International Journal of Agricultural Technology 2013 Vol. 9(6):1437-1445
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

In vitro antibacterial activity of tissue extracts from four channids against enteric pathogens

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M.A. Haniffa, P. Jeyasheela and M. James Milton (2013) *In vitro* antibacterial activity of tissue extracts from four channids against enteric pathogens. International Journal of Agricultural Technology 9(6):1437-1445.

Abstract The aim of this study was to identify the presence of antimicrobial activity in tissues of selected *Channa* species. Atotal of 20 solvent fractions from the tissue were obtained by solid-phase extraction and the fractions were assayed for antimicrobial activity. The screening of antimicrobial activity for all the fractions were tested against five enteric pathogens including *Salmonella typhi, Staphylococcus aureus, Klebsiella vulgaris, Shigella dysenteriae, Shigella boydii*. The activity was measured in terms of zone of inhibition in mm. The tissue that showed efficient antibacterial activity was subjected to SDS-PAGE to find out the molecular weights. Efficient antibacterial activity was observed from the methanol extract of all the *Channa* species. A maximum zone of inhibition was observed in *Channa striatus* against *Shigelladysenteriae*. It is therefore suggested that *Channa striatus* can be a potential source of an antimicrobial protein for specific human pathogens.

Keywords: Channa species, Antibacterial activity, Enteric pathogens, Tissue protein.

Introduction

Infectious diseases are the main cause of human death worldwide (Westh and Zinn, 2004). Inspite of modern improvements in chemotherapeutic techniques, infectious diseases are still an increasingly important public health issue (World Resources Institute, 2000). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multi drug resistant pathogens (Bandow *et al.*, 2003). There is a an urgent need to explore and discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action for new and re-emerging infectious diseases (Rojas and Brotz, 2003). Search of natural antibacterial agents are gaining importance over conventional antibiotics, because of its rapid action and potent bactericidal activity. Moreover, it overcomes the problem of resistance and side effects.

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Hence, several attempts have been made exploring new antimicrobial drugs from natural sources including plant and animal products.

Enteric or diarrhoeal infections are major public health problems in developing countries and contribute to the death of 3.3 to 6.0 million children, annually. Enteric bacteria comprised *Salmonella* spp., *Shigella* spp*., Proteus* spp*., Klebsiella* spp., *Escherichia coli, Pseudomonas* spp*., Vibrio cholera* and *Staphylococcus aureus* which are major etiologic agents of sporadic and epidemic diarrhea both in children and adults (Arockiaraj *et al.*, 2004).

Channa, commonly called as murrels are obligatory air-breathing and precious edible freshwater fish that reside in swamps, slow-flowing streams and in crevices near riverbanks, belonging to the family Ophiocephalidae / Channidae (Qasim and Bhatt, 1966). It was reported that epithelial tissues of fishes produce antimicrobial molecules which serve as the first line of a host's defense against microbial invasion in a variety of vertebrates including humans (Ganz, 1999). Earlier research reports showed that the muscle tissue of *Babylonia spirata* plays a role in the prevention of colonization by parasites, bacteria and fungi (Periyasamy *et al.,* 2012). Antibacterial activity in fish mucus has been demonstrated in several fish species (Palaksha *et al,* 2008; Kitani *et al.*, 2008; Dhanaraj *et al.,* 2008 and 2009; Raida and Buchmann, 2009; Tendencia *et al.,* 2004; Easy and Ross, 2009). Very limited studies have been carried out in the screening of antimicrobial activities in different tissues of freshwater fish species (Ruangsri, 2010). *Channa striatus* is both a popular food choice and a natural remedy in traditional medicine, particularly in post operative patients to induce wound healing. Its chemical composition includes high level of essential amino acids and a good profile of fatty acids that could directly improve tissue growth and would heal. Other pharmacological activities include antimicrobial, anti-inflammatory, cell proliferation, induction of platelet aggregation and antinociceptive properties (Mat Jais, 2007). With this background, the antimicrobial activity of the tissue extracts of four *Channa* species viz. *Channa striatus, C. punctatus*, *C. diplogramme* and *C. marulius* were evaluated against selected enteric pathogens.

