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ANTIBACTERIAL ACTIVITY OF CERTAIN CEPHALOPODS FROM ANDAMANS, INDIA

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ABSTRACT

Cephalopods are well known for their bioactive secondary metabolites like Ink and very few studies have been carried with respect to bioactivity of their tissues. This study focuses on the antibacterial activity of the methanolic extracts from the body tissues of selected cephalopod species Sepioteuthis lessoniana, Sepia brevimana and Octopus cyaneus against five human pathogens. Gram positive Staphylococcus aureus MTCC 96, Bacillus subtilis MTCC 441 and Gram negative: Escherichia coli MTCC 443, Vibrio cholerae MTCC 3906 and Klebsiella pneumoniae MTCC 109 were used for the activity by well diffusion method. The methanolic extracts of Sepia brevimana 100 µl concentrations was found to be effective against Klebsiella pneumoniae *(19mm), 100 µl concentration Octopus cyaneus had shown significant activity (17mm) against Escherichia coli and Vibrio cholerae. The result suggests that the methanolic extract of cephalopods tissues showed appreciable antibacterial activity against selected human pathogens and its potential can be used for the pharmaceutical and food industrial based applications.*

KEY WORDS

Antibacterial activity; Cephalopods; Pathogens.

INTRODUCTION

Cephalopods are a group of animals that are classified under the phylum Mollusca[1]. Marine invertebrates are potential source of natural antimicrobial compounds. Earlier studies by various researchers reported that molluscs have good antibiotic properties and besides this these animals are known for their behavior of camouflage. Several natural bioactive compounds like peptides, depsipeptides, sterols, sesquiterpenes, terpenes, polypropionates, nitrogenous compounds, macrolides, prostaglandins, fatty acid derivatives, miscellaneous compounds and alkaloids were reported from molluscs which were identified as essential with specific types of activities [2]. Cephalopods are also known for their bioluminescent properties. Previous studies shown that the muscle-derived lens of a Squid from the bioluminescent organ was found to be biochemically convergent with the ocular Lens[3]. Neuropeptide from *Sepia officinalis* was characterized[4]. Biosynthesis, localization and fate of catecholamines in the ink gland of *Sepia officinalis* revealed by[5]. Medicinal value of the cuttle bone and *Sepia's ink* [6]. Chemical composition of inks from different molluscs suggested convergent chemical defenses[7]. Cyclophosphamine, a well-known chemotherapeutic drug induced hemopoietic injuries it extracted from Squid ink[8]. The comparative study of lipid distribution and amino acid composition of cuttlefish *Sepia officinalis* and sardine *Sardina pilchardus* was carried out by[9]. Antimicrobial properties of polysaccharide extracted from cuttle bone and body tissue of *Sepia parshadi,* against ten human pathogenic bacteria and five fungi [10]**.** Effects of squid ink on growth performance, immune functions and antioxidant ability of broiler chickens[11]. Study of Ink

is a conspecific alarm cue in the Caribbean reef squid investigated by[12]. Cephalopods have a significant antimicrobial activity on clinical isolates[13].

Molluscan ink showed antimicrobial and plasma coagulation property[14]. Isolation and characterization of Lolduvin-S; a novel antimicrobial protein from the ink of Indian squid *Loligo duvauceli* [15]. Molluscs are not only known for their anti-microbial activity, but it even constitutes many classes of compounds which even act as antitumor, ant leukemic and antiviral agents that have been reported worldwide[16].

Studies carried out on antimicrobial activity revealed the valuable information for new antibiotic discoveries and have given a new vision into bioactive compounds in aqua cultured molluscs. In most of the publications concerning antimicrobial activity in molluscs, either single body compartments alone, like haemolymph and egg masses, or extracts of whole body have been tested for activity. Besides Ink, haemolymph, egg masses, cuttle bone and other body parts, a very few studies were carried out on the antibacterial activity of cephalopod tissues. This study focuses on the antibacterial activity of the methanolic extracts from the body tissues of selected Cephalopod species like *Sepioteuthis lessoniana, Sepia brevimana* and *Octopus cyaneus* against five human pathogens.

MATERIALS AND METHODS

Sampling and identification

Cephalopods samples *Sepioteuthis lessoniana* (Squid), *Sepia brevimana* (Cuttlefish) and *Octopus cyaneus* (Octopus) were collected from the sampling sites at Panighat ($11^0 41'$N, $92^0 43'$E), Sesostris Bay (11^0 40'N, $92^0$45'E), and North Bay (11^0 43'N, 92^0 $47'$E) which is situated near Port Blair in Andaman and Nicobar Islands, India. Squids were obtained by using unbaited lures called jigs, Cuttle fish were collected from landing center and Octopus by hand-picking with the help of local fisherman. Samples were brought to the laboratory and were identified using Cephalopods and FAO identification keys as described [1, 17].

