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Study on Various Properties of Partially Purified thermostable Superoxide Dismutase Obtained from *Curcuma longa*: A Medicinally Important Spice

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Abstract—*Superoxide dismutase (SOD, EC 1.15.1.1), an ubiquitous potent antioxidant enzyme primarily scavenges superoxide radical, which causes oxidative damage to a living organism's essential proteins, lipids and its 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. In the present work, Curcuma longa; one of the most renowned medicinally important spice of India, has been studied for SOD and its properties. The crude enzyme (SOD) isolated from Curcuma longa was found to have specific activity of 17.33units/mg. SOD enzyme from C. longa was partially purified into three fractions 0-30%, 30-60%, and 60-90% based on saturation level of ammonium sulphate. The 60-90% fraction was found to have higher specific activity (51.29 U/mg) as compared to other two fractions; 51.29 units/mg. SOD enzyme was characterized further with respect to various biochemical parameters viz; pH optima, temperature optima and temperature stability for maximum SOD activity. The optimum pH and temperature conditions for maximum enzyme activity were found to be 7.0 and 30°C. The enzyme was found to be thermally stable and retained its activity upto 60°C showing its great applicability in industries with high economical feasibility. From the results obtained, it may be concluded that Turmeric (C. longa) may serve as potential source of SOD, a powerful antioxidant enzyme, which has immense importance in various bioindustries.*

Keywords: *Curcuma longa, Antioxidant activity, Superoxide dismutase, Spice, Thermostable.*

1. INTRODUCTION

Superoxide (O_2^-) is a highly reactive free radical, mainly derived from oxygen (reactive oxygen species/ROS), and is generated in our body by various endogenous systems, exposure to different pathophysiological states or physiochemical conditions. There is extensive evidence to implicate free radicals in the development of degenerative diseases, such as cancer, heart disease, Alziemer's, Parkinson's and arthritis and muscular degeneration as well as aging (Cross, 1987). Free radical damage to protein results in loss of enzyme activity and may be a contributory factor in the decline of the entire immune system (Pike, 1995). 'Antioxidants' are substances that neutralize free radicals or their actions in damaging cellular components. Nature has provided us with protective antioxidant mechanisms-superoxide dismutase (SOD), catalase, glutathione peroxidases etc., which protect us from metabolic damages. Superoxide dismutase is powerful antioxidant enzyme which catalyzes the dismutation of the superoxide radical into hydrogen peroxide and molecular oxygen and once in circulation in the bloodstream, these powerful antioxidants go to work detoxifying potentially harmful substances such as free radicals and reducing oxidative stress that might otherwise contribute to aging and various other diseases (Vouldoukis, 2004). Therefore, research on SOD activity is important in understanding of various mechanism of life. Identification of antioxidants from various natural plant sources is a highly active research area to replace the synthetic antioxidants, to improve food quality and stability, and reduce the risk of various chronic diseases. High Intake of fruits and vegetable offers a number of health benefits against degenerative diseases and can promote longevity. Intake of medicinal plants in rats results in an increase in antioxidant enzyme activity (superoxide dismutase) which reduces the risk of various inflammatory and heart disease (Choi, 2005). Medicinal plants increase the activity of superoxide dismutase which demonstrates anti-oxidative effects and therefore, may be used in disorders associated with oxidative stress (Gometi, 2014). Therefore, there is need to get the enzyme as a oral supplement in condition of starvation of the enzyme in the metabolism. Various sources have been found to contain substantial amount of SOD viz; spices (Kaur et al 2013; Chaudhary et al., 2012), dry fruits (Chaudhary et al., 2013) and other plants (Chaudhary et al., 2012).

C. longa (Turmeric) is a very rich source of biologically active compounds. It has been demonstrated to promote multiple health benefits. In Indian traditional system of medicine (Ayurveda) the plant is used as antipyretic, laxative, diuretic, digestive, antidiabetic, anti-snake venom, antileprotic, blood

diseases, bronchitis, piles and anti-gonorrheal (Kirtikar, 1987). The Chinese use turmeric as traditional medicine to treat diabetes and is also used as poultice for broken bones (Pee, 1995). Some of the recent studies on Turmeric have shown distinct health benefits which include reduce blood pressure, cholesterol, blood sugar and weight, increase immunity, treat anemia, gastro-intestinal tract disorders. It also provides other benefits from its antioxidant, anti-inflammatory and anti-cancer properties. Keeping in view of the immense importance of the plant this work has been undertaken to explore its potential for SOD enzyme.

