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DRUG DISCOVERY

Antimicrobial potential of crude extracts from Sea slug, *Euselenops luniceps* (Cuvier, 1817)

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To evaluate the anti-microbial potential of *Euselenops luniceps*, locally known as 'Moon-headed sidegill slug', which one of the sea slug species. The in vitro anti-microbial assay was carry out by disc diffusion method. Ten bacterial pathogens and ten fungal pathogens were used for the study. Five types of extractions was used; acetone, butanol, ethanol, hexane and methanol. Among the various strains maximum zone of inhibition (20±1.76 mm) was recorded in against *P. aeroginosa* in butanol extract and minimum zone of inhibition (3±1.67mm) was observed in against *K. pneumoniae* in acetone extract. The butanol extracts was able to produce a moderate activity against *E. coli, S. paratyphi* and *V. cholerae.* Among the various strains maximum zone of inhibition (12±0.98mm) was recorded in *Rhizopus* strain and minimum zone of inhibition (1±0.65mm) was observed in *E. Floccosum* strain. Overall results provide information that sea slug may pave the way to explore the potential development of new antimicrobial drugs to be launched in the pharmaceutical industries.

INTRODUCTION

Marine environment have a plenty of organisms as rich sources which are evolved with potential secondary metabolites being used as medicine (Devi *et al*., 1997). Apart from the food that is derived from the marine environment, a wide variety of novel bioactive substances in larger proportion is being isolated and characterized with great promise for the treatment of human diseases (Proksch *et al*., 2002) but only less than 1% of the isolated compounds examined so far for pharmacological activities (Fusetani, 2000). The prevalent use of antibiotic has promoted the emergence of antibiotic resistant pathogens. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including natural sources from any terrestrial or marine source (Normark and Normark, 2002).

Perusal of literature revealed that a large number of works have been carried out in other groups of organisms but only a few studies were made in molluscs (Periasamy *et al.*, 2011; Ramasamy and Balasubramanian, 2012). Molluscs are considered as one of the important sources to derive bioactive compounds that exhibit antitumor, antimicrobial, anti-inflammatory, and antioxidant activities (Benkendorff *et al*., 2011, Mohanraj *et al*., 2014). Among the molluscs, cephalopods are very good sources of bioactive compounds. The fluid from the ink sac is found to have antibiotic effect and a pigment from the ink sac of the cuttlefish has been used in medicine, especially in homeopathy (Vino *et al*., 2014). The presence of antimicrobial activity in molluscs has been reported from the mucus of the giant snail *Achantia folica* (Yamazaki, 1993), from the egg mass and purple fluid of the sea hare *Aplysia kurodai* and the body wall of the sea hare *Dolabella auricularia* (Iijima *et al*., 2003) and body tissue of six species of cephalopods (Ramasamy *et al*., 2011). They are widely distributed throughout the world and have many representatives in the marine and estuarine ecosystem namely slugs, whelks, clams, mussels, oyster, scallops, squids and octopus.

Sea slugs are the group of marine gastropod molluscs, soft-bodied, slow moving and thus would be highly vulnerable to predators of a variety of defenses. They are constantly exposed to relatively high concentrations of bacteria, viruses, and fungi of which many may be harmful to the organism. The survival of these organisms depends on efficient antimicrobial mechanisms to protect themselves against microbial infections and fouling. Studies of antimicrobial compounds of marine invertebrates may provide valuable information for new antibiotic discoveries and give new insights into bioactive compounds in marine molluscs. The potential of marine mollusc sea slug *E. luniceps* as a source of biologically active products is largely unexplored. The objective of the present investigation was to evaluate the antimicrobial properties of sea slug *E. luniceps*

MATERIALS AND METHODS

Collection and extraction

Specimens of *E. luniceps* were collected from the trash in Pazhayar landing centre, Southeast Coast of India and immediately transported to the laboratory. The whole animal cut with fine scissors and suspended in different solvents for overnight. The tissues were homogenised using sterile mortar and pestle. The homogenate of sea slug extracts were centrifuged at 5000 rpm for 15 min and the supernatants were collected and then filtered through Whatman No.1 filter paper. The solvent from

