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BIOSYNTHESIS AND CHARACTERIZATION OF GOLD NANOPARTICLES FROM *CEIBA PENTANDRA* (L.) GAERTN BARK AND EVALUATION OF ITS ANTIBACTERIAL AND ANTICANCER ACTIVITY

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Keywords:

Antibacterial activity, Anticancer activity, *Ceiba pentandra*, Gold nanoparticles, Human pathogens

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ABSTRACT: The present investigation was aimed at synthesis, characterization and *in-vitro* antibacterial and anticancer activities of synthesized gold nanoparticles using bark extract of *Ceiba pentandra* (L.) Gaertn. The synthesized AuNPs were confirmed by a UV-vis spectrometer through observing the surface plasmon resonance peak at 535 nm. Various other techniques, such as Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Selected area electron diffraction (SAED), and High- resolution transmission electron microscopy (HR-TEM) were also employed in the demonstration of the AuNPs properties. The synthesized gold nanoparticles were highly stable, spherical in shape, while other particles had irregular circles without uniform edges with average sizes of 20-48 nm. By using the agar well diffusion method, an attempt was made to evaluate the antibacterial activity of the metal nanoparticles against human pathogenic bacteria. AuNPs showed efficient antibacterial activity, which was found to be more pronounced against gram-negative bacteria than gram-positive ones. Through MTT assay, cytotoxicity effects of the synthesized AuNPs were tested against HCT-116 colon cancer cell lines with an IC_{50} inhibitory concentration of 40 µg/ml.

INTRODUCTION: The outbreak of infectious diseases and the development of antibiotic-resistant pathogens are at an alarming rate, and on top of that it has become a serious health issue since the morbidity and mortality associated with the microbial infections still remains high despite the increased knowledge of microbial pathogenesis and application of modern therapeutics [1].

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Therefore, there is a pressing demand for pharmaceutical companies and the researchers to implement and discover novel strategies through the development of new and effective antimicrobial agents from natural and inorganic substances to control microbial infections [2].

Some strategies have been employed, for instance, the modification of the current antibiotics through the alternation of the molecular structure or even synthesis of new antibiotics [3]. However, the development of new antibiotics, involves high production cost and its time consuming [4]. Similarly, cancer treatment and management are being faced up with many hindrances, for instance, multiple drug resistance, less drug reaching the

tumor site, nonspecific systemic distribution of antitumor agents, high toxicity, and intolerable cytoxicity [5-7]. The most devastating factor about cancer is that the death rate is still on the rise, and there is no significant improvement in the past years, even though there are so many advanced high- tech solutions towards combating the disease.

For instance, according to the statistical data in the year 2015 in India concerning colon cancer, 12,483 males and 15,205 females were diagnosed. There is a prediction that by the year 2020, the numbers would skyrocket to 13,420 and 19,013 cases, respectively [8]. Therefore, this implies that great efforts are required in order to overcome both bacterial infections and cancer in order to increase patient survival, for instance, through the improvement and designing of new strategies, tools, and drugs [9]. Previously researches have proved out that gold nanoparticles have unique physical and chemical properties, such as biocompatibility, stability, controllable size dispersity, nontoxic, and strong adsorbing capacity and hence can be used in a wide range of biomedical applications [10-15].

The fight towards the human pathogenic infections and antibiotic resistance menace has been achieved and proven through the conjugation of the various metal nanoparticles with the antibiotics, gold nanoparticle, in particular, has been reported to enhance antimicrobial activity on vancomycin-resistant enterococci when treated with vancomycin coated gold nanoparticles [16]. Similarly, gold nanoparticles fabricated with plant materials as described out by various researches, have also proven to have a strong antimicrobial effect on a wide range of Gram-positive and Gram-negative bacteria [17, 18].

Additionally, AuNPs have shown to possess vital abilities for instance, selectively recognizing cancer cells and enhance apoptotic effect [19]. Another study by Brown *et al.*, through combination of AuNPs nanobioconjugates of PEG linked with Oxaliplatin, significantly suppressed cytotoxicity and increased its ability to penetrate tumor cells [20].

