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ORIGINAL ARTICLE

Extraction, Purification and Characterization of Antioxidant Enzyme Catalase (CAT) Present in Chilli (*Capsicum annuum*, Var. Azad Mirch-1) Plant

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In this study, extraction, partial purification and characterization of an antioxidant enzyme catalase present in chilli (*Capsicum annuum*, var. Azad mirch-1) plant. Catalase enzyme was collected from fresh leaves, fruits and roots of chilli plant in the Rama University UP, Kanpur (India). 100 g of fresh tissues were taken and grinded in the mortar pistil till the fine homogenous slurry formed. Then 10g of PVPP was added along with 50 ml buffer (Sodium phosphate, 0.1M, pH 7.2), and 1g ascorbic acid. The extracted protein was fractionated with ammonium sulphate and partially purified with the use of SG-150 gel filtration column. Chilli leaf catalase has shown its optimum activity in pH 7.2 at 30°C. The native molecular weight of catalase present in the chilli leaf was found 59 kDa. The Km value was recorded 9.2 mM for H_2O_2 and V_{max} value was found 1.41 IU/mg X 10-3 for the chilli leaf catalase. The enzyme has shown linearity after 8mg/ml enzyme concentration. Chlilli catalase was purified up to 50.83 purification fold. The catalase enzyme activity was also recorded in the chilli fruits and roots separately.

Key words: Antioxidant enzyme, catalase, enzyme activity, Specific enzyme activity, purification fold

Antioxidants are the diverse group of compounds which are used in two different ways; one is enzymes and other is antioxidant metabolites. The antioxidants are enzymes which stop the oxidation of different molecules inside living system and in industry (Roberfoid and Calderon, 1995). Oxidation is a chemical reaction that can create free radicals, leading to chain reaction that may cause cells death. These enzymes are existing in the nature in the several colourful fruits and vegetables such as guava, chilli, tomato, cauliflower etc. In the second group antioxidant compounds are known to play a vital role in the medical field and in the industry (Hobert Tietze, 1998). In this field these are used in the prevention of cancer, muscular degeneration, alzheimer's disease, arthritis etc. In the industry, antioxidants compounds are used to stop oxidation processes (Lobo *et al.*, 2010).

In nature, large numbers of antioxidants enzymes are presents in the fruits, leaf and roots of the plants. In *Rumex obtusifolius* antioxidant enzyme (polyphenol oxidase, peroxidase, catalase and superoxide dismutase) activities in the crude extract have been reported by Alici and Arabaci, 2016 (Alici and Arabaci, 2016). Similarly, Susmitha *et al.* (2014) reported about the purification of Catalase enzyme from the plant *Pleurotus ostreatus*. The report presented by Havir *et al*. (1990) about the purification and characterization of an isozyme of catalase with enhanced peroxidatic activity has been reported from the leaves of *Nicotiana sylvestris*. In 2013 the purification and characterization of catalase enzyme from *Agricus bisporus* has been reported by Susmitha *et al.* (2013). Similarly in the literature activity of catalase enzyme in plants *Geranium macrorrhium* (shoot system plant part, rhizome with roots) has been reported by Markovic *et al*. in 2015. In *Doronicum columnae* there is also catalase activity has been reported in the flower heads, stems, leaves, rhizome along with roots (Uysal *et al.*, 2018). Similarly, in *Aegopodium podagracia* antioxidant enzyme activity in the shoot, rhizome and in roots of the plant has been exposed (Uysal *et al.*, 2018). In the *Tussilago farfara* antioxidant activity in the shoot, rhizome and in roots has been discovered (Uysal *et al.*, 2018).

