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Free Radical Scavenging Activity of the Nigerian Leech (*Aliolimnatis michaelseni*) Saliva Extract

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Authors' contributions

This work was carried out in collaboration of all the authors. Authors ICJO and ECE designed the study. Authors ICJO, CCM and SSE wrote the protocol and performed the statistical analysis. While authors DU, MBB and PCO performed the laboratory analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Introduction: Antioxidant is a substance that is capable of restricting or frustrating the oxidation of other oxidizable molecules by suppressing the free radical- caused oxidation chain reaction. They occur naturally in the body or can be obtained from external (Exogenous) sources such as plants and animal products. Laboratory experimental studies using these exogenous sources have shown that they can prevent free radical damage associated with development of cancer, heart diseases, diabetes and other life threatening diseases.
Aim: The aim of this study was to determine the free radical scavenging activity of Nigeria Leech (*Aliolimnatis michaelseni*) saliva extract (LSE).
Place and Duration of Study: Leech samples were collected from fresh water ponds in Nasarawa

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State Nigeria between June and December 2014.
Methodology: Antioxidant activity of the salivary gland secretion was determined using DPPH free radical scavenging activity method.
Results: Results showed that Leech saliva extract expressed a free radical scavenging activity with IC_{50} of 8.169 ug/ml initially and 8.67 ug/ml after starvation for 1 month compared with 5.025 ug/ml of L-ascorbic acid as a positive control.
Conclusion: Therefore, this study revealed that the proteomic contents of LSE are promising natural antioxidants.

Keywords: Antioxidants; DPPH; free radical; leech saliva extract.

1. INTRODUCTION

Leech saliva extracts have received much attention because of their extensive uses in many medical fields [1]. The salivary gland secretions of leeches are known to contain a variety of peptides and proteins with a wide range of therapeutic benefits and chemical properties [2,3]. The concept of the medical application of leeches can be traced back to the beginning of civilization. Traditionally, in many countries including Nigeria, leech application had been used for many human body disorders starting from the conventional usage of leech for bloodletting. Moreover, many reports mentioned the usage of leech in skin diseases, nervous system abnormalities like brain congestion, urinary and reproductive system problems (nephritis, vaginitis). In addition, ocular inflammation, dental problems and haemorrhoids were also treated by leech therapy [4,5,6].

Antioxidants are substances which when present at low concentration are capable of preventing or delaying oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species (ROS). These ROS include reactive free radicals such as superoxide, hydroxyl, peroxyl, alkoxyl and non-radicals such as hydrogen peroxide, hypochlorous acid, etc. Antioxidants scavenge radicals by inhibiting the initiation and breaking chain propagation or suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen [4,7]. Antioxidants exhibit their activities by being involved in the oxidation process themselves rather than the biological targets [8]. Free radical-induced oxidative damage is involved in the pathogenesis of many chronic and degenerative diseases, such as cardiovascular disease, cancer, diabetes, neurodegenerative disease and ageing [9,10,11]. The harmful action of these free radicals can, however be blocked by antioxidant substances which scavenge the free radicals and detoxify the organism.

On the other hand, antioxidants have gained a remarkable interest from scientists because of their valuable potential applications in medicine and industries. They have very promising applications as prophylactic and therapeutic agents for many life-threatening illnesses [12]. Some reports suggested that people who routinely intake flavonoid, a typical natural antioxidant, from tea, fruits and vegetables are at lower risk of developing coronary heart diseases and stroke [13].

It is also well recognized that numerous natural and/or synthetic substances containing phenolic rings in their structure scavenge hydroxyl radicals by means of aromatic hydroxylation [14]. One of such recently discovered agents is resveratrol, present in red grapes and wine and shown to have beneficial effects in diabetes [15]. The most widely used class of drugs containing phenolic rings, salicylates and their derivatives, have been shown to protect against free radical injury *in vivo* [16]. The health benefits of free radical scavengers have also been emphasized by other Scientists in renal diseases [17,18]. Also many articles have shown the importance of fruit and vegetable diet in the prevention of cardiovascular disease, which has been attributed to their antioxidant properties [19,20].

The objective of this study was to determine the free radical scavenging activity of Nigeria Leech (*Aliolimnatis michaelseni)* saliva extract.

