Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

DOI:10.21474/IJAR01/7715
DOI URL: http://dx.doi.org/10.21474/IJAR01/7715

RESEARCH ARTICLE

BIOCHEMICAL ANALYSIS AND PEPTIDE MODELING OF LYSOZYME IN INDIAN *ENNEROPENAEUS INDICUS* SHRIMPSPECIES.

Shoba.K, Nithy. G and Deepa.L.
Department of Biochemistry, D.K.M college for women (Autonomous) , Vellore, Tamilnadu, India.

Manuscript Info

Manuscript History
Received: 20 July 2018
Final Accepted: 26 August 2018
Published: September 2018

Keywords:-
Fenneropenaeusindicus, Lysozyme, SDS-PAGE and HPLC, Motif, Peptide.

ABSTRACT

FAO defines aquaculture as "the farming of aquatic organisms, including fish, mollusks, crustaceans, and aquatic plants". Fanning here implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding and protection front predators. Aquaculture is the only alternative to mitigate the threats to the world's fisheries by taking the pressure off from wild fish stocks while supporting livelihoods and food production. Aquaculture production has increased tremendously over the past few decades and today accounts for almost a half of global fish production by weight, while production from wild fisheries has largely slowed or stagnated.
The enzyme lysozyme is extracted from *Fenneropenaeusindicus* and it is estimated by using Lowry's method. Lysozyme is biochemically analysed with SDS-PAGE and HPLC. The activity of the enzyme is assayed and characterized by determining the optimum pH and temperature. The effect of activator and inhibitor on lysozyme activity is obtained.The final results of our project clearly explain that the identified motif peptide sequence and the 3D peptide structure are potential candidates for drug docking studies and also act as novel molecular markers useful for pharmacoinformitics and clinical endocrinology studies.

Introduction:-

Fenneropenaeusindicus is a significant aquaculture species in Iran, Saudi Arabia, India, Indonesia, Vietnam and South Africa (Hoffman et al., 1998; UNEP, 2002). In 1999, 7043 MT of *F. indicus* were produced by aquaculture farms (FAO, 2001). *F. indicus* has a wide distribution, from the Indo-West Pacific - southeast and East Africa in the Indian Ocean, the Red Sea (Saudi Arabia), Sri Lanka (e.g. Negombo Lagoon), throughout India (the Arabian Sea and Bay of Bengal), through Malaysia, Iran, Bangladesh, Thailand, Indonesia, the Philippines, South China and northern Australia. The predominant species in Papua New Guinea appears to be *Fenneropenaeusmerguiensis* (L Evans, Ecotao Enterprises, South Africa, personal communication, 2004). In some areas, such as Thailand (Phongdara et al., 1999) and Australia, banana prawns comprise two species, *F. merguiensis* and *F. indicus*, which are very similar in morphology. They can be identified by a distinct difference in the rostrum. *F. indicus* is a marine shrimp (with estuarine juveniles) which likes mud or sandy mud habitats at depths of 2-90 metres (6-300 feet). It is one of the major wild caught commercial species of the world. Adults are marine and the juveniles are estuarine.

Corresponding Author:-Shoba.k.
Address:-Department of Biochemistry, D.K.M college for women (Autonomous) , Vellore, Tamilnadu, India.

F.indicus tolerates low water quality conditions and tolerates low dissolved oxygen better than *Penaeusmonodon* (Rosenberry, 2004). Ideally, oxygen levels should be between 5 and 10 ppm, though higher levels (up to 20 ppm) do not seem to cause any problem. At night, the minimum oxygen levels should be 3.6 ppm. Similar to *P. monodon* (Groth et al., 2001), it still survives when dissolved oxygen (DO) levels are around 1.5 ppm (mg/L). It tolerates salinities from 4 ppt (L Evans, Ecotao Enterprises, South Africa, personal communication, 2004) to 50 ppt (A Abedian, TarbiatModares University, Iran, personal communication, 2004), high temperatures and high densities, and it is readily available in the wild. *F. indicus* also reaches sexual maturity and spawns in ponds. It tolerates 45 ppt salinity in the Red Sea (De Bruin et al., 1994). The ideal growout water temperatures are from 25 to 32°C. Between 20 and 23°C, *F. indicus* grows too slowly for commercial production, although it will survive quite well at these temperatures. Between 18 and 20°C, the growth rate is roughly a seventh of that at 28°C. The animal can live at temperatures down to 15°C, but shows stress, such as a slow or lack of recovery from moulting at these low temperatures.

