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Investigation Activity and Stability of Peroxidase Plant Enzyme to Decolorize Azo Dyes

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ABSTRACT

The current work aimed to extract purified Peroxidase enzyme from red radish to determine the enzyme's specific activity at the best temperature and pH. The extracted enzyme was used to decolorize some Azo dyes like Congo red, Methyl red, and Methyl orange. The best experimental condition was sodium acetate buffer 0.1 M, pH 6 for buffer solution for peroxidase extraction from red radish with a specific activity of 44414.4 U/mg protein after using 1:2 (V:W) as the extraction ratio. Ion-exchange chromatography on a CM-cellulose column and 0-70 percent (W:V) saturated ammonium sulfate was used; with the enzyme purified process, the Peroxidase enzyme activity was 2913 U/ml, and protein concentration was 0.014 mg/ml, with a specific activity 208071 U/mg protein, a purification fold of 3.5, and a yield of 77%, partially purified Peroxidase. The partially purified Peroxidase's biochemical properties were examined. The maximum removal efficiencies of the azo dyes were 99.8, 42.9, and 50.4% for Congo red, Methyl red, and Methyl orange investigated in this study. The results showed the ability of plant enzymes to treat different kinds of Azo dyes.

Keywords: Extraction, Peroxidase enzyme, Ion-exchange chromatography, enzyme stability.

1. INTRODUCTION

Water is an important factor in continued life on earth, the freshwater available on earth forms less than 1% of the total exit water, and contaminated water is usually produced by various industries, which causes many dangers to the environment (Asses et al. 2018).

At present, biological treatments have become one of the important technologies for dealing with pollution problems in general, and enzyme therapy is one of these treatments that top the list of modern solutions; its applications are wide in various scientific fields, especially with the industrial development and the increase in human consumption of water. Enzymatic treatment is a successful method that does not leave negative effects on the environment and humans, Peroxidase enzyme is abundant in Organisms and many plant species and is used in research to determine how diseases which affect various plant species (Cheung, Set.al. 2007).The Peroxidase enzyme could be found and isolated from a variety of plants, including potatoes, yeast, beans, wheat, and Japanese radish. In the industrial field, plants enzymes have been used with high efficiency in bio-bleaching processes for the paper and textile industries some studies used the whole plant as soybean for purification, immobilization and application of this enzyme to the decolorization of textile dyes or degradation of phenolic compounds (Jasim et al. 2019). Extracting enzymes from plant sources was an easy process with a low cost that minimizes hole treatment costs (Al-Sa'ady, 2020). Nabila

B.et.al.(2012) was found the removal of azo dye Direct Yellow (DY106) was affected by pH, enzyme activity, temperature and dye concentrations when using Cucurbita pepo (courgette) peroxidase extract during 15 min, the decolorization of DY106 yield was 75% at pH 2, 20°C. Afaf et al. (2012) the enzyme peroxidase can clear aromatic and phenolic compounds and remove textile dye before disposal effluents to aquatic sources. A turnip (Brassica rapa) peroxidase was used for discoloring textile azo dyes such as Congo Red (CR). The removal efficiency is affected by pH, initial dye concentration, temperature, contact time, and enzyme concentration. The ideal conditions for 96% discoloration of 50 mg/l congo red concentration were pH 2.0, 40 °C, 0.45 U/ml peroxidase activity in 10 min; the product analysis by the enzymatic IR spectroscopy and UV-Vis and found the electron-withdrawing properties of the azo links obstruct the capability of dye's molecules to oxidative and found some reducing enzymes have been found to degrade azo dyes, proved that the use of an enzymatic treatment process is a viable approach for the degradation of azo dyes from aqueous solutions (Ahmedia et al. 2012). Another study found that the Peroxidase was extracted from Raphanus sativus and purified by ammonium sulphate purification 65.20 U/mg specific activity . A decolorization of 97% for Solar Blue and 77 % for Solar Flavine 5G (pH 4 , 50 °C) (Haq et al. 2012). Maddhinni et al. (2006) recorded 70% decolorization for Azo yellow 12 at pH 4 after 1 hr and 20.4% for Azo Pink and 22.6% for Azo Purple when using the horse radish peroxidase (HRP) with activity (1.8 units/ml). The decolourisation of Methyl Orange (MO) and Bismarck Brown (BB) by crude peroxidase from Armoracia rusticana (Horseradish) at optimum temperatures (28-32°C) and optimum pH for decolorisation was 4.0, the decolorization was (72.95 %) and 3.0 (79.24 %) for MO and BB, respectively. Dye decolorization a basic dye safranin by the Raphanus sativus (radish) (35.58 U/ml) The effect of the influencing parameters of dye concentration,enzyme concentration , temperature and pH, on safranin decolorization producing 30.07 % decolorization at an optimum pH of 7 ,30 °C. (Park et al.2015). Enzymatic treatments by synergy between the sonochemical and enzymatic techniques using immobilized horseradish peroxidase (HRP) enzyme on the decolourization of acid red dye (azo dye). There was a (6.5, 25°C) for immobilized HRP and (7.0, 20-25°C) for free HRP . The specific activity(0.272U/mg) of the free enzyme was found to be bigger than the immobilized enzyme (0.104U/mg), and the immobilized enzyme exhibited higher stability (up to 3 cycles) and degradation potential than the free enzyme in all experiments. decolourization of acid red (61.2%). However, the total decolourization done with combined technique was lesser than the sum of individual techniques, indicating negative synergy between the sonochemical and enzymatic techniques (Malani et al. 2013). Acid azo (Acid Black 10 BX) dye removal by Horseradish peroxidase (HRP) was extracted From horseradish and its performance was evaluated in both free and immobilized form , removal was observed by immobilized beads with alginate 54% of dye at (pH 4.5, 4C)(Mohan et al. 2004).

(Aziz et al.2021) Revealed that the specific activity for crude peroxidase activity extracted from soybean was 2919 U/mg proteins, and that the extraction of Peroxidase Ziziphus mauritiana was specific activity 17.6 U/mg, (Khan et al. 2018). While the results of Bania et al. (2012) revealed that the peroxidase activity extracted from radish, turnip, cabbage, and tomato gave a specific

activity of (1.46,1.74,1.09, and 1.14) U/mg proteins, respectively. These results showed that A sodium acetate buffer with a pH of 5.5 had the greatest effect for extracting peroxidase from ficus carica latex, with specific activity reaching 4489 U/mg (Elsayed et al. 2018) A (0.2 M) sodium phosphate buffer with a pH of 7.5 had the best results for extracting Peroxidase from various plants (Hussein et al. 2020). Ion-exchange chromatography was used in (Fahmy et al.2018) was purify Peroxidase from the Euphorbia tirucalli latex plant, and it was got it that the yield was 12 percent with a purification ratio of 2.0. While Victor (2018) discovered that the Peroxidase purified from green cabbage, at utilized (NH4)2SO4 precipitation gave a purification fold of 4.71with yield of 80.05 and gel filtrated enzyme purification fold 14.14 and a yield 9.44 percent. Horseradish had the best peroxidase extract ratio of 2:1 (W:V) , yielding 4,479 U/mg protein (AL-Sa'ady et al.2018). While in Hussein et al.(2020) the optimal ratio of plant peroxidase extract was 1:20 (W: V) after 90 min. The optimum pH range for barley Peroxidase stability was 4.0 to 5.5 (Basha, S. A.,2016). In contrast, Basha et al.(2016) discovered that the ideal pH range for the stability of the enzyme from green gram roots (Vigna radiata) was 5.0 to 7.0. According to the findings in (Ahmedia et al.2012), white turnip (Brassica rapa) Peroxidase activity was at its highest at 25 °C, with an enzyme activity of 3.406 U/ml. While the outcomes of Mamounata D.,et.al.(2018) indicated that 35 °C was the ideal temperature for the red radish Peroxidase enzyme extraction. In