Materials and methods

Live specimens of *Channa striatus, C. punctatus*, *C. marulius* and *C. diplogramme* were collected from local rivers and reservoirs of Kerala and Tamilnadu, India. They were immediately brought to the laboratory. The muscle tissue of *Channa* sp. (50 g) was cut into small pieces and the tissue sample was used for extraction with different solvents such as acetone, benzene, chloroform, methanol and water. The extracts were cold steeped over night at -18°C and filtered with Whatman No. 1 filter paper. The filtrate was poured in previously weighed petri plate and evaporated to dryness in rotary evaporator (Becerro *et al.*, 1994; Wright, 1998). The dried crude extracts were used for antimicrobial assay against human pathogens such as *Salmonella typhi, Staphylococcus aureus, Klebsiella vulgaris, Shigella dysenteriae* and *Shigella boydii*. All the pathogenic bacterial strains were obtained from Government Medical College, Tirunelveli. The bacterial strain was cultured in nutrient broth incubated at 37 °C and maintained on nutrient agar (HiMedia) slant at 4°C.

Antibacterial Assay

Antibacterial assay was carried out by disc diffusion method (Dulger and Gonuz, 2004) using microbial cell suspension whose concentration was equilibrated to 0.5 McFarland standards. For this, 0.1ml (10^{-5}cfu /ml) of 24 hrs old bacterial culture was placed on Mueller Hinton agar medium and spread throughout the plate by spread plate technique. The sterile filter paper disc of 6mm diameter loaded with 50 µl of muscle extract was placed on the surface of the medium and incubated at 37^{o}C for 24hrs. Antibacterial activity was recorded by measuring the diameter of zone of inhibition including the disc. Chlortetracycline (5 mcg / disc) was used as positive control. The entire test was performed in triplicate. The results are expressed as mean ±SD.

Molecular Size of Muscle Protein by SDS-PAGE

SDS-PAGE was performed as described by Laemmli *et al.* (1970) in 10% Polyacrylamide gel. Protein samples were then loaded and electrophoresis was performed at a constant voltage of 75 V. The run was stopped when the dye front was 2 to 3 mm away from the bottom edge of the gel. At the completion of electrophoresis, the glass sandwich was disassembled. The stacking gel was discarded and the resolving gel was stained using Coomassie Blue. Molecular weights of the proteins were determined by comparing relative mobility of protein bands to the standard protein markers.

Results

Five crude extracts from four *Channa* species namely, *C. striatus, C. punctatus, C. marulius* and *C. diplogramme* were screened against five enteric pathogens. Almost all the five extracts had efficient antibacterial activity against the pathogens. The zone of inhibition ranged from 3.6 to 12.6 mm which is depicted in Figure 1. The activity of all the five solvent extracts of four *Channa* species against the studied enteric pathogens has been represented in Fig. 1. The maximum zone of inhibition was found to be 12.6 mm for the methanol extract of *C. striatus* against *Shigella dysenteriae*. The methanol extract of *C. marulius* had highest inhibition zone of 11.8 and 11.1 mm against *Shigella boydii* and *S. aureus* respectively. Water extracts of all the species showed least activity against the pathogens. Water extracts of *C. striatus* had no activity against *S. aureus* and *Shigella boydii*, as well as *C. punctatus* against *K. vulgaris* and *Shigella boydii*, and *C. diplogramme* against *S. aureus* and *K. vulgaris*. In all the species the inhibition observed was in the order of methanol> chloroform> acetone> benzene> water against the bacteria.

The tissues of the *Channa* species which showed efficient antibacterial activity were subjected to SDS-PAGE to study the molecular weights of the tissue protein. Crude protein sample of four *Channa* species yielded 34 bands ranging from 16.64-232.76 k Da (Fig. 2). The highest number of band was found in *C. diplogramme* and least number of bands was found in *C. punctatus*. The total number of bands for each *Channa* species and its molecular weights has been recorded in Table 1.

