International Journal of Basic and Applied Biology
Print ISSN: 2394-5820, Online ISSN: 2349-2539, Volume 2, Number 5; April-June, 2015, pp. 315-318

http://www.krishisanskriti.org/ijbab.html

# Thermostable Superoxide Dismutase of Medicinally Important *Buchanania lanzan*: Partial Purification and Characterization

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**Abstract—***Oxidative stress is mainly caused by the free radicals, which stabilizes itself through electron pairing with biomolecules (proteins, DNA, lipids) and result in their damage. This damage results in various diseases such as Parkinson's disease, neurological disorders, aging and cancer. Human cells are competent enough to protect themselves against free radical damage but sometimes antioxidant supplements are required to reduce this damage. Dry fruits are important sources of dietary nutrients and antioxidants. Superoxide dismutase (SOD) is considered as one of the body's internal most powerful antioxidant. In the present work, Buchanania lanzan (Chironji); a dry fruit (seed), has been studied for the presence of SOD. Seeds are used as blood purifier to cure digestive disorders and various other medicinal uses. The specific activity of crude enzyme isolated from B. lanzan was found to be 0.52 units/mg. The enzyme was partially purified by salt fractionation into three parts; 0-30%, 30-60% and 60-90% based on $(NH_4)_2SO_4$ saturation level; out of which 0-30% showed the highest specific activity (4.72 units/mg) with purification fold of 9.07-. The dialyzed enzyme was further characterized on the basis of various biochemical parameters. The maximum activity was found at pH and temperature conditions corresponding to 8.0 and $30^0$C, respectively, in 30 minutes of incubation period. The pH and temperature stabilityvalues were 6.0-9.0 and $55^0$C, respectively. The $K_m$ and $V_{max}$ values were determined to be 0.185 mM and0.142 units/min/ml, respectively. Out of various metal ions tested $Mn^{2+}>Zn^{2+}>Na^+$ were found to be strong inhibitors in mentioned order. The results obtained suggest that Buchanania lanzan may act as potential source of SOD enzyme, an antioxidant, which further has many applications in various bio-industries.*

**Keywords:** *Buchanania lanzan, Superoxide dismutase, antioxidant.*

## 1. INTRODUCTION

*Buchanania lanzan* is a widely used plant with medicinal uses for various diseases. It is commonly known as chironji, charoli and found mostly in North western parts of India. It has various uses in improving immunity,diarrhea [1], respiratory problems, purifying blood and enhancing life span [2]. Its roots, fruits, seeds and leaves are useful for many medicinal purposes. Regular intake of chironji seeds are helpful in improving health and prevent many diseases. Methanolic root extract of *B. lanzan* with a focus on antimicrobial and anti-biofilm properties has wound healing activity [3]. Chironji oil is an effective remedy for treating problems relating to the reproductive system. The seeds of *B.lanzan* have aphrodisiac properties and hence are useful in treating sexual and reproductive problems such as premature ejaculation, impotence and loss of libido [4]. As is has been used in treating many diseases and have various health benefits the plant is found to be good candidate and therefore used as Superoxide dismutase (SOD) source. Normal metabolic processes such as the catalytic transformation of various molecules by enzymes result in production of Superoxide radicals, or anions (negatively charged atoms) which are produced when oxygen gains an excess electron [5]. The excess of reactive species result in damage of cell lipids, proteins and DNA by oxidative action, which further result in loss of function and even cellular death [6], which has linked some diseases with oxidative stress [7]. Superoxide anions are strongly implicated in the development of numerous degenerative diseases, including atherosclerosis [8], stroke [9], heart attack, chronic and acute inflammatory conditions, and various age-related disorders [10]. Antioxidants really act as bodyguard to prevent cells against oxidative stress. SOD is one of the body's primary internal antioxidant defenses and plays a critical role in reducing oxidative stress and various diseases. SOD2 has been proposed as a potential marker and a useful tool to predict metastatic potential of various cancer [11].Sometimes endogenous antioxidants are not able to prevent oxidative damages and hence require the supply of exogenous antiradicals. Thus, intake of dietary sources may support the prevention of oxidative stress .Various sources have been found to contain substantial amount of SOD viz; spices [13],dry fruits [14] and flowering plants [15]. SOD obtained from seeds of this plant may be a potential candidate for targeting many diseases. To live longer and enjoy better health, one of the most important way is to increase you SOD levels. Because of various benefits and importance of this enzyme the work has been undertaken to explore the potential of SOD enzyme.

## 2. MATERIALS AND METHODS

*B.lanzan* (Chironji) seeds were obtained from certified shop. 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 inphosphate 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. Partial purification of SOD isolated from *Buchanania lanzan:*** *Buchanania lanzan* extract was partially purified by downstreaming techniques.

