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Optimized Synthesis of Gold Nanoparticles using Green Chemical Process and its Invitro Anticancer Activity Against HepG2 and A549 Cell Lines

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ABSTRACT. In the present study, the biosynthesis of gold nanoparticles (AuNPs) was achieved by using marine brown seaweed *Padina tetrastrum* (PT) as a reducing and capping agent. The optimized Au Nps were performed by changing the concentration of algae extract, pH and temperature was analyzed by UV-vis spectrophotometer. The synthesized Au NPs were characterized by XRD, FTIR, SEM, TEM, EDX and SAED. The X-ray diffraction showed the Au NPs which can state by the presence of peaks at (1 1 1), (2 0 0), (2 2 0) and (2 2 2). The FTIR result clearly showed that the extracts containing -OH as a functional group (sugar molecules) act in capping the nanoparticles synthesis. SEM images revealed that all particles were spherical in shape. TEM image confirms the spherical in shape with an average size ranges from 8-10 nm. Synthesized Au NPs were evaluate for the *in vitro* cytotoxic activity of human liver cancer (HepG2) and lung cancer (A549) cell line at the different concentrations compared with standard drug cyclophosphamide.

Introduction. The Metal nanoparticles are acquired great interest in the field of nanotechnology and nanomedicine due to their unique properties such as optical, electrical, mechanical and chemical properties which have exhibit diverse sizes and shapes than the bulk state of the metal [1-3]. Thus, the activity of nanoparticles was mainly determined by their size, small size of nanoparticles exhibit high surface area due to the large fraction of the atoms and generates the active sites on the surface of nanoparticles [4]. The unique size and shape of nanoparticles were extensively used in catalysis, electronics, plasmonics and sensing [1, 5, 6]. Among the metal nanoparticles, gold has been used to cure various diseases in several centuries ago. Gold is inert, less toxic, highly thermal stability which has been used in various applications including biolabeling, gene and drug delivery system [7]. Many of the methods are available to synthesis of metal nanoparticles which has been exhibits inevitable due to the use of harmful and toxic chemicals [8]. Among the synthesis methods, green synthesis methods using biological probes predicted to avoid the using the toxic chemicals in the fabrication of nanoparticles. The biological probes are bacteria, fungi, yeast, actinomycetes, plants and algae involved in the green chemistry of nanoparticles; they provide large scale production, less time consumption and eco benign. Algae mediated synthesis nanoparticles were better reflection compare to the other biological probes. Algae have many phytochemicals could acts as both reducing and stabilizing agent for the nanoparticles and they are easily available marine sources [9-10]. The compounds isolated from *PT* like sulphated polysaccharides are having good medicinal properties such as anti-inflamtery activity, anti-oxidant effect, anti-hyper glycemic activity and hypo lipedemic properties [25-27]. In this study we report that green reduction of gold ions to AuNPs using the algae extract of *PT*, which is available throughout the year. This brown algae exhibits medicinal properties

such as anti hepatitis B virus [11] and with high value phytochemicals such as several fatty acids, phenols, terpenoids and sterols [12], which is motivating us to carry out the synthesis of AuNPs using *PT* biomass and evaluated anticancer property against lung and liver cancer cells.

Materials and Methods. All the analytical grade chemicals and media were purchased from Hi-media and Sigma.

Synthesis of AuNPs using PT. The brown algae *PT* was collected from coastal area of Tuticorin, TN, India. The algal extract and nanoparticles were prepared based on our previous studies [9, 10]

To study the effect of parameters such as algae extract concentration, pH and temperature on the nanoparticles synthesis was carried out by following experiments. Different concentrations of algae extract (5, 7.5 and 10ml) were added into the 1 mM of gold chloride solution. The pH of the extract was adjusted (6, 7, 8 and 9) to study the effect of pH on nanoparticles formation. The reaction mixture was incubated at different temperature conditions are 20 °C, 40 °C and 80 °C respectively. After incubation nanoparticles formation was measured by UV-vis spectrophotometer at different wavelength and time intervals.

