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RESEARCH ARTICLE

Optimum Condition for Peroxidase Activity in Crude Extract of *Loranthus europaeus*

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Abstract

In this study peroxidase was extracted from fruit part of Loranthus europaeus then peroxidase activity was detected and optimized. Optimum conditions for the activity of crude peroxidase were studied. Results showed maximum activity of peroxidase was achieved 0.5125U/ml when the enzyme was incubated with 1mM of 4-aminoantipyrine at 40 °C for 10 minutes in the presence of 0.2M of potassium phosphate buffer solution at PH7.

Keywords: *Loranthus europaeus, Peroxidase, enzyme activity.*

Introduction

The family Loranthaceae is a large family belonging to the grade santalales. It almost includes 75 genus belong to 1000 species, part of these species are parasites on plant roots while the remaining parasites on branches and stems trees and known as Mistletoe [1]. It is found mostly in Europe and as far as Iran, not found in America or Australia.

Mistletoe is growing in central Europe and China. The showy mistletoes can be found throughout the world [2]. Loranthus micranthus is characterized of the antioxidant activity of extracts aerial tissues, in the mainland of Greece was study the total phenolic content and antioxidant potential of Loranthus europaeus have reducing antioxidant power assay [3]. It possesses optimum antidiabetic activity [4].

Mistletoe extracts were introduced for the first time in a cancer treatment by [5], founder of anthroposophy. Peroxidase (EC.1.11.1.7) is a heme contain enzyme that oxidizes a variety of inorganic and organic compounds using the hydrogen peroxide and it belongs to class oxidoreductases.

Peroxidase is widely distributed in nature and can be easily extracted from most plant cell and from some animal organs and tissues [6]. Peroxidase is an enzyme that performs several biochemical and physiological roles in the plants [7].

Involve in the growth of plants, differeniiation and development processes, include auxin catabolism, biosynthesis of ethylene, plasma membrane oxidation systems and the generation of H2O2, Installation of cell membrane, lignification and suberization, as well as resettlement to pathogens [8]. Peroxidase is found during the plant regality an increase in peroxidase activity has been linked with environmental stresses on plants [9, 10, 11]. In this time the role of peroxidases is important in H2O2 - mediated signalling operation as the response to abiotic and biotic stresses in plants [12, 13].

Peroxidases can enter into many fields and replace the current chemical oxidation technology and meet the environmental requirements of future technologies due to its nature of oxidation [14]. The aim of this study is to extraction of peroxidase enzyme from Loranthus europaeus and determin optimum conditions for peroxidase activity from this plant.

Materials and Methods

Extraction of Peroxidase

Mistletoe were washed and homogenized in blender and mixed 200 gms with PH 7 phosphate buffer to prepare the crude extract. The enzyme was filtered using cloth and the enzyme place with buffer was

centrifuged at 8000 rpm for 10 minutes to remove other small impurities from supernatant. It was filtered with whatmann filter paper and stored as crude extract, stored in a refrigerator and was brought to room temperature before every experiment [15].

Enzyme Assay

Peroxidase activity was assayed according to [16] by colorimetric method, using phenol, 4-aminoantipyrine and H2O2 as the dye-generation compounds. Protein concentration in plant extracts and enzyme concentrates was determined according [17].

Optimization of Crude Peroxidase Activity

Effects of different factors on crude peroxidase activity were studies according to [18].

Effect of Substrate

The activity of crude peroxidase was determined by incubation it with different substrate concentration (0.1, 0.2, 0.5,1,2,5 and 10 mM), then peroxidase activity was determined according to [19].Optimum concentration was stated in the next experiments.

Effect of Reaction Time

Different incubation periods (5, 10,15,20,25 and 30 minutes) were done at 25 C^o, and then enzyme activity was determined.

Effect of Buffer PH

PH of the reaction mixture was adjusted to different values range (4, 5,6,7,8 and 9). Then enzyme activity was determined and optimum PH was stated in the next experiment.

Effect of Temperature

Optimal temperature for crude peroxidase activity was identified by incubation the mixture at different temperatures (10, 20,30,40,50 and 60 C^o). Then enzyme activity was determined and optimum temperature for activity was stated in the next experiments.

