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Original Article

Antimicrobial potentials of mucus mucin from different species of giant African land snails on some typed culture pathogenic bacteria

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Abstract

The study aims at evaluating the antibacterial activities of mucus mucin from three species of the giant African land snails; *Archachatina marginata*, *Achatina achatina*, and *Achatina fulica*. Snail slime was collected from forty-five snails comprising the three species of snails from the southwestern region of Nigeria. The antibacterial potential and bacteria growth rate (in hours) of the mucus mucin were determined using agar well diffusion method and liquid broth. Acetic acid (acid), ammonium bicarbonate (alkaline), and water (aqueous) were each used to extract the slime. The result showed that mucus secretions from the three snail species differed in color, degree of the sliminess, and volume. Snail mucus extract had antimicrobial effects on gram-positive and gram-negative bacteria. The inhibitory effects of mucus extracts differed depending on the treatment method and storage time, with acid extracts having a higher inhibitory capacity regardless of snail species or storage time. *A. marginata's* mucus secretions had a stronger antibacterial activity against *Bacillus subtilis* when compared to mucus from *A. achatina* and *A. fulica*. The zone of inhibition of the mucus mucin in solid agar ranged between 24.0–19.5mm for *A. marginata* and ranged between 21.0-17.5mm and 21.0–15.0mm for *A. achatina* and *A. fulica*, within 2-72 storage hours). Mucus mucin seems to lose its antibacterial potential with time; however, the antibacterial capability of the giant African snail species could provide the much-needed solution to antibiotic resistance.

Keywords: Land snail, Mucous mucin, Antibacterial activity, Pathogenic bacteria, Antibiotic resistance

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Introduction

Antibiotic resistance has been a prominent topic of discussion in numerous seminars and conferences the world over, yet clinical cases of antibiotic-resistant bacterial infections are a daily occurrence. This situation has contributed significantly to the difficulties in treating bacterial infections. The irresponsible use of antibacterial compounds and other chemicals allows bacteria strains to acquire resistance (Etim et al., 2016; Aslam, 2018). This condition has prompted the increase in research into alternative treatments for bacterial infections in animals, fish, and humans. The significance of zootherapy, herbal therapy, nature, and vaccine concepts increased as the search for the solution to antibacterial resistance progressed. On this note, numerous investigations on the antimicrobial capabilities of various terrestrial and aquatic plants (Daboor and Haroon, 2012; Khamene et al., 2019), marine animals (Kumari et al., 2019), bacteria (Onianwah, 2019), and a few terrestrial animals, have been done (Perumal et al., 2007).

The contents of the snail mucus, reported by Cilia and Fratini (2018), included a high level of metals, proteoglycans, copper peptides, glycosaminoglycans, hyaluronic acid, anti-bacteria, and glycoprotein enzymes. Despite the earlier reports on the composition and characteristics of the snail mucus, there is a need for more knowledge of its antibacterial properties. Further research into the component of snail mucus has shown that the mucous of *Helix aspersa* contains a variety of natural substances with medicinal and therapeutic properties for human skin, including allantoin and glycolic acid (El Mubarak et al., 2013). Abiona et al. (2013); Etim et al. (2016) evaluated the mucus mucin from giant African snail for its antibacterial and wound-healing capabilities (Abiona et al., 2013; Etim et al., 2016).

Iguchi et al., 1982; Kubota et al., 1985 and Otsuka-Fushino et al., 1992, discovered 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. According to the reports, achacin suppressed the growth of both gram-positive and -negative bacteria, suggesting that it is only effective against actively developing and dividing microorganisms. In *A. fulica* mucus, Zhong et al. (2013) discovered a peptide with an antibacterial action *against S. aureus, Bacillus spp., Klebsiella pneumoniae,* and *Candida albicans.* Santana et al. (2012) observed that *A. fulica* mucus suppressed the growth of *S. aureus and S. epidermidis* when tested against some microbial organisms. The antibacterial action of snails is due to the diversity of their mucus composition. However, there is a need for more extensive study on the antimicrobial activity of the giant African land snails. The study plan was to discover and compare the antimicrobial activity of the giant African land snails on some typed culture pathogenic bacteria that are problematic to man and livestock species.

Material and Methods

Source of snail species

Three snail species, *Archachatina marginata* (AM), *Achatina achatina* (AA), and *Achatina fulica* (AF), forty-five (45) snails in all (15 AM, 15AA, and 15AF), were obtained from the snail rearing unit of the Teaching and Research farms of Landmark University, Omu-Aran, Kwara State, Nigeria. The snails were kept in plastic cages (38 x 33 x 27cm) at room temperature (~25°C) with reduced lightning in the animal housing unit of the Animal science laboratory. The snails were fed with leaves and unripe fruits of *Carica papaya* (pawpaw), freshwater, and a source of calcium. The cages were regularly thoroughly cleaned.

