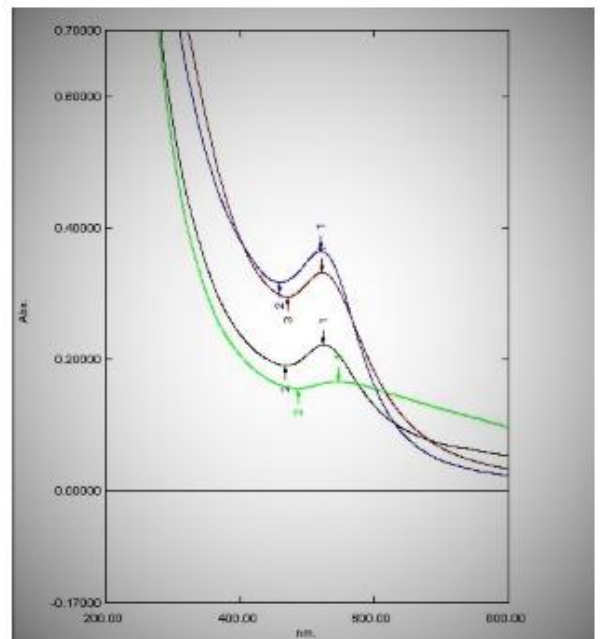
(a)



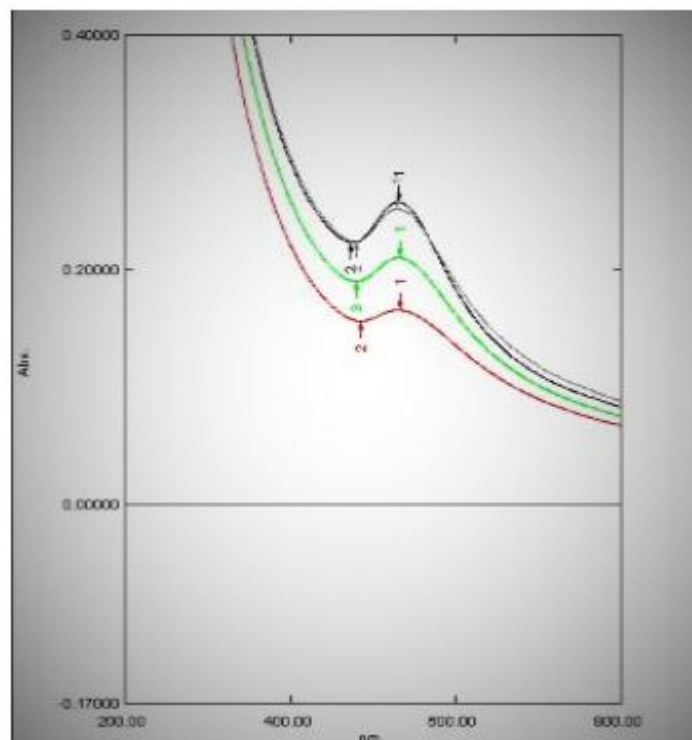
(b)



(c)



(d)



(e)

Figure No. 3: UV-Visible spectra of biosynthesized gold nanoparticles, (a) at different concentration of HAuCl_4 (b) at varying volume of *M. paradisiaca* flower extract (c) at different pH conditions (d) at different temperature conditions (e) at different incubation time.

Characterization of Silver nanoparticles

The shape of the synthesized metallic nanoparticles was analysed by the FEGSEM. The image obtained by the FEGSEM showed monodispersed spherical metallic nanoparticles as shown in figure no: 4 and 6. The TEM images revealed that the synthesized metallic nanoparticles are monodispersed with the spherical shape as shown in figure no: 5 and 7. The synthesized metallic nanoparticles are crystalline in nature. The Z-average, polydispersity index (PDI), zeta potential of synthesized metallic nanoparticles analysed by DLS method is given in table no: 1 and as shown in figure no: 8 and 9.

