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Research Article

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Biosynthesis of silver nanoparticles using tissue extracts of *Turbo brunneus* (R.), *Cypraea annulus* (L.), *Babylonia spirata* (L.) and their antibacterial potential

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Abstract

Living organisms have huge potential for the production of nanoparticles having wide applications. One of the most studied aspects of nanotechnology nowadays is their ability to offer the opportunity to fight microbial infections via synthesis of nanoparticles. In the present investigation synthesis of silver nanoparticles from *T. brunneus*, *C. annulus* and *B. spirata* extracts were analyzed. The appearance of brown colour in the reaction suggest the formation of silver nanoparticles. The synthesis of silver nanoparticles had been confirmed by the surface plasmon band and the specific wavelength of silver nanoparticles solution remains close to 460nm in *T. brunneus*, 450nm in *C. annulus* and 430nm in *B. spirata*. Among the three marine gastropods *B. spirata* showed the maximum activity against *E. coli* (17 mm). The least activity was observed in *C.annulus* against *P. aeruginosa* (1mm) at 10 µl concentration respectively. The results of the present study concluded that the tissue extracts of *Turbo brunneus*, *Cypraea annulus* and *Babylonia spirata* are a good source for the synthesis of silver nanoparticles.

Keywords: Silver nanoparticles, *Turbo brunneus*, *Cypraea annulus*, *Babylonia spirata*, Silver nitrate.

1. Introduction

Nanobiotechnology, an emerging field of nanoscience utilize nano-based systems for various biomedical application of nanoparticles which are being viewed as fundamental building block of nanotechnology. Nanoscale particles and molecules are a potential alternative for treatment of disease because they have unique biologic effects based on their structure and size, which differs from traditional small - molecule drugs (Wagner and Dullaart, 2006). Researchers in the last years have turned to biological systems for nanoparticles synthesis (Tsibakhashvili *et al*., 2010). Biosynthesis of nanoparticles is now established as an alternative to chemical and physical methods of synthesis (Peter Amaladhas *et al*., 2012). Green chemistry approach emphasizes that the usage of natural organisms as a reliable, simple, nontoxic and eco-friendly (Sathishkumar *et al*., 2010).

Silver nanoparticles are one of the most commonly used nanomaterials which gained increasing interest in the field of nanomedicine due to their unique properties and obvious therapeutic potential in treating a variety of disease (Kalishwaralal *et al*., 2009).

Living organisms have stupendous application, as rapid and green synthetic methods using biological extracts have shown a great potential in nanoparticles synthesis. Silver nanoparticles are used in molecular diagnostics, in therapies and in devices that are used in several medical procedures (Rai *et al*., 2009).

The mechanism of prevention of bacterial growth by antibiotics is quite different from the mechanisms by which nanoparticles inhibit microbial growth. Therefore, nanoparticles have the potential to serve as an alternative to antibiotics and to control microbial infections (Selvakumar *et al*., 2012). In particular, silver ions have long been known to exert strong inhibitory and bactericidal effects as well as to possess a broad spectrum of antimicrobial activities and effectively used for the treatment of various infectious diseases (Mudshinge *et al*., 2011). Some microorganisms are resistant to commercially available drugs and antibacterial agents often increasing serious health problems (Wright, 2005).

Therefore, it is very urgent and important to explore the other approaches to effectively suppress the variety of pathogenic bacteria. Silver nanoparticles synthesized with a green method have high antibacterial activity. It is of great interest to develop nanoparticles based antibiotics that decrease the toxicity and side effects of free drugs (Rajesh Kumar *et al*., 2014). In this scenario there is much scope for future drug discovery within this phylum, exploring novel compounds with newer mode of action. Hence an attempt has been made to study the nanoparticle synthesis by three marine gastropods *Turbo brunneus*, *Cypraea annulus* and *Babylonia spirata* belonging to three different orders.

