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Study of Antimicrobial Activity of Tissue Extracts from Bivalve *Geloina proxima* (Prime, 1864) from Dapoli Coast of Ratnagiri District Maharashtra (India)

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Abstract: *Mangrove bivalve Geloina proxima of family Corbiculidae is one of the most dominant indigenous giant mangrove bivalve of Dapoli coast. It has great commercial value and biodiversity importance. Despite their economic importance and food potential, this bivalve has received very little attention. Hence, its potential antibacterial activity against human pathogenic bacteria is assayed. The foot and gills tissue extracts were prepared in 06 different solvents and assayed for antibacterial activity by using disc diffusion method against 08 different human pathogenic bacteria. Among the extracts, the Butanol, Ethanol, Methanol, Acetone and Hexane crude extracts showed potential antibacterial activity for most of the pathogenic bacteria. The foot extracts in Butanol and gill extracts in Ethanol have showed higher activity with maximum inhibition zone.*

Keywards: *Bivalve, Geloina proxima, Antimicrobial, Activity.*

I. INTRODUCTION

Marine life includes diverse group of organisms that inhabit the pelagic and benthic realm. The ocean offers a great biodiversity of fauna and flora estimated at more than 5,00,000 species. This number exceeds the number of land species [1]. This rich diversity of marine organisms assumes a great opportunity for the discovery of new bioactive substances. Thus the marine environment is an exceptional reservoir for bioactive natural products; many of which exhibit structural features that are not found in terrestrial natural products [2]. Among these diverse marine organisms, bivalves are the most commonly used sea food, therefore having great demand in the world market as a food product. In addition various bivalve species have valuable pharmacological properties which attract attention of researchers as biologically active substances. There is growing interest in the marine natural products or marine secondary metabolites. Hence bivalves are now widely used in research institutes worldwide for various studies, but only recently they have been recognized as potential sources of anti-bacterial and anti-fungal substances. The potential of marine bivalves as a source of biologically active products is largely unexplored. Hence, a broad, based screening of marine bivalves for bioactive compounds is necessary. The first attempt to locate antimicrobial activity in marine organisms was initiated around 1950's [3]. Since then, a large number of marine organisms from a wide range of phyla have been screened for antimicrobial activity [4].From 1960's approximately 300 bioactive marine natural products were filed for patent. Approximately 6,500 bioactive compounds have been isolated from the marine organisms [1]. Many classes of bioactive compounds exhibiting anti-tumor, anti-leukemia, anti-bacterial and anti-viral activities have been reported [5]. Anti-microbial peptides are important in the first line of the host defense system of many animal species [6]. The demand for effective and nontoxic anti-bacterial therapeutics has become greater with the increased incidence of the bacterial infections. Rinehart, K.L. et.al. (1981)[7]reported that many marine organisms have anti-microbial properties, but most of the anti-bacterial agents that have been isolated from marine sources have not been active enough to compete with classical anti-microbial compounds obtained from microorganisms. Mary Elezabeth, K.G., C. Chellaram and P. Jamila, (2003) [8]studied theanti-microbial activity of reef associated gastropod, *Trochus radiatus* and observed maximum anti-bacterial activity against *S. aureus* and *E. coli*.B. Chandran, et.al.(2009) [9] estimated the anti-microbial and anti-fungal activity *Perna viridis* (Linnaeus, 1758).They also carried out SDS-PAGEto estimate the molecular weight of proteins and reported from gill extracts of the animal. They that the maximum zone of inhibition (19 mm) against *Staphylococcus aureus* and minimum activity (11 mm) against *Salmonella paratyphi*. In the study of anti-fungal activity, maximum zone of inhibition was observed against *Aspergillus flavus* (13mm) and minimum zone of inhibition was recorded against *Mucor* sp. (11mm). They claimed that the gill extracts of *P. viridis* may be potential source for antibiotic compounds. Ramasamy Mariappan (2010)[10]studied potential anti-bacterial activity of marine bivalves *Meretrix casta* and *Tridacna maxima*from south east coast of India and observed distinct anti-bacterial activity against twelve pathogenic bacteria namely, *Salmonella typhi, Salmonella paratyphi , Lactobacillus vulgaris, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumonia, Pseudomonas aerugenosa, Bacillus megatorium, Vibrio cholera, Staphylococcus aureus, Proteus vulgaris, Proteus milbaris.* He also concluded that the whole body extracts of the *Meretrix casta* and *Tridacna maxima* would be

