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Research Article

ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF SNAIL (*ARCHACHATINA MARGINATA*) SLIME ON SOME CLINICAL WOUND ISOLATES

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ABSTRACT

Objective: The objective of the study is to investigate the antibacterial activity of African giant land snail (*Archachatina marginata*) slime against some bacteria isolated from various types of wound.

Methodology: Six (6) bacterial isolates (*Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus* (MRSA) *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Klebsiella* sp) isolated from wound sample of patient obtained from Microbiology Department of Muhammad Abdullahi Wase Specialist Hospital Kano were involved in the study. The antibacterial activity of the slime extract was determined using agar well diffusion method while minimum inhibitory concentration of the slime was determined using broth dilution technique.

Results: The result showed that higher activity was shown by *E. coli* with average zone of inhibition of 15.2 mm, followed by *Klebsiella* sp (14.2 mm), *Proteus mirabilis* (13.3 mm), *Pseudomonas aeruginosa* (13.0 mm) and least activity was shown by *Staphylococcus aureus* (09.1 mm). No activity was shown by Methicillin Resistant *Staphylococcus aureus* (MRSA). There is no statistical difference in the activity of the slime against the test isolates ($p<0.05$).

Conclusion: It is concluded that the slime of *A. marginata* contain antibacterial agents.

Key word: *Archachatina marginata*, Antibacterial activity, bacteria, wound

INTRODUCTION

Wound is a breakdown in the function (protective) of skin, loss of continuity of epithelium with or without loss of underlying connective tissues such as nerves, muscles and muscles following skin injury [1,2]. Virtually all open wounds are colonized with microorganisms [3]. Some wounds are clearly infected with microorganism due to purulent secretion of inflammation that has classically defined the host response to tissue damage caused by pathogenic and invasive microorganisms [4]. The chance that a wound will become infected is related directly to the size of inoculum and virulence of colonizing isolates and inversely related to systemic and host resistance [5].

The occurrence of antibiotics resistant bacteria in wound cases is seems to be increasing daily and this bring about difficulties being faced in the treatment of such bacteria [6]. As result, there increasing need for the development of new and more effective alternative antimicrobial agents from readily available materials of plant or animal origin, an example include slime which is produced by snails [6]. Slime is a mucus produce by snails which has been found to contain glycosaminoglycans. The glycosaminoglycans have been reported to be of great importance in healing and repairing of wound [7]. Several studies conducted showed high resistance to antibiotics by bacteria responsible for deterioration of wound, thereby facing a challenge in the management of wound infection [8,9].

Archachatina marginata (African giant land snail) produce large quantity of mucin in their mucus secretion (slime) which has been reported to contain antimicrobial protein [8]. A bactericidal glycoprotein known as achacin was also reported to be obtained from the body surface mucus of African giant snail *(A. marginata)* [10]. Otosuka-Fuchino *et al*. [11] and Santana *et al*. [12] reported that the slime from snail contain antibacterial agents that's kill both Gram positive and negative bacteria by attacking their cell membrane. Base on these facts, this study was design to investigate the antibacterial activity of African giant land snail (*Achachatina marginata*) against some bacteria isolated from various types of wound.

MATERIALS AND METHODS

Study Area

The study area is Kano metropolis, samples from infected wound patients were collected from Muhammad Abdullahi Wase Hospital in the state capital. Kano State is located in the North-west Nigeria located at latitude 11^0 30 N and longitude 8^0 30 E. It share borders with Kaduna state to the south- west, Bauchi state to the South-East, Jigawa state to the East, Katsina state to the North [13]. It has a total area of 20,131km^2 (7,777sqm) and estimated population of 13.4 million [14].

Ethical approval

Ethical approval (Issue number: HMB/ GEN/488/Vol. I) was obtained from Health Service Management board (HSMB) Kano State based on the consent of Muhammad Abdullahi Wase Specialists Hospital (MAWSH) Ethical Committees.

Characterization of Bacterial Species from Wound Samples

Bacterial isolation and identification was conducted using methods described by Cheesbrough [5]. Samples of wound (pus) obtained from different types of different patients were inoculated on Nutrient agar, Mac Conkey agar, Mannitol salt agar, Blood agar and Chocolate agar using the streak plate method. The plates were incubated at 37^0C for 24 hours after which the isolates were isolated. Identification of the isolates was conducted using Gram-staining, morphological and sugar fermentation reactions on specified media, as well as biochemical reactions, such as Catalase, Coagulase, Oxidase, Urease and Indole tests [16].

