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RESEARCH ARTICLE

A STUDY ON ANTIMICROBIAL ACTIVITY OF THE INTERNAL SHELL OF *L.DUVAUCELI* AND *S.PHARAONIS* FROM THOOTHUKUDI COASTAL REGION

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ABSTRACT

Antimicrobial activity of the methanol extracts from the internal shell of *L. duvauceli* and *S. pharaonis* were tested against eight pathogenic bacteria namely *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Aeromonoas hydrophila, Streptococcus pyogenes, Vibrio fischeri, Klebsiella pneumonia, Escherichia coli* and one fungal strain *Candida albicans* by well diffusion method. The methanol extracts of *Loligo duvauceli* was found to be effective against *Streptococcus pyogenes*, *Klebsiella pneumonia*, *Aeromonoas hydrophila, Vibrio fischeri*, *Escherichia coli* and *Candida albicans*. The cuttle bone extract showed maximum antibacterial activity (19mm) against *Aeromonoas hydrophila* and *Escherichia coli* and a minimum activity of 15mm was recorded against *Streptococcus pyogenes*. The results suggested that the antimicrobial activity was higher in the bone extract of *S.pharaonis* than *L. duvauceli.* A correct understanding and utilization may lead to use this waste material as a valuable pharmaceutical agent and can control pathogenic bacteria which cause dreadful human diseases.

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INTRODUCTION

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health of human life since their introduction (Sarkar *et al.,* 2003). However, over the past few decades these health benefits are under threat due to the careless and indiscriminate use of antibiotics and the emergence of drug resistant bacteria. The multiple resistance mechanisms have severely limited the use of many classes of drugs. Hence a search for novel antimicrobial drugs with therapeutic potential for which the pathogens may not have developed resistance is the need of the hour (Patil *et al.,* 2001). In recent years the marine environment has been looked as a possible source of products for human or animal medicine. Many organisms from these environments produce a variety of metabolites, some of which can be used for drug development (Chellaram and Prem Anand 2010). Studies on antimicrobial compounds of marine invertebrates may provide valuable information for new antibiotic discoveries and give new insights into bioactive compounds in molluscs. Among marine invertebrates, cephalopods are considerably important as a food resource as well as a source to be considered in the discovery of new substances for drug development. Background research has shown that the ink, accessory nidamental gland, and body tissue possesses a wide range of biological role such as antibacterial (Nirmalae *et al.,* 2002, Venilla *et al*., 2011., Smiline Girija *et al.,* 2011.,), antioxidant (Lei *et al.,* 2007 and Ilamparithi et al., 2011), anticancer (Rajaganapathi *et al.,* 2000. Naraoka *et al.,* 2000, Zhong *et al.,* 2009 and Chen *et al*., 2010, and immunity promotion (Guan *et al.,* 2010).

Cuttlefish bone refers to the internal cartilaginous shell of the cuttlefish, squid and octopus. Cuttlefish bone is typically smooth and white, with small, wavy lines or ridges. Powdered cuttlebone is a good source of food for poultry and cage birds, as it is a rich source of calcium. Traditionally cuttlebone powder is used as a medicine for some ear ailments (Trivedi and Sarvaiya, 1976, Raje and Singh, 1992). Its functions are to stop bleeding, harmonize the stomach and improve kidney efficiency. Some patients with stomach problems may take it to combat acid reflux disease and some intestinal disorders.

Only very few studies have been carried out on the antimicrobial activity of the internal shell of cephalopod. Keeping this in mind, the present study has been designed to assess the antimicrobial activity of the internal bone of squid and cuttlefish.

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MATERIALS AND METHODS

Collection and Preparation of extract

In the present study the animals (*L.duvaucelii and S.pharaonis*) were collected from Gulf of Mannar, Thoothukudi coastal region (Long 78^0 8" to 79^0 30" E and Lat 8^0 35" to 9^0 25" N) by trawl catch, brought to the laboratory, cleaned and washed with fresh sea water to remove all impurities. The internal shells were dissected, washed, air dried and pulverized. 10g of pulverized bone powder was mixed with 100ml of methanol solvent and kept in rotary shaker at 100rpm overnight and filtered with Whatman No.1 filter paper and concentrated to dryness at 40^0C, lyophilized and stored at 4 ^{0}C until further use.