Nanoparticles can be synthesized by different methods physical, chemical, and biological [21-23]. However, the physical and chemical methods are expensive, use toxic solvents, which are hazardous and make them unsuitable for biomedical applications [24]. Therefore, there is an increasing demand to develop environmentally feasible, low-cost, energy-efficient, and high-yield procedures for their synthesis. So, the biological approaches (bacteria, fungi, yeasts, and plant extract) for synthesizing nanoparticles become more preferred alternative synthesis procedure [25].

Ceiba pentandra, also known as silk-cotton, belongs to a family of Bombacaceae [26]. This plant has been used in traditional medicine for the treatment of several ailments such as antifungal [27], and diabetes [28], antibacterial [29,] and anticancer [30]. The present investigation was aimed at synthesis, characterization, and antibacterial activities of gold nanoparticles synthesized using bark extract of *C. pentandra* against human pathogens.

MATERIALS AND METHODS:

Collection and Identification of Plant Material: The bark of *Ceiba pentandra* (L.) Gaertn was collected from Ellispettai, Erode, Tamil Nadu, India, and the specimen were identified and authenticated by the Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2018/Tech/2733). The specimens were stored in the Department of Biochemistry, Bharathidasan College of Arts and Science, Erode, Tamil Nadu, India.

Preparation of Plant Extracts: The bark of *Ceiba pentandra* sample was collected, cleaned, shade dried, and made into a coarse powder. 10 grams of each plant sample was immersed in 200 ml of different types of solvents of increasing polarity (petroleum ether, ethyl acetate, acetone, and ethanol). All samples were left at room temperature for three days. Then, samples were filtered using filter paper and concentrated using a vacuum rotary evaporator at 80 °C prior to drying process. Crude extracts were collected until thick and viscous paste or powder of extract is visible and stored at -20 ºC.

Synthesis of Gold Nanoparticles: Gold nanoparticles were synthesized by adding 5 ml of ethanol extract of *Ceiba pentandra* bark to 45 ml of 1mM $HAuCl_4 \cdot 3H_2O$ solution at room temperature. The reduction process was incubated for various time points and monitored by UV-visible absorption spectroscopy from day 1 to day [30].

The change in color of the solution from pale yellow to ruby red indicated the reduction of Au^{3+} ions to Au0. Gold nanoparticles were obtained by centrifuging the solution at 10,000 rpm for 30 min followed by repeated washing with water three times, and the lyophilized powder was stored for characterization.

Characterization of Synthesized Gold Nanoparticles: The spectral response of synthesized AuNPs was studied using a UV–visible spectrophotometer (Shimadzu UV-1800) in the range of 300-700 nm. Fourier transform infrared spectroscopy (FTIR) results were obtained from a Jasco 6300 spectrometer (ATR mode) in the range of 400–4000 cm^{-1}. The morphological studies, including size, shape, and distribution, were analyzed using HR-TEM, and selected area electron diffraction (SAED) (JEOL JEM-2000 EX microscope) patterns with an accelerated voltage of 200 KV. Powder X-ray diffraction (XRD; Rigaku, Miniflex-600, Japan) was performed using an X-ray diffractometer–Cu Kα radiation system to determine the structure of synthesized nanoparticles.

Antibacterial Activity:

Microorganisms used for the Study: The human pathogens microbial strains were acquired from the Institute of Microbial Technology [IMTECH], Chandigarh. Antibacterial activity was carried out for the biosynthesized AuNPs by the Agar-well diffusion method against human pathogens Gram-positive *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus* (MTCC-96) and Gram-negative *Pseudomonas aeruginosa* (MTCC-741) and Escherichia coli (MTCC-724) bacterium.

Antibacterial Screening of Biosynthesized AuNPs: Four bacterial strains - *Bacillus subtilis* MTCC-441, Staphylococcus aureus MTCC-96, *Pseudomonas aeruginosa* MTCC-741, and *Escherichia coli* MTCC-724-were inoculated into the nutrient broth under aseptic conditions and incubated at 37 ºC for 18 h.

After the incubation period, the bacteria to be tested were swabbed on the nutrient agar plate using a sterile cotton swab. Each bacterium was swabbed separately, and each plate is divided into five parts. Wells (6 mm) were made on a Muller Hinton Agar plate using a sterile cork borer under aseptic condition and were added with 25 µl, 50 µl, 75 µl and 100 µl of each extract solution, separately to each well. All the plates were incubated at 37 °C for 24 hr. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in mm). Ciprofloxacin was used as an antibacterial standard against all pathogens. Experiments were carried out in triplicates [31].

