ANTIMICROBIAL ACTIVITY IN THESTARFISH, *PENTACERASTER MAMMILLATUS* (AUDOIN, 1826).

[1]Arokya Jothi Blessy. A and [2]Sr. M.R. Basil Rose
[1]Research Scholar (MSU/RES/R4/Reg. No. 10085), [2]Associate Professor
[1]Department of Zoology, Holy Cross College (Autonomous), Nagercoil.
[1]Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli-627012,
Tamil Nadu, India.

ABSTRACT: Antimicrobial efficiency of the butanol, methanol and chloroform extracts derived from the digestive gland, tube feet, spine and gonad of the starfish, *Pentaceraster mammillatus* were examined against three human pathogenic bacteria (*Streptococcus mutans*, *Staphylococcus aureus* and *Klebsiella pneumoniae*) three fish pathogenic bacteria (*Vibrio harveyi*, *Proteus mirabilius* and *Aeromonous hydrphila*) and three fungal pathogens (*Aspergillus flavus, Aspergillus niger*and *Rhizopus stolonifer*). The antimicrobial activity was evaluated by measuring the zone of inhibition using disc diffusion method. The butanol extract of the digestive gland inhibited the growth of all the tested bacteria and a pathogenic fungus *R. stolonifer*, the tube feet arrested the growth of all the bacterial pathogen and two fungal pathogens *A. flavus and R. stolonifer*; the spine arrested the growth of *S. mutans* and *S. aureus* and the gonad inhibited the growth of *S. mutans* and all the three fish pathogenic bacteria. The chloroform and methanol extract of all the tissues could inhibit the growth of only a few pathogens compared tothat of the butanol extract. However, the chloroform extract of spineinhibited the growth of more number of pathogens than the butanol and methanol extract.

KEYWORDS- **Antimicrobial activity,fungal pathogen,bacterial pathogen,disc diffusion method,Zone of inhibition.**

I. INTRODUCTION

Microbial populations in seawater and sediments may be as high as 106 and 109 per milliliter, respectively (Austin, 1988). Marine invertebrates are therefore constantly exposed to high concentrations of bacteria, fungi and viruses, many of which may be pathogenic. Echinoderms are benthic organisms, which are constantly exposed to relatively high concentrations of the pathogens which can be harmful to the organism (Haug et al, 2002). During the last decade, there has been an increase in research on marine crustaceans, molluscs and echinoderms, particularly on their secondary metabolites with desirable antimicrobial properties (Casas et al, 2010; Haug et al, 2002). Sea stars, the benthic free living echinoderm have evolved rich sources of bioactive metabolites such as steroidal glycosides, steroids, anthraquinones, alkaloids, glycolipids and phospholipids (de Marino et al, 1997; Palagiano et al, 1995; Pathirana and Andersen, 1986). Steroidal glycosides and related compounds are predominant metabolites in sea stars and have a broad variety of biological activities such as cytotoxic, hemolytic, ichthyotoxic, repellent, antineoplastic, antimicrobial, antifungal, antiviral and anti-inflammatory (Andersson et al, 1989; Chludil et

al, 2002; Ivanchina et al, 2000; Prokofeva et al, 2003; Wang et al, 2002; Wang et al, 2004). The antimicrobial peptides have been also found in the gastrointestinal organs, in the eggs and in the body wall (Hauget al,2002). The present work was designed and carried out to assess the potential antimicrobial activity in the starfish *P. mammillatus* against a few human and fishpathogens.

II. MATERIALS AND METHODS

2.1 Collection and maintenance of animal

The starfish *P. mammillatus* were collected from the Arabian Sea at Pallam, Kanyakumari District, Tamilnadu, India. They were transported to the laboratory in plastic bucket containing aerated sea water and fed with snail and small fishes. They were maintained at room temperature (30ºC). Digestive gland, tube feet, gonad and spines were carefully dissected.

