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Biochemical Aspects of Superoxide Dismutase Isolated from *Amaranthus spinosus*: A Therapeutically Important Plant

S Sharma, S Bahuguna, N Kaur and N Chaudhary

Amity Institute of Biotechnology, Amity University Uttar Pradesh
Sector-125, Noida-201303, India

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, Amaranth; a highly nutritious grain of Indian subcontinent, has been studied for the presence of SOD activity. The crude enzyme (SOD) isolated from the seeds of *Amaranthus spinosus* was found to have specific activity of 0.54 units/mg and was characterized further with respect to various biochemical parameters viz; pH, temperature, time, pH stability, temperature stability, for maximum SOD activity. The enzyme was found to show its maximum activity at temperature and pH conditions corresponding to 30^0C and 8.0, respectively, in 30 minutes of incubation period. Temperature and pH stability was found to lie between 20-40^0C and 6.0-9.0, respectively. The results obtained suggest that *Amaranthus spinosus*, a traditional valued food and a medicinal source in India, may also act as potential source of SOD enzyme, an antioxidant, which further has great importance in therapeutics.

Keywords: *Amaranthus spinosus*, Antioxidant activity, Superoxide dismutase.

1. Introduction

Superoxide (O_2^-) are highly reactive free radicals, which are mainly derived from oxygen (reactive oxygen species/ROS), and are generated in our body by various endogenous systems, exposure to different physiochemical conditions or pathophysiological states. There is extensive evidence to implicate free radicals in the development of degenerative diseases, such as cancer, heart disease, Alzheimer's, Parkinson's and arthritis and macular degeneration as well as aging (Cross, 1987). Free radical damage to protein can result in loss of enzyme activity and may also be a contributory factor in a progress decline in the function of the immune system (Pike, 1995). 'Antioxidants' are substances that neutralize free radicals or their actions in damaging cellular components. Nature has endowed us with protective antioxidant mechanisms- superoxide dismutase (SOD), catalase, glutathione peroxidases etc, which protects 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 may reduce the risk of 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 an 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, 2013; Chaudhary, 2012), dry fruits (Chaudhary, 2013) and flowering plants (Chaudhary, 2012).

A. spinosus has been 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 venum, antileprotic, blood diseases, bronchitis, piles and anti-gonorrheal (Kirtikar, 1987). The Chinese use *A. spinosus* as traditional medicine to treat diabetes and seeds used as poultice for broken bones. Some tribes in India apply *A. spinosus* to induce abortion. *A. spinosus* is known as chaulai in states of Uttar Pradesh and Bihar. It is a genus of annual or short-lived perennial plants. People around the world value amaranths as leaf vegetables, cereals, and ornamental plants. Amaranthus also reported to contain beta-carotene, thiamine, riboflavin, niacin and ascorbic acid. Carotenoids serve as precursors of vitamin A, show antioxidant activity (Pee, 1995).

Some of the recent studies on amaranth seeds, seed oil and leaves 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 anti-cancer. 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 minutes. The filtrate was treated as crude extract.

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

***2.3. 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.

2.4. 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.

2.5.2. Temperature and pH stability: Suitable buffers (100 mM) 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).

3. Results and Discussion

3.1 Screening: Various therapeutically important plants for Superoxide Dismutase (SOD) with high specific activity were screened and *Amaranthus spinosus* 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 the seeds of *Amaranthus spinosus* was found to have specific activity of 0.54 units/mg. Further characterization was performed with respect to various biochemical parameters viz; pH, temperature, time, pH stability, temperature stability, for maximum SOD activity.

3.2 Time course: The enzyme was found to show its maximum activity under 30 minutes of incubation period.
3.3 Temperature and pH optima: Maximum activity of SOD enzyme was observed at temperature and pH conditions corresponding to 30^0C and 8.0, respectively.
3.4 Temperature and pH stability: Enzyme was found to be stable between 20-40^0C temperature and pH ranging from 6.0 to 9.0.