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Table 1 Antibacterial activity of various solvent extracts *E. Luniceps*

S. No	Pathogens	Zone of inhibition (mm)					
		Acetone	Butanol	Ethanol	Hexane	Methanol	Std
1	*E. coli*	7±1.26	12±1.02	6±1.06	-	-	26±0.21
2	*K. oxytoca*	-	16±1.38	-	5±1.42	8±1.73	21±0.40
3	*K. pneumoniae*	3±1.67	11±1.23	5±1.64	-	-	19±0.12
4	*P. aeroginosa*	-	20±1.76	9±1.18	-	10±1.75	28±0.21
5	*P. mirabilis*	-	16±1.69	8±1.83	6±1.35	-	22±0.21
6	*S. paratyphi*	6±1.94	-	-	4±1.28	-	19±0.21
7	*S. typhi*	5±1.98	14±1.92	-	-	6±1.82	25±0.21
8	*S. aureus*	-	17±1.81	-	-	8±1.09	24±0.21
9	*V. cholerae*	-	-	-	-	-	18±0.21
10	*V. parahaemolyticus*	-	16±0.98	-	7±1.43	-	26±0.21

Table 2 Antifungal activity of various solvent extracts *E. luniceps*

S. No	Pathogens	Zone of inhibition (mm)					
		Acetone	Butanol	Ethanol	Hexane	Methanol	Control
1	*A. alternata*	-	5±0.65	1±0.23	-	-	18±0.18
2	*A. flavus*	-	6±0.48	3±0.43	-	3±0.73	16±0.12
3	*A. niger*	2±0.56	6±0.75	-	3±0.43	-	16±0.09
4	*C. albicans*	-	9±1.09	4±0.42	2±0.76	4±0.76	21±0.28
5	*C. tropicalis*	-	5±0.86	-	-	-	14±0.16
6	*E. floccosum*	-	-	-	1±0.89	-	14±0.18
7	*Mucor* sp	-	-	3±0.67	-	-	12±0.21
8	*Pencillium* sp	-	6±0.43	-	-	2±0.43	18±0.42
9	*Rhizopus* sp	1±0.96	12±0.98	6±0.43	5±0.79	3±0.76	23±0.13
10	*T. rubrum*	-	2±0.76	-	-	-	12±0.42

combined filtrates was evaporated at reduced pressure and temperatures. The obtained residues were stored in refrigerator for further use.

Microbial strains

Ten bacterial pathogens *Escherichia coli, Klebsiella oxytoca, K. pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella paratyphi, S. typhi, Staphylococcus aureus, Vibrio cholerae* and *V. Parahemolyticus* and ten fungal pathogens viz. *Alternaria alternata, Aspergillus flavus,A. niger, Candida albicans, C. tropicalis, Epidermophyton floccosum, Mucor* sp., *Pencillium* sp., *Rhizopus* sp., and *Trichophyton rubrum* were used for the present study. All these pathogens were obtained from the Department of Medical Microbiology (Raja Muthiah Medical College hospital) Annamalai University, Annamalai Nagar, Chidambaram. Bacterial cultures were maintained on nutrient agar slants at 4ºC and regularly sub cultured every month. Sub culturing of fungi was done from broth to broth using Saboraud dextrose broth (SDB) media. The clinical isolates were grown overnight in respective broth before evaluating for antimicrobial activity.

Anti-bacterial Assay

The spectrum of antibacterial activity was studied using a range of 10 different strains of human pathogenic gram positive and gram negative bacteria of which there were one antibiotic agent (Tetracycline). *In-vitro* antibacterial assay was carried out by disc diffusion technique (Bauer *et al*., 1996) in Whatman No.1 filter paper discs with 4mm diameter were impregnated with known amount test samples of the sea slug and positive control contained of a standard antibiotic disc. Negative controls are comprised sterile disc only. The impregnated discs along with control (Tetracycline) were kept at the centre of Nutrient Agar Plates, seeded with test bacterial activity was expressed in terms of diameter of Zone of inhibition was measured in mm using Vernier caliper or a scale and recorded.