Preparation of methanolic extract

Cephalopods samples collected were brought to the laboratory specimens were dissected and skins were removed and cut in to small pieces. 5g of tissue of each cephalopod was weighed and homogenized with 5ml methanol using mortar and pestle following aseptic techniques. To this homogenate 100ml methanol was added and incubated at room temperature for 48 hrs. This methanolic extract was centrifuged at 27°C at 10000 rpm for 10 min (Eppendorf centrifuge 58000) and the supernatant was collected and concentrated under vacuum in a rotary evaporator (Buchi) at 30°C. The crude methanolic extract was analyzed for its antibacterial activity using standard well diffusion technique.

Human pathogenic cultures

Five pathogenic bacterial strains were used for this study. (Gram positive: *Staphylococcus aureus MTCC 96*, *Bacillus subtilis MTCC 441*, Gram negative: *Escherichia coli* MTCC 443*, Vibrio cholerae* MTCC 3906 and *Klebsiella pneumoniae* MTCC 109). All bacterial cultures were type strains and obtained from National Institute of Ocean Technology (NIOT) center at Port Blair and the Regional Medical Research Center (RMRC), Port Blair, Andaman and Nicobar Islands, India.

Inoculum preparation for bacteria

Standard Microbial techniques were followed for media preparation and other routine process. Nutrient broth (Himedia) was prepared and sterilized in an autoclave at 121°C, 15 lbs pressure for 15 min. The bacterial strains were individually inoculated in sterilized nutrient broth and were incubated at 37°C for 24 hrs. Mueller Hinton Agar (MHA, Himedia) plates were prepared and were inoculated with 24 hrs old bacterial broth cultures with the help of a sterile cotton swab and the plates were incubated at 37°C for 24 hrs.

Antibacterial assay using well diffusion method

Antibacterial activity was determined by following well diffusion technique. Suspension of each bacterial strain was carefully mixed in the test tube and the respective strains were cotton swabbed on petridishes containing Muller Hinton Agar (MHA-Hi-media). Wells (9mm) were prepared in these plates

using Cork-borer, by maintaining sterile conditions and the crude methanolic extract was prepared to a final concentration of 0.5mg/ml and 1mg/ml. Two concentrations were used for testing by loading 50 µl and 100 µl of samples to the wells separately. Methanol was used as a negative and Gentamicin was used as positive controls. All the plates were incubated at 37°C for 24 hrs. Growth inhibition halos produced by the methanolic extracts of cephalopods were examined and the diameter (mm) was measured and results were calculated by measuring the zone of inhibition in millimeters. All the tests were performed in triplicates and the data was expressed as mean values ± SD.

