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Comparative analysis of enzymatic defence mechanisms in *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC. using a Michaelis–Menten kinetic model

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This study compares the enzymatic antioxidant defence responses of two saponin-rich species, *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC. Dried fruit powders were analysed for catalase, peroxidase, and polyphenol oxidase activities using standard spectrophotometric assays. Distinct interspecific variations were observed *Acacia concinna* (Willd.) DC. showed higher enzymatic activity, whereas *Sapindus mukorossi* Gaertn. exhibited comparatively higher polyphenol oxidase activity. Enzyme kinetic parameters (*Vmax* and *Km*) were estimated using Michaelis–Menten modelling and Lineweaver–Burk plots. *Acacia concinna* (Willd.) DC. demonstrated lower *Km* and higher *Vmax* values for most enzymes, indicating superior catalytic efficiency and substrate affinity. Linear Discriminant Analysis (LDA) revealed that the first two discriminant functions accounted for 96.53% of the total variance, and post hoc test ($P = 0.05$) confirmed significant differences in enzymatic profiles. The present study highlights the residual enzymatic potential, kinetic adaptability and biochemical composition of these saponin-rich species, emphasizing the role of enzyme efficiency in physiological stress defence response.

Keywords Protein, Catalase, Peroxidase, Polyphenol oxidase, Kinetic, Defence, Physiological stress

Sapindus mukorossi Gaertn. (Aritha or Ritha) and *Acacia concinna* (Willd.) DC. are traditional medicinal plants widely used in India, particularly for their saponin-rich fruit pericarps. The pericarp of *Sapindus mukorossi* Gaertn. produces a natural soapy lather and contains mucilage, sugars, and approximately 11.5% saponins including seven known saponins, two newly identified dammarane-type saponins, and four oleanane glycosides contributing to its extensive use in ayurveda-based treatments[1,2]. *Acacia concinna* (Willd.) DC. fruits, commonly used as purgatives, emetics, expectorants, and hair-care agents, contain flavonoids and monoterpenoid-type saponins as major bioactive constituents[3,4].

Although both species are known for their rich phytochemical profiles and antioxidant potential, most previous studies have focused on qualitative phytochemical screening rather than quantitative assessments of enzymatic defence systems. Limited research has integrated Michaelis–Menten kinetic modelling with biochemical analyses to explain how defence enzymes function in *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC.[5]. This gap limits our understanding of how these species differ in terms of catalytic efficiency *Km*, *Vmax*, enzymatic adaptability, and biochemical responses under oxidative stress. A comparative kinetic evaluation of key antioxidant enzymes catalase, peroxidase, and polyphenol oxidase therefore holds substantial importance for elucidating species-specific defence strategies[6].

Phytochemicals such as saponins play a crucial role in modulating antioxidant activity. They can enhance the expression of enzymatic antioxidants and directly neutralize Reactive Oxygen Species (ROS), making them valuable for managing oxidative stress, anti-aging therapies, and protective skincare formulations[7–12]. Plants naturally employ a robust enzymatic antioxidant defence network comprising catalase, peroxidase, superoxide dismutase, and polyphenol oxidase to prevent oxidative damage[13,14]. Catalase converts hydrogen peroxide into water and oxygen, while polyphenol oxidase oxidizes phenolic compounds into quinones that

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polymerize into melanin, contributing to defence against stress and pathogens[15–18]. Catalase is present across cellular compartments including cytosol, mitochondria, and peroxisomes in both prokaryotes and eukaryotes[19], highlighting its fundamental biological importance.

Beyond their physiological roles, these enzymes also have extensive industrial applications. Catalase is used to remove excess hydrogen peroxide in food processing (e.g., cold pasteurization of milk), textile production, and various biotechnological processes[18,20,21]. Glutathione peroxidase reduces H_2O_2 using electron donors such as ascorbate and phenolics[22], while plant, fungal, bacterial, and animal peroxidases demonstrate functional diversity due to amino acid sequence variations[23]. Peroxidase functions in lignification, hormone modulation, indole acetic acid metabolism, and defence responses[24–26]. Polyphenol oxidase also plays a critical role in post-harvest physiology by catalysing the oxidation of phenolics, leading to enzymatic browning in fruits and vegetables[18]. In defence responses, it inhibits pathogens, alters plant proteins, and reduces nutritional availability for herbivores[27], reinforcing the plant's overall antioxidant system[28]. Enzymatic reactions in plants follow single- or multi-substrate kinetic mechanisms, with sequential and random binding modes influencing the complexity and efficiency of catalytic processes[29,30].

Research on plant defence and metabolism often focuses on key enzymes such as catalase, peroxidase, and polyphenol oxidase. The comparative kinetic profiling of these enzymes in *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC. 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 catalase, peroxidase and polyphenol oxidase in stress physiology but also highlight the ecological significance and pharmaceutical potential of *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC. as model plants for studying plant defence responses.

Material and methods

Collection of samples

Dry fruits of *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC. were procured from the local market of Junagadh, Gujarat, India. Botanical identification and authentication were performed by Dr. Sandip Gamit, Department of Life Sciences, Bhakta Kavi Narsinh Mehta University. A voucher specimen (Reference No. BKNMU 478) has been deposited in the departmental herbarium for future reference and documentation. The collected fruit pericarps were oven-dried, finely ground using a mechanical crusher, and the resulting powder was subsequently used for protein estimation and enzymatic activity assays.

Chemical and reagent

Chemicals used in this study were purchased from Hi-Media, Merck, and Sigma-Aldrich with the purity of chemicals are as follows: Bovine Serum Albumin (BSA), KH_2PO_4-monopotassium phosphate, K_2HPO_4-Dipotassium hydrogen phosphate, EDTA– Ethylenediaminetetraacetic acid**,** hydrogen peroxide (H_2O_2), guaiacol, and catechol etc.

Quantification of total protein

Folin-Lowry method was employed to estimate the total protein content where a 1 g fruit powder was macerated, with 20% trichloro-acetic acid. The homogenate was then centrifuged at 6000 rpm for 20 min and subsequently, the supernatant was discarded. Then, 5 mL of 0.1 N NaOH was added to the pellet and the mixture underwent a second round of centrifugation for 20 min. The resulting supernatant was preserved for protein estimation. For the protein estimation process, 20 µL of the extract was combined with 980µL of Milli-Q water to achieve a final volume of 1000 µL. Following these, 4500 µL of solution C was added to the mixture. Solution C is a combination of solution A and solution B, in a 50:1 ratio. solution A consists of 2% sodium carbonate in 0.1 N sodium hydroxide, while solution B comprises equal volumes of 0.5% copper sulphate and 1% sodium potassium tartrate. The reaction mixture was thoroughly mixed and incubated in dark conditions for 10 min at room temperature. Subsequently, 500 µL of diluted folin reagent (in a 1:1 ratio) was added to the solution and incubated for 30 min. The absorbance of the solution was measured at a wavelength of 660 nm using a spectrophotometer, and this measurement was compared against an appropriate blank. The protein content was quantified using bovine serum albumin as a standard within the range of 50–300 µg and calculated by the following formula: [31,32].

$$\text{Protein content (mg/g)} = \text{Samples OD} \times \text{Standard OD} \times \text{Dilution factor}$$

Enzyme extraction

1 g of dry fruits powder was mixed with 10 mL of extraction buffer (0.1 M phosphate buffer + 0.5 mM EDTA, pH adjusted to 7.5) at 4 ° C.The sample was centrifuged at 15,000 rpm for 20 min. The supernatant was collected and utilized for the enzyme assays, this enzyme was performed in triplicate[33].

Analysis of catalase activity

The OD value were evaluated by taking 1500 µl of 100 mM phosphate buffer with pH 7.0, 500 µl of (5–25 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 at 0, 5, 10, 15, and 20 min intervals using a UV–Visible spectrophotometer at 240 nm. The OD value was also registered in the blank, without the enzyme 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 (ε = 39.4 mM^{-1} cm^{-1}) oxidized min^{-1} mg^{-1} protein[34].

Analysis of peroxidase activity
The OD value were evaluated by taking 1000 µl of 100 mM phosphate buffer with pH 6.1, 500 µl of (92–100 mM) guaiacol, 400 µl of milli-Q 500 µl of (6–14 mM) H_2O_2 and enzyme supernatant in the quartz cuvette, the rate of guaiacol and H_2O_2 decomposition was estimated at 0, 5, 10, 15, and 20 min intervals using a UV–Visible spectrophotometer 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 (ε = 26.6 mM^{-1} cm^{-1})[35].

