

Evaluation of Antibacterial Potential of *Sepia aculeata* Extracts

Gayathri N¹, Veena Desai², Meenakshi Sundaresan³

^{1,2,3}Department of Zoology, D. G. Ruparel College of Arts, Science and Commerce, Mahim, Mumbai-16, Maharashtra, India

Abstract: Molluscs are one of the well-known phyla of invertebrate organisms which are studied for their antimicrobial, antitumor and antiviral properties. In the present study, the antibacterial activity of cephalopod *Sepia aculeata* was carried out. The digestive system, female reproductive system, male reproductive system and salivary glands of *S. aculeata* from Mumbai coast were extracted in various solvents. These extracts were screened against different pathogens for antibacterial activity. The maximum zone of inhibition was exhibited by the aqueous extract of digestive system against *Klebsiella*. There were no lethal effects observed on the brine shrimps.

Keywords: Antimicrobial, Bioactivity, Needle Cuttlefish

1. Introduction

Marine bioactive substances have great pharmacological value thus drawing attention of researchers in this field. In recent years, efforts have been made to bridge between marine natural products and clinical trials [1]. There is a huge scope for exploring the potential of bioactive substances from cephalopods in India. Antimicrobial studies have been done either using whole body extracts or on single body compartments like egg masses, haemolymph, ink sac, etc. [2]. Very few studies are being carried out on biological systems such as digestive system and reproductive system. The current study is carried out to explore the antimicrobial potential of different systems of *Sepia aculeata* extracted in solvents.

2. Materials and Methods

Sepia aculeata samples used in the study were freshly collected from fish landing centers at Mumbai and identified using FAO species catalogue, and confirmed by DNA barcoding. Samples were immediately dissected to separate the digestive system, reproductive system and salivary glands. The samples were homogenised, extracted in methanol, chloroform-methanol 2:1, n-hexane and distilled water and evaporated in rotary evaporator [3-6]. Seven bacterial strains viz. *Bacillus subtilis*, *Edwardsiella tarda*, *Escherichia coli*, *Klebsiella*, *Pseudomonas*, *Salmonella typhi* and *Staphylococcus aureus* were used for the assay. The antibacterial assay was carried out by disc diffusion assay [7,8]. Minimum Inhibitory Concentration (MIC) of the microorganisms was determined by resazurin microtitre

assay[9,10] To assess toxicity of extracts, Brine shrimp lethality assay was performed [11,12].

3. Results

The aqueous extract of digestive system showed highest zone of inhibition (12 mm) against *Klebsiella*. (Table 1). The minimum Inhibitory Concentration (MIC) of this extract was found to be 1 µg/ ml against all the tested microorganisms (Fig.1). The zone of inhibition of 10 mm against *Pseudomonas* was observed in the chloroform: methanol extract of female reproductive system and aqueous extract of male reproductive system (Fig.2). No lethal effect was observed on brine shrimp nauplii till 24 hours.

