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Heat Tolerant Superoxide Dismutase from Therapeutically Significant *Cuminum cyminum*: Partial Purification and Kinetic Studies

Shikha Agarwal[1*], Priyanka Sharma[2] and Nidhee Chaudhary[3]

[1,2,3]Amity Institute of Biotechnology, Amity University Uttar Pradesh Sector- 125, Noida- 201313, India
E-mail: [1]shikhu1904@gmail.com, [2]priyankasharma.bscmicro@gmail.com, [3]nchaudhary@amity.edu*

Abstract—*Superoxide dismutase (SOD, EC 1.15.1.1), an antioxidant enzyme; strengthens body's primary antioxidant system playing a key role in scavenging the free radicals (disease causing agents) and helps fighting the diseases such rheumatoid arthritis, neurological disorders, Alzheimer's disease, Diabetes, Parkinson's disease, heart disease and cancer. Since the digestive tract enzymes present in human body easily deactivate SOD molecules, great need develops for bioavailable forms of SOD. In the present investigation, Cuminum cyminum (Zeera) has been studied for the presence of high specific activity of SOD. C. cyminum or Cumin seeds, used in Indian food on almost daily basis, are well known for great health benefits such as enhancing appetite, digestion, vision, strength and lactation. The specific activity of the crude enzyme extracted from C. cyminum was found to be 0.57 Units/mg. The SOD enzyme obtained has been partially purified into three fractions based on 0-30%, 30-60% and 60-90% saturation level of $(NH_4)_2SO_4$; 0-30% fraction (5.46 Units/mg) having higher specific activitywith purification fold of 9.57. The screened enzyme fraction after dialysis was further characterized on the basis of various biochemical parameters. The enzyme was found to show maximum activity at pH 8.0, temperature 30^0C in 30 minutes of reaction time. The thermal and pH stability values of the enzyme were 50^0C and 6.0-9.0, respectively. The K_m and V_{max} values were determined to be 0.284 mM and 2.142 inhibition percent ml^{-1} min^{-1}, respectively. Various ions in the form of their respective salts were used to perform inhibition studies. They were found to inhibit SOD activity in the given descending order;* $Na^+ > Mg^{2+} > Ca^{2+} > Zn^{2+}$. *The results obtained suggest that C. cyminum, a medicinal important plant, can serve as potential source of heat tolerant SOD, which further have great importance in therapeutics and other bio-industries.*

Keywords: *Cuminum cyminum, Superoxide dismutase, Heat tolerant, Antioxidant, Therapeutics.*

1. INTRODUCTION

Reactive oxygen species (ROS) are small, highly reactive, oxygen-containing molecules (free radicals) that are naturally generated in small amounts during the body's metabolic reactions [1]. These free radicals can react and damage complex cellular molecules such as fats, proteins, or DNA. The cellular damage caused by free radicals, initiates accumulation of mutations in nucleic acids sequences, which increases in number by time and finally gives rise to rapid aging, rheumatoid arthritis, heart disease, Parkinson's disease, Diabetes, Alzheimer's disease, neurological disorders and cancer [2]. Because ROS form naturally during many metabolic processes, cells have developed several protective mechanisms to detoxify the ROS. Co-operative defense systems that protect the body from free radical damage include the anti-oxidant nutrients and enzymes [3]. Antioxidant plays an important role in human health because the biological defense mechanism cannot operate under severe oxygen stress. Nature has endowed us with protective antioxidant mechanisms- superoxide dismutase (SOD), catalase, glutathione peroxidases etc, which protects us from metabolic damages [4]. Superoxide Dismutase (SOD: 1.15.1.1) an antioxidant enzyme omnipresent in all living cell inner and outer membranes acts as catalyst in dismutation of Superoxide (O_2-) radical into hydrogen peroxide and molecular oxygen and helps in scavenging these free radicals to fight against the diseases [5]. Since the digestive tract enzymes present in human body easily deactivate SOD molecules, a high need is developed of finding bioavailable forms of SOD [6]. High Intake of fruits and vegetable can promote longevity and offers a number of health benefits against degenerative diseases. Intake of medicinal plants in rats results in an increase in of activity superoxide dismutase [7]. Plant producing significant amount of SOD enzyme play crucial role against various pathological diseases. Various sources have been found to contain substantial amount of SOD viz; spices [8], dry fruits [9] and flowering plants [10]. *Cuminum Cyminum*, belongs to family Apiaceae and is extensively used in culinary practices of the Indian Subcontinent and some other Asian, African and Latin American countries as a spice [11].Cumin (zeera) seeds have been used as antipyretic,