Characterization of synthesized AuNPs. The synthesized Nanoparticles were characterized by techniques are UV-vis spec, FT-IR (Perkin elmer), SEM (Philips XL-30), TEM and SAED (Philips CM200) and XRD (Philips PW 1830).

Anticancer activity of AuNPs against HepG2 and A549 cell lines. The viability of cells was assessed by MTT assay using HepG2 and A549 cell lines.

Results and discussion

Visual observation. Fig. 1 (a, b and c) shows the *PT* extract alone, *PT* and gold chloride solution at initial stage and *PT* after 24 h incubation respectively. After addition of *PT* extract to the aqueous gold chloride, the yellow colour of the gold chloride solution is changed to brownish pink colour. After that, the colour of the solution is vigorously changed to ruby red while increasing the incubation time from 30 min to 48 hr. After 48 hr incubation, the colour of the solution is stable reveals that the synthesis of gold nanoparticle process was completed. The formation of dark ruby red colour indicates the gold chloride is reduced into AuNPs by using the extract of *PT*. The gold nanoparticle synthesis process was started at 1 h and 4h the process was completed at 15 hr [13, 14]. However, in the present study, the gold nanoparticle synthesis process is rapidly started at 40 min and completed at 48 hr.

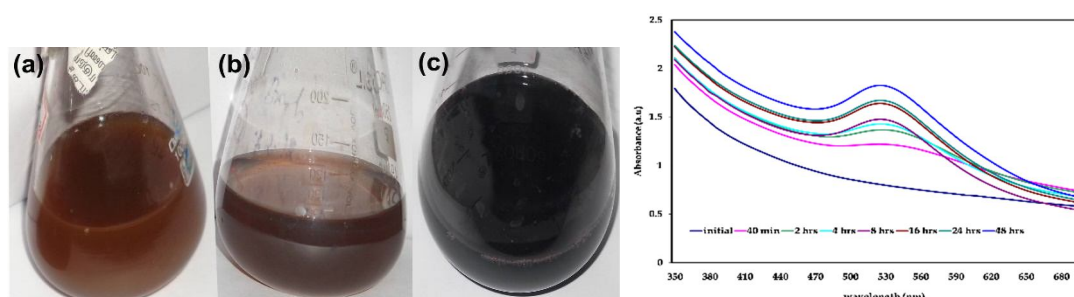


Fig. 1. Visual observation of synthesis of AuNPs by using *PT* (a) Algae extract (b) Initial and (c) final colour change and UV-vis absorption spectrum.

UV-vis spectroscopy analysis. Fig. 1 shows the reduction of gold chloride into AuNPs by using the extract of marine algae. The absorbance of AuNPs is monitored at different time intervals from 20 min to 48 h. Singh et al. [15] have reported the synthesis of AuNPs by using the *P. gymnospora*, a marine algae. In the report of UV-vis spectrophotometer, they have illustrated that the gold nanoparticle synthesis process was started at 1 hr and ended at 12 hr and the SPR band was occurred

at 527 nm. The formation of SPR peak is assigned to the oscillation of electrons at the surface of the nanoparticles is well matched for various metal nanoparticles with size ranging from 2 to 100 nm [16]. The synthesis of gold nanoparticle by *PT* is gradually increased while increasing the time of incubation from 20 min to 48 h.

Effects of algae extract concentration, pH and temperature. Fig. 2 shows the varying algae extract concentrations, pH and temperature on AuNPs synthesis. 7.5 ml concentration of algae extract was effective to synthesis of AuNPs, the maximum synthesis was attained in the concentration of 7.5 ml. At 5 ml and 10 ml concentration, the SPR band was broadened and obtained at 500-540 nm indicates formation of anisotropic gold nanoparticles.

