Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(2):598-606

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis of silver nanoparticles using Haemolymph of marine crabs (*Carcinus maenas* and *Ocypode quadrata*) and its influence on clinical pathogens

[*1]Packia Lekshmi N. C. J., [1]Brindha Shini A., [1]Jeeva S., [1]Rajabrindha J. and [2]Selva Bharath M.

[1]Department of Microbiology, Udaya College of Arts and Science, Vellamodi, KK District, Tamilnadu, India
[2]Suganthi Devadhasan Marine Research Institute, Tuticorin, Tamilnadu, India

---

ABSTRACT

In order to synthesize alternative drugs from the natural sources this work was carried out to synthesize the silver nanoparticles from the haemolymph of two marine crabs, Carcinus maenas and Ocypode quadrata and to determine their influence on human and fish pathogens. The silver nano particles were synthesized from haemolymph and analysed by UV spectroscopy and SEM. The antimicrobial activity of haemolymph and silver nanoparticles from haemolymph were tested against human and fish pathogens. Our recent world approaching an increasing commercial demand in nanoparticles (NPs) due to their broad applicability in various field such as catalysis, chemistry, electronics, energy, and medicine. These NPs can be synthesized by various physical, chemical and biological techniques for the development of NPs. In our research, we successfully synthesized the NPs from the haemolymph of Carcinus maenas and Ocypode quadrata. Ag synthesis was confirmed by the colour change, wave range of UV-Vis Spectroscopy and the shape and size of the NPs determined by the SEM. It shows moderate and high toxic to the human pathogens as well as the fish pathogens. UV–VIS spectrum showed absorption peak at around 380 and 420 nm. The synthesized silver nanoparticles were clustered and the size ranged from 45-50 nm. The present process showed that haemolymph is an excellent biomaterial for the synthesis of Ag NPs with antimicrobial activity for nanotechnology based industries.

Key words: Haemolymph, UV-Vis Spectroscopy, AgNPs, SEM, Nanotechnology.

INTRODUCTION

Oceanic organisms have been used for medicinal purpose in all over the world. In recent past numerous pharmacological substances of marine origin were developed. While mycobacterial and gram negative bacterial infections are still among the common series, antiviral drugs are high on priority and pathogenic diseases. So far in marine invertebrates approximately 7000 marine natural products have been established; 18% from coelenterates (sea whips, sea fans and soft corals), 33% from sponges, and 24% from representatives of ascidians (also called tunicates), Opisthobranch mollusks (nudibranches, sea horse etc.,), bryozoans (moss animals) and echinoderms (star fish, sea cucumber etc.)

Marine invertebrates frequently have high application against microorganisms. In crustaceans, haemocyte act as a major role in defence system against microbes rests largely on cellular activities, such as adhesion, phagocytosis, encapsulation, nodule formation and melanisation. The multimeric coagulation and phenoloxidase are considered to be important defences in these organisms. The first line of defense of arthropods against pathogens and parasites is of physical nature via their hard cuticle. Still, once this barrier is passed, a complex interaction of innate humoral

and cellular immune reactions is induced in both tissues and haemocoel, which results in a fast elimination of microorganisms [1].

In the presence study, we have selected two types of marine crabs *i.e.,Carcinus maenas and Ocypode quadrata*. *C.maenas* is a common littoral crab. It has a carapace up to 60 mm long and 90 mm wide, but can be larger outside its native range, reaching 101 mm. The colour of *C.maenas* varies greatly from the grey, brown or red. This variation has a hereditary component, but is largely due to local environmental physical and chemical factors [2]. By the environmental stresses moulting become red-colour rather than green. Red individuals are stronger and more aggressive, but are less tolerant of low salinity or hypoxia.

O. quadrata is a species of ghost crab. David Knott described this creature as an "occult, secretive alien from the ancient depth of the sea" [3]. Adults are greyish or the colour of straw, and approximately 5 cm. Young crabs are cryptically coloured to merge in with their sandy habitat. *O.quadrata* lives in burrows in sand above the strandline. Their abundance is decreased due to human behaviour.

Antimicrobial activity has been detected in several crustaceans, including lobster, crabs, shrimps etc [4, 5]. Pharmacological properties are rich in marine crabs [6, 7] but not in fresh water crabs. Antibacterial activity of haemolymph extracts from six different species of crabs was performed by Veeruraj *et al*. [6] and seven crab species was performed by Anbuchezian *et al*. [7]. The antimicrobial activity of crude haemolymph extract from *O.macrocera* was also reported [8], the shore crab *Carcinus maenas* [9, 10] fresh water crab, *Paratelphusa hydrodromous* [11] the blue crab *Callinectes sapidus*, mud crab *Scylla serrata* and *O.macroce* [8, 11] were also reported.

