Determination of SOD, POD, PPO and CAT Enzyme Activities in *Rumex obtusifolius* L.

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Authors’ contributions

This work was carried out in collaboration between both authors. Author GA designed the study, wrote the protocol and interpreted the data. Author EHA anchored the field study, gathered the initial data and performed preliminary data analysis. While authors GA and EHA managed the literature searches and produced the initial draft. Both authors read and approved the final manuscript.

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ABSTRACT

**Aims:** The purpose of this study was to measure antioxidant enzyme (polyphenol oxidase, peroxidase, catalase and superoxide dismutase) activities of crude extract of *Rumex obtusifolius* L. in order to gain insight about this plant's antioxidant potential.

**Study Design:** The study was composed of the collection of plant material, extractions of the antioxidant enzymes, activity measurements of the enzymes and finally evaluation of the experimental results.

**Place and Duration of Study:** Department of Chemistry (biochemistry laboratories), Faculty of Science and Arts of Sakarya University, between June 2015 and July 2015.

**Methodology:** Enzymatic antioxidant activity of this plant was investigated by carrying out catalase, superoxide dismutase, peroxidase and polyphenol oxidase enzyme activity assays. Enzyme activities of the crude extract were measured by using spectrophotometric method. Optimum pH and temperature values of each enzyme were also determined for measurement of enzyme activities in ideal conditions.

**Results:** Finally, our results showed that *Rumex obtusifolius* L. crude extract had good activity for all the enzymatic procedures tested. The activity levels of enzymatic antioxidants polyphenol oxidase, peroxidase, catalase and superoxide dismutase of the plant were found to be 12.8; 195.2;
38.7; 11.6 EU/mL, respectively. Optimum pH and temperature values of all the enzymes (except PPO: optimum temperature 30°C) tested were also found to be 7.0 and 25°C, respectively.

**Conclusion:** Our results demonstrate that this edible plant, *Rumex obtusifolius* L., might be a potential source of natural antioxidants with good antioxidant enzyme capacity.

**Keywords:** Antioxidant enzyme; catalase; peroxidase; polyphenol oxidase; *Rumex obtusifolius* L.; superoxide dismutase.

**1. INTRODUCTION**

In the course of normal metabolic functions, highly reactive compounds called free radicals can be generated in the body in the form of reactive oxygen species (ROS). Most of the environmental toxicants also are potential source of free radicals. These molecules are unstable because they have lone pair of electrons and therefore become highly reactive species. They easily react with cellular molecules such as proteins, lipids, carbohydrates and nucleic acids, and denature them. As a result of these reactions, these components lose their ability to function normally and ultimately resulting in various pathological conditions in living organisms [1].

Due to ROS are natural by-products of ongoing biochemical reactions, they are involved in the cell growth, differentiation, progression, and death. The concentration levels of ROS in the body are very important because low concentrations of ROS may be beneficial or even necessary in processes such as intracellular signaling and defense against micro-organisms; however, overproduction of ROS play a role in the aging process as well as in a number of human disease states, including cancer, ischemia, and failures in immunity and endocrine functions. As a safeguard against the accumulation of ROS, living organisms possess antioxidant defense systems including several non-enzymatic and enzymatic antioxidant components. Therefore, when ROS overproduction arises as a consequence of a pathologic event, antioxidant defense system promotes the regulation and expression of these enzymes [2].

Antioxidant enzymes are capable of stabilizing or deactivating free radicals before they oxidize the components of cells. There are various mechanisms for antioxidant enzyme preservation; they could reduce the energy of the free radicals or give up some of their electrons for their use, thereby causing them to become stable. Moreover, they may also interrupt the oxidizing chain reaction to minimize the damage caused by free radicals.

Our body’s potential of overcoming free radicals related health problems could be made more substantial by reducing exposure to free radicals and increasing the intake of antioxidant enzyme rich foods or antioxidant enzyme supplements. Consequently, antioxidant enzymes play a critical role for maintaining optimal cellular and systemic health and wellbeing [1]. In this connection, antioxidant enzyme rich fruits and vegetables are effective preservers of various diseases [3].

Plants have the effective enzymatic antioxidant defense system including catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and polyphenol oxidase (PPO) enzymes. This system allow for scavenging of ROS leading to protection of plant cells from oxidative damage [4,5]. Superoxide dismutase (EC 1.15.1.1) is the antioxidant enzyme that responsible for the dismutation of the highly reactive superoxide anion to O₂ and to the less reactive species H₂O₂. The released hydrogen peroxide can be reduced to water by catalase (EC 1.11.1.6) or glutathione peroxidase (EC 1.11.1.9) enzymatic reactions [6,7,8]. Polyphenol oxidase (EC 1.10.3.1) is a copper containing metalloenzyme that oxidizes phenolic compounds to quinones and eventually the quinones are polymerized (nonenzyme-catalyzed) to melanin pigments [9].

*Rumex obtusifolius* L. (*Polygonaceae*) is one of the most common plants that occur in silage fields, ditches and on river banks. It generally grows in Northern Ireland, Africa, temperate Asia, and Europe. This perennial and dietary plant has long been used in folklore medicine as an antidote to nettle, depurative, astringent, laxative, and tonic, and in the treatment of sores, blisters, burns, cancer and tumors [10].

