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Green Synthesis of Gold Nanoparticles using Adina Cordifolia Bark Extract and its Antimicrobial and in Vitro Anticancer Study

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ABSTRACT

In this study, green synthesis of gold nanoparticles were success fully synthesised by using *Adina cordifolia* plant bark aqueous extract which provides eco-friendly process, an environmentally benign, easy and proficient way for the synthesis of gold nanoparticles. The smaller size of gold nanoparticles have research on various dieses are very important. The green synthesized gold nanoparticles were characterized by UV-Visible spectroscopy, FT-IR, XRD, SEM, TEM and their antimicrobial activity was investigated. From UV-Visible spectrophotometer result was confirmed the formation of gold nanoparticles by color changed to ruby red color from pale yellow color indicates the reduction of Au^{3+} ions to Au^{o}. The antibacterial activity for the synthesized gold nanoparticles was confirmed by the antibacterial activity experiment against *Bacillus subtilis* and *Escherichia coli* by agar well method. The synthesized AuNPs was performed anticancer activity against MCF-7 breast cancer cell line. Compared to Adriamycin, Positive Control Compound AuNPs exhibited potent anticancer activity with the IC_{50}. The green synthesized gold nanoparticles proved to be potential candidates for medical application antimicrobial and anticancer activity is highly essential.

Keywords: Green Synthesis, Antibacterial Activity, In Vitro Anticancer Activity

I. INTRODUCTION

Green synthesis of metal nanoparticles by plant extract is a simple, eco-friendly and efficient method in comparison to chemical mediated synthesis method and plant extracts acts as stabilizing agents and low-cost reducing agents[1] and recently various types of metal nanoparticles are synthesized by different plant sources. Green synthesis of mono-dispersed nanoparticles with specific shapes and sizes has a challenge in bioscience and it has major advantages in a pharmacological industry to cure different viral and bacterial diseases[2] and this eco-friendly synthesis of nanoparticles is considered as building blocks of these generations to control various diseases. The important parameters controlled for the synthesis of gold nanoparticles by concentrations of reagents, temperature, pH, pressure, and time of reaction. Many reports related to this field states that biosynthesized nanoparticles will control oxidative stress, apoptosis-related changes, and Geno-toxicity[3]. First proposed by Mie by Maxwell equation AuNPs appear in red ruby color due to a surface Plasmon resonance and the reduction by reducing agents in the presence of stabilizer and the visible spectrum of wavelength of around 520 nm in different shapes such as rod-like, spherical, oblong etc, can be synthesized[26].

Parida has done the experiment of synthesised gold nanoparticles from *Allium cepa* L plant extract with MCF-7 cancer cell line for *in vitro* anticancer studies

and it showed AuNPs in the range of 100 nm in diameter and also showed that cytotoxic activity on MCF-7 cancer cell line was dose-dependent manner, Hence AuNPs effectively inhibits the growth of MCF-7 cancer cell line[4]. Boruah was successfully proved *in vitro* anti-cancer experiment in the study of cytotoxicity assay with different concentrations of AuNPs against MCF-7 cell lines from *Camellia sinensis* L plant extract and it was in the size range of 25 nm and results was a decrease in the percentage growth of cancer cells[5].

Adina cordifolia (Roxb.) commonly known as Haldu is large deciduous tree species of family Rubiaceae[6]. The tree may attain 30 m height and straight clean bole of 12 m and a girth of 7 m and over, with a large high crown, erect trunk and horizontal branches (Anon, 1985). The species of flower may be insignificant individually and attractive. The bark of the tree act as an antiseptics and capable of destroying microbes, virus particles and prevent or inhibit their growth. *Adina cordifolia* is a traditional healer for the treatment of cough, jaundice, diarrhea, stomachache, fodder, swelling in stomach, and roots are astringent and constipating[7].