A methodological understanding of biological activity will lead to the formation of useful drugs with specific actions. The potential of marine crabs as a source of biologically active products is largely unexplored. Hence this work was carried out to study the bioactivity of haemolymph of marine crab combined with nanotechnology using silver compound.

EXPERIMENTAL SECTION

Collection of pathogens

The human bacterial and fungal pathogens were isolated from the clinical samples collected from diagnostic laboratories, Kanyakumari district, Tamilnadu and identified following standard identification procedures. The fish pathogens were isolated from the infected fish collected from coastal areas of Kanyakumari district, Tamilnadu and identified following standard procedures.

Collection of animals

Two different species of crabs were collected from different areas along estuarine region in Kanyakumari district, Tamilnadu. They were collected using cast net. Healthy animals of different sizes were used throughout for experimental purposes.

Collection of haemolymph

Haemolymph were collected by cutting each walking legs of the animal with a fine sterile scissor. The haemocyte degranulation and coagulation was avoided, the haemolymph was collected in the presence of sodium citrate buffer, pH 4.6 (2:1 v/v). Then equal volume of physiological saline (0.85% w/v) was added to it. The haemolymph was centrifuged at 2000 rpm for 15 minutes at 4 $^{\circ}$C to remove haemocytes. Supernatant were collected by aspiration and stored at 4 $^{\circ}$C until use.

Synthesis of silver nanoparticles from haemolymph

25ml of crab haemolymph was added into 225ml of aqueous solution of 1mM silver nitrate ($AgNO_3$) and kept at room temperature for the reduction of silver nitrate into Ag+ ions. The solution was kept in the dark to avoid other biological changes. After 4 hours incubation, the colour change was observed. Samples showed change in colour from almost colourless to brown, which was a clear indication of the formation of nanoparticles produced through reduction of silver ions to metallic silver. Control showed no colour change, when incubated in the same conditions. Then the solutions were filtered and centrifuged three to four times at 10,000 rpm for 15minutes. Soon after the pellet was collected and washed three times with sterile deionised water.

Analysis of silver nanoparticles from crab haemolymph

Analysis by UV-Vis spectroscopy

UV-Vis spectral analysis was done by using UV-Vis spectrophotometer (Double Beam, I 2902). The bio reduction of Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium by periodic sampling of the aliquots (2ml). The wavelength of spectrophotometer was taken between 200nm-400nm.

SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-400 SEM machine. Thin films of the sample were prepared on a carbon coated paper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 minutes.

Antimicrobial activity by standard well diffusion method

The well of approximately 8mm in diameter were loaded with different quantity of haemolymph such as 10µl, 20µl, 30µl,40µl and 50µl respectively with the help of micropipette in mueller hinton agar plates which was swabbed with respective pathogens.

RESULTS

Collection and confirmation of crabs

The marine crabs were collected from the estuarine region in Kanyakumari district, tamilnadu. The crabs were identified as *Carcinus maenas* and *Ocypode quadrata* based on the morphological characters. *C.maenas* was a common littoral crab having five short teeth, along the rim behind each eye (Figure 1). The colour of *C.maenas* was reddish brown and its length was approximately 3.5 inches. *O.quadrata* was a species of ghost crab, greyish or straw coloured and approximately 5cm. Stalked compound eyes were present (Figure 2).

Figure 1.*Carcinus maenas*

Figure 2.*Ocypode quadrata*

Collection of haemolymph

Haemolymph collected from the crab, *C.maenas* was dark brown in colour. This may be due the presence of high amount of haem. The haemolymph collected from the crab, *O.quadrata* was pale colour. The haemolymph from both the crabs were further used for the determination of antimicrobial activity against human and fish pathogens.

Isolation of human and fish pathogens

The human pathogens such as *Staphylococcus* sp., *Proteus* sp., *Klebsiella* sp., *Pseudomonas* sp., *E. coli*, *Aspergillus* sp. and *Candida* sp. were collected from the laboratory which mentioned before and the fish pathogens used in this study was isolated from infected fish and identified as *Vibrio* sp., *Shigella* sp., *Klebsiella* sp. and *Pseudomonas* sp. based on morphological and biochemical characters.