Test Isolates Isolates of *Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus* (MRSA) *Escherichia coli*, *Proteus* sp,

Pseudomonas aeruginosa and *Klebsiella* sp isolated from wound samples of patients were obtained from Microbiology Department of Muhammad Abdullahi Wase Specialist Hospital Kano. The pure isolates of each of the test organisms were stored in peptone water and refrigerated at 4^0C before use [16].

Collection of Snails and Extraction of Slime

Fifteen (15) snails (*A. marginata*) were purchased from ″Sabon-Gari market″ Kano State, Nigeria. The snails were handled in accordance with the principles of animal welfare in scientific experiments [17]. Authentication and identification of the snails was done at Department Biological Sciences, Bayero University Kano by Prof. T.I. Oyeyi and Mr. Yakubu Ali. The slime specimens were extracted according to the method of Lawrence *et al.* [17] from the snail samples by removing the skin from the shell with a sterile sharpened metal rod in to a beaker and the slime secretions aseptically squeezed out from the soft body. The crude extracted slime secretion considered as 100% concentration was stored in the refrigerator at 4^0C [17].

Fig. 1: African giant snail (*Archachatina marginata*)

Preparation of Slime Extracts Solution

The stored extracted slime was prepared into various concentrations (25, 50, 75 and 100 v/v) with distilled water as diluents. This was done by dissolving the respective volumes of slime into corresponding volumes of sterile distilled water [18]. The crude extracted slime secretion was considered as 100% concentration [17].

Determination of Antibacterial Activity of Snail Slime

The agar well diffusion method was used to determine the antibacterial activity of the slime extracts as described by Ali *et al*. [16]. One milliliter of the different standardized organisms (0.5 McFarland) were introduced separately and thoroughly mixed with Mueller Hilton Agar, in a sterile Petri dish and allowed to set then labeled. A sterile cork borer 6mm was used to punch hole (i.e. 5 well) in the inoculated agar and the agar was then removed. Four wells formed were filled with different concentrations of the snail slime extract as follows; 25, 50, 75 and 100 v/v respectively while 5^{th} well at the centre was filled with Gentamicin (50mg/mL) as positive control. The plates were left for 1 hour to enable proper diffusion of the slime to take place, and then incubated at 37°C for 24 hours. After incubation, the plate observed for the zone of inhibition. The experiment was conducted in triplicate and average result was calculated [19].

Determination of Minimum Inhibitory Concentration (MIC) of the Slime

The MIC of the slime was determined using broth dilution technique. Two-fold serial dilutions of the slime were prepared by adding 2ml of 100v/v of the slime into a test tube containing 2ml of Nutrient broth, thus producing solution containing 50v/v of the slime extract. The process continues serially up to test tube No. 5, hence producing the following concentrations; 50, 25, 12.5, 6.25 3.125v/v. Test tube No. 6 do not contain slime extracts and serve as negative control. Exactly 0.1ml of 0.5 McFarland equivalent standards of test organisms were introduced into the test tubes and incubated at 37 °C for 24 hours. After incubation the test tubes were observed for growth by checking for turbidity [20].

Data Analysis

The data for zone of inhibition were subjected to one way Analysis of Variance (ANOVA) to indicate the most significant treatment on the bacterial species tested, where the ANOVA indicated significance, Least Significant difference (LSD) was used to separate means. ANOVA analysis was conducted with Open Start Statistical Software (version 08.12.14).

RESULTS

Antibacterial Activity of Snail Slime

The antibacterial activity of different slime concentration against the isolates recovered from different wound samples is presented in Table 1. The result showed that antibacterial activity of the slime increased with increase in concentration of the slime. Based on the result, higher activity was shown by E. coli with average zone of inhibition of 15.2 mm, followed by Klebsiella sp (14.2 mm), Proteus mirabilis (13.3 mm), ***Pseudomonas aeruginosa*** (13.0 mm) and least activity was shown by ***Staphylococcus aureus*** **(9.1 mm). No activity** was shown by Methicillin Resistant ***Staphylococcus aureus*** **(MRSA).** Zones of inhibition recorded by the control ranged from 17 - 22mm.