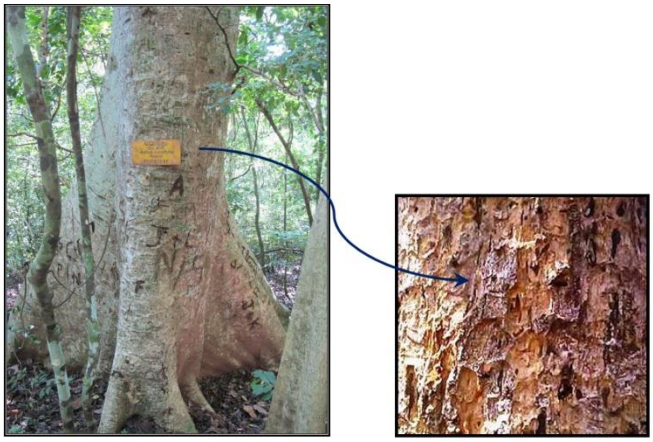

Figure 1. Bark of *Adina Cordifolia* plant

II. METHODS AND MATERIAL

2.1. Material

The plant Adina Cordifolia material was collected from sabarkantha district, which belongs to Gujarat, India. Analytical grade gold chloride was purchased from Sigma-Aldrich Chemicals. All glass wear was before used in research work washed with distilled water and dried in an oven. Ultra-pure deionised water used in entire research.

2.2. Preparation of A. Cordifolia bark extract

Collected plant bark first washes tap water then again washes with double distilled water and dry it at room temperature for 15-20 days. After dried bark, it was converting into powder form by using the grinder and collects it in neat and clean dry air tight bottle for use of research. Weigh 10 gm of powder takes in 250 ml conical flask and add 100 ml deionised water after that conical flask put on the magnetic stirrer and stir for 30 min at 60°C. The extract was cool down at room temp and filtered with Whatman no.1 filter paper and the filtrate obtained was store at room temp at the dark place for further use[8].

2.3. Synthesis of Gold Nanoparticles

The reaction mixture was prepared by 10 ml of M. parvifolia extract was added to 90 ml of an aqueous gold chloride solution in a 250 ml conical flask. The mixture of the solution was kept under vigorous stirring at room temperature for 1 hour. After that, the solution color changed to ruby red from pale yellow indicates the reduction of Au^{3+} ions to Au^{o}. Ruby red colored formation was established after the initial confirmation for the formation of plant-mediated gold nanoparticles.

2.4. Purification of Gold Nanoparticles

The reaction mixture was centrifuged at 12,000 rpm for 15 min and particles were washed twice with de-ionized water and dried at room temp.

2.5 Characterization of prepared Gold Nanoparticles

2.5.1. UV-Visible Spectrophotometer Analysis

The green synthesized AuNPs characterization was monitored by Shimadzu 1800 UV-Visible spectrophotometer in the wavelength range of 300-700 nm. 2mm caveat and double distilled water were utilized for blank reading.

2.5.2. FT-IR spectroscopy analysis

The Fourier Transform Infrared spectra were identified by an FT-IR spectrophotometer (Perkin Elmer Spectrum) using KBr. The sample powder was mixed with KBr and prepared pallet scanned at the range of 4000-450 cm^{-1}.

2.5.3. X-ray diffraction (XRD) analysis

The X-ray diffraction was used to obtaining the crystalline structure and data in the 2Θ range of 20°-80°.

The Debye Scherrer formula,

$$D=k\lambda/\beta\cos\theta$$

Where,

D = particle diameter size

K = constant equals

Λ = wavelength of the X-ray source

β = the full width at half maximum of the diffraction peak

Θ = the Bragg angle

2.5.4. Scanning Electron Microscopy (SEM) analysis

Scanning electron microscopy is one more commonly used technique for characterization of nanoparticles. The surface morphology of AuNPs was analyzed by scanning electron microscope it was performed by SIGMA model and an operating on the voltage of 20 kV and for operation need a very small amount of dry powder sample put on a grid and removed excess sample with the help of blotting paper.

2.5.5. Transmission Electron Microscopy (TEM) Analysis

TEM analysis characterized the size, shape and morphology of the gold nanoparticles and a prepared sample was dried under vacuum in desiccators before placing it in a specimen holder. A thin sample was irradiated with a sharp high-energy electron beam focused by magnetic lance and electron intensity distribute on the beam after interaction with sample and image was recorded by digital camera and display on a computer screen[9].