Antibacterial and fungal assay of haemolymph of *Carcinus maenas* against human pathogens

Antibacterial activity of haemolymph of crab was studied by well diffusion method. The antibacterial assay was determined on bacterial pathogens like *Staphylococcus* sp., *Proteus* sp., *Klebsiella* sp., *Pseudomonas* sp., and *E.coli;* fungal pathogens such as *Candida* sp., and *Aspergillus* sp.

Antimicrobial activity of haemolymph of *C.maenas* against human pathogens

Antibacterial activities of crab haemolymph of *C.maenas* against human pathogens were given in table 1. The best activity was found against *Staphylococcus* sp. (19.67±0.58 mm) in 50 µl concentration followed by *E.coli* (18±1 mm) and *Pseudomonas* sp. (15.33±0.58 mm) (Figure 3, 4 & 5). The lowest activity was found against *Proteus* sp., and *Klebsiella* sp. The activity against fungi was found to be less active when compared to bacterial pathogens. The zone of inhibition was 13.33±0.58 mm for *Aspergillus* sp. (Figure 6) and 12.33±0.58 mm for *Candida* sp. in 50 µl concentration. The antimicrobial pattern showed concentration dependent activity.

Table: 1 Antimicrobial activity of haemolymph of *C.maenas* against human pathogens

Organisms	Concentration of extract/ Zone of inhibition (mm)				
	10 µl	**20 µl**	**30 µl**	**40 µl**	**50 µl**
***Staphylococcus* sp.**	13.67±0.58	15±1	16.33±0.58	18±1	19.67±0.58
***Proteus* sp.**	11±1	11.67±0.58	12.33±0.58	13.33±0.58	14.33±0.58
***Klebsiella* sp.**	10.67±0.58	11.33±1.15	12.33±0.58	13.33±0.58	14.67±0.58
***Pseudomonas* sp.**	12.33±1.15	13.33±0.58	13.67±0.58	14.67±1.15	15.33±0.58
E.coli	12.66±0.58	13.33±1.15	14.33±0.58	15.67±0.58	18±1
***Candida* sp.**	8.66±0.58	9.33±1.15	10.67±0.58	11.67±1.15	12.33±0.58
***Aspergillus* sp.**	10.33±0.58	10.67±0.58	11.33±0.58	12.33±0.58	13.33±0.58

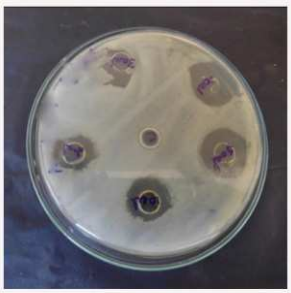

Figure 3.*C.maenas* haemolymph against *Staphylococcus* sp.

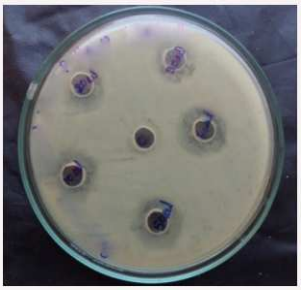

Figure 4.*C.maenas* haemolymph against *E.coli*

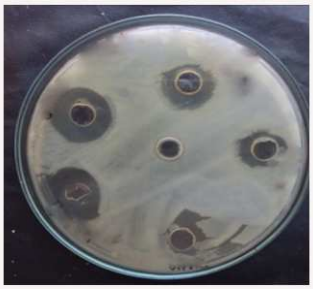

Figure 5.*C.maenas* haemolymph against *Pseudomonas* sp.

Figure 6.*C.maenas* haemolymph against*Aspergillus* sp.

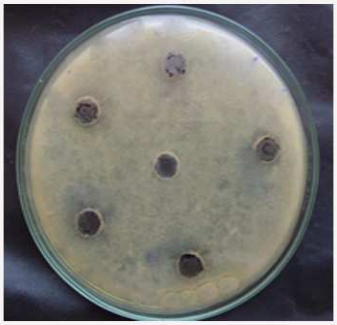

Figure 7.*C.maenas* hamolymph against *Shigella* sp.