*2.5.1 Ammonium sulphate fractionation*: crude extract was fractioned into 3 parts corresponding to 0-30%, 30-60% and 60-90% based on saturation level of ammonium sulphate. Specific activity of the fractions was calculated and the one having maximum value was studied further.

**2.6. Biochemical characterization of SOD:**The dialyzed SOD enzyme was characterized as follows:-

**2.6.1. 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.

**2.6.2. 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).

**2.6.3. Effect of various metal ions:** The effect of various metal ions like $Cu^{2+,}$ $Co^{2+,}$ $Mn^{2+,}$ $Ca^{2+,}$ $Zn^{2+,}$ $Na^{+}$, $Mg^{2+}$ as their respective salts $CuSo_4$, $CoCl_2$, $MnCl_2$, $CaCl_2$, $ZnSo_4$, NaCl, $MgSo_4$ were studied at 0.25 mM concentration.

**2.6.4.Effect of varying substrate concentration:** The varying substrate concentration in the range of 0.050 mM and 0.508 mM was used to study the effect on enzyme activity. The Michaelis-Menten constant (Km) and maximum velocity were calculated by using Lineweaver Burk plot (1/v vs 1/s).

## 3. RESULTS AND DISCUSSION

**3.1 Screening:**Various dry fruits were screened for Superoxide Dismutase (SOD) with high specific activity and *Buchanania lanzan* was 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 *Buchanania lanzan* was found to have specific activity of 0.52 units/mg. Further characterization was performed with respect to various biochemical parameters viz; pH, temperature, time, pH stability, temperature stability, effect of metal ions, effect of varying substrate concentration for maximum SOD activity.

**3.2 Ammonium sulphate fractionation:**Out of three fractions obtained after ammonium sulphate fractionation, 0-30 % fraction was found to have highest specific activity i.e. 4.72 units/mg as shown in Fig. 1.

**3.3 Time course:**The enzyme was found to show its maximum activity under 30 minutes of incubation period (Fig. 2).

**3.4 Temperature and pH optima*:*** Maximum activity of SOD enzyme was observed at temperature and pH conditions corresponding to 30°C and 8.0, respectively as shown in figures 3 and 4, respectively.

**3.5 Temperature and pH stability*:*** Enzyme was found to be stable up to 55°C temperature and pH ranging from 6.0 to 9.0 as shown in figures 5 and 6, respectively.

**3.6Effect of metal ions:** Out of various metal ions tested, $Mn^{2+}$>$Zn^{2+}$>$Na^{+}$ were found to be strong inhibitors in mentioned order as shown in Fig. 7.

**3.7 Determination of Michaelis-Menten constant ($K_m$ and $V_{max}$):** Using Lineweaver Burk plot thevalues of $K_m$ and $V_{max}$ were determined to be 0.185 mM and 0.142 units/min/ml (Fig. 8).

**Table 1: Specific activity of SOD extracted from various dry fruits.**

| Dry fruits | Specific activity(units/mg) |
|---|---|
| ***Buchanania lanzan*** **(Chironji)** | 0.52 |
| ***Phoenix dactylifera*** **(Khajoor)** | 0.36 |
| ***Vitis vinifera*** **(Raisins)** | 0.29 |
| ***Ficus carica*** **(Anjeer)** | 0.24 |
| ***Juglans*** **(Walnuts)** | 0.20 |
| ***Prunus dulcis*** **(Almonds)** | 0.15 |
| ***Anacardium occidentale*** **(Cashewnut)** | 0.08 |