2.6. Antimicrobial activity

2.6.1. Test organism for antibacterial activity

In this study, two type bacteria were collected from the microbiology department, HNGU. One was gram-positive bacteria and another one was gram-negative, Bacillus subtilis (+ve) and Escherichia coli (-ve). The bacterial strains were grown and maintained on nutrient agar at 38°C in incubation condition for 5 days and the culture was stored at 4°C for further experiment work.

2.6.2. Media preparation

In the media preparation, B. subtilis and E. coli bacteria were grown in a nutrient agar medium. 2.8 gm nutrient agar powder was added into 100 ml of distilled water for nutrient agar preparation then the prepared medium was kept in the cotton-plugged

glass container and sterilized in the autoclave at 120°C for 20 min.

2.6.3. Method for testing Antimicrobial Activity of Synthesised Copper Nanoparticles

Antimicrobial activity of green synthesised nanoparticles was carried out by agar disc diffusion method[10-12] against B. subtilis (+ve) and E. coli (-ve) bacteria.

The nutrient agar plates were prepared by 20ml for each of molten media into sterile Petri-plates. Plates were left standing for 10 minutes to let the culture get absorbed.

Using the micro-pipette, 100µl of sample of nanoparticles suspension was poured into different concentration (25µl, 50µl, 75µl) into each plate. Then Antibiotic-Ampicillin drug was used as positive control. After adding the samples in the wells, the dishes were kept in a refrigerator for an hour for absorption of the samples into the surrounding medium from the well. The plates were transferred into an incubator set at 37°C to allow bacterial growth on the medium. After 24 hrs the plates were taken out of the incubator and observed for the zone of bacterial growth inhibition around the wells. The zone of inhibition was measured in millimeters[13].

2.7. Anticancer

For this experiment, RPMI 1640 medium containing 10% fetal bovine serum and 2mM L glutamine for grown the cell lines and cells were incubated into 96 well microtiter plates in 100µl at plating densities depending on the doubling time of individual cell lines. In the microtiter plates added experimental drugs then incubated at 37°C temperature, presence of 5% carbon dioxide and 95% air and 100% relative humidity for 24 hours to the addition of experimental drugs after the procedure of cell incubation.

The experimental drugs were initiating solubilized in 100mg/ml dimethyl sulfoxide and diluted in 1mg/ml water then stored frozen prior to use. Preparation of diluted complete medium containing test article to 100µg/ml, 200µg/ml, 400µg/ml and 800µg/ml by frozen concentrate 1mg/ml for the time of drug addition.

Microtiter wells containing 90µl of medium and added 10µl of different drug dilution. The required final drug concentration was i.e. 10 µg/ml, 20 µg/ml, 40 µg/ml and 80 µg/ml. Prepared plates were incubated for two days in standard condition after the compound addition and assay was completed by the addition of cold TCA.

The cell were fixed in situ by the gentle addition of 50µl of cold 30% (w/v) TCA and incubated for 1 hour at 4oC. Now, discard the supernent from the plates and the plates were washed 5-6 times with tap water and air dried then added to each of the wells sulforhodamine B solution (50µl) at 0.4% (w/v) in 1% acetic acid and the plates were incubated for 20 minutes at room temperature. When staining was completed unbound dye and the residual dye was removed after staining with 1% acetic acid by washing five times then plates was air dried. The bond stain was evaluated with 10mM Trizma base and shown reading of absorbance was on the plate reader at 540-690 nm reference wavelengths[14-15].

