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Full Length Research Article

Antibacterial Efficacy of Cow Urine Distillate Supplemented Cultures of *Sinodiaptomus sarsi* Hemolymph against Infectious Bacterial Pathogens

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Abstract

In an aquatic environment rich in microorganisms, crustaceans have developed a mechanism for identifying and eradicating harmful microorganisms. The hemolymph, which is responsible for circulating nutrients, oxygen, hormones, and cells throughout the invertebrate body, may also possess a biological process similar to that of vertebrate antibodies. This process may activate the innate immune mechanisms of crustaceans, enabling them to combat and eliminate pathogens that may infect them. The current study aimed to assess the antibacterial activity of hemolymph extracted from the calanoid copepod, *Sinodiaptomus sarsi*, against clinically significant bacterial pathogens. The hemolymph of the studied organism, when cultured in cow urine distillate, exhibited enhanced antibacterial efficacy. These findings suggest that the hemolymph of *Sinodiaptomus sarsi* cultured with cow urine distillate may serve as an alternative to commercial antibiotics in the fight against infectious pathogens.

***Key words*:** *Sinodiaptomus sarsi*, Cow urine distillate, Antibacterial activity, Haemolymph, Copepods

The global population relies heavily on fishing and aquaculture as primary sources of sustenance, nourishment, income, and livelihoods. The consumption of fish has experienced a remarkable surge, accounting for 50% of the food industry's fastest-growing sector [1]. The fishing industry is a crucial contributor to a nation's economy and plays a significant role in human nutrition [2]. The success of aquaculture is contingent upon the cultivation of healthy stock. To maintain a disease-free and healthy stock, live food and supplementary artificial feed are necessary [3]. In the realm of aquaculture, a variety of live feed options are available for the nourishment of developing fish larvae. Among these options, zooplanktons are considered to be the most suitable due to their unique characteristics. Zooplanktons are heterotrophic planktonic organisms that are capable of thriving in a diverse range of habitats, including both marine and freshwater environments, as well as lentic and lotic aquatic systems. As a crucial component of the aquatic food chain, zooplanktons play a significant role in the material cycle and energy flow of aquatic ecosystems. They consume primary producers, such as phytoplankton, and subsequently transfer energy to higher trophic levels of the food chain, where they are preferred and preyed upon by developing fish larvae [4-5]. The biological indicators of aquatic bodies, including abundance, species composition, biomass, and community structure, are reflective of their polluted and eutrophicated nature [6]. The dominant classes of zooplankton, namely copepods, cladocera, and rotifers, have been extensively studied [7-9]. The population dynamics of these species are influenced by various environmental factors, such as water temperature, light, pH, dissolved oxygen, biochemical oxygen demand, and turbidity. Additionally, the chemical composition of water, including total nitrogen, total phosphate, NH_3-N, and NO_3^-, also plays a significant role in the growth and abundance of zooplankton [10].

Copepods are utilized as live feed in aquaculture due to their high nutritive value and digestibility. Previous research has emphasized the mass production of zooplankton species using waste as a nutrient source in mass culture. The promotion of environmentally friendly practices has been encouraged to improve quality of life, including the production of renewable energy from waste materials and the recycling of effluent water from human activities. The conversion of liquid and solid waste from animal and plant sources into a recyclable resource with potential for commercial and industrial application has been demonstrated in various studies. Animal excreta [11], Panchagavya [12], cow urine distillate [13-14], cattle urine [15], cow dung and chicken droppings [16] and fish faeces [17]

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have been employed as nutrients in the mass culture of zooplankton species.

Zooplankton belonging to the Arthropoda family and Crustacean subfamily possess an open circulatory system that is comprised of hemolymph, a liquid that shares functional characteristics with blood. Hemolymph contains hemocytes, nutrients, oxygen, hormones, and cells, which together form a crucial defense mechanism for zooplankton [17]. Hemocytes and hemolymph are responsible for clearing pathogens and debris, with antimicrobial peptides being the primary component of the hemolymph that exerts a defense response against invading microbes [18]. Antimicrobial peptides are the major component in the hemolymph that exerts the defence response against invading microbes [19]. The overuse of synthetic antibiotics has led to the rapid progression of resistance among bacterial pathogens, necessitating the search for alternatives from natural sources. Therefore, the present study aims to identify the antibacterial potency of hemolymph isolated from the calanoid copepod, *Sinodiaptomus sarsi*, collected from a freshwater pond.

