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ACID PHOSPHATASE FROM SCORPION VENOM: CHARACTERIZATION AND INHIBITION BY *Boswellia Dalzielii* AND *Bauhinia Rufescens* METHANOLIC EXTRACTS

H. Zanna, Y. Tijani, C. Chidiebere and A. Daniel
Department of Biochemistry, Faculty of Science
University of Maiduguri P.M.B. 1069, Maiduguri, Nigeria.
E-mail : zannahassan@yahoo.co.uk

ABSTRACT

Partial characterization of *Opisthacanthus capensis* venom acid phosphatase and inhibitory effects of methanolic extracts of *Boswellia dalzielii* and *bauhinia rufescens* on the venom enzyme were reported. Acid phosphatase from *O. capensis* venom had pH and temperature optima of 6 and 30°C respectively with an activation energy of 0.13 J/mol. The *O. capensis* venom acid phosphatase also displayed K_m, V_{max} and K_{cat} of 0.29 mg/ml, 0.0286 µmol/min and 0.10 min^{-1} respectively. Different concentrations (0.5, 1.0 and 1.5 mg/ml) of the methanolic extracts of stem and root barks of *B. dalzielii* and *B. rufescens* inhibited O. *capensis* acid phosphatase activity respectively with the extracts from the both plants displaying a mixed non-competitive inhibition pattern and a decrease in the computed index of efficiency (K_{cat}). This study provides some scientific basis for the use of these plants by the herbalists in the management of poisonous scorpion bites.

Keywords : Acid Phosphatase, *Opisthacanthus capensis*, Inhibition

INTRODUCTION

Scorpion envenomation is an occupational hazards for farmers, Farm labourers, villagers, migrating population and hunters**.** Except for *Hemiscorpius Lepturus*, all venomous scorpion species, belong to the large family *Buthidae* [1]. The most notorious ones are found in the genera *Buthus* (Mediterranean Spain to the Middle East), *Parabuthus* (western and southern Africa), *Hottentotta* (South Africa to South East Asia), *Tityus* (central America, South America and the Caribbean), *Leiurus* (northern Africa and middle east), *Androctonus* (northern Africa to southeast Asia), *Centruroides* (Southern United States, Mexico, central America and Caribbean) and *Mesobuthus* (throughout the Asia). Scorpions are generally found in dry, hot environments, although some species

also occur in forest and wet savannas. All species are nocturnal, hiding during the day under stones, wood or tree barks. The risk of scorpion sting is higher in rural areas, but some species have found close contact with man, and live around or inside human dwelling. They consist of sixteen (16) families and approximately 1500 different species and subspecies which conserved their morphology almost unaltered [2].

Scorpion envenomation is an important public health hazard in tropical and sub-tropical regions. Envenomation by scorpions can result in a wide range of clinical effects, including, cardiotoxicity, neurotoxicity and respiratory dysfunction. Out of 1500 scorpion species known to exist, about 30 are of medical importance. Although a variety of different scorpion species exist, majority of them produce similar cardiovascular effects. Scientists and clinicians have studied patho-physiology of scorpion envenomation by critical observations of clinical, neurotransmitters studies, radioisotope studies, echocardiography and haemodynamic patterns. Regimen including scorpion antivenom, vasodilators, intensive care management have been tried to alleviate the systemic effects of envenoming. In spite of advances in patho-physiology and therapy, the mortality remains high in rural areas due to lack of access to medical facilities [3].*Opisthacantus capensis* which belongs to the family liochelidae and is among the scorpion species dangerous to human and whose venom contains powerful neurotoxins and it is especially potent that it indirectly contributes to tissue damage. Acid phosphatase, an enzyme of scorpion venom is an anticoagulant enzyme which inhibit the prothrombinase complex by binding to coagulant factor X [3].

Acid phosphatase (ACP) is a family of enzymes that belongs to the hydrolase class. They are specifically grouped together because of the shared ability to catalyse the hydrolysis of orthophosphate monoesters under acidic condition [4]. They are produced by both prokaryotic and eukaryotic cells and are presumed to convert organic phosphorus onto available phosphate [5]. Phosphate is an important molecule for cellular growth that is involved in many different biological reactions. The hydrolysis of phosphomonoesters by phosphatases in biological system is an important process. This process is linked to energy metabolism, metabolic regulation and wide variety of cellular signal transduction pathways [6]. The role of acid phosphatase in phosphorus metabolism has been extensively studied in prokaryotic and eukaryotic system. The physiological function of acid phosphatase is to provide inorganic phosphate for cellular growth [7], since phosphorylation and dephosphorylation of protein is an

important phenomenon in cellular metabolism [8, 9]. Deficiency of phosphorus in human leads to metabolic disorder such as respiratory disease and retardation of growth [10].

