JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Antimicrobial, antioxidant and cytotoxic activities of hemolymph from marine spiny lobster, Panulirus homarus (Linnaeus, 1878) from Vizhinjam coast, Kerala.

Anoop Appu[1], Ratheesh Sadanandan[2] and Ravichandran Samudrapandian[3]
[1, 2] Research Scholar, [3]Associate professor
Centre of Advanced Study in Marine Biology,
Annamalai University,
Parangipettai, 608 502,
Tamilnadu, India

Abstract

P. homarus is a marine spiny lobster which is edible and widely cultivated species in Asian countries. Antimicrobial activity of haemolymph collected from; *P. homarus* (PHH) were tested against clinical pathogens. Antimicrobial activities of PHH were carried out by well diffusion method. PHH exhibit a significant activity *E.coli, S. aureus, S.typhi, S.pyogenes* and *P.chrysogenum.* Results were compared with standard antibiotic drugs Gentamycin and Clotrimazole. The antioxidant potential of the PHH was measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. After DPPH activity of PHH at different concentrations (1.56-1000µg) it exhibited antioxidant activity with IC_{50} value of 172.53. Cytotoxic activity at different concentrations (0.25-100 mg/mL) was tested against MCF-7 cell line by MTT assay. PHH exhibited potential cytotoxicity against these cell lines after 24 hours in a dose dependent manner. These findings indicate that PHH may be used in clinical applications.

Keywords: Marine lobster, *P. homarus,* antimicrobial activity, well diffusion method, DPPH radical scavenging assay, MTT assay, cytotoxic assay.

Introduction

According to research, crustaceans, especially lobsters with open circulatory systems and hemolymph, hav e tremendous immunological and therapeutic effects. They created a number of effective defences against invading diseases (Jiravanichpaisal., 2006). Some arthropods have a fluid called hemolymph in their circulatory systems. T he interior of the lobster's body is completely filled with hemolymph, which surrounds all of the cells and contains the protein hemocyanin, which is based on copper. Haemolymph is capable of phagocytosis, encapsulation, nodule formation, and mediating cytotoxicity, according to published research, and is engaged in the innate immune resp

onse of crustaceans (Söderhäll, 1992). The use of crustacean haemolymph in the pharmacological and cosmetic the rapy of viral and other neoplastic or preneoplastic mammalian tissue lesions was the subject of ongoing research.

Spiny lobsters are nutritionally rich high value marine fishery resources. Because of its high demand among domestic as well as international market, this resource is heavily fished in India. They are one among the world's most highly priced and valuable seafood species that are captured and marketed in more than 90 countries (Fitzgibbon *etal*., 2014). Farming of spiny lobsters had a significant positive impact on livelihoods in impoverished coastal communities, with almost all the economic development occurring within family businesses (Jones, 2010). They were highly expensive and sought after sea food due to their taste and nutritional value. Studies conducted in India on spiny lobsters were on taxonomy (Chakraborty *etal.,* 2015) , fishery and biology (Radhakrishnan *etal.,* 2008 , Thomas 1972) growth, moulting and breeding (Thomas., 1972., Vijayakumaran *etal*., 2005, Syda Rao *etal*., 2010) larval development (Radhakrishnan., 1995), lobster cage culture (Lipton *etal.,* 2010).

Spiny lobster, *P. homarus* is one among the major distributed and dominant species among the lobster species Indian and Pacific oceans (Radhakrishnan *etal.,* 2008). They are commonly known as rock lobsters because they inhabit along the rocky areas (Chakraborty and Radhakrishnan, 2015). *P. homarus* is an important tropical lobster of food value and known for their flavour and taste. The nutritional and biochemical content of the lobster *P. homarus* was studied by Arumugam *etal.,* (2020) and it composed of minimum moisture (83.52±1.52b), crude protein (56.38±1.52b g/100g), carbohydrate (7.26±0.52b g/ 100g), crude lipids (7.18±0.22b g/100g) and ash (36.19 ± 4.28b g/100g).

