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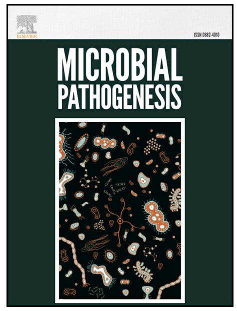

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Synthesis of nano-cuboidal gold particles for effective antimicrobial property against clinical human pathogens

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Abstract

Algae could offer a potential source of fine chemicals, pharmaceuticals and biofuels. In this study, a green synthesis of dispersed cuboidal gold nanoparticles (AuNPs) was achieved using red algae, *Gelidium amansii* reacted with $HAuCl_4$. It was found to be 4-7 nm sized cubical nanoparticles with aspect ratio of 1.4 were synthesized using 0.5 mM of $HAuCl_4$ by HRSEM analysis. The crystalline planes (111), (200), (220), (311) and elemental signal of gold was observed by XRD and EDS respectively. The major constitutes, galactose and 3,6-anhydrogalactose in the alga played a critical role in the synthesis of crystalline AuNPs with cubical dimension. Further, the antibacterial potential of synthesized AuNPs was tested against human pathogens, *Escherichia coli* and *Staphylococcus aureus*. The synthesized AuNPs found biocompatible up to 100 ppm and high concentration showed an inhibition against cancer cell. This novel report could be helped to exploration of bioresources to material synthesis for the application of biosensor and biomedical application.

Keywords: Algae; AuNPs; HRTEM; antimicrobial; pathogenesis

1. Introduction

The continuation of the copious research, which perpetuates to provide new illuminating scientific data, validates the desideratum for the research, development and analysis of the synthesis of nanoparticles by various routes for their application in general and biological way in particular. Green synthesis is an ideal method for the synthesis of nanoparticles without adding toxic chemical reagents and with no need of expensive instruments and more importantly, which follows eco-friendly procedures as compared to other conventional methods [1,2]. Various biomolecules were used to synthesis nanoparticles such as soluble starch [3], C-phycoerythrin [4], ascorbic acid [5]; plant extract [6]. Algae well known as eukaryotic aquatic photoautotrophs and oxygenic are prime producers in many aquatic environments [2,7]. Red algae have recognizable advantage due to its tremendous metal reducing nature, low cost and apparent structure. Rapid production of AuNPs over extracellular biosynthesis has been made appropriate form by red algae. Previously, a *Comparative nanomics*, a study was proposed to compare biological entity for the synthesis of nanoparticles and an anisotropic AuNPs have been synthesized based on the photosynthetic microalgae used [7].

Gold (Au) and AuNPs attracted much attention and universally adapted materials due to their unique and tunable surface plasmon resonance (SPR). The AuNPs are used for the development of biosensors and vapor sensing [8], antimicrobial [9-12], anticancer [13-15], DNA labelling [7], bioelectricity [16], catalytic and anti-helicobacterial and multifaceted applications [4,17-20]. Generally, reducing the size of the AuNPs to zero dimensional can promote the surface area and catalytic active sites. AuNPs of different sizes and shapes including spherical, octahedral, sub-octahedral, decahedral, icosahedral, irregular shape, nano- triangles and nanoprisms, tetrahedral, hexagonal platelets and nanorods have been reported [21].

To date, extensive studies revealed on the application of nanomaterials due to their small size and high surface area acts as strong antimicrobial agents against the pathogenic microbial species which are resistant to traditional antibiotics [1,22]. Cancer is a lethal disease and the available treatment options that include surgery, radiation, and chemotherapeutic drugs often kill healthy cells and cause toxicity to humans. Generally, nanoparticles attached to the metals, minerals and drugs could be a useful material to fight against the life threatening cancer cells [10]. Thus there is a need to develop various nanomaterials and nanodevices for the early diagnosis and treatment of cancer as a potential biocompatible therapeutic agent which was addressed in this study.

Most of the prokaryotic and eukaryotic microorganisms were studied in this direction. Algal cells are highly organized units, regarding morphology and metabolic pathways, capable of synthesizing reproducible particles with well-defined size and structure, more importantly; biogenic nanoparticles often exhibit water-soluble and biocompatible properties, which are essential for many applications [7,23]. In the present study, *Gelidium amansii*, a marine red alga is a rich source of polysaccharide and phycoerythrin has been studied for the synthesis of gold nanoparticles. The major advantages of polysaccharides are that its content in the algae is about 67 % of the total biomolecules, and it can be easily extracted from the algal cell. Further we have studied the antibacterial and anticancer potential of *G.amansii* synthesized AuNPs.

