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Snail Slime: Evaluation of Anti-Inflammatory, Phytochemical and Antioxidant Properties

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Authors' contributions

This work was carried out in collaboration among all authors. Author NCC co-designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author LCC edited the study design and protocol. Authors GG and CO managed the bench work and analyses of the study and wrote the protocol. Authors GU and OFO managed the literature searches and assay protocol. All authors read and approved the final manuscript.

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ABSTRACT

Snails posses numerous nutritional, medicinal and therapeutic properties, making it an essential component of human nutrition, medicinal and skin health. This study is aimed at evaluating the anti-inflammatory and antioxidant properties of snail slime obtained from giant African land snail (*Achatina fulica*). The slime was extracted by low heat method and anti-inflammatory properties was ascertained by inducing paw edema in day old chicks using carrageenan, the phytochemical screening was conducted to ascertain the level of phenols and flavonoids, while the antioxidant properties was determined by DPPH radical scavenging activity, using ascorbic acid as standard. Statistical analysis using ANOVA version 20.0 was conducted to analyze the data obtained from the study. Post study statistics of data (mean ± SD) indicates that the snail slime extract potentially posses anti-inflammatory, relative phytochemical and antioxidant properties at significant ($p \leq 0.05$) level, hence validating its use in nutrition, medicinal and cosmetic purposes, and preparations.

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1. INTRODUCTION

Most disease initiation and progression is greatly influenced by oxidative damage and chronic inflammation [1]. Natural anti-inflammators and antioxidants have been implicated as protective constituents that slow oxidative damage processes and inflammatory mediation, hence they provide a measure of protection [2]. Tissue injuries or trauma that is externally imposed not minding the remote cause results in inflammation, and as such is targeted at inactivation, destruction and elimination of the invaders (foreign body), followed by initiation of tissue repair [3]. Immune response (innate) against infection is a key response of inflammation and a broad spectrum of disease has as a result of inflammatory response been identified, managed and or treated [4-6].

Snails are one of the oldest known animal species on earth, existence close to 500 million years (https://www.snail-world.com/). Snails fundamentally differ and exist in many types due to the existence of aquatic (fresh water) and terrestrial (humid land) species. The giant African snail is a terrestrial gastropod mollusc belonging to the group octopuses and a part of the phylum Mollusca [7]. Numerous therapeutic properties of snail slime have been reported and harnessed since early history and scientific research in recent times has demonstrated its efficacy in a variety of health and skin related treatment and uses. There are also lots of beneficial attributes abound in snail slime which are yet to be explored. A growing interest on the possible role of snail slime in medicine and the industry (pharmaceutical and cosmetics) due to its ability to crawl on rough surfaces without abrasions and the resistance to bacterial and environmental pollutants attributed to its slime secretion has aroused scientific concerns [8]. This study aims at evaluating the anti-inflammatory and antioxidant properties of *Achatina fulica* (snail) slime.

2. MATERIALS AND METHODS

2.1 Reagents

Ethanol and methanol purchased from BDH chemical Ltd (Poole, England); 1,1-diphenyl-2-picrylhydrazin (DPPH), carrageenan and tween 80 purchased from Sigma Chemical Company (St. Louis, MO, USA); ascorbic acid, diclofenac and potassium tablets purchased from Juhel Pharmaceutical Ltd (Enugu, Nigeria) and other chemicals of analytical grade were employed for this research study.

3. METHODOLOGY

3.1 Procurement and Housing of Snails

Twenty (20) healthy *Achatina fulica* were purchased from a local food market in Elele, Rivers State, Nigeria. The snails were housed in a porous plastic bowel (native sieve) with moist dead leaves as bedding and were sprinkled with water intermittently daily in order to maintain adequate humidity. They were fed with green vegetables, ripe bananas and guavas *ad libitum*.

3.2 Extraction and Preparation of *Achatina fulica* Slime

The slime was extracted from *A. fulica* using heat method. Briefly, cleanly rinsed and dried snails were placed in a 500 ml measuring cylinder and placed in a water bath at 70^{o}C for 10 minutes to induce the snail into secretion of large volume of slime, and this was repeated 5 times on different set of snails. The collected slime was transferred into a clean sterile sample container for preservation at 4^{o}C. An aliquot of the extracted slime was collected into a clean test tube, allowed to thaw and centrifuged at 4000tr/min for 10 minutes. The supernatant was collected in a clean sample tube for use.

