Inhibitory Activity of Ink and Body Tissue Extracts of Euprymna Stenodactyla and Octopus Dollfusi Aganist Histamine Producing Bacteria

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Abstract: To assess the antimicrobial activity of the ink and body tissue extracts of the cephalopods, Euprymna stenodactyla and Octopus dollfusi against histamine producing bacteria. Methods: Euprymna stenodactyla and Octopus dollfusi were extracted using methanol, ethanol, acetone and chloroform and tested against selected human pathogenic and histamine producing Bacteria (HPB) Escherichia coli, Salmonella typhi, Klebsiella oxytoca, Klebsiella pneumonia, Vibrio parahaemolyticus, Aeromonas hydrophila, Pseudomonas aeruginosa, Bacillus cereus and Staphylococcus sp. by agar well diffusion method. Results: Highest inhibitory activity was observed against Pseudomonas aeruginosa in methanol ink extracts of E. stenodactyla and ethanol extracts of Salmonella typhi. Ethanolic extracts of O. dollfusi showed highest inhibitory activity against can be used in the seafood processing industries to enhance the shelf life of sea foods and control the histamine fish poisoning.

Key words: Antibacterial activity · Cephalopod ink · Euprymna stenodactyla · Histamine producing bacteria · Octopus dollfusi

INTRODUCTION

Sea food is considered as one of the good source of animal protein for its high content of Poly Unsaturated Fatty Acids (PUFA). Consumption of a variety of fish is associated with an increasing number of human intoxication and diseases, which pose public health problems worldwide. This is mainly because of the highly perishable nature of the seafood species, aided by the unhealthy mode of handling and improper preservation leading to histamine fish-poisoning. Scombroid fishes and some non-scombroid fishes are able to produce histamine due to improper preservation and handling. These fishes have high amount of free L-histidine in their body tissues, which is the precursor amino acid for histamine formation [1]. Histamine toxicity occur from several minutes to several hours after ingestion of toxic fish and the illness typically lasts few hours but may continue for several days which include cutenous rash, urticaria, burning itching, edema, gastrointestinal inflammation, nausea, vomiting, diarrhea, hypertension and neurological headache [2]. Due to the growing consumer demand for seafood products it is imperative to use natural antimicrobials and antioxidants as preservatives [3]. Cephalopods are well known for their defense mechanism to escape from their enemies by changing colour and inking to confuse them [4]. Cephalopods such as Octopus, squid and Loligo ink possess the antimicrobial, retroviral and anticancer properties [5].

Several studies pertaining to biomedical properties of the ink of cephalopods had been reported previously [5-9]. Effect of natural and traditional preservatives such as spices and oils have already been tested against the histamine producing bacteria [10,11]. But research related to the antibacterial activity of
cephalopod ink and body tissue extract against histamine producing bacteria were not available. In Cephalopods such as squid, only the mantle, fins and arms with tentacles were consumed and the other internal organs such as ink gland, gut and accessory nidamandal glands were discarded.

The objective of the present study was to single out the natural antibacterial agent from the cephalopod ink and body tissue extract which is potential against histamine producing bacteria, that may enhance the shelf life and preserve the fishery products without adversely affecting its quality and delicacy.

**MATERIAL AND METHOD**

**Cephalopod Sample Collection:** Cephalopods *Euprymna stenodactyla* (Orbigny, 1848) and *Octopus dollfusi* (Ropsin, 1929), were collected from Thondi coast, Palk Strait, Southeast coast of India. They were identified by using the Central Marine Fisheries Research Institute (CMFRI) Bulletin No. 37. Identify of common cephalopods of India [12] and [7].

**Bacterial Strains:** In the present study seven species of Gram negative histamine producing bacterial strains including *Escherichia coli*, *Salmonella typhi*, *Klebsiella oxytoca*, *K. pneumoniae Vibrio parahaemolyticus*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and two Gram positive histamine producing bacterial strains *Bacillus cereus* and *Staphylococcus* species were tested. The bacterial strains obtained from the Microbial culture collection from Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamilnadu, India.

