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Physicochemical properties of rhodanese: A cyanide detoxifying enzyme from *Pentadiplandra brazzeana* (Baill) root

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Rhodanese a ubiquitous sulphur transferase enzyme catalyses the detoxification of cyanide to a less toxic thiocyanate. Rhodanese from the root of *Pentadiplandra brazzeana* was purified by ammonium sulphate precipitation and affinity chromatography. The enzyme had a specific activity of 4.82 RU/mg of protein. The K_m values of the substrates (KCN and $Na_2S_2O_3$) were 11.76 and 10 mM, respectively. The optimum pH and temperature of the enzyme activity were 8.0 and 60°C, respectively. The enzyme was not inhibited by the heavy metals ($BaCl_2$, KCl, $NiCl_2$, NaCl, $MnCl_2$, and $ZnCl_2$). This study affirmed the presence of rhodanese in the root of *P. brazzeana*, an indication that the plant may be of useful biotechnological application, especially in bioremediation of cyanide intoxified farm sites.

Key words: Cyanide, rhodanese, detoxification, inhibition, *Pentadiplandra brazzeana*.

INTRODUCTION

Rhodanese (cyanide: thiosulphate sulphur transferase; E.C.2.8.1.1) is a multifunctional, mitochondrial sulphur transferase enzyme that catalyses the detoxification of cyanide by sulphuration in a double displacement (ping pong) mechanistic reaction (Smith and Urbanska, 1986). It occurs in varieties of plants and animals. Rhodanese is a sulphur transferase that catalyses, *in vitro*, the formation of thiocyanate from cyanide and thiosulphate or other suitable sulphur donors (Westley, 1980; Nagahara et al., 1996; Okonji et al., 2011).

$$S_2O_3^{2-} + CN^- \xrightarrow{\text{rhodanese}} SCN^- + SO_3^{2-}$$

The enzyme major function is cyanide detoxification

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(Smith and Urbanska, 1986; Buzaleh et al., 1990). This function is more prominent in mammals where highly cytotoxic cyanide is converted to a less toxic thiosulphate and excreted through the kidney (Bourdoux et al., 1980; Cagianut et al., 1984; Keith et al., 1989; Fagbounka et al., 2004). In plants, a close relationship exists between rhodanese activity and cyanogenesis, which suggests that the enzyme provides a mechanism for cyanide detoxification in cyanogenic plants (Smith and Urbanska, 1986). Some other functions of the enzyme are the transfer of sulfur from thiosulphate to a nucleophilic acceptor such as reduced lipoate (Volini et al., 1966; Tomati et al., 1974), reactivation of ferredoxin from apoferredoxin (Tomati et al., 1974) and supply of sulphide centre for the electron transport chain in mammalian tissues (Westley et al., 1981). Cyanide is one of the most toxic substances present in a wide variety of food materials that are consumed by animals and man (Egekeze and Oehme, 1980). Many plants and plant products used as food in tropical countries contain cyanogenic glycosides. These plants include cassava, linseed, beans and peas, which are known to contain Linamar coexisting with lotaustralin. Millet, sorghum, tropical grass and maize are sources of dhurin. Amygdalin is found in plums, cherries, pears, apple and apricots (Oke, 1979; Osuntokun, 1981; Saidu, 2004). It has been reported that ingestion of cyanogenic glycosides in forage crops can result in the death of grazing animals (Keeler et al., 1978). Most of these plants and their products are staple foods in the tropics. *Pentadiplandra brazzeana* Baill is the sole species in the genus *Pentadiplandra* (family Pentadiplandraceae). It has many medicinal uses such as: abortifacients, ecbolics, arthritis, rheumatism, subcutaneous parasitic infection, diarrhoea, dysentery, general healing, genital stimulants/depressants, diuretics, pain-killers, pulmonary troubles, venereal diseases and vermifuges (Burkill, 1985). The root of the *P. brazzeana* is used commonly in the Delta and Eastern part of Nigeria as food. Reports by Ndoye et al. (2016) showed that the essential oil obtained from the root of *P. brazzeana* constitutes mainly of benzyl isothiocyanate and benzyl cyanide. As *P. brazzeana* is a common plant in Eastern Nigeria used majorly for its spice (edible) and medicinal qualities, it is important to understand the role of rhodanese in the root of this plant. It is widely believed that the major function of rhodanese is in cyanide detoxification, however, according to Okonji and Agboola (2014), the wide distribution of rhodanese in different tissues is an indication that there may be other functions. Shahbazkia et al. (2009) elucidated the other functions of rhodanese to include the participation in energy metabolism, formation of iron sulphur centre in proteins, and detoxification of hydrogen sulphide.

