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RESEARCH ARTICLE

IN-VITRO ANTIBACTERIAL ACTIVITY AND BRINE SHRIMP LETHALITY TEST ON SELECTED THREE MARINE MOLLUSKS FROM VELLAR ESTUARY, PARANGIPETTAI

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ABSTRACT

Antibacterial activity was evaluated on crude extracts of marine bivalves, namely *Anadara granosa, Placenta placenta* and *Pinctada fucata* were collected from Vellar estuary, Parangipettai, Tamilnadu, India. The antibacterial activity was carried out against 10 pathogens. The human pathogen *S. aureus* showed 6 mm. against crude methanol extract of *A. granosa* and 5mm in *Escherichia coli, Vibrio cholorae* and *Vibrio parahaemolyticus* against methanol crude extract of *P. placenta.* In *P. fucata* 8 mm. was observed an against *Streptococcus pyogens*. In shrimp lethality test, the maximum mortalities were observed 80% against 500µg/ml of the crude extract of *A. granosa* and 0% mortality was observed against 100µg/ml concentration of *P. fucata*. The results strongly suggest that, the mollusk extracts can be used an antimicrobial agents and further studies.

INTRODUCTION

The marine surroundings could be a large supply for yet to be discovered bioactive natural bio-products. Apart from the food, plentiful diversity of bioactive compounds are being isolated and characterized with enormous assure treatment of human diseases. In the past century, however an increasing role has been played by microorganisms in the production of antibiotics (Fenical, 1993). Hence, there is an important interest in discovering new antimicrobial bioactive compounds with less environmental and toxicological risks to that there is no resistance developed by the pathogens. In general, The Mollusks are broadly distributed throughout the globe and it has several representatives in the ocean and estuarine ecosystem, particularly slugs, clams, mussels, scallops and a few more species. This wealthy diversity to marine organisms assumes an excellent chance for the detection of new bioactive compounds (Jhonson and Soderhall, 1985). Several investigations have recorded the bioactivity of the mollusks like *Aplysia sp*. (Stallard and Faulkner, 1974), *Phyllidae sp*. (Ilagedone *et al.,* 1999) bivalves (Jayaseeli *et al.,* 2001), gastropods (Emerson Kagoo and Ayyakannu, 1992) and their egg masses (Prem Anand *et al.,* 1997). *A. granosa* habitually found backwater estuaries in mud soil and commonly they are known as blood clams owing to presence of blood like fluid.

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P. fucata is a species of marine bivalve mollusk in the family Pteriidae, it is commonly called as pearl oysters. *P. placenta* is less known oyster, popularly called as windowpane oyster because the shell of the animal were made use of in window panes by people in the previous Portuguese Colonies in India. The present investigation was carried out to investigate the antibacterial activity and Brine Shrimp Lethality test of the body tissues of three selected marine mollusks from Vellar estuary, Parangipettai, Southeast coast of India.

MATERIALS AND METHODS

Sample collection

The Mollusks blood clam *A. granosa*, windowpane oyster *P. placenta* and pearl oyster *P. fucata* live specimens were collected from Vellar estuary, Parangipettai. The freshly collected samples were cleaned and washed with sea water and freshwater to remove all impurities. The Mollusks shells were removed and the tissues were sun-dried for one week.

Preparation of Extract

The dried mollusks samples were soaked in 100% methanol for 10 days at normal room temperature. After filtration with Whatman No. 1 filter paper, the methanol extracts were reduced by vacuum evaporation. The residues were re-suspended in 200 ml of 100 percent methanol and these

solutions were transferred to new vial to remove salt precipitates and stored at 0°C for additional use.

Test microorganisms and microbial culture

All bacterial strains (*Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Lactobacillus vulgaris, Proteus mirabilis, Pseusdomonas aeruginosa, Salmonella typhi, Salmonella paratyphi, Staphyloccus aureus and Vibrio sp*. were obtained from the Rajah Muthiah Medical College, Annamalai University, Chidambaram, Tamil Nadu, India.

Antibacterial assay of methanol crude extract

Antibacterial activity was carried out by victimization of standard disc diffusion method. The microorganism strains were sub cultured and swabbed on surface of the Muller Hinton (MH) agar plates. The prepared antibiotic discs were (Whatman No. 1 filter paper with 6 mm diameter) soaked with the 50 µl of six crude extracts and placed on the surface of the plate, control discs were placed with respective solvents and tetracycline (30 µg/ml) used as a positive control. All the plates were incubated at 37°C for 24 hours and measured the zone of inhibition. The susceptibility of the test organisms were determined by radius of the inhibition zone around each disc. All extracts were tested with triplicate at a concentration of 30 mg $disc^{-1}$.