Extraction and physical characteristics of mucus mucin from the snail species

Mucus extraction was done by taking each snail and gently stimulating the water moistened foot surface and mantle area with a small spatula. After the extraction, mucus was into clean 5ml Eppendorf tubes. The mucus physical characteristic observations made were for the following parameters: color, texture/thickness, sliminess, and mean volume per snail. The mucus texture and sliminess were determined by adopting the method of Billings and Westmore (1998), while the mucus extracted was measured with calibrated bottles. The mucus secretion was stored in the refrigerator at 4°C for bacteriological assay

Processing of crude mucus mucin

Three mucus types were employed for the study: crude slime, acid extract, alkaline extract, and aqueous extract. The raw crude mucus used in this study refers

to the extracted mucus placed in a centrifuge at 3000 rpm for 15 min. The supernatant was decanted and stored at4°C in a refrigerator until as needed.

For aqueous mucus extracts, we used the modification of the method described by Kumari et al. (2019). A known volume of raw slime mixed with an equal volume of distilled water was placed on a centrifuge at 3000 rpm for 15 min. The supernatant separated from the sediment was then stored at4°C in a refrigerator until when needed.

For the acid and alkaline extracts, we used the modification of the method described by Subramanian et al. (2008). To a known volume of a respective crude slime in a beaker, an equal amount of 10 % (v/v) of acetic acid (for acid extract) or 1 mg/mL ammonium bicarbonate (for alkaline extract) was mixed and boiled for 5 min in a water bath. The mixture was then allowed to cool and then centrifuged at 3000 rpm for 15 mins. The respective supernatant was decanted and stored in a refrigerator at 4 ºC until when needed.

Antibacterial bacterial activity

The antibacterial potential of the respective mucus mucin was determined using the agar well diffusion method, as reported by Ali et al. (2017). The respective mucus mucin was tested against the following bacterial pathogens: *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella typhi* (ATCC 20971), *Escherichia coli* (25922), and *Klebsiella pneumoniae*.

For the growth rate study in broth medium, we used two bacterial species (*Escherichia coli* and *Staphylococcus aureus*). To a 150 mL quantity of sterile nutrient broth in a 200 mL-capacity conical flask, was added 10 mL of a mucus mucin treatment (crude slime, acid extract, alkaline extract, or aqueous extract), followed by the addition of 1 mL of a 24-hold broth culture of a known bacterial isolate. The medium was incubated in a rotary shaker (100 rpm) at 30 °C. After the inoculation of the test bacterium, the optical density was determined, by taking 6 ml of each sample at 0 hours and every 1h thereafter, using a spectrophotometer at a wavelength of 750 nm for 10 hours. For each sample, a control experiment was set up, containing only the test bacteria without the mucus mucins. The experiments were all done in duplicate.

Growth was estimated as:

$$Growth = \frac{ln(C1) - ln\,(C0)}{ti - t0}$$

Where C1 means absorbance at a final time, C0 means absorbance at the initial time, t1 means final time, and t0 means absorbance at an initial time (Adeyemi et al., 2020).

Results

Physical properties of mucus mucin

The physical properties of the mucus secretions from the three species of snail were observed. Mucus mucin from *A. fulica* was a colorless fluid with high sliminess and low texture thickness. Mucus mucin from *A. achatina* was a yellowish, slightly cloudy fluid with moderate sliminess, and thickness. Mucus mucin from *A. marginata* was a brownish fluid, with a high texture thickness, but low sliminess compared to *A. achatina* mucus. The mucus secretion from *A. marginata* was thicker in texture than that from *A. achatina* which was thicker in texture than secretions from *A. fulica* which, though lighter in texture, was the slimiest when compared to mucus from the other two species. The thickness of the mucus texture reduced in the order: very thick sticky glob in *A. marginata* to thick slimy fluid in *A. achatina* and a stretchy, highly slippery fluid in *A. fulica*. The mucus color varied from brown color in *A. marginata* to cream color in *A. achatina* and colorless in *A. fulica*. The volume released at stimulation and the sliminess of the mucus increased in the order *A. marginata* < *A. achatina* < *A. fulica* (Table 1).

Table-1: Physical characteristics of mucus mucin from giant African land snails

Mucus parameter	Source of mucus	Mucus physical characteristics
Color	AM	Brown and cloudy
	AF	Cream and slightly cloudy
	AA	Colorless and clear
Texture/Thickness	AM	Very thick sticky globs
	AF	Thick slimy fluid
	AA	Stretchy and highly slippery fluid
Sliminess	AM	Low
	AF	Medium
	AA	High
MV/snail(ml)	AM	2.43±0.15
	AF	3.00±0.10
	AA	4.43±0.40

Legend- AM= *Archachatina marginata*, AF= *Achatina fulica*, AA= *Achatina achatina*, MV= Mucus mean volume

Antibacterial potential of the mucus mucin

When the different mucin extracts were used for testing 2h after collection, all the treatments showed inhibition against the *Pseudomonas aeruginosa* except the aqueous extracts from the AA species and crude slimes from AF and AA species. None of the mucin extracts showed inhibition after storage of more than 48 h. Both the crude slime and aqueous extracts showed no inhibition when used after more than 24 h of storage. These observations were irrespective of the snail species that produced the mucous mucin (Table 2).

