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Antifungal Activity of *Channa striatus* (Haruan) Crude Extracts

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Abstract: The present study was carried out to screen the *in vitro* antifungal activity of various extracts of *Channa striatus* (Haruan) against 13 filamentous fungus and 2 non-filamentous fungus (yeast) obtained from the China General Microbiological Culture Center (CGMCC) using the filter paper, Oxford cylinder and inoculation techniques. Both extracts 1 and 2 were prepared in distilled water under pressured cooking, but extract 2 was added with special blend of soup spices. The mucus extract was collected in distilled water as extract 3, after 1 h hypothermal shocked being introduce at -20°C to the fish. In addition, extracts 4, 5, 6 and 7 were obtained by soaking the fillet separately in methanol, ethanol, chloroform and chloroform: methanol (2:1; w v^{-1}). Additional experiments on the fish fillet extracts' antibacterial activity against various strains of *Staphylococcus aureus* were also carried out using the single concentration microdilution method. The results obtained demonstrated that only extract 5 (ethanol) inhibited the growth of *Neurospora crassa* 3.1601, *Aleurisma keratinophilum* 3.3544 and *Cordyceps militaris* 3.4655 after 24, 46 and 64 h of treatment. The same extract also inhibited the growth of *Botrytis pyramidalis* 3.1930 and *Paecilomyces fumosa-roseus* 3.3727, but only for the first 24 and 46 h. All extracts did not show any signs of growth inhibitory effect when tested against the *S. aureus* strains. This is very important for although the inhibition was not strong enough to kill some of the affected fungus, the partial inhibition by an animal (fish) based extract will be of better use for human consumption to avoid unnecessary repercussion.

Key words: *Channa striatus*, crude extracts, antifungal, fish fillet extracts, pathogenic fungus

INTRODUCTION

Fish by-products contain potentially valuable proteins, enzymes etc., (Ingram, 1980) and thus, research on biologically active compounds from fish could be an interesting field to explore. Despite an intimate contact with high concentrations of pathogens (bacteria and viruses) in their environment, the fish can still maintain a healthy system under normal conditions. This could be attributed to a complex system of innate defense mechanisms within the fish themselves, particularly the production of broad-spectrum antimicrobial compounds. According to Andreu and Rivas (1998) endogenous antimicrobial peptides are widely distributed in nature and are considered as the earliest components in the evolution of innate immunity. Other innate mechanisms that have been reported include the acute-phase proteins, non-classical complement activation, release of inflammatory mediators and phagocytosis (Cancre *et al*., 1999; Fouchereau-Peron *et al*., 1999; Hellio *et al*., 2002).

Channa striatus (Haruan) is a freshwater fish indigenous to the Southeast Asian countries, including Malaysia (Wee, 1982) and its extracts have been proven to possess antinociceptive (Mat Jais *et al*., 1997) and anti-inflammatory (Somchit *et al*., 2004) activities. Traditionally used to alleviate post-operative pain and enhance wound healing process (Wee, 1982), *C. striatus* contain high amount of arachidonic acid (ARA) and all of the essential amino acids (Zuraini *et al*., 2005) that are necessary for the process of wound healing tissue growth.

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Subsequently, we are herein to look at the antifungal activity of the fish extracts on selected filamentous and non-filamentous fungal obtained from the Institute of Microbiology, Chinese Academic of Sciences, Beijing, China. This collaborative research is specifically looking at the potential of *C. striatus* extracts to inhibit the fungal growth. This may be providing us a new alternative, for most of the antifungal agent that now available are mostly of plant based and very limited of the animal based. Based on the pharmacological activities reported on the fish, it will be interesting to screen whether *C. striatus* extracts contain bioactive compound (s) that inhibit (s) or at least, retard the growth of some pathogenic fungus.

MATERIALS AND METHODS

Preparation of fresh fillet of *C. striatus:* Throughout the study a market size of 250 g *C. striatus*, obtained from Pontian, Johore, Malaysia, were used and acclimatized in the Universiti Putra Malaysia, Selangor, Malaysia for at least 3 days prior to experiments. The fish were verified by the State Fisheries Department, Ministry of Agriculture, Malaysia and its' catalogue number (59.1.2) can be found in the Sarawak Museum, Sarawak, Malaysia. Preparation of fresh haruan fillet was carried out using the method described by Mat Jais *et al.* (1997). Precleaned live fish were weighed and sacrificed by an hour hypothermal shock. The fillets were obtained by carefully cutting the fish lengthwise along the backbone to gain the maximum amount of flesh without any backbone.