Brine Shrimp Lethality Test

Brine shrimp eggs were obtained from CP aquaculture, Chennai, Tamil Nadu, India. The artificial sea water was prepared by dissolving 38 g. of sea salt in 1000 ml of Disc. H_2O for hatching the shrimp eggs in to plastic container contained dark and light areas separately. Then allowed two days for the shrimp to hatch and mature as larva. Briefly, 4 ml of the artificial seawater and 10 brine shrimps were introduced into each test tube, made the total volume in to 5 ml. The number of surviving shrimps were counted and recorded after 24 hours. Using probit analysis, the lethality concentration (LC50) was assessed at 95% confidence intervals. LC50 value of less than 1000 µg/ml is toxic while LC50 value of greater than 1000 µg/ml is nontoxic. The percentage of mortality (%) calculated by dividing the number of dead larva by the total number and then multiplied by 100%.

RESULTS

The present investigation is to find out the antibacterial and cytotoxic effects of selected marine molluscs on methanol crude extract of *A. granosa, P. placenta* and *P. fucata*. The *A. granosa* showed the highest antibacterial activity (6 mm) against *S. aureus* and lowest activity (1mm) was observed an against *K. pneumonia* and *V. parahaemolyticus*. In *P. placenta* crude methanol extract has produced maximum activity against *E. coli*, *V. parahaemolyticus* and *V. cholera* (5 mm) followed by *S. paratyphi, S. aureus* and *S. pyogenes*. In contrast the *A. granosa* caused (0 mm.) nil activity against *E. coli*, *V. cholera* (4 mm). The antibacterial activity results were shows at Figure 1.

In the current study, highest mortalities (80%) were observed on 500 µg/ml concentration of *A. granosa* methanol crude extract. Minimum mortalities 20% observed on 100µg/ml of the same crude extract of *P. placenta* were exhibited. The maximum mortalities (70%) were observed at a concentration of 500 µg/ml and minimum mortalities were observed 10% observed in 100 µg/ml of the same extract.

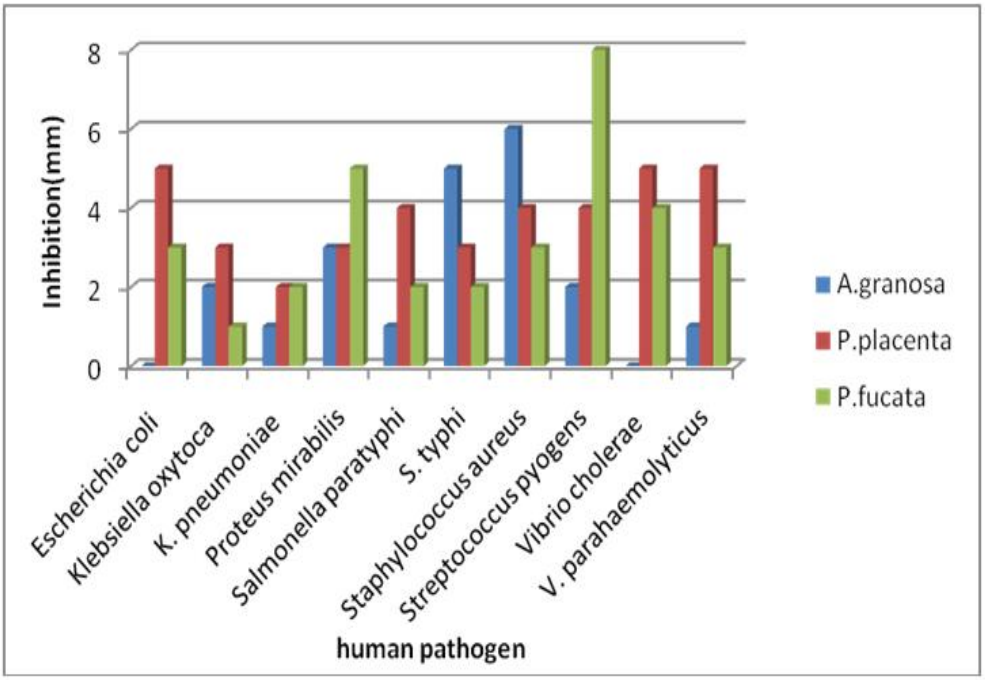

Fig. 1. Antibacterial activity of methanol extract from selected three Marine mollusks species