2. Materials and methods

2.1 Algal culture, chemicals and microorganisms

Gold (III) chloride trihydrate ($HAuCl_4.3H_2O$: ≥99.9%; Sigma Aldrich, USA). All other chemicals required for antibacterial, and anticancer studies were of the highest purity available and of analytical grade. Marine red alga, *G. amansii* was collected and determined the properties according to the Sustainable Environmental Process Research Institute (SEPRI), Korea and washed using tap water several times for removal of sea salts and dusts. The algal mat was then dried and grind into fine powder using mesh (0.3 mm). The bacterial cultures of *S. aureus*, and *E. coli* were used for antibacterial studies.

2.2 Synthesis of gold nanoparticles

In a typical synthesis, an aliquot of algal raw powder (2 mg) added was mixed with varying the concentration of 1 ml of $HAuCl_4.3H_2O$ (0.5 mM, 1 mM) solution and kept at room temperature for 1 h. The color of the solution was changed to dark brown. The solution was then washed with milli-Q water and AuNPs was separated by centrifugation at 10, 000 rpm for 1 hr.

2.3 Characterization of gold nanoparticles

The absorption of AuNPs was analyzed in UV-Vis-NIR spectrophotometer V-570 (JASCO, USA) in the λ 800 to 400 nm, band width 2.0 nm. The synthesized AuNPs were characterized by powder x-ray diffractometry using CuKα radiation (λ = 0.1548 nm) in a diffraction angles from 20 to 90 degrees. The sample was placed on the single crystalline Si plate to minimize the background. The particle size and atomic arrangements of the AuNPs was observed by scanning transmission electron microscope (JEM 2100F, Japan) with accelerating voltage at 200 kV equipped with a CCD camera (Gatan Orius, 200D). The samples for TEM analysis were prepared by dropping methanol suspensions of the AuNPs on the Cu grid coated with carbon collodion film. Samples were dried thoroughly by evacuation before the TEM analysis. The surface topography, shape and size of the AuNPs were also examined by scanning electron microscope (Hitachi SU8000, Japan) with parameters of 5 kV electron energy and 10 µA vacuum levels.

2.4 Screening for antimicrobial activity

The antimicrobial activity of *G. amansii* synthesized AuNPs was carried out with both Gram Negative and Gram Positive bacterial cultures such as *E. coli* and *S. aureus*. The nutrient agar medium was prepared with different concentration of synthesized AuNPs (20–300 ppm) and was used to cultivate bacteria. *G. amansii* extract used as control. Inoculated cultures incubated at 37 °C for 24 h and its turbidity was measured under 600 nm of wavelength using ELISA reader (Tecan, Switzerland).

2.5 *In-vitro* cytotoxicity assay

The *in-vitro* cytotoxicity test of *G. amansii* synthesized AuNPs was evaluated to investigate biocompatibility and suitability in biomedical applications. The cell viability was checked using MTT assay [14,19] against rat glial tumor C6 cell lines procured from American Type Culture Collection. Briefly, 200 mL of cell suspension containing approximately 1.25×10^4 cells per well was added into each well of a 96-well culture plate and incubated for 48 h at 37^oC in a 5% CO_2 humidified atmosphere. At the end, 20 μL of MTT reagent (10%) was added in each well and incubated for 4 h at 37 °C in a water bath. Then, DMSO (10%) was added to each well and shaken for 30 min on a plate shaker in the dark. The absorbance was read at 590 nm using a microplate reader (Epoch microplate spectrophotometer; Biotek, Winooski, USA). Cells without nanoparticles were used as a control. Cell viability for a well was calculated by the following equation: CV (%)= (the OD value of treated well/the OD value of non- treated control well) x 100.

2.6 Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparisons test.

3. Results and discussion

The nanoparticles synthesis reactions were started after the algal powder was introduced into aqueous $HAuCl_4$ at 0.5 and 1.0 mM solution. The pale yellow color solution turned to ruby red color which corroborates the synthesis of AuNPs (Fig. 1a). It has been reported that the color change was occurring because the active biomolecules present in *G. amansii* reduced Au^+ into Au^0 [7]. Furthermore, cyanophycean and chlorophycean biomass treated with 1 mM of $HAuCl_4$ solution initiated to turn purple within an hour of exposure, in contrast to the control sets which remained the same, which is corroborate with rhodophycean used in this study.