a good source of antibacterial agents would replace the existing inadequate and cost effective antibiotics. Periyasamy et.al. (2012)[11] investigated the antimicrobial activity of the tissue extracts of *Babylonia spirata* against nine bacterial and three fungal pathogens and reported that great medicinal value of the gastropod muscle of *B. spirata*may be due to high quality of antimicrobial compounds.Girija A.S., et.al. (2012) [12] studied anti-bacterial activity of squid ink on Extended – Spectrum β-Lactamase (ESBL) producing strains of *E. coli*. and *Klebsiella pneumonia*e and concluded that hexane extract of the squid ink scored high anti-bacterial activity against the ESBL producing strains of *E. coli*. and *Klebsiella pneumonia*e. Their study suggested that the squid ink can be an enigmatic pigment of therapeutic value in near future for treatment of dreadful infections caused by ESBL strains**.**

Keeping in mind all above research reference, *G. proxima* has been screened for its anti-bacterial activity using foot and gill extracts obtained with various solvents.

II. MATERIALS AND METHODS

Sterilization of Apparatus:

Glassware used were of Borosilicate glass to withstand high temperature of sterilization. All the glassware was soaked overnight in a container of water with detergent added for cleaning. Next day glassware was scrubbed internally and externally with a brush and rinsed thoroughly at least 2–3 times in running tap water to remove traces of the soap. O nefourth rinsing in hot water is always advisable and facilitates drying. Glassware were set on a clean surface to drain and dried perfectly. Non-absorbent cotton wool plugs were placed extending into tubes at least an inch and not more, in each of the test tubes and were wrapped with newspaper. After washing, the glass wares were dried, a small piece of cotton stub about 1.5 inches was introduced inside the pipette from the end used for sucking (in the top) to avoid accidental contamination while pipetting cultures. Each pipette was wrapped in newspaper and secured with a piece of thread. Petridishes were wrapped in multiples with paper. Similarly conical flasks and bottles were plugged with cotton and plugs covered with newspaper and tied securely with thread. Plugs should be tight enough to prevent the glassware from slipping off. Scalpel and Cork borer were also wrapped in paper.

After packing the glass wares, they were placed in a hot air oven with temperature set at 182ºC for dry heat sterilization for 1 hr. for dry heat sterilization. Dry heat kills all vegetative bacteria, fungal and bacterial spores. Care has to be taken that hot materials get sufficient time to cool before opening the door, to prevent cracking by too sudden contraction of the glass.

Culture Media:

The ability to grow and maintain microorganisms in a laboratory requires synthetic nutritive media containing a precise composition of nitrogen, carbohydrates (as an energy source), lipids, various organic materials, mineral salts and water. Vitamin-like substances are the accessory growth factors of many pathogenic species for fastidious growth. Blood, serum, ascetic fluid, fresh vegetable extracts containing these growth factors (eg. Blood agar), are specialized media essential in the isolation and identification of microorganisms, testing of antibiotic sensitivities, water and food analysis, industrial microbiology and other activities. Culture media may be solid (agar, gelatin, coagulated serum or egg) or liquid (broth). Today a number of dehydrated media are commercially available for culture of various bacterial strains. They may be selective, differential or complex media. They are convenient to use routinely and are of pure quality grade which gives highly satisfactory results. In general, an ideal medium should have nutritive substances and electrolytes in right concentrations, should be able to provide support, should contain no toxic substances, should be isotonic and stable for sterilization process.

Preparation of Culture Media:

In the present experimental work Nutrient agar medium (HIMEDIA, Mumbai) was employed.

Ingredients (gm/litre)	**Composition**
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50
Agar	15.0
Final pH (at 25ºC)	7.4±0.2

Each ingredient was weighed accurately in a two litre flask and 1000 ml distilled water was added to it with intermittent shaking. The medium was digested by boiling at 110 º C to dissolve the contents completely. This was then distributed in narrow necked bottles, cotton plugged and autoclaved at 121ºC for 15 min. (15 lbs) for sterilization. At this high pressure, all bacteria and their resistant spores are killed. In case of nutrient broth with no addition of agar the test tubes containing broth were sterilized as above. For preparation of slants, after the nutrient agar medium was autoclaved, it was placed in water bath at 50ºC to equilibrate for 30 min. This prevented excessive water condensation on glass and kept agar in liquid state. The medium was aseptically transferred to test tubes. The tubes were then kept slanting on a tabletop inclined by resting it to a support using a glass rod or pipette. The agar medium on solidifying produces slant with increased surface area. (Elliptical shape). The length of the tube occupied by the slant must not be more than half. In case of blood agar, the autoclaved nutrient agar media was cooled to 50ºC and for complete medium 5 % sterile defibrinated sheep blood was added to it aseptically, mixed and distributed.