Mandujano et al. (2016), When Peroxidase was incubated with phosphate buffer pH 7.4, discovered the best pH was 5.5 for the enzyme activity of soybean, while in (Aghelan et al. 2015) the optimum pH was 6.0 for (0.3 M, pH 6.0) sodium phosphate buffer. In contrast, found Rathnamsamy et al.(2014) that pH equal to 6.0 for horse radish Peroxidase activity. The outcomes of Aziz. et al.(2021) showed that 5.0 pH with a specific activity of 1750 U/mg protein was the ideal pH for the Peroxidase activity from Nigella seeds. The optimum temperatures for Peroxidase stability from green cabbage were between 40 to 50 ^{o}C (Bania,Ila et al. 2012), while Basha et al.(2016) found the optimum temperatures stability of Peroxidase enzyme from Ficus carica latex was 30 to 50 ^{o}C. The purpose of this study was to use the Peroxidase enzymatic extract , purify, and characterize .The red radish was choose for this then useing in removing azo dyes such as (Congo red ,Methyl red , Methel Orang) from wastewater produced from many from industries, such as the textile industry to do biodegradation by a batch system.

2.Chemical materials:

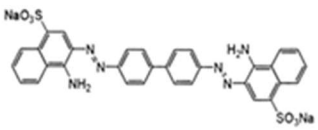
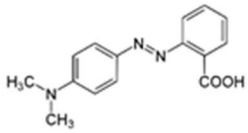
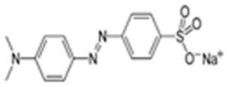
2.1. materials

The materials used in this work analytical grade and were purchased from local market (Ethanol (chemical formula), phosphoric acid (H_3PO_4), Sodium acetate ($H_3CCOONa \cdot 3H_2O$), Ammonium Sulfate $(NH_4)_2SO_4$, BDH-England), (Hydrochloric acid(HCl), Hydrogen Peroxide H_2O_2, Sodium Hydroxide(NaOH), Congo red (CR) (chemical formula), Methyl red(MR) (chemical formula), Orange red (OR) (chemical formula), Fluka-Switzerland), (Na-alginate, Potassium dihydrogen phosphate |(KH2PO4), Calcium chloride ($CaCl_2$), Merck-Germany), (Guaiacol(chemical formula), Tris- HCl, Coomassie brilliant blue G-250, Sigma, USA)

2.2. Main properties of dyes used in this study:

Table 1 shows the characterization of three dyes that used in the experimental work of this study:

Table 1 characterization of three dyes that used in the experimental work of this study

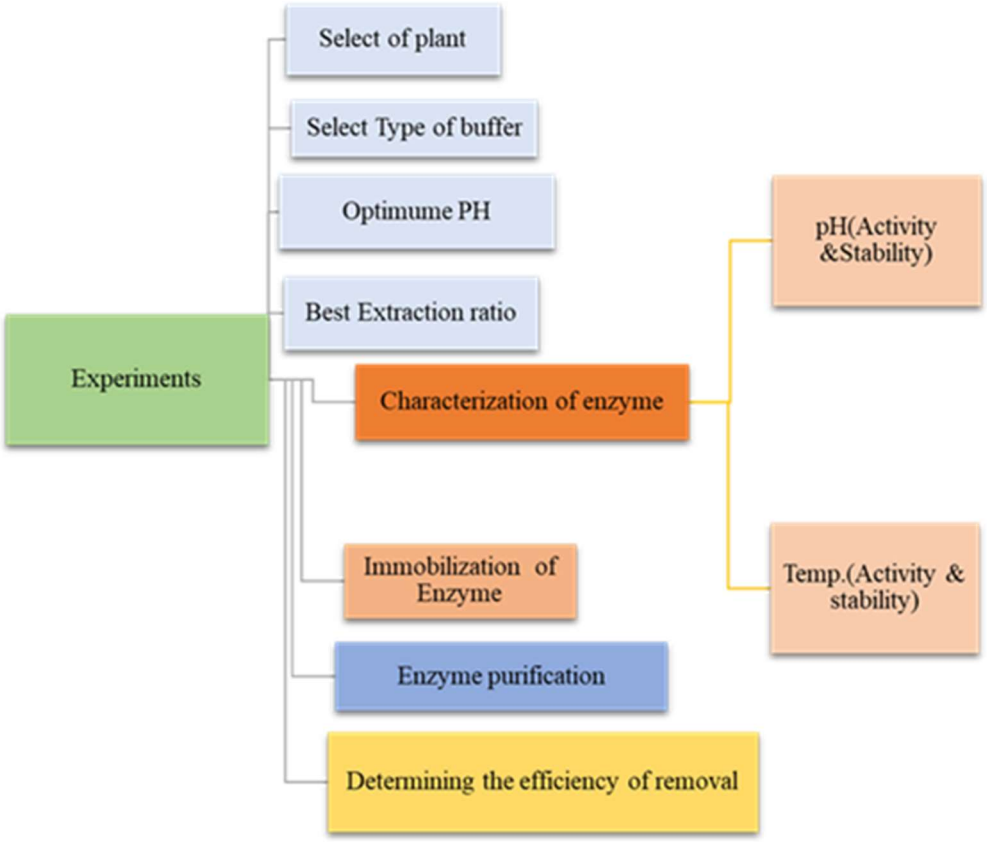
Properties	Congo red dye	Methyl red dye	Methyl Orang dye
Symbol	CR	MR	MO
Molecular formula	$C_{32}H_{22}N_6Na_2O_6S_2$	C15H15N3O2	C14H14N3NaO3S
Chemical structure			
Chemical name	1-napthalenesulfonic acid, 3,3-(4, 4-biphenylenebis (azo)) bis(4-aminodisodium) salt.	2-{(4-(Dimethylamino)phenyl)diazenyl}benzoic acid	Sodium 4-{(4-(dimethylamino) phenyl) 5iazinyl} benzene-1-sulfonate
			
Molecular weight (g/mole)	696.7	269.304	327.33
Melting point °C	>360	179–182	300
Main hazards	Toxic(T), Xi Irritant	Health Hazard, Environmental Hazard, warning	Toxic (T)
UV detection wave length (nm)	498 nm	409nm	510 nm