Analysis of polyphenol oxidase activity
The OD value were evaluated by taking 2900 µl of (100–500 mM) catechol in the 10 mM phosphate buffer with pH 6.0, 100 µl milli-Q and enzyme supernatant in the quartz cuvette the rate of catechol oxidation to o-quinone decomposition was estimated for 0, 5, 10, 15 and 20 min at time at 0, 5, 10, 15, and 20 min intervals using a UV–Visible spectrophotometer at 420 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 (ε = 1.0 mM^{-1} cm^{-1}) at 420 nm spectrophotometrically[36].

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

$$\mathrm{SA} = \frac{\text{Change in OD per minute}}{\text{Molar extinction co} - \text{efficient of enzyme } (\varepsilon) \times \text{Volume of enzyme in sample}} \times \mathrm{TPC}$$

where, SA = Calculation of Enzyme Specific Activity, TPC = Total Protein Content, 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.

The Michaelis–Menten kinetic equation

The Michaelis–Menten kinetic equation is one of the most established models describing the relationship between the reaction velocity and substrate concentration in enzyme catalysed reactions[37,38]. The equation is expressed as:

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

In this case, *Km* is the Michaelis–Menten constant, *Vmax* is the maximum reaction velocity, and V is the initial velocity. enzyme catalytic efficiency and substrate affinity are reflected in these parameters. While a higher *Vmax* indicates an increased catalytic capacity to neutralize Reactive Oxygen Species (ROS), a lower *Km* indicates a higher substrate affinity, which is necessary for effective antioxidant defence under oxidative stress. Conventional non-linear regression was used to determine the kinetic parameters (*Vmax* and *Km*), and Lineweaver–Burk linearization standard methods for enzymatic kinetics was used to validate the results. Lineweaver–Burk plot differential equations governing enzyme kinetics with high accuracy and stability. When evaluating the oxidative stress response, these methods promise methodological transparency and accurate interpretation of enzyme kinetics [39,40].

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 (LDA) as well as post hoc test constructing.

Results

Total protein content

An analysis was conducted to determine the total protein content in saponin rich fruits *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC. at various concentrations. Figure 1 depicts the varying protein content ranges found in different concentration of saponin rich fruit extracts. The total protein content of saponin-rich fruit extracts from *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC. was quantified at different concentrations (0.02, 0.04, 0.06,0.08 and 0.1 mg/mL). As shown in Fig. 1, protein levels increased progressively with rising extract concentration in both the species. In *Sapindus mukorossi* Gaertn., protein content ranged from 0.231 mg/g at 0.02 mg/mL to 0.549 mg/g at 0.1 mg/mL. In contrast, *Acacia concinna* (Willd.) DC. exhibited comparatively higher protein levels, increasing from 0.639 mg/g at 0.02 mg/mL to a maximum of 1.259 mg/g at 0.1 mg/mL. Compare both species *Acacia concinna* (Willd.) DC. showed consistently higher protein content across all concentrations, with the highest value recorded at 0.1 mg/mL, whereas the lowest protein content was observed in *Sapindus mukorossi* Gaertn. at 0.02 mg/mL. The positive correlation between extract concentration and total protein content is evident in both species.

Enzyme assay

Three type of enzyme perform in fruits *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC. of such as catalase, peroxidase, and polyphenol oxidase were performed in triplicate.

Catalase activity
This scientific investigation examined the catalase activity of *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC. fruits extract with different concentrations (0.2, 0.4, 0.6, 0.8, and 1 mg/ml) with respect to different

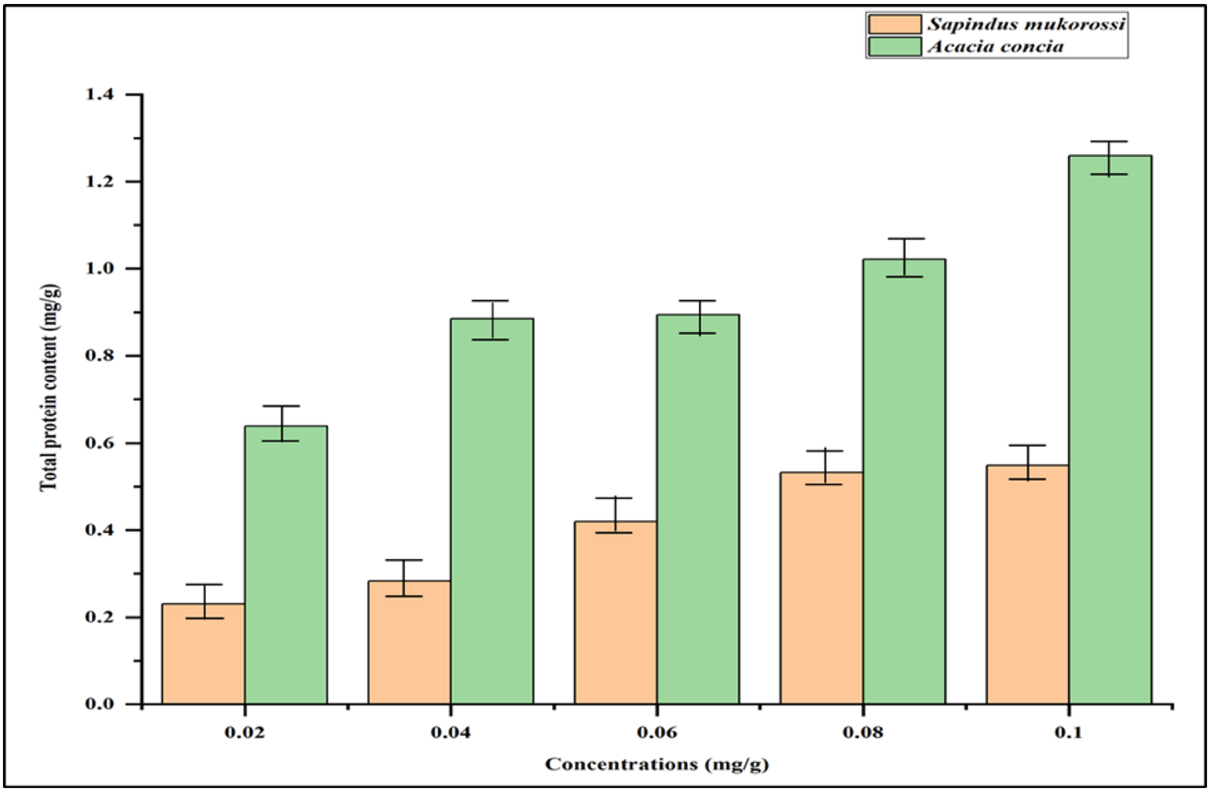


Fig. 1. Total protein content (mg/g) in selected plant species at different concentrations.

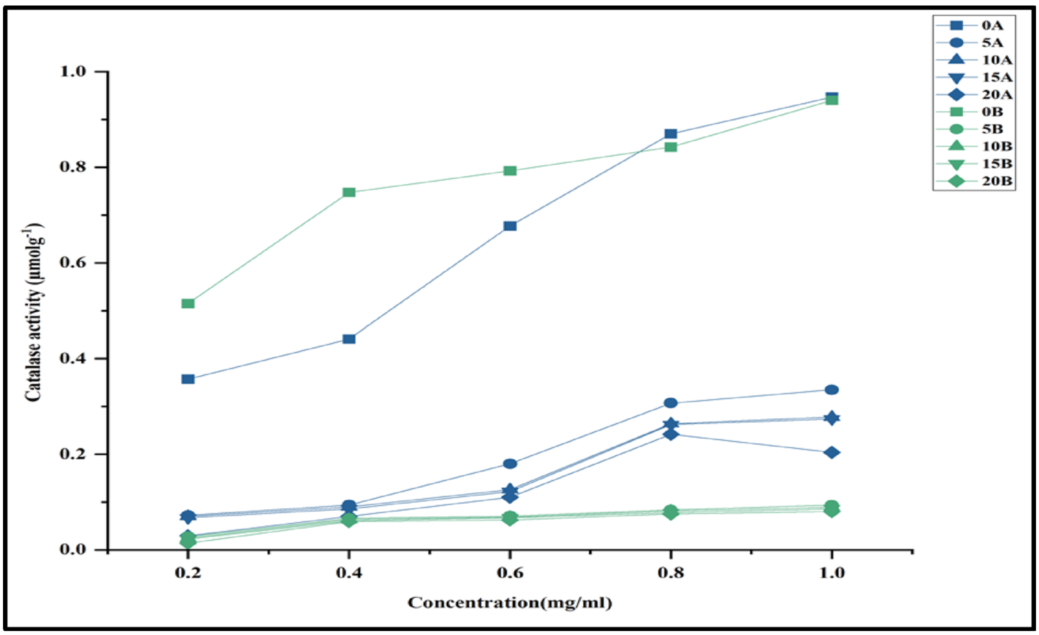


Fig. 2. Catalase enzyme activity in *Sapindus mukorossi* Gaertn. (A) & *Acacia concinna* (Willd.) DC. (B) at different time duration with various concentrations.

