RESEARCH ARTICLE

OPTIMUM CONDITIONS OF SUPEROXIDE DISMUTASE EXTRACTION FROM TAMARIXAPHYLΛ L. (TAMARISK).

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Abstract

The present study was conducted to investigate the optimum conditions of superoxide dismutase (SOD) extract from Tamarixaphylla L. (tamarisk) parts included (Leaves, Flowers, Fruits, and Seeds). The results indicated that the highest superoxide dismutase specific activity concentrated in the crude extract of the leaves reaching 24.98 unit / mg protein, and followed by flowers 17.53 unit / mg protein , and fruits 8.99 unit / mg protein , while the seeds have lowest specific activity 5.66 unit / mg protein. using potassium phosphate buffer 0.1 M pH 7.8 containing 1mM EDTA–Na₂ and 2% PVP, and pyrogallol as substrate, Therefore, the leaves was used as source of SOD . The study was amid to determine the optimum conditions of the enzyme extraction . The result indicated that the optimum concentration of EDTA–Na₂, PVP , extraction ratio, pH, buffer concentration, and the extraction time were 2mM, 2%, 1:3, 0.1 M and 20 min, respectively.

Introduction:-

Superoxide dismutase SOD (EC 1.15.1.1) is a metalloenzymes which is Known to accelerate spontaneous dismutation of superoxide radicales to molecular oxygen and hydrogen peroxide [1]. SOD is wildly distributed among aerobically living organisms and has been inferred to play active role in controlled superoxide levels in cellular compartments[2,3]. There are three types of SODs in plants, classified according to the metal at the catalytic centre: CuZnSOD, FeSODand MnSOD[4].Cu/ZnSOD are chiefly located in chloroplast, also in the cytosol. And also found in the watermelon cotyledons [5].FeSODs located in the chloroplasts, but also located in the peroxisomes and mitochondria of Dianthus caryophyllus L.together with a Mn-isozyme[6]. Also found in Hybrid agave [7]. MnSOD in mitochondria [8].MnSODis essential to a biotic live [9,10].Specific inhibitors used to detect isoenzymes of SOD include H₂O₂ and (KCN). Cu/Zn SOD inhibited by both inhibitor, unlike MnSOD, while FeSOD inhibited only by H₂O₂ [11,12,13].

All types of superoxide dismutase are abundant in different organisms, including tobacco, pea, Pisumsatifivum, Ginkgo biloba,Nupharluteum, RauwolfiaserpentinaBenth, Methanobacteriumumbryantiand Escherichia coli[14-21]. SOD extract from different plant species , Bambusaoldhamii[22], leaves and roots of Deschampsia Antarctica[23], and Manihotesculenta[24].

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Material and Methods:

Plant and Chemicals Materials:

*Tamarixaphylla* L. plants were harvested from the farmland surrounding the university of Qadisiyah province of Diwaniyah.

The source of chemicals were as follows: Potassium phosphate Pyrogallol, Bovine serum albumin, PVP, EDTA-Na₂, HCl, (BDH(England)). Coomassie brilliant blue G-250 (Sigma( USA))., Tris-base, , (Fluka (Switzerland)). Tris-HCl(Oxoid(England)).

SOD Extraction:

Superoxide dismutase extracted from *Tamarixaphylla* L. according to [25], with some modification. Fresh plants tissues (5g) was pulverized in a cold mortar and pestle with (15ml) of 0.1M potassium phosphate buffer pH (7.8) containing 1mM EDTA-Na₂ and 2% (w/v) insoluble PVP. The homogenate was strained through four layers of miracloth and centerfuged at 15,000 rpm for 20 min at 0º.

SOD activity:

The activity of superoxide dismutase was determined using a Pyrogallol auto-oxidation . One unit of activity is defined as the amount of SOD required to inhibit the 50% of pyrogallol auto-oxidation [26].

Protein determination:

The total protein concentration was measured by the Bradford method [27], by using bovine serum albumin as the standard.

