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ANTIBACTERIAL ACTIVITY OF MARINE PUFFER FISH CHELONODONPATOCA FROM THOOTHUKUDI COAST

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ABSTRACT

The antibacterial activity of puffer fish *Chelonodonpatocaagainst* ten bacterial strains has been evaluated.Liver and muscle tissue extracts of *Chelonodonpatocaprepared* by using acetic acid. The antibacterial test was done by Agar diffusion technique. The liver extract showed maximum activity with zone of inhibition 12 mm radius at 10 mg /10µl in *Pseudomonas sp* .In thecase of muscle extract, the zone of inhibition extended up to 11 mm radius in S*higellaflexneriat* 10mg/10 µl. The results indicated that the liver and muscle extracts of *Chelonodonpatocamay* have potent antibacterial compounds that can be further explored and utilized for the welfare of mankind.

KEYWORDS : Chelonodonpatoca, antibacterial activity, Bacillus ceseusand Zone of inhibition.

INTRODUCTION

Marine ecosystem has drawn attention of many scientists due to its biological and chemical diversity (Chew *et al*., 2007). The number of natural products isolated from marine organisms increases rapidly and now exceeds with hundreds of new compoundsbeing discovered every year(Faulkner.,2002; Proksch*etal* ., 2006). Many bioactive compounds have been extracted from various marine animals like tunicates, sponges, soft corals, sea hares, nudibranchs, bryozoans, sea slugs and marine organisms (Doniaet *al* 2000 andHaefner., 2003). Many class of bioactive compounds exhibiting antitumour, antileukemia, antibacterial and antiviral activity have reported worldwide (Pettit *et al.*,1991).So far approximately 7000 natural products have been reported from the marine

organisms(Venkataraman K,2005). Among them, fishes are the largest classes of vertebrates.Due to the aquatic environment, fishes have some unique characteristics (Ellis 2001and Plouffeet al., 2005). Fishes are evolving with innate immune response to protect themselves against the infection (Noguchi, 2008)

The puffer fishes are commonly known of all type of fish poisoning and has been recognized from ancient times.It is probably the most common fish poisoning along the coasts of Asia.There are as many as 120 species of puffer fish that live mostly in tropical seas.All belong to the order Tetraodontiformes.They also called as blow fish, toad fish, swell fish, globe fish and balloon fish(Tordaetal., 1973).They are named after their habit of inflating themselves with water or air when threatened, making it difficult for a predator to swallow them.

This fish is known to carry tetrodotoxin (TTX) (Bilecenogluet al.,2006) which is known a nonprotein organic compound and one of the strongest marine paralytic toxins today.TTX named after the order of fish from which it is most commonly associated, the Tetraodontiformes (tetras – four and odontos -tooth) or the tetraodon puffer fish (Halstead, 1978).TTX can be found in the liver, gonad, intestines and skin of these fish and cause death in approximately 60% of persons who ingest it (Ellenhorn and Barceloux 1988).The toxin has only occasionally beendetected in the muscle of these fishes.

Antimicrobial activity ofepidermal mucus extracts against a broad range of microbial pathogens was observed by Hellioet *al*.,2002. These experiments supported the hypothesis that the epidermal mucus plays a protective function against microbial infection in fish. The antimicrobial function of epidermal mucus appears to result from its mechanical and biochemical properties.The mucus layer on the surface of fish is continuously replaced, which possibly prevents stable colonizationby parasites, bacteria and fungi(Pickering, 1974).

Antibacterial proteins are assumed to form ion channels in bacterial membrane and kill both gram positive and gram negative bacteria (Ebranet *al.*, 2000).Knouftet *al.*, (2003) has reported endogenous peptides with antimicrobial activity from fish mainly from the skin and its secretions.

In India, studies on the antibacterial activity of puffer fish are very limited. The aim of the present study is to evaluate the antibacterial activity of muscle and liver of *Chelonodonpatoca* collected from Thoothukudi coast.

MATERIALS AND METHODS

COLLECTION OF SPECIMEN

Specimens of puffer fish *Chelonodonpatoca* were collected from fish landing centre at fishing harbour, Thoothukudi. Then they werewashed with sea water and transported to the laboratory in dry ice and was stored in the deep freezer at -20^{0}c.

ANTIBACTERIALACTIVITY

Preparation of acetic acid extract

Specimens of *Chelonodonpatoca* was thawed and dissected into tissues like muscleand liver. Ten grams of each tissue was homogenized with 50 ml of 0.1% acetic acid and were boiled in hot water bath around 45^{0}C for 10 minutes, cooled and centrifuged off. Then it wascondensed using rotary evaporator at 60^{0}C. Then it was stored at the deep freezer for further useat -200C (Kawabata., 1979).

BACTERIAL STRAINS

The reference strains used to test anti-microbial activity includes *Bacillus ceseus ,Vibrio cholerae(01),Vibrio cholerae (0139),Escherichia coli, Pseudomonas aerogenosa , Aeromonashydrophila , Salmonella typhi ,Shigellaflexneri , Pseudomonas spand Staphylococcus aureas.*

AGAR DIFFUSION TECHNIQUE (ACAR, 1980)

1.0ml of 12 h old nutrient broth culture was transferred into the sterilized petridishes. About 15-20ml of antibiotic agar medium was poured into each petridish. Sterilized paper discs prepared from Whatmann No.1 were used for loading acetic acid extract of puffer fish. The paper discs were loaded with different concentration viz., 10mg/10µl, 1mg/10µl and 0.1mg/10µl. The zone of inhibition was measured after 24hrs.

