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RESEARCH ARTICLE

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

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Abstract

The present study was conducted to investigate the optimum conditionsof 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-Na_2 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-Na_2, PVP , extraction ratio, pH, buffer concentration, and the extraction time were 2mM, 2%, 1:3, 0.1M and 20 min, respectively.

Introduction:-

Superoxide dismutase SOD (EC 1.15.1.1) is a metaloenzymeswhich 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.togather 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 H_2O_2 and (KCN). Cu/Zn SOD inhibited by both inhibitor, unlike MnSOD, while FeSOD inhibited only by H_2O_2 [**11,12,13**].

All types of superoxide dismutase are abundant in different organisms, including tobacco, pea, *Pisumsatfivum, Ginkgo biloba,Nupharluteum, Rauwolfiaserpentina*Benth, *Methanobacteriumbryantii*and *Escherichia coli***[14-21].** SOD extract from differents 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. plant 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-Na_2, , 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-Na_2 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-Na_2:-

SOD from *Tamarixaphylla* L. leaves extract at different concentration of EDTA-Na_2 (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.Therefor, it has been used as a source of SOD in further studies.

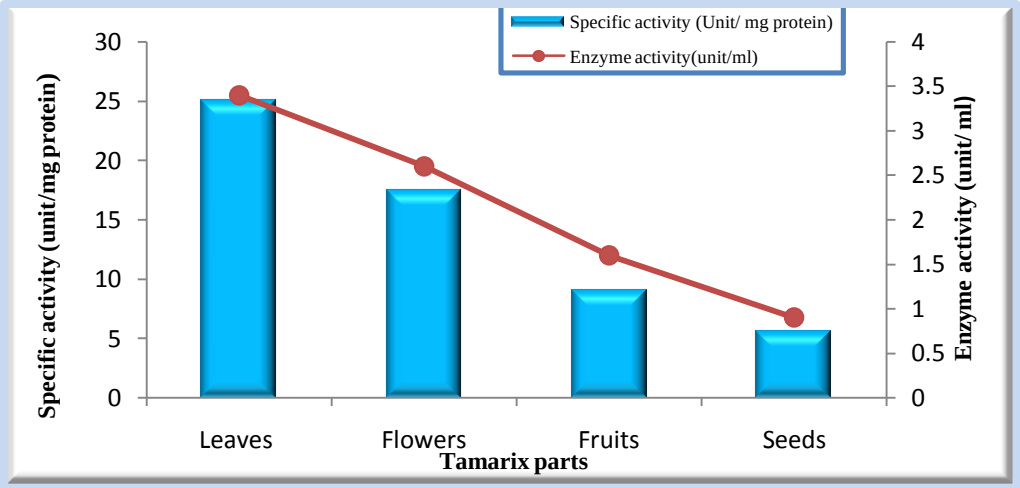

Figure 1:- SOD activity in *T. aphylla* L. parts

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 *Eleusinecoracana*L. by added 1mM EDTA-Na_2**[28]**. The optimum concentration of EDTA-Na_2added in the extraction of SOD from roots and leaves of *Medicagotruncatula*was 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.

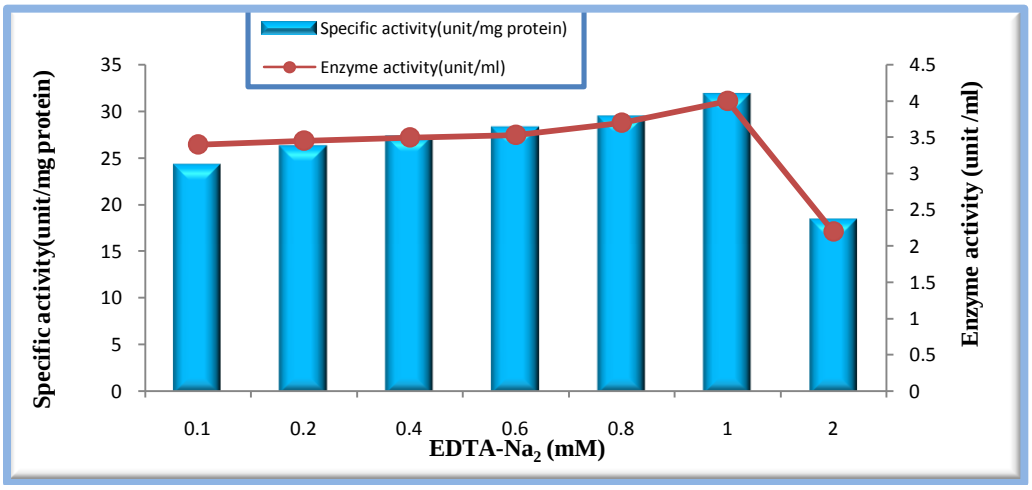

Figure 2:- Effect of EDTA-Na_2on the specific activity of SOD from *T. aphylla*L.

Concentration of PVP:-

The high SOD activity showed at concentration 2% (w/v) . The PVP added to extract SOD from *Fritillariameleagris*was 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]**.