2. MATERIALS AND METHODS

2.1 Leech Sampling and Saliva Collection

Leeches, *Aliolimnatis michaelseni*, were collected from the natural lake and ponds in

Nasarawa State, North central Nigeria. They were maintained in well-aerated plastic containers filled with un-chlorinated water. Water was regularly changed every two days. The collected leeches were kept in a room under 12h:12h light and dark cycle at the room temperature (25ºC).

Leech saliva extract (LSE) was collected without sacrificing the animal as described by Abdualkader et al. [21]. One hundred and fifty-eight (158) starved leeches' at different periods (weeks) vomited colorless salivary fluids which was centrifuged at +4ºC and at 2500 rpm for 10 min [22] and the supernatant was filtered using 0.45 µm Sartorius® filter paper. The resultant LSE was aliqouted in 1-ml glass tubes and lyophilized for 24 hrs. The resultant lyophilized LSE was used during the experimental procedures.

2.2 Free Radical Scavenging Activity

Antioxidant activity was measured by the method of Blois [23] in terms of radical scavenging ability using DPPH method in a methanolic (MeOH) medium [21,24,25]. The lyophilized LSE was dissolved in MeOH yielding a 3-time concentrated LSE, and the resultant methanolic solution was termed as 3×mLSE. Volumes of 100 µl of serial double-fold dilutions of the 3×mLSE were pipetted into 96-well plate. Ten, all volumes were brought to a final volume of 300 µl by MeOH. Then, 15 µl of methanol DPPH solution 84 (0.002 M) was added and the absorbance were taken after 15min at 516nm. The same procedures were performed using serial step-wise dilutions of L-ascorbic acid (50 µg/ml) in methanol as a positive control. The PHS was used as a negative control. Finally, a volume of 15 µl of DPPH was added to 300 µl of Me OH and absorbance was measured immediately at 516 nm as control reading. All measurements were repeated in triplicates, and the mean ± standard error of the mean (SEM) was considered. The free radical scavenging activity (% antiradical activity) was estimated from the equation:

$$\% \text{ antiradical activity} = \frac{\text{Control absorbance} - \text{Sample absorbance} \times 100}{\text{Control absorbance}}$$

This method for measuring antioxidant activity is both the same before and after starvation.

2.4 Data Analysis

The collected data were analyzed using SPSS version 15.0. The relationship before and after starvation periods were compared by Students t-test.

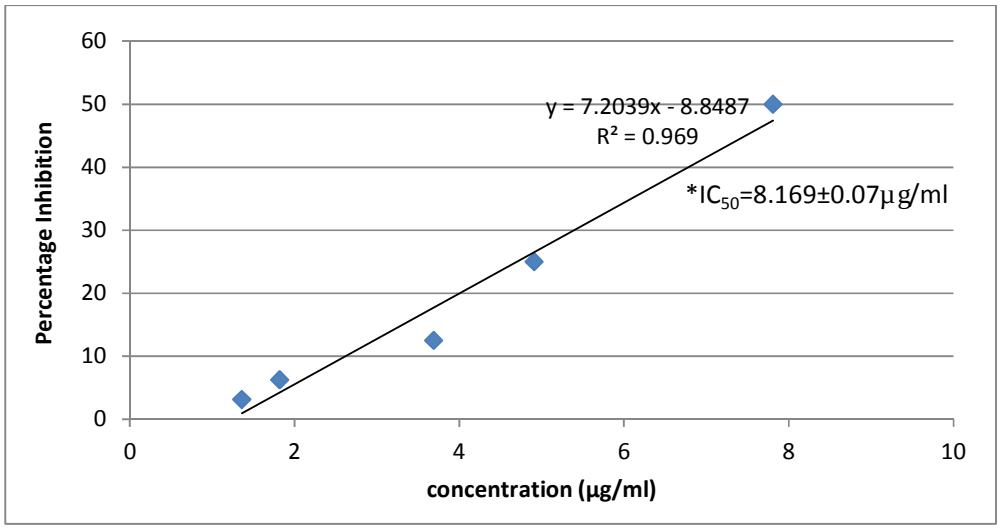
3. RESULTS

Results showed a free radical scavenging activity of Leech saliva extract with IC_{50} of 8.169±0.07 µg/ml compared with 5.025 µg/ml of L-ascorbic acid as a positive control before starvation and IC_{50} of 8.67±0.07 µg/ml after starvation for 1 month also compared with 5.025 µg/ml of L-ascorbic acid as a positive control (Fig. 1a and b). There was a slight increase in difference in the activities before and after starvation though not significant at $p>0.05$.