Inoculum Preparation:

An ideal inoculum after overnight incubation gives even semi-confluent growth. Too heavy an inoculum reduces the size of inhibition zones produced by many antibiotics. Isolation of organisms in pure culture is of utmost importance. Using a sterile inoculating loop (Nichrome No. 24) inocula were prepared by picking colonies from 24 hrs. old cultures. The overnight broth cultures were diluted 1:100 in sterile saline to obtain density of bacterial suspensions of 10^6 -

10^7cfu/ml, by comparing its turbidity with that 0.5 McFarland opacity standard.

Bacterial Strains:

All bacterial strains used were procured from K.E.M Hospital, Parel, Mumbai and Bombay Veterinary College, Parel, Mumbai. The bacterial strains used were- *Shigella shmittzi, Salmonella typhi, Salmonella paratyphi B, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgaris*.

All organisms were clinically isolated strains. These strains were routinely sub cultured on nutrient agar slants and were used for the present study.

Bioassay for disc Diffusion method:

Live animals collected and cleaned thoroughly. The desired tissues were dissected and chopped to small pieces. They were washed thoroughly with distilled water and air dried.Extraction of bioactive compounds from 1% tissue samples were done with Water, Butanol, Ethanol, Methanol, Acetone and Hexane. 3 gm of tissues samples (Foot and gills) with 5 ml of water and solvents were ground well with morter and pestle separately. They were centrifuged at 15000 rpm. for 30 minutes. The supernatants were stored at - 20 C^0 until use.Bacterial Strainswere inoculated in sterile nutrient broth and incubated at 37 C^0 for 24 hrs. Pathogens were swabbed on the surface of agar plates, disc impregnated with extracts were placed on the surface. Control disc were placed with water and solvents to assess the effect on pathogens. The anti-bacterial activity was investigated by using the standard techniques [13]. The plates were incubated at 37 C^0 for 24 hrs. Anti-bacterial activity was recorded in terms of the zone of inhibition. While determining zone of inhibition precaution has been taken by subtracting value of solvent used for the extraction and results have been measured using Vernier Caliper Scale and expressed in mm.

Well diffusion method:

The glassware and its sterilization were done as mentioned earlier. The culture media also prepared as mentioned earlier.

Preparation of Standard Drug:

Ciprofloxacin tablets, a bactericidal quinolone (Oflox – 400, Cipla Ltd.) having a broad-spectrum activity were used as standard reference antibiotic for comparative evaluation. A stock solution of 1000 µg/ml (1mg/ml) was prepared in sterile distilled water. Further dilutions of concentration in range of 0.5, 1, 2, 3 and 5 µg/ml were tested.

Bioassay for agar – well diffusion method: [14-17]

Principle:

This method is based on diffusion of the drug extracts from the well into the surrounding agar medium. The growth inhibition indicates susceptibility of the organisms. The concentration of the drug decreases as the distance from the well increases. Visible growth of bacteria occurs on the surface of the agar where the concentration of drug has fallen below its inhibitory level for the test strain. For testing, 7 ml of melted nutrient agar media was aseptically poured into sterile petri plates, which formed the based layer. 0.1 ml of broth culture was added in 100 ml of nutrient agar media, which was melted and cooled to 50ºC. It was mixed well and 4 ml was added to each plate forming the seed layer over already containing 7 ml of the basal layer. The plates were tilted to distribute the inoculum evenly and allowed to harden. With the help of a sterile cork borer, wells 8 mm in diameter were punched on the agar media plates. Each time the cork borer was flamed by passing it momentarily through a Bunsen flame. 50 µl. of the tissue extract of respective concentrations were added in each well using a sterile tip with a micropipette. A pre–diffusion time for 1 hr. at room temperature was allowed before incubation at 37ºC. Similarly 50 µl. of standard reference antibiotic Ciprofloxacin at respective concentrations were added for comparative evaluation. Each set of experiment was performed in triplicates. All the inoculated plates were kept for incubation for 24 hr. at 37º C. In case of *Streptococcus pneumoniae*, the blood agar plates were incubated in CO_2 enriched atmosphere (5%) at 37ºC for 24 hr. After incubation, the plates were observed to locate and measure the zones of inhibition of growth of microorganisms in each well.

