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ANTIBACTERIAL POTENTIAL OF SEA STAR *PROTOREASTER LINCKII* FROM MANDAPAM, SOUTHEAST COAST OF INDIA

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ABSTRACT: The present study was aimed to evaluate the antibacterial potential of the commonly available sea star *Protoreaster linckii*. The samples were collected from Mandapam, the Gulf of Mannar, southeast coast of India. Four different extracts like methanol, acetonitrile, dichloromethane and ethanol at varying concentrations ranging from 250 to 1000 µg/ml was used for the experiment to study the inhibitory effects on ten selected human urinary tract infectious pathogenic bacteria. A concentration dependent inhibitory effect was observed in all the extracts of star fish. The results of the study suggested that most of the extracts have strong inhibitory activity against different pathogens similar to the standard drugs. Methanolic extracts exhibited a maximum zone of inhibition (17.00±0.1mm) against gram positive *Staphylococcus saprophyticus* at the higher concentration. Minimum zone of inhibition (0.700±0.35 and 0.800±0.15 mm) were reported in gram positive *S. aureus* while using the median and higher concentrations of dichloromethane extracts.

KEYWORDS: Starfish, *Protoreaster linckii*, Antimicrobial activity, UTI

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1.INTRODUCTION

Marine environment is the most productive ecosystem in the world that consists of abundant faunal and floral diversity [1]. Marine organisms contain numerous organic bioactive compounds including secondary metabolites [2]. These secondary metabolites were having applications in agrochemical, cosmeceutical, food, nutraceutical and pharmaceutical industries [3-5]. Secondary metabolites

having desirable antimicrobial properties were isolated from marine organisms [6-8] and they are also a rich realm comprising various antioxidant and anticancer natural compounds with low toxicity, high efficiency and none drug resistance [9]. Starfish commonly known as sea stars are marine benthic organisms belonging to the class Asteroidea that comes under the phylum Echinodermata. They have numerous tube feets, exhibits pentameral symmetry and have high regenerative ability. Starfish contains a number of biologically active compounds and molecule having pharmacological properties including anticoagulant, antifouling, antifungal, anti-inflammatory, antimicrobial, antiviral, cytotoxic, feeding deterrent, haemolytic, hemotoxic and wound healing activities [10-19]. Marine ecosystem of India consists of 7517 km coastline area spread across nine coastal states and two union territories that are covered by Arabian Sea, Bay of Bengal and Indian Ocean. Drug discoveries from marine non-chordates have an important research field since decades and Indian marine natural product research gained momentum only in the late seventies [20]. The data from available literatures revealed that taxonomical studies were conducted regarding some of the starfish in Indian coast, whereas studies on their pharmacological potential are very scare [21-22]. The sea star *Protoreaster linckii* also named as 'Red-knobbed Starfish' are widely distributed throughout the Indo-Pacific region. It has a gorgeous appearance with bright red or orange reticulate patterns on their dorsal sides and it feeds on sponges, sea anemones and soft corals [23]. The Gulf of Mannar, located along the southeastern part of Tamil Nadu, is a marine biosphere reserve extending from Rameswaram to Kanyakumari acts as a nursery ground for this starfish. Hence the sea star *P. linckii* was choosed as the experimental animal for the present study with an aim of identifying their antibacterial potential against the human urinary tract infectious pathogens.

2. MATERIALS AND METHODS

2.1. Sample collection and preparation of extracts

Live specimens of the starfish *Protoreaster linckii* were collected by scuba diving from an intertidal zone of Mandapam (Lat. 9°16'N; Long. 79°8'E), Tamil Nadu in the Gulf of Mannar along the southeast coast of India during January 2018. The samples were thoroughly washed with sea water and cleaned to remove debris and are immediately stored in ice box and transported to the Zoology laboratory of Jamal Mohamed College, Tiruchirappalli. The extraction process was carried out following the standard methodology [24]. Whole body of star fish samples were placed on different polar solvents such as methanol, acetonitrile, dichloromethane and ethanol separately in the ratio of 1:3 (w/v) for 72 hrs at normal room temperature. Then the extracts were filtered through Whatman No.1. filter paper and the solvents were concentrated by rotary evaporator (VC100A Lark Rotavapor®) at 30°C with reduced pressure to give predominantly an aqueous suspension and concentrated under reduced pressure to give a residue. The crude extracts were stored at 4ºC and used for further analysis.