Anticancer Activities of Synthesized AuNPs on HCT-116 Cells: The estimation of the potential cytotoxicity effect of the synthesized AuNPs on cancerous HCT-116 cell lines was done by MTT assay as described by Mosmann [32]. HCT-116 cells (1×10^4) were seeded in a 96-well plate. In an increasing concentration of (5-100 µg/ml) the cells were treated with CP-AuNPs and followed by incubation for 24 h at 37 ºC in the presence of 5% CO_2 in an incubator. MTT solution (5 mg/ml) was added to the treated cell and further incubated at 37 ºC for 4 h after which the formazan crystals were dissolved by adding 200 µl of DMSO. The viability of the cells was carried out by using a scanning multi-well microplate reader at 570 nm. Phase-contrast microscopy was used to analyze the morphological changes in the cells after treatment with AuNPs. HCT-116 cells were exposed to AuNPs at 5–100 µg/ml for 24 h.

RESULTS AND DISCUSSION:

Color Change and UV–vis Spectroscopy: The present study attributes towards the synthesis of plant-mediated metallic nanoparticles as one of the viable substitutes for combating human pathogens. In the present study, the color change from pale yellow to ruby red, as shown in **Fig. 1A** was the first initial confirmation indicating the reduction of Au^{3+} ions to Au0 due to excitation of surface plasmon vibrations in AuNPs from *Ceiba pentandra* bark extract [33]. The reduction process was incubated for various time points and monitored by UV-visible absorption spectroscopy from day 1 to day [30]. CP- AuNPs showed a maximum peak at 535 nm after 30 days as shown in **Fig. 1B** was indicating the completion of the reduction process, which amplifies out that the increase of reaction time between the gold ions and biological reductant, lead to the synthesis of stable plasmonic peaks. Similar results were obtained by [34].

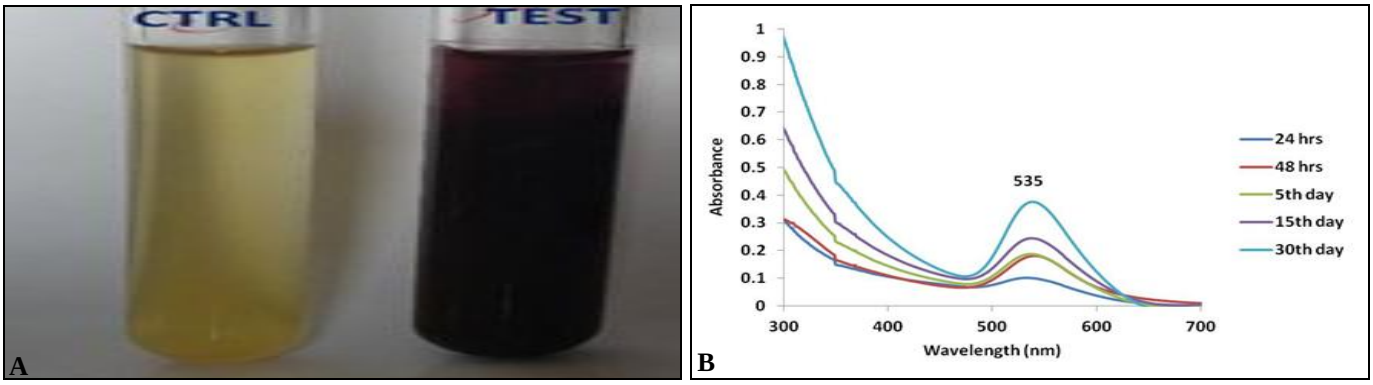


FIG. 1: SYNTHESIS OF AUNPS USING *C. PENTANDRA* BARK EXTRACT. : THE FIGURE REPRESENTS AuNPs EXHIBITING COLOR CHANGE FROM PALE YELLOW TO REDDISH BROWN DUE TO EXCITATION OF SURFACE PLASMON VIBRATIONS, INDICATING THE FORMATION OF AuNPs. (B) UV–VIS SPECTRA OF AUNPS

