

Note

Antibacterial activity in the extracts of accessory nidamental gland of the Palk Bay squid *Sepioteuthis lessoniana* (Lesson, 1830) (Cephalopoda: Decapoda)

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ABSTRACT

The present study illustrates antibacterial activity from the accessory nidamental glands (ANGs) of Palk Bay squid, *Sepioteuthis lessoniana* (Lesson, 1830). ANGs from squids at different maturity stages (immature, maturing, ripe and spent) were used for extraction using various solvents viz., acetone: alcohol, ethanol, butanol and methanol. The antibacterial activity of the extracts was evaluated by disc-diffusion method, using four pathogenic strains of bacteria (*Escherichia coli*, *Aeromonas hydrophila*, *Staphylococcus aureus* and *Bacillus megaterium*). The extracts from different maturity stages (maturing, ripe and spent) showed different levels of antibacterial activity against the tested bacterial strains, except in *B. megaterium*. The immature stage ANG-extracts did not exhibit any antibacterial activity while the other stages showed pronounced activity. Among the four extracts, butanol extract showed the maximum antibacterial activity, followed by methanol extract. Maximum antibacterial activity was found in ripe stage, especially in butanol extract against *E. coli* (10.1 ± 1.65 mm) and minimum activity was found in ethanol extract against *A. hydrophila* (3.0 ± 0.94 mm) in the maturing stage.

Keywords: Accessory nidamental glands, Antibacterial activity, Palk Bay, *Sepioteuthis lessoniana*

The accessory nidamental gland (ANG), is an organ in the reproductive system of females of certain cephalopod taxa (loliginids, sepiids and sepiolids) associated with egg laying. ANGs are located at the anterior end of the nidamental glands (NG) forming the nidamental gland complex (Fig. 1). Antimicrobial activity of ANG extracts from sepiids viz., *Sepiella inermis*, *Sepia aculeata* and *Sepia pharaonis* (Sherief *et al.*, 2004) and from loliginids viz., *Loligo pealei* (Barbieri *et al.*, 1997) and *Loligo duvaucelii* (Gomathi *et al.*, 2010) have been reported earlier.

S. lessoniana belonging to the family Loliginidae, is a commercially important species distributed in the Indo-Pacific region. The colour of the ANGs in this squid changes from colourless to white in young female and in sexually mature animals it changes to yellow, orange (sub-adults) and finally to intense orange red. After spawning, the colour fades away to pink (Silas *et al.*, 1982). The present study investigates potential antibacterial activity from the ANG extracts of the squid *S. lessoniana*.

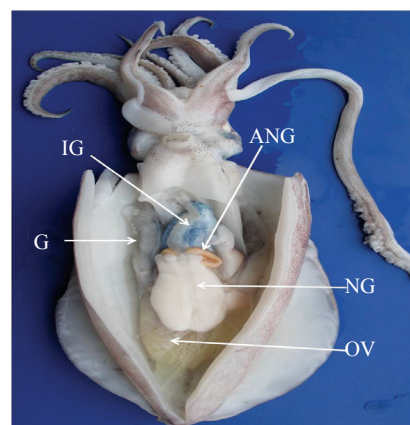


Fig. 1. *Sepioteuthis lessoniana* -Viscera (IG – Ink gland; ANG : Accessory nidamental Gland; NG: Nidamental Gland; G : Gill; OV : Ovary)

Females of *S. lessoniana* at different maturity stages (immature, maturing, ripe and spent) were collected from the Rameswaram Fish Landing Centre (lat. 09°16'52.3" N and long.79°18'54.3" E), south-east coast of India

during January - December 2009. The animals were dissected, their ANGs were removed aseptically and the colour recorded. The ANGs were surface sterilised and the extraction was done in four different solvents, such as acetone: alcohol (7:2 v/v), ethanol, methanol and butanol. About 3.5 g of tissue was extracted with 10 ml of each solvent and the extract was centrifuged at 10,000 rpm for 20 min at 4°C and the clear supernatant was used immediately for the antibacterial assay. The antibacterial activity was tested by disc diffusion technique (Collins and Lyne, 1967). Four species of pathogenic bacteria *viz.*, *Escherichia coli*, *Aeromonas hydrophila*, *Staphylococcus aureus* and *Bacillus megaterium*, were used to screen the antibacterial activity of the ANG extracts. Well isolated colonies of pathogenic bacterial strains were inoculated in sterile nutrient broth and incubated at 37°C for 24 h. Pathogens were swabbed on the surface of Muller Hinton agar plates and sterile filter paper discs (Whatman No. 1), of 6 mm dia impregnated with 250 µl of ANG extracts were placed on the surface. The activity was measured as the diameter of the inhibition zone in mm.