The Equipment:

(Bench centrifuge ,L.K.B.(U.S.A) (INCUBATOR,MEMMERT ,GERMANY) (Magnetic Stirrer,Scintific Industries -USA)(Refrigerator,KONCORD,Korea)
(Distillator,Controls ,ENGLAND),(PH-Meter,Crison,USA), (ShakerIncubator ,Type LSI-3016A, Korea),(Water Bath,GFL-Germany), (Dialysis tubes ,Sigma ,USA) (Micropipettes ,Volac ,Germany), (Electronic Balance ,A.N.D., Japan),
(Sensitive balance,Sartorius ,Germany),(CM-Cellulose column Chromatography, Ranch) ,(UV-Vis spectrophotometer APEL PD-303 UV Spectrophotometer - Japan), (Millipore filter unit,GallenKamp – England) ,(Beakers, cylinders, flask, pipet ,France)

3. Methodology:

The methodology of this work as follow:



3.1 Peroxidase enzyme Sources:

The Peroxidase enzyme was extracted from seven different plants, including red radish, white radish, apple, banana, green onion, potato, and aloe-vera; These plants were selected according to their low price and availability in the local market. All the plants were washed with tap water and used for Peroxidase extraction.

3.2 Extraction of Peroxidase Enzyme:

Using the blender, fifty grams of each plant (red radish, white radish, apple, banana peel, potato, green onion, aloe vera) were homogenized in 30 ml of sodium acetate (pH 6, 0.1 M) for 5 minutes; The extract was filtered, the filtrate centrifuged at a speed of 10,000 rpm for ten minutes at four degrees Celsius, the supernatant represents the crude Peroxidase enzyme, also was calculated the Peroxidase activity and protein content (Aziz et al.2016) .

3.3 Determination of Peroxidase activity and protein concentration:

Peroxidase activity is assessed using the following methods (Silva 2010) : The substrate solution was made by guaiacol combining the volumes according to the following ratios (1: 1: 1: 7) (V: V: V: V) Guaiacol, Hydrogen Peroxide solution, acetate sodium, distilled water, and was prepared 2.9 ml of the substrate at pH 6.0, and 0.1 ml of the enzyme made up the reaction solution; also 3 ml of the substrate volume was used in the blank sample. Al-Sa'ady (2020) A spectrophotometer was utilized to measure an increase in absorbance at 540 nm after three minutes in order to identify

the oxidation of guaiacol. The amount of enzyme that defines as an absorbance of 0.001/min was evaluated as one unit of Peroxidase activity Aziz et al. (2021) . To calculate Peroxidase activity, the following equation was used AL-Sa'ady,A.J.R.,et.al.2018):

$$Activity\ of\ peroxidase\ (U.ml{-}1) = \frac{((A2\ sample - A1\ sample) - (A2\ blank - A1\ blank))}{(0.001 \times t)}$$

Where:
A2 sample: is the sample's final absorbance (1 U = 1 µmol/min); A1 sample: is The sample's starting absorbance (1 U = 1 µmol/min); A2 blank: is the control's final absorbance (1 U = 1 µmol/min) ; A1 blank: is The control's initial absorbance (1 U = 1 µmol/min); and t is the reaction time in minutes (3 minutes). Using bovine serum albumin as a reference, the Bradford method Bradford (1976), which measures protein content, was applied.

3.4 Optimum pH and extraction buffer type for Peroxidase extraction:

To prepare red radish for Peroxidase extraction, various buffer types were mixed and homogenized. The buffers were (0.1M , pH (4.0, 5.0, 6.0)) Sodium acetate, (0.1M, pH 7.0) Potassium Phosphate, (0.1M, pH 7.0) Sodium Phosphate, (0.1M, pH 8.0) Tris-HCl. Furthermore, distilled water was utilized to extract the enzyme. Estimates were made of the protein content and activity of the enzyme (Aziz et al.2016) .

3.5 Extraction ratio:

To extract the Peroxidase enzyme, the red radish was mixed in varying quantities of (0.1M, pH 6.0) sodium acetate buffer (0.5:1, 0.75:1, 1:1,1.25: 1,1.5:1:,1.75:1, and 2:1) were the extraction ratios (W:V). Estimates were made on the protein content and enzyme activity
(Al-Sa'ady 2020).

3.6 Purification of the Peroxidase enzyme:

3.6.1 Ammonium sulfate precipitation:

The precipitation phase was done by adding ammonium sulfate ($NH_4)_2SO_4$) for the Enzyme extract at 0-70 as a saturation rate. At 4 ^{O}C, solid ammonium sulfate was gradually added, mixed for 60 minutes, and centrifuged at 10000 rpm for 30 minutes. Precipitates were solved in a small volume of 0.02 M Tris-HCl buffer pH 8.0, for 24 hours was dialyzed by (1000 ml, pH 8.0)Tris-HCl solution. Protein content and activity were estimated (Aghelan et al.2015) , (Park et al.2015).

3.6.2 Purification of Peroxidase by ion-exchange chromatography:

Following the directions provided by Pharmacia Company, a Carboxymethyl cellulose (CMC) column (25 x1.7 cm) was produced, packed, and equilibrated using (0.02 M, pH 8.0) Tris-HCl buffer. A pasture pipette was used, the dialyzed protease volume (13 ml) was gently passed in a (CMC), thereafter was washed by(0.005 M, pH 8.0) Tris-HCl buffer (Whitaker 1972) , then used the same buffer to elute bound proteins, and a gradient of NaCl 0.1–1 M was fed over the exchanger at a flow of 30 ml/hr. Protein fractions were gathered in three milliliters. The absorbance of each fraction, including the washed and eluted fractions, was measured after it had been collected at a wavelength of 280 nm. After calculating the enzyme activity in each fraction,

gathering the activation components, and estimating the enzyme activity and protein concentration (Aghelanetal.2015), (Park.et.al.2015).

3.7 Characterization of enzyme:

3.7.1 Optimum pH for partially purified Peroxidase activity

The substrate solution was created with various pH levels (3-9). For establishing the optimum pH for enzyme activity. Enzyme activity for the produced substrate was measured for various pH (AL-Sa'ady et al. 2018) . The relationship between enzyme activity and pH was then drawn.

3.7.2 Optimum pH for enzyme stability

In test tubes, 2 ml of the enzyme and 2 ml of buffers previously made at a pH range of 3 to 9 were combined, and the mixture was incubated at 40 ^{O}C for 30 min in a water bath. After cooling immediately in an ice bath, the enzyme activity was estimated for each treatment (AL-Sa'ady et al.2018) , and a relationship between the percentage of remaining activity and pH was drawn to determine the pH of Peroxidase stability.