Microbial cultures

Eight bacterial strains (*Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis (*MTCC 441*)*, *Pseudomonas aeruginosa (*ATCC 27853*)*, *Aeromonoas hydrophila, Streptococcus pyogenes, Vibrio fischeri, Klebsiella pneumonia (*ATCC 15380*)*, *Escherichia coli (*ATCC 25922) and one fungal strain *Candida albicans* (MTCC 227*)* were used for antimicrobial activities. (All the bacterial and fungal strains were clinical isolates, obtained from the Madras Medical College, Chennai).

Inoculum preparation for bacteria

Nutrient broth was prepared and sterilized in an autoclave at 15lbs pressure for 15 minutes. All the eight bacterial strains were individually inoculated in the sterilized nutrient broth and incubated at 37^0c for 24hour. Nutrient agar (Himedia) was prepared, sterilized in an autoclave at 15lbs pressure for 15 minutes and poured into sterile petridishes and were left at room temperature for solidification. The 24 hour old bacterial broth cultures were inoculated in the petridishes using a sterile cotton swab.

Inoculum preparation for fungi

Sabouraud Agar (Himedia) broth was prepared and sterilized in an autoclave at 15lbs pressure for 15 minutes. The sterilized sabouraud agar was poured into sterile petridishes and incubated at 37^0c for three days. The fungal strain *Candida albicans* was inoculated in the broth using a sterile cotton swab and incubated at 37^0C for 72 hours.

Antimicrobial assay

The antibacterial and antifungal activity was carried out by following agar well diffusion method (Perez *et al*., 1990). Each plate, a single well of 6mm diameter was made using a sterile borer. The extracts were freshly reconstituted with suitable solvents and was introduced into the well using a micropipette. The plates were incubated overnight at 37^0C. Microbial growth was determined by measuring the diameter of the zone of inhibition. For each strain a control was also maintained where pure solvents were used instead of extract. The experiment was done three times for confirmation of activity and the mean values are presented.

RESULTS

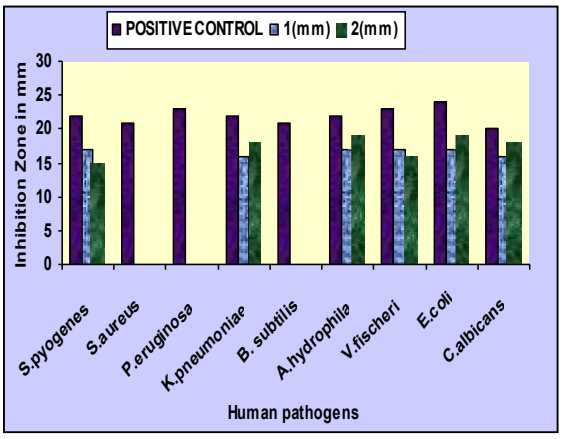
The antimicrobial activity of the internal bone from *L.duvauceli* and *S.pharaonis* are presented in Fig -1.

Methanol extract from the internal shell of *L.duvauceli*

Methanol extract from the internal shell of *L.duvauceli* showed activity against several bacteria and the range was 16 to 17 mm. Maximum antibacterial activity (17mm) was recorded against *Streptococcus pyogenes, Aeromonoas hydrophila, Vibrio fischeri, Escherichia coli,* and a minimum (16mm) was against *Klebsiella pneumonia.* The fungal strain *Candida albicans* showed a inhibition zone of 16mm.