The intensity of the color change was increased is due to the excitation of SPR and reduction of $HAuCl_4$ [24]. The surface plasmon band for AuNPs usually has a range of 510–560 nm in aqueous solutions, depending on the nanoparticles morphology. Figure. 1b showed absorbance spectra of AuNPs using algal powder with 0.5 and 1.0 mM, a maximum absorbance at 540 nm was observed 0.5 mM (Fig 1b). It is due to availability of reluctant in the reaction mixture and excess metal ion in the 1 mM. Interestingly, we recorded an increase in the absorbance of AuNPs while decreasing $HAuCl_4$ concentration from 1.0 to 0.5 mM. The dosage of reducing agent and incubation time has direct proportion with rate of AuNPs synthesis [25]. The further investigation was dealt with AuNPs synthesized from 0.5 and 1 mM concentration.

3.1 Characterization studies

XRD pattern strongly evidenced according to JCPDS standard data File No. 893697, that the synthesized AuNPs were crystalline and showed prominent Bragg reflections are (1 1 1), (2 0 0), (2 2 0) and (3 1 1) indicated the AuNPs fcc structure (Fig. 1c). The biochemical constitutes of the *G.amansii* has been reported with high amount of polysaccharide and galactose and 3,6-anhydrogalactose, an analogue of glucose and fructose [26]. The sugar moiety played a significant role in AuNPs synthesis. Usually, plant proteins and polyphenols, which could be stabilized the nanoparticles in more crystalline [13]. In the analysis by EDS of the AuNPs, the presence of an elemental gold signal was confirmed and showed at high percentage (Fig. 1d).

TEM images revealed that the cuboidal AuNPs were synthesized at the first time among the studies reported elsewhere [9]. Isotrophic nano-cubes of AuNPs synthesized by the critical role of galactose in the *Gelidium* sp. An oblong shaped nanocubic AuNPs showed an aspect ratio of 1.4 with 5 nm width and 7 nm in height (Fig. 2a-c). The non-spherical AuNPs exhibit multiple absorption bands, correlated with their multiple axes, in comparison to the single one for isodiametric particles. Previously, two discrete surface plasmon bands were observed in *Phormidium* sp., indicating possible synthesis of non-spherical AuNPs are about 50 nm and 25 nm by HRSEM and TEM analysis [7]. Our result also suggests that a high reduction rate was observed at lower concentration may lead to the formation of nanocubes. HRTEM analysis of AuNPs showed the crystalline and closely arranged electronic fringes with less than 1 nm (Fig. 2d). In algae, as AuNPs are recorded either an intracellular or extracellular production, as in *Phormidium willei*, *Coelastrella* sp., *Lyngbya majuscula* and *Spirulina subsalsa* [25].

According to the HRSEM, the topography AuNPs was observed and solidly cuboidal, in which the AuNPs is in well dispersed form (Fig. 3a). In the present study two discrete surface plasmon bands were observed in the case of *Gelidium* sp. indicating possible synthesis of AuNPs about 5-25 nm by HRSEM analysis (Fig. 3b,c) and it was later confirmed that these were average of 5 nm nano cube by HRTEM study of the size ranges about 4-7 nm. In this synthesis, AuNPs was observed without any agglomeration and the closed view of AuNPs showed three-dimensional oblong shaped AuNPs (Fig. 3d). Above results suggested that the nano-cuboidal AuNPs are synthesized due to the action of algae, *Gelidium*, which act as good bioreductant for synthesis of well discrete nano-cubes. The nanoparticles with discrete, cystalline have high potential applications particularly in medical and material chemistry in particular.

3.2 Antibacterial activity of AuNPs

In the present investigation, the antibacterial potential of *G. amansii* synthesized AuNPs against pathogenic bacteria like *S. aureus* and *E. coli*, which are commonly found in water. Figure. 4a,b shows relative bacterial colony count with high dose against both *E. coli* and *S. aureus*, propose that *G. amansii* synthesized AuNPs have significant biocompatibility up to 100 ppm. Increasing concentration of AuNPs (200–300 ppm) had significant effect on both *E. coli* and *S. aureus* growth (Fig. 4a,b). It was supposed that polysaccharide components may involve in the formation AuNPs due to which it does not prevail any significant antibacterial activity. However to increase antibacterial potential we can make prepare a mixture with AuNPs but the process become more expensive.

