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Der Pharma Chemica, 2013, 5(2):224-229
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Antibacterial activity of algae mediated synthesis of gold nanoparticles from *Turbinaria conoides*

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ABSTRACT

Green synthesis of metal nanoparticles is an important technique in improved methods of eco-friendly nanoparticles production. In this investigation, the biomedically valid gold nanoparticle was synthesized by using marine brown algae Turbinaria conoides. The colour changes from brown to pinkish red confirmed the gold nanoparticles synthesis. The triangle, rectangle and square shaped and 60 nm average sized gold nanoparticles were observed by Scanning Electron Microscope (SEM). The nature of elemental gold was analysed by using Energy dispersive analysis (EDS). Finally the antibacterial activity of gold nanoparticles was performed; it shows Streptococcus sp having the maximum inhibition and medium range of inhibition was examined against Bacillus subtilis and Klebsiella pneumoniae.

Keywords: gold nanoparticles, green synthesis, marine algae, antibacterial activity, *Turbinaria conoides.*

INTRODUCTION

Bioinspired nanoparticles play an important role in the field of biomedical technology. There are various microbes, plants, algae and biochemical compounds are play an important role in the field of green nanoparticles synthesis. In orderly the bacterial isolates used for the synthesis of NPs are *Desulfovibrio desulfuricans* [1], *Shewanella algae* [2], *Geobacter sulfurreducens* [3], *Bacillus subtilus* [4], *Plectonema boryanum* UTEX 485 *Cyanobacterium* [5], *Serratia nematodiphila* [6]. The fungal strains are like *Thermomonospora* sp. [7], *F. oxysporum* [8], *F. oxysporum F.* sp. *Lycopersici* [9], *A. flavus* [10], *F. semitectum* [11], *Cladosporium cladosporioides* [12], *Trichoderma asperellum* [13], *Neurospora crassa* [14].

The plants are major sources of nature involved vigorously for the synthesis of nanoparticles now a day. In this the different parts of plants such as leaves [15], seeds [16], and bark [17] are also used for the synthesis protocol. Some plants involved for the procedure are like *Pelargonium graveolens* (*Geranium leaf*) [18], *Cinnamomum camphora* [19], *Magnolia kobus and Diopyros kaki* leaf extracts [20], Anti-maglinant guava leaf (*Psidium guava*) [21], Pear fruit extract [22], Rosa hybrid petal extract [23], *Nyctanthes Arbortristis* ethanolic flower extract [24], natural precursor Clove (*Szygium aromaticum*) [25], *Chenopodium album* leaf extract [26].

Algae are the major source of the marine having a lot of applications in various fields. Among the marine sources, the macroalgae (seaweeds) occupy a major place as a source of biomedical Compounds. The compounds derived from macroalgae especially brown seaweeds are reported to have broad range of biological activities such as antibacterial [27], anticoagulant [28] and antifouling activity [29]. The brown algae are used for the synthesis of various nanoparticles now a days such as silver and gold nanoparticles synthesis by using *Sargassum wightii* Greville [30, 31], silver nanoparticles by *Padina tetrastromatica* and *Turbinaria conoides* [32, 33] and *Padina gymnospora* [34].

In this investigation we used marine brown algae *Turbinaria conoides* for the green synthesis of gold nanoparticles. The synthesized gold nanoparticle was characterized by using Scanning Electron Microscope and Energy Dispersive analysis. Finally the synthesized nanoparticles were used for the antibacterial activity against *Bacillus subtilis, Klebsiella pneumoniae* and *Streptococcus* sp.

MATERIALS AND METHODS

Preparation of algae extract

Leaves of *T. conoides* were used to prepare the aqueous extract. Leaves weighing 5 g were washed several times with double distilled water to eliminate the waste and dust materials. The leaves were cut into fine pieces and were boiled in an Erlenmeyer flask with 100 ml of sterile double distilled water for 15 min. algae extract broth was filtered through Whatman No 1 filter paper and stored at 4°C for further experiments and used within a week.