Table: 2 Antimicrobial activity of haemolymph of *C. maenas* against fish pathogens

Organisms	Concentration of extract/ Zone of inhibition (mm)				
	10 µl	**20 µl**	**30 µl**	**40 µl**	**50 µl**
***Vibrio* sp.**	11.33±0.58	12	12.66±0.58	14	14.33±0.58
***Shigella* sp.**	13.66±0.58	15±1	16.33±1.52	17	17.67±1.15
***Klebsiella* sp.**	11.66±0.58	13	14±1	14.33±0.58	15±1
***Pseudomonas* sp.**	10.67±0.58	12±1	11.67±0.58	12	12.67±0.58

Antibacterial activity of haemolymph of *C. maenas* against fish pathogens

Antibacterial activities of *C.maenas* against fish pathogen were tabulated in table 2. The activity was limited in *Pseudomonas* sp., (12.67±0.58 mm) and *Vibrio* sp., (14.33±0.58 mm) in 50 µl concentrations. The best activity was

achieved for *Shigella* sp. (17.67±1.15 mm) (figure 7) followed by *Klebsiella* sp. (15±1). *Pseudomonas* sp. was found to be less sensitive.

Antibacterial and antifungal activity of silver nanoparticles from haemolymph of *C. maenas* against human pathogens

Antimicrobial activity of silver nano particles from haemolymph of *C.maenas* was shown in table 3. The best activity was achieved for all the tested organisms and the activity was determined for *Staphylococcus* sp., was 21.67±1.15 mm (figure 8), *E.coli* (19.33±0.58 mm), *Pseudomonas* sp., (17.67±0.58 mm), *Proteus* sp., (16.33±0.58 mm) (figure 9) and *Klebsiella* sp., (17±1 mm) in 50 µl of concentration (Figure 10).

Figure 8. Antimicrobial activity of *C.maenas* Ag nanoparticle against *Staphylococcus* sp.

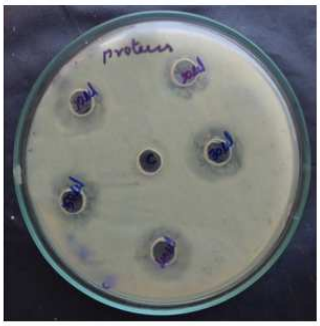

Figure 9. Antimicrobial activity of *C.maenas* Ag nanoparticle against *Proteus* sp.

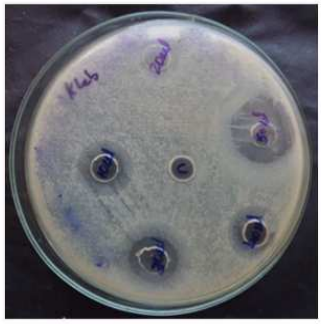

Figure 10. Antimicrobial activity of *C.maenas* Ag nanoparticle against *Klebsiella* sp.

Figure 11. Antimicrobial activity of *C.maenas* Ag nanoparticle against *Aspergillus* sp.

Figure 12. Antimicrobial activity of *C.maenas* Ag nanoparticle against *Candida* sp.

Table: 3 Antibacterial activity of Ag nano from *haemolymph* of *C. maenas* against human pathogens

Organisms	Zone of inhibition (mm)				
	10 µl	**20 µl**	**30 µl**	**40 µl**	**50 µl**
***Staphylococcus* sp.**	13.66±0.58	15±1	17	18.67±0.58	21.67±1.15
***Proteus* sp.**	12.67±0.58	13.67±0.58	14.67±1.15	15	16.33±0.58
***Klebsiella* sp.**	13.66±0.58	15.66±1.15	15.67±0.58	16.33±0.58	17±1
***Pseudomonas* sp.**	14	15.67±0.58	16.33±0.58	17.67±1.15	17.67±0.58
E.coli	12.67±0.58	14.33±0.58	15.67±0.58	17.67±1.15	19.33±0.58
***Candida* sp.**	9.67±1.15	10.33±0.58	12.33±0.58	12.67±0.58	13.66±1.15
***Aspergillus* sp.**	10.33±0.58	11	11.67±0.58	13.33±0.58	14.67±0.58

In this study, *Aspergillus* sp. was inhibited by this nanoparticle in moderate level, ie., 14.67±0.58 mm (fig 11) followed by *Candida* sp. (13.66±1.15 mm) (Fig 12).

Table: 4 Antibacterial activity of Ag nano from haemolymph of *C. maenas* against fish pathogen

Organisms	Zone of Inhibition (mm)				
	10µl	**20 µl**	**30 µl**	**40 µl**	**50 µl**
***Vibrio* sp.**	12.67±0.58	13.33±0.58	14.33±0.58	16.67±1.15	17±1
***Shigella* sp.**	13.67±0.58	14.66±0.58	15.33±0.58	18±1	18.67±1.15
***Klebsiella* sp.**	12.67±0.58	13.67±0.58	14.33±0.58	15.33±0.58	16.67±1.15
***Pseudomonas* sp.**	10.33±0.58	12.67±0.58	13.33±0.58	13.67±0.58	14.33±0.58

Antibacterial activity of silver nanoparticles from haemolymph of *Carcinus maenas* against fish pathogens

In the antimicrobial assay of Ag nano from haemolymph of *C.maenas* against fish pathogens showed that the higher zone of inhibition was observed in *Shigella* sp. (18.67±1.15 mm) followed by *Klebsiella* sp. (16.67±1.15 mm) and *Vibrio* sp. (17±1mm). The lowest activity was observed in *Pseudomonas* sp. (14.33±0.58 mm) (Table. 4).