diuretic, digestive, anti-diabetic, anti-spasmodic, blood diseases, bronchitis, piles and anti-gonorrheal in Ayurvedic medicine. The recent studies on cumin seeds and oil have shown that it is an efficient detoxifier. It removes toxins, including those which are produced by the body, such as some excess hormones and metabolic byproducts (free radicals), as well as those which get into the blood stream through food, such as uric acid, insecticides, synthetic colors, and fertilizers. It promotes sweating and urination, thereby removing the toxins with them [12]. In the present study, we have tried to explore the anti-oxidant potential of *Cuminum cyminum* in the form of SOD enzyme.

2. MATERIALS AND METHODS

C. cyminum (zeera) was purchased from the local market. All chemicals were of reagent grade and obtained from standard commercial firms.

2.1 Screening of various spices for SOD enzyme: Screening of various spices viz; *C.cyminum, Cinnamomum tamala, Myristica, Cinnamomum* Zeylanicum and *Chrysopogon Zizanioides* on the basis of their SOD activity was performed by using standard protocols mentioned below.

2.2Extraction of SOD enzyme: The preweighed and washed seeds were crushed in Phosphate buffer pH 7.0, and further centrifuged at 10,000 rpm for 15 minutes. Thefiltrate was treated as crude extract.

2.3 Protein determination: The protein was estimated by Lowry method (1951) using Bovine serum albumin (BSA) as standard [13].

2.4 SOD Assay: Superoxide dismutase (SOD) activity was determined using the protocol (NBT assay) described by Kakkar*et al.*(1984) [14]. One unit of SOD is defined as the amount of enzyme, which gave 50% inhibition of NBT reduction in one minute under standard assay conditions.

2.5 Determination of specific activity: Specific activity was determined by using thefollowing relationship:

Specific activity= Total enzyme units /Total protein (mg).

2.6 Partial purification of SOD isolated from *C. cyminum*: The crude extract was partially purified by downstreaming techniques.

2.6.1 Ammonium sulphate fractionation: The crude extract was subjected to precipitation using salting out process. Ammonium sulphate fractionation of the crude extractwas performed at three saturation levels viz; 0-30%, 30-60% and 60-90%. Ammonium sulphate was added to the extract accordingto required saturation level slowly, while keeping on ice. Theice cold solution of the protein was stirred continuously and precipitation at 0-4°C for an hour. Then the precipitate was centrifuged at 10,000 rpm for 30 min. The pellet was separated and the process was repeated for next saturation levels (30-60% and 60-90%) with the supernatant. The separated pellets were dissolved in minimum amount of sodium acetate buffer (pH 8.3, 0.17 M) and specific activity for each fraction was determined and the one having maximum value was studied further.

2.7 Kinetic and Biochemical characteristics of SOD: The dialyzed SOD enzyme was characterized with respect to the following parameters:

2.9.1 Time course: The mixture was incubated at different time intervals ranging from 10 min to 90 min to study the effect of time course on enzyme activity.

2.9.2 Temperature and pH optima: Suitable buffers of various pH values ranging from 3.0 to 11.0 were used to study the effect of pH on the enzyme activity. The optimum temperature for the enzyme activity was determined by incubating the reaction mixture in 100 mM buffer (appropriate pH) up to 90°C.

2.9.3 Temperature and pH stability: Suitable buffers of various pH values ranging from 3.0 to 11.0 were used to give shock to the enzyme for 2 hours under suitable temperature to study the effect of pH stability on the enzyme activity. The thermal stability for the enzyme activity was determined by incubating the enzyme at different temperatures for 2 hours (10-90°C).