2.2. Antibacterial susceptibility assay

The clinical isolates of selected human urinary tract infectious pathogens were obtained from the Govt. Medical College Hospital, Tiruchirappalli. Antibacterial activity of the crude extracts of star fish were evaluated by well diffusion method [25]. The bacterial strains were enriched in nutrient broth overnight at 37°C. Then they were streaked over Mueller Hinton agar surface using sterile cotton swabs. Then wells were loaded with 50µl of different extracts at various concentrations (250, 500 and 1000 µg/ml). Streptomycin 400µl was used as positive control and negative control was prepared using distilled water. The plates were incubated at 37°C for 24 h. Antimicrobial activities were determined after 72h by measuring the diameter of inhibition zone around the disc and the results were expressed in millimeters.

2.3. Statistical analysis

All the assays were conducted in triplicates and the data were expressed as mean with standard error (SE). Bar diagrams was plotted using Origin software (Version 8.0).

3. RESULTS AND DISCUSSION

The four different extracts of *Protoreaster linckii* were subjected for their antibacterial activities and the results are summarized in Table 1-4. It is evident that the methanolic extract (Fig.1) has comparatively higher antibacterial potential against the selected gram positive and gram negative bacterial isolates followed by acetonitrile (Fig. 2), ethanol (Fig. 3) and dichloromethane (Fig. 4) extracts in a dose dependent manner. Higher concentration of methanolic extract exhibited a maximum zone of inhibition (17.00±0.14) against Gram positive *Staphylococcus saprophyticus,* whereas medium to moderate inhibitions were reported for other three extracts such as acetonitrile (11.00±0.36), dichloromethane (12.49±0.65) and ethanol (14.00±0.76) at the same concentration. Lower concentrations of extracts did not exhibited any zones of inhibition against gram positive *Staphylococcus aureus*, whereas minimum zone of inhibition was observed while using the median and higher concentration of all the four extracts with corresponding values of 1.000±0.32 and 1.400±0.20 (methanol), 1.200±0.40 and 1.400±0.47 (acetonitrile), 0.700±0.35 and 0.800±0.15 (dichloromethane), 0.900±.02 and 1.100±0.31 (ethanol) respectively. The methanolic, ethanolic and acetonitrile extracts of this starfish at a concentration of 1000 µg/ml showed excellent inhibitory effects against the gram negative *Klebsiella pneumoniae* with higher zones of inhibition 8.500±0.16, 8.500±0.42 and 7.500±0.47 respectively and the values were higher than that of the standard drug (6.800±0.36), however dichloromethane extract showed moderate zones of inhibition (4.500±0.42) in the same species.

Table 1: Antibacterial activity of the methanolic extract of *Protoreaster linckii*

	Inhibition Zone (mm)				
	Concentrations of the extract µg/ml				
Pathogens	**250**	**500**	**1000**	**Standard**	**Control**
Staphylococcus Saprophyticus	15.23±0.42	16.30±0.10	17.00±0.14	20.00±0.12	----
Escherichia coli	10.15±0.28	12.13±0.23	12.59±0.32	18.21±0.31	----
Klebsiella pneumonia	6.900±0.47	7.300±0.16	8.500±0.16	6.800±0.36	----
Pseudomonas aeruginosa	6.500±0.36	7.100±0.27	7.000±0.36	18.52±0.16	----
Enterobacter cloacae	----	2.800±0.25	3.400±0.31	5.100±0.19	----
Enterococcus faecalis	2.100±0.20	2.600±0.42	2.800±0.17	5.300±0.23	----
Staphylococcus aureus	----	1.000±0.32	1.400±0.20	6.400±0.35	----
Klebsiella oxytoca	2.300±0.20	2.700±0.30	3.200±0.23	6.700±0.18	----
Mycoplasma genitalium	8.900±0.12	9.300±0.20	9.500±0.24	10.10±0.18	----
Proteus mirabilis	4.600±0.31	4.800±0.14	5.400±0.34	6.500±0.35	----

Table 2: Antibacterial activity of the acetonitrile extract of *Protoreaster linckii*

