Original Research Article

Purification and Characterization of Superoxide Dismutase Enzyme from *Punica Granatum* L.

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ABSTRACT

Superoxide dismutase (SOD) constitutes a very important antioxidant defense against oxidative stress in the body. SOD is found aplenty in many organisms, from microorganisms to plants and animals. Pomegranate (*Punica granatum* L.) peels and fruits have been used in traditional medicine. In this study, SOD (E.C 1.15.1.1) was purified from *Punica granatum* L. For this purpose, *Punica granatum* L. was homogenized centrifuged, fractioned with ammonium sulfate to precipitate, and then DEAE chromatography separation was applied. *Punica granatum* L. was purified 16.60-fold. SOD enzyme-specific activity was found as 166 U/mg protein.

Keywords: Punica granatum L., Pomegranate, Purification, Superoxide dismutase

1. INTRODUCTION

Superoxide dismutases (SOD) are a group of metalloenzymes that are found in all kingdoms of life. SOD form the front line of defense against reactive oxygen species (ROS)-mediated injury [1]. These proteins catalyze the dismutation of superoxide anion-free radical (O_2^-) into molecular oxygen and hydrogen peroxide (H_2O_2) and decrease O_2^- a level that damages the cells at excessive concentration [2]. This reaction is accompanied by alternate oxidation-reduction of metal ions present in the active site of SOD [3,4]. Based on the metal cofactors present in the active sites, SOD can be classified into four distinct groups: Copper-Zinc-SOD (Cu, Zn-SOD), Iron SOD (Fe-SOD), Manganese SOD (Mn-SOD), and Nickel SOD [5,6]. The different forms of SOD are unequally distributed throughout all biological kingdoms and are located in different subcellular compartments. SOD constitutes a very important antioxidant defense against oxidative stress in the body [7]. SOD enzyme has been reported to be able to protect against the harmful effects of reactive oxygen species involved in inflammatory joint diseases. The SOD enzyme has been explored for use in the treatment of various diseases, including the superoxide radical [8,9]. Several studies have been performed that reveal the therapeutic potential and physiological importance of SOD [10,11]. SOD is aplenty in many organisms, from microorganisms to plants and animals. Superoxide radicals are toxic to living cells, these radicals oxidize and break down biologically important molecules such as lipids and proteins. SOD removes superoxide radicals in cells [12-15].

The plant belongs to the Punicaceae family and grows in warm climate areas of South East Asia, the Mediterranean, the Americas, and other parts of the world. Fruits, peels, and roots of pomegranate have been commonly used in herbal remedies by local healers in many

countries. Pomegranate peels have been used in traditional medicine for treating diarrhea and dysentery [16-19].

In Yemen and other countries of the Arabian Peninsula, dried peels have been traditionally used for treating diarrhea, stomachache, and for healing wounds. In this regard, astringency is a known pharmacological property of tannins [20,21]. The tannin-rich peels are the products of the food industry, and are only used in animal feeds in many developed countries such as the U.S.A. The antimicrobial activity of peels has been demonstrated against pathogenic bacteria [16-19, 22-26].

2. MATERIAL AND METHODS

All the chemicals used in the research are analytical purity and Sigma, St. Louis, MO. provided by Merck. Pomegranate (*Punica granatum* L.) samples used as enzyme sources were obtained from the Cukurova University Faculty of Agriculture Department of Horticulture.

In the homogenate preparation, 10 g *Punica granatum* L. seeds, 10 g *Punica granatum* L. peel, and 10 g *Punica granatum* L. grains and membrane were homogenized in 25 ml KH_2PO_4 buffer with a pH of 7.0 containing 50% of PVP with 0.5% PVP after separately milling. The highest activity was obtained in *Punica granatum* L. grains. Since the highest activity was observed in *Punica granatum* L. seeds, all studies were carried out in *Punica granatum* L. seeds. Samples were centrifuged at 12,000 rpm for 20 minutes in a cooled centrifuge and the supernatant formed was separated from the precipitate. The supernatant obtained was stored at 4°C until use [27].

