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Study of Antibacterial Activity of Freshwater crab *Oziotelphusaravi* against some human pathogens

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Abstract

In the present study, experiment has been made to find the antimicrobial activity of haemolymph collected from a freshwater *Oziotelphusaravi*. The haemolymph collected was tested to antimicrobial activities against some of human pathogens viz., *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis, Proteus vulgaris, Salmonella typhi, Vibrio cholera, Enterobacter aerogenes, Bacillus subtilis,* and *Staphylococcus aureus.* The highest zone of inhibition was observed against *Bacillus subtilis*, while least in *Enterobacter aerogenes*. The result shows the antibiotic resistance of crab against human pathogens which confirm the resistant potential of haemolymphof *o.ravi*

Key words: Antimicrobial, Haemolymph, Human pathogens.

Introduction

Many infections in humans are caused by viruses or bacteria.Infectious diseases have become more common in recent years, and Staphylococcus aureus infections are among them. Bacterial infections-related diseases pose a serious risk to human health and account for around 50,000 fatalities worldwide each day (Ahmad and Beg, 2001). Around the world, people have been using crabs as medicine for a very long time. (Magalhaes et al., 1991) reported on their use as human food, and (Yeo et al., 2008) provides ample evidence of their economic and medical significance. Crustaceans

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have evolved a useful method for identifying and getting rid of harmful bacteria because they live in an aquatic habitat that is abundant in microorganisms. Because of their close interaction with a microbially rich aquatic environment, crabs are susceptible to infection at different stages of their maturation, which can result in significant losses from disease (Hudson and Lester, 1994). Although the researchers studied the pharmacological characteristics of marine crabs (Veerurajet al., 2008; Anbuchezianet al., 2009) but not those of freshwater crabs, crabs are significant sources of bioactive chemicals. Furthermore, it was suggested by Leena and Geni (2021) that the crab Scylla serrata has the ability to quickly create antimicrobial compounds in order to fight bacterial infections. Therefore, the purpose of the current study was to ascertain the haemolymph's antimicrobial effectiveness after it was extracted from Oziotelphusaravi, a freshwater crab.Supplies and Procedures

An assortment of fauna.

In Pottalkulam village, Tirunelveli District, Tamilnadu, India, healthy freshwater crabs (Oziotelphusaravi) were gathered from several paddy field locations and transported to the laboratory for the purpose of collecting hemolymph.

assemblage of hemolymph

During the course of the investigation, healthy crabs in various developmental stages were used, and each crab underwent a single bleed collection. By using sharp, sterile scissors to cut the leg of a live animal, hemolymph (about 3 mL) was extracted. The sodium citrate buffer pH 4.6 (2.1 v/v) was present when the hemolymph was collected in order to prevent haemoglobin degranulation and coagulation. Additionally, an equivalent volume of physiological saline (0.85% Nacl, w/v) was added. The hemolymph was centrifuged at 2000 g for 15 minutes at 4°C in order to extract the

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haemoglobin from it. Before being used, the supernatant was collected and kept at 4°C.

Use of Microbial Strains.

Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Proteus vulgaris, Salmonella typhi, Vibrio cholera, Enterobacter aerogenes, Bacillus subtilis, and Staphylococcus aureus were the ten bacterial strains against which the antimicrobial activity of freshwater crab haemolymph was tested.

Antimicrobial Test

The agar well diffusion method was used to assess the antibacterial activity of haemolymph (Ahmad et al., 1988). Pre-sterilized Petri plates were filled with 0.1 ml of molten Mueller Hinton Agar medium containing bacterial cultures that had been cultured for 12–16 hours. An instrument with a 6 mm diameter cork borer is used to punch walls in a solidified media and is filled with varying amounts of hemolymph (10µl, 20µl, and 30µl, respectively). A positive control that was employed was erythromycin. The plates were placed in a Biological Oxygen Demand (BOD) incubator and incubated for 24 hours at 37 °C. The widths of the zones of inhibition were measured in millimetres. Three duplicate assays were performed on each sample, and the mean SD data were recorded.

Result

Antimicrobial activities of haemolymph extracted from fresh water crab *O. ravi* showed its potential against human pathogenic bacteria strains. Reports obtained through well diffusion method exposed upright results. Haemolymph exerts its potential to suppress the growth and multiplication of bacterial cultures. Activities of

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haemolymph from *O. ravi* showed dose dependent manner of inhibition. Strains from gram negative and gram positivebacteriawere susceptible towards the haemolymph extracted from *O. ravi*.

Human pathogenic bacteria's such as *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Proteus vulgaris, Salmonella typhi,Vibrio cholera, Enterobacter aerogenes* of gram negative strains and *Bacillus subtilis, Staphylococcus aureus* of gram positive strains which was studied against extracted haemolymph of *O. ravi* wield its potential. An antimicrobial activity which was studied at 25, 50, 75 and 100µl showed diverse in their activities against different strains in dose dependent manner. At lower concentration of 25µlinhibition against strains was minimal and was against *E. coli* by showing a zone size of 15mm. Inhibition was nil or no zone of inhibition was obtained from 25µlconcentrations in any other strains.