3.3 Cytotoxicity of AuNPs

In the present investigation we have evaluated NPs dose dependent cell viability which is considerably vital to know the biocompatibility and suitability in biomedical application. In this concern in vitro cytotoxicity of the synthesized AuNPs was evaluated against tumor rat C6 cell line at different concentrations (10–300 μg/mL). After treatment with AuNPs, cells showed more than 75% viability at concentrations up to 100 ppm as shown in Fig. 5. However it has confirmed that AuNPs were not lethal to the C6 cells, since more that 50% of viability retained at high concentrations from 100 to 300 ppm (Fig. 5). I*n-vitro* toxicity studies are dependent on particle properties, dose, and culture conditions [27]. Biocompatibility of newly synthesized nanoparticles significantly important factor to be assessed before implying for the biomedical applications and it was supposed that by adding ligand or suitable drug molecule it can be used as nanodrug to treat the cancer.

4. Conclusion

Nano cubical AuNPs was synthesized by molecules in the *G. amensii*. AuNPs were synthesized in ambient conditions and characterization was carried out by HRTEM, HRSEM equipped with EDS, XRD and UV-Vis spectroscopy. It is believed that algal galactose, the main sugar monomer of red algae have reduced the Au ions into AuNPs. This may be the first report on the synthesis of nano-cubic AuNPs using photosynthetic algae. Three-dimensional oblong shaped, dispersed AuNPs was observed with size ranges of 5-25 nm and that an aspect ratio of 1.4 by HRSEM and HRTEM analysis respectively. The crystalline structure was confirmed by XRD analysis. The synthesized AuNPs showed less antibacterial activity as well as anticancer effect the results confirms biocompatibility of newly synthesized nanoparticles and which is significantly important factor for the biomedical applications These findings could be a promising a new generation of algal based nanoparticles synthesis and eventually for novel catalytic and therapeutic applications.

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Figure Legends

Fig 1. Bioreduced AuNPs showing the color changes from pale yellow to ruby red color confirming the formation of AuNPs in the solution (a); UV-Vis spectra of AuNPs synthesized by the red algae (b); a strong crystalline planes (111), (220), (311) showing in AuNPs by XRD analysis (c); EDS spectra of AuNPs showing a elemental gold signal at high percentage (d).

Fig 2. TEM analysis showing an well dispersed cuboidal AuNPs with size ranges from 5-15 nm by bioreduction of $HAuCl_4$ precursor (0.5mM) with algal extract. HRTEM image showing the regularly arranged electron fringes less than 1 nm.

Fig 3. SEM analysis showing well distinct, isotropic cuboidal AuNPs with ranges from 5-25 nm by bioreduction of $HAuCl_4$ precursor (0.5mM) with algal extract. This image was taken at various magnifications.

Fig 4. Antibacterial activity of *G.amansii* synthesized AuNPs against pathogenic bacteria (A) *E. coli* and (B) *S. aureus*

Fig 5. *In vitro* cytotoxicity activity of *G. amansii* synthesized AuNPs against rat glial tumor C6 cell line

