Pakistan J. Zool., vol. 46(6), pp. 1747-1751, 2014.

In Vitro Evaluation of Antimicrobial Activity of Cat Fish Slime Mucin on Selected Micro-organisms by Agar Diffusion Method

M.A. Momoh,[1*] A.T. Mora,[2] J.D.N. Ogbonna[1] and A.A. Agboke[3]
[1]*Drug Delivery Unit, Department of Pharmaceutics, University of Nigeria, Enugu State, Nigeria*
[2] *Department of Clinical and Pharmacy Management, Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna State, Nigeria*
[3]*Department of Pharmaceutical Technology and Pharm. Microbiology, University of Uyo, Akwa-Ibom State, Nigeria*

Abstract.- Treatments of bacterial infections have become a difficult task in the medical practice due to high level of resistance to the existing marketed antibiotics. This problem evoked us to search for new antimicrobial agent for better management of bacterial infections. The present study was carried out to evaluate antimicrobial properties of epidermal mucus (soluble mucin or s-mucin). s-mucin was extracted using cold precipitation method and its antimicrobial properties were tested against four pathogenic bacteria at different concentrations. Results showed s-mucin have remarkable antimicrobial activity. Particularly, s-mucin was more effective against *Staphylococcus aureus* and *Eschericia coli* than *Pseudomonas aeruginosa* and *Salmonella typhi.* Its activity was comparatively better than the reference drug. Thus, s-mucin from cat fish's mucus may serve as a remedy to the current antibiotics failure. Its formulation into pharmaceutical preparation is therefore, encouraged after further *in vivo* evaluation.

Key words: Catfish, mucus, s-mucin, antimicrobial activity.

INTRODUCTION

Treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance against existing marketed antimicrobial agents. These antimicrobial agents are often categorized according to their principal mechanism of action (Esimone *et al.*, 2005).

Penicillin and streptomycin have been widely employed in combating post-operative infections in man and animals (Esimone *et al.*, 2005). The resistance to these important molecule and many other newer antibacterial have drawn the attention of researchers to look for the alternative antibiotics (Kenechukwu *et al.*, 2013).

The marine environment contains a diverse number of plants, animals, and microorganisms, which, due to the unique adaptations to their habitat, produce a wide diversity of natural products (Constantino *et al.*, 2004). Many of the substances isolated from marine organisms have demonstrated to possess interesting biological and pharmacological activities (Bhadury *et al.*, 2006; El-Gamal, 2010). Cat fishes are an excellent source of bioactive compounds (slim or mucus), which demonstrate a broad range of biological activities, such as, anti-inflammatory, antibiotics and wound healing properties (Ramasamy *et al.*, 2011; Shahid *et al.*, 2014).

In an earlier study Adikwu and Ikejiuba (2005) indicated that employing mucin from snail fortified with honey was a novel strategy for wound healing and thus relatively fast wound healing activity were achieved. The author related the activity on the wound healing effect to its likely antimicrobial effect, considering the negative role of bacteria in wound healing. However, no *in vivo* or *in vitro* study on the mucin to further confirm it antibacterial activities. Nevertheless, for the purpose of drug formulation and better utilization of this agent, there is need to study the activities of the s-mucin against some clinical isolates to justify it further recommendation for possible formulation into pharmaceutical dosage form.

Mucin or mucus which are secreted as massive aggregates of protein is high-molecular weight glycoproteins found on the surface of epithelial tissues where they act as lubricants and

* Correspondence: jointmomoh@yahoo.com
0030-9923/2014/0006-1747 $ 8.00/0

protectants (Mortazavi *et al.*, 1992). It has been shown that mucus plays a role in the prevention of colonization by parasites, bacteria and fungi (Lemaitre *et al.*, 1996). They are mainly secreted in the intestine but also in airways and other body membranes (Adikwu *et al.*, 2005). Mucin protein backbones are characterized by numerous tandem repeats that contain proline and are high in serine asparagine, hydroxylysine and/or threonine residues (Adikwu and Ndu, 2006). Owing to the viscoelastic consistency of mucin, it controls the diffusion of molecules across the mucus membrane and also protects the mucus membrane against the harsh conditions of the gastric environment due to hydrochloric acid (Philip *et al.*, 2008; Momoh and Adikwu, 2008).

Our current knowledge on the antimicrobial properties of the secretory mucus of the epidermis in catfish skin is very limited. However, most mucus secreted from snail species and bovine mucous has been investigated; its content characterized, drug delivery as well as antibacterial effect evaluated (Momoh *et al.*, 2013; Adikwu and Ndu, 2006). In this study, the effect of s-mucin has been studied on clinical isolated microorganism.

MATERIALS AND METHODS

Materials

The materials *viz.*, Ketalar® injection (Effechem, Italy), ethanol (Sigma Aldrich, Germany), fused calcium chloride, monobasic potassium phosphate, sodium hydroxide (BDH, Poole, UK), nutrient agar (Antec Diagnostic Products, UK), gentamicin (Juhel, Nigeria Ltd) and distilled water (Lion Water, University of Nigeria Nsukka, Nigeria) were of analytical grade and sed without further purification.

