GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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# Biochemical and microbiological properties of haemolymph from African land snail (*Achatina achatina*) in Akure metropolis

Ekaete Elizabeth Gbolahan-Ayoade [1,*], Titus Adeniyi Olusi [1] and Wahab Gbolahan Ayoade [2]

[1] *Department of Biology, School of Life Sciences, Federal University of Technology, P.M.B 704, Akure, Ondo State, Nigeria.*
[2] *Department of Chemistry, School of Physical Sciences, Federal University of Technology, P.M.B 704, Akure, Ondo State, Nigeria.*

GSC Biological and Pharmaceutical Sciences, 2022, 21(03), 196–202

Publication history: Received on 19 November 2022; revised on 22 December 2022; accepted on 25 December 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.21.3.0491

## Abstract

Haemolymphs were aseptically collected from matured African Land Snails (*Achatina achatina*) obtained from four villages in Akure metropolis and examined for biochemical and microbiological properties using standard methods. Four villages (Ibulesoro, Aule, Igoba and Oda) within Akure metropolis were purposefully selected for this study, Twenty (20) snails were obtained in each village and a total of eighty (80) snail samples were collected from the four villages. The results of biochemical constituents of the haemolymph revealed that the mean values of pH ranged from 6.68 - 6.97; total protein (mg/dl) 24.27 - 30.33; glucose (mg/dl) 13.94 - 19.33; lipid (mg/L) 1.38 – 1.71; urea (g/dl) 3.63 – 3.92; albumin (g/dl) 1.74 – 1.96 and globulin (g/dl) 3.13 – 3.52. Mineral components of the haemolymph from the snails in all locations revealed the concentration (mg/L) of sodium in the ranged of 138.02 – 209.54; calcium 30.63 – 38.01; phosphate 54.36 – 65.74; potassium 32.15 – 40.22; bicarbonate 21.08 – 27.31; magnesium 16.41 – 21.52 and chloride 83.07 – 118.92. The results of microbiological components of the haemolymph from the snail samples revealed the mean plate count of various bacterial and fungi isolate. The amount (cfu/ml) of bacterial isolates in the haemolymph are *Staphylococcus aureus* which ranged from 1.92 x 103 – 2.86 x 103; Faecal *strephtoccoci* 2.48 x 103– 4.26 x 103 and *Shigella dysentriae* 0.83 x 103– 2.23 x 103; others include *Pseudomonas aeroginosa*, *Bacillus spp*, *Escherichia coli* and *Klebsiella pneumonia*. The predominant fungi isolate in the haemolymph was mucor spp with the mean total count ranged from 4.31 x 103– 9.33 x 103; followed by *Aspergilus fumigatus* 3.21 x 103– 5.43 x 103 , others are *Rhizopus spp*, *Aspergillus flavus*, and *Aspergillus niger*. The study revealed that the snail fluid (haemolymph) contained adequate amount of biochemical components and nutritionally valuable minerals such as sodium, potassium, calcium and other ions that could enhance the body normal physiological function when consumed. However, the prevalent of some pathogenic bacterial and fungi in the haemolymph proof it to be unsafe for human consumption in its raw form. The study therefore suggest that the haemolymph should be adequately pasteurized and disinfect before consumption as herbal remedies for medicinal purpose.

**Keywords:** Biochemical; Microbiological; Haemolymph; African land snail

## 1. Introduction

Land snails are pulmonates and vary greatly in size. The largest species being the giant African land snail (GALS) usually referred to as Tiger snail (*Achatina achatina*) which can grow up to 30cm in length [1]. Land snails live in habitats that are often damp or wet. Many such as *Archachatina* and *Achatina* species are normally confined to humid forested areas where they occur in great numbers. Snails hibernate during dry seasons in the tropics but are usually abundant in the rainy periods when fresh vegetation is available. Many gastropods particularly aquatic species are known to serve as intermediate hosts for different parasites especially trematodes that cause diseases such as *fascioliasis*, *schistosomiasis*

* Corresponding author: Ekaete Elizabeth Gbolahan-Ayoade
Department of Biology, Federal University of Technology, P.M.B 704, Akure, Ondo State, Nigeria.