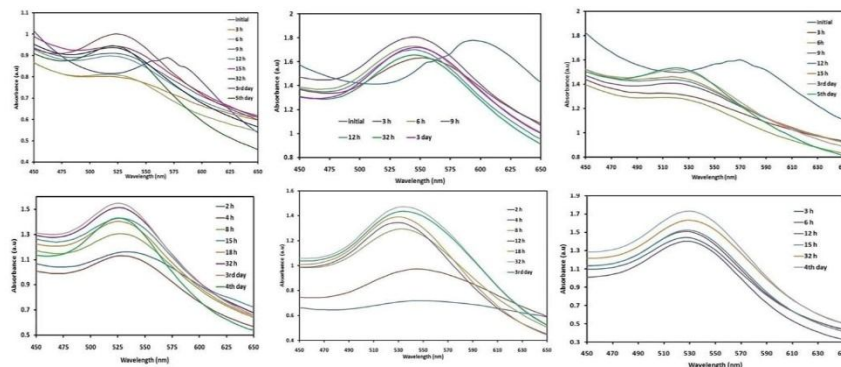


Fig. 2. Effect of Algal extract concentration, pH and temperature on AuNPs synthesis.

The narrow SPR band formed at 545 nm at 7.5 ml concentration of algae extract indicates even distribution small size nanoparticles. In low concentration, size reduction was slowly occurred due to the insufficient quantity of reducing agent and at high concentration synthesis process was hasty and cause competing for metal ions due to highly availability of reducing agent in the extract. The color intensity and the peaks for nanoparticles is pH dependent [17]. A sharp and symmetric peak is obtained at pH 6. At high pH broad peak was formed at 550 nm indicates large sized particles [18]. More stable and small size nanoparticles was synthesized at pH 6 and 7. In this study the position of SPR band does not show much variation, but their absorbance increases with pH.

Maximum conversion rate of gold ions to nanoparticles was occurred at higher temperature and the narrower SPR band was formed at 525 nm. At low temperature 20 and 40° C, the SPR band was broad and positioned at 545 and 530 nm respectively indicates formation of larger nanoparticles. Rapid and maximum synthesis of AuNPs with small size was obtained at 80° C. The maximum synthesis and faster rate of reduction was achieved at high temperature [19].

XRD and SEM. The XRD spectrum (Fig. 3) bragg reflection of *PT* derived AuNPs were observed at the 2θ values of 38°, 44°, 64° and 77° which are corresponding to the set of lattice planes (1 1 1), (2 0 0), (2 2 0) and (3 1 1), respectively and it was indexed for fcc gold. The peak of synthesized AuNPs is compared with the standard pure gold which was published by JCPDS (File no. 04-0784). The association of bimolecular with synthesized nanoparticles could be avoided by continuous centrifugation process. The synthesized AuNPs are mostly spherical in shape and its size ranges were varied from 40-90 nm for *PT* (Fig. 3a). The AuNPs are predominantly aggregated with each other and some of the individual monodispersed AuNPs are also viewed under TEM (Fig. 4). The SEM images exhibited that different shape of AuNPs obtained in the bark extract of *C. fistula*. [20]. The EDS spectra of *PT* synthesized AuNPs were shown in Fig. 3b. The EDS profile shows a strong signal at 3 keV reveals the presence of AuNPs.

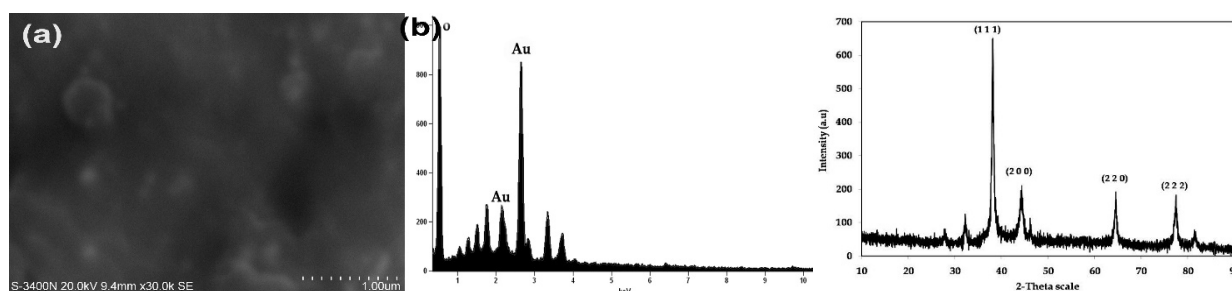


Fig. 3. SEM image of AuNPs synthesized using PT (a) and its corresponding EDS spectrum (b) and XRD spectrum.

