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Synthesis of gold nanoparticles using hibiscus and curcumin extractions and their anticancer activities

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Synthesis of gold nanoparticles using hibiscus and curcumin extractions and their anticancer activities

Abstract

Gold nanoparticles (Au-NPs) have shown multiple applications, whereby particles are usually prepared by chemical routs. In this study, we syntheized the Au-NPs by employing the eco-friendly non-chemical rout using Hibiscus and Curcumin extractions as reducing and stabilizer agents, and investigated their anticancer activities. The bonding, structrure and morphology of the prepared Au-NPs were studied by spectroscopy, and electron microscopy techniques. Ultraviolet-visible spectroscopy analysis confirms the characteristics absorption peak of gold for both specimens. Electron microscopy (SEM and TEM) analyses showed that the particles were predominantly spherical in shape. The particles were well dispersed when they were prepared with Hibiscus extraction with average size ~ 13 nm. An interesting morphology was observed for the specimen which was prepared with curcumin, where particles were found interconnected to each other (size ~18 nm). The anticancer cell activity of the as-synthesized Au-NPs was studied against human colorectal carcinoma cells (HCT-116) and breast cancer cells (MCF-7). It was observed that the treatment of cancer cells with Au-NPs decreased the number of cells significantly as compared to control cells. The Au-NPs -Hibiscus specimen showed the better inhibiting properties than Au-NPs -Curcumin, which is attributed to their uniform dispersion and small size.

Keywords: gold nanoparticles, green synthesis, hibiscus, curcumin, anticancer

1. Introduction

The field of nanotechnology is a multidisciplinary science, design and innovation at nanoscale, extending from 1 to 100 nm [1]. Nanoparticles, the materials between 1-100 nm can be connected with biological systems by means of diverse strategies, depending on the cell sort and targeting different organelles, through utilizing their surface properties and structures, the molecule measure and state of accumulation [2].

Among the different sort of nanomaterial applications within the field of medication and science, gold nanoparticles (Au-NPs) show significant future promise. Recently, Au-NPs and their extraordinary features have been broadly examined for their productive part within the development of biomedicine, principally due to their special biological, natural and physico-chemical properties that can be easily manipulated at a size less than 100 nm. The nanoscale structures, size, shape, and coating of Au-NPs are the most important parameters in nanoparticles properties due to their effect on particle interaction and surface-to-volume ratio changes [3]. The electronic structure of Au-NPs offers a large number of opportunities for their utilize in clinical applications, specifically in radiotherapy and radiography fields [2].

The Au-NPs have gained considerable attention in nanomedicine owing to high biocompatibility and expanded its applications which include biomedical imaging, radiation dose enhancement, biosensor, detection of human pathogens, gene transfer, nucleic acid labelling, therapeutic agents, sensing agents, drug delivery, antibacterial activity, and as molecular theranostics [2, 4].

Generally, there are two essential techniques of synthesizing nanoparticles, either top-down or bottom-up approach. They can be also categorized into chemical, physical and biological methods [5]. Recent endeavors have been committed to controllable processes including

morphology, structure, size, solubility, stability and functionality [6]. The conventional chemical preparation routes of Au-NPs often include the usage of toxic chemicals materials and costly process, such as sonochemical, sol-gel, hydrothermal, etc, which raise environmental hazard and render its clinical applications despite its attractive properties [4, 7]. On the other hand, biosynthesises of Au-NPs can be achieved by employing either microorganisms (fungi, bacteria, actinomycetes, yeast, etc.) or plant tissues (root, leaf, pectin, stem, seed, peel, fruit, flower, etc.). This type of synthesis gains more significance interest due to its simplicity, and quick production of nanoparticles [8]. Amongst alternative methods, plant extracts are simpler for mass-production applications, evinces to be eco-friendly, relatively cheaper and safe for humans due to biogradeable organic agents [9]. Hibiscus leaf extraction was utilized to synthesis Au-NPs of several forms and morphologies with average particle diameter around 13 nm [8]. A green approach to synthesize Au-NPs applying curcumin acting as reducing and stabilizing agents at room temperature is reported. Through varying the loading of curcumin and its derivatives, the size and the dispersity of Au-NPs can be controlled [10].

The plant extracted Au-NPs the were investigated in the area of medical, such as utilized as an antioxidant, antibacterial agents, antimicrobial and anticancer proliferations [11]. Due to their significant quenching efficiencies, surface modifiability and biocompatibility, Au-particles have become exceedingly doable materials for tumor treatment and for the purpose of inventive systems for cancer therapy [12]. In addition, Au-NPs could be used for diagnostics (theragnostic) and combined therapy, such as radiation sensitizers, photo thermal ablation of tumors and real time imaging, owing to their multifunctional properties [12].

The size, distribution, surface modification and morphology of the Au-NPs couls effect on their antibacterial properties [13]. For example, by some preparation methods, the molecules can be

associated with the surface of Au-NPs to create special biological activity [14]. In this study, Au-NPs particles are syntheized using hibiscus and curcumin extractions, and evaluate their anticanter activities against: (1) human colorectal carcinoma (HCT-116) and (2) human breast adenocarcinoma cells (MCF-7). The chemical bondings, optical properties and morphological features of the prepared Au-NPs are characterized using FTIR, UV-Vis spectroscopy, SEM/EDS and TEM/SAED techniques. The anticancer capabilities of the prepared Au-NPs are tested against HCT-116 and MCF-7cells using morphometric and bioassay. The important morphological features of the treated and untreated cells are examined under inverted microscope and calculate their cell viability.