2. Materials and Methods

2.1 Collection of experimental organisms

In the present study the gastropods *Turbo brunneus*, *Cypraea annulus* and *Babylonia spirata* were collected from the Thoothukudi ($8^0$45'N ; $78^0$46'E) coastal region. *Turbo brunneus*, a benthic marine animal belonging to order archaeogastropoda was collected from the rocks and dead corals in the coastal area of Thoothukudi. The mesogastropod *Cypraea annulus*, was collected by hand-picking along the sea shore and rocks of Hare Island region. The neogastropod *Babylonia spirata* was collected from the landed by-catch from fishing trawlers operated for crabs and prawns along the Thoothukudi coastal region. The freshly collected samples were brought to the laboratory, cleaned and washed with fresh sea water to remove all impurities. The shells were broken, tissues were removed and used for further studies.

2.2 Synthesis of silver nanoparticles

2.2.1 Preparation of bio-extracts

Five gram of tissue samples were 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 filter paper and the residue was again ground with 20 ml of water and filtered (Inbakandan *et al*., 2010). The filtered extract of three gastropod samples were stored in deep freezer at -40°C for further analysis.

2.2.2 Silver nanoparticle synthesis using silver nitrate

The aqueous solution of 1mM silver nitrate solution was prepared and used for the synthesis of silver nanoparticles. 5 ml of gastropod extracts were taken in a screw tube separately and to this equal volume of 1mM silver nitrate solution was added drop wise with constant stirring and observed for color change. The color of the solution was checked periodically and then the screw tube was incubated in dark room at room temperature of 24 hours. The water with the addition of 1mM silver nitrate served as control.

2.2.3 Visual identification

The filtrate treated with silver nitrate were observed for the change in color from light yellowish to reddish brown and finally to colloidal brown indicating silver nanoparticle formation. The colour change of the medium from colorless to brown after 24 hrs was observed and compared with the control, which forms a visual method of detection of silver nanoparticle synthesis.

2.2.4 UV - Visible spectroscopic analysis

The formation and completion of silver nanoparticles synthesis was characterized by UV-Visible spectroscopy by using Shimadzu UV-Visible spectrophotometer, model 1800. The bioreduction of the silver ions in the solution was monitored by periodical sampling of aliquots. The absorbance was recorded from 200-600 nm range operated at a

resolution of 0.5nm for detection of synthesized silver nanoparticles. The peak is known as plasmon resonance peak. Distilled water was used as a blank.

2.2.5 Antibacterial activity of silver nanoparticles

The silver nanoparticles synthesized using *T. brunneus*, *C. annulus* and *B. spirata* were tested for antibacterial activity by disc diffusion method against human pathogenic bacteria. Gram negative *Escherichia coli*, *Pseudomonas aeruginosa* and Gram positive *Bacillus subtilis*, *Staphylococcus aureus* were used for antibacterial screening. Twenty four hours old pure cultures were prepared for use each time.

The 20 ml of sterilized agar medium was poured into each sterile petriplates and allowed to solidify. Then different cultures of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* were evenly spread over agar media by using a sterile cotton swab. The sterile 6 mm paper disc was impregnated into the agar plates for the inoculation of synthesized silver nanoparticles and control. The different concentrations of 10 µl, 25 µl and 50 µl of silver nanoparticle was inoculated into the discs of all the plates. The plates were incubated for 24 hrs at 37°C. After incubation period, the different levels of zone of inhibition were observed.

3. Results

3.1 Synthesis of silver nanoparticles

3.1.1 Visual identification

Preliminary identification of nanoparticle formation was carried out by observing the colour change of the reaction solution. Diminution of silver ions to silver nanoparticles was visually distinguished by colour change from yellow to brown and dark brown which indicates the formation of silver nanoparticles (Figure – 1, 2 and 3). The intensity of the colour was increased during the period of incubation. The control showed no change in colour of the mixture when incubated in the same conditions.

Reaction mixture containing silver nitrate and extracts of *T. brunneus*, *C. annulus* and *B. spirata* showed brown colouration indicating formation of silver nanoparticles in the reaction mixture. The colour change was due to excitation of surface plasmon vibrations in the nanoparticles. This formation indicates that silver ions in reaction medium have been converted to elemental silver having the size of nanometric range. Furthermore, the nanoparticles formation by experimental organisms were confirmed by UV-Visible spectroscopy at different wavelengths.