Table-2: Effect of storage duration on the antibacterial potential of the mucous mucin treatments against *Pseudomonas aeruginosa*

Treatments	Source of mucin	Zone of inhibition at different hours of storage (mm + standard deviation)			
		2 h	24 h	48 h	72 h
Aqueous Extract	AM	11.0 ±2.0	-	-	-
	AF	11.0 ±1.0	11.0 ±1.7	-	-
	AA	-	-	-	-
Crude slime	AM	17.0 ±3.0	-	-	-
	AF	-	11.0 ±2.0	-	-
	AA	-	-	-	-
Acid Extract	AM	19.3 ±0.9	19.0 ±3.0	21.0 ±4.0	-
	AF	19.5 ±1.8	19.0 ±2.0	-	-
	AA	19.3 ±1.7	19.0 ±4.0	21.0 ±2.0	-
Alkaline extract	AM	11.0 ±2.0	-	-	-
	AF	12.8 ±1.9	-	11.5 ±0.7	-
	AA	10.5 ±1.8	-	-	-

Legend- AM = *Archachatina marginata*, AF = *Achatina fulica*, AA = *Achatina achatina*

When applied within 2 hours of collection, practically all of the treatments exhibited inhibitory action against Staphylococcus aureus. The growth of *Staphylococcus aureus* was observed to be uninhibited in the presence of aqueous, and alkaline extracts from AA species, crude slime from AA, and AM species. The lack of inhibition observed in presence of the treatments was irrespective of the duration of storage. The crude slime and aqueous extract used after 2 h of storage showed inhibition but showed none from 24 h of storage (Table 3).

Table-3: Effect of storage duration on the antibacterial potential of the mucous mucin treatments against *Staphylococcus aureus*

Treatments	Source of mucin	Zone of inhibition at different hours of storage (mm + standard deviation)			
		2 h	24 h	48 h	72 h
Aqueous Extract	AM	22.0 ±1.0	-	-	-
	AF	23.5 ±2.0	-	-	-
	AA	-	-	-	-
Crude slime	AM	-	-	-	-
	AF	21.8 ±2.0			
	AA	-	-	-	-
Acid Extract	AM	21.5 ±1.0	24.5 ±3.0	-	-
	AF	20.0 ±2.0	-	-	-
	AA	20.3 ±0.7	27.0 ±3.0	15.5±1.0	-
Alkaline extract	AM	12.2 ±2.0	-	-	-
	AF	19.0 ±2.0	23.5 ±2.5	-	-
	AA	-	-	-	-

Legend- AM= *Archachatina marginata*, AF= *Achatina fulica*, AA= *Achatina achatina*

Table-4: Effect of storage duration on the antibacterial potential of the mucous mucin treatment against *Escherichia coli*

Treatments	Source of mucin	Zone of inhibition at different hours of storage (mm + standard deviation)			
		2 h	24 h	48 h	72 h
Aqueous Extract	AM	-	-	-	-
	AF	-	-	-	-
	AA	-	-	-	-
Crude slime	AM	-	-	-	-
	AF	-	11.5 ±0.5	-	-
	AA	-	-	11.0±1.0	-
Acid Extract	AM	21.5 ±1.0	24.0 ±2.0	17.5±1.5	-
	AF	21.0 ±2.0	22.0 ±1.0	19.5±1.0	-
	AA	20.8 ±1.0	21.0 ±1.0	17.0±1.0	21.5±2.0
Alkaline extract	AM	-	-	-	-
	AF	-	-	-	-
	AA	-	-	-	-

Legend- AM= *Archachatina marginata*, AF= *Achatina fulica*, AA= *Achatina achatina*

Aqueous and alkaline extracts exhibited minimum inhibition against *E. coli*, and this was irrespective of the duration of storage or source of the mucus extracts. The crude slimes from AF (stored for 24 h) and AA (stored for 48 h) showed inhibitory activities, but none of the crude slimes showed inhibition against *E. coli.* However, significant inhibition was observed in the presence of the acid extracts for up to 72 h of storage time (Table 4).

As shown in Table 5, none of the treatments showed inhibition against *Salmonella typhi* except aqueous and alkaline extracts from AA, which was stored for up to a 2h period. However, remarkable inhibition was observed when the acid extract was used. These observations were irrespective of the source of the mucin treatments and their period of storage (Table 5).