Table 1. Molecular weights (k Da) of the tissues of *Channa* species

Bands	***C. striatus***	***C. punctatus***	***C. marulius***	***C. diplogramme***
1	-	232.65	185.45	232.76
2	179.10	148.56	-	163.26
3	116.17	-	-	107.71
4	85.88	95.80	-	-
5	59.08	-	-	63.23
6	-	-	55.08	-
7	-	-	39.29	37.70
8	-	28.43	30.71	-
9	22.78	23.10	25.53	22.25
10	20.57	20.11	21.26	-
11	-	-	19.55	19.56
12	-	-	18.03	18.00
13	17.81	-	-	17.63
14	-	-	17.17	17.31
15	-	-	16.85	16.64

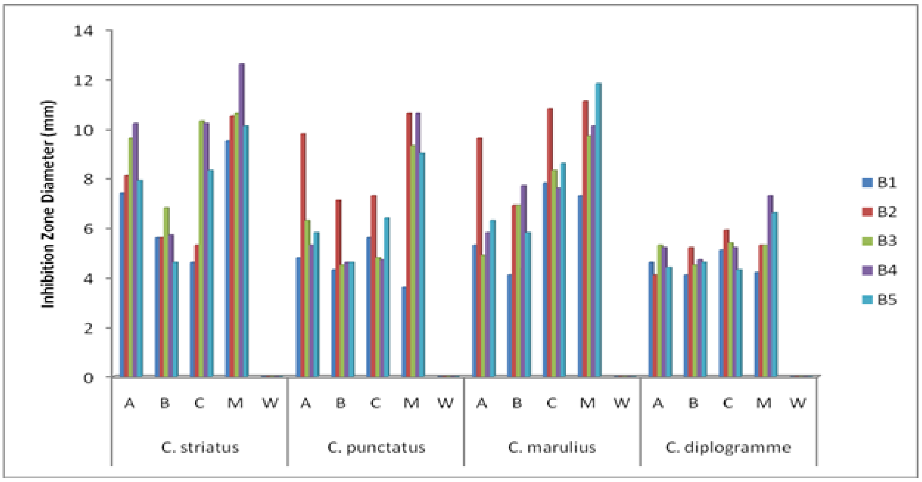

Fig. 1. Antibacterial activity of the tissue extract of *Channa* species.
B_1 - *Salmonella typhi*, B_2 - *Staphylococcus aureus*, B_3 - *Klebsiella vulgaris*, B_4 – *Shigella dysenteria*and B_5 – *Shigella boydii;* A – Acetone, B – Benzene, C – Chloroform, M – methanol and W – Water

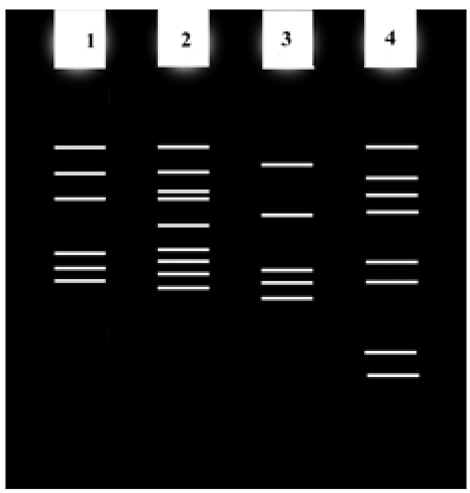

Fig. 2. Tissues of *Channa* sps expressing antibacterial activity. SDS-PAGE analysis showing the molecular weight of the tissue protein.

Discussion

Animals have been used as medicinal resources for the treatment and relief of a myriad of illnesses and diseases in practically every human culture (Costa, 2005). Innate antimicrobial compounds with a broad antimicrobial effect have been identified in a variety of multicellular organisms (Zasloff, 2002), ranging from insects (Bullet *et al.*, 1999) to several groups of vertebrates e.g. fishes (Cole *et al.,* 1997), amphibians (Zasloff, 1987) and mammals (Harder *et al*., 1997).