III. RESULTS AND DISCUSSION

3.1. Color change

Pale yellow to ruby red color

Figure 2. Showing color change of *Adina cordifolia* plant bark extracts containing before and after synthesis of AuNPs

3.2. UV-visible Spectroscopy

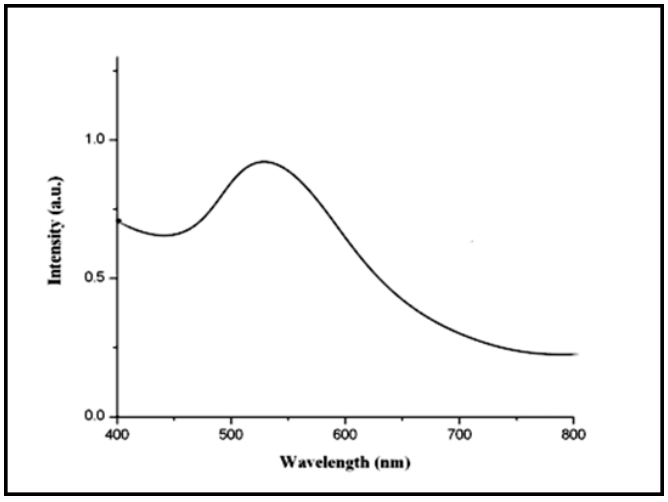

Figure 3. UV-visible spectra of gold nanoparticles

The characterization of gold nanoparticles by UV-Vis spectra from the range of 400-800 nm the absorption spectra were obtained at 525-530 nm in graph represent.

3.3. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR gives the information about present functional groups in synthesised copper nanoparticles and it shows in (fig. 4) clearly. In the spectra the peak at 3298.28 cm^{-1} and 3324.21 cm^{-1} indicating the presence of –NH or –OH group stretching in amino acids alcohols and phenols, Stretching at 2926.01 cm^{-1} corresponds to C-H stretching in alkanes and aldehydes, stretching at 1648.12 cm-1 indicate the presence of >C=O group and the peak at 1103.28 cm^{-1} corresponds to C-O stretching and the weak peaks in between 850.61 cm^{-1} to 526.57 cm^{-1} are associated to halo compounds stretching[16-17]. Hence these observations indicated the formation of AuNPs associated with metabolites protein like terpenoids contain functional groups as alcohols, phenols aldehydes, ketones and carboxylic acids. Kulkarni et al reported the bio-entities could probably play a double role of fabrication and stabilization of gold nanoparticles in the aqueous solution[18].

