



## Determination of in Vitro Antioxidant Enzyme Capacity and Oxidative Stress Levels in Mazi Meşesi (*Quercus infectoria*)

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### ARTICLE INFO

### ABSTRACT

#### Research Article

Received : 10/02/2021

Accepted : 10/03/2021

#### Keywords:

*Quercus infectoria* gal  
Catalase  
Superoxide dismutase  
Malondialdehyde  
Antioxidants

South East Anatolia Region has a large genetic plant diversity due to its physical and different climatic characteristics. These plants are potential sources of antioxidants that prevent oxidative stress caused by oxygen and photons. In recent years, it has become important to study the antioxidant capacity of many molecules found naturally in foods and biological systems. The reason for this is that it is believed that when the consumption of food rich in antioxidants is increased, the risk of developing different degenerative diseases will be reduced. In this study, it was aimed to measure the antioxidant capacity of *Quercus infectoria*, *G. olivier* gal seeds grown in Southeastern Anatolia. *Q. infectoria* gal seeds from Şanlıurfa province were used in our study. *Q. infectoria* gal seeds were extracted with water, ethanol and methanol and then antioxidant enzyme activities (catalase and superoxide dismutase) and malondialdehyde levels, which are indicators of oxidative stress were determined by spectrophotometric methods. It was found that the antioxidant capacity (catalase and superoxide dismutase activities) of extracts obtained from ethanol and methanol were higher and their malondialdehyde levels were statistically lower than those obtained from water. However, it was determined that there was no statistically significant difference between the antioxidant capacity and malondialdehyde levels of the extracts obtained from methanol compared to the extracts obtained from ethanol. It has been concluded that *Q. infectoria* gal seed has a effective antioxidant effect. In addition, it was observed that extracts obtained from ethanol and methanol have higher antioxidant capacity than extracts obtained from water.

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## Introduction

Thanks to the pharmacological compounds and bioactive substances found in the structure of the plants, it is forms the origin of many drugs used today (Sevindik et al., 2017). Therapeutic use in plants BC.it is based on the Mesopotomian civilization in the 5000s and it was determined that approximately 250 herbal drugs were used (Mohammed et al., 2018). It is reported by World Health Organization that the treatment with herbs will increase all over the World by the coming years (WHO, 2015). For this reason, obtaining and evaluating the main active ingredients of the extracts obtained from plants is of great importance in terms of economy and health.

The Thuja (*Q. infectoria*), which spread in the Southeastern Anatolia Region in our country, is a plant belonging to the Fagaceae family. *Q. infectoria* is referred

to as a oriental thuja in its varieties (Böhner, 1934). Thuja seeds are called gal. According to literature studies, gal extracts have used in many settlements as medicanal drugs for different purposes since ancient times. For example, it has been used in India for a long time to treat chronic diarrhea and söre throat (Camus, 1934). At the time of the ancient Greeks, Hippocrates prepared a special incense by mixing thuja seed with honey and oil. This incense has given succesfull results in the treatment of Hysteria for many years ((İnal.,1955).). The seeds of thuja called gal, are 1.5-2 cm. spherical in diameter and randomly rough on top (Shresthka, 2014). In the southeastern Anatolia Region, thuja seeds have been used by local people for centuries to treat burn wounds. Powdered thuja seeds applied regularly burned area.

Antioxidants are molecules responsible for reducing the formation of free radicals, ending radical reactions, neutralizing the formed radicals and removing damaged molecules (Machlin et al., 1985; Pehlivan et al., 2018). Free radicals can cause serious damage to the body if not neutralized (Yigit, 2013; Sevindik, 2018). According to the researches, it has been determined that the consumption plenty of vegetables and fruits reduces the risk of developing diseases, there is a significant decrease in cardiovascular diseases, cancer cases and mortality rates (Ariduru, 2013).

In recent years, there have been about the use of antioxidant plants in areas such as medicine and food. These studies are still continuing intensively. The reason for this is that the plants are very rich in terms of carotenoids, flavonoids and phenolic compounds, which are antioxidants, and these compounds do not have any side effects (Özkan, 2000; Salehi et al., 2019). It is known that the thuja used in this study is used by the public in the treatment of various diseases. Today, the raw materials of many drugs are composed of compounds synthesized by medicinal and aromatic plants. In literature searches, no in vitro study examining the antioxidant activity of thuja plant was found. For this reason, we aimed to measure the antioxidant enzyme activity of thuja plant and MDA levels, which are indicators of oxidative stress in methanol, ethanol and water extracts.

## Material and Method

The seeds of thuja that grow naturally in the province of Sanliurfa in the Southeastern Anatolia Region were used. The fruit outer skins of all the samples were removed and dried in a thin layer under room conditions, out of sunlight. Then the samples were pulled in the mill and stored at room temperature until they were used.