3.1.2 UV-Visible spectroscopic analysis

UV-Visible spectroscopic analysis confirmed the formation of the biosynthesized silver nanoparticles using the extract of *T. brunneus*, *C. annulus* and *B. spirata*. The above solutions were subjected to optical measurements by UV-Visible spectrophotometer. In *T. brunneus* 460nm wavelength was reported, in *C. annulus* 450 nm wavelength and in *B. spirata* 430nm wavelength suggested the presence of silver nanoparticles in the solution (Figure - 1, 2 and 3). This is the specific wavelength which indicates synthesized silver nanoparticles. Among all the three species, *T. brunneus* showed maximum wavelength of 460 nm. The occurrence peak at absorption intensity between 300 to 700 nm indicated the presence of surface plasmon resonance.

Figure 1 : Synthesis of silver nanoparticles and UV-Visible spectrum of *Turbo brunneus*

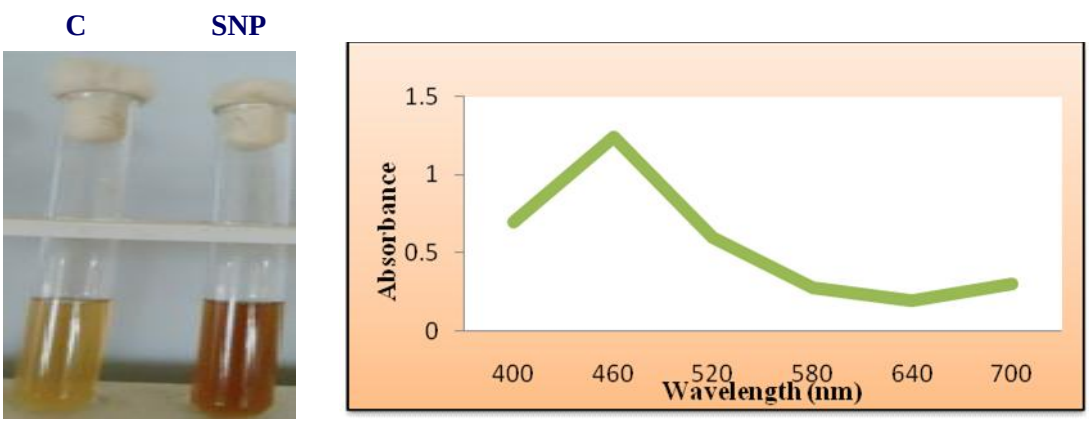


Figure 2: Synthesis of silver nanoparticles and UV-Visible spectrum of *Cypraea annulus*

