SCREENING OF CEPHALOPODS FOR BIO-ACTIVITY

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ABSTRACT

Extracts prepared from the various parts of the cephalopods, *Octopus vulgaris*, *Loligo duvaucelii* and *Sepia pharaonis* were tested against nine human pathogens for antibacterial activity. The aqueous ink extracts from *Loligo duvaucelii* and *Sepia pharaonis* exhibited broad antibacterial activity against all nine human pathogens tested. The posterior salivary gland extract of *Octopus vulgaris* showed highest activity against *Proteus mirabilis* and similar activity was observed for aqueous ink extract from *Sepia pharaonis* against *Proteus mirabilis* and that of *Loligo duvaucelii* against *Escherichia coli*.

INTRODUCTION

The term biological activity refers to "a change in the base-line function of an organism (or) part of an organism brought about by the administration of a test substance" (WHO 1993). During the past decade, there has been a boom in natural product's research from marine source. Scientists around the world have discovered novel bioactive compounds from both macro and micro organisms. Molluscs, which are widely distributed throughout the world have many representatives in the marine and estuarine ecosystem. But only a moderate number of studies have so far been published on the antimicrobial activity in molluscs. In gastropods, *Haliotis rufescens* (abalone), *Tegula gallina* (sea snail) and *Strombus gigas* (queen conch) were studied for the antimicrobial activity against bacteria as well as virus (Prescott & Li 1966). The Spanish dancer, a nudibranch in which the egg capsule substance was found to inhibit microbial growth (Pawlik 1992). Prem Anand *et al.* 1997) studied the antibacterial activity in the gastropod *Chicoreus virgineus*, *Rapana rapiformis* (egg capsule) which showed highest activity against the human pathogens. The haemolymph of the bivalves *Mytilus galloprovincialis*, *Ostrea edulis* and *Crassostrea gigas* inhibit bacterial growth (Hubert *et al.* 1996). In India, collaborative work with a Russian team showed that marine mussels were a very good source of antiviral drugs (The Hindu 1999).

Among molluscs, the class Cephalopoda has been little studied compared to the other classes. However, cephalopods are of special interest to biotoxicologists because most of them possess a well-developed venom apparatus involving the salivary glands. The cuttle fish ink of *Sepioteuthis lessoniana* showed antiseptic effect on *Staphylococcus aureus* (Mochizuki 1979). An antitumor compound was isolated from the squid ink *Illen argentinus* by Takaya *et al.* (1994).

The present study has been taken up to assess the antibacterial properties of *Octopus vulgaris*, *Loligo duvaucelii* and *Sepia pharaonis*.

MATERIALS AND METHODS

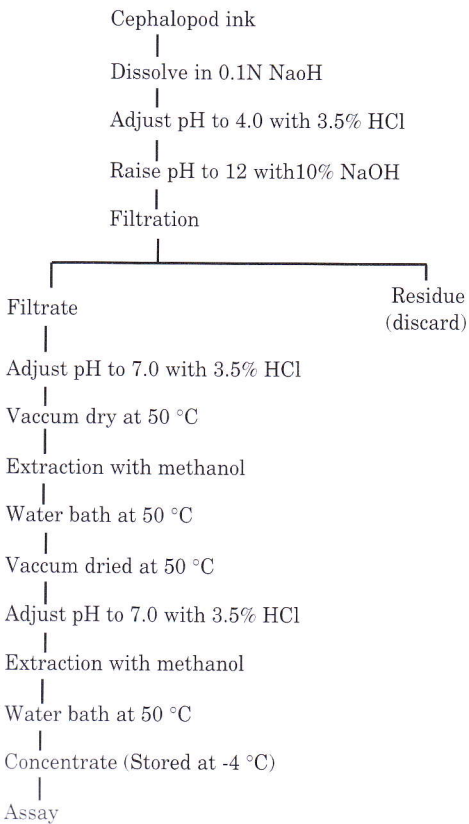
Specimens of three species of cephalopods were collected off Cuddalore coast, southeast coast of India and were screened for antibacterial activity. The species screened were (i) *Octopus vulgaris* (Posterior salivary gland) (ii) *Loligo duvaucelii* (posterior salivary gland and ink) (iii) *Sepia pharaonis* (posterior salivary gland, ink and internal shell)

The posterior salivary glands of *Octopus vulgaris*, *Loligo duvaucelii* and *Sepia*

pharaonis were dissected out. The crude extracts were prepared by homogenizing the sample with 0.9% saline solution and subsequent filtration through Whatman No:1 filter paper. This filtrate was kept at -4 °C for antibacterial assay.

