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Screening for Superoxide Dismutase Activity in Certain Food and Medicinal Plants of Mongolia

Bayarmaa Jambalsuren[1,*] Purev Dondog[1]

[1] *Department of Biology, School of Arts and Sciences, National University of Mongolia, Ulaanbaatar 14201, Mongolia*

**Corresponding author. Email: bayarmaa@num.edu.mn*

ABSTRACT

The purpose of the present work was to perform a screening for SOD activity in plants traditionally used as food and medicine in Mongolia. The SOD activity was measured by their effects on pyrogallol autoxidation. In the studied plants, the SOD activity varied from 0.42±0.01 to 10.47±0.1 U/mg protein. Plants with high SOD activity can serve as a potential source of antioxidant supplements in the human diet. Research is currently ongoing.

Keywords: *Antioxidant, Reactive oxygen species, Superoxide dismutase*

1. INTRODUCTION

Free radical production occurs continuously in all cells as part of normal cellular function. However, excess free radical production originating from endogenous or exogenous sources plays a role in many chronic and degenerative diseases [1]. Plants are an important source of dietary nutrients, biologically active compounds, and antioxidants. In addition, plants have a higher activity of antioxidant enzymes that protect cells from damage by reactive oxygen species (ROS). One of them is superoxide dismutase (SOD). It is an enzyme (EC1.15.1.1) that catalyzes dismutation reaction on the superoxide anions (O_2^-) - the partitioning of the radical into ordinary molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). Within a cell, the superoxide dismutase constitutes the first line of defense against superoxide [2], therefore acts as a good therapeutic agent against diseases mediated by ROS such as cancer, inflammatory diseases, ischemia, aging, rheumatoid arthritis, neurodegenerative diseases, and diabetes. Besides, SOD supplementation reduces lactic acid and prevents muscle fatigue, protects against UV rays, accelerates hair growth, heals wounds, reduces facial wrinkles and skin depigmentation [3].

The addition of SOD from plants to human and animal diets is a new approach in terms of improving health in pathological conditions. Compared to the population of other eastern countries, Mongols consume fewer types of plant food in their diet. For us, this is the cause of many diseases. There are no data on the activity of SOD in plants growing in Mongolian. Therefore, the aim of this study was a screening of superoxide dismutase activity in relatively common and traditionally used plants as food and alternative therapeutic tools for the selection of sources with high SOD activity.

2. EXPERIMENTAL

2.1. Plant Material

Two species of *Pulsatilla*, *P. turczaninovii* and *P. flavescens* were collected in May 2019, *Urtica dioica* L., *Rosa acieularis* Linde., *Plantago major* L. *Rheum undulatum* L.were collected in June-July 2021 from the Taijiin Am of Mt. Bogd Khan (N47°37’18” E107°17’42” and N47°37’02” E107°18’25”), 60 km from the Ulaanbaatar city. Species were identified by Dr. Sh. Dariimaa. *Allium cepa* L. was purchased from the vegetable market. Plant samples were packed in polyethylene bags, kept in ice, transported to the laboratory, and stored in a freezer until further studies. The aerial parts were separated into stems, leaves and flowers, crushed and mixed with distilled water for extraction, and then filtered. In the filtrate, SOD activity was determined.

2.2. Enzyme Assay

The activity of SOD was measured spectrophotometrically according to the SOD assay procedure Marklund & Marklund, 1974 [4]. The autoxidation of pyrogallol (1,2,3-benzenetriol) was observed in the presence of EDTA at pH 8.2. The spectrophotometer was adjusted to read zero using Tris-EDTA buffer (pH 8.2), and the absorbance of the control and sample solutions were measured. The control solution contained 1 mL of distilled water, 2 mLTris-EDTA buffer, and 2 mL of pyrogallol, while the sample solution contained 1 mL of plant extract, 2 mL Tris-EDTA buffer, and 2 mL of pyrogallol.