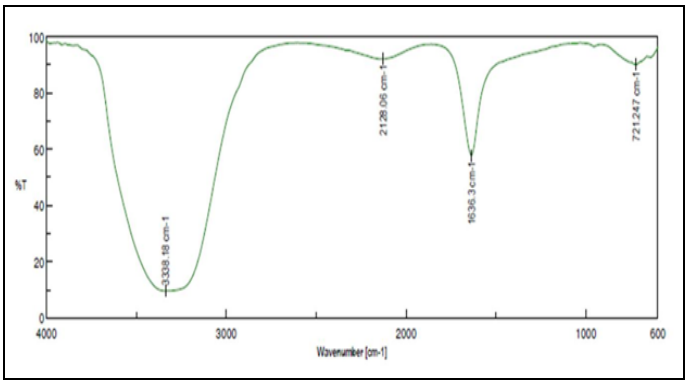


FIG. 2: FT-IR SPECTRUM OF *CEIBA PETANDRA* GOLD NANOPARTICLES

Characterization of CP-AuNPs:

FTIR Studies: The FTIR spectroscopy was used to identify the major compounds that are responsible for the biological reduction of Au^{3+} ions to Au0 from the bark extract of *C. pentandra*. The IR spectrum of gold nanoparticles **Fig. 2** showed a distinct peak in the range of 3336, 2128, 1636, and 722 cm^{-1} representing the presence of capping molecules with the nanoparticles. The FTIR a major peak, was obtained at 1,636 cm^{-1} indicating the presence of amide-I and amide II bonding from capped peptides; Annamalai *et al.*, reported similar results [35].

HR-TEM Analysis: HR-TEM analysis, as shown in **Fig. 3**, clearly illustrated that the shape and morphology of AuNPs which were variable shapes. Some particles were spherical while other particles had irregular circles without uniform edges.

The average sizes of the particles were found to be 20-40 nm. These differences in shape and size of nanoparticles synthesized by biological systems are common [36, 37].

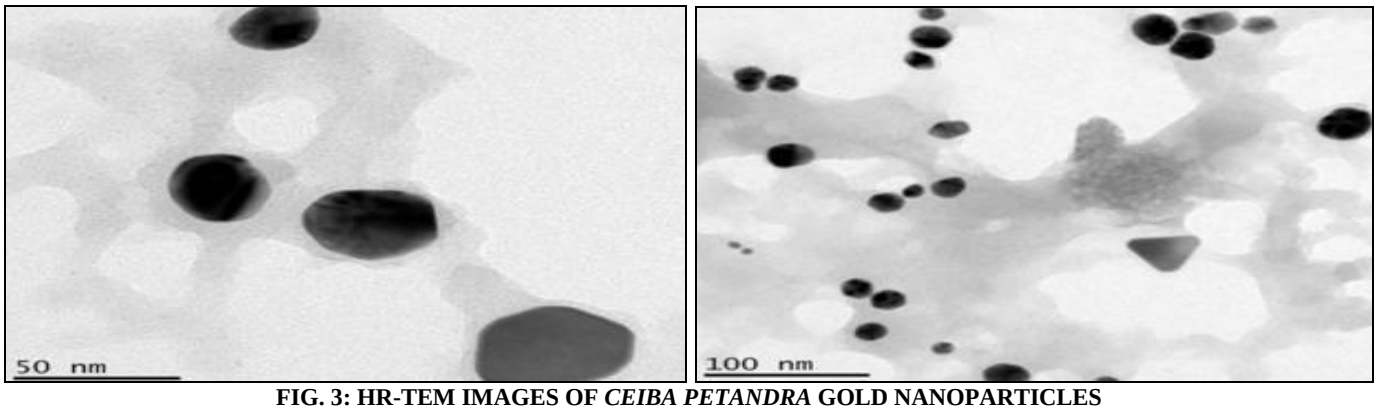


FIG. 3: HR-TEM IMAGES OF *CEIBA PETANDRA* GOLD NANOPARTICLES

XRD Studies and SAED Analysis: The XRD spectrum of the *C. pentandra* AuNPs, as shown in **Fig. 4** confirmed that the NPs formed were in the form of nanocrystals. The AuNPs, demonstrated the peaks at 2θ values corresponding to [111], [200] and [220], three important peaks related to their standard Bragg reflections. The diffraction peaks obtained by Scherrer's equation were at 38.41 [111], 44.40 [200], and 67.57 [220]. The crystalline nature of AuNPs was also confirmed by SAED **Fig.**

5. In this study, we observed that bright circular pattern rings in the nanoparticles. This might be the reflection from the lattice planes of crystalline Au nanoparticles. These similar results have been previously reported [38].