The organic solvent extracts prepared from the ANGs of *S. lessoniana* exhibited weak to pronounced antibacterial activity in accordance with the maturity phases of the animals (Table 1). In immature stage, ANG-extracts did not show any activity against the tested pathogens whereas in ripe stage, it showed good antibacterial activity. ANG of *S. lessoniana* was virtually rudiment and colourless in the sexually undifferentiated immature individuals while it was found well developed during maturing stage onwards. Because of poor development of ANG in immature stage,

the extracts did not show antibacterial activity against the tested pathogens. The activity was correlated well with ripe stage of females collected during the peak breeding season. Antibacterial activity was moderate in maturing stage, while poor inhibition was observed in case of spent stage. The different ANG extracts in *S. lessoniana* showed strong antibacterial activity against Gram-negative bacteria *E. coli* and *A. hydrophila*. Benkendroff *et al.* (2001) observed pronounced antibacterial activity of the egg masses of marine molluscs against *E. coli*. Among the four extracts, butanol extract showed pronounced activity followed by methanol extracts in all the three stages except immature stage. Preliminary experiments by earlier researchers also showed that butanol-ANG extracts from squids *viz.*, *Loligo pealei* (Barbeiri *et al.*, 1997), *Loligo duvaucelii* (Gomathi *et al.*, 2010) inhibited the growth of various bacterial pathogens. Most of the antibacterial activity studies in molluscs reported that methanol extract showed pronounced activity than butanol, but both extracts exhibited significant activity against *E. coli*. (Prem-Anand *et al.*, 1997). Ethanol extract from the four maturity stages did not show activity against *B. megaterium* while methanol extract from maturing and ripe stages showed activity against *A. hydrophila*. Barbeiri *et al.* (1997) noticed antibacterial activity against marine pathogens like *Vibrio anguillarum* and *Streptomyces griseus* and they attributed this antibacterial activity to the symbiotic ANG-bacteria, *Alteromonas* sp. and *Shewanella* sp.

During sexual maturation of *S. lessoniana*, the ANGs change from colourless in immature females, to coral

Table 1. Antibacterial activity (zone of inhibition in mm) of ANG- solvent extracts from different maturity stages of *S. lessoniana*

| Maturity stage | ANG-extracts | Bacterial strains | | | |
|----------------|-----------------|-------------------|----------------------|------------------|----------------------|
| | | <i>E. coli</i> | <i>A. hydrophila</i> | <i>S. aureus</i> | <i>B. megaterium</i> |
| Immature | Acetone:alcohol | Nil | Nil | Nil | Nil |
| | Ethanol | Nil | Nil | Nil | Nil |
| | Methanol | Nil | Nil | Nil | Nil |
| | Butanol | Nil | Nil | Nil | Nil |
| Maturing | Acetone:alcohol | 7.1 ± 2.42 | 5.9 ± 1.62 | 4.9 ± 0.42 | 4.8 ± 0.42 |
| | Ethanol | 5.2 ± 0.28 | 3.0 ± 0.94 | 5.1 ± 1.12 | Nil |
| | Methanol | 6.4 ± 1.65 | 4.5 ± 0.65 | 4.7 ± 1.42 | 4.6 ± 1.66 |
| | Butanol | 5.8 ± 1.78 | 6.1 ± 0.75 | 5.9 ± 1.28 | 5.5 ± 0.45 |
| Mature | Acetone:alcohol | 8.4 ± 1.78 | 8.1 ± 4.37 | 7.4 ± 1.42 | 7.1 ± 0.45 |
| | Ethanol | 6.5 ± 0.64 | 6.4 ± 5.63 | 6.3 ± 1.66 | Nil |
| | Methanol | 9.4 ± 0.28 | 8.3 ± 1.12 | 7.9 ± 4.24 | 7.9 ± 1.22 |
| | Butanol | 10.1 ± 1.65 | 9.3 ± 2.42 | 8.3 ± 0.24 | 8.0 ± 5.63 |
| Spent | Acetone:alcohol | 6.0 ± 0.24 | 4.2 ± 1.43 | 3.4 ± 0.45 | 4.0 ± 0.28 |
| | Ethanol | 4.0 ± 1.45 | Nil | 4.6 ± 1.63 | Nil |
| | Methanol | 5.3 ± 1.78 | 3.6 ± 0.94 | 3.5 ± 1.14 | 3.8 ± 1.37 |
| | Butanol | 5.0 ± 0.89 | 5.2 ± 1.65 | 4.7 ± 0.63 | 4.9 ± 0.62 |

Values are means of five replicates. 'Nil' - No activity

red in mature females. Colour of the ANGs was one of the major characters for accounting ovarian maturation cycle in *Loligo forbesi* (Lum-Kong, 1992). The red colouration of the ANGs of sexually mature squid, *L. pealei* was attributed to the bacterial residents (Bloodgood, 1977). Symbiotic bacteria (*Alteromonas* strain) in *L. pealei* were orange-red pigmented. ANGs bacteria accumulate carotenoid pigments during sexual maturation of *Sepia officinalis* (Van den Branden *et al.*, 1979) and *L. pealei* (Lum-kong and Hastings, 1992). In *S. officinalis*, Declair and Richard (1972) found that the pigments were tightly bound to granules which were actively secreted by the gland. and they concluded that the ANG bacterial community responds to the maturity stages of the host.

Acknowledgements

The authors thank the Director, Central Marine Fisheries Research Institute and the Dean, Faculty of Marine Sciences, CAS in Marine Biology for all the facilities extended.

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Date of Receipt : 04.07.2012

Date of Acceptance : 05.06.2014