Preparation of *C. striatus* extracts

Preparation of various extracts of the fillet of *C. striatus* for antifungal study: Seven different types of extractions were performed using the fresh fillet of *C. striatus*. Sample 1, where 400 g of fillet was soaked in 1600 mL of distilled water (dH_2O) and then boiled for 1 h, was prepared to imitate the traditional way of preparing *C. striatus* in soup or porridge. Similarly, sample 2 was prepared by boiling the fillet (600 g) with additional soup spices in 2000 mL dH_2O for 1 h under pressure. Sample 3 was the mucus extract collected after exposure of 250 g fillet in 1000 mL to the hypothermal shock (-20°C) for 1 h. Preparation of the mucus extract was described in detail by Mat Jais *et al.* (1997). In addition, samples 4, 5, 6 and 7 were prepared separately by soaking 10 g of fillet in 50 mL of methanol, ethanol, chloroform and chloroform:methanol (2:1; w v^{-1}) overnight, respectively. The supernatants obtained were evaporated under vacuum. Samples 8, 9, 10 and 11 were actually similar to those of samples 4, 5, 6 and 7, respectively, but were tested using different technique which was paper-sliced method.

Preparation of various extracts of the fillet of *C. striatus* for antibacterial study: The fillet (40 g) of *C. striatus* was separately soaked in 2:1 or 1:2 (v:v) chloroform:methanol (CM) systems as described in detail by Zakaria *et al.* (2007). This will yielded four types of extracts namely aqueous (2:1) (A), aqueous (1:2) (B), CM (2:1) (C) and CM (1:2) (D). The chloroform (E) and methanol (F) extracts were obtained by soaking the fillet (40 g) separately in chloroform and methanol in the ratio of 1:2 (w v^{-1}) for 24 h and evaporating the collected supernatant at 40°C under vacuum. The aqueous extracts were freeze-dried to obtain crude dried extracts, which were later dissolved in dH_2O to the desired dosage. The 2:1 or 1:2 (v v^{-1}) CM, chloroform and methanol extracts were separately evaporated (40°C) under vacuum to yield various types of pastes, which were later dissolved in Dimethyl Sulfoxide (DMSO) to the desired dosage before used.

Types of microorganisms: Thirteen species of filamentous and 2 species of non-filamentous fungi were used in the present study and were obtained from the China General Microbiological Culture Center (CGMCC). The filamentous fungi were 3.1601 *Neurospora crassa*, 3.1930 *Botrytis pyramidalis*, 3.2496 *Mucor racemosus*, 3.2545 *Mucor rouxianus*, 3.3544 *Aleurisma keratinophilum*, 3.3575 *Beauveria* sp., 3.3727 *Paecilomyces fumosa-roseus*, 3.4032 *Penicillium sclerotiorum*, 3.4392 *Rhizopus oligosporus*, 3.4398 *Eupenicillium reticulisporum*, 3.4523 *Verticillium psalliotae*, 3.4610 *Fusarium ventricosum* and 3.4655 *Cordyceps militaris* while the non-filamentous were 2.1490 *Filobasidiella neoformans* (Kwong-Chung) and 2.4990 *Rodotorula glutinis* (Fresenius Harrison)

The bacteria tested in this study were those in the collection of Forest Research Institute of Malaysia (FRIM) and belong to the *Staphylococcus aureus* strains, namely *S. aureus* 29213α, *S. aureus* 33591, *S. aureus* 700699, Vancomycin-Intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA).

Antifungal assay: Three different bioassays were used in the present study namely the filter paper, Oxford cylinder and inoculation methods. As for the filter paper method, 1 cm diameter discs, prepared using the Whatman No. 1 and pre-loaded with 50 μL of extracts, were placed onto the culture medium containing each fungal species.

In the Oxford cylinder method, a cylinder with inner diameter of 0.6 cm, outside diameter of 0.8 and 1 cm height, which gave a total volume of 0.3 mL, was used. Extracts, in the volume of 0.3 mL, was carefully poured into the tube with maximum meniscus and left over night on the fungus cultured medium at 25°C (for filamentous fungi) and 35°C (for non-filamentous fungi), respectively.