Anti-fungal assay

Stock cultures were maintained in Sabouraud Dextrose Agar and 10 different species of fungal pathogen were maintained in Sabouraud Dextrose broth for 24hrs until used for antifungal activity. *In-vitro* antifungal activity was determined by using the technique of Bauer *et al*., 1996 using 0.1ml of 24 hrs old cultures, maintained in Sabouraud Dextrose broth. Whatman No.1 filter paper (4mm) discs impregnated with test samples of the sea slug and positive contained a standard antifungal disc (Flucanozole). The inhibition zone was measured after 32hrs at 30ºC for the fungal plates. Antifungal activity was measured in term of diameter of zone (including the disc within) in mm.

Statistical analysis

The experimental results are expressed as means ± standard deviation (SD) of three measurements at least. Statistical Package for Social Science (SPSS 13) was used to analyse the variance (ANOVA). P-values < 0.05 were regarded as significant.

RESULTS

Antibacterial activity

In the present study, different solvent extracts from the species of *E. luniceps* were tested for the antibacterial activity. Tetracyclin was used as a positive control. The zone of inhibition in different bacterial strains against *E. luniceps* extraction is shown in (Table.1). Among the various strains maximum zone of inhibition (20±1.76) was recorded in against *P. aeroginosa* in butanol extract and minimum zone of inhibition

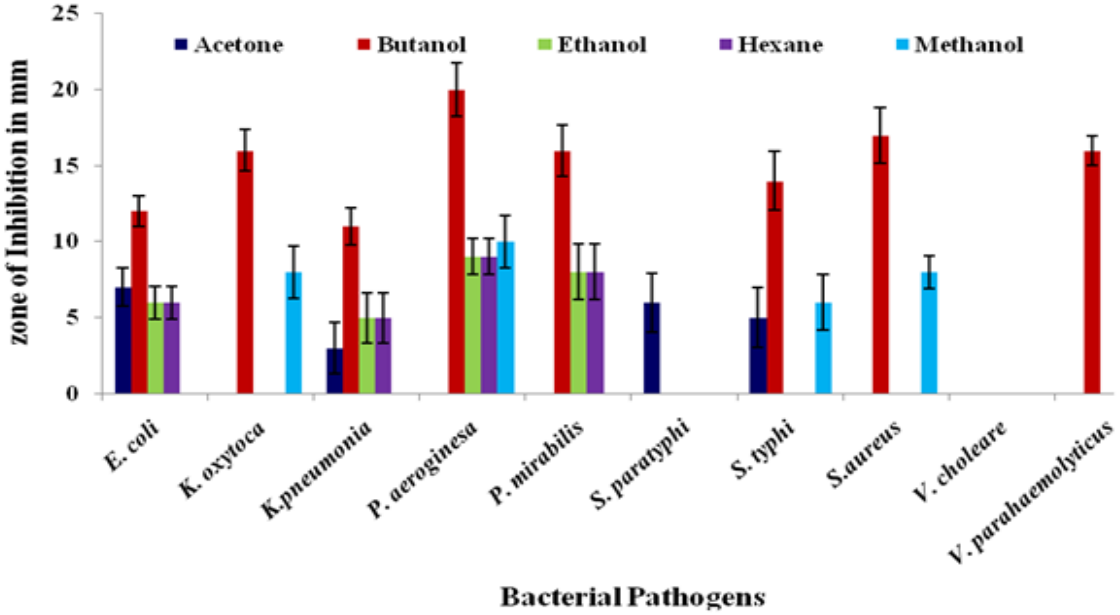

Figure 1 Antibacterial spectrum of various solvent extracts from *E. luniceps* against Bacterial pathogens