Optimum Conditions of SOD:

Concentration of EDTA-Na₂:

SOD from *Tamarixaphylla* L. leaves extract at different concentration of EDTA-Na₂ (0.1, 0.2, 0.4, 0.6, 0.8, 1 , 2) mM.

Concentration of PVP:

SOD extract at different concentration of PVP (0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5) % (w/v).

Optimum ratio for SOD extract:

SOD extract from *Tamarixaphylla* L. extracted at different ratio (1:2, 1:3 , 1:4 , 1:5 , 1:6) (w:v)

Concentration of buffer solution:

SOD extract at different concentration of potassium phosphate buffer range between (0.1-0.7) M pH 7.8.

Optimum pH:

SOD extract at different pH range between (4.0-9.0)

Optimum extraction time:

SOD extract at different time (5 , 10 , 15 , 20 , 25 , 30 ) min.

Results and Discussion:

SOD activity in *Tamarixaphylla* L. parts:

High SOD specific activity concentrated in the crude extract of *Tamarixaphylla* L. leaves compare with the other parts of plant, reached 36.55 unit/ mg protein. Therefore, it has been used as a source of SOD in further studies.
The optimum conditions of SOD:

Concentration of EDTA-Na$_2$: The enzyme was found to show maximum activity at concentration 1 mM. SOD extract from the leaves Eleusinecoracanala by added 1mM EDTA-Na$_2$[28]. The optimum concentration of EDTA-Na$_2$added in the extraction of SOD from roots and leaves of Medicagotruncatulawas 0.1mM [29]. The used of EDTA in the extraction of the enzyme maintained the stability of enzyme being chelating agent prevent overlapping contaminated ions of the buffer during the work of enzyme [30]. So it added in concentration 1 mM in the current study to get rid of the harmful effects of metal ions and to maintain the stability of the enzyme.

![Figure 1: SOD activity in T. aphylla L. parts](image1)

Concentration of PVP:
The high SOD activity showed at concentration 2% (w/v) . The PVP added to extract SOD from Fritillariameleagriswas 200 mg[31]. While the PVP ratio to extract SOD from roots and leaves of Kandeliacande L was 4% [32]. PVP used in the extraction of enzymes from plants tissues to adsorption of phenolic compounds and reduced the impact on the stability of protein and their effectiveness [33].

![Figure 2: Effect of EDTA-Na$_2$on the specific activity of SOD from T. aphyllaL.](image2)
Extraction ratio:-
Maximum activity of the enzyme showed at 1:3 (W:V). SOD extracted from the leaves of *Brassica napus* L. in 1:3 ratio [34]MnSOD extracted from the leaves of *Pisumsativum* L. in 1:5 ratio [8].

Concentration of buffer solution:-
The enzyme show high activity at 0.1 M of potassium phosphate buffer. The ionic strength of 0.1 M of potassium phosphate buffer sufficient to decode the correlation between cellular enzyme and other materials, While the difference in extraction buffer concentrations used in the study included a decrease in the efficiency of extraction increased emphasis this is because the increase of the buffer solution concentration increases the liberation of protein and non-protein, thereby increasing protein concentration and decrease the specific activity. Optimum SOD specific activity of SOD extract from seeds of Chickpea by used potassium phosphate buffer 0.1 M [35].SOD specific activity extract from Spinachby used 50mM potassium phosphate [36].
**Figure 5:** Effect of concentration of buffer solution of SOD specific activity

**pH buffer Solution:**

The highest SOD activity was determined at pH (8.0) by used potassium phosphate buffer 0.1M, therefore it considered the optimum pH. Optimum pH of SOD extract from the seeds of *Brassica napus* L. was 7.8[37]. The optimum activity of SOD extract from the leaves of *Triticum aestivum* L.[38].

**Figure 6:** Effect of pH of SOD from *T. aphylla* L.leaves

**Extraction Time:**

The high SOD activity determined at 20 min. with specific activity reached 38.11 unit/ mg protein ,then followed by 15, 10, 25.5 and 30 , respectively. 20 min was enough for liberation of the enzyme , Therefore it is considered the optimum period of the extraction.
Figure 7: Effect of extraction time on SOD specific activity

References:


