Original Research Article

Preparation, Characterization and Screening of Gold Nanoparticles Using Phenolic Rich Fractions of *Amaranthus gangeticus* L. for its *in-vitro* Anti-oxidant,Anti-Diabetic and Anti-Cancer Activities.

Abstract

Aim: In this present study, Gold Nanoparticles are prepared by biosynthesis method using Phenolic Rich Fractions of ethanolic leaf extract of *Amaranthus gangeticus* L. which was characterized and evaluated for their *In-vitro* antioxidative, antidiabetic, and anti-cancer potential against HeLa cells.

Materials and methods: Plant mediated AuNPs((gold nanoparticles) were synthesized using phenolic rich fractions of ethanolic extract of *Amaranthus gangeticus* L. and characterization was done by UV–visible spectroscopy, X-ray diffraction (XRD), Fourier Transform infrared (FT-IR) spectroscopy, Scanning Electronic Microscopy (SEM) and TEM analysisand Selected Area Electron Diffraction SAED pattern. The synthesized AuNPs were assessed for its pharmacological studies.

Results: Initially the formation of AuNPs has been identified by its change of colour followed by UV–visible spectroscopy (at 550 nm) due to surface Plasmon resonance. Using XRD pattern the crystalline property of AuNPs were confirmed. The functional group existing in Phenolic Rich Fractions of *Amaranthus gangeticus* L. responsible for the reduction of gold ion and the stabilization of AuNPs was investigated through FT-IR. The spherical shape of AuNPs were studied by SEM and TEM analysis. From the results, it is confirmed that the synthesized AuNPs having good antidiabetic potential at a very low concentration as well as potent anti-cancer activity against the HeLa cancer cells in a dose-dependent manner. It also observed with potent antioxidant activity.

Conclusion: From the present study, the synthesized AuNPs using *Amaranthus gangeticus* L. were found to be very potent to treat the major diseases like cancer and diabetes.

Keywords: Gold Nanoparticles, *Amaranthus gangeticus*, anti-oxidant, anti-cancer, anti-diabetic activity.

1. Introduction

Recent years have witnessed unprecedented growth of research and applications in the area of Nano science and nanotechnology. There is increasing optimism that nanotechnology, as applied to medicine, will bring significant advances in the diagnosis and treatment of disease.[1] In ancient Indian medical system (ayurveda), gold is used as medicine in the preparation of novel level *swarna bhasma* to treat tuberculosis, anemia and cough and also believed to prevent ageing.[2]Recently, the studies are focused towards the preparation and characterisation of nano particles. During the past several years, production of metallic nanoparticles using low-cost biological resources such as plants, algae, fungai and bacteria are reported.[3]The biosynthesis of metal nanoparticles (NPs) using medicinal plants has received considerable attention as a proper alternative to using hazardous chemical and physical synthetic techniques.[4] Plants are being exploited for their unique metal tolerance and effective production of gold metal NPs. A single medicinal plant contains an orchestra of chemical elements that phytocompounds such as terpenoids, polysaccharides, polyols and flavones take part in the bio-reduction, stabilization and bio-capping mechanisms to form stable gold and silver NPs.[5]Despite recent improvements made in the treatment of cancer and diabetes, there is a growing interest in herbal drugs and their formulations due to the undesired side effects associated with chemotherapy and oral hypoglycaemic agent.[6]Natural compounds are now being screened for treating several major diseases, including cancer, diabetes, cardiovascular, inflammatory, and microbial diseases. This is mainly because natural drugs possess unique advantages, such as lower toxicity and side effects, low-price, and good therapeutic potential.[7]However, the green nanoparticles offer an alternative route utilizing the natural ingredients present in plant extracts. Some of potential plant extracts used for green nanosynthesis are *Coriandrum sativum, Bischofia javanica* L., *Daucusc arota,Solanum lycopersicums*, *Hibiscus*, *Cannabinus* leaf, lemongrass, *Moringa oliefera* flower, *Bacopa monnieri*, *Citrus unshiu* peel, lemongrass *Cymbopogonflexuosus, Aloe Vera*, *Anana scomosus*.[8,9]*Amaranthus tricolor* Linn. commonly known as *Lal Chaulai* or Joseph's coat is an important medicinal plant belonging to the family Amaranthaceae.It is a promising food crop mainly due to its resistance to heat, drought, diseases and pests, and the high nutritional value of both seeds and leaves. Leaves are rich in proteins and micronutrients such as iron, calcium, zinc, vitamin C and vitamin A. This plant reported in ayurveda as astringent in menorrhagia, leucorrhoea, dysentery, diarrhoea, haemorrhagic colitis; also used in cough, bronchitis and externally used as emollient. It has been used for the treatment of piles, blood disorders, bladder distress, tooth ache, dysentery and as astringent, diuretic, haemorrhage and hepatoprotective action.[10] Considering these aspects, the present study focuses on the potential anticancer, anti-diabetic and antioxidant activity of Phyto-synthesized gold NPs.

2. Material and Methods

2.1 Collection of plant, extract preparation and phytochemical screening:

The leaves of plant of *Amaranthus gangeticus* were collected from Tirunelveli district, Tamilnadu Which was identified and authenticated by V. Chelladurai, Research officer – Botany, (Retired) Central council for research in Ayurveda & Siddha. The powdered leaf material was extracted by successive solvent extraction using different solvents like Petroleum Ether, chloroform, ethanol & water. The phytochemical screening was carried out by standard protocols.[11,12]

2.2 Total Phenolic Content Determination:

The Total Phenolic Contents of the various extracts of *Amaranthus gangeticus* determined using the Folin Ciocalteau reagent as mentioned by Singleton and Rossi.[13]

2.3 Preparation of Phenolic Rich Fraction from Ethanolic Extract of *Amaranthus gangeticus*:

The ethanolic extract (179g) was suspended in 525mL water and subsequently fractionated with petroleum ether (Boiling range 30-60^{0}C) and ethyl acetate. The ethyl acetate extract was evaporated and dried in vacuum to yield a yellowish green extract, which was named the phenolic compounds – rich fraction from *Amaranthus gangeticus*. [14] and total phenolic content has been determined using the Folin Ciocalteau reagent.

2.4 Preparation and Characterization of AuNPs:

90ml of aqueous solution of 1 mM chloroauric acid (HAuCl4) was treated with 10ml of phenolic rich fractions of *Amaranthus gangeticus* which was vigorously stirred and kept at room temperature. Reduction takes place rapidly and is completed in 5min which is confirmed by its colour change from pale-yellow colour to ruby red indicating the formation of gold nanoparticle. [15] further the solution was centrifuged at 10000rpm for 15min. The separated nanoparticles settled at the bottom were collected and washed with water, then dried using oven at 55^{0}C for two hours. The stabilized powder forms of the nanoparticles were stored for further studies.[16]

The formation of gold nanoparticles using phenolic rich fraction of plant extract is monitored by various analytical techniques like UV–Visible Spectroscopy UV–Vis(Shimadzu UV-2700), X-Ray Diffractometer XRD, Scanning Electron Microscopy SEM (GEMINI 500 SEM machine), Transmission Electron Microscopy TEM (FEI TECNAI G2 TEM @200KV) and Selected Area Electron Diffraction SAED pattern andFourier-Transform Infrared Spectroscopy FT-IR.[17]

2.5 In-vitro Anti-Oxidant Activity of Biosynthesized Au-Nps

2.5.1 Free radical scavenging activity on 2, 2-diphenyl-2-picrylhydrazyl (DPPHmethod):

To assess the scavenging ability on DPPH, Phenolic rich fraction of *A. gangeticus* and synthesized AuNPs (10–100 µg/ ml) in water was mixed with 1 ml of methanol solution containing DPPH radicals (0.1mM). The mixture was shaken vigorously and left to stand for 30 min in the dark before measuring the absorbance at 517 nm against a blank.[18] Then the scavenging ability was calculated using the following equation (1).

$$\% \text{ Scavenging activity} = \left[\frac{\text{Abs (control)} - \text{Abs (standard)}}{\text{Abs (control)}}\right] \times 100$$

Where, Abs (control): Absorbance of DPPH radical + methanol

Abs(standard): Absorbance of DPPH radical +AuNPs/Extract /standard.

