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RESEARCH ARTICLE

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Screening of Bioactive Metabolites from the Starfish *Pentaceraster mammillatus* against Human Urinary Tract Infectious Pathogens

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ABSTRACT

Presence of different bacterial pathogens in the urinary tract results in the occurrence of infectious diseases among the human beings. The emerging multi drug resistance capacities of pathogens are the major constrain in the treatment of bacterial diseases. Researchers are trying to develop new antimicrobial agents from natural resources including marine organisms to combat the issue. This research work has carried out to investigate the anti-bacterial activity of sea star *Pentaceraster mammillatus* against ten common UTI pathogens. Four different polar solvents *viz;* methanol, acetonitrile, dichloromethane and ethanol were used for the experiment. At the lowest concentration (250µg/ml), none of the above said extracts has showed any inhibitory effects in the pathogens; however the concentrations such as 500 and 1000µg/ml of extracts inhibited the bacterial growth. Acetonitrile and dichloromethane showed higher inhibitory effects on gram positive bacterial strains *Mycoplasma genetalium* (4.3 ± 0.23 mm) and *Staphylococcus aureus* (4.1 ± 0.21 mm) respectively. Ethanolic extract showed some good inhibitory effects on *M. genetalium* (3.5 ± 0.14 mm) and the methanolic extract demonstrated relatively lower inhibition.

Keywords: Starfish, *Pentaceraster mammillatus*, Antimicrobial activity, UTI.

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INTRODUCTION

Sea stars or starfishes are diverse group of animals belonging to the class Asteroidea, comes under the phylum Echinodermata. They are prevalent among the coral reefs, sea grass meadows, deep-sea floor, inter-tidal zones, coastal areas and in rocky sea shores. [1-2] They feed on micro-organisms (algae) and certain small animals (bivalves, sponges, snails) and also absorb organic nutrients and fecal matters from the surrounding water. Starfishes are used for food preparation in China due to the abundance of nutritional metabolites. They are well known for their pharmacological and biological characteristics and they were employed in traditional medicines as a remedial measure for asthma, bronchitis, diabetes and heart problems. [3-4]. Presently they are also used as anti-cancer, anti-bacterial, anti- fungal and antifouling agents. [5] It was also reported that a number of novel

marine natural products were elucidated from sea stars. [6] Studies revealed the presence of glycosylceramides [7], asterosaponins, polyhydroxysteroids [8] and polyhydroxysteroidal glycosides [9-10] in different sea stars across the world.

India is blessed with a rich coastal and marine biodiversity. [11] The Gulf of Mannar, which comprised of 140 km area from Rameshwaram island to Tuticorin is recognised as a marine national park and is noted for the rich diversity of various marine organisms having economic importance, that includes different starfishes, in which *Pentaceraster mammillatus* were considered as a relatively new one. [12-13] Only a few studies conducted earlier in the sea stars of this coastal area for the elucidation of potential compounds having desirable antimicrobial properties. [14-16] A previous study was conducted to purify and characterize a compound (lectins) from the digestive glands of *P. mammillatus* [17] and not any single work was performed on their antibacterial efficacy.

The higher incidence of Urinary tract infection (UTI) is a major threat for human beings and the consumption of antibiotics were found effective in controlling the pathogens. [18-20] Even though antibiotics at low-doses are effective in their treatment, increased antibiotic resistance in microorganisms is a major constrain for their control. [21] Hence the work was carried out to study the antibacterial efficacy of commonly available sea star *P. mammillatus* against UTI pathogens.

MATERIALS AND METHODS

Study area

The peninsular Tuticorin (Lat. 8° 81'N; Long. 78° 14' E) commonly known as 'Pearl City' is a major harbour in Tamil Nadu that lies in the South-east Indian coast. It is a part of Gulf of Mannar marine national park located in the Bay of Bengal. About 36,000 marine species including some rare floral and faunal groups exists in the study area.

Experimental animal and sample collection

Pentaceraster mammillatus is widely distributed in the East coast of Africa, including Madagascar and Mauritius, through Red Sea and Arabian Gulf to Sri Lanka and the Bay of Bengal into the tropical Indo-Pacific. They live in the subtidal on coarse sand or coral rubble. Live specimens of the starfish *P. mammillatus* were collected by scuba diving during January 2018. Using the sea water, the collected samples were cleaned and were immediately stored in ice box and transported to the Zoology laboratory of Jamal Mohamed College, Tiruchirappalli.

Extracts preparation

The standard methodology of Malla Reddy *et al.* [22] was followed for the extraction. Different polar solvents namely methanol, acetonitrile, dichloromethane and ethanol was used for extraction. Whole body of starfish samples were placed in the polar solvents and were kept at 22°C for a period of 72 hours. This was filtered through Whatman No. 1. filter paper and then the solvents were kept in rotary evaporator at 30°C. The aqueous suspension were further concentrated and the resultant residues were used as the crude extracts. The extracts were kept at 4°C until the commencement of the experiment.

