

Full Length Research Paper

***In vitro* antibacterial screening of methanolic extract of whole body tissue and ethylene diamine tetra acetate (EDTA) extract of cuttlebone of *Sepia pharaonis* (Ehrenberg, 1831) against selected clinical isolates**

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The need for the discovery of new and novel antibiotics is imperative because evidence suggests that development and spread of resistance to any new antimicrobial agent is inevitable. In the present study, the *in vitro* antibacterial activity of methanolic extract of whole body tissue and ethylene diamine tetra acetate (EDTA) extract of cuttlebone (polysaccharide) of *Sepia pharaonis* was investigated against ten bacterial species including Gram-positive species (*Staphylococcus aureus* and *Streptococcus pyogenes*) and Gram-negative species (*Salmonella typhi*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Klebsiella oxytoca*, *Escherichia coli*, *Salmonella paratyphi*, *Vibrio parahaemolyticus* and *Proteus mirabilis*) with different concentrations such as 25, 50, 75 and 100% using disc diffusion method. The highest inhibition zone was recorded against *P. mirabilis* for methanolic extract (18.3±0.1 for 100% concentration) and against *S. pyogenes* for EDTA extract (polysaccharide) (15.5±0.06 for 100% concentration) of cuttlebone. But the activity was totally absent in negative control. For minimum inhibitory concentration (MIC) technique, various ranges of concentrations between 20 and 100 mg/ml were prepared and tested. MIC values were found ranging from 40 and 100 mg/ml. All assays were carried out in triplets. A wide spectral and concentration dependent antibacterial activity was recorded in both extracts.

Key words: Antibacterial activity, polysaccharide, cuttlebone, methanolic extract, ethylene diamine tetra acetate (EDTA), minimum inhibitory concentration (MIC).

INTRODUCTION

In nature, animals are provided with their own protective response against their predators and pathogens. Marine

molluscs are exposed to microbial pathogens in their environment, which can number up to 10⁶ bacteria/ml of

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seawater (Ammerman et al., 1984). In order to defend themselves against such condition, molluscs have developed very effective mechanisms that are part of their innate immunity (Tincu and Taylor, 2004). Molluscs are widely distributed throughout the world and have many representatives in the marine and estuarine ecosystem such as slugs, whelks, clams, mussels, oysters, scallops, cuttlefishes, squids and octopods. Bioactive substances from marine biota have been used as special tools in pharmacological/biomedical research. Discovered bioactive compounds in molluscs were identified essentially as peptide, depsipeptide, sterols, sesquiterpene, terpenes, polypropionate, nitrogenous compounds, macrolides, prostaglandins and fatty acid derivatives, sterols, antimicrobial peptides (AMPs), miscellaneous compounds and alkaloids; they all presented specific types of activities (Balcazar et al., 2006; Destoumieux et al., 1997; Jones, 1971). The presence of antimicrobial activity in Mollusca has been reported from the mucus of the giant snail *Achatina fulica* (Kubota et al., 1985; Iguchi et al., 1982) from the egg mass and purple fluid of the sea hare, *Aplysia kurodai* (Yamazaki, 1993; Kamiya et al., 1984) and from the body wall of the sea hare *Dolabella auricularia* (Iijima et al., 2003).

Among marine invertebrates, cephalopods belong to a molluscan group comprising of 700 species in which bacterial associations have been known for a long time (Pierantoni, 1917; Bloodgood, 1977; Romanenko et al., 1995) which include the reproductive organs (accessory nidamental glands) of myopsids, sepiolids and sepiids (Kaufman et al., 1998; Grigioni et al., 2000; Pichon et al., 2005) and the light organ of sepiolids (McFall-Ngai and Ruby, 1991; Nishiguchi, 2002) ink extracts of *D. auricularia*, *O. vulgaris* and *S. aculeata* (Vennila et al., 2011) polysaccharide extract of *Sepia aculeata* and *Sepia brevimana* and heparin and heparin-like glycosaminoglycans (GAGs) from the cephalopod *Euprymna berryi* was reported against the human pathogenic microorganisms (Shanmugam et al., 2008a, b). Antibiotics are one of the most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction.