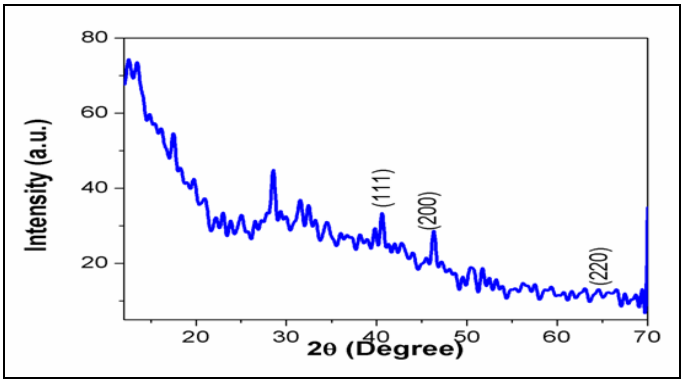


FIG. 4: XRD PATTERNS OF SYNTHESIZED GOLD NANOPARTICLES USING BARK EXTRACT OF *CEIBA PENTANDRA*

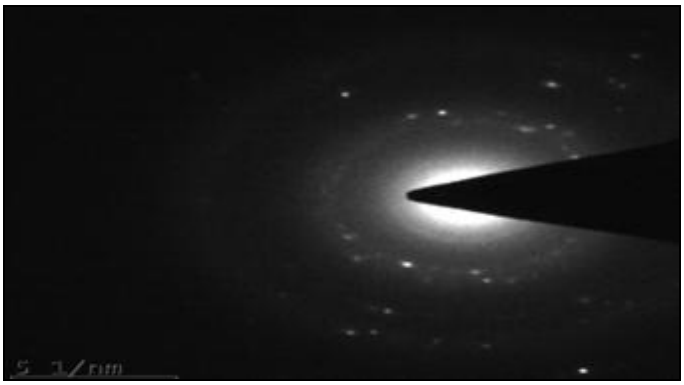


FIG. 5: SAED OF *CEIBA PENTANDRA* GOLD NANO-PARTICLES

Antibacterial Activity of AuNPs: The results of our study showed that the synthesized AuNPs had a significant inhibiting activity on all four bacteria in tested concentrations **Fig. 6**. Among the gram-positive organism, *B. subtilis* was found to be more sensitive with the inhibition of 12.7 mm, while *S. aureus* was slightly less sensitive with 12.5 mm. There was no zone of inhibition for *S. aureus* at a concentration of 25 µl. Further from the study, it was observed that among gram-negative organisms, *P. aeruginosa* was found to be more sensitive with the inhibition of 12.2 mm, and *E. coli* was slightly less sensitive with 10.3 mm as shown in **Table 1**.

Previous researches have shown out gold nanoparticles containing well-defined chemical stability and being with a smaller size than the bacteria penetrate easily into the bacterial organism [39]. From our results, gram-negative bacteria had a higher zone of inhibition in comparison to gram-positive, since gram-negative posses a thin peptidoglycan cell wall [40].