2.3. Calculation of SOD Activity

SOD activity was expressed in units (U/g). One unit of SOD activity is being defined as an amount of enzymes required causing 50% inhibition of pyrogallol autoxidation (IPA).

$$\%\ \mathrm{IPA} = \frac{\Delta \mathrm{A\ test}}{\Delta \mathrm{A\ control}} \mathrm{x}\ 100\% \quad (1)$$

$$\mathrm{SOD\ activity\ (U/g)} = \frac{\%\ \mathrm{IPA}}{50\%} \quad (2)$$

SOD specific activity was calculated per mg of protein and expressed in U/mg.

2.4. Estimation of Soluble Protein

The concentration of soluble protein was measured by an improved version of the Biuret method, with Benedict reagent. The calculations were carried out using standard BSA (0.2-1 mg/mL) solutions of calibration. 0.5 g of plant material was extracted in 10 mL of 0.9% NaCl after which was filtered. To 0.5 mL filtrate, 2 mL of 6% NaOH, and 0.2 mL Benedict reagent were added. After 15 min the absorbance was measured at 364 nm. Control solution contained 0.5 mL of distilled water, 2 mL 6% NaOH and 0.2 mL Benedict reagent [5]. All the experiments were performed in triplicate, and the results were expressed as mean ± SD.

3. RESULT AND DISCUSSION

The studied plants are traditionally used for food and medicine in Mongolia Table 1. The research results on the soluble protein content and SOD activity are shown in Table 2. The content of soluble protein in the studied samples varied from 0.92% (*Allium cepa* L. stem) to 8.74% (*Rosa acieularis* Linde. leaves).

Table 1. Plants selected for estimation of SOD activity

№	Plant species	Traditional uses
1	*Urtica dioica* L.	Young leaves used for food as a source of vitamins and anti-inflammatory remedies for rheumatoid arthritis [6].
2	*Plantago major* L.	The leaves used for wound healing [7, 8].
3	*Rosa acieularis* Linde.	Fruits and leaves have traditionally been used to make tea, fruits - as a source of vitamins [7].
4	*Pulsatilla flavescens*	Commonly called *Shar yargui* by the nomads. The plant is used to improve bone fusion, as well as antimicrobial and antipararasitic agent [8].
5	*Pulsatilla turczaninowii*	Commonly called *Hoh yargui* by the nomads. The plant is used to improve bone fusion, as well as antimicrobial and antipararasitic agent [8].
6	*Rheum undulatum* L.	Stems used for constipation. Rhubarb leaves and stems are used for food [7, 8].
7	*Allium cepa* L.	Used for food as a source of vitamins and taste improver [7].

All studied plants demonstrated SOD activity. The enzyme activities varied and the activity ranged from 0.42±0.01 to 10.47±0.1 U/mg protein as shown in Figure 1.

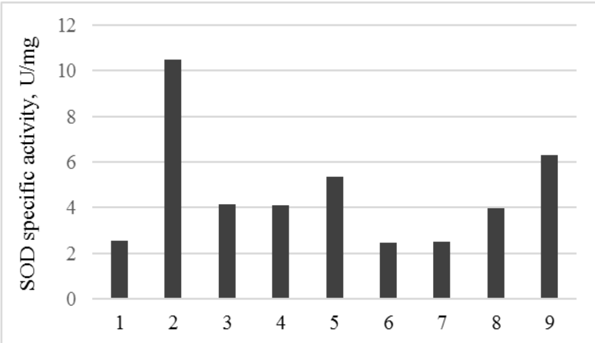

Figure 1. Comparison of SOD activity in the studied plants. 1 - *Urtica dioica* L. leaves; 2 - *Plantago major* L. leaves; 3 - *Rosa acieularis* Linde. flowers; 4 - *Rosa acieularis* Linde. leaves; 5 - *Pulsatilla flavescens* flowers; 6 - *Pulsatilla turczaninowii* flowers; 7 - *Rheum undulatum* L. leaves; 8 - *Rheum undulatum* L. stem; 9 - *Allium cepa* L. leaves

From the measured SOD activities in plants, *Plantago major* L. (10.47±0.1 U/mg) leaves extract showed the highest SOD specific activity followed by *Allium cepa* L. leaves (6.29±0.26 U/mg) *Pulsatilla flavescens* flowers (5.36±0.24 U/mg), *Rosa acieularus* Linde. flowers (4.14±0.21 U/mg) and *Rosa acieularis* Linde. leaves (4.12±0.18 U/mg) extracts.