Table-5: Effect of storage duration on the antibacterial potential of the mucous mucin treatments against *Salmonella typhi*

Treatments	Source of mucin	Zone of inhibition at different hours of storage (mm + standard deviation)			
		2 h	24 h	48 h	72 h
Aqueous Extract	AM	-	-	-	-
	AF	-	-	-	-
	AA	13.0±1.0	-	-	-
Crude slime	AM	-	-	-	-
	AF	-	-	-	-
	AA	-	-	-	-
Acid Extract	AM	18.8±1.0	17.0±2.0	17.5±1.5	-
	AF	10.3±1.0	14.5±1.0	17.0±1.0	-
	AA	17.3±2.0	19.5±1.0	-	-
Alkaline extract	AM	-	-	-	-
	AF	-	-	-	-
	AA	10.5±1.5	-	-	-

Legend- AM= *Archachatina marginata*, AF= *Achatina fulica*, AA= *Achatina achatina*

When tested against the *Bacillus subtilis*, none of the mucin treatments (except the acid extracts) showed inhibitory activity, this was irrespective of the source and duration of storage of the mucus treatments. However, the acid t had remarkable inhibitions against *B. subtilis*, a trend that was seen irrespective of the storage duration or the source of the mucus treatment (Table 6).

All the acid treatments (irrespective of source and storage period) showed remarkable inhibition against the growth of the *Klebsiella pnuemoniae.*

However, no inhibitory activity was observed against *Klebsiella pnuemoniae* for the aqueous extract, crude slime, and alkaline extracts obtained from the AA (except the crude slime stored for up to 48h). In addition, the mucus treatments from the AF only showed inhibition when stored for 2h (aqueous, extract), 72h (crude slime), and 48h (alkaline extract), as shown in Table7.

Table-6: Effect of storage duration on the antibacterial potential of the mucous mucin treatments against *Bacillus subtilis*

Treatments	Source of mucin	Zone of inhibition at different hours of storage (mm + standard deviation)			
		2 h	24 h	48 h	72 h
Aqueous Extract	AM	-	-	-	-
	AF	-	-	-	-
	AA	-	-	-	-
Crude slime	AM	-	-	-	-
	AF	-	-	-	-
	AA	-	-	-	-
Acid Extract	AM	24.0±1.0	21.0±3.0	19.5±2.5	23.5±1.0
	AF	21.0±3.5	19.0±1.0	15.0±2.0	19.5±1.0
	AA	20.3±1.7	21.0±1.0	17.5±1.5	19.0±2.0
Alkaline extract	AM	-	-	-	-
	AF	-	-	-	-
	AA	-	-	-	-

Legend- AM= *Archachatina marginata*, AF= *Achatina fulica*, AA= *Achatina achatina*

Table-7: Effect of storage duration on the antibacterial potential of the mucous mucin treatments against *Klebsiella pnuemoniae subtilis*

Treatments	Source of mucin	Zone of inhibition at different hours of storage (mm + standard deviation)			
		2 h	24 h	48 h	72 h
Aqueous Extract	AM	13.0±1.5	-	-	10.5±2.0
	AF	14.5±1.5	-	-	-
	AA	-	-	-	-
Crude slime	AM	-	-	10.5±2.0	-
	AF	-	-	-	13.5±1.0
	AA	-	-	12.0 ±2.0	-
Acid Extract	AM	23.5±2.5	22.5±0.5	19.5±2.5	23.5±2.5
	AF	20.0±2.0	22.5±0.5	15.5±3.0	20.0±2.0
	AA	23.0±1.0	22.5±1.5	15.0 ±0.5	23.0±1.0
Alkaline extract	AM	-	-	-	12.5±1.7
	AF	-	-	12.5±1.0	-
	AA	-	-	-	-

Legend- AM= *Archachatina marginata*, AF= *Achatina fulica*, AA= *Achatina achatina*

Growth inhibition in liquid medium

Escherichia coli showed consistent increases in growth with time in the presence of all the mucus extracts from the AA species however, alkaline and crude mucus extracts from the AA species, inhibited the growth of *Escherichia coli* better than others. Also, the crude mucus, acid, and alkaline extracts inhibited the growth of *Staphylococcus aureus* remarkably (Fig. 1).

When the mucus mucin from the AF species was used for the investigation, the growth rate of *E. coli* and *Staphylococcus aureus* was inhibited in the presence of the acid extract and this was observed throughout incubation. In the presence of the other mucus extracts, however, the growth of the *E. coli* was controlled in the following order: without slime > alkaline extract > aqueous extract > crude slime. For *Staphylococcus aureus*, the growth order in presence of the mucus mucin was as follows: crude > alkaline > aqueous > control (Fig. 2).

For AM species mucus, the growth of the bacteria organisms was remarkably inhibited in presence of the acid extract. This observation was evident for both the E. coli *and* S. aureus organisms. Although growth was observed in presence of the mucus mucin extracts, for the *E. coli* species, the order was as follows: acid extract < crude slime < aqueous extract < alkaline extract < control (slime). For the *S. aureus*, the growth pattern was acid extract < control < crude extract < aqueous extract < alkaline extract (Fig. 3).