3.3 Procurement of Chicks

Twenty (20) white cockerels (*Gallus gallus domesticus*, strain shaver 5790) weighing between 40-55 g were procured from Jocarl Breeder Farm, Umudioga road, Elele, Rivers State, Nigeria, conveyed to the animal house of pharmacognosy, Madonna University, Nigeria. They were properly housed with soft wood shavings as beddings and free access to clean water and commercially available starter pellet diet (Top feed, Nigeria) *ad libitum* prior to the study, with a 12/12 hours light and dark cycle maintained.

3.4 Preliminary Qualitative Phytochemical Screening

Quantitative phytochemical screening for the detection of secondary metabolites (phenols and

flavonoids) was carried out on the snail slime extract according to the methods adopted from Sofowara, [9] and Trease & Evans [10].

3.4.1 Test for phenolic compound (tannins)

1. Lead acetate test: 1 ml of lead acetate solution was added to a test tube containing 1 ml of the snail slime and the formation of a white precipitate is indicative of the presence of phenolic compound.
2. Brayer's test: 1 ml of ferric chloride solution was added to a test tube containing 1 ml snail slime and the formation of a dark blue colour indicates the presence of Phenolic compound.
3. Potassium ferricyanide test: 1 ml of potassium ferricyanide and ammonia solution was added to a test tube containing 1 ml of snail slime, the formation of a deep red colour indicates the presence of Phenolic compound.
4. Potassium dichromate test: 1 ml of concentrated potassium dichromate solution was added to 1 ml of snail slime, the formation of a yellow coloured precipitate indicates the presence of Phenolic compounds.

3.4.2 Tests for flavonoids

1. Sodium hydroxide (NaOH) test: an aliquot of the snail slime extract was treated with NaOH solution and then dilute HCl was added and the formation of a yellow or orange colour indicates the presence of flavones.
2. Sulphuric acid (H_2SO_4) test: an aliquot of snail slime extract was treated with concentrated H_2SO_4 and observed for the formation of yellow or orange colouration.
3. Shinoda test: an aliquot of the snail slime was treated with magnesium foil and concentrated HCl, the formation of an intense cherry red colour indicates the presence of flavones while an orange red colouration indicates the presence of flavonols.
4. Sodium chloride (NaCl) test: an aliquot of the snail slime extract was treated with 10% NaCl and the formation of yellow colour indicates the presence of coumarins.
5. Lead acetate test: an aliquot of the snail slime extract was treated with lead acetate and observed for the formation of a white precipitate.

3.4.3 Anti-inflammatory assay

Carrageenan-induced paw edema is the most common method employed in the evaluation of anti-inflammatory drugs. The 20 cockerels (*Gallus gallus domesticus*) were divided into five (5) groups of 5 chicks each. Group 1, negative control (received normal saline solution only); group 2, positive control (received [10 mg/kg of diclofenac] standard drug); group 3, test group (received [50 mg/kg p.o.] snail slime extract); group 4, test group (received [100 mg/kg p.o.] snail slime extract); group 5, test group (received [200 mg/kg p.o.] snail slime extract). All administrations were done 30 minutes prior to carrageenan injection. Thereafter, 0.1 ml of 1% (w/v) solution of carrageenan in 0.9% normal saline solution was injected subcutaneously (s.c.) into the plantar region of the right leg paw of each chick and the paw volume measured at 0, 60, 120, 180 and 240 minutes after carrageenan challenge according to their respective groups.

3.4.4 Antioxidant assay

The snail slime extract was solubilised using tween80 and diluted with distilled water (dH_2O) to a concentration of 1 mg/ml. At varying concentrations (50, 75, 100, 200 and 300 µg/ml) of the snail slime solution, methanol dissolved ascorbic acid was introduced into the respective test tubes. Methanol was used to adjust the volume to 3 ml. A solution of methanol and DPPH (0.1mM) was prepared and 1 ml of this solution was added to each test-tube, vigorously shaken and allowed to stand under room temperature (25^oC) for 30 minutes. Similarly, a control was prepared as described above but without sample or standard. A mixture of 3 ml tween80 and 1 ml of methanol was used for the baseline correlation. A spectrophotometric absorbance (Abs) was measured at 517 nm in triplicate and the average taken as the final result. Low absorbance of reaction mixture indicates high free radical scavenging activity (i.e. absorbance is inversely proportional to scavenging activity).