Antibacterial susceptibility assay

The clinical isolates of selected human urinary tract infectious pathogens were obtained from the Govt. Medical College Hospital, Tiruchirappalli. Antibacterial efficacy of the starfish extracts were assessed by well diffusion technique. [23] 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 (250µg, 500µg and 1000µg/ml). The positive control for the experiment was Streptomycin with the concentration of 400µl and distilled water was used as the negative control. The plates were then incubated for a period of 24 h at 37°C. Antimicrobial activities were determined after 72 h and results were expressed in millimeters.

RESULTS

The antibacterial efficacy of *Pentaceraster mammillatus* was examined against the selected human UTI pathogens on the basis of zone of inhibition. All the four extracts does not exhibited any kind of inhibitory effects at the lowest (250µg/ml) concentration, whereas an inhibition was recorded in the range of 0.8 to 4.3 mm for different extracts at the median and higher concentrations (500 and 1000µg/ml), and the results were only below the level of the standard drug.

The acetonitrile and dichloromethane extracts showed relatively more inhibition against the gram positive bacterial strains like *Staphylococcus saprophyticus* (3.8 ± 0.11 and 3.5 ± 0.41 respectively), *Entercoccus faecalis* (3.1 ± 0.18 and 3.0 ± 0.10 respectively), *S. aureus* (3.5 ± 0.41 and 4.1 ± 0.21 respectively) and *Mycoplasma genitalium* (4.3 ± 0.23 and 3.7 ± 0.21 respectively) (Table 2 and 3).

Ethanolic extracts of the starfish exhibited good inhibitory activity against the gram positive bacterial strains like *S. saprophyticus* (3.0 ± 0.18), *E. faecalis* (2.8 ± 0.30), *M. genitalium* (3.5 ± 0.14) and gram negative bacterial strain *Escherichia coli* (2.5 ± 0.34**)** (Table 4).

Methanolic extracts showed minimal zones of inhibition than the other extracts against the gram positive bacterial strain *S. aureus* (2.5 ± 0.56) and gram negative bacterial strain *Enterobacter cloacae* (2.5 ± 0.40) (Table 1).

DISCUSSION

The available literatures revealed the pharmacological properties of marine derived compounds. [24] A number of divers novel bioactive secondary metabolites with potential pharmaceutical and therapeutic properties were obtained from marine organisms and are now being utilized as a source for new drug discovery. [25-27] Recent studies demonstrated that the Echinoderms have unique biological properties. [28]

Table 1: Antibacterial efficacy of *Pentaceraster mammillatus* (Methanolic extract)

Type of Pathogens	Name of Pathogens	Zone of inhibition (mm)				
		Concentrations of the extract µg/ml				
		250	500	1000	Standard	Control
Gram positive	*Staphylococcus Saprophyticus*	---	1.8 ± 0.25	2.2 ± 0.26	20.0 ± 0.12	---
	Enterococcus faecalis	---	1.8 ± 0.41	2.0 ± 0.18	5.30 ± 0.23	---
	Staphylcoccus aureus	---	2.3 ± 0.51	2.5 ± 0.56	6.40 ± 0.35	---
	Mycoplasma genitalium	---	2.1 ± 0.19	1.9 ± 0.31	10.1 ± 0.18	---
Gram negative	*Escherichia coli*	---	1.5 ± 0.14	1.3 ± 0.16	18.2 ± 0.31	---
	Klebsiella pneumonia	---	1.6 ± 0.34	2.4 ± 0.19	6.80 ± 0.36	---
	Pseudomonas auroginosa	---	1.9 ± 0.24	2.3 ± 0.38	18.5 ± 0.16	---
	Enterobacter cloacae	---	2.3 ± 0.37	2.5 ± 0.40	5.10 ± 0.19	---
	Klebsiella oxytoca	---	2.0 ± 0.25	2.2 ± 0.19	6.70 ± 0.18	---
	Proteus mirabilis	---	0.9 ± 0.29	1.3 ± 0.11	6.50 ± 0.35	---

Table 2: Antibacterial efficacy of *Pentaceraster mammillatus* (Acetonitrile extract)

Type of Pathogens	Name of Pathogens	Zone of inhibition (mm)				
		Concentrations of the extract µg/ml				
		250	500	1000	Standard	Control
Gram positive	*Staphylococcus Saprophyticus*	---	3.1 ± 0.18	3.8 ± 0.11	20.0 ± 0.12	---
	Enterococcus faecalis	---	2.7 ± 0.21	3.1 ± 0.18	5.30 ± 0.23	---
	Staphylcoccus aureus	---	2.9 ± 0.28	3.5 ± 0.41	6.40 ± 0.35	---
	Mycoplasma genitalium	---	3.4 ± 0.12	4.3 ± 0.23	10.1 ± 0.18	---
Gram negative	*Escherichia coli*	---	---	1.5 ± 0.51	18.2 ± 0.31	---
	Klebsiella pneumonia	---	---	1.1 ± 0.42	6.80 ± 0.36	---
	Pseudomonas auroginosa	---	---	1.3 ± 0.34	18.5 ± 0.16	---
	Enterobacter cloacae	---	---	1.2 ± 0.14	5.10 ± 0.19	---
	Klebsiella oxytoca	---	---	1.5 ± 0.32	6.70 ± 0.18	---
	Proteus mirabilis	---	---	1.8 ± 0.14	6.50 ± 0.35	---