time 0, 5, 10, 15 and 20 min interval (Fig. 2). Legend of the figure detailed in Table 1. In *Sapindus mukorossi* Gaertn., catalase activity increased with extract concentration but declined progressively over time. At 0.2 mg/mL, activity decreased from 0.357 µmol·g^{-1} at 0 min to 0.029 µmol·g^{-1} at 20 min. Higher activities were observed at 0.4 and 0.6 mg/mL reaching 0.441 and 0.667 µmol·g^{-1} at 0 min respectively, followed by a gradual reduction across the 20-min interval. *Acacia concinna* (Willd.) DC. displayed substantially higher catalase activity at all

Legend	Times(min)	Species codes
0A	0	A- *Sapindus mukorossi* Gaertn
5A	5	A- *Sapindus mukorossi* Gaertn
10A	10	A- *Sapindus mukorossi* Gaertn
15A	15	A- *Sapindus mukorossi* Gaertn
20A	20	A- *Sapindus mukorossi* Gaertn
0B	0	B- *Acacia concinna* (Willd.) DC
5B	5	B- *Acacia concinna* (Willd.) DC
10B	10	B- *Acacia concinna* (Willd.) DC
15B	15	B- *Acacia concinna* (Willd.) DC
20B	20	B- *Acacia concinna* (Willd.) DC

Table 1. Caption of enzyme activity in different time duration.

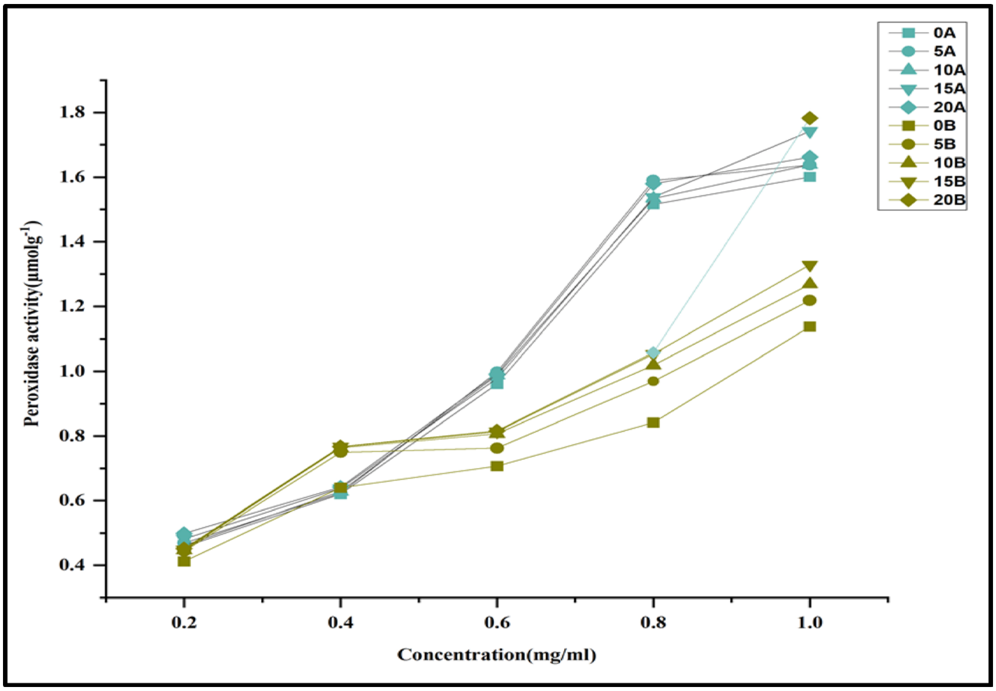


Fig. 3. Peroxidase enzyme activity in *Sapindus mukorossi* Gaertn. (A) & *Acacia concinna* (Willd.) DC. (B) at different time duration with various concentrations.

concentrations compared to *Sapindus mukorossi* Gaertn. Activity increased from 0.875 μmol·g^{-1} at 0.2 mg/mL to a maximum of 1.598 μmol·g^{-1} at 1.0 mg/mL at 0 min. Similar to *Sapindus mukorossi* Gaertn., activity declined steadily with increasing incubation time. Study findings *Acacia concinna* (Willd.) DC. exhibited the highest catalase activity across all tested concentrations, whereas *Sapindus mukorossi* Gaertn. showed comparatively lower activity. In both species, catalase activity decreased consistently with time regardless of concentration (Fig. 2).

Peroxidase activity

In (Fig. 3), peroxidase activity increased with both extract concentration and incubation time in *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC. fruit extracts. For *Sapindus mukorossi* Gaertn., peroxidase activity showed a gradual rise across concentrations. 0.2 mg/ml, activity increased from 0.457 μmol·g^{-1} at 0 min to 0.499 μmol·g^{-1} at 20 min. 0.4 mg/ml, activity rose from 0.623 to 0.642 μmol·g^{-1}, while at 0.8 mg/ml it ranged between 1.516 and 1.580 μmol·g^{-1}. The highest concentration (1 mg/ml) showed activities from 1.601 to 1.742 μmol·g^{-1}, indicating a moderate but consistent increase with time. For *Acacia concinna* (Willd.) DC., peroxidase activity was markedly higher at all concentrations. At 0.2 mg/ml, activity increased from 0.977 to 1.798 μmol·g^{-1}. 0.4 mg/ml, values rose from 1.440 to 1.699 μmol·g^{-1}, and at 0.8 mg/ml from 1.850 to 2.289 μmol·g^{-1}. The highest activity was recorded at 1 mg/ml, increasing from 2.451 μmol·g^{-1} at 0 min to 3.762 μmol·g^{-1} at 20 min. these finding highlight *Acacia concinna* (Willd.) DC. exhibited significantly higher peroxidase activity than *Sapindus mukorossi* Gaertn. at all tested concentrations. In both species, enzyme activity increased progressively with longer incubation time and higher extract concentration.

Polyphenol oxidase activity
In (Fig. 4), polyphenol oxidase activity increased progressively with extract concentration and incubation time in both *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC. fruit extracts. For *Sapindus mukorossi* Gaertn., activity increased steadily across all concentrations. 0.2 mg/ml, values rose from 1.055 μmol·g^{-1} at 0 min to 1.382 μmol·g^{-1} at 20 min. At 0.4 mg/ml, activity increased from 1.302 to 1.379 μmol·g^{-1}, while at 0.6 mg/ml it ranged from 1.345 to 1.382 μmol·g^{-1}. The highest concentration 1 mg/ml displayed the greatest activity, increasing from 1.758 μmol·g^{-1} at 0 min to 1.979 μmol·g^{-1} at 20 min. For *Acacia concinna* (Willd.) DC., polyphenol oxidase activity was comparatively lower. At 0.2 mg/ml, activity increased slightly from 1.171 to 1.189 μmol·g^{-1}. At 0.4 mg/ml, values rose from 1.224 to 1.373 μmol·g^{-1}, and at 0.6 mg/ml activity increased from 1.432 to 1.452 μmol·g^{-1} over the 20-min incubation period. These findings *Sapindus mukorossi* Gaertn. exhibited higher polyphenol oxidase activity than *Acacia concinna* (Willd.) DC. at all tested concentrations. In both species, enzyme activity generally increased with longer incubation time and higher extract concentration.

During stress condition plant produce toxic Reactive Oxygen Species (ROS), These enzymes catalase, peroxidase, and polyphenol oxidase neutralize reactive oxygen species. In enzyme kinetics, protein content determines catalytic potential, while enzyme activity reflects functional efficiency. Together, they play a crucial role in antioxidant defence by regulating reactions that neutralize reactive oxygen species under stress conditions.

