European Journal of Technique

journal homepage: https://dergipark.org.tr/en/pub/ejt

Vol.13, No.1, 2023

# Determination of Antioxidant Activity of Dietary Selenium, Oleuropein, Glutathione Mixture

**Feryal Zeliha Akay**[1*], **Göksel Kızıl**[2]

[1*] University of Dicle, Faculty of Science, Chemistry Department, Diyarbakır, Turkey. (e-mail: feryalakay@hotmail.com.tr).
[2] University of Dicle, Faculty of Science, Chemistry Department, Diyarbakır, Turkey. (e-mail: gokselk@dicle.edu.tr).

ARTICLE INFO

Received: May., 26. 2023
Revised: Jun., 04. 2023
Accepted: Jun, 30. 2023

Keywords:
Free radicals
Selenium
Oleuropein
Gluthatione
ROS(reactive oxygen species)

Corresponding author: *Feryal Zeliha Akay*

ISSN: *2536-5010* | e-ISSN: *2536-5134*

DOI: https://doi.org/10.36222/ejt.1303042

## ABSTRACT

Free radicals are a unit involved with cellular disorders through their damaging actions on proteins, lipids and DNA and area unit causative factors for an outsized variety of chronic diseases and therefore the aging method. Antioxidants of plant origin hold nice significance and have so gained utmost importance within the recent past. The current research spills the inhibition effect of selenium, glutathione and oleuropein mixtures against free radicals and supermolecule chemical action. It was determined that the mixture of oleuropein, glutathione and selenium used in the study inhibited free radicals. It was determined that it competed with the positive control at increasing concentrations. The mixture is intended to be used as a daily support supplement.
The aim of the study is to use the prepared mixture as an alternative to drugs known to have artificial antioxidant properties in the market. One of the aims of this study is to have both drug therapy in the literature and inhibition of ROS-induced damage in the biological system at an early stage.

## 1. INTRODUCTION

Phenolic compounds, which are the most effective phytochemical bioactive, and flavonoids and stilbenes, which are a group of phenolics, are important minor components of many fruits, vegetables and herbal products. The flavonoids, which form a large group of phenolic compounds, are divided into nine parts: flavones, isoflavones, flavonols, flavanols, flavanones, anthocyanidins, anthocyanins, flavonols and chalcones. Flavonoid antioxidant compounds are bioactive compounds with phenol chemical structure and contain polar hydroxyl ($OH^-$) substituents in their structures[1]. Phenolic substances are radical scavenging natural antioxidants. It is known that flavonoids inhibit lipid peroxidation and are radical scavenging [2].

The antioxidant effects of phenols have been explained by three mechanisms [3].
In the first mechanism (HAT), polyphenols (ArOH) free radical ($R^{\cdot}$); neutralizes it by giving hydrogen.

In the second mechanism (SET) (ArOH) free radical ($R^{\cdot}$); It forms the more harmless $ArOH^+$ radical by donating electrons.
Transition metals involved in free radical formation in the third mechanism (TMC); It forms stable compounds by being chelated with polyphenols. The formation of the $^{\cdot}OH$ radical, which is formed as a result of the Fenton reaction between $H_2O_2$ and the metal ion, is prevented(Figure 1.)

### 1.1. ROS (Reactive Oxygen Species)

They are forms of oxygen with high chemical reactivity compared to the normal oxygen molecule due to their chemical structure. Reactive oxygen species are thought to be an important factor in aging and most of the diseases are related to aging[4](Figure 2.).

### 1.2. Selenium

(Se) is an essential micronutrient in our diet and can reduce diabetic symptoms [5]. Se; It acts like insulin in streptozotocin (STZ)-induced diabetic mice, where it can regulate the activity of several enzymes involved in gluconeogenesis and glycolysis and facilitate the transport of glucose into cells[6]. However, high Se blood levels can cause toxicity.

Selenium affects oxidative stress, inflammation, apoptosis, uncontrolled cell proliferation, hormone production, angiogenesis and immune function, and DNA methylation. Selenium has insulin-like effects both in vitro and in vivo. Selenium ameliorates the treatment of models involving heart, kidney and platelet defects and alleviates disease symptoms in the diabetes animal [7].