and *paragonimiasis*, in both humans and other mammals. In parts of West Africa, specifically Nigeria, snails are served as a delicacy [2]. *Achatina achatina*, Ghana tiger snails, are also known as some of the largest snails in the world. Snail, called "igbin" in Yoruba language is a delicacy, widely eaten in Nigeria, especially among the Yorubas and Igbos. In Cameroon, snails, usually called 'nyamangoro' and 'slow boys' are a delicacy especially to natives of the South West region of Cameroon. The snails are either eaten cooked and spiced or with a favourite dish called 'eru'.

Reports also have shown that snail meat has medicinal value and can be used to treat ailments such as whooping cough, anaemia, asthma and high blood pressure due to its relatively low Cholesterol level but high mineral content [3; 4]. Haemolymph is regarded as a blood analogue found in all arthropods and most molluscs [5]. It is composed of water, inorganic salts (mostly Na, Cl, k, Mg and Ca) and organic compounds (mostly carbohydrates, proteins and lipids) [6; 7; 8; 9]. Since the haemolymph directly bathe snail organs in an open circulation [10], it has been associated with snail growth performance and susceptibility to infection and aggression [6].

Consumption of African land snails otherwise known as Terrestrial snails are common in most part of Nigeria with exception of view tribe due to traditional believe [11]. The nutritional quality of the snail meat makes it a better choice of animal protein, however, the infectious diseases associated with the various part of the snail including intestine which can be transmitted from the snail to human were not aware of by most consumers, especially the fluid (heamolymph) which is widely consumed for the treatment of various diseases. This study is therefore designed to evaluate the biochemical components of the fluid and scientifically examine the various microorganisms that are associated with the land snail species generally consumed in the study areas to create awareness on various type of diseases that can be infected by human through consumption of this land snail fluid.

## 2. Material and methods

### 2.1. Snail sample collection

Four villages within Akure north and south local government areas of Ondo State were purposefully chosen for this study. Twenty mature snails were purchased directly from farmers in each village. Therefore a total of eighty (80) snails with average weight of 158 gram were purchased from the four villages. The villages are Ibulesoro, Aule, Oda and Igoba all in Akure South local government area of Ondo State.

### 2.2. Snail sample Identification

The snail sample purchased were brought to the animal section of Biology Department for proper identification and authentication, then subsequently caged in separate house packed with various plant materials such as Tallilum triagulae, watermelon peel and ripe pawpaw so as to be feed through day and night prior analysis.

### 2.3. Biochemical Analysis of the Snail Haemolymph (Fluid)

The apex of the snails' shell was broken and the haemolymph was aseptically collected using the method described by [12]. The protein concentration of each sample was determined immediately using the biuret method described by [13]. The glucose content was determined by the colorimetric method of [14]. The lipids assay was done following the method of [15].

The haemolymph sodium ($Na^+$), potassium ($k^+$), calcium ($Ca^{2+}$), phosphate ($PO_4^-$), magnesium($Mg^{2+}$), bicarbonate ($HCO_3$) and chloride ($Cl^-$) were determined by the method described in the Association of Official Analytical Chemists [16].

### 2.4. Microbial analysis of haemolymph

The microbiological examination of snail haemolymph was examined for the presence of bacterial and fungi count including identification using the standard method of [17] as described by [18].

### 2.5. Data Analysis

Data were analysed using the Statistical Package for Social Sciences (SPSS) version 20.0. The Analysis of Variance test (ANOVA) was used to test for significant differences in the measured variables of the snail fluid obtained from the four different villages in Akure metropolis of Ondo States in southwest Nigeria. A post-hoc test was conducted using Duncan Multiple Range test, with the p value set at 0.05.

