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ORIGINAL ARTICLE **OPEN ACCESS**

Green Synthesis of Gold Nano-Particles from *Curcuma longa* and Its Anticancer Activity in Cancer Cell

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ABSTRACT

Nanotechnology has vast applications in almost all fields of Science and technology. The use of medicinal plants for the synthesis of metallic nanoparticles has gained much attention now a days. The gold nanoparticles were using as a bioactive compound from the aqueous extract of Curcuma longa and characterized by ultraviolet-visible (UV-vis) spectroscopy, Scanning Electron Microscopy (SEM), Energy Dispersive X-ray analysis (EDAX), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) for size and shape. The characterized leaves extract of Curcuma longa AuNPS were tested forits in-vitro anticancer activity against human colon adenocarcinoma cells. Curcuma longa AuNPS showed the Surface Plasmon Resonance (SPR) band at 540 nm. The SEM Images showed the spherical shaped nanoparticles at an average of 71.8 nm and further determined using the Scherrer equation. In vitro cytotoxic activity of the AuNPS indicated that the sensitivity of human cancer cell line for cytotoxic drugs is higher than that of Vero cell line for the same cytotoxic agents. By understanding the mechanistic action of AuNPS responsible for their therapeutic efficacy will help to device customized therapies and treatment against cancer as a potential cancer therapeutic tool.

Keywords: *Curcuma longa, HT29 cell line, Anticancer activity, Gold nano particles, SEM*

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INTRODUCTION

Uncontrolled growth and spread of abnormal cells lead to cancer and finally results in death. Ethno-pharmacological process on the synthesis of nanoparticles is an amazing technology beneath construction symbiosis between nanoscience and medical sciences. In this regard, the idea of functionalizing gold nanoparticles for antidiabetic nanomaterial by synthesizing pharmacologically key plant materials often been considered [1]. Advances beneath nanotechnology have identified possible candidates for biological and biomedical programs on pharmaceutics, for novel diagnostics and medical agents. The nanoparticle drug delivery system has the advantages of accumulating large amounts of therapeutic drugs in the tumor tissues through the passive and active targeting approach. Colon adenocarcinoma is the most common type of gastrointestinal cancer, and the National Institute of cancer has reported about 140,000 cases each year in the United States. This type of cancer begins in the inner layers of glandular structures of the colon cells and spreads into the wall of the colon and potentially into the lymphatic system and other organs. *Curcuma longa* plant used in the Indian Ayurvedic medicine for treating diabetes mellitus, has been known from antiquity also to have an antisaccharin taste effect [2]. Many researchers have reported that the leaves of *Curcuma longa* lowers blood sugar, stimulates the heart, uterus, and circulatory systems, and exhibit antisweet and hepatoprotective activities. *Curcuma longa* has been used in the treatment of diabetes since ages in folk, ayurvedic and homeopathic systems of medicine. In addition, it also possesses antimicrobial , antitumor ,obesity, anti- Inflammatory , and Anti-hyperglycemic Activity . Also in our previous studies we have reported that the bioactive compounds present in *Curcuma longa* possess the anti-stress, anti-allergic, and antiulcer activity[3]. Administration of *Curcuma longa* extract to diabetic rats increased superoxide dismutase activity and decreased lipid peroxide by directly scavenging the reactive oxygen species, due to the presence of various antioxidant compounds, or by increasing the synthesis of antioxidant molecules (albumin and uric acid) .[4] Presently, numerous methods namely physical, chemical substance, natural, as well as crossbreed techniques can be found in order to synthesize various kinds of precious metal Nanoparticles such as silver, gold, silicon, zinc and platinum nanoparticles.[5] Even though actual physical as well as chemical substance techniques tend to be more well-known as well as popular with regard to activity associated with nanoparticles, the actual associated environment degree of toxicity as well as nonbiodegradable character from the items restricted their own

programs.Therefore the actual "green" path with regard to nanoparticle activity through natural source is actually associated with excellent attention because of eco-friendliness, financial potential customers, feasibility as well as broad variety of applications. Thus we have chosen the one step synthesis of the Nanoparticles in the Green Way with the aqueous plant extract and called as green Synthesis. [6]In this study, an attempt was made to synthesize the gold nanoparticles from aqueous extract of the *Curcoma longa* and analyzed by ultravioletvisible (UV-vis) spectroscopy, scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) for size and shape. The green synthesized, characterized and bio-functionalized gold Nanoparticles from *Curcuma longa* were tested for in vitro anticancer activity against human colon adenocarcinoma cells. [7]Our present findings clearly demonstrated that it is indeed possible to have a much greener way to synthesize AU-NPs without compromising their medicinal properties and thus plant extracts may prove to be a good alternative to obtain Au-NPs with improved antibacterial and anticancer properties.[8,9,10]

