ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Antibacterial Effect of Freshwater Crab Powder against Pathogenic Bacteria

Nebrass F. Chachain

College of Science, Mustansirya University, Iraq.

Abstract

The objective of this study was to examine the antibacterial effect of crab powder on *Klebsiella pneumonia*, *Pseudomonas fluorescens* and *Escherichia coli*. Four concentration (250,300,400 and 500) mg /ml was used ,all bacteria giving the inhibition zone in all concentrations .Powder of freshwater Crab were show the highest inhibition zone (18mm) in bacteria in *P. fluorescens* at concentration 500 mg/ml and lowest inhibition zone to same species of bacteria was (11mm) at 250 mg/ml concentration .

Keywords: *Pathogenic Bacteria; Freshwater Crab Powder; Antibacterial Effect.*

Introduction

Aquatic invertebrates possess ant parasite, antibacterial, antiviral and anticancer activities. They contain biologically active compounds for diseases acquired immunodeficiency syndrome (AIDS) [1, 2]. Crustaceans are the most numerous, diverse and prevalent animals on earth along with insects .They are rich source of biologically active substances with anti-microbial cytotoxic and antineoplastic activities [3].Crabs are return to Arthropoda phylum and crustacean class used for the medicinal purpose all over the world since long time ago, their use as human food [4].

The first line of defense is constitute by cuticle, however, once pathogen cross it, a complex interaction of innate humeral and cellular immune reaction is induced in both the tissues and haemocoel thereby helping to eliminate the pathogen at rapid pace [5]. *Klebsiella pneumoniae* s a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium.

It appears as a mucoid lactose fermenter on MacConkey agar. Found in the normal flora of the mouth, skin, and intestines. [6]. *Pseudomonas* Genus includes large diversity of microorganisms living in various environmental niches as free-living organisms in soil and water, and, mainly, associated to the plant rhizosphere and endosphere. It can be found as human pathogenic bacteria [7,8].Finally *Escherichia coli* bacteria is part of natural intestine flora in human and animals and consider bioindicator of fecal contamination of environment and food [9].

Aim of the Study

This work was to study the effect of Crab powder on different isolates of pathogenic bacteria.

Material and Methods

Sample Collection

Freshwater crab (*Liocarcinus vernalis*) was purchased from local AL- ghazeel market and kept in glass tank with water then take into Invertebrate laboratory in University of Mustansirya in Baghdad , after that crab were identified depended on [10].The crabs that collected recorded weight between 40-90g.

Preparation Crab Powder

Freshwater crab *L. vernalis* washed several time with distilled water then euthanized by thermic shock in -20 C^0 for 20 minutes .Then killed by spiking to destroy the nerve center. tissues of the Crab broken up in to small pieces and separated two equal parts , then placed in oven at 220 C^0 for 3hour .After that ground to powder. Powder was soaked in 1:10 methanol for three days then filtered using no.1 whatman. The solvents were evaporated

from crude extract using rotator evaporated then powder stored in the refrigerator (- 10 C^0) temperature [11, 12].

Preparation of Culture Media

The media that used in this study were prepared by the instructions of the manufacturing companies and sterilized by autoclaving at 121 °C for 15 minutes under pressure 15bar/ *Inch2* , then poured in to sterilized petridishes after cooling to 45°C, incubated at 37°C for 24 hours for sterility then stored at 4°C until used .

Test Microorganisms

The test bacteria used in this study were obtained from higher studies Laboratory / department of biology /college of science / Mustansirya University. Which included [3] bacterial isolates (*Pseudomonas fluorescens*, Klebsiella *pneumonia* and *Escherichia coli*).

Antimicrobial Activity of Crab Powder against Bacteria

Agar well diffusion method was used for the assessment of antimicrobial activity. This method is based on the observation the inhibition growth of microorganisms in plate. Bacteria used in this test are: (*Pseudomonas fluorescens*, *Klebsiella pneumonia* and *E. coli).* Test bacteria were prepared by sub culturing of an active isolate on nutrient agar after 18-24 h. incubation at 37°C, then added 100 µl of this isolates to nutrient broth to reach the turbidity equivalent to the McFarland turbidity standard (1.5x 10^8 CFU/ml). Wells of (5 mm) diameter were punched in agar with sterile cork-borer wells were filled with (100 µl) of bacteria incubated at 37°C for 24 hours [13, 11].