Gram negative bacterial strains such as *P. aeruginosa, P. mirabilis* and *S.typhi* showed no zone of inhibition at 50 and 75µlconcentrations. Maximum inhibition was obtained from treated concentration of 100µlfrom all the strains. Positive controls showed maximum zone of inhibition than the extractedhaemolymph. Gram positive strains also respond to the extracted haemolymph by showing 22mm and 20 mm against *B. subtilis, S.aureus* respectively at 100 µlconcentration. No zone was evident at 50 µl concentration at *S.aureus.*

All the strains are susceptible towards the extracted haemolymphfrom fresh water crab *O. ravi*. This implies the potential of fresh water crab against human pathogenic bacterial strains. Most efficient activity against gram negative strains of 25 mm of inhibition was obtained from *E. coli*, followed by 19, 16, 14, 12, 10 mm

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against *V.cholera, K. pneumonia, P. mirabilis, S. typhi,* and *P. aeruginosa* respectiviely. Gram negative strains *P.vulgaris and E. aerogenes* revealed similar zone of inhibition of 15 mm. Results obtained against gram negative bacterial strains was displayed in figure 1 and table 1.

Table 1 Antimicrobial activities of Haemolymph of *O. ravi* against Gram negative bacterial strains.

Sl NO	Human Pathogens	Positive Control	25 µl	50 µl	75 µl	100 µl
1	*Escherichia coli*	29 mm	15 mm	17 mm	20 mm	15 mm
2	*Klebsiella pneumonia*	20 mm		12 mm	13 mm	16 mm
3	*Pseudomonas aeruginosa*	16 mm	-	-	-	10 mm
4	*Proteus mirabilis*	19 mm	-	-	-	14 mm
5	*Proteus vulgaris*	25 mm	-	11 mm	14 mm	15 mm
6	*Salmonella typhi*	18 mm	-	-	-	12 mm
7	*Vibrio cholera*	23 mm	-	-	13 mm	19 mm
8	*Enterobacter aerogenes*	19 mm	-	10 mm	13 mm	15 mm

Gram positive strains against haemolymph of fresh water crab *O. ravi* showed prominent activities by their zone of inhibition. *B. subtilis* was more susceptible and displayed maximum of 22 mm and *S.aureus* displayed 20mmagainst 100µlconcentration. Results obtained weretabulated in table 2 and figure 2.

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Table 2: Antimicrobial activities of Haemolymph of *O. ravi* against Gram Positive bacterial strains

Sl NO	Human Pathogens	Positive Control	25 µl	50 µl	75 µl	100 µl
1	*Bacillus subtilis*	26 mm	-	-	15 mm	20 mm
2	*Staphylococcus aureus*	25 mm	-	-	13 mm	19 mm

Discussion

Microbes which are present in the universe are diverse in their activities in which few are beneficial and harmful to living organisms. Antibiotics or a synthetic drug which was used to suppress the microbial activities has shown its potential and later become inactive against strains. This may due to development of resistance against strains due to repeated usage because of broad spectrum activity. Resistance can also acquire through signal transmission among bacterial species (*Blair et al.*, 2015), receptor modification (*Abreu et al.*, 2012).

Further benefits come from studying the effects of antibiotics derived from edible materials or biological sources. Having immunity against food-borne strains is more advantageous than using synthetic medications to combat microorganisms. According to Hoq et al. (2003), crabs are an enhanced source of nutrients and produce antimicrobial chemicals in response to wounds or infections in the cuticle layer. Haemolymph secretes the antimicrobial peptides that have been synthesised (Lee et al., 1995).Therefore, eating freshwater crabs can strengthen our resistance to a variety of microbial illnesses. Numerous studies indicate that the haemolymph of some crustacean species, such as crabs, possesses antibacterial qualities (Veerura et al., 2008).

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According to Kaper et al. (2004), E. coli is a pathogenic bacteria that can infect the gastrointestinal tracts of both humans and warm-blooded animals.According to Johnson et al. (2012), it is known to be resistant to penicillin G and other medications. The haemolymph of O. ravi exhibited promising growth-inhibiting properties. At a 100 µl concentration of 25 mm, it demonstrated a considerable suppression of growth, while the positive control displayed a 29 mm zone of inhibition. The haemolymph of Paratelphusahydrodromous was found to exhibit considerable suppression of bacterial growth against E. coli, K. pneumonia, P. aeruginosa, P. mirabilis, and S. aureus. Specifically, E. coli displayed a 14.8 mm zone of inhibition, according to Arul-Prakash et al. (2011).

