



## ANTIMICROBIAL POTENTIAL OF MUCIN EXTRACTED FROM GIANT AFRICAN LAND SNAIL (*ACHATINA FULICA*) ON MICROORGANISMS ISOLATED FROM SOKOTO WHITE COWPEA (*VIGNA UNGUICULATA* (L.) WALP.)

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**Abstract:** Cowpea (*Vigna unguiculata*), especially the Sokoto white variety, is a vital legume widely consumed in Nigeria due to its nutritional and economic value. Despite this importance, stored grains are vulnerable to microbial contamination, which undermines quality and food safety. Mucin extracted from the giant African land snail (*Achatina fulica*) has been noted for antimicrobial properties, yet its effect on cowpea-associated microbes remains insufficiently studied. This research evaluated the antimicrobial activity of snail mucin against microorganisms isolated from stored Sokoto white cowpea. Samples were collected from five major markets in Ogun State and subjected to standard microbiological analyses. Bacteria were identified through morphological, Gram, and biochemical tests, while fungal isolates were characterized using lactophenol cotton blue staining. Five bacterial species (*Shigella* sp., *Enterococci* sp., *Staphylococcus* sp., and *Pseudomonas* sp.) and five fungal species (*Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Fusarium* sp., and *Trichoderma* sp.) were detected. Bacterial counts ranged from 30 to 292 cfu/g, while fungi recorded 52–384 cfu/g, with some plates yielding uncountable colonies. Aflatoxigenicity screening revealed *A. flavus* and *Fusarium* sp. as toxin producers. Antimicrobial testing indicated that snail mucin showed no inhibition against most isolates, except for *A. flavus*, which displayed a minimal inhibition zone ( $6.10 \pm 0.05$  mm). In contrast, conventional antibiotics demonstrated significant efficacy against bacterial isolates. These results underscore the microbial risks associated with cowpea storage and reveal that snail mucin has limited antifungal activity. Improved storage and handling practices remain essential for minimizing contamination and safeguarding food safety.

**Keywords:** legume, cowpea, storage, snail mucin, antimicrobial

### 1. Introduction

Snail mucus, commonly referred to as slime, is a rich source of mucins, large glycoproteins secreted by mucous glands that provide essential protective functions. These secretions safeguard delicate epithelial surfaces against microbial invasion and physical stress, while also imparting the characteristic viscoelastic properties of mucus [1]. Certain mucins are membrane-associated, a feature that enhances their retention at epithelial interfaces [2]. Beyond

structural and protective roles, snail mucus exhibits diverse biological activities, including antimicrobial, anti-inflammatory, anticancer, and wound-healing effects, largely attributed to its complex composition of bioactive molecules such as allantoin, collagen, elastin, glycolic acid, and antimicrobial proteins [3,4,5]. The antimicrobial potential of snail mucus has been widely investigated. Achacin, a bactericidal glycoprotein isolated from the mucus of *Achatina fulica*, disrupts the

integrity of bacterial membranes and has been shown to inhibit both Gram-positive and Gram-negative organisms through hydrogen peroxide generation [6,7]. Additionally, mucus-derived lectins enhance the antibacterial activity of achacin by increasing oxidative stress in target pathogens [8]. Early studies demonstrated that both the soluble and mucin-rich fractions of *A. fulica* mucus inhibited common pathogens such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, with the mucin fraction exhibiting stronger antimicrobial effects [9]. These findings underscore the potential of snail mucus as a reservoir of bioactive compounds for the natural antimicrobial development. Legumes, particularly cowpea (*Vigna unguiculata*), represent a major dietary protein source across sub-Saharan Africa. Cowpea is highly adaptable to diverse agro-ecological zones and is widely consumed in Nigeria, where local varieties differ in seed size, color, and sensory attributes [10,11]. Among these, the Sokoto white variety is extensively cultivated and consumed, forming a staple in household diets. However, post-harvest contamination of cowpea by spoilage organisms and pathogenic microbes remains a significant concern, reducing its quality, safety, and market value [12]. The heavy reliance on cowpea as a food security crop highlights the importance of developing safe, sustainable methods for reducing microbial contamination. Conventional use of synthetic antimicrobial agents has contributed to the growing challenge of antimicrobial resistance, creating an urgent need for natural alternatives that are both effective and eco-friendly. Snail mucus, with its proven antimicrobial potential, presents a promising candidate for post-harvest protection of cowpea and other legumes.