2.2 Preparation of tissueextract

Themodified method of Chellaramet al, (2004) is used for the process of extraction. 1gm of digestive gland/tube feet/gonad/spines samples were extracted with high polar solvent methanol, medium polar solvent butanol and chloroform separately in the ratio of 1:3 (w/v) for 24 hrs at normal room temperature (30ºC), then extracts were filtered by Whatman filter paper No.1 and the solvents were concentrated by rotary evaporator under reduced pressure and temperature (30ºC). The resultant residues were stored at -4ºC for further analysis.

2.3 Microorganisms used for screening

For screening, three human bacterialpathogens(*Streptococcus mutans, Staphylococcus aureus* and *Klebsiella pneumoniae*) three fish pathogenic bacteria (*Vibrio harveyi, Proteus mirabilius* and *Aeromonous hydrophila*) and three fungal pathogens (*Aspergillus flavus,Aspergillus niger*and *Rhizopus stolonifer*) were used.

2.4 Antimicrobial assay

The antimicrobial and antifungal effects were investigated using the standard protocols by disc diffusion method (Bauer et al, 1996). Pathogenic microbial strains were inoculated in sterile nutrient broth and incubated for 24 hrs at 37ºC. The pathogens were swabbed on the surface of Muller Hinton Agar and fungal strains were cultured using Saboroud Dextrose Agar. Discs impregnated with 25µl of the samples were placed on the surface of the agar plateandwere incubated at 37ºC. Antibiotics, steptomycin, amikacin and fluconazole were used as positive control for the antimicrobial assay.

III. RESULTS

Analysis of the antibacterial and antifungal activity of the butanol, methanol and chloroform extract of the digestive gland, tube feet, spines and gonad of the starfish *P.mammillatus* showed, that the butanol extract of the digestive gland inhibited the growth of all the tested bacteria and a pathogenic fungus *R. stolonifer* and that of the tube feet inhibited the growth of all the tested microbes except *A. niger*. The maximum zone of inhibition was observed (20 mm) for*R. stolonifer* in thebutanol extract of the digestive gland. Moderate activity was found (12-17 mm)in the butanol extract of digestive gland, tube feet and gonad against all the tested pathogenic bacteria except *S. aureus*. The butanol extract of the spine inhibited the growth *S. aureus* and *S. mutans* and no other tested pathogens. None of the extracts inhibit the growth of *A. niger* (Table 1; Plate 1).

Depending on the number of pathogens inhibited the inhibitory potency of the chloroform extract of the tissues can be stated as spine > tube feet > digestive gland = gonad (Table 2; Plate 2).The methanol extract of the digestive gland of *P. mammillatus* could inhibit the growth of *S. mutans* and *R. stolonifer*; spine could inhibit *R. stolonifer* and gonad could inhibit *S. mutans* and *S. aureus*. The methanol extract of the tube feet failed to inhibit the growth of all the tested pathogens (Table 3; Plate 3).

Table 3.1:Antimicrobial activity of butanol extract of starfish, *P.mammillatus*.

Microbes tested		Zone of inhibition (mm)					
		Digestive gland	Tube feet	Spine	Gonad	+ control	- control
Human Bacterial Pathogen	*S. mutans*	15	9	17	14	27	-
	S. aureus	7	8	10	-	28	-
	K. pneumoniae	12	14	-	-	24	-
Fish Bacterial Pathogen	*V. harveyi*	13	12	-	12	25	-
	P. mirabilis	16	16	-	14	27	-
	A. hydrophila	16	12	-	13	18	-
Fungal pathogen	*A. flavus*	-	7	-	-	14	-
	A. niger	-	-	-	-	15	-
	R. stolonifer	20	9	-	-	23	-

Table3. 2: Antimicrobial activity of chloroform extract of starfish, *P.mammillatus*