Due to the presence of carotenoids, primarily asetaxanthin, in their bodies, lobsters can range in colour from greeni shbrown to black. *P. homarus* is a species with relatively limited mobility, which makes it more vulnerable to fish ing (Mohamed and George, 1968). Alka *etal.*,(2016),examined the diet and feeding of the spiny lobster, P. homaru s, along the Vizhinjam Coast of Kerala .

Therefore, the purpose of the current study was to look into the biological functions of haemolymph taken f rom the spiny lobster, *P. homarus*.

2. Materials and Methods

2.1. Chemicals

Muller Hinton agar, Nutrient broth, Gentamycin, Fetal bovine serum (FBS), Dulbecco´s modified eagles medium (DMEM), 3-(4, 5-dimethylthiazol-2-yl) -2,5 diphenyltetrazolium bromide (MTT), Penicillin-Streptomycin was purchased from Himedia (Mumbai). Trypsin was purchased from Gibco (USA). All other chemicals used in this study were of the analysis grade of good quality commercially available in the market.

2.2. Collection of *P. homarus*

Healthy live spiny lobster, *P. homarus* were brought from local fisherman in Vizhinjam, Trivandrum District, Kerala, India and brought to the laboratory for the collection of haemolymph.

2.3. Collection of Haemolymph

A sterile syringe was used to collect hemolymph from the ventral sinus at the base of the fifth lobster walking leg a nd transfer it to an Eppendorf tube. The clot was then macerated, followed by a 20minute centrifugation at 2000 g of the fluid. The supernatant was gathered and kept at 4°C until needed.

2.4. Microbial Strains

Antimicrobial activity of PHH were determined against 10 bacterial strains *E. coli, K. pneumonia, S. pyogenes, V.cholerae, S. typhi, P. aeruginosa, B.subtilis, S. marcescens*, *M. smegmatis and S. aureus* and 5 fungal strains, namely, *A. flavus, M.mucedo, P. chrysogenum, C. albicans* and *R.stolonifer.*

2.5. Antibacterial assay

It is standard procedure to assess the antibacterial activity of PHH using the agar well diffusion method (Va lgas *etal.*, 2007). PHH (10 mg/ml stock) was added in the amount of 100 L to the wells. Tris buffer and gentamyci n (40 l from a 4 mg/ml stock) were added as positive and negative controls, respectively. Under aerobic condition s, the plates were incubated for 24 hours at 36°C + 1°C.The plates were incubated for 24 h at 27ºC ± 1ºC, under aerobic conditions. After incubation, the plates were observed and the zone of bacterial growth inhibition around the wells was measured in mm.

2.7. Antioxidant activity

Antioxidant activity of PHH against stable 2, 2- diphenyl 2- picrylhydrazyl hydrate (DPPH) was determined according to the method of Brand-William *etal.*, (1995) with slight modification. A 60µM solution of DPPH in methanol was freshly prepared and a 3.9 ml of this solution was mixed with 1ml of the test sample at various concentrations (1.56 - 1000 µg/ml). The tubes were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured at 515 nm. Control was prepared with DPPH solution only, without any extract. 95% methanol was used as blank.

The percentage scavenging of DPPH by the extracts was calculated according to the following formula:

$$\% \text{ DPPH Radical scavenging} = [(Ac - At) / Ac] \times 100$$

Ac is the absorbance of the control (DPPH),
At is the absorbance of a test sample.

2.8. Cell line

MCF-7 (Human breast cancer cell line) was purchased from NCCS, Pune, India. The cells were maintained in DMEM, supplemented with 10% fetal bovine serum (FBS) and 100 U/l penicillin. Cells were cultured at 37ºC in a humified atmosphere of 5% CO_2 and 95% air. The cells were sub cultured at regular intervals.