Fenneropenaeusindicus is a significant aquaculture species in Iran, Saudi Arabia, India, Indonesia, Vietnam and South Africa (Hoffman et al., 1998; UNEP, 2002).In 1999, 7043 MT of *F. indicus* were produced by aquaculture farms (FAO, 2001). *F. indicus* has a wide distribution, from the Indo-West Pacific - southeast and East Africa in the Indian Ocean, the Red Sea (Saudi Arabia), Sri Lanka (e.g. Negombo Lagoon), throughout India (the Arabian Sea and Bay of Bengal), through Malaysia, Iran, Bangladesh, Thailand, Indonesia, the Philippines, South China and northern Australia. The predominant species in Papua New Guinea appears to be *Fenneropenaeusmerguiensis* (L Evans, Ecotao Enterprises, South Africa, personal communication, 2004).

In some areas, such as Thailand (Phongdara et al., 1999) and Australia, banana prawns comprise two species, *F. merguiensis* and *F. indicus*, which are very similar in morphology. They can be identified by a distinct difference in the rostrum.

F. indicus is a marine shrimp (with estuarine juveniles) which likes mud or sandy mud habitats at depths of 2-90 metres (6-300 feet). It is one of the major wild caught commercial species of the world. Adults are marine and the juveniles are estuarine.

F.indicus tolerates low water quality conditions and tolerates low dissolved oxygen better than *Penaeusmonodon* (Rosenberry, 2004). Ideally, oxygen levels should be between 5 and 10 ppm, though higher levels (up to 20 ppm) do not seem to cause any problem. At night, the minimum oxygen levels should be 3.6 ppm. Similar to *P. monodon* (Groth et al., 2001), it still survives when dissolved oxygen (DO) levels are around 1.5 ppm (mg/L). It tolerates salinities from 4 ppt (L Evans, Ecotao Enterprises, South Africa, personal communication, 2004) to 50 ppt (A Abedian, TarbiatModares University, Iran, personal communication, 2004), high temperatures and high densities, and it is readily available in the wild. *F. indicus* also reaches sexual maturity and spawns in ponds. It tolerates 45 ppt salinity in the Red Sea (De Bruin et al., 1994). The ideal growout water temperatures are from 25 to 32°C. Between 20 and 23°C, *F. indicus* grows too slowly for commercial production, although it will survive quite well at these temperatures. Between 18 and 20°C, the growth rate is roughly a seventh of that at 28°C. The animal can live at temperatures down to 15°C, but shows stress, such as a slow or lack of recovery from moulting at these low temperatures.

Methods:-

The enzyme lysozyme is extracted from the *F. indicus.* The amount of protein present in the sample is estimated by plotting OD value at 640nm against the standard protein using Lowry's method. The biochemical analysis of the enzyme is done by using Sodium Dodecyl Sulphate - Poly Acrylamide Gel Electrophoresis and High Performance Liquid Chromatography. The proteins get separated on the basis of molecular weights. When the electrophoretic run is completed the gel is immersed in 0.25% Coomassie blue solution for 30minutes for staining. The gel is destained with 40% methanol and 10% acetic acid. The protein bands are observed clearly under UV- Trans illuminator. The mobile phase used in HPLC is 0.1% phosphoric acid and acetonitrile in the ratio 80:20. Sample is precipitated by 60% ammonium sulphate and the precipitate is recovered and purified further on G 200 sephadex. The sample is placed in auto sampler tray and run the HPLC. High pressure liquid chromatogram is obtained with the help of computer. Bioinformatics analysis were carried out for lysozyme protein , motif were identified and identified motif are modeled using pepfold server.