3.7.3 The optimum temperature for partially purified Peroxidase activity

In order to determine the best temperature for Peroxidase activity, the substrate solution (2.9 ml) was combined with 0.1 ml of the enzyme solution and incubated for 3 min at various temperatures (15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, and 75) °C (Mizobutsi et al.2010). The activity was then estimated for each treatment and drawn the relation for the remaining activity (in percent) with optimum pH for Peroxidase stability.

3.7.4 Thermal stability of partially purified Peroxidase

A water bath was used to incubate one ml of partially purified Peroxidase for 30 min at various temperatures (15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, and 75) °C. The enzyme test tubes were immediately placed in an ice bath, after which the enzyme activity was calculated (AL-Sa'ady et al.2018) .In addition, a relationship between residual activity (in percent) and temperatures was drawn to test the thermal stability of the enzyme.

3.8 The Enzyme Immobilization:

The (crude and purified) Peroxidase enzyme was immobilized by dissolving five grams of 0.1M Na-alginate (pH 6) with distilled water and mixed with the enzyme in the ratio of (Enzyme: Na-alginate) 0.25:1 (V: V) at 25 °C , and (0.2 M,100 ml)Cacl2 solution was prepared (Jasim,A.M.,et.al.2019). The immobilized enzyme was prepared as white beads by distillation in Cacl2(0.2M) solution (Jasim et al.2019).

3.7.6 Decolourization by (crude and purified) Peroxidase enzyme immobilization:

Congo red (CR), Methyl red (MR) and Methyl Orange dyes with (400 mg/l and 1000 mg/l) concentrated were used in two tests,the crude and purified Peroxidase beads were added to the 1:3 (W: V) for each solution, the solutions were stirred at (25 °C,120 rpm) using a thermostatic shaker and then screen 10 ml of each dye after 24 hours at 540 nm. The decolorization efficiency of inactivated Peroxidase was assessed by monitoring the decrease in absorbance and the decolorization activity was calculated (AL-Sa'ady et al.2018) ,as follows:

Decolorization %: is the removal efficiency of dye at time t.

Ai: is the initial absorbance of dye before enzymatic treatment (U/ ml).
Af: is the final absorbance of dye after enzymatic treatment (U/ ml).

4. RESULTS AND DISCUSSION

4.1 Peroxidase sources:

Figure (1) illustrates the various specific Peroxidase activity for diverse plant sources. This graph demonstrates that red radish Peroxidase produced the highest specific activity 9271 (U/mg), while white radish, bananas, apples, potatoes, aloe vera, and green onions produced specific activities that were 8195, 3596, 913, 726, 405, and 267 U/mg, respectively. Because it is readily available and affordable, red radish was chosen as a source of Peroxidase (Rosario Goyeneche et.al.2015) Since red radish is affordable and widely accessible, it was chosen as the source for extracting the Peroxidase enzyme.

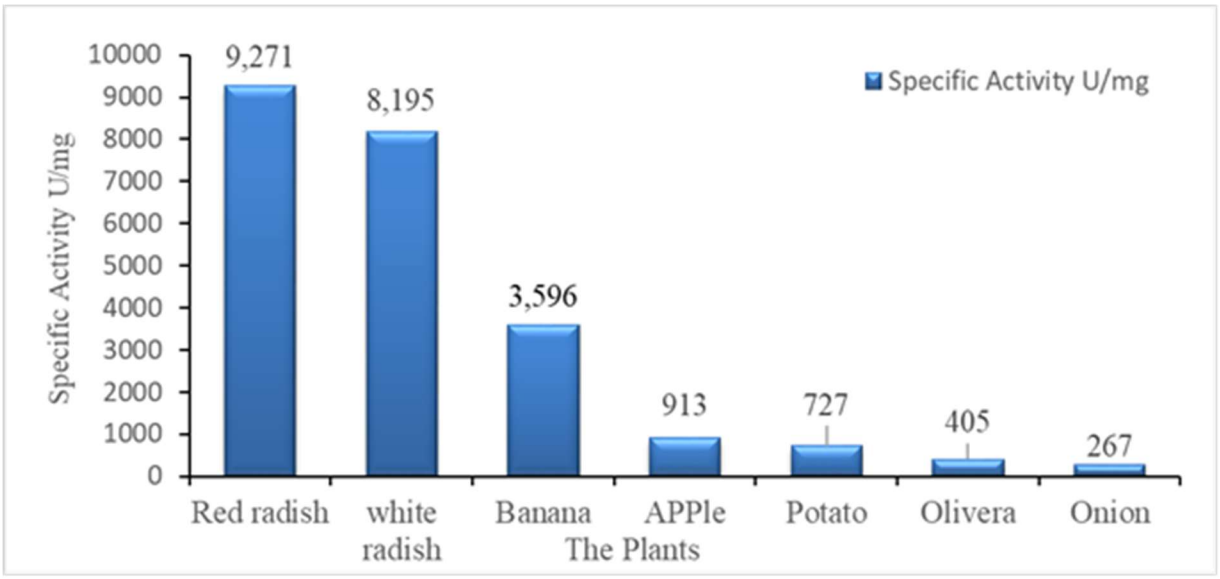


Figure (1): The different activity for Peroxidase extract from plant sources at pH 7, 25˚C.

4.2 Extract buffer type:

After extracting the enzyme using various buffers, the specific peroxidase activity was calculated, as shown in Figure (2). These findings demonstrate that the sodium acetate buffer 0.1 M, pH 6 was the better buffer to extract enzymes having specific activity reaching 16638.5 U/mg, whereas the specific activity of the other buffers was in the order of (16638,16488,13169,13773,11218,9003),respectively.

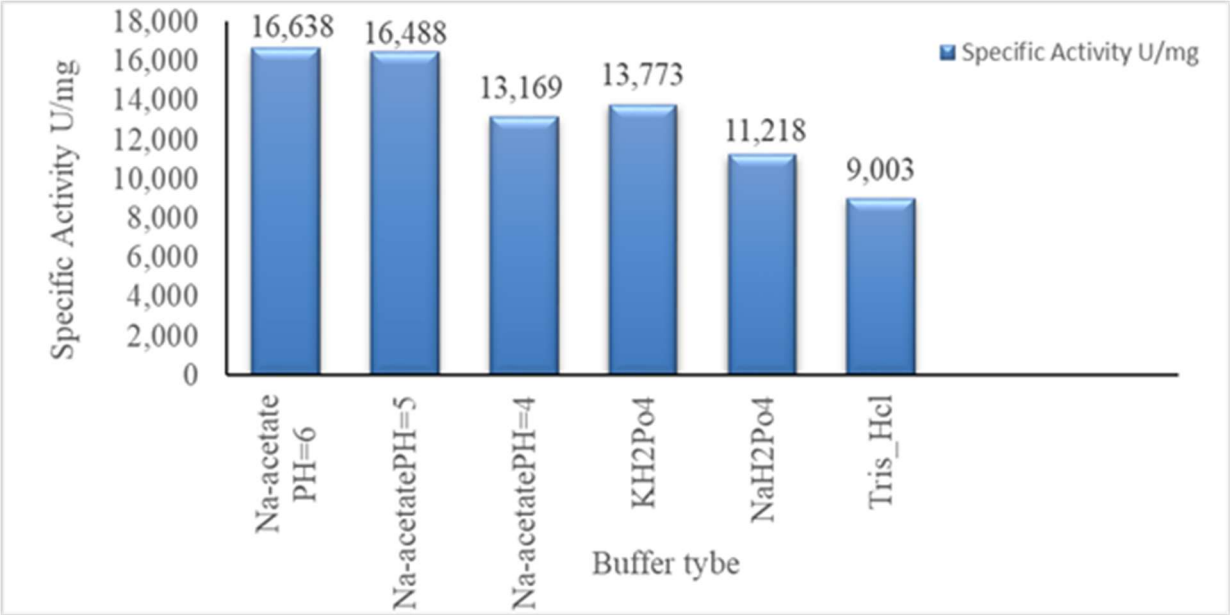


Figure (2): Experiment to determine the best buffer and best pH for peroxidase extraction from red radish at 25˚C.