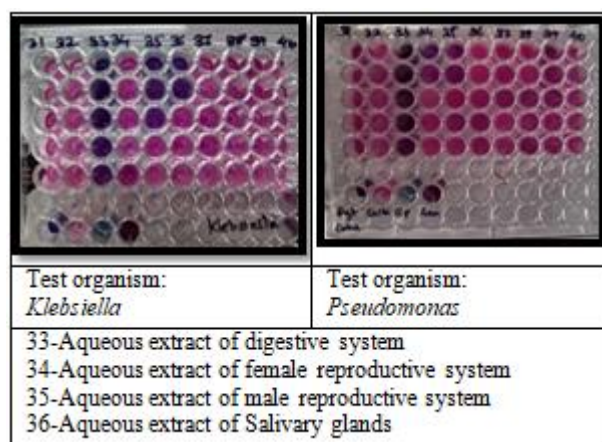


Figure 1: Estimation of the minimum inhibitory concentration of the extracts

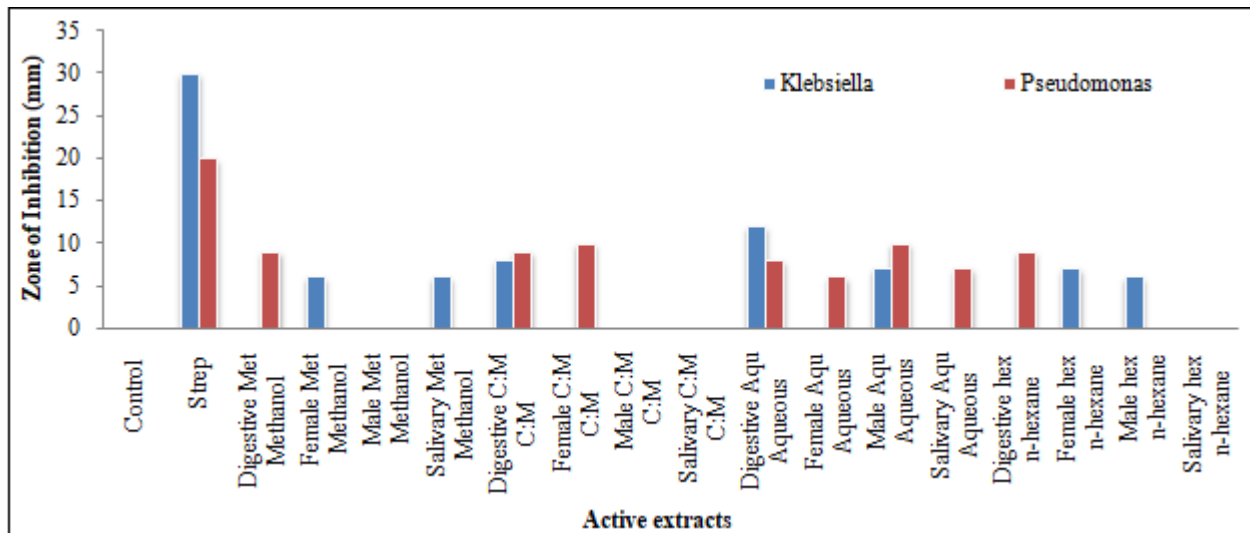


Figure 2: Effect of the extracts on *Klebsiella* and *Pseudomonas*

Table 1: Antibacterial activity of the extracts of *Sepia aculeata*

Solvent		Zone of inhibition (In mm)						
		<i>B.subtilis</i>	<i>E.coli</i>	<i>Klebsiella</i>	<i>Pseudomonas</i>	<i>S.Typhi</i>	<i>S.aureus</i>	<i>E.tarda</i>
	Control	0	0	0	0	0	0	0
	Strep	20	28	30	20	25	27	25
Methanol	Digestive	9	0	0	9	7	0	8
Methanol	Female	9	0	6	0	7	6	0
Methanol	Male	0	0	0	0	0	0	0
Methanol	Salivary	8	0	6	0	9	7	0
C:M	Digestive	6	6	8	9	7	7	8
C:M	Female	8	0	0	10	8	0	0
C:M	Male	0	0	0	0	0	0	0
C:M	Salivary	0	0	0	0	0	0	0
Aqueous	Digestive	6	8	12	8	7	7	6
Aqueous	Female	0	0	0	6	0	7	0
Aqueous	Male	9	6	7	10	6	6	8
Aqueous	Salivary	7	7	0	7	0	0	0
n-hexane	Digestive	9	0	0	9	0	0	0
n-hexane	Female	7	0	7	0	0	0	0
n-hexane	Male	0	0	6	0	0	0	0
n-hexane	Salivary	0	0	0	0	0	0	0

4. Discussion

The present study was aimed to evaluate the effect of the extracts of various systems of *Sepia aculeata* against bacteria. The Chloroform: methanol (2:1) extract of female reproductive system, aqueous extract of digestive and male reproductive systems have shown potential activity. Maximum activity was shown against *Klebsiella* followed by *Pseudomonas*. Moderate antibacterial activity was seen against *B.subtilis* and *S.typhi*. The protein content of the aqueous extract of digestive system was estimated to be 0.769 mg/ml. The maximum antibacterial activity exhibited by this extract could be due to proteinaceous component (s) present in it. The carbohydrate and lipid content of this extract was found to be 0.012 mg/ml and 23% respectively.

The methanolic extracts of *Sepia brevimana* was reported to exhibit antibacterial activity against *Klebsiella pneumoniae* [13]. Potent antibacterial and antifungal activity against human pathogens were observed against the polysaccharides extracted from cuttlebone of *S.aculeata* and *S.brevimana*. [14]. The methanolic extracts of the whole body

tissue of *Sepiella inermis* shows antibacterial activity against *K. pneumoniae* and *S.aureus* [15]

The study for bioactivities of the ink, ink gland, nidamental glands, shell, etc. have been extensively carried out in *Sepia* species. The methanol extract of ink of *Sepia officinalis* was reported to exhibit inhibitory activity against *Pseudomonas aeruginosa* and *Salmonella typhi*. [16]. The antibacterial effect of methanolic extract of the ink has been demonstrated against *Aeromonas hydrophila* [17]. Antibacterial activity of methanolic extract of ink of *Sepia pharaonis* has been studied against *Bacillus*, *Pseudomonas*, *Escherichia coli*, *Staphylococcus* and *Klebsiella*[18]. The chloroform extract of the ink gland of *Sepia officinalis* has been reported to possess antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexineri* and *Proteus vulgaris* [19].

The aqueous and the chloroform:methanol extracts of *Sepia aculeata* exhibits potential antibacterial activity. Purification and characterization is being carried out.