Whereas, the other signals oxygen and carbon with elemental gold indicates the accumulation of algal biomass with gold nanoparticles. The biomolecules of algal extract play an important role in the reduction of gold metal ions into gold nanoparticles. It is one of the advantages for the green synthesis of metal nanoparticles when compared to other processes.

TEM analysis. The PT synthesized AuNPs are monodispersed and mostly spherical in shape. The aggregation of NPs leads to the formation of large sized particles. The spherical and undefined shapes are also found in the TEM images indicated that these particles are synthesized at the beginning of the reaction (i.e.) at 6-18 hr incubation. After 24 hr, incubation the particles are aggregated and form a bulk structure. Due to the absence of stabilizing agent in the brown algal extracts, the particles are aggregated with each other and form a bulk structure. The sizes of the AuNPs are found in the range of 8 to 10 nm for PT. The SAED pattern also suggested that the synthesized AuNPs are crystalline in nature. The result of SAED pattern was coincided with the result of XRD. The appearance of rings is attributed to set of diffraction planes (1 1 1), (2 0 0), (2 2 0) and (3 1 1) of fcc gold (Fig. 4b and 3b). Similar SAED pattern was obtained by using the algae and plants [13, 24]. The synthesis of metallic gold nanoparticle using the marine sponge and plant leaves of *Mangifera indica* they reported that the synthesis of gold nanoparticle are crystalline in nature and had ring at (111), (200), (220) and (311) of fcc gold [14, 21].

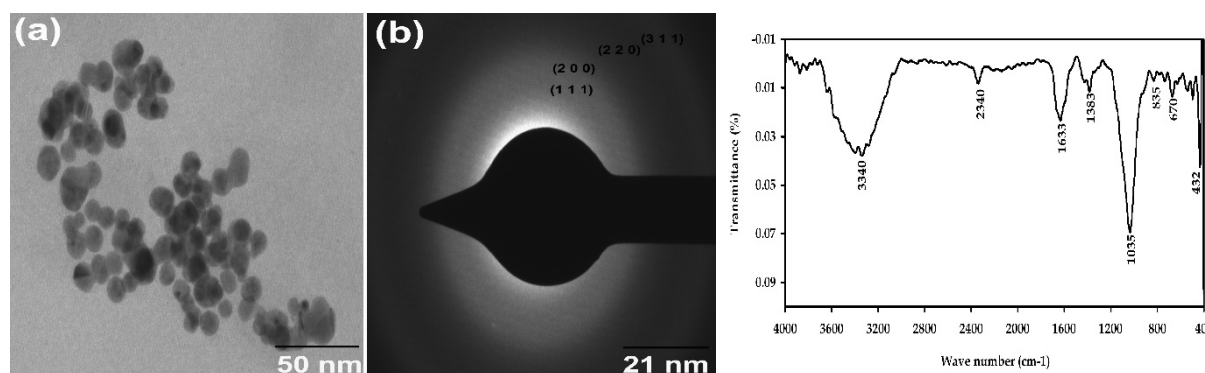


Fig. 4. Transmission electron microscopy images of AuNPs synthesized using PT (a) 50 nm (b) SAED pattern and FT-IR spectrum.