### Extraction of Samples

The cold extraction method applied by Fernández Agulló (2013) in the extraction of the samples was modified. In the extractions made with water, 10 g of sample was weighed and 100 mL of water was added on it. Then, after being kept in the shaking water bath at 60°C for 12 hours, the samples passed through the coarse filter and then through whatman 4 filter paper were centrifuged and the supernatant was taken and used as stock solution.

To obtain extracts using ethanol and methanol as solvents, 10 g of sample was weighed, 90 mL of solvent and 10 mL of water were added. Then, the samples passed through the coarse filter and then through whatman 4 filter paper were centrifuged, and finally the supernatant was taken under vacuum until the ethanol or methanol was removed according to the solvent used in the rotary evaporator. The extract-water mixture obtained was used as the stock solution. Biochemical analyzes (SOD, superoxide dismutase; CAT, catalase and MDA, malondialdehyde) were performed on all three extracts (ethanol, methanol and water) obtained.

### Determination of SOD Activity

SOD enzyme in tissue samples was measured according to the method described by Fridovich. SOD accelerates the dismutation of toxic superoxide radicals into hydrogen

peroxide and molecular oxygen during oxidative energy production. In this method, the red colored formazan dye formed by 2- (4-iodophenyl) -3- (4-nitrophenol) -5 phenyltetrazolium chloride (pydonitrotetra zolium violet: NT) produced by the use of xanthine and xanthine oxidase with a wavelength of 505 nm. It is based on the reading of optical density (OD). The OD of the red color formed decreases according to the color formed in the absence of SOD, and SOD activity is measured by determining the difference here (Fridovich, 1974).

### Determination of CAT Activity

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

### MDA Levels

For each plant extract; MDA level was determined using the Ohkawa method (Ohkawa, 1979). It is based on the principle of forming a pink colored complex with MDA, which is the secondary product of lipid peroxidation, which occurs as a result of incubation of the sample with thiobarbituric acid (TBA) at pH 3.4 under aerobic conditions. The color intensity formed is directly proportional to the MDA concentration in the environment; It was evaluated spectrophotometrically at 532 nm.

## Results

As seen in Table 1, the antioxidant capacity (CAT and SOD activities) of extracts obtained from ethanol and methanol are higher than those obtained from water ( $P < 0.05$ ). Moreover, the highest CAT and SOD activities were found in methanol extracts ( $P < 0.05$ ). It was determined that the MDA levels of extracts obtained from ethanol and methanol were statistically lower than those obtained from water ( $P < 0.05$ ) (Figure 1). However, it was determined that there was no statistically significant difference between MDA levels of extracts obtained from methanol compared to extracts obtained from ethanol ( $P > 0.05$ ).

Table 1. CAT and SOD activity in extracts obtained with ethanol, methanol, water

Groups	CAT Activity Average± SD	SOD Activity Average ± SD
Ethanol Group	558.05±230.31**	76.55±17.97***
Methanol Group	652.93±179.68**	138.73±35.10***
Water Group	219.47±44.18*	40.33±9.80***

CAT: catalase; SOD: superoxide dismutase; SD: Standard deviation

\* CAT activity was statistically significantly higher in ethanol and methanol compared to water ( $P < 0.05$ ).

\*\* No significant differences were found between methanol and ethanol extracts in terms of CAT activity ( $P > 0.05$ ).

\*\*\* While SOD activity showed significant differences between methanol, ethanol and water extracts ( $P < 0.05$ ), the highest SOD activity was found in methanol extract ( $P < 0.05$ ).