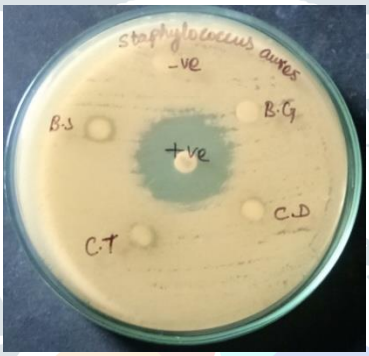
Microbes tested		Zone of inhibition (mm)					
		Digestive gland	Tube feet	Spine	Gonad	+ control	- control
Human Bacterial Pathogen	*S. mutans*	-	-	-	-	23	-
	S. aureus	-	7	10	9	27	-
	K. pneumoniae	15	9	10	-	21	-
Fish Bacterial Pathogen	*V. harveyi*	-	-	11	-	18	-
	P. mirabilis	-	-	10	-	18	-
	A. hydrophila	-	-	-	-	22	-
Fungal pathogen	*A. flavus*	-	-	-	-	14	-
	A. niger	-	-	-	-	16	-
	R. stolonifer	19	12	8	9	25	-

Table 3.3: Antimicrobial activity of methanol extract of starfish, *P.mammillatus*

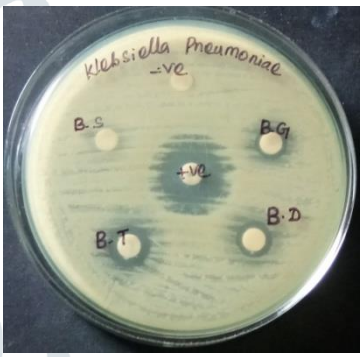
Microbes tested		Zone of inhibition (mm)					
		Digestive gland	Tube feet	Spine	Gonad	+ control	- control
Human Bacterial Pathogen	*S. mutans*	8	-	-	7	28	-
	S. aureus	-	-	-	10	23	-
	K. pneumoniae	-	-	-	-	22	-
Fish Bacterial Pathogen	*V. harveyi*	-	-	-	-	26	-
	P. mirabilis	-	-	-	-	26	-
	A. hydrophila	-	-	-	-	20	-
Fungal pathogen	*A. flavus*	-	-	-	-	12	-
	A. niger	-	-	-	-	17	-
	R. stolonifer	18	-	11	-	24	-

Plate - 3.1: Antimicrobial activity of butanol extracts of starfish *P. mammillatus* tissues.