## 3. Results and discussion

**Table 1** Biochemical components of haemolymph from the snail sample

| Biochemical | Ibulesoro | Aule | Igoba | Oda |
|---|---|---|---|---|
| pH | $6.87^{b} \pm 0.07$ | $6.68^{a} \pm 0.02$ | $6.81^{b} \pm 0.03$ | $6.97^{c} \pm 0.04$ |
| Total protein (mg/dl) | $24.27^{a} \pm 0.05$ | $26.39^{c} \pm 0.08$ | $25.35^{b} \pm 0.06$ | $30.33^{d} \pm 0.08$ |
| Glucose (mg/dl) | $13.94^{a} \pm 0.03$ | $15.81^{b} \pm 0.05$ | $15.83^{b} \pm 0.08$ | $19.33^{c} \pm 0.02$ |
| Lipid (mg/L) | $1.38^{a} \pm 0.03$ | $1.69^{c} \pm 0.01$ | $1.71^{c} \pm 0.02$ | $1.58^{b} \pm 0.05$ |
| Urea (g/dl) | $3.63^{a} \pm 0.02$ | $3.86^{b} \pm 0.05$ | $3.87^{b} \pm 0.03$ | $3.92^{c} \pm 0.03$ |
| Albumin (g/dl) | $1.74^{a} \pm 0.01$ | $1.83^{b} \pm 0.01$ | $1.85^{b} \pm 0.01$ | $1.96^{c} \pm 0.02$ |
| Globulin (g/dl) | $3.13^{a} \pm 0.03$ | $3.41^{b} \pm 0.01$ | $3.52^{c} \pm 0.02$ | $3.38^{b} \pm 0.01$ |

Mean ± SEM of triplicate determinations; Mean followed by the same superscript are not significantly different @ $p \leq 0.05$

### 3.1. Biochemical constituents

Biochemical composition of the haemolymph of *Archachatina archatina* obtained from different locations in Akure metropolis is presented in Table 1. The mean pH of the haemolymph varied significantly (6.87, 6.68, 6.81 and 6.97) for snails from Ibulesoro, Aule, Igoba and Oda villages respectively. The least pH value (6.63) was obtained in snail from Aule village while the highest pH 6.97 was obtained in snails from Oda village. The pH value reported in this study are lower than 7.27 and 7.07 reported by [19] for haemolymph from snails obtained from Lagos and Osun State respectively. pH is a measure of acidity and alkalinity of a solution, however, the pH of the haemolymph from the snails in all the locations fell around neutral pH 7 which implies that the fluid was not too acidic or basic and may not posse any adverse effect to human body when consumed. Total protein (mg/dl) contents of the haemolymph were (24.27, 26.39, 25.35 and 30.33) for snails from Ibulesoro, Aule, Igoba and Oda villages respectively. The least protein content was obtained in snails from Ibulesoro while the highest was obtained Oda village. The protein contents of the haemolymph in this study was in the range of 24.27 to 30.33mg/dl, these was lower than 35.23 and 52.03 reported by [19] for haemolymph from snails obtained from Lagos and Osun State respectively. The values also lower than 39.10 and 49.60 mg/dl reported by [8] for juvenile and adult African giant land snail (*Acrhachatina marginata*). Snails are known to be a good source of protein which can be consumed to ameliorate the problem of protein deficiency in diets [20; 21; 22] and the haemolymph also proof the presence of appreciable amount of protein than can be used as a regulators in human physiological processes especially, growth and repaired of worn-out tissue. The glucose (mg/dl) levels of the haemolymph obtained from the snails ranged from 13.94 in Ibuesoro village to 19.33 in Oda village and there was no significant ($p<0.05$) difference in the values 15.81 and 15.83 obtained for snails from Aule and Igoba villages respectively. However, these values are within the range of 21.27 and19.32 reported by [19] for snails from Lagos and Osun States respectively, the results are also in agreement with 20.00 and 15.75 mg/dl reported by [23] for haemolymph in snails obtained from Ondo and Osun States respectively. This showed that snail fluid can also provide some energy for normal body function when consumed. Just like glucose, lipid also provide adequate energy to human body in form of Adenosine triphosphate (ATP) even more than glucose. The lipid contents (mg/L) of haemolymph from snails obtained in four locations varied significantly as the least 1.38 was obtained in snail from Ibulesoro and the highest value was obtained in snail from Oda village, while 1.69 and 1.71 were obtained from snails from Aule and Igoba respectively which are not significantly different. The amount of urea (mmol/L) contents of the haemolymph of snails in all locations also varied significantly as the highest value 3.92 was obtained in Oda snail and the least 3.63 was obtained from Ibulesoro snail, however, there was no significant difference in the values 3.86 and 3.87 in snails obtained from Aule and Igoba villages respectively. These values are in agreement with 3.76 and 3.64 mmol/L reported by [19] for haemolymph of *Archachatina marginata* obtained from Lagos and Osun States respectively.