2.9.4 Effect of varying substrate concentration: The varying substrate concentration were in the range of 0.5mM to 10mM to study the effect of varying enzyme activity.

2.9.5 Determination of Michaelis-Menten constant (K_m) and maximum velocity (V_{max}): The reciprocal values of V_0 and S_0 were plotted by Line weaver Burk method to determine the accurate values of Michaelis-Menten constant and maximum velocity.

2.9.6 Effect of metal ions: The effect of various metal ions like Cu^{2+}, Co^{2+}, Mn^{2+}, Ca^{2+}, Zn^{2+},Mg^{2+} and Na^{+} in the form of their respective salts i.e. $CuSO_4$, $CoCl_2$, $MnCl_2$, $CaCl_2$, $ZnSO_4$, $MgSO_4$ and NaCl were studied at 0.25 mM concentration.

3. RESULTS AND DISCUSSION

SOD activity has been determined spectrophotometrically for over twenty years ever since its function was first understood (McCord and Fridovich, 1969) [15].

3.1 Screening: Out of the five sources screened, the SOD enzyme isolated from the seeds of *C. cyminum* was found to have maximum specific activity of 0.57 Units/mg as illustrated in Table 1 and was further characterized with respect to various biochemical parameters.

3.2 Ammonium sulphate fractionation: Out of three fractions obtained, 0-30 % fraction was found to have highest specific activity i.e. 5.46 Units/mg as shown in table 2.

3.3 Time course: Fig. 1 illustrates the time course for the SOD-catalysed reaction showing 30 minutes to be the optimum time period

3.4 Temperature and pH optima:The enzyme was found to show maximum activity at pH 8.0 and temperature 30^0C as shown in Fig. 2 and 3, respectively.

3.5 Temperature and pH stability: Fig. 4 depicts the thermal stability curve of the isolated SOD showing activity retention up to 50^0C. The pH stability range of the enzyme was 6.0-9.0, as shown in Fig. 5. Many SODs are known to show high thermal stability may be due to hydrophobic regions of the protein. Thermostablility up to 50°C shows great potential for future research with high economic feasibility[16].

3.6Effect of metal ions: Out of various metal ions studied, Na^{+}>Mg^{2+}>Ca^{2+}>Zn^{2+}were found to be strong inhibitors in mentioned order as shown in Fig. 6. Sodium and Magnesium ions showed about 50% reduction in enzyme activity.

3.7 Determination of Michaelis-Menten constant (K_m and V_{max}):Using Lineweaver Burk plot the values of K_m and V_{max} were determined to be 0.284 mM and 2.142 inhibition percent ml^{-1} min^{-1}, respectively(Fig. 7).

Table 1: Specific activity of SOD extracted from various sources.

S. no	Plant source	Specific activity (Units/mg)
1	Cuminum cyminum (zeera)	0.57
2	Cinnamomum tamala (Tejpatta)	0.46
3	Myristica (Jaiphal)	0.42
4	Cinnamomum zeylanicum (Dalchini)	0.33
5	Piper nigrum (Black pepper)	0.31

Table 2: Specific activity of SOD obtained after salt precipitation.

S. No	Sample	Specific activity (Units/mg)
1	0-30%	5.46
2	30-60%	4.34
3	60-90%	3.39

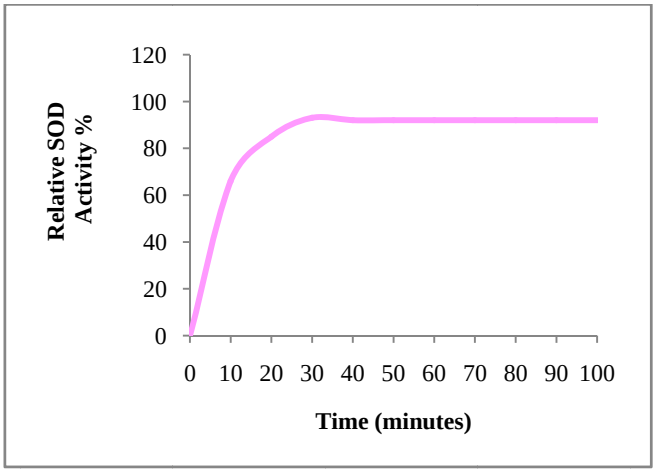

Fig. 1: Time course of SOD catalyzed reaction.