2. Experimental Details

2.1. Synthesis of Au-NPs using Hibiscus and Curcumin extractions

Sample 1 (Au-NPs – Hibiscus specimen): prior to make the powder, the commercially available Hibiscus sabdriffa (Roselle) flowers in dried state were washed, and rinsed several time with distilled water to get rid of dust or other contaminants if any. The washed flowers were then dried in an oven for two hours at temperature around 60 ^{o}C. The dried flowers were ground with a blender for making the fine powder. Took 5 grams of fine power and added 100 ml water to the powder. Double-ditilled water (DD-H_2O) was used throughout the experiments for the synthesis of Au-NPs. The powder/water mixture was then boiled, stirred for 10 minutes and filtered using filter paper (Whatmann filter paper No.1). Now, 0.4 gram of chloroauric acid ($HAuCl_4.3H_2O$) powder was dissolved in 100 ml of DD-H_2O in order to prepare 10 mM of $HAuCl_4.3H_2O$ solution. The prepared extract was diluted four times, 100 ml from the diluted extract was placed in an Erlenmeyer flask and added 120 ml gold salt solution into it. The extract/gold salt mixture

was heated in a Microwave oven of 1000 W for 20 sec. The immediate colour change of the solution to purple was observed. The colour change is an indication for the formation of Au-NPs. The pictorial representation of the preparation of Au-NPs using Hibiscus extract, referered as Au-NPs -Hibiscus (sample 1) is depicted by ***Figure 1a***.

Sample 2 (Au-NPs-Curcumin): The turmeric extract was prepared by adding 25 mg of dried powder bought from a local supermarket to 100 ml of water and vortexed for 5 minutes Note: double-ditilled water (DD-H_2O) was used throughout the experiments for the preparation of Au-NPs using curcumin. The solution was left untouched for10 minutes to allow undissolved turmeric to precipitate and then filtered in a similar way as described above for sample 1.To prepare a 10 mM of $HAuCl_4 \cdot 3H_2O$ solution, 0.3958 g of $HAuCl_4 \cdot 3H_2O$ was dissolved in 100 mL of DD-H_2O and vortexed for 3 minutes at a medium speed. To synthesise the gold nanoparticles (Au-NPs), 10 ml of the 10 mM $HAuCl_4 \cdot 3H_2O$ solution was added to the 3 .5 ml turmeric solution in a clean sterilized Erlenmeyer flask. The solution was then heated for 20 seconds in a microwave oven of 1000 W. An alteration (change) in the color was observed, the solution became dark purple. The colour change in an indication of reduction reaction which confirmed the formation of Au-NPs. The pictorial representation of Au-NPs preparation using Curcumin extract, the specimen referered as Au-NPs -Hibiscus (sample 2) is depicted by ***Figure 1b.*** Further characterizations of the prepared Au-NPs-hibiscus and Au-NPs-curcumin specimens are carried out using widely used tools, such as SEM/EDX, TEM/SAED, FTIR and UV-Vis spectrometry before performing the anticancer experiments.

2.2. SEM and TEM characterization

The morphology and the structure of the colloidal nanoparticles (Au-NPs -Hibiscus and Au-NPs -Curcumin) was evaluated using scanning electron microscopy (SEM) (Model: Inspect S50, FEI, at 20 kV). For SEM samples, the colloidal nanoparticles of each sample were dropped onto the SEM metallic stubs covered with conducting doubled sided tape. The prepared stubs were air-dried and transport into the SEM chamber for examination. The SEM micrographs were taken at two magnifications, namely x50, 000 and x100, 000. In addition, transmission electron microscopy (TEM) (Model: Morgagni 268, FEI, at 80 kV) was used at 80 kV in bright-field imaging mode for detailed morphology and structure of the prepared Au-NPs . The size histograms were drawn by untilizing the particles as measured their sizes from the TEM images. The crystalline structure of Au-NPs -Hibiscus and Au-NPs -Curcumin specimens were investigated by SAED patterns (selected area electron diffraction pattern), which were taken in the TEM. The detailed description of SEM and TEM method is given elsewhere[15, 16]. For detailed morphology and structure,

2.3. FTIR and UV-Vis spectroscopy characterization

The functional groups of the absorption bands of the specimens (Au-NPs -Hibiscus and Au-NPs -Curcumin) prepared using Hibiscus and curcumin were studied by Fourier transform infrared spectroscopy (FTIR: Nicolet 6700 with FTIR spectrometer, specetra range 400- 4000 cm^{-1}). The spectra were taken. For clarity, the close-up spectra of the Au-NPs prepared using Hibiscus and curcumin extracts were also shown between the wavenumner of 400 – 1200 cm^{-1}. The optical properties of Au-NPs-Hibiscus and Au-NPs -Curcumin specimens were studied using ultraviolet-

visible (UV-Vis) spectroscopy (Double-beam Shimadzu UV-1800 spectrophotometer). The absorption spectra of the specimens were taken between 350-800 nm with 1 nm resolution.

2.4. Anticancer characterization

2.6.1 Treatment of Au-NPs -Hibiscus and Au-NPs -Curcumin: Two cancer cell lines: HCT-116 (human colorectal carcinoma) and MCF-7 (human breast adenocarcinoma cells) were taken to study the impact of Au-NPs on the cell viability. The cells of both the cell lines were cultured by applying the method as reported in our previous reports [29-31]. In this method, the cells were cultured in the cultured media using 96-well plates in a CO_2 incubator. The chemicals in the cultured media are mentioned in our previous experiments. The grown cells were then treated with prepared products; Au-NPs -Hibiscus and Au-NPs -Curcumin for 48 hours. In the control group, Au-NPs -Hibiscus and Au-NPs -Curcumin were not added. To examine the specificity of the Au-NPs -Hibiscus and Au-NPs -Curcumin specimens, non-cancer human cell line such as, HEK-293 (embryonic kidney cells) were also included in our studies.