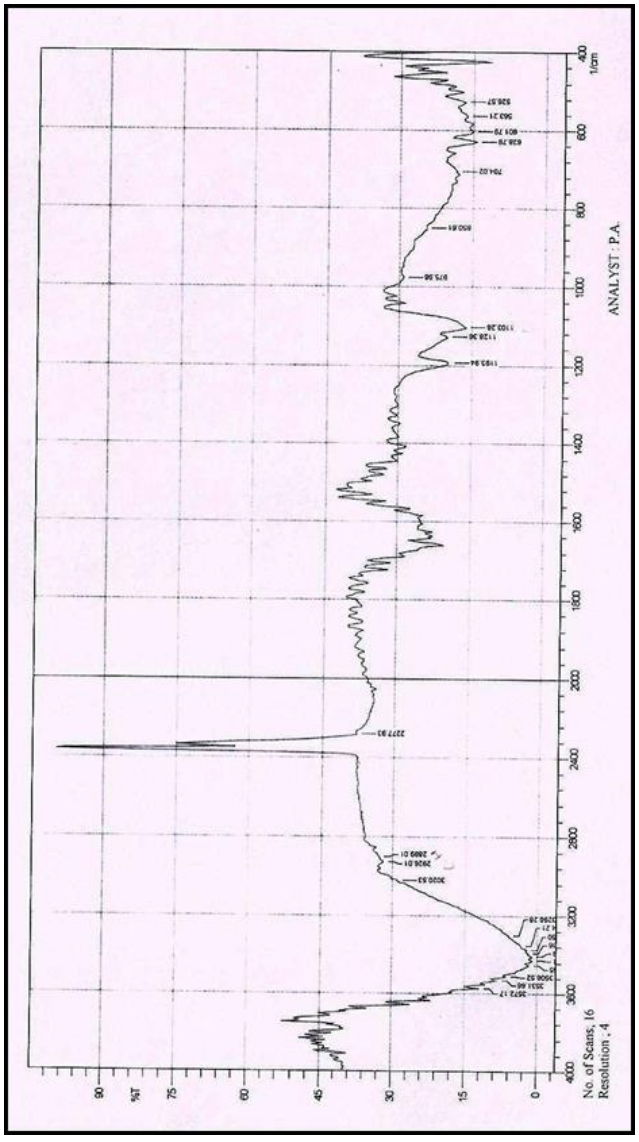

Figure 4. FTIR spectra of copper nanoparticles

3.4. X-ray diffraction

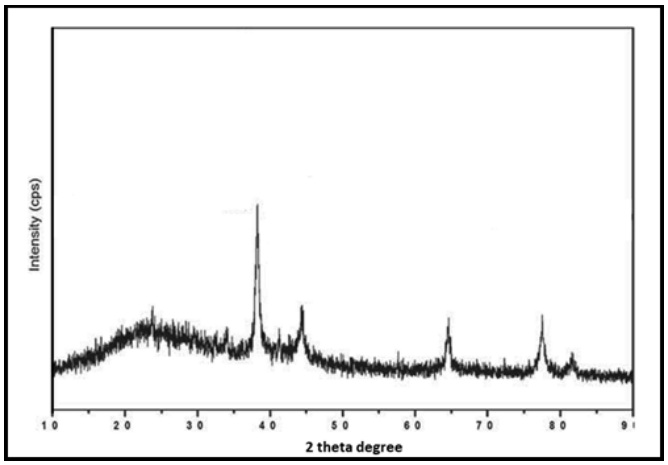

Figure 5. XRD pattern of gold nanoparticles synthesized from an extract

X-ray diffraction pattern study confirmed the phase of AuNPs and the peaks observed at 2θ values of 38.3º, 44.6º, 64.4º, 78.2º and 81.5º correspond to crystal planes of (111), (200), (220), (311) and (222) of gold nanoparticles[19-20]. The diffraction peaks denote as crystalline phase, fcc crystal structure in (Fig. 5). The particle average size was calculated by the Scherrer formula and determines 15.3 nm. *Citrus reticulate*, *Citrus sinensis* and *Chrysopogon zizanioides* have a similar pattern reported of XRD for gold nanoparticles[21-23].

3.5. Scanning Electron Microscopy (SEM) analysis

The gold nanoparticles size determined by scanning electron microscope image, in the surface morphology study of AuNPs average size was 14 – 32 nm; (fig.6) shows the existence of symmetrical spherical shape[24].