Results And Discussion:-

As with all forms of gel electrophoresis, molecules may be run in their native state, preserving the molecules' higher-order structure or a chemical denaturant may be added to remove this structure and turn the molecule into an unstructured linear chain whose mobility depends only on its length and mass-to-charge ratio. In most proteins, the binding of SDS to the polypeptide chain imparts an even distribution of charge per unit mass, thereby resulting in a fractionation by approximate size during electrophoresis. The result of SDS-PAGE is shown in Fig 1. High-performance liquid chromatography is a technique in analytical chemistry used to separate, identify and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column. The chromatogram obtained for standard and sample collagenase is given in Fig 2 and 3.

The target sequence is retrieved from NCBI in FASTA format, presence of motif in the lysozyme were identified using scan prosite motif server. The motif region of target sequence was modeled using pep fold server(Fig 4,5).

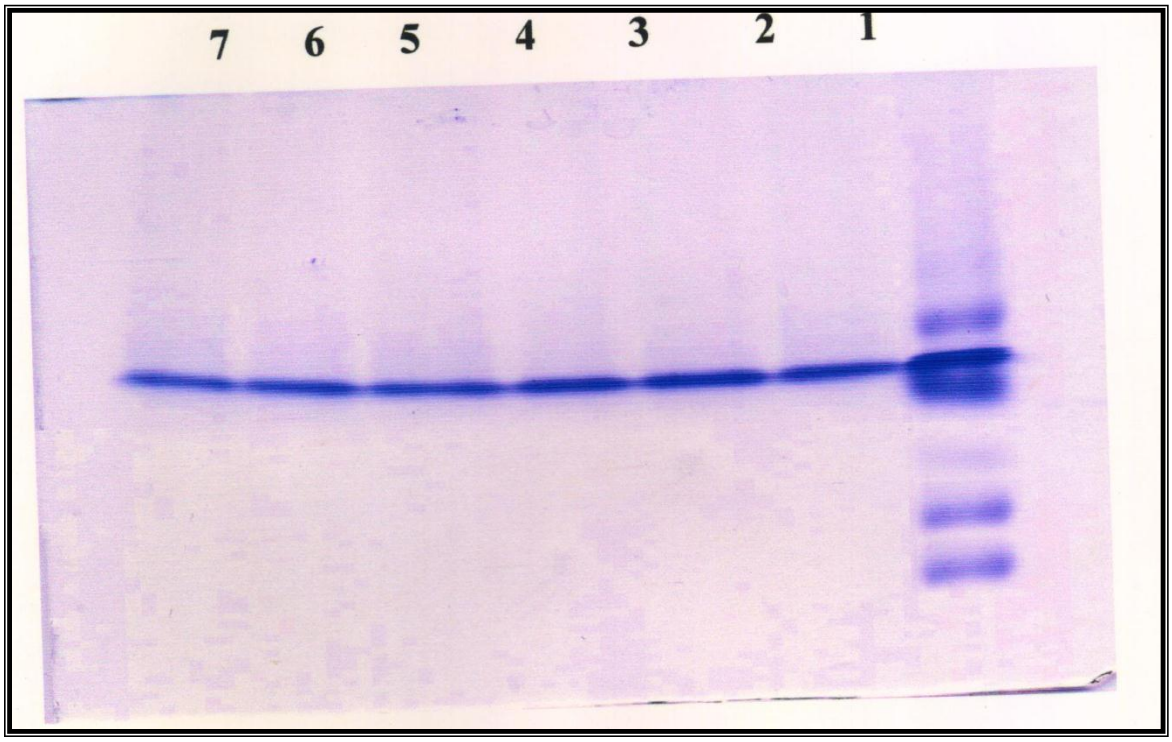

Fig. 1:-Purification profile of lysozyme by sds page in *Fenneropenaeusindicus*

Lane: 1 Protein Marker (14,000, 29, 000, 43, 000, 66, 000 Da)
Lane: 2 Crude enzymes
Lane: 3 Supernatant obtained from centrifugation.
Lane: 4 Extract obtained from Ion exchange chromatography
Lane: (5-7) Extract obtained from after analysis

