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Free Radical Scavenging Activity and Protein Concentration and Profile of the Blowfly Maggot (*Lucilia robineau*) Excretion/Saliva Extract

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ICJO and ECE designed the study. Authors ICJO, KA and SI wrote the protocol. Authors CH, SSE and EDU performed the laboratory analysis. Authors AA and AAB 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

Aim: The aim of this study was to determine the free radical scavenging activity, Protein concentration and profile of Blowfly maggot (*Lucilia robineau*) excretion/secretion (ES).
Place and Duration of Study: Maggot samples were collected from first generation of blowflies reared between June 2014 and July 2015.
Introduction: Maggots of the blowfly have been successfully used as a debridement agent for chronic and infected wounds through history.
Methodology: Antioxidant activity, protein concentration and profiles of the maggot ES of *Lucilia robineau* was determined using DPPH free radical scavenging activity and Bradford methods
Results: Results showed that maggot ES expressed a free radical scavenging activity with IC_{50} of 152.66 µg/ml compared with L-ascorbic acid with IC_{50} of 108.99 µg/ml as a positive control while

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total protein concentration was 430.51 mg/ml. Protease and specific activities are $1.5x10^{-2}$ mg/ml and 28,700.67 IU while the protein fractions are Albumins 36.96%, Alpha Globulins 21.32%, Beta Globulins 31.69% and Gamma Globulins 10.02%.

Conclusion: Therefore, this study revealed that the proteomic content of maggot ES of *Lucilia robineau* has promising natural antioxidants. This is the first study on antioxidant properties, protein concentration and profile of Blowfly maggot (*Lucilia robineau*).

Keywords: Antioxidants; DPPH; free radical; maggot ES and protein fractions.

1. INTRODUCTION

Maggots have long been used as a traditional way of cleansing and healing gangrenous wounds. The first modern clinical study of maggot therapy was initiated in 1989, at the University of California. The result of many clinical trials and studies has indicated that maggot therapy is still useful today as a safe and effective treatment tool for some types of wounds. Those studies also demonstrated that there is no reason to withhold maggot therapy until all other modalities has been exhausted, nor use it only as a "last resort". While published accounts of "pre-amputation maggot therapy" show a limb salvage rate of over 40%, the success of maggot debridement therapy MDT when used earlier in the course of treatment (say a 2^{nd} or 3^{rd} or 4^{th} line treatment) is even more dramatic [1].

In recent decades, there has been an increasing world-wide spread of hospital-acquired pathogens exhibiting resistance against one or more of the clinically applied antibiotics. This serious problem attracted renewed attention to the old-fashioned and almost forgotten therapy of Maggot Debridement Therapy (MDT). The use of medicinal maggots in USA was approved by the Food and Drug Administration as a medical device in 2004 [2]. Over the last few years, several comparative clinical trials investigating the efficacy of MDT have been performed [3]. In terms of debridement, MDT is more effective than conventional therapies [4]. On the other hand, in terms of wound healing and remodeling, the outcomes of clinical trials are controversial [5]. MDT has attracted great attention due to its successful application and efficacy in the elimination of multi-drug resistant wound pathogens [6].

Antioxidant is a substance capable of frustrating or restricting the oxidation of other oxidizable molecules by suppressing the free radical caused oxidation chain reaction. Antioxidants exhibit their activities by being involved in the oxidation process themselves rather than the biological targets [7].

Free radical-induced oxidative damage is involved in the pathogenesis of many chronic and degenerative diseases, such as cardio-vascular disease, cancer, diabetes, neuro-degenerative diseases and ageing [8-10]. The harmful action of the free radicals can, however be blocked by antioxidant substances which scavenge the free radicals and detoxify the organism.

The objective of this study was to explore the properties of natural products related to cultural uses and ethnic medicine by determining the free radical scavenging activity of maggot excretion/ saliva extract.

2. MATERIALS AND METHODS

2.1 Blowfly (Maggot) Sampling and Maintenance

Raw meat were exposed to blowflies in Minna Abattoir for a period of one to two hours after which the eggs deposited in meat was placed in a nylon bag usually between 25-34ºC and kept till it decayed and maggots emerged. Maggots at the third instar pupated for adult emergence. These new adults were transferred to a fresh meat for egg laying and emergence of second generation maggots for the extraction of excretion/secretion of saliva.