The objective of the present study was to quantify four different antioxidant enzyme activities (SOD, POD, PPO and CAT) of crude extract of *Rumex obtusifolius* L. plant from Sakarya city of Turkey.
2. MATERIALS AND METHODS

2.1 Chemicals and Reagents
All chemicals used for the analysis were of analytical grade and were purchased from Sigma-Aldrich.

2.2 Plant Material
*Rumex obtusifolius* L., a wild and edible plant from Turkey, was the material for this work. Fresh samples were collected from agricultural wasteland of Sakarya city during August 2015 and stored at -20°C.

2.3 Extraction for Antioxidant Enzyme Assays
Crude extract was prepared by homogenization of frozen plant sample in buffer medium. *Rumex obtusifolius* L. plant which is stored at -20°C was used for the enzyme extraction with all parts. The same plant sample was washed with distilled water two times. 10 g of the sample was cut quickly into thin slices and homogenized in 50 mL of 100 mM sodium phosphate buffer (pH 7.0) containing 1 mM ascorbic acid and 0.5% (w/v) polyvinylpyrrolidone for 5 min at 4°C. The homogenate was filtered through three layers of cheesecloth and then the filtrate was centrifuged at 5,000 x g for 15 min, and the supernatant was collected.

2.4 Measurement of Antioxidant Enzyme Activity

2.4.1 Catalase
Catalase (CAT) activity was measured spectrophotometrically at room temperature by monitoring the decrease in absorbance at 240 nm resulting from the decomposition of H₂O₂. Catalase activity was measured according to the method of Aebi [11]. One unit (U) of catalase activity was defined as the amount of enzyme that caused an absorbance change of 0.001 per min under assay conditions. The reaction mixture contained 100 mM sodium phosphate buffer (pH 7.0), 30 mM H₂O₂ and 100 µL of crude extract in a total volume of 3.0 mL.

2.4.2 Peroxidase
The peroxidase (POD) activity was determined using 4-methylcatechol as substrate. The increase in the absorption caused by oxidation of 4-methylcatechol by H₂O₂, was measured at 420 nm spectrophotometrically. The reaction mixture contained 100 mM sodium phosphate buffer (pH 7.0), 5 mM 4-methylcatechol, 5 mM H₂O₂ and 500 µL of crude extract in a total volume of 3.0 mL at room temperature. One unit of enzyme activity was defined as 0.001 change in absorbance per min, under assay conditions [12].

2.4.3 Polyphenol oxidase
Polyphenol oxidase (PPO) activity assay was carried out by measuring the increase in absorbance at 420 nm for 4-methylcatechol spectrophotometrically. The assay was performed with 100 mM sodium phosphate buffer (pH 7.0), 5 mM 4-methylcatechol and 500 µL of crude extract at room temperature. Total volume of reaction mixture was 3.0 mL. One unit (U) of enzyme activity was defined as the amount of the enzyme that caused a change of 0.001 in absorbance per min [13].

2.4.4 Superoxide dismutase
Superoxide dismutase (SOD) activity was determined by measuring the inhibition in photoreduction of nitroblue tetrazolium (NBT) by SOD enzyme [14]. The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 0.1 mM EDTA, 50 mM sodium carbonate, 12 mM L-methionine, 50 µM NBT, 10 µM riboflavin and 100 µL of crude extract in a final volume of 3.0 mL. A control reaction was performed without crude extract. The SOD reaction was carried out by exposing the reaction mixture to white light for 15 min at room temperature. After 15 min incubation, absorbance was recorded at 560 nm using a spectrophotometer. One unit (U) of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT.

2.5 Effect of pH
The optimal pH value for *Rumex obtusifolius* L. activity was determined in buffers of pH values ranging from 3.0 to 9.0. The buffer systems used were citrate buffer for pH 3.0-5.0; phosphate buffer for pH 6.0-7.5; Tris-HCl buffer for 7.5-9.0. The enzyme activity of four antioxidant enzymes (CAT, POD, PPO and SOD) was measured according to protocols mentioned above.
2.6 Effect of Temperature

Enzyme activity was measured at temperature values ranging from 10 to 70°C to determine the optimal temperature value of *Rumex obtusifolius* L. for antioxidant enzymes (CAT, POD, PPO and SOD). Temperature effect was determined by heating the substrate solution in buffer to the appropriate temperature in a water bath (or cooling in an ice bath) and then the reaction started by adding extracted enzyme solution.

2.7 Statistical Analysis

All experiments were conducted in triplicate and all results were expressed as the average ± standard deviation of the measurements. Excel (Microsoft Co, Redmond, WA, USA) was used to calculate the standard deviation.

3. RESULTS AND DISCUSSION

Medicinal plants are used in traditional medicine as natural healing drugs with therapeutic effects such as prevention of cardiovascular diseases, inflammation disorders, or reducing the risk of cancer. Furthermore, medicinal plants have a special place in pharmacological industry because of the presence of active chemical substances as agents for drug synthesis. Also, they are substantial for food and cosmetic industry as additives, due to their protective effects because of the existence of antioxidants and antimicrobial components [15].