However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illness not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria. It is essential to investigate newer drugs with lesser resistance. In most of the findings concerning antimicrobial activity in molluscs, either single body compound alone, like haemolymph and egg masses, or extracts of whole body tissues have been tested for activity. The present study has been focused on the antibacterial activity of methanolic extract of the whole body tissue and also EDTA extract (polysaccharides) from the cuttlebone of pharaoh cuttlefish (*Sepia pharaonis*) on ten important clinically isolated human pathogenic bacteria.

MATERIALS AND METHODS

Sampling and identification

The cuttlefish, *S. pharaonis* was obtained from Thengaithittu landing centre of Puducherry (Lat.11° 54' 44" N; Long. 79° 49' 13" E), South-east coast of India. The publications of Roper et al. (1984), Jothinayagam (1987) and Shanmugam et al. (2002) were used for identification.

Preparation of methanolic extract from body tissues

The methanolic extract of the body tissues was prepared by following the method of Ely et al. (2004). *S. pharaonis* was brought to the laboratory; skin, visceral organs, cuttlebone and ink sac were removed. Remaining edible body tissues were separated and cut into small pieces and homogenized (REMI, RQ-127 A) and extracted with 100% methanol for 24 - 48 h by incubating at room temperature. Then, the methanolic extract was centrifuged to collect the supernatant and concentrated under vacuum in a rotary evaporator (LARK, Model: VC- 100A). The crude methanolic extract of whole body tissue was assayed for antibacterial activity using standard disc diffusion method.

Preparation of EDTA extract from cuttlebone

The EDTA extract was obtained from the internal shell (cuttlebone) of *S. pharaonis* by following the method of Okutani and Morikawa (1978). The air-dried cuttlebones were pulverized and washed with acetone. The powder was extracted with hot 10 mM EDTA solution and filtered with Whatman No. 1 filter paper with hyflosuper cel. Then saturated barium hydroxide solution was added to the filtrate. The precipitate obtained after standing overnight was collected on a filter paper (Whatman No.1) with hyflosuper cel and washed with distilled water. The precipitate was dissolved in 10 mM EDTA solution and was dialyzed against deionised water. The dialyzate solution present in the dialysis membrane was then freeze-dried and a pure white coloured powder was obtained. The lyophilized powder was used for assaying the antibacterial activities.

Microbial cultures

Ten bacterial species including Gram positive species (*Staphylococcus aureus* and *Streptococcus pyogenes*) and Gram negative species (*Salmonella typhi*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Klebsiella oxytoca*, *Escherichia coli*, *Salmonella paratyphi*, *Vibrio parahaemolyticus* and *Proteus mirabilis*) were used as test organisms. All the bacterial species were clinical isolates, obtained from Raja Muthiah Medical College Hospital, Annamalai University, Annamalai Nagar, South India.

Preparation of inoculum

Nutrient broth was prepared and sterilized in an autoclave at 15 lbs pressure for 15 min. All the ten bacterial strains were individually inoculated in the sterilized nutrient broth and incubated at 37°C for 24 h. Mueller Hinton Agar (MHA, Himedia) was prepared, sterilized in an autoclave at 15 lbs for 15 min pressure and poured into sterile Petri dishes and incubated at 37°C for 24 h. The 24 h-old bacterial broth cultures were inoculated in the Petri dishes by using a sterile cotton swab.