2.5.2 Hydrogen peroxide scavenging activity:

The H_2O_2 scavenging activity was assayed, in brief; different concentrations (10, 20, 40, 60, 80 and 100 µg/ ml) of Phenolic rich fraction of *A. gangeticus* and AuNPs and ascorbic acid (control) were mixed with 0.6ml of 50 mM H_2O_2 solution(2 mM H_2O_2 in phosphate buffer, 50 mM, and pH-7.4) and incubated at room temperature (26 ± 2 °C) for 10 min. The absorbance was measured at 230 nm. [19] The percentage of H_2O_2 scavenging was calculated using Eq. (1)

2.5.3 Hydroxyl radical scavenging activity:

Exactly, 0.2 mL of different concentrations (10, 20, 40, 60, 80 and 100 µg/ ml) of Phenolic rich fraction of *A. gangeticus* and synthesized AuNPs and ascorbic acid (control) were added with 1.0 mL of EDTA solution and added with 1.0 mL of DMSO (0.85%) in 0.1 M phosphate buffer (pH 7.4). The reaction mixture was kept in a water bath at 90°C for 15 min and the reaction was terminated by adding 1.0 mL of ice-cold 17.5% trichloroacetic acid. Further 3.0 mL of Nash reagent (75 g of ammonium acetate, 3.0 mL of glacial acetic acid and 2.0 mL of acetyl acetone in 1.0 L of water) was added to all the test tubes and incubated for 15 min for color development. The absorbance was observed at 412 nm. [20] The ability to scavenge hydroxyl radical was calculated using Eq. (1)

2.6 In-vitro Anti-Diabetic Activity of Biosynthesized Au-Nps

2.6.1 Alpha-Amylase Inhibitory Activity:

In 96-well plate, 50 µL of phosphate buffer (100 mM, pH = 6.9) was added followed by 20 µl alpha-amylase (2 U/mL) and 20 µl of varying concentrations of above solutions (500,400,300,200 and 100 µg/mL) were pre-incubated at 37 ˚C for 20 min. Thereafter, 20 µL of 1% soluble starch (100 mM phosphate buffer pH 6.9) was added as a substrate and incubated again at 37 ˚C for 30 min. Then, 100 µL of the DNS(3,5-dinitrosalicylic acid)colour reagent was added and boiledin a waterbath for 10 min. The absorbance of the resulting mixture was measured at 540 nm using a plate reader.[21] Acarbose at various concentrations (500,400,300,200 and 100 µg/mL) was used as a standard. The results were expressed as percentage inhibition, which was calculated using the formula below(Eq.2);

$$\text{Inhibitory activity (\%)} = (1 - A/B) \times 100$$

where A is the absorbance in the presence of test substance and

B is the absorbance of control

2.6.2 Alpha-glucosidase inhibitory assay:

Briefly, 5 µl of phenolic rich fraction of ethanolic extract of plant and their corresponding gold nanoparticles (prepared at concentration of (500,400,300,200 and 100 µg/mL) was added to 20 µl of 1.0 U/mL alpha-glucosidase solution into a well of a 96-well plate. Thereafter, 60 µl of 67 mM potassium phosphate buffer (pH 6.8) was then added. After 5 min of incubation, 10 µl of 10 mM p-Nitrophenyl α-D-glucopyranosidesolution (PNP-GLUC)(substrate) was then added and further incubated for 20 min at 37°C. After incubation, 25 µl of 100 mM Na_2CO_3 (sodium carbonate)as a stopsolution was added and absorbance was measured at 405 nm. Mixtures without enzyme, sample extract and Acarbose served as blanks. while in positive controls Acarbose replaced the sample extract. Each test was repeated thrice and the calculation was done as (Eq.2). [22]

2.6.3 Glucose diffusion inhibition:

In a dialysis tube (6 cm×15 mm), 6 ml (50g/L) of phenolic rich fraction of ethanolic extract of the plant and their corresponding gold nanoparticles and 2 ml of 0.15 M NaCl containing 1.65 mM D-glucose were added. The dialysis tube was sealed at each end and placed in a centrifuge tube containing 45 mL 0.15 M NaCl. The tubes were shaken occasionally and incubated at 37 °C for 3 h. Concentration of glucose within the dialysis tube was measured and control tests were conducted in the absence of samples. The movement of glucose into the external solution was monitored at set time intervals by glucose oxidase kit method. All the tests were carried out in triplicate.[23]

2.7 In Vitro Anti-Cancer Activity of Biosynthesized Au-Nps

2.7.1 MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) Assay:

Cytotoxicity of the phenolic rich fraction of ethanolic extract of the plant and their corresponding gold nanoparticles was examined by using MTT assay. In brief, HeLA cells obtained from (The National Centre for Cell Science)NCCS, Pune, India, were plated in 96-well plates at a density of 1 x 10^4 cells/well. Cells were exposed to 2,5,10,25,50 and 100 µg/mlphenolic rich fraction of ethanolic extract of the plant and their corresponding gold nanoparticles for 48 hrs at 37°C in a 5% CO_2 atmosphere. Following this, MTT was added in the wells, and plates were incubated for 4 h further. The reaction mixture was taken out and 100 μl/well DMSO was added and mixed several times by pipetting up and down. The absorbance of plates was measured at 570nm. The results were expressed as percentage of control.[24]

2.7.2 Morphological Analysis:

The changes in the morphology are observed under the microscope to determine the alterations induced by different samples in HeLa cells treated with 1 μg/ml to 100 μg/ml (2 μg/ml, 5 μg/ml, 10, 25, 50, and 100 μg/ml) of different samples. Images of the cells are grabbed at 20x by using the phase contrast inverted microscope.[25]

3. RESULTS

The plant of *Amaranthus gangeticus* shown in Figure.1.The leaf shows the presence of glycosides, alkaloids, carbohydrates, proteins, amino acids, phenolic compounds, flavonoids, steroids, tannins.The total phenolic content (Gallic acid equivalents, mg/g) in the chloroform, ethanolic and aqueous extracts were 45.6 $\pm$ 1.33, 105.6 $\pm$ 1.10, 99.2 $\pm$ 0.95 Mg of GAE /g of extract, respectively.

Fig 1. *Amaranthus gangeticus* L.

Preparation of Phenolic Rich Fraction and its Total Phenolic Content Determination:

The phenolic compounds – rich fraction from *Amaranthus gangeticus*. (47.256g) was prepared and its Total phenolic Content was calculated as 104.4 mg of Gallic Acid Equivalent (GAE) /g.

Synthesis of Gold nanoparticles:

Plant mediated gold nanoparticles has been synthesized using phenolic rich fraction of ethanolic extract of *Amaranthus gangeticus* (Fig 2). The purple colour that appeared after mixing the leaf extract of *A.gangeticus*with gold chloride solution confirmed the formation of gold nanoparticles.

