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Research Article

Antimicrobial Susceptibility Test of Snail Mucin on *Staphylococcus aureus* and *Candida albicans*

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Abstract

This research work was done to ascertain the antimicrobial susceptibility of snail mucin on *Staphylococcus aureus* and *Candida albicans*. Solution of measured amount of citric acid was used to stimulate the slime production from the snail. Agar in well diffusion method was adopted using Muller Hilton agar. Eight plates of the media was used after inoculum development of the isolates. Ten (10%) and thirty (30%) concentration of both the slime extract and standard drugs (Chloramphenicol and fluconazole) for bacterial and fungal isolates respectively. After 24 hrs and 48 hrs incubation zone of inhibitions were measured. For the 30%, *Staphylococcus aureus* and *Candida albicans* gave 17 and 12 mm respectively while chloramphenicol and fluconazole gave 16 and 10 mm respectively. For the 10% concentration, *Staphylococcus aureus* and *Candida albicans* gave 3 and 1 mm while chloramphenicol and fluconazole gave 10 and 7 mm respectively. Since the organisms are sensitive to the snail mucin more the standard drug at 30%. The research recommends that the slime can the used to treat infections due to the organisms. It can also be used as additive in cream and probiotics production. These can be done after purification.

Keywords: *Mucin; Purification; Incubation; Sensitive; Inhibition; Zone*

Introduction

Snail mucin also known as snail slime, snail secretion and mucus used to describe the thick fluid produced by snails to protect their skin and shell. While they are often used interchangeably, snail mucin is specifically a type of snail secretion that is rich in nutrients, minerals and glycoproteins and is often used in skincare products. It is produced by snails in their foot, specifically in the pedal gland. The pedal gland is a specialized gland located in the snail's foot, which produces thick, protective mucus that helps the snail to reduce friction and prevent injury as it moves, protect its skin from dehydration and damage, aid in locomotion and movement, defend against pathogens and predators.

This slime is composed of various compounds, including zinc, carbohydrates, calcium, glycoproteins, glycolic acid, hyaluronic acid, and other nutrients, which are beneficial for skin health [Lee., *et al*. 2020]. The human skin being home to a complex and dynamic community of microorganisms, collectively known as the skin microbiota (microorganisms that reside on the skin and promotes skin health). This

microbiota plays a crucial role in maintaining skin health, preventing infection, and regulating the immune system [1]. However, an imbalance of the skin microbiota, also known as dysbiosis, has been linked to various skin disorders such as acne, eczema, and psoriasis [2]. Microorganisms including *Staphylococcus* spp and *Candida* spp. tend to play important roles on the skin. Snail mucin can be preserved and stored by: Freeze-drying (lyophilization), spray-drying, microencapsulation and refrigeration (4°C).

Materials and Methods

Sample collection

A total of 47 same species of snails were bought from a snail. The snails were handled in accordance with the principles of animal welfare in scientific experiments.

Collection of the test organisms

The test organisms were obtained from a facility; Nucleometrix Molecular Laboratory Yenegoa, Bayelsa State Nigeria, where they have already been purified and identified as *Staphylococcus aureus* and *Candida albicans*. The two isolates were then transferred to the laboratory and were sub-cultured into nutrient broth for inoculum development (to enable growth of organism) and was stored in the incubator at 37°C for 24hrs.

Sample processing

The snails were thoroughly washed clean with distilled water and placed on a sieve in a sterilized stainless bowl.

Stimulating solution preparation

A 38.0g of citric acid was weighed out using weighing balance by Hanna which was then mixed with 125 ml of sterile distilled water and put into a sterile spray bottle.

Snail mucin extraction

The snails were weighed and the weight was 370.6g. Mucin extraction was done by applying the snail stimulating solution on the snail for 10 seconds and allow to sit for 10 minutes which induces them to produce their slimes. This process was repeated several times in order to produce required slime.

After the extraction, mucin was filtered using a 0.5 micrometer sieve and put into clean 5 ml sterile container. The mucus physical characteristic observations were made which include the following parameters: color, texture/thickness, sliminess. The mucus texture and sliminess were determined by adopting the method of Billings and Westmore [3], while the mucus extracted was measured with calibrated bottles. For aqueous mucus extracts, the method described by Kumari., *et al*. [4] was adopted. A 60 ml volume of raw slime mixed with an equal volume of distilled water was placed on a centrifuge at 3000 rpm for 15 minutes. The supernatant separated from the sediment. At the end, a total snail mucin of 55 ml was obtained. The mucus secretion was stored in the refrigerator at 4°C for anti-microbiological assay.

Extract dilution

Two different concentration of the snail slime were prepared. That include 10% and 30%. The following formula was used.

$$\frac{\text{Desired percentage concentration X total volume of slime}}{100}$$

For 10% concentration = $\frac{10 \times 55}{100}$ = 5.5 ml then 90 ml of sterile distilled water added to make it up

For 30% concentration = $\frac{30 \times 55}{100}$ = 16.5 ml then 70 ml of sterile distilled water added to make it up

Microbial assay of snail slime extract on Staphylococcus aureus and candida albicans

The determination of mucus antibacterial activity by Agar well diffusion method as reported by Ali., *et al*. [5].