MATERIAL AND METHODS

The leaves of The leaves of plants *Curcuma longa* were collected from different parts of Krishnagiri. The Leaves of Plantswere first washed with distilled water to remove the dirt and further washed with mild soap solution and rinsed thrice with distilled water. The leaves were blotted with tissue paper and shade dried at room temperature for at least 2 weeks. After complete drying, the leaves were cut into small pieces, powdered in a mixer, and sieved using a 20µ mesh sieve to get a uniform size range for further studies. 20.0 g of the sieved leaf powder was added to 100 mL of sterile distilled water in a 500 mL Erlenmeyer flask and boiled for 5 minutes and kept in continuous dark conditions at 30°C. The extract was filtered and stored in an airtight container to protect from sunlight until further use .[11]

Preliminary phytochemical activity

The qualitative phytochemical analysis of *Curcuma longa* extracts were performed following the methods of Parekh and Chanda [11,13]to determine the presence of alkaloids (Mayer's, Wagner, Dragendorff's test), flavonoids (alkaline reagent, Shinoda), phenolics (lead acetate, alkaline reagent test), triterpenes (liberman - burchard test), saponins (foam test), tannins (gelatine). The results were qualitatively expressed as positive (+) or negative (−) .The chemicals used for the study were purchased from Sigma-Aldrich (Chennai, India)

Quantitative phytochemical analysis

Estimation of total phenolic content

The total phenolic content of each extract was measured using an adapted Folin Ciocalteu colorimetric method. About 200µl of 10% (v/v) Folin Ciocalteu reagent was mixed with 100 µl of aqueous extract in phosphate buffer (75 mM, pH 7.0). Gallic acid as a positive control and phosphate buffer as a negative control and the absorbance was measured at 765 nm after 30 minutes of incubation using UV-Vis 3000+ double beam spectrophotometer (LabIndia,Maharashtra, India). A standard curve was calculated using gallic acid concentrations ranging from 0.05 to 0.5 mM and the results were expressed as mg/g gallic acid equivalents (GAE) of dried weight[14,15].All the experiment was carried out in triplicate and results averaged expressed as mean ± SD.

Estimation of total flavonoids

The aluminum chloride colorimetric method was used for flavonoid determination. 250 µl of each sample was mixed with 1.25 ml of deionized water and 0.075 ml of 5% sodium nitrite. After 6 min, 0.15 ml of 10% aluminum chloride was added and after another 6 min the product was mixed with 0.5 ml of 1M sodium hydroxide and 2.5ml of deionized water. Total flavonoids were measured at 510 nm using UV-Vis 3000+ double beam spectrophotometer (Lab India, Maharashtra, India). The results were given in mg CE/ g plant extract of cathequin equivalent [16].

Total antioxidant capacity

For total antioxidant capacity assay, 0.3 ml of the *Curcuma longa* extract (10 mg/ml) dissolved in water was mixed with 3ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated and absorbance was measured at 695 nm against reagent blank. Gallic acid was used as the standard and the total antioxidant capacity was expressed as equivalents of ascorbic acid [17].

DPPH of radical scavenging assay

The method of Blios was used for the determination of scavenging activity of the DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) free radical. The reaction mixture (DPPH and extract) was vortexed, incubated and its absorbance measured at 517 nm. The scavenging ability ofthe plant extract was calculated using the following equation: DPPH Scavenging activity (%) = [(Abs control – Abs sample)]× 100 **(1)**(Abs control)]

Where, *Abs control* is the absorbance of DPPH without sample; *Abssample* is the absorbance of DPPH with sample[18].

Preparation of gold nanoparticles

Chloroauric acid (HAuCl4) from Sigma-Aldrich (St Louis, MO, USA)and the aqueous leaf extract of *Curcuma longa* were used for the synthesis of gold nanoparticles. Five ml of aqueous leaf extract of *Curcuma longa* was added to 10 mL of 1 mM aqueous HAuCl4 solution in 250 mL Erlenmeyer flasks and incubated in a rotary shaker at 150 rpm in dark. The color change in the colloidal solutions occurred showed the formation of GNPs. [19]. The Bio functionalized gold nano particles synthesized using aqueous leaf extract of *Curcuma longa and* was characterized by the previously described methods. Nanoparticles are generally characterized by their size, shape, surface area, and dispersity the bioreduction process was monitored by the UV-visible spectroscopy, size of the synthesized gold nanoparticles were characterized by scanning electron microscopy (SEM). EDAX analysis was carried out to confirm the elemental gold in the *Curcuma longa* gold nanoparticles and Fourier transformation infrared spectroscopy (FTIR) was used to understand the biomolecules responsible for the biosynthesis, and the crystalline structure was investigated by the X-ray diffraction (XRD) technique. The characterization methods are described briefly in our previous studies. Similarly the Gold nanoparticles can be prepared and the stability of particles was evaluated without any additional stabilizing chemicals/and or physical steps also it is observed that the colloidal solution maintains its stability and uniformity, similar results were reported by other researchers.;[20]

UV-vis absorbance of *Curcuma longa* AuNPS .

