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Research Article

Catalytic Properties and Immobilization Studies of Catalase from *Malva sylvestris* L.

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Catalase was partially purified from *Malva sylvestris* L. and immobilized onto chitosan. Then, its catalytic properties were investigated. $(NH_4)_2SO_4$ precipitation and dialysis were performed in the extracted enzyme. Further purification was performed with sephadex G-200 column. Kinetic studies of the purified enzyme activity were measured and characterized. The inhibitory effects of KCN, NaN_3, $CuSO_4$, and EDTA on *M. sylvestris* L. catalase activity were observed except NaCl. Furthermore, *M. sylvestris* L. catalase was immobilized covalently with glutaraldehyde onto chitosan particles. The pH and temperature optima as well as the changes in the kinetics (Km, Vmax) of the immobilized and free *M. sylvestris* L. catalase were determined. The Km value for immobilized catalase (23.4 mM) was higher than that of free enzyme (17.6 mM). Optimum temperature was observed higher than that of the free enzyme. The optimum pH was the same for both free and immobilized catalases (pH 7.50). Immobilized catalase showed higher storage and thermal stabilities than free catalases. Free catalase lost all its activity within 60 days whereas immobilized catalase lost 45% of its activity during the same incubation period at 4°C. The remaining immobilized catalase activity was about 70% after 8 cycles of batch operations.

1. Introduction

Reactive oxygen species (ROS) play an important role in survival of all living organisms. Highly reactive and reduced metabolites of O_2 such as superoxide anion ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2) are formed during cellular respiration in organisms. When ROS are formed excessively in the cell, they damage DNA, proteins, and lipids which causes to loss of cell function, oxidative stress, and programmed cell death (PCD) [1–5]. To regulate oxidative stress, the eukaryotic cell produces different ROS-scavenging enzymes such as superoxide dismutase (which reduces $O_2^{-\bullet}$ to H_2O_2), glutathione peroxidase, and catalase [6–10].

Catalase (oxidoreductase, EC 1.11.1.6; CAT) is a tetrameric heme containing intracellular enzyme that is widely distributed in animals, plants, and all aerobic microorganisms. The typical catalase reaction is the rapidly degradation of two molecules of H_2O_2 to water and molecular oxygen [11–13]. Catalase has been biochemically, genetically studied, purified, and characterized from many plants such as black gram (*Vignamungo*) seeds [14], dill (*Anethum graveolens*) [15], van apple [16], parsley [17], *Nicotiana tabacum* [18], cotton [19], *Pinus taeda* [20], sunflower [21], and pumpkin [22].

Catalase has also very high industrial importance with its industrial applications in removal of hydrogen peroxide used as oxidizing, bleaching or sterilizing agent and in the analytical field as a component of hydrogen peroxide [23–25]. Using soluble enzyme is restricted because of certain properties like nonreusability, instability, high cost, and denaturation. On the other hand, immobilized enzymes improve thermostability, operational stability, recovery, reusability, high purity, and high product yields in their industrial applications [26].

Catalase has been extensively immobilized on numerous carrier materials such as chitosan [27], dextran [28], asymmetric cellulose membrane [29], nylon membrane [30], eggshell [31], magnesium silicate [32], Eupergit C [33], microbeads with organic polymers [34], and characterizations of the immobilization enzyme were studied systematically. Catalase enzymes used in these immobilization studies

were usually obtained from bovine liver and microorganisms (*Aspergillus niger*) [32–36]. Immobilization of catalase from plant origins has not been studied widely yet.

Malva sylvestris L. leaves are used as a food and also as a traditional medicine [37]. Furthermore, previous studies have been reported that *M. sylvestris* L. leaves have powerful antioxidant properties including high radical-scavenging activity [38]. These properties of *M. sylvestris* L. leaves are very important for an antioxidant enzyme such as catalase. There is no available information on the basic aspects of the catalase enzyme from *M. sylvestris* L. purification and its immobilization process in literature.