In the present study we have exposed the presence of catalase (EC 1.11.1.6) activity in the commonly eatable plant chilli. The aim of the study was to identify, isolate, purify and characterize the catalase present in the leaf, fruit and root of chilli plant. People are using green and ripen chilli since ancient times mainly for its antioxidant activity but the systematic knowledge of the presence of anti-oxidants especially catalase activity is not attempted so for. General reaction of catalase enzyme is mentioned below;

MATERIALS AND METHODS

The entire work has done in the department of biotechnology, Rama University, Kanpur, Uttar Pradesh, (India). Chilli plants were grown in the experimental form of the University. Plant tissues were collected from the young tissues of the plant. The enzyme catalase was extracted from the leaves, roots, and fruits of this plant in vitro conditions by using buffer (Sodium Phosphate, 0.1M, pH-7.2) and PVPP (polyvinyl pyrophosphate) 0.1% fresh weight of tissue etc. Fresh harvested tissues (100 g from each) were ground with liquid nitrogen and homogenized with buffer and PVPP. After the extraction of crude protein it was centrifuged with 10000 rpm for 30 min at 4°C. Then crude protein was fractionated with ammonium sulphate (30-50%) and desalted by the running SG-25 column. Enzyme assay Assessment of enzyme activity by the use of spectrophotometric based enzyme assay (Hadwan, 2018). Partial purification of catalase enzyme was done with the SG-150 (Sigma Aldrich) size exclusion chromatography. All the reagents such as buffer, substrates were procured from Sigma Aldrich, some chemicals such as PVPP, H_2O_2 and SDS-PAGE were brought from Thermo Fisher Scientific India Pvt. Ltd.

Catalase enzyme assay (DPPH method)

The catalase enzyme activity (spectrophotometric method) was determined by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) which is a stable free (Garcia *et al.,* 2012). The delocalization on the DPPH was confirmed

for the generation of a purple colour at the maximum absorption 240 nm. The maximum absorbance was decrease linearly according to the catalase concentration. Trolox was used as standard antioxidant. 10mM H_2O_2 was used for the enzyme activity and total enzyme assay was for 3ml. The concentration of protein was estimated by Lowry method (Lowry *et al.,* 1951) for the determination of specific activity and BSA was used as standard.

RESULTS

The fresh leaves of chilli plant (100 gm) were collected and washed twice with distilled water and grinded in the mortar pistil with the use of liquid Nitrogen till the fine homogenous slurry formed. 0.2 gm of PVPP and 3 ml buffer (Sodium Phosphate, pH 7.0) were added along with 0.1 g Ascorbic acid. Homogenate was mixed proper and stored it at 4°C for 20 min. Then homogenate was filtered through three layers of muslin cloth and squeeze out crude enzyme. Then isolated crude enzyme was centrifuged at 10000 rpm for 30 min at 4°C. Supernatant was collected and stored in 4˚C. Then the crude protein was fractionated with ammonium sulphate (30-50%) and desalted by the running SG-25 column. The fraction bearing catalase enzyme activity were pooled and subjected for partial purification with the use SG-150 size exclusion chromatography. The column was equilibrated with the use of equilibrium buffer and concentrated proteins were loaded into the column. Then the protein was eluted with the use of elution buffer. The following elution chromatogram was obtained Fig. 1.

The fractions obtained (partial purified) from the 80ml to 110ml were pooled and subjected to characterization of catalase enzyme of chilli leaf.

Kinetics of partial purified enzyme:

First of all, 0.1M Tris-HCl buffer was used to obtain the optimum pH for the enzyme but obtained result was not found significant. Next pH optimum was determined with the use of sodium phosphate buffer. We obtained significant optimum catalase activity in the pH 7.2 of 0.1M sodium phosphate buffer (Fig. 2).

Similarly, we also set up an enzyme assay to determine temperature optimum for the Chilli leaf catalase. The maximum catalase activity was obtained at 30˚C as shown in the Fig. 3 below.

For the determination of linearity of enzyme activity, we obtained that linearity was obtained at the concentration of enzyme beyond 8 mg/ml onward and it was maintained till 17 mg/ml (Fig. 4).

Native molecular weight of chilli catalase was determined with use of SG-100 gel filtration column.

In the present study molecular weight of native chilli catalase was found 59 kDa proteins as shown at Table 2 and Fig. 5.

For the calculation of Km and Vmax values, substrate saturation plot was drawn. The plot was applied to estimate the enzyme activity with the H_2O_2. The Km value was 9.2 mM for H_2O_2 and Vmax value was 1.41 IU/mg X 10^{-3} were obtained for the chilli catalase activity (Fig. 6).