2.6.2 MTT assay: After 48 hours, the cells were processed for the MTT assay as per previous studies [24-27]. Both control, and Au-NPs -Hibiscus and Au-NPs -Curcumin -treated groups were treated with 20 µl of MTT, and treated cells were then further incubated. After 4 hours incubation in CO_2 incubator, the cell culture media was changed with 1% of DMSO. The cell-well plates of both treated cells, HCT-116 and MCF-7 were analyzed under ELISA plate reader (Model: Biotek Instruments, United States of America). Thereafter, the cell viability was find out for the staistics analysis using the following simple relation (1).

$$Cell\ viability\ (in\ \%) = \left[\frac{(\text{Optical density})_{\text{treated cells with Au–NPs}}}{(\text{Optical density})_{\text{untreated cells}}}\right] x\ 100 \qquad (1)$$

2.6.3 Cancer DNA staining: DNA of the cancer cells was studied using DAPI (4′,6-diamidino-2-phenylindole) staining assay [25-27]. In this assay, the grown cells were distributed into two groups: (1) control grou, where treatment of cells was not done with any nanoparticles and (2) experimental group, where cells were treated with Au-NPs -Hibiscus (0.8 µg/ml) and Au-NPs -Curcumin (0.8 µg/ml) in two separate batches. After 48 hour treatment, both groups (treated and untreated) were placed in the ice-cold paraformaldehyde enviroment and then with Triton X-100 in phosphate buffered saline (PBS). Thereafter, the cells were stained with DAPI staining chemicals for 5 min under no-light environment. The cells were finally washed with PBS and cover slipped. In order to find the effect of the nanoparticles on the cancerous cells, the morphology of the treated and untreated cells was visulized under confocal scanning microscope (Model, Zeiss, Germany) after DAPI staining.

2.6.4 Statistical analysis: The otanied data was displayed in the form of mean (±), standard deviation (SD) from triplicate experiments. The statistical data was obtained by suing the one way ANOVA and Dennett's post hoc test for statistical discussion.

3. Results and Discussion

3.1. Chemical, physical and optical analyses

FTIR spectroscopy was carried out to study the active functional groups available in the samples, the results of FTIR are shown in ***Figure 2a***. The broad bands at ~3203 and ~3314 cm^{-1} are due to O-H stretching bond, for Au-NPs -Hibiscus and Au-NPs -Curcumin, respectively [17]. The

peaks observed at approximately 2920 and 2850 cm^{-1} are due to the C-H stretching mode [18]. For Au-NPs -Hibiscus, the strong peak at 1646 cm^{-1} represents the vibrational modes of C=C, which becomes small and shifts to low wavenumbers for Au-NPs -Curcumin specimen. Within the zoomed spectra (1200 – 800 cm^{-1}) (***Figure 2b***), at 1069 cm^{-1} and 1018 cm^{-1} two additional peaks can be assigned to the C-O stretching frequency [19]. The intensity of the peaks reduced obviously for Au-NPs-Curcumin and shifted to low wavenumber.

The formation of the colloidal Au-NPs was judged by colour change within the solution which changed from light yellow to purple-red immediately after heating the reactants in the microwave. The growth of Au-NPs was further verified by conducting the UV-Vis spectroscopy and analyzing the surface plasmon resonance (SPR) peak for both the prepared solutions, Au-NPs -Hibiscus and Au-NPs -Curcumin. ***Figure 3*** displays the UV-Vis spectroscopy results of aqueous dispersion of Au-NPs-hibiscus and Au-NPs-curcumin. For Au-NPs -Hibiscus, the spectra illustrates a sharp SPR peak at ~ 520 nm, while a strong but relatively a broad peak at ~ 546 nm for Au-NPs using Curcumin [20–22]. The SPR peak in the spectra of Au-NPs is produced by oscillation of surface electrons, causing a strong excitation of light. The appearance of a single absorption peak suggested that the colloidal Au-NPs are nearly spherical [23, 24]. The Au-NPs synthesised with curcumin shows a broad absorption with a redshift (about 15 nm) compared to nanoparticles synthesised with Hisbiscus extraction which shows the sharp and narrower SPR peak. The redshift of SPR is due to the size (crystallites) increase of the gold nanoparticle as confirmed by TEM as shown below.

3.2. Morphological, elemental and structural analyses

The morphology and structure of the syntheiszed gold nanoparticles (Au-NPs) using Hibiscus and Curcumin extraction were analyzed by SEM, EDS and TEM. ***Figure 4*** shows the SEM and EDS results of the gold nanoparticles of sample 1 (Au-NPs -Hibiscus) and sample 2 (Au-NPs - Curcumin) specimens. Sample 1 was prepared by adding of Hibiscus extraction into 10 mM of $HAuCl_4.3H_2O$ solution. A colour change of the solution was observed upon heating the mixture into a Microwave oven, indication of the formation of gold nanoparticles. Similarly, sample 2 was prepared by using curcimin exraction and $HAuCl_4.3H_2O$ solution. Both the samples exhibited a compact mprphology, confirming the presence of particles in the range of nanometer. The electronic images showed that the layer structure or sheet like morphology composed pf individual colloidal nanoparticles. However, the resolusion of the SEM is limited to identify the size, shape and structure of the nanoparticles. EDS spectra showed the obvious peak for the gold, near to 2.4 keV, confirming the successful preparation of Au-NPs. Furthermore, TEM was carried out to reveal the size, shape and structure of the colloidal specimens at high resolution. By TEM images as shown in ***Figure 5***, it can be seen that the particles displayed the perfect spherical shaped morphology. It was noted that particles were well dispersed on the support film in case of sample 1. In the case of sample 2, the particles were interconnected in the form of chain upto several nanoparticles. A slightly smaller size was found for specimen 1 as compared to specimen 2, the average particle size was estimated ~13 and 18.3 nm, respectively (***Figure 5c & f***). SAED analysis further confirms the nature of the particles, particles in both cases showed the crystalline structure as suggested by well-separated diffraction rings (signature of crystalline structure) with first four lattice planes were indexed as: (111), (200), (220) and (311), indicating face centered cubic (fcc) structure of gold (JCPDS: 04-0784) (***Figure 5b & e***) [25, 26].

