INVITRO ANTIBACTERIAL EFFICACY OF *ASTROPECTEN INDICUS* AGAINST SELECTED HUMAN URINARY TRACT PATHOGENS

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Abstract: **The present study shows the antibacterial activity of acetonitrile, methanol, dichloromethane and ethanol extracts of *Astropecten indicus* against ten selected clinical pathogenic bacterial isolates. All the bacterial strains selected for the experiment are well known for their ability to infect the human urinary tract. The present investigation showed that the extracts of starfish *A. indicus* have moderate inhibitory effects against the clinical isolates at the higher concentration (1000µg/ml), whereas lower inhibitions were recorded at the median concentration (500µg/ml) and lower concentration (250µg/ml) of all the extracts were not enough to inhibit the growth of any bacterial pathogens. Among the four different extracts, acetonitrile extracts showed higher inhibitory effect against the gram positive *Staphylococcus aureus* (4.2±0.14) and *Mycoplasma genitalium* (3.7±0.23) at higher concentration.**

Index Terms **- Starfish, *Astropecten indicus*, Antimicrobial activity, UTI**

I. INTRODUCTION

Coastal bay areas are considered as nursery grounds for marine biota and it serves as an ecosystem for a variety of species including many benthic and nektonic organisms that are explored as economic resources [1-4]. The marine organisms produce diverse groups of bioactive compounds and are well known for their chemical diversity, biochemical specificity, less side effects, binding efficiency and propensity to interact with biological targets [5]. They plays a crucial role in biomedical research and drug development, either specifically as drugs or as lead structure for bioinspired chemical drug synthesis [6]. These compounds recovered from marine organisms and their synthetic analogues were capable of exhibiting anesthetic, repellent and settlement inhibition properties without any toxic effects to the non-target organisms [7]. Owing to the pharmaceutical properties, marine natural products are being utilized for production of life saving drugs [8].

Urinary tract infection (UTI) is a common health problem among infants, childrens, pregnant and older womens due to the bacterial proliferation in urinary tract and it results in fever, chills, urinary urgency, dysuria, frequency and cloudy urine [9-13]. Vaginal sex, usage of cervical cap and Nonoxynol-9–coated male condoms are some of the important factors associated with urinary tract infections [14-16].

Scientific interest over secondary metabolites having antimicrobial potential, has led to the increased utilization of marine organisms such as Crustaceans, Molluscs and Echinoderms [17-18]. It is quite worth to note that only 5% of marine living resources are tapped for drug discovery and the remaining unexplored resources will be the promising source for novel bioactive macromolecules [19]. Only few studies have been conducted using some of the Indian sea stars for their pharmacological screening [20].

The Gulf of Mannar marine biosphere reserve, located along the southeast coast of India, extending from Rameswaram to Kanyakumari has blessed with a rich diversity of different starfishes, which has not much utilized for bioprospecting or pharmacological studies. Hence the purpose of the present study was to ascertain the antibacterial efficacy of methanol, acetonitrile, dichloromethane and ethanol extracts of star fish *Astropecten indicus* that are available in the Mandapam coast.

II. MATERIALS AND METHODS

Study area and sample collection

The study area Mandapam (Lat. 9° 16'N; Long. 79° 8' E) Tamil Nadu, located at southeast coast of India has the Gulf of Mannar on southern side and the Palk Bay on it's northen side. Live specimens of the starfish *Astropecten indicus* were collected by scuba diving during the month of January 2018. The samples were thoroughly rinsed with sea water to remove debris and sand at site of collection. Then the samples were immediately stored in ice box and transported to the Zoology laboratory of Jamal Mohamed College, Tiruchirappalli.

Preparation of extracts

The extraction process was carried out following the standard methodology [21]. 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.

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 [22]. 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 20µl of different extracts at various concentrations (250µg, 500µg 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.