4.3 Optimum ratio for Peroxidase extraction:

Seven ratios were used for the purpose of estimating the optimum ratio of peroxidase extraction from red radish, which is: 0.5:1, 0.75:1, 1:1, 1.25:1, 1.5:1, 1.75:1, and 2:1 (W: V). 0.1 M sodium acetate (pH 6.0) was used and it was found that the highest specific activity of the crude extract was in a 2:1 (W: V) ratio with a specific activity of 44414.4 units/mg protein. While the results of the other ratios were as follows (8561, 19207, 16638, 23010, 25110, and 39969) U/mg protein, respectively, as shown in Figure (3).

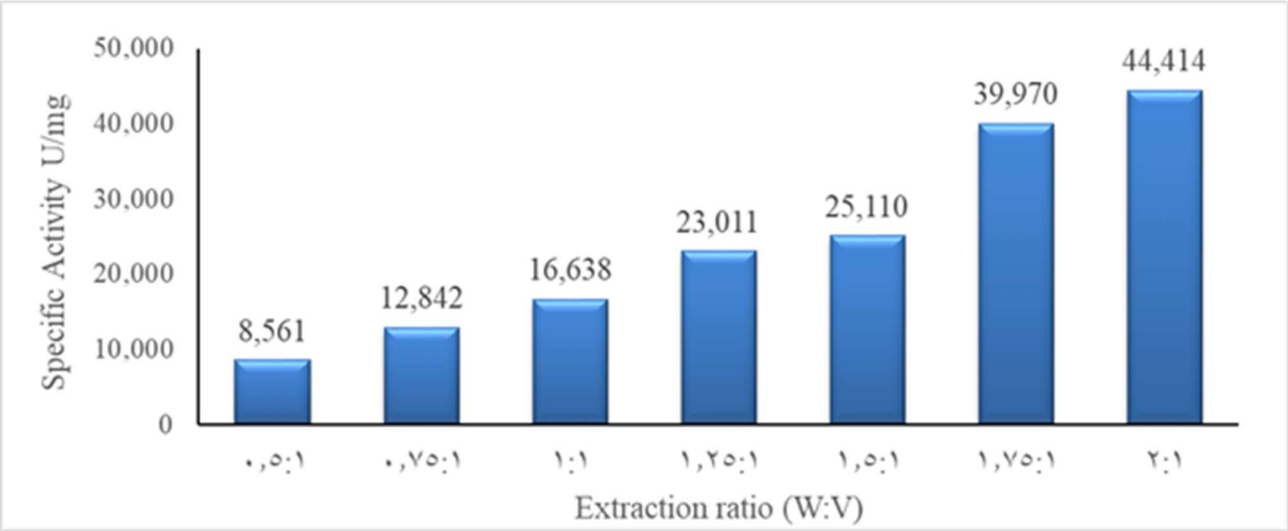


Figure (3):Optimum ratio for Peroxidase enzyme extraction from red radish at pH 6, 25˚C.

4.4 Purification of enzyme:

Ion-exchange chromatography was used to purify the Peroxidase from red radish using a (CMC) column. The test demonstrated that used (0.05 M, pH 8.0) of Tris-HCl to wash, which is represented by fractions 12-47, permits the emergence of one peak (Figure 4).Three peaks are visible after adding 170 ml of Tris-HCl to a NaOH gradually (the elution stage), and they are represented by the fractions 59-81, 83-87 and 89-89. The protease activity and protein content were evaluated for each fraction. The wash step 12-47 fractions showed Peroxidase activity. The outcomes further demonstrate that the red radish-derived Peroxidase had negative charges that repel each other to the negatively charged (CMC); as a result, the Peroxidase was eluted and get it in the wash step. Protein concentration is 0.014 mg/ml, activity is 2913 U/ml, specific activity is 208071 U/g, purification fold is 3.5, and yield is 77 percent, as shown in Table 1.

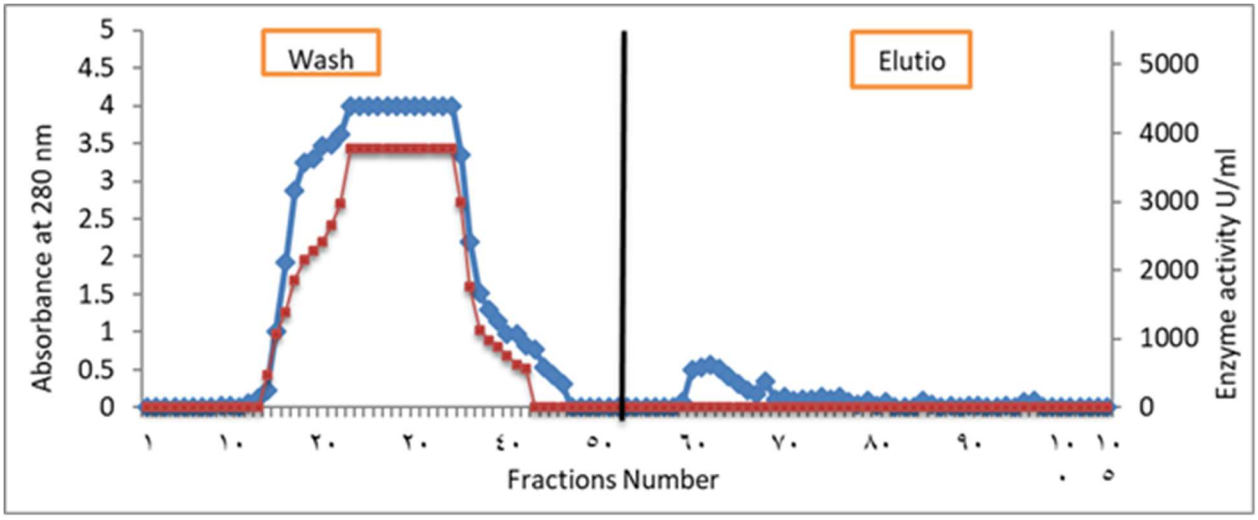


Figure (4): Purification of the enzyme by Ion exchange chromatography using (CMC) column (25 -1.6) cm equilibrated with (0.05 M, pH8.0) Tris-HCl, eluted with NaCl gradient (0.1-1 M) and Tris-HCl, inflow 30 ml/ hr., 3 ml for each fraction.

