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ANTIBACTERIAL ACTIVITY OF SQUID *LOLIGO DUVAUCELI* AGAINST HUMAN PATHOGENS

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ABSTRACT

The present study was carried out for antibacterial activity of *Loligo duvauceli* to prevent the emergence proliferation of resistance microbial strain that can make a significant impact in treating microbial infection. The increase in resistance is a global problem, no country is immune to this condition and all major bacterial pathogens have acquired resistance to at least one or more drugs. As resistance is increased, patients are on high risk of severity because of untreated pathogens. Natural bioactive substances have least quantum of side effect than compared to synthetic product. Squid have been known for their bioactive compound like ink and very few studies are done with respect to the bioactivity of their tissue. The present study focus on evaluation of the antibacterial activity of cold methanolic extract and crude ink gland extract from Cephalopod species *Loligo duvauceli* against four human pathogen *Staphylococcus aureus* NCIM 5021, *Escherichia coli* NCIM 2931, *Bacillus subtilis* CIM 2063, *Salmonella typhi* NCIM 2501 were used for the activity by agar cup method. The *S. aureus* strain was found to be more effective than other selected strains. The overall results suggest that the methanolic extract of *Loligo duvauceli* showed appreciable antibacterial activity against selected human pathogens and it efficacy can be used for pharmaceutical industry.

KEYWORD: Antibacterial activity, *Loligo duvauceli*, methanolic extract, inhibition zone.

INTRODUCTION

Cephalopods are group of animals classified under the phylum Mollusca (Boyle *et al.*, 2005.). Squid form the major group of Cephalopods. Several natural bioactive compounds like peptide, sterols and terpenes are reported from mollusca (Blunt *et al.*, 2006.). Different types

of bioactive compounds exhibiting anti-tumor, anti-leukemic, antibacterial and antiviral activities have been reported worldwide. 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 (Montgomery *et al.*, 1992). The potential antibacterial activity of the squid ink has already been reported against biofilm bacteria. The present study was carried out to evaluate the antibacterial activity of entire body of *L. duvauceli* against *Staphylococcus aureus* NCIM 5021, *Escherichia coli* NCIM 2931, *Bacillus subtilis* CIM 2063, *Salmonella typhi* NCIM 2501.

MATERIALS AND METHODS

Sample collection

Squid sample was collected in the month of January from Malad jetty, Mumbai, Maharashtra, India. The animal was collected from local fishermen and brought to laboratory in ice condition.

Extract preparation

The cuttle bone, eyes and alimentary canal was removed and the entire body was chosen for the study.

Methanol Extract of Body tissue and Crude Ink Gland

The extract of squid body and ink gland was prepared according to Mohanraju (2013) method. The sample was thoroughly washed three to four times with distilled water. Postero-ventral was dissected with sterile scissors and the ink gland was removed. Soft body tissue (85gm) was weighed and homogenized in mortar and pestle using 170 mL of cold methanol. Centrifugation was carried at 10,000 rpm for 30 minutes. The supernatant was collected and concentrated by air drying in evaporating dish at room temperature and then stored in a sterile bottle for further use. Same procedure was followed for crude ink gland extraction.

Test Organisms

The test organisms used for antibacterial testing were obtained in pure culture from Department of Life Sciences, University of Mumbai, Kalina as shown in Table (1). These microorganisms were inoculated in nutrient broth to obtain 16-18 hrs. growth culture for bioassay.

Antibacterial assay using Agar cup method

The bioassay of the extracts was carried out by agar cup method. This is a fairly simple method in which only small amount of sample is required. Sterile nutrient agar media were cooled to approximately 42 ^{o}C, inoculated with 0.6mL culture and poured onto in sterile 90 mm petri dish. The plates were kept on flat surface and allowed to solidify. When the agar had set, wells were punched using a sterile 2 mm cork borer. The number of well punched into the agar never exceeded 4 per plate. 100 µL extract were filled into the well using a micropipette. One of the well contained equal amount of methanol as negative control. Another well was seeded with ampicillin as positive control. The rest of the well contained equal amount of different extract of sample. The pertidishes were kept at 4^{o} C for an hour to allow diffusion of the test sample. All the test were carried out in duplicate and the average value for zone of inhibition in millimeter were taken after 24 hrs and 48 hrs of incubation at 37^{o}C.