The presence of rhodanese enzyme in the root of *P. brazzeana* (Baill), a plant common in the Eastern part of Nigeria used mostly as spicing ingredient in food preparation is reported in this study.

MATERIALS AND METHODS

Chemicals

Potassium cyanide, sodium thiosulphate, boric acid, sodium borate, mercaptoethanol, nitric acid, ferric nitrate and formaldehyde were purchased from Sigma Chemical Company, St. Louis, USA. All other reagents were of analytical grade.

Collection and preparation of sample

The *P. brazzeana* root was collected from the Eastern part of Nigeria and it was identified in Department of Botany, Obafemi Awolowo University, Ile-Ife. The dried plant root was cut into smaller pieces after which it was blended into a powdery form. One hundred and fifty grams of the powder was then soaked in 3 volumes of 0.1 M phosphate buffer, pH 7.2. This was allowed to stay for 48 h in the refrigerator. The suspension was then centrifuged at 4,000 rpm for 30 min in a Microfield Centrifuge Model 800 D. The supernatant was collected and precipitated with 80% ammonium sulphate and stored in the refrigerator for 24 h after which it was centrifuged and the pellet was collected and desalted by dialysis. After the dialysis, the sample was centrifuged again and supernatants were used as the source of enzyme.

Protein and enzyme assays

Bradford method (1976) was used to measure the protein concentration of the enzyme using bovine serum albumin (BSA) as standard. Rhodanese activity was assayed by the method of Agboola and Okonji (2004). The reaction mixture contained 0.25 M sodium thiosulphate, 0.25 M potassium cyanide, 50 mM borate buffer, pH 9.4 and 100 µl of enzyme solution in a final volume of 1.0 ml. The reaction was carried out for 1 min at 37°C and stopped by adding 0.5 ml 15% formaldehyde and 1.5 ml of Sorbo reagent (which was made up of ferric nitrate solution containing 0.025 g $Fe(NO_3)_3.9H_2O$ in 0.74 ml water and 0.26 ml concentrated nitric acid). Absorbance was measured at 460 nm. One unit of activity of the enzyme was represented as RU, and 1 RU was defined as the amount of enzyme that gave an optical density of 1.08 (Sorbo, 1953).

Reactive Blue-2 agarose affinity chromatography

Reactive Blue-2 agarose resin was packed into a 1.5 × 10 cm column and equilibrated with 0.05 M citrate buffer (pH 5.0). Three milliliters of enzyme solution from the preceding step was then layered on the column. The column was then washed with the buffer to remove unbound protein, followed by a step-wise elution with 1.0 M NaCl solution in the same buffer. Fractions of 2 ml were collected from the column at a rate of 12 ml/h. The active fractions from the column were pooled and dialyzed against 50% glycerol in 0.1 M phosphate buffer, pH 7.5.

Kinetic studies

The enzyme's Michealis-Menten constant (K_m) for each of the substrates, thiosulphate and KCN, was determined by varying the concentration of each compound per time. For KCN, its concentration was varied between 10 and 100 mM at constant 250

Table 1. Purification process of rhodanese from *Pentadiplandra brazzeana* root.

Fractions	Total activity (RU)	Total protein (mg)	Specific activity (RU/mg)	Yield (%)	Purification fold
Crude extract	78.48	72.96	1.08	100	1
Ammonium sulphate (80%)	71.80	31.12	2.31	91.49	2.14
Reactive Blue-affinity chromatography	15.60	3.24	4.82	19.88	4.46

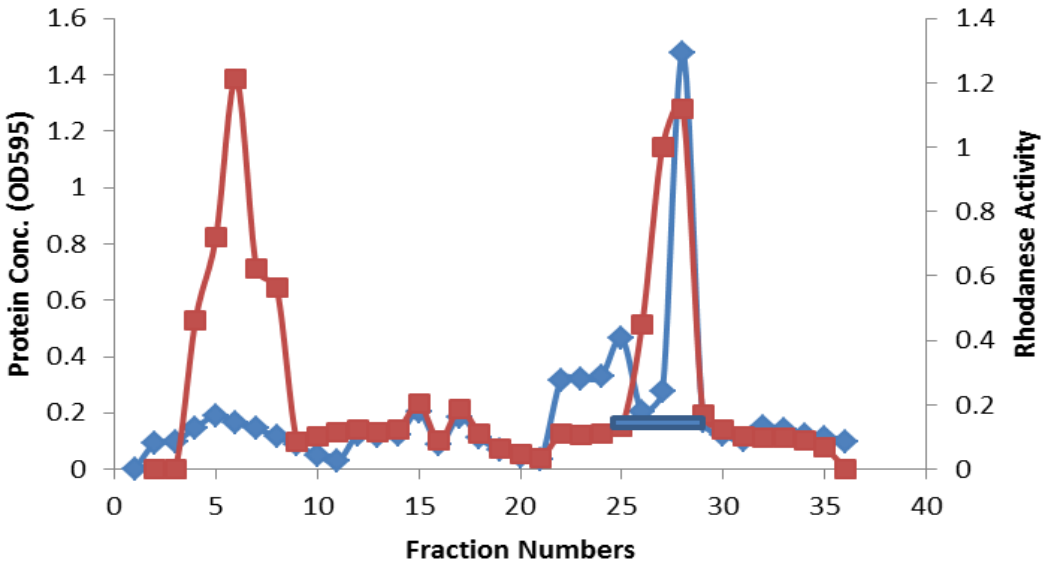

Figure 1. Affinity chromatography profile of *P. brazzeana* root rhodanese. Pooled fractions (▬), Rhodanese activity (- ■-■-■- ■-), Protein concentration (-■-■-■-■-■-■).

mM $Na_2S_2O_3$, while for sodium thiosulphate, its concentration was varied between 25 and 250 mM at constant 250 mM KCN. Estimation of rhodanese activity was carried out at the assay conditions of pH 9.4 and 37°C. The kinetic constants of the *P. brazzeana* root rhodanese were also determined in the presence of two other sources of sulphur, namely; $(NH_4)_2S_2O_8$ and $Na_2S_2O_5$ at concentrations varied between 25 and 250 mM and at a fixed concentration of 250 mM KCN, respectively.

Effect of pH and temperature on *P. brazzeana* root rhodanese

The effect of pH on the *P. brazzeana* root rhodanese was determined by assaying for the enzyme activity using buffers of different pH [citrate buffer (pH 3 to 5), phosphate buffer (pH 6 to 8), and borate buffer (pH 9 to 11)]. For determination of optimum temperature of enzyme activity, 100 µl of the enzyme was assayed at temperatures between 30 and 70°C. The reaction mixture was first incubated at the selected temperature for 1 min before initiating the reaction by the addition of the enzyme that had been equilibrated at the same temperature.

Thermal stability of the Brazilian *Pentadiplandra* root rhodanese was studied by incubating aliquots of enzyme at various temperature (40, 50, 60 and 70°C) up for 60 min in a thermostatic water bath and measuring their activity at room temperature after brief cooling in ice. The incubation was carried out in sealed vials.