This study seeks to investigate the antimicrobial activity of mucin extracted from the giant African land snail (*Achatina achatina*) against bacterial and fungal species isolated from the Sokoto White

cowpea (*Vigna unguiculata*). The Sokoto White cowpea has been reported to be susceptible to contamination by several microorganisms, including bacterial species such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., and *Bacillus* spp., as well as fungal species such as *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp., and *Fusarium* spp., particularly during post-harvest handling and storage. Findings from this work will expand current knowledge on the antimicrobial potential of snail mucin as a natural bioactive agent and contribute to global efforts aimed at reducing dependence on synthetic antimicrobials, thereby promoting food safety, public health, and agricultural sustainability.

## 2. Materials and methods

### 2.1 Study area and sample collection

The study was carried out in Abeokuta, Ogun State, Nigeria, using cowpea (*Vigna unguiculata*) variety “Sokoto white.” Ten representative samples were obtained from five major markets: Eleweeran, Osiele, Iyana Mortuary, Adatan, and Lafenwa. Each sample was collected aseptically into sterile ziplock bags, clearly labeled, and transported immediately to the laboratory for microbiological analysis. Mucin was extracted from the Giant African land snail using the method of stimulation of the body surface according to the method of [13].

### 2.2 Sample preparation

In the laboratory, 25 g of Sokoto White cowpea (*Vigna unguiculata* (L.) Walp.) seeds were aseptically weighed and homogenized using a sterile mortar and



Fig. 1. Beans



Fig. 2. Giant Snails

pestle. The resulting ground material was transferred into sterile sample containers and properly labeled. A 1:10 (w/v) suspension

was prepared by adding 225 mL of sterile distilled water to the homogenized sample and thoroughly mixing to ensure uniform microbial distribution. Serial ten-fold dilutions were subsequently prepared using sterile distilled water to reduce microbial load before plating [14].

### 2.3 Preparation of media

The culture media used in this study included Nutrient Agar (NA), Mueller–Hinton Agar (MHA), Sabouraud Dextrose Agar (SDA), Rose Bengal Agar (RBA), Neutral Red Desiccated Coconut Agar (NRDCA), Sulfide–Indole–Motility (SIM) medium, and Triple Sugar Iron (TSI) agar. All dehydrated media were obtained from Oxoid Ltd. (Basingstoke, Hampshire, United Kingdom). Media were prepared in accordance with the manufacturer's instructions. Prepared media were sterilized at 121 °C for 15 min using an autoclave (Model: LS-50L; Labtron Equipment Ltd., Camberley, Surrey, United Kingdom). After sterilization, sterile molten agar was aseptically dispensed into labeled sterile Petri dishes and allowed to solidify at room temperature.

### 2.4 Preparation of snail mucus from African giant snails (*Achatina fulica*)

Snails were sourced from Alabata in Ogun State, Nigeria, and transported to the laboratory in sealed zip-lock bags. Their shell heights measured between 3.0 and 5.0 cm. Mucus secretion was induced by applying a mild electric stimulus (5–10 V) for 30–60 seconds at intervals, after which the mucus from 10–20 snails was pooled; each snail contributed mucus 2–3 times. The total volume of raw mucus collected prior to dilution was approximately 45–60 mL, which was transferred into sterile containers and kept on ice for further processing.

