International Journal of Innovative Research and Reviews, 8(2) 42-49

International Journal of Innovative Research and Reviews
ISSN: 2636-8919
Website: www.injirr.com

Research paper, Short communication, Review, Technical paper

RESEARCH ARTICLE

Biochemical Content Analysis of the Preparation in a Storage Container Excavated from a Grave in Daskyleion Ancient City

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ARTICLE INFO

Received : 10.07.2024
Accepted : 11.14.2024
Published : 12.15.2024

Keywords:
Daskyleion
Alabastron
Catalase
Superoxide dismutase
Peroxidase

ABSTRACT

This study aims to shed light on the content of the remains found in an alabaster alabastron that was excavated from T4 Tumulus in the Daskyleion Ancient City in the Ergili village of the Aksakal Town of Bandırma, and is thought to have been used for the storage of essences or drug-like preparations. Daskyleion; the excavations carried out in the city, which is thought to have hosted great civilizations such as Phrygians and Lydians and one of the centers of the Satrapal administration system established by the Persians in Anatolia, dating back to 6th century BC, have unearthed remains from many different periods. The Lydian culture, which existed in the ancient city of Daskyleion, is famous for its cosmetics and there was a concept of "Lydian cosmetics" that has survived to the present day. Research was conducted in this direction, based on the container found in the burial chamber. This study aims to investigate the content of organic residues found in situ in an alabaster alabastron during the excavations of Tumulus T4 (Koru Tumulus) at the ancient city at Daskyleion and to shed light on the burial customs of the period to which the tomb belongs. As a result of the analysis, it was concluded that the perfume left in an alabastron as a grave gift was of plant origin.

Contents

Cite this article Demir N, Demir Y, İren K, Kasar Ö. Biochemical Content Analysis of the Preparation in a Storage Container Excavated from a Grave in Daskyleion Ancient City. *International Journal of Innovative Research and Reviews (INJIRR)* (2024) 8(2) 42-49
Link to this article: http://www.injirr.com/article/view/231

1. Introduction

Daskyleion is an ancient city located within the borders of modern Ergili neighborhood of Bandırma, Balıkesir, in the region known as Mysia in ancient times. Founded in the 9th century BC, the city hosted Mysians, Phrygians, Lydians, Persians, Macedonians and Byzantines [1,2].

There are many tumulus tombs built for the elites of the city during the Lydian and Persian Periods. It is known that there are perfume samples in the consistency of ointment or cream placed in the containers of the period, especially in the tombs belonging to the elite and administrators of the city [3–5] One of these examples was found in a stone alabastron in Daskyleion. One of these in the ancient city, T4 tumulus, is a family tomb consisting of a burial chamber, antechamber and a dromos. Skeletal parts belonging to two men and a woman were found inside the room [6]. The remains of another man were found in the antechamber.

Tumulus T4, which was understood to have been built in the 5th century BC, has the tomb architecture and burial gifts that we are familiar with from Lydian Tumuli. There are two marble klinai with decorated feet that we see in the tumuli of the Lydian Region in the tomb chamber (Figure 1).

Figure 1 Grave chamber of the T4 Tumulus

A wooden klinai was made in the tomb chamber and the third burial was placed here [7]. Traces of paint belonging to the purple shroud in which the corpse was wrapped are preserved on the marble klinai. Numerous alabastrons were found scattered throughout the tomb chamber and dromos, in whole and in pieces. In addition to the alabastrons, prestigious tomb gifts such as lydions, Lydian type lekythoi, coins of Cyzicus and a rython made of quartz were left.

Alabastrons, which were understood to have been placed in perfume, were found in the T4 tumulus. One of these alabastrons, carved from a stone called alabaster, contained residues of the product it contained (Figure 2a-b) [8,9].

Figure 2 Alabaster alabastron and its content b. Drawing of alabastron

People have always had two basic desires within them in a process extending from the past to the present. One of these is the desire to be immortal and the other is the desire for eternal youth and beauty. In this direction, they have prepared various chemical mixtures, sometimes hoping that they would be a healing and sometimes a beauty elixir. Their devotion to these has reached the level of wanting them to be with them after death. Sometimes, they have also used some mixtures to ward off evil spirits and to be protected in some way after death.