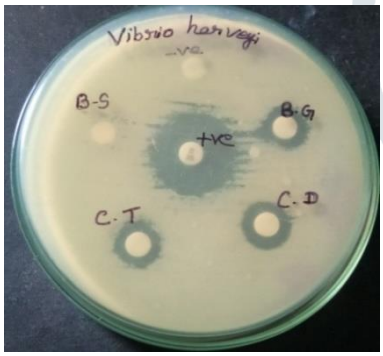
S. mutans



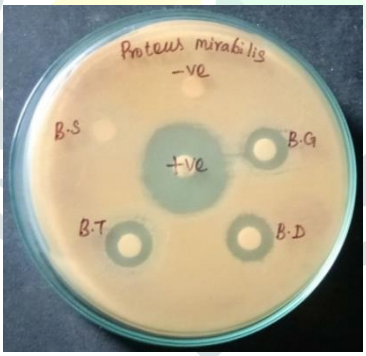
S. aureus



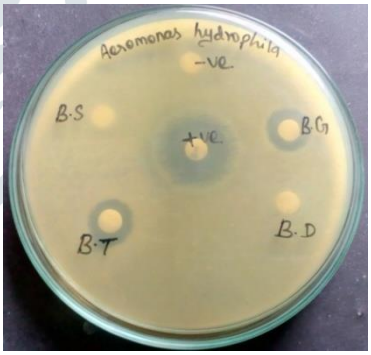
K. pneumoniae



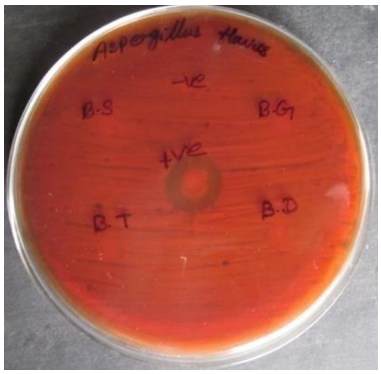
V. harveyi



P. mirabilis

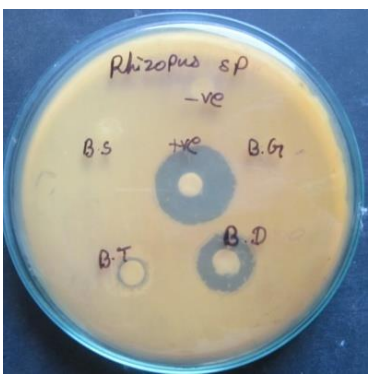


A. hydrophila



A. flavus

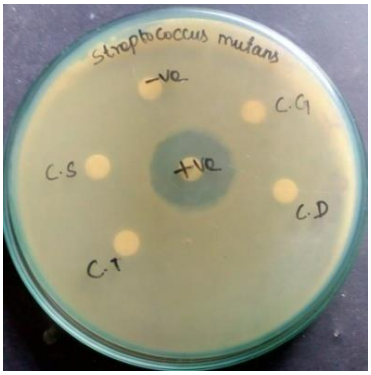
A.niger



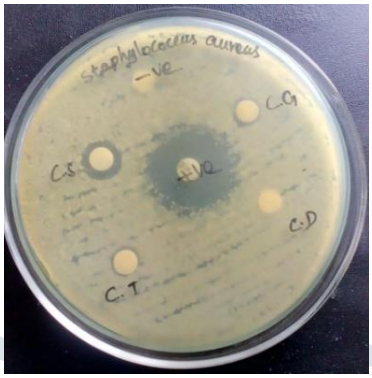
R. stolonifer

B.G - Gonad
B.D - Digestive gland
B.T - Tube feet
B.S - Spine

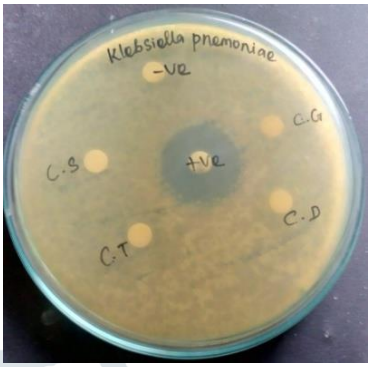
Plate -3.2:Antimicrobial activity of chloroform extracts of starfish *P. mammillatus* tissues.