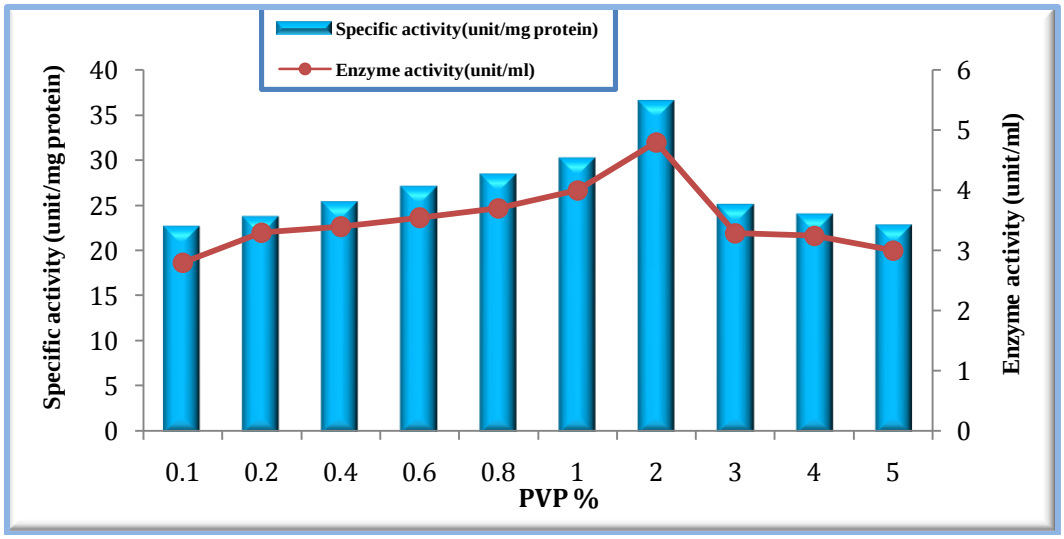

Figure3:-Effect of PVPon the specific activity of SOD from *T. aphylla*L.

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]**.

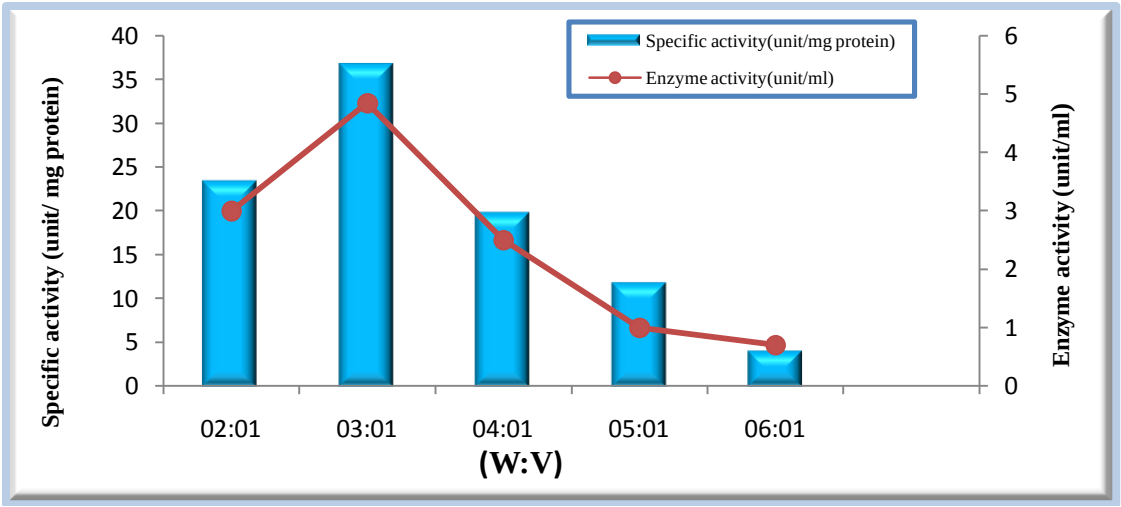

Figure 4:-Extraction ratio of SOD from *T. aphylla*L. leaves

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 50mMpotassium phosphate **[36]**.