 

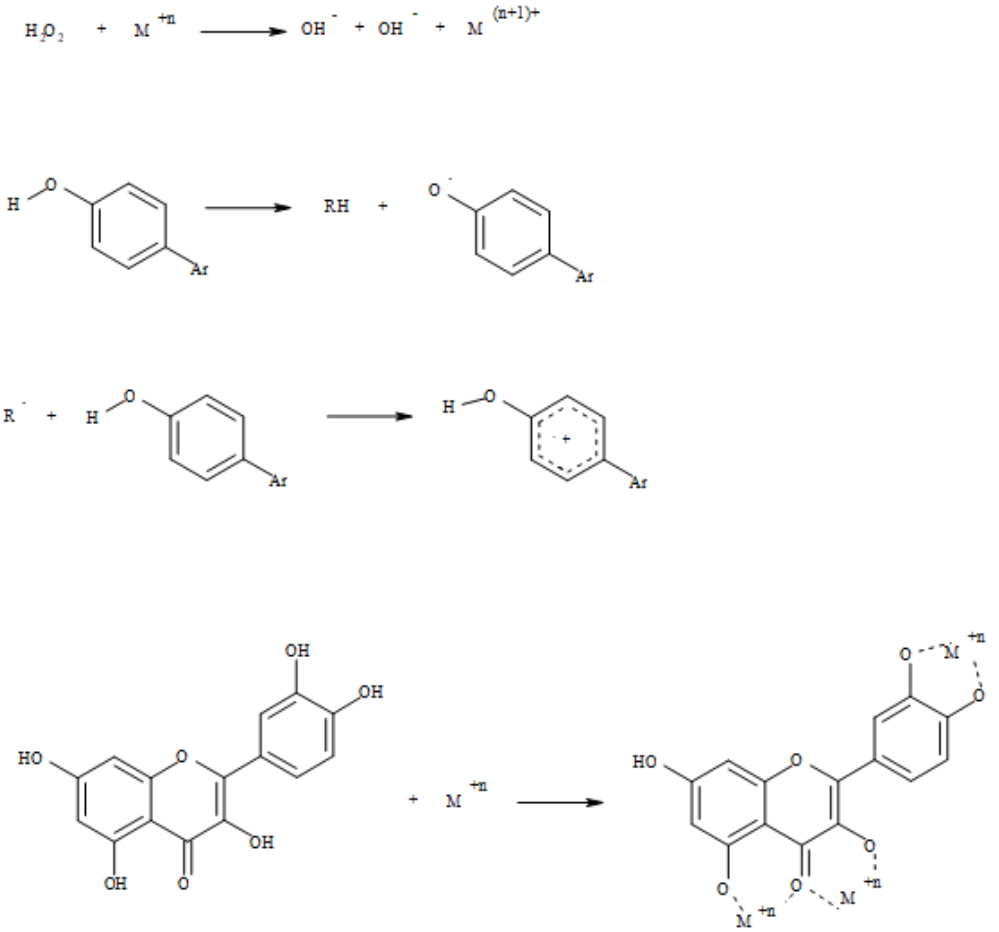

**Figure 1.** Antioxidant mechanism of polyphenols

### *1.3. Glutathione*

Free radicals formed during glycation; They damage the decline of antioxidant defense mechanisms, cell organelles and enzymes. Thus, oxidative-chronic stress causes the formation and development of diseases that reduce the quality/duration of life such as diabetes, atherosclerosis, Alzheimer's and cancer.

Glutathione extinguishes oxidation agents[8]. Oxidation agents play a role in the formation, development and acceleration of aging in many diseases. It preserves hemoglobin. S-H groups have reducing properties against oxidizing agents and prevent oxidation of hemoglobin. Glutathione; It is depleted as a result of excessive consumption of drugs, chemicals, and pesticides. It is irreversibly damaged. (Figure 3).