SEM/EDS and TEM/SAED analyses confirmed the formation of gold nanopartilces suing Hibiscus and curcumin extraction.

3.3. Anticancer activities of Au-NPs

3.3.1. Impact of Au-NPs on cancer cells viability

The treatment of Au-NPs -Hibiscus and Au-NPs -Curcumin caused significant decreased in the number of cancer cells, as the number of DAPI stained cells were found to be significantly less in the Au-NPs -Hibiscus and Au-NPs -Curcumin -treated cells as compared to control cells (***Figure 6***). The decrease in the cancer cells for the treated specimens are due to the cell death which are due to systematic cell death (loss of life), also known as apoptosis. There are several reports available which suggested that treatment of nanoparticles caused the loss of cancer cells due to programmed cell death [27–29].

The impact of Au-NPs (Hibiscus) and Au-NPs (Curcumin) on HCT-116 and MCF-7 was invetigatged using cell vialibilty assay. The results of the cell viability assay are represented in ***Figure 7***. The results of this assay displayed a substantial decrease in the cell viability after the treatments of Au-NPs -Hibiscus and Au-NPs -Curcumin. However, Au-NPs -Hibsicus is better in inhibiting the cancer cells as compared to Au-NPs -Curcumin as observed by ***Figure 6b & e***. It was observed that the inhibitory response on cancerous cells was dose-dependent in both Au-NPs -Hibiscus and Au-NPs -Curcumin specimens. We have also calculated the IC_{50} for both Au-NPs -Hibiscus and Au-NPs -Curcumin specimens, and found that IC50 for Au-NPs -Hibiscus was 0.04 µg/ml; whereas for Au-NPs -Curcumin was 0.6 µg/ml. In addition, the impact of Au-NPs -Hibiscus and Au-NPs -Curcumin on non-cancerous cells, HEK-293 was also studied. The results did not show any significant inhibitory action on both non-cancerous cells and HEK-293 cells. There are

several studies which reported the successful inhibition of the cancer cells after treatments with different types of nanoparticles [30–32].

Conclusions

In this study, Au-NPs were synthesized by employing an eco-friendly rout, using Hibiscus and Curcumin extractions as a reducing and stabilizer agents.The prepared Au-NPs specimens (Au-NPs -Hibiscus and Au-NPs -Curcumin) were characterized by FTIR spectroscopy, UV–Vis spectroscopy, SEM and TEM techniques. UV-Vis spectrum of both the specimens exhibited a surface plasmon resonance peak at ~ 520 nm (546 nm), the characteristics absorption peak for gold. SEM and TEM analyses revealed the spherical morphology of the particles with average size about 13 and 18.3 nm, respectively for Au-NPs -Hibiscus and Au-NPs -Curcumin specimens. However, it was observed the particles were well dispersed and perfect in shape when they were prepared with Hibiscus. On the other hand, a slight aggregation or interconned morphology was observed for Au-NPs -curcumin. The anticancer cell activity of the Au-NPs was studied against HCT-116 and MCF-7 cells. It was observed that the treatment of cancer cells with Au-NPs decreased the no. of cell significantly as compared to control cells. The Au-NPs -Hibiscus specimen showed the better inhibiting properties than Au-NPs -Curcumin, which is attributed to their uniform dispersion and small size. No significant inhibitory action was noted on health (non-cancerous) cells and HEK-293 cells. Disintegration of cancer DNA study suggested that the decrease in the cancer cells are due to cell death which is described by programmed cell death or apoptosis. The results of the cell viability assay showed a substantial decrease in the cell viability after treatment with both types of Au-NPs.

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Conflict of interest: The authors declare that they have no conflict of interest.

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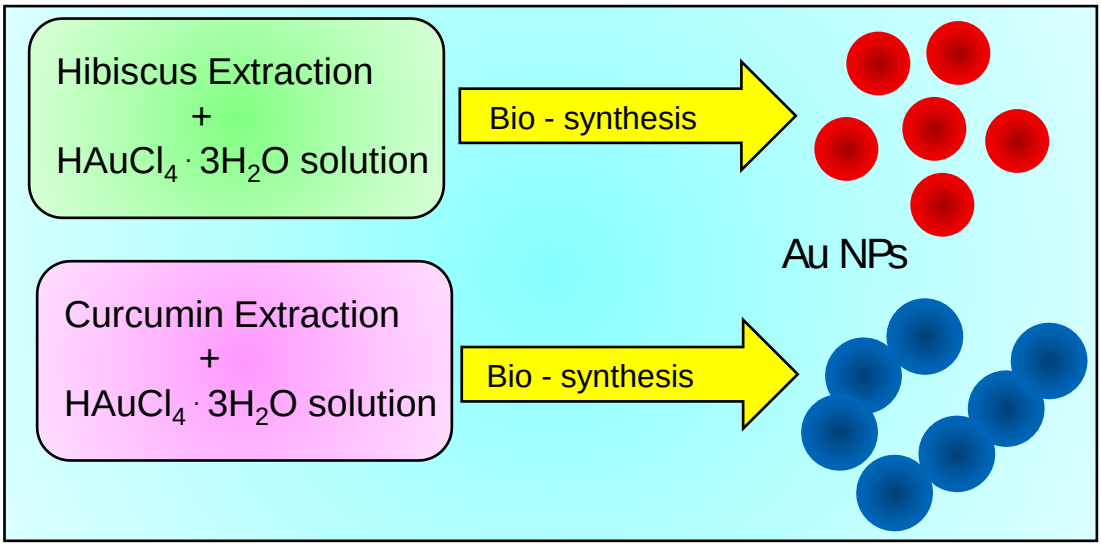
Graphical Abstract



Gold nanoparticles *(Au-NPs)* are syntheized by eco-friendly non-chemical rout using Hibiscus and Curcumin extractions and investigated their anticancer activities against colorectal carcinoma (HCT-116) and breast cancer cells (MCF-7). The Au-NPs -Hibiscus specimen showed the better inhibiting properties than Au-NPs -Curcumin, which is attributed to their uniform dispersion and small size.

