



## Investigation of Antioxidant Properties of *Spartium junceum* L.: Effect of Plant Parts and Storage Conditions

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### ABSTRACT

*Spartium junceum* L. is a plant traditionally used for different medicinal purposes. While limited research data explicates its antioxidizing ability, interest in this plant is induced mainly due to its possible role, especially against stress-causing oxidative effects. The objectives of this study were to compare antioxidant activity in flowers and leaves of *Spartium junceum* L., as well as time under different storage conditions implemented for antioxidative mechanisms. *Spartium junceum* L. plants were obtained from the Kahramanmaraş, Turkey region; subsequently, the flowering and leaf parts of the plant were separated and analyzed. Plant homogenates were prepared, and the activities of SOD and CAT enzymes, as well as MDA levels, were determined using spectrophotometric methods. Enzyme activity upon storage at +4°C, -20°C, and -70° temperature enzyme samples were carried out separately and operated for less than one month in our laboratory. Flowers exhibited higher SOD and CAT activities than leaves. Flowers also showed higher levels of MDA. It may be due to the structural and biochemical differences, where flowers experience extra oxidative stress. The optimal enzyme retention under storage conditions was at -70°C, and a decrease in temperature increased the stability of this biocatalyst. In contrast, MDA levels increased at low temperatures at total capacity. The antioxidant properties of the flower extract had stronger antioxidant potential than those of the leaf part, which also means that chemically active substances show much higher concentrations in this plant section. Storage temperature significantly affects the stability of enzymes, and it was stated that low temperatures mainly maintain antioxidant activity. The results obtained from this study recommend *Spartium junceum* L. as a valuable antioxidant food resource.

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## Introduction

*Spartium junceum* L. is a perennial herb that grows in the Mediterranean region. It's been used for thousands of years in traditional medicine and is highly considered an antioxidant. *Spartium junceum* L., for instance, has been reported to have a range of pharmacological properties, including anti-inflammatory and cytotoxic activities, based on its biological activity studies. The aromatic juice of the plant has been reported to have a cytotoxic effect (Cerchiara et al., 2012; Teresa Cerchiara et al., 2013), and its flower extracts were studied for their anti-inflammatory and analgesic activities (Menghini et al., 2006; Zengin et al., 2019). It has been reputedly described as a sedative and diuretic for the flowers (Menghini et al., 2006). Studies *in vivo* have shown that *Spartium junceum* L. has antiulcerogenic effects on gastric ulcers, highlighting the possible role of this plant for therapeutic use in stomach ulcers (Yesilada, Takaishi, et al., 2000). Also, extracts from the plant were revealed as very toxic against some cancer cell lines and might potentially be a natural anti-tumor agent (Cerchiara et al., 2012).

This antioxidant capacity of plants is important as it can protect cells from the harmful effects of oxidative stress. Oxidative stress leads to free radical-induced damage of cells and is a causal agent in the pathogenesis of aging and chronic diseases (e.g. diabetes, cardiovascular disease) / inflammation. Antioxidants prevent harmful free radicals from interfering with cell integrity and lower your risk of chronic diseases including heart disease, diabetes, and even cancer (Aune, 2019; Çelik & Pepe, 2024; Jena et al., 2023; Karaman & Türkay, 2023). Various studies on the antioxidant action of *Spartium junceum* L. have drawn attention to its phenolic compounds and free radical scavenging activity (Eruygur et al., 2022; Habibatni et al., 2016). Its extracts have been shown to scavenge free radicals and relieve oxidative stress (Yesilada, Tsuchiya, et al., 2000). The plant is known for its antioxidant properties, with flavonoids playing an important role. Flavonoids are reputed to be free radical scavengers and can protect cells from toxic injury due to survival antioxidant properties. Many studies showed that *Spartium junceum* L. contains

flavonoid glycosides with free radical scavenging activity (Roy et al., 2022). It may be helpful in its antioxidant effect on parameters measured in this study.