Microorganisms

The following strains of bacteria were used: *Ps. aeruginosa* ATCC 27853, *S. typhi* ATCC 19421, clinical isolate, *E. coli* ATCC 25925 and *Staph. aureus.* The strains were incubated at 37°C for 24 h and maintained at the Pharmaceutical Microbiological Unit, Department of Pharmaceutics, University of Nigeria. The isolates were propagated on nutrient agar plate (Oxoid, 40, England) according to the manufacturers' specification. The stock cultures were stored at 4°C on nutrient agar. They were then subcultured in nutrient broth (Oxoid, 40, England) at 37°C for 8h prior to antimicrobial testing.

Extraction and purification of the cat fish slime

Twenty catfish of average weight 180 kg were obtained from Nsukka market. The fish were allowed to stay in a make shift water for a week, after which the fish were electrocuted and the slime harvested. Briefly, by using a microscope slide, the mucus layer was gently scraped off and diluted four times with distilled water. The gel was homogenized for 2h at 4°C and exhaustively dialyzed against distilled water using a 12 kDa (MwCO) dialysis membrane. The dialysate was finally centrifuged at 10,000 rpm for 30 min to yield a supernatant of water-soluble mucus glycoprotein layer and lower layer of insoluble mucus glycoprotein. The supernatants were collected separately, pooled and lyophilized at –40°C for 48 h to obtain flakes of soluble mucin (s-mucin), which were powdered and used for the study. The plan of work in this study was approved by our institution's Animal Ethics Committee in compliance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of November 24, 1986 (86/609/EEC) (European Community Council Directive, 1986).

Preparation of antimicrobial s-mucin

Five different concentrations (100, 75, 50, 25, 10 mg%) of cat fish slime (mucin) were prepared by dissolving known weight of the extracted mucin in known volume of distilled water. For the purpose of comparison, a reference drug (gentamicin) 100mg% of the standard antibacterial agent (gentamicin, Juhel, Nigeria Ltd) was similarly prepared and served as positive control. Normal saline was also used as the negative control.

Antimicrobial screening tests of s-mucin

The sterility of s-mucin was evaluated by the cup-plate agar diffusion, for which approximately 200 mg portion of the dried mucin was dissolved in 2 ml distilled water and dispersed on the surface of the agar. The agar was incubated for 48 h.

For preliminary sensitivity test a 100 mg of s-mucin, dissolved in 1 ml of sterile water was inoculated in molten nutrient agar in a bottle, mixed and aseptically poured into Petri dish and allowed to solidify. Each plate was divided into four portions according to the number of the organisms used. The organisms were aseptically inoculated and incubated at 37^{o}C for 24 h.

For determination of antimicrobial activity as a function of inhibition zone diameter (IZD), the plate agar diffusion method was used (Nisar *et al*., 2008). This method depends on the diffusion of s-mucin from holes on the surface of the microbial seeded agar. Briefly, four cups were aseptically bored at equal distances from each other with the help of sterile cork borer. Using a sterile applicator, one drop of the formulation of a batch was applied in the holes. This was done after seeding the agar with different test organisms separately. The plates were incubated at 37.5±1°C for 24 h and the zone of inhibition was then determined to evaluate the antimicrobial activity. The assay was performed in triplicate and the mean diameter was recorded.

Data and statistical analysis

All experiments were performed in replicates (n= 3) for validity of statistical analysis. Results were expressed as mean ± SD. Student's t test was performed for parametric studies and a comparison of the results between two samples. For a comparison of the results among three or more groups, one-way analysis of variance and the post-hoc Tukey B test was performed for homoscedastic groups. Differences were considered significant for p values < 0.05.

RESULTS AND DISCUSSION

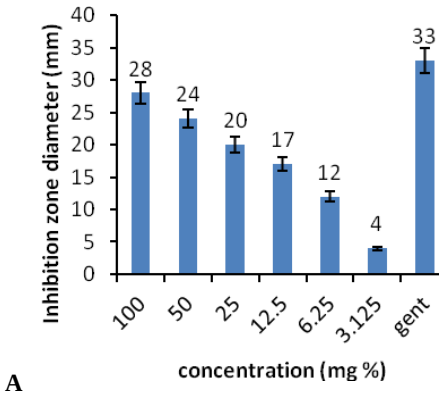
On the subject of the *in vitro* evaluation study on the sterility of the s-mucin, result showed no growth after incubation for 48 h, this indicate that the s-mucin after isoloation and storage contain no micro-organism. This evaluation was carried out to ensure that the s-mucin was not contaminated before being used for antimicrobial study. Consequently, any result obtained from the antimicrobial evaluation can be attributed to the activity of mucin and can be generalized within the limit of experimental error. The result of this evaluation also showed that the processing method used in the extraction and storage was capable of protecting the s-mucin from microbial contamination. The preliminary sensitivity test result showed that the s-mucin has activity against all the tested clinical isolates. However, that activities were more on the *E. coli* and *Staph. aureus.*