Cal:% radical scavenging activity = $[A_{control} - A_{sample} / A_{control}] \times 100$

Where: $A_{control}$ = Abs of control
A_{sample} = Abs of sample

The antioxidant activity was also expressed as IC_{50}. The IC_{50} value is the concentration (µg/ml) of test sample that inhibits the formation of DPPH radical by 50%.

3.5 Data Analysis

Data obtained were statistically analyzed for mean ± standard deviation (SD), comparison of mean and LSD correlation was done using analysis of variance (ANOVA) version 20.0 and significance set at $p<0.05$.

4. RESULTS

The results of findings from the phytochemical and anti-inflammatory analysis carried out were expressed statistically as mean results of triplicate evaluation.

Table 1. Qualitative phytochemical screening of *Achatina fulica* slime extract

Phytochemicals	Inference
Phenols	Present (++)
Flavonoids	Present (++)

Key: ++ = relatively present

4.1 Anti-inflammatory Activity of *A. fulica* Slime Extract on Carrageenan-Induced Edema in Chicks

The anti-inflammatory activity of *A. fulica* slime extract on carrageenan-induced edema in chicks from obtained result (Table 2) showed a significant ($p<0.05$) difference in IVP, VAI and various time variations (60-240 mins) when compared within each group. However, there was no difference in the group comparison when considering the positive control (group 2) and treated groups (3-5) as against the negative control (group 1).

4.2 Percentage (%) DPPH Radical Scaven ging Activity of *A. fulica* Slime Extract

The percentage (%) DPPH radical scavenging activity of *A. fulica* slime extract was assessed against vitamin C (standard) to ascertain its antioxidant property and the result revealed a capability of the slime extract to scavenge DPPH radical in proportion to concentration (Fig. 1). There was an observed significance ($p<0.05$) in its scavenging activity although not as effectively compared with vitamin C (Vit. C) scavenging capability been that vitamin C is well known exogenous antioxidant vitamin.

5. DISCUSSION

The use of snail as food has been a practice over hundreds of years with little or no attention given to the efficacy of its slime as medicinal and cosmetic therapies. Hence the scientific evaluation of the anti-inflammatory and antioxidative properties of the giant African land snail (*Achatina fulica*) slimes extract.

The preliminary qualitative phytochemical analysis result revealed the presence of phenols and flavonoids. This result is similar to the report of phytochemical analysis done by Bhosale et al. [11] which indicated the presence of tannins, flavonoids and alkaloids. On the contrary, tannins, flavonoids, and alkaloids were absent in the report of Mandefro et al. [12]. Variations in extraction solvents and/or techniques could be responsible for the differences in result [13].

The slime extract presented a moderate anti-inflammatory effect on acute edema induced by sub-plantar injection of carrageenin on paw of chicks in comparison with diclofenac (standard drug) administration. This finding was supported by some clinical studies on the safety and efficacy of green lipped mussel extract (GMLE) for use in humans [12-20].

Antioxidant property of the slime (*A. fulica*) was evaluated by DPPH assay method which is associated with the DPPH radical scavenging effect of the antioxidants present in the slime and this is achieved by the ability of the slime donating hydrogen ion. The snail slime exhibited

Table 2. Anti-inflammatory activity of *A. fulica* slime extract on carrageenan-induced edema in chicks

Groups	IVP	VAI (0 Mins)	60 Mins	120 Mins	180 Mins	240 Mins
Group 1	2.32±0.20	$3.28±0.26^{a}$	$2.92±0.08^{a}$	$2.7±0.16^{c}$	$2.54±0.18^{c}$	$2.44±0.22^{c}$
Group 2	2.14±0.21	$3.16±0.34^{a}$	$2.84±0.27^{a}$	$2.6±0.23^{b}$	$2.42±0.23^{b}$	$2.28±0.23^{b}$
Group 3	2.02±0.18	$3.12±0.29^{a}$	$2.98±0.29^{a}$	$2.78±0.26^{a}$	$2.56±0.18^{c}$	$2.36±0.15^{b}$
Group 4	2.22±0.19	$2.98±0.16^{a}$	$2.8±0.21^{a}$	$2.58±0.19^{c}$	$2.46±0.11^{b}$	$2.38±0.13^{b}$
Group 5	2.28±0.33	$3.06±0.21^{a}$	$2.92±0.18^{a}$	$2.74±0.15^{a}$	$2.56±0.05^{b}$	$2.54±0.11^{b}$

