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BIOSYNTHESIS OF SILVER NANOPARTICLES USING OYSTER *SACCOSTREA CUCULLATA* (BORN, 1778): STUDY OF IN-VITRO ANTIMICROBIAL ACTIVITY

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ABSTRACT
The field of nanotechnology is the most active area of research in modern materials science. Though there are many chemical as well as physical methods, green synthesis of nanomaterial is the most emerging method of synthesis. We report the synthesis of antimicrobial silver nanoparticles (AgNPs) using oyster *Saccostrea cucullata* belonging to the marine mollusk. The synthesized AgNPs have been characterized by UV-Vis spectroscopy and X-ray diffractometry. The UV–vis spectrum of the aqueous medium containing silver ion showed peak at 430 nm corresponding to the plasmon absorbance of silver nanoparticles. XRD results have shown that these nanoparticles exhibit a face-centered cubic crystal structure. X-ray diffraction (XRD) spectrum of the synthesized nanoparticles exhibited Bragg reflections corresponding to silver nanoparticles. Such AgNPs synthesized by oyster extract were found to have enhanced antimicrobial activity against well-known pathogenic strains. To the best of our knowledge, this is the first report of production of silver nanoparticles are synthesized by oyster *Saccostrea cucullata*.

KEY WORDS: oyster *Saccostrea cucullata*, Silver nanoparticles, UV-Spec, XRD, Anti-microbial activity

INTRODUCTION

Nanotechnology is enabling technology that deals with nano-meter sized objects. It is expected that nanotechnology will be developed at several levels: materials, devices and systems. Nanoparticles are being viewed as fundamental building blocks of nanotechnology. An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different size, shape and controlled disparity. Synthesis of nanoparticles using biological entities has great interest due to their unique shape dependent optical, electrical and chemical properties have potential application in nanobiotechnology. Many biological organisms, both unicellular and multicellular, are known to produce inorganic materials either intra or extra-cellularly (Simkiss and Wilbur, 1989) often of nanoscale dimensions and of exquisite morphology and hierarchical assembly. Thus, there is a need for green chemistry that includes a clean, non-toxic and environment friendly method of nanoparticles synthesis (Mukherjee *et al*., 2001). Preparation of nanoparticles using green technologies is advantageous over chemical agents due to their environmental consequences. Green synthetic procedures include mixed-valence polyoxometallates (Zhang *et al*., 2009), polysaccharide (Kemp *et al*., 2009), tollens (Panacek *et al*., 2009), irradiation (Abid *et al*., 2002), and biological methods (Maliszewska *et al*., 2009). In the biological method, extracts from living organisms may act both as reducing and capping agents in synthesis of nanoparticles. The reduction of metal ions by combinations of biomolecules found in these extracts such as enzymes/proteins, amino acids, polysaccharides, and vitamins is environmentally benign, yet chemically complex. Living organisms have huge potential for the production of nanoparticles having wide applications. By using the organisms from simple bacteria to highly complex eukaryotes in the reaction mixture, the production of nanoparticles with desired shape and size can be obtained (Sharma *et al*., 2009). Rapid and green synthetic methods using biological extracts have shown a great potential in nanoparticles synthesis. However, understanding the mechanism of involvement of biomolecules is lacking. Better understanding will give new green paths in the development of controlled shape and size. Thus tailoring of materials at the atomic level to attain unique properties, which can be suitably manipulated for the desired applications, is a major task in nanotechnology (Hubenthal, 2009). With the terrestrial resources being greatly explored and exploited, researchers turn to the oceans for numerous reasons. The oceans cover more than 70% of the world surface housing 34 living phyla out of the 36 and more than 300,000 known species of fauna and flora. The marine environment is known to contain over 80% of world's plant and animal species. In recent years, many bioactive compounds have been extracted from various marine plants, marine animals and marine organisms (Bhimba *et al*., 2010). The oceans are the source of a large group of structurally unique natural products which are mainly accumulated in invertebrates such as sponges, tunicates, bryozoans, and molluscs. Several compounds show pronounced pharmacological activities and are interesting candidates for new biotechnological applications. Literatures revealed that

synthesis of nanoparticles using oyster has been unexplored, which aroused our interest. Therefore in the present study, the formation of silver nanoparticles was investigated using silver nitrate in the presence of the oyster (*Saccostrea cucullata*).

MATERIALS AND METHODS

Collection of Marine Oyster

Oyster (*Saccostrea cucullata*) was collected from the Mandapam coast (Lat. 09° 17.417'N; Long. 079° 08.558'E) along the south east coast of India, during the month of March 2012. Collected Oysters were cleaned, opened aseptically and shucked as recommended by Hunt *et al*., 1984. The oyster tissue samples were thoroughly washed with sea water and then with distilled water. Then they were kept in an ice box at 4°C, transferred to the laboratory and stored in deep freezer at -40 °C.