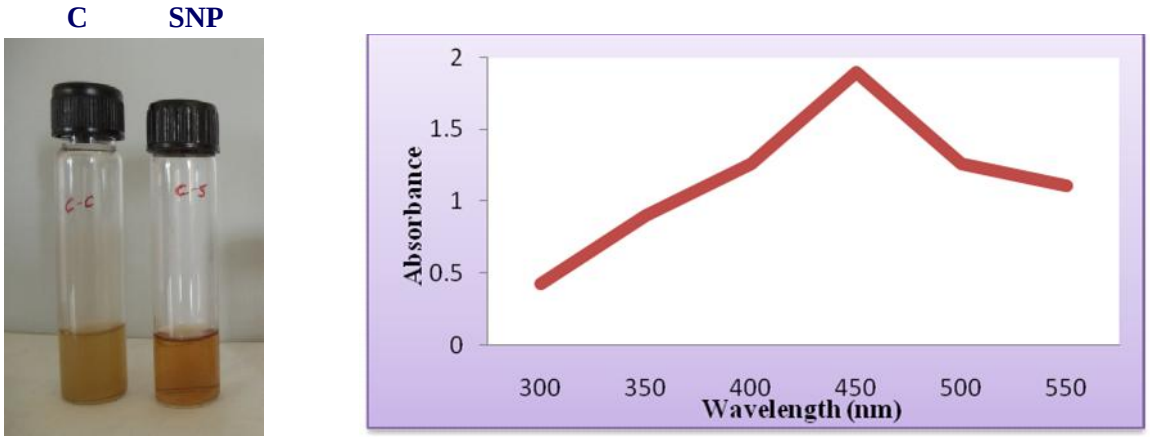
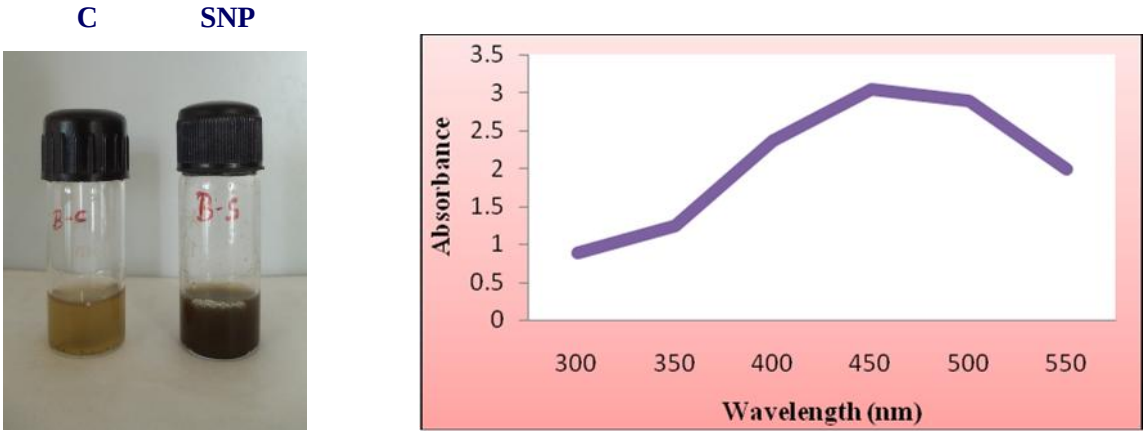


Figure 3 : Synthesis of silver nanoparticles and UV-Visible spectrum of *Babylonia spirata*



3.1.3 Antibacterial activity of silver nanoparticles

The antibacterial efficacy of synthesized silver nanoparticles of *T. brunneus*, *C. annulus* and *B. spirata* were investigated against some selected gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) human pathogenic bacteria by disc diffusion method. Compared with control, the silver nanoparticles synthesized by the body tissues of all the experimental gastropods developed maximum inhibition zone against all pathogens tested. It has been reported that antibacterial effect was dose dependent.

In *T. brunneus* the antibacterial activity of silver nanoparticles showed maximum zone of inhibition against *P. aeruginosa* (15 mm) at 50µl concentration followed by *B. subtilis* (12 mm) and *E. coli* (10 mm). The minimum zone of inhibition was observed against *S. aureus* (2 mm) (Fig. 4). In *C. annulus* the highest activity was observed against *E. coli* (8 mm) followed by *S. aureus* and *B. subtilis* (7 mm) and trace activity was observed against *P. aeruginosa* (1 mm) (Fig. 5). In *B. spirata* the maximum activity was found against *E. coli* (17 mm) followed by *P. aeruginosa* (16 mm), *S. aureus* (14 mm) and *B. subtilis* (13 mm) respectively. The least activity was found against *B. subtilis* (7 mm) at 10 µl concentration (Fig. 6). Among the three experimental organisms, silver nanoparticles synthesized from the whole body tissues of *B. spirata* developed maximum zone of inhibition against *E. coli* (17 mm) and *C. annulus* developed minimum zone of inhibition against *P. aeruginosa* (1 mm). The results of maximum inhibitory concentration revealed that among the concentrations, 50 µl concentration showed maximum activity and 10 µl concentration showed minimum activity.