The above mentioned results were for the chilli leaf along these results we also obtained the data from the fruits and roots catalase activity in this plant. The catalase activity in the fruits was found 0.958 IU/mg X 10^{-3} while in the case of roots it activity was quite low 0.105 IU/mg X 10^{-3}.

Table 1: Purification table of chilli catalase enzyme.

S.N.	Fraction	Specific enzyme activity (IU/mg X 10^{-3})	Protein (mg/ml)	Total Volume (ml)	Total Sp. enzyme activity	Yield (%)	Purification fold
1.	Crude protein	1.41	3.51	152	752.26	100	1
2.	30-50% $(NH_4)_2SO_4$ fraction	18.70	3.64	9	612.61	81.44	13.26
3.	SG-150 eluted protein	71.68	1.08	4	309.66	41.16	50.83

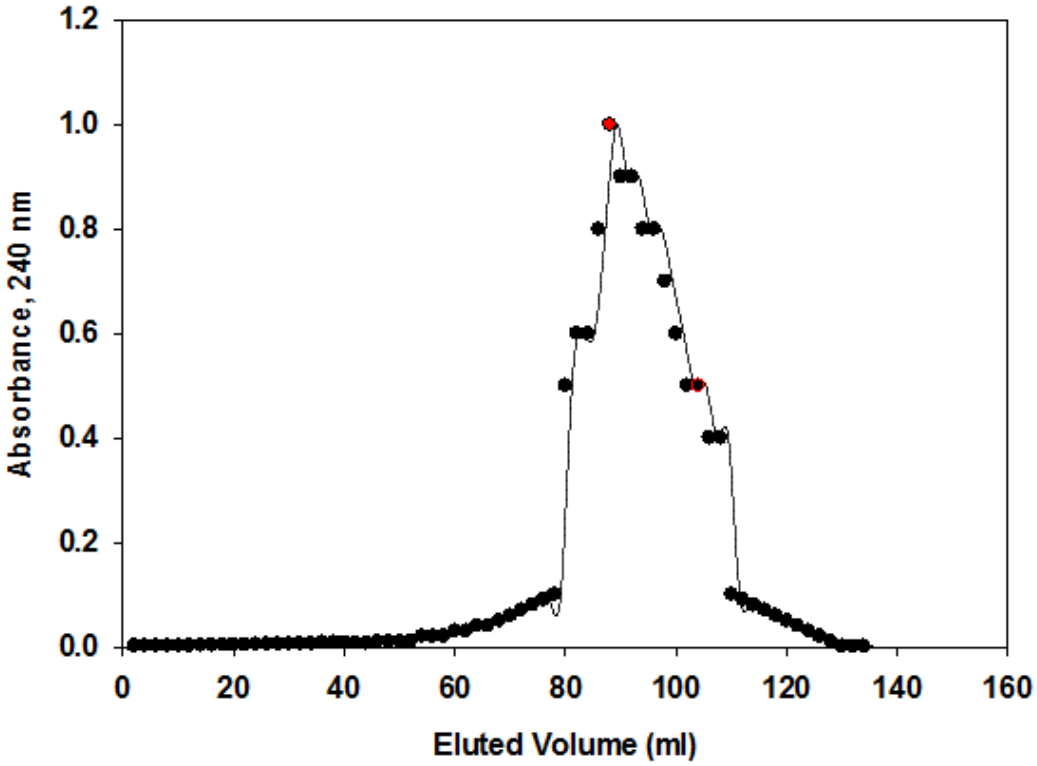

Figure 1: SG-150, chromatogram of eluted catalase protein

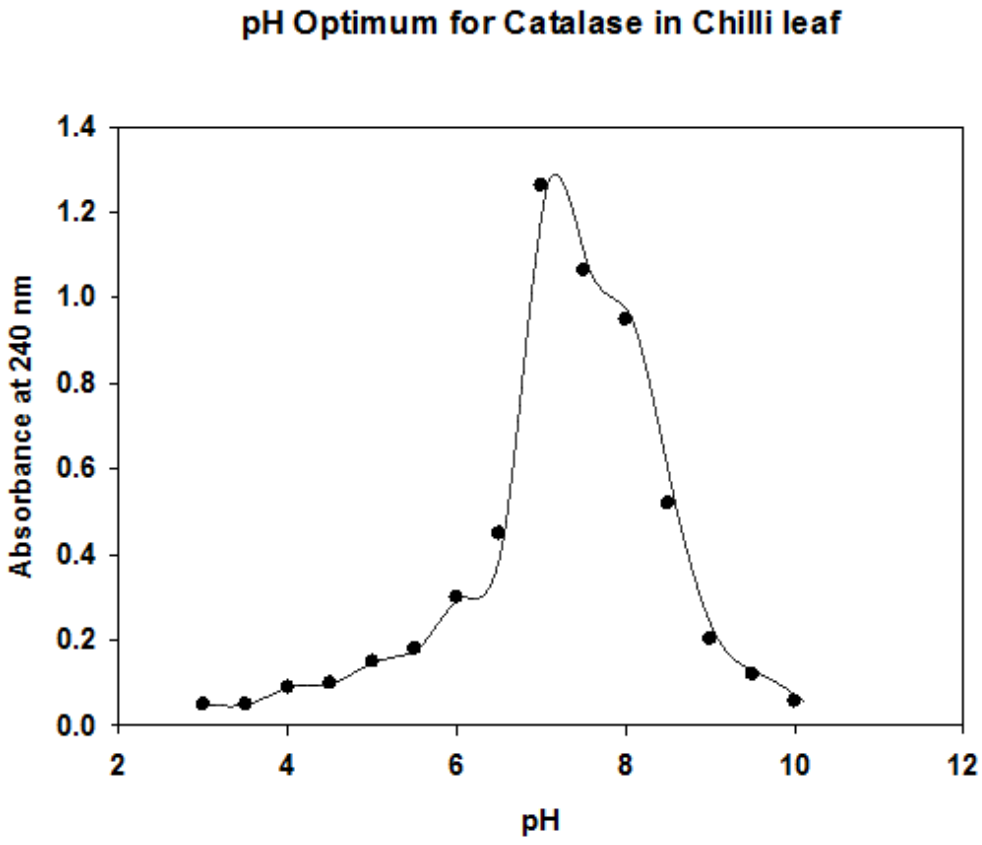

Figure 2: pH optima for chilli catalase

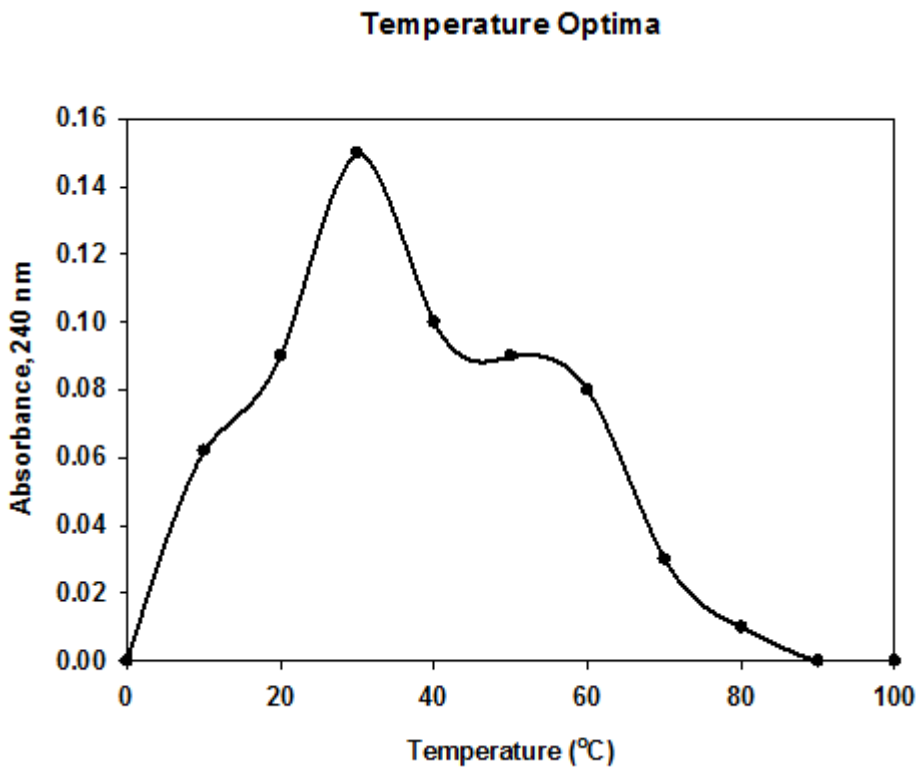