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[41].

$$\frac{1}{V} = \frac{K_m}{V_{max}.\,[S]} + \frac{1}{V_{max}} \quad (2)$$

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 intercept 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 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 [28,42].

In this kinetic study, from Table 2, this study shows a value of *Vmax* and *Km* is varies in various substrates (H_2O_2, guaiacol, catechol) in catalase, peroxidase and polyphenol oxidase enzyme activity of *Sapindus mukorossi* Gaertn. *and Acacia concinna* (Willd.) DC. fruit.

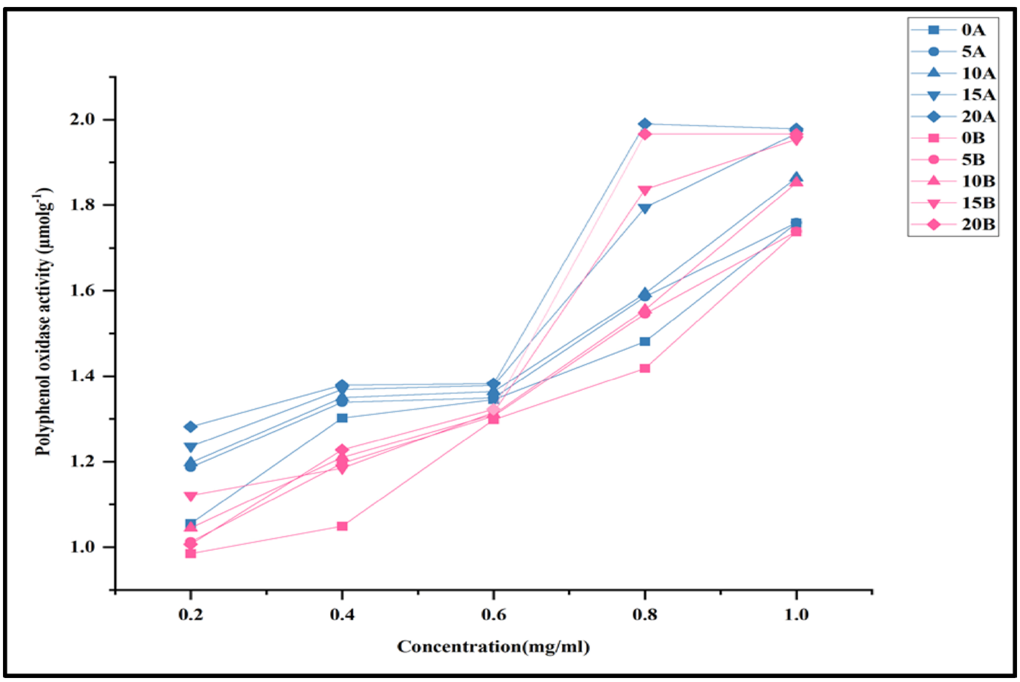


Fig. 4. Polyphenol oxidase enzyme activity in *Sapindus mukorossi Gaertn.* (A) & *Acacia concinna* (Willd.) DC. (B) at different time duration with various concentrations.

Species name	Enzyme	Substrate	*Km* (mM)	*Vmax* (μmol min^{1}mg^{-1} protein)
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

Table 2. Determination of enzyme kinetics parameter of selected species.

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* Gaertn. fruits. In the single substrate model, the Lineweaver–Burk plot will result in a *Km* value of 45.4862 mM and *Vmax* value of 10.9769 μmol min^{-1} mg^{-1} protein. Similarly, in *Acacia concinna* (Willd.) DC. fruit, the *Km* value will be 32.2093 mM and the *Vmax* value will be 14.1442 μmol/min^{-1} mg^{-1} protein. The process's low reaction rate was indicated by a low *Vmax* value shown in (Fig. 5). The reaction time for a low-reaction rate process would be longer than that of a high reaction rate process. Regarding the *Km* value, it indicated the ability of the enzyme to bind to the substrate. A lower *Km* value would suggest more substrate enzyme binding than in a reaction with a higher *Km* value. The binding of substrate enzyme was reported to be affecting the reaction rate at negative way. The value of *Km* obtained in this study is considered quite high compared to other studies from (Table 2). Catalase is a crucial antioxidant enzyme that protects plant cells from oxidative damage caused by various stress conditions.

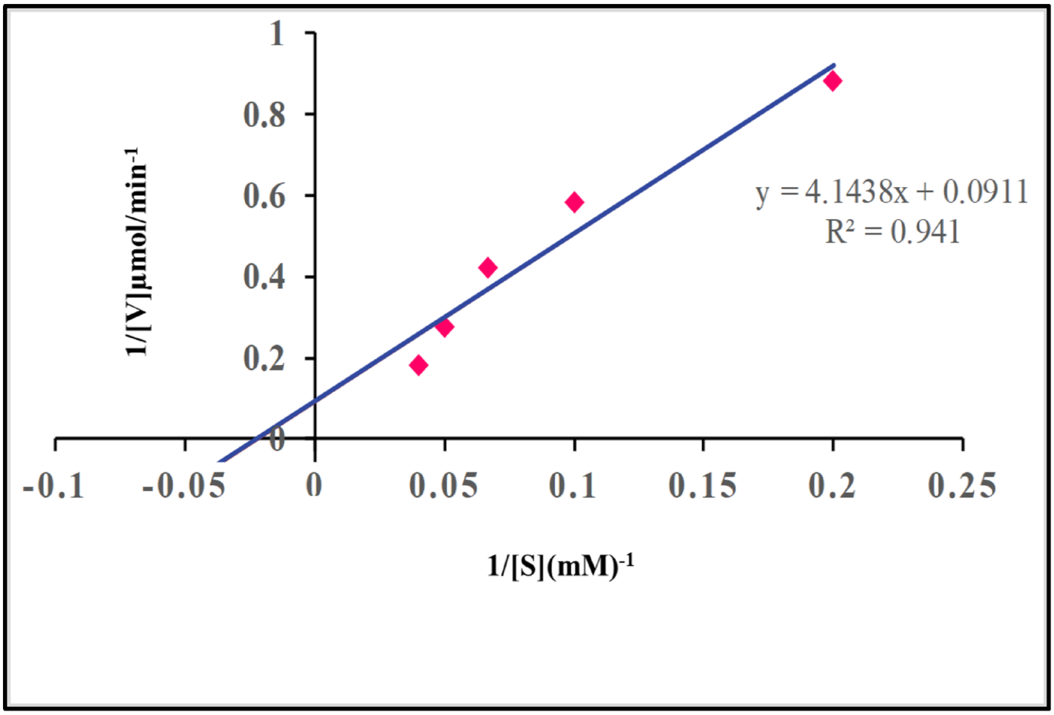
Peroxidase enzyme kinetic model in two Substrate (Random bi–bi model)

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 25 mM) versus different time (0, 5, 10, 15 and 20 min) in *Sapindus mukorossi* Gaertn. fruits. Applying the Lineweaver–Burk plot to two substrate models (guaiacol and H_2O_2) will also result in *Km* values of 100.757, 0.02071 mM and *Vmax* values of 0.12809, 4.56829 μmol min^{-1} mg^{-1} protein. Similarly, in *Acacia concinna* (Willd.) DC. fruit, *Km* values will be 82.5937 and 43.0420 mM and *Vmax* values will be 0.0966, 0.48019 μmol min^{-1} mg^{-1} protein shown in (Figs. 6 and 7). The process's low reaction rate was indicated by a low *Vmax* 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 *Km* value, it indicated the ability of the enzyme to bind to the substrate. A lower *Km* value would suggest more substrate enzyme binding than in a reaction with a higher *Km* value. Reaction rate was discovered to be negatively impacted by substrate enzyme binding. The value of *Km* obtained in this study is considered quite high relative to other studies from Table 2.

