Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 6, Issue 2, 2013 ISSN - 0974-2441

Research Article

BIOLOGICAL SYNTHESIS OF GOLD NANOPARTICLES USING MARINE ALGAE *GRACILARIA CORTICATA* AND ITS APPLICATION AS A POTENT ANTIMICROBIAL AND ANTIOXIDANT AGENT

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Received: 16 February 2013, Revised and Accepted: 10 March 2013

ABSTRACT

The synthesis, characterization and application of biologically synthesized nanomaterials are an important aspect in nanotechnology. Biological synthesis of gold nanoparticles has received a tremendous attention and has been a focus of research due to their high chemical, thermal stability and promising applications in medicinal field due to its environmental friendly approach and also low cost techniques. In the present study gold nanoparticles were synthesized using the aqueous extract of red marine algae *Gracilaria corticata* as the reducing agent. The formation of gold nanoparticles was confirmed by UV-Visible Spectroscopy and Scanning Electron Microscopy (SEM). The synthesized gold nanoparticles were screened against bacterial pathogens gram positive *Staphylococcus aureus, Enterococcus faecalis* and gram negative *Escherichia coli, Enterobacter aerogenes* and also studied for its antioxidant activity by DPPH free radical scavenging assay and Ferric- ion reducing ability antioxidant power assay. The antibacterial and antioxidant activity of the gold nanoparticles showed a considerable activity in comparison with the standards. These results not only provide a green approach for the synthesis of nanoparticles but also open a door for new pharmaceutical leads.

Keywords: Nanotechnology, marine algae, *Gracilaria corticata*, gold nanoparticles, antibacterial activity, antioxidant activity.

INTRODUCTION

Nanotechnology is an escalating field of modern research with desired applications in electronic and medicine [1]. Nanotechnology involves synthesis of nanoparticles of size ranging from 1 to 100 nm [2]. A new branch of nanotechnology is nanobiotechnology which combines biology principles with physical and chemical procedures to generate nano-sized particles with specifics functions. Nanobiotechnology represents an economic alternative for chemical and physical methods of nanoparticles formation. One of the most important criteria of nanotechnology is that of the development of clean, non toxic and eco friendly green chemistry procedures [3]. Nanoparticles are synthesized by physical, chemical and biological methods [4]. Metallic nanoparticles are most promising and remarkable biomedical agents. Due to their large surface volume ratio, they govern interest of researchers on microbial resistance.

Silver, Aluminum, Gold, Zinc, Carbon, Titanium, Palladium, Iron, Fullerenes and Copper have been routinely used for the synthesis of nanoparticles. However, former three metals are most popular metals in bionanomaterial synthesis. Biosynthesis of nanoparticles is an exciting recent addition to the large repertoire of nanoparticles synthesis methods and now, nanoparticle has entered a commercial exploration period. Gold and silver nanoparticles are pertaining to have a wide range of application in the fields of physical, chemical and biological science.

The antimicrobial activity of different nanomaterials such as gold nanoparticles has recently reported [5]. Gold nanoparticles have a great bactericidal effect on a several range of microorganisms; its bactericidal effect depends on the size and shape of the particle [6]. Nanoparticles can act as antimicrobial and antifungal agents, due to their ability to interact with microorganisms [7-9]. Nanoparticles attach to the cell surface causes structural changes and damage disturbing the vital cell functions and finally leading to cell death [10, 11].

Antioxidants are the substances which act as free radical scavengers by preventing and repairing damages caused by reactive oxygen species, and therefore can enhance the immune defense and lower the risk of cancer and degenerative diseases [12]. Recent studies have demonstrated that gold nanoparticles are potential antioxidants [13]. Previous literature revealed that the nanoparticle synthesis using algae as source has been unexplored and underexploited. Recently there are a few, reports that algae is being used as a biofactory for synthesis of metallic nanoparticles [14]. Keeping the above information in mind, we report on the extracellular biosynthesis of gold nanoparticles from the marine algae *Gracilaria corticata* for its antimicrobial and antioxidant activity. *Gracilaria corticata* is a predominant red microalga species found in coastal regions of Indian subcontinent, belonging to the family Gracilariacea. It possess several biomedical properties such as antibacterial, antiviral, antifungal, antiprotozoal, anti- inflammatory, antioxidant, cytotoxic, contraception, gastrointestinal, cardiovascular, hypoglycemia, anti-enzymes, spasmolytic and allelophatic effects [15].