The aim of this work was to investigate the isolation and characterization of catalase from *Malva sylvestris* L. leaves and then, immobilization of this plant catalase on glutaraldehyde-pretreated chitosan films. The resultant immobilized catalase was characterized, and its thermal and operational stability, catalytic properties and reusability aspect were compared to free catalase. Immobilization of *Malva sylvestris* L. plant catalase will improve its thermostable, operational, reusable properties compare to that of its free counterpart. These properties of immobilized plant catalase will give more advantages than soluble-free catalase. Thus, the immobilized catalase might have useful applications in food and textile industries in removal of hydrogen peroxide used as oxidizing, bleaching, or sterilizing agents.

2. Experimental

2.1. Materials. *Malva sylvestris* L. in Sakarya region, chitosan, glutaraldehyde, hydrogen peroxide, polyvinylpolypyrrolidone (PVPP), sodium phosphate buffer, acetate buffer, $(NH_4)_2SO_4$, acetic acid, KCN, EDTA, NaN_3, NaCl, $CuSO_4$, sephadex G_{200}.

2.2. Extraction and Purification. *Malva sylvestris* L. catalase enzyme extraction was prepared as described in a previous work [15]. Fifteen g of *M. sylvestris* L. was obtained from local Sakarya region. After that samples were added to 100 mL 50 mM sodium phosphate buffer (pH; 7.0) with 0.3 g polyvinylpolypyrolidone (PVPP). The mixture was homogenized with blender. Then, the filtrate was centrifuged at 14.000 g for 30 min and supernatant was collected [39]. Extraction was fractionated with $(NH_4)_2SO_4$, solid $(NH_4)_2SO_4$ was added to the supernatant to obtain 80% saturation. The mixture was centrifuged at 14,000 g for 30 minutes and the precipitate was dissolved in a small amount of phosphate buffer and then dialyzed at 4°C in the same buffer for 24 h with three changes of the buffer during dialysis. The dialyzed enzyme extract was centrifuged and loaded onto sephadex G_{200} column (1 × 10 cm) previously equilibrated with extraction buffer and washed with the same buffer to remove unbound proteins. The eluate was used as the CAT enzyme source in the following experiments.

2.3. Determination of Protein. The amount of CAT was performed according to method of Bradford with bovine serum albumin as standard [40].

2.4. Preparation of Chitosan Beads. Three grams of chitosan powder were suspended into 99 mL of distilled water by stirring for 10 min. One milliliter of glacial acetic acid was then added, and stirring was allowed for 3 h at room temperature. The solution was filtered and dried. Some 2.0 wt. % NaOH aqueous solution was added to neutralize the acetic acid in the chitosan film. The films were repeatedly washed with deionized water and finally dried again.

2.5. Reinforcement of Chitosan Beads by Glutaraldehyde Treatment. The crosslinking was carried out by immersing the dried chitosan into 0.05% (w/v) glutaraldehyde solution in 0.05 M cold phosphate buffer (pH 7.0) for 1 h. The brownish reinforced beads were washed several times by 0.05 M cold phosphate buffer (pH 7.0) to remove the excess of glutaraldehyde [41].

2.6. Immobilization of Catalase into Chitosan Beads. Chitosan beads were mixed with 2 mg/mL *Malva sylvestris* L. catalase solution in 0.05 M phosphate buffer (pH 7.0) for 5 h with slight stirring and washed at 4°C. Then, the beads dried in a vacuum incubator at room temperature and stored at 4°C. The amount of immobilized enzyme was estimated by subtracting the amount of protein determined in the supernatant after immobilization from the amount of protein used for immobilization.

2.7. Activity Assays of Catalase. Catalase (CAT) activity was determined at 25°C according to Aebi [42]. The reaction mixture contained 40 mM H_2O_2 in a 50 mM phosphate buffer pH 7.0 and 0.1 mL enzyme in a total volume of 3 mL. CAT activity was estimated by decreased in absorbance of H_2O_2 at 240 nm. Activities were carried out at optimum conditions. Approximately 100 mg of catalase immobilize chitosan beads were mixed 10 mL of 10 mM H_2O_2 solution in 0.05 M phosphate buffer (pH 7.0) 25°C. After 5 min, the reaction was determinated by removal of the chitosan beads from the reaction mixture. The absorbance of the reaction mixture was determined, and the immobilized catalase activity was calculated. The kinetic constant was determined using Lineweaver-Burk plot by initial reaction rates of free or immobilized enzyme catalase.