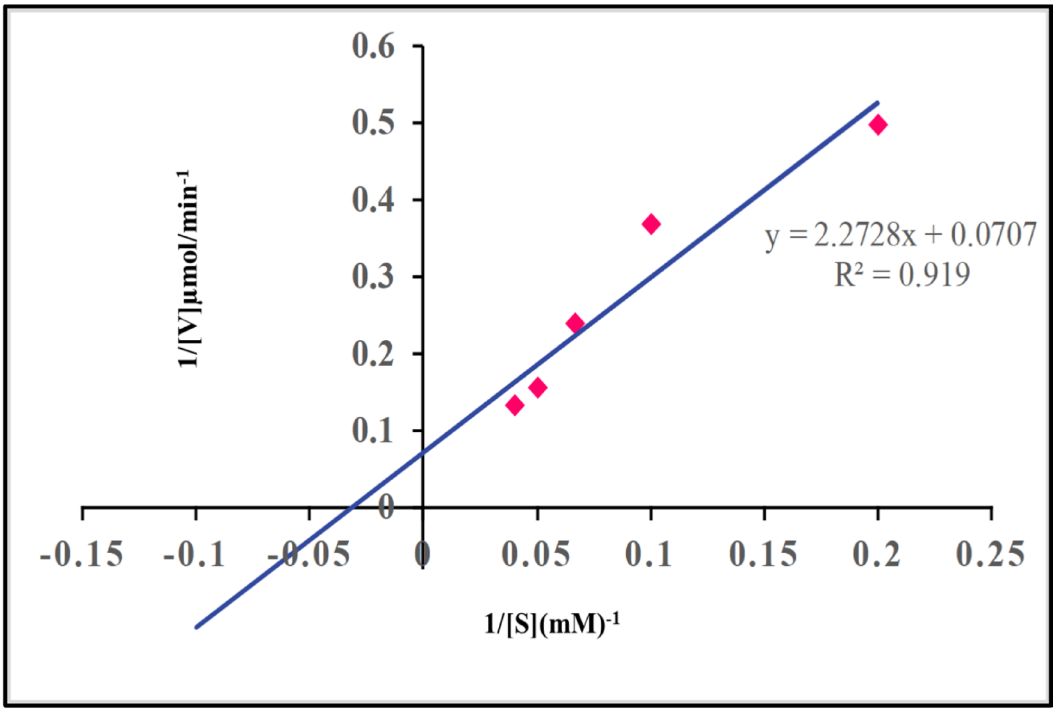
Polyphenol oxidase enzyme kinetic model in single substrates (Michaelis–Menten model)

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* Gaertn. fruits. When using the Lineweaver–Burk plot in a single substrate model, the *Km* value will be 3.45237 mM and the *Vmax* value will be 1.415428 μmol min^{-1} mg^{-1} protein. Similarly, in *Acacia concinna* (Willd.) DC. fruit, the *Km* value will be 0.31491 mM and the *Vmax* value will be 4.032258 μmol min^{-1} mg^{-1} protein shown in (Fig. 8). The process low reaction rate was indicated by a low *Vmax* value. The reaction time for a low reaction rate process would be longer than that of a high reaction rate process. Regarding the *Km* value, it indicated the ability of the enzyme to bind to the substrate. A lower *Km* value would suggest that there was more substrate-enzyme binding than in a reaction with a higher *Km* value. The binding of substrate enzyme was reported to be affecting the reaction rate at negative way. The value of *Km* 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 value of *Km* and *Vmax* 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. *Km* and *Vmax* provide a functional understanding of how well defence-related enzymes utilized their substrates under different stress intensities. Enzymes with low *Km* and high *Vmax* are typically more efficient in rapid and strong defence responses, contributing to a plant's biochemical comparision and adaptive capacity, according to current research. A high *Km* 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 *Vmax* may reflect a slower but sustained defence response, suitable for long-term protection or in species with moderate metabolic rates[40].



(a) ***Sapindus mukorossi*** **Gaertn.**



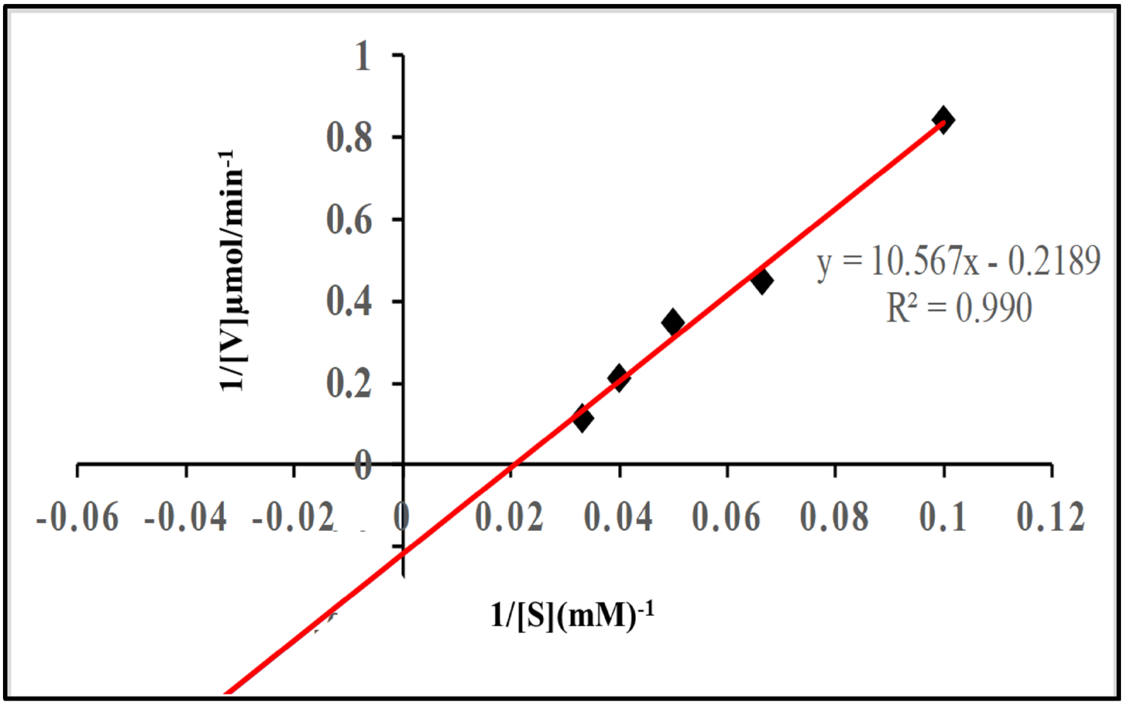
(b) ***Acacia concinna*** **(Willd.) DC.**

Fig. 5. Michaelis–Menten model for catalase enzyme of species (**a**) & (**b**) H_2O_2 as a Substrates.

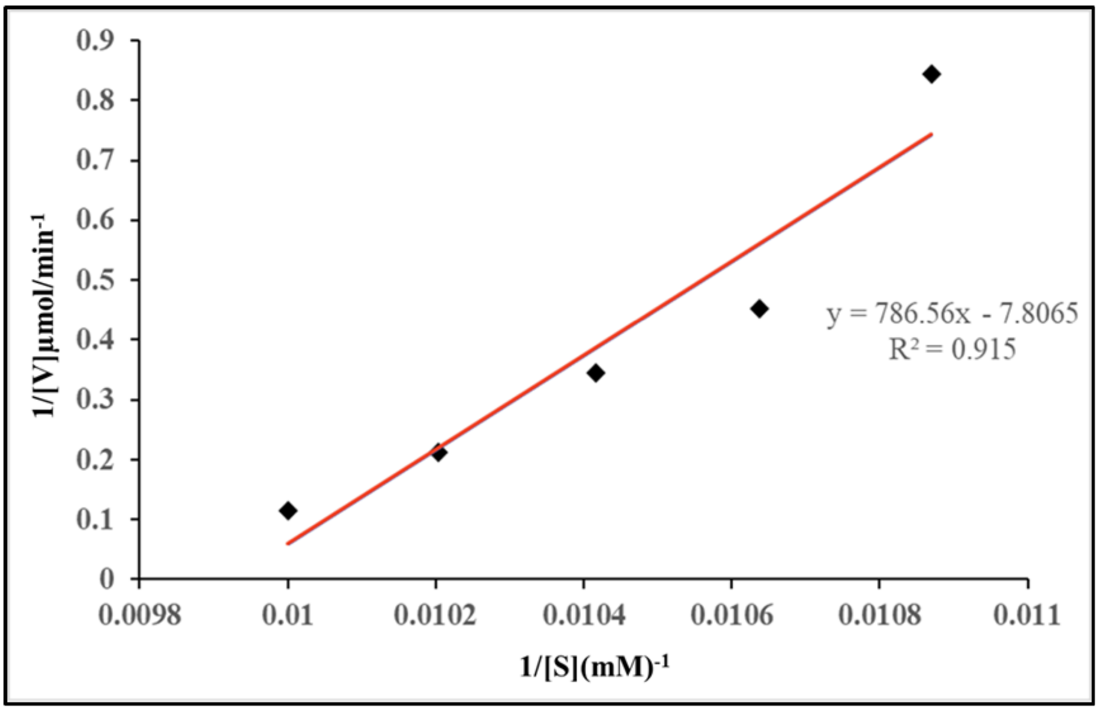
Statistical analysis

Linear discriminant analysis (LDA)