In the Lydian Period, a cosmetic called *bakkaris* was mentioned, which was placed in alabastrons and lydions (Athenaeus, Deipnosophistae, 15.690e). It is known that the cosmetic called *bakkaris* was of plant origin and was made from the plant with the same name (Plinius, Naturalis Historia, 21.16.29-30). Although it is not known exactly which plant the Lydian perfumes were made from, some archaeometric studies have been conducted on perfume residues found in other centers. One of these studies is the analysis conducted on the sediments found in containers called lydions in the ancient cities Gordion and Sardis. In these analyses, it was determined that the residues in the lydion contained a mixture of ruminant animal fats and plant molecules [10].

In this study, the presence of CAT, SOD and POX enzymes was investigated on the residues found in the bottle, which was found during the excavations carried out in the Daskyleion Ancient City and was thought to have been used for the purpose of storing essences.

2. Materials and Methods

2.1. Sample Procurement

The sample used for the study was obtained from the residues found in a bottle that was found during excavations at the Daskyleion Ancient City and was thought to have been used for storing essences.

2.2. Preparation of Homogenate

The residues taken from different points in a bottle that was found during excavations at the Daskyleion Ancient City in the Ergili village of Bandırma's Aksakal Town and was thought to have been used for storing essences were crushed and pulverized. Then, it was washed several times with alcohol (5%) to remove possible bacterial contamination. After this process, it was washed with salt water to remove the alcohol in the environment. No fungi or bacteria were found as a result of the microscopic examination of the sample.

The pulverized sample (5 g) was treated with liquid nitrogen and mixed with 50 ml of 0.1 M phosphate buffer in a mixer and centrifuged at 6,000 x g for 20 minutes. The homogenate was centrifuged and the precipitate was discarded [11].

2.3. Determination of Protein by Coomassie Blue Method

This method was developed by taking advantage of the fact that Coomassie brilliant blue G-250 dye gives a blue color of varying intensity in protein solutions of different concentrations. It was observed that the dye tends to bind especially to basic amino acids such as arginine and some aromatic amino acids such as tyrosine and tryptophan. Coomassie brilliant blue G-250 binds to proteins in phosphoric acid medium and the formed complex shows maximum absorbance at 595 nm. The sensitivity of the method is between 1-100 mg [12].

2.4. Ammonium Sulfate Precipitation

Ammonium sulfate precipitation was performed between 0% and 100% in the homogenate at 0-20, 20-40, 40-60, 60-80 and 80-100. The gram amount of ammonium sulphate used was calculated from the formula below.

$$\text{Ammonium Sulfate Amount (Gram)} = \frac{1{,}77\ X\ V\ X\ (S-So)}{3{,}54-S}$$

2.5. Catalase and Superoxide Dismutase Studies

Catalase enzyme (H_2O_2: H_2O_2 oxidoreductase E.C.1.11.1.6) is one of the oxidase enzymes that reduces or breaks down H_2O_2 and is a structural component of perixisomes [13]. Catalase enzyme found in plant sources consists of four heme-containing subunits and the molecular weights of the subunits are between 54 and 59 kDa, respectively [14]. The basic function of CAT is to prevent irreversible damage that may occur especially in membranes by removing hydrogen peroxide and a peroxide such as ROOH, which are synthesized in some stages of metabolism in the presence of molecular oxygen [15]. Because hydrogen peroxide is a potential source of singlet oxygen and hydroxyl radical [16]. CAT is a protective enzyme that catalyzes the conversion of hydrogen peroxide to water and oxygen, thus preventing hydrogen peroxide from damaging cellular compounds. If hydrogen peroxide is not broken down by CAT, it acts as a precursor to the hydroxyl radical, a very dangerous free radical for the body, and this radical causes permanent damage to the cell. CAT uses hydrogen peroxide as a substrate, both as an electron acceptor and electron donor [17–19].

Superoxide dismutase enzyme (superoxide oxido reductase, EC 1.15.1.1) is found in all cells that metabolize oxygen. It is an important defense against oxygen toxicity. The function of superoxide dismutase (SOD) is to protect aerobic organisms against the harmful effects of superoxide. It catalyzes the rapid dismutation of superoxide radicals into H_2O_2 and oxygen. SOD is an enzyme with very high catalytic activity.