III. RESULTS

Ethanolic gill extracts showed antibacterial activity against five human pathogenic bacteria with highest antibacterial activity against *Salmonella typhi* (11.5mm) and lowest activity against *Staphylococcus aureus* (6mm). Butanolic gill extracts showed anti-bacterial activity against three human pathogenic bacteria. Hexane gill extracts showed highest anti-bacterial activity only against *Staphylococcus aureus*. However, No any anti-bacterial activity found in the Water, Methanol and Acetone foot extracts against all pathogens.

TABLE 1. Antibacterial activity of foot extract of *G. proxima* against human pathogens

Pathogens	Zone of Inhibition					
	Water	Ethanol	Methanol	Acetone	Hexane	Butanol
Shigella shmittzi	-	-	-	-	-	10mm.
Salmonella typhi	-	-	-	-	-	10.5mm.
Salmonella paratyphi B	-	10.5mm.	-	-	11mm.	7.7mm.
Escherichia coli	-	6mm.	8.25mm.	-	-	12mm.
Klebsiella pneumonia	-	-	-	11.5mm.	-	10mm.
Pseudomonas aeruginosa	-	-	-	-	-	7mm.
Staphylococcus aureus	-	6.5mm.	7.5mm.	-	-	7mm.
Proteus vulgaris	-	8mm.	-	7mm.	-	10.5mm.

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TABLE 2. Antibacterial activity of gill extract of *G. proxima* against human pathogens

Pathogens	Zone of Inhibition					
	Water	Ethanol	Methanol	Acetone	Hexane	Butanol
Shigella shmittzi	-	-	-	-	-	-
Salmonella typhi	-	11.5mm	-	-	-	-
Salmonella paratyphi B	-	10 mm	-	-	-	-
Escherichia coli	-	-	-	-	-	8.5 mm
Klebsiella pneumonia	-	8mm	-	-	-	8 mm
Pseudomonas aeruginosa	-	7.5mm	-	-	-	-
Staphylococcus aureus	-	6 mm	-	-	11mm	5mm
Proteus vulgaris	-	-	-	-	-	-

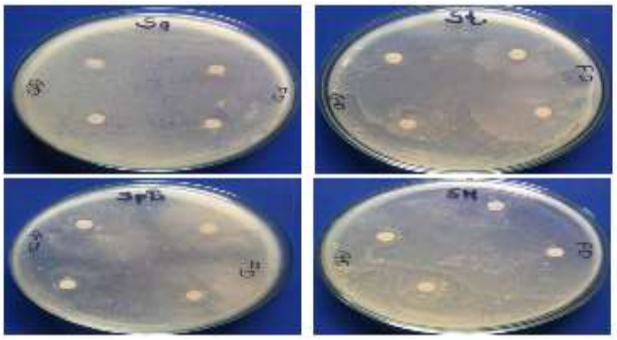

Fig. 1. Zone of Inhibition in Water extract as control.
(Sa- *Staphylococcus aureus*, St- *Salmonella typhi*, SpB- *Salmonella paratyphi B*, SH- *Shigella shmittzi*, FD-Foot, GD-Gill)

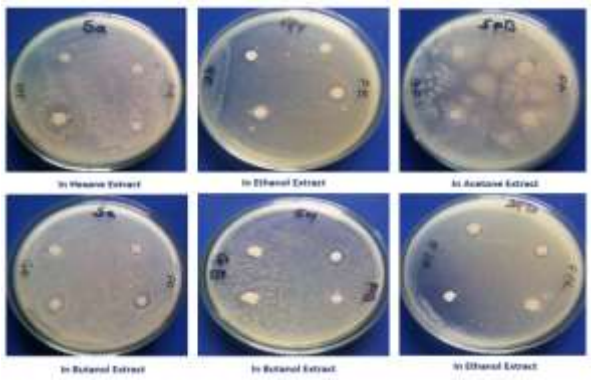

Fig. 2. Zone of Inhibition in different extracts
(Sa- *Staphylococcus aureus*, Pr-*Proteus vulgaris* , SpB- *Salmonella paratyphi B*, SH- *Shigella shmittzi*, GH- Gill in Hexane, FH-Foot in Hexane, GB- Gill in Butanol, FB-Foot in Butanol, GE-Gill in Ethanol, FE-Foot in Ethanol, GA-Gill in Acetone, FA-Foot in Acetone, G Eth-Gill in Ethanol, F Eth-Foot in Ethanol)

Results of well diffusion method:

TABLE 3. Antibacterial activity of gill, foot and hepato pancreas extract of *G. proxima* against human pathogens