Methanol extract from the internal shell of *S.pharaonis*

The methanol extracts from internal shell of *S. pharaonis* showed activity varied between 15mm to 19mm. Maximum inhibition zone (19mm) was recorded against *Aeromonoas hydrophila, Escherichia coli* and a minimum zone (15mm) was developed against *Streptococcus pyogenes.* The extract showed no activity against *Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The extract developed an inhibition zone of 18mm against *Candida albicans*. Among the methanolic extracts of the two species tested *S.pharaonis* showed more susceptibility than *L.duvauceli.*



1– Methanol fraction of *L. duvauceli*
2– Methanol fraction of *S. pharaonis*

Figure 1. Antimicrobial activity - zone of inhibition for *L.duvauceli* and *S. pharaonis*

DISCUSSION

In the present study internal shell of *L.duvauceli* showed maimum activity of 17mm against *Aeromonoas hydrophila, Vibrio fischeri, Streptococcus pyogenes* and *E.coli* and a minimum zone of 16mm against *Klebsiella pneumonia*. Similar study was carried out by Barwin Vino *et al.,* 2014 in the crude extract of *Doryteuthis sibogae* and *L.duvauceli* gladius against nine species (B. *subtilis, E.coli, K.pnemoniae, S.aureus, V.parahaemolyticus, V.cholerae, S.typhii, P.aeroginosa* and *Shigella sp*) of clinically isolated human pathogenic bacteria and four fungal strains (*Candida sp., Rhizopus sp., A.fumigatus* and *A.flavus*) at four different concentrations.

Polysaccharide extract of *D.sibogae* showed activity against eight strains except *V.cholerae* but *L. duvauceli* showed activity against 7 bacterial strains except *V. Cholera* and *B. subtilis*. Both extracts showed no activity against *Candida sp*.whereas in the present study *L.duvaucelii* developed an inhibition zone of 16mm against the fungal strain *Candida albicans.*

Shanmugam *et al*. (2008a) studied the antibacterial activity in the cuttlebone extract of *S.aculeata and S.brevimana* against nine pathogenic bacterial strains viz., *B. subtilis, E.coli, K.pnemoniae, S.aureus, V.parahaemolyticus, V.cholerae, S.typhii, P.aeroginosa* and *Shigella sp.* The activity was recorded in almost all the concentrations except in negative control. Further the EDTA extract showed good activity against four fungal pathogens (*Candida sp., Rhizopus sp., A.fumigatus* and *A.flavus*) at different concentrations. *S.aculeata* recorded a inhibition zone of 12mm against *A.flavus* and *Candida species* whereas *S.brevimana* showed no activity against *candida albicans*. The activity was predominant in the cuttlebone extract of *S.aculeata* than *S.brevimana*. Similarly in the present investigation the cuttlebone extract of *S.pharaonis* showed maximum activity against *A.hydrophila* and *E.coli* (19mm) and a minimum of 16 mm zone against *S.pyogenes* (15mm). The extract developed an inhibition zone of 18 mm against the fungal strain *C.albicans*

Ramasamy *et al.,* 2011 reported the antimicrobial activity of polysaccharide from cuttlebone and methanolic extract from body tissue of *Sepia prashadi*. The highest inhibition zone of 13mm was recorded against *V. parahaemolyticus* in polysaccharide extract. Vairamani *et al.,* 2012 evaluated the antimicrobial potency of methanolic extract of whole body tissue of *Sepiella inermis* and EDTA extract of cuttlebone against ten human pathogens at different concentrations. EDTA extract showed highest activity of 11mm against *E.coli* . The extracts have good antimicrobial activity depending on the concentration. Jeyalakshmi *et al.,* 2014 studied the EDTA extract of cuttlebone of *Sepia pharaonis* against ten bacterial species and observed the maximum inhibition zone of 15.5 for 100% concentration. This findings coincide with the present investigation and at the same time the zone of inhibition was relatively higher than that of the previous study.

Conclusion

In the present study, the methanol extract from the internal bone of squid and cuttlefish showed promising antibacterial and antifungal activity against the human pathogenic strains. The results enforces the idea that cephalopod bone are a source to be considered in the discovery of drug development to control microbial diseases.

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