2.6. Preparation of Sephadex G-25 Column

3g of Sephadex G–25 gel was taken and added to 90 ml, 25 mM pH=8.0 (4 °C) Tris-HCl buffer. The column (1.7x10 cm) was filled with gel and equilibrated with 25 ml, 25 mM pH=8.0 (4 °C) Tris-HCl buffer. The column was waited for one day to equilibrate.

2.7. Application of Proteins to Sephadex G-25 Column

1 ml of enzyme extract containing catalase and superoxide dismutase enzymes was applied to the column. Samples were taken from the column with 25 mM pH=8.0 (4 °C) Tris-HCl buffer. Protein samples were collected in 1 ml tubes and the amount of protein, catalase and superoxide dismutase enzyme activities were examined for each collected tube.

2.8. Determination of Catalase Activity

The method used for the determination of catalase (CAT) activity is the method applied by [20] based on Luck [21]. Activity measurement with this method is based on the principle of monitoring the absorbance decrease at 240 nm that occurs while H_2O_2 in the CAT activity measurement medium is converted to O_2 and H_2O [20].

For activity measurement, 1.475 mL of 103 mM KH_2PO4 buffer and 1.5 mL of 40 mM H_2O_2 substrate solution were placed in a 3 mL spectrophotometer cuvette and 25 µL of enzyme extract was added. After the cuvette was placed in the spectrophotometer, its absorbance against the blank was read at 240 nm for 3 minutes at 1-minute intervals. In the measurements, the absorbance decrease per minute was calculated from the range in which the absorbance decreased linearly. The amount of enzyme that reduces the absorbance by 1 µmol in 1 minute at 25 °C was accepted as 1 enzyme unit [22].

2.9. Optimum pH Study of Catalase Enzyme

For the optimum pH study, 40 mM H_2O_2 solution was used as a substrate and the phosphate buffer range from pH: 5.0 to 9.0 was studied. The results were shown in a table and graphed.

2.10. Investigation of The Effect of Temperature on Catalase Enzyme

The investigation of the effect of temperature on catalase enzyme was carried out with H_2O_2 substrate. It was studied at the optimum pH of the enzyme and at temperatures between 0-90°C. The desired temperatures were adjusted in an ice bath below room temperature and using a heated and circulating water bath above room temperature. The results are given in a graph.

2.11. Determination of Superoxide Dismutase (SOD) Enzyme Activity

Superoxide dismutase (SOD) activity is based on the spectrophotometric determination of the photochemical reduction reaction of nitro blue tetrazolium (NBT) to blue colored formazan by superoxide radicals by SOD enzyme [23,24].

The reaction mixture (3 mL); contains 50 mM KH_2PO_4 (pH: 7.8), 13 mM methionine, 75 mM NBT, 2 mM riboflavin and 0.1 mM EDTA. The color change intensity of NBT was read against the blank at 560 nm within 15 min. The blank consists of the same process without enzyme. 1 unit of SOD activity, the enzyme amount causing 50% inhibition of NBT reduction observed at 560 nm, was accepted as 1 enzyme unit and the values were presented as EU/g leaf [23,24].

2.12. Optimum pH Study of Superoxide Dismutase Enzyme

SOD enzyme activity was determined using buffers prepared at pHs ranging from 4 to 100. In activity determinations, the spectrophotometer was set to 560 nm and absorbance values were read and calculated.

2.13. Examination of The Effect of Temperature on Superoxide Dismutase Enzyme

In order to determine the optimum temperature of SOD enzyme, enzyme activity was examined between 0–100 °C. In activity determinations, the spectrophotometer was set to 560 nm and absorbance values were read and calculated. Water bath was used for high temperatures and ice bath was used for low temperatures.

2.14. Peroxidase Studies

Peroxidase (EC 1.11.1.7) is a monomeric heme-containing enzyme with a molecular weight between 32 and 45 kDa. Peroxidases use peroxide as an electron acceptor and oxidize a large number of donor compounds. Peroxides are widely distributed in nature and are expressed in eukaryotic and prokaryotic cells [25–28].