Antimicrobial Activity Powder of Crab against Test Bacterial

The crab powder were prepared and four concentration prepared by adding distilled water to powder. These concentrations prepared as following

- Two hundred and fifty mg/ml were prepared by adding 12.5mg of powder in 5 ml distilled water.
- Three hundred mg/ml were prepared by adding 15mg of powder in 5 ml distilled water
- Four hundred mg/ml were prepared by adding 20mg of powder in 5 ml distilled water
- Five hundred mg/ml were prepared by adding 25mg of powder in 5 ml distilled water Antimicrobial activity of crab powder with different concentration were determined using agar well diffusion method. About 100µl of each concentration were loaded in to the walls of agar plates inoculated previously with test bacterial separetalley .Blank well fill with distilled water used as control. The plate kept in room temperature and incubation period 37°C.The zone of inhibition were measured in (mm).

Results and Discussion

Crab powder was tested for antibacterial activities. Four different concentration was used (250.300.400 and 500) mg/ml. For antimicrobial assay three isolates of tested bacteria (*Pseudomonas fluorescens*, *Klebsiella pneumonia* and *E.coli*) were used. The results of antimicrobial activities of crab powder against bacteria were recorded in Table (1).

Table 1: Inhibition effect of different concentrations of crab powder against different isolates bacteria

Bacteria	Concentration (mg/ml)				
Pseudomonas fluorescens (mm)	0	11	12	16	18
Klebsiella pneumonia (mm)	0	13	14	14	15
Escherichia coli (mm)	0	13	15	16	17

From table (1) we conclude inhibition zone of P. *fluorescens* at concentration 500 mg/ml was 18 mm and to *K. pneumonia* was 15 mm while *E.coli* was 17 mm. and at a concentration 400 mg/ml inhibition zone of P. *fluorescens* was (16mm), while in K. *pneumonia the* activity were less effect (14mm) and recorded 16mm to *E. coli* at same concentration. In concentration 300 mg/ml show highest inhibition (15mm) in bacteria *E. coli* , while *K. pneumonia the* inhibition zone was (14mm) and least one was (12mm) seen in bacteria *P. fluorescens*. Finally at 250 mg/ml concentration *less* inhibition zone was 11 mm of *P. fluorescens* while *K. pneumonia and E. coli* were recorded same inhibition zone (13 mm) that agree with [14] they found powder of crab have antibacterial activity against medically important pathogens. Crabs act as an antimicrobial activity against a variety of bacterial strains of both positive and negative

bacteria [15, 16]. Cancer has many antimicrobial and peptide proteins that have inflammation function, wound repair, and adaptive immune system regulation [17, 19].Reported that Crustaceans contain good source of Antimicrobial potency, that agree with many studies reported there are an antimicrobial agent from crabs against many pathogens [20, 23]. Explain that Crustacean extracts disabling the outer membrane barrier properties of gram negative bacteria [24]. Suggesting increase in antimicrobial activity of the crab powder with increased chitosan concentration and that agree with [25] he pointed out when extract hymolymph from freshwater crab inhibition zones were [14 ,10, 11, 8 and 8] mm against *E. coli*, Staphylococcus aureus, K.pneumonia ,Streptococcus and Bacillus respectively Figure (1).