Medicinal plants are the backbone of traditional medicine in Nigeria and are indispensable for many reasons. One of such reasons is inadequacy of health centres in the rural areas and victims of scorpion bite mostly depend on traditional healers and herbal antidotes as an alternative treatment [11]. *Boswellia dalzielii* hutch and Bauhinia rufescen are plants commonly used in the treatment of scorpion bite. *Boswellia dalzelii* hutch (family *Burseraceae*) commonly known as frankincense tree is a tree of the savannah forest recognized by its papery bark peeling off in a ragged manner. The plant has several medicinal uses. The decoction of the stem bark is used to treat rheumatism, septic sores, venereal diseases and gastrointestinal ailment [12, 13]. Phytochemical studies of the plant revealed the absence of alkaloids while saponin, tannins, flavonoids, cardiac glycoside, steroids and *terpenes* were found to be present [14,15]. The methanolic and aqueous extracts showed antibacterial and antifungal activities [16, 15]. Studies of the aqueous extract of the stem bark of *Boswellia dalzielii* showed no antimicrobial activity but produced some anti-ulcer activity [17]. In another study incensole was found to be part of the chemical composition of the stem bark of *Baswellia dalzielii*. Incensole was found to be only moderately active against the microbes used for studies [18]. *Bauhinia rufescens* is used as a traditional medicine in Nigeria, sub-saharan tropical Africa Mauritania to Sierra Leone and sudan. The root is antipyretic and astringent, it is used in the treatment of intermittent fevers. The leaves and fruit are used in the treatment of diarrhea and dysentery [19]. The leaves are used externally in a decoction for treating eye diseases.

MATERIALS AND METHODS

Chemicals

All chemicals used for this study were of analytical grade. methanol, citric acid, Comassie Brilliant Blue G-250, Bovine Serum Albumin (Sigma-Aldrich) sodium citrate, Hydrogen chloride (HCL) from BDH laboratories, P-*Nitrophenyl* phosphate (PNPP) (Glanson chemicals)

SAMPLE COLLECTION

Plant Collection and Preparation

The plant, *Boswellia dalzielii* and *Bauhinia rufescens* were collected from *kaltungo* and Difa areas, Gombe State. They were first identified by Mallam

Sadiq of the Department of Botany, Gombe State University and was authenticated by prof. S.S. Sanusi of the Department of Biological science, University of Maiduguri. The stem bark of *B. dalzielii* and root bark of *B. Rufescens* were separately washed, air dried and pounded into powder using pestle and mortar. 20g was weighed and extracted with 200ml methanol using soxhlet extraction method. The extracted mixture was ovum dried to paste and five different concentrations of 0.5, 1.0 and 1.5 mg respectively in 2ml of distilled water were prepared and used for the inhibition studies.

Scorpions

The scorpions were collected from Gamboru area, Maiduguri, Nigeria and were authenticated by Dr. A.M. Kokori of the Department of Biological Science, University of Maiduguri, Nigeria as Opisthacantus capensis. The scorpions were fed with cow dung, insects and water adlibitum.

Venom Extraction

The venom was extracted from the tail of the scorpion into a 90 mM Citrate buffer pH 4.8.

Enzyme Assay

Acid phosphatase was assayed as described by Bergmeyer *et al*., [20]. To 5ml of venom, 5 ml 90 mM Citrate buffer, pH 4.8 and 5 ml 15.2 mM P-Nitrophenyl phosphate (PNPP) were added. The solution was mixed by inversion, equilibrated at 37$^{\circ}C$ and incubated for exactly 10 minutes. Then 4 ml 100mM NaOH was added to stop the reaction and absorbance was taken at 410 nm.

Effect of pH on venom Acid Phosphatase Activity

This was determined by assaying enzyme activity at varying pH ranging from 4.8-9.0.

Effect of Temperature on Venom Acid Phosphatase activity

This was done by incubating a mixture of enzyme and its substrate at varying temperature (30, 40, 50, 60, 70 and 80°C) for 10 minutes and activity assayed.

Activation Energy

This was carried out by preincubating the enzyme and its substrate at various temperatures for 10 minutes before assaying for activity. Logarithm of initial velocity was plotted against reciprocal of the temperature in Kelvin (Arrhenius plot) and the slope was used in determining Ea.

Determination of Km and Vmax of Acid Phosphatase

This was carried out by incubating various substrate concentrations with fixed enzyme concentration and corresponding initial velocity (V_o) was recorded which was then used for the double reciprocal plot (Line-weaver Burk plot) for the determination of K_m and V_{max}.