Preparation of bio-extracts

Five gram of oyster tissue sample was weighed and ground in 30 ml of water using sterile mortar and pestle. After a systematic grinding, the crude extract was filtered using Whatman No. 1 (42 µm) filter paper and the residue was again ground with 20 ml of water and filtered (Inbakandan *et al*., 2010). The filtrate (50 ml) or the crude filtered extract of oyster sample was stored in deep freezer at -40 °C for further analysis.

Silver nanoparticle synthesis using silver nitrate

For synthesis of silver nanoparticles, 100 ml of 1mM $AgNO_3$ aqueous solution was mixed with 10 ml of stored filtrate in a 250 ml Erlenmeyer flask and agitated at 45°C in dark. Control (without the filtrate, only silver nitrate) was also run along with the experimental flask. Sample of 1 ml was withdrawn at different time intervals and the absorbance was measured at a resolution of 1 nm using a UV-Visible spectrophotometer.

Characterization of nanoparticles

UV-Vis Spectroscopy

1 mM (final concentration) $AgNO_3$ was mixed with 10 ml of filtrate in a 250 ml Erlenmeyer flask and agitated in dark. Then after 24 h, the optical density of synthesized nanoparticles suspended in distilled water was measured by UV-Visible spectrophotometer (Spectra Max Plus^{384}, USA) at different wavelength ranging from 300 to 700 nm and plotted the values on a graph.

X- Ray Diffraction Analysis

The formation and quality of nanoparticles were checked by XRD technique. X-ray diffraction (XRD) measurement of the oyster (*S.cucullata*) filtrate reduced Ag nanoparticles was carried out on films of the respective solutions drop coated onto glass substrates were recorded in a wide range of Bragg angles 2θ at a scanning rate of 0.388/min on a X-ray diffractometer (X'pert Pro, Netherlands) operating at a voltage of 40 kV and a current of 30 mA with Cu K radiations.

Antimicrobial assay

The silver nanoparticles synthesized using *S.cucullata* was tested for antimicrobial activity by agar well-diffusion method against human pathogenic bacteria *Staphylococcus aureus*, *Klebsiella pneumonia*, *Klebsiella* oxytoca, *Proteus mirabilis*, *Vibrio cholera*, *Salmonella paratyphii*, human pathogenic fungi *Aspergillus niger*, *Aspergillus flavus, Candida albicans,*. The pure cultures of bacterial and fungal pathogens were subcultured on nutrient agar and Potato Dextrose Agar (PDA) respectively. Wells of 5 mm diameter were made on nutrient agar and PDA plates using gel puncture. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Using a micropipette, different concentrations of the sample of nanoparticles solution (10 µl, 20 µl and 50 µl) was poured onto each well on all plates. After incubation at 37°C for 24 hours, the different levels of zone of inhibition of bacteria were measured. The fungal plates were kept at room temperature for 48 hr and the clear zones were measured.

RESULTS AND DISCUSSION

Silver nanoparticles have applications in spectrally selective coating for solar energy absorption, optimal receptors in intercalation material for electrical batteries, polarizing filters, catalysts in chemical reaction, bio-labeling and as antimicrobial agents (Kuber and D'Souza, 2006). There are several physical and chemical methods for synthesis of metallic nanoparticles (Edelstein and Cammarata, 1996). However, biological methods may be relatively simple, reliable, eco-friendly and promising (Deendayal *et al*., 2006). Formation of silver nanoparticles by reduction of silver nitrate during exposure to oyster (*Saccostrea cucullata*) extract can be easily monitored from the change in colour of the reaction mixture. The change in colour of the reaction mixture after 24 hr at 45°C is presented in Figure 1. The colourless solution changed into brown colour which indicates the formation of silver nanoparticles. In case of control (silver nitrate solution alone), no change in color was observed. The colour change was due to excitation of surface plasmon vibrations in the metal nanoparticles. This formation indicates that silver ions in reaction medium have been converted to elemental silver having the size of nanometric range. This observation was reconfirmed by UV-Visible spectrum and XRD analysis.

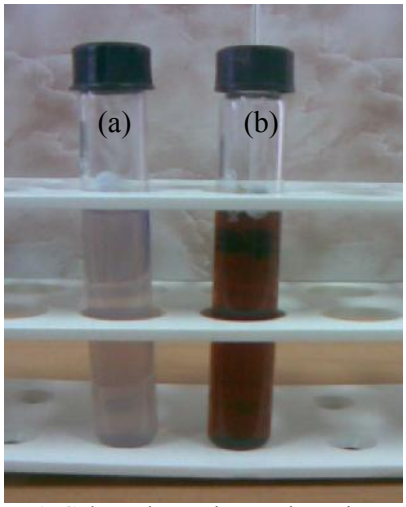


FIGURE 1. Colour change in reaction mixture (silver nitrate + oyster extract) (a) at 0 hours (b) after 24 hours.