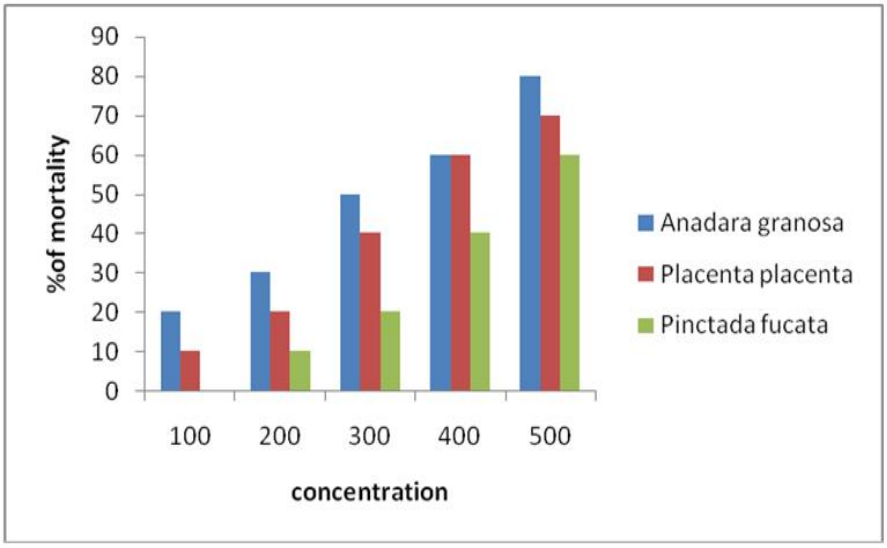

Fig. 2. Brine Shrimp lethality assay of methanol extract from selected three marine mollusks species

The crude extracts of *P. fucata* produced maximum mortalities (60%) were observed at a concentration of 500 µg/ml. and minimum were observed in 10% in 200 µg/ml. 0% of activity also recorded in same extract at100 µg/ml concentration. Results were showed at Figure 2.

DISCUSSION

In the present study pronounced inhibition was conferred by the methanol extract of *A. granosa, P. Placenta* and *P. fucata* against ten bacterial strains. On marine organisms plenty of bioactive works has been done to visualize the bioactivity of natural products of their potentially pharmacological utilization. A screening of antibacterial activity in cuttlefishes extracts of *Sepia sp* and *Loligo sp.* and marine snails of Tibia *Insulaechorab-curta* were conducted (Zainab Dakhil Degiam and Ali Tahar Abas, 2010). Mariappan *et al.* (2010) were reported the antibacterial activity on clam *Meretrix casta* and *Tridacna maxima* that out of the six solvents used the extract obtained from the ethanol and methanol exhibited higher antibacterial activity against twelve human pathogens (Mariappan *et al.,* 2010). Some more marine mollusks species like *Mytilus edulis, Mytilus galloprovincialis, Crassostrea virginica* and *Crassostrea gigas* conjointly reported by (Tincu Andy and Taylor Steven 2004; Mitta, Guillaume *et al.,* 1999). The results show that in-vitro antibacterial in cuttlefishes and marine snail extract's antibacterial activity has previously described in wide selection of mollusk species (Shanmugam *et al.,* 2008; Benkendorff *et al.,* 2001; Gunthorpe and Cameron 1987; Constantine *et al.,* 1975). Annamalai *et al.* (2007) were studied the antimicrobial activity of green mussel *Perna viridis* and edible oyster *Crassostrea madrasensis* extract of marine bivalves (Annamalai *et al.,* 2007). Estari *et al.* (2011) were reported the antimicrobial activity of fresh water mussel *Lamellidens marginalis* extract against gram positive and gram negative bacteria (Estari *et al.,* 2011). The acetone extracts showed numerous activities against bacterial pathogens in almost like that of here the methanol extracts showed various activities against human pathogens. In brine shrimp lethality the crude extract of three marine bivalves has been exposed at the cytotoxicity of brine shrimp lethality assay at all different concentration. The larval mortality has been enhanced with increasing concentration and also the three mollusks bivalve extracts were caused strong cytotoxic and antibacterial activity against microorganism pathogens.

Conclusion

In this present work proved that the biological activity is made from 3 marine bivalve mollusks. Therefore, the crude or purified methanol extract of marine bivalve mollusks *Anadara granosa*, *Placenta placenta* and *Pinctada fucata* had medicament and cytotoxic activities.

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