Enzymatic defence mechanisms in Sapindus mukorossi and Acacia concinna: A Michaelis Menten model approach

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Abstract

Sapindus mukorossi (soapnut) and *Acacia concinna*, belonging to the families Sapindaceae and Leguminosae respectively, are medicinal plants known for their high saponin content as part of their adaptation and defence strategies against environmental stresses. This study investigates and compares the enzymatic antioxidant defence responses of these two saponin rich species, focusing on key enzymes involved in plant stress role in plant adaptation. Enzymatic assays displayed notable differences in the activities of catalase, peroxidase, and polyphenol oxidase; *Acacia concinna* possessed higher overall enzymatic activity, while *Sapindus mukorossi* possessed higher polyphenol oxidase activity reflecting their ecological adaptation and biochemical resilience. The comparative kinetic profiling highlights the enzymatic adaptability of both species under oxidative stress, emphasizing the ecological and biochemical roles of saponins in defence. Linear Discriminant Analysis (LDA), which captured 96.53 percent of the total variation, proved a clear isolation based on enzymatic profiles. Post hoc analysis confirmed statistically significant differences ($p \leq 0.05$) in enzyme activity between the two species. These findings provide insights into the metabolic resilience of saponin-rich plants and contribute to understanding plant defence mechanisms in stressful environments.

1. Introduction

Sapindus mukorossi, also known as Aritha or Ritha, is a soap nut native to India. Its pericarp is used for washing clothes and cleaning due to its soapy lather. The fruit contains mucilage, sugars, and 11.5% saponins, which are glycosides with foaming properties. It produces seven known saponins, two new dammarane type saponins, and four oleanan Ayurveda-based treatments [1,2] *Acacia concinna*, a medicinal plant in tropical rainforests. The plant's fruits are used for purgative, emetic, expectorant, and hair growth, with chemical evaluations focusing on flavonoids and monoterpenoids[3,4].

Phytochemicals, such as saponins, can enhance the activation of antioxidant enzymes and neutralize reactive oxygen species (ROS), making them useful in antioxidant therapies for oxidative stress-related conditions, anti-aging, and protective skincare formulations. These compounds can directly scavenge ROS or enhance the body's natural antioxidant Defence system [5,6,7]. Plants have a powerful antioxidant defence mechanism consisting of enzymes like catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and polyphenol oxidase (PPO). These enzymes scavenge reactive oxygen species (ROS) to protect cells from oxidative damage [8,9]. Catalase, a key antioxidant enzyme, converts hydrogen peroxide into water and polyphenol oxidase converts phenolic compounds into quinones, which are polymerized to melanin pigment[10,11,12,13]. Catalase (CAT), present in the cytosol, mitochondria, and peroxisomes, dismutates H_2O_2 into H_2O and O_2, and is present in both prokaryotes and eukaryotes[14]. This system helps reduce oxidative damage caused by ROS[9].

The industrial sector uses catalase for a variety of purposes, from producing porous materials for the textile industry to removing excess H_2O_2 from milk used in the cold pasteurization process to make

cheese[13,15]. Every cell contains peroxidases, APX and GPX in particular, which catalyse the conversion of H_2O_2 to H_2O. Because it uses ascorbate as an electron donor in the first phase of the ascorbate glutathione cycle, APX is considered the most crucial plant peroxidase in H_2O_2 detoxification[16]. H_2O_2 gets broken down enzyme GPX with less specificity to electron donor substrates where co-substrates such as ascorbate and phenolic compounds are used as oxidants[17]. Peroxidases are categorized as animal, plant, fungal, and bacterial peroxidases based on their structure and amino acid arrangement. Variation in amino acid sequences are the cause of these peroxidase species[18]. The peroxidase enzyme is essential for Defence mechanisms, hormone modulation, however, lignin production, and reducing the amount of indole acetic acid in fruits and vegetables[19,20,21].

Phenolic compound oxidation by polyphenol oxidase (PPO) causes enzymatic browning in food processing and post-harvest physiology of plant products[22]. PPO plays a role in defence mechanisms against insect and plant pathogen attacks, preventing bacterial growth and acting as polyphenolic barriers. It alters plant proteins, reducing nutritional availability to herbivores or intruders[23]. The effective enzymatic antioxidant Defence of plants relies on enzymes like catalase, peroxidase, superoxide dismutase, and polyphenol oxidase[24]. Single-substrate kinetic mechanisms are the first steps in evolutionary processes, while enzymes can catalyse complex reactions using sequential and non-sequential mechanisms. Sequential mechanisms involve a displacement reaction between substrates, while random mechanisms have no obligatory binding sequence, making the reaction more complex[25,26].

Research on plant defence and metabolism often focuses on key enzymes such as catalase, peroxidase, and polyphenol oxidase, The comparative kinetic profiling of catalase, peroxidase, and polyphenol oxidase in *Sapindus mukorossi* and *Acacia concinna* highlights their enzymatic adaptability under oxidative stress conditions. These responses underscore the critical role of saponins in modulating enzyme activity, enhancing stress tolerance, and contributing to the plants' ecological fitness. By applying the Michaelis-Menten kinetic model, this study aims to elucidate the catalytic efficiency and substrate specificity of these key enzymes, thereby providing deeper insights into the biochemical and ecological defence strategies of saponin-rich species. These findings not only underscore the importance of CAT, POD, and PPO in stress adaptation but also highlight the ecological significance and pharmaceutical potential of *Sapindus mukorossi* and *Acacia concinna* as model plants for studying plant defence responses.

2. Material and Methods

2.1 Collection of Samples

The fruits of *Sapindus mukorossi* and *Acacia concinna* used in this enzyme study were procured from a local market in Junagadh, Gujarat, India.

2.2 Quantification of total protein

Protein estimation was determined using the method described by Folin lowery using bovine serum albumin as the standard[27].

2.3 Enzyme kinetic modelling

1g of Fruits powder was mixed with in 10 mL of extraction buffer (0.1 M phosphate buffer + 0.5 mM EDTA, pH adjusted to 7.5). The sample was centrifuged at 15000 rpm for 20 min. Supernatant was collected and utilized for the enzyme assays[28].

2.3.1 Analysis of catalase activity

The OD value were evaluated by taking 1500µl of 100mM phosphate buffer with pH 7.0, 500µl of (5 mM to 25 mM) H_2O_2, 500 µl of milli-Q and enzyme supernatant in the quartz cuvette the rate of H_2O_2 breakdown was determined for 0, 5, 10, 15 and 20 minutes at an interval between the samples in the ultraviolet light spectrum of the spectrophotometrically at 240nm. The OD value was also registered in the blank, without the enzyme's supernatant. The catalase activity was determined spectrophotometrically at room temperature by monitoring the decrease in absorbance resulting from the decomposition of H_2O_2 at 240 nm. The enzyme activity was expressed in µM H_2O_2 (= 39.4 mM^{-1} cm^{-1}) oxidized min^{-1} mg^{-1} protein[29].

2.3.2 Analysis of Peroxidase activity

The OD value were evaluated by taking 1000µl of 100mM phosphate buffer with pH 6.1, 500µl of (92 mM to 100mM) Guaiacol, 400µl of milli-Q 500µl of (6 mM to 14 mM) H_2O_2 and enzyme supernatant in the quartz cuvette, the rate of Guaiacol and H_2O_2 decomposition was estimated for 0, 5, 10, 15 and 20 minutes at time interval between the samples in the ultraviolet light spectrum of the spectrophotometrically at 470 nm. The OD value was also registered in the blank, without the enzyme supernatant. The increase in the absorption caused by oxidation of guaiacol by H_2O_2 (= 26.6 mM^{-1} cm^{-1})[30].