The bioreduction of HAuCl4 by the aqueous leaf extract of *Curcuma longa*was recorded periodically using a UV-Vis 3000+ double beam spectrophotometer (Lab India, Maharashtra, India). The sampleswere diluted with 2 mL of deionized water and measured for UV-Visspectrum at regular time intervals. The deionized water was used as a blank for background correction of all UV-VIS spectra. All samples were loaded into a 1cm path length quartz cuvette for UV-Vis spectrometric readings and scanned from 200 to 800 nm at a scanning speed of 0.5 nm interval. The UV-VIS spectra were fit with Gaussian curves correcting for a cubic background for full-width at half maximum (FWHM) and wavelength of maximum absorbance measurements. The Gaussian fits to the UV-VIS spectra all hadgoodness of fit values (52 ~ 1), showing accurate curve analysis.[21]

SEM and EDAX analysis of *Curcuma longa AuNPS*

Electron microscopy is another commonly used method of characterization. Scanning electron microscopy and transmission electron microscopy are used for morphological characterization at the nanometer to micrometer scale. The leaves of *Curcuma longa* GNPs were characterized using high resolution Scanning Electron Microscope (JSM-5600LV; JEOL, Tokyo, Japan).[22] The samples were prepared by a simple drop coating ofsuspended gold solution on to an electric clean glass and allowing the solvent (water) to evaporate and the samples were left to dry at room temperature. EDAX analysis was carried out to confirm the presence of elemental gold the *Curcuma longa AuNPS*using the drop coated AuNPS of *Curcuma longa* on to carbon film, and analyzed using EDAX (S-3400N; Hitachi, Tokyo, Japan).

FTIR spectroscopy analysis of *Curcuma longa AuNPS*

To identify the biomolecules present in the leaf extract of *Curcuma longa* and the phytocompuonds capped on the GNPs after synthesis were analysed by FTIR. (*PerkinElmer RX1; PerkinElmer, Waltham, MA,USA)* The bioreduced chlorauric solutions were centrifuged at10,000 rpm for 15 minutes, and the pellets were washed three timeswith 20 mL of deionized water. The resulting purified suspensions were dried and ground with KBr pellets and the FTIR spectrum was recorded in the range of 400–4000 cm−1, and a good signal and noise ratio, were recorded at 512 scans. [23]

XRD Analysis of bio functionalized *Curcuma longa AuNPS*

The purified *Curcuma longa* AuNPS, were characterized by XRD measurements using an XRD-6000 X-ray diffractometer (Shimadzu, Kyoto, Japan) operated at a voltage of 40 kV and 30 mA with Cu Kα radiation in θ–2θ configurations. The crystallite domain size was calculated from the width of the XRD peaks by assuming that they were free from non-uniform strains using the following Scherer formula,Where, D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the FWHM, and θ is the diffraction angle expel the added instrumental broadening, theFWHM was corrected using the FWHM from a large-grained Si sample. This modified formula is valid when the crystallite size is smaller than 100 nm.

***in -vitro* cytotoxicity studies of *Curcuma longa AuNPS*Cell culture**

Human HT-29 cells were routinely grown at 37°C in a humidified atmosphere of 5% CO2 and 95% air, in Dulbecco's Modified Eagle Medium (DMEM) Glutamax supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS) and 1 mM Anti– -Inc A antibody (Invitrogen). This medium is considered as complete DMEM. MTT assay was performed to determine the cytotoxic properties of biofunctionalyzed