2.9. Cytotoxic activity

Cytotoxicity of MCF-7 were carried according to the method of Joseph *etal*.,(2012). Untreated wells were kept as control. The absorbance at 570 nm was measured with micro plate reader. Two wells per plate without cells served as blank. All experiments were done in triplicates. The cell viability was expressed using the following formula:

$$\textbf{Percentage of cell viability} = \frac{\textbf{Average absorbance of treated}}{\textbf{Average absorbance of control}} \times \mathbf{100}$$

The IC_{50} value is the half maximal inhibitory concentration of the sample. The IC_{50} values were calculated using the equation for slope (y = mx + C) obtained by plotting the average absorbance of the different concentrations of the test sample (6.25-100 µg/mL) in Microsoft Excel.

2.10. Statistical analysis

All the experiments were performed in triplicate (n = 3). The data were expressed as mean ± standard deviation.

3. Results & Discussion

Crustacean haemolymph constitutes of a diverse of defensive molecules by which have immense application in pharmacology. PHH showed antibacterial activity against gram negative organisms such as *E.coli* (15.67±0.58), *S. aureus* (12.67±1.15), *S.typhi* (12.33±0.58) and *S.pyogenes* (10.67±1.15) (Table-1). *In vitro* antifungal susceptibility by PHH showed significant inhibition (10mm) of mycelial growth against *P.chrysogenum* among tested fungi (Table-2). On the other hand, the growth of all these microorganisms was totally inhibited by antibiotics Gentamycin and Clotrimazole. Similarly antibacterial activity of the lobsters such as, *G. strigosa*, *N. norvegicus*, the common shrimp, *C.crangon*, hemolymph reduced the viable count of test bacteria *P.immobilis*, *G. antarcticus*, *P. citreus*. Investigations of unidentified antibacterial factors have been conducted in several lobster species, such as *Panulirus argus* (Evans *etal.*, 1968, 1969b; Weinheimer *etal.*, 1969), *Panulirus interruptus* (Evans *etal.*, 1969a), *Homarus americanus* (Rabin, 1965; Stewart and Zwicker 1972). Previous works show that decapod crustaceans contain factors with antibacterial activity, particularly in the hemolymph or in the hemocytes. Antibacterial activity was reported in different body-parts of *Pagurus bernhardus* (Hermit crab), *Pandalus borealis* (Northern shrimp), *Hyas araneus* (Spider crab) and *Paralithodes camtschatica* (King crab) (Licona-Jain *etal.*, 2020)

PHH have antioxidant activity up to 800 mg/ml with IC_{50} value of 172.53 (**Fig-1**). The antioxidant potential of *Thenus unimaculatus* extract by methanolic and chloroform was studied by Gunalan *etal*., (2019). The DPPH radical scavenging effect has showed concentration depended and the results have been recorded 13, 21, 33, 41 and 54% in methanol and 4, 6, 11, 19 and 24% in chloroform at the concentration of 1-3mg/mL. Also there were reports of antioxidant activity crustacean antioxidant activity from marine crab *Charybdis lucifera* (Zakzok *etal.*, 2021) and *Ocypoda macrocera* (Sivaperumal *etal.*, 2013).

Antiproliferative activity of PHH was checked against MCF-7 cell lines by MTT assay. PHH found to induce growth inhibition in a dose dependent manner, with IC_{50} of 93µg/mL (**Fig-2**). The haemolymph of crabs exhibits potent anticancer activities that have been investigated in *Atergatis roseus* and *Eriphia verrucosa* (Salama and Mona, 2018), *Dromia dehanni* (Priya and Ravichandran, 2015a; Rethnapriya *etal*., 2019) and *Calappa calappa* (Priya and Ravichandran, 2015b).

Table-1. Antibacterial activity of PHH

Microorganism	Gentamycin (160 mg) (mm)	PHH(100µl from 10 mg/ml) (mm)
E. coli	**24.33 ±1.15**	**15.67±0.58**
S. aureus	**31.33±1.15**	**12.67±1.15**
P. aeruginosa	**25.33±0.58**	**0 ± 0**
K.pneumoniae	**21.33±1.54**	**0 ± 0**
B. subtilis	**25.67±0.58**	**0 ± 0**
M. smegmatis	**30.33±0.58**	**0 ± 0**
V. cholerae	**25.67±0.58**	**0 ± 0**
S. typhi	**30.67±1.15**	**12.33±0.58**
S.marcescens	**25.33±0.58**	**0 ± 0**
S. pyogenes	**37.33±1.15**	**10.67±1.15**