Moreover, *Spartium junceum* L. extracts have been found to exhibit superoxide dismutase (SOD) activity and might act like this enzyme in converting toxic superoxides into less harmful forms (Yesilada, Tsuchiya, et al., 2000). These results suggest that the plant could be used to prevent oxidative stress-associated cellular damage. Given the high content of flavonoids and other bioactive components in this plant, we may assume it has significant antioxidant activity. This property might generate protective effects against oxidative stress (Nanni et al., 2018).

Nevertheless, the detailed antioxidant action of each organ in plant parts such as leaves and flowers from *Spartium junceum* L. and its differences among different conditions that might occur during storage still need to be studied to a great extent. However, the information regarding biochemical stability and activity of antioxidant compounds at various temperatures (both higher (+4 °C) and lower than this range (-20°, -70° C)) during their storage for different periods like 1 to up to a maximum of 30 days is less represented in published literature. The storage conditions of plants directly affect the antioxidant activities and stabilization of biochemical components, which is why studies in this field are insufficient.

This study was intended to analyze the antioxidative ability of *Spartium junceum* L., and it included measurement of SOD, catalase (CAT), and malondialdehyde (MDA) content in leaves as well as flowers. Also, to evaluate how they are impacted by the storage conditions (at three different temperatures: +4°C, -20°C, and -70°C) at specific periods (1, 3, 5, 15 and 30 days). The focus of this research is to provide a more detailed source of information on the particular antioxidant activity (in terms of leaves and flowers) and explore the various health benefits by which relatively fewer scientific studies with respect to *Spartium junceum* L remain.

## Materials and Methods

The naturally growing *Spartium junceum* L. plant used in this study was collected from the borders of Kahramanmaraş province in the Mediterranean Region. Plant description was carried out by researchers at the Faculty of Agriculture of our university. Plant samples were sterilized, sectioned, separated into flowers and leaves, and prepared for analysis.

### Biochemical Analysis

#### Plant preparation for biochemical analysis

Plant sampling is processed in the Kahramanmaraş Sütçü İmam University Faculty of Medicine, Medical Biochemistry Research Laboratory. Fresh plant samples delivered to the laboratory were mixed and separated from flowers and leaves with a sterile scalpel and milled using a mechanical cutter Lavion company Brand. Plant homogenate ground with 1/10 KCl was rendered and filtered. The filtrate was centrifuged (Hettich 420 R) at 5000 rpm for five minutes, and the supernatant was used in the analysis. Analyses were carried out in five replicates for fresh plant leaves and flowers and single measurements for storage conditions.

### Determination of SOD Activity

The enzymic activity was assayed by following Fridovich method (1995). The process depends on the formation of red formazan dye (Fridovich, 1995) due to an interaction of superoxide radicals produced by xanthine and additionally by xanthine oxidase with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride which is called as pydonitrotetrazolium violet (NBT). The spectrophotometer readings are made by determining the optical density (OD) of formazan that has been formed, which is read at a wavelength of 505 nm. The intensity of color decreases under SOD action, which in turn is known to indicate the amount of activity.

### Determination of CAT activity

CAT activity was determined by measuring the decreased hydrogen peroxide concentration at 230 nm (Beutler, 1984). CAT activity was expressed as U/mg protein. CAT catalyzes the degradation of hydrogen peroxide. The hydrogen peroxide degradation rate by CAT was measured spectrophotometrically using the light absorption of hydrogen peroxide at 230 nm.

### Determination of MDA level

It is based on the principle that MDA, which is the secondary product of lipid peroxidation formed as a result of incubation of the sample at 90-95 °C with thiobarbituric acid (TBA) at pH 3.40 under aerobic conditions, forms a pink complex with TBA. This color intensity is directly proportional to the MDA concentration in the medium and is evaluated spectrophotometrically at 532 nm (Ohkawa et al., 1979).

### Protein level

The levels of protein and polyphenol compounds can be determined by the Folin Cioacalteu method. The Folin technique is based on the absorbance measurement at 750 nm by the spectrophotometric method of the color reaction of tyrosine and tryptophan residues contained in proteins with phosphotungstic-phosphomolybdic acid (Lowry et al., 1951). Bovine serum albumin was used as standard. SOD, CAT, and MDA levels were calculated and then divided by the measured protein levels, and the results were given as SOD, CAT activity, and MDA level per mg protein.