AuNPs against HT29 and Vero cell lines. The cell lines were seeded in 96-well tissue culture plates and the appropriate concentrations of Au-NPs stock solutions were added to the cultures to obtain respective concentration of Au-NPs and incubated for 48 hrs at 37°C. The non-treated cells were used as control. The incubated cultured cell was then subjected to MTT (3- (4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, atetrazole) colorimetric assay. MTT assay was based on the measurement of the mitochondrial activity of viable cells by the reduction of the tetrazolium salt MTT (3-(4,5-dimethyathiazol-2-yl)-2,5-diphenyl tetrazolium bromide) toform a blue water-insoluble product, formazan. MTT (5 mg/mL, 20 µL) was added to respective set of cells and the plates were incubated for an additional 4 h. After 4 h of incubation, the medium was removed and DMSO (200 µL, Sigma–Aldrich, USA) was added to dissolve the formazan crystals resulting from the reduction of the tetrazolium salt only by metabolically active cells MTT is reduced in metabolically active cells to yield an insoluble purple formazon product. The cells were harvested during the exponential phase and counted by a hemocytometer after staining with trypan blue solution. The cell suspensions were dispensed (100µl) in triplicate into 96-well culture plates at optimized concentrations of 1 × 105/well for each cell lines, after a 24- hr recovery period. Assay plates were read using a spectrophotometer at 520 nm[52]. The spectrophotometrically absorbance of the samples was measured using a micro plate (ELISA) reader. The cytotoxicity data was standardized by determining the absorbance and calculating the correspondent AuNPs concentration. The data generated were used to plot a dose-response curve of the concentration of extract which was required to kill 50% of cell population (IC50) was determined from formula 3 as described below .Since the absorbance was directly correlated with the number of viable cells, the percent viability was calculated from the absorbance[55]. The IC50, the concentration of the drug at which 50% cell growth is inhibited, was calculated by the curve fitting of the cell viability data using Prism 5.2. After *Curcuma longa* AuNP Streatment, the plates were observed under an inverted microscope to detect morphological changes and photographed.

RESULTS AND DISCUSSION

Phytochemical screening of *Curcuma longa* leaf extract

The results of the preliminary phytochemical screening of aqueous extracts of *Curcuma longa* revealed the presence of alkaloids, phenols, flavonoids, sterols, tannins and triterpenes (Table 1). As tabulated in the table 2 the total flavonoids were 135.62 ± 27.84 µg/g, total phenol content was 275.23± 1.12 µg/g and tannin 113.43 ± 15.13 µg/g were present in the water extract of *Curcuma longa.* The flavonoids and phenolic compounds exhibited a wide range of biological activities like antioxidant and lipid peroxidation inhibition properties.

TABLE 1: PHYTOCHEMICAL SCREENING OF LEAF EXTRACT OF *CURCUMA LONGA*

Compounds	Aqueous extract
Alkaloids	+
Triterpenoids	+
Glycosides	-
Saponins	+
Tannins phenols	+
Flavonoids	+
Steroids	+

+ Present -Absent

The total antioxidant activity was 9.13 }0.04 µg/g and DPPH radical scavenging activity was 52.14 } 0.32% respectively (Table 2). Our results indicated that the flavonoids could be a significant source of antioxidant activity which may be depending upon their molecular structure and the presence of hydroxyl groups.

TABLE 2: ESTIMATION OF PHYTOCHEMICAL COMPOUNDS OF LEAF EXTRACT OF *CURCUMA LONGA*

Bioactive compounds	(µg/g)
Total antioxidantant	9.13 ±0.04
Flavonoids	135.62 ±27.84
Tannin	113.43± 15.13
Total phenol content	275.23 ± 1.12
Freeradical scavenging	50.14 ± 0.30

All the values given are means of triplicates determinations. Data presented as the mean ± standard deviation; Gallic acid equivalent; Tannic acid equivalent, Quercetin equivalent, Catechin equivalent

Uv vis spectroscopy

Due to the abundance of carboxyl, carbonyl, hydroxyl and phenolic groups, the natural-source extracts reduce Au III and stabilize the Au-NPs with these groups. The non-toxic chemicals from natural source extracts were used in the syntheses in order to avoid adverse
effects in medical and biology applications. These Au-NPs syntheses using natural-source extracts are simple, just mixing the extracts with aqueous HAuCl4 until the color changes to red or purple. The aqueous plant extract when mixed with aqueous chloroauric acid, the color of the solution changed from pale yellow to a vivid ruby red, by the formation of gold nanoparticles. This change in color was due to the collective coherent oscillation of conduction electrons at the surface of the gold nanoparticles that interact with the oscillating electric field of the incident light, a phenomenon called Surface Plasmon Resonance (SPR). This change in color indicates the reduction in AuCl4- ions was traced with UVvis spectroscopy.