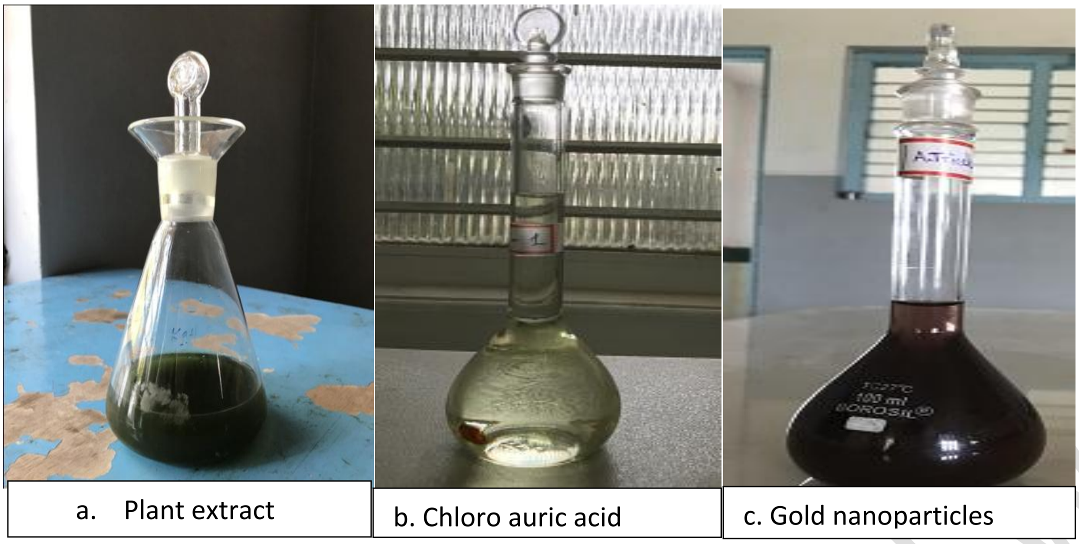

Figure 2(a-c): Colour changes in the plant extract after adding Gold Chloride solution.

Characterization of biosynthesized AuNPs

After visual confirmation by detecting a colour change in the biosynthesis of AuNPs, the samples were exposed to spectral analysis. In this study *Amaranthus gangeticus* gold nanoparticles shows the SPR peak from 520-570nm shown in Fig 3. Broadening of peak indicated that the particles are polydispersed.[26] The biosynthesized AuNP's XRD pattern is shown in Fig 4. It showed three well-resolved diffraction peaks at 2θ angles of 38.16 ^{0}, 44.43 ^{0}, and 24.26^{0}corresponding to (2.35646), (2.03738) and (3.66582) respectively (Fig 4). The broad line peaks are because of the small particle size. [27]Figure 5 show the SEM images of the synthesized AuNPs. The morphology of AuNPs isirregular, some possess spherical shapes, White particles are observed at different magnifications. [28] Morphology of the biogenic gold nanoparticles was investigated by TEM and it is almost sphericalranging from 10-200 nm. [29]showed in Figure. 6(a-d). Further, FT-IR analysis were carried out for the plant extract and AuNPs showed in Figure. 7(a&b). The phenolic rich fraction of ethanolic extract of *Amaranthus gangeticus* showed intense peaks at 3370.14 cm-1, 1625.88 cm-1, 1397.69 cm-1, 1347 cm-1, 1237 cm-1, 1053.97 cm-19(Figure 7(a)) And in stabilized gold nanoparticles the strong bands were observed at 3308.81 cm-1, 1637.29 cm-1, 1437.62vcm-1,1312.11 cm-1, 1140.97 cm-1, 1055.40 cm-1, (Figure 7(b)). In the Plant extract the peak was broad and blends, but after encapsulation of nanoparticles the peak was narrow and sharper. The absorption peak at at 3370.14 cm-1observed in control extract, which is due to OH stretching vibration, 1625.88 cm-1 is due to C=O stretching, 1397.69 cm-1and 1347 cm-1 is due to C-H stretching of aromatic ring, 1237 cm-1 and 1053.97 cm-1 is for CO stretching which indicates the Control extract may have the phenolic substances.