### Statistical Analyses

GraphPad Prism 10 software was used to analyze the data obtained in this study. Normality tests were applied to determine whether the data fit normal distribution. An unpaired t-test for independent groups was used to evaluate whether the differences between the groups were statistically significant. The significance level was accepted as  $p < 0.05$ . The results obtained are presented as mean  $\pm$  standard error.

## Results and Discussion

This study demonstrated that the flowers of *Spartium junceum* L. exhibit an enhanced antioxidant potential starting from extracts than leaves. Results showed that the floral tissues possessed higher activities of enzymatic antioxidants and malondialdehyde (MDA) levels, which indicate an enhanced action for antioxidative defense. Then, this result associated with the phenol-richness of flowers would have promising implications for preventing oxidative stress.

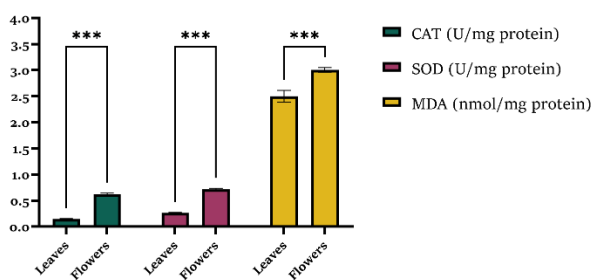


Figure 1. Comparison of catalase (CAT), superoxide dismutase (SOD) activities, and malondialdehyde (MDA) levels in *Spartium junceum L.* flowers and leaves.

CAT (U/mg protein), SOD (U/mg protein), and MDA (nmol/mg protein) levels were measured in flower and leaf extracts. CAT, SOD activities, and MDA levels were significantly higher in flowers than in leaves (\*\*p<0.01).

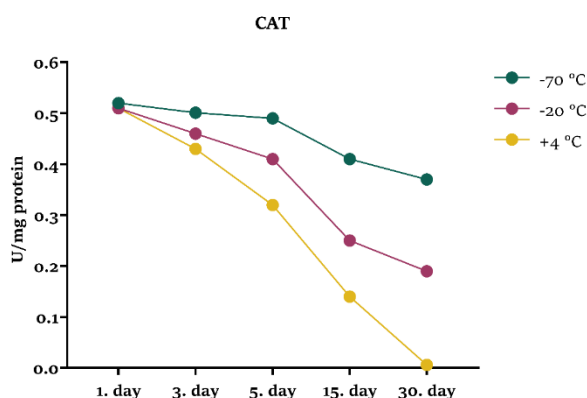


Figure 2. Time-dependent catalase (CAT) activity variation in *Spartium junceum L.* samples at different storage temperatures.

CAT activity measured in the plant's flower and leaf parts (pool) was monitored at +4°C, -20°C, and -70°C for periods of 1, 3, 5, 15, and 30 days. While the maximum decrease was observed at +4°C, CAT activity was maintained at the highest levels in samples stored at -70°C.

CAT enzyme activity was determined according to 0.626 U/mg protein in flower extracts and 0.146 U/mg protein of leaf extract, respectively (Figure 1). By scavenging hydrogen peroxide into water and oxygen, the CAT enzyme protects cells against reactive oxygen species (ROS), which cause cellular damage (Nandi et al., 2019). The result shows that the antioxidative defense system in flowers is more sensitive than in leaves. This high CAT activity accords with a compensatory phenolic system in response to oxidative stress, given that the concentration of these compounds and such enzymatic capacity would provide a greater degree of detoxification. These findings conform to the literature where plant antioxidants are frequently reported as positive protectants against ROS (Çelik, 2023; Eruygur et al., 2022; Habibatni et al., 2016). In addition, various enzymes that include CAT are critical in protecting from oxidative stress-associated diseases (Menghini et al., 2006). Hence, there is an interest in *Spartium junceum L.* to prevent damage induced by oxidative stress (Cerchiara et al., 2012; Nanni et al., 2018).