Table 3: Antibacterial efficacy of *Pentaceraster mammillatus* (Dichloromethane extract)

Type of Pathogens	Name of Pathogens	Zone of inhibition (mm)				
		Concentrations of the extract µg/ml				
		250	500	1000	Standard	Control
Gram positive	*Staphylococcus Saprophyticus*	---	2.3 ± 0.32	3.5 ± 0.41	20.0 ± 0.12	---
	Enterococcus faecalis	---	2.1 ± 0.18	3.0 ± 0.10	5.30 ± 0.23	---
	Staphylcoccus aureus	---	2.8 ± 0.26	4.1 ± 0.21	6.40 ± 0.35	---
	Mycoplasma genitalium	---	2.7 ± 0.18	3.7 ± 0.21	10.1 ± 0.18	---
Gram negative	*Escherichia coli*	---	---	1.5 ± 0.12	18.2 ± 0.31	---
	Klebsiella pneumonia	---	---	0.8 ± 0.11	6.80 ± 0.36	---
	Pseudomonas auroginosa	---	---	1.0 ± 0.12	18.5 ± 0.16	---
	Enterobacter cloacae	---	---	0.9 ± 011	5.10 ± 0.19	---
	Klebsiella oxytoca	---	---	0.9 ± 0.19	6.70 ± 0.18	---
	Proteus mirabilis	---	---	0.9 ± 0.19	6.50 ± 0.35	---

Table 4: Antibacterial efficacy of *Pentaceraster mammillatus* (Ethanolic extract)

Type of Pathogens	Name of Pathogens	Zone of inhibition (mm)				
		Concentrations of the extract µg/ml				
		250	500	1000	Standard	Control
Gram positive	*Staphylococcus Saprophyticus*	---	2.5 ± 0.18	3.0 ± 0.18	20.0 ± 0.12	---
	Enterococcus faecalis	---	2.1 ± 0.34	2.8 ± 0.30	5.30 ± 0.23	---
	Staphylcoccus aureus	---	---	1.1 ± 0.18	6.40 ± 0.35	---
	Mycoplasma genitalium	---	2.3 ± 0.41	3.5 ± 0.14	10.1 ± 0.18	---
Gram negative	*Escherichia coli*	---	--	2.5 ± 0.34	18.2 ± 0.31	---
	Klebsiella pneumonia	---	--	1.5 ± 0.25	6.80 ± 0.36	---
	Pseudomonas auroginosa	---	--	1.3 ± 0.14	18.5 ± 0.16	---
	Enterobacter cloacae	---	--	1.0 ± 0.28	5.10 ± 0.19	---
	Klebsiella oxytoca	---	---	1.4 ± 0.61	6.70 ± 0.18	---
	Proteus mirabilis	---	---	---	6.50 ± 0.35	---

The work was carried out to understand antibacterial efficacy of *Pentaceraster mammillatus* extracts against ten human urinary tract pathogens. Previous reports were also revealed that the common bacterial pathogens of urinary tract can be controlled using different extracts of various sea stars such as *Astropecten indicus* [16], *Protoreaster linckii* [29], *Ophiocnemis marmorata* and *Stellaster equestris.* [30] Marine organisms including the Ascidians such as *Phallusia arabica* [31] and *Microcosmus exasperatus* [32] extracts also exhibited antibacterial activity against UTI pathogens. In addition to this, various other marine species were also proven to be effective against the UTI pathogens. They include sea urchin such as *Echinometra mathaei* [33], *Tripneustes gratilla* [34] and sea sponge *Clathria indica.* [35] Previous studies have also described the antibacterial activity of marine algae *Sargassum whitti* [36], *Ulva lactuca*, *Laurencia optusa* and *Turbinaria triquatra.* [37]

The starfish *Pentaceraster mammillatus* were evaluated for their antibacterial potential against human Urinary tract infectious pathogens using the methanol, acetonitrile, dichloromethane and ethanol extracts. Results revealed that the acetonitrile and dichloromethane extracts were having relatively higher inhibitory effects against the pathogens. Hence further studies are required using the purified fractions of acetonitrile and dichloromethane extracts. Isolation, purification and characterization of the compound may recommended for greater effects on UTI pathogens with the minimal dose and this marine natural product will receive overwhelming response from the pharmaceutical industries in the coming future as an alternative method of novel drug development.

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