Pathogens	Control	Foot Extract	Gill extract	H.P. extract
Shigella shmittzi	-	-	-	-
Salmonella typhi	-	-	-	-
Salmonella paratyphi B	-	-	-	-
Escherichia coli	-	-	-	-
Klebsiella pneumonia	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-
Staphylococcus aureus	-	-	-	-
Proteus vulgaris	-	-	-	-

Bioassay of well difussion method was carried out by making the use of bacterial 8 strains such as *Shigella shmittzi*, *Salmonella typhi*, *Salmonella paratyphi B*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*. The results of well diffusion method were found negative including control. Three tissue extracts namely foot,gill and hepatopancreas were prepared along with control but surprizingly the results were negative without any zone of inhibition.

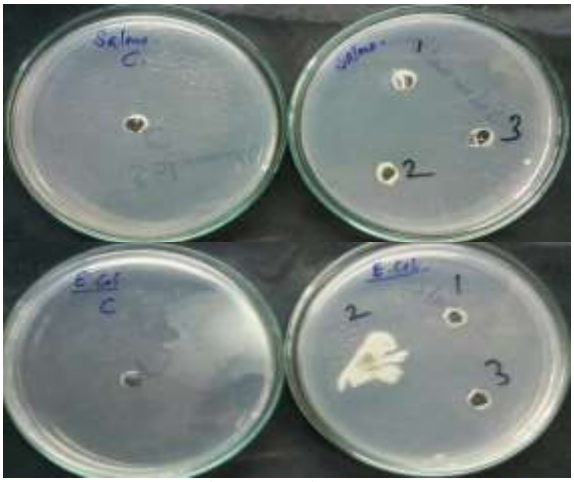

Fig. 3. Zone of Inhibition for bacterium *E. coli.*

Fig. 4. Zone of Inhibition for bacterium *Shigella*.
(C- Control, 1- Foot, 2- Gill, 3- Hepatopancreas)

Fig. 5. Zone of Inhibition for bacterium *Pseudomonas aeruginosa*
(C- Control, 1- Foot, 2- Gill, 3- Hepato pancreas)

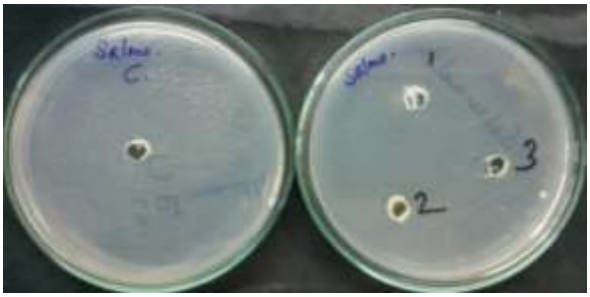

Fig. 6. Zone of Inhibition for bacterium *Salmonella typhi*
(Salmo-Salmonella, C- Control, 1- Foot, 2- Gill, 3- Hepatopancreas)

IV. DISCUSSION

Molluscs are well-known seafood and popular for their high delicacy. They are an excellent source for bio-medically important products. Among the molluscs, gastropods and bivalves have pronounced pharmacological activities.

In the present investigation, the disc diffusion method showed distinct anti-bacterial activity against most of the pathogenic bacteria.

The antibacterial activities of ethanol extracts of gastropods *Babylonia spirata* and *Turbo brunneus* was observed maximum activity against *E. coli, K. pneumoniae, P. vulgaris* and *S.* **[18].** This study corroborates the results of the present investigation. Very similar to this maximum anti-bacterial activity against *S. aureus* and *E. coli.* on *Trochus radiatus* was reported **[8]**.Butanolic foot extracts showed highest anti-bacterial activity against *E. coli* (12mm). Acetone foot extracts showed highest anti-bacterial activity against *Klebsiella pneumonia* (11.5mm). Hexane foot extracts showed anti-bacterial activity against only one pathogen namely *Salmonella paratyphi B* with highest inhibition zone (11 mm). Aqueous extracts were ineffective against all the selected organisms. Difference in anti-bacterial activity found with bivalve extracts may depend upon extracting capacity of solvents and compound extracted. However, it was surprising that no inhibitory activity was observed against the test organisms, when studied by the well- diffusion method.

V. CONCLUSION

Thisantimicrobial study indicates that as per the paper disc method, the foot tissue extracts of *G. proxima* would be a good source of an effective anti-bacterial agent. Further studies are needed to elucidate structure and mechanism of action of the mangrove clam extracts.