Ink was collected from both *Loligo duvaucelli* and *Sepia pharaonis*. The extraction was carried out following the method of Mochizuki (1979) with some modifications which are summarized in Fig. 1. Prior to extraction, cephalopod ink was dissolved in 0.1N NaOH and the pH was adjusted to 4.0 with 3.5% HCl and the pH was raised to 12 suddenly with 10% NaOH. The samples were filtered through Whatman No:1 filter paper and the pH of the filtrate was adjusted to 7.0 with 3.5% HCl. These extracts were vaccum dried at 50 °C. Methanol extracts were obtained from the dried samples and subsequently, the supernatant was separated by centrifugation. The supernatant solutions thus obtained were pooled and left for 2 hrs in water bath at 50 °C for evaporation and the remaining residue was collected and stored under -4 °C for antibacterial assay.

Fig. 1. Scheme of extraction of antibacterial substance from cephalopod ink.

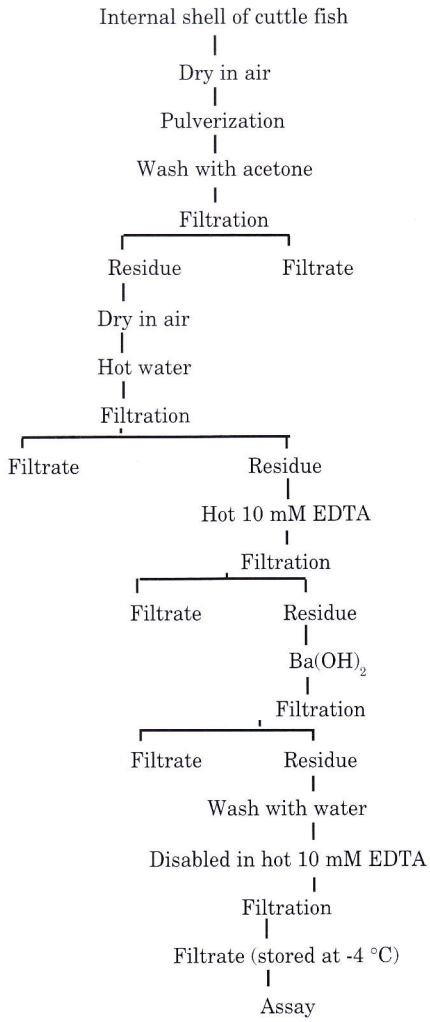


Sepia pharaonis's internal shell crude extraction was obtained by following the method of Okutani & Morikawa (1978) with some modifications (Fig. 2). The air dried internal shell of the cuttlefish was pulverized and washed with acetone. The residue was air dried and washed in hot water and filtered. The powder was extracted with hot 10 mM EDTA solution and filtered through Whatman No:1 filter paper. The saturated $Ba(OH)_2$ solution was added to the filtrate and left overnight. The precipitate was collected on a filter paper (Whatman No:1) and washed with water. The precipitate was dissolved in 10 mM EDTA solution and filtered through Whatman No:1 filter paper. The filtrate was stored at -4 °C and used in the present investigation as the crude extract. The crude extracts were tested against nine human pathogens, *Salmonella typhi, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus faecalis, Vibrio cholerae, Proteus vulgaris, Proteus mirabilis, Shigella flexneri* and *Escherichia coli*. For antibacterial assay, the agar diffusion method was followed (Prem Anand *et al.* 1997). Simultaneous controls were run along with test samples.