2.8. Effect of Inhibitors. Potassium cyanide (KCN), EDTA, NaCl, NaN_3, and $CuSO_4$ were tested as inhibitors for catalase enzyme.

2.9. Influence of pH. The activity assays were carried out over the pH range 3.00–9.00. Reaction rates of free and immobilized enzyme preparations depending on pH were investigated using 50 mM acetate buffer pH 3.5, 4.0, and 5.0 50 mM phosphate buffer at pH 6.0, 7.0, 7.5, 8.0, and 9.0. Activity of pH profiles was determined at various pH in 10 mM H_2O_2 solution at 25°C.

2.10. Influence of Temperature. The effect of temperature on enzyme activity was investigated in the range of 4–70°C for both free and immobilized catalases. Activity of temperature

profiles was determined at indicated temperatures in 10 mM H_2O_2 solution (pH 7.5).

2.11. Storage Stability of Free and Immobilized Catalase. The activity of free and immobilized catalase was investigated at 25°C and 4°C in 50 mM phosphate buffer (pH 7.5).

2.12. Reuse of the Immobilized Catalase. The residual activity of the immobilized enzyme was determined under standard assay conditions. The same immobilized enzyme was reused after it was thoroughly washed.

3. Results and Discussion

3.1. Purification of Catalase. Typical results of the partial purification procedure of CAT from *Malva sylvestris* L. are summarized in Table 1. After the enzyme extraction from *M. sylvestris* L., the extracted enzyme solution was reacted with several $(NH_4)_2SO_4$ (0–20%, 20–40%, 40–50%, 50–60%, and 60–80% saturated solutions) to purify CAT by altering its solubility and to find out the almost completely precipitation of CAT with maximum activity. In conclusion, CAT activity of the precipitate of 30–60% $(NH_4)_2SO_4$ saturation was found to be 53.8% purification, and this saturation was used all the extraction processes.

After ammonium sulfate precipitation, the dialyzed enzyme extract was loaded onto Sephadex G200 gel filtration column and eluted with 50 mM phosphate buffer (pH 7.0). As a result, CAT enzyme was purified 123.6 fold with a yield 42.7%.

3.2. Effect of Inhibitors on Catalase Activity. The percentage of the catalase activity was determined with different concentrations of inhibitors (Figure 1). KCN, EDTA, sodium azide (NaN_3), copper sulphate ($CuSO_4$) and NaCl were used for the inhibition studies. Only 5.2% of catalase activity was retained in the presence of 0.5 mM sodium azide which was also observed by Kandukuri [14]. In presence of 3 mM EDTA, 55% activity was retained. EDTA inhibited the enzyme activity because it was known a metal chelator that could have complex with iron ion in the active site of catalase [37]. NaCl had almost no effect to *M. sylvestris* L. catalase. KCN also inhibited the enzyme activity 80% in 3 mM concentration.

3.3. Immobilization of Malva sylcestris L. Catalase on Chitosan Beads. Enzymes have been used in several industrial processes for a long time. Textiles, food, animal feed, detergent, pulp and paper are among the industries that use enzymes. Catalase has been used especially in the textile industry as a bleaching material [35, 36]. Many studies on immobilization of catalase have been reported [43, 44].

In this study, we investigated immobilization of *M. sylvestris* L. catalase onto chitosan beads with gluteraldehyde. There is no report yet about *M. sylvestris* L. catalase immobilization in the literature.

The catalase was incubated with glutaraldehyde-pretreated chitosan beads for 5 hours. The Michaelis constants, Km and Vmax, for free and immobilized catalase were determined using H_2O_2 as substrate. The amount of bound enzyme and kinetic parameters were shown in Table 2.