Effect of metals

The inhibition effects of various metal ions such as $BaCl_2$, KCl, $NiCl_2$, NaCl, $MnCl_2$ and $ZnCl_2$ at concentrations of 0.01 and 0.001 M were carried out on the rhodanese enzyme under the standard condition.

RESULTS AND DISCUSSION

Rhodanese from *P. brazzeana* root was purified by ammonium sulphate precipitation and reactive blue-2 agarose affinity chromatography (Table 1; Figure 1). Specific activity of 4.82 RU/mg of protein and a yield of 19.88% was obtained for the enzyme. Different values of specific activities of rhodanese have been reported. Aladesanmi et al. (2009) obtained 14.43 RU/mg for land tortoise liver rhodanese. Earlier, Sorbo (1953a, b) reported 256 RU/mg for bovine liver rhodanese. Lee et al. (1995) and Agboola and Okonji (2004) obtained 1,076 RU/mg and 131 RU/mg for mouse and fruit bat liver rhodaneses, respectively.

The K_m and V_{max} values for rhodanese from *P.*

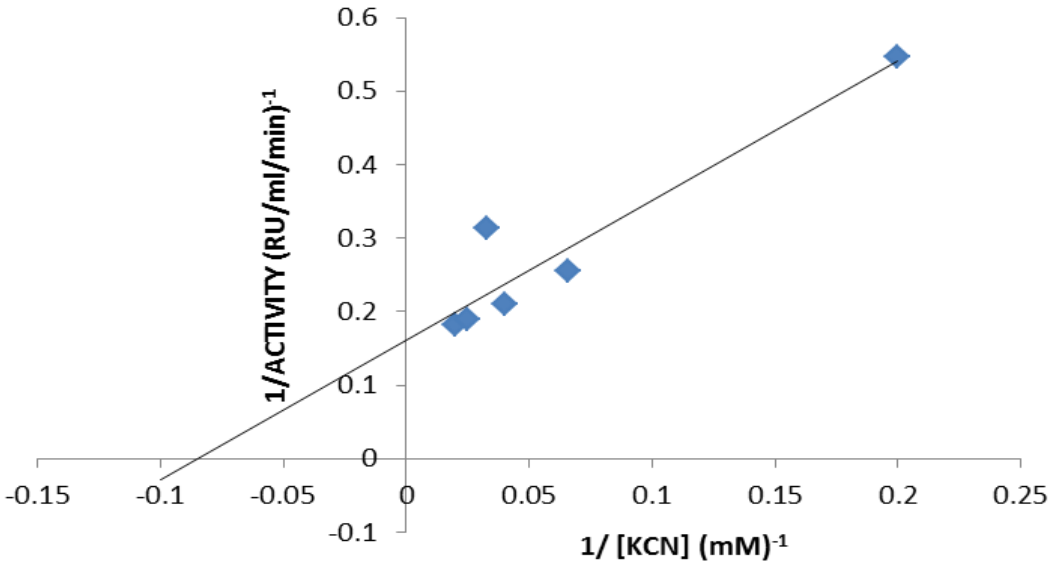

Figure 2. Lineweaver-Burk plot for *P. brazzeana* root rhodanese using KCN as substrate.

