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ANTIBACTERIAL SUSCEPTIBILITY TEST OF *Channa striatus* FILLETS AND MUCUS AGAINST *Staphylococcus aureus* AND *Pseudomonas aeruginosa*

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Abstract

Channa striatus is an indigenous fresh water carnivorous air breathing fish species and widely distributed in Malaysia. This white boneless meaty and tender taste edible fish is both a popular food of choice and a natural remedy in traditional medicine due to its pharmacological activities such antimicrobial, anti-inflammatory, cell proliferation and many more. Due to these salutary values of this natural product, *Channa striatus* often being studied in countless times in order to determine valuable information which researcher may gain. Therefore, the determination of effect exerted from both extracts particular in antibacterial activity was conducted to provide antibacterial effectivity for future formulation development. In this study, we followed the method of Wei et al. (2015) for the collection of *Channa striatus* mucus. Whilst for the fillets, the extraction method of Morachis-Valdez *et al. (*2017) and Susakate, S. *et al. (*2019) were referred. As both products were strongly claimed to have beneficial towards wound healing, the antibacterial investigation was carried out against two important pathogens causing wound infection. Comparing the two, *Channa striatus* has shown a better inhibition zone against *Pseudomonas aeruginosa* compared to *Staphylococcus aureus*. As conclusion, the potential values of *Channa striatus* fillets and mucus extracts need to be studied in greater detail covers the aspects of raw materials, extraction standardization to the formulation development in order to create a new natural sources product that potentially could act as alternative agent to fight bacterial infection and heal wound much quicker than natural healing process.

INTRODUCTION

Search for antibacterial candidates from natural sources always remained a potential area of investigation due to evolving resistance of microorganisms to the existing antibiotics. Aquatic organisms are believed to offer a rich source of potential new antibacterial drugs. *Channa striatus* Bloch (Family: Channidae), commonly known as Snakehead murrel is a fresh water carnivorous air breathing fish species and commonly known as ' Haruan' in Malaysia [1]. The species is believed to be native of Malaysia and other Southeast Asian countries. *C.striatus* is rich with proteins and considered as an excellent source of health food among Malaysian community. It has been a subject of renewed interest in Malaysian traditional medicine in the search for a better cure for diseases and ailments. Its consumption by women during postpartum is believed to be beneficial in promoting wound healing and reducing postoperative pain [2].

Several studies such as anti-inflammatory, analgesic, antimicrobial and anticancer properties have been reported by previous authors [3]. In the present study, we report the antibacterial susceptibility test of the fillet and mucus extracts of *C. striatus* on two common bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* that are involved in causing wound infection.

MATERIALS AND METHODS

Materials

Fresh fish (*C. striatus*) were procured from the local market in Ipoh, Perak. Suitable strains of *Staphylococcus aureus* (ATCC 49775) and *Pseudomonas aeruginosa* (ATCC 27853) were purchased from American Type Culture Collection (ATCC), USA.

Methods

Fish Collection and Maintenance

The fish were stocked into large plastic tank containing tap water in order to acclimatize them to laboratory conditions and

maintained for one week. During the period of acclimatization, the fish were fed with commercial feed once daily and 50% of the water was changed every day. After one week of acclimatization, only healthy fish was used for the mucus collection while the dead or fish with skin lesions were removed out from the tank.

Collection of fillets

The selected fish were cleaned and slaughtered. The fillets were obtained by carefully cutting the fish lengthwise along the backbone to gain the maximum amount of flesh without any backbone (**Figure 1**).