Linear Discriminant Analysis (LDA) is a multivariate statistical technique used to analyse datasets that are arranged into several groups or categories. It expands upon the standard Principal Component Analysis (PCA)



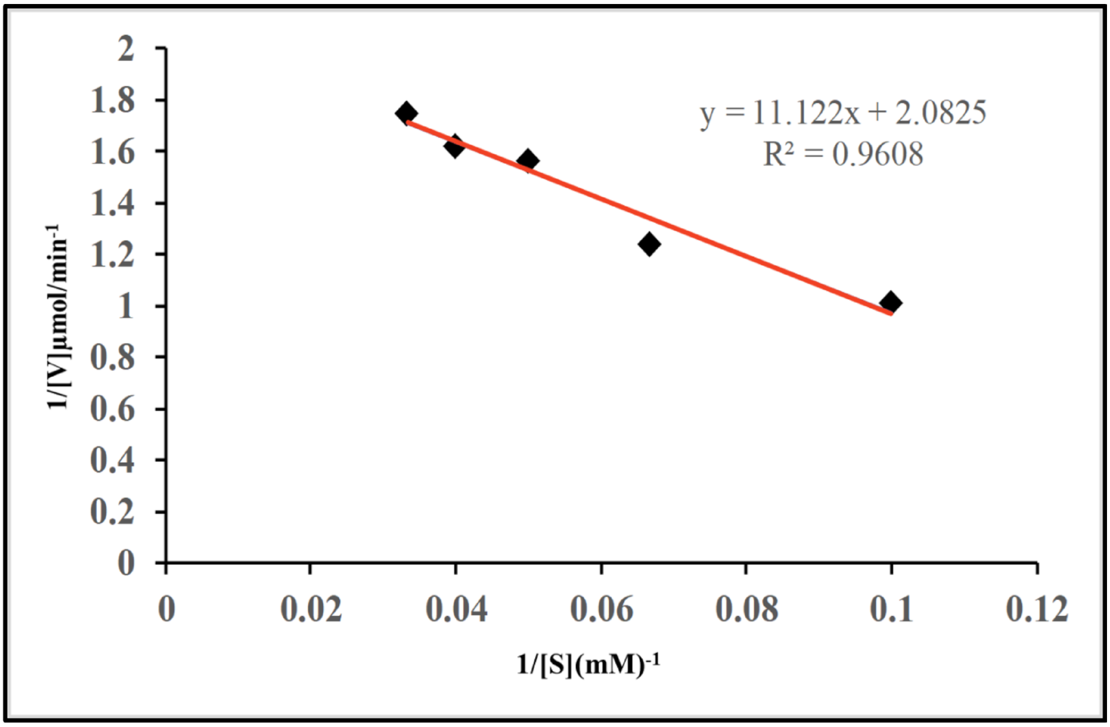
(a) *Sapindus mukorossi* Gaertn.



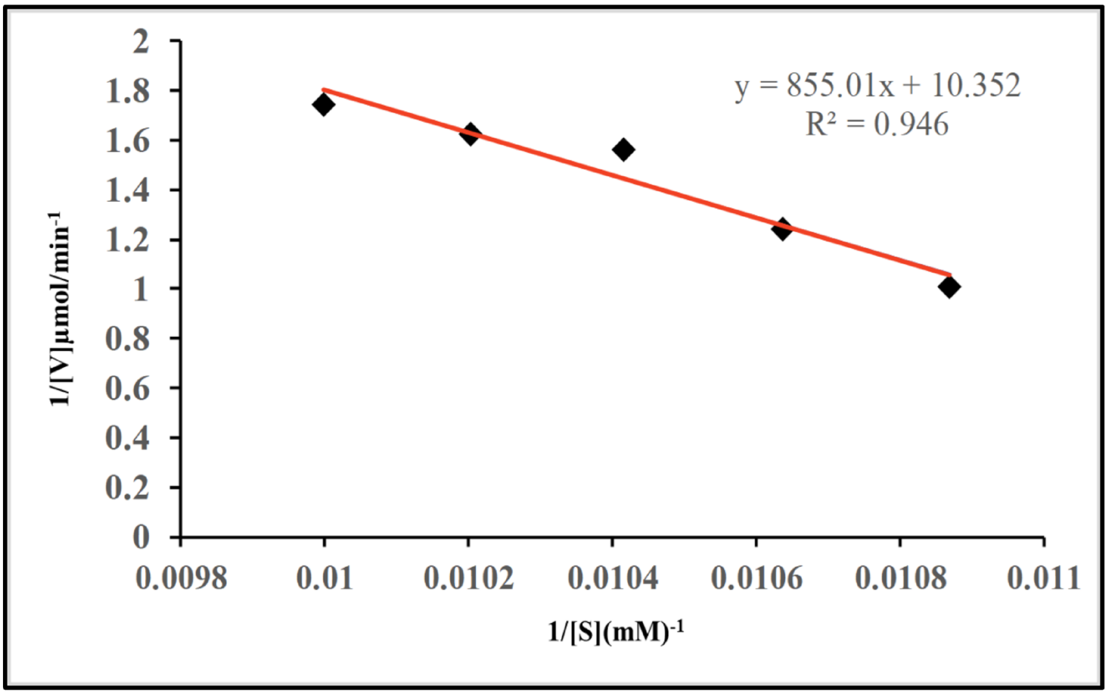
(b) *Acacia concinna* (Willd.) DC.

Fig. 6. Random bi–bi model for peroxidase enzyme of species (**a**) and (**b**) guaiacol as a substrates.

by enabling the analysis of several groups at once within a dataset. LDA was performed using enzyme activity variables (catalase, peroxidase, and polyphenol oxidase) to differentiate between species. Assumptions of normality and homogeneity of variance, and triplicate enzyme activity was performed to give a more significant result. Figure 8 indicates that component 1 explains 96.53% of the total variance in the data, indicating that it



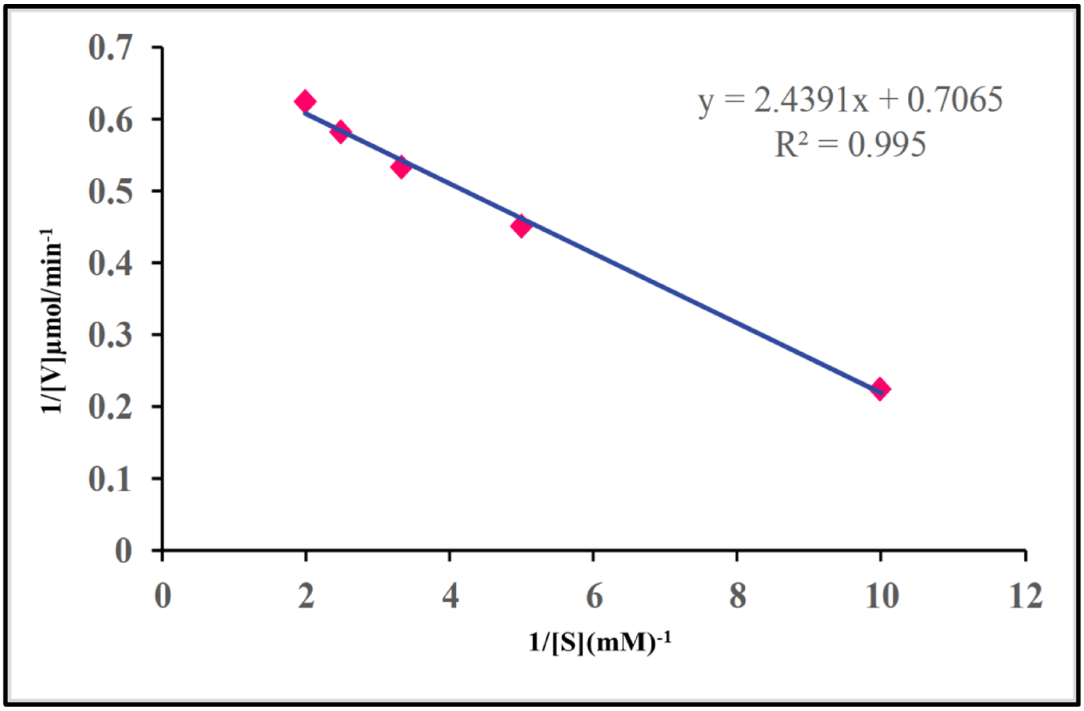
(a) ***Sapindus mukorossi*** **Gaertn.**