Biosynthesis and characterization of gold nanoparticles

From the stored filtrate, typically 10 ml of algae extract was added 100 ml of 1 mM aqueous solution of gold chloride and kept at room temperature. A colour change to pinkish ruby red of the surrounding medium was observed by visual observation confirming the reduction of gold ions to nanoparticles. The size and shape of the algae mediated synthesized gold nanoparticles was employed by Scanning Electron Microscope (Hitachi, Model: S-3400N). Qualitative elemental analysis, standard less quantitative analysis, X-ray line scans and mapping can be performed with SEM-EDS combination

Antibacterial activity of gold nanoparticles

Antibacterial activity of gold nanoparticles was done against *Bacillus subtilis, Klebsiella planticola* and *Streptococcus sp* by well diffusion method. Fresh overnight cultures of inoculums of each culture were spread on to sterilized Muller Hinton agar plates and made 3 wells with 5 mm diameter. Different concentration of gold nanoparticles were added into the each well of agar plates and incubated at 37°C for 24 hr. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well and finds the statistical analysis by performing triplicate experiments.

RESULTS AND DISCUSSION

Visual observation

The reaction mixture of 1 mM aqueous solution of gold chloride with algae extract exhibit pink colour indicates reduction of gold ions to gold nanoparticles (Figure 1). Initially the reaction mixture was turned into brownish pink. After that the colour of the solution vigorously changed into pink while increasing the incubation time from 30 min to 48 h. the colour of the solution is stable without change of intensity indicates the reduction was completed. Colour arising was occurred in the reaction mixture due to the excitations of surface plasmon resonance in the nanoparticles. This important observation indicates the reduction of the Au^{+} ions and the biosynthesis of gold nanoparticles. Previously, Singaravelu et al. [31] reported that the gold nanoparticle synthesis process was started at 1 h and the process was completed at 15 hr. However, in the present study, the gold nanoparticle synthesis process is rapidly started at 50 min for *T. conoides* respectively and completed at 48 h of incubation time.

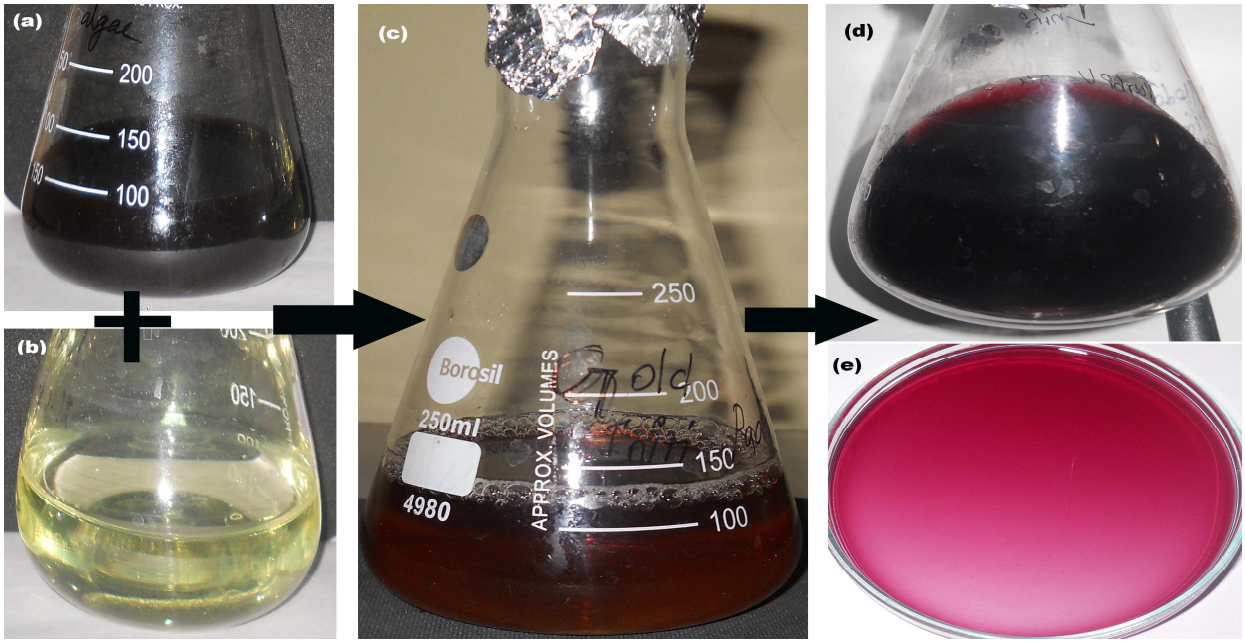

Fig. 1: Visual observation of gold nanoparticles green synthesis (a) *T. conoides* Extract (b) 1mM Gold chloride solution (c) After the addition of a & b Initial (d &e) synthesized gold nanoparticles solution in flask and plate

Scanning Electron Microscope

Figure 2 is SEM image, obtained with *T. conoides* brown seaweed extract and 1 mM Gold chloride solution at room temperature. It is shown that relatively square, rectangle, cubic and triangle shaped nanoparticles are formed with average diameter of 60 nm with some divergence. Daisy and Saipriya [35] have reported the synthesis of gold nanoparticles by using the *Cassia fistula*. The SEM images exhibited that different shape of gold nanoparticles obtained in the bark extract of *C. fistula*.