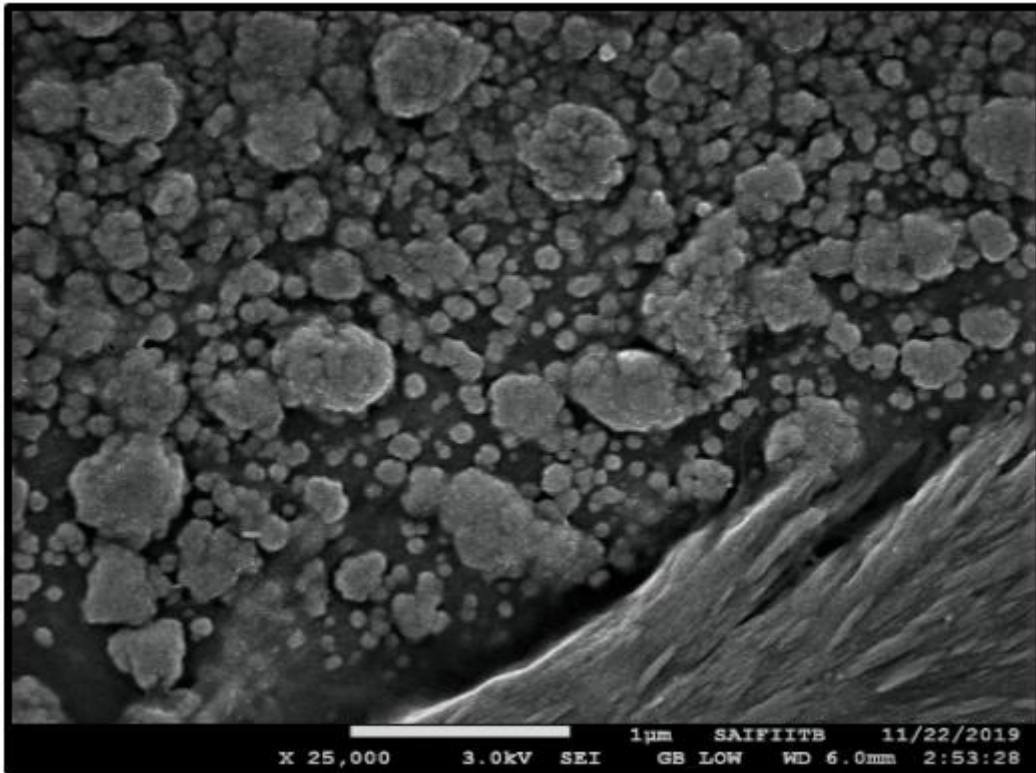


Figure No. 4: FEGSEM images of biosynthesized silver nanoparticles

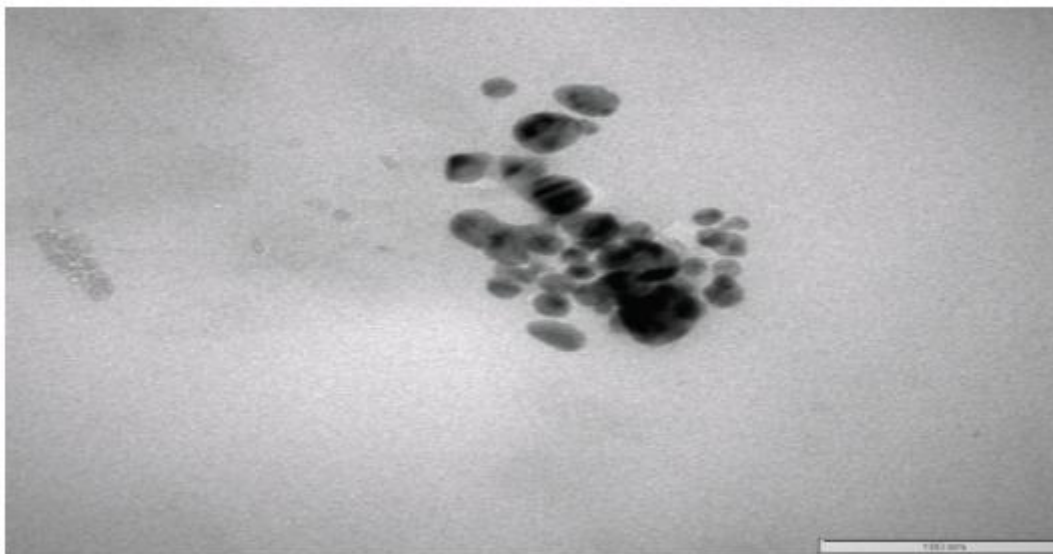