Antibacterial assay

In vitro antibacterial activity was determined by disc diffusion

technique of Gunthorpe and Cameron (1987). The stocks for methanolic extracts were prepared in the concentration of 100 mg/ml. 60 mg of lyophilized cuttlebone powder of crude extract was dissolved in 0.6 ml of solvent (10 mM EDTA) to prepare stock solution. From this 0.24, 0.18, 0.12 and 0.06 ml of sample was taken and each was made up to 0.24 ml with respective (10 mM EDTA) solvent. The control with respective solvent (10 mM EDTA) was also prepared. These different concentrations (0.24, 0.18, 0.12 and 0.06 mL known as 100, 75, 50 and 25% respectively) of the extract were applied to 6 mm sterile disc, allowed to dry at room temperature and placed on agar plate seeded with bacterial strains. Positive control disc containing 50 μ l of tetracycline (1 mg/ml) and as negative control containing 50 μ L of methanol and 10 mM EDTA each were used. These impregnated discs were allowed to dry at laminar air flow chamber for 3 h, and were placed at the respective bacterial plates and incubated at 37°C for 24 h. Result was calculated by measuring the zone of inhibition in millimeters. Each extract was tested thrice for the confirmation of activity.

Determination of the minimum inhibitory concentration (MIC)

The methanolic extract and EDTA (polysaccharide) extract that showed significant antibacterial activity was selected for the determination of MIC followed by the turbidimetric method of Rajendran and Ramakrishnan (2009). A stock solution of 100 μ g/mL⁻¹ was prepared and was serially diluted to obtain various ranges of concentrations between 20 and 100 μ g/mL⁻¹. To 0.5 mL of each of the dilutions of different concentrations was transferred into sterile test tube containing 2.0 mL of nutrient broth. To the test tubes, 0.5 mL of test organism previously adjusted to a concentration of 10⁵ cells /mL⁻¹ was then introduced. A set of test tubes containing broth alone was used as control. All the test tubes and control were then incubated at 37°C for 24 h. After the period of incubation, the tubes were studied for visible signs of growth or turbidity. The lowest concentration of methanolic and EDTA extract that inhibited the growth of bacteria was taken as the minimum inhibitory concentration. All assays were carried out in triplicates and the control test was carried out with the broth alone.

Statistical analysis

Data on the inhibitory effect of methanolic extract and the EDTA extract of *S. pharaonis* were analyzed by one-way analysis of variance (ANOVA) using SPSS-16 version software followed by Duncun's multiple range test (DMRT). *P* values <0.05 were considered as significant.

RESULTS

The methanolic extract of whole body tissue and the EDTA extract of polysaccharides from cuttlebone of *S. pharaonis* showed wider activity against pathogenic organisms. In general, the activity was higher in 100% concentration and lower in 25% concentration but activity was totally absent in negative control whereas the positive control showed activity against all pathogenic organisms (Table 1).

In 100% concentration, the maximum inhibition zone was observed against *P. mirabilis* (18.3 \pm 0.1mm) for methanolic extract and against *S. pyogenes* (15.5 \pm 0.06mm) for EDTA extract of polysaccharide. The minimum inhibition zone of 9.9 \pm 0.36mm was recorded against *S. aureus* for methanolic

extract and against 8.3 \pm 0.12 mm *V. parahaemolyticus* for EDTA extract; 75% concentration of methanolic extract showed highest activity against *P. mirabilis* (16.5 \pm 0.06 mm), and against *S. pyogenes* (15.47 \pm 0.12 mm) for EDTA extract. The lowest activity against *S. aureus* was 8.9 \pm 0.36 mm for methanolic extract and 7.4 \pm 0.17 mm against *V. parahaemolyticus* for EDTA extract. Whereas, in 50% concentration of methanolic extract, highest activity of 13.8 \pm 0.06 mm was found against *P. mirabilis*, and 14.5 \pm 0.21 mm against *S. pyogenes* for EDTA extract. The lowest activity of 8.3 \pm 0.12 mm was recorded against *S. aureus* for methanolic extract and 9.23 \pm 0.06 mm against *S. typhi* for polysaccharides; for 25% concentration, the methanolic extract showed maximum activity of 12.47 \pm 0.06 mm against *P. mirabilis*, and 13.27 \pm 0.2mm for EDTA extract against *S. pyogenes*. The minimum activity was 7.9 \pm 0.36 mm against *S. aureus* for methanolic extract and 7.2 \pm 0.2 mm against *S. typhi* for EDTA extract.