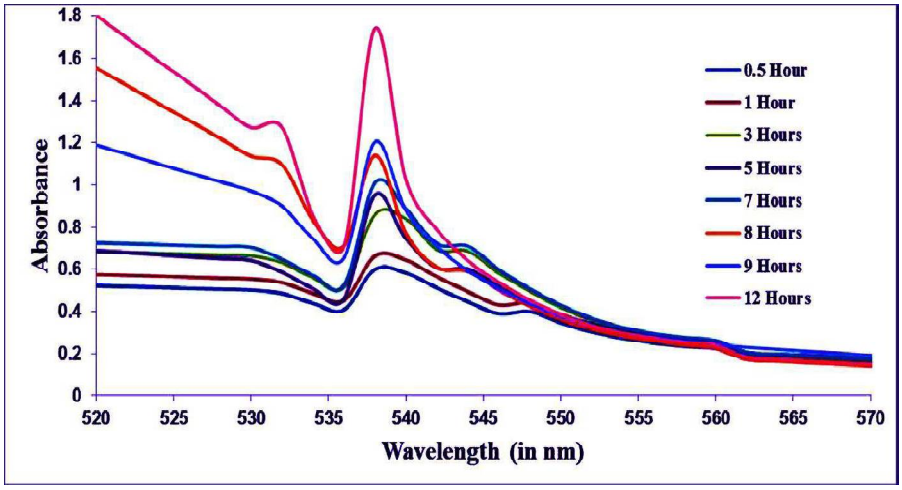

Fig. 1: UV–vis spectra of the reaction of aqueous leaf extract of

***Curcuma longa* with aqueous gold recorded as a function oftime**

The ultraviolet-visible (UV-vis) spectra recorded as a function of time and reaction of this solution at room temperature showed the appearance of a Surface Plasmon Resonance (SPR) band at 540 nm, which then shifts to 538 nm and increased in intensity with time and accompanied by an increase in absorption in the near-infrared (NIR) region of the electromagnetic spectrum. These time dependent features were characteristic of formation of spherical gold nanoparticles that aggregate with time. The formation of anisotropic particles whose ratio increases with time or a combination of both processes are shown in Figure 1. This sharpness in absorbance peak depends on the size and dimensions of the produced nanoparticle, as with higher concentration of plant extract the particle size may be smaller, which results in sharpness of the plasmon resonance band of AuNPs. The absorption peak intensity increased rapidly with increase in reaction time from 60minute because of the continuous formation of AuNps in the reaction system. It was, therefore, remarked that an optimum time is needed for completing reaction because of the instability of formed gold nanoparticles. The optimum time required for completing reaction was recorded to be 12 hours. After 12 hours no further increase in the Intensity was observed. The intensity of the absorption band increased with time and reached a maximum after 12 h. after which no further change in the spectrum was observed indicating that the precursors had been consumed. From our previous studies the gold nanoparticle synthesis using aqueous extract of *M. edule, M umbellatum,C. zizanioides* and *I. aspalathoides*. we have observed that the color of the reaction mixture on formation of GNPs changed to ruby red color from a colorless/straw color. Also our results are comparable with the other available reports for plant extract mediated synthesis. Similarly Jayaseelan *et al*, 2013. has reported that the aqueous extract of *Abelmoschusesculentus seeds* showed the SPR at 536 nm. The narrow SPR was observed at lower quantities of the extract due to the formation of large anisotropic particles. Functionalized gold nanoparticles have been used to target drugs and biomolecules to specific cell types and organelles such as the nucleus or mitochondria.

SEM image of bio functionalized *Curcuma longa AuNPS*

A scanning electron microscope was employed to analyze the structure of the bio functionalized *Curcuma longa GGNPS* that were formed and represented in Figure 2. To understand the particle size, morphology and the periodicity of the bio functionalized *Curcuma longaGGNPS* synthesized by aqueous extract of

Curcuma longa the SEM Investigation was carried out. The nano particles formed is aggregated whereby solvated gold salt is reduced in the presence of surface capping ligands. This aggregation of the particles can be reduced or prevented by increasing the amount of the plant extract.