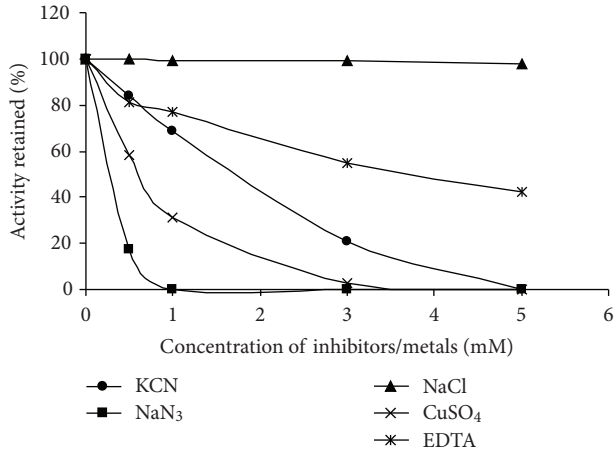

Figure 1: Effects of various compouns on CAT from *Malva sylvestris* L.

The Km values of free and immobilized enzyme were found to be 17.6 mM and 23.4 mM (Table 1). The Km value of immobilized enzyme was higher than that of free enzyme, which meant immobilized enzyme had less affinity to its substrate. Change in the affinity of the enzyme to its substrate was probably caused by structural changes in the immobilization procedure of the enzyme and by lower accessibility of the substrate to the active site of the immobilized catalase enzyme.

3.4. Effect of pH on Catalase. The effect of pH on the free and immobilized enzyme for hydrogen peroxide degradation was investigated in the pH range between 3.00 and 9.00 in acetate or/and phosphate buffers at 25°C. The results are presented in Figure 2. At the end of the time, the activity measurements of the enzymes were made under the optimum assay conditions (Figure 2). As it was shown from the figure, pH stabilities of the both enzymes had some similarities. Both free and the immobilized catalase gave an optimum at pH 7.50; however, the immobilized catalase gave a much broader pH stability than the free enzyme. This suggested that immobilized enzyme was less sensitive to pH changes than free enzyme.

3.5. Effect of Temperature on Catalase. Temperature profiles of free and immobilized catalase are shown in Figure 3. Optimum temperature was found at about 25°C for free and 40°C for immobilized catalase. Figure 3 showed that the activity of immobilized catalase was more stable than the free catalase around 25–40°C. The activity loss of immobilized enzyme was less than the free enzyme for higher temperature. Chitosan support might have a protecting effect at the high temperatures at which enzyme deactivation takes place. Immobilization of catalase in chitosan beads can cause an increase in the enzyme rigidity which is commonly reflected

Table 1: Partial purification of catalase from *Malva sylvestris* L.

Fractions	Protein concentration (mg/mL)	Activity (U/mL)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude extract	15.2 ± 0.2	3767 ± 531.3	451,629 ± 43,15	247.53 ± 29.8	1	100
$(NH_4)_2SO_4$	13.1 ± 0.16	5960 ± 213.5	238, 340 ± 6733.4	458.41 ± 25.8	1.81 ± 0.27	53.8 ± 2.7
Sephadex G_{200}	0.12 ± 0.31	3678 ± 194.0	187.12 ± 4221.3	30650 ± 523.1	123.6 ± 13	42.7 ± 5.8

Table 2: Kinetic parameters of free and immobilized *M. sylvestris* L. catalase.

	Km (mM)	Vmax (U/mL)	Bound protein (mg/g chitosan)	Optimum pH	Optimum temperature (°C)
Free CAT	17.6	2631	—	7.50	25
Immobilized CAT	23.4	3333	0.088	7.50	40

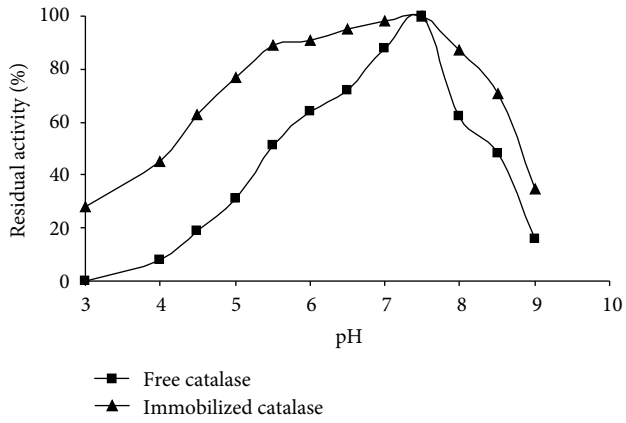

Figure 2: Effect of pH on activity.