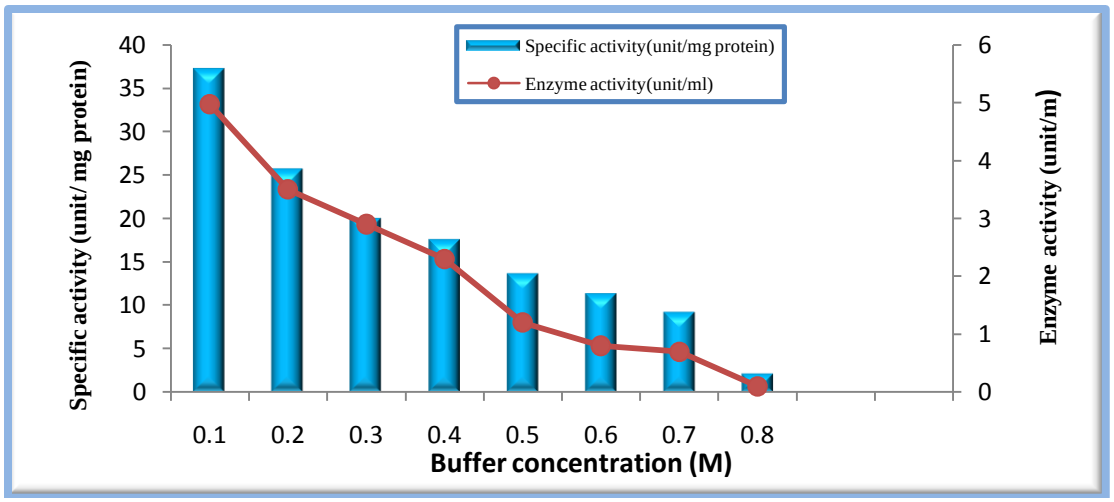

Figure 5:-Effect of concentration of buffer solution of SOD specific activity

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 *Triticumaestivum* L **[38]**.

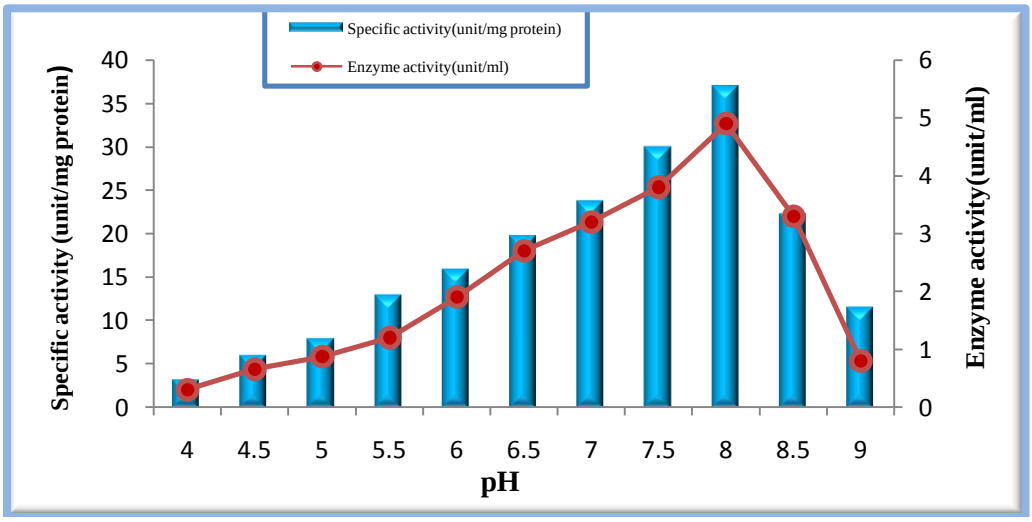

Figure 6:-Effect of pH of SOD from *T. aphylla* L.leaves

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.

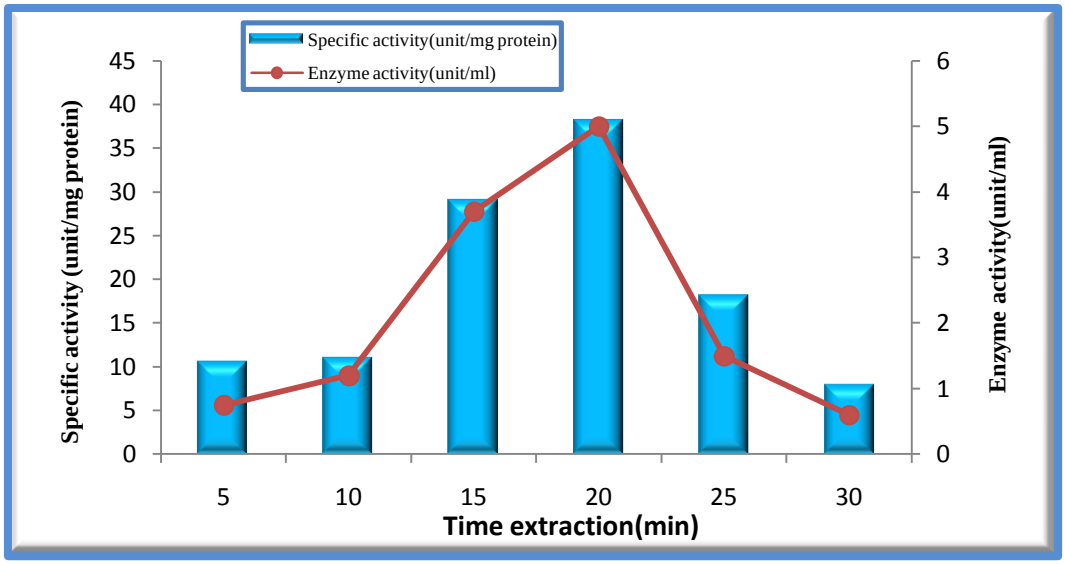

Figure 7:-Effect of extraction time on SOD specific activity

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