Results and Discussion

Peroxidase Activity in Crude Extract

The crude samples were subjected to spectrophotometric analysis at 510 nm wavelength for designation of enzyme activity, which are given in Table (1) whereas the activity of crude peroxidase extract from Loranthus Europe us was 0.5125 U/ml and specific activity of 0.1580 U/mg.

Table 1: Crude peroxidase activity in L.europeus

Crude extract	Activity (U/ml)	Total protein(mg/ml)	Specific activity(U/mg)
L. europeus	0.5125	3.243	0.1580

Optimum Conditions for Peroxidase activity

Optimum conditions for peroxidase activity were studied after extraction of the enzyme from Loranthus europaeus plant. These conditions include the optimum time of reaction, PH of the reaction mixture and temperature of the reaction. Changing in any of these parameters may affect the enzyme activity [20].

Effect of Substrate Concentration

Results presented in Figure (1) showed that the activity of peroxidase was increased gradually with the increase in substrate concentration. Maximum activity of peroxidase was obtained when the substrate concentration was 1mM, at this concentration; peroxidase activity was (0.2509U/ml).

This concentration of substrate was regarded as the optimum for peroxidase activity. These results were agreed with [21] Who found that there is a positive relationship between the enzyme activity and substrate concentration, the reaction was increased with the increase of substrate concentration when the peroxidase activity was constant until the maximum rate was achieved (steady state), then the increase of substrate concentration does not affects the rate of reaction and does not significantly affect the formation of the product [22].

At the steady state, There is no any enzyme molecule free to act on extra-substrate molecule, substrate inhibition will sometimes occur when excessive amounts of substrate are present in the reaction mixture [23]. According to results mentioned in Figure (1) peroxidase produced by L. europaeus reached

the steady state when the substrate concentration was 1Mm.

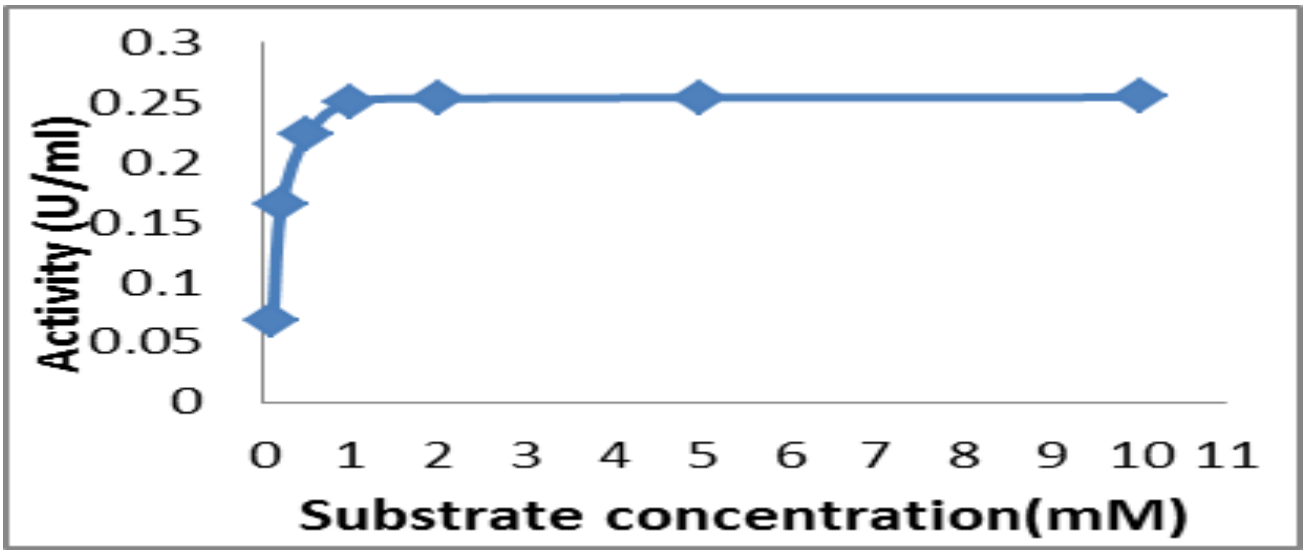

Figure 1: Effect of substrate (4- aminoantipyrine) concentration on peroxidase activity extracted from Loranthus europaeus

Effect of Reaction Time

Result indicated in Figure (2) showed that the optimum reaction period was 10 minutes; the enzyme activity was 0.4652 U/ml. According to these results, It has been concluded 10 minutes of incubation was enough for peroxidase to bind substrate perfectly in reaction mixture reaching maximum enzyme activity. The time-scale is an important factor in determining the enzyme activity, and it was preferred to use methods with short time incubation to determine the enzyme activity [24].