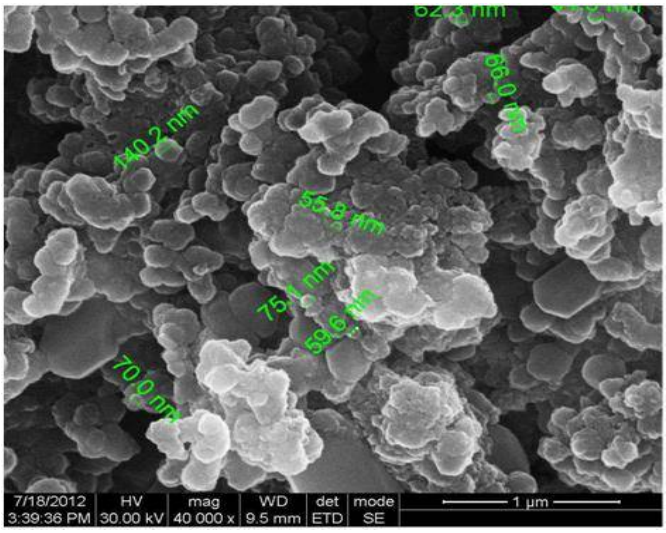

Fig. 2: Scanning electron microscopic image of *Curcuma longa*AuNPS

But the SEM Images of our earlier research has revealed that this biologically eco-friendly synthesis of GNPs utilizing the leaf extracts of *M. edule , C. zizanioides* and *M. Umbellatum* has not aggregated due to the biomolecules from the plant extract. And the mechanism behind these particles formation and not aggregated was presumed to proceed via spontaneous nucleation and isotropic growth of Nanoparticles along with the plant Extract. As these chains grow in diameter with increasing Au deposition, spherical particles break off from these structures, forming the Nano sphere product typically observed from this synthesis.

EDAX spectrum of*Curcuma longa AuNPS*

The EDAX attachment present with the SEM is known to provide information on the chemical analysis of the fields that are being investigated or the composition at specific locations (spot EDAX). The elemental composition of the bio functionalized *Curcuma longa. GGNPS* was determined by Energy Dispersive X-ray Analysis (EDXA) and shown in figure-3. The area-profile analysis of the synthesized nanoparticles showed strong peaks of Au at 2.114 keV that are characteristic of Au-NPs, along with the C, N, and O signatures at below 1keV. It is most likely that the C, N, and O signals in the EDXA spectrum are due to X-ray emission from proteins bound to the nanoparticles surface since the unbound proteins and biomolecules were removed by centrifugation followed by repeated washing.

The EDAX of bio- functionalized gold nanoparticles showed strong signals for gold atoms and the weaker signals for carbon, oxygen and chloride were provenients from biomolecules of plant extracts. Similar strong signals were obtained around 2.00 & 9.5 KeV for green synthesized gold nanoparticles of *M. umbellatum* from our earlier studies.

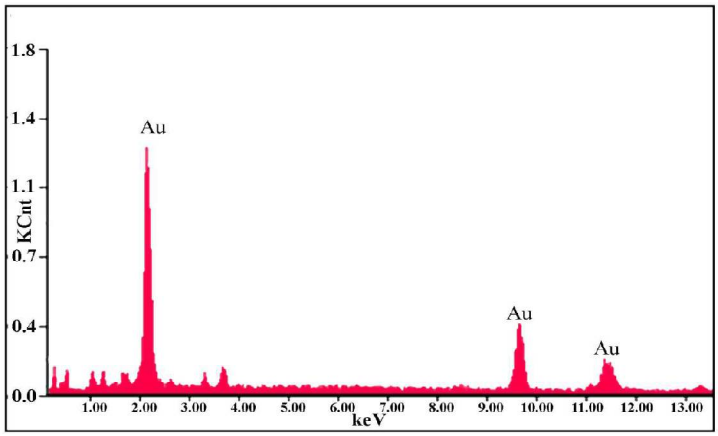

Fig. 3: Energy dispersive X-ray spectrum of *Curcuma longa AuNPS*

FTIR spectrum of bio-functionalized *Curcuma longa AuNPS*

Fourier transform infrared (FTIR) spectroscopy is a chemical analytical technique, which measures infrared intensity versus wavelength (wavenumber) of light. It is used to determine the nature of associated molecules of plants or their extracts with nanoparticles. The FTIR of the bio-functionalized gold nanoparticles using the aqueous extract of *Curcuma longa* is shown in figure -4. The flavonoids present in the plant extract are powerful reducing
agents which may be responsible for the reduction of chloroauric acid. The carboxylate group present in proteins can act as surfactant to attach on the surface of AuNPs and it stabilizes AuNPs through electrostatic stabilization. Thus it is found that Aqueous Plant extract has the ability to perform dual functions of reduction and stabilization of AuNPs.