SOD activity in several points was lower in *Rheum undulatum* L. stem (3.69±0.18 U/mg), *Urtica dioica* L. leaves (2.53±0.11 U/mg), *Rheum undulatum* L. leaves (2.52±0.12 U/mg), *Pulsatilla turczaninowii* flowers (2.46±0.1 U/mg) extracts and found to be the lowest in *Allium cepa* L. stem (0.42±0.01 U/mg).

Table 2. Soluble protein content and SOD activity in plant samples

№	Plant species	Soluble protein, %	SOD, U/g
1	*Urtica dioica* L. leaves	5.42±0.22	137.3±6.5
2	*Plantago major* L. leaves	1.28±0.06	134.0±4.8
3	*Rosa acieularis* Linde. flowers	6.28±0.25	260.0±11.2
4	*Rosa acieularis* Linde. leaves	8.74±0.42	360.0±15.2
5	*Pulsatilla flavescens* flowers	4.48±0.24	240.0±12.1
6	*Pulsatilla turczaninowii* flowers	3.68±0.16	90.7±4.4
7	*Rheum undulatum* L. leaves	3.15±0.1	79.41±3.8
8	*Rheum undulatum* L. stem	2.22±0.09	90.7±4.4
9	*Allium cepa* L. leaves	4.18±0.18	263.1±12.8
10	*Allium cepa* L. stem	0.92±0.02	3.85±0.15

We find only a few works on the determination of SOD activity for the *Rosa*, *Plantago* and *Allum* genus. Previously, Mileva et al. determined SOD activity in essential oils of *Rosa* species cultivated in Bulgaria, Moldova and China (*Rosa alba* L.; *Rosa damascena* Mill. F. Trigintipetala Dieck; *Rosa gallica* L.; Rosa IX-4, a hybrid of *Rosa damascena* Mill. F. Trigintipetala Dieck X *Rosa gallica* L.; *Rosa rugosa* Thunb.; *Rosa damascena* Mill) by SOD assay Kit-WST. All of the essential oils demonstrated superoxide-scavenging activity [9]. In the leaves and flowers of *Rosa acieularis* Linde., belonging to the same genus as these species the SOD activity was relatively high.

Grigore *et al.* determined SOD activity by inhibition of photoreduction of nitroblue tetrazolium (NBT) in three Plantago species (*P.lanceolata* L., *P.media* L. and *P.schwarzenbergiana* Schur) during phenophases. The results showed that the SOD activity was lower at vegetative stage in *P.media* and *P.schwarenbergiana* compared to *P.lanceolata*. In *P.lanceolata* SOD activity slightly decreased from vegetative to the fruiting stage [10]. The result of our work shows that the SOD activity in the leaves of *Plantago major* L. is higher compared to other plants.

For Allum species, Csiszar et al. determined SOD activity by the above-mentioned method (NBT) in leaves and roots of *Allium cepa* L., *Allium ascalonicum* auct. hort. and *Allium sativum* L. artificially creating drought conditions. The results show that after water stress elevated SOD activities were detected in plant roots [11]. Unfortunately, these works did not cover the plant species we studied; therefore, comparison of the obtained data is impossible (excluding *Allium cepa* L). The results for *Allium cepa* L. are very close to the results of previous authors.