Figure 3: Temperature optima of chilli catalase

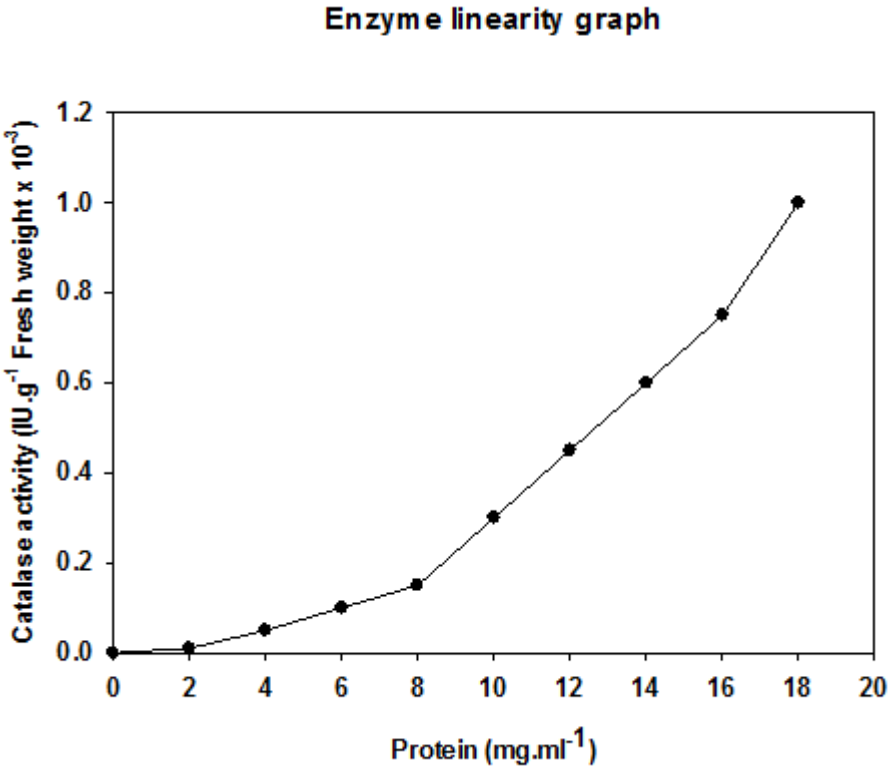

Figure 4: Establishment of linearity in chilli leaf catalase

Table 2: Determination of molecular weight with the use of SG-100 with elution of standard molecular weight marker proteins

S.N.	Standard	Eluted volume (ml)	Molecular weight (kDa)	Log $_{10}$ (MW)
1.	BSA.	74	66.0	1.81
2.	Oval albumin	78	43.7	1.64
3.	Ribonuclease A	90	13.7	1.13
4.	Chilli catalse	75	59	1.75