2.15. Purification of Enzyme by CM-Sephadex A50 Ion-Exchange Chromatography

4 g CM-Sephadex was transferred to 100 ml distilled water and incubated in a thermostatic water bath at 90°C for 5 hours to swell. The swollen gel was incubated in 0.5 N 100 ml cold NaOH for 1 hour and then neutralized with 0.5 N HCl. After the air was removed from the gel material and packed into a 5x30 cm^2 x cm column, it was equilibrated with 100 mM phosphate buffer (pH: 7.0). The buffer level on the gel was lowered to the gel level and the enzyme solution obtained from dialysis was applied to the column using an automatic pipette. Then, washing was performed with 10 mM phosphate buffer (pH: 7.0) and the washing process was continued until the pH and 280 nm absorbance values of the wash buffer added from the top and the eluates taken from the bottom became equal.

After the washing was completed, 250 ml of 100 mM phosphate buffer (pH: 7.0) was filled into the chamber of the gradient mixer connected to the column and mixed with a mechanical mixer, and 250 ml of 1 M NaCl prepared with 100 mM phosphate buffer (pH: 7.0) was filled into the other chamber opening to this chamber, and linear gradient elution was started with increasing ionic strength.

2.16. Determination of Peroxidase Enzyme Activity

Peroxidase (POD) activity determination is based on the principle of monitoring the absorbance increase caused by the colored compound, which is the product of the reaction in which guaicol and H2O2 are substrates, at 470 nm [29].

For activity measurement, 10 mL of substrate solution containing 100 mL of 0.1 M, NaH2PO4 (pH: 5.5) and 5 mM guaicol will be placed in the spectrophotometer cuvette, and 10 µL of enzyme extract will be added. The absorbance increase at 470 nm for 5 minutes will be recorded at 1 minute intervals and the absorbance increase in the part where the absorbance increases linearly will be proportional to 1 minute. The amount of enzyme that increases the absorbance by 0.01 in 1 minute at 25 °C was accepted as 1 enzyme unit.

2.17. Optimum pH study of Peroxidase Enzyme

For the optimum pH study, pH was studied in the range of 3.0–9.0. 0.1 M acetate buffer was used for pH 3.0–4.5, 0.1 M phosphate buffer was used for pH 4.5–7.5, and 0.1 M Tris/HCl buffer was used for pH 8.0–9.0. The activity of the enzyme was measured spectrophotometrically in these buffer solution ranges and the findings were given in graphs.

2.18. Examination of the effect of temperature on peroxidase enzyme

Studies conducted to examine the effect of temperature on enzyme activity were studied at the optimum pH of the enzyme for each substrate and in the range of 0–80°C. The desired temperatures were adjusted using a circulatory water bath. The enzyme solution was transferred as quickly as possible and activity measurements were made. The findings were given in graphs.

Determination of Molecular Weight by Gel Filtration Sephadex G100 column (3 x 70 cm) was prepared. The column was equilibrated with buffer (0.05 M Na_2HPO_4, 1 mM dithioerythritol, pH 7) until the absorbance at 280 nm became zero. Standard protein solution (bovine serum albumin, 66 kDa; egg ovalbumin, 45 kDa; pepsin, 34 kDa; trypsinogen, 24 kDa; β-lactoglobulin and lysozyme, 14 kDa) was added to the column. Purified protease enzyme was added to the separation column and then eluted under the same conditions. The flow rate was set as 20 mL/h throughout the column. The elution volume was compared with standard proteins.

Table 1 Catalase purification process from residues and the obtained results

Steps	Volume mL	Activity EU/mL	Total Activity EU	%	Protein amount (mg/mL)	Specific Activity EU/mg	Purification rate
Crude extract	70	0.055	3.85	100	1.98	0.028	-
40% $(NH_4)_2SO_4$	7	0.421	2.95	76.6	1.22	0.45	12.32
Sephadex G-25	5	0.170	0.85	22.08	0.08	2.125	75.89

Table 2 Superoxide dismutase purification process from residues and the results obtained

Steps	Volume mL	Activity EU/mL	Total Activity EU	%	Protein amount (mg/mL)	Specific Activity EU/mg	Purification rate
Crude extract	60	1,08	64,8	100	3,82	0,28	-
60% $(NH_4)_2SO_4$	20	0.95	19	29.32	1.07	0.89	3.18
Sephadex G-25	10	0.61	6.1	9.41	0.10	6.1	21.79

The data regarding the results obtained using all purification techniques are given in Table 1 and Table 2. Ammonium sulfate fractionation, a widely used technique in enzyme purification, was used as the first step. Catalase enzyme was obtained from the crude extract at 40–60% $(NH_4)_2SO_4$ saturation. For superoxide dismutase enzyme, 60-80% $(NH_4)_2SO_4$ saturation was obtained from the crude extract. Catalase and superoxide dismutase activity peak enzymes were applied to ion exchange chromatography. Protein determination was performed in the obtained eluates by Bradford method and then the purification degree of both enzymes was calculated separately for these two steps. The purification degree of catalase enzyme was calculated as 75.89%. The purification degree of superoxide dismutase enzyme was calculated as 21.79%.