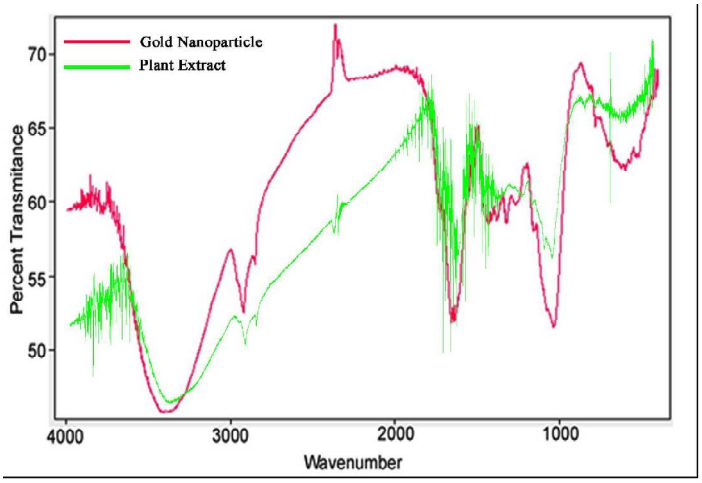

Fig. 4: FTIR spectrum of the *Curcuma longa AuNPS*

The active site of protein(s) involved in reduction of Au ions and AuNPs formation was investigated and the spectrum of the protein extract exhibited intense and distinct absorption bands at 1720 and 3317 cm−1, which correspond to the amide bands of the polypeptides/proteins in the aqueous plant extract. As plant molecules adsorbed on the surface of the GGNPs, the amide groups intend to form stronger bonds with Au atoms, which will break most of the H-bonds between the N–H groups and lead to the narrowing and blue-shifts of the amide bond. The band appearing at 1387 cm−1 corresponds to C–N stretching of amine group and in the raw extract, the peak was broad and blends, but after encapsulation of
nanoparticles the peak was narrow and sharper. The presence of the amide linkage suggests that there are some proteins in the reaction mixture. This protein might be responsible for the formation of the nanoparticles and may play an important role in the stabilization of the formed nanoparticle The other band at 1443 cm-1 was assigned to the methylene scissoring vibrations of proteins The band located at 1318 cm-1and 1089 cm-1 was due to C–N stretching vibration of aromatic and aliphatic amines, respectively, which is similar to the Earlier reports of Suman *et al*, 2014. The positions of these bands are close to those reported for native phytochemicals reported in the *Curcuma longa* extract. Thus, we can confirm that the nano capping of the phytochemicals from *Curcuma longa* extract are responsible for reduction and subsequent stabilization of Au-NPs. The absorption bands that appear in the IR spectrum of the aqueous extract could also be seen in the IR spectra of phytocapped AuNPs. This shows that the phytoconstituents protect the AuNPs fromaggregation.[25]

XRD spectrum of bio-functionalized *Curcuma longa*AuNPS

The XRD pattern of the synthesized gold nano particles were form aqueous extract of *Curcuma longa* is shown in figure -5. The diffraction peak at 2θ = 33° and subsequent higher order reflections can be indexed to the Au (1 1 1) and other facets of gold nanoparticles. The XRD spectrum also reveal a weak peak around 2θ = 28°, which can be attributed to the phytochemical components from the plant extract. This further confirmed that the particles formed on the membrane consisted of crystalline gold particles having a structure with lattice constants of 12 nm. The average grain size of the SNPs formed in the bioreduction wasdetermined using the Scherrer equation is 71.8 nm.

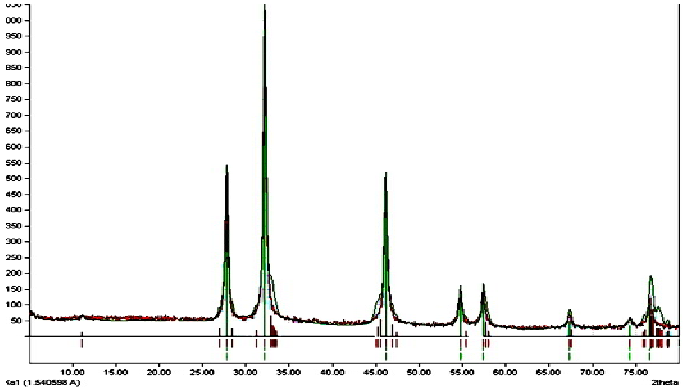

Fig. 5: X-ray diffraction spectrum of of bio-functionalized *Curcuma longaAuNPS*

The XRD pattern clearly explains the crystalline structure of the SNPs formed by green biosynthesis. The XRD peaks at 38.2°, 44.5°, 64.7° and 77.7° can be indexed to the and indicates gold nanoparticles which is highly crystalline. Similar results were reported in *Abelmoschus esculentus*, *Citruslimon*, *Citrus reticulata* and *Citrus sinensis*. Simillary, In ourprevious studies using *Chrysopogon zizanioides* similar XRD Peaks were Reported.

Anticancer Activity of *Curcuma longa AuNPS*

In vitro cytotoxic activity of the bio-functionalized *Curcuma loga AuNPS* against HT29 and Vero cell line at different concentration was evaluated.