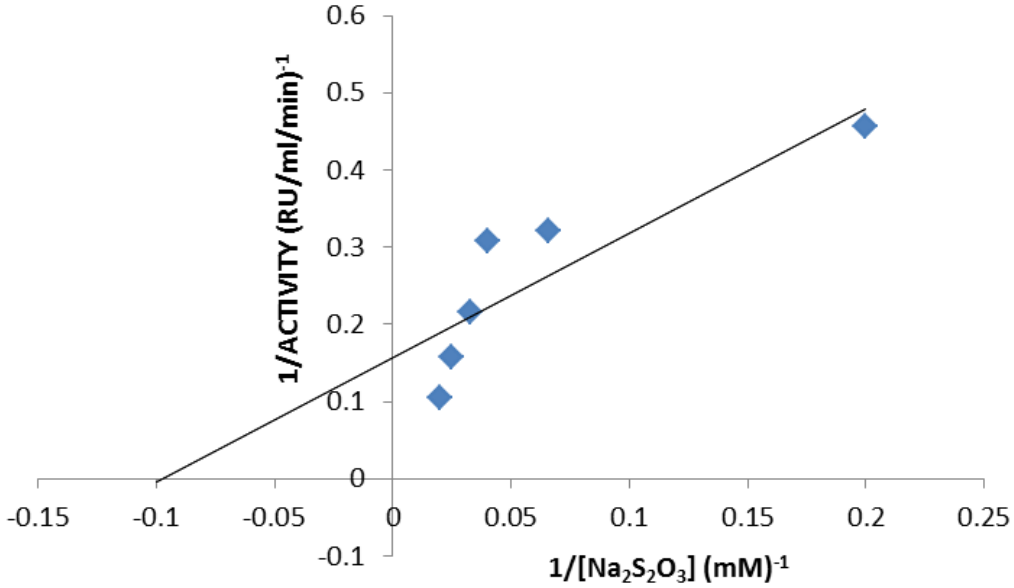

Figure 3. Lineweaver-Burk plot for *P. brazzeana* root rhodanese using $Na_2S_2O_3$ as substrate.

brazzeana root were obtained from the double reciprocal plots (Figures 2 to 5). The enzyme's K_m values for KCN and $Na_2S_2O_3$ were 11.76 and 10.0 mM, respectively (Table 2). In the presence of other substrates (sulphur compounds), namely, $Na_2S_2O_5$ and $(NH_4)_2S_2O_8$, used as alternative sources of sulphur donor, the enzyme had K_m values of 20 and 10 mM, respectively. The K_m values obtained with the substrates, KCN and $Na_2S_2O_3$, are close to those of previous works done. Jarabak and Westley (1974) obtained 9.5 and 4.5 mM, respectively for human liver rhodanese, while Lee et al. (1995) obtained values of 12.5 and 8.3 mM, respectively for mouse liver rhodanese. The fruit bat liver rhodanese was shown to have K_m values of 13.5 and 19.15 mM, respectively (Agboola and Okonji, 2004). These values indicate that the *P. brazzeana* rhodanese has almost similar affinity for its substrates as reported by other authors mentioned earlier. In comparison of the K_m values of the enzyme for