2.3.3 Analysis of Polyphenol oxidase activity

The OD value were evaluated by taking 2900µl of (100 mM to 500 mM) Catechol in the 10mM phosphate buffer with pH 6.0, 100µl milli-Q and enzyme supernatant in the quartz cuvette the rate of Guaiacol and H_2O_2 decomposition was estimated for 0, 5, 10, 15 and 20 minutes at time interval between the samples in the ultraviolet light spectrum of the spectrophotometrically at 490 nm. The OD value was also registered in the blank, without the enzyme supernatant. The polyphenol oxidase activity was determined by measuring the increase in absorbance resulting from the oxidation of catechol (= 1.0 mM^{-1} cm^{-1}) at 490 nm spectrophotometrically[31].

Specific activity (U mg^{-1} of protein)

$$\text{S.A} = \frac{\text{Change in OD per minute}}{\text{Molar extinction co}-\text{efficient of enzyme } (\upvarepsilon) \times \text{Volume of enzyme in sample}} \times \text{Total protein content}$$

Where,

S.A = Specific activity,

Molar extinction co-efficient of enzyme:

Catalase = 39.4 U/ μ mols/g,

Peroxidase = 26.6 U/ μ mols/g,

Polyphenol oxidase = 1.0U/μmols/g,

2.4 The Michaelis Menten kinetic equation

The kinetic equation in Michaelis-Menten One of the earliest and still useful mathematical models for a straight forward enzyme catalysed reaction[32,33]. Michaelis–Menten equation can be expressed as the equation below (Eq. 1).

$$V = \frac{V_{max} \cdot [S]}{K_m + [S]} \text{ [1]}$$

Eq. 1 represents the Michaelis-Menten equation. The relationship between the initial rate of product formation and the concentrations of substrates can be expressed in Eq. 1. It is essential to calculate the maximal velocity (*Vmax*) and the Michaelis constant (*Km*). The amount of enzyme in the reactor may also have an impact on *Vmax*. The RK4 method was applied to determine the kinetic parameters. kinetics models of this study on enzyme's affinity for the substrate and its catalytic efficiency. A lower *Km* indicates a higher substrate affinity, which is necessary for an effective defence response under oxidative stress. *Vmax* represents the maximum catalytic capacity of the enzyme, reflecting the plant's potential to rapidly neutralize reactive oxygen species (ROS) during stress conditions. *Km* is used in the context of plant defence as an indicator of the enzyme's affinity for its substrate. Together, these parameters provide insights into the efficiency, speed, and robustness of the enzymatic antioxidant system, which plays a critical role in enhancing plant resilience and adaptation to biotic and abiotic stressors[34].

2.5 Statistical analysis:

Origin (pro) software, version 2024, MS Excel-2019, Minitab® (version 19.2020.1) was used in the present research study for the conducting the Linear Discriminant analysis as well as Post hoc test constructing.

3. Results

3.1 Total protein contents

Figure 1 Total Protein content (mg/g) in selected plant Species at different concentrations.

An analysis was conducted to determine the total protein content in saponin rich fruits (*Sapindus mukorossi* and *Acacia concinna*) at various concentrations. Figure 1 depicts the varying protein content ranges found in different concentration of saponin rich fruits extracts. *Sapindus mukorossi* exhibited protein content levels of 0.231 mg/g in 0.02 mg/g, 0.283 mg/g in 0.04 mg/g, 0.420 mg/g in 0.06 mg/g, 0.532 mg/g in 0.08 mg/g and 0.549 mg/g in 0.1 mg/g, of concentration. The protein content of *Acacia concinna* was determined to be 0.639 mg/g in 0.02 mg/g, 0.885 mg/g in 0.04 mg/g, 0.894 mg/g in 0.06 mg/g, 1.022 mg/g in 0.08 mg/g and 1.259 mg/g in 0.1 mg/g, of concentration. If compare two species *Sapindus mukorossi* and *Acacia concinna* maximum protein contents was observed in *Acacia concinna* 0.1 mg/g (1.259 mg/g) and minimum protein content was observed in *Sapindus mukorossi* 0.231 mg/g in 0.02 mg/g concentrations. If concentration of fruit extract increase total protein content of both the species also increases shown in (Fig. 1).

3.2 Enzyme assay

Three type of enzyme perform in fruits *Sapindus mukorossi* and *Acacia concinna* of such as Catalase, Peroxidase, and Polyphenol oxidase.

3.2.1 Catalase activity

This scientific investigation examined the catalase activity of *Sapindus mukorossi* and *Acacia concinna* fruits extract with different concentrations (0.2, 0.4, 0.6, 0.8, and 1 mg/ml) with respect to different time (0, 5, 10, 15 and 20 minute) interval. In (Fig. 2a), The catalase activity of *Sapindus mukorossi* fruits extracts was measured at various concentrations. The activity ranged from 0.357 μmols/g^{-1} in 0 minutes to 0.029 μmols/g^{-1} in 20 minutes at 0.2 mg/ml fruit extract concentration. The activity increased to 0.441 μmols/g^{-1} in 0 minutes, 0.094 μmols/g^{-1} in 5 minutes, 0.090 μmols/g^{-1} in 10 minutes, 0.086 μmols/g^{-1} in 15 minutes, and 0.070 μmols/g^{-1} in 20 minutes at 0.4 mg/ml fruit extract concentration. The activity also increased to 0.667 μmols/g^{-1} in 0 minutes, 0.180 μmols/g^{-1} in 5 minutes, 0.126 μmols/g^{-1} in 10 minutes, 0.122 μmols/g^{-1} in 15 minutes, and 0.110 μmols/g^{-1} in 20 minutes at 0.6 mg/ml fruit extract concentration.

(Fig. 2a), The catalase activity of *Acacia concinna* (B) fruits extracts was measured at various concentrations. The activity ranged from 0.875 μmols/g^{-1} in 0 minutes to 0.024 μmols/g^{-1} in 20 minutes at 0.2 mg/ml fruit extract concentration. The activity increased to 1.271 μmols/g^{-1} in 0 minutes at 0.4 mg/ml fruit extract concentration. The activity also increased to 1.347 μmols/g-1 in 0 minutes at 0.6 mg/ml fruit extract concentration. The activity increased to 1.432 μmols/g^{-1} in 0 minutes at 0.8 mg/ml fruit extract concentration. The activity reached 1.598 μmols/g^{-1} in 0 minutes at 1 mg/ml fruit extract concentration.

Compare both fruits species the most significant catalase enzyme activity was recorded in the *Acacia concinna* fruits extract and minimum observed in *Sapindus mukorossi* fruits extracts. If compare different time duration the majority the catalase enzyme activity will decreased with increase in incubation time but increase with respect to concentration.