Table-2. Antifungal activity of PHH

Microorganism	Clotrimazole (400 µg)	PHH (100µl from 10 mg/ml) (mm)
C. albicans	**23.67±0.58**	**0 ± 0**
A. niger	**20.67±1.15**	**0 ± 0**
P. Chrysogenum	**29.33±1.15**	**12.33±0.58**
M. mucedo	**30.33±0.58**	**0 ± 0**
R. stolonifer	**18.33±0.58**	**0 ± 0**

Fig-1- ***P. homarus***

Fig-2- Antioxidant activity of PHH

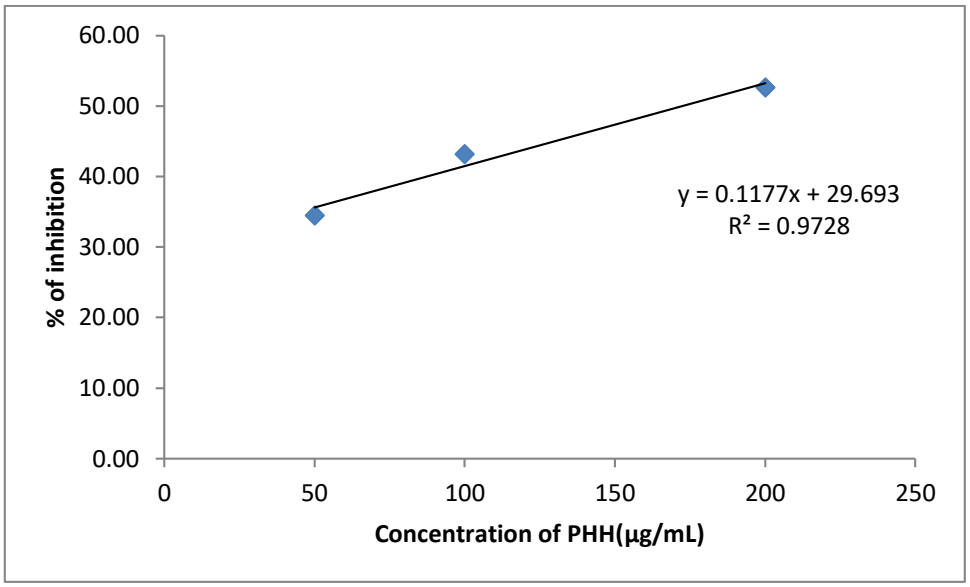
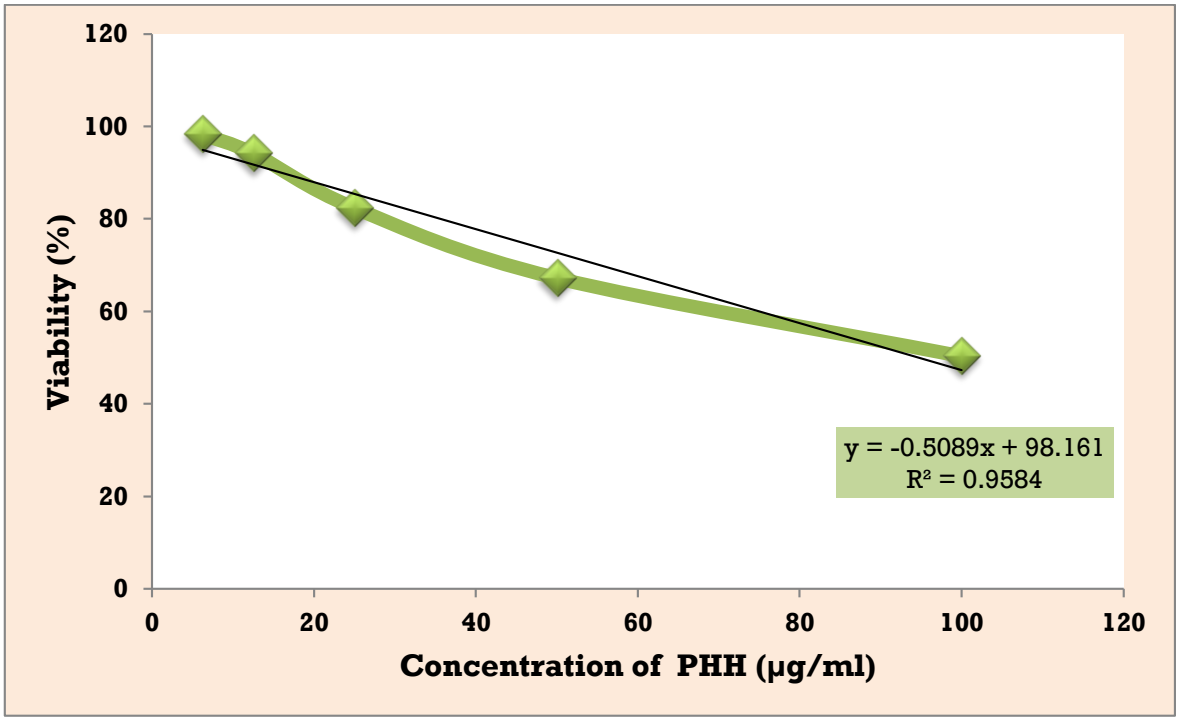


Fig-3- MTT assay of PHH



References

[1] Arasukumar, Balu & Prabakaran, Gopal & . B, Gunalan. (2020). Extraction and Antioxidant Potential of Metabolites from Sand Lobster (Thenus unimaculatus). 10.21276/ijpbs.2019.9.3.4.

[2] Arumugam, A., Dineshkumar, R., Rasheeq, A.A., Prabakaran, G., Sampathkumar, P. and Murugan, S. (2020). Biochemical Profile of Spiny Lobsters P.homarus and P.ornatus. Agricultural Science Digest. 40(4): 411-417.

[3] Brand-William, W., Cuvelier ME., Berset C., "Use of free radical method to evaluate antioxidant activity" LebensmWiss Technology, 28, 25-30, 1995.

[4] Chakraborty, R. D., and Radhakrishnan, E. V., 2015. Taxonomy, Biology and Distribution of Lobsters. Summer School on Recent Advances in Marine Biodiversity Conservation andManagement, pg.: 100-110.

[5] Fitzgibbon, Q.P., Jeffs, A.G. and Battaglene, S.C. (2014). The Achilles heel for spiny lobsters: the energetics of the non feeding post larval stage. Fish and Fisheries. 15(2): 312-326.

[6] Jiravanichpaisal, Pikul & Lee, Bok & Söderhäll, Kenneth. (2006). Cell-mediated immunity in arthropods: Hematopoiesis, coagulation, melanization and opsonization. Immunobiology. 211. 213-36. 10.1016/j.imbio.2005.10.015.

[7] Jones, C.M. 2010. Tropical rock lobster aquaculture development in Vietnam, Indonesia and Australia. Journal of the Marine Biological Association of India, 52:304-315.

[8] Joseph, M.M., Aravind, S.R., Varghese, S., Mini, S. and Sreelekha, T.T., 2012. Evaluation of antioxidant, antitumor and immunomodulatory properties of polysaccharide isolated from fruit rind of Punica granatum. *Molecular medicine reports*, *5*(2), pp.489-496.

[9] June R.S. Chisholm, Valerie J. Smith,Comparison of antibacterial activity in the hemocytes of different crustacean species,Comparative Biochemistry and Physiology Part A: Physiology,Volume 110, Issue 1,1995,Pages 39-45,ISSN 0300-9629,https://doi.org/10.1016/0300-9629(94)00157-O.

[10] Licona Jain, Alan & Campa-Córdova, Ángel & Luna, Antonio & Racotta, Ilie & Tello, Marlene & Angulo, Carlos. (2020). Dietary supplementation of marine yeast Yarrowia lipolytica modulates immune response in Litopenaeus vannamei. Fish & Shellfish Immunology. 105. 10.1016/j.fsi.2020.07.043.