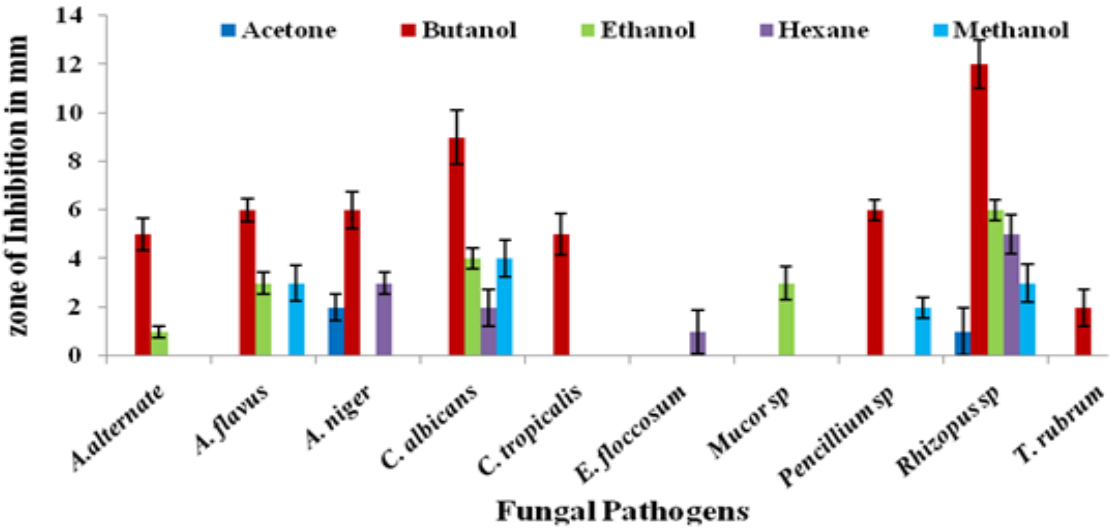

Figure 2 Antifungal spectrum of various solvent extracts from *E. luniceps* against fungal pathogens

(3±1.67mm) was observed in against *K. pneumoniae* in acetone extract. The butanol extracts was able to produce a moderate activity against *E. coli, S. paratyphi* and *V. cholerae*. The lowest activity was found with hexane, acetone, methanol and ethanol extracts against *V. cholerae* and *Proteus mirabilis* (Fig. 1). *S. aureus, K. pneumoniae, P. aeruginosa, Pseudomonas* spand *S. typhi* were highly resistant to most of the extract.

Antifungal activity

Different solvent extracts of *E. luniceps* were tested for the antifungal activity. Fluconazole was used as a positive control. The zone of inhibition in different fungal strains against *E. luniceps* extraction is shown in (Table. 2). Among the various strains maximum zone of inhibition (12±0.98mm) was recorded in *Rhizopus* strain and minimum zone of inhibition (1±0.65mm) was observed in *E. floccosum* strain. The lowest activity was found with hexane, acetone, methanol and ethanol extracts against *Alternaria alternata, Aspergillus flavus, Aspergillus niger, C. Albicans, Candida tropicalis, E. floccosum, Mucor* sp, *Pencillium* sp, and *T. rubrum* were highly resistant to most of the extract (Fig. 2).

DISCUSSION

Antimicrobial agents are essential drugs for human and animal health and welfare. More than 1,100 antibiotic substances have been isolated from invertebrates. Among these, 50 have found widespread use in the prevention and treatment of bacterial diseases in animal and man (Gale and Kiser 1967). Marine organisms contain much undiscovered bioactive compounds; the number of new active compounds isolated

from marine organisms are estimated at 10,000 (Kelecom, 2002). In the present study various solvent extracts of *E. Luniceps* were screened for its antimicrobial activity against human bacterial and fungal pathogens. All the solvent extracts showed a broad spectrum of activity against the tested pathogens. In antibacterial activity maximum zone of inhibition was observed against *P. aeroginosa* by butanol extract and acetone extracts showed minimum activity against *K. pneumoniae.* Similar observations were found with fractions showed significant activity extracts of *Meretrix casta* against *P. Aeruginosa P. mirablis, E.coli,* and *S. aureus* (Ramasamy, 2010).