Table 2: Purification steps of Peroxidase extract from red radish

Step	Volume	Activity U/ml	Protein concentration mg/ml	Specific activity U/mg	Total activity	Fold	Yield %
Crude extract	200	1,649	0.028	58,893	329,800	1	100
Ammonium sulphate precipitation	15	20,570	0.078	263,718	308,550	4.4	93.9
Purification of enzyme by ion-exchange chromatography (CMC)	87	2,913	0.014	208,071	253431	3.5	77

4.5 Optimum pH for partially purified Enzyme activity:

The optimum pH for partially pure Peroxidase activity was pH 6.0 and the activity was depressed at pH levels lowest and highest from this value, As shown in the figure (5).

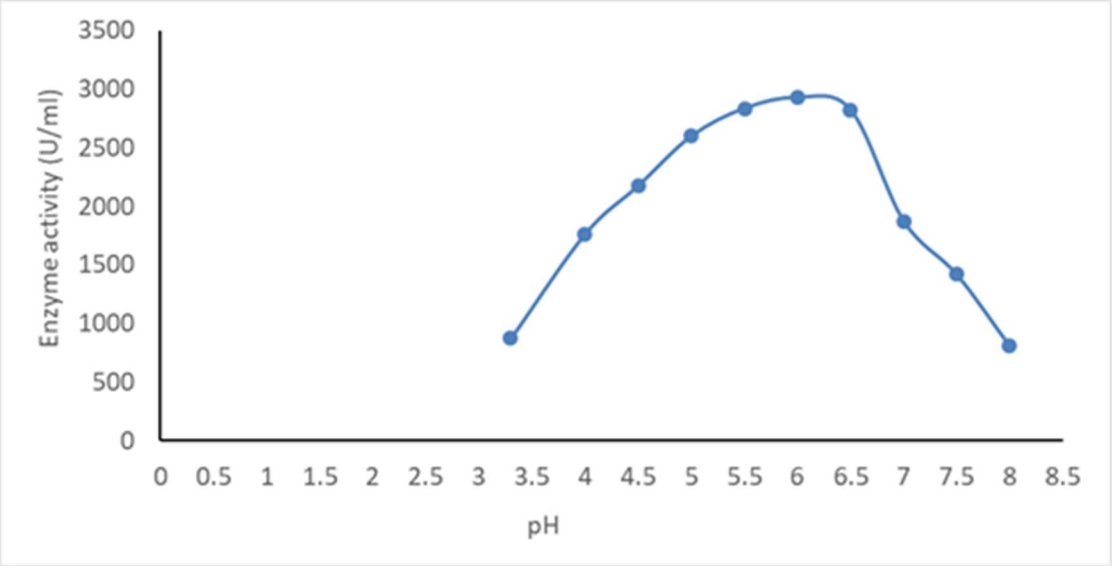


Figure (5): Effect of different pH on partially purified Peroxidase activity at 25˚C.

4.6 Partially purified Peroxidase stability:

The pH for partially purified Peroxidase stability is crucial for figuring out the best conditions for storing and purifying enzymes. According to the findings in Figure (6), Peroxidase was least active at pH values of 8.5 and had a stability at range of pH values of 4.0 to 8.0.

When is a change in the secondary and tertiary textures of the remains catalytic and the ionic case for the active site, that causes a decrease in enzymatic activity under acidic and basic circumstances ((Aghelan et al.2015), (Lehmacher et al.1990)

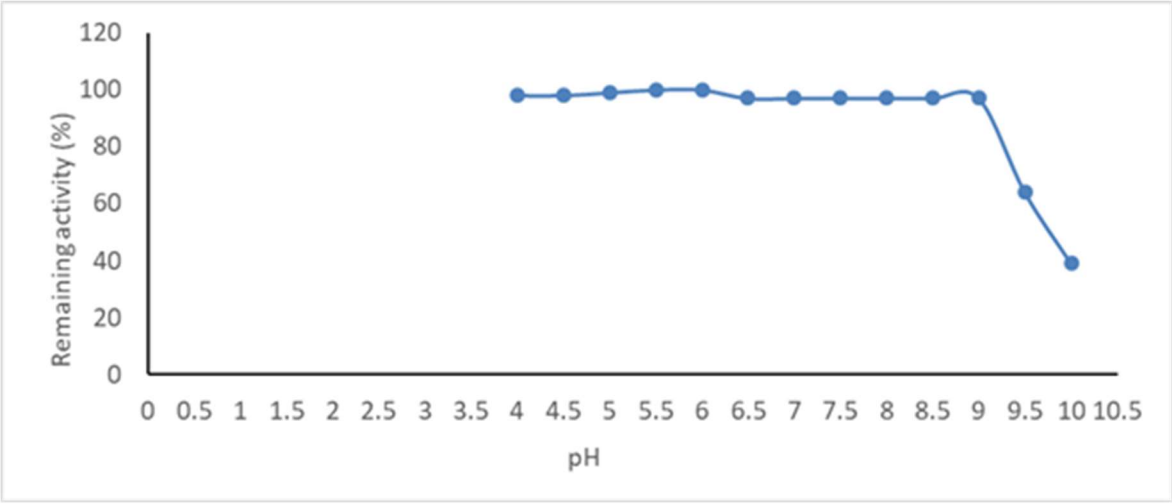


Figure (6): The effect of pH on the partially purified Peroxidase stability extracted from red radish.

4.7 The effect of optimum temperature on partially purified Peroxidase activity:

The Peroxidase and substrate were incubated for 3 minutes at various temperatures between 15 and 70 °C in order to determine the ideal temperature for enzyme activity. According to Figure (7), the best temperature for red radish partly purified Peroxidase activity was 25 °C, with a maximum activity of 2917 U/ml. After that, the activity decreased until it was completely lost at 60 °C.

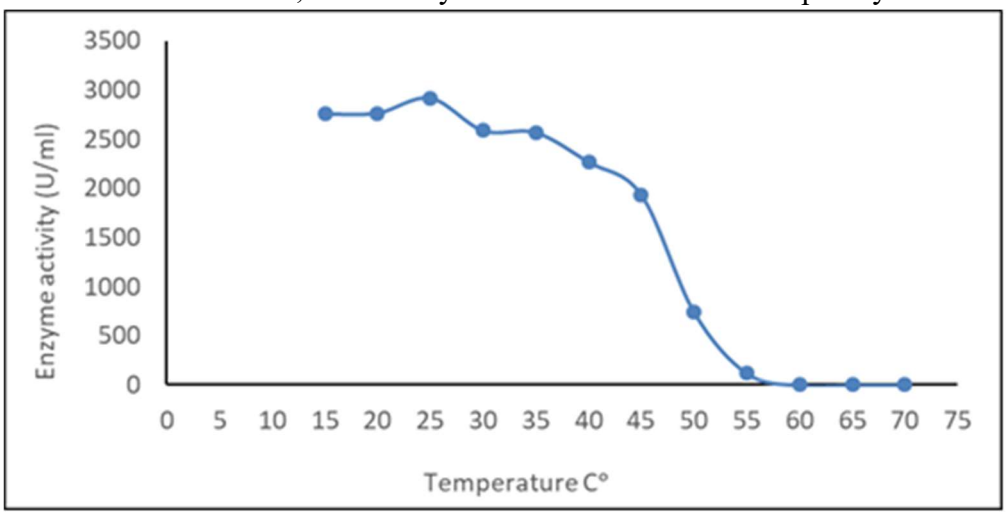


Figure (7): Influence of temperature on partially purified Peroxidase activity from red radish at pH 6.