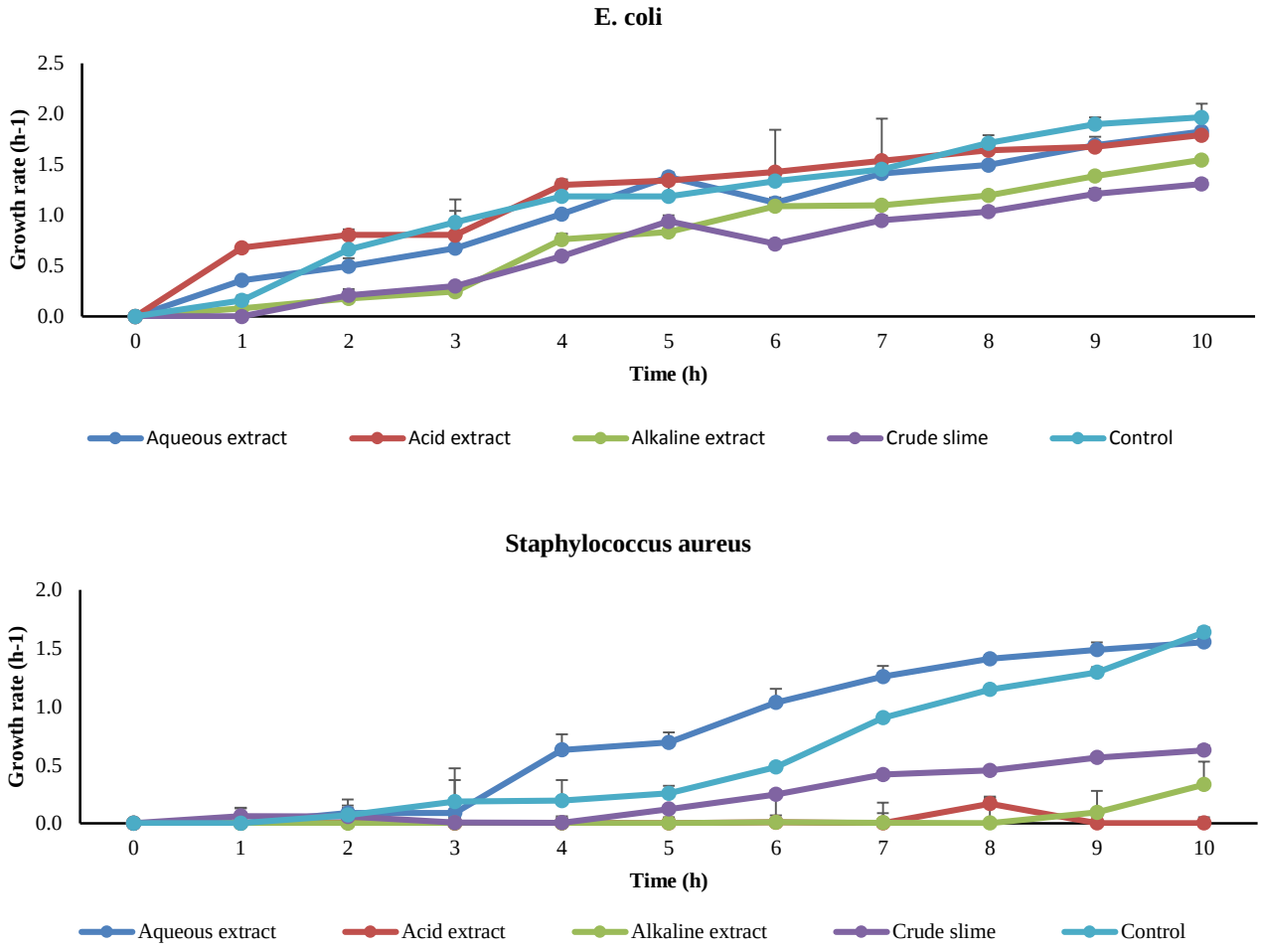

Figure-1: Growth of the bacterial species in presence of the respective mucous mucin from the AA species