UV-Visible spectrum

The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for

structural characterization of silver nanoparticles (Sun *et al*., 2001). UV-visible spectral analysis confirmed the formation and stability of the biosynthesized silver nanoparticles using the extract of oyster *Saccostrea cucullata*. A peak was observed in UV-visible spectrum (Figure 2) corresponding to the surface plasmon resonance occurs at 430 nm with absorbance of 0.7 optical densities and clearly indicates the formation of silver nanoparticles in solution as the exact position of absorbance depends on a number of factors such as the dielectric constant of the medium and size of the particle. In order to assess the stability of the newly formed silver nanoparticles UV-vis spectral analysis was made which shows that the surface plasmon absorbance did not change even after six months indicating the stability of the silver nanoparticles. The surface plasmon band in the silver nanoparticles solution remains close to 430 nm throughout the reaction period, suggesting that the particles are dispersed in the aqueous solution with no evidence for aggregation.

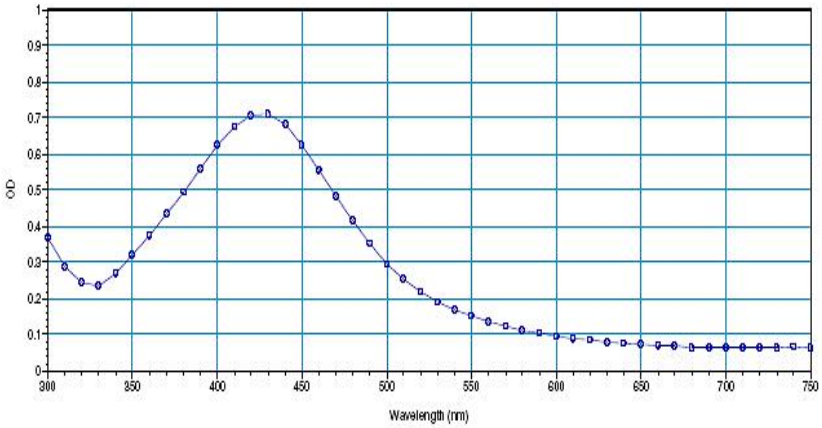
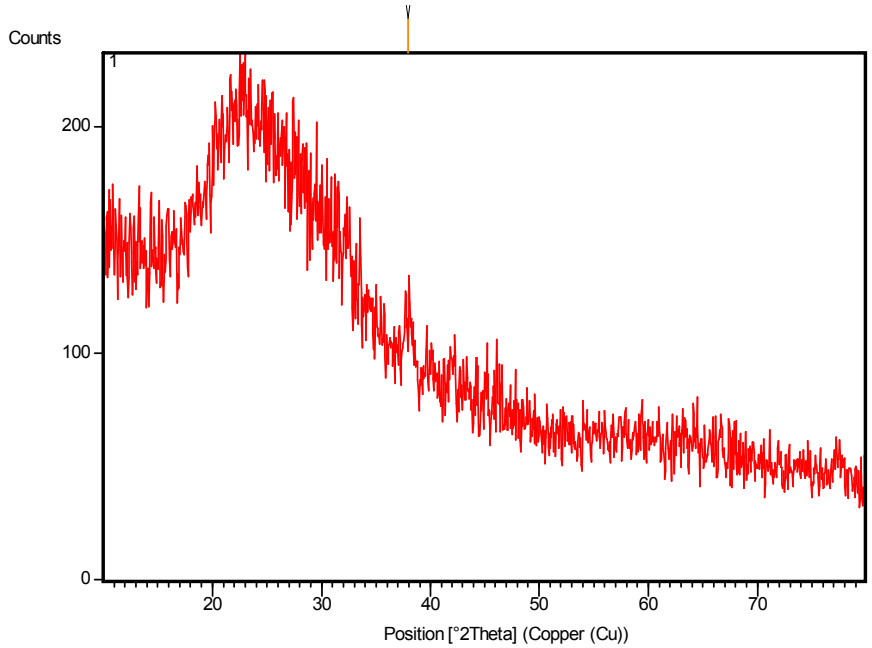


FIGURE 2. UV-Vis absorption spectrum of obtained silver nanoparticles

X-ray diffraction pattern

The X- ray diffraction patterns obtained for the silver nanoparticles synthesized using oyster (*S. cucullata*) extract is shown in Figure 3. The presence of intense peak of silver nanoparticles corresponding to the (111) which is indexed as crystalline silver face-centered cubic (fcc) phase (Leff *et al*., 1996). According to Scherrer's formula (Jeffrey, 1971), $t = 0.9\ \lambda/(\beta \cos\theta)$, an average crystal size (t) of the silver nanoparticles can be estimated from the X-ray wavelength of the Cu Ka radiation, the Bragg angle (θ), and the width of the peak at half height (maximum) (β) in radians. The average size of the silver nanoparticles as calculated using the peak at 37.93° (which is the characteristic (111) peak of silver) is 10.5 nm.



Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
37.93(7)	15(6)	0.8(2)	2.37049	100.00

FIGURE 3. X-ray diffraction patterns of prepared silver nanoparticles

Antimicrobial activity of silver nanoparticles (Ag-NPs)

The antimicrobial activity of oyster (*S. cucullata*) mediated silver nanoparticles was determined against human pathogenic bacteria and fungi. In our study, the AgNPs synthesized using oyster (*S. cucullata*) extract exerted a fairly significant antimicrobial action on the tested bacterial and fungal pathogens. This is evident by the values of diameter of zone of inhibition obtained during assessment of antimicrobial activity (Table 1). Results clearly demonstrate that newly synthesized silver nanoparticles are promising antimicrobial agent against the pathogens employed. The maximim antibacterial activity was observed against *Staphylococcus aureus* followed *by Klebsiella oxytoca*, *Salmonella paratyphii*, *Klebsiella pneumoniae* and the least was noticed against *Vibrio cholera*. In the anti-fungal activity, synthesized nanoparticles showed maximum activity against *Aspergillus niger*. Similarly, Selvakumar *et al*., (2012) also reported antimicrobial activity of silver nanoparticles synthesized by marine derived *Streptomyces rochei* against common human pathogens.

The mechanism of the bactericidal effect of silver nanoparticles against bacteria is not very well-known (Panacek *et al*., 2008). Silver nanoparticles may attach to the surface of the cell membrane and disturb its power function such as permeability and respiration. It is reasonable to state that the binding of the particles to the bacteria depends on the surface area available for interaction. Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles (Panacek *et al*., 2008). Morones *et al*., (2005) demonstrated using the Scanning Tunneling Electron Microscopy (STEM) and the X-ray Energy Dispersive Spectrometer (EDS), showed silver nanoparticles not only at the surface of cell membrane, but also inside the bacteria. This then suggests the possibility that the silver nanoparticles may also penetrate inside the bacteria and fungi, causing damage by interacting with phosphorus- and sulphur-containing compounds such as DNA. Silver tends to have a high affinity to react with such compounds. One more possibility would be the release of silver ions from nanoparticles, which will have an additional contribution to the antimicrobial properties of silver nanoparticles. Currently, the increase of bacterial resistance to antimicrobial agents poses a serious problem in the treatment of infectious diseases as well as in epidemiological practice. Increasingly, new bacterial strains have emerged with dangerous levels of resistance, including both of Gram-positive and Gram-negative bacteria. Dealing with bacterial resistance will require precautions that lead to prevention of the emergence and spreading of multi resistant bacterial strains, and the development of new antimicrobial substances (Panacek *et al*., 2008). Our results demonstrate the ability of the oyster (*S. cucullata*) on synthesizing silver nanoparticles and their antimicrobial activity represent a significant advancement in the nanomaterial with realistic implications.

TABLE 1. Zone of inhibition (mm) of oyster *Saccostrea cucullata* mediated silver nanoparticles.

	Nanoparticles in µl		
Test organism	10 µl	20 µl	50 µl
Staphylococcus aureus	8.6	12.3	16.7
Klebsiella pneumoniae	7.3	10.6	13.9
Klebsiella oxytoca	7.8	11.7	15.6
Proteus mirabilis	7.2	10.4	13.4
Vibrio cholera	6.9	9.8	12.8
Salmonella paratyphii	7.5	11.5	14.2
Aspergillus niger	8.2	9.3	10.1
Aspergillus flavus	7.4	8.2	9.5
Candida albicans	6.1	6.8	7.2

CONCLUSION

A critical need in the field of nanotechnology is the development of reliable and eco-friendly processes for synthesis of metallic nanoparticles. The present study reported extra-cellular synthesis of silver nanoparticles by oyster (*S. cucullata*). The characteristics of the obtained silver nanoparticles were studied using UV-Vis, XRD techniques. The results indicated that the natural marine derived oyster (*S. cucullata*) is a good source for the extracellular synthesis of silver nanoparticles. Investigation on the antimicrobial activity of AgNPs against bacterial and fungal pathogens reveals high potential of oyster extract stabilized AgNPs to be used as antimicrobial agent in medicinal field as well as food and cosmetic industries.

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