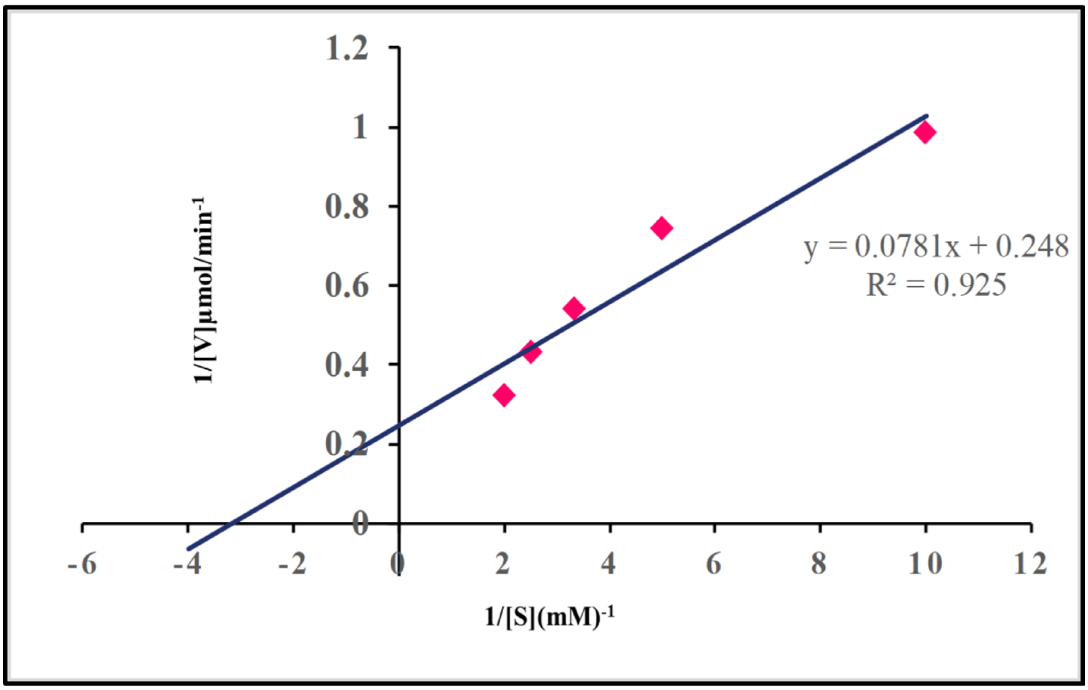
(a) ***Acacia concinna*** **(Willd.) DC.**

Fig. 7. Random bi–bi model for peroxidase enzyme of species (**a**) and (**b**) H_2O_2 as a Substrates.

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



(a) ***Sapindus mukorossi*** **Gaertn.**



(b) ***Acacia concinna*** **(Willd.) DC.**

Fig. 8. Michaelis–Menten model for polyphenol oxidase enzyme of species (**a**) and (**b**).

Component 2. While catalase enzyme activity demonstrates 3.468 variance negative loading value on Component 2, LDA found that the combinations of variables had different strengths of association. (Fig. 9) also shows the relationship between a specific enzyme activity of the two species in various time durations [43].

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 [32,44]. The outcomes derived from Tukey's HSD post hoc test concerning the examined characteristics revealed significant discrepancies in enzyme activity across plant species at $P \leq 0.05$, ensuring the robustness and reliability of the results, and the experiment set-up was triplicate justify a more significant *P* value on statistical analysis. A statistically notable distinction ($P = 0.05$) was noted in *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC., indicating variations in all three enzymes (catalase, peroxidase, and polyphenol oxidase) displayed significant discrepancies in activity of *Sapindus mukorossi* Gaertn. catalase (SMC) and *Sapindus mukorossi* Gaertn. peroxidase (SMP) at *P* value 0.004399, *Acacia concinna* (Willd.) DC. catalase (ACC) and *Acacia concinna* (Willd.) DC. peroxidase (ACP) at *P* value 0.002157, *Sapindus mukorossi* Gaertn. catalase (SMC) and *Sapindus mukorossi* Gaertn. polyphenol oxidase at *P* value 0.000174 and *Acacia concinna* (Willd.) DC. catalase (ACC) and *Acacia concinna* (Willd.) DC. polyphenol oxidase (ACPO). In contrast, when comparing two enzymes of same species, the *Sapindus mukorossi* Gaertn. peroxidase (SMP) and *Sapindus mukorossi* Gaertn. polyphenol oxidase (SMPO) at *P* value 0.100903 and *Acacia concinna* (Willd.) DC. peroxidase (ACP) and *Acacia concinna* (Willd.) DC. polyphenol oxidase (ACPO) at *P value* 0.31313, no statistically remarkable differences were observed. The results from 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. 10).

Discussion

Antioxidant enzymes play a central role in maintaining cellular redox homeostasis by scavenging excess Reactive Oxygen Species (ROS). Hydrogen peroxide (H_2O_2), a major ROS generated during oxidative stress, can diffuse across cellular compartments, causing widespread oxidative damage. In aerobic organisms, superoxide dismutase (SOD), catalase, and peroxidases function sequentially to detoxify ROS. SOD converts superoxide radicals into H_2O_2, which is subsequently degraded to water and oxygen by catalase and glutathione peroxidase, thereby reducing oxidative stress and preventing lipid peroxidation[45].

Previous work on *Vigna mungo (L.)* demonstrated high catalase activity during germination, with a specific activity of 25,704 U/mg on day four and kinetic parameters $Km = 16.2$ mM; $Vmax = 2.5$ µmol min^{-1} mg^{-1} protein indicative of strong substrate affinity and catalytic efficiency[34,46]. In comparison, the catalase activities measured in *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC. fruits were comparatively lower 0.667 and 1.598 µmol·g^{-1} respectively. Michaelis–Menten modelling revealed higher *Km* values in both species

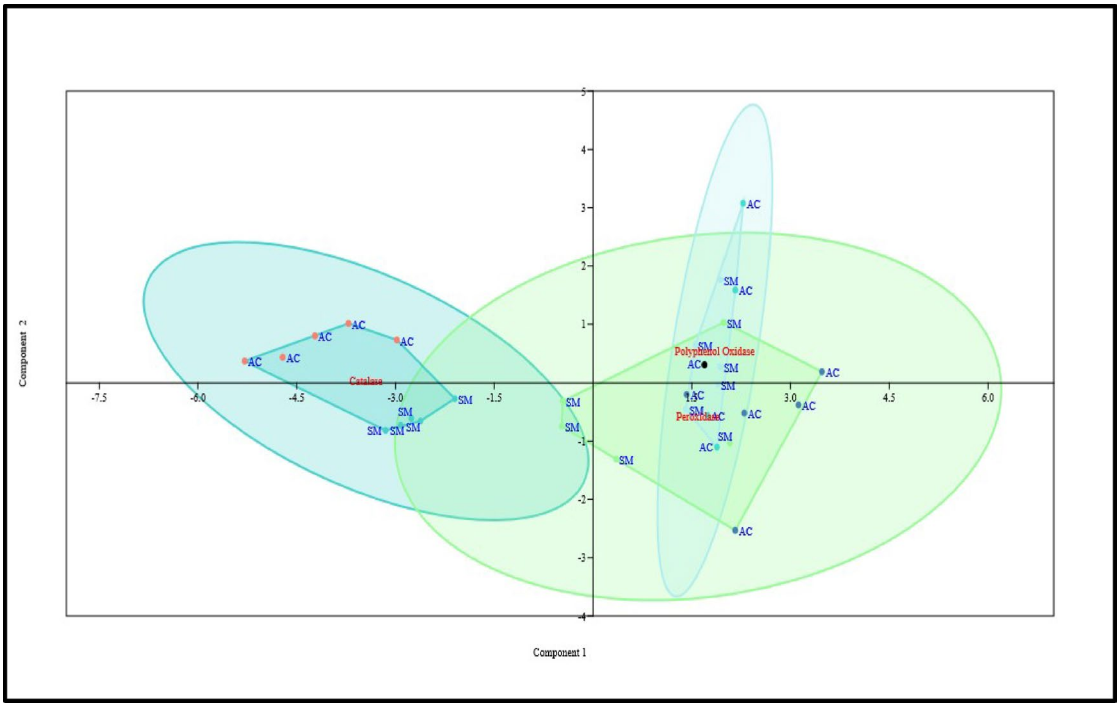


Fig. 9. Linear Discriminant Analysis (LDA) of catalase peroxidase and polyphenol oxidase enzyme activity in selected species.