4.8 Optimum temperatures for partially purified Peroxidase stability:

The effect of temperature on partially purified Peroxidase stability from red radish was studied with various temperatures between 15– 75 °C for 30 min. the outcomes of this experiment showed that the partially purified Peroxidase was stable at temperatures between 15-50 °C, then the enzyme activity decreased and was lost at 75 °C (Figure 8).

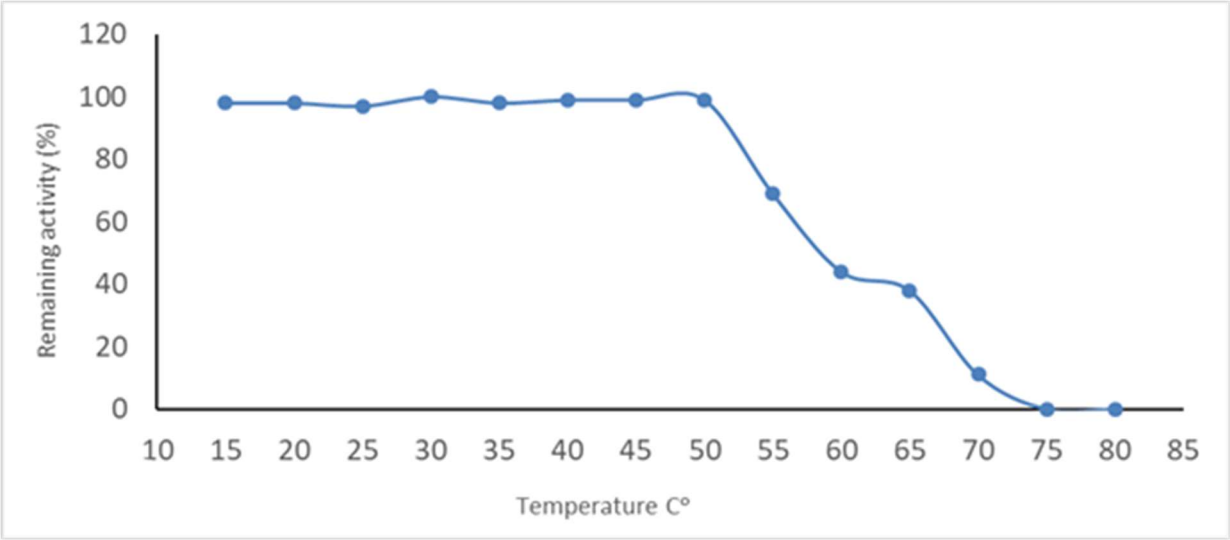


Figure (8): Influence of temperature on partially purified Peroxidase stability from red radish at pH 6.

The reduction in enzyme activity due to the thermal effect on Peroxidase structure maybe led to denaturation of protein. The temperature may do effect at the protein construction by fraction the bonds which set the secondary and tertiary construction for the protein that conduct to denaturation (Chesworth, J. M., et.al.1998).

4.9 Decolourization by the immobilized (crud and purified) Peroxidase enzyme:

The results showed that the removal efficiency of Congo red (CR), Methyl red (MR) and Methyl orange (MO) dyes at a concentration of (400 mg/l and pH 6, at 25 °C using (crude and purified) immobilized Peroxidase enzyme, where removal efficiency was following: 99%, and 94 % for the decolorization of Congo red dye, 11% and 93% for decolorization of Methyl red and 10%, 20% for Methyl orange, respectively. As shown in Figure (9).

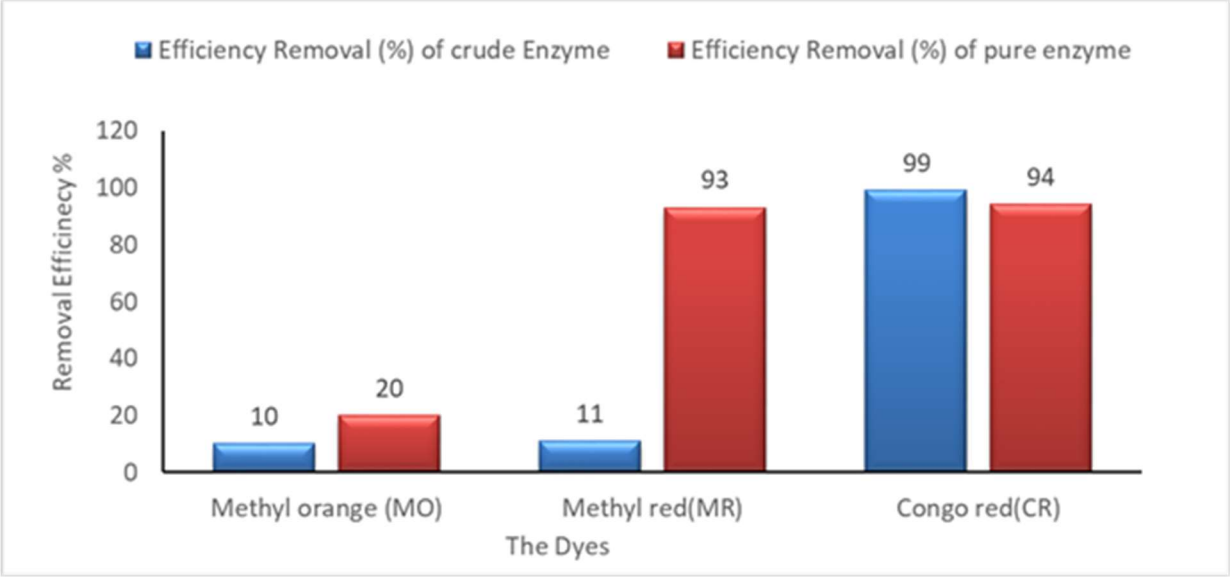


Figure (9): The removal efficiency (%) by(crude and purified) at a concentration of 400 mg/l at pH 6 , 25˚C.