Inhibition Studies

Inhibition studies were carried out by determining the effect of various concentrations of methanolic extracts of *Boswellia dalzelii* and *Bauhinia rufescens* on crude acid phosphatase. Michaelis constant (K_m) and V_{max} was determined in the presence and absence of varying concentrations of the extract.

Estimation of Total Protein

Total protein was estimated as described by Bradford [21].

Statistical Analysis

Results are mean ± standard deviation for triplicate determination. Students' t-test was used to compare paired means and a diference was considered statistically significant at $p \leq 0.05$.

RESULTS

Figure 1 depicts the effects of different pH on *O. capensis* venom Acid Phosphatase activity. Optimum Acid Phosphatase activity was recorded at pH 6. The effects of different temperature on *O. capensis* Venom Acid Phosphatase activity is presented in Figure 2. Highest Acid Phosphatase activity was recorded at 30°C. The enzyme also displayed an activation energy of 0.13 J as calculated from the slope of the plot of log V_o against the reciprocal of absolute temperature (K) (Figure 3).

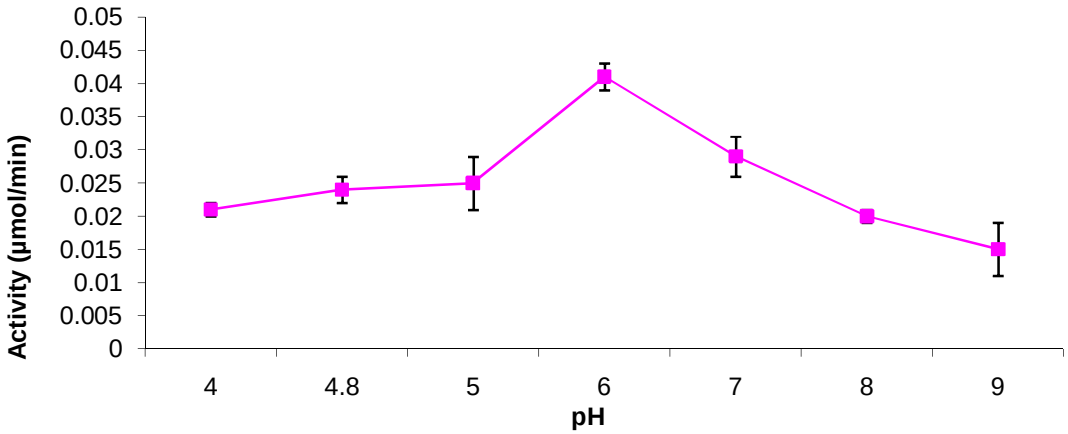

Figure 1. Effects of pH on O. capensis Venom Acid Phosphatase Activity

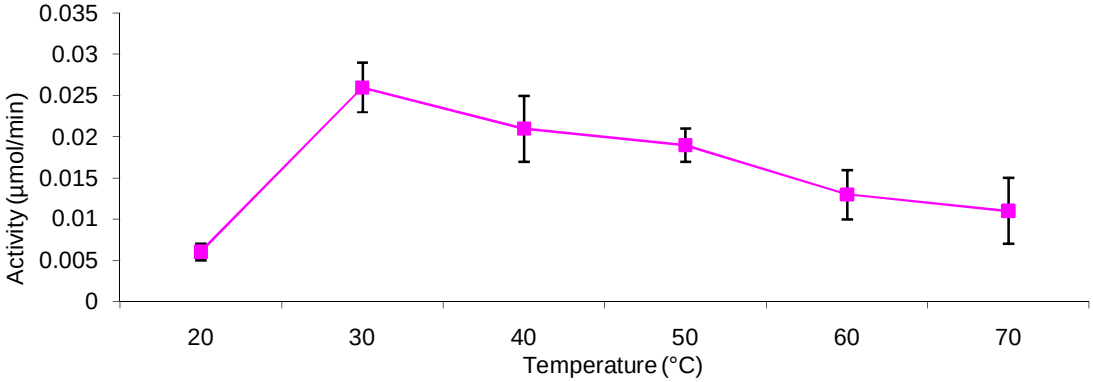

Figure 2. Effects of Temperature on O. capensis Venom Acid Phosphatase Activity

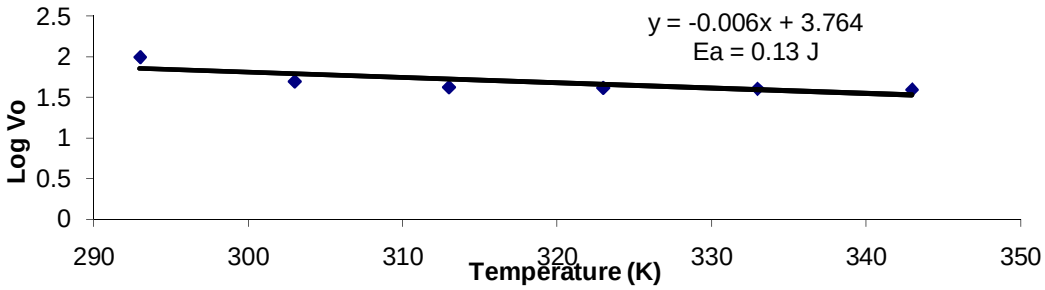

Figure 3. Arrhenius Plot for O. capensis Venom Acid Phosphatase

Table 1 shows the effects of different concentrations of methanolic extract of *Boswelia dalzielii* on *O. capensis* venom acid phosphatase activity. Michaelis constant (K_m) increased while V_{max} and K_{cat} decreased in the presence of different concentrations of methanolic extract of *Boswelia dalzielii* compared to the control.

Table 2 shows the result of different concentrations of methanolic extract of *Bauhinia rufescens on O. capensis* venom acid phosphatase activity. Michealis constant (K_m) increased while V_{max} and K_{cat} decreased in the presence of the various concentrations of the extract compared to the control.

Table 1: Effects of Different Concentrations of Methanolic Extract of *Boswelia dalzielii* on *Opisthacanthus capensis* Venom Acid Phosphatase Activity

Kinetic parameter	Control	0.5mg/ml	1.0mg/ml	1.5mg/ml
K_m(mg/ml)	0.29[a]	0.33[b]	0.98[c]	0.92[d]
V_{max}(µmol/min)	0.0286±0.0013[a]	0.0210±0.0011[a]	0.0134±0.009[b]	0.0058±0.0005[c]