MATERIALS AND METHODS

Sample Collection

In the present study, red marine algae, *Gracilaria corticata* was collected from Muttom coastal region, Kanyakumari district, Tamilnadu, South India. Samples were brought to laboratory in polythene bags and cleaned thoroughly with fresh water to remove adhering debris and associated biota. The algae were cleaned using brush for the removal of the epiphytes with distilled water. After cleaning, algae were dried in shade at room temperature for one week.

Extraction

Dried sample (25 g) were cut into fine pieces and boiled with 100 ml of sterile distilled water for 5 min. The crude extract was passed through Whatmann No.1 filter paper and the filtrates were stored at 4°C for further use.

Synthesis of Gold Nanoparticles

In the synthesis of gold nanoparticles, 10 ml of the aqueous extract of *Gracilaria corticata* was added to 90 ml of 10^{-3} M aqueous HAuCl4 solution in 500ml Erlenmeyer flask and stirred for 4 hr at 120 rpm at 40° C. Suitable controls were maintained throughout the conduct of experiments.

Characterization of Nanoparticles

UV- Visible Measurement: The color change in the reaction mixture (metal ion solution + marine algae extract) from yellow color due to

aqueous auric chloride to pink- red color indicates the formation of gold nanoparticles. The color change was recorded through visual observation. The bio reduction of gold ions in aqueous solution was monitored by subsequently measuring UV-Vis spectra of the solution. Aliquots of the reaction solution were removed absorptions were measured using UV- Visible spectroscopy model Lasany I - 2902. UV- Visible measurement was carried out using deionized water as reference.

SEM analysis: The synthesized gold nanoparticles were characterized for their size using Scanning Electron Microscope (JEOL-JSM 6390, Japan).

Antimicrobial Activity

Ciprofloxacin is the antibiotic drug used to conjugate with the gold nanoparticles. An aqueous stock solution of ciprofloxacin was prepared. The antibiotic conjugated gold nanoparticles were prepared by mixing the gold nanoparticles with the antibiotic stock solution of concentration 1:1 ratio. The antimicrobial activity was checked for the antibiotic, gold nanoparticles and conjugated gold nanoparticles by agar well diffusion method. The levels of inhibition zones were measured after 24hrs incubation at 37^0C.

Estimating Antioxidant Activity

DPPH free radical scavenging assay: The free radical scavenging activity of gold nanoparticles was measured by the DPPH method [16]. Percentage inhibition or DPPH scavenging activity was calculated by

Percentage of Inhibition of DPPH Activity = Abs Blank-Abs Sample x 100 Abs Blank Where, Abs Blank = Optical density of Control, Abs Sample = Optical density of sample extract.

FRAP assay: Ferric- ion reducing ability antioxidant power assay is a technique to determine the total antioxidant power interpreted as the reducing capability. The antioxidant power was measured in this method using ascorbic acid as standard [17]. FRAP value is calculated by

FRAP value of sample (μM) = (Change in absorbance 86 of sample from 0 to 4 minute / change in absorbance of standard from 0 to 4 minutes) x FRAP value of standard (1000 μM)

Note: FRAP value of Ascorbic acid is 1.01

RESULTS AND DISCUSSION

In the present study the biosynthesis of gold nanoparticles using the red marine algae *Gracilaria corticata* species, its antibacterial effect on common human pathogens and its antioxidant activity was ascertained in this work.

After the addition of the extract to the auric chloride solution, the solution changed from yellow colour to pink- red colour within 4 hrs. Fig. 1 shows the aqueous extract of *Gracilaria corticata*, the Gold precursor ($HAuCl_4$) and the formation of pink- red colour which confirms the synthesis of gold nanoparticles. Recently there are a few, reports that algae is being used as a biofactory for synthesis of metallic nanoparticles. The synthesis of silver bionanoparticles using *Sargassum wightii, Kappaphycus alvarezii* and *Gelidiella acerosa* crude extracts, respectively has been reported [18, 19].