3.2.2 Peroxidase activity

In (Fig. 2b), The peroxidase activity of *Sapindus mukorossi* (A) fruits extracts was measured at various concentrations. The activity ranged from 0.457 µmols/g in 0 minutes to 0.499 µmols/g^{-1} in 20 minutes at 0.2 mg/ml fruit extract concentration. The activity increased to 0.623 µmols/g^{-1} in 5 minutes, 0.628 µmols/g^{-1} in 10 minutes, 0.638 µmols/g^{-1} in 15 minutes, and 0.642 µmols/g^{-1} in 20 minutes at 0.4 mg/ml fruit extract concentration. The activity also increased to 1.516 µmols/g^{-1} in 0 minutes, 1.590 µmols/g^{-1} in 5 minutes, 1.534 µmols/g^{-1} in 10 minutes, 1.538 µmols/g^{-1} in 15 minutes, and 1.580 µmols/g-1 in 20 minutes at 0.8 mg/ml fruit extract concentration. The activity was also measured at 1.601 µmols/g^{-1} in 0 minutes, 1.638 µmols/g^{-1} in 5 minutes, 1.639 µmols/g^{-1} in 10 minutes, 1.742 µmols/g^{-1} in 15 minutes, and 1.662 µmols/g^{-1} in 20 minutes at 1 mg/ml fruit extract concentration.

In (Fig. 2b), The peroxidase activity of *Acacia concinna* (B) fruits extracts was measured at various concentrations. The activity ranged from 0.977 µmols/g^{-1} in 0 min to 1.798 µmols/g^{-1} in 20 min at 0.2 mg/ml fruit extract concentration. The activity increased to 1.440 µmols/g^{-1} in 0 min, 1.663 µmols/g^{-1} in 5 min, 1.692 µmols/g-1 in 10 min, 1.698 µmols/g^{-1} in 15 min, and 1.699 µmols/g^{-1} in 20 min at 0.4 mg/ml fruit extract concentration. The activity also increased to 1.850 µmols/g^{-1} in 0 min, 2.110 µmols/g^{-1} in 5 min, 2.210 µmols/g^{-1} in 10 min, 2.280 µmols/g^{-1} in 15 min, and 2.289 µmols/g^{-1} in 20 min at 0.8 mg/ml fruit extract concentration. The activity also increased to 2.451 µmols/g^{-1} in 0 min, 2.616 µmols/g^{-1} in 5 min, 2.718 µmols/g^{-1} in 10 min, 2.840 µmols/g^{-1} in 15 min, and 3.762 µmols/g^{-1} in 20 min at 1 mg/ml fruit extract concentration. Compare both fruits species the most significant Peroxidase enzyme activity was recorded in the *Acacia concinna* fruits extract and minimum observed in *Sapindus mukorossi* fruits extracts. If compare different time duration the majority the Peroxidase enzyme activity will increase with increase in incubation time and various concentration.

3.2.3 Polyphenol Oxidase activity

In (Fig. 2c), The polyphenol oxidase activity of *Sapindus mukorossi* (A) fruits extracts was measured at various concentrations. The activity ranged from 1.055 µmols/g^{-1} in 0 min to 1.382 µmols/g^{-1} in 20 min at 0.2 mg/ml fruit extract concentration. The activity increased to 1.302 µmols/g-1 in 0 min, 1.339 µmols/g^{-1} in 5 min, 1.350 µmols/g-1 in 10 min, 1.369 µmols/g^{-1} in 15 min, and 1.379 µmols/g-1 in 20 min at 0.4 mg/ml fruit extract concentration. The activity also increased to 1.345 µmols/g^{-1} in 0 min, 1.349 µmols/g^{-1} in 5 min, 1.364 µmols/g^{-1} in 10 min, 1.378 µmols/g^{-1} in 15 min, and 1.382 µmols/g^{-1} in 20 min at 0.6 mg/ml fruit extract concentration. The activity was also observed at 1.758 µmols/g^{-1} in

0 min, 1.759 µmols/g^{-1} in 5 min, 1.864 µmols/g^{-1} in 10 min, 1.968 µmols/g^{-1} in 15 min, and 1.979 µmols/g^{-1} in 20 min at 1 mg/ml fruit extract concentration.

In (Fig. 2c), The polyphenol oxidase activity of *Acacia concinna* (B) fruits extracts was measured at various concentrations. The activity ranged from 1.171 µmols/g^{-1} in 0 minutes to 1.189 µmols/g^{-1} in 20 minutes at 0.2 mg/ml fruit extract concentration. The activity increased to 1.224 µmols/g^{-1} in 0 minutes, 1.347 µmols/g^{-1} in 5 minutes, 1.358 µmols/g^{-1} in 10 minutes, 1.337 µmols/g^{-1} in 15 minutes, and 1.373 µmols/g^{-1} in 20 minutes at 0.4 mg/ml fruit extract concentration. The activity also increased to 1.432 µmols/g^{-1} in 0 minutes, 1.439 µmols/g^{-1} in 5 minutes, 1.442 µmols/g^{-1} in 10 minutes, 1.446 µmols/g^{-1} in 15 minutes, and 1.452 µmols/g^{-1} in 20 minutes at 0.6 mg/ml fruit extract concentration. Compare both fruits species the most significant Polyphenol oxidase enzyme activity was recorded in the *Sapindus mukorossi* fruits extract and minimum observed in *Acacia concinna* fruits extracts. If compare different time duration the majority the Polyphenol oxidase enzyme activity will increase with incubation time and various concentration. During stress condition plant produce toxic reactive oxygen species (ROS), this enzyme Catalase, Peroxidase and polyphenol Oxidase neutralized this reactive oxygen species.

3.3 Determination of kinetics Parameter

The Michaelis-Menten equation was transformed into linear form using the Lineweaver-Burk plot, a method used for the first time to estimate kinetic parameters, utilizing Eq. 1 to express the plot[35].

$$\frac{1}{V} = \frac{K_m}{V_{max} \ . \ [S]} + \frac{1}{V_{max}}$$

1

The Michaelis-Menten constant *Km*, substrate concentration [S], maximum product formation rates, and product formation rates were all examined in the study. The y-axis interception point of a straight line was inverted to calculate *Vmax*, and the negative reciprocal of the x-intercept was used to calculate *Km*. The values were utilized in the Microsoft Excel solver for the RK4 method. The number of enzymes added affects *Vmax* value, but substrate concentration has no effect. *Km* gauges the substrate-enzyme affinity. *Km* and *Vmax* are important parameters in enzyme kinetics because they are strongly impacted by substrate concentration [24,36].

Table 1
Determination of enzyme kinetics parameter of selected species.

Species name	Enzyme	Substrate	K_m(mM)$^{-1}$	V_{max} (µmol/min^{-1})
Sapindus mukorossi	Catalase	H_2O_2	45.4862	10.9769
	Peroxidase	Guaiacol	100.757	0.12809
		H_2O_2	0.02071	4.56829
	Polyphenol Oxidase	Catechol	3.45237	1.415428
Acacia concinna	Catalase	H_2O_2	32.2093	14.1442
	Peroxidase	Guaiacol	82.5937	0.0966
		H_2O_2	43.0420	0.48019
	Polyphenol Oxidase	Catechol	0.31491	4.032258

In this kinetic study, From Table 1, this study shows a value of V_{max} and K_m is varies in various substrates (H_2O_2, Guaiacol, Catechol) in Catalase, Peroxidase and Polyphenol oxidase enzyme activity of *Sapindus mukorossi and Acacia concinna* fruit.