Figure 4: Antibacterial activity of synthesized silver nanoparticles by *Turbo brunneus*

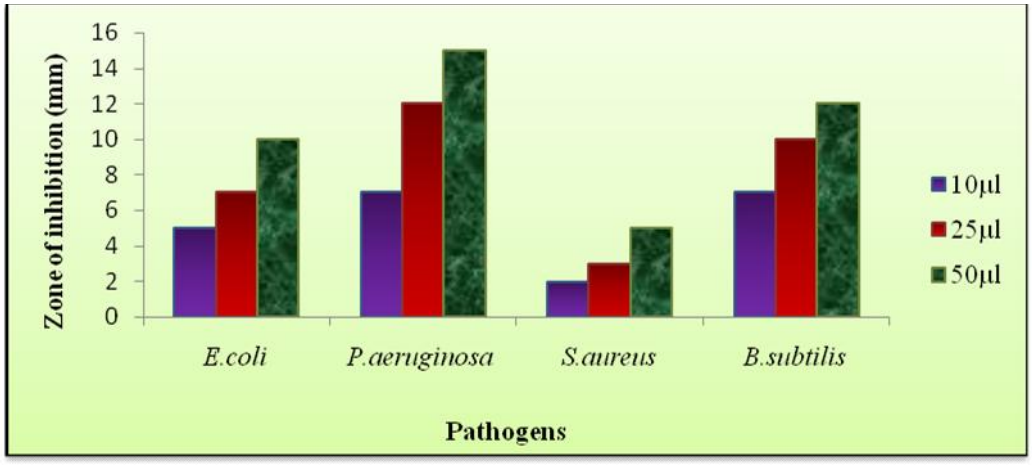


Figure 5: Antibacterial activity of synthesized silver nanoparticles by *Cypraea annulus*

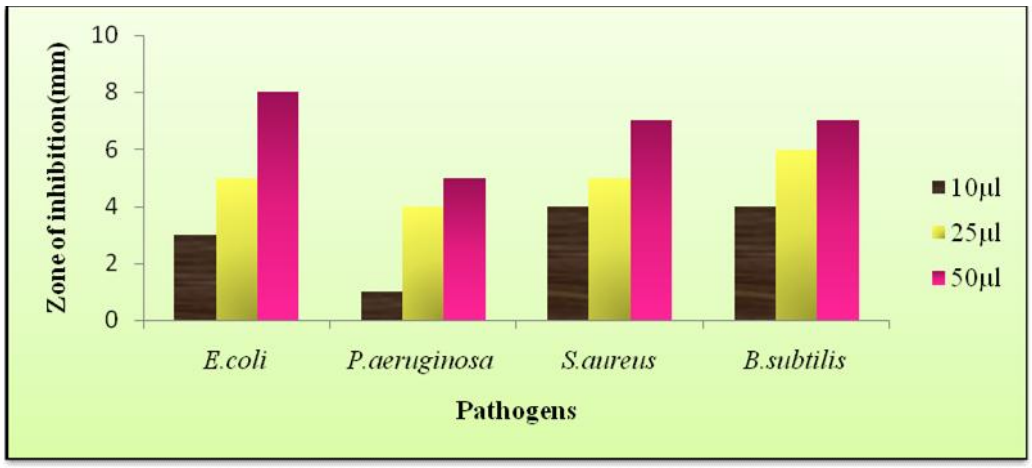
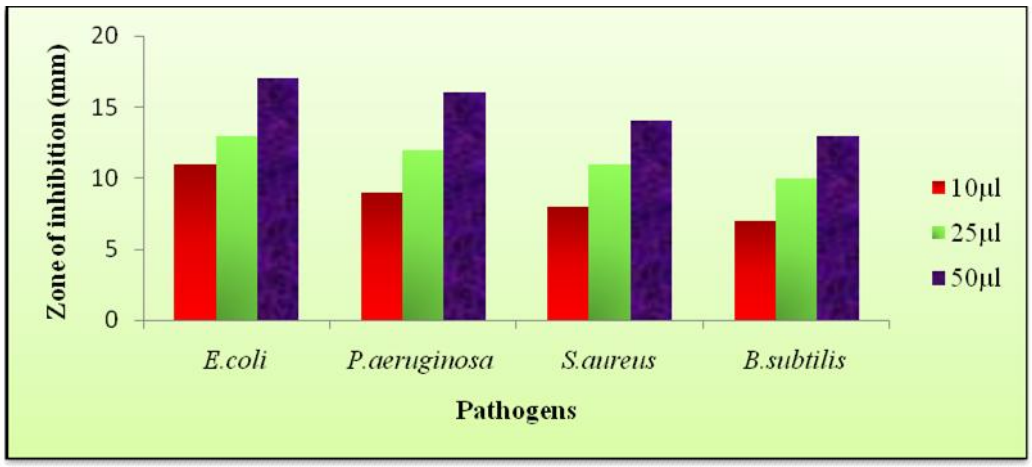