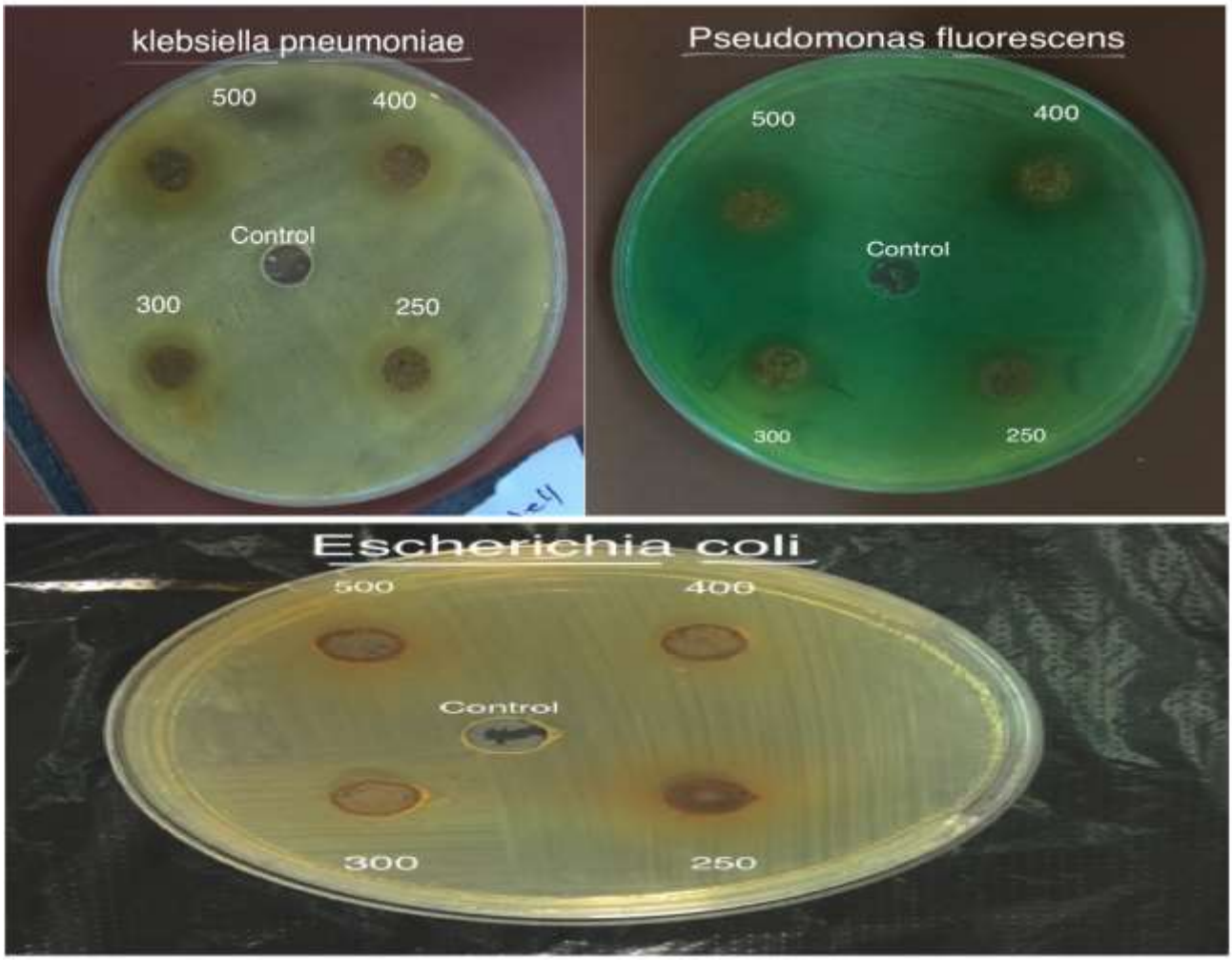

Figure 1: Inhibition zones to different concentrations of *L. vernalis* powder against three species of pathogenic bacteria

References

1. Chalupniak A, Waszczuk K, Halubek K, Piasecki T, Gotszalk T, Rybka J (2014) Application of quartz tuning fprks for detection of endotoxins and gram-Biosens. Bioelectron., 53: 132- 137.
2. Senthilkumar K, Kim S (2013) Marine invertebrate natural products for anti-inflammatory and chronic diseases .Evil-Based Complement .Altern. Med., 1-11.
3. Thomas RF (2010) Antibiotic resistance and the threat to public health. Centers for Disease Control and Prevation. U.S. Department of Health and Human Services.
4. Magalhaes C, Barbosa UC, Daniel V (2006) Decapod crustaceans used as food by the Yanomami Indians of the Balawa-ú village, State of Amazonas, Brazil. Acta Amazonica, 35: 369-74.
5. Ravichandran S, Sivasubramaniana K, Anbuchezhiab RM (2010) Antimicrobial activity from the haemolymph of the crab Ocypode macrocera (H. Milne-Edwards, 1952). World Applied Sciences Journal, 11(5): 578-581.