Antibacterial and antifungal activity of haemolymph of *O. quadrata* against human pathogens

The zone of inhibition was found high against *Staphylococcus* sp. (15.33±0.58 mm) followed by *E.coli* (14.67±0.58 mm) (figure 13), *Pseudomonas* sp. (14.33±0.58 mm) and *Proteus* sp. (13.33±0.58 mm) and finally by *Klebsiella* sp. (12.67±0.58 mm) (Table.5).

Table: 5Antibacterial activity of haemolymph of *O. quadrata* against human pathogen

Organisms	Zone of inhibition (mm)				
	10 µl	20 µl	30 µl	40 µl	50 µl
***Staphylococcus* sp.**	11.67±0.58	12.33±0.58	13.33±0.58	14	15.33±0.58
***Proteus* sp.**	9.33±0.58	10	10.667±0.58	12.33±0.58	13.33±0.58
***Klebsiella* sp.**	9	10.67±0.58	11.33±0.58	12.67±0.58	12.67±0.58
***Pseudomonas* sp.**	11.67±0.58	12.33±0.58	13	13.67±0.58	14.33±0.58
E.coli	9.67±0.58	10.67±0.58	12	12.67±0.58	14.67±0.58
***Candida* sp.**	9	9.33±0.58	9.33±0.58	9.67±0.58	9.67±0.58
***Aspergillus* sp.**	9	9	9.33±0.58	9.33±0.58	10.67±0.58

Both the tested fungal pathogens were inhibited at a lesser extent. The activity was observed to be only 10.67±0.58 mm for *Aspergillus* sp., (figure 14) and 9.67±0.58 mm for *Candida* sp.

Antibacterial activity of haemolymph of *O. quadrata* against fish pathogens

The haemolymph of *O.quadrata* inhibits *Shigella* sp. and *Vibrio* sp. effectively and the zone of inhibition was 18.67±1.15 mm and 17±1 mm respectively followed by both *Klebsiella* sp. (16.67±1.15 mm) (figure 15) and *Pseudomonas* sp. (14.33±0.58 mm) (Table 6).

Figure 13.Antimicrobial activity of *O.quadrata* haemolymph against *E.coli*

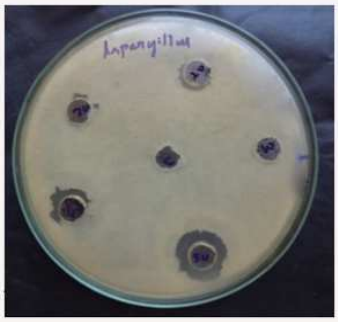

Figure 14. Antimicrobial activity of *O.quadrata* haemolymph against *Aspergillus* sp.

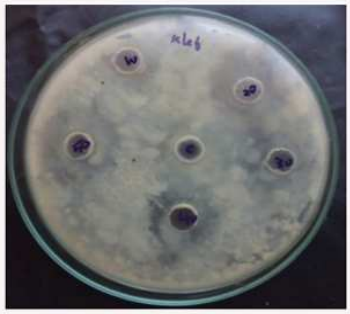

Figure 15. Antimicrobial activity of *O.quadrata* haemolymph against *Klebsiella* sp.

Figure 16. Antimicrobial activity of *O.quadrata* Ag nanoparticles against *Staphylococcus* sp.

Figure 17. Antimicrobial activity of *O.quadrata* Ag nanoparticles against *Shigella* sp.

Figure 18. Antimicrobial activity of *O.quadrata* Ag nanoparticles against *Klesiella* sp.

Figure 19. Antimicrobial activity of *O.quadrata* Ag nanoparticles against *Pseudomonas* sp.