There was no activity in all concentrations for methanolic extract against *S. paratyphi* and *V. parahaemolyticus* and for EDTA extract against *K. pneumoniae*.

MIC of the active extract against the test organisms

The MIC results found ranging from 40 and 100 mg/ml are given in Tables 2 and 3. MIC values for methanolic extract of *S. pharaonis* against bacterial species such *S. aureus*, *K. pneumoniae*, *S. typhi*, *V. cholerae*, *K. oxytoca*, *E. coli*, *P. mirabilis* and *S. pyogenes* showed 100, 100, 80, 80, 60, 100, 60 and 80 mg/ml respectively. Whereas for EDTA extract of polysaccharides, the MIC values were found to be 100, 80, 80, 80, 100, 80, 60 and 40 mg/ml against bacterial species such *S. aureus*, *S. typhi*, *V. cholerae*, *K. oxytoca*, *E. coli*, *S. paratyphi*, *P. mirabilis* and *S. pyogenes*, respectively.

DISCUSSION

This study dealt with the antibacterial activity of crude methanolic extract of a whole body tissue and EDTA extract (polysaccharides) of cuttlebone of *S. pharaonis* and compared them with different concentrations (0.24, 0.18, 0.12, and 0.06 mL of samples made as 100, 75, 50, and 25% concentration, respectively). The activity was recorded for both extracts in all the four concentrations for majority of bacterial species. The effects of the extracts were different for different bacterial species.

Many studies on bioactive compounds from mollusks exhibiting antitumor, antileukemic, antibacterial and antiviral activities have been reported worldwide (Hochlowski et al., 1983). Antibacterial activity has previously been described in a wide range of molluscan species such as oyster (*Crassostrea virginica*), mussel (*Mytilus edulis* and *Geukensia demissa*), muricid mollusks (*Dicathais orbita*) and sea hare (*Dolabella auricularia*) (Anderson and Beaven, 2001; Benkendorff et al., 2001; Gunthorpe and Cameron,

Table 1. Zone of inhibition showed by the methanolic extract of whole body tissue and EDTA extract (polysaccharides) from cuttlebone of *S. pharaonis*.

Name of the species/concentration	Methanolic extract zone of inhibition (mm)				EDTA extract-polysaccharides zone of inhibition (mm)				Positive (tetracycline) (mm)
	25	50	75	100	25	50	75	100	
<i>S. aureus</i>	7.9±0.36	8.9±0.2	8.9±0.36	9.9±0.36	11±1	9.96±0.45	11.2±0.25	12±0.3	24.55±0.14
<i>K. pneumoniae</i>	9.2±0.2	10.3±0.12	11.1±0.15	12.2±0.2	-	-	-	-	21.11±0.22
<i>S. typhi</i>	10±0.2	10.1±0.36	12.23±0.25	13±0.2	7.2±0.2	9.23±0.06	10.17±0.15	10.27±0.16	20±0.12
<i>V. cholera</i>	10.27±0.12	12.23±0.15	12.47±0.06	16.4±0.27	7.4±0.06	9.3±0.1	10.27±0.12	10.73±0.12	24.22±0.26
<i>K. oxytoca</i>	10.5±0.1	13.4±0.2	15±0.2	17.6±0.1	12.5±0.1	12.6±0.2	13.6±0.1	13.7±0.12	26.31±0.56
<i>S. paratyphi</i>	-	-	-	-	8.2±0.13	9.3±0.1	10.2±0.12	11.3±0.12	22.44±0.21
<i>E. coli</i>	9.5±0.06	10.7±0.1	12.6±0.06	13.37±0.02	10.2±0.12	10.73±0.12	11.1±0.16	11.3±0.12	21.11±0.11
<i>P. mirabilis</i>	12.47±0.06	13.8±0.06	16.±0.06	18.3±0.1	12.6±0.06	12.6±0.06	13.3±0.02	14.2±0.12	25.62±0.31
<i>V. parahaemolyticus</i>	-	-	-	-	-	-	7.4±0.17	8.3±0.12	20.34±0.22
<i>S. pyogenes</i>	10.47±0.12	11.4±0.2	12.5±0.06	15.3±0.1	13.27±0.21	14.5±0.21	15.47±0.12	15.5±0.06	24.12±0.16

*The statistical significance: P values ≤0.05 (DMRT).