In present study the ethanol extracts showed a moderate activity against *E. coli* and *K. pneumonia.* According to McQuaid *et al*. (1999) the species of *Siphonaria* showed defensive chemical mechanisms against their predators by producing biologically active substances. Earlier different metabolites present in the extract of *Siphonaria* species were found to exhibit antimicrobial activity (Hochlowski and Faulkner, 1983). Most species of invertebrates suffer disease from a wide range of microbial pathogens and parasites. Certain levels of immunity can be found in all living things. However, invertebrates lack many features of the vertebrate immune system (Beck and Habicht, 1996) and studies on invertebrate immunity have revealed some novel types of defence, such as antibacterial peptides and proteins (Hoffmann and Hetru, 1992). Similar study was carried out by (Jayaseeli *et al*., 2001) they found antibacterial activity of four bivalves against few pathogens and the extracts showed significant activity against *Bacillus subtills.* Antibacterial activity of gastropods against *S. typhi* was reported by (Rajaganapathi, 1996) also supporting present study on antibacterial activity of bivalve extracts. (Anand and Patterson Edward, 2001) studied the antibacterial activities in ethanol extracts of gastropod *Babylonia spirata* and *Turbo brunneus* and observed highest activity against *E. coli, K. pneumonia, P. vulgaris and S. typhi.*

Marine molluscs are highly delicious seafood and also very good source of bioactive compounds. Sea slugs are widely used in world research institution for various studies, but only recently they have been recognized as potential sources of antibacterial and antifungal substances. As an earlier report has been performed the antimicrobial activity of the opisthobranch mollusc *Kalinga ornata* with five different solvents used and it has showed good activities against bacterial pathogens and maximum zone 8 mm of inhibition were recorded against *S. typhi* and *P. mirabilis* from butanol extract. Suresh *et al.* (2012) were reported that ethanol extract of *B. zeylanica* and *H. conoidalis* shows the maximum inhibition zone against *K. Pneumonia* and *S. paratyphi*. As an earlier report has been made, the crude ethanol extracts of *B. spirata* showed good activities against *Pseudomonas aeruginosa*. The methanol extract of *Hemifusus pugilinus* possessed the highest activity against *E. coli* and lowest activity was observed against *Klebsiella oxytoca* (Periyasamy *et al*., 2012). The methanolic extract of *Chicoreus virgineus* and *C. ramous* experimentally analyzed and observed the broad spectrum antimicrobial activities of body tissue extract. These results lend support to the present findings of the antimicrobial activity of *K. ornata* and *B. leachi*. From the above result express that the aqueous extracts of mollusc was shows predominant activity. The knowledge of the self-defense mechanism of molluscs is extremely limited compared to that of vertebrates and arthropods. Very similar to the present study, Mohanraj *et al.*, (2014) noticed highest antibacterial activity with extracts of *K.ornata* against *S. aureus* and *E. coli*. From the present study the butanol extracts showed better antimicrobial activity against all strains used when compared to the other solvent extracts.

CONCLUSION

This is the first report of an anti-microbial activity of sea slug, *Euselenops luniceps* extracts. Hence, there could be probability of new bioactive compound in the crude extract, which might provide a basis for further development of novel compound from, *Euselenops luniceps*. This also provided a new insight towards the development of good candidates for pharmaceutical and bioactive natural products.

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Mrs. Sivaprakasam Ramya collected the sea slug species and performed the crude extractions, antimicrobial activities and initial wrote the manuscript. Dr. Samuthirapandian Ravichandran, and Dr. Kannan Mohan contributed to the discussion and edited the final manuscript.

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