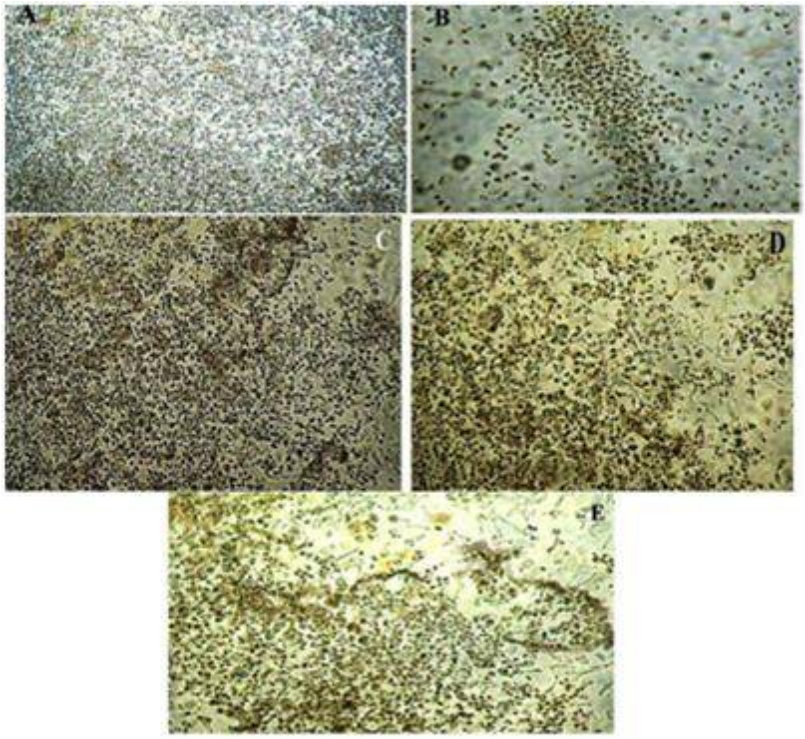

Fig.6: Inverted microscopic Image of HT29 Cell Lines treated with different Concentrations of bio-functionalized *Curcuma longa* AuNPS. A-control, B-TestC-90 µg, D-89.5 µg, E-85.5.5 µg:

Hai Feng Tang et al, 2009. Has reported that the saponin could induce apoptosis of U251 cells, and both the BAD-mediated intrinsic apoptotic signaling pathway and the caspase-8-mediated extrinsic apoptotic signaling pathway were involved in the apoptosis. Apparently, the promising saponins further study as potential anticancer agents. Jun Ai et al, 2014, has proposed a method which can be used as a qualitative method to recognize the presence or absence of the cancer cells with gold nanoparticles for targeted cancer cell imaging and efficient photodynamic therapy.

TABLE 3: RESULTS OF MTT ASSAY TEST ON HT 29 AND VERO CELL LINES AFTER TREATMENT WITH CONCENTRATIONOF SYNTHESIZED AuNPs

	Cell concentration		% Of inhibition	
Concentration in (µg)	**Vero cells (×104)**	**HT 29 (×104)**	**Vero cells**	**HT 29**
Control	5	5.5	0	0
Test	0	0	100	100
90	1.5	0.8	65	85
89.5	2.6	1.7	45	75
85.5	4.3	2.1	25	65

Similarly, a simple method for the fabrication of water soluble Curcumin conjugated gold nanoparticlesto target various cancer cell lines.Thus this one step synthesis of Gold nanoparticles using aqueous extract of *Curcuma longa* mayserve as a potential anticancer drug for cancer therapy. Thus further studies have to be carried out to understand the nature of cytotoxicity and the death or proliferation of cells caused by nanoparticles. Therefore, the goal of this research is to provide an important basis for applying nanoparticles in vitro anticancer activity against human colon adeno carcinoma cells. In addition to the anticancer activity, *Curcuma longa* leaves possess the antiulcer activity , thus we proposed the Gold Nano synthesis from plant extract for anticancer activity against human colon adeno carcinoma cells.

CONCLUSION

The biosynthesis of gold nanoparticles using the extract of *Curcuma longa* was economical, non-toxic, and environmentally. The formation of gold nanoparticles was characterized by UV-visible spectrophotometer. The bio-functionalized nanoparticles were stable due to the reducing and capping nature of phyto-constituents present in the aqueous extract of *Curcuma longa* analyzed by FTIR spectra. The particle size of the synthesized silver nanoparticles is less than 75 nm, which was confirmed by XRD and SEM analysis. Nanogold shows good cytotoxic activity against cancer cells and may serve as a potential anticancer drug for cancer therapy. This research is to provide an important basis for applying nanoparticles *In vitro* anticancer activity against human colon adeno carcinoma cells. Most of these protocols employ reduction of gold ions from gold salt solution in the presence of a capping agent. The role of capping agent is to prevent the aggregation of nanoparticles. Though nanoparticles have potential application in various applications, their xenobiotic nature restricts its application. There have been reports stating that chemically synthesized nanoparticles causing toxicity.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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