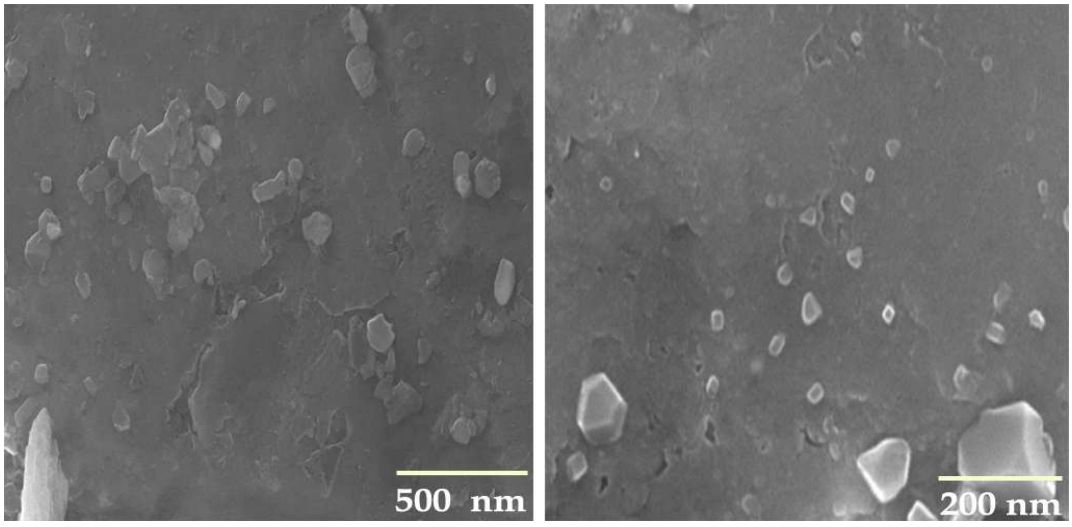

Fig. 2: SEM images of functionalized gold nanoparticles synthesized by using *Turbinaria conoides*

Energy dispersive analysis

The EDS spectra recorded from the AuNps are shown in Figure 3. The EDS profile shows a strong gold signal along with weak oxygen, carbon, magnesium and silica peaks, which may have derived from the biological and chemical molecules and bound to the surface of the gold nanoparticles. It has been reported that nanoparticles synthesized

using brown algal extracts are surrounded by a biochemical material from the seaweed and it is play a important role in the stability [18] it is an one of the advantage for the green synthesis of metal nanoparticles when compare to others.

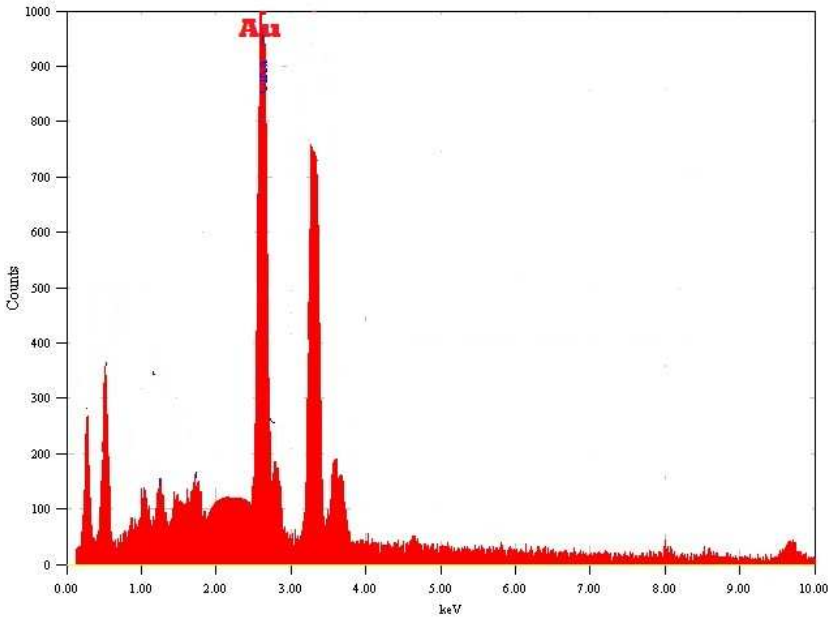

Fig. 3: EDAX Spectra of gold nanoparticles synthesized by algae extract of *T. conoides*