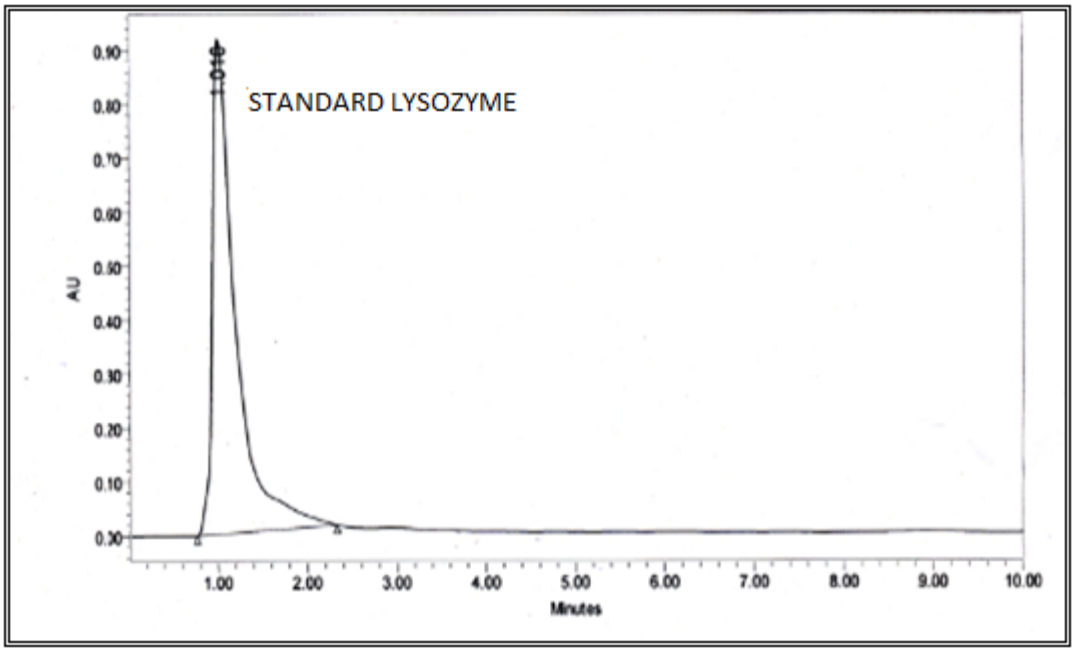

Fig 2:-Chromatogram for standard lysozyme

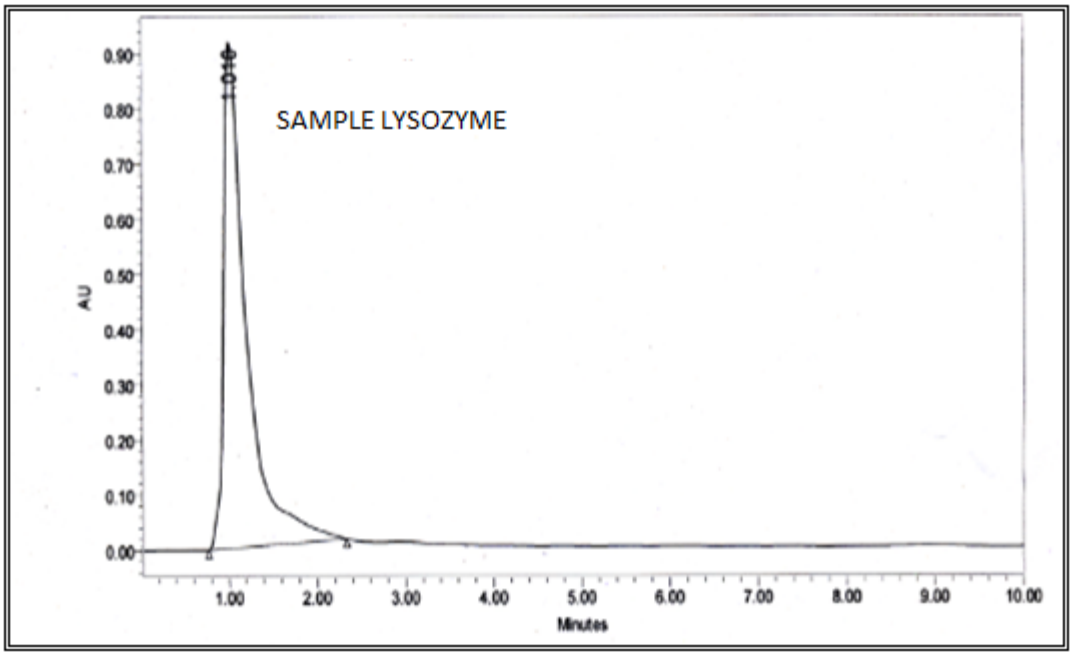

Fig 3:-Chromatogram for sample lysozyme

Bioinformatic analysis:-
Sequence retreival – ncbi:-
NCBI
>ACV49870.1 LYSOZYME [FENNEROPENAEUS INDICUS]
MRVLPLALLVGLLAVSDAKVFKKCEFARLLETRYHLSRNDIKNWVCIAEFESSFNTAAINRNRNRSTDYGIF
QINNKYWCGSDYGKNVCGIPCSDLMRDDITASLRCAETVRRETERYSGRGNGYTAWVAYNSKCKNRNLD
QYMADCWSRGSNSIFQF