3.3.1 Catalase enzyme kinetic model in single substrates (Michaelis-Menten Model)

Catalase enzyme activity in H_2O_2 as substrates with various substrate concentration (5, 10, 15 20 and 25 mM) versus different time (0, 5, 10, 15 and 20 min) in *Sapindus mukorossi* fruits. In the single substrate model, the Lineweaver burk plot will result in a K_m value of 45.4862 mM^{-1} and V_{max} value of 10.9769 µmol/min^{-1}. Similarly, in *Acacia concinna* fruit, the K_m value will be 32.2093 mM^{-1} and the V_{max} value will be 14.1442 µmol/min^{-1}. The process's low reaction rate was indicated by a low V_{max} value shown in (Fig. 3). The reaction time for a low-reaction rate process would be longer than that of a high reaction rate process. Regarding the K_m value, it indicated the ability of the enzyme to bind to the substrate. A lower K_m value would suggest more substrate enzyme binding than in a reaction with a higher K_m value. The binding of substrate enzyme was reported to be affecting the reaction rate at negative way. The value of K_m obtained in this study is considered quite high compared to other studies from (Table 2). Catalase (CAT) is a crucial antioxidant enzyme that protects plant cells from oxidative damage caused by various stress conditions.

3.3.2 Peroxidase enzyme kinetic model in two Substrate (Random bi–bi model)

Peroxidase enzyme activity in Guaiacol and H_2O_2 as substrates with various substrate concentration (92, 94, 96, 98 and 100 mM and 5, 10, 15, 20 and 25mM) versus different time (0, 5, 10, 15 and 20 min) in *Sapindus mukorossi* fruits. Applying the Lineweaver burk plot to two substrate models (Guaiacol and H_2O_2) will also result in K_m values of 100.757, 0.02071 mM^{-1} and V_{max} values of 0.12809, 4.56829 $\mu mol/min^{-1}$. Similarly, in *Acacia concinna* fruit, K_m values will be 82.5937 and 43.0420 mM^{-1} and V_{max} values will be 0.0966, 0.48019 $\mu mol/min^{-1}$ shown in (Fig. 2a & 2b). The process's low reaction rate was indicated by a low V_{max} value. The reaction time for a low reaction rate process would be longer than that of a high-reaction-rate process. With respect to the K_m value, it indicated the ability of the enzyme to bind to the substrate. A lower K_m value would suggest more substrate enzyme binding than in a reaction with a higher K_m value. Reaction rate was discovered to be negatively impacted by substrate enzyme binding. The value of K_m obtained in this study is considered quite high relative to other studies from Table 2.

3.3.3 Polyphenol oxidase enzyme kinetic model in single substrates (Michaelis-Menten Model)

Polyphenol oxidase enzyme activity in Catechol as substrates with various substrate concentration (0.1, 0.2, 0.3, 0.4 and 0.5 mM) versus different time (0, 5, 10, 15 and 20 min) in *Sapindus mukorossi* fruits. When using the Lineweaver burk plot in a single substrate model, the K_m value will be 3.45237 mM^{-1} and the V_{max} value will be 1.415428 $\mu mol/min^{-1}$. Similarly, in *Acacia concinna* fruit, the K_m value will be 0.31491 mM^{-1} and the V_{max} value will be 4.032258 $\mu mol/min^{-1}$ shown in (Fig. 5). The process's low reaction rate was indicated by a low V_{max} value. The reaction time for a low reaction rate process would be longer than that of a high reaction rate process. Regarding the K_m value, it indicated the ability of the enzyme to bind to the substrate. A lower K_m value would suggest that there was more substrate-enzyme binding than in a reaction with a higher K_m value. The binding of substrate enzyme was reported to be affecting the reaction rate at negative way. The value of K_m obtained in this study is considered quite high compared to other studies from Table 2.

In the current investigation, there are several factors that may affect the values of K_m and V_{max} in a in specific enzyme activity Two of These elements that were especially significant compared to others are the difference in substrate type. The enzyme activity (U) changed when different substrates were used due to the different amounts of enzyme released during substrate degradation. The value of kinetic constants is heavily influenced by the substrate used in the process. K_m and V_{max} provide a functional understanding of how well defence related enzymes utilize their substrates under different stress intensities. Enzymes with low K_m and high V_{max} are typically more efficient in rapid and strong defence

responses, contributing to a plant's biochemical resilience and adaptive capacity, current research A high K_m implies the enzyme requires a higher substrate concentration to function effectively, which may be beneficial during prolonged or high-intensity stress when substrate accumulation is higher. low V_{max} may reflect a slower but sustained defence response, suitable for long-term protection or in species with moderate metabolic rates[34].

3.4 Statistical analysis:

3.4.1 Linear Discriminants analysis

Linear Discriminants analysis (LDA) is a multivariate statistical technique used to analyze datasets which are arranged into several groups or categories. It expands upon the standard Principal Component Analysis (PCA) by enabling the analysis of several groups at once within a dataset. The Fig. 5 indicated that component 1 explains 96.53 of the total variances in the data, indicating that it captures a significant amount of the variability. Hence, component 1 is the dominant principal component, explaining the majority of the variance in specific enzyme activity Other variables exhibit relatively low loadings on component 2, indicating lesser contributions to the variation. Similarly, Component 1 is characterized by strong positive loadings for Peroxidase and polyphenol oxidase activity that these are closely associated with Component 2. While Catalase enzyme activity demonstrates 3.468 variance negative loading value on Component 2 So, LDA found that the combinations of variables had different strengths of association. (Fig. 6) also shows the relationship between a specific enzyme activity of the two species in various time duration [37].

3.4.2 Post hoc Test

Science research frequently employs Post hoc test as a statistical method due to its critical role in addressing alpha-level inflation errors, which increase the likelihood of errors (false positives) resulting from the two group comparisons inherent in research studies[38]. The outcomes derived from post hoc test concerning the examined characteristics revealed significant discrepancies in enzyme activity across plant species at $P \leq 0.05$.

A statistically notable distinction ($P = 0.05$) was noted in *Sapindus mukorossi* and *Acacia concinna*, indicating variations in their all three enzymes (catalase, peroxidase, and polyphenol oxidase) displayed significant discrepancies in activity of *Sapindus mukorossi* catalase (SMC) and *Sapindus mukorossi* peroxidase (SMP) at *P* value 0.004399, *Acacia concinna* catalase (ACC) and *Acacia concinna* peroxidase (ACP) at *P* value 0.002157, *Sapindus mukorossi* catalase (SMC) and *Sapindus mukorossi* polyphenol oxidase at *P* value 0.000174 and *Acacia concinna* catalase (ACC) and *Acacia concinna* polyphenol oxidase (ACPO). In contrast, when comparing two enzyme of same species the *Sapindus mukorossi* peroxidase (SMP) and *Sapindus mukorossi* polyphenol oxidase (SMPO) at *p* value 0.100903 and *Acacia concinna* peroxidase (ACP) and *Acacia concinna* Polyphenol oxidase (ACPO) at *p* value 0.31313 no statistically remarkable differences were observed. The results from (Fig. 7) unequivocally demonstrate that distinct species possess unique enzyme profiles. Among the two distinct species analysed,

significant differences were identified between SMC-SMP, ACC-ACP, SMC-SMPO and ACC-ACPO. underscoring significant variations in these defensive responses shown in (Fig. 7).