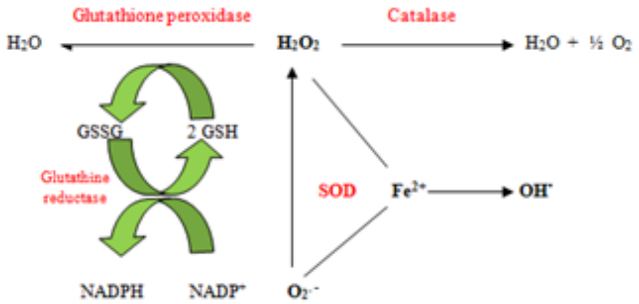


**Figure 2.** Free Oxygen Detoxification System

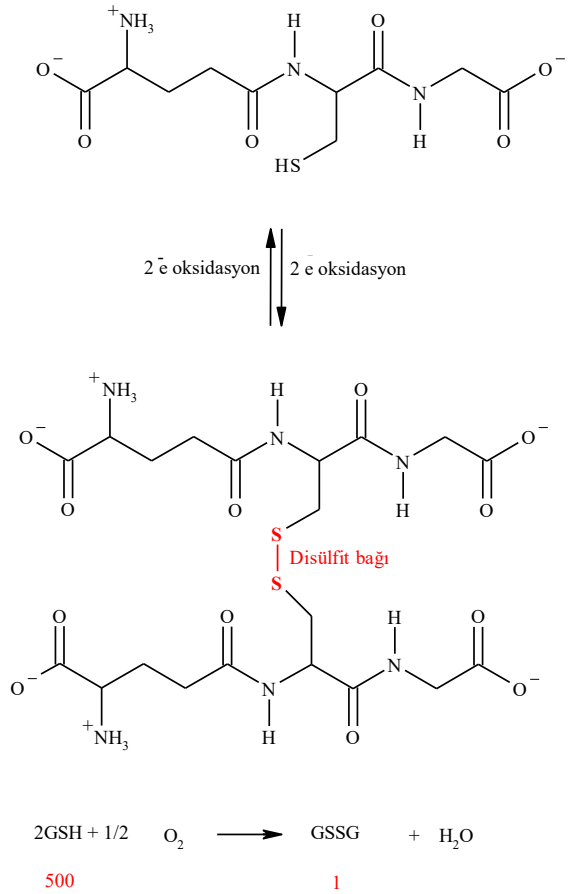


**Figure 3.** Oxidation and Reduction of Glutathione

### *1.4. Oleuropein*

It is obtained from olive leaves and olive oil. (Figure 4.). Various studies have shown that oleuropein contains a high antioxidant activity[9].

Oleuropein inhibits low-density lipoproteins[10]. It scavenges free radicals formed in in vitro oxidation and in vivo lipid peroxidation. Oleuropein has a protective neuroprotective effect on spinal cord injury and oxidative spinal cord [11].

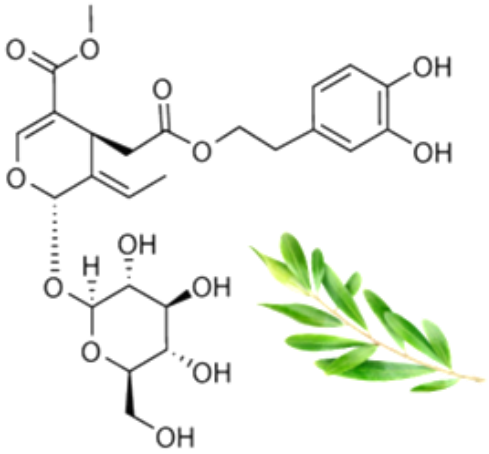

**Figure 4.** Oleuropein Chemical Structure

In the study, a mixture of oleuropein, selenium and glutathione substances with antioxidant properties known to inhibit reactive oxygen species (ROS) was prepared. The mixture was calculated according to the daily requirement of the body. The effect of the mix on scavenging DPPH and OH-radical was investigated.

## 2. MATERIALS AND METHODS

### *2.1. Chemicals Used*

Selenium, L-glutathione, oleuropein were bought from Sigma-Aldrich. Gallic acid, ethanol, 1,1-diphenyl-2-picryl-hydrazil(DPPH), butylated hydroxytolue(BHT), butylated hydroxyanisole(BHA), α-tocopherol, quercetin, folin&ciocalteu's phenol reagent, ascorbic acid sodium carbonate, iron-2-chloride, iron-3-chloride, ethylenediaminetetraacetic acid (EDTA), hydrogen peroxide, ferrozine, ferricyanide, trichloroacetic acid(TCA), $KH_2PO_4$, $K_2HPO_4$ deoxyribose, 2-thiobarbituric acid(TBA), sodium hydroxide.