	Inhibition Zone (mm)				
	Concentrations of the extract µg/ml				
Pathogens	**250**	**500**	**1000**	**Standard**	**Control**
Staphylococcus Saprophyticus	10.12±0.28	10.30±0.72	11.00±0.36	20.00±0.12	----
Escherichia coli	11.36±0.20	11.40±0.45	11.69±0.31	18.21±0.31	----
Klebsiella pneumonia	6.900±1.04	7.700±0.35	8.500±0.42	6.800±0.36	----
Pseudomonas aeruginosa	6.300±0.20	7.400±0.56	9.700±0.53	18.52±0.16	----
Enterobacter cloacae	----	2.100±0.40	2.600±0.81	5.100±0.19	----
Enterococcus faecalis	2.600±0.31	2.800±0.56	3.400±0.70	5.300±0.23	----
Staphylococcus aureus	----	1.200±0.40	1.400±0.47	6.400±0.35	----
Klebsiella oxytoca	2.300±0.20	2.600±0.21	3.100±1.10	6.700±0.18	----
Mycoplasma genitalium	8.900±0.12	9.500±0.72	9.800±0.70	10.10±0.18	----
Proteus mirabilis	4.000±0.10	4.300±1.06	4.700±0.76	6.500±0.35	----

Table 3: Antibacterial activity of the dichloromethane extract of *Protoreaster linckii*

	Inhibition Zone (mm)				
	Concentrations of the extract µg/ml				
Pathogens	**250**	**500**	**1000**	**Standard**	**Control**
Staphylococcus Saprophyticus	11.50±0.36	12.31±0.31	12.49±0.65	20.00±0.12	----
Escherichia coli	10.59±0.81	11.46±0.45	12.13±0.31	18.21±0.31	----
Klebsiella pneumonia	2.900±0.76	3.300±0.56	4.500±0.42	6.800±0.36	----
Pseudomonas aeruginosa	12.50±0.81	13.10±0.67	13.70±0.53	18.52±0.16	----
Enterobacter cloacae	----	1.600±0.40	1.800±0.31	5.100±0.19	----
Enterococcus faecalis	2.500±0.64	2.600±0.40	2.800±0.59	5.300±0.23	----
Staphylococcus aureus	----	0.700±0.35	0.800±0.15	6.400±0.35	----
Klebsiella oxytoca	1.200±0.12	1.500±0.40	1.900±0.36	6.700±0.18	----
Mycoplasma genitalium	8.500±0.53	8.900±0.50	9.300±0.70	10.10±0.18	----
Proteus mirabilis	4.100±0.36	4.600±0.50	5.100±0.10	6.500±0.35	----

Table 4: Antibacterial activity of the ethanol extract of *Protoreaster linckii*

	Inhibition Zone (mm)				
	Concentrations of the extract µg/ml				
Pathogens	**250**	**500**	**1000**	**Standard**	**Control**
Staphylococcus Saprophyticus	11.12±0.36	12.30±0.42	14.00±0.76	20.00±0.12	----
Escherichia coli	11.56±0.31	12.13±0.31	12.39±0.64	18.21±0.31	----
Klebsiella pneumonia	6.300±0.56	6.700±0.76	7.500±0.47	6.800±0.36	----
Pseudomonas aeruginosa	6.700±0.32	7.100±0.47	8.700±-0.43	18.52±0.16	----
Enterobacter cloacae	----	2.500±0.42	3.100±0.11	5.100±0.19	----
Enterococcus faecalis	2.400±0.81	2.900±0.81	3.800±0.15	5.300±0.23	----
Staphylococcus aureus	----	0.900±.02	1.100±0.31	6.400±0.35	----
Klebsiella oxytoca	2.500±0.42	2.800±0.42	3.200±0.59	6.700±0.18	----
Mycoplasma genitalium	8.500±0.47	8.900±0.36	9.300±0.53	10.10±0.18	----
Proteus mirabilis	4.200±0.42	4.600±0.34	5.100±0.10	6.500±0.35	----