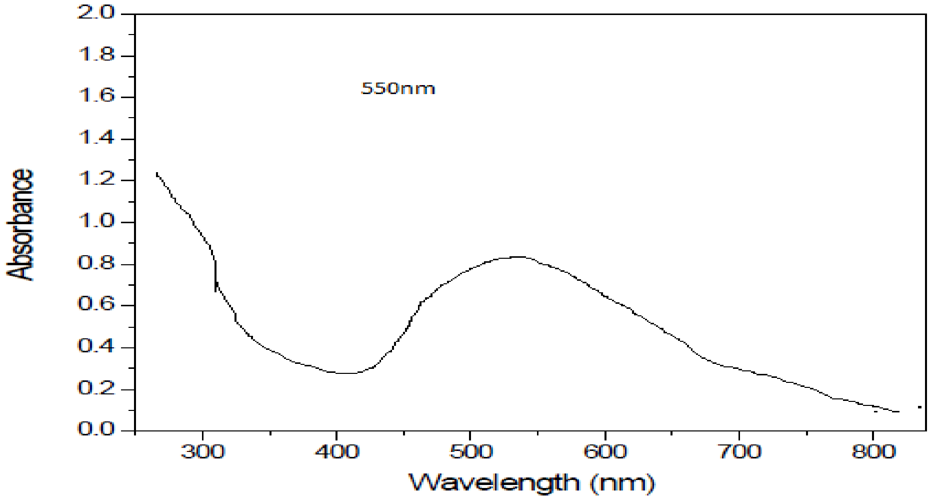

Figure 3: UV-VIS absorption spectra of biosynthesized Au NPs

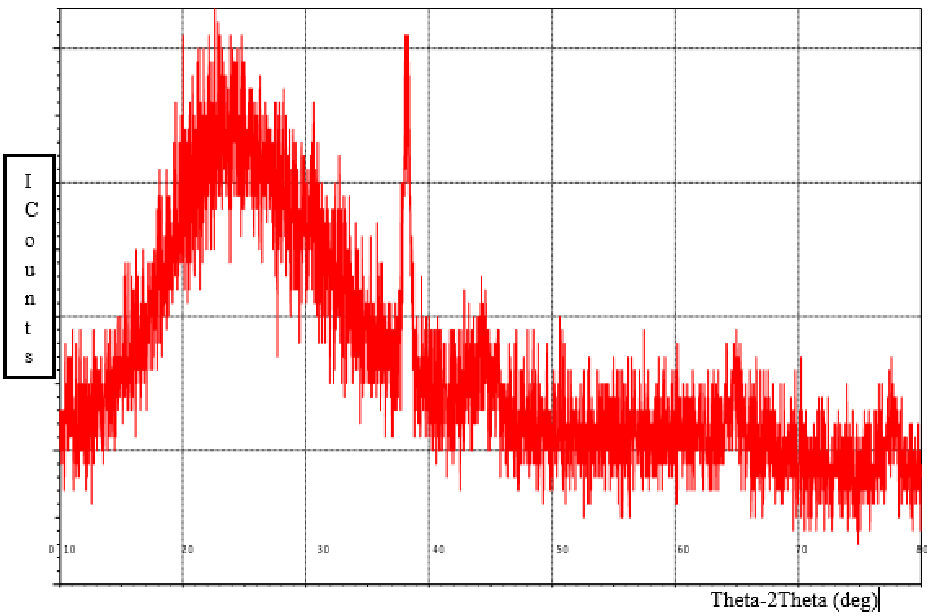

Figure 4: XRD pattern ofbiosynthesized Au NPs

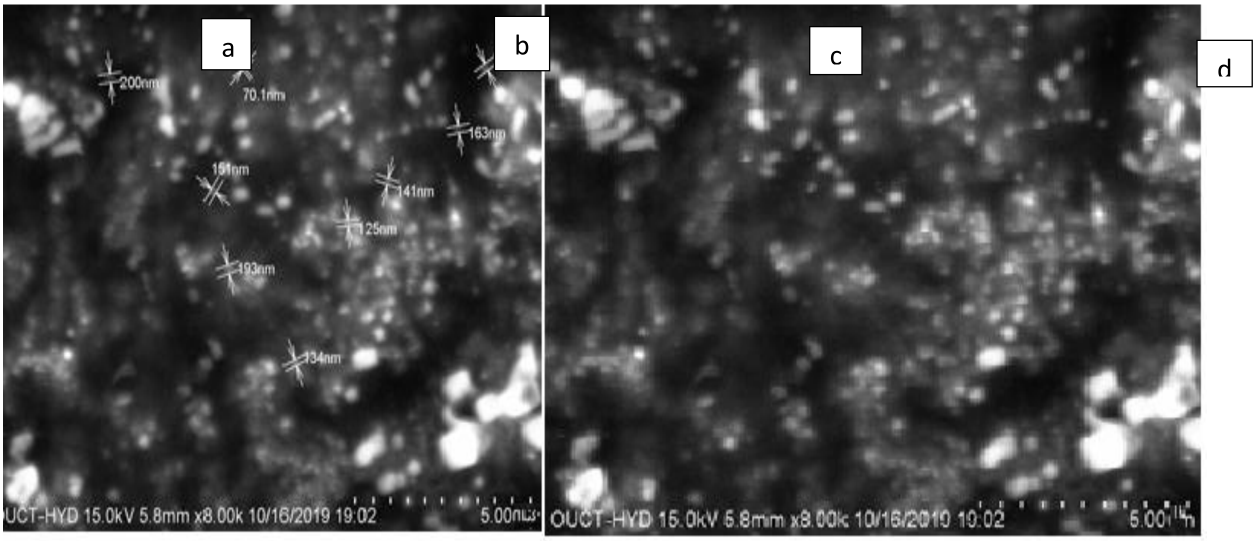

Figure5: SEM images of the biosynthesized AuNPs at different magnifications

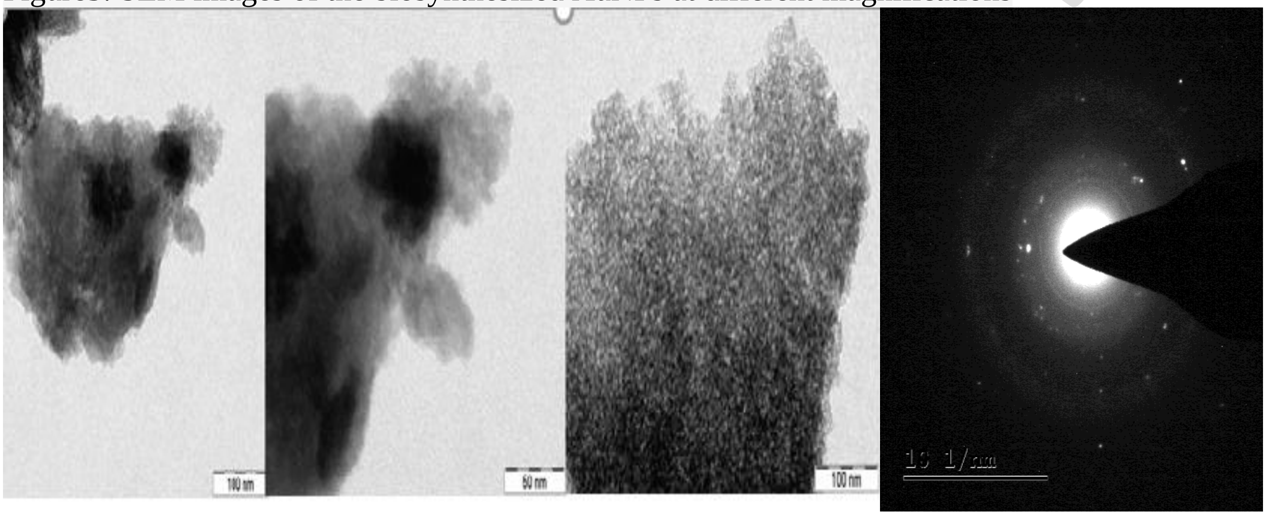

Figure 6:(a-c) TEM images of the bio synthesized AuNPs(d)SAED picture

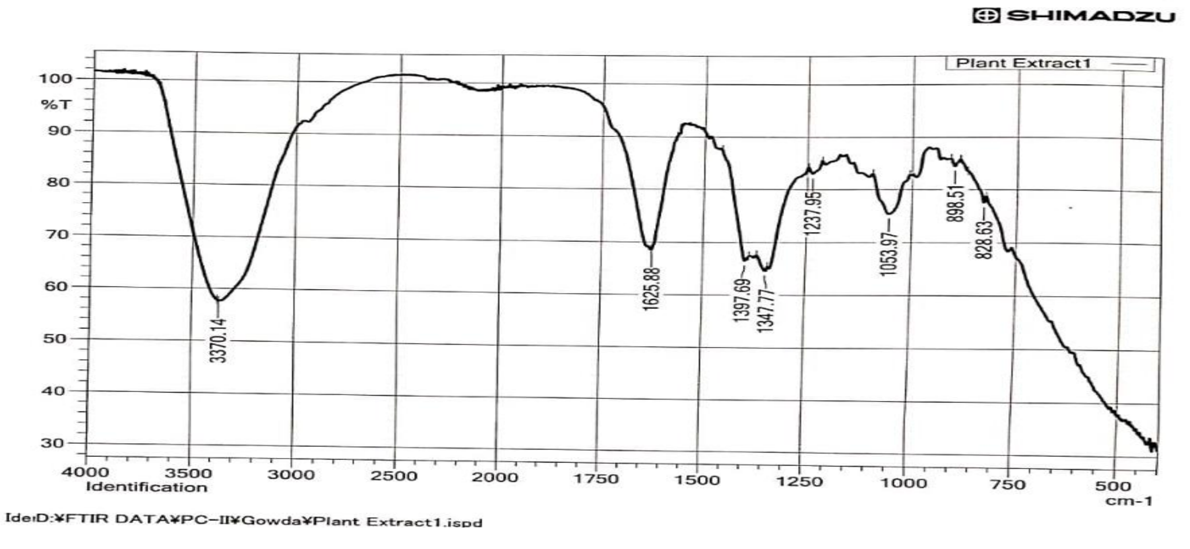

Figure 7(a)**:** FTIR Spectra forphenolic rich fraction of Plant extract

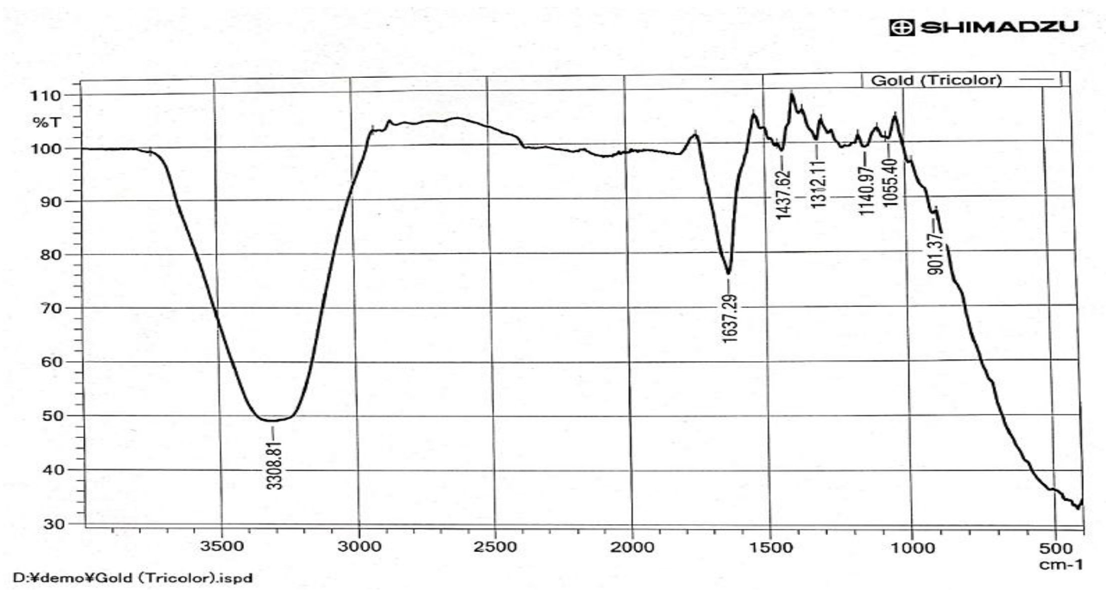

Figure 7(b)**:** FTIR Spectra of Biosynthesized AuNPs

In-vitro anti-oxidant activity:

The Anti-oxidant potential of biosynthesized AuNPs were examined by DPPH free radical scavenging, Hydrogen peroxide scavenging and Hydroxyl radical scavenging assays and the graphically the results were shown in Figure. 8(a-c). IC_{50} values for AuNPs and phenolic rich fraction of *A.gangeticus* were tabulated in Table.1.