2.2 Extraction of Maggot ES

The method described by Shuchi et al. [11] was used. Live adult specimens collected from the field were anaesthetized by chloroform for a short time for identification. After identification, the species were transferred to new cages for oviposition. Eggs laid on the meat were treated with 70% ethanol and sterile distilled water successively three times. The treated eggs were deposited on fresh meat and allowed to hatch to maggots for 2-3 days in an incubator at 35ºC.

Late second or early third instar maggots were aseptically transferred to a flat petri-plate and washed with ethanol and sterile distilled water successively three times and soaked in filter paper. Treated larvae were incubated in sterile distilled water (5 µl/larva) for 60 minutes at ambient temperature in the dark [12]. Resultant Maggot Extract (ME) obtained were transferred to another tube using a pipette and autoclaved for 20 minutes at 121ºC. Subsequently, the maggot extract is allowed to cool to room temperature. Any remaining were stored at -20ºC for analysis and future use.

2.3 Free Radical Scavenging Activity

Antioxidant activity was measured by the method of Blois (1958) [13] in terms of radical scavenging ability using DPPH method in a methanolic medium [14,15]. The lyophilized maggot ES was dissolved in MeOH yielding a 3-time concentration, and the resultant methanolic solution was termed as 3×m maggot ES. Volumes of 100 µl of serial double-fold dilutions of the 3×m maggot ES were pipetted into 96-well plate. Then, all volumes were brought to a final volume of 300 µl by MeOH. Then, 15 µl of methanol DPPH solution (0.002 M) was added and the A516 were taken after 15 min. 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 MeOH and A516 was measured immediately as a control reading. The free radical scavenging activity (%antiradical activity) was estimated from the equation:

% antiradical activity= control absorbance - Sample absorbance/ control absorbance x 100

2.4 Total Protein Estimation, Profiles and Fractions

Total protein concentration assay was executed as described by Bradford (1976) and the standard protocols of Bradford reagent kit [16] using BSA as a standard protein and the PHS as a blank. Concentration was measured in mg/l.

The different protein fractions were quantified by selective precipitation by passing it through a pH gradient i.e. changing the pH over a range of values [17].

Protease activity was determined by measuring the amount of amino acids released upon hydrolysis proteins using bovine serum albumin as a substrate.

Protease activity = amino acid released / Time

Specific activity = protein conc. / protease activity

2.5 Data Analysis

The collected data were analysed using SPSS version 15.0.

3. RESULTS

Results showed a free radical scavenging activity of maggot excretion/secretion with IC_{50} of 152.66±0.69 µg/ml. Similarly, L-ascorbic acid which is the standard was found to be a free radical scavenger with IC_{50} of 108.99±0.34 ug/ml (Fig. 1).

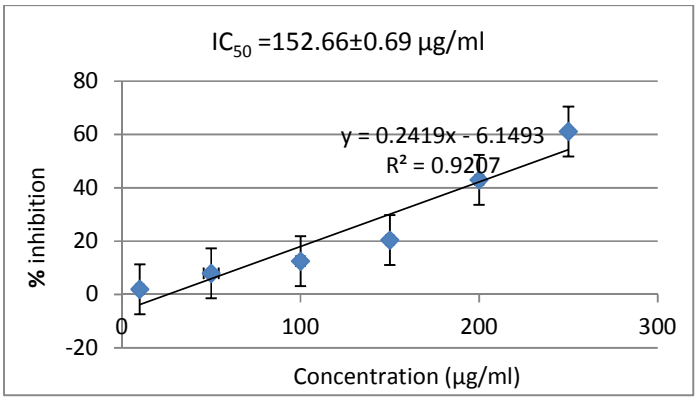
The protein concentration was 430.51 mg/ml (Fig. 2); protease and specific activities are $1.5x10^{-2}$ mg/ml and 28,700.67 IU. Protein fractions are Albumins 36.96%, Alpha Globulins 21.32%, and Beta globulins and Gamma globulins are 31.69% and 10.02% respectively (Table 1). The major protein was albumin, followed by beta, alpha and gamma globulins.