The highest activity of superoxide dismutase (SOD) was detected in flowers, and its specific activity was 0.718 U/mg protein also. In leaves, this value was 0.262 U/mg protein (Figure 1). The SOD enzyme serves in the antioxidant defense, removing superoxide radicals to

hydrogen peroxide (Younus, 2018). The high SOD activity in flowers may reflect the effect of flavonoids and other polyphenols to increase the activity of these enzymes (Beslo et al., 2023). The scant information found in the literature regarding SOD activity of *Spartium junceum L.* points to this species being a good contender for oxidative stress (Eruygur et al., 2022). Consumption of food with high activities of SOD can be essential to prevent cardiovascular and neurodegenerative diseases (Cerchiara et al., 2012; Teresa Cerchiara et al., 2013).

MDA levels (an index of lipid peroxidation) were reported at 3.006 nmol/mg protein in flowers and 2.5 nmol/mg protein in leaves (Figure 1). The flowers exhibited the highest MDA contents in this experiment, indicating increased oxidative processes and metabolic rates. This agrees with the prior study: flowers undergo higher oxidative stress than fruits and leaves, retaining their structural conformationality involving biochemical pathways.

The ABTS radical scavenging activity of *Spartium junceum L.* methanol extracts was reported in the literature as indicating a potent radical-scavenging ability against ABTS. The fact that this effect was especially evident in the flowers supports that this part may be a richer source of antioxidant compounds. In superoxide radical scavenging tests, the plant extracts also showed good potency. Flower extracts were found to be the most active, and this activity was also notably higher when the compounds were redissolved in methanol (Zengin et al., 2019). This could indicate that the biological properties of *Spartium junceum L.* might be due to its antioxidant properties as well.

*Spartium junceum L.* has been studied for its anti-herpesviruses activity, and the aqueous extracts exhibited potent activities against herpes simplex virus type 1 (HSV-1) *in vitro* experiments (Duman et al., 2019). This provides the basis that the antiviral activity is probably related to the high antioxidant content of plants' properties. Furthermore, with the cytotoxic effect it has against glioblastoma cell lines (Abusamra et al., 2015), this plant could be suitable for cancer treatment. These plants' antioxidant activities and anticancer properties have been associated with their high radical scavenging effects, which point to structural features responsible for each activity that will lead to future research.

We also studied how different storage temperatures and durations impacted the stability of antioxidant enzymes along with oxidative markers. Therefore, this work is significant in optimizing preservation methodologies so that a return to plants retains bioactivity. The temperatures used for the assay of enzyme activities between 30 days showed that high CAT and SOD activities were maintained at -70°C up to 30 days: CAT 0.37 U/mg protein and SOD 0.501 U/mg protein (Figure 2 and 3). With increasing temperatures, CAT 0.19 U/mg protein and SOD 0.39 U/mg protein were measured at -20°C, while at +4°C CAT 0.006 U/mg protein and SOD 0.11 U/mg protein were associated with decreasing enzymatic activities. This supports the effect of temperature on slowing enzymatic degradation and metabolism and maintaining enzyme activity over time (Stajner et al., 2010).

When the enzyme activities were analyzed over time, CAT and SOD showed a significant reduction in activities with increasing storage period at -70°C, beginning on day 1 (0.52 U/mg protein for CAT, 0.68 U/mg protein for SOD)

to day 30 (0.37 U/mg protein for CAT, 0.501 U/mg protein for SOD) (Figure 2 and 3). This could be due to the apparent preservation of enzyme structure in low temperatures stimulating short-term activity, which is lost during long-term storage. Factors such as prolonged freeze-thaw cycles may lead to structural changes in enzyme molecules and affect their activity (Teresa Cerchiara et al., 2013).