FTIR. The FT-IR spectrum shown in Fig. 4. The AuNPs synthesized from PT having the strong and broad intense peak formed at 3340 cm^{-1} indicating the presence of O–H stretching or H-bonded alcohols and phenols, weak band at 2340 cm^{-1} was occurred due to the C–O stretching vibrations of carboxylic acid, 1633 cm^{-1} is assigned to N–H bending of primary amines, 1383 cm^{-1} indicates presence of aliphatic NO_2 groups of nitro compounds, sharp narrow band at 1035 cm^{-1} arise from C–O stretching of carboxylic acids or C–N stretching of aliphatic amines, the intense band at 835 cm^{-1}

characteristics to CH bending of aromatics, the bands are located at 670 and 432 cm^{-1} may be attributes to alkyl halides. Sugar molecules such as l-fucose, l-rhamnose, d-xylose, d-arabinose, d-galactose and d-glucose were identified and half-ester sulphate and d-mannuronic acid and a neutral laminaran were present in the PT was proved [22].

Cytotoxic activity of gold nanoparticles. Anticancer activity AuNPs was increased while increasing the concentration of AuNPs (Fig.5). Maximum cytotoxicity activity 47% was observed at 100 μg of gold nanoparticles. AuNPs efficiently facilitate into the cancer cell lines to suppress the cell proliferation. AuNPs enter into the cells cause oxidative stress, this elevated stress reduced the cell viability and increased DNA damage. Increased Reactive oxygen species (ROS) generation cause cell death in different type of cells. AuNPs interact with functional groups of intracellular groups with the nitrogen bases and phosphate groups in DNA cause cell damage [23] and also suppress the signaling of proteins [24].

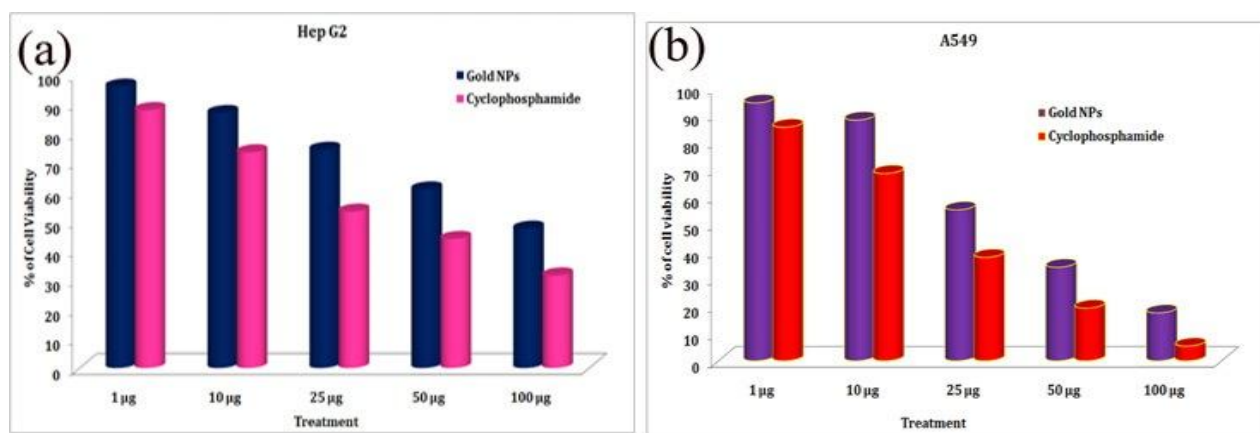


Fig. 5. Anticancer activity of AuNPs.

Summary. In this study, eco-friendly green synthesis of AuNPs was carried out by using algae extract of PT. Size and shape were controlled by different concentration of algae extract, pH and temperature and analyzed by UV-vis spectrophotometer. High quantity of algae extract concentration leads to formation of larger sized nanoparticles and the absorbance intensity was increased with increased pH. The temperature affect the nanoparticles synthesis was identified by UV-vis spectrophotometer. The optimum conditions for AuNPs synthesis is 7.5 ml algae extract concentration, pH 6 and temperature is 80°C. SEM shows spherical shape of nanoparticles and the size ranges from 8 to 10 nm. FTIR indicates to presence of amine and carboxyl groups in algae extract may be responsible for reduction of gold ions to gold nanoparticles. Highest cytotoxicity of AuNPs was assessed against (HepG2) and lung cancer (A549).

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