### *2.2. Selenium, oleuropein and glutathione mixture used*

Selenium, oleuropein and glutathione were calculated according to the daily need of the body (Selenium 10 µg/kg, L- glutathione 15 µg/kg, Oleuropein 7mg/kg). A mixture of these substances was prepared. This mixture was used in all experimental studies.

### *2.3. Biochemical analysis*

#### *2.3.1. DPPH Radical Scavenging Activity*

Antioxidants donate their hydrogen to the radical in order to scavenge the DPPH radical. The DPPH radical is free and stable. They become stable by gaining electrons or hydrogen. DPPH radical scavenging activity is more widely used as it provides the opportunity to compare the antioxidant activity in a short time compared to other methods[12]. The basic principle of this method is based on the reduction of absorbance.

Unpaired electrons on DPPH give maximum absorbance at 517 nm in the visible region. The reaction between the antioxidant molecule and DPPH causes a decrease in the concentration of DPPH in the environment, thus reducing the absorbance. The resulting structure is non-radical DPPH-H.

During the reaction, the color of the mixture changes from purple to yellow.

1 mg/ml stock solutions of oleuropein, gluthatione and selenium mix used in the study were prepared. Dilutions were made from stock solutions at concentrations of 10-100 µg/ml. A solution of 0.1 mM DPPH in 96% ethanol was prepared. 3 ml of mix extract was taken and 1 ml of 0.1 mM DPPH solution was added to them. After mixing the tubes thoroughly by vortexing, they were incubated for 30 minutes at room temperature in the dark. Then, absorbance values were measured in a UV device at 517 nm. BHA and were used as positive control. The negative control is the mix or the test sample without the positive control. The % inhibition values were calculated using the formula below.

$$\% I = [\ (A_{Control} - Sample)\ /\ A_{Control}\ ] \times 100 \qquad (1)$$

#### *2.3.2. OH Radical Scavenging Activity(Deoxyribose Assay)*

The hydroxy radical scavenging activity of the mix used in the study was investigated in the $Fe^{2+}$/ascorbate/EDTA/$H_2O_2$ system by the deoxyribose method. Malondialdehyde (MDA) is formed after the hydroxy radical attacks deoxyribose. The resulting MDA reacts with TBA (2-thiobarbituric acid) to form a pink colored MDA-TBA complex[13].

Respectively; 100µl 1mM EDTA, 10 µl 0.1 mM $FeCl_3$, 100µl 50 mM $H_2O_2$, 360 µl 2.8 mM deoxyribose, 1 mg/ml stock solutions of oleuropein, glutathione and selenium mix(10-100µg/ml), 330 µl 50 mM pH 7.4 phosphate buffer and 100 µl of 0.3 mM ascorbic acid was used. The mixture was incubated for 1 hour in the dark and in a water bath at 37 $^oC$. After incubation, 1 ml was taken from the mixtures, respectively; 1 ml of 10% TCA and 1 ml of 0.5% TBA (containing 0.025% BHA in 0.025M NaOH) were added. It was boiled in a 100 $^oC$ water bath until the pinkish color turned yellow (approximately 5 minutes). As soon as the color transformation was observed, it was cooled on ice. The absorbance value was measured in UV spectroscopy at 532 nm[14-15].

There is no mix in the negative control and no positive control. The % inhibition values were calculated using the formula below.

$$\% I = [\ (A_{Control} - Sample)\ /\ A_{Control}\ ] \times 100 \qquad (2)$$

### *2.4. Statistical analysis*

Data are presented as mean±SD. Statistical significance of the data was determined by Student's test, and a value of $p < 0.05$ was considered as statistically significant.

## 3. EXPERIMENTAL RESULTS AND DISCUSSION

### *3.1. Biochemical analysis*

#### *3.1.1. DPPH Radical Scavenging Activity*

Antioxidant substances scavenge the DPPH radical by giving their own hydrogen to the radical. DPPH is a free and stable radical. The effect of scavenging DPPH radical is determined in a short time compared to other antioxidant methods.

Therefore it is used more widely. While determining the DPPH radical scavenging activity 5 concentrations of mix was studied in the range of 10-100 µg/ml.