Fig 3:-The above result shows the fastaformat protein sequence of Lysozyme from *Fenneropenaeusindicus prawn.*

Motif Analysis – Scanprosite:-
Scan prosite:-

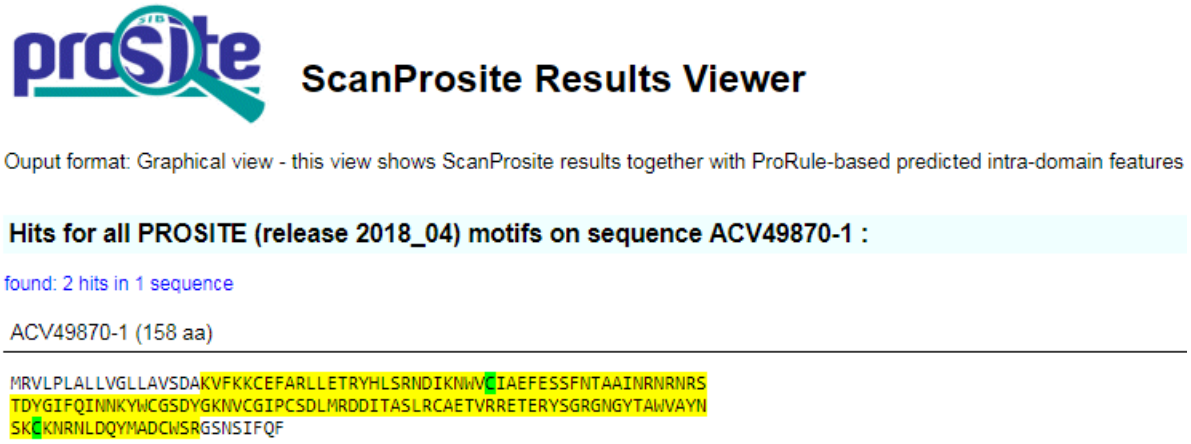

Fig 4:-The above result shows the motif in yellow colour and conserved domain region in green colour.

Hree dimensional structure prediction of motif:-
Pepfold server:-

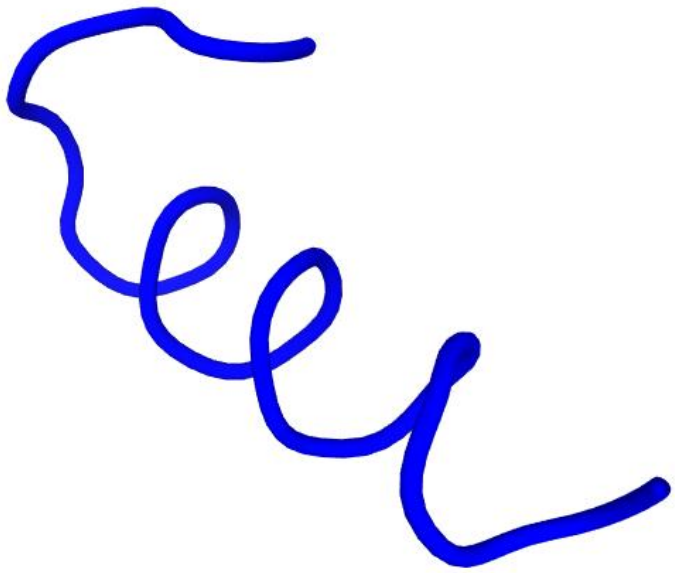

Fig- 5:-The above result shows the motif region of Lysozyme protein which is modeled by pep fold server.

