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Protein profile and free radical scavenging activity of scorpion venom *Pandinus imperator*

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ABSTRACT

Scorpion venom has been used in traditional medicine for thousands of years mainly in Asia and Africa. It is a highly complex mixture of nucleotides, proteins and enzymes and it was ascertained that scorpion venom components with these valuable potentials can be used to counter the emerging global health crisis. The aim of this study is to determine the protein profile and free radical scavenging activities of scorpion venom *Pandinus emperator.* Scorpions were randomly sampled from their natural habitat between the month of April to December 2020. Extraction of the venom was done by electrical stimulation, concentration of proteins and peptides in the venom extract was estimated by Bradford proteins assay and free radical scavenging activity of the scorpion venom by DPPH method. Results showed that venom extract has concentrations of 1.19±0.35, 1.45±0.21 and 0.89±0.08 during September, October and November respectively. Also, specific activity is $9.18x10^{-4}$, $9.94x10^{-4}$ and $9.54x10^{-4}$ for the 3 months. Protein profile revealed the following fractions and their percentage concentration. The γ-Globulin shows the highest concentration of 40.7%, Albumin 31.5%, α-Globulin 17.6% and β-Globulin 10.2%. Antioxidant activity of venom was obtained at different concentrations of the venom extracts, 50 (46.17%), 100 (84.51%), 150 (96.71%), 200 (97.26%) and 250 (97.98%). The final result shows I.C50=99.04ug/ml indicating that the venom contains antioxidants capable of fighting diseases in the body.

Keywords: Scorpion venom, proteins, peptides, Antioxidant activity

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NTRODUCTION

Pandinus imperator, Emperor scorpion, belongs to the family of Scorpionidae and is native to rainforests and savannas in West Africa. Scorpions are the most primitive arthropods belonging to order Scorpiones and class Arachnida (Lena *et al.*, 2015). They are classified into 18 families and about 1500 different species and subspecies (Shultz, 2007). According to an estimate, frequency of deaths caused by scorpion sting is higher in comparison to that of snakebite (Mohammed, 2003).

Scorpion varies in size according to age and species from 1-20 cm in length. These can be found outside their normal territory when they accidentally crawl into luggage, boxes, containers, or shoes, and are transported home via human unwillingly (Sharif *et al.*, 2020). Scorpions when stimulated secrete venom which is a cocktail of variable concentrations of neurotoxins, cardiotoxins, nephrotoxins, hemolytic toxins, phosphodiesterases, phospholipases, hyaluronidase, glucosaminoglycans, histamine,

seratonin, tryptophan and cytokine releasers (Barrett *et al.*, 2008).

Venom which is nature's gift provided to scorpions comes into play. A second advantage of venom is the presence of enzymes as venom's constituents with diverse activity. These enzymes initiate the process of digestion in the tissue of the prey stung before consumption. The venom is an effective defensive device, which serves to deter and incapacitated the opponent (Li *et al.*, 2011). Even though, hissing and aggressive defence posture is usually enough to deter most animals including humans. When deterrence proves inadequate scorpions defend themselves by injecting venom into the body of enemy (Miyashita *et al.*, 2007). The main purpose of production of venom in scorpions is to secure food and self-protection. Venom glands, the factory of scorpion's venom are located on the lateral side of tip of sting. These are made of different types of tall columnar cells. Of these cells, one type produces toxins while others produce mucus (Luna-Ramirez *et al.*, 2016).