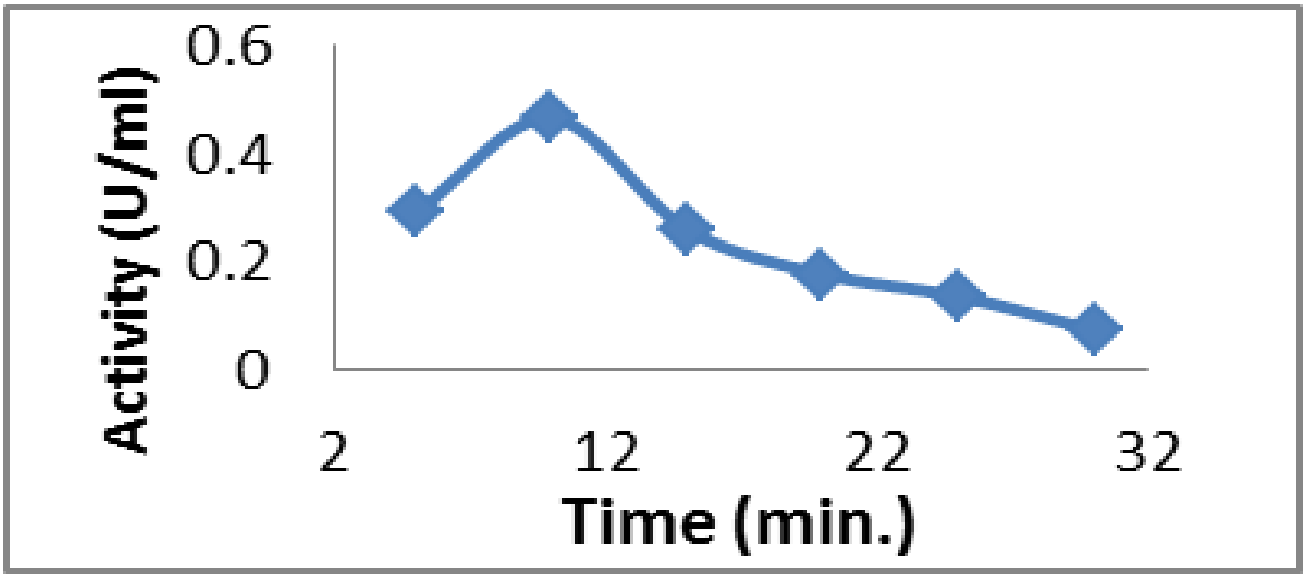

Figure 2: Effect of reaction time on peroxidase activity extracted from Loranthus europaeus

Effect of Buffer PH

Effect of PH on the activity of peroxidase activity produced by Loranthus europeus was studies. Result indicicated in Figure (3) showed that maximum peroxidase activity was obtained when PH of the reaction mixture was adjusted to 7, at this value; the activity was 0.5066 U/ml. Generally most plants gave maximum enzyme activity at or near neutral PH [25]. The substrate in PH7 has greater affinity to the active site of the peroxidase. Any increase or decrease in hydrogen ions (H+) concentration causes a change in PH of the reaction mixture which may leading to changes in three-dimensional structure of protein, resulting in the enzyme denaturation [26]. The effect of PH on activity resulted from its effect on the ionization state of the substrate [27], it becomes a competitive inhibitor [28]. Reported that the optimum PH of peroxidase in Rrumex obtusifolius L. Was 7.