Figure. 1

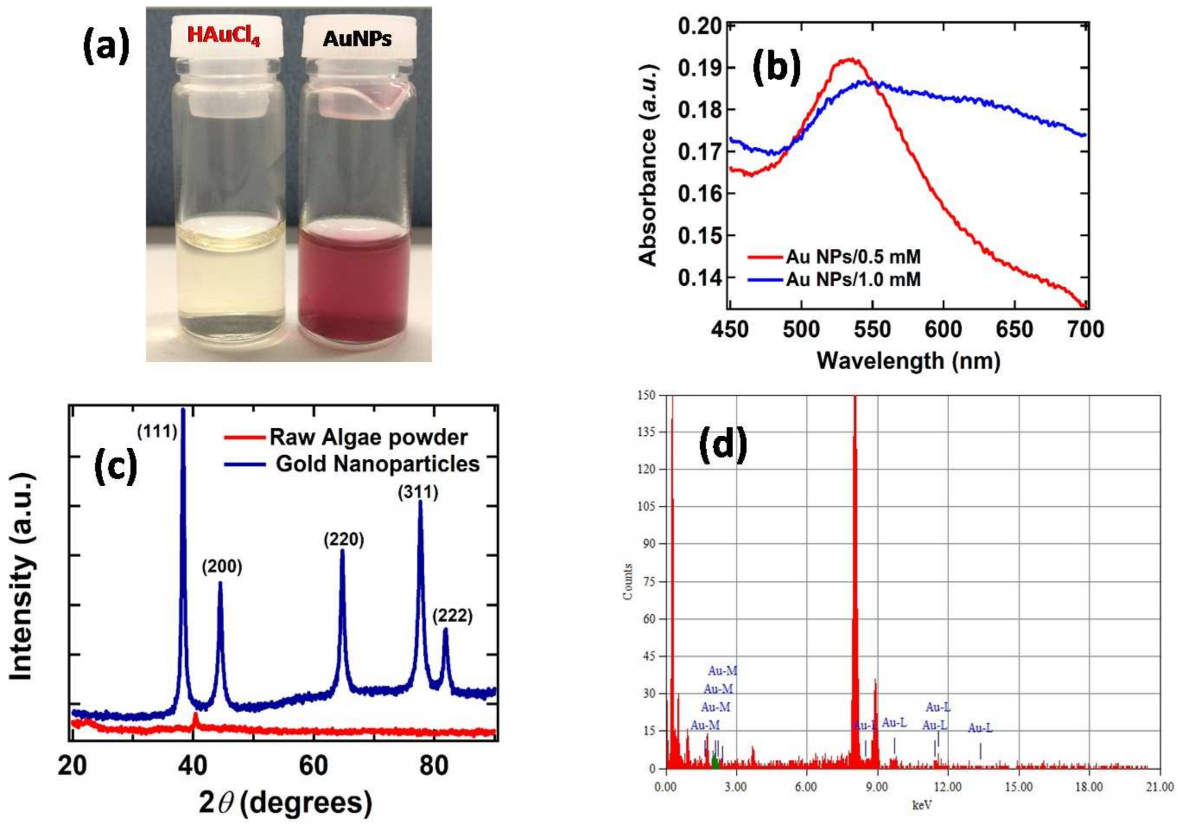

(a)
HAuCl4
AuNPs
(b)
Absorbance (a.u.)
Wavelength (nm)
Au NPs/0.5 mM
Au NPs/1.0 mM
(c)
Raw Algae powder
Gold Nanoparticles
(111)
(200)
(220)
(311)
(222)
Intensity (a.u.)
2θ (degrees)
(d)
Counts
keV
Au-M
Au-L