The results of the purification of peroxidase by the three-phase separation system prepared from the oleander flowers in optimized conditions are given in Table 2. 40% (w/v) ammonium sulfate saturation and in the 1:1.5 (v/v) homogenate: t-butanol ratio, the enzyme was purified from the mid-phase of the UFA system with an activity yield of 372,09% and purification coefficient of 1.064.

In the TPP system to efficiently collect the enzymes at the interface, studies were carried out in the presence of 40% ammonium sulfate and 1.0: 0.5, 1.0: 1.0, 1.0: 1.5, 1.0: 2.0 homogenate: t-butanol (v / v). After the process, the phases were carefully separated. The amount of protein was most observed in the medium phase [30,31]. The supernatants were discarded. The homogenate was brought to 40% (w / v) ammonium sulfate saturation and different amounts of t-butanol (1: 0.5, 1: 1, 1: 1.5, 1: 2, v / v) were added. Phase separation at room temperature was expected to occur. In each medium and sub-phase obtained from the homogenate: t-butanol ratios, peroxidase activity and protein were determined and the phase with the highest yield and purification coefficient was determined. The highest activity was observed in the middle phase.

In this TPP system, while the ammonium sulfate saturation was 40% (w / v) and the homogenate: t-butanol ratio was 1: 1.5, the enzyme predominantly preferred to remain in the middle phase. The results are given in Table 3.

The activity-absorbance graph of the catalase enzyme purified from the residues by ion-exchange chromatography is shown in Figure 3.

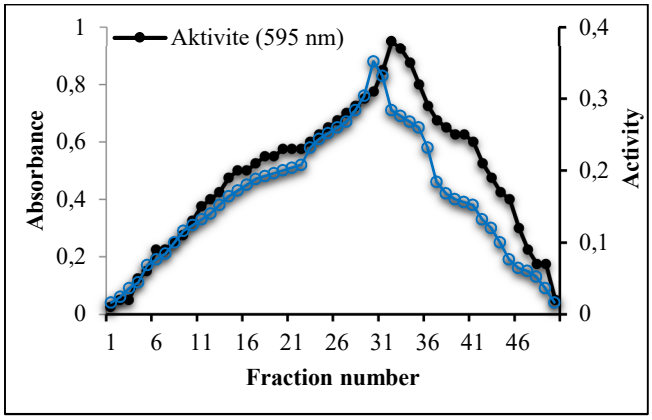

Figure 3 Activity-absorbance graph of catalase enzyme purified from residues

3. Results

3.1. Purification Enzymes

The presence of catalase and superoxide dismutase enzymes on the residues found in the bottle found during the excavations carried out in Daskyleion Ancient City and thought to have been used for essence storage was investigated using ammonium sulfate precipitation and Sephadex G–25 ion exchange chromatography techniques. H_2O_2 was used as a substrate in determining the activity of the protein eluted from Sephadex G–25 column.

The molecular weight of catalase enzyme purified from residues was determined as 56.0 kDa using gel filtration chromatography and compared with known standard proteins.

3.2. Effects of pH on Protease Activity

In order to determine the optimum pH, activity measurements were made in the range of pH 5–9. 0.1 M acetate buffer was used for pH 4–5, phosphate buffer for pH 6–7, and Tris-HCl buffer for pH 8–9. The optimum pH was found to be 5.5 as shown in Figure 4.