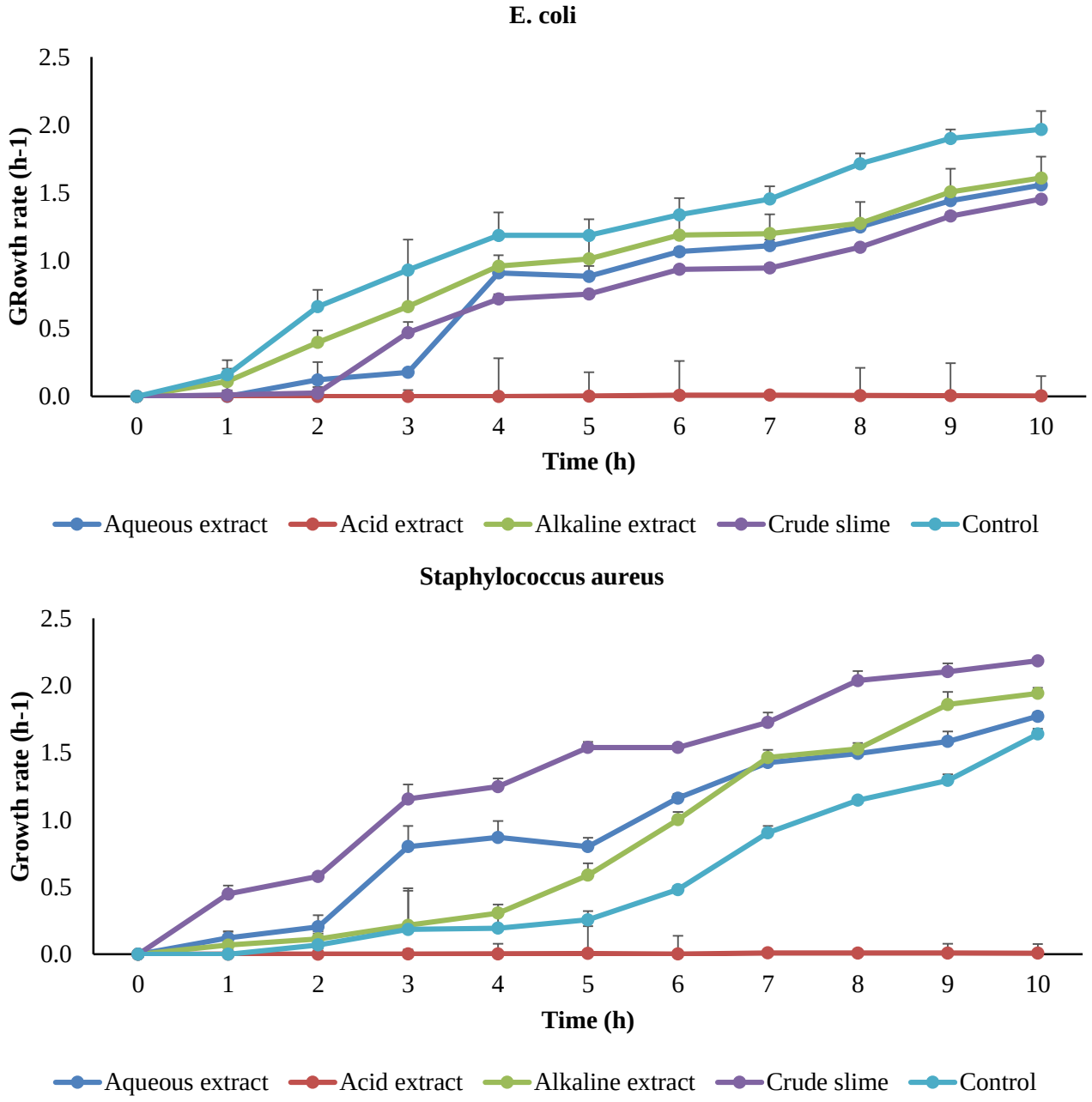

Figure-2: Growth of the bacterial species in presence of the respective mucous mucin from the AF species