Figure. 2

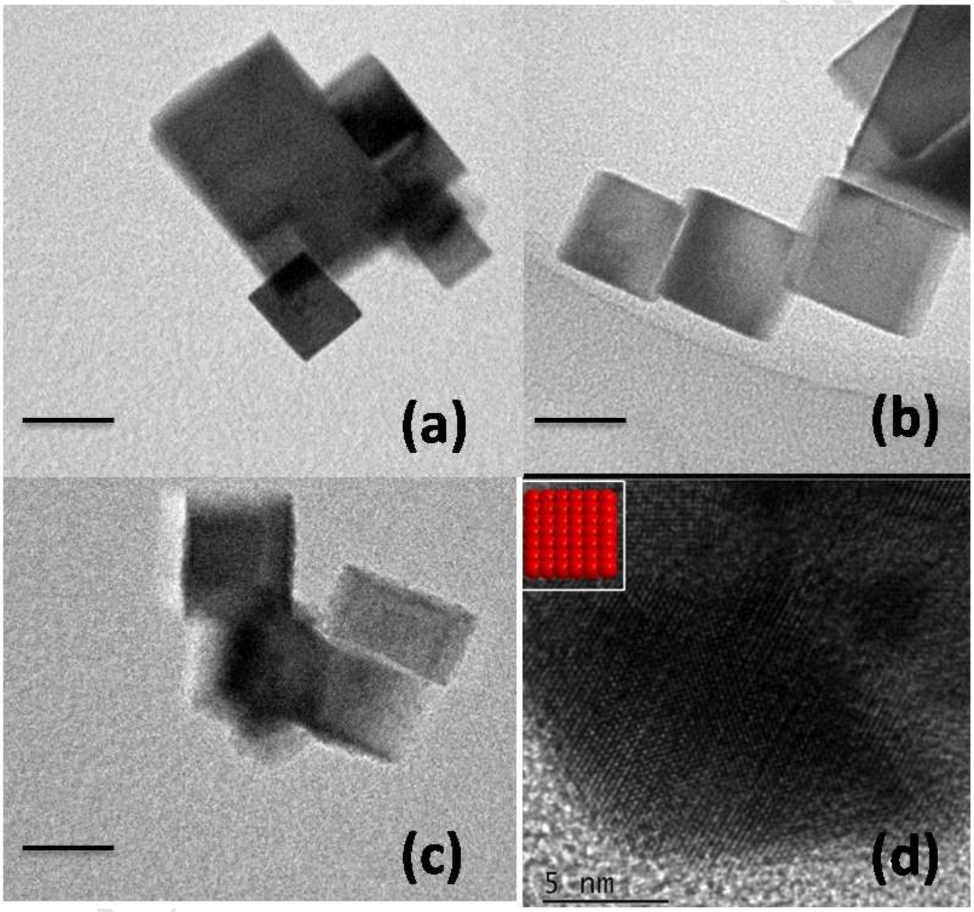


Figure. 3