% Inhibitions [ ($A_{Control}$ – Sample) / $A_{Control}$ ] x 100 was calculated with this formula

Mix showed scavenging activity between 28.08% and 91.41%.

BHA showed scavenging activity between 93.50% and 96.30% (Figure 5.).

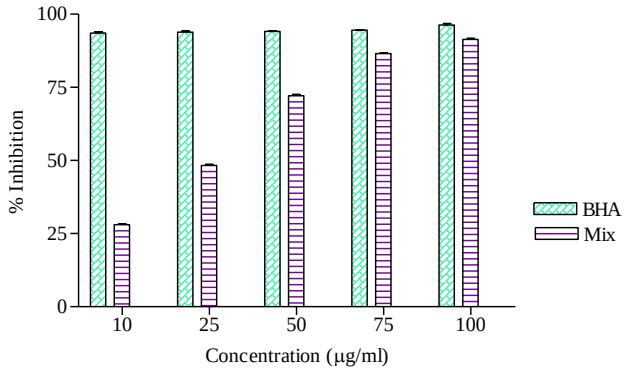


**Figure 5.** Scavenging effect of different concentrations(10, 25, 50, 75, 100 µg/ml) of *mix* on the DPPH radical. Each value is given as the average of 3 test results and ± standard deviations (SD) (n=3)

### *3.1.2. OH Radical Scavenging Activity(Deoxyribose Assay)*

The OH radical scavenging activity of the mix was studied at 5 concentrations in the range of 10-100µg/ml.

% Inhibitions [ ($A_{Control}$ – Sample) / $A_{Control}$ ] x 100 was calculated with this formula

BHA was used as positive control. Mix showed hydroxy radical scavenging activity in the range of 39.68% and 99.67%. BHA showed hydroxy radical scavenging activity in the range of 38.21% and 97.39%(Figure 6.).

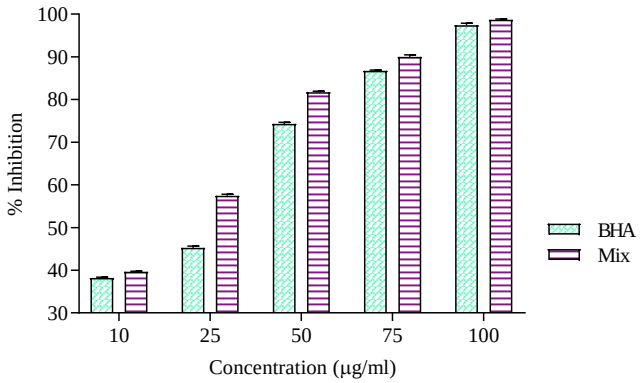


**Figure 6.** The hydroxy radical scavenging activity (%) of mix of different concentrations (10, 25, 50, 75, 100 µg/ml) Each value is averaged over 3 test results and given ± standard deviations (SD) (n=3)

## 4. CONCLUSION.

Antioxidant defense elements produced by the body can be damaged due to unnecessary drug use, chemical exposure and bad eating habits. In order for our body's defense elements to work, we need an antioxidant system. As a support element for our damaged antioxidant system, we need to take antioxidant supplements from the diet. It was determined that the mixture of oleuropein, glutathione and selenium used in the study inhibited free radicals. It was determined that it competed with the positive control at increasing concentrations. The mixture is intended to be used as a daily support supplement.

The prepared mixture was used as a protective agent in immunoglobulin G protein and lymphocyte damage in the follow-up study. Quite efficient results have been obtained.

## ACKNOWLEDGEMENT

This study was carried out as a thesis in the YOK 100/2000 Priority Thematic Area Biomaterials and Tissue Engineering doctoral program. Dicle University Scientific Research Project Coordinatorship was supported by the Ph.D. project numbered Science-22.004.

## AUTHOR CONTRIBUTIONS

Author A: Conceived and designed the analysis. Collected data and performed the analysis. Performed statistical analysis and wrote the paper.

Author B: She was the PhD thesis advisor and provided the execution of the project.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

[1] Pietta PG. Flavonoids as antioxidants. Journal of natural products, 63(2000) 1035–1042. https://doi.org/10.1021/np9904509

[2] Tokusoglu, O. (2016). Effect of high hydrostatic pressure processing strategies on retention of antioxidant phenolic bioactives in foods and beverages–a review. Polish Journal of Food and Nutrition Sciences, 66(4).