Albumin is the most common protein found in the bloodstream. Its main function is to maintain osmotic pressure, the mechanism that prevents fluids from leaking out of the blood vessels and into surrounding tissues. It is an important transporter in the blood, albumin also binds fats and helps body with fat metabolism [24]. The albumin contents (g/dl) of the haemolymph obtained in snails from four locations differ significantly and ranged between 1.83 in snails from Aule village to 1.96 from Oda village respectively. Globulins are another class of proteins found in the blood which come in several forms such as alpha and beta which act as transporters and can inhibit some enzymes, gamma globulins / immunoglobulins act as antibodies thereby plays a vital roles in the immune system [25]. Globulin contents (g/dl) of the haemolymph obtained from snails in all the four locations ranged from 3.13 in Ibulesoro and 3.52 in Igoba villages

respectively. The normal Albumin globulin ratio is 0.8 – 2.0 [26]. The albumin globulin ratio is a measure to identify a causes of change in total serum protein. It usually go out of normal range, if one component increases or decreases relative to other. A low albumin / globulin ratio (A/G) may reflect or indicate overproduction of globulin and if higher, is an indication of overproduction or high concentration of albumin in serum protein [25].

**Table 2** Mineral Components (mg/L) of Haemolymph from the Snail sample

| Minerals | Ibulesoro | Aule | Igoba | Oda |
|---|---|---|---|---|
| Sodium $Na^+$ | $163.48^b \pm 0.15$ | $138.02^a \pm 0.12$ | $194.61^d \pm 0.21$ | $209.54^c \pm 0.23$ |
| Calcium $Ca^{2+}$ | $30.63^a \pm 0.21$ | $34.64^c \pm 0.33$ | $32.58^b \pm 0.37$ | $38.01^d \pm 0.32$ |
| Phosphate $PO_4^{2-}$ | $65.74^d \pm 0.81$ | $61.02^c \pm 0.71$ | $58.67^b \pm 0.92$ | $54.36^a \pm 0.59$ |
| Potassium $K^+$ | $32.15^a \pm 0.28$ | $34.98^b \pm 0.21$ | $40.22^d \pm 0.91$ | $37.12^c \pm 0.42$ |
| Bicarbonate $HCO_3^-$ | $21.08^a \pm 0.03$ | $24.18^b \pm 0.01$ | $27.31^c \pm 0.03$ | $24.13^b \pm 0.02$ |
| Magnesium $Mg^{2+}$ | $16.41^a \pm 0.06$ | $19.62^b \pm 0.02$ | $21.52^c \pm 0.01$ | $19.58^b \pm 0.03$ |
| Chloride $Cl^-$ | $83.07^a \pm 0.12$ | $86.11^b \pm 0.13$ | $97.44^c \pm 0.11$ | $118.92^d \pm 0.16$ |

Mean ± SEM of triplicate determinations; Mean followed by the same superscript are not significantly different @ $p \leq 0.05$