Data are presented as the mean ± standard deviation (SD). $^{*}P<0.05$ vs. Group 1 (NC); $^{\#}P<0.05$ vs. Group 2. $^{a}P<0.05$ vs. IVP (NC); $^{b}P<0.05$ vs. VAI; $^{c}P<0.05$ vs. IVP & VAI. IVP, Initial volume of paw; VAI, volume after inoculation

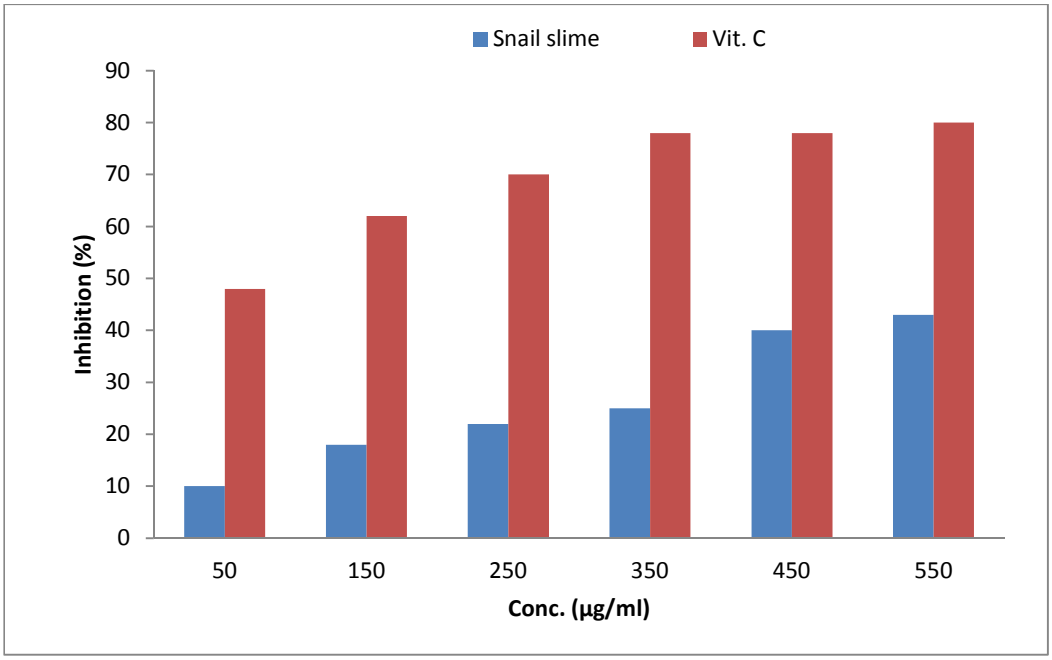

Fig. 1. Percentage (%) DPPH scavenging activity of *A. fulica* slime extract

antioxidant properties as compared with ascorbic acid (vit. C) as illustrated (Fig. 1), although not as effective as vitamin C which is proven scientifically as an antioxidant vitamin [21]. The decreased in absorbance of DPPH radical was compared to that of vitamin C (control), and the antioxidant property in the snail slime resulted in scavenging activity due to the reaction between antioxidant and free radical progression attributed to donation of hydrogen ion (Fig. 1).

6. CONCLUSION

Anti-inflammatory, medicinal, cosmeceutical and nutraceutical property of giant African land snail (*Achatina fulica*) slimes extract has span for centuries with little or paucity of scientific evidence. This investigation demonstrates the possibility of a promising anti-inflammatory and antioxidant properties, cosmeceutical and nutraceutical efficacy of snail slime as a natural source of remedy for key health conditions such as inflammations and free radical scavenging, hence the need for its characterisation to justify these outlined properties.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products as they are not intended for use or as an avenue for any litigation but for the advancement of research and knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Strict compliance with National Institute of Health (NIH) Nigeria guideline for the care and use of laboratory animals was maintained and permission sought from the animal handling and ethics committee of the Institution.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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