2. MATERIALS AND METHODS

Plant materials were obtained from Navdanya Pvt. Ltd., New Delhi. All chemicals were of reagent grade and obtained from standard commercial firms.

2.1. Extraction 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 mins. The filtrate was treated as crude extract.

2.2. Partial purification of SOD enzyme from C. longa: Ammonium sulphate fractionation of the crude extract was performed at three saturation levels viz; 0-30%, 30-60% and 60-90%. Ammonium sulphate was added to the extract according to the required saturation level slowly, while keeping on ice. The ice cold solution of the protein was stirred continuously for further 1 h and then centrifuged at 14,000 rpm for an hour. The pellet was separated and the process was repeated for next saturation level in similar fashion. The separated pellets were dissolved in minimum amount of sodium phosphate buffer (pH 8.3, 0.05 M).

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

2.4. SOD Assay: Superoxide dismutase (SOD) activity: Superoxide dismutase (SOD) activity was determined using the protocol (NBT assay) described by Kakkar *et al.* (1984). 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 the following relationship:

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

2.5. Biochemical characterization of SOD: The crude SOD enzyme was characterized as follows:-

2.5.1. Temperature and pH optima: Suitable buffers (100 mM) 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.

Temperature stability: The thermal stability for the enzyme activity was determined by incubating the enzyme at different temperatures for 2 hours (10-90°C).

3. RESULTS AND DISCUSSION

3.1 Screening: Various therapeutically important plants for Superoxide Dismutase (SOD) with high specific activity were screened and *C. longa* has found to possess higher activity than others as illustrated in Table 1. Therefore, it has been used as SOD source in further studies. SOD enzyme isolated from *C. longa* was found to have specific activity of 17.33 units/mg. Further characterization was performed with respect to various biochemical parameters viz; pH optima, temperature optima and temperature stability for maximum SOD activity.

3.2 Partial purification of SOD enzyme from C. longa: Superoxide Dismutase extracted from *C. longa* has been partially purified into three saturation levels of ammonium sulphate viz; 0-30%, 30-60% and 60-90% with purification levels being, 1.62-fold, 1.83-fold and 2.96-fold respectively as illustrated in Table 2. The 60-90% $(NH_4)_2SO_4$ fraction having the highest specific activity.

3.3 Biochemical characterization of partially purified SOD from *C. longa:* Many SODs from various plant sources have been characterized using various parameters viz; *Capsicum annuum* (Kaur et al., 2013), *Jatropha curcas* (Chaudhary et al., 2012; Chaudhary et al., 2012), *Radix lethospermi* (Haddad et al., 2005), Spinach (Asada et al., 1973), tobacco (Sheng et al., 2004) *Haberlea rhodopensis* (Apostolova et al., 2012) *Amaranthus spinosus* (Sharma et al., 2014) and dry fruits *Juglans regia* and *Ribes nigrum* (Chaudhary et al., 2013; Chaudhary et al., 2013).

3.3a Temperature and pH optima: The optimum pH and temperature conditions for maximum enzyme activity were found to be 7.0 and 30°C (Kaur et al., 2013; Chaudhary et al., 2013; Kaur et al., 2013) and enzyme retained most of its activity in the range of 4.0-7.0. It can thus be concluded that a positive

charge area in the active site region of an enzyme is very important for the electrostatic facilitation of the catalyzed dismutation reaction (Donnelly et al., 1989; Mavelli et al., 1983; Benov et al., 1994; Benovic et al., 1983). The positive charge on the surface, in combination with electrostatic repulsion by negatively charge areas on the surface, serves to guide $O_2^{\cdot-}$ radicals to active site channel.

3.3b Temperature stability: The enzyme was found to be thermally stable and retained its activity upto 60°C (Kaur et al., 2013; Chaudhary et al., 2013; Chaudhary et al., 2013; Kaur et al., 2013). Many SOD's are known to have high thermal stability (Trainer et al., 1982; Rao et al., 1988). It is therefore, suggested that the hydrophobic regions of a protein plays an important role in thermal stability (Mozheav, 1993).