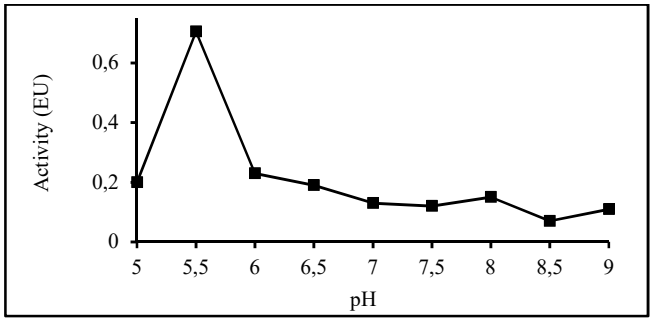

Figure 4 Effect of pH on the activity of catalase enzyme purified from residues.

3.3. Effect of Temperature on Purified Catalase Enzyme

Catalase activity of the enzyme purified from residue was determined in the temperature range of 0-90°C. The amount of activity against temperature change is shown in Figure 5. The temperature at which the highest activity measurement was taken was determined as 60 °C.

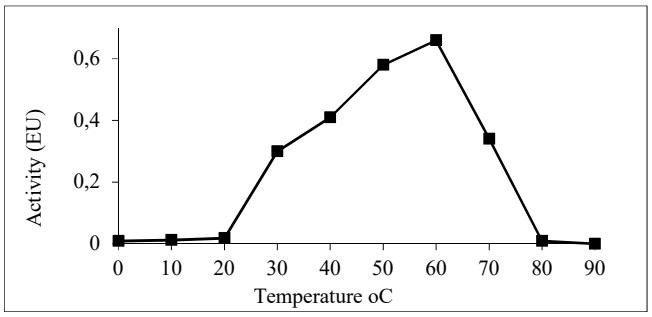

Figure 5 Effect of temperature on the activity of catalase enzyme purified from residues

The activity-absorbance graph of the superoxide dismutase enzyme purified from the residues by ion-exchange chromatography is shown in Figure 6.

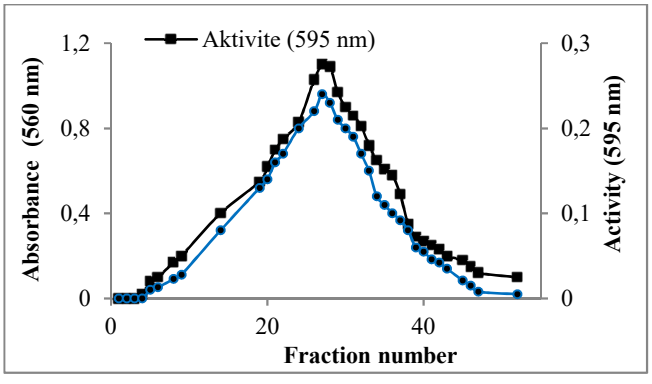

Figure 6 Activity-absorbance graph of superoxide dismutase enzyme purified from residues

The molecular weight of superoxide dismutase enzyme purified from residues was determined as 34.5 kDa using gel filtration chromatography.

3.4. Effects of pH on Superoxide Dismutase Activity

Activity measurements were made in the pH 5–9 range to determine the optimum pH. 0.1 M acetate buffer was used for pH 4–5, phosphate buffer for pH 6–7, and Tris-HCl buffer for pH 8–9. The optimum pH was found to be 7.0 as shown in Figure 7.

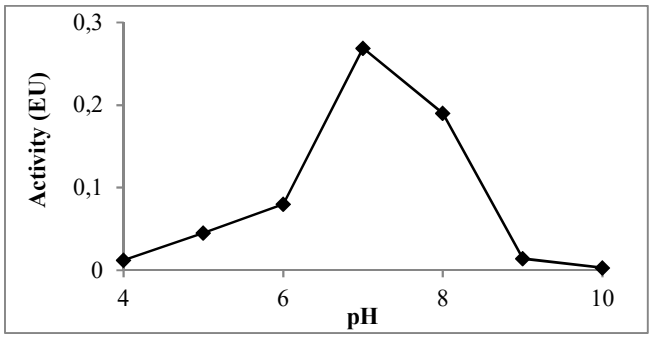

Figure 7 Effect of pH on the activity of superoxide dismutase enzyme purified from residues.

3.5. Effect of Temperature on Purified Superoxide Dismutase Enzyme

The activity of superoxide dismutase purified from residues was determined in the temperature range of 0-100 °C. The amount of activity against temperature change is shown in Figure 8. The temperature at which the highest activity measurement was taken was determined as 50 °C.