UV- Visible spectrophotometer results showed a peak at 540nm (Fig.2). It is the surface

Plasmon resonance of metallic gold nanoparticles exhibit pink- red

color and gives rise to an absorption peak at 540nm. The peak at 540nm confirms that the nanoparticles were well dispersed without

any aggregation. Gold nanoparticles with very good monodispersity have been reported [20]. The technique outlined above proved to be very useful for the analysis of nanoparticles [21]. As illustrated a strong absorption band with a maxima located at 540 *nm* was observed due to formation of gold nanoparticles (Fig. 2).

The SEM micrographs of nanoparticles obtained showed well distributed gold nanoparticles with a size ranging from 45- 57nm (Fig. 3).

In the present work, the synthesized gold nanoparticles and ciprofloxacin conjugated gold nanoparticles were checked for its antibacterial activity against some selected gram positive *Staphylococcus aureus, Enterococcus faecalis* and gram negative *Escherichia coli, Enterobacter aerogenes* pathogenic bacteria by agar well diffusion method. (Fig.4). 'A' indicates zone of inhibition of gold nanoparticles; 'B' indicates zone of inhibition of antibiotic conjugated gold nanoparticles and 'C' indicates zone of inhibition of antibiotic(Ciprofloxacin) respectively (Fig.4). The maximum zone of inhibition was measured in the antibiotic conjugated gold nanoparticles well than the antibiotic (Ciprofloxacin) well. In the pure gold nanopartilces well no zone of inhibition was measured. The maximum antimicrobial activity was observed in the antibiotic conjugated gold nanoparticles well against *E. Coli* (24mm), *Enterobacter aerogenes* (21mm), and the moderate activity was observed in the *Staphylococcus aureus* (19mm) and the least was noticed against *Enterococcus faecalis* (14mm) as shown in Table1. The antibacterial properties of drugs coated gold nanoparticles were higher when compared with the pure drugs. The small size of gold nanoparticles, large surface area and high penetrating power might be the reason for the enhanced activity and hence such nanoparticles could effectively bind to the substrates on the outer membrane and cell membranes of organisms. From Fig.4 it can be seen clearly that the antibiotic coated gold nanoparticles is more effect for gram negative organisms. Gram negative organisms possess a thin cell wall with peptidoglycans where as gram positive organisms generally have thick cell wall made of peptidoglycans. Thus, an easier permeability could be achieved in the case of Gram negative organisms [22].

DPPH is widely used for testing preliminary radical scavenging activity of a compound or a plant extract. In the present study the synthesized gold nanoparticles showed potential free radical scavenging activity. The use of DPPH provides an easy and rapid way to evaluate antioxidant activity. Results of DPPH reduction is shown in Table 2. The synthesized gold nanoparticles showed a good capacity of scavenging the DPPH free radical.The antioxidant activities of the individual compounds, present in the extract may depend on structural features, such as the number of phenolic hydroxyl or methoxyl groups, flavones hydroxyl, keto groups, free carboxylic groups and other structural features. FRAP is an antioxidant assays often used to measure the antioxidant capacity of foods, beverages and nutritional supplements containing polyphenols. It is reduced inthe presence of an antioxidant molecule. Use of FRAP provides an easy and rapid way to evaluate antioxidant activity [23]. Results of FRAP reduction is shown in Table 3.

TABLE 1:TABLE SHOWS THE ANTIBACTERIAL ACTIVITY OF BIOLOGICALLY SYNTHESIZED GOLD NANOPARTICLES

MICROORGANISMS	ZONE OF INHIBITION (mm)Antibiotic (Ciprofloxacin) Conjugated gold nanoparticles.	Antibiotic (Ciprofloxacin)
Gram Positive		
Enterococcus faecalis	14	13
Staphylococcus aureus	19	17
Gram Negative		
Enterobacter aerogenes	21	20
E.coli	24	19

TABLE 2: TABLE SHOWS DPPH FREE RADICAL SCAVENGING ACTIVITY OF GOLD NANOPARTICLES

SAMPLE	CONCENTRATION (µl/ ml)	BLANK	TEST ABSORBANCE I	II	III	%
1	100	0.500	0.120	0.120	0.132	75.2
	200	0.500	0.098	0.098	0.094	80.6
Standard (BHT)	1.6	0.500	0.013	0.013	0.013	97.4