Figure No. 5: TEM images of biosynthesized silver nanoparticles

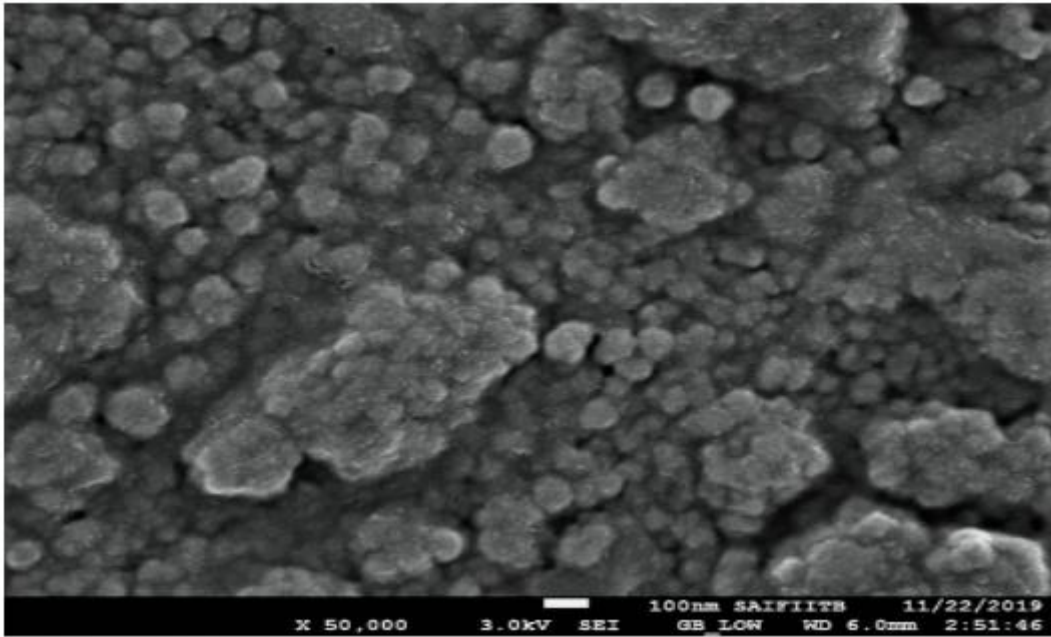


Figure No. 6: FEGSEM images of biosynthesized gold nanoparticles