4. Discussion

Since antioxidant enzymes eliminate excess free radicals from cells, they are thought to be the most crucial component of cellular Defences. One significant mediator of the cytotoxicity brought on by oxidative stress is thought to be H_2O_2. It can diffuse in and out of cells and tissues and is made from almost every source of the oxidative cycle. Aerobic cells are protected from O_2 toxicity and lipid peroxidation by antioxidant Defence enzymes like SOD and CAT. Oxygen and the less reactive H_2O_2 are produced when SOD combines with the superoxide anion radical. Since CAT and GPx subsequently transform H_2O_2 into water, SOD may shield cells from the harmful effects of superoxide radicals[39].

This work studied catalase, a principal antioxidant enzyme from black gram seeds. Day four sprouted black gram seeds were found to have a significant catalase high specific activity of 25,704 U/mg was obtained. Applying the Lineweaver burk plot using one substrates Michaelis constants (K_m) and maximum reaction velocities (V_{max}) were determined using these substrates at various concentrations. The K_m and V_{max} of the purified catalase were found to be 16.2 mM and 2.5 µmol/min, result compare with catalase activity in *sapindus mukorossi* and *acacia concinna* fruits Specific activity of catalase 0.667 µmols/g^{-1} obtained in *sapindus mukorossi* fruits and 1.598 µmols/g^{-1} in *acacia concinna* fruits. Apply Michalis mention kinetics models in the catalase activity H_2O_2 as substrate K_m value 45.4862 mM^{-1} and V_{max} value of 10.9769 µmol/min^{-1} and *Acacia concinna* fruit, the K_m value will be 32.2093 mM^{-1} and the V_{max} value will be 14.1442 µmol/min^{-1}. The value of K_m and V_{max} is obtained in this study is considered quite high compared to black gram seeds[40]. Specific peroxidase activity 2.13 (EU/mg protein) obtained from leaves of rocket (*Eruca vesicaria* sbsp. *Sativa*). Specific peroxidase activity 1.662 µmols/g^{-1} obtained in *Sapindus mukorossi* and 3.762 µmols/g^{-1} *Acacia concinna* fruit. Compare specific activity of this two fruits maximum obtained in leaves of rocket (*Eruca vesicaria* sbsp. *Sativa*). The V_{max} and K_m values were determined by Lineweaver-Burk plot using Guaicol as a substrates K_m 375.74 mM and V_{max} 0.314 µmol/L. compare this result with Peroxidase enzyme activity in Guaiacol and H_2O_2 as substrates with various substrate concentration. Applying the Lineweaver burk plot to two substrate models (Guaiacol and H_2O_2) will also result in K_m values of 100.757, 0.02071 mM^{-1} and V_{max} values of 0.12809, 4.56829 µmol/min^{-1}. Similarly, in *Acacia concinna* fruit, K_m values will be 82.5937 and 43.0420 mM^{-1} and V_{max} values will be 0.0966, 0.48019 µmol/min^{-1} if compare result with leaves of rocket (*Eruca vesicaria*) the value of K_m is low and V_{max} is high significant result was obtained in selected fruits[41]. Polyphenol oxidase enzyme activity from artichoke (*Cynara scolymus L*) heads, catechol as a substrate. Polyphenol oxidase enzyme specific activity was obtained 10,400 (U/ml min) this activity compare with *Sapindus mukorossi* fruits extracts was 1.979 µmols/g^{-1} and *Acacia concinna* fruits 1.452 µmols/g^{-1} for catechol as a substrate. Applying the Lineweaver burk plot to one

substrate models Catechol as a substrate will also result (K_m and V_{max} values were 10.2 mM and 19,662 U/ml min result of artichoke plants leaves compare with *sapindus mukorossi* fruits K_m value will be 3.45237 mM^{-1} and the V_{max} value will be 1.415428 µmol/min^{-1}. Similarly, in *Acacia concinna* fruit, the K_m value will be 0.31491 mM^{-1} and the V_{max} value will be 4.032258 µmol/min^{-1} significant K_m and V_{max} value obtained in *Cynara scolymus L* leaves compare to selected fruits[42].

5. Conclusion

The current investigation revealed noteworthy variations among two distinct species of exposed to catalase, peroxidase and polyphenol oxidase enzyme activity. These inspections encompassed the total protein content as well as the specific enzyme activity of catalase, peroxidase, and polyphenol oxidase. Comparative enzymatic profiling revealed that both species exhibit significant catalase and peroxidase activity in *Acacia concinna* species, polyphenol oxidase activity in *Sapindus mukorossi* as well as significant protein contents were displayed in *Acacia concinna* species. *Acacia concinna* species evolved **s**uperior enzymatic defence systems that contribute to greater ecological adaptability and tolerance to environmental challenges, compared to *Sapindus mukorossi.* This underscores the importance of integrating biochemical profiling with enzyme kinetics to understand stress adaptation in selected plant species. Linear discriminant analysis displayed of the total variances in the data, indicating that it captures a significant amount of the variability in enzyme activity. Post hoc test confirmed statistically significant variances in enzyme activity among the species, an important discrepancy significant differences were identified between *Sapindus mukorossi* and *Acacia concinna* catalase and peroxidase enzyme and catalase and polyphenol oxidase enzyme underscoring significant variations in these defensive responses in plants. These fruits are ecologically important for sustainable environmental adaptation and ecologically valuable for pharmacological innovation.