To obtain the water-soluble fraction, twice the volume of water was added to the pooled mucus, mixed overnight, and centrifuged at 8000 g for 30 minutes ( $25 \pm 2$  °C). The resulting supernatant was designated as the water-soluble fraction. The mucin fraction was isolated from the water-soluble fraction

by ethanol precipitation. Cold absolute ethanol (99.5% v/v) was added to the water-soluble fraction at a ratio of 3:1 (ethanol: supernatant, v/v), followed by centrifugation at 2500 g for 30 min. The resulting precipitate was dissolved in sterile distilled water and labelled as the mucin fraction. The reconstituted mucin was adjusted to a final concentration of 10 mg/mL and used as the stock solution for antimicrobial activity evaluation [9].

### 2.5 Bacteriological analysis

Aliquots (1 mL) from serial dilutions ( $10^{-3}$  to  $10^{-5}$ ) were inoculated into sterile Petri dishes, followed by the addition of 15 mL molten nutrient agar. Plates were gently rotated to ensure even distribution, then incubated at 37 °C for 24 h. Representative colonies were purified by sub-culturing on NA and MacConkey agar, followed by incubation at 37 °C for another 24 h.

### 2.6 Gram Staining

Isolates were heat-fixed onto sterile glass slides and sequentially stained with crystal violet (1 min), Gram's iodine (1 min), ethanol (decolorizer), and safranin (1 min). After air-drying, smears were observed under oil immersion at 100× magnification.

### 2.7 Biochemical characterization

Purified isolates were subjected to standard biochemical tests:

**Catalase test:** to detect the presence of catalase enzyme through the breakdown of hydrogen peroxide.

**SIM test:** to evaluate hydrogen sulfide production, indole formation, and motility.

**TSI test:** to determine fermentation of glucose, lactose, and sucrose, and production of hydrogen sulfide.

### 2.8 Fungal isolation and identification

The Pour-Plate Method was used: From  $10^{-3}$  and  $10^{-5}$  dilutions, 1 mL aliquots were transferred into sterile petri dishes. Containing approximately 15 mL of molten Sabouraud Dextrose Agar (SDA) and Rose Bengal Agar (RBA) plates. SDA plates were incubated at room temperature ( $28 \pm 2$  °C) for 48 h. The plates were gently swirled to

maintain a uniform distribution of the inoculum throughout the agar medium before solidification. After which, the solidified plates were inverted and incubated. RBA plates were similarly incubated to restrict fast-growing fungi. Purified fungal isolates were obtained by repeated sub-culturing on SDA. This technique, utilizing the 1 mL volume, ensures that the fungal spores and hyphal fragments are embedded within the medium.

### 2.8.1 Macroscopic examination

Fungal colonies were evaluated for morphology, including pigmentation, texture, growth rate, and colony margins.

### 2.8.2 Microscopic examination

Slides were prepared by placing a small portion of fungal growth in a drop of Lactophenol Cotton Blue stain.

A covers-lip was gently placed over the preparation to avoid air bubbles. The slides were initially examined microscopically under the 10X objective for overall orientation and arrangement, and then under the 40X objective to assess general hyphal morphology and the presence of reproductive structures. To resolve fine morphological details crucial for identification (such as spore structure, conidial attachment, and detailed hyphal arrangement), the preparations were finally examined under the 100X objective using oil immersion.

### 2.8.3 Qualitative screening for aflatoxin production

The aflatoxigenic potential of fungal isolates was assessed using Neutral Red Desiccated Coconut Agar (NRDCA) [15]. The medium was prepared by boiling 50 g of desiccated coconut flakes in 500 mL of distilled water for 10 min, followed by blending and filtration to obtain the coconut extract. The filtrate was supplemented with 5 g of agar, and Neutral Red dye (0.002% w/v) was added until a color shift was observed. The pH of the medium was adjusted to 6.0 using dilute acetic acid (1–2 mL). The medium was then autoclaved at 121 °C for 15 min, cooled, and aseptically dispensed into sterile Petri dishes. Fungal isolates were inoculated and

incubated at room temperature for 72 h. Plates were subsequently exposed to 365 nm UV light in a dark chamber, and the presence of characteristic fluorescence indicated aflatoxin production.