Figure 6: Antibacterial activity of synthesized silver nanoparticles by *Babylonia spirata*



4. Discussion

Nanoparticles are the fundamental building blocks of nanotechnology (Sharma *et al*., 2009). Synthesis of nanoparticle is basically a reduction process. This reduction is carried out by chemical or biological reducing agent. In the case of animal extract, short chain peptides or proteins are responsible in the synthesis of nanoparticles. In the present study green synthesis of silver nanoparticles from *T. brunneus*, *C. annulus* and *B. spirata* extracts were carried out. The appearance of brown colour in the reaction suggest the formation of silver nanoparticles. Very similar to the present study the biological synthesis of silver nanoparticles has been observed by Vigneshwaran *et al*. (2006); Khandelwal *et al*. (2010) and Saxena *et al*. (2010). They also suggested that the color change appeared due to the surface plasmon resonance of deposited silver nanoparticles.

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). The synthesis of silver nanoparticles had been confirmed by measuring the UV-Vis spectrum of the reaction media. In the present study the surface plasmon band in the silver nanoparticles solution remains close to 460nm in *T. brunneus* (Figure - 1), 450nm in *C. annulus* (Figure - 2) and 430nm in *B. spirata* (Figure - 3), suggesting that the particles are dispersed in the aqueous solution with no evidence for aggregation. This is the specific wavelength which indicates synthesized silver nanoparticles in experimental organisms.

The broadening of peak indicated that the particles are poly-dispersed. The weak absorption peak at shorter wavelengths is due to the presence of several organic compounds which are known to interact with silver ions (Savithramma *et al*., 2011). The frequency and width of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium (Bijanzadeh *et al*., 2012). Umayaparvathi *et al*. (2013) reported that UV-Visible spectrum surface plasmon resonance occurs at 430nm in *Saccostrea cucullata*.

The silver nanoparticles provide significant pharmaceutical, clinical, biological and immunological applications. They are used to prevent infection, in (burn and traumatic) wound dressings, diabetic ulcers, coating of catheters, dental works, scaffold and medical devices (Thomas *et al*., 2007 and Rai *et al*., 2009). Silver nanoparticles are non-toxic to humans, but most effective against bacteria, virus and other eukaryotic microorganism at low concentrations (Krutyakov *et al*., 2008). Moreover several salts of silver and their derivatives are commercially manufactured as antimicrobial agents (Kasthuri *et al*., 2009).

With respect to the microbes, the silver nanoparticles get attached to the cell wall, thereby disturbing the permeability of cell wall and cellular respiration. The nanoparticles may also penetrate deep inside the cell wall, thus causing cellular damage by interacting with phosphorous and sulphur containing compounds, such as DNA and protein. The bactericidal properties of silver nanoparticles are due to the release of silver ions from the particles, which confers the antimicrobial activity (Amarendra, 2010). Besides, the potency of the antibacterial effects corresponds to the size of the nanoparticle. The smallest particles have higher antibacterial activities.