RESULT

The crude tissue extracts (Liver and Muscle) of *Chelonodonpatocawere* screened against ten human pathogenic bacteria for testing their antibacterial activities. The maximum zone was observed against the *Pseudomonas spin* the liver extract of *Chelonodonpatocaand* minimum zone was observed against *Vibrio cholerae* (0139) in the muscle extract as shown in Table 1 andFigure 1&2.

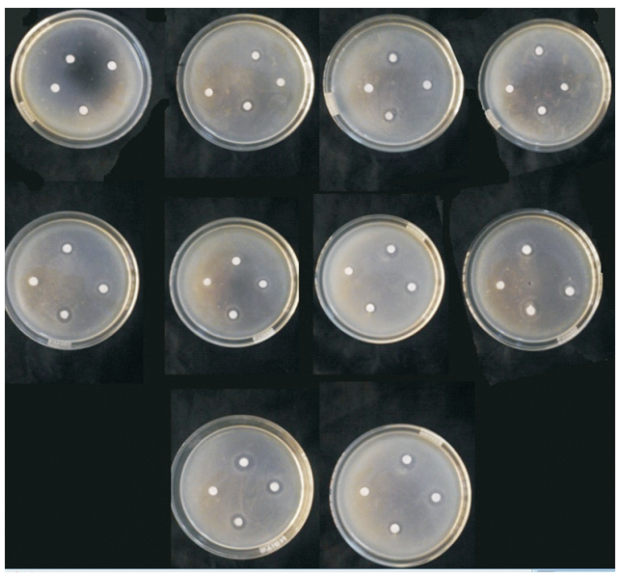
TABLE – 1

ACTIVITY OF ACETIC ACID EXTRACT OF MUSCLEAND LIVER OF CHELONODON PATOCA AGAINST BACTERIAL STRAINS

Bacterial Strains	Control	10 mg/ 10µl.		1 mg/ 10µl.		0.1 mg/ 10µl.	
		Muscle	Liver	Muscle	Liver	Muscle	Liver
Bacillus ceseus (BC)	-	+++	+++	++	+++	++	++
Vibrio cholerae(01) VC (01)	-	++	+++	++	++	+	++
Vibrio cholerae (0139) VC (0139)	-	+++	+++	++	++	+	+
Escherichia coli(EC)	-	++	+++	++	+++	+	++
Pseudomonas aerogenosa (PA)	-	++	+++	++	++	++	++
Aeromonashydrophila (AH)	-	++	+++	++	+++	+	++
Salmonella typhi (ST)	-	+++	+++	++	+++	++	++
Shigellaflexneri (SF)	-	+++	+++	++	++	++	+
Pseudomonas sp (P.sp)	-	++	++++	++	+++	+	++
Staphylococcus aureas (SA)	-	+++	+++	++	++	++	++

Zone of inhibition mm (radius), +5.5 – 7.5, ++ 7.5 – 9.5, +++ 9.5 – 11.5, ++++ 11.5 – 13.5.

FIGURE - 1
ANTIBIOTIC AGAR PLATE SHOWING ANTIBACTERIAL ACTIVITY OF ACETIC ACID EXTRACT OF MUSCLE OF CHELONODONPATOCA AGAINST BACTERIAL STRAINS



z1 - Zone of inhibition
a – 10mgl / 10ml
b - 1 mg / 10ml
c – 0.1 mg/ 10ml
d - control

FIGURE - 2
ANTIBIOTIC AGAR PLATE SHOWING ANTIBACTERIAL ACTIVITY OF ACETIC ACID EXTRACT OF LIVER OF CHELONODONPATOCA AGAINST BACTERIAL STRAINS

z1 - Zone of inhibition
a – 10mgl / 10ml
b - 1 mg / 10ml
c – 0.1 mg/ 10ml
d - control

DISCUSSION:

The emergence of new infectious diseases and resistance to the antibiotics by existing ones led to the new sources for drug discovery. Many organisms have been antibacterial properties, although most of the antibacterial agents that have been isolated from marine sources have not been active enough to complete with classical antimicrobial obtained from microorganisms (Rinehart *et al*., 1981).

Antimicrobial peptides are important in the first line of the host defence system of many animal species (Boman, 1995). In recent years, the therapeutic drugshas been widely recognized that many organisms use antibacterial peptides as part of their host defence system against the invasion of microorganisms (Boman, H.G 1991 and Zasloff. M, 1992).

The mode of action of antimicrobial agents also plays a vital role in the bacterial susceptibility. This is because different antimicrobial agent affects the microorganisms differently.Most of reported antimicrobial peptides typically have strong antimicrobial activity against a wide range of Gram – positive bacteria but very weak or no activity against Gram – negative bacteria(Mitta et al., 2000).

The antimicrobial property of mucus against the various pathogens has been demonstrated previously in rock fish(Kitani *et al*., 2008).

The antibacterial activity was observed maximum againstthe Pseudomonas spin the liver extract of *Chelonodonpatocaand* minimum was found against *Vibrio cholerae* (0139) in the muscle extract. The results clearly indicate the toxins present in the fish are having bioactive compounds that may be used for therapeutic needs.

CONCLUSION

In the present study, the muscle and liver extracts of puffer fish *Chelonodonpatocahave* been examined for their bioactivity. These results revealed that the muscle and liver has some valuable bioactive compound. So this study paves the way for further investigation on the pharmacological composition of puffer fish to discover potential chemotherapeutic agents.

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