The homogenates of the SOD enzyme in the *Punica granatum* L. were precipitated with ammonium sulfate precipitation at 0-10%, 10-20%, 20-30%, 30-40%, 40-50%, 50-60% respectively. After the supernatant was dialyzed after 20% ammonium sulfate precipitation, SOD activity was obtained, the sample was passed through the DEAE-Cellulose column (0.82 cm x 25 cm).

The effect of temperature on the purified SOD was determined at 5, 10, 15, 20, 25°C. SOD samples were incubated at 5°C and 50 mM pH 7.8 Tris base buffer to determine their stability. Activity values were observed at intervals of 1, 3, 5, 8, and 16 hours at 40°C and 50°C for 1, 3, 7, and 14 days using the same enzyme homogenate.To determine the thermal stability, the activities of the SOD enzyme were measured using xanthine concentrations of 0.01 mM and 0.12 mm.

The Lineweaver-Burk graph was used to calculate the Michaelis-Menten constant (K_m), maximum velocity (V_{max}), and catalytic activity (k_{cat}/K_m) for SOD determination.

3. RESULTS

The specific activity of SOD in the purified samples was determined as 166 U/mg protein. The effect of temperature on the SOD enzyme, temperatures between 5ºC and 25ºC were studied and the results are given in (Fig. 1).

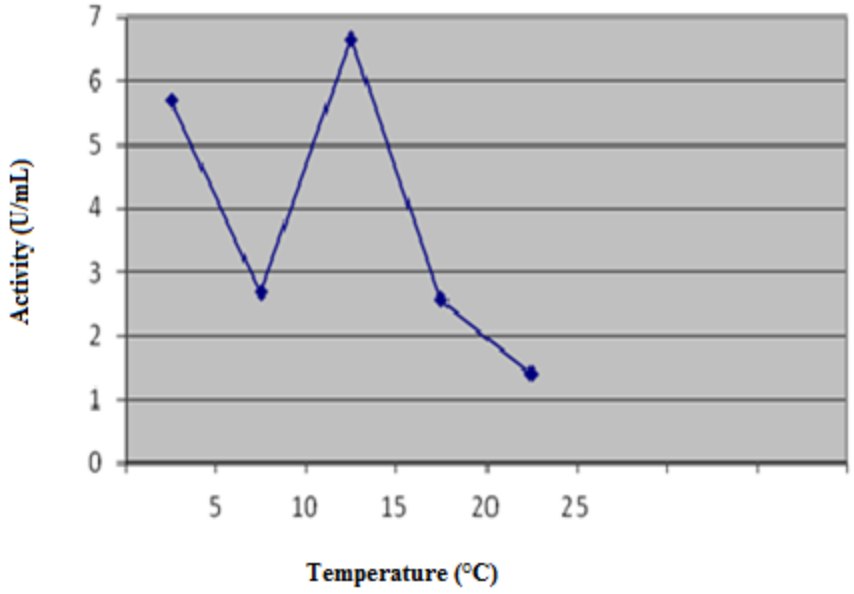

Fig. 1. Effect of temperature on SOD activity purified from *Punica granatum* L.

The optimum temperature of the enzyme was determined as 15ºC. After 20% ammonium sulfate precipitation, the supernatant solution containing SOD was dialyzed to the DEAE-cellulose column. The absorbances of the fractions eluted with NaCl solutions between 25-450 mM at 280 nm were measured and SOD activity was investigated in protein-containing eluates. The absorbances of the eluates at 280 nm and the SOD activity values measured at 560 nm were plotted in (Fig. 2).