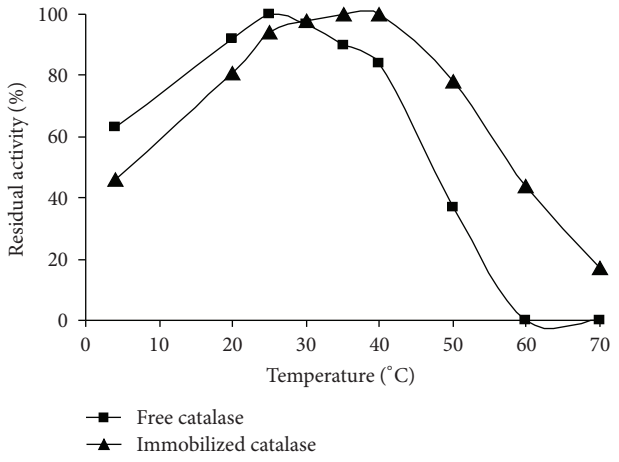

Figure 3: Temperature stability.

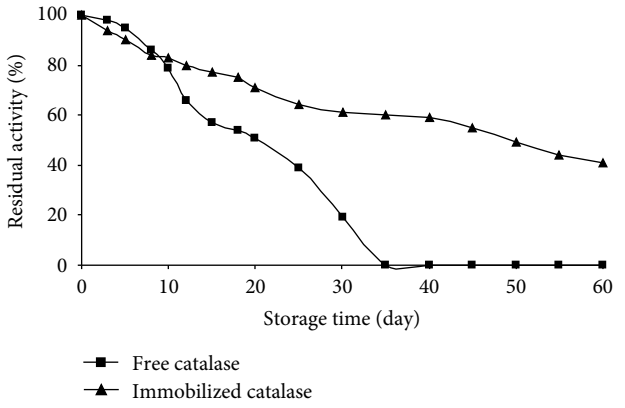

Figure 4: Storage stability at 4°C.

by increase in stability towards denaturation by raising the temperature [45, 46].

3.6. Storage Stability and Reusability. Free and immobilized catalase were stored in a phosphate buffer (50 mM, pH 7.0) at 4°C and the activity measurements were carried out for a period of 60 days (Figure 4). The free enzyme lost all of its activity within 35 days. On the other hand, immobilized catalase lost 45% of its activity during the incubation period (Figure 4). Free and immobilized catalase were stored in a phosphate buffer (50 mM, pH 7.0) at 25°C and the activity measurements were carried out for a period of 60 days. When the temperature was raised to 25°C (Figure 5), there was a significant decrease in the activity of the enzyme over a 3 days period. After 3 days, only 15% of the activity was remained. Immobilized enzyme was preserved %96 activity after 3 days. At 25°C, the immobilization process provided an advantage in the stability of enzyme over free enzyme activity, especially at longer durations.

It was shown that immobilized enzyme could be reused (Figure 6). The activity of immobilized enzyme remained 50% after it was reused for ten times. The results confirmed that the stability of immobilized enzyme was good.

4. Conclusions

Catalase enzyme form *Malva sylvestris* L. was successfully characterized and immobilized onto chitosan with glutaraldehyde. Our work showed that the catalase enzyme was

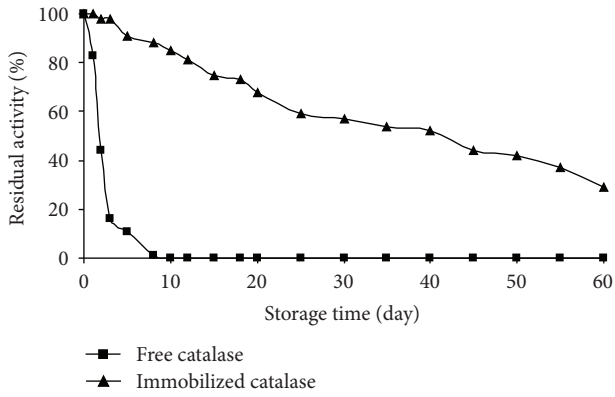

Figure 5: Storage stability at 25°C.