When the MDA levels were analyzed according to storage conditions, paradoxically, it was observed that MDA levels increased with decreasing temperatures, and the highest level at day 30 was 2.35 nmol/mg protein at -20°C (Figure 4). Furthermore, MDA levels increased from day 1 to day 30 at all temperatures. These findings suggest that lipid peroxidation may increase under cold storage while enzyme activity is maintained, possibly due to membrane phase changes that trigger oxidative stress. This highlights a critical balancing act required under storage conditions where maintenance of enzymatic activity may inadvertently increase oxidative damage (Teresa Cerchiara et al., 2013).

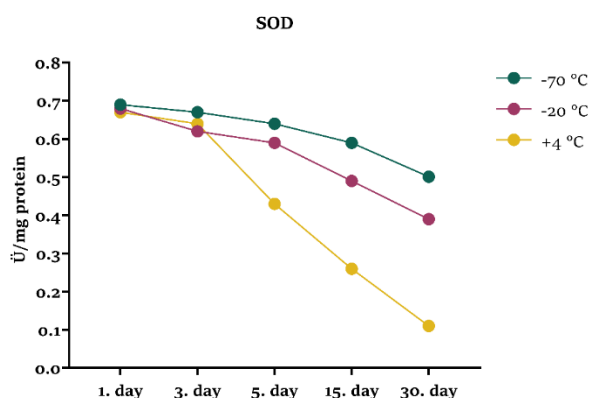


Figure 3. Time-dependent variation of superoxide dismutase (SOD) activity in *Spartium junceum L.* samples at different storage temperatures.

SOD activity measured in both flower and leaf parts (pool) of the plant was monitored at +4°C, -20°C, and -70°C for periods of 1, 3, 5, 15, and 30 days. While the maximum decrease was observed at +4°C, CAT activity was maintained at the highest levels in samples stored at -70°C.

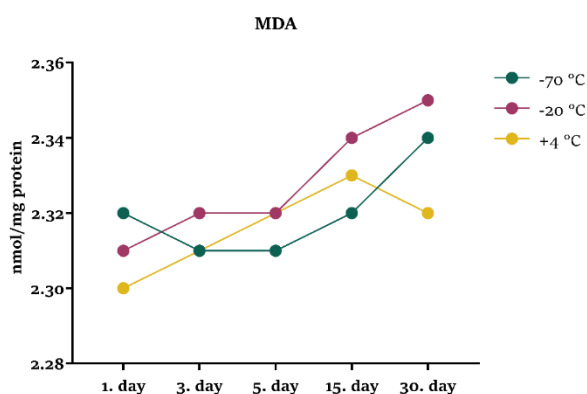


Figure 4. Time-dependent malondialdehyde (MDA) level variation in *Spartium junceum L.* samples at different storage temperatures.

MDA analyses of the plant's flower and leaf parts (pool) were monitored at +4°C, -20°C, and -70°C for periods of 1, 3, 5, 15, and 30 days. The results showed that MDA levels, an indicator of oxidative stress, increased significantly over time, especially in samples stored at -20°C. The increase in MDA levels was more limited in samples stored at -70°C.

## Conclusion

In conclusion, this study compared the antioxidant enzyme activities between the flowers and leaves of *Spartium junceum L.* at different storage temperatures. Flowers demonstrated higher antioxidant enzyme activity and lipid peroxidation than leaves, indicating their more significant antioxidative potential. Storage conditions significantly influenced enzyme stability, with -70°C identified as the optimal temperature for preserving the functional stability of antioxidant enzymes such as SOD and CAT over time. These findings highlight the importance of low-temperature storage to maintain the antioxidant properties of plant-based extracts. Furthermore, the higher antioxidant activity observed in the flowers of *Spartium junceum L.* underscores their potential as a natural source of antioxidants, warranting further evaluation for nutraceutical or therapeutic applications.

## Declarations

### Ethical Approval Certificate

This research does not involve any data or materials related to humans, animals, or other living organisms. As a result, ethical committee approval and informed consent is not required for this study.

### Author Contribution Statement

Muhammed Mehdi Üremiş: Data analysis, formal analysis, and writing the original draft  
Ergül Belge Kurutaş: Data collection, conceptualization, methodology, and review.

### Fund Statement

Not applicable

### Conflict of Interest

The authors declare no conflict of interest.

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