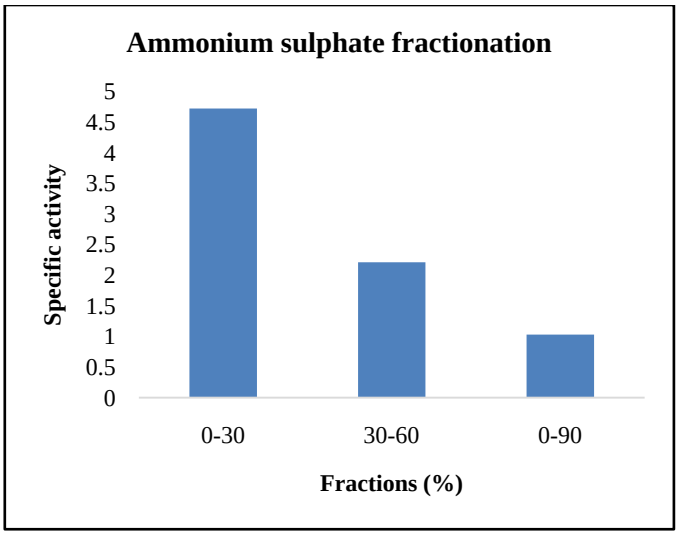


**Fig. 1: Specific activity of SOD fractions obtained afterammonium sulphate fractionation.**

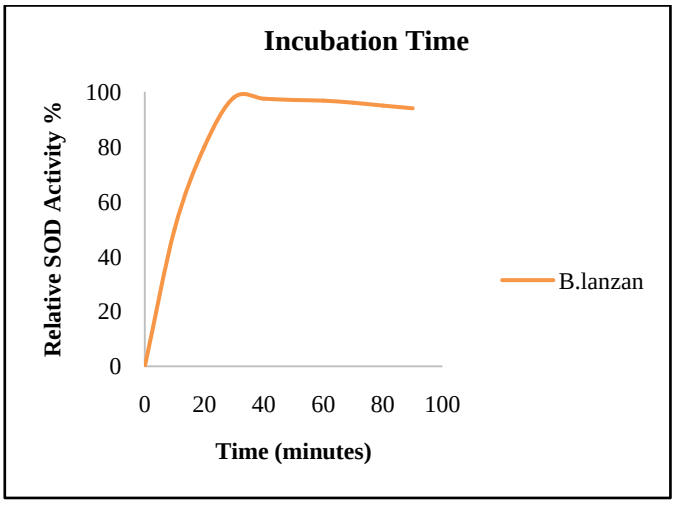


**Fig. 2: Time course of SOD activity isolated from *B. lanzan.***

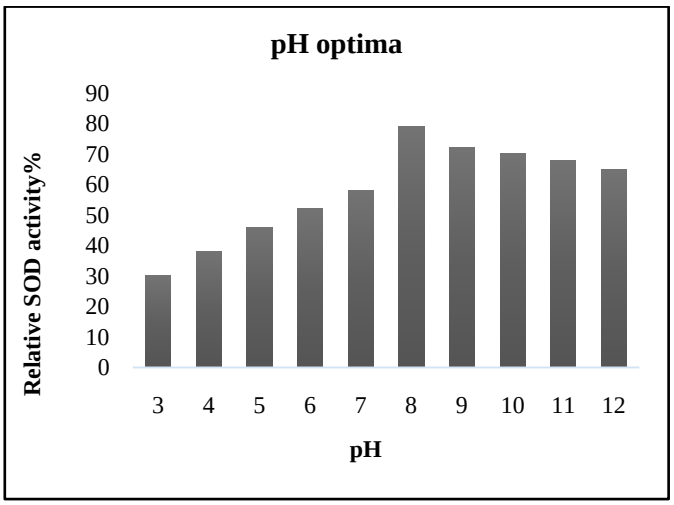


**Fig. 3: pH optima of SOD enzyme isolated *B.lanzan*by incubating the reaction mixture at pH ranging from 3.0- 12.0.**

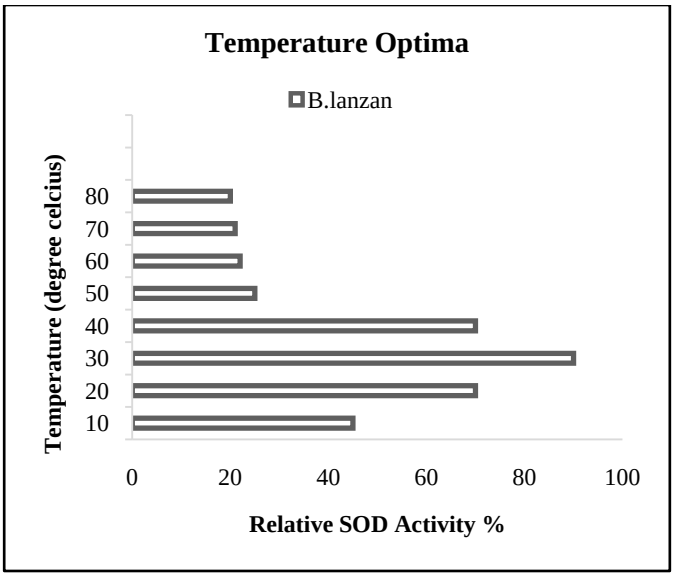


**Fig. 4: Temperature optima of SOD isolated from *B.lanzan* by incubating the reaction at different temperatures.**

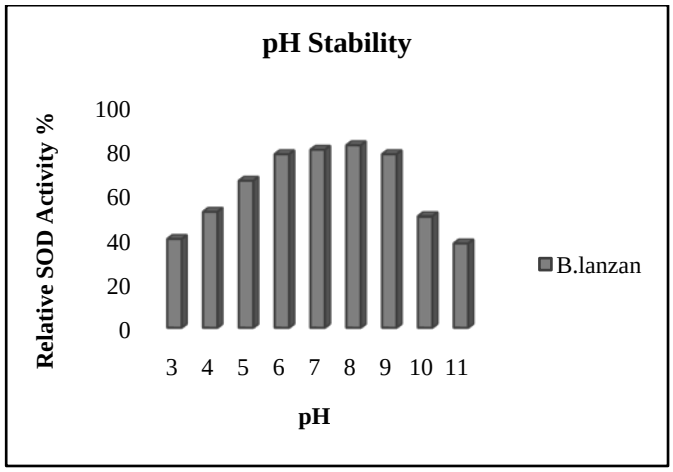