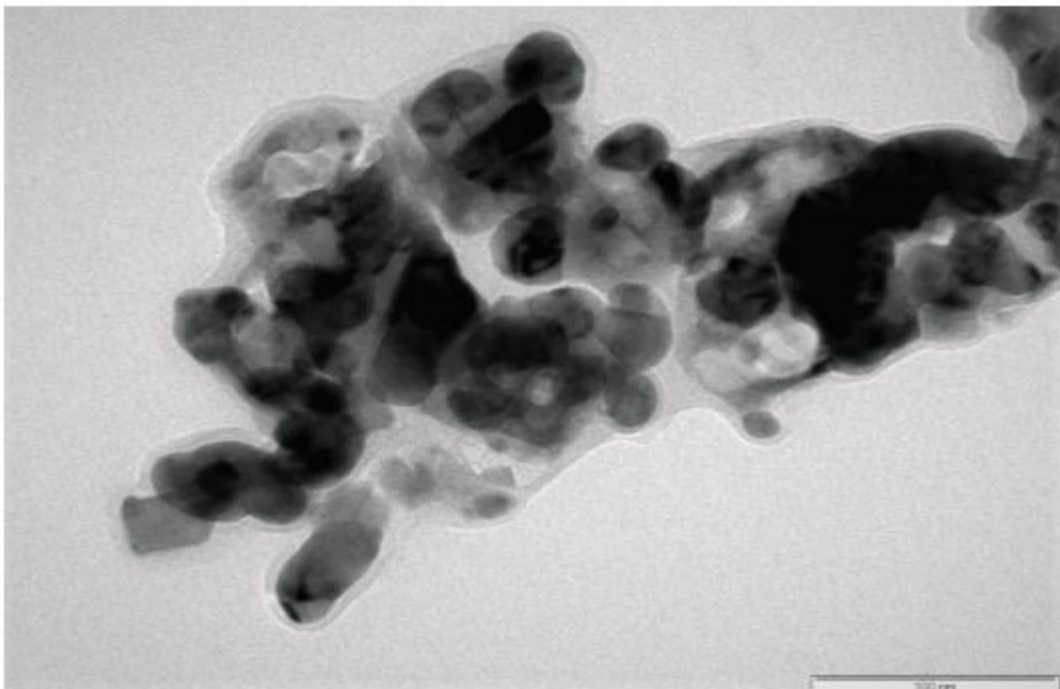
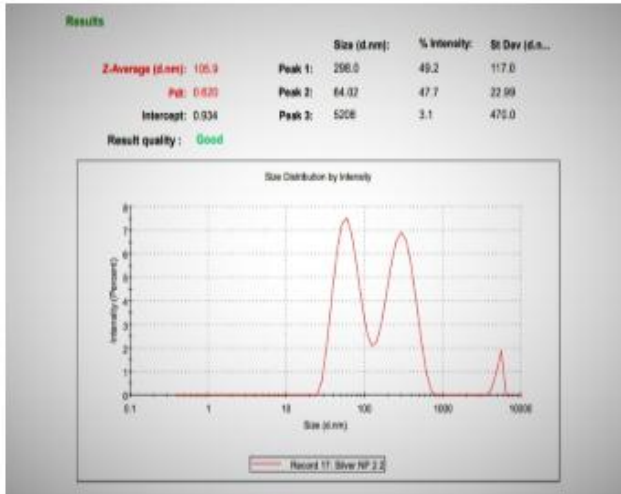


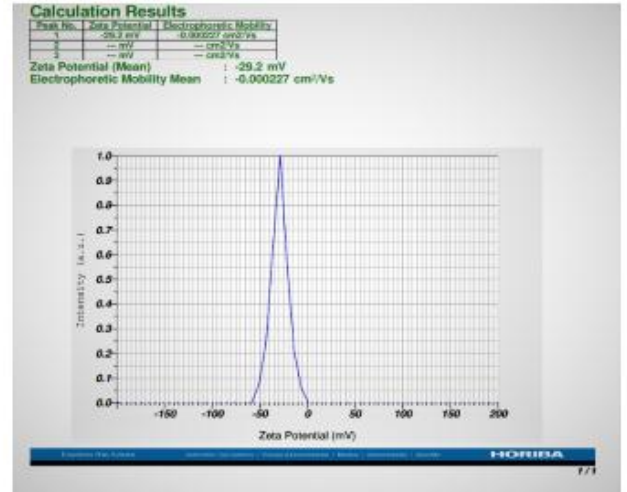
Figure No. 7: TEM images of biosynthesized gold nanoparticles