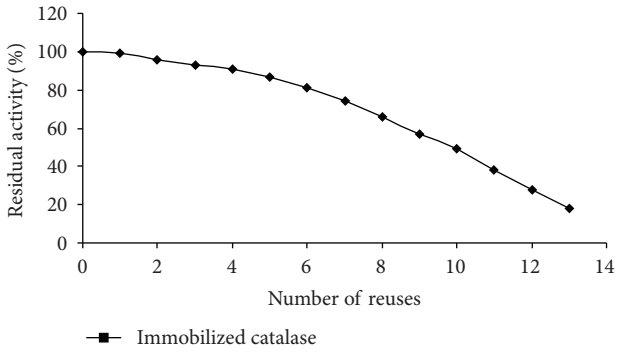

Figure 6: Operational Stability.

first time isolated and characterized from *Malva sylvestris* L. leaves as a plant source. Then, this plant catalase successfully immobilized onto chitosan material. Thus, the plant enzyme was gained strength against thermal denaturation by this successful immobilization of plant catalase on chitosan beads. It was found that thermal stability and storage stability of immobilized catalase were better than that of free catalase. It is possible to increase the degradation of hydrogen peroxide and can be used many times in the processes. Our results show that the immobilization of *Malva sylvestris* L. catalase on chitosan makes the enzyme more useful in degradation of hydrogen peroxide in various industrial applications such as food and textile.

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References

[1] C. H. Foyer and G. Noctor, "Oxygen processing in photosynthesis: regulation and signalling," *New Phytologist*, vol. 146, no. 3, pp. 359–388, 2000.

[2] K. Apel and H. Hirt, "Reactive oxygen species: metabolism, oxidative stress, and signal transduction," *Annual Review of Plant Biology*, vol. 55, pp. 373–399, 2004.

[3] R. Mittler, S. Vanderauwera, M. Gollery, and F. Van Breusegem, "Reactive oxygen gene network of plants," *Trends in Plant Science*, vol. 9, no. 10, pp. 490–498, 2004.

[4] C. Laloi, K. Apel, and A. Danon, "Reactive oxygen signalling: the latest news," *Current Opinion in Plant Biology*, vol. 7, no. 3, pp. 323–328, 2004.

[5] I. C. Mori and J. I. Schroeder, "Reactive oxygen species activation of plant Ca^{2+} channels. A signaling mechanism in polar growth, hormone transduction, stress signaling, and hypothetically mechanotransduction," *Plant Physiology*, vol. 135, no. 2, pp. 702–708, 2004.

[6] J. G. Scandalios, "Oxygen stress and superoxide dismutases," *Plant Physiology*, vol. 101, no. 1, pp. 7–12, 1993.

[7] J. A. Baum and J. G. Scandalios, "Developmental expression and intracellular localization of superoxide dismutase in maize," *Differentiation*, vol. 13, no. 2, pp. 133–140, 1979.

[8] H. Willekens, D. Inze, M. Van Montagu, and W. Van Camp, "Catalases in plants," *Molecular Breeding*, vol. 1, no. 3, pp. 207–228, 1995.

[9] K. Asada, "The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons," *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 50, pp. 601–639, 1999.

[10] K. J. Dietz, "Plant Peroxiredoxins," *Annual Review of Plant Biology*, vol. 54, pp. 93–107, 2003.

[11] A. Mhamdi, G. Queval, S. Chaouch, S. Vanderauwera, F. Van Breusegem, and G. Noctor, "Catalase function in plants: a focus on *Arabidopsis* mutants as stress-mimic models," *Journal of Experimental Botany*, vol. 61, no. 15, pp. 4197–4220, 2010.

[12] M. E. Percy, "Catalase: an old enzyme with a new role?" *Canadian Journal of Biochemistry and Cell Biology*, vol. 62, no. 10, pp. 1006–1014, 1984.

[13] J. Switala and P. C. Leuwen, "Diversity of properties among catalases," *Archives of Biochemistry and Biophysics*, vol. 401, no. 2, pp. 145–154, 2002.