Fish by-products are rich in potentially valuable proteins, minerals, enzymes, pigments and also contain antimicrobial agents. Among the fish by products, fish mucus, gills and blood ~~is~~ are considered more valuable and has been reported to contain several antimicrobial proteins (Subramanian *et al.*, 2008; Hellio *et al.,* 2002). According to Boman (1995) and Andreu and Rivas (1998), most of the antimicrobial peptides kill bacteria by a common mechanism, which involves direct electrostatic interactions with negatively charged phospholipids on microbial cell membranes followed by physical disruption and solubilization. Fish contain naturally occurring proteins and glycoproteins of non immunoglobulin nature (transferrins, metallothionein) that react with environmental antigens and confer an undefined natural immunity to fish. Hence, several endogenous peptides with antimicrobial activity have been purified from fish especially from the skin and intestinal mucus. The metal ion chelating mechanism exhibited by these compounds deprives the microbes of essential inorganic ion sources and thus inhibiting the microorganisms. Fish also contain lectin which has antifungal and antibacterial activities (Alexander *et al*., 1992). Protein quantification results revealed that *Channa striatus* contains a high amount of proteins that may be a potential antimicrobial source.

Several antimicrobial studies have been done earlier using the mucus of fishes and the tissue extracts of gastropods. But this was the first report on the antibacterial activity of the various tissue extracts of *Channa* species. The maximum zone of inhibition was found to be 12.6 mm for the methanol extract of *C. striatus* against *S. dysenteriae*. Similar studies have been done using the methanol extracts of *Hemifusus pugilinus,* which showed highest activity against *E.coli* and the lowest activity against *Klebsiella oxytoca*. The methanol extracts of *Anadara granosa* showed good response against *E. coli* and least response against *Salmonella typhi* (Srinivasan, 2008). The methanol extracts of *Sepia officinalis* showed the maximum inhibition zone against *E.coli* and *Lactobacillus vulgaris* and minimum zone was recorded against *Salmonella paratyphi* (Reddy, 2008). The maximum antibacterial inhibition zone was exhibited by acetone crude extract of *Trochus tentorium* against human pathogen *S. pneumonia* (Anbuselvi *et al.,* 2009). The acidic mucus extract of *Channa striatus* inhibited the growth of three human pathogens, *Bacillus subtilis*, *Klebsiella pneumoniae*and *Pseudomonas aeruginosa* but no activity was observed against the fish pathogen *Aeromonas hydrophila* (Ong *et al.*, 2010). In our investigation, aqueous extract of none of the species showed activity against the pathogens. The absence of antimicrobial activity of the aqueous extracts could be due to the presence of low levels of enzymes. Earlier studies also have reported that, no microbial growth inhibition observed in aqueous fish mucus extracts of a wider range of fish species including Arctic char (*Salvelinus alpinus*), brook trout (*Salvelinus fontinalis*), koi carp (*Cyprinus carpio*), striped bass (*Morone saxatilis*), haddock (*Melanogrammus aeglefinus*) and hagfish (*Myxine glutinosa*) (Subramanian *et al*., 2008).

Conclusion

The results of this study support the folkloric usage of fishes and suggest that the fishes possess certain constituents with antimicrobial agents for new drugs for the therapy of infectious diseases caused by pathogens. The *Channa* species which shows broad spectrum of antibacterial activity can be subjected to further evaluation to analyze the chemical composition as well as to reveal the mode of action on bacteria.

Acknowledgement

We acknowledge the financial assistance received from Indian Council of Agricultural Research – National Agricultural Innovation Project (ICAR-NAIP F.No. 1(5)/2007-NAIP dt. 22 August 2008) to carry out this study. We are grateful to Rev.Dr.A.Joseph, S.J., Principal and Consortium Leader, for providing necessary facilities.

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(Received 15 July 2013; accepted 31 October 2013)