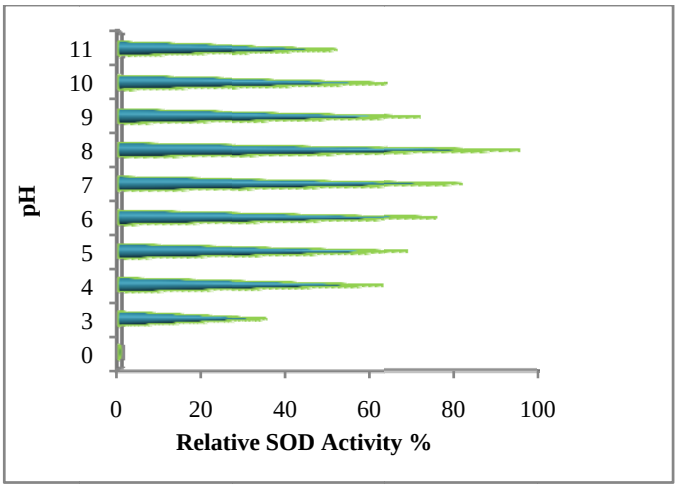

Fig. 2: Effect of pH on activity of SOD enzyme isolated from *C.cyminum* seeds.

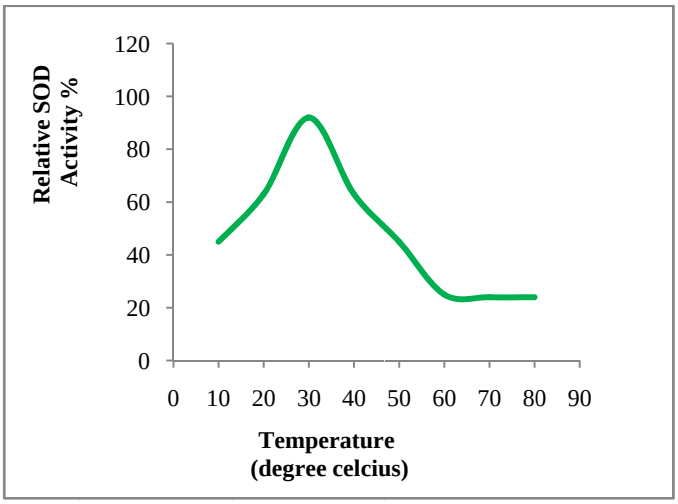

Fig. 3: Effect of temperature on activity of SOD enzyme isolated from *C.cyminum* seeds.

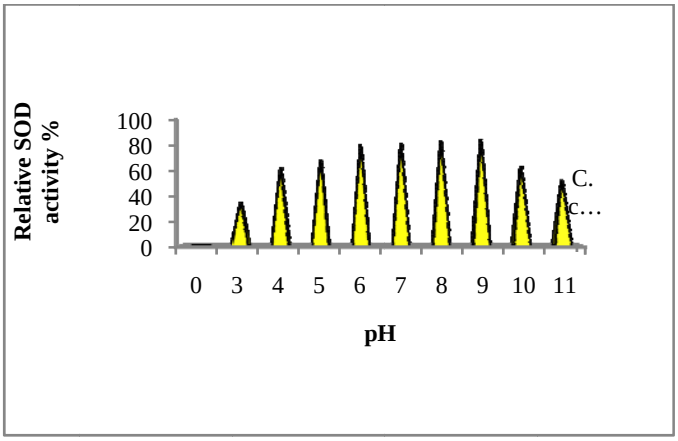

Fig. 4: pHstability curve of SODenzymeisolated from *C.cyminum*.