Table 2. MIC of the methanolic extract of whole body tissue from *S. pharaonis*.

Name of the species	Concentrations (%) / activity							
	100	80	60	40	20	+ve	-ve	Normal
<i>S. aureus</i>	*	+	++	+++	+++	-	+++	Nil
<i>K. pneumoniae</i>	*	+	++	+++	+++	-	+++	Nil
<i>S. typhi</i>	-	*	+	++	+++	-	+++	Nil
<i>V. cholera</i>	-	*	+	++	+++	-	+++	Nil
<i>K. oxytoca</i>	-	-	*	+	++	-	+++	Nil
<i>E. coli</i>	*	+	++	+++	+++	-	+++	Nil
<i>P. mirabilis</i>	-	-	*	+	++	-	+++	Nil
<i>S. pyogenes</i>	-	*	+	++	+++	-	+++	Nil

*MIC concentration, -No growth, +Cloudy solution (slight growth), ++Turbid solution (strong growth), +++Highly turbid solution (dense growth).

1987; Prem Anand et al.,1997). Antibacterial peptides have been isolated and characterized from the hemocytes of *M. edulis* (Charlet et al., 1996) and from sea hare, *D. auricularia* (Iijima et al., 2003). Patterson and Murugan (2000) spectrum

of antibacterial activity for aqueous ink extract reported broad of the cephalopods *L. duvacei* and *S. pharaonis* against nine human pathogens.

However, majority of marine organisms are yet to be screened for discovering useful antibiotics.

The hypobranchial gland extracts of *Chicoreus ramosus* was found inhibiting the growth of ten bacterial strains; out of this, the broad inhibition zone was formed against *Streptococcus faecalis* and *S. aureus* (Emerson and Ayyakkannu, 1992b).

Table 3. MIC of the EDTA (polysaccharides) extract of the cuttlebone from *S. pharaonis*.

Name of the species	Concentrations (%) / activity							Normal
	100	80	60	40	20	+ve	-ve	
<i>S. aureus</i>	*	+	++	+++	+++	-	+++	Nil
<i>S. typhi</i>	-	*	+	++	+++	-	+++	Nil
<i>V. cholera</i>	-	*	+	++	+++	-	+++	Nil
<i>K. oxytoca</i>	-	*	+	++	+++	-	+++	Nil
<i>E. coli</i>	*	+	++	+++	+++	-	+++	Nil
<i>S. paratyphi</i>	-	*	+	++	+++	-	+++	Nil
<i>P. mirabilis</i>	-	-	*	+	++	-	+++	Nil
<i>S. pyogenes</i>	-	-	-	*	+	-	+++	Nil

*MIC concentration, -No growth, +Cloudy solution (slight growth), ++Turbid solution (strong growth), +++Highly turbid solution (dense growth).

