Ethiopian Journal of Environmental Studies and Management Vol. 6 No.2 2013

COMPARATIVE EVALUATION OF INHIBITORY ACTIVITY OF EPIPHGRAM FROM ALBINO AND NORMAL SKINNED GIANT AFRICAN LAND SNAIL (*Archachatina marginata)* AGAINST SELECTED BACTERIA ISOLATES

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http://dx.doi.org/10.4314/ejesm.v6i2.8

Received 2nd January 2012; accepted 26th February 2013

Abstract

A study was conducted on evaluation of inhibitory activity of epiphgram from albino and normal skinned giant African land snail (Archachatina marginata). After aestivation, epiphgram were collected from twenty snails (10, albino and 10 normal skinned). The epiphgram were washed, air dried and ground into powder form. Minimum inhibitory concentration (MIC) of epiphgram from both albino and normal skinned species were determined by standard broth micro-dilution method using four (4) clinical bacteria Isolates which include: Eschericia coli, Pasteurella species, Salmonella species and Staphylococcus aureus. Ciproflaxacin antibiotic was used as control. Result showed that epiphgram from both albino and normal skinned snails had higher microbial activity for both Eschericia coli and Salmonella species as shown by minimum inhibitory concentration (MIC: 0.050 and 0.098 vs 0.098 and 0.049) compared to streptomycin (MIC: 78 and 1.95 vs 15.63 and 1.95) which is the control. However, normal skinned epiphgram had higher antimicrobial activity considering the MIC values for Salmonella species, Staphylococcus aureus and Pasteurella species. While MIC value recorded for Eschericia coli showed that albino snail epiphgram had higher antimicrobial activity in the elimination of this bacteria species better than the normal skinned. It can be concluded from this study that substance which eliminate bacteria especially the four bacteria isolates used in this study are present in the epiphgram of both normal and albino snails in varying proportion with the highest amount present in normal skinned snail's epiphgram except for Eschericia coli.

Keyword: *Albino snail, Archachatina marginata, Inhibitory activity, Epiphgram, Bacteria isolate*

Introduction

Bacteria have survived for millions of years by developing resistance to new stressors. They do this by simple modification of one or more of their enzymes thus breaking the link between a target protein and the antibiotics (Sanae *et al.*, 2003). The appearance of a growing number of bacteria resistant to conventional antibiotics has become a serious problem, because of the abuse. The development of new effective antibiotics is a pressing issue. Antimicrobial peptides are not only the potent antibiotics, but also have activity of killing viruses and cancerous cells. They are gaining attention as antimicrobial alternatives to antibiotics (Dolashka *et al*., 2011).

Giant African land snails are known to play significant roles in the life and culture of rural dwellers. They are known to be a very good delicacy among villagers, urban dwellers and among international hoteliers. Recently, there has been much emphasis on their products been used for both medicinal and pharmaceutical purposes. Cosmetic industry has also shifted ground to their products as a useful source of raw materials in cubing some skin diseases (Brieva *et al*., 2008).

The efficacy of these products outside the living body of this animal is traceable to their immunity. This also suggests that the nature has made their body to resist and survive under severe foreign environment. Adikwu and Ikejiuba (2005) reported wound healing property of Snail mucin which depict efficacy of their product against microbial activities.

This animal has a complex response to changes in environmental condition. Pulmonate land snails respond to unfavourable conditions such as drop in temperature and dehydrating periods by going into a state of inactivity called aestivation (Rizzatti and Romero, 2001; Fields, 1992; Storey and Storey, 1990; Schmidt-Nielsen, 1996). In temperate region, hibernation is considered to be the response which is characterised by extreme cold

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(Abeloos, 1965; Aupinel, 1990; Bailey, 1981; Jeppesen, 1977; Jeppesen and Nygard, 1976; Riddle, 1983). Under unfavorable environmental condition , land snails secrete a mucus secretion that hardens into a calcareous epiphram (Li and Graham, 2007). This tough membrane covers the mouth of the shell and serves as a barrier against desiccation, infection and other damage during the period of dormancy (Block, 1971; Struthers *et al*., 2002). If this animal is intact after period of inactivation or dormancy, then it is an indication that epiphgram could be another protein product produced by this animal that could be used for medicinal purpose. This study therefore aimed at studying inhibitory microbial activity of normal and albino skinned epiphgram.

Materials and Method

Experimental Area

The research was carried out at the Snail Physiology Research Unit of the College of Animal Science and Livestock Production and Department of Veterinary microbiology, University of Agriculture, Abeokuta, Ogun State. The location lies within the rainforest belt of Western Nigeria, latitude 7 ^{o}N, longitude 3 ^{o} 2' E and altitude 76 m.a.s.l. The climate is humid with a mean annual rainfall of 1,037 mm, mean temperature of 28.7 ^{o}C and mean relative humidity of 82 %.

Materials

A total of twenty snails (20) *Archachatina marginata* weighing between 150g to 180g were used for this experiment. Ten (10) of them were albino while the other ten (10) were normal skinned type. Ten (10) plastic cages with each having a dimension of 30 cm by 40 cm by 24 cm, with small plastic feeding and drinking troughs in each cage, also, mixture of dried pawpaw leave and Poultry layers mash in ratio 50:50 v/v, w/w together with morta, pestle and universal bottles were used for this study.

Snails and their management

The plastic cages were clean prior to the commencement of the experiment, two weeks was set aside as a period of acclimatization. The snails were fed *ad libitum*. Drinking water was also provided daily *ad-libitum* in drinking troughs. Feed and water troughs were washed daily while the cages were also clean daily.

Aestivation, Epiphgram Collection and Preparation

At the fourth week, feed and water were withdrawn from the snails. After four to seven days, epiphgrams were fully formed. Collection was made at the second week. Epiphgrams were removed with the use of sterile spatula by lifting the edge of the snail aperture. Collected epigrams were collected in sterile universal bottles and stored at room temperature. The epiphgrams obtained were ground to powder in sterile morta with pestle. One gram of powdered epiphgram was weighed and thoroughly dissolved in 100ml of sterile water to make 0.001g/mL (1mg/mL).

Minimum Inhibitory Concentration (MIC)

Standard broth micro-dilution method was used to determine MIC of epiphgram obtained from both albino and normal skinned snail specie (*A. marginata*) to clinical bacteria isolates which include; *Eschericia coli, Pasteurella* sp., *Salmonella* sp. and *Staphylococcus aureus*. MIC to streptomycin (0.5–64 ug/mL) was also determined. Each epiphgram solution and ciprofloxacin antibiotic were serially diluted in accordance to their respective ranges in sterile 1% glucose peptone and equal volume of 0.5 Mac Farland broth inoculum of each clinical isolates was prepared after 18–20 hrs incubation at 37°C in ambient temperature; and was added to all the dilution ranges and incubated at 37°C in ambient temperature for 24hours. A drop of 0.25% phenol was added to all the wells after incubation to ascertain the end point showing no growth after incubation. Pink colouration indicates growth due to glucose fermentation of the isolates changing it to acidic medium while yellow colouration indicates no growth after incubation. The MIC of each sample was determined as the highest dilution showing yellow colouration as a result of no growth.

Result

Table 1 and 2 show the minimum inhibitory concentration of both albino snail and normal skinned epiphgram against selected bacteria Isolates (*Eschericial coli, Salmonella* sp. *Staphylococcus aureus, Pasteurella* sp.). Minimum inhibitory concentration (MIC) values gotten were lower in normal skinned type snails for all the bacterial isolate tested except for *E. coli* compared to albino snails. Values recorded for

Salmonella in normal skinned type snail is 0.049 as against 0.098 for albino. Others include: Pasteurella (50 vs 100), *Staphylococcus* (50 vs 100) and E. coli (0.098 vs 0.050). Considering the minimum inhibitory concentration of the control (Streptomycin), values recorded were higher in both albino and normal skinned snails (Plate 1) except for staphylococcus where values recorded in epiphgram in both type of snails were higher (albino: 100 vs 1.95 and normal : 50 vs 3.91).