6. Ryan KJ, Falkow S (2004) Haemophilus and Bordetella. Sherris Medical Microbiology 4th ed. Ryan KJ; Ray CG, eds. Medical Publishing Division, McGraw-Hill, USA, 396-401.
7. Loper JE, Hassan KA, Mavrodi DV, Davis EW, Lim CK, Shaffer BT (2012) Comparative genomics of plant-associated pseudomonas spp.: Insights into diversity and inheritance of traits involved in multitrophic interactions. PLoS Genet, 8(7):1-27.
8. Cullen L, Mcclean S (2015) Bacterial Adaptation during Chronic Respiratory Infections. Pathogens, 4 (1):66-89.
9. Osinska A, Korzeniewska E, Harnisz M, Niestepski S (2018) The prevalence of virulence gene specific for Escherichia coli in waste water samples from wastewater treatment plants with the activated sludge process. In EDP sciences.
10. Pretzmann G (1968) .Die Familie Trichodactylidae (Milne-Edwards 1853) Smith 1870 .Entomologische Nachrichtenblatt, 15(7-8):70- 76.
11. Vinayavekhin N, Saghatelian A (2010) Untargeted metabolomics. Curr. Protoc .Mol. Biol., 30: 1-24.
12. Laith A, Ambak M, Abol-munafi A, Nurhafizah W, Najiah M (2017) Metabolomic analysis of marine and mud crabs based on antibacterial activity .Aquaculture Reports, 7: 7-15.
13. Stockes Y, Nagase H, Ose Y, Sato T, Yamaada A, Hibi M (1986) .An-mutagenicity of extracts from crude drugs in chins medicine .Mullet. Res, 17(4):1-4.
14. Varadharajan D, Soundarapandian P (2013) Antibacterial activity of crab shell extracts against human pathogenic bacteria and usage of new drugs. Journal of Developing drugs, 2: 1000110.
15. Manhas P, Gupta RK, Langer S (2017) Antibacterial activity from the haemolymph of freshwater crab, Himalayapotamon emphysetum on some of clinical pathogens. International Journal of Creative Research Thoughts, 5(4): 2320-2882.
16. Hikima S, Hikima J, Rojtinnakorn J, Hirono I, Aoki T (2003) Characterization and function of Kuruma shrimp lysozyme possessing lytic activity against Vibrio species .Gene, 316: 187-195.
17. Chekmenev E, Vollmar B, Cotton M (2010) Can antimicrobial peptides scavenge around a cell in less than a second? Biophys. Acta 1798: 228-234.
18. Sylvester F, Ravichandran S (2012) Hemolymph proteins in marine Crustaceans .Asian J. Trop. Biomed., 2: 496-502.
19. Anbuchezhian RM, Ravichandran S, Rameshkumar G, Ajithkumar TT (2009) Influence of crab haemolymph on clinical pathogens .Adv. Biol. Res., 3(3-4): 104-109.
20. Soundarapandian P, Roy S, Varadharajan D (2014) Antioxidant activity in hard and soft shell crabs of Charybdis lucifera (Fabricius ,1798) ,J. Aquacult .Res. Dev., 5: 7.
21. Lekshmi N, Viveka S, Anusha S, Jeeva S, Brindha R, Selva B (2015) Antibacterial activity of freshwater crab and snail and isolation of antibacterial peptides from haemolymph by SDS-PAGE. Int. Pharm. Sci., 7(1):109-114.
22. Singh S, Arya P, Bahuguna S, Mehta J, Bhatt G, Ghowdhury A, Bahuguna V (2016) Antibacterial activity from haemolymph of mud crab of genus may de lliathlphusa against respiratory tract pathogens .Int. J. Pharm. Sci., 8(2): 324-325.
23. Othakara A, Izume M, Mitsutomi M (1983) Action of microbial chitosanases on chitosan with different degrees of decatylation .Agric. Biol. Chem., 52: 3181-3182.
24. Gokilavani S, Vijayabharathi V, Parthasarathy R (2014) Physico-chemical characteristics and antibacterial activity of chitosan extracted from shell of crab paratelphusa hydrodromous .Asian .J. Res. Pharm. Sci., 4: 125-128.
25. Sumalatha D, Jayanthi J, Raghunathan M (2016) Antimicrobial potential of hemolymph of a freshwater crab Oziotelphus senex (Fabricius 1798) .Int. J. Pharm. Tech. Res., 9: 156-160.