In the broad spectrum (7 species of gastropods, 1 bivalve and 5 cephalopods) study of Rajaganapathy (2001), the methanol and the saline extracts of ink gland, salivary gland, body mucus and internal shell of cephalopods such as *Loligo duvaucelli*, *S. pharaonis*, *Sepeilla inermis*, *Octopus dollfusi* and *Cistopus indicus* recorded varying antibacterial activity against different bacterial strains viz., *B. subtilis*, *E. coli*, *K. pneumoniae*, *P. vulgaris*, *P. mirabilis*, *S. typhi*, *S. flexnari*, *S. faecalis* and *V. cholerae*. All the cephalopod extracts exhibited activity against at least three bacteria and the highest activity of 10.5 mm was recorded in the ink gland extracts against *P. mirabilis*. The saline extract of salivary gland and the methanol extracts of body mucus of *L. duvauceli*, *S. pharaonis*, *S. inermis*, *O. dollfusi* and *C. indicus* showed significant activities against *K. pneumoniae*, *B. subtilis*, *S. flexnari*, *S. typhi* and *S. faecalis* with the maximum activity (8 mm) recorded in the salivary gland extracts against *S. typhi*. The body mucus extracts were reported to have promising activities against *S. typhi* with the inhibition zones measuring as high as 6 mm (Rajaganapathy, 2001). The maximum zone of inhibition (19 mm) in antibacterial activity from the gill extraction of *Perna viridis*, against *S. aureus* and minimum activity (11 mm) was observed against *S. paratyphi* (Chandran et al., 2009).

So far, there are only a few studies carried out on the antibacterial activity of the internal bone of cephalopods. Barwin Vino (2003) for EDTA extract (polysaccharides) of *Doryteuthis sibogae* gladius recorded 10 mm inhibition zone against *E. coli* and *K. pneumoniae*, 9 mm inhibition zone against *S. aureus* and 7 mm against *S. typhi*. Whereas the EDTA extract of *L. duvauceli* extract showed only low activity, that is, 5 mm against *P. aeruginosa*, 4 mm against *S. typhi* and *E. coli*. At the same time, the gladius extract of both species showed no activity against *V. cholerae*. The polysaccharide extract from the gladius of *D. sibogae* recorded potent antibacterial activity against the bacterial strains mentioned above and at the same time the polysaccharide of *L. duvauceli* gladius extract recorded only low activity. Further, the methanol extracts

of the cuttlebone of *S. pharaonis* showed activity against *S. flexnari* (5 mm), *S. faecalis* and *V. cholerae* (4.5 mm) and *S. typhi* (3.5 mm); whereas *S. inermis* extracts of cuttlebone showed activity only against *K. pneumoniae* and *V. cholerae* (3.5 mm). Such similar activities were found only in 50, 75 and 100% concentrations of *S. aculeata*, but the *Sepia brevimana* extracts showed highest activity against all the strains at all concentrations (Mahalakshmi, 2003). In comparison, the activity was predominant in the cuttlebone extract of *S. aculeata* than *S. brevimana* (Shanmugam et al., 2008b).

Ramasamy et al. (2011a) screened the antimicrobial activity of polysaccharide from cuttlebone and methanolic extract from body tissue of *Sepia prashadi*. The antibacterial activity was predominant in cuttlebone extracts (using EDTA) of the cuttlefish, (*S. prashadi*) against almost all the 10 pathogenic bacterial species tested viz., *E. coli*, *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, *V. cholerae*, *V. parahaemolyticus*, *V. alginolyticus*, *Streptococcus* sp., *S. pneumoniae* and *Salmonella* sp. The activity was recorded in almost all the concentrations except in negative control. The highest inhibition zone of 13 mm was recorded against *V. parahaemolyticus* in polysaccharide extract and 13 mm inhibition zone was recorded against *S. aureus* in methanolic extract. Further Ramasamy et al. (2011b) assayed the antimicrobial activity (*in vitro*) of methanolic extracts from *S. inermis*, *S. kobeensis*, *S. lessoniana*, *O. aegina*, *O. dollfusi* and *O. aerolatus* against 10 bacterial species and fungal strains and reported good (10-15 mm diameter) microbial activity was seen in the extracts of *S. inermis*, *S. lessoniana* and *O. dollfusi* which indicates the presence of potent antimicrobial compounds in them. Similarly, Vairamani et al. (2012) studied the antibacterial activity of polysaccharide from cuttlebone and methanolic extract from body tissue of *S. inermis* against 9 bacterial strains and found activity against 8 strains except *Vibrio alginolyticus*.