RESULTS

The results of antibacterial activity have shown in the Table (2). On the basis of zoological assortment characters, the squid designated for the study was *L. duvauceli*. Dissection was performed successfully and the technique used for obtaining the crude extract was satisfactory. Methanol was used for extract preparation. Zone of inhibition was measured across the well.

Methanol extract of body tissue

Antibacterial activity of the crude methanol extract showed zone of inhibition against all selected strains. The zone of inhibition ranges from a mean value of 25mm for *S. aureus*, 22mm for *E. coli*, 19mm for *S. typhi* and 11mm for *B. subtilis* respectively. The highest zone of inhibition was recorded in *S. aureus* whereas minimum zone of inhibition was recorded in *B. subtilis.*

Crude ink gland extract

Antibacterial activity of the ink gland extract showed zone of inhibition against selected bacterial strains such as *S. aureus* (28mm), *B. subtilis* (26mm), *E. coli* (24mm), and *S. typhi* (23mm). *S. aureus* was found to be the most sensitive one exhibiting a lager zone of inhibition followed by *B. subtilis* and *E. coli* showing intermediate zone of inhibition, followed by *S. typhi* showing small zone of inhibitions.

Table 1: List of test microorganism used and their media.

Sr.No.	Microorganism	Media	Incubation Tempreature
1.	*Staphylococcus aureus* NCIM 5021	Nutrient agar	R.T.
2.	*Escherichia coli* NCIM 2931	Nutrient agar	37^{o}C
3.	*Bacillus subtilis* CIM 2063	Nutrient agar	37^{o}C
4.	*Salmonella typhi* NCIM 2501	Nutrient agar	37^{o}C

Table 2: Antibacterial activity of crude methanol extracts and crude ink gland extract of *L. duvauceli* against *S. aureus, E. coli, B. subtilis* and *S. typhi.*

Sr. No.	Test organism	Positive control (ampicillin)	Negative control (methanol)	Crude methanol extract	Crude ink gland extract
1.	*S. aureus*	26mm	-	25mm	28mm
2.	*E.coli*	26mm	-	22mm	24mm
3.	*B.subtilis*	25mm	-	11mm	26mm
4.	*S. typhi*	25mm	-	19mm	23mm

DISCUSSION

The present investigation showed that crude methanol extract and crude ink gland extract of *L. duvauceli* possess antibacterial activity towards four selected bacterial strains (*S. aureus, E. coli, B. subtilis* and *S. typhi*). The maximum zone of inhibition was observed against *S. aureus.* Ampicillin was used as positive control and methanol was used as negative control. It did not inhibit the growth of tested strains. Marine natural products are having pharmaceutical value and extensive studies have been carried out using many marine plants and animal. There is little report on the bioactivity of entire body of squids *L. duvauceli* crude methanol extract from selected geographical region. Thus it is selected for the present investigation. There are many studies done on squid ink which shows that squid ink constitutes the pigment melanin and the process of melanogenesis was well explained in the gland of *Sepia* sp. (Palumbo, 2003).

Previous studies have shown that Ink gland contain a variety of melanogenic enzymes as tyrosinase, dopachrome tautomerase and peroxidase (Prota, 2000). Takaya *et al* (1994) also reported the activity of crude extract of ink gland. Patterson and Murugan (2000) reported broad spectrum of antibacterial activity for aqueous ink extract of the cephalopods *L. duvauceli*. The present study is in agreement with the previous reports. Earlier reports suggest that the extract with alcohol such as ethanol, methanol and butanol yields salt from the extract (Ram Kumar *et al.,* 2005). Thus the activity shown by this extract could be affected by presence of salt. Overall result indicates that there are active biomolecules in the body of the *L. duvauceli* which is effective against selected bacterial strains. However there is a

requirement to further determine the biomolecule responsible for antibacterial activity present in which body part. Once the biomolecule will be extracted it could be used in the future therapeutic scenario in the treatment of dreadful infections caused by these pathogen and can serve as an alternating source against antimicrobial resistance.

CONCLUSION

More recent studies on marine organisms have focused mainly on their application for the treatment of human diseases. It is found that the marine ecosystem offers a huge potential as a natural based bioactive compounds. Many researches have shown that products from marine organisms have served as a source of useful drug. Present investigation showed potent antibacterial activity against selected human pathogenic strains. Thus this study recommends the use of entire body of squid including ink gland as a valuable biopharmaceutical product with antibacterial property. Further analysis of elucidating the bioactive molecule responsible for the antimicrobial activity is to be done. This study concludes by stating that squid will definitely aid in the eradication of these resistant strains in near future.

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