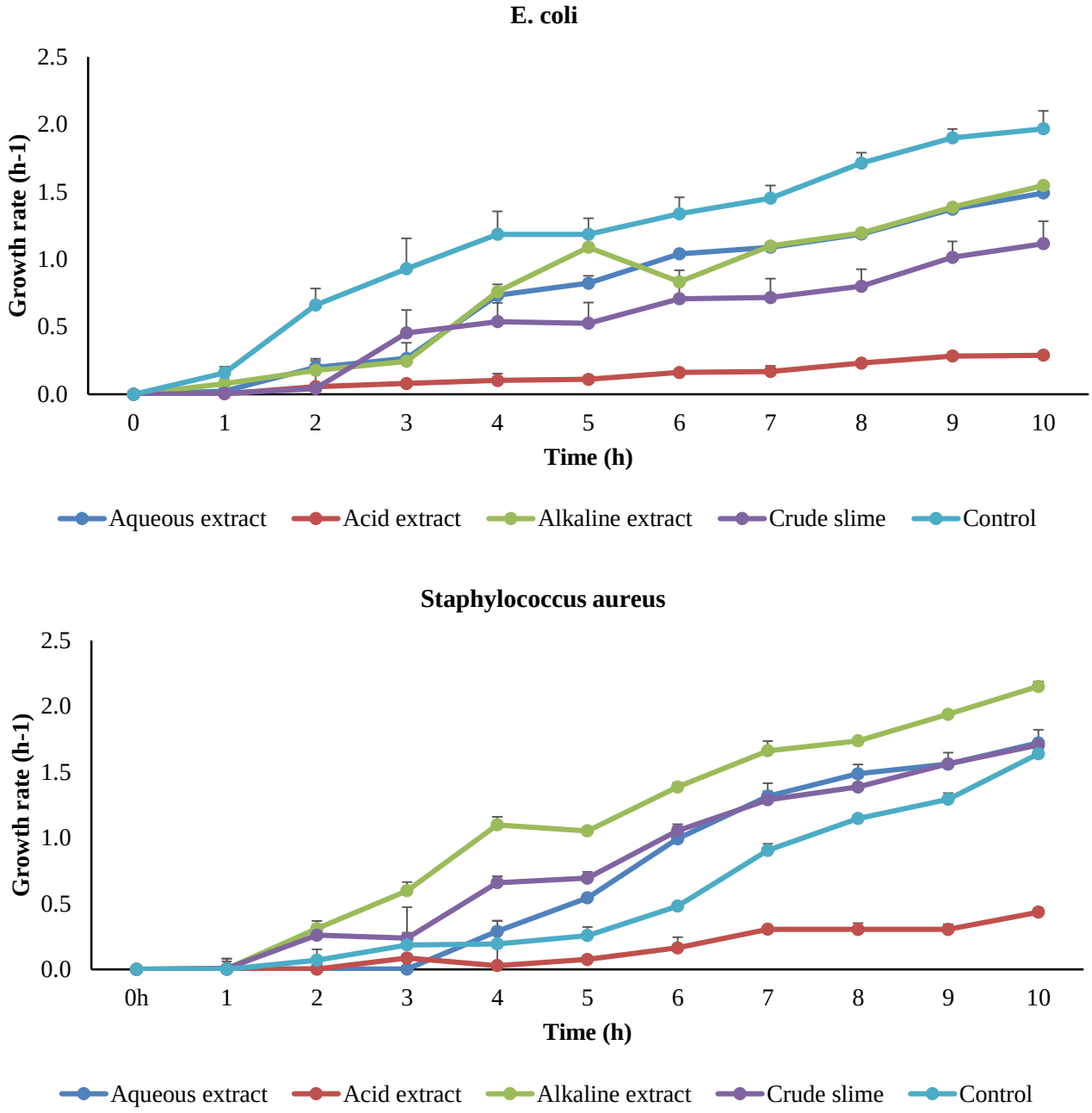

Figure-3: Growth of the bacterial species in presence of the respective mucus mucin from the AM species

Discussion

The differences observed in mucus color and texture in this study may be attributed to the nutrients utilized from the feed materials consumed by the experimental snails. The feed preferences of the different snail species have been implicated as a possible cause of the differences in the physical characteristics of snail mucus reported earlier (Fagbuaro et al., 2006; Ubua et al., 2013). However, because the snails in the present study were fed similar diets, the differences in mucus properties could not be attributed to the snails' feed preferences, but rather to the species' innate ability to consume and digest nutrients from the ingested feed materials. Mucus production involves a considerable cost to the animal, utilizing up to 70% of all consumed

energy (Davies and Hawkins, 1998). The variation in the volume of mucus released by the experimental snails could be attributed to the capacity of the different species to consume and or retain water. Feed type preference has been implicated in the composition of the snail flesh, hemolymph, and the mucus volume produced (Ajiboye, 2011; Ademolu et al., 2015). The variation in the degree of mucus viscosity observed may be species-dependent. In agreement with the observation in the present study, *A. fulica* has been reported to produce highly viscous mucus mucin which has been reported to help create a barrier between the snail and its environment, reduce moisture loss, and provide protection from bacterial infection (Etim et al., 2016; Cilia and Fratini, 2018). Fagbuaro et al. (2006) and Ademolu et al. (2004) suggested that the differences in mucus physical characters might be a reflection of the feeding preferences of the snails, which could affect their nutritional intake, the volume, and composition of their mucus.

The mucus mucin from the terrestrial snails of study all showed considerable antibacterial activity against tested pathogenic bacteria cultures, even with the increase in storage time. The potentials of these land snails to limit the growth of pathogenic bacteria cultures may be a reason for their survivability in the environment. Etim et al. (2016) and Iguchi et al. (1982) reported that snail mucus secretion provides survival aids, heals wound, and prevents microbial contamination. Other reports (Adikwu and Enebeke, 2007; Santana et al., 2012) also supported the antibacterial potential of the snail mucus mucin.

The giant African land snails possess inherent potentials that could be useful in the fight against antimicrobial resistance since the bacterial organisms inhibited by the mucus mucin secretions from the experimental snails in the study consisted of both gram-positive and gram-negative bacteria. According to reports, mucus from the giant African land snail inhibited both gram-positive and gram-negative bacteria (Otsuka-Fushino et al., 1992; Santana et al., 2012). Other authors (Etim et al., 2016; Abiona et al., 2013) also reported that some African land snail mucus secretions inhibited the test organism more strongly than commercial antibiotics. On the contrary, Santana et al. (2012) found no significant difference in antimicrobial activities between mucus secretions of giant African land snails and commercial antibiotics.