**Extract Preparation:** *E. stenodactyla* and *O. dollfusi* were washed with sterile water. The abdomen of Cephalopods was cut open and the ink glands and mantle tissue were carefully removed and air dried. These samples were kept at room temperature in a glass bottle with Methanol, Ethanol, Acetone and Chloroform for 7 days. Then it was filtered using Whatman No. 1 filter paper and the filtrate was evaporated. The solvent extract were collected in petriplates and allowed to evaporate the residual ethanol, methanol, acetone, chloroform solvents [7].

**Antibacterial Assay:** Antibacterial activity of the sample was assayed by the standard Nathan agar well diffusion method [13, 14]. Sterile cotton swab was dipped into the bacterial suspension. Then the swab was used to spread the entire dried surface of the Mueller Hinton Agar (MHA) (Himedia, Mumbai) plates. A constant amount of 0.7 mg of the dried extract in 50 µl solvent was placed on to each well. The well at the center without the extract served as control. After 24 hrs of incubation at 37°C zone of inhibition around the well was measured.

**RESULTS**

The crude ink extract of *E. stenodactyla* showed the highest inhibitory activity 6 mm against *P. aeruoginosa* in methanolic extract and chloroform extract. *E. coli* growth was inhibited up to 3 mm in chloroform extract and 2 mm in ethanolic extract. *S. typhi* growth was inhibited up to 4 mm in the ethanolic extract. *B. cereus* growth was inhibited up to 2 mm in chloroform extract. Mantle tissue extract of *E. stenodactyla* inhibited the growth of *E. coli* up to 2 mm in methanolic extract *P. aeruoginosa* and *A. hydrophila* inhibited at 2 mm in acetone extracts. *B. cereus* growth was inhibited up to 4 mm in chloroform extract and the *Staphylococcus* sp. growth inhibited up to 2 mm in ethanol extract.

The ink of *O. dollfusi* was inhibited the growth of *V. parahaemolyticus* at 5mm in ethanol extract, the *B. cereus* growth at 4 mm in acetone extract and *P. aeruoginosa* up to 3 mm in acetone extract. Ink extract showed very less activity in different solvents against other organisms tested whereas the body tissue extracts of *O. dollfusi* showed the highest activity against *P. aeruf* up to 4 mm and 3 mm against *A. hydrophila* in methanol and acetone extracts respectively (Table 1).

**DISCUSSION**

Marine environment is highly interesting for its many hidden resources till now unknown to man. Those resources were not fully explored and utilised so far. Now a days more attention has been paid by the researchers to explore the potential drugs from the marine sources. Several bio active molecules extracted from marine invertebrates, including molluscs, possesses broad spectrum antibacterial activity that inhibit the growth of bacteria, fungi and yeasts [15]. The main objective of
Table 1: Antibacterial activity of the crude ink and body tissue extract of Euprymna stenodactyla and Octopus dollfusi against the histamine producing bacteria (inhibition zone mm)

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>E. coli Ink</th>
<th>E. coli Body tissue</th>
<th>O. dollfusi Ink</th>
<th>O. dollfusi Body tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S. typhi</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>K. oxytoca</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>T</td>
</tr>
<tr>
<td>V. parahaemolyticus</td>
<td>1</td>
<td>T</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>A. hydrophila</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>B. cereus</td>
<td>1</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>Staphylococcus sp.</td>
<td>1</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
</tbody>
</table>

(T: Trace), (-: Nil), (E: Ethanol), (M: Methanol), (C: chloroform), (A: Acetone), (1-6: Zone of inhibition in mm)

The present study was to compare the ability of Ethanol, methanol, chloroform and acetone extract against the growth of histamine producing bacteria. The result of the present study indicates that the ink extracts and body tissue extracts were inhibited the growth of histamine producing bacteria in methanol and ethanol extracts.