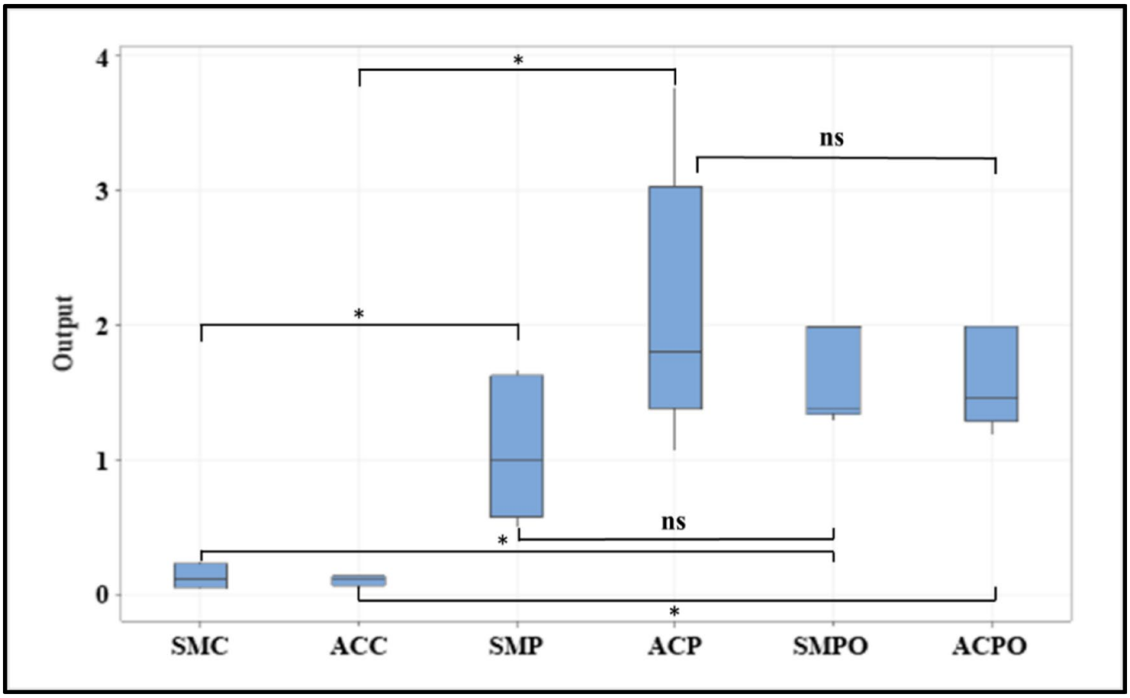


Fig. 10. post hoc test of catalase peroxidase and polyphenol oxidase activity in selected species.

45.4862 mM and 32.2093 mM for *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC. respectively, suggesting reduced substrate affinity relative to *Vigna mungo (L.)*. The corresponding *Vmax* values (10.9769 and 14.1442 µmol min^{-1} mg^{-1}protein) indicate moderate catalytic capacities, reflecting species-specific physiological stress adaptations.

Peroxidase activity also exhibited species-dependent variation. In *Eruca vesicaria* subsp. *sativa* (rocket) leaves, peroxidase activity reached 2.13 EU/mg protein[26], whereas fruit extracts of *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC. showed activities of 1.662 and 3.762 µmol·g^{-1}, respectively. Kinetic analysis using guaiacol and H_2O_2 as substrates yielded *Km* values of 100.757 and 0.02071 mM, and *Vmax* values of 0.12809 and 4.56829 µmol min^{-1} mg^{-1} protein in *Sapindus mukorossi* Gaertn. In *Acacia concinna* (Willd.) DC., the *Km* values were 82.5937 and 43.0420 mM, with *Vmax* values of 0.0966 and 0.48019 µmol min^{-1} mg^{-1} protein. When compared to rocket leaves *Km* = 375.74 mM, *Vmax* = 0.314 µmol min^{-1} mg^{-1} protein both fruit species showed lower *Km* and higher *Vmax*, indicating stronger substrate affinity and more efficient catalytic performance, possibly due to differences in tissue type and metabolic state.

Polyphenol oxidase activity also demonstrated notable interspecific variation. Artichoke (*Cynara scolymus L.*) heads exhibit exceptionally high polyphenol oxidase activity 10,400 U/ml min and kinetic parameters *Km* = 10.2 mM; *Vmax* = 19,662 U/ml min[47]. In contrast, *Sapindus mukorossi* Gaertn. and *Acacia concinna* (Willd.) DC. fruits showed lower specific activities 1.979 and 1.452 µmol·g^{-1}, respectively. *Km* values for *Sapindus mukorossi* Gaertn. 3.45237 mM and *Acacia concinna* (Willd.) DC. 0.31491 mM indicated higher substrate affinity than artichoke, but their *Vmax* values 1.415428 and 4.032258 µmol min^{-1} mg^{-1} protein were substantially lower, reflecting reduced catalytic turnover in fruit tissues.

Mechanistically, the enhanced catalase and peroxidase activities in *Acacia concinna* (Willd.) DC. may be linked to saponin-rich phytochemistry, as saponins have been reported to stimulate antioxidant enzyme expression and modulate intracellular ROS levels[48,49]. This may contribute to an efficient ROS-scavenging system and stronger oxidative defence. Conversely, the higher polyphenol oxidase activity in *Sapindus mukorossi* Gaertn. appears consistent with its phenolic-based defence mechanism, where in PPO converts phenolics into antimicrobial quinones that mitigate oxidative and pathogen-induced damage[50].

Simultaneously, the kinetic parameters *Km* and *Vmax* across species demonstrate clear biochemical signatures of physiological stress adaptation. Lower *Km* values indicate enhanced substrate affinity, while higher *Vmax* values correspond to greater catalytic efficiency both essential for rapid ROS detoxification under stress[39]. These enzymatic profiles highlight species-specific defence strategies *Acacia concinna* (Willd.) DC. relies predominantly on saponin-mediated enzymatic ROS modulation, whereas *Sapindus mukorossi* Gaertn. employs phenolic-driven oxidative defence pathways. Collectively, these mechanisms reflect adaptive metabolic responses contributing to biochemical mechanism.

Conclusion

The present study provides valuable insights into the enzymatic defence responses associated with saponin metabolism under oxidative stress in *Sapindus mukorossi* Gaert. and *Acacia concinna* (Willd.) DC.. Distinct enzymatic efficiencies were observed between the two species *Acacia concinna* (Willd.) DC. exhibited higher catalase and peroxidase activities, indicating greater physiological stress response, while *Sapindus mukorossi* Gaertn. showed elevated polyphenol oxidase activity, reflecting a phenolic-based defence mechanism. Michaelis Mention kinetics proved that, *Acacia concinna* (Willd.) DC. demonstrates greater catalytic efficiency and substrate affinity for most enzymes, indicating stronger residual enzymatic biochemical content. However, *Sapindus mukorossi* Gaertn. shows exceptional efficiency specifically in peroxidase activity using H_2O_2, suggesting it has a specialized mechanism for hydrogen peroxide detoxification. Linear Discriminant Analysis (LDA) and post hoc tests further supported these findings by revealing significant interspecific variation in enzymatic profiles. These species-specific enzymatic strategies highlight adaptive mechanisms that enhance biochemical comparison. The enhanced antioxidant enzyme activities further suggest that saponins contribute to cellular protection and stress mitigation. In physiologically such defence responses support plant survival in stress-prone environments, biotechnologically, they offer potential for developing stress-tolerant crops and utilizing saponin-rich species in ecological restoration and sustainable agriculture used for future study.

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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Rushita Parmar: Investigation and Experimental data analysis, drafting the manuscript, Vaishali Varsani: Writing-review and editing, Dushyant Dudhagara: Data curation, Visualization, Writing-review and editing, Sandip Gamit: Writing-review and editing, Nisha Naghera:Writing-review and editing Ramesh Kothari: Data analysis, and Editing, Suhas Vyas: Conceptualization, Designing, Supervision, Writing-review & editing.

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