### 2.9 Antimicrobial susceptibility testing

A fresh bacterial suspension was adjusted to 0.5 McFarland ( $\approx 1-2 \times 10^8$  CFU/mL), and a sterile cotton swab was used to streak the surface of a Mueller–Hinton agar plate to create a uniform lawn. Antibiotic disks were evenly (maintain recommended spacing), pressed gently for full contact, and then the incubated plates were inverted at  $35 \pm 2$  °C for 16–18 h. Zone diameters (mm) and interpret results using the current CLSI (or EUCAST) breakpoint tables were used to report susceptible, intermediate, or resistant [16].

## 3. Results

### 3.1 Viable microbial count

Tables 1 and 2 show the viable bacterial and fungal count of the cowpea samples

The bacterial load of cowpea samples ranged from  $30 \times 10^{-3}$  to  $292 \times 10^{-3}$  cfu/g, while fungal counts were generally higher, with several samples showing TNTC (Too Numerous to Count) at  $10^{-3}$  dilution.

Overall, Eleweeran and Adatan samples had the highest microbial contamination, whereas Osiele samples recorded comparatively lower counts.

### 3.2 Morphological and biochemical characteristics of bacteria isolated from the cowpea

Table 3 gives a detailed report on the morphological characteristics of bacteria isolated from the cowpea. The bacterial isolates displayed diverse morphological features, ranging from irregular to round shapes, with variations in edge type, elevation, and pigmentation (white, yellow, and green colonies).

Gram staining revealed a mix of Gram-negative rods and Gram-positive cocci/clusters, indicating heterogeneity among the isolates. Table 4 shows the biochemical characteristics of the bacterial

isolates. The biochemical tests revealed varying reactions among the bacterial isolates, with differences in motility, catalase, and lactose fermentation. Based on these characteristics, the isolates were presumptively identified as *Shigella* sp., *Enterococci* sp., *Staphylococcus* sp., and *Pseudomonas* sp.

### 3.3 Morphological characteristics of fungi isolated from the cowpea

Table 5 gives a detailed report on the morphological characteristics of fungi isolated from the cowpea. The fungal isolates exhibited distinct colony morphologies, with variations in color (olive green, dark green, white, dark brown, bluish green), elevation (flat, raised, elevated), and forms (irregular, filamentous, conidia). Based on these features, the isolates were identified as *Aspergillus flavus*, *Trichoderma* sp., *Fusarium* sp., *Aspergillus niger*, and *Aspergillus fumigatus*.

### 3.4 Aflatoxicogenicity of the fungi isolated from the cowpea

Table 6 reveals the aflatoxicogenicity of fungi isolates: among the fungal isolates tested,

*Aspergillus flavus* and *Fusarium* sp. demonstrated positive aflatoxicogenic potential. In contrast, *Trichoderma* sp., *Aspergillus niger*, and *Aspergillus fumigatus* showed no evidence of toxin production.

### 3.5 Antimicrobial susceptibility testing

Table 7 shows the antimicrobial effect of the snail mucin on each of the fungi and bacteria isolates. Snail mucin extract exhibited no inhibitory effect on most of the bacterial and fungal isolates tested. However, a slight antifungal activity was observed against *Aspergillus flavus*, with a mean zone of inhibition of  $6.10 \pm 0.05$  mm. Fig. 3 shows the percentage of inhibition. Table 8 shows the antimicrobial susceptibility testing of bacteria isolates with antibiotic discs. The bacterial isolates displayed varied susceptibility, with *Shigella* sp. and *Pseudomonas aeruginosa* sensitive only to levofloxacin (LEV), while *Staphylococcus* sp. responded to LEV and ciprofloxacin (CPX). *Enterococci* sp. showed the broadest susceptibility, responding to multiple antibiotics including PEF, CN, APX, Z, AM, R, CPX, S, SXT, and E.