Statistical analysis

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

III. RESULTS

The four different extracts of *Astropecten indicus* were subjected for their antibacterial activities against four gram positive and six gram negative pathogens and the results were summarized in **Table 1-4**. Present investigation revealed that the starfish extracts were having low level inhibitory effects against the clinical isolates. All the four extracts with low concentration were not enough to inhibit the growth of any bacterial pathogens, whereas low level of inhibitions were recorded for certain pathogens at the median concentration of extracts and the growth of all the pathogens were inhibited in a low level at all the highest concentrations of each extracts. **Table 1** depicts that the acetonitrile extracts had relatively more inhibition against gram positive bacterial strains like *Staphylococcus saprophyticus* (2.1±0.16), *Enterococcus faecalis* (2.5±0.21), *S. aureus* (3.0±0.18) and *Mycoplasma genitalium* (2.8±0.31) at median concentration. Antibacterial potential were further increased when higher concentration of acetonitrile extract was used and recorded the maximum zone of inhibition against *S. aureus* (4.2±0.14) followed by *M. genitalium* (3.7±0.23), *E. faecalis* (3.3±0.24) and in *S. saprophyticus* (3.1±0.21).

Methanolic extract at higher concentration showed moderate inhibitory effects against the gram positive *S. aureus* (3.3±0.17) and gram negative *Enterobacter cloacae* (3.1±0.18) and a minimum zone of inhibition (0.7±0.14) in gram negative *E. faecalis* as shown in **Table 2**. Dichloromethane extracts had moderate inhibitory effects on gram positive *S. saprophyticus* (2.1±0.31), *E. faecalis* (1.9±0.21), *S. aureus* (1.4±0.14) and *M. genitalium* (1.1±0.11) at a concentration of 500µg/ml (**Table 3**). The corresponding zones of inhibition recorded for these strains revealed that the antibacterial potential of dichloromethane extracts at the concentration of 1000µg/ml in the following order: *S. saprophyticus* (3.2±0.12) > *E. faecalis* (2.8±0.12) > *S. aureus* (2.3±0.21) > *M. genitalium* (1.9±0.34). **Table 4** demonstrated the lower inhibitory action of ethanolic extract when compared to other three extracts. Ethanolic extract exhibited higher inhibition (3.5±0.34) in gram positive *M. genitalium* and minimum level of inhibition (0.6±0.23) in gram negative *Klebsiella oxytoca*. It was also evident that the recorded zones of inhibitions for all pathogens for different extracts were found below the level of standard drug Streptomycin at a concentration of 400µl.

Table 1: Antibacterial activity of the acetonitrile extract of *Astropecten indicus*

	Inhibition Zone (mm)				
	Concentrations of the extract µg/ml				
Pathogens	**250**	**500**	**1000**	**Standard**	**Control**
Staphylcoccus Saprophyticus	--	2.1±0.16	3.1±0.21	20.00±0.12	----
Escherichia coli	--	--	1.3±0.32	18.21±0.31	----
Klebsiella pneumonia	--	--	1.7±0.31	6.800±0.36	----
Pseudomonas auroginosa	--	--	1.7±0.23	18.52±0.16	----
Enterobacter cloacae	--	--	1.9±0.23	5.100±0.19	----
Enterococcus faecalis	--	2.5±0.21	3.3±0.24	5.300±0.23	----
Staphylcoccus aureus	--	3.0±0.18	4.2±0.14	6.400±0.35	----
Klebsiella oxytoca	--	--	2.3±0.13	6.700±0.18	----
Mycoplasma genitalium	--	2.8±0.31	3.7±0.23	10.10±0.18	----
Proteus mirabilis	--	--	2.5±0.12	6.500±0.35	----

Table 2: Antibacterial activity of the methanol extract of *Astropecten indicus*

	Inhibition Zone (mm)				
	Concentrations of the extract µg/ml				
Pathogens	**250**	**500**	**1000**	**Standard**	**Control**
Staphylcoccus Saprophyticus	--	--	2.5±0.11	20.00±0.12	--
Escherichia coli	--	--	0.9±0.14	18.21±0.31	--
Klebsiella pneumonia	--	--	0.8±0.11	6.800±0.36	--
Pseudomonas auroginosa	--	--	1.0±0.12	18.52±0.16	--
Enterobacter cloacae	--	--	3.1±0.18	5.100±0.19	--
Enterococcus faecalis	--	--	0.7±0.14	5.300±0.23	--
Staphylcoccus aureus	--	--	3.3±0.17	6.400±0.35	--
Klebsiella oxytoca	--	--	1.1±0.21	6.700±0.18	--
Mycoplasma genitalium	--	--	2.9±0.16	10.10±0.18	--
Proteus mirabilis	--	--	1.3±0.14	6.500±0.35	--

Table 3: Antibacterial activity of the dichloromethane extract of *Astropecten indicus*