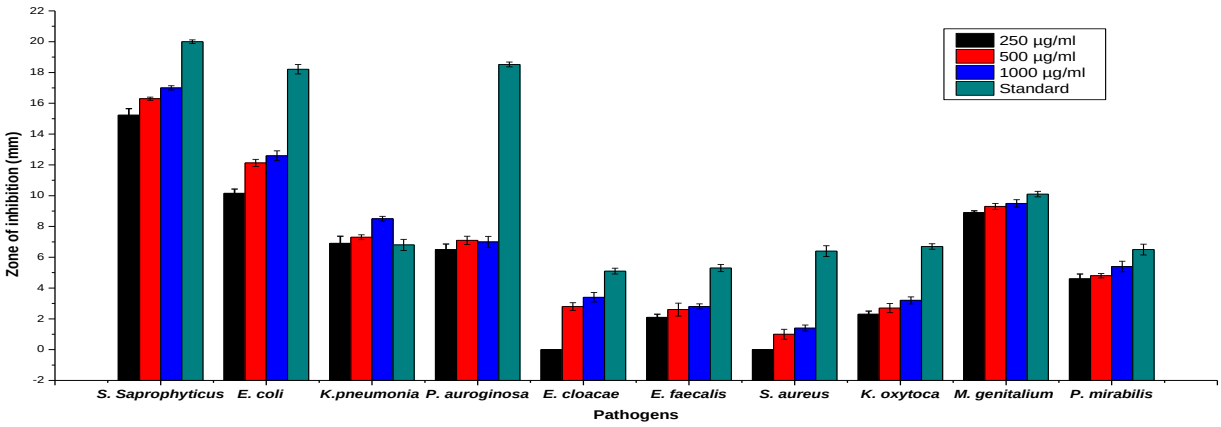

Figure 1.Antibacterial activity of the methanolic extract of *Protoreaster linckii*

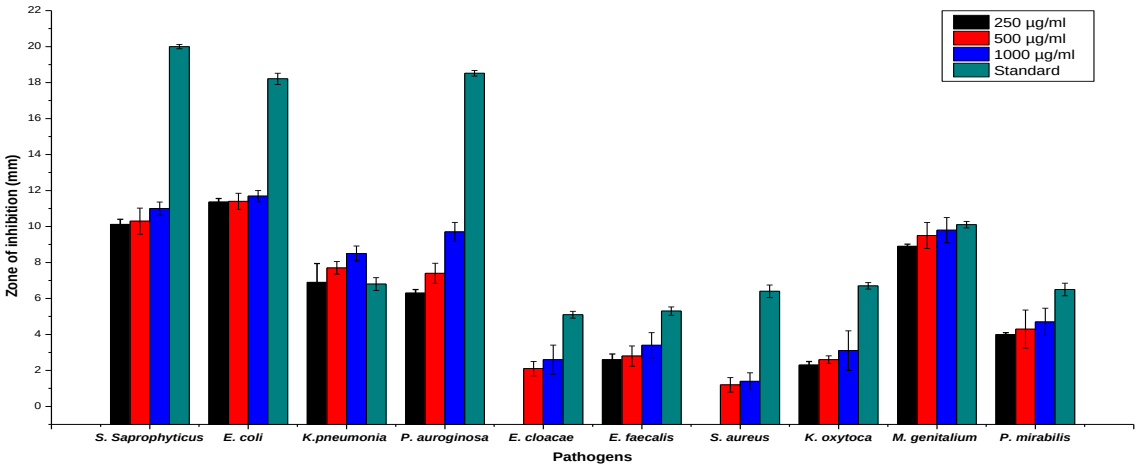

Figure 2. Antibacterial activity of the acetonitrile extract of *Protoreaster linckii*

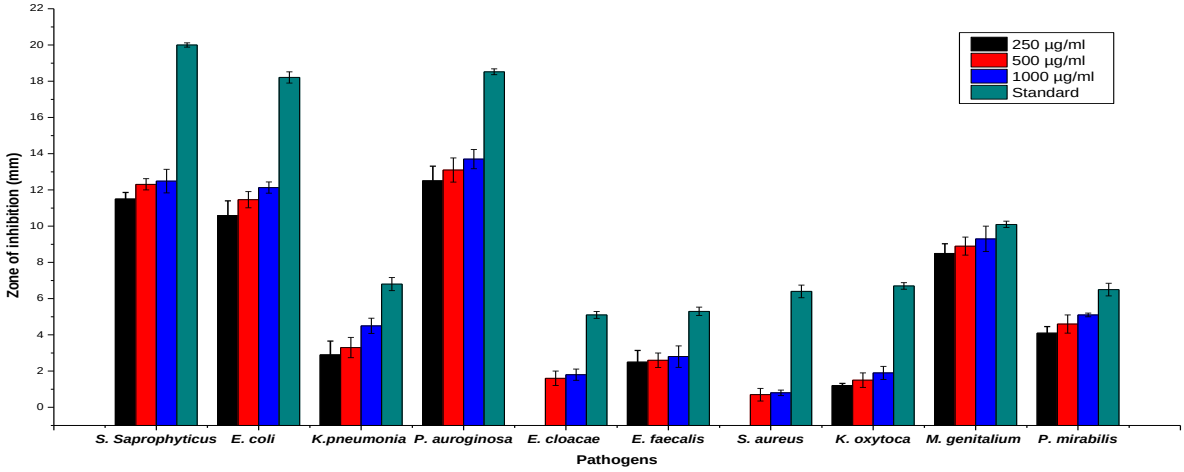

Figure 3. Antibacterial activity of the dichloromethane extract of *Protoreaster linckii*