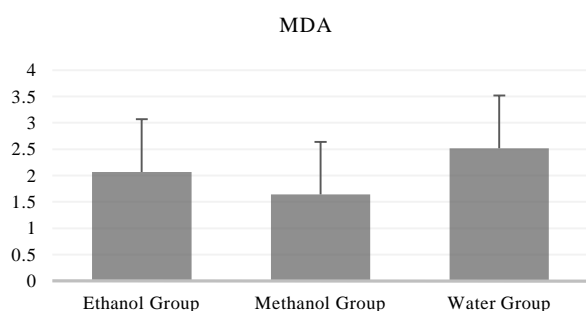


Figure 1. MDA levels in extracts obtained with ethanol, methanol, water

## Discussion

As in all countries of the world, medicinal plants have been used by the public for centuries in the treatment of diseases in our country. Today, herbs and herbal medicine raw materials make up 25% of prescription drugs. The antioxidant effects of plants and their important properties for human health have been investigated in laboratories since 1926. In recent years, many studies have been done on the antioxidant effects of medicinal plants (Salehi et al., 2020a). Nowadays, herbs and herbal medicine raw materials constitute a large part of medicines used in treatment. The inadequacy of synthetic drugs and therapeutic agents against the increasing diseases in recent years and the detection of their side effects have increased the necessity of using natural products. For this purpose, plants have been investigated in terms of biochemical, microbiological and pharmacological aspects, and even in terms of plant defense mechanisms in recent years when biological warfare is on the agenda (Kalaycıoğlu and Öner, 1994; Mohammed et al., 2020). There are many different phenolic compounds found in all plant metabolisms as secondary metabolites that are thought to play a role in protecting the plants against some pests (Saldamlı, 2007; Mohammed et al., 2019). Phenolic compounds defined as secondary metabolism products of plants are widely found in plants and today the structure of thousands of phenolic compounds has been defined (Kafkas et al., 2006). Newly defined phenolic compounds are continuously added to these every day. Phenolic compounds can be found in fruits, vegetables, seeds, flowers, leaves, branches and stems of plants (Coşkun, 2006; Aydın et al., 2007).

These metabolites, which are generally in the class of phenolic compounds, can show their antioxidant activities in the human body when consumed by humans through the diet (Chaudiere, 1999). For this reason, in recent years, people have been encouraged to take natural food-derived antioxidants. On the other hand, the use of plant extracts containing abundant antioxidant compounds (Karagözler et al., 2008) as a preservative in the food industry is also a common practice in recent years. However, in food technology, synthetic antioxidants are also used as additives to ensure the long-term durability of foods. Although these synthetic antioxidants have very high protective properties, there is also controversy that they may be harmful to human health due to their unnatural nature (Pokorny, 2007). It has been reported in various studies that the antioxidant properties of natural antioxidants are related to their molecular structure (Rice-

Evans et al., 1997; Pannala et al., 2001; Apak et al., 2007; Salehi et al., 2020b).

There are many studies showing that *Q. infectoria gal* seeds contain ellagic acid (Quideau, 2009). In some studies on ellagic acid, it has been observed that it has antioxidant, antiviral, anticarcinogenic, antimutagenic and anti-inflammatory properties (Singh, 2005). Studies conducted today show that defense systems originating from the organism alone are not sufficient in removing oxygen radicals. Especially in researches, it has been determined that antioxidants taken from outside can be quite effective against oxidative damages in the organism (Kurutaş, 2015). An increasing number of scientific studies show that nutritional components have positive effects on health and contribute to the prevention of diseases such as cardiovascular diseases, cancer and osteoporosis (Kurutaş, 2015). Phytochemicals found in vegetables and fruits that we consume frequently are effective by capturing oxidants as well as by regulating the activation of detoxifying enzymes, stimulation of the immune system, cell proliferation and apoptosis, hormone metabolism, and antibacterial and antiviral effects (Kurutaş, 2015).

*Q. infectoria* plant belongs to the group of plants exposed to high levels of oxidative stress. On the other hand, the presence of a powerful antioxidant that protects the plant has been revealed by various researchers (Dicko, 2006). Kaur et al. (2008) reported that as a result of the incubation of *Q. infectoria gal* extract with macrophages, cells were protected against oxidative stress and seeds showed a high level of antioxidant activity. In this study, it can be said that *Q. infectoria gal* seeds show high levels of antioxidant activity (SOD and CAT) due to ellagic acid and polyphenol compounds.

When the literature is searched, studies on antioxidant activity measurement specific to *Q. infectoria gal* are very few. Today, antioxidant measurements can be made using different methods. In our study, we aimed to obtain results on antioxidant enzyme activities using spectrophotometric methods. In this sense, SOD and CAT activities and MDA levels were measured as oxidative stress parameters in the plant. When SOD and CAT enzyme activities were evaluated, it was observed that *Q. infectoria gal* plant seed had high antioxidant capacity. This result we found supports the literature studies. Unver et al. (2008) in a study of antioxidant activity of some plants with application in medicine, Turkey, acetone, ethanol, was determined using various solvents such as water. According to the data obtained, it was found that the free radical scavenging efficiency of plant extract, whose solvent is water, is higher than extracts whose solvents are ethanol and acetone (Unver et al., 2008). In our study, we used methanol, ethanol and water. Because methanol, ethanol and water are a universal solvent traditionally used to extract most plant products with antioxidant activity. Among the solvents we prepared using methanol, ethanol and water, the highest antioxidant capacity was found in the methanol extract, while the lowest antioxidant activity was found in the extract we prepared with water. It has been observed that the active substances existing in plants change depending on the solvent used.

In addition, it is thought that determination of possible active substances in our plant and elucidation of their chemical structures will be pharmacologically important.

Today, the risk of chronic and degenerative diseases is increasing day by day due to changing life conditions and eating habits. From this point on, reducing this risk is one of the main issues of the scientific world. In this context, plants and their biological activities are used in the development of new drug and food formulations. As a result of our work on this basis, methanol extract exhibited effective antioxidant effects. *Q. infectoria gal* can be considered as an important candidate for natural agents, as it is important to identify safe and effective functional agents.

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