[14] S. S. Kandukuri, A. Noor, S. S. Ranjini, and M. A. Vijayalakshm, "Purification and characterization of catalase from sprouted black gram (*Vigna mungo*) seeds," *Journal of Chromatography B*, vol. 889- 890, pp. 50–54, 2012.

[15] G. Arabaci, "Partial purification and some properies of catalase from dill (*Anethum graveolens L.*)," *Journal of Biology and Life Sciences*, vol. 2, no. 1, pp. 11–15, 2011.

[16] I. H. Yoruk, H. Demir, K. Ekici, and A. Sarvan, "Purification and properties of catalase from Van Apple (Golden Delicious)," *Pakistan Journal of Nutrition*, vol. 4, no. 1, pp. 8–10, 2005.

[17] L. Öztürk, M. Bülbül, M. Elmastaş, and M. Çiftçi, "Purification and some kinetic properties of catalase from parsley (*Petroselinum hortense Hoffm., Apiaceae*) leaves," *Preparative Biochemistry and Biotechnology*, vol. 37, no. 3, pp. 229–238, 2007.

[18] E. A. Havir and N. A. Mchale, "Biochemical and developmental characterization of multiple forms of catalase in Tobacco leaves," *Plant Physiology*, vol. 84, no. 2, pp. 450–455, 1987.

[19] W. Ni, R. B. Turley, and R. N. Trelase, "Characterization of a cDNA encoding cotonseed catalase," *Biochimica et Biophysica Acta*, vol. 1049, no. 2, pp. 219–222, 1990.

[20] R. T. Mullen and D. J. Gifford, "Purification and characterization of catalase from Loblolly Pine (*Pinus taeda L.*) megagametophytes," *Plant Physiology*, vol. 103, no. 2, pp. 477–483, 1993.

[21] R. Eising, R. N. Trelease, and W. Ni, "Biogenesis of catalase in glyoxysomes and leaf-type peroxisomes of sunflower cotyledons," *Archives of Biochemistry and Biophysics*, vol. 278, no. 1, pp. 258–264, 1990.

[22] J. Yamaguchi, M. Nishimura, and T. Akazawa, "Purification and characterization of heme-containing low-activity form of catalase from greening pumpkin cotyledons," *European Journal of Biochemistry*, vol. 159, no. 2, pp. 315–322, 1986.

[23] M. Y. Arıca, H. A. Öktem, Z. Öktem, and S. A. Tuncel, "Immobilization of catalase in poly(isopropylacrylamide-*co*-hydroxyethylmethacrylate) thermally reversible hydrogels," *Polymer International*, vol. 48, no. 9, pp. 879–884, 1999.

[24] N. Ertaş, S. Timur, E. Akyılmaz, and E. Dinçkaya, "Specific determination of hydrogen peroxide with a catalase biosensor based on mercury thin film electrodes," *Turkish Journal of Chemistry*, vol. 24, no. 1, pp. 95–99, 2000.

[25] T. Santoni, D. Santianni, A. Manzoni, S. Zanardi, and M. Mascini, "Enzyme electrode for glucose determination in whole blood," *Talanta*, vol. 44, no. 9, pp. 1573–1580, 1997.

[26] J. F. Kennedy, E. H. M. Melo, and K. Jumel, "Immobilized enzymes and cells," *Chemical Engineering Progress*, vol. 86, no. 7, pp. 81–89, 1990.

[27] Ş. A. Çetinus and H. N. Öztop, "Immobilization of catalase on chitosan film," *Enzyme and Microbial Technology*, vol. 26, no. 7, pp. 497–501, 2000.

[28] J. J. Marshall and M. L. Rabinowitz, "Stabilization of catalase by covalent attachment to dextran," *Biotechnology and Bioengineering*, vol. 18, no. 9, pp. 1325–1329, 1976.

[29] E. Selli, A. D'Ambrosio, and I. R. Bellobono, "Enzymatic activity under tangential flow conditions of photochemically grafted membranes containing immobilized catalase," *Biotechnology and Bioengineering*, vol. 41, no. 4, pp. 474–478, 1993.