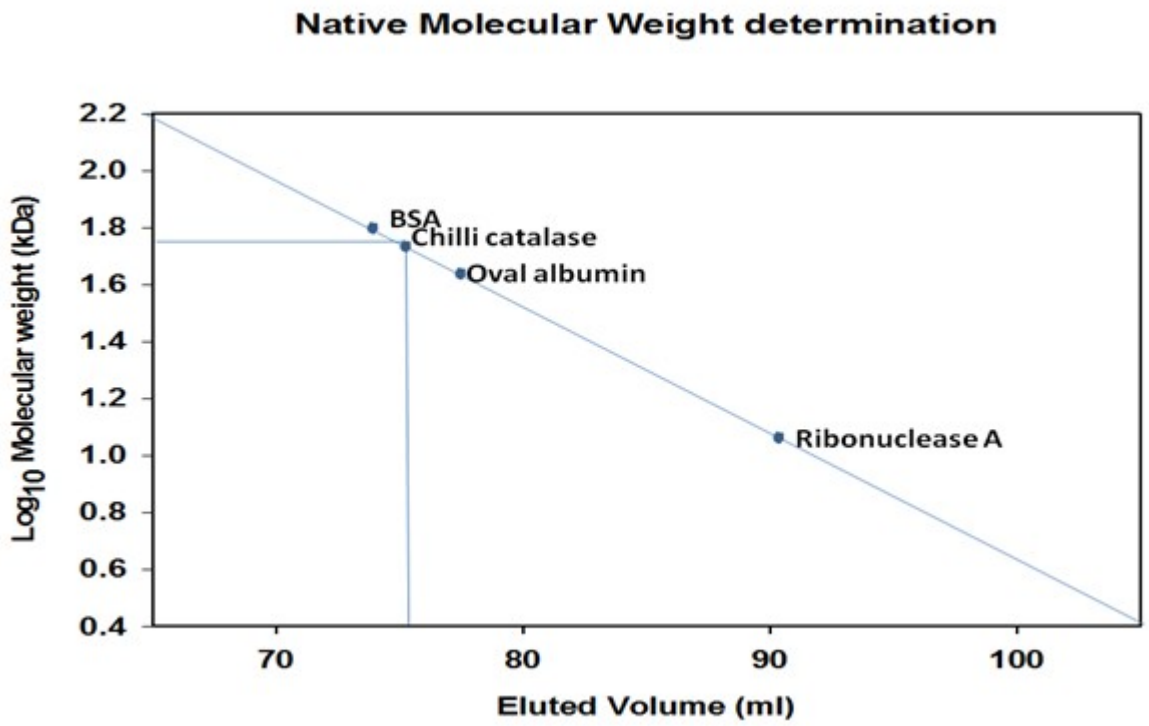

Figure 5: Graph showed molecular weight for chilli leaf catalase.

DISCUSSION

The catalase enzyme is found largely in livings system but most of the scientific studies were reported mainly in the microbes. The catalases extracted from different sources have been shown similar type of biological activities as in the present study. First report of catalase in plants was reported in *Spinacia oleracia* by Galeston *et al*., (1955). So far, none of the study has been focused on the purification and characterization of native catalase in the leaves, fruits and roots of the chilli plant. Most of the studies have been focused about the catalase under the stress conditions (Verma and Mishra, (2005) and Tiwari, (2003)) in other plants. In the present study, native catalase from chilli plant was obtained. The plant tissues were ground in the liquid nitrogen with ice cold 0.1 M Sodium phosphate buffer. About 13.26 fold of purification was achieved with the ammonium sulphate precipitation. With the use of column chromatography 85% purification fold was obtained. The obtained purification fold was 10 times more than on apple catalase (Yoruk *et al*., 2005). After the partial purification of the catalase, the enzyme activity was found very low; this might be due to presence of isoenzyme for the catalase as reported in Wheat germ (Garcia *et al*., 2000). The chilli catalase was found most active at pH 7.2 and this pH range (6.8-7.5) for the catalase had been demonstrated by most of the authors (Aebi, 1984). In this study there was a direct relationship between the enzyme concentration and the enzyme activity was

reported, this might be due to low stability of enzyme at low concentration (Jacob and Johnson, 1979). In the present study, 10mM H_2O_2 was used as a substrate and this concentration was also used to estimate the Km and Vmax values. In the present study the Km vale was found 9.2mM which is similar to Km value (6.7 mM) obtained by Tayefi-Nasrabadi *et al*. 2011. In the present study the obtained Vmax value is 0.0014 IU/mg for chilli catalase very similar Vmax value of catalase (0.0014 U/ mg) has been reported by Su and Li in 2007.

CONCLUSION

Catalase from chili leaves, fruits and roots was partially purified and characterized. The data obtained from this study will help to fill the gaps in this area. For the authentication of this study regular researches are needed due to fluctuating agronomic practices and climatic changes.

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