The potency of scorpion's venom varies from species to species with some producing only mild flu while others producing death (Kievit *et al.*, 2010). Scorpion venoms exert their action mainly by affecting specific functions of the ion channels (Lawal *et al.*, 2016). The venoms have been employed in medication for thousands of years in Asia (Batista *et al.* 2004). It is an extremely advanced mixture of salts, nucleotides, biogenic amines, enzymes, mucoproteins, peptides and proteins (e.g. Neurotoxins). Scorpion venoms contain peptides that block or modify ion-channel function and present some doable applications to regulate cell excitability (Beckheet *et al.*, 2013). As a result of their necessary structural and purposeful diversity, it's projected that scorpion-derived peptides might be accustomed to developing new specific medicines. This research aims to determine the protein profile and free radical scavenging activity of scorpion venom *Pandinus imperator.*

MATERIALS AND METHODS

Collection of Scorpion Species

Scorpions were randomly sampled from their natural habitat between the month of April to December 2020 within the study area and are usually kept in a white plastic bucket perforated to allow proper aeration. The Scorpions were fed with insects regularly and water kept at a corner in the plastics (Oukkache *et al.*, 2008). Temperatures and relative humidity of the locations were also recorded.

Identification/Determination of Species Composition and Abundance

The scorpions were later identified as described by Qi and Zhu (2005). hand lens was used to view the animals in order to determine their colour, body size and length. Numbers of scorpions found in each habitat were recorded according to their species, family and sex.

Extraction of Venom

The extraction of the venom was done by electrical stimulation method as described by Oukkache *et al.* (2008). The body of the scorpion was immersed in a saline solution for better electrical conduction and given a shock with electrode of 20volt. As much as 10ml of venom content of the telsons emitted were collected in a collecting bottle diluted with double distilled water and centrifuged at 15,000 rpm for 15 minutes at 4°C. The supernatant was transferred to new bottle, lyophilized and stored at −80°C.

Estimation of Protein Concentration
Concentration of proteins and peptides in the venom extract was estimated by using Bradford proteins assay. A 2.3ml of scorpion venom extract was added to 5ml of Bradford reagent and left to stand for 5-10minutes. The sample was then poured into a cuvette and absorbance taken at 595nm in ultra-violet (UV) visible spectrophotometer and extrapolated on the standard curve of Bovine-serum albumen. This was done three times at different intervals for precision (Varsel *et al.*,1960).

Activity bioassay of the scorpion venom.
The protein profile of the scorpion venom was determined by turbidity of the venom. This method was based on the fact that separated protein fraction of the scorpion venom extract precipitate as finely disperse suspension in phosphate solution of definite concentration. The degree of turbidity of the solution is proportional to the protein fraction concentration (Barona *et al.*, 2006).

Protease Activity of the scorpion venom
This was determined according to the method described by Hernandez-Betancourt *et al.*, (2009). To 1ml of 1% casein was added to 0.1ml of venom extract and 0.1ml of phosphate buffer of PH 9.0 was incubated at 50^{0c} for 30minutes. Enzymes activity was terminated by placing test tube in boiling water bath to which 2ml of Ninhydrin solution was added and heated in the boiling water bath for 5minutes waiting for colour to developed. Absorbance was read on the spectrophotometer at 540nm.

Free Radical Scavenging Activity of the scorpion venom
This was determined by a method described by Wheeler *et al.* (1982). The frozen scorpion venom extract was dissolved in methanol (MEOH) and made into a 3-times concentration of the scorpion venom extract. This produced a 3-time methanol scorpion venom extract solution of volume of 100µl of this solution serially diluted. This was pipette into each well in a 96-well plate. By adding MEOH, all volumes were made up to 300µl. A 15µl of 2,2-Diphenyl- 1-picrylhydrazyl (DPPH) was added into each well. After 15 minutes, absorbance was read at 516nm. The same processes were followed using serially increased dilution of L-ascorbic acid in methanol as positive control. 0.001m arginin in 0.15m of DPPH was put into 300µl of MEOH and absorbance taken immediately at 516nm as control. These were done in triplicates and the antioxidant activity was calculated from the equation:

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

RESULTS

Results of protein concentration reveals that on September 0.40ml of venom extract show a concentration of 1.19 ± 0.35^{a}, while 0.30ml of venom extract shows 1.45 ± 0.21^{a} and in 0.50ml of venom extract shows 0.89 ± 0.08^{a} in October and November respectively using the same quantity of distil water of 0.70ml for dilution. The result was expressed as mean ± Standard Error. Different alphabets along the column indicate significant difference at $P<0.05$. Using Duncan Multiple Range Test (DMRT) Table 1.