RESULTS AND DISCUSSION

The saline crude posterior salivary gland extracts of *Octopus vulgaris* inhibited antibacterial activity against *Bacillus subtilis* (5 mm) and *Staphylococcus faecalis* (10 mm). Significant activity against *Proteus mirabilis* (19 mm) was observed. But activity against both *Salmonella typhi* and *Vibrio cholerae* was less and there was no activity against the other four pathogens tested namely, *Klebsiella pneumoniae, Proteus vulgaris, Shigella flexneri* and *Es-*

Fig. 2. Schematic diagram of extraction of antibacterial substance from internal shell of cuttle fish



cherichia coli (Table 1).

The saline (PSG) extracts of *Loligo duvaucelii* exhibited antibacterial activity against all pathogens except *Escherichia coli*. Significant activity was observed against *Klebsiella pneumoniae* (12mm) and *Proteus mirabilis* (8mm) (Table 1).

The saline (PSG) extracts from *Sepia pharaonis* showed negative result against *Escherichia coli* and showed significant activity against both *Proteus vulgaris* (11 mm) and *Proteus mirabilis* (11 mm) (Table 1).

In poisonous cephalopods all bacteriological tests against human pathogens including *Clostridium botulinum* and *C. perfringens*, proved to be negative (Kawabata *et al.* 1957). But, in this study posterior salivary gland extracts of *Octopus vulgaris*, *Loligo duvaucelii* and *Sepia phara- onis* exhibited a broad spectral activity against eight bacterial pathogens (Table 1).

In general, the crude ink extracts of both *Loligo duvaucelii* and *Sepia pharaonis* exhibited activity against all nine pathogens. More significant activity was noticed against *Proteus mirabilis* (21 mm). The *Loligo duvaucelii* ink extract showed significant activity against *Klebsiella pneumoniae* (13 mm), *Vibrio cholerae* (13 mm) and *Escherichia coli* (14 mm). The cuttlefish ink of *Sepioteuthis lessoniana* showed antiseptic effect on *Staphylococcus aureus* (Mochizuki, 1979). An antitumor compound was isolated from the squid ink of *Illex argentinus* by Takaya *et al.* (1994). In the present study, the ink extract of both *Loligo duvaucelii* and *Sepia pharaonis* exhibited broad spectral activity against all 9 bacterial pathogens (Table 1).

The internal shell extract of *Sepia Pharaonis* exhibited activity against all pathogens except two strains, *Bacillus subtilis* and *Escherichia col.* Significant activity was observed against *Proteus mirabilis* (13 mm) and *Shigella flexneri* (10 mm). Simultaneous controls for all extracts showed negative results. The internal shell extract of *Sepia pharaonis* exhibited activity against seven pathogens (Table 1). An antitumor substance was obtained from the internal shell of squid (Okutani 1976).

In this study, a wide spectral antibacterial activity has been recorded in almost all the extracts, which is significant and showed promising trends. The results of the present study revealed the broad spectral antibacterial activity of cephalopod crude extracts and further investigation to isolate and characterize the active compounds would certainly introduce new potential drugs to coun-

Table 1. Antibacterial activity of different extraction of the cephalopods

Name of pathogens	Extracts (Inhibition zone diameter (mm))					
	Posterior salivary gland			Ink		Internal shell
	Octopus vulgaris	*Loligo duvaucelii*	*Sepia pharaonis*	*Loligo duvaucelii*	*Sepia pharaonis*	*Sepia pharaonis*
Salmonella typhi	Trace	6	5	8	7	7
Bacillus subtilis	5	7	7	9	Trace	-
Klebsiella pneumoniae	-	12	5	13	6	5
Staphylococcus faecalis	10	Trace	Trace	8	7	9
Vibrio cholerae	Trace	Trace	Trace	13	Trace	9
Proteus vulgaris	-	5	11	Trace	5	Trace
Proteus mirabilis	19	8	11	5	21	13
Shigella flexneri	-	5	Trace	9	7	10
Escherichia coli	-	-	-	14	8	-

ter the deadly pathogens.

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