Discussions

Snails have been reported to be one of the oldest species around the globe that have survived extreme environmental conditions for more than 600 million years (IRIS, 2010). This fact is an indication that snails have some special adaptive proteins with which they survive in their environment. Antimicrobial activity of epiphgram from normal and albino skinned giant African land snail (*A. marginata*) as shown in Table 1 and 2 from this study is a further evidence of their survival. This observation showed that epiphgram contains one or more of such protein that are used for protective purpose. Furthermore, some forms of protein are known to be of importance in the protection of this animal against some form of bacterial attack. Boman (1995) assert that antimicrobial peptides are among the earliest developed molecular effectors of innate immunity and are significant in the first line of host defense response of diverse species. Many different families of molecules have been found throughout the animal and plant kingdoms that display similar modes of action against a wide range of microbes (Boman, 1995). This study also showed that overall minimum inhibitory concentration of epiphgram from normal skinned *A. marginata* compared to those from albino snails were higher for most of the four bacteria isolates used in this study. The reason for this observation may be as a result of differences in rate and amount of secretion of those proteins which perform this protective action. Ademolu *et al.* (2011) discovered in their study that normal skinned had significantly higher protein, glucose and lipids than albino snails under similar experimental condition. Differences may also be as a result of deficiency of enzyme responsible for metabolism of melanin in albino snail during prenatal development (Allegretti *et al*., 2009). Considering the four bacteria isolates used, antimicrobial activity of epiphgram from both normal skinned and albino were seen to be higher than the control (Streptomycin) in the elimination of *Eschericia coli*, and *Salmonella* sp. This is an indication that giant African land snail (*A. marginata*) secrets antimicrobial protein/proteins whose action is better than the conventional antibiotic streptomycin.

According to Alexander *et al.* (1997), many marine species possess a mucosal barrier to microbe-laden external environment. The mucosal are very rich in antimicrobial peptide which facilitate the removal of some bacteria. Differences in rate of elimination of different types of bacteria may be as a result of structural type of binding of anionic phospholipid-rich membranes with which antimicrobial peptide dissolves bacteria membranes which are quite similar in some bacteria and some with little differences. Munozo-Crego *et al.* (1999) and Schmid *et al.* (2003) reported that pathogen surfaces bear a large number of oligoglucides that may be bound by specific proteins example of which are lectins. Study by Abiona (2010) revealed that substances which bind the four bacteria isolates used in this study were present in the haemolymph of giant African land snails (*A. marginata* and *A. achatina*). MIC values recorded for both *Salmonella species* and *Staphylococcus aureus* is a further indication that antimicrobial proteins secreted by this animal still have capacity to control this pathogen but not as strong as in the first two species of bacteria isolate. This observation could still be attributed to changes in the membrane architecture of these bacteria which may offer slight resistance to the antimicrobial proteins secreted by this animal. Despite this resistance, it was still clear that epiphgram from normal skinned *A. marginata* recorded much better MIC values compared to albino snails. The import of this observation is that normal skinned *A. marginata* had better gene expression for those antimicrobial proteins than the albino snails.

Conclusion

This study showed clearly that substance which could eliminate *Eschericia coli*, *Pasteurella specie*, *Salmonella species* and *Staphylococcus aureus* are present in the epiphgram of both normal skinned and albino giant African land snail

(*Archachatina marginata*). It was obvious from the study that epiphgram from normal skinned had higher antimicrobial activity compared to albino snail. This showed that epiphgram from both species could be of use for medicinal application.

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Table 1 Minimum inhibitory concentration of Albino snail (*A. marginata*) epiphgram against selected bacteria Isolate

Isolate	Epiphgram MIC (µg/ml)	Streptomycin MIC (µg/ml)
Eschericia coli	0.050	7.8
Salmonella specie	0.098	1.95
Staphylococcus aureus	100	1.95
Pasteurella specie	100	125

Note: The lower value of MIC means more antimicrobial activity

Table 2 Minimum inhibitory concentration of normal skinned (A. marginata) epiphgram against selected bacteria Isolate

Isolate	Epiphgram MIC (µg/ml)	Streptomycin MIC (µg/ml)
Eschericia coli	0.098	15.63
Salmonella specie	0.049	1.95
Staphylococcus aureus	50.0	3.91
Pasteurella specie	50.0	250

Note: The lower value of MIC means more antimicrobial activity