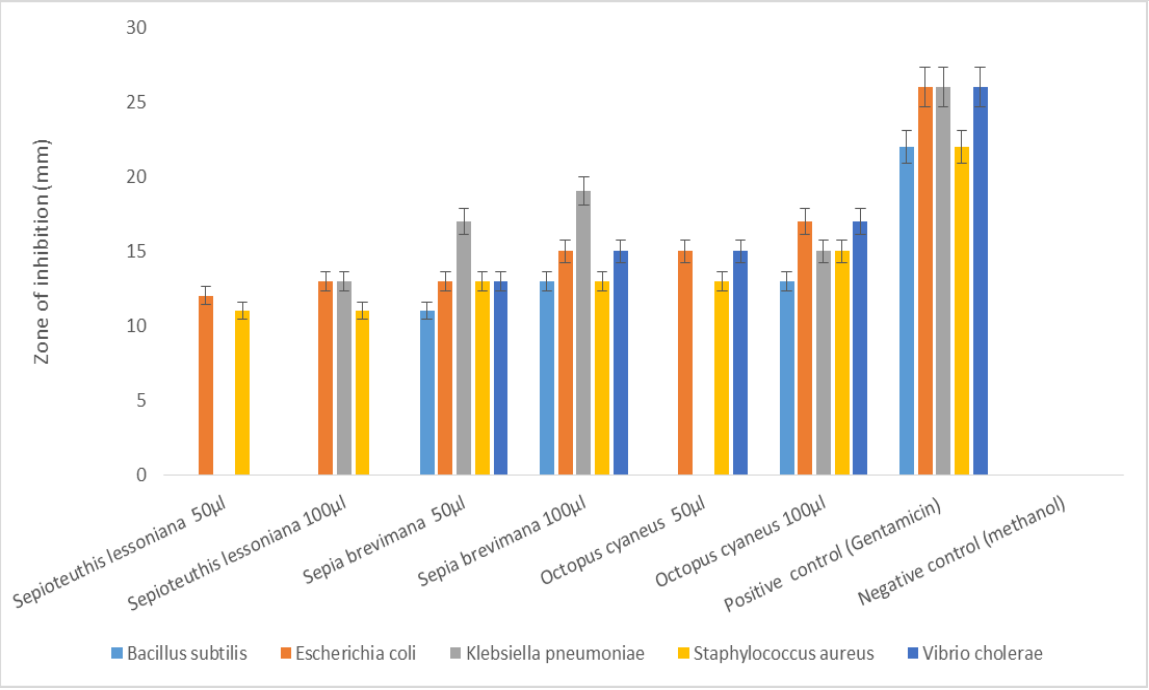
RESULTS AND DISCUSSION

The present determination showed that methanolic extract from different cephalopods possessed antibacterial activity towards all five pathogenic strains with two concentrations 50µl and 100 µl. The maximum zone of inhibition was observed against *K. pneumoniae* (17mm) in *Sepia brevimana* extract, extracts of *Octopus cyaneus* exhibited moderate zone of inhibition against *E. coli* and *V. cholerae* (15mm) respectively and *Sepia brevimana* extract showed meager activity against *E. coli, V. cholerae* and *S. aureus* (13 mm). The lowest inhibition zone of 12mm, 11mm was observed against *E.coli* and *S. aureus* respectively with extracts of *Sepioteuthis lessoniana.* 100µl concentration showed the highest zone of inhibition against *K. pneumoniae* (19mm) in *S. brevimana* extract. *O. cyaneus* extract showed significant activity against *E. coli* and *V. cholerae* (17mm) and moderate activity was observed against *K. pneumoniae* and *S. aureus* (15mm). *S. brevimana* extract exhibited moderate activity against *E. coli* and *V. cholerae* (15mm). Gentamicin was used as positive control and methanol was used as negative control it does not inhibited the growth of any of the tested strain **(Table 1) Figure 1**.

Table 1: Antibacterial activity of methanolic extracts of Cephalopods at 50µl and 100µl concentrations.

BACTERIAL STRAINS	Sepioteuthis lessoniana		Sepia brevimana		Octopus cyaneus		Positive control (Gentamicin)	Negative control (methanol)
	50µl	100µl	50µl	100µl	50µl	100µl		
Bacillus subtilis	_	_	11mm	13mm	_	13mm	22mm	_
Escherichia coli	12mm	13mm	13mm	15mm	15mm	17mm	26mm	_
Klebsiella pneumoniae	_	13mm	17mm	19mm	_	15mm	26mm	_
Staphylococcus aureus	11mm	11mm	13mm	13mm	13mm	15mm	22mm	_
Vibrio cholerae	_	_	13mm	15mm	15mm	17mm	26mm	_

Figure 1: Antibacterial activity of methanolic extracts of Cephalopods representing mean values ± SD.



Marine natural products have drawn the attention of researchers in recent years due to their pharmacological value. Extensive studies have been carried out with many marine plants and animals. As the marine plants and animals gets adapted to different types of habitats in the marine environment they show several adaptations, to overcome the difficulties they face in an ecosystem such as predation, surface fouling, space, reproduction [10]. Defensive mechanism is one such adaptation which is well studied in most of invertebrates. The antibacterial assay have been reported long back in several molluscan species such as *Crassostrea virginica* (oyster), *Mytilus edulis* and *Geukensia demissa* (mussel)[18], *Dicathais orbita* (muricid mollusks)[19], *Dolabella auricularia* (sea hare)[14, 20], 21 species of Dorid Nudibranchs tissues exhibited significant antibacterial and antifungal properties[21]. Novel neuropeptide was isolated from *Sepia officinalis*[4]. Significant antimicrobial activity was observed from the polysaccharide extracted from cuttle bone and methanolic extract of body tissue of *Sepia parshadi,* against ten human pathogenic bacteria and five fungi[10]. Methanolic tissue extract of Cephalopods 100% concentration shows the maximum activity against human pathogens[3]. The present investigation of 50 µl and 100 µl concentration of *Sepia brevimana* methanolic extract have shown maximum zone of inhibition against gram negative *K. pneumoniae.* Whole body extraction of *Meretrix casta* and *Tridacna maxima* showed a good source of antibacterial agent's[22]. Different solvent extracts of Gastropods had shown significant antibacterial activity[23]. Sea Star, *Astropecten indicus* was reported to have a potential antimicrobial source against human pathogens[24]. Methanol and chloroform tissue extract of *Siphonaria* possessed antibacterial activity [25]. Cephalopods are a good food source for coastal communities being important in their food menu.

CONCLUSION

Hence the present finding reveals that the methanolic extract of the Cephalopods showed appreciable antibacterial activity against five human pathogens. It shows a different source of metabolites that inhibited the pathogens and further studies are under way to explore the compound present in the tissue extracts that may lead for a source of drug from these marine animals.

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