	Inhibition Zone (mm)				
	Concentrations of the extract µg/ml				
Pathogens	**250**	**500**	**1000**	**Standard**	**Control**
Staphylcoccus Saprophyticus	--	2.1±0.31	3.2±0.12	20.00±0.12	----
Escherichia coli	--	--	0.8±0.10	18.21±0.31	----
Klebsiella pneumonia	--	--	0.6±0.11	6.800±0.36	----
Pseudomonas auroginosa	--	--	0.9±0.19	18.52±0.16	----
Enterobacter cloacae	--	--	1.1±0.11	5.100±0.19	----
Enterococcus faecalis	--	1.9±0.21	2.8±0.12	5.300±0.23	----
Staphylcoccus aureus	--	1.4±0.14	2.3±0.21	6.400±0.35	----
Klebsiella oxytoca	--	--	1.0±0.12	6.700±0.18	----
Mycoplasma genitalium	--	1.1±0.11	1.9±0.34	10.10±0.18	----
Proteus mirabilis	--	--	1.0±0.23	6.500±0.35	----

Table 4: Antibacterial activity of the ethanol extract of *Astropecten indicus*

	Inhibition Zone (mm)				
	Concentrations of the extract µg/ml				
Pathogens	**250**	**500**	**1000**	**Standard**	**Control**
Staphylcoccus Saprophyticus	--	1.3±0.13	1.7±0.18	20.00±0.12	----
Escherichia coli	--	--	0.8±0.21	18.21±0.31	----
Klebsiella pneumonia	--	--	0.7±0.11	6.800±0.36	----
Pseudomonas auroginosa	--	--	0.8±0.12	18.52±0.16	----
Enterobacter cloacae	--	--	0.9±0.25	5.100±0.19	----
Enterococcus faecalis	--	1.6±0.41	2.0±0.14	5.300±0.23	----
Staphylcoccus aureus	--	1.8±0.32	2.3±0.21	6.400±0.35	----
Klebsiella oxytoca	--	--	0.6±0.23	6.700±0.18	----
Mycoplasma genitalium	--	2.0±0.21	3.5±0.34	10.10±0.18	----
Proteus mirabilis	--	--	0.7±0.12	6.500±0.35	----

IV. DISCUSSION

Sea stars have a tough skin which acts as a solid shield over them giving them a protective covering (Sumithaa *et. al.,* 2017). Sea stars have different mechanisms to prevent them against microbial attack. It includes the physical barriers such as tough skin, presence of thorn, pedicellariae and paxillae over the epidermis [23] and chemical barriers such as the release of surface associated compounds and secondary metabolites [24-25]. The antibacterial macromolecules from the marine organisms can be broadly classified into the following classes such as terpenes, polypeptides, quinones, steroids, protein and alkaloids [19]. Presences of antimicrobial peptides (AMPs) are also regarded as another innate immune response in starfish [26-27].

The sea star *A. indicus* known as comb star or sand star are found in intertidal and subtidal sandy areas and they feeds on molluscs and crustaceans [28]. The present study was conducted to evaluate antibacterial potential of the starfish *A. indicus* that are commonly available along the Mandapam coast. Previous studies has also reported similar antibacterial activity [28] as well as hemotoxic and wound healing potential of *Astropecten indicus* [29]. Similar antibacterial studies were conducted using the star fishes like *Asterias rubens* [30], *A. amurensis* and *Asterina pectinifera* [31] *Pentaceraster affinis* [32], *Protoreaster lincki* [33-34], *Stellaster equestris* [35-36]. Besides the antibacterial potential, starfish extracts possess anticancer [37], antifungal [38], antiinflammatory [39] and anticoagulant [40] properties, hence extensive studies were conducted on the isolation of bioactive compounds from starfish [41-42].

V. CONCLUSION

Results of the present experiment demonstrated moderate antibacterial activity of starfish *Astropecten indicus* for the acetonitrile extracts followed by methanol, dichloromethane and ethanol extracts at their higher concentrations. Growth of gram positive pathogens *Staphylococcus aureus* and *Mycoplasma genitalium* were found highly inhibited, when the acetonitrile extracts were used at the concentration of 1000µg/ml. Zones of inhibition recorded for all the extracts were found below the level of standard drug. However, there should be more focus on searching for new antimicrobial compound in star fishes by newly developed high-througthput screening systems since there is limited information about antimicrobial compounds from star fishes. Further studies like purification, structural elucidation and further evaluation is also required in this field of study.

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