[11] Lipton.A.P, Syda Rao.,G,Jose Kingsly,H.,Imelda Joseph.,Suresh Kumar Mojada.,Hanumantha Rao and Rajendran,P.2010.Open sea floating cage farming of lobsters. Successful demonstration by CMFRI off Kanyakumari coast.Fishing Chimes,30(2)pp11-13.

[12] Magaldi, S., Mata-Essayag, S., Hartung de Capriles C., et al. Well diffusion for antifungal susceptibility testing Int. J. Infect. Dis., 8 (2004), pp. 39-45.

[13] Vijayakumaran, M., T. Senthil Murugan, M.C. Remany, Mary Leema, J. Dileep Kumar, J. Santhanakumar, R. Venkatesan and M. Ravindran 2005. . Captive breeding of the spiny lobster, Panulirus homarus. New Zealand journal of marine and Freshwater Research, 2005, Vol. 39: 325-334.. New Zealand Journal of Marine and Freshwater Research. 39. 325-334.

[14] Mohamed, K. H.; George, M. J. 1968: Results of the tagging experiments on the Indian spiny lobster, Panulirus homarus (Linnaeus)—movement and growth. Indian Journal of Fisheries 15: 15-26.

[15] Mona, Mahy & Salama, Wesam. (2018). In vitro anti-tumor effects of hemocyanin isolated from Atergatis roseus and Eriphia verrucosa crabs. Journal of Cancer and Biomedical Research. 1. 28-36. 10.21608/jcbr.2019.34742.

[16] Priya, E.R. and Ravichandr, S. (2015a). Anti-cancer activity of brachyuran crab Dromia dehaani (Rathbun, 1923). Asian J. Biotechnol., 7: 119–128. https://doi.org/10.3923/ajbkr.2015.119.128.

[17] Priya, E.R. and Ravichandr, S. (2015b). Anticancer compounds of Calappa calappa L. (1758). Int. J. Zool. Res., 11: 107. https://doi.org/10.3923/ijzr.2015.107.111.

[18] Radhakrishnan, E V and Vijayakumaran, M (1995) *Early larval development of spiny lobster, Panulirus homarus (Linnaeus, 1758) reared in the laboratory.* Crustaceana, 68 (2). pp. 151-159.

[19] Radhakrishnan, E.V., and Thangaraja R., 2008. “Sustainable Exploitation and Conservation of Lobsters Resources in India- A Participatory Approach”. Glimpses of Aquatic Biodiversity- Rajiv Gandhi Chair Spl. Pub., **7**: 184-192.

[20] RethnaPriya, E.; Ravichandran, S.; Gobinath, T.; Tilvi, S. and Devi, S.P. (2019). Functional characterization of anti-cancer sphingolipids from the marine crab Dromia dehanni. Chem. Phys. Lipids, 221: 73–82. https://doi.org/10.1016/j. chemphyslip. 2019.03.010.

[21] Söderhäll K., Cerenius L. 1992 - Crustacean immunity. Ann. Rev. Fish Dis., 3-23.

[22] Syda, Rao & George, Rani & Mk, Anil & Saleela, K & Jasmine, Suryavamsi & Kingsly, H. & Rao, G. (2010). Cage culture of the spiny lobster Panulirus homarus (Linnaeus) at Vizhinjam, Trivandrum along the south-west coast of India. Indian Journal of Fisheries. 57. 23-29.

[23] Thomas, M M (1972) Growth of the spiny lobster, Panulirus homarus (Linnaeus), in captivity. Indian Journal of Fisheries, 19 (1&2). pp. 125-129.

[24] Zakzok, Somaia & Alkaradawe, Rabab & Mohammad, Samya & Tawfik, Mohamed. (2021). Antiproliferative and antioxidant activities of the edible crab Callinectes sapidus hepatopancreas and hemolymph extracts. Egyptian Journal of Aquatic Biology and Fisheries. 25. 10.21608/EJABF.2021.179659.