Antibacterial activity of Ag nano from haemolymph of *O. quadrata* against human pathogens

The higher zone of inhibition was observed for *Staphylococcus* sp., (16±1 mm) (figure 16) in the antimicrobial activity of Ag nano from haemolymph of *O.quadrata* (Table. 7). The sensitive pattern of this extract was followed by *E.coli* (15.67±1.15mm), *Klebsiella* sp. (14.67±1.15mm) *Pseusdomonas* sp. (14.67±0.58mm) and *Proteus* sp. (13.67±1.15mm) in 50 µl of concentration. Similar to silver nanoparticles from *C. maenas*, silver nano particles

from *O.quadrata* showed good antimicrobial activity against two fungal pathogens. It showed 14.67±1.15 mm activity for *Aspergillus* sp. and 13.66±1.15 mm activity for *Candida* sp.

Table: 6 Antibacterial activity of haemolymph of *O. quadrata* against fish pathogen

Organisms	Zone of inhibition (mm)				
	10 µl	20 µl	30 µl	40 µl	50 µl
***Vibrio* sp.**	12.67±0.58	13.33±0.58	14.33±0.58	16.67±1.15	17±1
***Shigella* sp.**	13.67±0.58	14.66±0.58	15.33±0.58	18±1	18.67±1.15
***Klebsiella* sp.**	12.67±0.58	13.67±0.58	14.33±0.58	15.33±0.58	16.67±1.15
***Pseudomonas* sp.**	10.33±0.58	12.67±0.58	13.33±0.58	13.67±0.58	14.33±0.58

Table: 7 Antibacterial activity of Ag nano from haemolymph of *O. quadrata* against human pathogen

Organisms	Zone of inhibition (mm)				
	10 µl	20 µl	30 µl	40 µl	50 µl
***Staphylococcus* sp.**	11.33±0.58	12.33±0.58	13.67±1.15	14.33±0.58	16±1
***Proteus* sp.**	8.67±0.58	9.67±0.58	11±1	14±1	13.67±1.15
***Klebsiella* sp.**	9	11.67±0.58	12.33±0.58	13.33±0.58	14.67±1.15
***Pseudomonas* sp.**	10.67±0.58	11	13±1	13.33±1.15	14.67±0.58
E.coli	9.67±0.58	10.67±0.58	13±1	12.67±0.58	15.67±1.15
***Candida* sp.**	8.66±0.58	8.66±0.58	11.33±0.58	13.66±1.15	13.66±1.15
***Aspergillus* sp.**	9.66±0.58	11.67±0.58	12.33±0.58	13.67±1.15	14.67±1.15

Antibacterial activity of Ag nano from haemolymph of *O. quadrata* against fish pathogen

Among the fish pathogens, *Shigella* sp. (fig 17) showed only 15.33±1.15 mm of inhibition zone followed by other two fish pathogen, *Vibrio* sp. and *Klebsiella* sp. (fig 18) showed 14.33±0.58 mm inhibition of zone was given in the table.8. The least activity was found against *Pseudomonas* sp. (13.66±0.58) (Fig 19). In this study, the Ag nano particle does not showed any remarkable result when compared to haemolymph of *C. maenas.*

Table:8 Antibacterial activity of Ag nano from haemolymph of *Ocypode quadrata* against fish pathogen

Organisms	Zone of inhibition (mm)				
	10 µl	20 µl	30 µl	40 µl	50 µl
***Vibrio* sp.**	9.67±0.58	11±1	12.33±0.58	13.67±1.15	14.33±0.58
***Shigella* sp.**	11±1	11.68±0.58	13±1	13.67±0.58	15.33±1.15
***Klebsiella* sp.**	11	11.67±0.58	12.67±0.58	13.33±0.58	14.33±0.58
***Pseudomonas* sp.**	9.67±0.58	11	12±1	13±1	13.66±0.58

Confirmatory analysis of Ag synthesis by UV-Vis spectrophotometer

The synthesized silver NPs was confirmed by the formation of brown colour after incubation. The reduced brown coloured molecules was analysed by UV-Vis spectrophotometer. The nanoparticles from haemolymph from *C.maenas* showed strong peak was observed at 380 nm (Fig20). The strong peak for silver nanoparticles from *O.quadrata* was observed at 420 nm (Fig21).