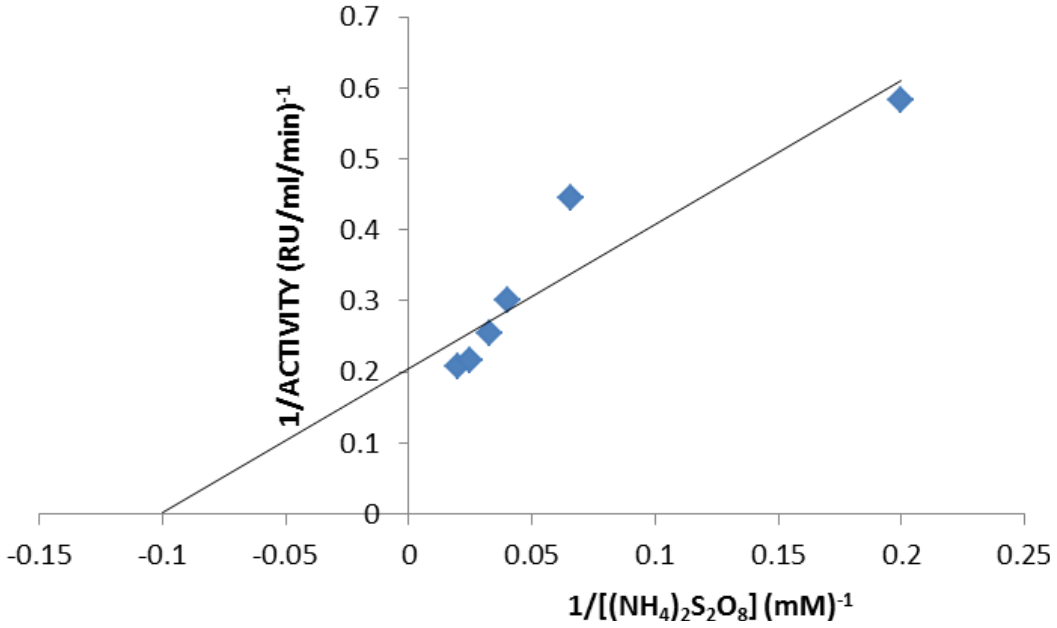

Figure 4. Lineweaver-Burk plot for *P. brazzeana* root rhodanese using $(NH_4)_2S_2O_8$ as substrate.

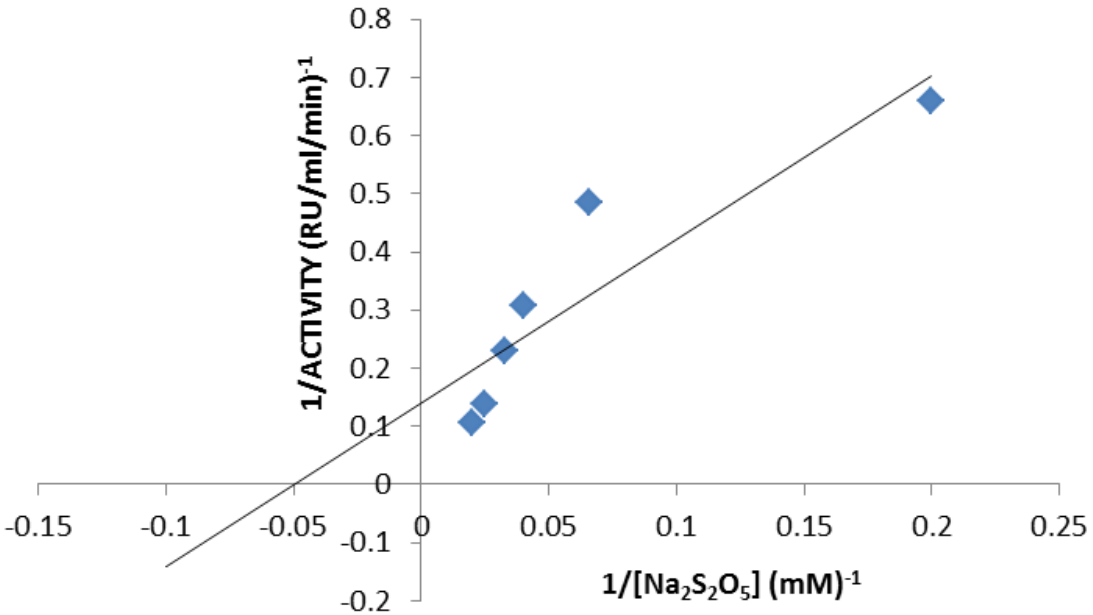

Figure 5. Lineweaver-Burk plot for *P. brazzeana* root rhodanese using $Na_2S_2O_5$ as substrate.

each of the sulphur donors, the enzyme exhibited a K_m value of 10, 20 and 10 mM for $Na_2S_2O_3$, $Na_2S_2O_5$, and $(NH_4)_2S_2O_8$, respectively (Table 2). Lower K_m value is an indication of strong affinity of the enzyme for the substrate and high efficiency of the catalysis of the detoxification reaction. The K_m values obtained indicate that the *P. brazzeana* root rhodanese binds to $Na_2S_2O_3$ and $(NH_4)_2S_2O_8$ with similar affinity and to $Na_2S_2O_5$ with less affinity when all three sulphur donors are compared. Sodium thiosulphate ($Na_2S_2O_3$) has been reported to be highly specific for rhodanese (Sorbo, 1953b; Westley, 1980) although the enzyme can utilize other sulphur

Table 2. Kinetic parameters of KCN and various sulphur compounds used as substrates.