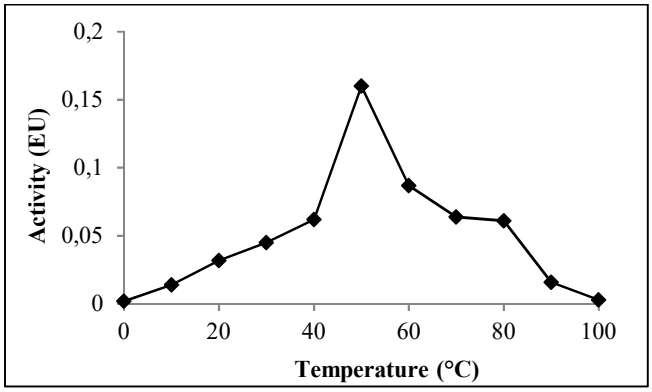

Figure 8 Effect of temperature on the activity of superoxide dismutase enzyme purified from residues

3.6. Purification of Peroxidase Enzyme

The presence of peroxidase enzymes on residues found in a bottle that was found during excavations at Daskyleion Ancient City and was thought to have been used for essence storage was investigated using ammonium sulfate precipitation and CM-Sephadeks A50 ion exchange chromatography techniques. Guaicol and H2O2 were used as substrates to determine the activity of the protein eluted from the CM-Sephadeks A50 column.

Data regarding the results obtained using all purification techniques are given in Table 3. Ammonium sulfate fractionation was used in the first step. Peroxidase enzyme

was obtained from the crude extract at 20-40% $(NH_4)_2SO_4$ saturation.

Enzymes with peak peroxidase activity were applied to ion exchange chromatography. Protein determination was performed in the obtained eluates by Bradford method and then the purification degree of peroxidase enzyme was calculated separately for each step.

The purification degree of peroxidase enzyme was calculated as 47.13%.

Table 3 Peroxidase purification process from residues and the obtained results

Steps	Volume mL	Activity EU/mL	Total Activity EU	%	Protein amount (mg/mL)	Specific Activity EU/mg	Purification rate
Crude extract	50	0,125	6,25	100	1,57	0,080	-
40% $(NH_4)_2SO_4$	10	0,531	5,31	84,96	1,27	0,418	5,23
Sephadex G-25	5	0,452	4,52	72,32	0,12	3,77	47,13

The activity-absorbance graph of the peroxidase enzyme purified from the residues by ion-exchange chromatography is shown in Figure 9.

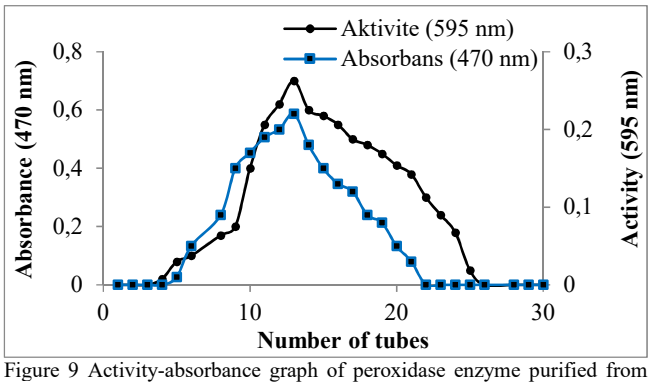

Figure 9 Activity-absorbance graph of peroxidase enzyme purified from residues

The molecular weight of catalase enzyme purified from residues was determined as 43.0 kDa using gel filtration chromatography and compared with known standard proteins.

3.7. Effects of pH on Peroxidase Activity

For optimum pH study, pH range was studied in pH 3.0–9.0. 0.1 M acetate buffer was used for pH 3.0–4.5, 0.1 M phosphate buffer was used for pH 4.5–7.5 and 0.1 M Tris/HCl buffer was used for pH 8.0–9.0. Optimum pH was found to be 6 as shown in Figure 10.