Declarations

6. Acknowledgments

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7. Funding Declaration

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8. Data availability

The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

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Figures

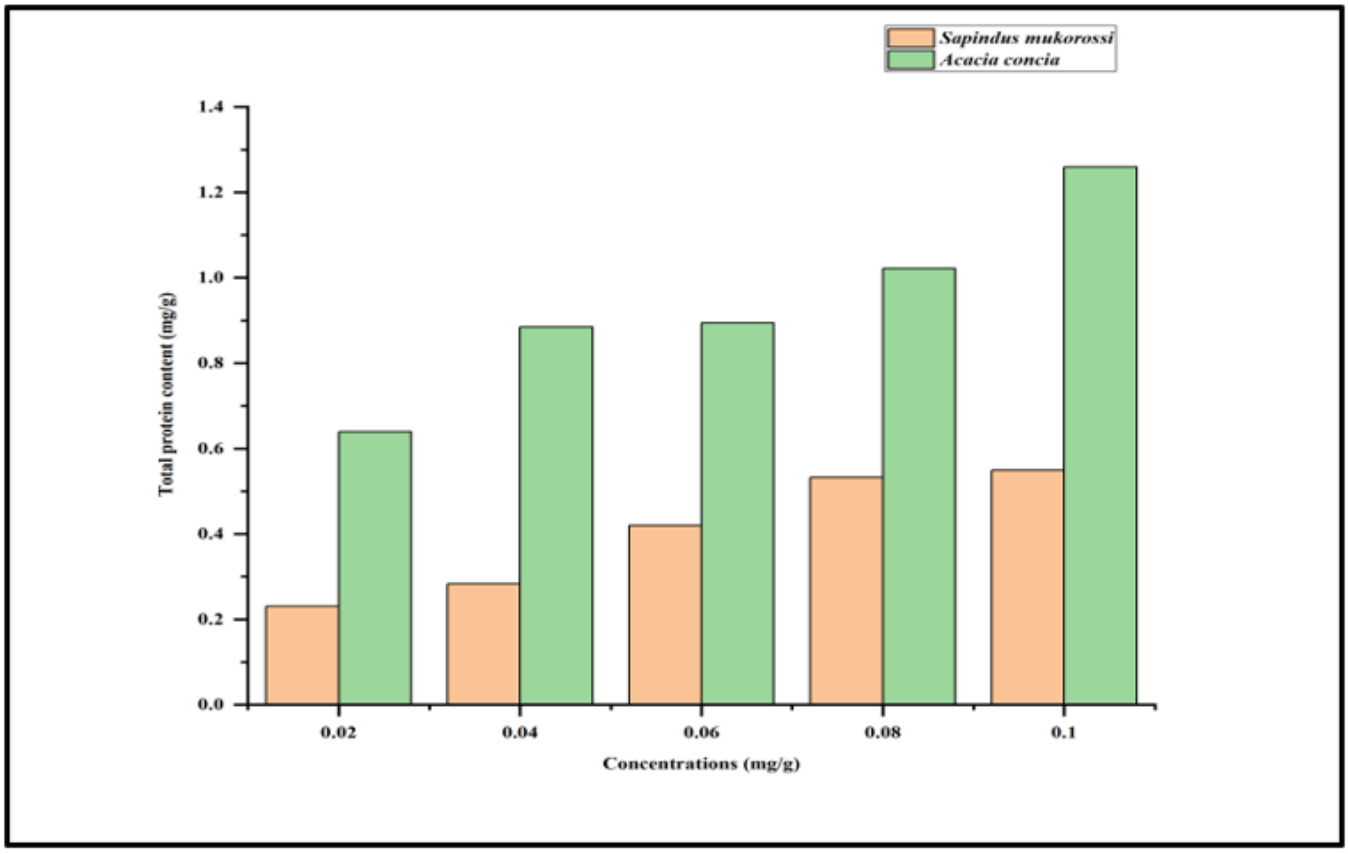


Figure 1

Total Protein content (mg/g) in selected plant Species at different concentrations.

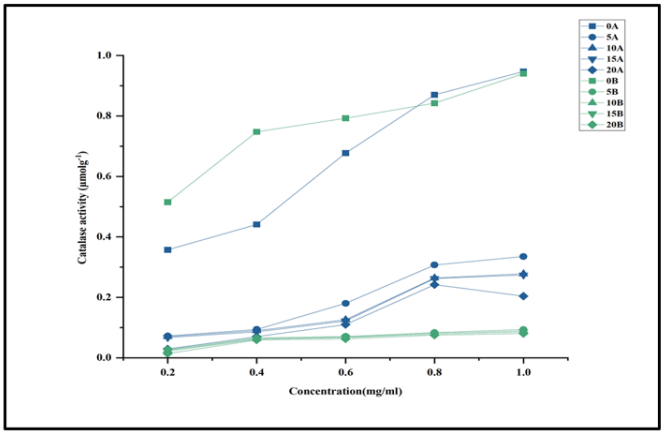


Fig. 2 (a)

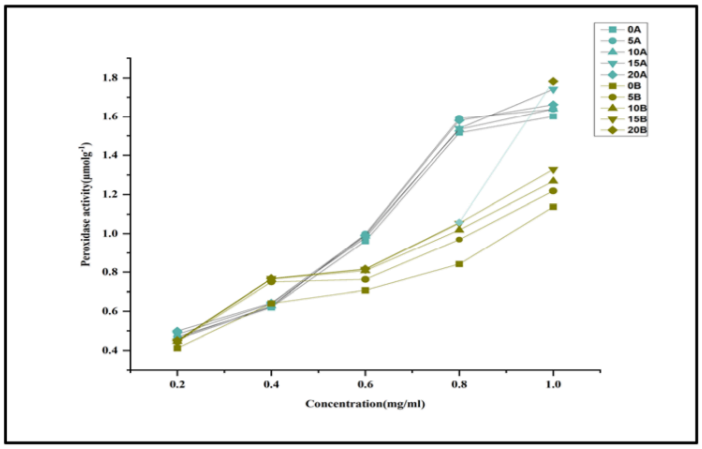


Fig.2 (b)

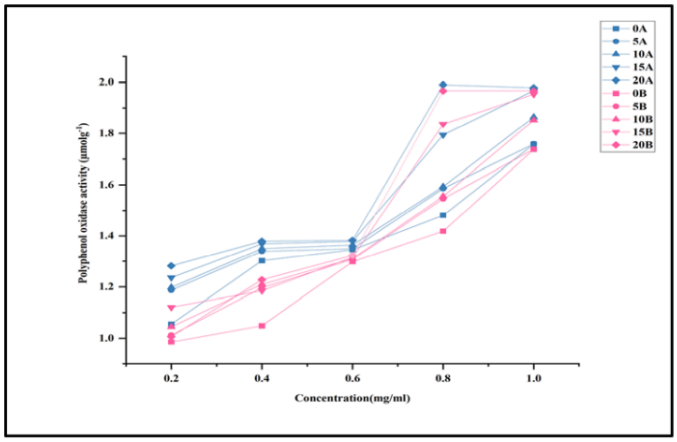


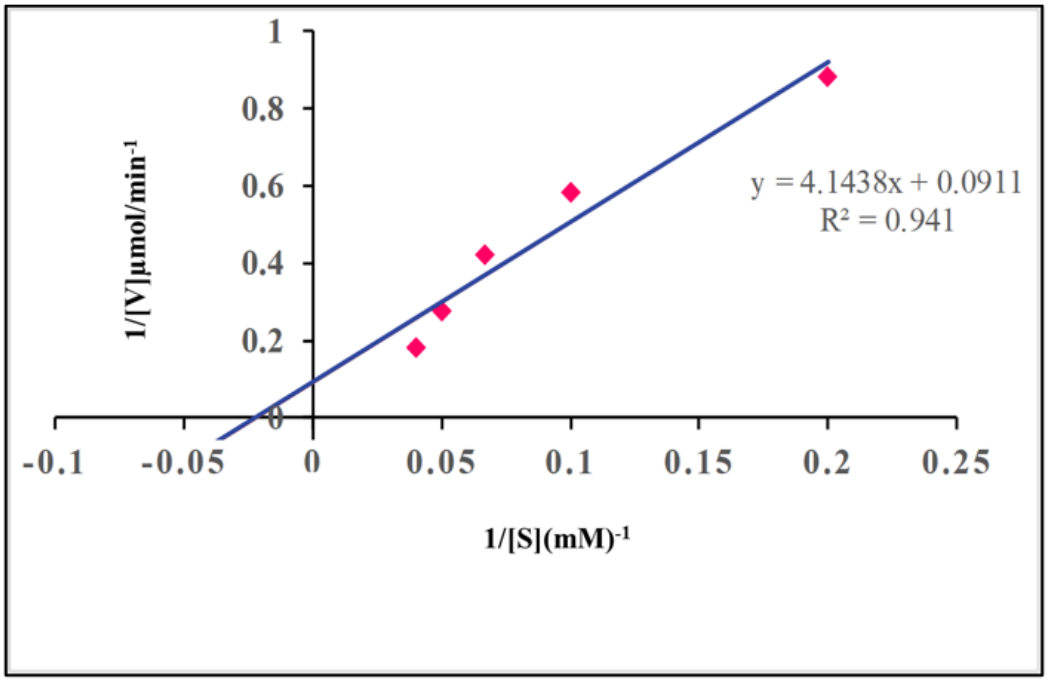
Fig. 2 (c)