### 3.2. Mineral components

The function of the haemolymph in the open circulatory system of snail is to serve as transport of nutrient to the various body parts [10], hence it is expected that the haemolmph should contain higher concentrations of minerals for absorption into body tissue. Haemolymph biochemical properties of giant African land snails have been reported to influence physiological processes [9], growth performance [7, 27] of the land snail. Sodium and chloride contents are the most abundant ions in the haemolymph of all the snails obtained from the four locations in the study areas. The sodium contents of the haemolymph ranged between 138.02 in the snails from Aule village to 209.54 in the snails from Oda village respectively, while that of chloride showed a lowest concentration (83.07mg/L) in snails from Ibulesoro and the highest value (209.54mg/L) was obtained in the snails from Oda village. However, this report was in agreement with that of [27, 8] who reported that $Na^+$ and $Cl^-$ were the most abundant ions in the haemolymph of the slug Arion ater and the land snail, *A. marginata*. The role of Na+ in nervous communication is significant. Therefore, higher concentrations of Na+ recorded in the haemolymph of snails in the study areas could have been responsible for the control of the contraction of the snail muscular foot [10]. However, the low concentration of Sodium is desirable for muscle contraction and nerve transmission; maintenance of water balance and blood pressure [28]. Phosphate plays an important role in formation of ATP, a higher energy form [8]. The phosphate concentration of the haemolymph in the snails varied significantly and showed that the lower value 54.36mg/L was obtained in the snails from Oda village while the highest value 65.74mg/L was obtained in the snails from Ibulesoro. This implies that consumption of the snail haemolymph could supply adequate energy that body required. Calcium and magnesium are another important mineral that plays a significant role in healthy bone and teeth formation and potassium also plays an important role in lowering the risk of high blood pressure [29]. The calcium contents of the haemolymph varied significantly among the locations and ranged between 30.63mg/L in snails from Ibulesoro to 38.01 mg/L from snails from Oda villages respectively, while the potassium contents in the haemolymph showed that the least value 32.15mg/L was obtained in the snails from Ibulesoro village and the highest value 40.22mg/L was obtained in the snails from Igoba village respectively, while the magnesium contents ranged from 16.41mg/L in the snails from Ibulesoro village and 21.52mg/L in the snails from Igoba village and there was no significant difference in the magnesium contents of the snails from Aule 24.18mg/L and Oda 24.13mg/L villages respectively. Bicarbonate maintained and regulates proper acid – base balance, the bicarbonate contents in the haemolymph showed that the least value 21.08mg/L was obtained in the snails from Ibulesoro village and the highest value 27.31mg/L was obtained in the snails from Igoba village. The study revealed that the snail fluid (haemolymph) contained adequate amount of nutritionally valuable minerals such as sodium, potassium, calcium and other ions that could enhance the body normal physiological function when consumed.

**Table 3** Microbiological components of Haemolymph from snail samples

| **Bacterial count (cfu/ml)** | **Ibulesoro** | **Aule** | **Igoba** | **Oda** |
|---|---|---|---|---|
| *Staphylococcus aureus* | 2.21 x $10^3$ | 2.68 x $10^3$ | 1.92 x $10^3$ | 2.86 x $10^3$ |
| *Escherichia coli* | 0.42 x $10^3$ | 0.67 x $10^3$ | 0.33 x $10^3$ | 0.54 x $10^3$ |
| *Faecal strephtoccoci* | 4.26 x $10^3$ | 3.44 x $10^3$ | 2.48 x $10^3$ | 3.18 x $10^3$ |
| *Pseudomonas aeruginosa* | 1.41 x $10^3$ | 1.27 x $10^3$ | 1.03 x $10^3$ | 1.64 x $10^3$ |
| Bacillus spp | 0.91 x $10^3$ | 0.62 x $10^3$ | 0.84 x $10^3$ | 0.53 x $10^3$ |
| *Klebsiella pneumonia* spp | 1.58 x $10^3$ | 2.06 x $10^3$ | 1.92 x $10^3$ | 2.36 x $10^3$ |
| *Shigella dysentriae* | 1.31 x $10^3$ | 1.96 x $10^3$ | 0.83 x $10^3$ | 2.23 x $10^3$ |
| **Fungi count (cfu/ml)** | | | | |
| *Aspergillus niger* | 3.36 x $10^3$ | 5.41 x $10^3$ | 1.62 x $10^3$ | 2.48 x $10^3$ |
| *Mucor spp* | 6.18 x $10^3$ | 9.33 x $10^3$ | 6.54 x $10^3$ | 4.31 x $10^3$ |
| *Aspergillus flavus* | 5.63 x $10^3$ | 5.38 x $10^3$ | 2.17 x $10^3$ | 3.46 x $10^3$ |
| *Aspergillus fumigatus* | 4.42 x $10^3$ | 3.67 x $10^3$ | 3.21 x $10^3$ | 5.43 x $10^3$ |
| *Rhizopus spp* | 2.68 x $10^3$ | 2.27 x $10^3$ | 3.12 x $10^3$ | 2.07 x $10^3$ |