Table 1. Analysis of blowfly maggot *(Lucilia robineau)* excretion/saliva extract protein

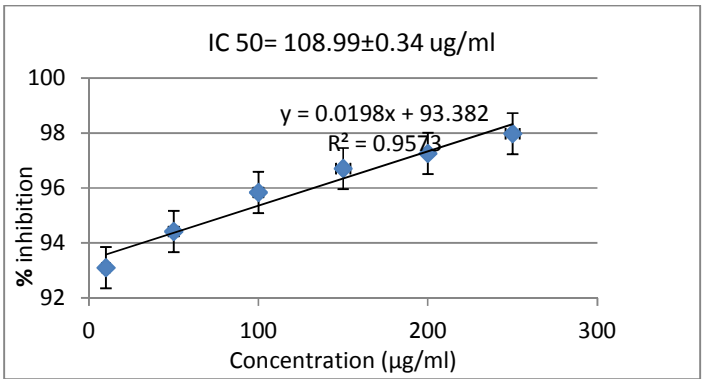
Activity	Unit
Bradford assay	430.51 mg/ml
Protease activity	1.5x10-2 mg/ml
Specific activity	28,700.67 IU
Protein fraction	
Albumins	36.96%
Alpha globulins	21.32%
Beta globulins	31.69%
Gamma globulins	10.02%

4. DISCUSSION

Antioxidants are biochemicals, vitamins and other nutrients that protect our cells from damage caused by free radicals. The result of the antioxidant properties of maggot excretion/ secretion was high compared to the standard L-ascorbic acid. This is the first study on the free scavenging activities of maggot ES to the best of our knowledge as such we are unable to compare our result with others studies. Leeches that are also animal products have very high antioxidant properties as seen in Malaysian leeches [18] and Nigeria leeches (*Aliolimnatis michaelseni*) [19].

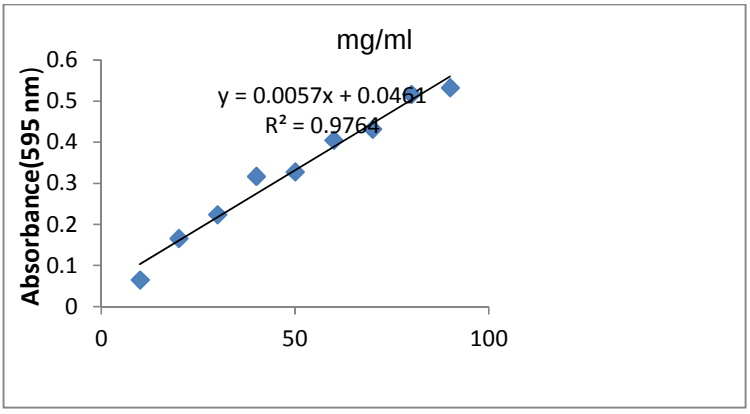


(a)



(b)

Fig. 1. DPPH free radical scavenging activity of maggot ES (a) and L-ascorbic acid (b)



Concentration (mg/ml)

Fig. 2. The standard curve for total protein estimation of maggot ES performed according to Bradford assay

The search for raw 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.

This result has explored the potentials of natural animal product related to cultural uses and ethnic medicine for the treatment of diseases, since antioxidants have valuable potential applications in medicine and industries. They have very promising applications as prophylactic and therapeutic agents for many life-threatening illnesses [20].

This study also revealed that Maggots ES has a high protein concentration of compared to other animal products like leech saliva [18,19]. Determination of the total protein concentration is an important tool among the procedures used in enzyme and protein characterization and purification. Physiological studies of protein expression and clinical diagnoses of altered protein levels in body fluids, is indicative of a variety of diseases.

Fractionation of proteins is often employed to quantify protein types within food materials. The results of the extracted protein fractions from maggot ES showed that albumin, beta, alpha and gamma globulins were observed and quantified accordingly. The data showed that albumin was the major protein in the maggot ES.

5. CONCLUSION

The excretion/secretion from blowfly maggot, *Lucilia robineau* had a high free radical scavenging activity and a high protein content indicating that it may have some therapeutic properties for the treatment of various diseases.

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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