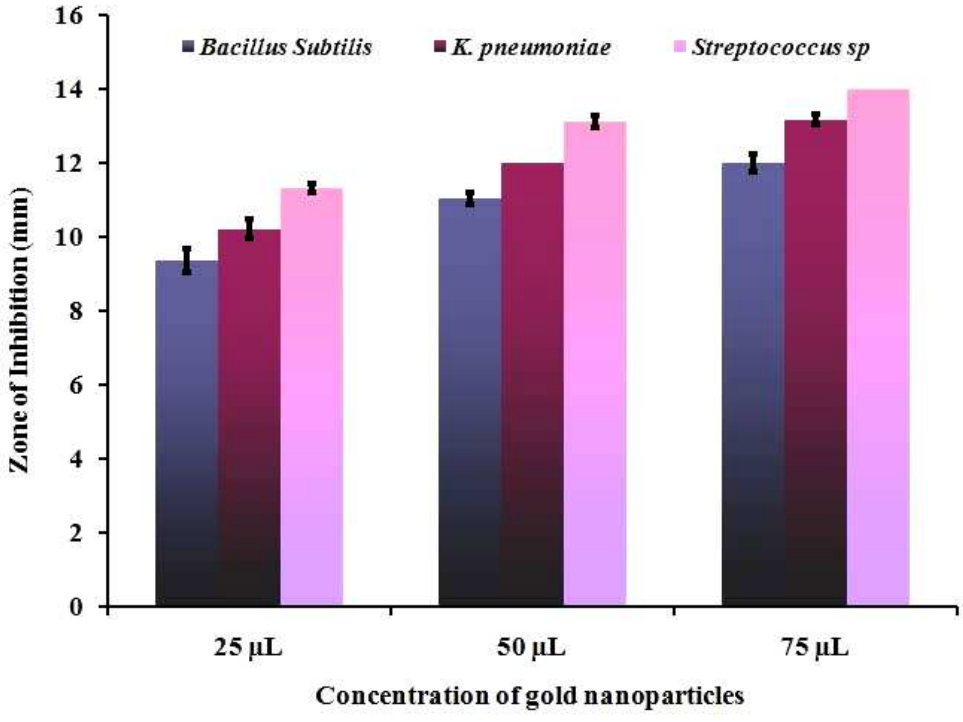

Figure 4: Antibacterial activity of gold nanoparticles synthesize from *Turbinaria conoides*

Antibacterial activity

Three pathogenic bacteria isolates such as *Bacillus subtilis*, *Klebsiella pneumoniae* and *Streptococcus sp*. were used for the antibacterial activity of gold nanoparticles (Figure 4). In this study, the disease causing bacteria

Streptococcus sp shows the maximum zone of inhibition (14 mm). The opportune bacteria *B. subtilis* have the minimum range of inhibition (12 mm). The pneumonia fever causing bacteria *K. pneumoniae* have medium range of inhibition (13mm) because of the addition of 90 µl of colloidal gold nanoparticles. In the year of 2012, Nazari et al [36] investigated that the gold nanoparticles against *P. aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* by disk diffusion method with minimum inhibition. But our study illustrates good zone of inhibition against all the pathogenic bacterial strains. This study also shows that increased zone of inhibition were observed at high concentration of gold nanoparticles. So the zone of inhibition is direct proportion to concentration of gold nanoparticles.

CONCLUSION

Gold nanoparticles have great potentially active in the field of physics, chemistry and biomedicine due to their functionally active properties. In biomedicine gold nanoparticles were used in drug delivery, cancer therapy and antimicrobial agent. In this study we concluded that green synthesis of gold nanoparticles using seaweed extract of *T. conoides* and its antibacterial activity against pathogenic bacteria. A gold nanoparticle synthesis was started at 30 min and completed at 48 h. thus synthesized gold nanoparticles was stable for several months. The SEM image shows the poly dispersed nanoparticles. The presence of gold at binding enegy was confirmed by EDS analysis. Resulted green synthesized gold nanoparticles shows high antibacterial activity against *B. subtilis*, *K. planticola* and *Streptococcus sp* revealed by zone of inhibition.

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