Table No. 1: Z- average, PDI and zeta potential of biosynthesized silver and gold nanoparticles from *M. paradisiaca* flower extract

Metallic nanoparticles	Z-average (nm)	PDI	Zeta potential (mV)
Silver nanoparticles	105.9	0.620	-29.2
Gold nanoparticles	85.3	0.796	-26.1

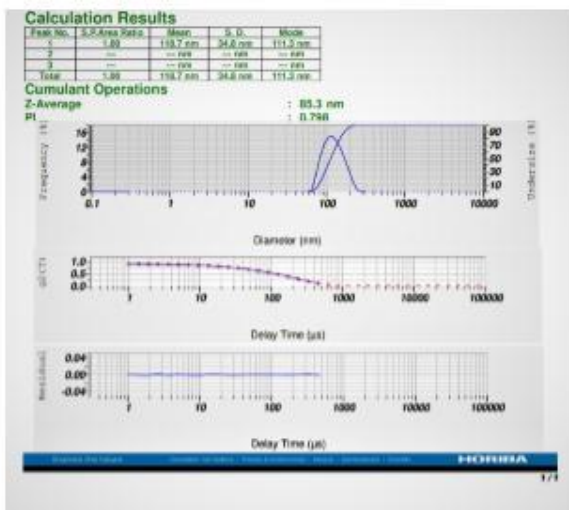


(a)

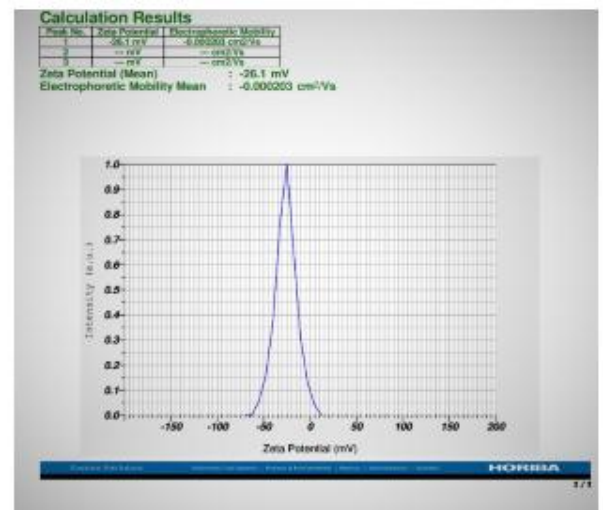


(b)

Figure No. 8: (a) Particle size of biosynthesized silver nanoparticles (b) Zeta potential of biosynthesized silver nanoparticles



(a)



(b)

Figure No. 9: (a) Particle size of biosynthesized gold nanoparticles (b) Zeta potential of biosynthesized gold nanoparticles

Antibacterial activity of metallic nanoparticles

The antibacterial activity of the metallic nanoparticles was accessed by the agar well diffusion method. Metallic nanoparticles showed antibacterial activity against pathogenic microorganisms. Metallic nanoparticles displayed varying degrees of antibacterial activity against pathogenic microorganisms which was accessed by the diameter of zone of inhibition while *M. paradisiaca* flower extract didn't showed any antibacterial activity.

Several mechanisms have been postulated the bactericidal properties of metallic nanoparticles against pathogenic microorganisms. First, metallic nanoparticles have the tendency to attach to the negatively charged cell surface by altering the physical and chemical properties of the cell wall and the cell membrane and disrupting the main functions of the cell such as electron transport and respiration, osmoregulation and permeability³⁴⁻³⁷. Second, metallic nanoparticles have the tendency to interact with DNA, proteins and other phosphorus and sulphur containing cell components by permeating the cell and thereby causing further damage to bacterial cells^{38,34}. Third, metallic nanoparticles generate an amplified bactericidal effect by releasing metal ions which is size and dose-dependent^{39,34}.