[30] C. Dajun, W. Xiaqin, and Z. Zuxin, "Biosensor of hydrogen peroxide using triethyloxonium fluoroborate as alkylating reagent before enzyme immobilization," *Sensors and Actuators B*, vol. 14, no. 1–3, pp. 554–555, 1993.

[31] U. Chatterjee, A. Kumar, and G. G. Sanwal, "Goat liver catalase immobilized on various solid supports," *Journal of Fermentation and Bioengineering*, vol. 70, no. 6, pp. 429–430, 1990.

[32] S. S. Tukel and O. Alptekin, "Immobilization and kinetics of catalase onto magnesium silicate," *Process Biochemistry*, vol. 39, no. 12, pp. 2149–2155, 2004.

[33] Ö. Alptekin, S. S. Tükel, D. Yıldırım, and D. Alagöz, "Immobilization of catalase onto Eupergit C and its characterization," *Journal of Molecular Catalysis B*, vol. 64, no. 3-4, pp. 177–183, 2010.

[34] G. Bayramoglu and M. Y. Arica, "Reversible immobilization of catalase on fibrous polymer grafted and metal chelated chitosan membrane," *Journal of Molecular Catalysis B*, vol. 62, no. 3-4, pp. 297–304, 2010.

[35] F. Horst, E. H. Rueda, and M. L. Ferreira, "Activity of magnetite-immobilized catalase in hydrogen peroxide decomposition," *Enzyme and Microbial Technology*, vol. 38, no. 7, pp. 1005–1012, 2006.

[36] S. A. Costa, T. Tzanov, A. Paar, M. Gudelj, G. M. Gübitz, and A. Cavaco-Paulo, "Immobilization of catalases from Bacillus SF on alumina for the treatment of textile bleaching effluents," *Enzyme and Microbial Technology*, vol. 28, no. 9-10, pp. 815–819, 2001.

[37] P. M. Guarrera, "Traditional phytotherapy in Central Italy (Marche, Abruzzo, and Latium)," *Fitoterapia*, vol. 76, no. 1, pp. 1–25, 2005.

[38] L. Barros, A. M. Carvalho, and I. C. F. R. Ferreira, "Leaves, flowers, immature fruits and leafy flowered stems of *Malva sylvestris*: a comparative study of the nutraceutical potential and composition," *Food and Chemical Toxicology*, vol. 48, no. 6, pp. 1466–1472, 2010.

[39] A. Gholamhoseinian, N. Ghaemi, and F. Rahimi, "Partial purification and properties of catalase from *Brassia oleracea capitata*," *Asian Journal of Plant Sciences*, vol. 5, no. 5, pp. 827–831, 2006.

[40] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Analytical Biochemistry*, vol. 72, no. 1-2, pp. 248–254, 1976.

[41] S. H. Chiou, T. C. Hung, R. Giridhar, and W. T. Wu, "Immobilization of lipase to chitosan beads using a natural cross-linker," *Preparative Biochemistry and Biotechnology*, vol. 37, no. 3, pp. 265–275, 2007.

[42] H. Aebi, "Catalase *in vitro*," *Methods in Enzymology*, vol. 105, pp. 121–126, 1984.

[43] Ş. Akkuş Çetinus and H. N. Öztop, "Immobilization of catalase into chemically crosslinked chitosan beads," *Enzyme and Microbial Technology*, vol. 32, no. 7, pp. 889–894, 2003.

[44] Ö. Alptekin, S. S. Tükel, D. Yıldırım, and D. Alagöz, "Characterization and properties of catalase immobilized onto controlled pore glass and its application in batch and plug-flow type reactors," *Journal of Molecular Catalysis B*, vol. 58, no. 1–4, pp. 124–131, 2009.

[45] B. Jiang and Y. Zhang, "Immobilization of catalase on crosslinked polymeric hydrogels—effect of anion on the activity of immobilized enzyme," *European Polymer Journal*, vol. 29, no. 9, pp. 1251–1254, 1993.

[46] M. A. Abdel-Naby, "Immobilization of *Aspergillus niger* NRC 107 xylanase and β-xylosidase, and properties of the immobilized enzymes," *Applied Biochemistry and Biotechnology*, vol. 38, no. 1-2, pp. 69–81, 1993.