On the clinical applications of nanoparticle, organisms producing nanoparticles through biological synthesis were found to be more biocompatible (Guidelli *et al*., 2011). The antibacterial activity of the nanoparticles synthesized by three gastropods *T. brunneus*, *C. annulus* and *B. spirata* was determined against human pathogenic bacteria. In the present study, silver nanoparticles synthesized using gastropod extracts exerted a fairly significant antibacterial action on the tested bacterial pathogens. In *T. brunneus* the antibacterial activity of silver nanoparticles showed maximum zone of inhibition against *P. aeruginosa* (15 mm) at 50 µl concentration followed by *B. subtilis* (12 mm) and *E. coli* (10 mm) (Fig. 4). The minimum zone of inhibition was observed against *S. aureus* (2 mm). In *C. annulus* the highest activity was observed against *E. coli* (8mm) followed by *S. aureus* and *B. subtilis* (7 mm) and trace activity was observed

against *P. aeruginosa* (1mm) (Fig. 5). In *B. spirata* the maximum activity was found against *E. coli* (17 mm) followed by *P. aeruginosa* (16 mm), *S. aureus* (14 mm) and *B. subtilis* (13 mm) respectively. The least activity was found against *B. subtilis* (7 mm) at 10 µl concentration (Fig. 6).

It is found that the silver nanoparticles have many inhibitory and bactericidal effects and so its application is extended as an antibacterial agent. The antibacterial activity of silver nanoparticles is estimated by the zone of inhibition. The silver nanoparticles have relatively higher anti-bacterial activity against gram negative bacteria than gram positive bacteria (Durga Praveena and Vijayakumar, 2014).

Antimicrobial activities of silver nanoparticles have been studied by various researchers especially on *E. coli* and *S. aureus* (Baker *et al*., 2005 and Sarkar *et al*., 2007). Selvakumar *et al*. (2012) studied the antibacterial activity of silver nanoparticles. They observed the maximum antibacterial activity against *S. aureus*, *P. aeroginosa*, *Enterobacter faecalis*, *E. coli* and the least was noticed against *K. pneumoniae* which is in agreement with the present work. Ghassan *et al*. (2013) observed dose dependant antimicrobial activity of synthesized nanoparticles from *Rosmarinus officinalis*. Umayaparvathi *et al*. (2013) studied the biosynthesis of silver nanoparticles using oyster *Saccostrea cucullata*. The maximum antibacterial activity was observed against *S. aureus* followed by *K. oxytoca*, *S. paratyphii*, *K. pneumoniae* and minimum activity was noticed against *V. chloerae*.

Vijayan *et al*. (2014) reported that silver nanoparticles synthesized using aqueous extract of *Turbinaria conoides* inhibits the growth of marine biofilm forming bacteria. Packia Lekshmi *et al*. (2015) described the silver nanoparticles synthesized by haemolymph of *Carcinus maenas* and *Ocypode quadrata* showed excellent antimicrobial activity against human and fish pathogens. The higher zone of inhibition was observed by *Staphylococcus sp*. (21.67 mm) followed by *E. coli* (19.33 mm) and *Pseudomonas sp*. (17.67 mm) in *C. maenas*. The maximum zone of inhibition was observed by *Staphylococcus sp*. (16 mm) followed by *E. coli* (15.67 mm) and *Klebsiella sp.* (14.67 mm) in *O. quadrata* at 50 µl concentration. The result of the present study coincides with the findings of the above authors. Among the three experimental organisms, *B. spirata* showed maximum zone of inhibition against *E. coli* (17 mm) and *C. annulus* showed minimum zone of inhibition against *P. aeruginosa* (1 mm).

5. Conclusion

The results of the present study revealed that the natural marine derived extracts of *Turbo brunneus*, *Cypraea annulus* and *Babylonia spirata* are a good source for the extracellular synthesis of silver nanoparticles. The characteristics of the obtained silver nanoparticles were studied using UV-Vis spectral analysis. The silver nanoparticles showed antibacterial activities against both gram negative and gram positive bacteria. The gram negative bacteria showed higher activity than gram positive bacteria. It is concluded that the formulation of silver nanoparticles could be used as an effective antibacterial agent. The biologically synthesized nanoparticles from marine compound offers stabilized nanoparticles suitable for both biomedical and industrial application.

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