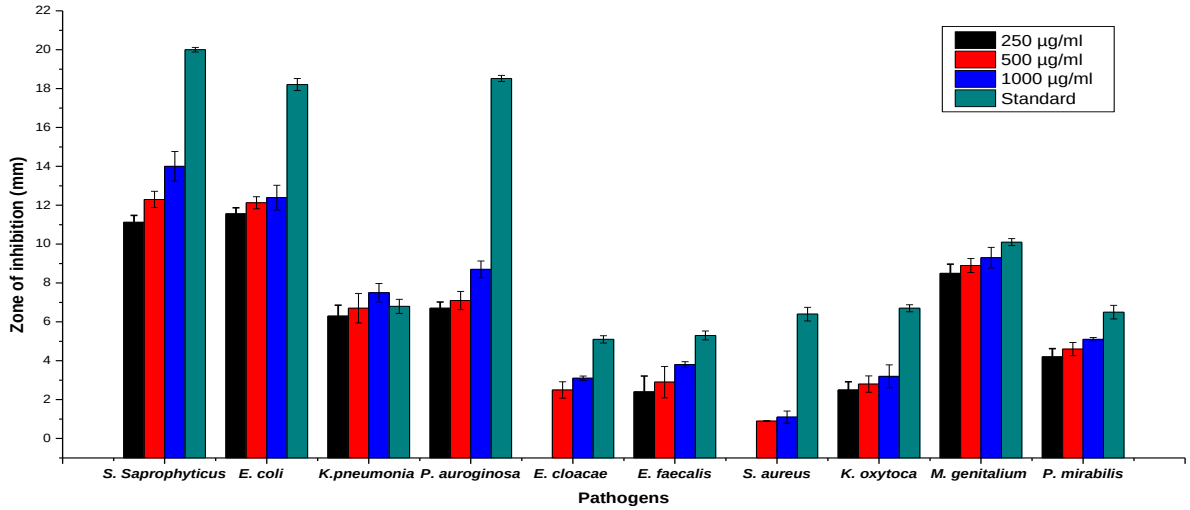

Figure 4. Antibacterial activity of the ethanol extract of *Protoreaster linckii*

DISCUSSION

Urinary tract infection (UTI) is the most potent bacterial infection characterized by the proliferation of bacteria in urinary tract [26] and it results in fever, urinary urgency, dysuria, urinary frequency, cloudy and malodorous urine [27]. It is prevalent among infants and children, pregnant and older womens [28-30]. Higher incidence of UTI was reported in females than the males [31]. Gram positive and gram negative pathogens including *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis* and *Staphylococcus saprophyticus* are the most common causative agents of urinary tract infection [32]. Numerous studies were conducted regarding the antibiotic resistance of urinary tract infectious pathogens [33-35], which highlights the need of identifying new classes of natural compounds for clinical use. The present study was carried out to assess the antimicrobial activity of different extracts of starfish *P. linckii* against ten human UTI pathogens. Some of the earlier studies have also reported similar antibacterial activity in the sea star *P. linckii* [36-37]. Previous reports have also revealed that the antibacterial potential of certain other invertebrates [38-40]. The previous studies on the anti-microbial properties of starfishes along with compound analysis stated that steroidal glycosides, saponins and saponin like steroid derivatives were responsible for antimicrobial activity [18, 41]. Antimicrobial peptides (AMPs) present in Echinoderms were responsible for their humoral immunity[42]. Hence the elucidation and fractionation of compounds responsible for antimicrobial activity of the *P. linckii* should be taken into account.

4. CONCLUSION

The aim of the current study was to screen the extracts derived from starfish *Protoreaster linckii* for their activity against selected bacterial pathogens that are responsible for urinary tract infection. All the four extracts inhibited the growth of tested gram positive and gram negative pathogens. Methanolic extracts were found to be highly potent against all the pathogens and the maximum zone

of inhibition was reported against *Staphylococcus saprophyticus* and the minimum zone of inhibition was observed in the median concentration of dichloromethane extract against *S. aureus.* However further studies has to be conducted using other extracts with different concentrations. Considering the abundance of starfish *P. linckii*, results of the present study could be used as a baseline data for bioprospecting of the species for pharmaceutical applications.

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CONFLICT OF INTEREST

None

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