MATERIALS AND METHODS

Collection of plankton samples

Planktonic specimens were procured from the subsurface stratum of the Mahamaham reservoir, at a profundity ranging from 50 to 100 cm, as reported by Davis [20]. These specimens were subjected to morphological identification through employment of a compound microscope, following staining with Eosin, as described by Manickam *et al*. [21]. The isolated copepods were subsequently housed in a beaker, which was filled with 500 ml of tap water.

Pure culture preparation

The morphological attributes of *Sinodiaptomus sarsi* were discerned through the utilization of a 10x microscope, as reported by Witty [22]. Subsequently, the copepod species were segregated, inoculated, and nourished with yeast media, as documented by Nandakumar [23].

Collection cow urine and preparation of CUD

Urine specimens were procured from six healthy cows (Tag Nos. 0414, 0437, 0468, 0474, 0479, 0484) residing in Goshala, Sri Vittal Rukmini Samsthan, Govindapuram, near Kumbakonam. The collection of urine samples was conducted during the early morning hours, between 4:00 and 5:00 a.m., and was subsequently placed in hermetically sealed, sterile containers for transportation to the laboratory [24]. The cow urine samples were subjected to distillation for a duration of 5 to 6 hours at a temperature range of 50 to 60 °C, utilizing a distillation apparatus [25]. The distilled urine was utilized immediately for treatment without any storage.

Isolation of haemolymph

In this study, a total of twenty fully developed *Sinodiaptomus sarsi* adults were isolated and subjected to homogenization using a tissue homogenizer with sterile physiological saline solution. The resulting homogenate was then subjected to centrifugation at a speed of 6000 rpm for a duration of 10 minutes. The supernatant obtained, which contained the hemolymph, was collected and utilized for the purpose of conducting an antibacterial assay, as described by Praveena *et al*. [26].

Antimicrobial activity of hemolymph

The present study aimed to investigate the antimicrobial activity of the hemolymph of *Sinodiaptomus sarsi* against four bacterial pathogens, namely *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Rhodococcus rhodochrous*, using the agar well diffusion method on nutrient agar media. The solidification of the nutrient agar was achieved by creating wells using a well borer. The bacterial pathogens were swabbed onto the surface of Nutrient agar plates, and 25 µl of the test sample was injected into each well. The plates were then incubated for 30 minutes to allow the samples to diffuse into the medium, followed by a 24-hour incubation period at 37 °C. The diameters of the inhibition zones in millimeters were measured, and the assay was performed three times, with the mean results being reported [27].

RESULTS AND DISCUSSION

The current research pertaining to the life cycle culture methods of copepods and their potential use as natural food for fish larvae has the potential to contribute to the development of more precise techniques for large-scale culture of copepods. This, in turn, could facilitate the use of copepods as a high-quality and easily digestible food source. The diets employed in the study were found to be effective. Morphological identification of the calanoid copepod, *Sinodiaptomus sarsi*, collected from Mahamaham pond was conducted. Sinodiaptomus species was distinguished by the presence of a triangular, chitinous projection on the dorsal surface of the last prosomal somite, with moderately developed wings. The left wing was observed to have two lobes, with the innermost lobe being circular, while the right-wing extremity was relatively pointed. Both lobes were found to possess a robust hyaline spine. The urosome of the specimen under study is composed of three somites. The genital somite exhibits a slight degree of asymmetry and is characterized by the presence of moderately long hyaline spines on each side. The intermediate somite, on the other hand, is relatively short in length. Notably, the antennules extend beyond the caudal setae. Additionally, the coxopodite of the fifth leg is distinguished by a protracted and robust distolateral projection, as depicted in (Fig 1) [28].