Substrates	K_m (mM)	V_{max} (RU/ml/min)
KCN	11.76	0.16
$Na_2S_2O_3$	10.00	0.16
$Na_2S_2O_5$	20.00	0.14
$(NH_4)_2S_2O_8$	10.00	0.20

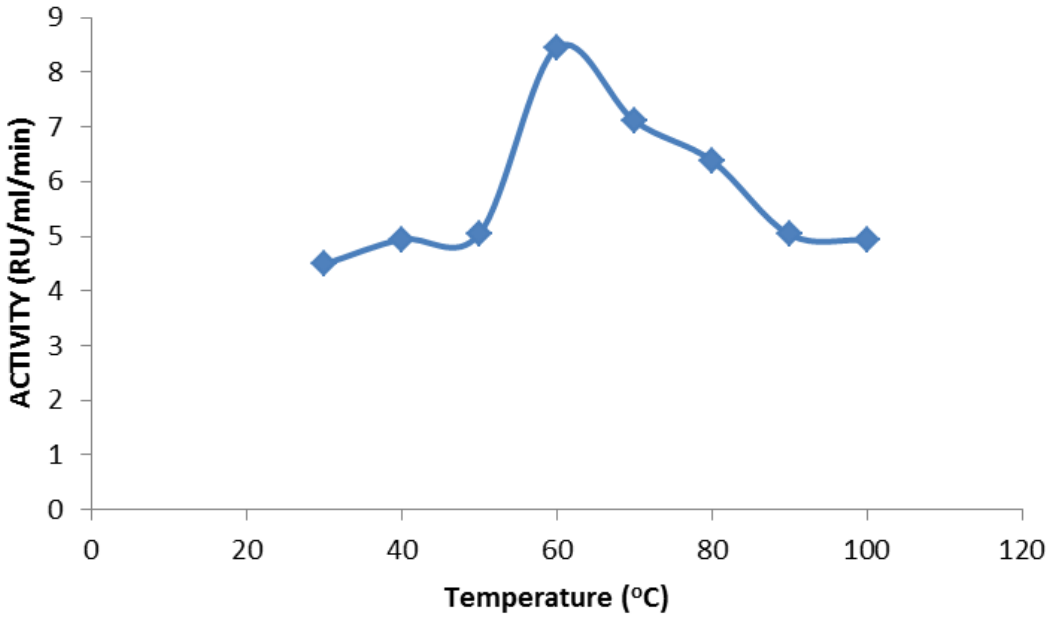

Figure 6. Effect of temperature on *P. brazzeana* root rhodanese.

donors as substrates.

The optimum temperature of 60°C for the *P. brazzeana* root rhodanese is similar to the results obtained by most researchers for rhodaneses from different sources (Figure 6). Optimum temperature of 50°C was obtained for land tortoise liver rhodanese (Aladesanmi et al., 2009). Chew and Boey (1972) obtained optimum temperature of 59°C for cassava leaf rhodanese. Agboola and Okonji (2004) also reported an optimum temperature of 35°C for the fruit bat liver rhodanese. The thermal stability experiment showed that the enzyme was heat labile, losing about 50% of relative activity after only 10 min of incubation at 60°C.

The maximum activity of *P. brazzeana* root rhodanese was observed at a pH of 8.0 (Figure 7). A pH range of 8.0 to 11.5 has been reported in various works (Chew and Boey, 1972; Lee et al., 1995; Agboola and Okonji, 2004; Okonji et al., 2010). The pH optimum of 8.5 was obtained for rhodanese from the liver of land tortoise (Aladesanmi et al., 2009). It has also been reported that at low pH (4 to 6) rhodanese is stabilized against inactivation (Kurban and Horowitz, 1991).