Table 1.

Bacterial count of the cowpea (cfu/g)

S/N	SAMPLES	$10^{-5}$	$10^{-3}$
1	OSIELE 1	132	64
2	OSIELE 2	70	43
3	ELEWERAN 1	180	102
4	ELEWERAN 2	292	112
5	ADATAN 1	92	45
6	ADATAN 2	75	44
7	LAFENWA 1	30	17
8	LAFENWA 2	35	22
9	MORTUARY 1	30	18
10	MORTUARY 2	56	30

Table 2.

Fungal count of the cowpea (cfu/g)

S/N	SAMPLES	$10^{-5}$	$10^{-3}$
1	OSIELE 1	140	52
2	OSIELE 2	320	106
3	ELEWERAN 1	TNTC	200
4	ELEWERAN 2	TNTC	372
5	ADATAN 1	TNTC	380
6	ADATAN 2	TNTC	304
7	LAFENWA 1	TNTC	384
8	LAFENWA 2	TNTC	312
9	MORTUARY 1	TNTC	368
10	MORTUARY 2	TNTC	360

Table 3.

**Morphological characteristics of the bacteria isolates**

S/N	SHAPE	EDGES	ELEVATION	COLOR	GRAM STAIN
1	Irregular	Rough	Flat	White	Negative rod
2	Round	Smooth	Raised	White	Positive cocci
3	Round	Smooth	Raised	Yellow	Negative rod
4	Round	Rough	Raised	White	Positive cocci
5	Round	Smooth	Raised	Green	Positive clusters

Table 4.

**Biochemical characteristics of the bacteria isolates.**

S/N	COAGULASE	SULPHUR	INDOLE	MOTILITY	CATALASE	LACTOSE	POSSIBLE BACTERIA
1	Negative	Negative	Negative	Negative	Positive	Positive	<i>Shigella</i> sp.
2	Negative	Negative	Negative	Positive	Positive	Positive	<i>Staphylococcus</i> sp.
3	Negative	Negative	Negative	Negative	Negative	Positive	<i>Enterococci</i> sp.
4	Positive	Negative	Negative	Negative	Positive	PNegative	<i>Pseudomonas</i> sp.

Table 5.

**Morphological characteristics of the fungi isolates.**

S/N	COLOR	ELEVATION	FORM	MICROORGANISM
1	Olive green	Flat	Irregular	<i>Aspegillus flavus</i>
2	Dark green	Flat	Conidia	<i>Trichoderma</i> sp.
3	White	Raised	Filamentous	<i>Fusarium</i> sp.
4	Dark brown	Raised	Conidia	<i>Aspergillus niger</i>
5	Bluish green	Elevated	Conidia	<i>Aspergillus fumigatus</i>

Table 6.

**Aflatoxigenicity of the fungi isolates.**

S/N	TOXIGENICITY	FUNGI
1	Positive	<i>Aspegillus flavus</i>
2	Negative	<i>Trichoderma</i> sp.
3	Positive	<i>Fusarium</i> sp.
4	Negative	<i>Aspergillus niger</i>
5	Negative	<i>Aspergillus fumigatus</i>

Table 7.

**Antimicrobial susceptibility testing of the snail mucin on bacteria and fungi isolates.**

S/N	MICROORGANISMS	ZONE OF INHIBITION
1	<i>Shigella</i> sp.	None
2	<i>Enterococci</i> sp.	None
3	<i>Staphylococcus</i> sp.	None
4	<i>Enterococci</i> sp.	None
5	<i>Pseudomonas</i> sp.	None
6	<i>Aspergillus flavus</i>	6.10 ± 0.05 <sub>a</sub>
7	<i>Trichoderma</i> sp.	None
8	<i>Fusarium</i> sp.	None
9	<i>Aspergillus niger</i>	None
10	<i>Aspergillus fumigatus</i>	None

Table 8.