Preparation of media

Eight plates of Mullen Hilton Agar were prepared by dissolving 4.75g of powder in 100 ml of distilled water and it was then sterilized by autoclaving at 121°C for 15 minutes. It was allowed to cool and poured on the petri dish, after solidifying. The test organisms (*Staphylococcus aureus* and *Candida albicans*) from the inoculum developed flask were inoculated on 4 plates each by spreading the on the surface using sterile swab stick. After 30 minutes, a sterile cork borer was used to make 2 holes adjacent to each other.

Snail mucin extract of 10% and 30% concentrations were added to one hole each of the 4 plates. And 10% and 20% chloramphenicol were added to designated 10% and 30% concentration plates inoculated with *Staphylococcus aureus.* Also, 10% and 30% fluconazole were added to designated 10% and 30% concentration plates inoculated with *Candida albicans*. All plates were incubated for 24 hours for *Staphylococcus aureus* and 48 hours for *Candida albicans*. Results were recorded and zone of inhibition was measured using a millimeter ruler.

Qualitative phytochemical and proximate analysis of the extract or sample

All the proximate (ash, crude protein, crude fat, moisture, acids and carbohydrate) analysis were carried out using the standard AOAC methods [6]. All determinations were done in duplicates. The proximate values were reported in percentage.

Results

The result of the bacteriological analysis of the snail mucin on the *Staphylococcus aureus* and *Candida albicans* were shown below.

Test Organism	30% Snail mucin (mm)	30% Chloramphenicol/Fluconazole (mm)	Mucin reading
S. aureus	17	16	Sensitive
C. albicans	12	10	Sensitive

Table 1: *Comparison of antimicrobial effect of 30% snail mucin and antibiotics on Staphylococcus aureus and Candida albicans.*

Test Organism	10% Snail mucin (mm)	10% Chloramphenicol/Fluconazole (mm)	Mucin reading
S. aureus	3	10	Sensitive
C. albicans	1	7	Sensitive

Table 2: *Comparison of antimicrobial effect of 10% snail mucin and antibiotics on Staphylococcus aureus and Candida albicans.*

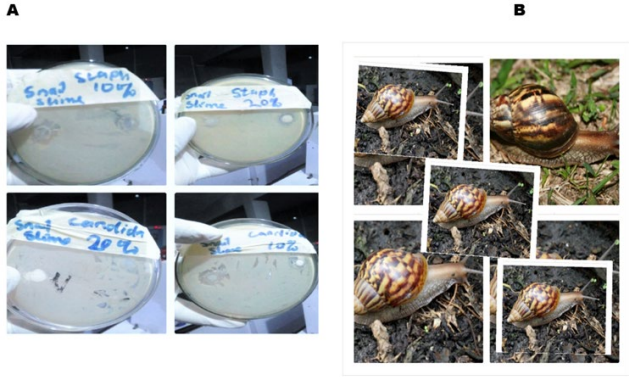

Figure A and B: *Incubated plates showing different zones of clearing and snail sample.*

Discussion

From the results, and picture shown above, the 30% concentration of *Archachatina marginata* mucin showed great antibacterial activity against *Staphylococcus aureus* with high inhibition diameter, compared to 10% concentration of chloramphenicol expressed in mm.

Also, the 30% concentration of *Archachatina marginata* mucin showed great antifungal activity against *Candida albicans* with inhibition diameter, compared to 10% concentration of fluconazole expressed in mm. The results of this research showed varied inhibitory and antimicrobial potency against *Staphylococcus aureus* and *Candida albicans*. This result agreed on the one done by Abiona., *et al*. [7]; Etim., *et al*. [8] which evaluated the mucus mucin from giant African snail for its antibacterial and wound healing capabilities. After their work, they discovered the antibacterial properties of *Achatina fulica* mucus when they analyzed the supernatant from centrifuged snail mucus and found a glycoprotein known as 'Achacin' as the active component after further biochemical investigation.

Conclusion and Recommendations

Therefore, snail mucus secretions could be a source for antibacterial and antifungal agents that can serve as an alternative to the expensive conversational and synthetic antimicrobial agents used because it exhibits a promising antimicrobial activity against *S. aureus* and *Candida albicans* supporting its potential as a natural antimicrobial substituent especially at a 30% concentration. This research recommends as follows:

1. Snail mucin should be used as a natural prebiotic alternative for promoting gut and skin health.
2. It can be incorporated into creams, lotions and soaps to take care of most skin infections.
3. It should be used as additive in food (e.g. fermented products), and pharmaceutical industries but this will be after purification.

Conflict of Interest

There is no conflict of interest in this research work.

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