Finally, for the inoculation method, 1.0 mL of each extract was mixed separately with 4 mL of agar in a slanting test tube to create a slope and left to aggregate at room temperature. Subsequently, each of the fungal strain was smeared evenly on the slope to grow for a period of 24, 46 and 64 h.

The medium used (Potato Dextrose Agar-PDA) was prepared using the extract liquid of 20% potato, 1% glucose and 1.5% agar.

Antimicrobial assay: The cultures of antibiotic-susceptible *S. aureus* 29213 alfa, *S. aureus* 33591 and *S. aureus* 700699 were grown in Muller Hinton Broth (Difco) while those of VISA and VRSA were grown in Tryptic Soy Broth (Bucto™) at 37°C. The screening procedure for antibacterial activity as well as the MIC and MBC determination was carried out according to the liquid microdilution method described by the Society for Japanese Chemotherapy (1990) with slight modifications in which the single concentration test was performed prior to the determination of the MICs and MBCs.

RESULTS AND DISCUSSION

Table 1 shows the antifungal activity of various extracts of *C. striatus* assessed using different types of assays. Only Sample 5 (ethanol extract) of the *C. striatus* extract demonstrated antifungal activity via the inoculation method and inhibited five species of filamentous (3.1601 *N. crassa*, 3.1930 *B. pyramidalis*, 3.3544 *A. keratinophilum*, 3.3727 *P. fumosa-roseus* and 3.4655 *C. militaris*) and two non-filamentous fungi (2.1490 *Filobasidiella neoformans* and 2.4990 *Rodotorula glutinis*) (Table 1).

Table 1: Inhibitory effect on the growth of filamentous and non-filamentous fungi in three bioassays after exposure to various types of *C. striatus* extracts

	Types of extracts																	
	1		2		3		4		5		6		7		8	9	10	11
Fungi strains no.	A	B	A	B	A	B	A	B	A	B	A	B	A	B	C	C	C	C
2.1490	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
2.4990	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
3.1601	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
3.1930	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
3.2496	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.2545	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.3544	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
3.3575	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.3727	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
3.4032	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.4392	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.4398	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.4523	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.4610	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.4655	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+

A-Oxford-cylinder method; B-Paper-disc method; C-Inoculation method, – -Inhibition on fungi growth observed, + -No inhibition on fungi growth observed

Table 2: Antifungal profiles of various types of *C. striatus* extracts against different types of filamentous fungi assessed using the inoculation method at different culture time

	Types of extracts																				
	1			2			3			4			5			6			7		
	Culture time (h)																				
Fungi strains no.	24	46	64	24	46	64	24	46	64	24	46	64	24	46	64	24	46	64	24	46	64
3.1601	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	+	+	+
3.1930	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	+	+	+
3.2496	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.2545	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.3544	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	+	+	+
3.3575	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
3.3727	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+
3.4032	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.4392	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.4398	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.4523	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.4610	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.4655	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	+	+	+

– Inhibition on fungi growth observed, + No inhibition on fungi growth observed

Table 3: Antifungal profiles of sample 5 (ethanol extract) of *C. stiatus* against two types of non-filamentous fungi a various dilution fold assessed using the inoculation method

Dilution (fold)	5	10	20	100
2.1490	–	–	+	+
2.4990	–	–*	+	+

– Inhibition on fungi growth observed at 24, 46 and 64 h of the culture time, + No inhibition on fungi growth observed, * Inhibition was observed only at the first 24 h of the culture time

Table 2 shows the antifungal activity of various extracts of *C. striatus* assessed at various exposure times. Sample 5 inhibited the growth of 3.1601 *N. crassa,* 3.3544 *A. keratinophilum* and 3.4655 *C. militaris* at the exposure time of 24, 46 and 64 h, but inhibited the growth of 3.1930 *B. pyramidalis* and 3.3727 *P. fumosa-roseus* only up to the first 46 h of exposure (Table 2). Interestingly, serial dilution of Sample 5-5 and 10 fold was also found to inhibit the growth of *Filobasidiella neoformans* and *Rodotorula glutinis* (Table 3).