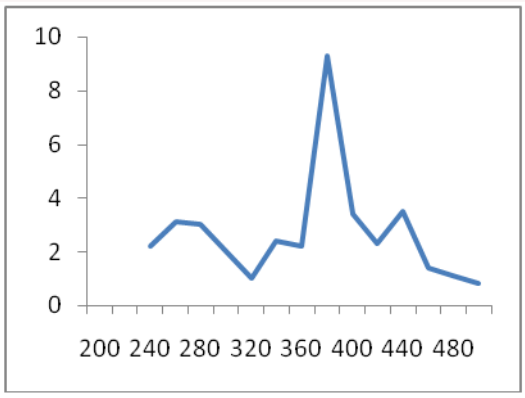

Figure.20 UV-vis spectrograph of Ag nanoparticle synthesized from haemolymph of *C.maenas*

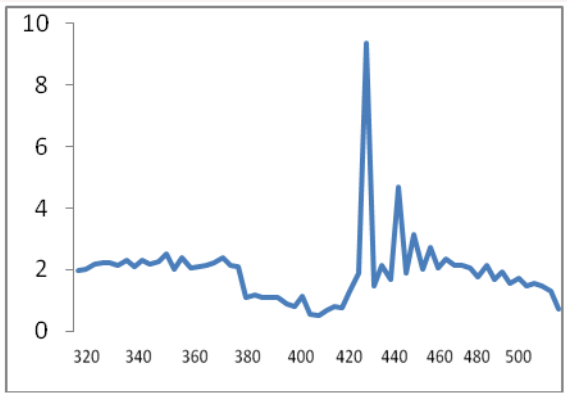

Figure.21 UV-vis spectrograph of Ag nanoparticle synthesized from haemolymph of *O.quadrata*

SEM analysis of silver nanoparticles

In this the magnification was possible, from about 10 times more than 500,000 times. The magnification was done in 30,000 and 40,000 X. This study showed the surface morphology of the silver nanoparticles synthesized by aqueous haemolymph of *C. maenas* confirmed the presense of Ag nanoparticles. From the image, (Fig.22) we conclude that the nanoparticles were clustered. The surfaces of the aggregates were rough. The particles were more or less spherical with sizes in the range of 45-50nm.

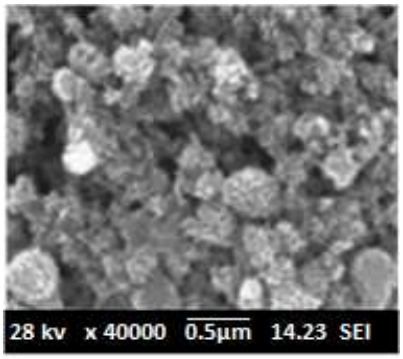

Figure 22. SEM analysis of silver nano particles from haemolymph of crab

DISCUSSION

Mainly new infectious disease and resistance to the antibiotics leads to the fresh sources for innovative drug discovery. The antibacterial agents that have been isolated from marine invertebrates have not been active to struggle with conventional antimicrobials obtained from microbes [12]. Still, the research on marine organisms is in its infancy, marine organisms yet to be screened for the discovery of useful antibiotics.

The presence of naturally occurring haemolymph of several crustaceans was well known since the beginning of the 20th century. In the present investigation, haemolymph were collected from two different crabs viz., *C.maenas, O.quadarata* was subjected to antibacterial and antifungal assay. In our report, haemolymph of *C.maenas* showed the excellent activity against all the pathogens tested.

Kumaravel *et al*., [13] in their study, reported only 13 mm of inhibition zone against *Candida* sp. The highest zone of inhibition was observed in the haemolymph of *P.monodon* against *Klebsiella* sp. (5 mm). Thus in fungal activity, the highest zone of inhibition was observed in the haemolymph of *P.monodon* against *Aspergillus fumigatus, P.indicus* against *C. neoformans,P. semisulcatus* against *A. fumigatus* and *C. neoformans*. Moreover, the other strains were showed negative result by these three shrimps. In our report, the antimicrobial activity of haemolymph of *C. maenas* against two fungal strains, *Candida* sp. (9.67mm) and *Aspergillus* sp. (10.33mm) were effective.

As we know, production of nanoparticles can be achieved through different methods. Chemical approaches are the most popular methods for the production of nanoparticles. Since they are costly, pathway changes to the biological synthesis [14]. But in our recent research we have tried the synthesis of silver naoparticles from the haemolymph of crab. In the study of Ravichandran *et al*. [8], *O.macrocera* haemolymph showed maximum zone of inhibition by methanolic extract of haemolymph against *S. typhi*. But the bacteria *P. aeruginosa, S. flexineri, V. cholera* and multi-drug resistant *S. aureus* and fungal strains, *Rhodotorula* sp., *A. niger* were insensitive to all extract of the haemolymph. The extracts of haemolymph tested show good activity against microbial strains. Different extract of *O. macrocera* pathogenic bacterial, fungal and multi-drug-resistant antimicrobial effect of six brachyuran crabs revealed the maximum antibacterial effect of crude haemolymph is shown *Dromia abrolhensis* against *E. coli* the minimum against the *S.serrata* [15]. A similar result was observed with the haemolymph of some brachyuran crabs against clinical pathogens [15]. In our approach we have observed best antimicrobial activity against human bacterial and fungal pathogens and fish bacterial pathogens.