Table 1: Protein concentration of Scorpion venom extract

Months	Volume of Extract (ml)	Volume of Distilled water (ml)	Dilution Factor (ml)	Protein Conc. (mg/ml)
September	0.40 ± 0.05^{ab}	0.70 ± 0.00^{a}	1.82 ± 0.26^{ab}	1.19 ± 0.35^{a}
October	0.30 ± 0.00^{a}	0.70 ± 0.00^{a}	2.30 ± 0.00^{b}	1.45 ± 0.21^{a}
November	0.50 ± 0.06^{b}	0.70 ± 0.00^{a}	1.44 ± 0.17^{a}	0.89 ± 0.08^{a}

Data is expressed as mean ± Standard Error; Different alphabets along the column indicate significant difference (P>0.05) using Duncan Multiple Range Test (DMRT).

The protease activity of the *P. emperator* venom was carried out three times using 0.1ml of extracted venom. The specific activities are $9.18x10^{-4}$, $9.94x10^{-4}$ and $9.54x10^{-4}$ for September, October and November respectively. There was no significant difference within the three months of testing (Table 2).

Table 2: Protease activity of Scorpion venom

Months	No.of Scorpions	Volume of Venom (ml)	Protease activity (mg/sec)	Specific activity (i.u)
September	29	0.1	$1.53x10^{-2}$	$9.18x10^{-4}$
October	29	0.1	$1.49x10^{-2}$	$8.94x10^{-4}$
November	29	0.1	$1.59x10^{-2}$	9.54x10-4

Data is expressed as mean ± Standard Error; Different alphabets along the column indicate significant difference (P>0.05) using Duncan Multiple Range Test (DMRT).

The protein profile revealed the following fractions and their percentage concentration. The γ-Globulin shows the highest concentration of 40.7%, Albumin 31.5%, α-Globulin 17.6% and β-Globulin 10.2% (Table 3).

Table 3: Protein Profile of the extracted scorpion venom

Fractions	Percentage (%)
Albumin	31.5
α-Globulin	17.6
β-Globulin	10.2
γ-Globulin	40.7

During the test of antioxidant activity of venom, the following results were obtained at different concentration of the venom extracts, 50 (46.17%), 100 (84.51%), 150 (96.71%), 200 (97.26%) and 250 (97.98%). The final result shows I.C50=99.04ug/ml indicating that the venom contains antioxidants capable of fighting diseases in the body (Table 4) and Figure 1 shows the inhibition curve at different levels of concentration.

Table 4: Scavenging Radical Activity/antioxidant of the Venom Extract.

Standard Ascorbic Acid	
Conc.ug/ml	%inhibition
0	0
50	46.17
100	84.51
150	96.71
200	97.26
250	97.98
I.C50 =99.04ug/ml	