Figure 2

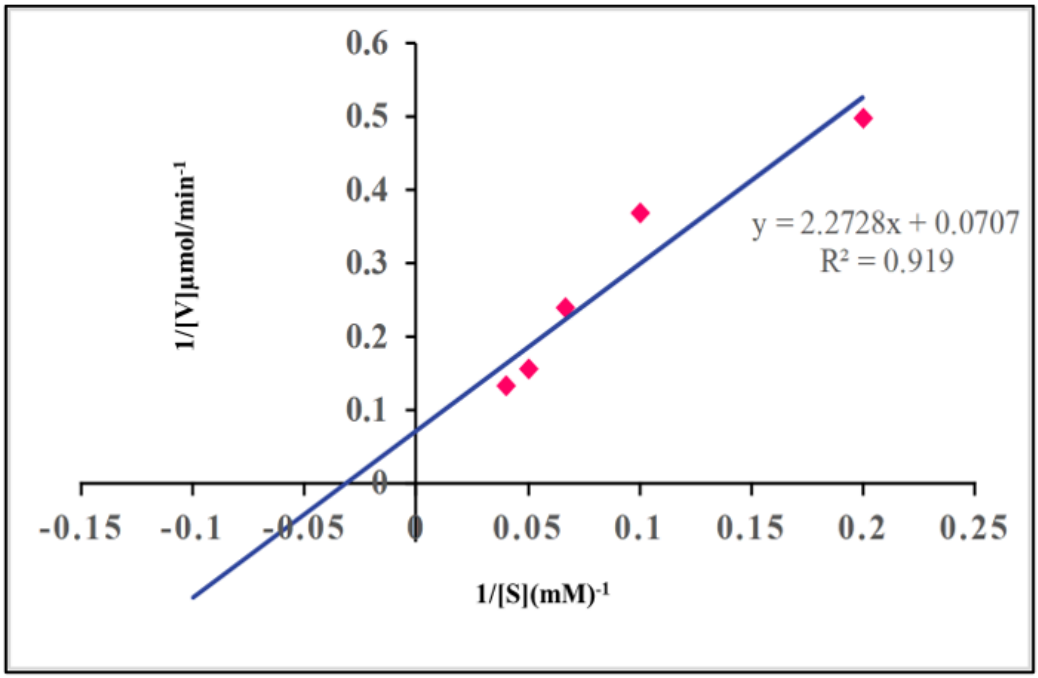
(a) Catalase enzyme activity in *Sapindus mukorossi* (A) & *Acacia concinna* (B) at different time duration with various concentrations.

(b) Peroxidase enzyme activity in *Sapindus mukorossi* (A) & *Acacia concinna* (B) at different time duration with various concentrations.

(c) Polyphenol oxidase enzyme activity in *Sapindus mukorossi* (A) & *Acacia concinna* (B)at different time duration with various concentrations.



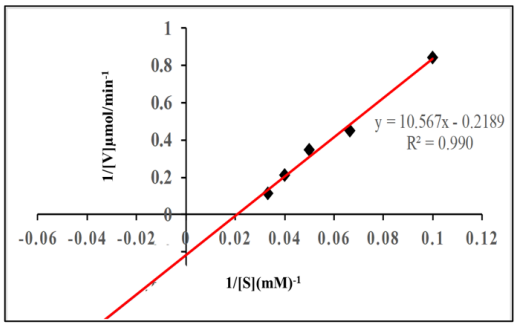
(a) *Sapindus mukorossi*



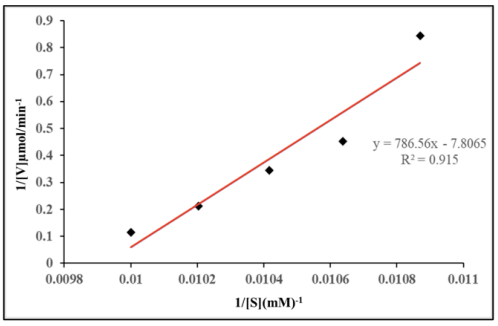
(b) *Acacia concinna*

Figure 3

Michaelis-Menten model for catalase enzyme of species (a) & (b) H_2O_2 as a Substrates.

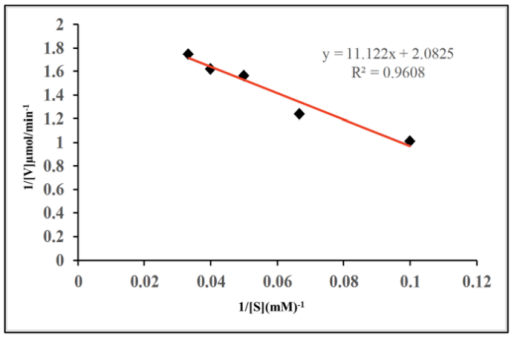


(a) *Sapindus mukorossi*

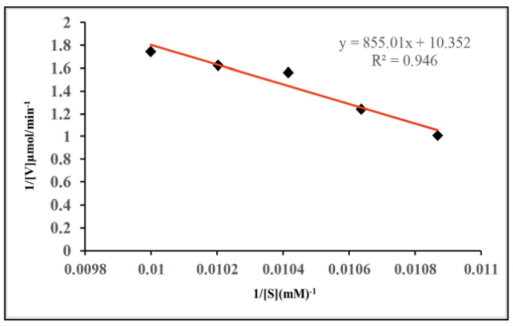


(b) *Acacia concinna*

Fig. 4 (a)



(a) *Sapindus mukorossi*



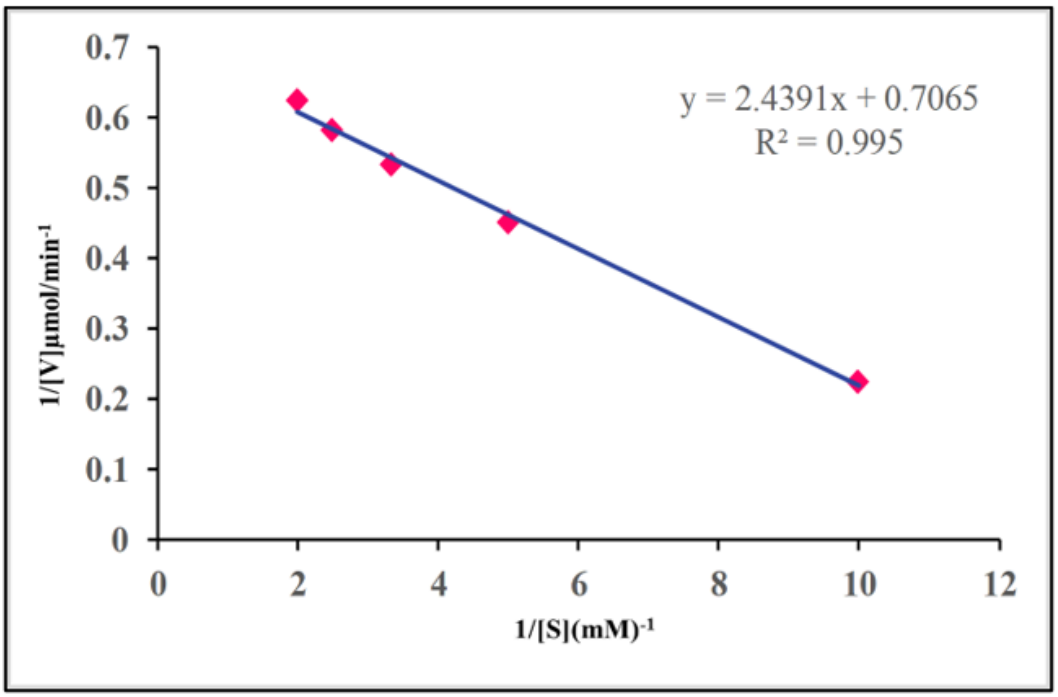
(b) *Acacia concinna*

Fig. 4(b)