Antibacterial activity has been reported from many mollusc species viz. Oyster (Crossastrea virginica), mussel (Mytilus edulis and Geukensia demissa), murcid mussel (Dicathais orbita) and sea hare (Dolabella auricularia) [16-18]. The broad spectrum of antibacterial activity for Loligo duvaucelii and Sepia pharaonis aqueous extract of the ink against human bacterial pathogens [19]. Inhibitory activity of Nerita albicilla and Nerita oryzarum, Chicoreus virgineus, Chicoreus ramosus against biofilm forming bacteria from Tuticorin coast [20]. The antimicrobial activities of Sepia prashadi methanolic extract against human pathogenic bacteria and antibacterial activity of marine bivalves Meritrix casta and Tridacna maxima with different solvents were investigated [9, 21].

Only meager studies have been carried out on antibacterial activity of cephalopod ink and cephalopod body extract with ethanol, methanol, chloroform and acetone. Antibacterial activity of sea hare Aplysia extraordiraria, Dolabella auricularia and the cephalopod Sepioteuthius lessoniana and Sepia pharaonis ink extracts against 40 biofilm forming bacteria. The ink extract of two gastropods and two cephalopod screened antibacterial activity [5]. The methanolic extracts of Aplysia extraordiraria and Dolabella auricularia ink shown activities against some biofilm forming bacteria.

Antibacterial activity of the extracts of the ink samples of cuttle fish, Sepia pharaonis against selected human pathogens such as Salmonella paratyphi B, Pseudomonas aeruginosa and Shigella dysenteriae 5mm each in toluene extract. Diethyl ether column purified fractions showed prominent activity 6.5 mm against Klebsiella pneumoniae and Staphylococcus epidermis 5mm [5].

The in vitro antimicrobial activity of the crude methanolic extracts of six species of cephalopods, Sepia locobiensis, Sepiella mermis, Sepioteuthis lessoniana, Octopus aegina, Octopus aerolatus, Octopus dollfusi were screened against clinically isolated human pathogenic bacteria viz. Vibrio cholae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Vibrio alginolyticus, Staphylococcus aureus, Vibrio parahaemolyticus, Streptococcus sp., Salmonella sp. and E.coli [22]. The body tissue methanolic extract against Bacillus cereus was 4 mm and against E. coli 2mm. The antibacterial activity of the crude diethy ether ink extracts of Sepia pharaonis against Salmonella paratyphi and Klebsiella pneumoniae exhibited an inhibitory zone of 5mm and 4mm respectively [5]. The inhibitory activity of methanolic extract of Sepia prashadi body extract at 100 % concentration observed against organisms K. pneumoniae 8mm, V. Parahaemolyticus 10 mm, Salmonella sp. 7 mm and E. Coli 11 mm. Activity has been observed from Streptococcus sp. There is very few studies were carried out to inhibit the histamine producing bacteria by actinomycetes, among the 41 strains of actinomycetes tested S. aurofusciculatus, S. chattanoogenensis and S. hawaiiensis strains showed good inhibitory activity [23].
CONCLUSION

On comparing it was found that, *E. stenodactyla* and *O. dollfusi* ink extract showed moderate activity against the histamine producing bacteria. The ink and body tissue of *E. stenodactyla* and *O. dollfusi* extract has shown promising antibacterial activity against histamine producing bacteria. The growth of *P. aeruginosa*, *S. typhii*, *E. coli* and *B. cereus* were inhibited at different level by *E. stenodactyla* ink extracts. This study concluded that ink and body tissue extract showed inhibitory effects on Gram positive and Gram negative histamine producing bacteria. These findings are giving an idea that cephalopods may be considered as seafood preservative to enhance the shelf life of the fishery products. Further attempts have to be made thereby purification and characterization of these extracts is necessary to discover the efficacy of potent compound against the fish spoilage bacteria.

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