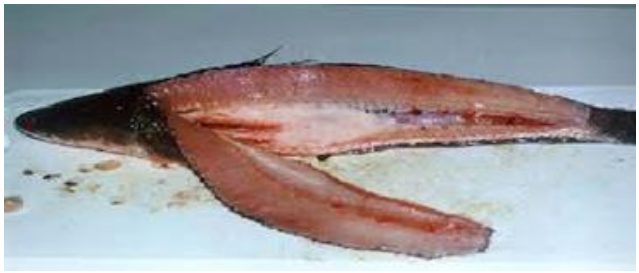

Figure 1. Fillet obtained by cutting the fish lengthwise along the backbone

Extraction of fillets

The method of Morachis-Valdez *et al.,* 2017 and Susakate, S. *et al.,* 2019 [3,4] was followed. Briefly, about 100 g of the fillet was homogenized in a blender for 4 min with a mixture of methanol (200ml) and chloroform (100ml). Then about 100 ml of chloroform was added to the mixture. After blending for additional 1 min, 100 ml of distilled water was added to the mixture. The homogenate was stirred and filtered. About 40 ml chloroform was further used to rinse the exhausted fillets. The liquid extract was allowed to stand until two layers (organic and aqueous) separated out. The chloroform layer was collected and evaporated to dryness.

Mucus collection

The method of Wei et al., 2010 [5] was followed to collect the mucus from the fish. Fish was starved for one day prior to mucus collection (**Figure 2**). On the day of mucus collection, the fish was washed and transferred into a sterile polyethylene bag for 10 to 20 min and the mucus was slough off from the fish by moving the bag front and back direction. The step was repeated for five fish and the mucus was pooled and stored in a refrigerator at 4°C.

Figure 2. Fish starved one day prior to mucus collection

Preparation of mucus extract

About 30 ml of fish mucus was mixed with 30 ml of 3% acetic acid and homogenized using a vortex machine (Figure 3). The mixture was then placed in boiling water bath for 5 min and cooled in ice and again homogenized using polytron homogenizer. Next, the mixture was centrifuged at 18,000 rpm for 35 min at 4°C. Finally, the supernatant liquid was collected and filtered. The filtrate was then stored in refrigerator at 4°C.

Figure 3. Mixture of mucus and 3% acetic acid were homogenized using a vortex machine

Antibacterial investigation: Screening for antibacterial activity

The fillet extract was studied for antibacterial activity by using disc diffusion method.

Preparation of bacterial culture

The bacterial slant was incubated overnight at 37°C. Initially, 9 ml of normal saline was filled in each of 10 glass tubes and labelled from 1 to 10. Then, 10 ml of bacteria culture solution was shaken uniformly using a vortex machine and 1 ml had taken and mixed into tube no 1. The tube was labelled with $1x10^{-1}$. Step 3 then repeated with tube no 2 to 10 accordingly. The tubes were labelled with 1x ($10^{-1\ to-5}$).

Determination of zone of inhibition

Antimicrobial susceptibility test discs are used for determination of susceptibility of bacteria to antimicrobial agents, applying in vitro diffusion method (Andrews, 2005) [6]. The antibacterial activity of the extracts was performed by disc diffusion method on nutrient agar plates. The extracts were separately dissolved in DMSO (10 mg/ml) and discs were prepared at concentrations of 20 µl/disc. Standard antibiotic discs of ciprofloxacin (5 µg/disc) were used for the comparison of the activity. Solvent control (DMSO) was also maintained throughout the experiment. The test organisms were inoculated on the medium and the plates were incubated at 37^0C for 24 h. The zone of inhibition was calculated by measuring the minimum dimension of zone of no bacterial growth around the disc.

RESULTS AND DISCUSSION

In the present investigation, the mucus extract showed better degree antibacterial activity against *S. aureus* and *P. aeruginosa*. However, the fillet extract was found to be effective only against *S. aureus* at the tested concentration. The results are tabulated in **Table 1**.

Table 1. Antibacterial activity of *C. striatus*

Treatment	Concentration	Zone of inhibition (mm)	
		S. aureus	***P. aeruginosa***
Fillet extract	20 µl/disc	4.2	6.3
Mucus extract	20 µl/disc	16.1	19.9
Ciprofloxacin	5 µg/disc	38.7/39.3	42.6/44.1

The spectrum of activity observed by the mucus extract may be an indicative of the presence of broad spectrum antibacterial compounds in the mucus. On contrary, no inhibition *P. aeruginosa* by the fillet extract indicated that it was either ineffective at the tested concentration or maybe the extract is effective against only Gram positive bacteria.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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