Highlights

- Nanoparticles of gold (Au-NPs) were prepared using hibiscus and curcumin.
- The characterization results confirmed the successful preparation of spherical shaped Au-NPs with Dia. ~ 20 nm.
- Anticancer behavior of Au-NPs-Hibiscus and Au-NPs-Curcumin was studied against HCT-116 and MCF-7 cells.
- Our results show the inhibition properties of Au-NPs as potent anti-cancer nanomaterials.

Figure and captions

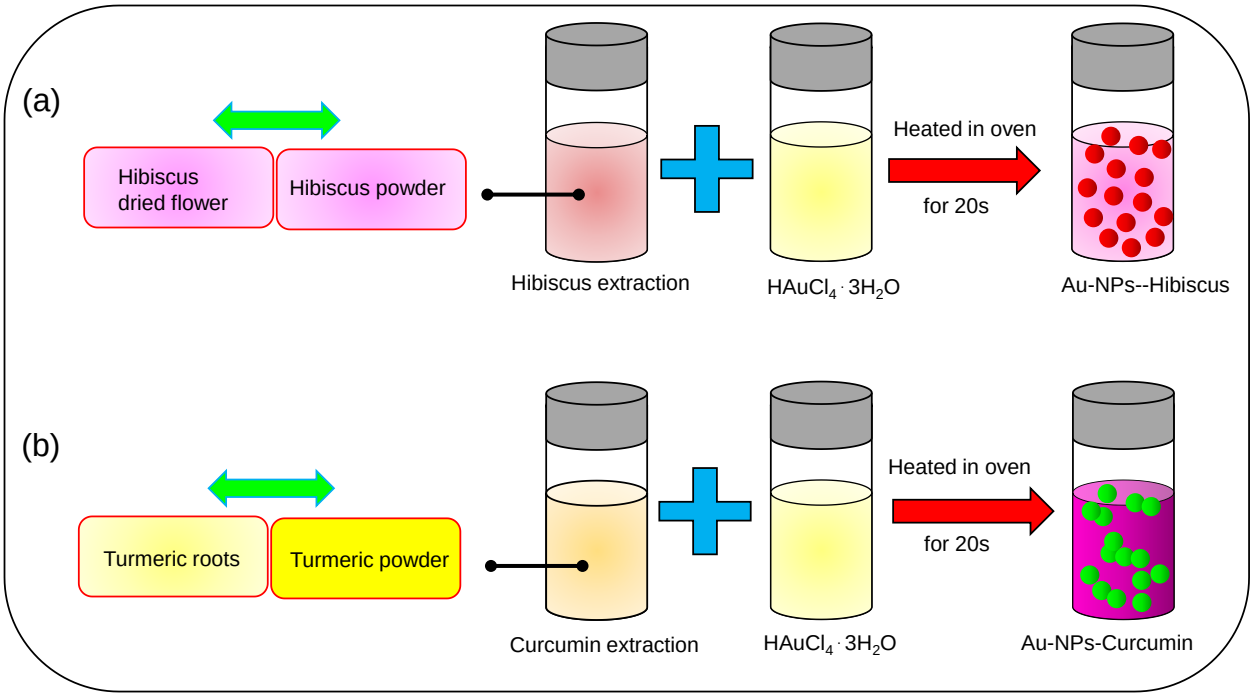


Figure 1. Schematic illustration of gold nanoparticles (Au-NPs) preparation using (a) Hibiscus and (b) Curcumin extractions.

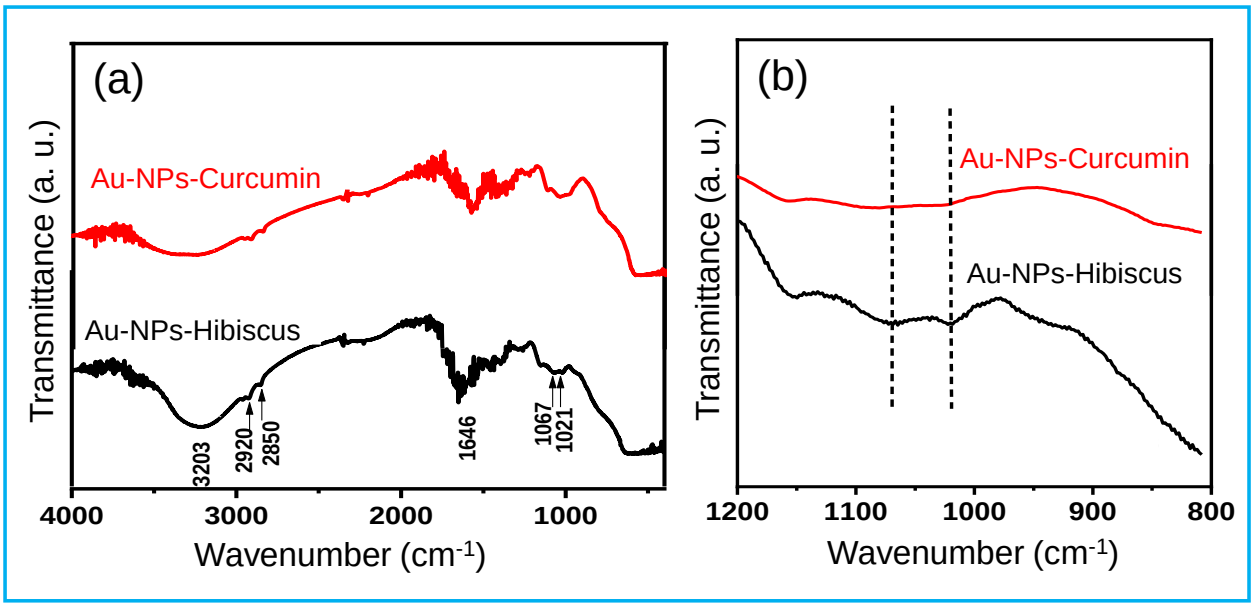


Figure 2. (a) Fourier transform infrared (FTIR) spectra of Au-NPs-Hibiscus and Au-NPs-curcumin. (b) Close-up spectra between 1200 and 800 cm^{-1}. The characteristics bands are marked.