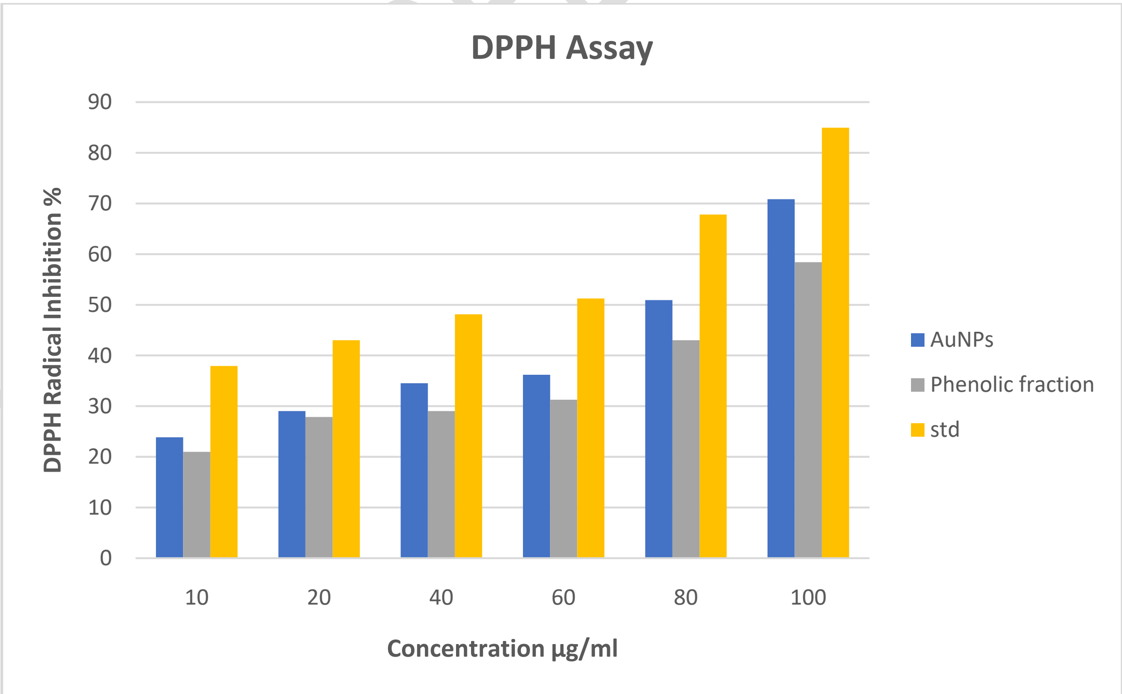

Figure 8(a): DPPH scavenging effect of Biosynthesized AuNPs

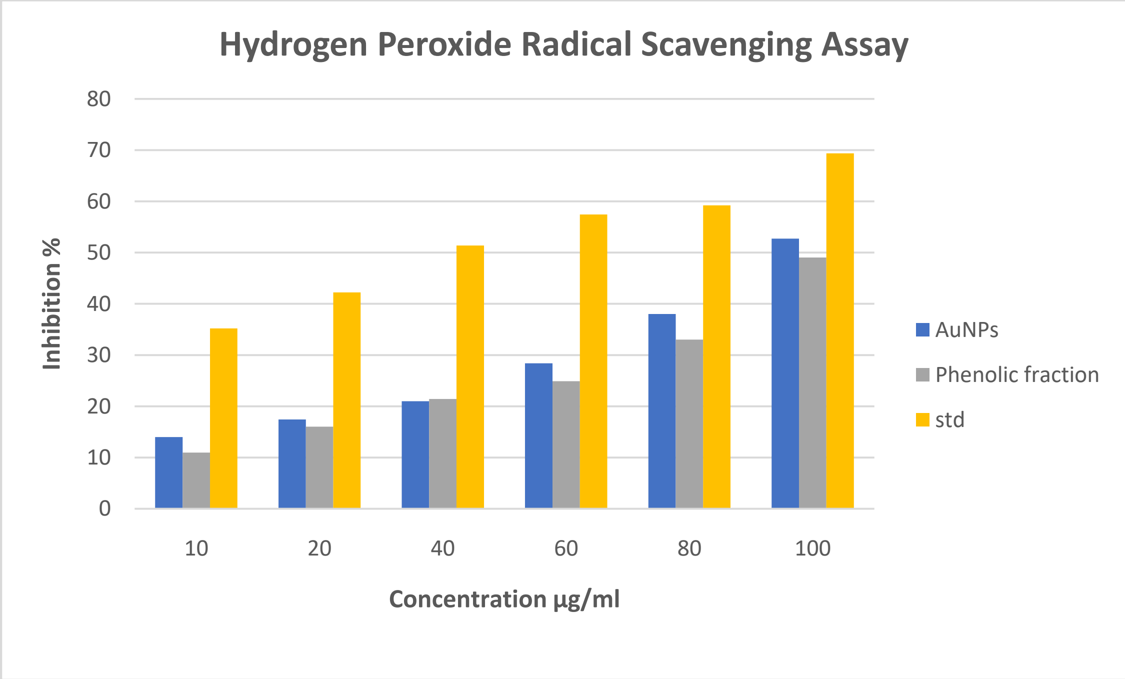

Figure 8(b): Hydrogen Peroxide scavenging effect of Biosynthesized AuNPs

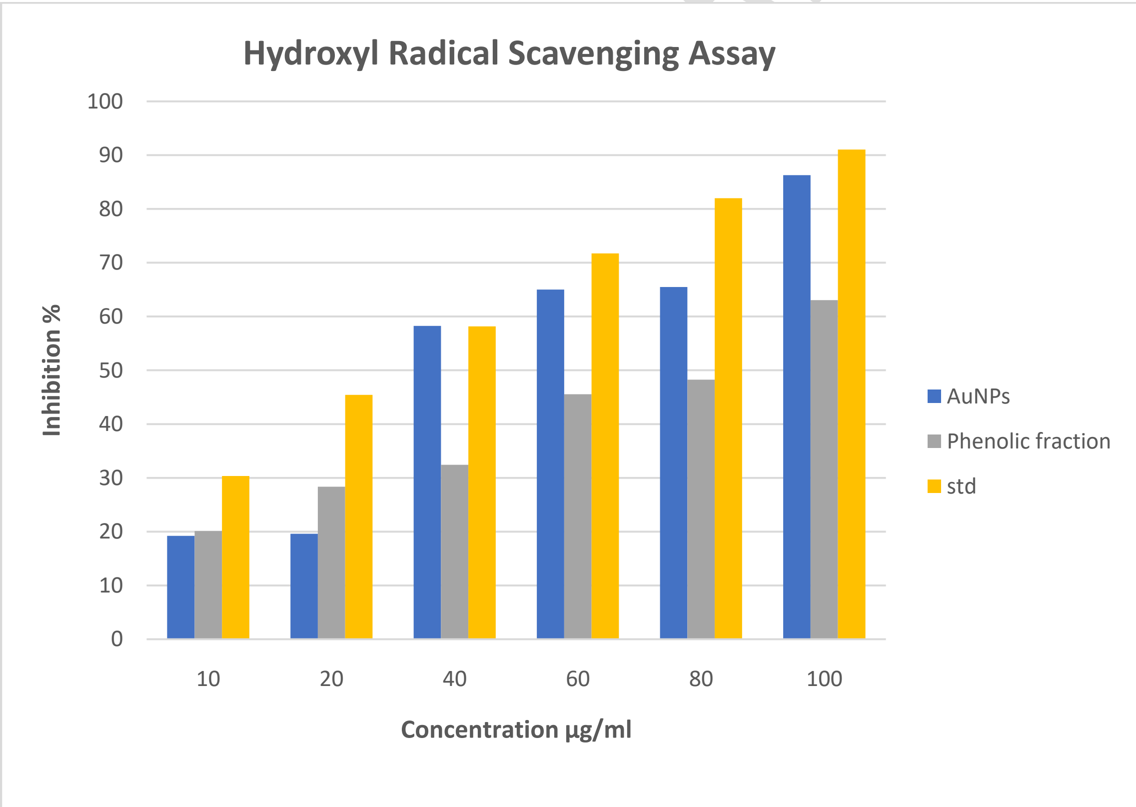

Figure 8(c): Hydroxyl Radical scavenging effect of Biosynthesized AuNPs

Table 1: IC_{50} value of Phenolic rich fraction of *A.gangeticus* mediated synthesized Gold nanoparticles