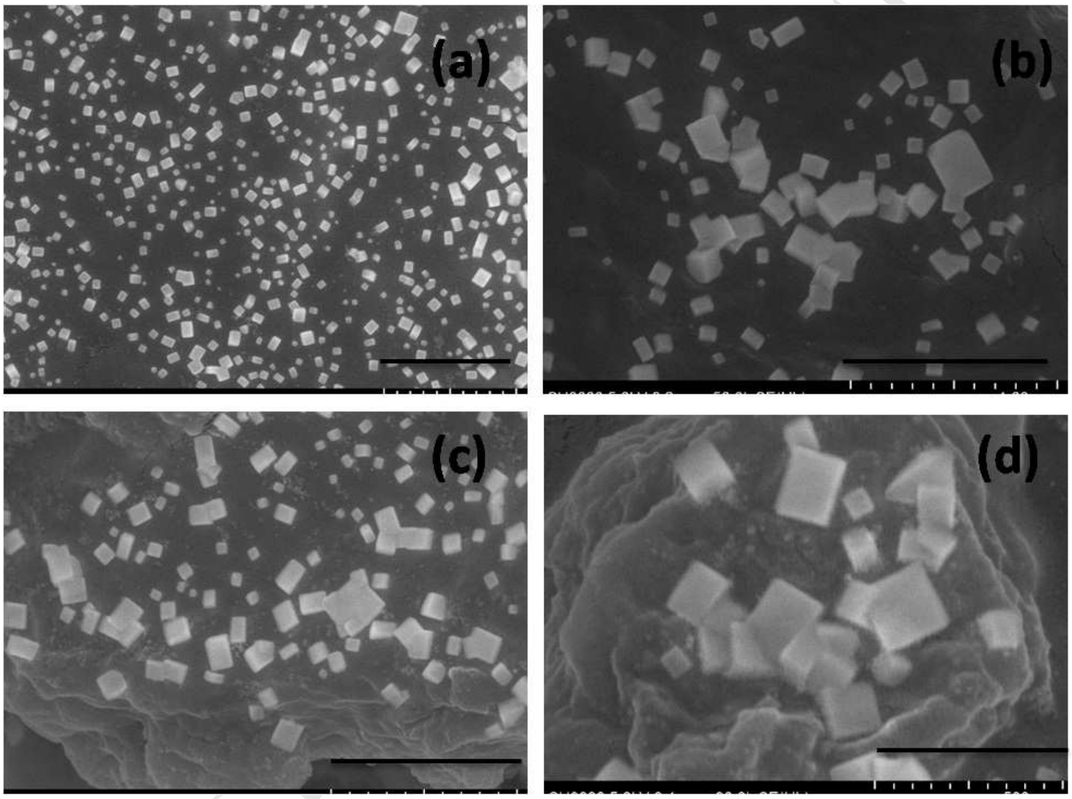
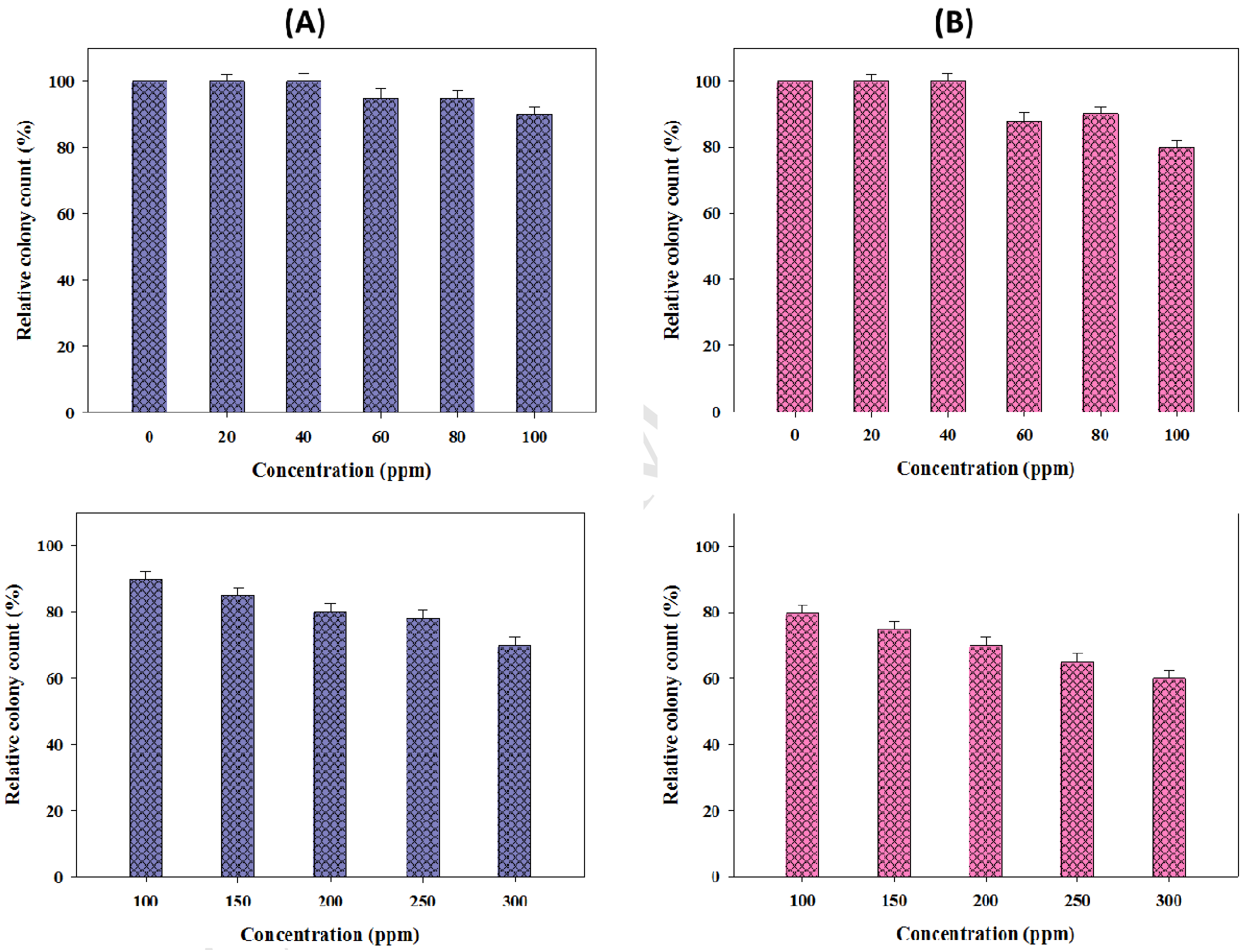