Conclusion:-

Shrimps as invertebrates offer an interesting system to investigate defense echanisms as they operate through innate immune responses. Though hemocytes are the cells primarily involved in containing pathogens, the immune response within shrimps is orchestrated by Ps that functions as the first line of defense against invading microorganisms (Brown &ancock 2006; Hancock et al 2006). Several AMPs have been demonstrated to have in

vitro ntimicrobial activity and modulate immunity to protect the host from infection (Brown & Hancock 2006; Hancock & Diamond 2000; Yang et al 2002). There are many groups of shrimp AMPs synthesized primarily in the hemocytes and are released in response to infection (Munoz et al 2004). Various species of shrimps have been varying capability for resistance to infection, particularly WSSV, which could be due to the structural and functional differences of the AMP's. Biological activity of shrimp AMPs had been characterized in vitro using their recombinant proteins and synthetic peptides (Bachere et al 2004; Smith et al 2008; Zhao & Wang 2008). Two other immune genes TCTP and peroxiredoxin from F. indicus have been reported for its multifunctional role in shrimp innate immunity (Kiruthiga et al 2012; Rajesh et al 2010). Lysozyme takes part in the degradation of microbes within and outside shrimp hemocytes and has been known as an important molecule in shrimp defense mechanism. (Dela Re Vega et al 2004). Further very little information is available on the wide spectrum activity of shrimp lysozyme, particularly from F. indicus. This forms the basis of the present study where in lysozyme has been identified from Indian shrimp F. indicus and the functional characterization has been carried out. The future studies on this enzyme may lead to find out more insights.

References:-

1. Altschul, SF, Gish, W, Miller, W, Myers, EW &Lipman, DJ 1990, 'Basic local alignment search tool', Journal of Molecular Biology, vol. 215, pp. 403-410.
2. Amparyup, P, Kondo, H, Hirono, I, Aoki, T &Tassanakajon, A 2008, 'Molecular cloning, genomic organization and recombinant expression of a crustin-like antimicrobial peptide from black tiger shrimp *Penaeusmonodon*', Molecular immunology, vol. 45, no. 4, pp. 1085-1093.
3. Brown, KL & Hancock, RE 2006, 'Cationic host defense (antimicrobial) peptides', Current opinion in immunology, vol. 18, no. 1, pp. 24-30.
4. Chae, HZ, Chung, SJ & Rhee, SG 1994, 'Thioredoxin-dependent peroxide reductase from yeast', Journal of Biological Chemistry, vol. 269, no. 44, pp. 27670-27678.
5. Chang, CF, Su, MS, Chen, HY & Liao, I 2003, 'Dietary -1, 3-glucan effectively improves immunity and survival of *Penaeusmonodon* challenged with white spot syndrome virus', Fish and shellfish immunology, vol. 15, no. 4, pp. 297-310.
6. Davis, KM, Akinbi, HT, Standish, AJ, Weiser, JN 2008, 'Resistance to mucosal lysozyme compensates for the fitness deficit of peptidoglycan modifications by *Streptococcus pneumoniae*', PLoS pathogens, vol. 4, no. 12, pp. e1000241.
7. Guaní Guerra, E, Santos Mendoza, T, Lugo Reyes, SO &Terán, LM 2010,'Antimicrobial peptides: general overview and clinical implications in human health and disease', Clinical Immunology, vol. 135, no. 1, pp. 1-11.
8. Karunasagar, I, Otta, SK &Karunasagar, I 1997, 'Histopathological and bacteriological study of white spot syndrome of *Penaeusmonodon* along the west coast of India', Aquaculture, vol. 153, no. 1, pp. 9-13.
9. Kyte, J & Doolittle, RF 1982, 'A simple method for displaying the hydropathic character of a protein', Journal of Molecular Biology, vol. 157, no. 1, pp. 105-32.
10. Lavilla-Pitogo, CR, Baticados, MCL, Cruz-Lacierda, ER & de la Pena, LD 1990, 'Occurrence of luminous bacterial disease of Penaeusmonodon larvae in the Philippines', Aquaculture, vol. 91, no. 1, pp.1-13.
11. Primavera, JH 1978, 'Induced maturation and spawning in five-month-old (Penaeusmonodon) Fabricius by eyestalk ablation', Aquaculture, vol. 13, no. 4, pp. 355-359.
12. Radek, K & Gallo, R 2007, 'Antimicrobial peptides: natural effectors of the innate immune system', Seminars in Immunopathology, vol. 29, no. 1, pp. 27-43.
13. Saurabh, S, &Sahoo, P K 2008, 'Lysozyme: an important defence molecule of fish innate immune system', Aquaculture Research, vol. 39, no. 3, pp. 223-239.
14. Scott, MG & Hancock, RE 2000, 'Cationic antimicrobial peptides and their multifunctional role in the immune system', Critical Reviews in Immunology, vol. 20, no. 5.
15. Sengupta P, Meena K, Mukherjee R, Jain SK &Maithal K 2008,'Optimised conditions for high level expression and purification of recombinant human interleukin-2 in E.coli', Indian journal of Biophysics &Biochemistry,vol. 45, pp 91-97.
16. Shoba k and Vanitha S, Gene expression analysis and molecular mechanics studies on collagenase protein in *fiddler crab (uca)* using insilico protocols. International journal of novel trends in pharmaceutical sciences, ISSN: 2277 – 2782, volume 7, number 2, April 2017.
17. Shoba.k and Dr. Mazher sultana, Three - dimensional structure and motif prediction studies on collagenase protein in fiddler crab, International journal of novel trends in pharmaceutical sciences, Issn: 2277 – 2782,volume 6, issue 4, pages 79 – 83.