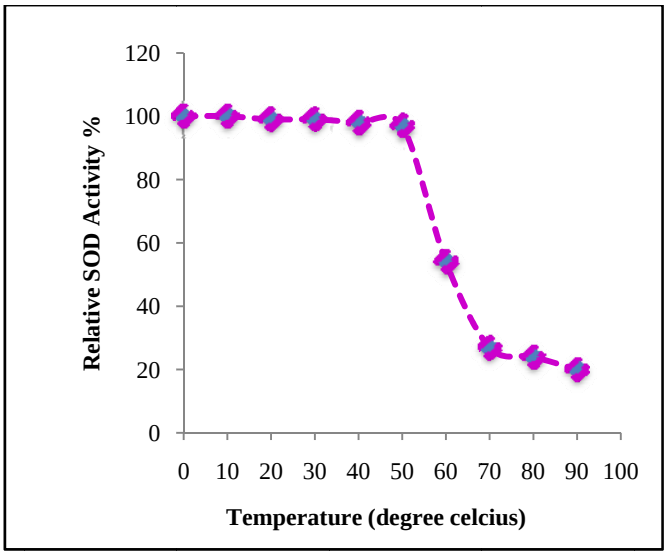

Fig. 5. Thermal stability profile of SOD enzyme isolated from *C.cyminum*after 2h of incubation.

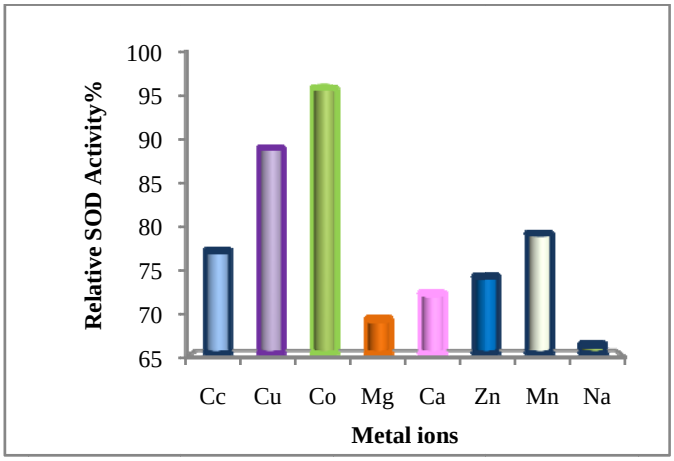

Fig. 6: Effect of metal ions on activity of SOD enzyme isolated from *C.cyminum.*

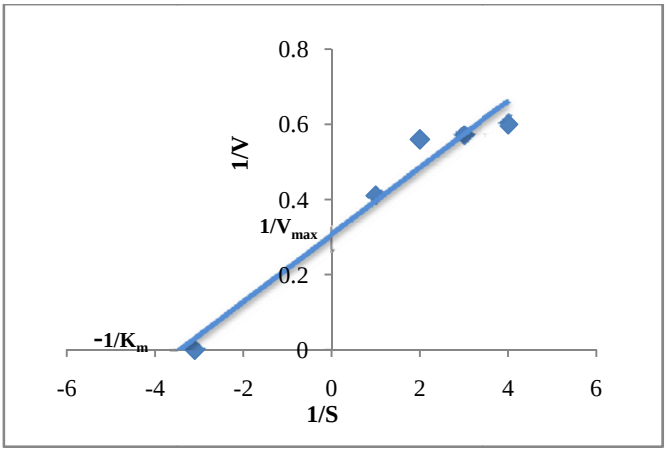

Fig. 7: Lineweaver plot showing K_m and V_{max} values of SOD isolated from *C.cyminum.*

4. CONCLUSION

According to results it has been concluded that *C. cyminum*, a therapeutically potential source of SOD enzyme used traditionally in Ayurvedic medicines for curing many diseases, act as multi-flavored spice in different cuisines which have its medicinal value which is very much beneficial for health. The thermo stability of the isolated enzyme makes it industry friendly with high economical feasibility. In this field further studies are very much require keen attention to develop and make safe supplements to cure many more infectious diseases.

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