IC_{50} Value (µg/ml)	DPPH Assay	Hydrogen peroxide Assay	Hydroxyl Radical Assay
STD (Ascorbic acid)	29.02	34.21	28.53
Phenolic Rich Fraction of *A.gangeticus*	131.42	239.04	87.18
AuNPs	70.28	178.12	55.96

In-vitro anti-diabetic activity of biosynthesized Au-NPs:

Alpha-amylase and α-glucosidaseinhibitory activity:

As the results showed, the α-amylase inhibitory activities of all the samples were varied IC_{50} values from 372.31 ± 1.09 and 234.71 ±1.32 µg/mL and showed the α- glucosidase inhibitory activitiy with varied IC_{50} values from 445.7 ± 1.09 and 238.31 ±1.15 µg/mL, respectively. Concentration-dependent inhibition was observed.

Figure 9(a & b) shows the α-amylase and α- glucosidase inhibitory activity of the AuNPs and plant extract.

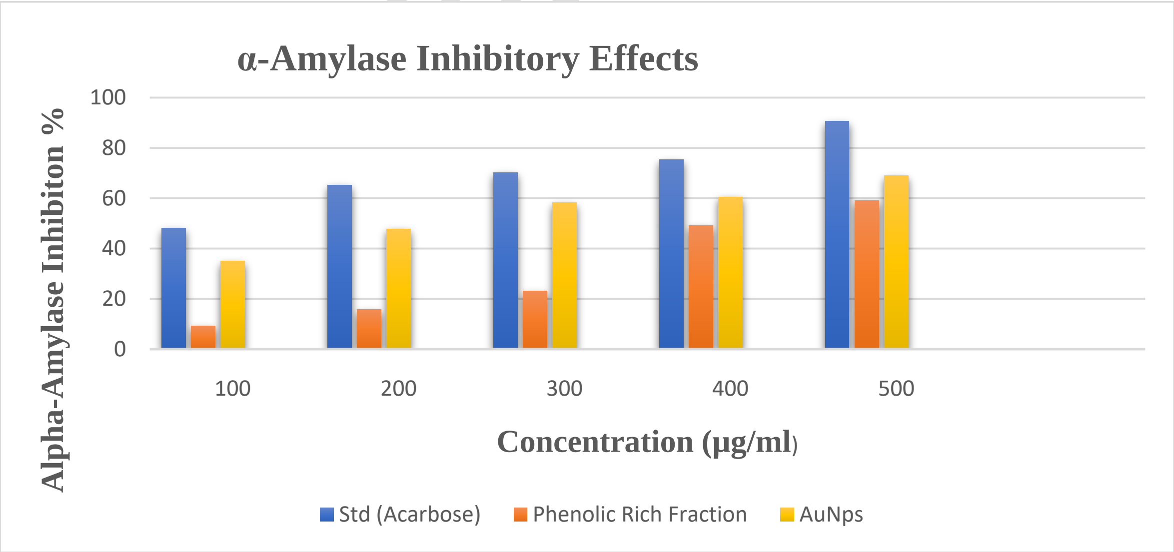

Figure 9(a): α-Amylase Inhibitory Activities of Biosynthesized AuNPs

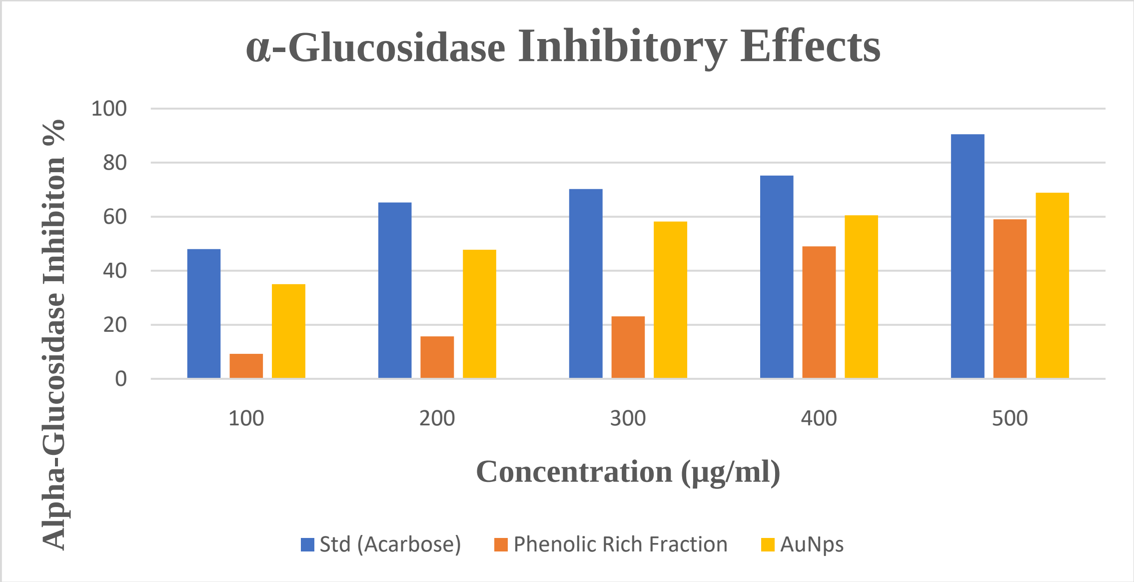

Figure 9(b): α-GlucosidaseInhibitory Activities of Biosynthesized AuNPs

Glucose diffusion potential of AuNPs:

The effect of phenolic rich fraction of *A.gangeticus*, AuNPs as antidiabetic agents has been studied. The effects of phenolic rich fraction of *A.gangeticus*, AuNPs on glucose diffusion inhibition were summarized in Table.2 . At the end of 27 hrs, glucose movement of control (without plant extract) in the external solution had reached a plateau with a mean glucose concentration above 300mg/dl (311.2±2.72). It was evident from the table that the AuNps were potent inhibitors of glucose diffusion.

Table 2: Effect of phenolic rich fraction of *A.gangeticus*, AuNPs (50g/litre at 27hr) on the movement of glucose out of the dialysis tube, glucose level in external solution.

Samples	**1h**	**3h**	**5h**	**24h**	**27h**
Control (in the absence of extract)	130.13±1.01	205.13±2.32	232.13 ±1.71	301.15±1.58	311.2±2.72
Phenolic Rich Fraction of *A.gangeticus*	106.36±2.18	146±1.19	192.12±1.61	242.11±1.48	282.26±1.68
AuNPs	98.17±1.19	148±0.33	178.55± 0.86	226.12±2.56	240±1.26

The values are expressed as mean ± SEM.

In-vitro anticancer activity:

MTT Assay:

The cytotoxic effect of AuNPs and Phenolic rich fraction was studied by MTT assay. The percentage growth inhibition was increasing with increasing concentration of test compounds which is graphically represented in Figure 10(a & b).