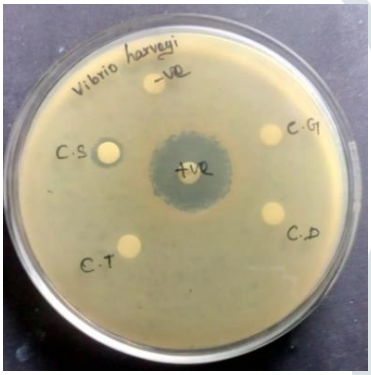
S. mutans



S. aureus

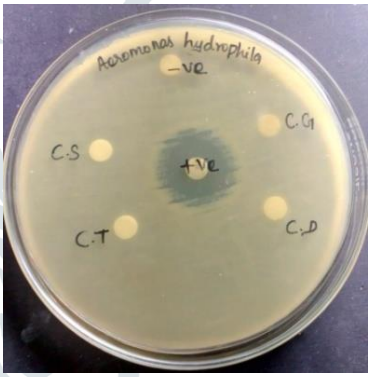


K. pneumoniae

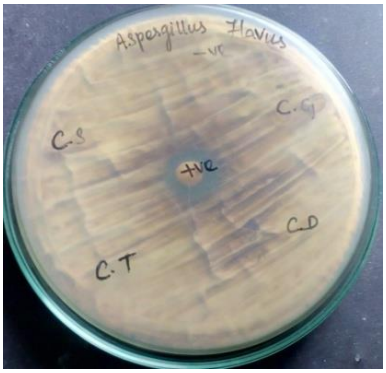


V. harveyi

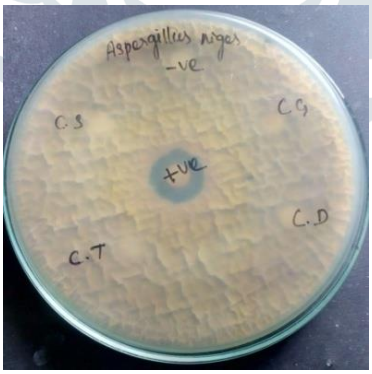
P. mirabilia



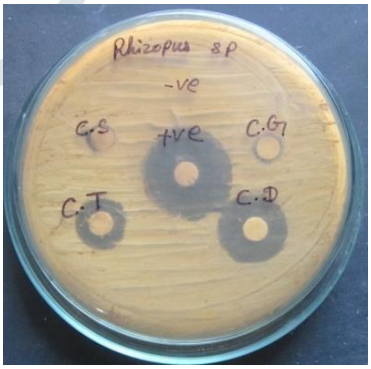
A. hydrophila



A. flavus



A. niger

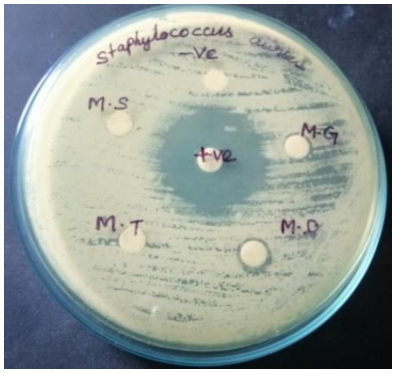


R. stolonifer

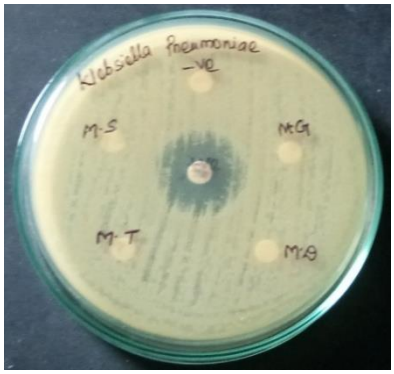
C.G - Gonad
C.D - Digestive gland
C.T - Tube feet
C. S - Spine

Plate - 3.3: Antimicrobial activity of methanol extracts of starfish *P. mammillatus* tissues.