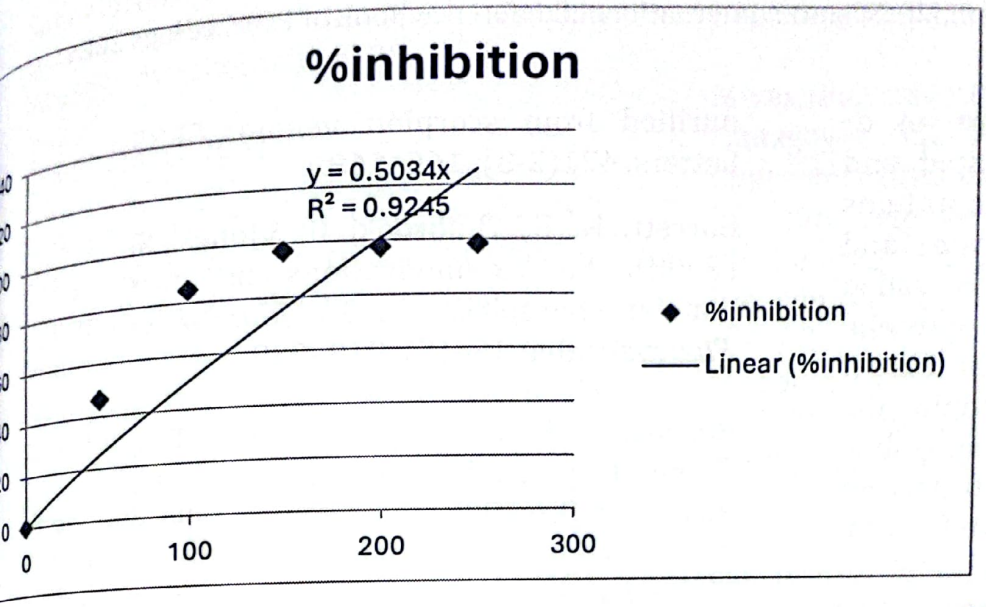

Figure 1: Graph of inhibition at different levels of concentration of the venom Extract.

DISCUSSION

Scorpion venoms contain complex mixture of bioactive substances that include proteins, free amino acids, heterocyclic, inorganic salts, components, and peptides. Scorpions produce this venom first as a result of secondary metabolic activities and as have been described mainly to contain enzymes that are used for self-defences, the capture of prey and possibly to ward of infection (Chippaux *et al.,* 2012). Some of these scorpion venom bioactive metabolites have already been purified and characterized and estimated that these venoms approximately contain 100,000 different components, but only 1 % of these molecules have been isolated and characterized (Cordeiro *et al.,* 2015)

The current study revealed that the examined scorpion venom contained proteins of little variation in relation to the extraction months. The slight variation could be attributed to the slight environmental factors which in turn could influence metabolic activities which may be responsible to the current variation observed. A high proportion of protein component were found in the species of scorpions analyzed. This is similar to the venom of most scorpions previously reported (Khoobdel *et al.,* 2013; Erdes *et al.,* 2014; Xu *et al.,* 2010). Experts have described that Sorpion venom contains varieties of chemical compounds with different biomedical and pharmacological potencies. These therapeutic properties (i.e., Biochemical and pharmacological) have been well captured in studies during the past few decades and have resulted in numerous polypeptidyl toxins isolation and characterization.

The presence of Albumin, Globulin and its subsets including α-Globulin, β-Globulin and Gamma Globulin in the extracted scorpion venom indicate that the protein components in rich in bioactive components. Albumin serves in the transport of hormones, bilirubin, vitamins, metals, and drugs. It has an important role in fat metabolism by binding fatty acids and keeping them in a soluble form in the plasma. The alpha-globulin fraction of the scorpion venom is a mixture of several conjugated

proteins. The best known are an α-lipoprotein (combination of lipid and protein) and two mucoproteins (combinations of carbohydrate and protein). One mucoprotein is called orosomucoid, or α1-acid glycoprotein; the other is called haptoglobin because it combines specifically with globin, the protein component of haemoglobin. Haptoglobin contains about 20 percent carbohydrate. The beta-globulin fraction of serum contains, in addition to lipoproteins and mucoproteins, two metal-binding proteins, transferrin and ceruloplasmin, which bind iron and copper, respectively. They are the principal iron and copper carriers of the blood. The gamma-globulins are the most heterogeneous globulins.

Several antimicrobial peptides have been described from scorpions, including several cysteine-containing defensin-typepeptides from haemolymph of the scorpions *Leiurus quinquestriatus hebraeus* and *Androctonus australis* (Atanasov *et al.,* 2021). The current study revealed the inhibition activity of the venom at different levels of concentrations (50-46.17, 100-84.51, 150-96.71, 200-97.26, 250-97.98 and finally the, I.C50=99.04ug/ml).

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