Fig 1 Calanoid copepod, *Sinodiaptomus sarsi* identified in Mahamaham freshwater pond

The *Escherichia coli* species is a crucial constituent of the normal intestinal microflora in humans and other mammals. However, it also encompasses various pathotypes that are accountable for a broad spectrum of diseases, including enteric ailments such as diarrhea or dysentery, as well as extra-intestinal infections like urinary tract infections and meningitis

[29]. On the other hand, *Pseudomonas aeruginosa* is responsible for severe and often fatal nosocomial infections, particularly in immunocompromised hosts. It is the most significant pathogen in cystic fibrosis and a frequent cause of hospital-acquired urinary tract infections. *Pseudomonas aeruginosa* is primarily found in soil, but it can also be detected in various other environments, including freshwater environments, sinks, showers, respiratory equipment, and contaminated distilled water. This microorganism is classified as an opportunistic pathogen and is known to exhibit natural resistance to multiple classes of antimicrobial agents. Furthermore, it has the ability to acquire resistance genes from other organisms, as reported by Wisplinghoff *et al*. [30]. *Staphylococcus aureus* is a prevalent human pathogen that can cause a range of infectious diseases, such as skin and soft tissue infections, endocarditis, osteomyelitis, bacteremia, and fatal pneumonia. This microorganism can be categorized into methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA), based on its sensitivity to antibiotic drugs. Guo *et al*. [31] have reported on the prevalence of MRSA. *Rhodococcus rhodochrous* has been associated with various infections, including pneumonia, bacteremia, pericarditis, skin lesions, meningoencephalitis, infection of the ventriculoperitoneal shunt, and chronic corneal ulceration. Meningitis caused by non-equine has been reported in immunocompetent hosts, as reported by Mandell *et al*. [32].

Table 1 Antibacterial zone of inhibition of hemolymph of *Sinodiaptomus sarsi*

Bacterial species	Zone of inhibition (mm)	
	Antibiotic	Hemolymph (25µl/ml)
E. coli	24.6±0.3	18.6±0.6
P. aeruginosa	24.8±0.3	18.4±0.4
S.aureus	24.2±0.3	22.5±0.2
R. rhodochrous	18.8±0.4	17.1±0.6