K_{cat} (Min^{-1})	0.10±0.01[a]	0.06±0.03[b]	0.014±0.02[c]	0.006±0.03[d]

Values are Mean±SD for triplicate determination.Values with different superscripts in the same horizontal row are significantly ($p \leq 0.05$) different.

Table 2: Effects of Different Concentrations of Methanolic Extract of *Bauhinia rufescens* on Venom Acid Phosphatase Activity.

Kinetic Parameter	Control	0.5mg/ml	1.0mg/ml	1.5mg/ml
K_m (mg/ml)	0.29±0.09[a]	0.31±0.05[b]	0.33±0.08[c]	0.30±0.04[d]
V_{max} (µmol/min)	0.0286±0.012[a]	0.016±0.005[b]	0.011±0.09[c]	0.0169±0.012[d]
K_{cat} (Min^{-1})	0.06±0.01[a]	0.05±0.03[b]	0.03±0.02[c]	0.06±0.04[a]

Values are mean±SD for triplicate determination. Values with different superscripts in the same horizontal row are significantly ($p \leq 0.05$) different.

DISCUSSION

The PH of 6 displayed by the *O. capensis* venom phosphatase indicates that the phosphatase is indeed an acid phosphatase. Phosphatases that operate at pH below 7 are classified as acid phosphatases and those that operate at pH above 7 are classified as alkaline phosphatase [22]. The optimum temperature of 30°C recorded for the scorpion venom acid phosphatase shows that the enzyme can thrive under physiological condition. The increase in the K_m decrease in V_{max} and K_{cat} of *O. capensis* venom acid phosphatase in the presence of various concentrations of methanolic stem and root bark extracts of B. dalzielii and B. rufecens is indicative of a mixed non-competitive inhibition pattern (table 1 and 2). The hydrolysis of phosphomonoesters by acid phosphatase in biological system is linked to energy metabolism, metabolic regulation and wide variety of cellular signal transduction pathways [6].

CONCLUSION

The methanolic extracts of stem bark of *B. dalzielii* and root bark of *B. rufescens* have demonstrated some beneficial effects on *O. capensis* venom toxicity by inhibiting acid phosphatase activity. Hence, the efficacy of these plants in the management of scorpion bite by the herbalists may have some scientific basis.

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Biographical Notes:

H. Zanna: BSc Biochemistry, 1990 (University of Maiduguri), MSc Biochemistry, 1997(University of Jos), PhD Enzymology, 2010 (ABU, Zaria).

Y. Tijani: BSc Biochemistry 2010 (University of Maiduguri), MSc Biochemistry, 2015 (University of Maiduguri).

C. Chidiebere: BSc Biochemistry, 2015 (University of Maiduguri)

A. Daniel : BSc Biochemistry, 2015 (University of Maiduguri)