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References

- [1] Glaser KB and Mayar AMS. "A renaissance in marine pharmacology: From preclinical curiosity to clinical reality," *Biochemical pharmacology*. 70, pp. 440-448, 2009.
- [2] Ramasamy, P., Subhapradha, N., Srivasan, A., Shanmugam, V., Krishnamoorthy, J. and Shanmugam, A. "In vitro evaluation of antimicrobial activity of methanolic extract from selected cephalopods on clinical isolates", *African Journal of Microbiology Research* 5(23), pp. 3884-3889, 2011.
- [3] Kawashima Y, Nagashima Y and Shiomi K. "Determination of tetramine in marine gastropods by liquid chromatography /electrospray ionization-mass spectroscopy," *Toxicon* 44 (2), pp. 185-191, 2004.
- [4] Miyamoto T, Sakamoto K, Arao K, Komoria T, Itiguchi R and Sasaki T. "Dorisenones, Cytotoxic Spongian Diterpenoids from the Nudibranch *Chromodoris obsoleta*," *Tetrahedron Journal* 52 (24), pp. 8187-8198, 1996.
- [5] Karthikeyan P, Cikesh PC, Bindiya ES, Raghul Subin, Tina KJ, Chandrasekaran M and Sarita G B "Characterization of a bioactive Protein with Antimicrobial property from *Loligo* Spp.," *World Journal of Fish and Marine Sciences* 1 (4), pp. 262-267, 2009
- [6] Pawankumar, Venkateshvaran K, Srivastava PP, Nayak SK, Shivaprakash SM and Chakraborty SK "Pharmacological studies on the venom of the marine snail *Conus lentiginosus* Reeve," *International Journal of Fisheries and Aquatic studies*.1(3), pp. 79-85, 2014.
- [7] Choudhury S, Pattnaik P, Sree A, Bapuji M and Mukherjee SC "Antibacterial activity of sponge extracts against fish pathogens," *Aquaculture Research*. 34, pp. 1075-1077, 2003.
- [8] Bragadeeswaran S, Sri Kumaran N, Prasath S and Prabahar R "Bioactive potential of sea urchin *Temnopleurus toreumaticus* from Devanampattinam, Southeast coast of India," *Journal of Pharmacy and Alternative Medicine* 2, pp. 9-17, 2013.
- [9] Mann CM and Markham JL "A new method for determining the Minimum inhibitory concentration of essential oils," *Journal of applied microbiology* 84, pp. 538-544, 1997.
- [10] Sarker SD, Nahar L and Kumarasamy Y. "Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals," *Methods* 42, pp. 321-324, 2007.
- [11] Meyer N, Ferrigi R, Putnam E, Jaconson B, Nicolas E and Mclaughlin L "Brine shrimp convenient general bioassay for active plant constituents," *Plant Medica* 45, pp. 31-34, 1982.
- [12] Sarah Q, Chowdhury F and Misbahuddin M "Brine shrimp lethality assay, " *Bangladesh J Pharmacol* 12, pp. 186-189, 2017.
- [13] Mohanraju R, Dayanand BM, Karthick P, Sumantha N, Narayana MK and Ramesh. "Antibacterial activity of certain cephalopods from Andamans, India," *International Journal of Pharmacy and Biological Sciences* 3(2), pp. 450-455, 2013.
- [14] Shanmugam A, Mahalakshmi TS and Barwin VA "Antimicrobial Activity of Polysaccharide Isolated from the Cuttlebone of *Sepia aculeata* (Orbigny, 1848) and *Sepia brevimana* (Steenstrup, 1875): An Approach to Selected Antimicrobial Activity for Human Pathogenic Microorganisms," *Journal of Fisheries and Aquatic Science*, 3, pp. 268-274, 2018.
- [15] Vairamani S, Subhapradha N, Ramasamy P, Barwin VA, Raveendran S and Shanmugam A. "Antibacterial Activity of Methanolic Extract of Whole Body Tissue and Ethylene Diamine Tetra Acetate Extract of Cuttlebone of *Sepiella inermis* (Orbigny, 1848)," *Research Journal of Microbiology* 7(5), pp. 263-272, 2012.
- [16] Ismail M and Riaad R.m "Screening the antimicrobial activity of different *Sepia officinalis* (Cephalopoda: Sepioida) Parts collected from Alexandria Mediterranean Waters, Egypt Against some human pathogens," *Singapore journal of Science and Research* 8(1), pp. 1-7, 2018.
- [17] Adharyan IR. "Antibacterial Activity of Cuttlefish *Sepia* sp.(Cephalopoda) Ink Extract Against *Aeromonas hydrophila*," *Traditional medicinal journal* 24(3), pp. 184-188, 2019.
- [18] Senan VP. "Antibacterial activity of methanolic extract of the ink of cuttlefish, *Sepia pharaonis* against pathogenic bacterial strains," *International journal of pharmaceutical sciences and research* 6(4), pp. 1705-1710, 2015.
- [19] Sheela MJ, Dhanya S and Pugazhendhi A. "Antibacterial effect of Ink gland extracts from Indian squid *Loligo duvauceli* and cuttlefish *Sepia officinalis* from North Chennai," *Indian Journal of Applied Microbiology*, 17(1), pp 32-37, 2014.