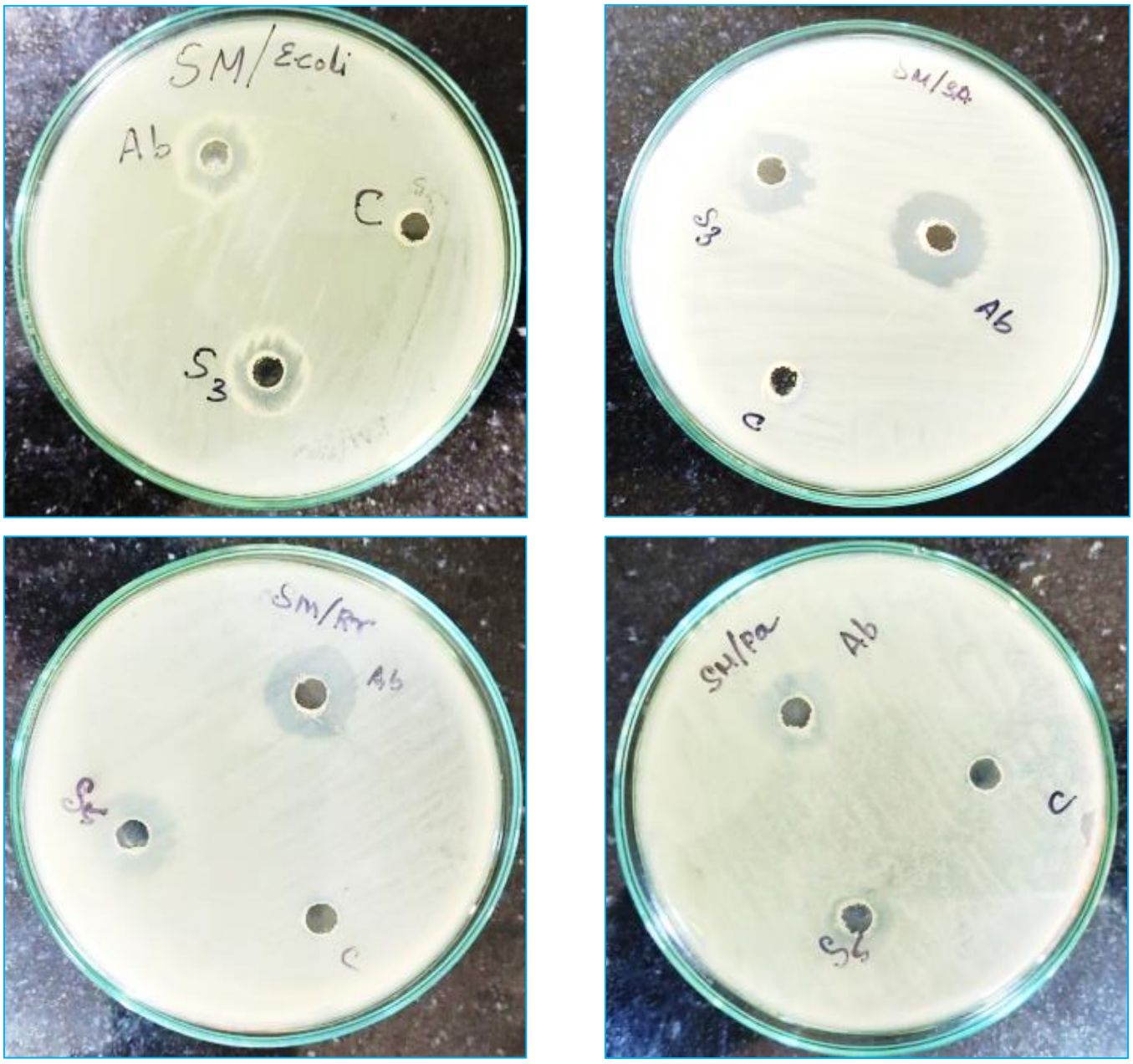

Fig 2 Inhibition zone produced byhemolymph of *Sinodiaptomus sarsi* against bacterial pathogen

Ojewole and Olusi [33] and Chauhan *et al*. [34] have documented the beneficial effects of CUD in the treatment of cardiac, respiratory, and renal diseases, as well as its antimicrobial properties. The presence of CUD in culture has

been shown to enhance the antibacterial activity of hemolymph proteins against certain human pathogenic bacterial species. In the current investigation, the hemolymph of *Sinodiaptomus sarsi* was found to be efficacious in suppressing the growth of all bacterial species tested. The antibacterial activity of the hemolymph was particularly potent against *Staphylococcus aureus*, with an observed inhibition zone of 22.5±0.2 mm. Conversely, the least susceptibility was observed in *Rhodococcus rhodochrous*, with an inhibition zone measuring 17.1±0.6 mm. The present study observed a zone of inhibition of 18.6± 0.6 mm and 18.4± 0.4 mm for *E. coli* and *P. aeruginosa*, respectively (Fig 2, Table 1). Praveena *et al*. [35] investigated the antimicrobial activity of antimicrobial proteins isolated from the hemolymph of *Mesocyclops leuckarti* cultured with cow urine distillate. The authors reported that the maximum antibacterial activity was observed against *Klebsiella pneumonia*, while the minimum activity was observed against *Shigella flexneri*, with the zone of inhibition measuring 27 mm and 17 mm, respectively. Salgado *et al.* [36] reported on the antibacterial activity of hemolymph from *Oratosquilla orateria* and *Scylla serrate* against *Staphylococcus epidermis* and *Pseudomonas aeruginosa*. The authors evaluated hemolymph concentrations ranging from 10 to 40 µl and observed significant antibacterial activity against the selected pathogens. This enhanced antibacterial activity may be attributed to the inherent antibacterial, antifungal, and antimicrobial properties of cow urine distillate (CUD) [37-38]. Plankton cultured in CUD media exhibited similar characteristics by absorbing the active ingredients from CUD into the hemocoel and hemolymph, as determined by Badadani *et al.* [39].

CONCLUSION

Based on the findings of the current investigation, it can be inferred that the utilization of cow urine distillate as a nutrient source in the cultivation of S. sari results in the inhibition of antimicrobial activity against *E. coli*, *P. aeruginosa*, *Staphylococcus aureus* and *R. rhodochrous* in the hemolymph. The heightened antibacterial efficacy of the hemolymph may be attributed to the constituents present in the cow urine distillate that were assimilated by the zooplankton during its laboratory-based cultivation.

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