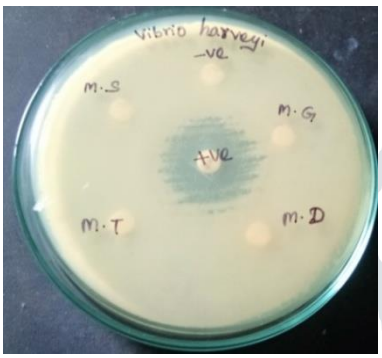
S. mutans



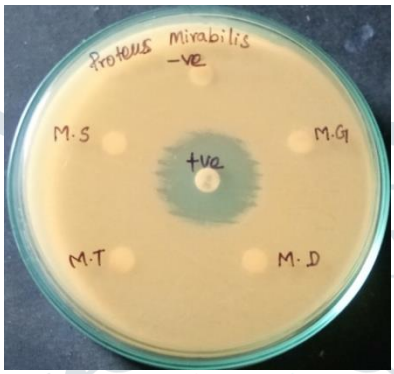
P. mirabilis



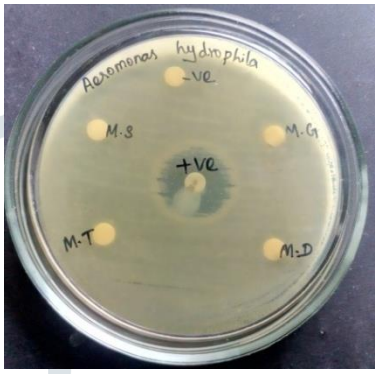
K. pneumoniae



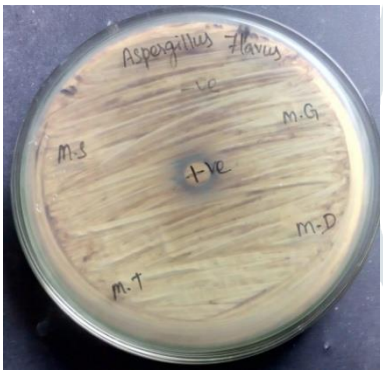
V. harveyi



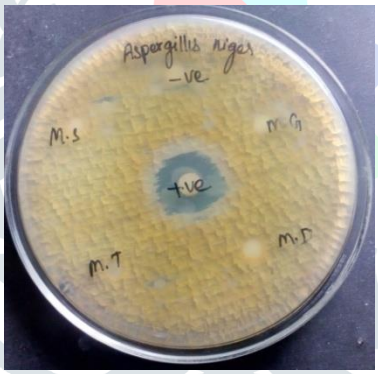
P. mirabilis



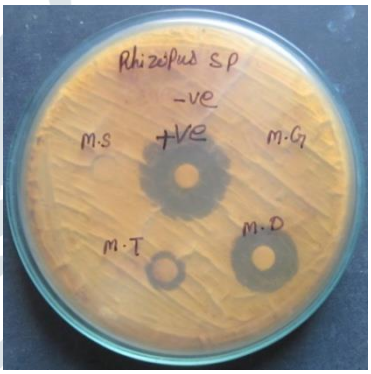
A. hydrophila



A. flavus



A. niger



R. stolonifer

M.G - Gonad
M.T - Tube feet
M.D - Digestive gland
M.S - Spine

IV.DISCUSSION

In the present investigation, antimicrobial efficacy of thebutanol, chloroform and methanol extract of thedigestive gland, tube feet, spine and gonad of the starfish*P. mammillatus* was studied by disc diffusion method. Antibacterial activity has previously been described in a wide range of echinoderm species (Anderson et al, 1983, 1989; Bryan et al, 1994; Ridzwan et al, 1995). The results of the present

investigation revealed, that the butanol and chloroform extract were highly efficient in inhibiting the growth of most of the pathogens when compared to the methanol extract.

The butanol extract of the digestive gland and tube feetinhibited the growth of all the tested human and fish pathogenic bacteria and fungi *R. stolonifer*. The chloroform extract of the all the tissues were effective against the fungi *R. stolonifer* and all except that of the gonad was found to inhibit the growth of the bacteria *K. pneumoniae*.The differential inhibitory potential of the extracts with different solvents enabled us to determine the presence of antibacterial substances potentially in the lipid or the water-soluble fraction.The antimicrobial activity may be due to the antimicrobial compounds including steroidal glycosides, polyhydroxylated steroids, lysozymes, complement-like substances or antimicrobial peptides which have been reported from echinoderms (Beauregards, 2002; chludilet al,2002; Levinaet al, 2009; Kuwanaraet al, 2009). Since the antibacterial effects with organic solvents has been found, protein factors may be responsible for growth inhibition.

Antimicrobial peptides (AMPs) are crucial immune effector molecules for invertebrates which lack a vertebrate-type adaptive immune system. Antimicrobial peptides (AMPs) are evolutionarily conserved small molecular weight proteins of the innate immune response, with a broad spectrum of antimicrobial activities against bacteria, viruses, and fungi (reviewed by Mookherjee and Hancock, 2007). AMPs appear naturally throughout all three domains of life from unicellular to multicellular organisms (Zasloff, 2002; Riley and Chavan, 2007; Wang et al, 2009).They do not only inactivate bacteria in vitro and in vivo thereby protecting host organisms against a variety of infections, but they also modulate immunity (Hancock and Diamond, 2000; Zasloff, 2002; Hancock et al, 2006).As activity has been demonstrated against human and fish pathogenic bacteria as well as against selected fungal pathogens, it may therefore be reasonable to assume that multiple factors are responsible for the antimicrobial activities detected. Further study is required to establish if this observed activity is attributable to proteinaceous (including lysozyme-like) or non-proteinaceous factors.

V.CONCLUSION

Different solvent extracts of the digestive gland,tube feet, spine and gonad of the starfish, *Pentaceraster mammillatus* were screened for antimicrobial agents. The butanol extracts of the digestive gland, tube feet, gonads and the chloroform extract of the spine showed strong antibacterial effect against the tested pathogens. The extracts should be further analyzed to isolate and purify as well as determine the chemical structure of the antibacterial compounds to use as novel antibiotics.

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