Figure No. 10: Antibacterial activity of biosynthesized silver nanoparticles against *E. coli*, *S. aureus*, *P. aeruginosa* and *B. subtilis*



Figure No. 11: Antibacterial activity of biosynthesized gold nanoparticles against *E. coli*, *S. aureus*, *P. aeruginosa* and *B. subtilis*

The bactericidal activity with varying concentration of silver and gold nanoparticles (50, 100 & 150 $\mu\text{g/ml}$) was accessed in terms of diameter of inhibition zone against the pathogenic microorganisms as shown in figure no: 10 and 11. The diameter of inhibition zone (in mm) displayed by silver and gold nanoparticles against pathogenic microorganisms is given in the below table no: 2 and table no: 3 respectively.

Table No. 2: Antibacterial activity of biosynthesized silver nanoparticles against representative pathogenic microorganisms and its evaluation in terms of the diameter of zone of inhibition (in mm).

For Silver nanoparticles	Diameter of zone of inhibition (in mm)			
	<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>
Microorganisms used				
Concentration ($\mu\text{g/ml}$)				
50	18	23	22	15
100	21	24	23	17
150	22	26	24	21

Table No. 3: Antibacterial activity of biosynthesized gold nanoparticles against representative pathogenic microorganisms and its evaluation in terms of the diameter of zone of inhibition (in mm).

For Gold nanoparticles	Diameter of zone of inhibition (in mm)			
Microorganisms used	<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>
Concentration (µg/ml)				
50	20	22	20	18
100	23	24	22	20
150	25	27	23	22

Antioxidant activity of biosynthesized metallic nanoparticles:

The metallic nanoparticles possess free radical scavenging activity against the DPPH as it is indicated by the colour change from purple to yellow. The antioxidant activity of metal nanoparticles (test) was compared with the antioxidant activity of ascorbic acid (standard). It was found that the antioxidant activity of biosynthesized metallic nanoparticles increased as the concentration of metallic nanoparticles increases from 20µg/ml to 100µg/ml. The comparative study has been illustrated in table no: 4 and 5 and figure no: 12 and 13.

Table No. 4: Antioxidant activity (in % inhibition) shown by biosynthesized silver nanoparticles as a test in comparison to ascorbic acid as a standard

Sr. No.	Concentration (µg/ml)	Antioxidant activity of biosynthesized silver nanoparticles (in %)	Antioxidant activity of Ascorbic acid (in %)
01	20	68.15	71.42
02	40	79.02	83.09
03	60	86.28	88.03
04	80	90.71	91.07
05	100	92.08	94.06

Table No. 5: Antioxidant activity (in % inhibition) shown by biosynthesized gold nanoparticles as a test in comparison to ascorbic acid as a standard

Sr. No.	Concentration (µg/ml)	Antioxidant activity of biosynthesized gold nanoparticles (in %)	Antioxidant activity of Ascorbic acid (in %)
01	20	57.23	71.42
02	40	64.27	83.09
03	60	69.21	88.03
04	80	73.27	91.07
05	100	80.78	94.06