Preliminary screening of Sample A, B, C, D, E and F against various strains of *S. aureus* using the single concentration microdilution methods failed to demonstrate those extract antistaphylococcal activity (data not shown).

The present study demonstrated that only the ethanol extract of *C. striatus* exhibited antifungal activity. However, none of the extracts exhibited antistaphylococcal activity. The antifungal activity observed was not strong enough to kill the respective fungus, but this partial inhibition is good for human used as it will not promote serious side effects.

The failure of the filter paper and Oxford cylinder techniques, or the microdilution method to provide a consistent antifungal or antistaphyococcal results, respectively, could be due to the lower content of bioactive compounds loaded/poured and the different sensitivities of those strains.

Fish tissues and body fluids contain naturally occurring proteins or glycoproteins of non-immunoglobulin nature (e.g., transferrins, caeruloplasmin and metallothionein) that react with a diverse array of environmental antigens and may confer an undefined degree of natural immunity to fish. Those compounds exhibited microbial growth inhibitory activity via simple metal ions chelating mechanism, which deprive microbes of essential inorganic ion sources. Fish also contain serum and cellular interferons which are anti-viral proteins, enzyme-inhibitors (e.g., α_2-macroglobulin and other α-globulins) that inhibit the extra cellular proteases secreted by pathogens (Alexander and Ingram, 1992) and, a variety of relatively specific lytic molecules, like hydrolase enzymes (lysozyme, chitinase and chitobiase) that act on fungi and bacteria. Other than that, fish also contain lectins that possessed antifungal and antibacterial activities (Alexander and Ingram, 1992).

In addition to the above compounds, several endogenous peptides with antimicrobial activity have recently been purified from fish, especially from the skin and skin mucus (Oren and Shai, 1996; Park *et al*., 1997; Park *et al*., 1998). These endogenous peptides were suggested to play an important role in fish defense. One class of endogenous antimicrobial peptides that have been described by Lauth *et al*. (2002) was peptides with an overrepresentation of certain amino acids (e.g., glycine, histidine, tryptophan and proline). It is interesting to note that the extracts of *C. striatus* have been shown to contain all the essential amino acids (Mat Jais *et al*., 1994; Zuraini *et al*., 2005; Zakaria *et al*., 2007) including those mentioned above.

However, despite the claims by Oren and Shai (1998) and Scott and Hancock (2000) that most endogenous antimicrobial peptides possessed broad spectrum antimicrobial activity against bacteria, yeast and filamentous fungi and that microbial killing is a result of the interaction of the peptides with the microbial outer membrane that leads to membrane destabilization and channel formation, our finding seems to indicate that the *C. striatus* extract possessed a narrow, instead of broad, spectrum of antimicrobial activity. The failure of most of the crude extracts of *C. striatus* to exhibit antifungal or antistaphylococcal activities and the narrow antimicrobial spectrum of the fish crude ethanol extract seen in the present study should not be used to disregard the potential of *C. striatus* as a potential source of antimicrobial peptides. This statement is based on the finding that even the prepurified fractions of various parts of the Hybrid Striped Bass (derived from parental species, *Morone chrysops* and *Morone saxatilis*), failed to exhibit antimicrobial activity and that the methods of fractionation also influenced the types of compounds obtained (Lauth *et al*., 2002). Interestingly, most antimicrobial peptides are often amphipathic and/or hydrophobic (Hancock, 2001; Hancock and Lehrer, 1998; Lüders *et al*., 2005), which might explain the ability of ethanol extract of *C. striatus* to exhibit antifungal activity.

Although, the precise mechanism of action for antimicrobial peptides is yet to be explained, studies have shown that many antimicrobial peptides recognized the prokaryotic membranes as targets (Sarmasik, 2002). One of the models proposed to explain the mechanism of action of those peptides described that the mechanism involves several steps: Electrostatic contact between a negatively

charged membrane and positively charged peptide; conformation of helical structure and insertion of the peptide into the membrane and aggregation of several helices to form a pore, large enough to kill a target microbe (Merrifield *et al*., 1994).

CONCLUSION

In conclusion, the results obtained indicate the potential of ethanol extract of *C. striatus* as narrow spectrum antifungal agent. However, further in-depth antimicrobial studies using fractions (prepurified) or purified compounds are required and are being planned before final conclusion could be drawn on the antimicrobial properties of *C. striatus*.

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