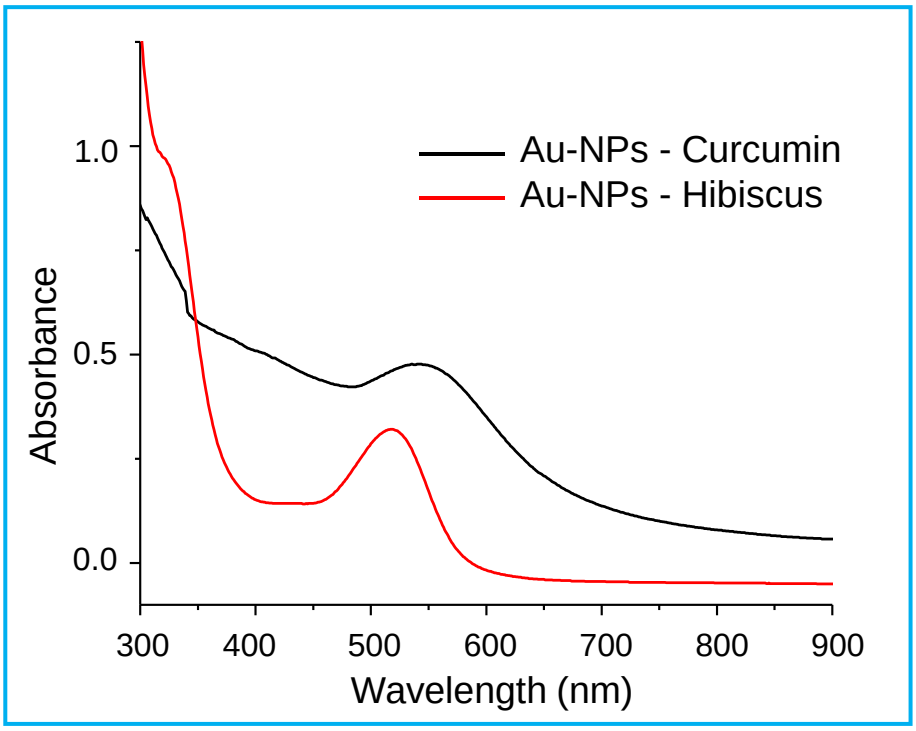


Figure 3. UV-Vis spectroscopy of Au-NPs-Hibiscus (red spectrum) and Au-NPs-Curcumin (black spectrum).

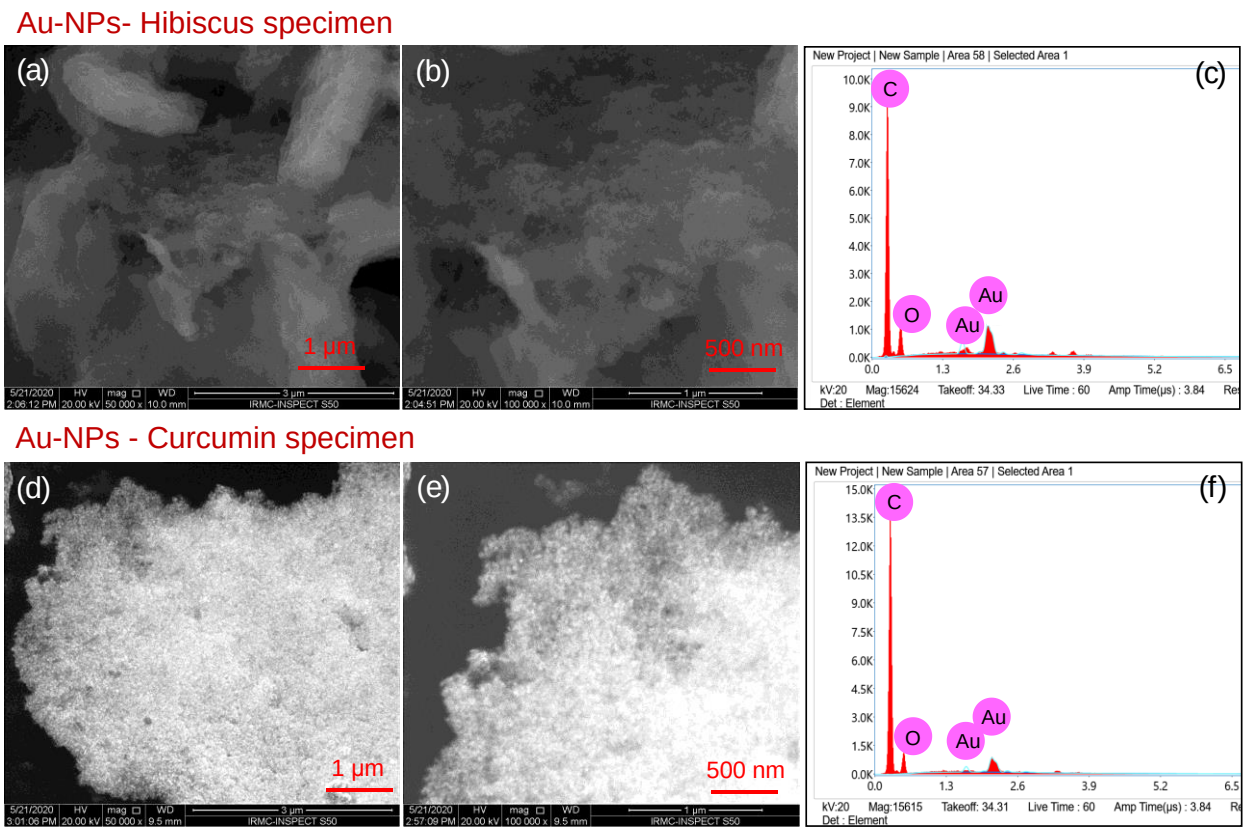


Figure 4: SEM images of the prepared Au-NPs using Hibiscus and Curcumin at two magnifications (x50, 000 and x100, 000) along with EDS spectra.