4. CONCLUSION

In plants traditionally used in folk medicine and food, the content of soluble protein and the activity of SOD were determined. The soluble protein content showed the following decreasing trend: *Rosa acieularis* Linde. leaves > *Rosa acieularis* Linde. flowers > *Urtica dioica* L. leaves > *Pulsatilla flavescens* flowers > *Allium cepa* L. leaves > *Pulsatilla turczaninowii* flowers > *Rheum undulatum* L. leaves > *Rheum undulatum* L. stem > *Plantago major* L. leaves and *Allium cepa* L. stem. For SOD activity, we obtained the following decreasing trend: *Plantago major* leaves > *Allium cepa* L. leaves > *Pulsatilla flavescens* flowers > *Rosa acieularus* Linde. flowers > *Rosa acieularis* Linde. leaves > *Rheum undulatum* L. stem > *Rheum undulatum* L. leaves > *Urtica dioica* L. leaves > *Pulsatilla turczaninowii* flowers and *Allium cepa* L. stem. Based on our initial studies, it is possible to choose the most suitable plant sample from these analyzed plant samples and tissues for further study.

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CONFLICT OF INTEREST

We are, the authors of this article, declare that this work was carried out by us in the laboratory of Biochemistry of National University of Mongolia. We have no conflict of interest / competing interests under this article.

REFERENCES

[1] Young I.S., Woodside J.V., (2001) Antioxidants in Health and Disease. Journal of Clinical

Pathology, Vol. 54(3), 176-186. DOI: 10.1136/jcp.54.3.176

[2] Perrya J.J.P., Shina D.S., Getzoffa E.D., Tainer J.A., (2010) The structural biochemistry of the superoxide dismutases. Biochimica et Biophysica Acta, Vol. 1804(2), 245-262. DOI: 10.1016/j.bbapap.2009.11.004

[3] Yasui K., Baba A., (2006) Therapeutic potential of superoxide dismutase (SOD) for resolution of inflammation. Inflammation Research, Vol. 55(9), 359-63. DOI:10.1007/s00011-006-5195-y

[4] Marklund S., Marklund G., (1974) Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenientassay for superoxide dismutase. European Journal of Biochemistry, Vol. 47, 469-474. DOI: 10.1111/j.1432-1033.1974.tb03714.x

[5] Henry R.J., Sobel C., Berkman S., (1957) Interferences with Biuret Methods for Serum Proteins Use of Benedict's Qualitative Glucose Reagent as a Biuret Reagent. Analytical Chemistry, Vol. 29(10), 1491-1495. DOI: 10.1021/ac60130a028

[6] Ligaa U., (1982) Useful plants of Mongolia. Ulaanbaatar, Printing house of Academy of Science. 123-124 (in Mongolian).

[7] Lamjav Ts., Dorjjantsan D., Tsedenbaljir D., (1971) Medicinal plants of Mongolia. Ulaanbaatar, State printing house. 104-108, 128-132, 279-280, 334-335 (in Mongolian).

[8] Sanchir Ch., Bathuu J., Boldsaikhan B., (2003) Pictorial atlas of useful plants of Mongolia. Ulaanbaatar. 170, 184, 201-202 (in Mongolian).

[9] Mileva M., Krumova E., Miteva-Staleva J., Kostadinova N., Dobreva A., Galabov A.S., (2014) Chemical compounds, *in vitro* antioxidant and antifungal activities of some plant essential oils belonging to *Rosaceae* family. Comptes rendus de l'Acadamie bulgare des Sciences mathematiques et naturelles. Vol. 67(10), 1363-1368.

[10] Grigore M.N., Ivan M., Verdes A., Oprica L., (2017) Enzymatic Activity and Non - enzymatic Antioxidants Content in Several Plantago Species (from Valea Ilenei Nature Reserve), During Different Phenophases. Revista de Chimie, Vol. 68(7), 1539-1543. DOI:10.37358/RC.17.7.5712

[11] Csiszar J., Lantos E., Tari I., Madoşă E., Wodala B., *et. al.*, (2007) Antioxidant enzyme activities in *Allium* species and their cultivars under water stress. Plant, Soil and Environment. Vol. 53(12), 517-523. DOI: 10.17221/2192-PSE