As shows as , the figures (10) (11), the efficiency of removing the three dyes every hour at 1000 mg /l dye concentration, where the results for the crude enzyme of (CR) was 95.7% , 52.9 % for (MR) and 43.8% (MO), while the decolorization using the purified enzyme of CR , MR and MO was (94.8 , 50.4 ,42.9) %, respectively. The pH factor may be influencing the decolorization of the methyl orange solution because the pH of methyl orange ranges between (3.1-4.4), or because of the density of the dye or the presence of metals in the crude peroxidase enzyme as in the case of decoloring methyl red. Also, some previous studies on the treatment of azo dyes with radish extract obtained the results shown in the table 3 below:

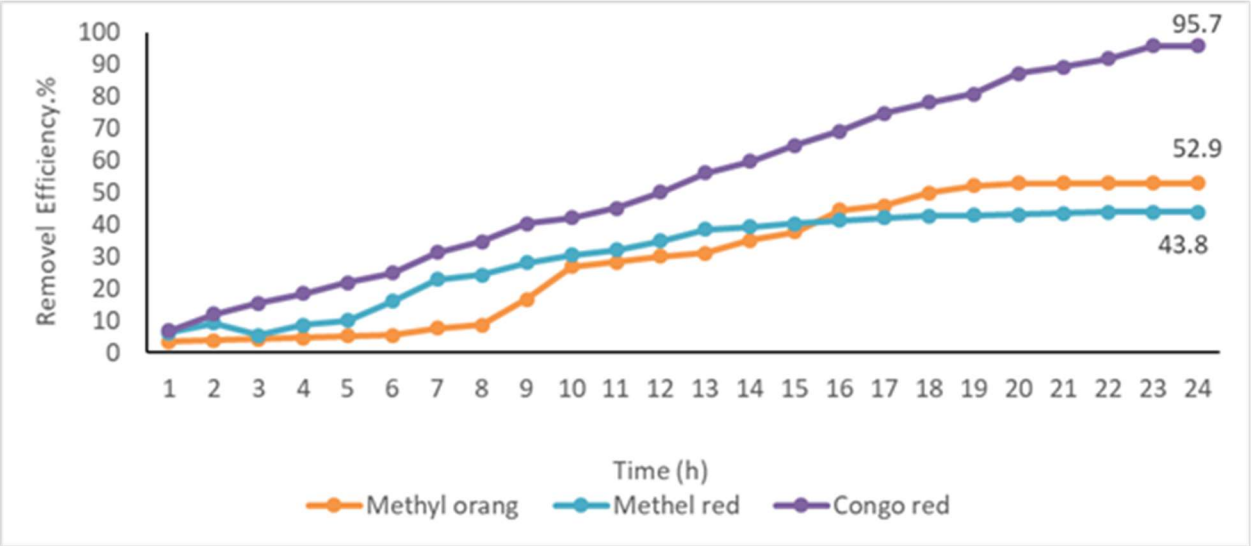
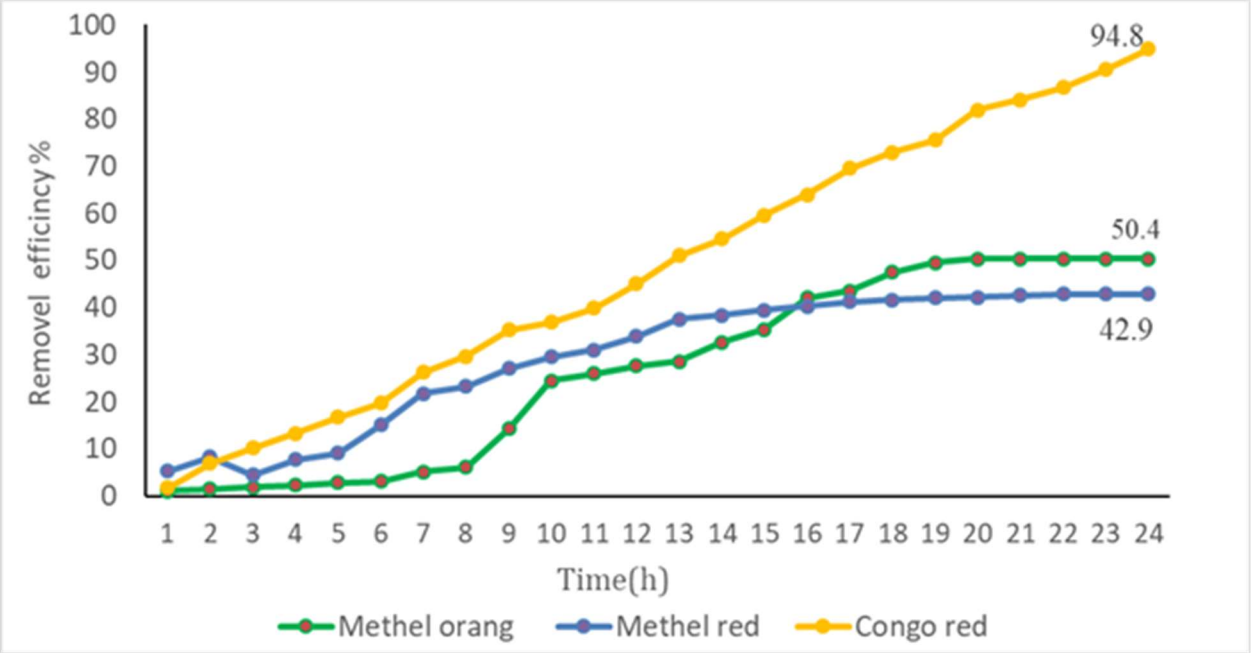


Figure (10): The removal efficiency (%) by crude enzyme for concentration 1000 mg/l at pH 6 , 25˚C.



Figure(11):The removal efficiency(%) by purification enzyme a concentration of 1000 mg/l at pH 6 , 25˚C.

Plant	Dye	Decolorization	Reference
Peroxidases	Congo red	96	Ashwini Rane et al.2020.
Horseradish peroxidas	RemazolBrilliant BlueR, ReactiveBlack 5, , Crystal Violet and CongoRed	82.17–97.82 % By chitosan beads	Bilal M,et.al.2017
Brassicajuncea root	Methyl Orang	92% in 4 days by Enzymatic Activity	Telke A A et, al. 2011
Peroxidase Brassica rapa	Crystal Ponceau 6R Soluble state	97%	Almaguer et al. (2018)
Peroxidase Brassica rapa	Reactive blue 21 Soluble state	57%	Silva et al. (2012)

Table 3: The results of previous studies about the treatment of azo dyes with radish extract

5. Conclusion:

This study concluded that Red radish is a good source of Peroxidase enzyme extraction and can partially purify by ion exchange chromatography. The optimal pH for free and immobilized purified Peroxidase was 6.0, and the stability pH for free and immobilized purified Peroxidase was 4 to 8; the optimal temperatures for free and immobilized purified Peroxidase was 25 ^{o}C, and the stability temperatures range for free and immobilized purified Peroxidase between (15-50 ^{o}C). Good results can be obtained when using the Peroxidase enzyme to remove the Congo red dye present in wastewater resulting from many industries which cause healthy and environmental serious problems.

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