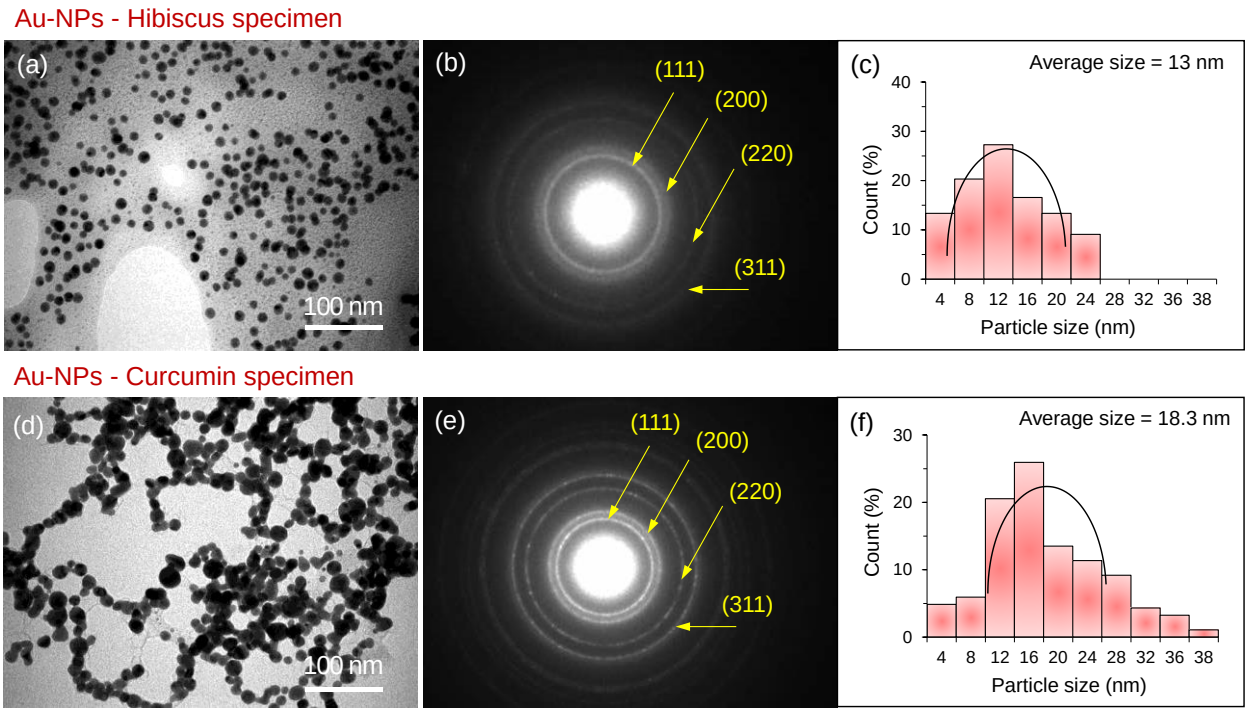


Figure 5: TEM images, SAED patterns and size histogram of gold nanoparticles (Au-NPs); (a-c) sample 1: Au-NPs using Hibiscus flowers as extract, and (d-f) sample 2: Au-NPs using curcumin extract. The scale bars are 100 nm (a, d).