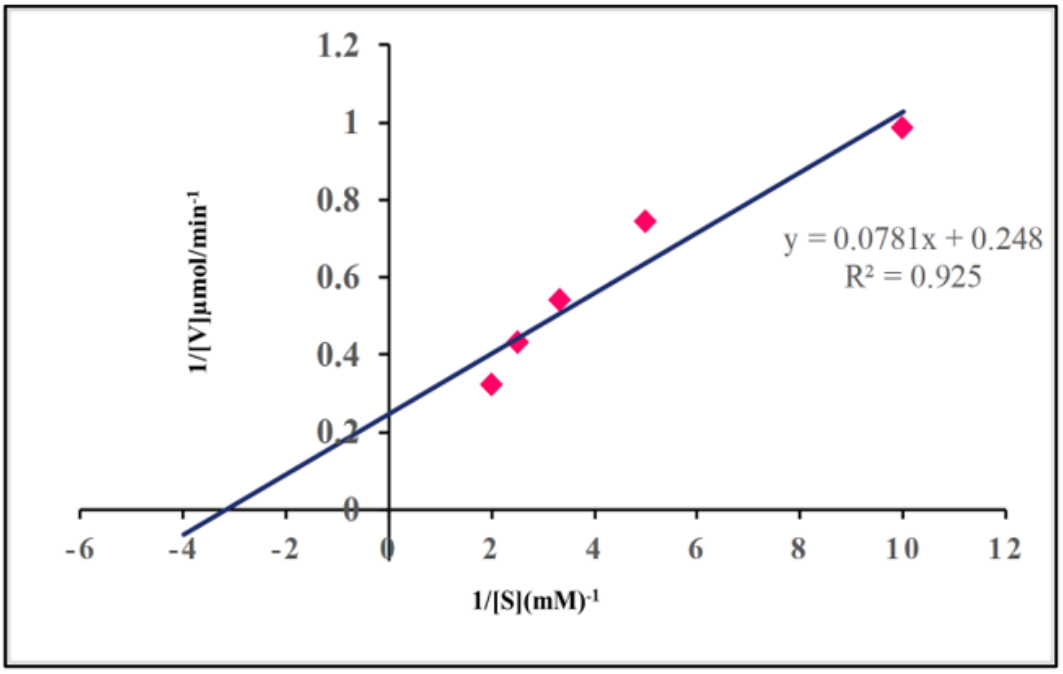
Figure 4

(a) Random bi–bi model for Peroxidase enzyme of species (a) and (b) Guaiacol as a substrates.

(b) Random bi–bi model for Peroxidase enzyme of species (a) and (b) H_2O_2 as a Substrates.



(a) *Sapindus mukorossi*



(b) *Acacia concinna*

Figure 5

Michaelis-Menten model for Polyphenol oxidase enzyme of species (a) and (b).

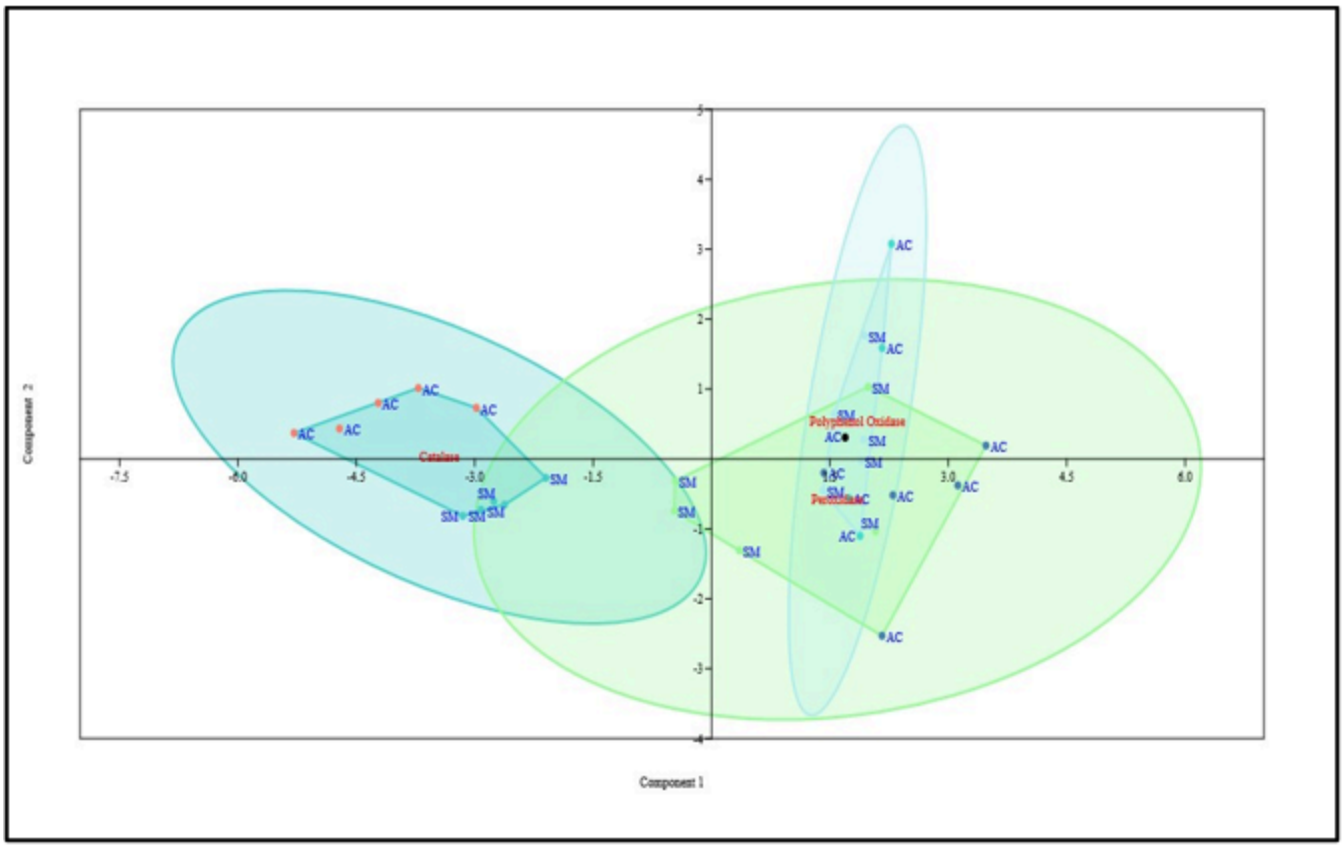


Figure 6

Linear Discriminants analysis of catalase peroxidase and polyphenol oxidase enzyme activity in selected species.