**Antimicrobial susceptibility testing of bacteria isolates with antibiotic discs.**

S/N	MICROORGANISMS	SUSCEPTIBILITY PROFILE
1	<i>Shigella</i> sp.	LEV
2	<i>Enterococci</i> sp.	PEF, CN, APX, Z, AM, R, CPX, S, SXT, E
3	<i>Staphylococcus</i> sp.	LEV, CPX.
4	<i>Enterococci</i> sp.	PEF, CN, APX, Z, AM, R, CPX, S, SXT, E
5	<i>Pseudomonas aeruginosa</i>	LEV



Fig. 3. Antimicrobial susceptibility disc

#### 4. Discussion

Cowpea (*Vigna unguiculata*) is one of the most important legumes in sub-Saharan Africa, yet it remains highly susceptible to microbial contamination during cultivation, storage, and handling. Although the crop provides essential proteins and amino acids for human diets and also contributes to soil fertility, its production is hindered by various pests and pathogens. In particular, fungal and bacterial infections are major contributors to post-harvest losses, reducing both seed quality and market value [17]. These pathogens are often introduced through spore dispersal, carryover from contaminated seeds, or unfavorable storage practices that create a conducive environment for microbial growth [18]. In this study, both bacterial and fungal organisms were successfully isolated from the Sokoto White cowpea variety. The isolates included *Pseudomonas* spp., *Staphylococcus* spp., *Shigella* spp., *Enterococcus* spp., and fungal species such as *Aspergillus flavus*, *Fusarium* spp., *Trichoderma* spp., *A. niger*, and *A. fumigatus*. These findings are consistent with earlier reports that identified similar spoilage

and pathogenic organisms from stored legumes [19,20]. The presence of these microorganisms highlights the vulnerability of cowpea seeds to contamination and the need for improved storage and handling practices. The antimicrobial screening of mucin extracted from the giant African land snail (*Achatina fulica*) showed selective inhibitory effects on the isolates. Interestingly, while most bacterial isolates, such as *Shigella*, *Enterococcus*, *Staphylococcus*, and *Pseudomonas* were not inhibited, *Aspergillus flavus* recorded a measurable zone of inhibition ( $6.10 \pm 0.05$  mm). This aligns with reports by [21], who demonstrated antifungal properties of snail mucin extracts against common seed-borne fungi. The reduced susceptibility of Gram-negative bacteria observed in this study can be attributed to the complex outer membrane of these organisms, which restricts the entry of antimicrobial agents [22]. The results further indicate that, based on the observed zones of inhibition, the mucin's activity was limited to the tested fungus, *A. flavus*, suggesting a stronger antifungal potential compared to the lack of effect observed

against the tested bacterial species. This suggests the presence of bioactive peptides and glycoproteins capable of disrupting fungal growth. Similar observations were made by [23,24], who described snail mucin as a natural reservoir of antimicrobial compounds. This highlights its potential role as an alternative to synthetic fungicides in managing fungal contamination in stored cowpea seeds.

Overall, the study confirms that the Sokoto White cowpea is susceptible to microbial infestation during storage, but snail mucin extract shows promise as a natural antimicrobial agent. Considering the cost and limited accessibility of commercial seed treatments for smallholder farmers, mucin-based interventions may provide a sustainable, affordable, and eco-friendly option for reducing post-harvest losses. Further research is required to optimize its formulation, assess its safety for human use, and evaluate its efficacy under realistic storage conditions.

## 5. Conclusions

Sokoto White cowpea is prone to bacterial and fungal contamination during storage. Snail mucin exhibited selective antifungal activity against *Aspergillus flavus* but showed no inhibition against *Fusarium* spp., which remains a major food safety concern. These findings highlight the potential of snail mucin as a natural preservative while emphasizing its limitations. Future studies should focus on optimizing its formulation, exploring synergistic combinations with other natural or conventional preservatives, and developing practical application strategies to enhance cowpea storage safety, reduce reliance on synthetic chemicals, and support public health.

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