In the present investigation, the crude methanolic extract from the whole body tissue and the EDTA extract of polysaccharides from cuttlebone of *S. pharaonis* were used

to study the antibacterial activity against selected human pathogens. The methanolic extract showed prominent antibacterial activity against eight bacterial species except *S. paratyphi* and *V. parahaemolyticus* with the activity ranging from 7.9 ± 0.36 mm of inhibition zone against *S. aureus* (25% concentration) to 18.3 ± 0.1 mm against *P. mirabilis* (100% concentration). Among the tested ten species highest inhibition zone of activity (for all four concentrations) was found against *P. mirabilis*; also it was found noteworthy in ascending order (12.47 ± 0.06 for 25%; 13.8 ± 0.06 for 50%; 16.5 ± 0.06 for 75% and 18.3 ± 0.1 for 100%); for the lowest inhibition activity similar ascending attitude was followed against *S. aureus* (7.9 ± 0.36 for 25%; 8.3 ± 0.12 for 50%; 8.9 ± 0.36 for 75% and 9.9 ± 0.36 for 100% concentration).

Further the EDTA extract showed good activity against nine bacterial strains tested, except *K. pneumoniae* with the activity ranging from 7.2 ± 0.2 mm against *S. typhi* (25% concentration) to 15.5 ± 0.06 mm against *S. pyogenes* (100% concentration). Similarly, high inhibition zone of activity in ascending manner (for all four concentrations) was recorded against *S. pyogenes* (13.27 ± 0.21 for 25%; 14.5 ± 0.21 for 50%; 15.47 ± 0.12 for 75% and 15.5 ± 0.06 for 100%), also low inhibition activity against *S. typhi* and *V. parahaemolyticus* was found. In general, the increased concentration showed increase in the activity of the extracts (Shanmugam et al., 2008b).

Emerson and Ayyakkannu (1992a, b), Shanmugam et al. (2008b) and Ramasamy et al. (2011a) reported a broad spectral activity in the hypobranchial gland extract of *Chicoreus ramosus* against 10 bacterial species; good activity of the cuttlebone (EDTA) extract of *S. aculeata* and *S. brevimana* and *S. prashadi* against all bacterial strains. Although different species and experimental procedures were used in the different studies, they indicated the high frequency of detectable antibacterial activity in marine molluscs. However, there exists a difference in the activity shown by the compounds present in the extracts in laboratory studies and natural environments, which may be due to their varying concentration present in the extracts used in both places (Kelman et al., 2006; Mirnijas et al., 2011).

When compared with the above mentioned studies, whole body tissue methanolic extract as well as cuttlebone EDTA extract (polysaccharide) exhibited better activity and the activity was found dose dependent. The effect of extracts was different with different bacterial strains. The level of activity measured by disc diffusion assay is dependent on both the rate of diffusion of extract into the agar and the potency of extract. The deference in response may be due to species-specific characteristics. Extracts that contain highly active compounds (more potent), but have physical properties that generate a lower diffusion rate, may reappear to have low activity in the assay (Kelman et al., 2006). Further, the dose as well as the concentration of the active principle in the extract shows either 'bactericidal' or 'bacteriostatical' effects shows either 'bactericidal' or 'bacteriostatical' effects against the

bacteria (Zarakolu et al., 1999).

Conclusion

The present study of *S. pharaonis*, exhibiting a wide spectral antibacterial activity has been recorded in both extracts, as compared to the studies of Emerson and Ayyakkannu (1992a), Shanmugam et al. (2008b) and Ramasamy et al. (2011a). It also revealed that the cuttlebone, thrown as a waste in the landing centers and processing plants, might be used for the extraction of polysaccharides that have antibacterial activity. The activity was found dose dependent and supporting the presence of bioactive principle. Further investigation on the purification and chemical elucidation of the bioactive principles present shall pave the way for the development of either the base or a new drug itself in the future.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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