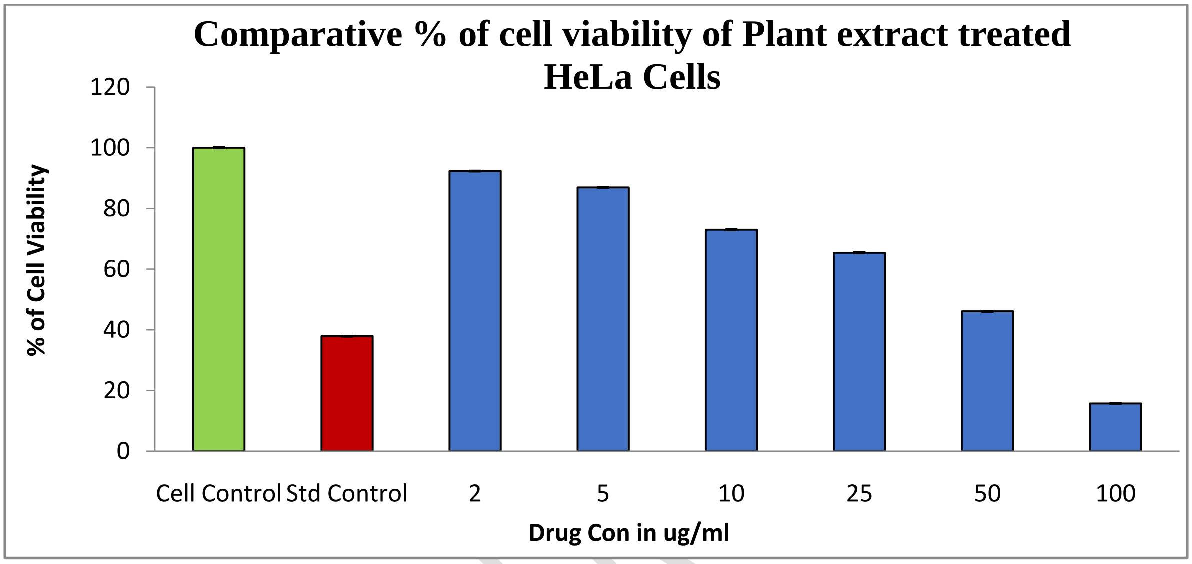

Figure 10(a): Comparative % of cell viability of Phenolic Rich Fraction of *A. gangeticus* for HeLa Cell Lines by MTT Assay

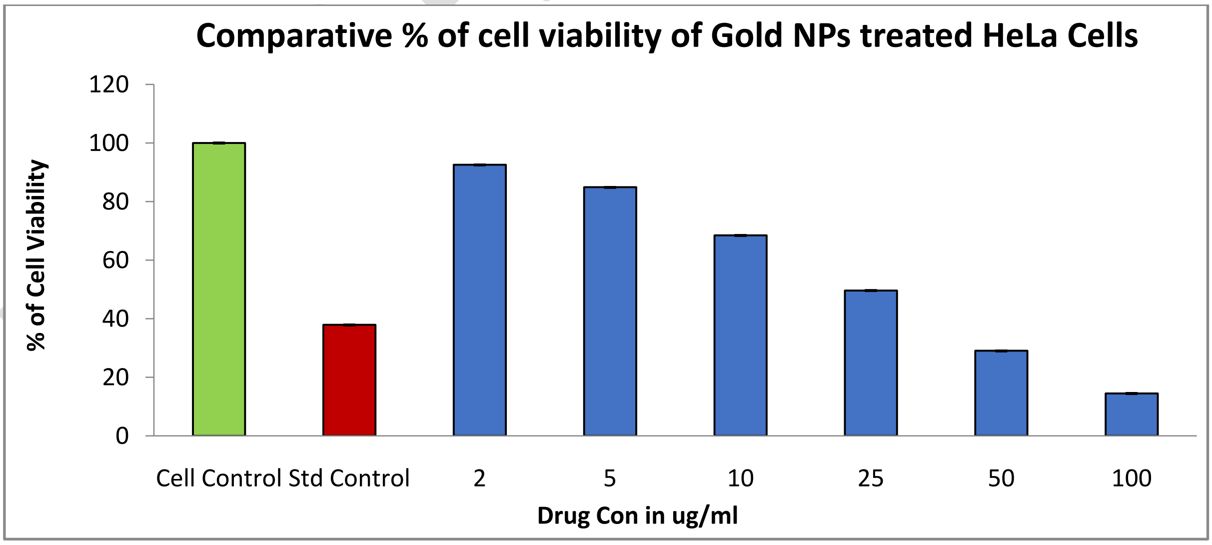

Figure 10(b): Comparative % of cell viability of AuNPs for HeLa Cell Lines by MTT Assay

Morphological Analysis

Morphological changes of HeLa cells treated with Phenolic Rich Fraction of *A.gangeticus* and AuNPs are shown in Figure 11 & 12.Morphological study shows significant effect on the morphology of HeLa cells treated with the AuNP's and plant extract compared to vehicle treated ones; The cells were reduced in size and abnormally shrunken, with their vehicle treated counterparts. These results suggested that the toxicity of the AuNP's is highly specific to cancer cells with non-toxicity to normal cells at a specific concentration.

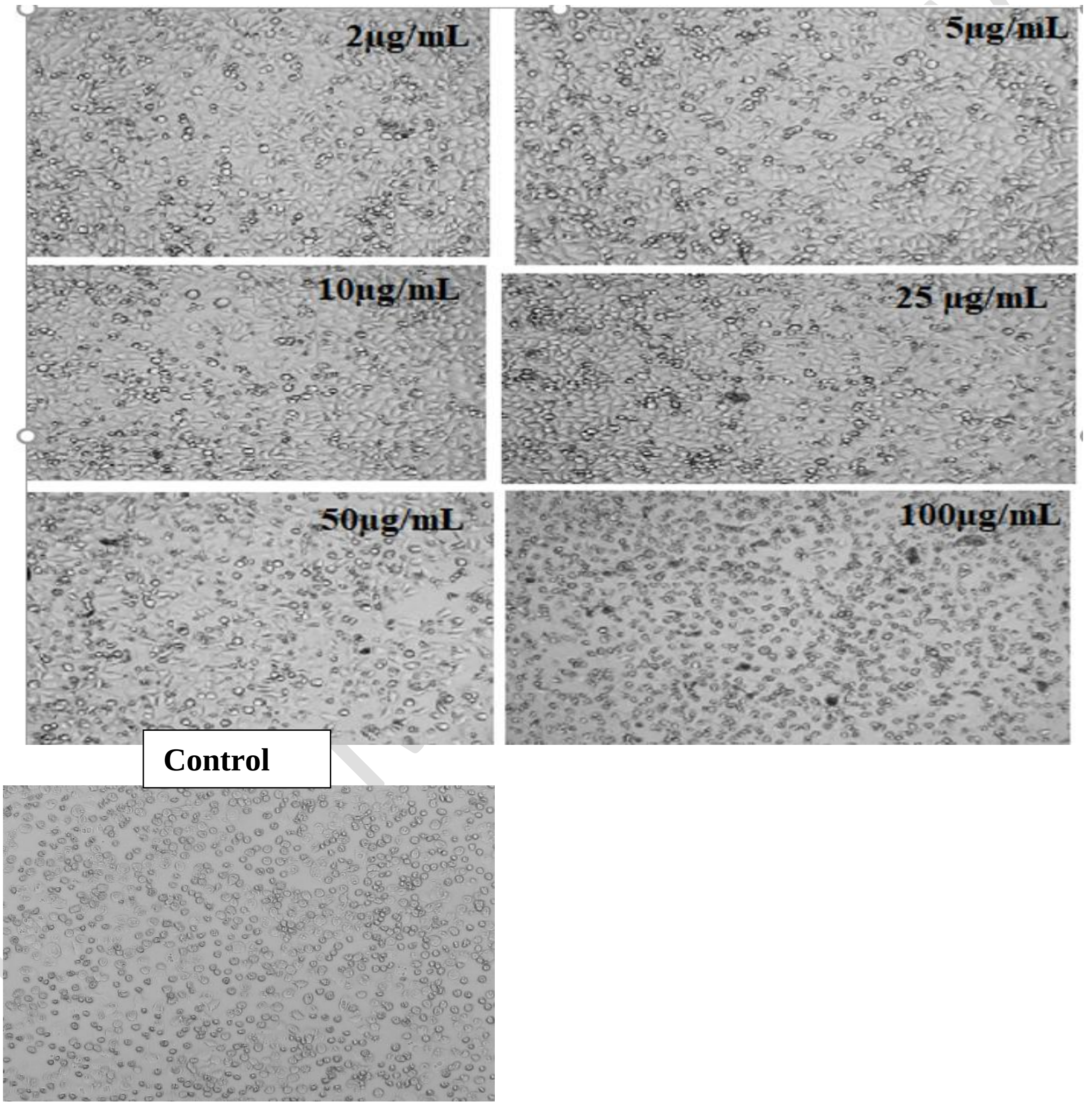

Figure 11: Morphological changes of HeLa cells treated with Phenolic Rich Fraction of *A.gangeticus*