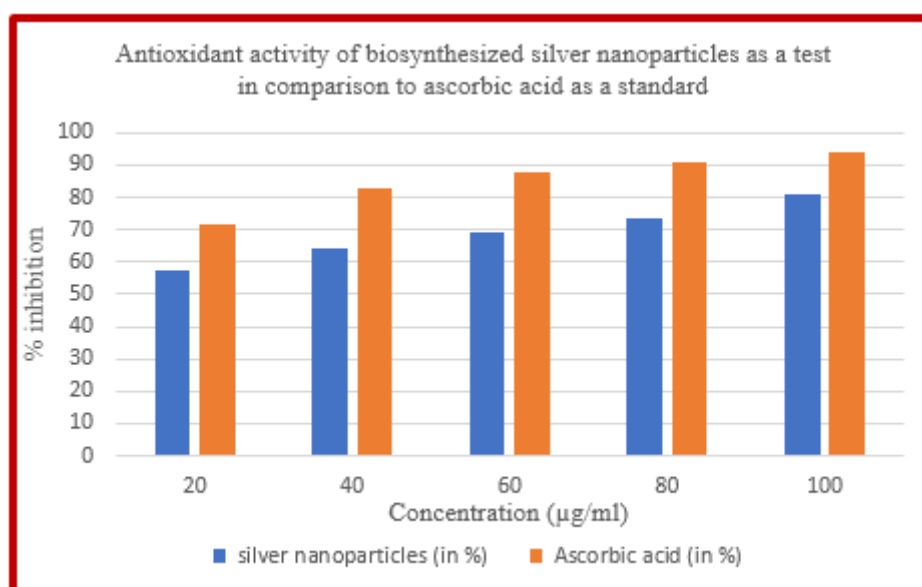


Figure No. 12: Antioxidant activity of biosynthesized silver nanoparticles as a test in comparison to ascorbic acid as a standard

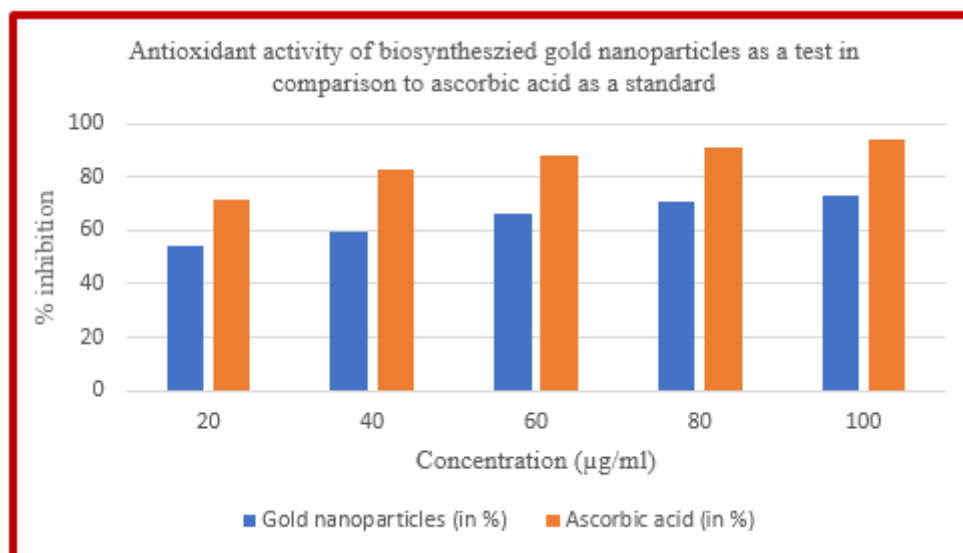


Figure No. 13: Antioxidant activity of biosynthesized gold nanoparticles as a test in comparison to ascorbic acid as a standard

CONCLUSION

M. paradisiaca flowers are successful used for the rapid and consistent synthesis of metallic nanoparticles. The biosynthesized metallic nanoparticles using *M. paradisiaca* flower extract were then characterized. The Z-average size and zeta potential of biosynthesized silver nanoparticles was found to be 105.09 nm and -29.2 mV respectively whereas for gold nanoparticles it was found to be 85.3 nm and -26.1 mv respectively. The biosynthesized metallic nanoparticles were found to be spherical, uniform, crystalline and monodispersed. Antimicrobial study revealed that the biosynthesized metallic nanoparticles showed good antimicrobial activity against the selected representative microorganisms. DPPH study showed that the biosynthesized metallic silver and gold nanoparticles possessed good antioxidant activity against the DPPH and its activity was compared with the standard ascorbic acid. Thus, green synthesis approach is considered to be one of the most feasible and superior process in comparison to other conventional methods due to its non-toxic, eco-friendly and cost-effective process and this biological process could be used for large scale production.

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