Table1: Antibacterial Activity of Snail Slime against test isolates

	Concentration (v/v)/zones of inhibition (mm)				
Isolates	**25**	**50**	**75**	**100**	**Control**
Staphylococcus aureus	00.00 ± 0.0^a	10.34 ± 0.9^b	11.67 ± 1.2^b	14.34 ± 1.3^c	20
MRSA	0.00 ± 0.0^a	0.00 ± 0.0^a	0.00 ± 0.0^a	0.00 ± 0.0^a	17
E. coli	13.67 ± 0.8^a	14.67 ± 1.2^a	16.00 ± 0.0^b	17.34 ± 1.2^b	22
Proteus mirabilis	11.67 ± 1.2^a	12.67 ± 0.8^a	13.34 ± 0.8^a	15.34 ± 1.7^b	19
Pseudomonas aeruginosa	10.67 ± 1.1^a	12.34 ± 0.8^a	13.34 ± 1.3^b	15.67 ± 1.4^c	20
Klebsiella sp	12.34 ± 1.3^a	13.67 ± 1.7^a	14.47 ± 1.2^a	16.34 ± 1.4^b	21

Key: Values having different superscript in the same row are considered significantly different at probability level of p<0.05.

Table2: Minimum Inhibitory Concentration (MIC) of the Slime against isolates

Isolates	**MIC (v/v)**
Staphylococcus aureus	50
MRSA	NA
E. coli	25
Proteus mirabilis	25
Pseudomonas aeruginosa	25
Klebsiella sp	25

Key: NA = Not available

Minimum Inhibitory Concentration (MIC) of the Slime

The minimum inhibitory concentration (MIC) of the slime is represented in Table 2. The result showed dilutions of various concentrations of the slime against test organisms. Lower MIC was recorded by E. coli, Klebsiella sp, Proteus mirabilis, ***Pseudomonas aeruginosa*** (25v/v each) while highest MIC value (50v/v) was recorded by S aureus.

DISCUSSION

Land snails are able to produce mucus which has been reported to demonstrate antibacterial properties against both gram positive and negative bacteria [21,22,23]. In addition to that, the mucus secretion has been tested in surgical wounds of experimental animal and proved to improve the repair of dermal cicatricial [21,22]. In the present study, the slime of *A. marginata* showed antibacterial activity against both Gram negative and positive bacteria but more pronounced among Gram negative bacteria. However, no activity was recorded among Methicillin Resistant *Staphylococcus aureus* (MRSA). The result of this study was in conformity with the finding of Santana *et al*. [12] who reported antibacterial activity of snail (*A. marginata*) slime against *S. aureus* and *S. epidermidis*, and that of Periyasamy *at el*. [24] who noticed the activity of crude snail skin methanol extract against *S. aureus* and as well that of Lawrence *et al*. [17] reported that *Staphylococcus* sp was susceptible to mucus from *A. marginata*. On the other hand, this result was contrary with that of Ajiboye, [25] who study the secretion and antibacterial activity of snail slime (*A. marginata*) against some pathogenic bacteria isolates namely *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. His results showed that no evidence of antibacterial activity in the snail slime found. The result of this study also contradict the work of Sodipe *et al*. [26] who evaluate the antibacterial activity of haemo-lymph of giant African land snail against some bacterial isolates namely *Salmonella* sp., *Staphylococcus aureus*, *Escherichia coli*, *Pasteurella* sp and no antibacterial activity was found. This differences in the antibacterial efficacy of the slime could be related to different environmental factor of and possibly the strains of the bacterial species.

Evidence of antibacterial activity in snail slime and mucin obtained from snail slime has been reported by several researchers [12,17,24]. Snails have specific proteins that help their survival in their environment, including preventing bacterial contamination. Their musus secretion contains antimicrobial proteins [27]. The antibacterial activity of mucin found in the mucous secretions of land snails was found to be related to antibacterial factors in the protein component, instead of its activity on the cell surface of bacteria [28]. Another antibacterial protein common to snail mucus is called achacin. The achacin found in snail mucus of land snail can bind to bacteria, thereby causing effects [28,29]. Achacin is a member of the L-amino acid oxidase family, and is antibacterial through its production of hydrogen peroxide [30]. The minimum inhibitory concentration of mucus secretions from *A. marginata* against the test organisms were observed at mucus concentrations of 25v/v for E. coli, Klebsiella sp, Proteus mirabilis, ***Pseudomonas aeruginosa*** each while highest MIC value of 50v/v was recorded by S aureus.

CONCLUSION

The study was aimed to investigate the antibacterial activity of snail (*A. marginata*) slime against some bacteria associated with different types of wound. The slime was active against some isolates E. coli, Klebsiella sp, Proteus mirabilis, ***Pseudomonas aeruginosa*** and Staphylococcus aureus. However, the slime was not active against MRSA. The antibacterial activity of the slime is attributed to the presence of antibacterial protein such mucin. Therefore, the slime of snail (*A. marginata*) slime is effective for the treatment of wound infection.

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CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTION

The authors contributed equally in the conduction of the experiment and development of the manuscript.

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