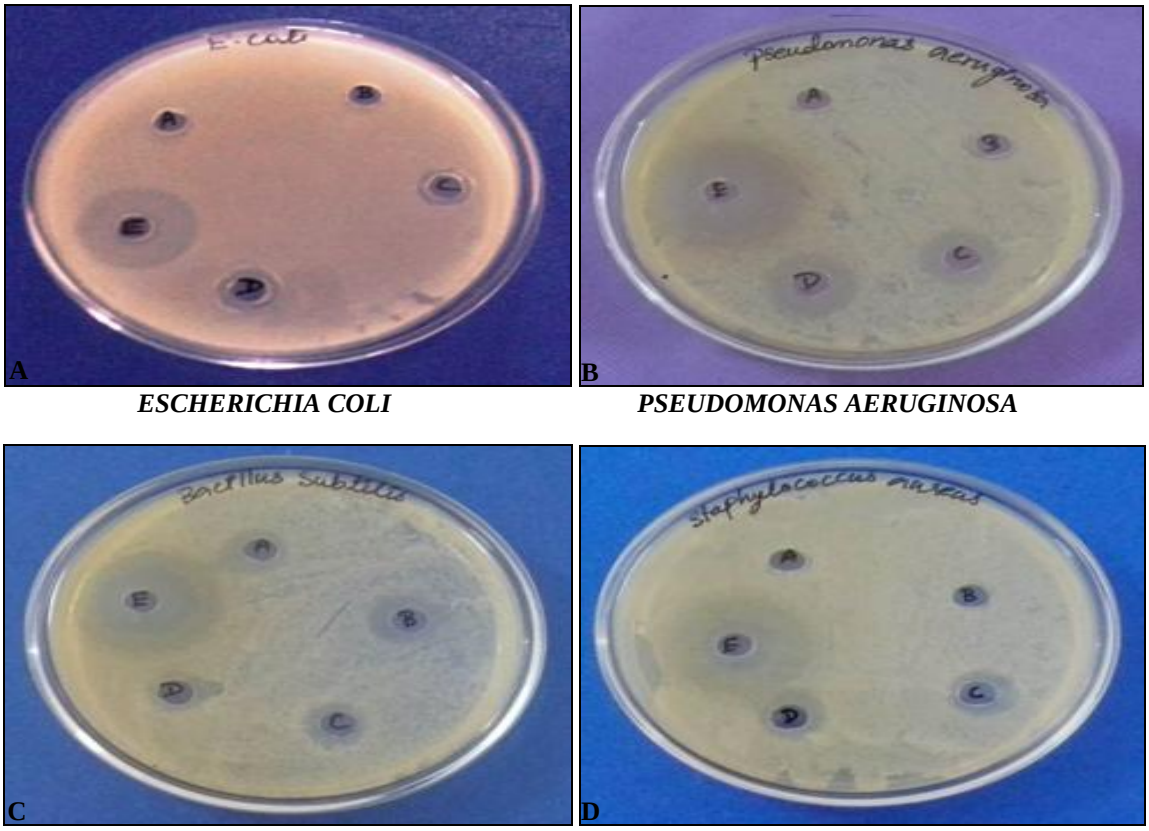
The exact mechanism is still unknown, though some researchers have proposed out for instance, that the reaction between gold ions and SH groups of protein leads to bacterial inactivation [41]. The ability of AuNPs to bind and penetrate the bacterial cell wall, hence attacking the respiratory mechanism, leading to cell division, thereby causing cell death, might be another factor [42].

TABLE 1: GOLD NANOPARTICLES AGAINST HUMAN PATHOGENS

	Zone inhibition (mm in diameter)				
Bacterial strains	**25**	**50**	**75**	**100**	**Standard Ciprofloxacin (5 µg)**
Escherichia coli	--	--	8.2±0.11	10.3±0.42	17.8±0.43
Pseudomonas aeruginosa	--	8.6±0.34	9.4±0.25	12.2±0.36	23.5±0.62
Bacillus subtilis	8.1±0.85	9.1±0.18	10.1±0.16	12.7±0.51	19.6±0.54
Staphylococcus aureus	--	9.3±0.25	11.2±0.09	12.5±0.48	18.9±0.35