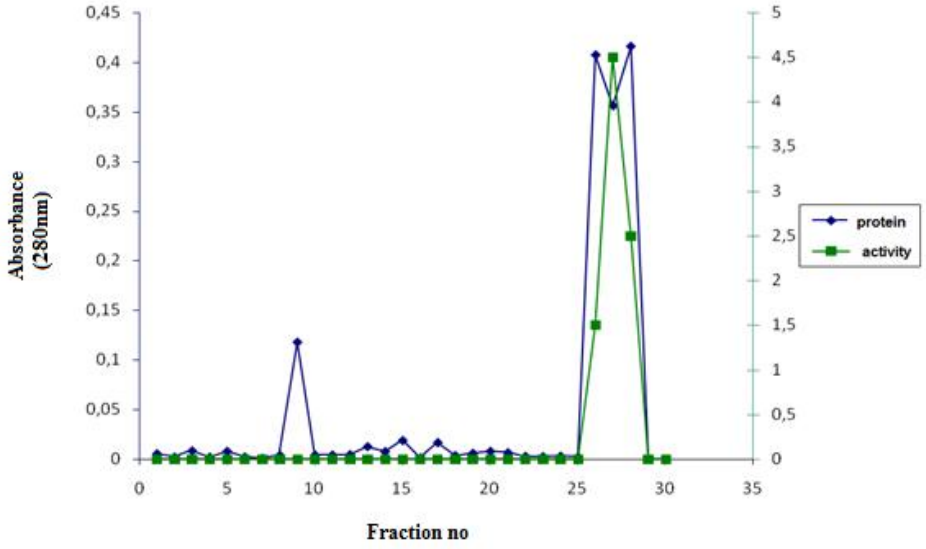

Fig. 2. Fractions from the DEAE-Cellulose column

In the determination of SOD enzyme activity studies with pomegranate, the samples showing the highest activity after the DEAE column were used to determine the stability of the enzyme. It was seen that SOD which was kept in 50 mM Tris base buffer lost its initial activity at 5**°C** and 25°C after 14 days. A schematic representation is shown in (Fig. 3).

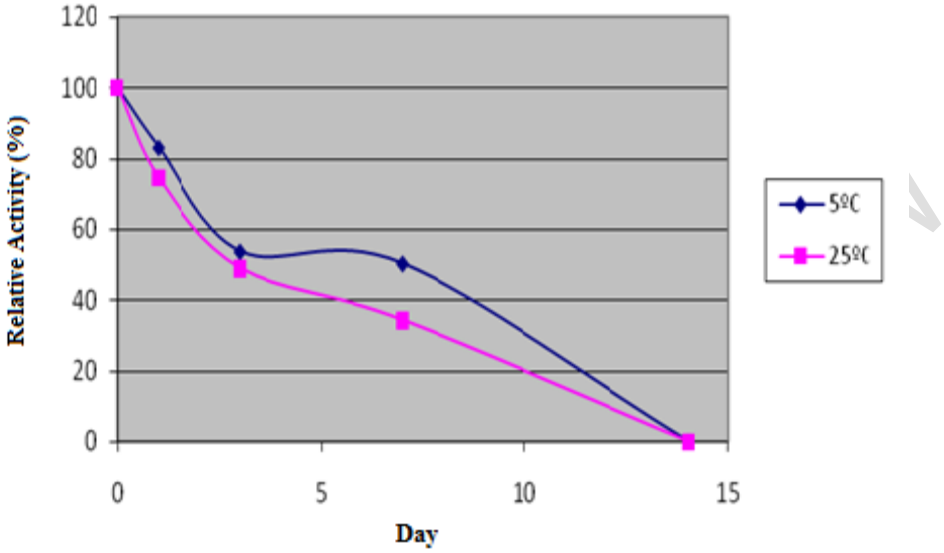

Fig. 3. Storage stability

To determine the thermal stability of purified SOD, the activity of SOD was measured at 15°C at 40°C and 50°C for 1, 3, 5, 8, and 16 hours. At the end of 16 hours at 15°C, it maintains 90% of its initial activity, while at 40°C and 50°C it has lost 100% of its initial activity in (Fig. 4).