In the baseline report it is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [16]. In our investigation silver NPs was confirmed by the formation of brown colour after incubation. Normally Ag NPs was synthesised by the raw material such as plant, microbes, etc. It is a first approach synthesizing the Ag NPs in haemolymph of *Carcinus maenas*. Varshneya *et al*. [17] shootout the research in the plant material and it gives the nm range at 420 in *Stevia rebaudiana* leaves. In over report UV-spectrometric analysis peak value formation in the range of 400-450nm shows the Ag NPs is in present thus we have received the peak value of 380nm in Ag NPs from *C.maenas* and 420nm in

Ag NPs from *O.quadrata*. SEM analyses express morphology of the Ag NPs. In 2012 Panneerselvam *et al*. [18] reported that *Catharanthus roseus* has the Ag NPs of 35-55nm range. But in our investigation nanoparticles were clusters and the surface was rough and the size ranges from 45 to 50nm.

CONCLUSION

The critical need in the field of nanotechnology is the development of reliable and ecofriendly process for synthesis of metallic nanoparticles. The present study reported extra-cellular synthesis of nanoparticles by crabs *C.maenas* and *O.quadrata* can be used as a therapeutic agent for many bacterial infections in the form of nano drugs or in the combinational therapeutics. The characteristics of the obtained silver nanoparticles were studied using UV-Vis and SEM techniques.

REFERENCES

[1] P Bulet; C Hetru; J Dimarcg; D Hoffmann, *Dev Com Immunol*., **1999,** 223, 329-341.
[2] JV Brian; T Fernandes; RJ Ladle; PA Todd, *J. Exp. Mar. Biol.,* **2005**, 329(1), 47-54.
[3] David knott, South Carolina Department of Natural Resources. **2009**.
[4] JEM Stewart; B Zwicker, *Canadian J. Microbiol.,* **1972,** 18, 1499-1509.
[5] MJ Noga; TA Arroll; Fan Zhigin, *Fish shellfish immune.,* **1966**, 6(6), 403-12.
[6] A Veeruraj; S Ravichandran; G Rameshkumar, *Trends Applied Sci. Res.,* **2008**, 3(2), 174–181.
[7] RM Anbuchezhian; S Ravichandran; G Rameshkumar; TT Ajithkumar, *Advan. Biol. Res.,* **2009**, 3(3-4), 104-109.
[8] S Ravichandran; K Sivasubramaninan; RM Anbuchezhian, *WASJ.,* **2010,** 11(5), 578-581.
[9] JRS Chisholm; VJ Smith, *J. Mar. Biol. Ass. U. K.,* **1992**, 72 (4), 529–542.
[10] JRS Chisholm; VJ Smith, *Comp.Biochem Physiol.,* **1995,** 110A, 39-45.
[11] AA Prakash; S Balasubramaniam; G Gunasekaran; M Prakash; P Senthilraja, *ISRN Pharmacol.,* **2011,** 642768, 1-4.
[12] KL Rinchart; PD Shaw; LS Shield; JB Gloer; GC Harbow; KoKer, *Pure Appl.Chem.,* **1981,** 53, 795-817.
[13] K Kumaravel; S Ravichandran; S Sritamabose, *African J of Microbiol.,* **2010,** 4, 2592-2596.
[14] M Singh; S Manikandan; AK Kumaraguru, *Research J nanosci and nanotechnol.,* **2011,** 1(1), 1-11.
[15] G Rameshkumar; S Ravichandran; T Aravindhan, *Middle East J Sci. Res.,* **2009,** 4, 40-43.
[16] SS Shankar; A Rai; B Ankamwar; A Singh; A Ahmad; M Sastry, *Nat. Mater.,* **2004,** 3(7), 482-488.
[17] R Varshneya; S Bhadauriaa; MS Gaurb, *Adv. Mat. let.,* **2010,** 1(3), 232-237.
[18] C Panneerselvam; S Ponarulselvam; K Murugan; K Kalimuthu; S Thangamani, *Asian Pac. J Trop. Biomed.,* **2012,** 2(7), 1-7.