18. Shoba K. , Manjuladevi M , Dr. Mazher sultana, Biochemical analysis and gene expression profiling on collagenase protein in fiddler crab, World journal of pharmacy and pharmaceutical sciences, issn 2278 – 4357, volume 6, issue 3, 747-756
19. Shoba K. , Sowmiya S and Dr. Mazher sultana, World Journal of Pharmaceutical and Life Sciences, ISSN 2454-2229, Vol. 3, Issue 1, 427-436.
20. Shoba.K, Hebsibahelsie.B, insilico homology modeling ofribulose-1, 5-bisphosphate carboxylase protein in gracilariaedulis, world journal of pharmacy and pharmaceutical sciences, 2017,volume 6, issue 8, 396-406, issn 2278 – 4357.
21. Shoba.K, Lavanya.G, Identification Of De Novo Peptide And Motif Prediction On Porphyria Protein (Hmbs) Using Insilico Tools, Universal Journal Of Pharmacy ,2018, Volume 8, Issue 1, Issn2320-303x.
22. Shoba.K, Lavanya.G, Tertiary Structural Prediction And Drug Binding Studies On Mutated Gene (Hmbs) In Human Porphyria,International Journal Of Novel Trends In Pharmaceutical Science,2018,Issn 2277 - 2782,Volume 8,Issue 1
23. Shoba K., Hebsibah Elsie B. And Bavyasri S. Insilico Peptide Modeling Stuidies And Structural Analysis On Ribulose -1, 5 Bisphosphate Carboxylase In GracilariaEdulis, World Journal Of Pharmacy And Pharmaceutical Sciences, 2018,Volume 7, Issue 3, 1086-1095, Issn 2278 – 4357.
24. Shoba. K, Hebsibah Elsie. B and Jayakumari. S. Sathya. R. (2018); Insilico Structural Analysis and Drug Docking Stuidies On Ribulose -1, 5 Bisphosphate Carboxylase In *Gracilaria Edulis*. International journal of advanced research. **6** (9). 159-165] (ISSN 2320-5407).
25. Hebsibah Elsie B, Subashini.G, Nithya.G and Shoba.K. (2018); Purification and Identification of Antioxidant Peptides from the Skin Protein Hydrolysate of Marine Fish (*Aurigequula Fasciata*). European journal of Pharmaceutical and Medical Research.5 (10). 371 – 378.Issn No 2394-3211.