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

S. No.	Plant Source	Specific Activity (Units/mg)
I	*Amaranthus spinosus*	0.54
II	*Petroselinum crispum*	0.35
III	*Cymbopogon citratus*	0.24
IV	*Psoralea corylifolia*	0.21
V	*Ocimum sanctum*	0.12

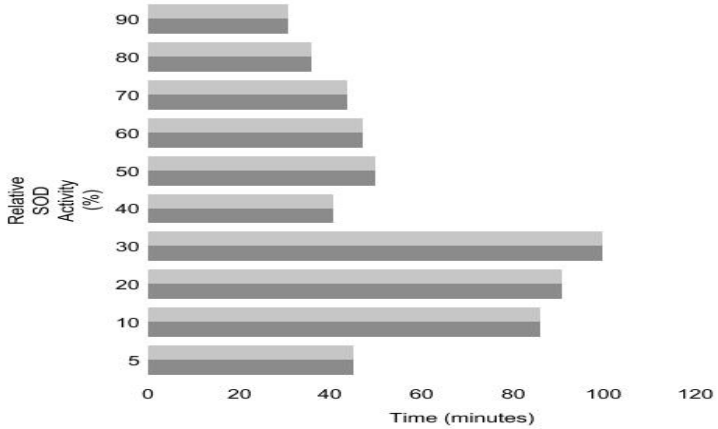

Figure 1: *Time course of SOD catalyzed reaction.*

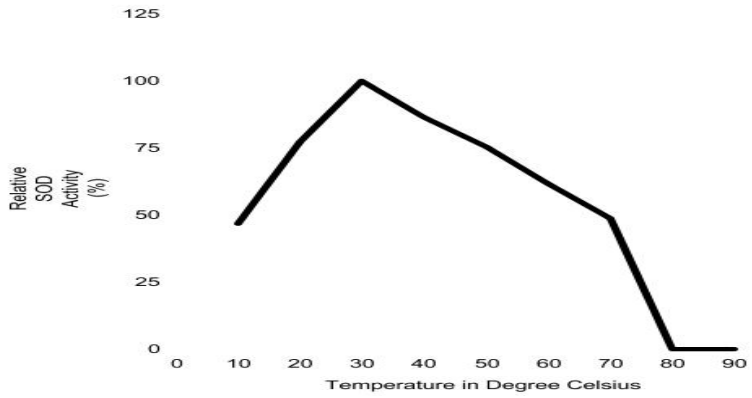

Figure 2: *Effect of temperature on activity of SOD enzyme isolated from A. spinosus seeds*

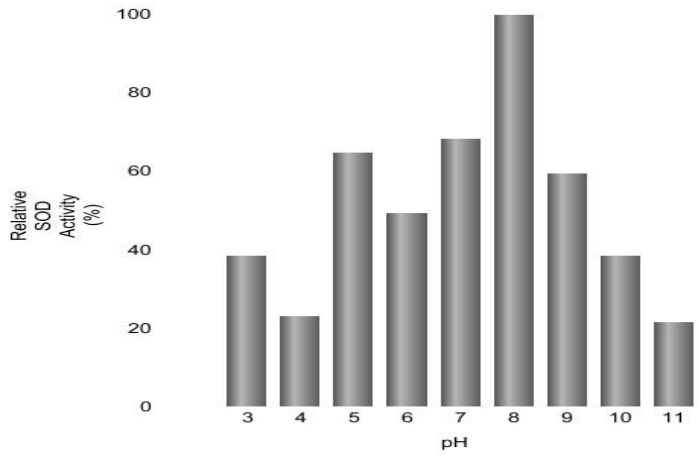

Figure 3: *Effect of pH on activity of SOD enzyme isolated from A. spinosus seeds.*

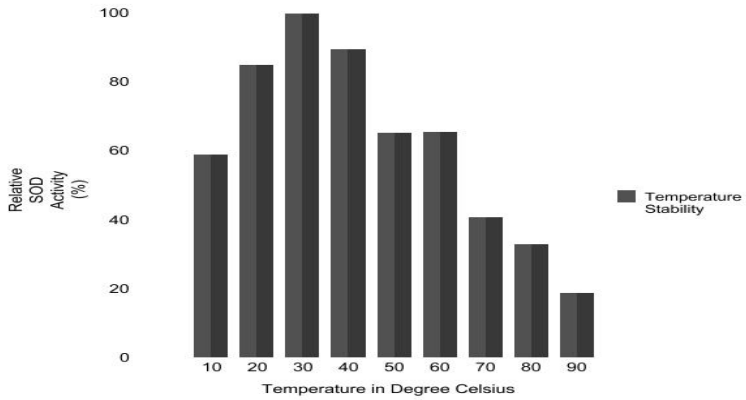

Figure 4: *Thermal stability of SOD enzyme isolated from A. spinosus seeds after 2 hrs. of incubation.*