### 3.3. Microbiological properties

Table 3 showed the microbiological activities of the haemolymph from the snail samples. It revealed the microbial loads of various bacteria and fungi isolates that present in the haemolymph of the snails obtained from different locations in the study area. The amount (cfu/ml) of bacterial isolates in the haemolymph are *S. aureus* which ranged from 1.92 x $10^3$ – 2.86 x $10^3$; *E. coli* ranged from 0.33 x $10^3$ – 0.67 x $10^3$; *F. strephtoccoci* ranged from 2.48 x $10^3$– 4.26 x $10^3$; *P. aeroginosa* ranged from 1.03 x $10^3$– 1.64 x $10^3$; *Bacillus spp* ranged from 0.53 x $10^3$– 0.91 x $10^3$; K. *pneumonia* ranged from 1.58 x $10^3$– 2.36 x $10^3$and *S. dysentriae* ranged from 0.83 x $10^3$– 2.23 x $10^3$. The prevalent isolated species are *S. aureus, K. pneumonia* and *F. strephtoccoci* The results indicates that the snail fluid (haemolymph) contained various type of pathogenic bacteria at different concentrations and this implies that consumption of the haemolymph could cause various type of disease such as dysentery, diarrhea, pneumonia and nausea.

The amount (cfu/ml) of Fungi isolates are *A. niger* which ranged from 1.62 x $10^3$ – 5.41 x $10^3$; *Mucor* spp ranged from 4.31 x $10^3$– 9.33 x $10^3$; *A. flavus* ranged from 2.17 x $10^3$– 5.63 x $10^3$; *A. fumigatus* ranged from 3.21 x $10^3$– 5.43 x $10^3$ and *Rhizopus* spp ranged from 2.07 x $10^3$ – 3.12 x $10^3$ . The results revealed that *mucor spp* was the predominant fungi in the snail fluid followed by *Aspergilus fumigatus*. The presence of these fungi isolates is an indication that consumption of snail fluids has a potential risk of contracting various fungi infections or diseases by the consumers. The dominance of *Aspergillus spp* in the examined samples (haemolymph) was in accordance with the results of [30] who stated that *Aspergillus* and *penicillium* spp were the main moulds in herbal drugs. The results obtained in this study agreed with that of [31; 32] who observed that snails' haemolymph did not resist the growth of fungal and bacterial isolates. This is in tandem with earlier observation of [9] that the physiological state of the snails is reflected in the haemolymph as it has open circulatory system where the fluid bathes the entire organs and tissues.

## 4. Conclusion

The haemolymph collected from African land snails (*Archatina achcatina*) obtained from selected four villages in Akure metropolis were evaluated for biochemical, mineral and microbial constituents. The results of biochemical constituents of the haemolymph revealed a neutral pH values, low fat content, high glucose and protein with normal range of albumin / globulin. The study also revealed that the snail fluid (haemolymph) contained adequate amount of nutritionally valuable minerals such as sodium, potassium, calcium and other ions that could enhance the body normal physiological function when consumed. However, the prevalent of some pathogenic bacterial and fungi in the haemolymph proof it to be unsafe for human consumption in its raw form. The study therefore suggest that the haemolymph should be adequately pasteurized and disinfect before consumption as herbal remedies either as a whole or as a supplement in herbal mixture for the treatment of diseases.

## Compliance with ethical standards

*Acknowledgments*

The authors give thanks to God Almighty for the success of this work. We also acknowledged the Department of Chemistry and Microbiology of the Federal University of Technology, Akure for providing all necessary equipment and reagent used for this research.

*Disclosure of conflict of interest*

The authors declared no conflict of interest.

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