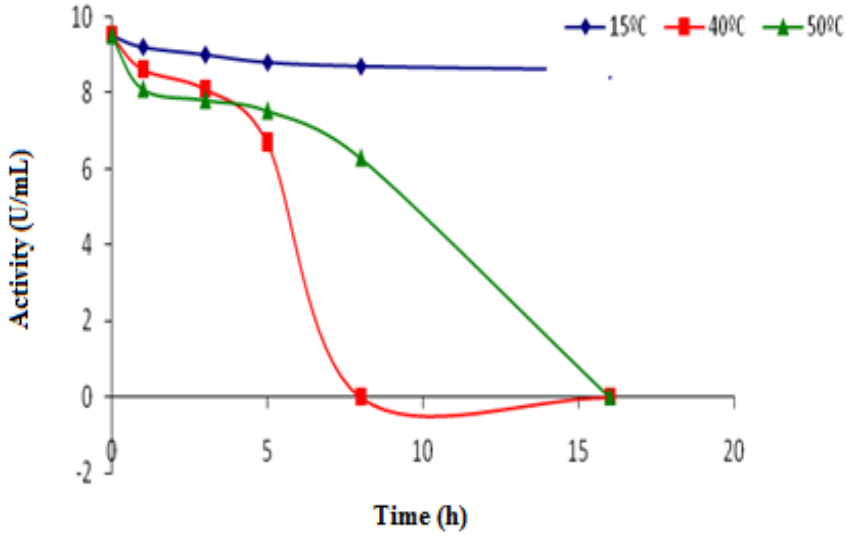

Fig. 4. Thermal stability graph

To calculate the K_m and V_{max} values of the SOD enzyme, activity values at different substrate (xanthine) concentrations were measured and the Lineweaver-Burk graph was drawn using the Sigma Plot Enzyme Kinetic Module program. K_m and V_{max} values calculated using this program were calculated as 0.6 mM and 1803.6 U/mg protein, respectively.

4. DISCUSSION

10 g of pomegranate (*Punica granatum* L.) was floured then homogenized in 25 ml KH_2PO_4 buffer with a pH of 7.0 and 50 mM containing 0.5% PVP. Homogenize solution was centrifuged at 12,000 rpm for 20 minutes and separated from the supernatant precipitate. In the studies performed with the obtained supernatant, the protein amount was 0.69 mg/mL and the specific activity of the SOD enzyme was 10 U/mg protein The supernatant 20% ammonium sulfate fraction showed the highest SOD activity.
This fraction after dialysis was loaded with the DEAE-Cellulose column. The optimum temperature of the enzyme was determined as 15°C (Fig. 1). In storage stability studies were seen that SOD which was kept in 50 mM Tris base buffer lost its initial activity at 5˚C and 25˚C after 14 days (Fig. 3).
In thermal storage studies at the end of 16 hours at 15°C, it maintains 90% of its initial activity, while at 40°C and 50°C it has lost 100% of its initial activity (Fig. 4). In the calculations, K_m and V_{max} values were calculated respectively, as 0.6 mM and 1803.6 U/mg protein.
Malgorzata et al., soybean roots, and branches were found that the activity of the SOD enzyme. Bozdemir purified catalase and superoxide dismutase enzymes from flaxseed and found the specific activities of the enzymes as 15.94 U/mg protein and 2.05 U/mg protein. Wang et al. were found that the molecular weight of the SOD enzyme extracted from buckwheat leaves is about 31,000. This enzyme is a homodimer of Cu/Zn-SOD. During our literature survey, we couldn't find the *Punica granatum* L. study of SOD enzyme purification. Therefore, we could not discuss our results with previous studies. We suggest that SOD purified from *Punica granatum* L. can be used commercially as cosmeceuticals, pharmaceuticals, and nutraceuticals industry.

ETHICAL APPROVAL

This article does not contain any studies done with human or animal participants performed by any of the authors.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

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