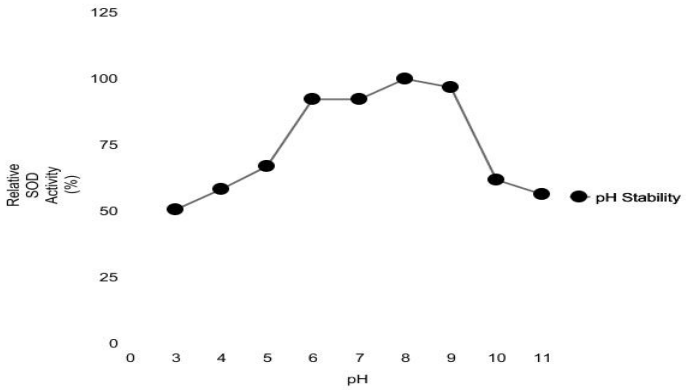

Figure 5: *pH stability of SOD enzyme activity isolated from A. spinosus seeds after 2 hrs. of incubation.*

Conclusion

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

References

[1] A S Gometi, V N Ogugua, P E Joshua, C E Odo, F O Nduka and I O Orhonigbe (2014), Anti-oxidative potentials of some medicinal plants on lipid peroxidation in alloxan-induced diabetic rats, *Afri. J. Bio.*, **13**, *1*, pp. 156-162.

[2] C E Cross (1987), Oxygen radicals and human disease, *Annal. Int. Med.*, **107**.

[3] E M Choi and J K Hwang (2005), Effect of some medicinal plants on plasma antioxidant system and lipid levels in rats, *Phytother Res.*, **19,** *5*, pp. 382-286.

[4] H Lowry, J Rosebrough, L Farr and J Randall (1951), Protein measurement with the Folin pheol reagent, *J. Biol. Chemistry,* **193**, pp. 265-275.

[5] I Vouldoukis, M Conti, P Krauss, et al. (2004), Supplementation with gliadin-combined plant superoxide dismutase extract promotes antioxidant defences and protects against oxidative stress, *Phytother. Res.,* **18**, pp. 957-962.

[6] J Pike and R K Chandra (1995), Effect of vitamin and trace element supplementation on immune indices in healthy elderly, *Int. J. Vit. Nutr. Res.*, **65**, pp. 117-120.

[7] K R Kirtikar and B D Basu (1987), Indian Medicinal Plants, *Inter- national book distributors*, Dehra Dun, India, **3,** pp. 2057-59.

[8] N Kaur, S Chand and N Chaudhary (2013), Characterization of Superoxide Dismutase Isolated from Culinary-Medicinal Indian Chilli (*Capsicum annuum L.*), *Protein Science*, **22,** *1*.

[9] N Chaudhary, S Chand and N Kaur (2013), Some Aspects of Thermostable Superoxide Dismutase Isolated from *Juglans regia* and *Ribes nigrum:* Therapeutically Important Dry Fruits, *Protein Science,* **22,** *1*.

[10] N Chaudhary, S Chand and N Kaur (2013), Kinetic and Biochemical Parameters of Thermostable Superoxide Dismutase isolated from *Ribes nigrum*: A Functional Food, *GSTF Journal of Biosciences,* **2**, *2*.

[11] N Chaudhary, N Kaur, N Jabalia and H Bansal (2012), Biochemical investigations on thermostable superoxide dismutase isolated from *Jatropha curcas* stem, *International Journal of Environmental Engineering and Management,* **3**, *5*, pp. 323-326.

[12] N Chaudhary, N Kaur, N Jabalia and H Bansal (2012), Studies on thermostable superoxide dismutase enzyme isolated from *Zingiber officinale* peel: A therapeutically important enzyme, *Journal of Proteins and Proteomics,* **3,** *2*.

[13] N Chaudhary, N Kaur , N Jabalia and H Bansal (2012), Studies on biochemical aspects of thermostable superoxide dismutase isolated from *Jatropha curcas* root, *Biologix,* **1**, pp. 173-176.

[14] S B Kakkar and P Viswanathan (1989), A modified spectrophotometric assay of superoxide dismutase, *Indian J. Biochem. Biophysics*, **21**, pp. 130-132.

[15] S D Pee , C E West, D M Daryadi and J G A J Hautvast (1995), Lack of improvement in vitamin A Status with increased consumption of dark green leafy vegetables, *Lancet*, **346**, pp. 75-81.