TABLE 3: TABLE SHOWS FRAP REDUCTION BY THE SYNTHESIZED GOLD NANOPARTICLES

SAMPLE	CONCENTRATION (mm/ml)
100µl	178.2
	188.1
	188.1
200µl	237.6
	227.7
	237.6

FIGURE 1:Gold nanoparticles synthesized from the marine algae *Gracilaria corticata* Formation of pink- red colour confirms the synthesis of gold nanoparticles.A is Culture supernatant, B is Gold precursor (HAuCl4) and C is gold nanoparticles.

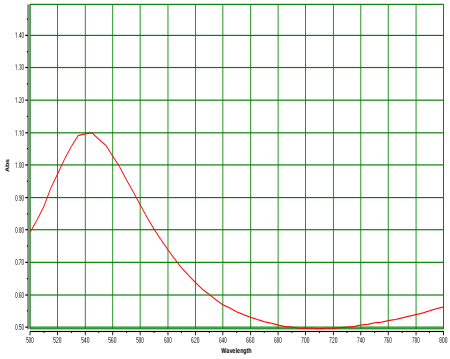

FIGURE 2: UV-VIS Spectra absorbance value of synthesized gold nanoparticlesUV- Visible spectrophotometer graph showing a peak at 540nm.

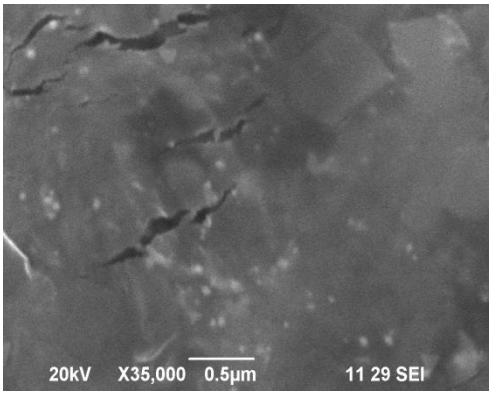

FIG. 3: SEM Micrograph of the synthesized gold nanoparticles

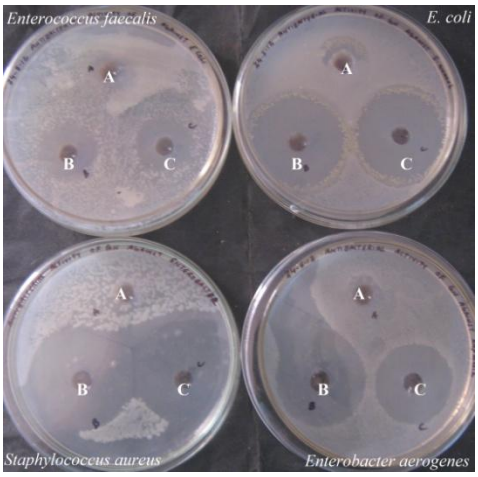

FIGURE 4: Antibacterial activity of gold nanoparticles against pathogenic bacteria

Maximum zone of inhibition was measured in the antibiotic conjugated gold nanoparticles against the gram negative bacteria.

A is Gold Nanoparticles, B is Gold Nanoparticles Conjugated with Ciprofloxacin and C is Antibiotic (Ciprofloxacin).

CONCLUSION

In conclusion, the bio-reduction of aqueous gold ions by the aqueous extract of *Gracilaria corticata* has been demonstrated. This green chemistry approach towards the synthesis of gold nanoparticles has many advantages such as ease with which the process can be scaled up and economic viability. Applications of such nanoparticles in medical and other applications make this method potentially use for the large-scale synthesis of other inorganic nano materials. Toxicity studies of gold nanoparticles open a door for a new range of antibacterial and antioxidant agents.

ACKNOWLEDGEMENT

The authors are sincerely grateful to the management, Udaya School of Engineering, Ammandivilai, Kanyakumari, Tamil Nadu, India – 629 204, for providing all the facilities to carry out this research work.

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