The effect of metal ions on the activity of *P. brazzeana* root rhodanese did not show any much inhibition, instead, the enzyme was markedly activated with $BaCl_2$ showing 100% activation at 0.001 M concentration (Table 3). Inhibition studies on rhodanese from different sources present different trends. Agboola and Okonji (2004) reported the inhibition of fruit bat rhodanese by Ba^{2+} and Zn^{2+}. This is also supported by the report of Aladesanmi et al. (2009) on the tortoise liver rhodanese which was also inhibited by Ba^{2+} and Zn^{2+} but was not affected by Mn^{2+}, Co^{2+}, Sn^{2+}, $Ni^{2+}$and NH_4^+. Nok et al. (1993) reported on the modulation of rhodanese activity by Cu^{2+}, Zn^{2+} and Ca^{2+}. Inhibition by salts and certain monovalent anions may be due in part, to an increase in the ionic strength of the medium (Mintel and Westley, 1966). Some metal ions with strong affinity for ligands (such as phosphate

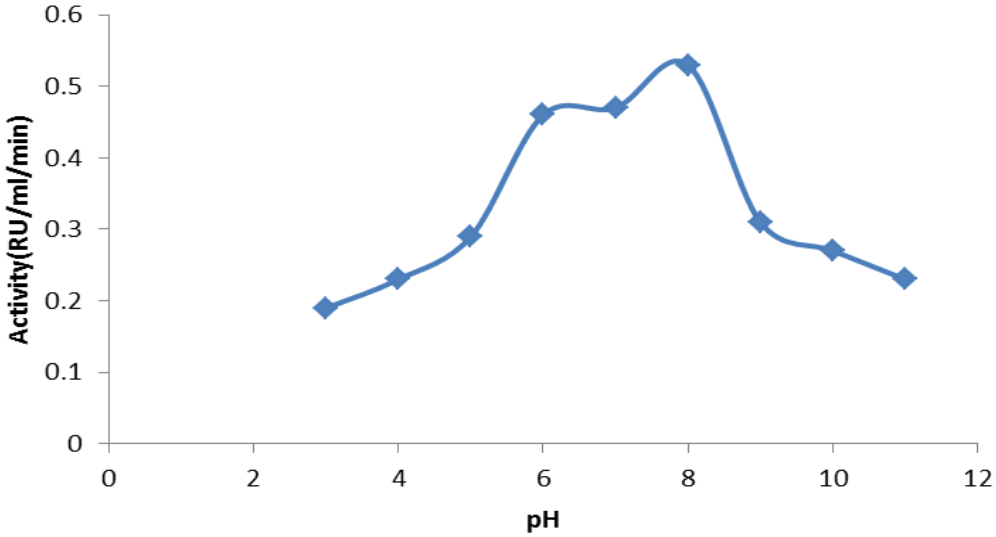

Figure 7. Effect of pH on *P. brazzeana* root rhodanese.

Table 3. Effect of metals on *Pentadiplandra brazzeana* root rhodanese.

Metals ions	Enzyme activity (%)	
	0.01 M	0.001 M
$BaCl_2$	92.35 ± 7.7	100 ± 0.2
KCl	85.75 ± 14.2	83.18 ± 8.7
$NiCl_2$	98.60 ± 1.4	89.78 ± 10.2
NaCl	95.31 ± 4.7	85.53 ± 6.4
$MnCl_2$	88.51 ± 9.8	91.06 ± 8.9
$ZnCl_2$	82.85 ± 0.8	87.4 ± 1.5

cysteinyl and histidyl side chains of protein) have been reported to inhibit rhodanese activity considerably (Ulmer and Vallee, 1972). Inhibition may also be as a result of the metal ions interacting with the sulfhydryl groups at the enzyme catalytic site or an induced change in the secondary and tertiary structure of the enzyme (Oyedeji et al., 2013). Tayefi-Nasrabadi et al. (2006) reported such structural changes after incubation of protein with metal ions.

This study showed the presence of rhodanese in the root of *P. brazzeana* and elucidated the physicochemical properties of the enzyme. The presence of rhodanese in the root of the plant is a suggestion that the enzyme may be functional in many physiological activities, one of them being the detoxification of cyanide.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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