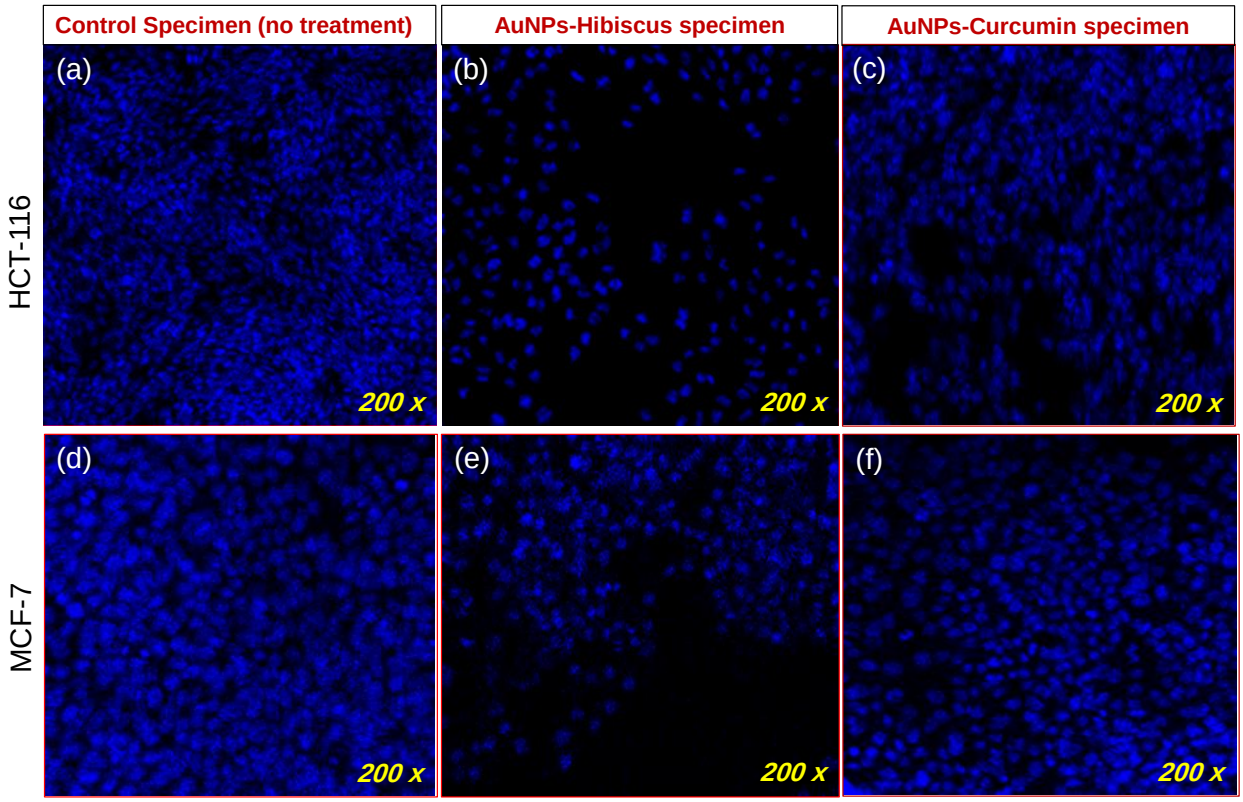


Figure 6: Disintegration of colon and breast cancer cell DNA (200x): (a-c) Impact of Au-NPs-Hibiscus and Au-NPs-Curcumin on colon cancer cells (HCT-116), post 48 hours treatment (0.8 µg/ml). (d-f) Impact of Au-NPs-Hibiscus and Au-NPs-Curcumin on breast cancer cells (MCF-7) after treatment of 48 hours (0.8 µg/ml).

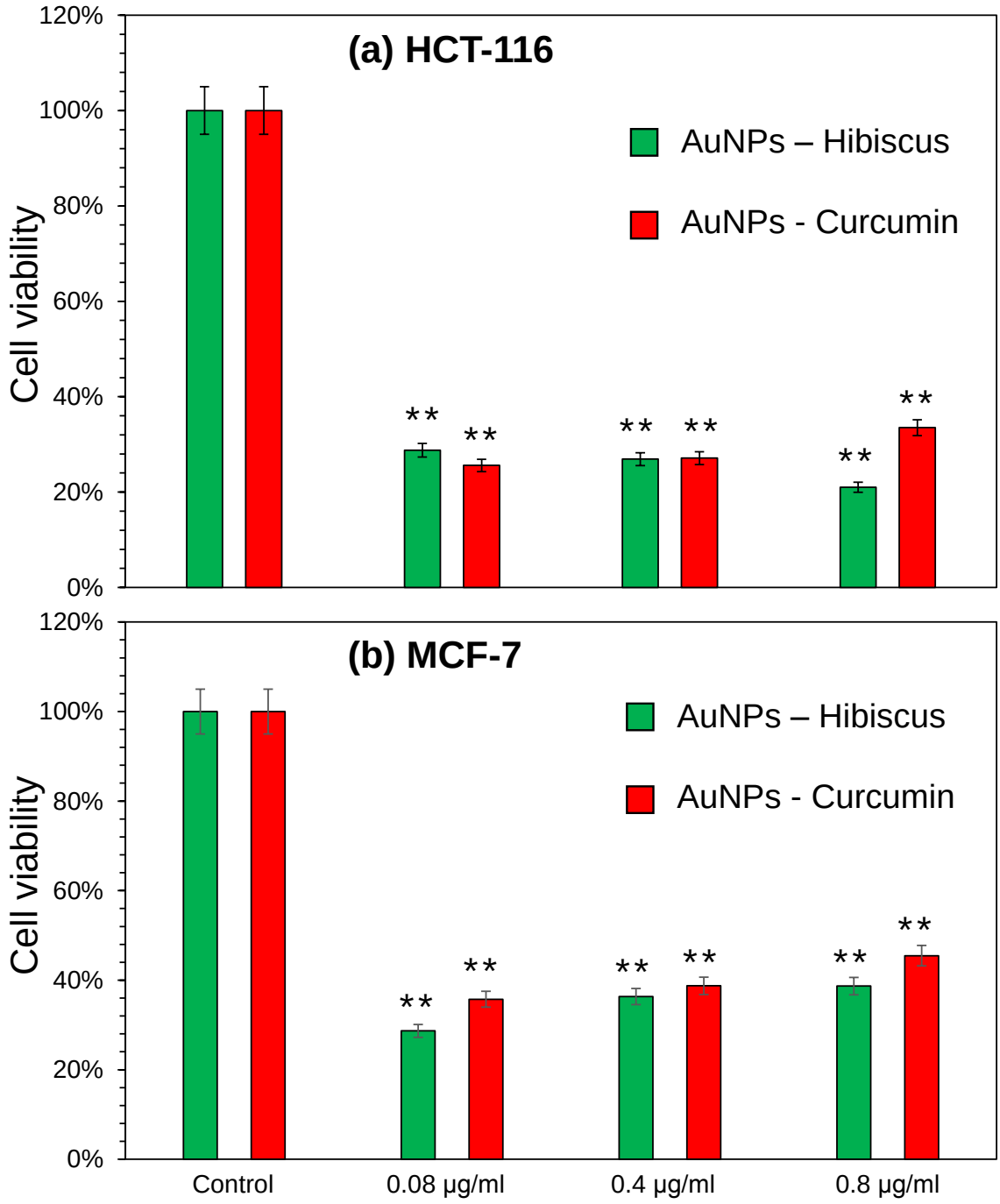


Figure 7: Cell viability assay: Impact of Au-NPs-Hibiscus and Au-NPs-Curcumin on (a) colon cancer cells (HCT-116) and (b) breast cancer cells (MCF-7). * $P < 0.05$; ** $P < 0.01$.