Antibacterial activity

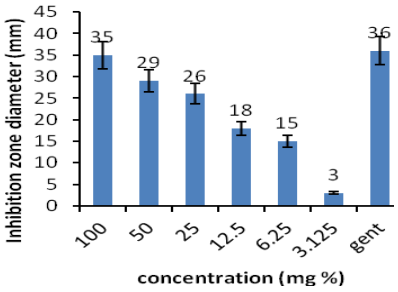
In vitro antibacterial activity studies showed that the s-mucin had effect on the test organisms comparable to reference drug (gentamicin), indicating that the bioactivity of s-mucin was not changed during the isolation and preparation procedures. Throughout the preparation method the use of any organic solvent was avoided so as to ascertain the natural activity of the mucin and at the same time, maintained the mucin in low temperature to avoid degradation or denaturisation.

The pharmaceutical and science researchers have accelerated their efforts and re-direct their attention on the natural sources of drug especially from the marine environment. The microbiological test was carried out to determine the sensitivity of the organisms such as *Staph. aureus*, *P. aeruginosa*, *S. typhi* and *E. coli* on the s-mucin and to compare the activity with the reference drug, gentamicin. The result of the sensitivity test showed that both the s-mucin and the reference samples produced very significant inhibitions against *Staph. aureus* and *E. coli*, with greater effect on the former than the latter. This finding indicated that the mucin extracted had activity against the test organisms, and this justified its use in the research.

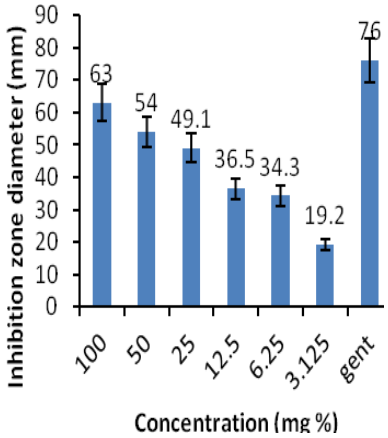
Figure 1, showed the *in vitro* antibacterial activity of s-mucin as a function of the IZD against *P. aeruginosa, Staph. typhi, E. coli* and *S. aureus* respectively. A significant increase in the IZD was observed in comparisons with control ($p < 0.001$). In the reference drug (positive control) there was a slight increase in the IZD compared with the s-mucin. The size of the IZD varied proportionately with the concentrations of the s-mucin (Fig. 1). The result showed that the s-mucin was able to diffused through the agar and exert it antimicrobial effect. The preparations gave these zones of inhibition in decreasing order of magnitude: 100 > 50 > 25 >



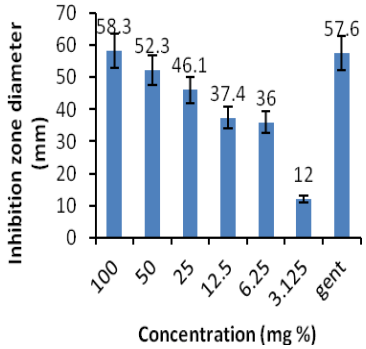
A



B



C



D

Fig. 1. IZD of mucin in *P. aeruginosa* (A), *S. typhii* (B), *E. coli* (C) and *S. aureus* (D).

12.5 > 6.25 > 3.125 mg % of s-mucin. A very small zone of inhibition was observed for preparation with least concentration (3.125 mg %) of S-mucin, probably because the concentration of S-mucin in this batch was low to yield concentrations equal to or above the minimum inhibitory concentration (MIC) to cause significant inhibition. Also normal saline, which served as the negative control, had no inhibition since it contained no test agent (S-mucin). The reference drug sample (gentamicin) 100 mg % gave higher zone of inhibitions in all cases. However, there was no significant difference ($p>0.005$) observed in the activity of the reference drug and S-mucin (Fig. 1C,D).

The determination of IZD using agar plate method is based on the diffusion of an antimicrobial agent or formulation thereof through a solidified nutrient agar. From the result presented in Figure 3, in all cases, the S-mucin concentrations showed activities against the micro-organism and the activities were concentration dependent.

CONCLUSIONS

In this study, different concentration of mucin was successfully prepared by dispersion method. The permeation of mucin from this dispersion system was significantly higher than that from commercial drug sample. However, it activities were comparatively better than the reference sample considering the concentration used in this study. More so, the activities of the dispersion mucin were concentration dependent. The results of this study indicate that cat fish mucin could be further explore to serve as an alternative to the current antibiotic failure or resistance currently affecting the effective management of infections in the clinical practice.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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(Received 3 September 2013, revised 2 November 2014)