Table 1: Specific activity of SOD isolated from various spices

S. No.	Sample Name	Specific Activity*(units/mg)
1	Turmeric (C. longa)	17.33
2	Fennel (F. vulgare)	3.40
3	Nigella (N. sativa)	4.67
4	Ginger (Z. officinale)	10.03
5	Tejpatta (L. nobilis)	3.12

Table 2: Specific activity and purification-fold of various fractions of SOD obtained from

Sample	Specific Activity (units/mg)	Purification-fold
Crude	17.33	1.0
0-30% (NH4)2SO4 fraction)	28.07	1.62
30-60% (NH4)2SO4 fraction)	31.71	1.83
60-90% (NH4)2SO4 fraction)	51.29	2.96

C. longa after ammonium sulphate precipitation

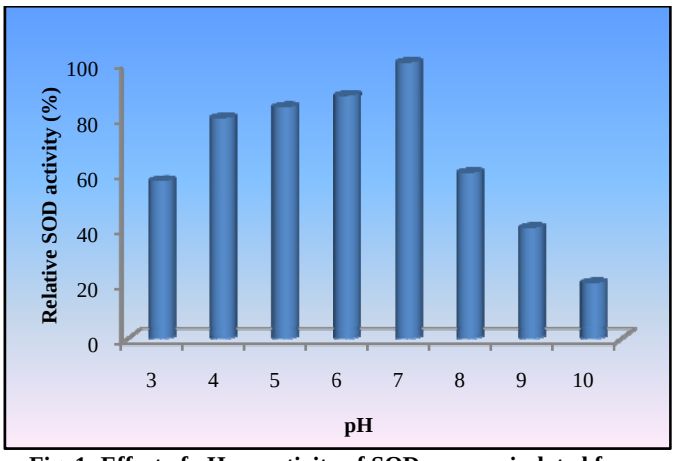

Fig. 1: Effect of pH on activity of SOD enzyme isolated from *C. longa.* Suitable buffers (100 mM) of various pH values ranging from 3.0 to 11.0 were used to study the effect of pH on the enzyme activity.

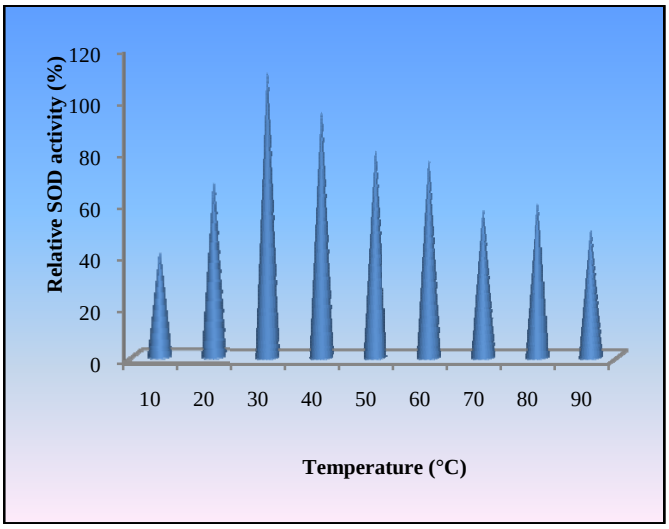

Fig. 2: Effect of temperature on activity of SOD enzyme isolated from *C. longa.* The optimum temperature for the enzyme activity was determined by incubating the reaction mixture in 100 mM buffer at temperature ranging from 10°C to 90°C.

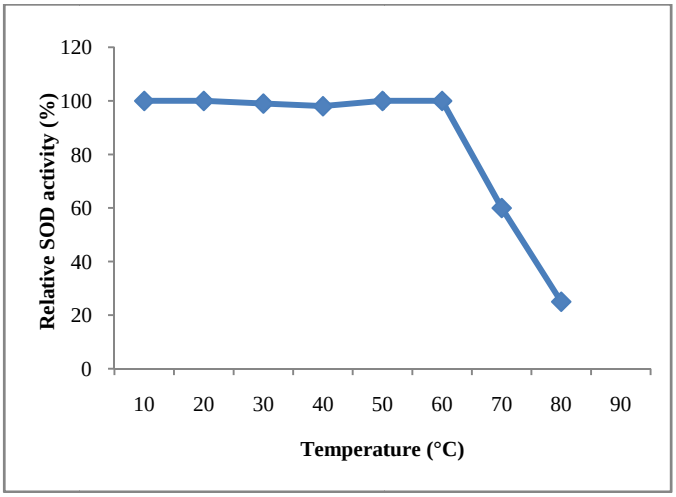

Fig. 3: Thermal stability of SOD enzyme isolated from *C. longa*. The thermal stability for the enzyme activity was determined by incubating the enzyme at different temperatures (shown) for 2 hours (10-90°C),followed by assay under standard conditions.

4. CONCLUSION

It can be inferred from the results obtained that *C. longa* is a potential source of SOD; an antioxidant enzyme, which is of immense importance in cosmetics and pharma industry amongst others. Further studies are required in this direction to establish and develop safe food supplements, therapeutics and cosmetic products.

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