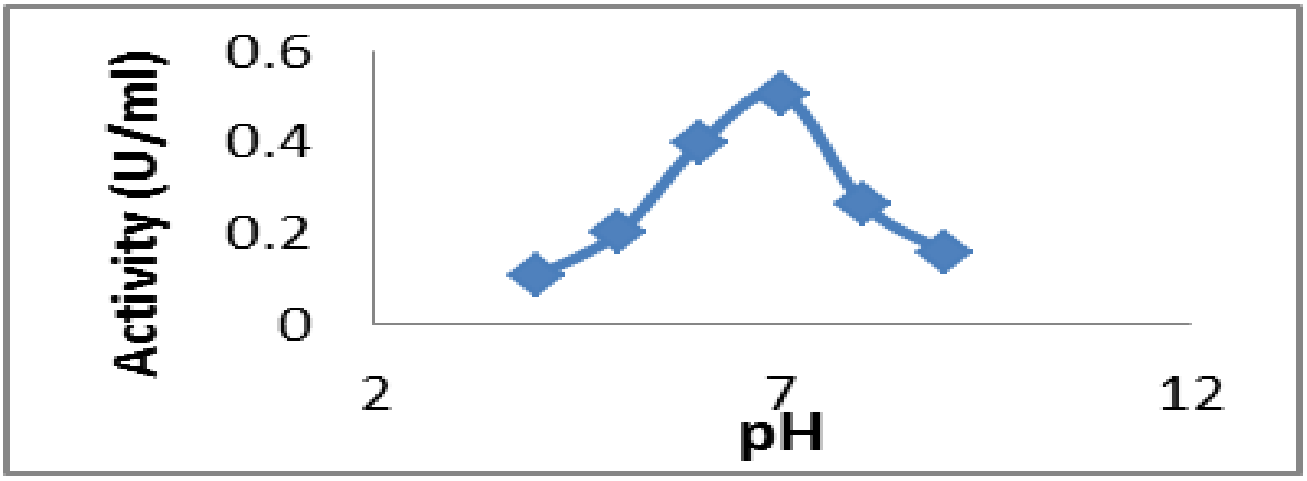

Figure 3: Effect of buffer PH on peroxidase activity extracted from Loranthus europaeus

Effect of Temperature

Results illustrated in figure (4) showed that the maximum activity of peroxidase was obtained when the temperature of the reaction mixture was 40C°.

At this temperature, enzyme activity was increased to 0.5125 U/ml. Enzyme activity increased with increasing temperature because the kinetic energy of the enzyme molecules and the substrate increases and becomes faster, the more often they collied with one another and the greater the rate of enzyme [29]. Then the activity of enzyme decreases with temperature up to 40C°. The most atoms make the enzyme molecules vibrate; leading to broken the hydrogen bonds and other forces, the enzyme was denatured and loses its catalytic activity [30, 31].Reported that the optimum temperature of peroxidase was 40C°.

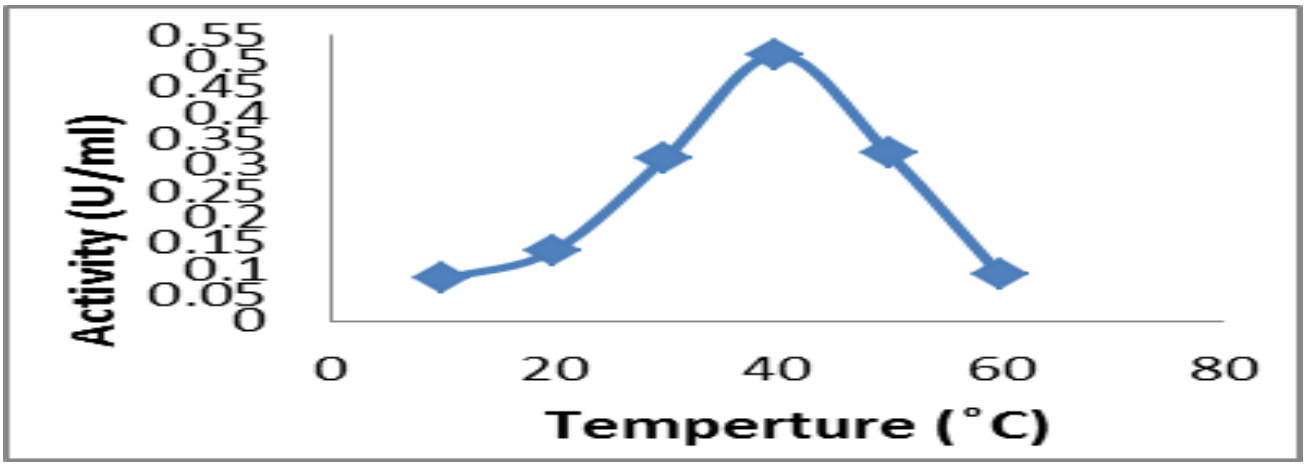

Figure 4: Effect of temperature on peroxidase activity extracted from Loranthus europaeus

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