REFERENCES

1. Kamboj, V.P., (1999): Bioactive agents from the ocean Biota. In Oceans Science Trends Future Directions. Somayajuly BLK (Ed.), Indian National Science Academy, New Delhi India, pp.197-227.
2. Jhonson, M.W. and Sonderhall, K., (1985): Exocytosis of the Prophenoloxidase activating system from cray fish haemocytes. J.Com. Physiol., 15: 175-181.
3. Berkholder, P.R. and L.M. Burkholder, 1958. Anti-microbial activity of horny corals. Science, pp: 127:1174.
4. Shaw, P.D., W.O. McClure, G. Van Blaricom, J. Sims, W. Fenical and Rude, (1976). Anti-microbial activities from marine organisms. In: Food-drug from sea, 25: 55-60.
5. Pettit, G.R., C.L. Herald, M.R. Boyd, J.E. Leet, C. Burfrese, D.L. Doubek and Schimidt (1991) : Antineoplastic agents. 219. Isolation and structure of the cell growth inhibitory constituents from the Western Pacific marine sponge *Axinella* sp. J. med. chem., 34: 3339 - 3340.
6. Boman, H., (1995): Peptide antibiotics and their role in innate immunity. Annu. Rev. Immunol. 13: 61-92.
7. Rinehart, K.L., P.D. Shaw, L.S. Shield, J.B. Gloer, G.C. Harbour, M.E.S. Koker, D. Samain, R.E. Schwartz, A.A. Tymiak, E.G. Swynenberg, D.A. Stringfellow, J.J. Vavva, J.H. Coasts, G.E. Zurenko, S.L. Kuentel, L.H. Li, G.J. Bakus, R.C. Brasca, I.I. Craft, D.N. Young and J.L. Connot (1981) : Marine natural products as a source of anti-viral, anti-microbial and anti-neoplastic agents, Pure. Appl. Chem., 53: 795-817.
8. Mary Elezabeth, K.G., C. Chellaram and P. Jamila, (2003): Anti-microbial activity of reef associated gastropod, *Trochus radiatus*. National Seminar on Ecosystem, Remediation, pp: 68.
9. B. Chandran, G. Ramesh Kumar and S. Ravichandran(2009): Anti-microbial Activity from the Gill Extraction of *Perna viridis* (Linnaeus, 1758) Global Journal of Biotechnology & Biochemistry 4 (2): 88-92.
10. Ramasamy Mariappan, V. Sukumaran and M. Ayyaoo (2019): Potential antibacterial activity of marine bivalves *Meretrix casta* and *Tridacna maxima*from south east coast of India.Advances in bioresearch Vol.1, (1): 92 - 96.
11. Periyasamy N., Srinivasan M, Balakrishnan S. (2012) : Anti-microbial activities of the tissue extracts of *Babylonia spirata*Linnaeus, 1758 (Mollusca: Gastropoda) from Thazhanguda, southeastcoast of India. Asian Pacific Journal of Tropical Biomedicine 36-40.
12. Girija A.S., Vijayshree priyadarshini J., Pandi suba P., Hariprasad P., and Raghuraman R. (2012): Anti-bacterial activity of squid ink on ESBL producing strains of *E. coli*. and *Klebsiella pneumonia*e Indian Journal of Geo marine – sciences Vol. 41(4),.pp. 338- 343.
13. Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M.(1996): Antibiotic susceptibility testing by a standardized single disc method Am.J.Clini.pathol.24:493-496.
14. Cruikckshank R. (1968): Medical Microbiology. 11th ed. The English Language Livingstone Ltd., Society and E. & S., London.
15. Report of the Working Party on Antibiotic Sensitivity Testing of the British Society of Anti-microbial Chemotherapy. (1991): A guide to sensitivity testing. *J. Antimicrob. Chemother.***27**: 1 – 50..
16. National Committee for Clinical Laboratory Standards (2000): Methods for dilution anti-microbial susceptibility tests for bacteria that grow aerobically. Wayne, PA, USA: Approved Standard M7-A4.
17. National Center for Infectious Disease, Center for Disease Control and prevention, WHO (1999): Laboratory Methods for the Diagnosis of Epidemic Dysentery and Cholera, Center for Disease Control and prevention. Atlanta, Georgia, USA: 71–72.
18. Prem Anand, T., J. Rajaganapathy and Patterson Edward, (1997): Anti-bacterial activity of Marine mollusks from Portonova region. Indian J. Mar. Sci., 26:206-208.