**Fig. 5: pH stability of SOD isolated from *B.lanzan* (after 2 hr. of pre-incubation).**

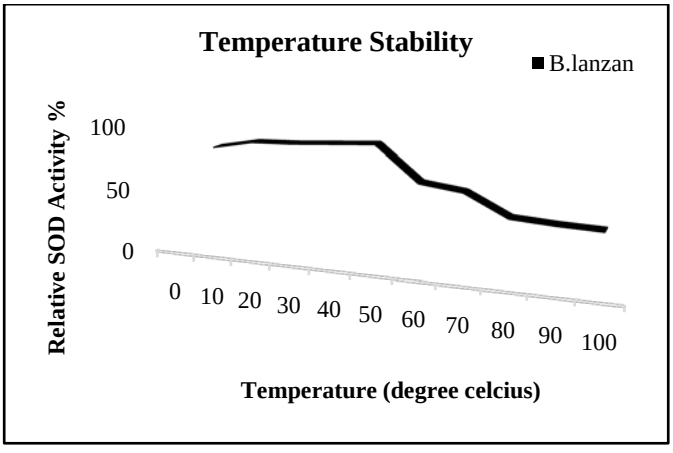


**Fig. 6: Temperature stability of SOD enzyme isolated from B.lanzan(after 2 hr. of pre-incubation).**

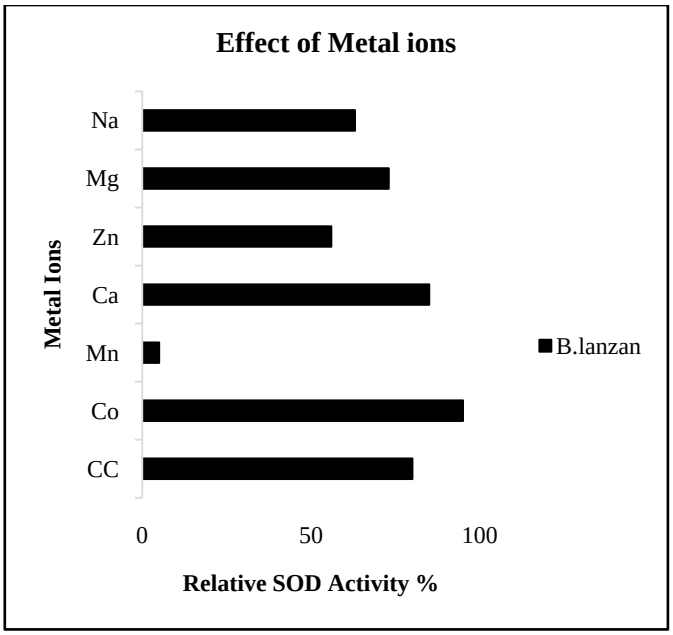


**Fig. 7: Effect of metal ions on SOD activity.**

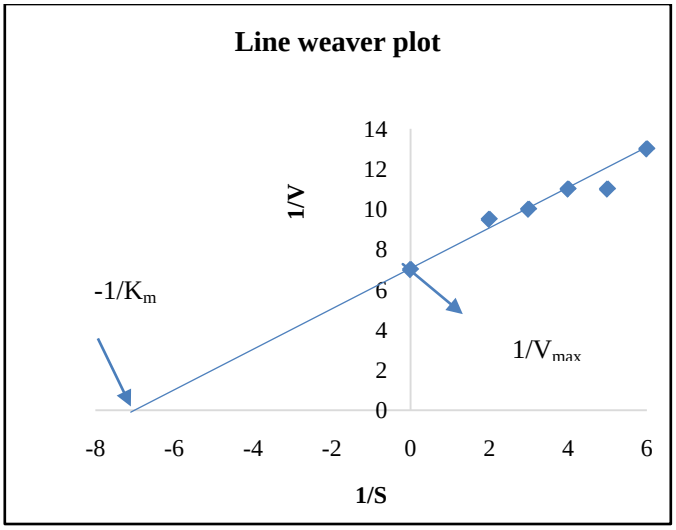


**Fig. 8: Lineweaver plot of SOD showing $K_m$ and $V_{max}$.**

## 4. CONCLUSION

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

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