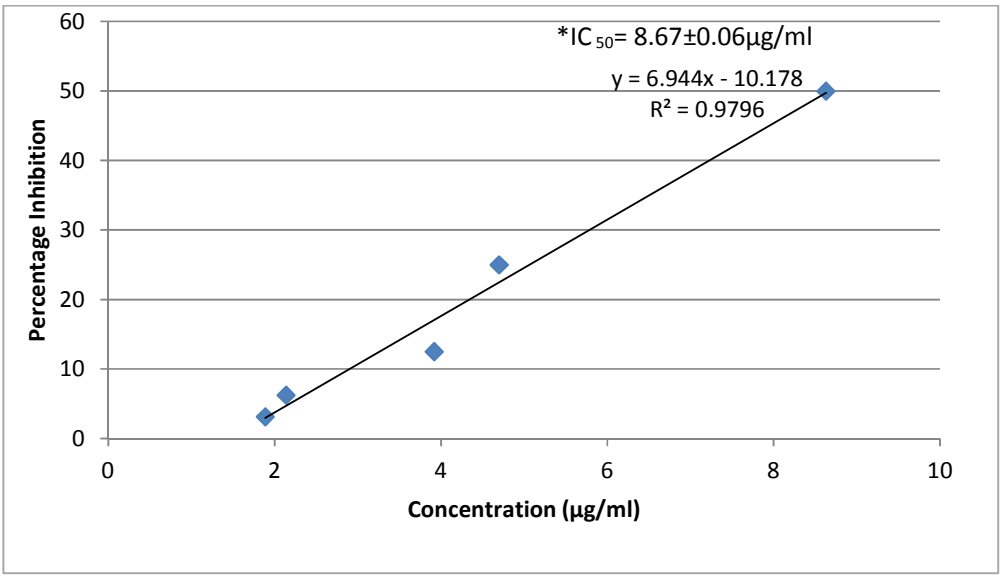
4. DISCUSSION

Results from this study revealed that the Nigeria Leech, *Aliolimnatis michaelseni* saliva extract with IC_{50} of 8.169 µg/ml had a high free radical scavenging activity in which is in agreement with the work of Ghawi et al. [26] who had first reported the antioxidant activity of Malaysian Leech saliva extract with IC_{50} of 7.282 µg/ml compared with 5.803 µg/ml of L-ascorbic acid. The difference in activities could be due to difference in geographical locations as leeches are localized. Rabiat et al. [27] have also shown that antioxidant activities of some Nigerian fruits depend on the dose taken. Reactive oxygen species (ROS), including superoxide free radical, hydrogen peroxide, hydroxyl free radical and oxygen, play a key role in the elimination of diseases, which may result in DNA mutations, protein inactivation, lipid peroxidation, cell apoptosis or abnormal proliferation, inducing the occurrence of diseases from the cellular and molecular levels [6,7]. The Antioxidants from Nigerian leech saliva are capable of scavenging ROS and protecting cells from oxidative damage.

The search for natural materials containing potent antioxidants continues to attract the attention of researchers. Vegetables, fruits and spices are known to be rich sources of natural antioxidants, and medicinal plants and animals are another important source for a wide variety of natural antioxidants. These medicinal plants and animals have high anti-inflammatory activities which could be from, in part, their antioxidant properties.



(a)



(b)

Fig. 1. DPPH free radical scavenging activity of LSE before (a) and after starvation for one month (b)

** Lethal concentration at 50%*

This study also revealed that starvation of leeches for one month showed an increase on their antioxidant activities but not significant. However, studies have shown that starvation of leeches over time has led to a significant increase in the concentration of protein of leeches [26], which will also lead to increase in the antioxidant properties. It is likely that further studies with starvation for up to 5 or 6 months will show a significant increase in the antioxidant properties.

5. CONCLUSION

The salivary gland secretion from the medicinal Nigeria leech, *Aliolimnatis michaelseni* had a significantly higher free radical scavenging activity compared to that of L-ascorbic acid and

as such may be a natural source for treating diseases arising from oxidative damage.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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