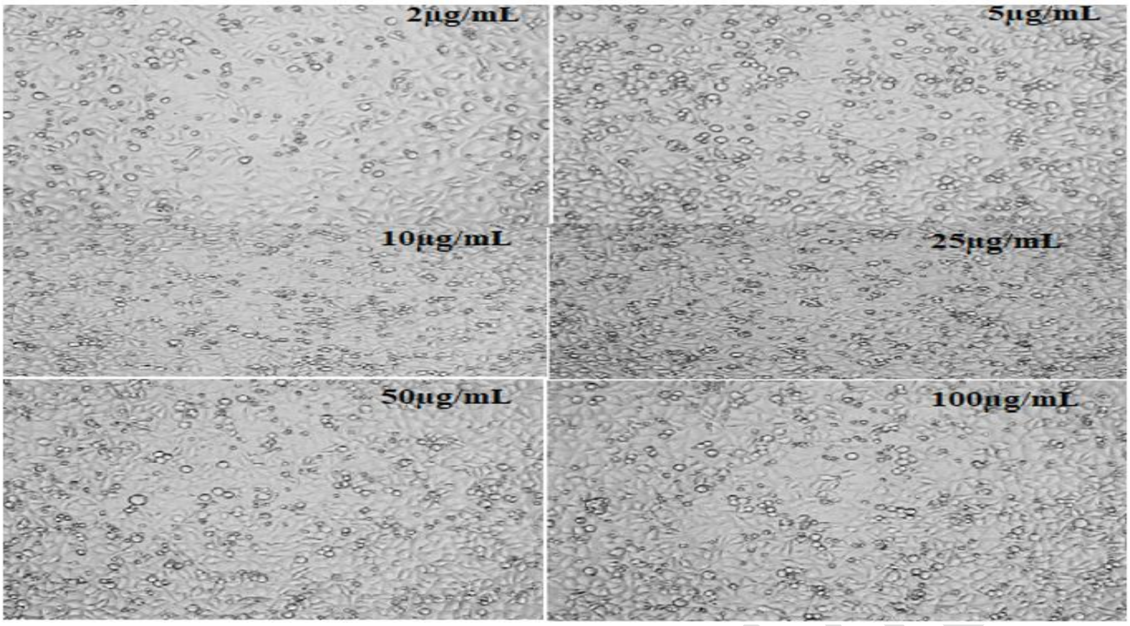

Figure 12: Morphological changes of HeLa cells treated with AuNPs

DISCUSSION

During the past several years, production of metallic nanoparticles using low-cost biological resources such as plants, algae, fungi and bacteria are reported.

Plant mediated gold nanoparticles has been synthesized using phenolic rich fraction of ethanolic extract of *Amaranthus gangeticus*. The purple color that appeared after mixing the leaf extract of *A.gangeticus*with gold chloride solution confirmed the formation of gold nanoparticles.In this study, UV-Vis analysis confirmed the presence of this material at a wavelength of 550nm.The crystalline nature of gold nanoparticles was confirmed from X-ray diffraction.Three peaks were observed at 38.16 ^{0}, 44.43 ^{0}, and 24.26^0 in the 2θ range 10-80^0. The observed peak broadening and noise were probably macromolecules present in the plant extract which may be responsible for the reduction of gold ions. Hence XRD pattern thus clearly illustrated that the gold nanoparticles formed in this present synthesis are crystalline in nature.SEM technique was employed to visualize the size and shape of gold nanoparticles.The overall morphology of most of AuNPs may be described as irregular, some possess spherical shapes.Morphology of the biogenic gold nanoparticles of was investigated by TEM and it is is almost spherical ranging from 10-200 nm. It is quite obvious from the image that the AuNPs were capped with phyto constituents i.e phenolic rich fraction of *Amaranthus gangeticus* leaves extract.A crystalline structure of the biosynthesized gold nanoparticles was also evidenced by the SAED pattern.ATR FTIR measurements were carried out to identify the possible biomolecules responsible for reduction, capping and efficient stabilization of the Au nanoparticles. The FTIR studies have confirmed the fact that the Phenolic group has the stronger ability to bind metal indicating that the phenolic constituents could possibly form a layer covering the metal nanoparticles (i.e., capping of gold nanoparticles) to prevent agglomeration and thereby stabilize the medium.

This study gives the details on the synthesis of gold NPs using plants to exhibit the potent anticancer, anti-diabetic and antioxidant activity. The Anti-oxidant activity of biosynthesized AuNPs were examined by DPPH free radical scavenging, Hydrogen peroxide scavenging and Hydroxyl radical scavenging assays. The DPPH free radical scavenging activity of AuNPs at the five different concentrations (10–100 μg/mL) was found in the extent of 23.85%– 70.85% (Figure. 8a) whereas that of Hydrogen peroxide scavenging assay was in the extent of 14.01%–52.72% at the same concentration. (Figure. 8b). The Hydroxyl radical scavenging activity of AuNPs at a concentration of 10–100 μg/mL ranged from 19.21% to 86.27%.Thus, the free radical scavenging potential of Biosynthesized AuNPs was higher than the phenolic rich fraction of *A.gangeticus*. The improved antioxidant activity of nanoparticles compared with the phenolic rich fraction could have been recognized as the adsorption of bioactive compounds of the plant over spherically shaped nanoparticles. In-vitro anti-diabetic activity of biosynthesized Au-NPs also studied. Among the samples, the gold nanoparticles showed the highest alpha–amylase enzyme inhibition activity with an IC_{50} value of 234.71$\pm$1.32and alpha–glucosidase enzyme inhibition activity with an IC_{50} value of 238.31 $\pm$1.15. The cytotoxicity study was carried out by MTT assay for thephenolic rich fraction of ethanolic extract of *A. gangeticus*and their corresponding gold nanoparticles.These test samples were screened for its cytotoxicity against HeLa cell lines at different concentrations to determine the IC 50 (50% growth inhibition). The IC_{50} value the phenolic rich fraction of ethanolic extract of *A. gangeticus* and their and gold nanoparticles were 49.82μg/ml and 20.86μg/ml. The percentage growth inhibition depends on the increasing concentration of test compounds.

CONCLUSION

A simple one-pot green synthesis of stable AuNPs were prepared by the phenolic rich fraction of *Amaranthus gangeticus* L. (Leaves) at room temperature. It is an eco-friendly, rapid green approach which is a low cost and an better way for the preparation of goldnanoparticles. In this study, AuNPs exhibited excellent anti-oxidant, anti-diabetic and anti-cancer potential as compared to phenolic rich fraction of *A.gangeticus.* Thus, *A.gangeticus* mediated AuNPs could serve as a viable source for natural anti-diabetic and anti-cancer drug in the pharmaceutical industry.

The study highlights the efficacy of " Ayurveda " which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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