Aside from the fact that snail mucus secretions aid the animal's movement as it glides along, this study showed that terrestrial mollusks can keep themselves protected from microbial contamination and environmental damage only for a minimum time, hence the need for continuous slime secretions. Observations show that mucus mucin seems to lose its antibacterial potential with time. Berniyanti et al. (2007) observed that the mucus secretion by mollusks is a defense mechanism protecting their epithelial surfaces but may also be necessary for feeding, reproduction, locomotion and osmoregulation.

Snail mucus seems to possess unique proteins that help them survive in the wild by limiting bacterial contamination. The antibacterial action of the mucus secretions of *Achatina fulica*, according to Iguchi et al. (1982), is related to the antibacterial components present in its protein moiety rather than its activity on the cell surface of bacteria. The antibacterial factor, a component of proteins found in snail mucus, could protect snails from external infection (Abiona et al., 2013). Achacin, found in the mucus of the giant African snail, is an L-amino acid oxidase enzyme that generates hydrogen peroxide to kill bacteria. It could bind both gram-positive and gram-negative bacteria (Ehara et al., 2002). In addition, Ito et al. (2011) discovered lectin, a high molecular weight protein, in *A. fulica* mucus. By boosting the local concentration of hydrogen oxides in the mucus, the lectin released by the collar tissue appears to speed up the antibacterial activity of achacin (Ito et al., 2011). After digestion with pronase and heating to 75°C for 5 minutes, the activities of the snail antibacterial factor, a two-subunit glycoprotein, were diminished, indicating that it was dependent on the protein or glycoprotein's protein moiety. The antibacterial ability of the snail antibacterial factor was linked to the higher-order protein structures or glycoprotein protein subunits (Yasushi et al., 1985). Despite differences in the cell wall structure, the snail mucus antibacterial factor showed a high growth inhibitory effect against both gram-positive and negative bacteria therefore, the crucial location or metabolic pathway that is responsive to the snail antibacterial factor may be located in the bacterial cell walls, cell membranes, or cytoplasm (Yasushi et al., 1985)

This study showed that mucus extracts from the three snail species could inhibit the growth of *S. aureus*, *P. aeruginosa*, *E coli*, *S. typhii*, *B. subtilis*, and *K. pneumonia*. The ability of these snails to prevent the activities of these pathogenic organisms is a further proof that the mucus mucin is an unusual adaptive tool

in the survival strategy of the snails. Earlier, authors (Iguchi et al., 1982; Kubota et al., 1985; Otsuka-Fushino et al., 1992) also reported that snail mucus mucin inhibited the growth of *S. aureus, B. subtilis, E. coli*, and *P. aeruginosa*. Santana et al. (2012) and Zhong et al. (2013) reported antimicrobial activity of mucus mucin against *S. aureus*, several *Bacillus* spp., *K. pneumoniae*, and *Candida albicans.*

The higher antibacterial activity of mucus secretion from *A. marginata* against *Bacillus subtilis* compared to *A. achatina* and *A. fulica;* may be due to differences in the amount of the protein components required for these protective measures produced by each snail species. These results indicated that mucus mucin from the different experimental snail varied in their antibacterial potential against bacterial organisms. This observation agrees with earlier reports (Abiona et al., 2013; Etim et al., 2016) that the mucus mucin from *A. marginata* shows more antibacterial activity against some bacterial organisms compared to other species of African land snails. In addition, the acid medium extracted mucus seems to have a higher capacity to inhibit the test organisms compared to the other mucus extraction media. The antibacterial activity of each mucus type varied with the extraction medium, possibly due to differences in the components of the media used. This implies that the media used for mucus extraction may affect the antibacterial potential of the snail mucus. Lopez et al. (2012); Sugesh et al. (2013); Gayathri and Sanjeevi (2014) used various mucus extraction methods to evaluate the antimicrobial activity of mollusks with varied outcomes. Mucus mucin from the giant African land snails is a potential source of antibacterial components which could be harnessed for human use.

Conclusion

This study revealed that mucus mucin from the giant African land snails (namely; *A marginata, A. achatina*, and *A. fulica*) can inhibit the proliferation of the pathogenic bacteria organisms evaluated (namely; *P. aeruginosa, S. aureus, E. coli, S. typhi, B. subtilis,* and *K. pnuemoniae*). The notable differences in the snail mucus characteristics were mainly in the capacity of each mucus type to sustain the inhibitory potential against bacterial growth over time. In addition, mucus mucin exhibited variations in the bacteria growth inhibition strength, based on the snail species and medium of mucus extraction. There is the need to isolate the active ingredients in the mucus of each species for further studies and possible application in the development of pharmacological and therapeutic products.

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Contribution of Authors

Okeniyi FA: Planned and conducted the experiment, collected, analysed & interpreted data & wrote the manuscript

Oghenochuko OM, Olawoye SO, Animashahun RA, Adeyonu AG & Akpor OB: Participated in data collection & analysis at different stages, literature review and manuscript editing