In this work, a medicinal plant used in traditional medicine, *Rumex obtusifolius* L., was investigated for its antioxidant potency in terms of antioxidant enzymatic potentials to support its traditional medicinal usage. The activity results of antioxidant enzymes of *Rumex obtusifolius* L., namely, catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and polyphenol oxidase (PPO) were shown in Table 1. The plant extract had good enzyme activities for all the enzymes tested. Thus, we can say that *Rumex obtusifolius* L. possess the antioxidant enzymes in its tissues and we were also able to extract the enzymes by providing the necessary conditions.

The enzyme activity was found to be 1159.6±262 Unit/g tissue for superoxide dismutase, 3866.9±36 Unit/g tissue for catalase, 19533.8±436 Unit/g tissue for peroxidase, and 1284.6±56 Unit/g tissue for polyphenol oxidase, respectively (Unit activity definitions were given in material and methods section).

Superoxide dismutase is one of the key enzymes which become involved in cellular defense against reactive oxygen species in living organisms; hence it is an important indicator of antioxidant capacity [16]. P. Rani et al. reported that, SOD activity was found to be 21.02 Unit/g tissue for goose berry, 6.58 Unit/g tissue for grapes, 30.45 Unit/g tissue for orange, and 6.91 Unit/g tissue for tomato, respectively [17]. *Rumex obtusifolius* L. showed significantly higher SOD activity than these samples. According to enzyme activity results, it can be supposed that *Rumex obtusifolius* L. has good antioxidant capacity.

### Table 1. Antioxidant enzyme activities of *Rumex obtusifolius* L.

<table>
<thead>
<tr>
<th>Antioxidant enzyme</th>
<th>Activity* (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxidase</td>
<td>195.24±0.0044</td>
</tr>
<tr>
<td>Catalase</td>
<td>38.65±0.00026</td>
</tr>
<tr>
<td>Polyphenol oxidase</td>
<td>12.84±0.00025</td>
</tr>
<tr>
<td>Super oxide dismutase</td>
<td>11.59±0.01600</td>
</tr>
</tbody>
</table>

Units of enzyme activities were expressed as: SOD- One unit of activity was defined as the amount of enzyme causing 50% inhibition of photoreduction of NBT. POD- One unit of enzyme activity was defined as 0.001 changes in absorbance per min, under assay conditions. PPO- One unit of enzyme activity was defined as the amount of the enzyme that caused a change of 0.001 in absorbance per min. CAT- One unit of catalase activity was defined as the amount of enzyme that caused an absorbance change of 0.001 per min under assay conditions. *± value is standard error.

### Table 2. Optimum pH and temperature values from literature

<table>
<thead>
<tr>
<th>Sample</th>
<th>Enzyme</th>
<th>Substrate</th>
<th>Optimum pH</th>
<th>Optimum temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana (Musa sapientum L.)</td>
<td>PPO</td>
<td>Dopamine</td>
<td>6.5</td>
<td>30</td>
</tr>
<tr>
<td>Olive (Olea europaea L.)</td>
<td>POD</td>
<td>Phenol</td>
<td>7.0</td>
<td>35</td>
</tr>
<tr>
<td>Curcuma aromatica</td>
<td>SOD</td>
<td>Riboflavin</td>
<td>7.8</td>
<td>20</td>
</tr>
<tr>
<td>Black gram (Vigna mungo)</td>
<td>CAT</td>
<td>Hydrogen peroxide</td>
<td>7.0</td>
<td>40</td>
</tr>
</tbody>
</table>
Fig. 1. Effect of pH on *Rumex obtusifolius* L. antioxidant enzymes
*Mean ± S.E.M = Mean values ± Standard error of means of three experiments*

Fig. 2. Effect of temperature on *Rumex obtusifolius* L. antioxidant enzymes
*Mean ± S.E.M = Mean values ± Standard error of means of three experiments*

Also, optimal temperature and pH values of the *Rumex obtusifolius* L. antioxidant enzymes were investigated. Optimum pH values for the enzyme activity were determined between pH 3.0 and 9.0. It was found that the optimum pH value was 7.0 for all the enzymes tested for the used substrates. Optimum pH results were given in Fig. 1. The optimum temperature value was found 25°C for all the enzymes tested, except PPO. The optimum temperature of PPO was determined 30°C. Optimum temperature results were shown in Fig. 2. The
optimum pH and temperature values of the each tested enzyme from *Rumex obtusifolius* L. were similar to the values given in literature (Table 2).

4. CONCLUSION

In the present study, we determined four different antioxidant enzyme activities (CAT, POD, SOD and PPO enzymes) in *Rumex obtusifolius* L. plant. Our results showed that *Rumex obtusifolius* L. has a good antioxidant potential. Based on the results, we can conclude that the plant is a promising source of natural antioxidants and might be used in the treatment of diseases associated with oxidative stress.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES