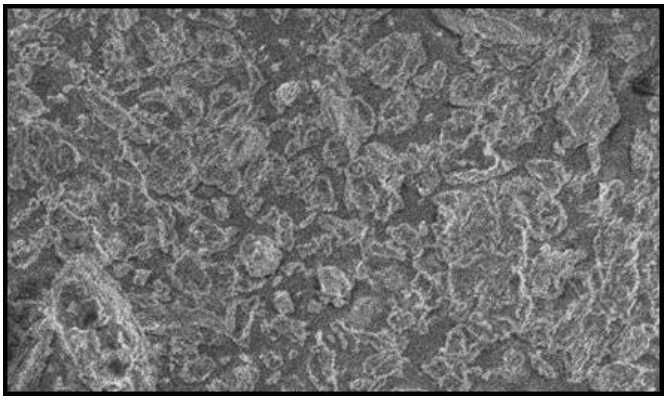

Figure 6. SEM analysis of gold nanoparticles

3.6. Transmission Electron Microscopy (TEM) analysis

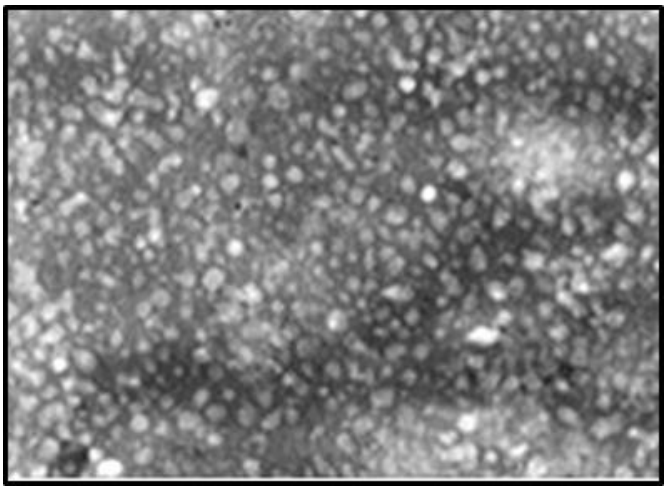

Figure 7. TEM analysis of copper nanoparticles

The image of Silver nanoparticles synthesised using an aqueous extract of (plant name) shown in (fig.7) the synthesised AuNPs was spherical in shape and an average diameter of 8-17 nm. Singh et al. have reported a similar geometry of synthesized silver and gold nanoparticles using natural precursor clove[25].

3.7 Antimicrobial activity

The antimicrobial activity of green synthesised gold nanoparticles against two human pathogenic bacteria such as *Bacillus subtilis* and *Escherichia coli*. Here *Bacillus subtilis* is gram +ve and *Escherichia coli* is gram –ve bacteria were evaluated and compared to a

commercial antibiotic drug Ofloxacin. Synthesised AuNPs showed the clear diameter of the zone of inhibition around the well wherein the suspension of AuNPs was applied. The obtained result was presented in table and figure.

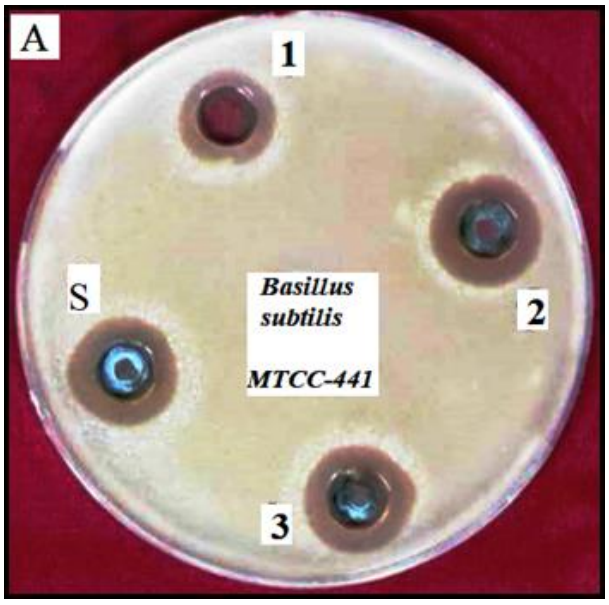

Figure 8. Bacterial growth inhibition against Bacillus subtilis MTCC-441 by using 1. 25µl, 2. 50µl, 3. 75µl and S. control sample (Ofloxacin)

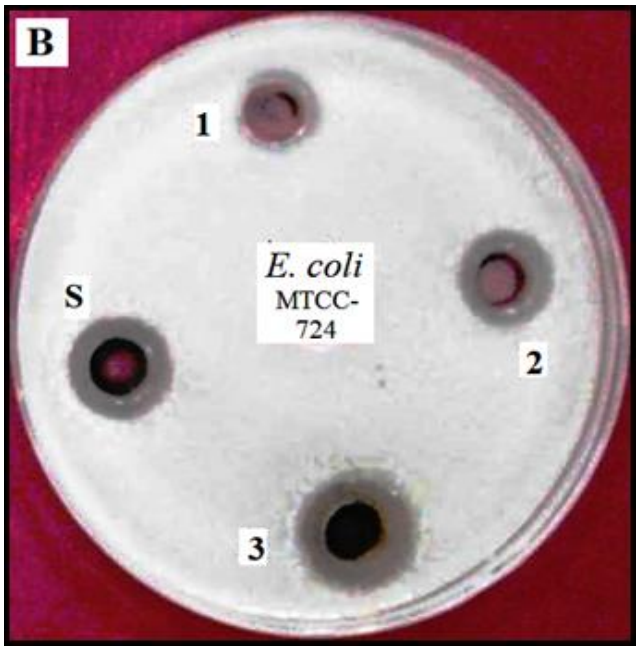

Figure 9. Bacterial growth inhibition against E. coli MTCC-724 by using 25µl, 2. 50µl, 3. 75µl and S. control sample (Ofloxacin)

Table 1. Zone of diameter area (mm) exhibited by the formed gold Nanoparticles against pathogenic bacteria

Concentration (µl)	Diameter of zone of inhibition (mm)	
	Bacillus subtilis MTCC-441	*Escherichia Coli* MTCC-724
25 µl	7.3	6.5
50 µl	9.5	8.0
75 µl	10.7	10.2
Standard Drug Ofloxacin	13.5	12.6

3.8. Anticancer activity

Table 2. % control growth of Human Cancer Cell line MCF-7

Human Breast Cancer Cell Line MCF-7				
	% Control Growth			
	Drug Concentrations (µg/ml)			
Experiment	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml
Sample	-64.8	-59.1	-56.6	-43.1
ADR	-71.0	-69.1	-80.5	-69.8

Per cent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Per cent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100.

Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as: [Ti/C] x 100 %

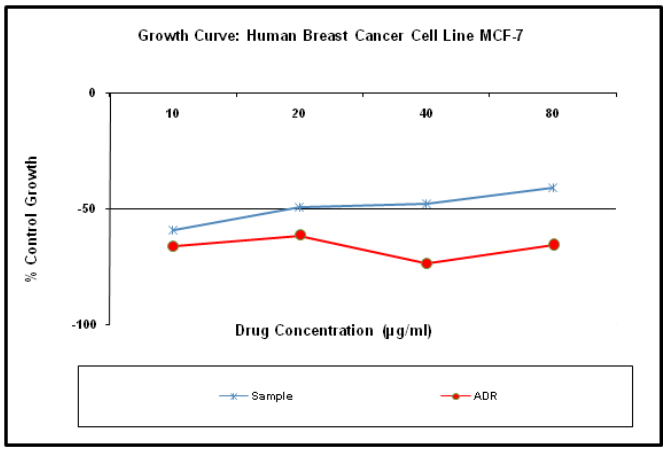

Figure 10. Growth curve: Human Breast Cancer cell line MCF-7

Table 3. Drug concentrations (µg/ml) calculated from graph

MCF-7	Drug concentrations (µg/ml) calculated from graph
	GI50*
Sample	<10
ADR	<10

Where,

GI50 = Concentration of drug causing 50% inhibition of cell growth

ADR = Adriamycin, Positive Control Compound

GI50 value of ≤ 10^-6 molar (i.e. 1µmolar) or ≤ 10µg/ml is considered to demonstrate activity in case of pure compounds. For extracts, GI50 value ≤ 20 µg/ml is considered to demonstrate activity

IV. CONCLUSION

In this study, green synthesis of gold nanoparticles were success fully synthesised by using *Adina cordifolia* plant bark aqueous extract which provides eco-friendly process, less time consuming, an environmentally benign, easy and proficient way for the synthesis of gold nanoparticles. The green synthesised gold nanoparticles were characterized by UV-Visible spectroscopy, FT-IR, XRD, SEM, TEM and their antimicrobial activity was investigated. From UV-Visible spectrophotometer result was confirmed the formation of gold nanoparticles by color changed to ruby red color from pale yellow color indicates the reduction of Au^{3+} ions to Au^{o} . FTIR analysis was confirmed the bending vibrations and stretching bonds present in the sample. The particle size of the metal nanoparticles is 15.3 nm, which was confirmed by XRD and SEM analysis. Spherical in shape and size in the range 8-17 nm of gold nanoparticle was confirmed by TEM analysis. The antibacterial activity for the synthesised gold nanoparticles was confirmed by the antibacterial activity experiment against *Bacillus subtilis* and *Escherichia coli*. Here *Bacillus subtilis* is gram positive and *Escherichia coli* is gram negative bacteria were evaluated and compare to a commercial antibiotic drug Ofloxacin by agar well method and the maximum zone of inhibition was higher in gram positive bacteria compared to gram negative bacteria. The synthesised AuNPs showed anticancer activity against MCF-7 breast cancer cell line. The green synthesised gold nanoparticles proved to be potential candidates for medical application antimicrobial and anticancer activity is highly essential.

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