Figure. 4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(B)
Relative colony count (%)
Concentration (ppm)
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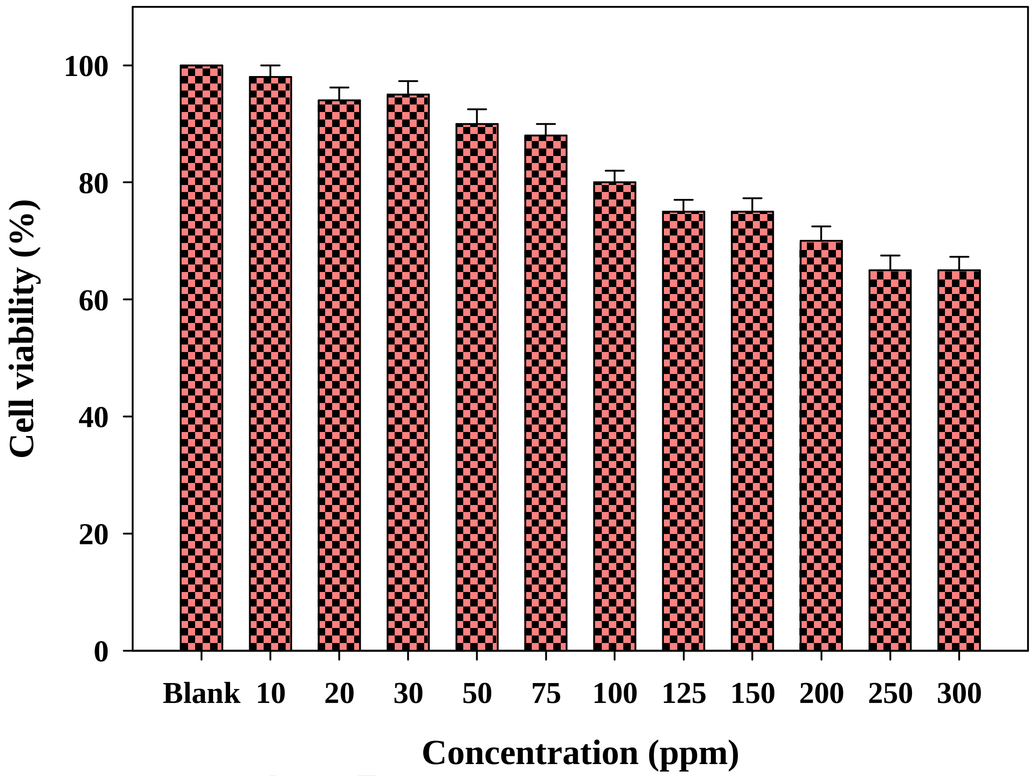
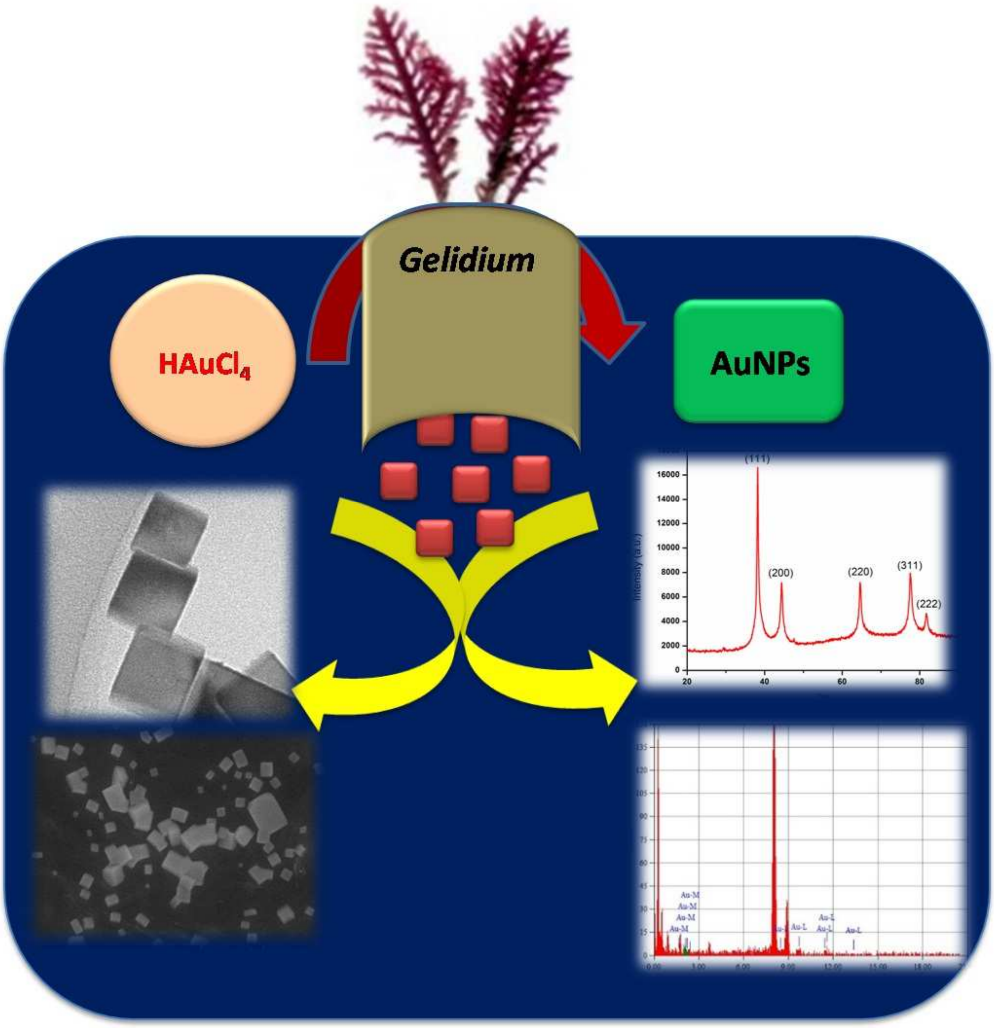
20
40
60
80
100
150
200
250
300

Figure. 5


Cell viability (%)
0
20
40
60
80
100
Blank
10
20
30
50
75
100
125
150
200
250
300
Concentration (ppm)

Graphical Abstract



Research Highlights:

- ✓ Rapid and one pot synthesis of AuNPs using alga
- ✓ Synthesis of nano-cuboidal structures and well defined characterization
- ✓ AuNPs exhibits antimicrobial and anti-cancer property with biocompatible limits