Values were expressed in mean ± Standard deviation. -- : No zone of inhibition

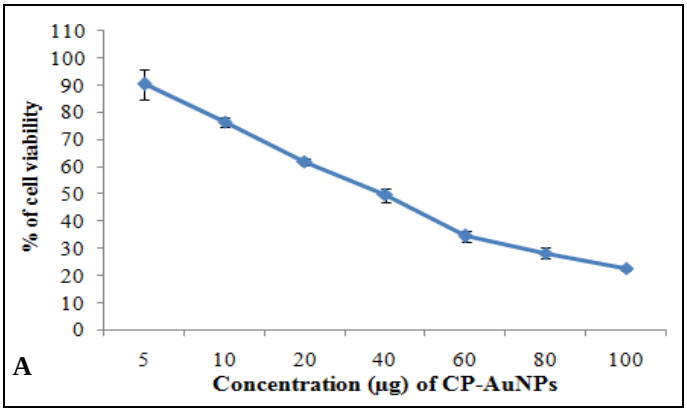
Effect of AuNPs cytotoxicity on HCT-116 Cells: Through MTT assay, the cytotoxic effect of the synthesized AuNPs, at different concentrations of (5-100 µg/ml) on HCT-116 cell lines were confirmed. After 24 h incubation variations were observed between the control and treated cell lines. The IC_{50} inhibitory concentration was found to be at 40 µg/ml **Fig. 7A**. Therefore, for this study, 20 µg/ml and 40 µg/ml concentrations were selected for the optimal treatment for the other anticancer studies. Similar studies to this were carried out by various researchers using gold nanoparticles synthesized from various plant extracts and different cell lines [43, 44]. There was no significant change observed in the morphology of control HCT-116 cells. The control cells appeared in normal shape and were attached to the surface **Fig. 7B**. However, the HCT-116 cells exposed to AuNPs showed morphological changes, including cell shrinkage and formation of apoptotic bodies, which were due to the cytotoxic effects and the ability of the synthesized nanoparticles to induce stress hence leading to cell death [45, 46].

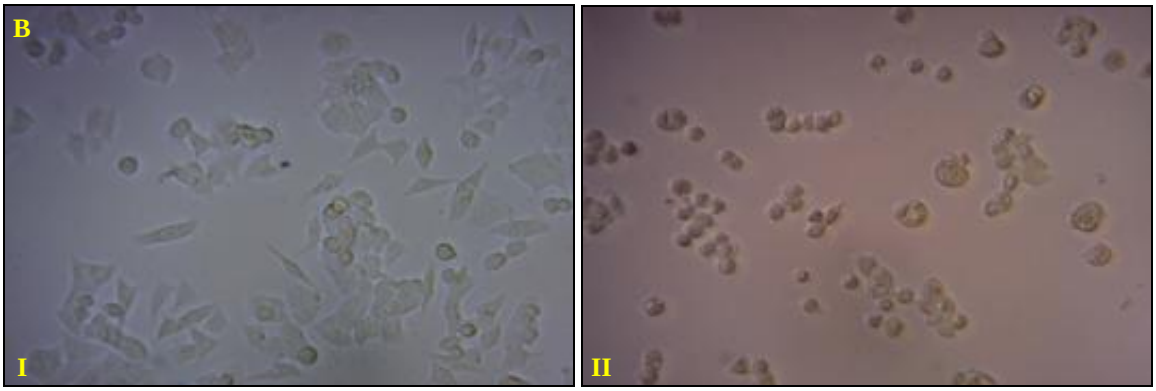


ESCHERICHIA COLI ***PSEUDOMONAS AERUGINOSA***

BACILLUS SUBTILIS ***STAPHYLOCOCCUS AUREUS***

FIG. 6: ANTIBACTERIAL ACTIVITIES OF *CEIBA PETANDRA* GOLD NANOPARTICLES AGAINST HUMAN PATHOGENS A- 25 µl; B- 50 µl; C- 75 µl;D- 100 µl; E- Positive





CONTROL **40 µg/mL CP-AUNPS**

FIG. 7: (A) CYTOTOXICITY POTENTIAL OF CP-AuNPs (5-100 µG/ML) ON HCT-116 CELLS USING MTT ASSAY. ALL DATA WERE EXPRESSED IN MEAN ± SD OF THE THREE EXPERIMENTS. (B) MICROSCOPY IMAGES SHOWING THE EFFECT OF CP-AuNPs MORPHOLOGICAL CHANGES IN HCT-116 CELLS. (I) CONTROL (II) 40µg/mL OF CP-AuNPs

CONCLUSION: The present study attributes towards the synthesis of plant-mediated gold nanoparticles as one of the viable substitutes for combating human pathogens and cancer activities. The results obtained in the present investigations are promising enough for future studies to elucidate the exact mechanisms responsible for the antibacterial and anticancer activities.

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