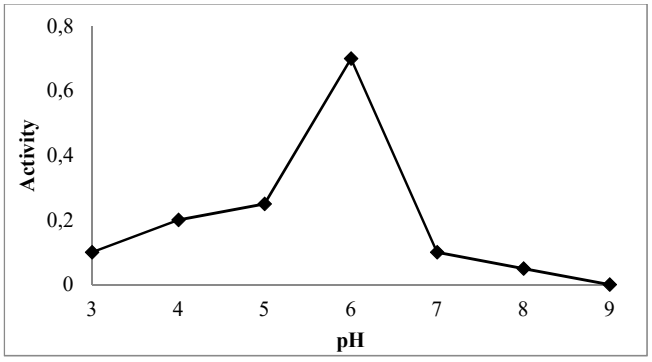

Figure 10 Effect of pH on the activity of peroxidase enzyme purified from residues

3.8. Effect of Temperature on Purified Peroxidase Enzyme

Peroxidase activity of the enzyme purified from residues was determined in the temperature range of 0–80 °C. The amount of activity against temperature change is shown in Figure 11. The temperature at which the highest activity measurement was taken was determined as 40°C.

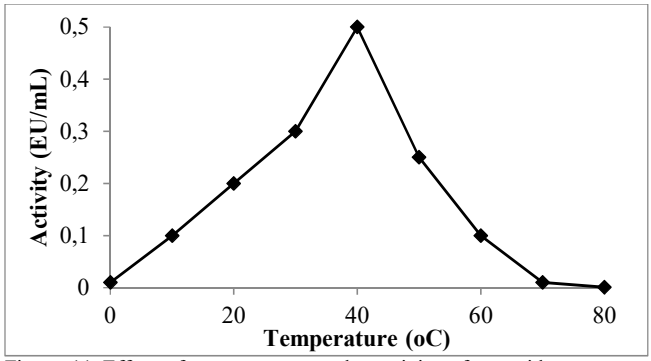

Figure 11 Effect of temperature on the activity of peroxidase enzyme purified from residues.

4. Discussion

The enzymatic properties and molecular characteristics of the residues analyzed from the bottle discovered in the Daskyleion Ancient City show intriguing parallels with known plant-derived enzyme preparations. The catalase enzyme's molecular weight of 56 kDa and its optimum pH (5.5) and temperature (60°C) are consistent with catalase enzymes reported in plant extracts, such as those isolated from Brassica species, which exhibit similar thermostability and acidic pH optima. Likewise, the superoxide dismutase enzyme, with a molecular weight of 34.5 kDa and an optimum pH of 7 and temperature of 50°C, aligns well with plant SOD enzymes, such as those found in Spinacia oleracea (spinach), which demonstrate comparable molecular weights and neutral pH activity ranges.

The peroxidase enzyme's molecular weight of 43 kDa, along with an optimum pH of 6 and temperature of 40°C, is indicative of plant-derived peroxidases such as those reported from Horseradish peroxidase (HRP), which share similar physicochemical characteristics. These results are compatible with the hypothesis that the residues originated from a preparation potentially derived from Baccharis species, a genus known for its antioxidant and enzymatic properties.

Moreover, the purification approach employed in this study, involving ammonium sulfate precipitation followed by ion exchange and gel filtration chromatography, mirrors methodologies widely documented in enzymology literature, further validating the robustness of the findings. These biochemical and molecular parameters contribute to a broader understanding of ancient bioactive formulations, suggesting that the preparation was purposefully optimized for specific enzymatic stability and activity, potentially for medicinal or preservative applications. Such findings emphasize the sophistication of ancient practices in harnessing plant biochemistry, as also evidenced in historical texts and previous studies of ancient herbal remedies.

5. Conclusion

Prof. Dr. During the excavations carried out in the Daskyleion Ancient City under the directory of Kaan İren, the presence of CAT, SOD and POX enzymes was detected on the residues found in the bottle that was found and thought to be used for essence storage. In the purification of all three enzymes, firstly ammonium sulfate precipitation and then ion exchange chromatography were used. Their molecular weights were found with the help of gel filtration chromatography. In the studies conducted, the molecular weight of the catalase enzyme was determined as 56 kDa, optimum pH as 5.5 and optimum temperature as 60 °C; for the superoxide dismutase enzyme, optimum pH as 7, optimum temperature as 50 °C and molecular weight as 34.5 kDa; for the peroxidase enzyme, molecular weight as 43 kDa, optimum temperature as 40 °C and optimum pH as 6. The data obtained are compatible with an herbal preparation which is possibly originated from *bakkaris.*

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