According to Li et al. (2014), K. pneumonia is an opportunistic pathogen that can cause severe pneumonia, phylogenic liver abscess, meningitis, necrotizing fasciitis, and endophthalmitis in healthy individuals.Good action against K. pneumonia was demonstrated by our studies, which showed inhibition of the 12 mm, 13 mm, and 16 mm zones against 50, 75, and 100 µl concentrations. In accordance with this, mud crab Scylla tranquebarica tested against K. pneumonia revealed a 13–14 mm zone of inhibition in response to 50–100 mg/ml treatment concentrations (Laith et al., 2017).

*P.aeruginosa*causes infection towards immune compromised patients and mostly hospital acquired infections, the antibiotics which are used to control become ineffective due to development of resistance (Bassetti *et al*., 2018).Even though many drugs are resistance against *P.aeruginosa* strain our reports revealed its potential against the strain through its zone of inhibition 10 mm at 100 µconcentration. In the same way freshwater crab haemolymph of *Maydelliathelphusamasoniana* showed 8.5mm zone of inhibition against *P.aeruginosa* (Gupta *et al*., 2017).

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P. mirabilis causes catheter-associated urinary tract infections (CAUTIs) such as urolithiasis and also alkalize the urine and develop kidney stones (Armbruster *et al*., 2018). The harmful effects of the species against human can be suppressed or inhibited with the haemolymph of fresh water crab *O. ravi*, which was evident by its inhibition at 100µl concentration. Likewise study of haemolymph of *Paratelphusahydrodromous*studied against *P.mirabilis* showed its potential but was less sensitive by exhibiting 12 mm in diameter (Arul-Prakash *et al*., 2011)

P. vulgaris is an emerging multidrug resistant pathogenic bacteria strain (Mandal*et al*., 2015). Drug resistance mechanism among the strains differs in various geographical locations (McGregor *et al*., 2014; Bilal *et al*., 2019). Reports from our results divulge significant activity at 50,75 and 100 µl of showing zone of inhibition of 11, 14 and 15 mm correspondingly positive control show evidence of 25 mm. earlier studies on antimicrobial potential of haemolymphfrom male and female *Uca triangularis* showed 9mm and 5mm zone of inhibition (Kavitha *et al*., 2019)

*S. typhi*is a pathogenic bacterium causing life threatening diseases (Song *et al*., 2010), such as food and waterborne gastroenteritis and typhoid fever in human beings (Mathur *et al*., 2012). This pathogenic bacteria species are suppressed by the haemolyph of fresh water crab *O. ravi* through its 100 PPM concentration showing zone of inhibition 12 mm. Studies by (Ravichandran *et al*., 2010) reports *S.typhi* is susceptible towardshaemolymph of brachyuran crabs (*Hyas araneus, Podopthalmus vigil, Dromiadehanni, Charybdis helleri, Portunussanguinolentu*and*Portunuspelagicus*)

V. cholera is a facultative human pathogenic bacterium causing acute gastrointestinal disease, cholera as a major health issues (Maheshwari *et al*., 2011).

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Mostly associated with aquatic environment and peoples who are in contact with aquatic animals, plants can easily affect with this pathogenic bacteria (Vezzulli *et al.*, 2010).Result exposed to have potential activity against *V. cholera* by showing inhibition of 13mm and 19 mm against 75 and 100 µl concentrations. Similarly notable activity was obtained from cell free haemolymph from *Rapanaraiformis*against *V. cholera* (Amruthalakshmi and Yogamoorthi, 2017).

Enterobacter aerogenes is opportunistic and multiresistant pathogen against human in past decades in hospital (Davin-Regli and Pages, 2015). It is emerged nosocomial pathogen in intensive care unit, patients who are supported with ventilators (Mezzatesta *et al*., 2012).Haemolymph of fresh water crab *O. ravi* showed significant activity at 50, 75 and 100 µl treated concentrations of showing 10, 13 and 15 mm zone of inhibition. In the same way haemolymph of crab *Grapsustenuicrustatus* showed activity against *E. aerogenes* of 8 mm zone of inhibition (Rathika *et al*., 2020).

B. subtilis is enteric human pathogenic bacteria causing diarrheal infection in children's and adults through impact on immune system (Shamaila *et al.*, 2016). Inhibition on growth of *B. subtilis* is suppressed by haemolymph of *O. ravi* which shows maximum of 22 mm zone of inhibition at 100 µl concentration. Haemocyaninisolated from haemolymph of *Eriphia verrucosa* showed strong antimicrobial potential against *B. subtilis* (Kizheva *et al*., 2019).

S. aureus is a human pathogen which spread through community or hospital acquired and causes infections in internal tissues or bloodstream (*Taylor and Unakal.*, 2022). As the strain is resistance against multiple drugs MRSA (Methicillin-Resistant *Staphylococcus aureus*) development of antibiotics is challenging (Ripari *et al*.,

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2023). Our reports reveal its potential against *S.aureus* strain by exhibiting zone of inhibition 15 mm and 20 mm against 75 and 100 µl concentrations. Earlier reports in study of haemolymph of *P.pelagicus* against *S. aureus* revealed 15 mm of zone of inhibition (Anbuchezhian *et al*., 2009).

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