[3] Leopoldini, M., Marino, T., Russo, N., & Toscano, M. (2004). Antioxidant properties of phenolic compounds: H-atom versus electron transfer mechanism. The Journal of Physical Chemistry A, 108(22), 4916-4922. 2

[4] Bonnefont-Rousselot D, Thérond P, Beaudeux JL, Peynet J, Legrand A, Delattre J. High density lipoproteins (HDL) and the oxidative hypothesis of atherosclerosis. Clin Chem Lab Med. 37(1999) 939-948 doi:10.1515/CCLM.1999.139

[5] Pillai, S. S., Sugathan, J. K., & Indira, M. (2012). Selenium downregulates RAGE and NFκB expression in diabetic rats. Biological trace element research, 149, 71-77.

[6] Hwang, J. Y., Zhang, J., Kang, M. J., Lee, S. K., Kim, H. A., Kim, J. J., & Kim, J. I. (2007). Hypoglycemic and hypolipidemic effects of Saururus chinensis Baill in streptozotocin-induced diabetic rats. Nutrition Research and Practice, 1(2), 100-104.

[7] Ayaz, M., Ozdemir, S., Ugur, M., Vassort, G., & Turan, B. (2004). Effects of selenium on altered mechanical and electrical cardiac activities of diabetic rat. Archives of Biochemistry and Biophysics, 426(1), 83-90.

[8] Liu, H., Wang, H., Shenvi, S., Hagen, T. M., & LIU, R. M. (2004). Glutathione metabolism during aging and in Alzheimer disease. Annals of the New York Academy of Sciences, 1019(1), 346-349

[9] Hassen, I., Casabianca, H., & Hosni, K. (2015). Biological activities of the natural antioxidant oleuropein: Exceeding the expectation–A mini-review. Journal of Functional Foods, 18, 926-940.

[10] Ahamad, J., Toufeeq, I., Khan, M. A., Ameen, M. S. M., Anwer, E. T., Uthirapathy, S., ... & Ahmad, J. (2019). Oleuropein: A natural antioxidant molecule in the treatment of metabolic syndrome. Phytotherapy Research, 33(12), 3112-3128.

[11] Khalatbary, A. R., & Ahmadvand, H. (2012). Neuroprotective effect of oleuropein following spinal cord injury in rats. Neurological research, 34(1), 44-51.

[12] Gülçin I, Küfrevioglu OI, Oktay M, Büyükokuroglu ME, Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (Urtica dioica L.). Journal of ethnopharmacology, 90(2004) 205–215. https://doi.org/10.1016/j.jep.2003.09.028

[13] Wang L, Yen JH, Liang HL and Wu MJ. "Antioxidant effect of methanol extracts from lotus plumule and blossom (Nelumbo nucifera

Gertn.)," Journal of Food and Drug Analysis 11 (2003) https://doi.org/10.38212/2224-6614.2726.

**[14]** Shimada K, Fujikawa K, Yahara K, Nakamura T, Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of agricultural and food chemistry, 40(1992) 945-948.

**[15]** Mazumder A, Gerlt JA, Absalon MJ., Stubbe J, Cunningham RP, Withka J, Bolton PH, Stereochemical studies of the. Beta.-elimination reactions at aldehydic abasic sites in DNA: endonuclease III from Escherichia Coli, sodium hydroxide, and Lys-Trp-Lys. Biochemistry, 30(1991) 1119-1126.

## BIOGRAPHIES

**Feryal Zeliha Akay** obtained her BSc degree in Chemistry from Dicle University in 2015. She received the MSc. diploma in Biochemistry from the Dicle University in 2018 and make PhD in two programs; Biochemistry, Biomaterials and Tissue Engineering of the same university. She is active in teaching and research in the AGEs(Advanced glycation and product, biological macromolekules damage and cell culture system.

**Göksel Kızıl, Prof. Dr.** obtained her BSc degree in Chemistry from Dicle University in 1990. She received the PhD diploma in Biochemistry from the University of Nottingham in 1998 She is active in teaching and research in the immune system cells, vaccine and biological macromolekules damage and cell culture system