ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Araştırma Makalesi *Research Article* DOI: 10.5281/zenodo.10712659

Oxidative stress in chronic diseases: An Overview of Orange Peel Extracts

Kronik Hastalıklarda Oksidatif Stres: Portakal Kabuğu Ekstrelerine Bakış

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ABSTRACT

Aim: Oxidative stress is an important factor involved in the pathogenesis of various chronic diseases, from cardiovascular disorders and neurodegenerative diseases to metabolic syndrome and cancer. Antioxidants derived from natural sources have gained considerable attention due to their potential to combat oxidative stress and prevent disease progression. Orange peel, in particular, have emerged as promising candidates for their rich content of bioactive compounds with potent antioxidant properties. In this study, we aimed to determine the antioxidant capacity of ethanol and methanol extracts of orange peel.
Methods: Ethanol and methanol extracts from orange peel were obtained. The Folin-Ciocalteu Reagent (FCR) was used to determine the total phenolic component levels in the extracts of orange peel. By using the DPPH (1,1-diphenyl-2-picrylhydrazil), FRAP (Iron ion reducing antioxidant power), and CUPRAC (Cu2+ ions reducing) techniques, antioxidant activities were assessed. To calculate the extract's equivalent antioxidant capacity, different reference sample concentrations ranging from 250 to 1000 g/mL were made.
Results: The greatest concentration of the phenolic component in the methanol and ethanol-extracted orange peel extracts was 1000 µL/mL. The maximum values for the extracts' FRAP, CUPRAC (Trolox Eq g/mL), and DPPH radical scavenging capacity (inhibition%) were determined at a concentration of 1000 µL/mL. Ethanol extract showed higher antioxidant capacity compared to methanol extract.
Conclusion: Orange peel extracts demonstrate considerable promise as natural therapeutic agents for alleviating oxidative stress and its associated burden on chronic diseases. Our findings could improve the way orange peels are used in the food, cosmetics, and pharmaceutical industries. However, further research is warranted to elucidate the precise mechanisms of action, optimal extraction method, optimal dosages, and potential side effects of the extracts.
Key words: Antioxidant, chronic diseases, ethanol, extract, methanol, orange peel.

ÖZ

Amaç: Oksidatif stres, kardiyovasküler bozukluklar ve nörodejeneratif hastalıklardan metabolik sendrom ve kansere kadar çeşitli kronik hastalıkların patogenezinde yer alan önemli bir faktördür. Doğal kaynaklardan elde edilen antioksidanlar, oksidatif stresle mücadele etme ve hastalığın ilerlemesini önleme potansiyelleri nedeniyle büyük ilgi görmektedir. Özellikle portakal kabuğu güçlü antioksidan özelliklere sahip zengin biyoaktif bileşik içerikleri sayesinde umut verici adaylar olarak ortaya çıkmaktadır. Bu çalışmada portakal kabuğunun etanol ve metanol ekstraktlarının antioksidan kapasitelerini belirlemeyi amaçladık.
Yöntem: Portakal kabuğunun etanol ve metanol ekstreleri elde edildi. Ekstrelerin toplam fenolik bileşen seviyelerini belirlemek için Folin-Ciocalteu Reaktifi (FCR) kullanıldı. DPPH (1,1-difenil-2-pikrilhidrazil), FRAP (Demir iyonu indirgeyici antioksidan gücü) ve CUPRAC (Cu^{2+} iyonları indirgeyen) teknikleri kullanılarak antioksidan aktiviteleri değerlendirildi. Ekstraktın eşdeğer antioksidan kapasitesini hesaplamak için 250 ile 1000 g/mL arasında değişen farklı referans numune konsantrasyonları hazırlandı.
Bulgular: Portakal kabuğunun etanol ve metanol ekstrelerindeki fenolik bileşenin en yüksek konsantrasyonu 1000 µL/mL olarak bulundu. Ekstrelerin FRAP, CUPRAC (Trolox Eq g/mL) ve DPPH radikal süpürme kapasitesi (% inhibisyon) için maksimum değerler 1000 µL/mL'lik konsantrasyonda görüldü. Etanol ekstraktı, metanol ekstraktına kıyasla daha yüksek antioksidan kapasite gösterdi.
Sonuç: Portakal kabuğu ekstreleri, oksidatif stresi ve bununla ilişkili kronik hastalıkların etkisini hafifletmek için doğal terapötik maddeler olarak umut vaat etmektedir. Bununla birlikte, ekstrelerin kesin etki mekanizmalarını, optimal ekstraksiyon yöntemini, optimal dozajları ve potansiyel yan etkileri aydınlatmak için daha fazla araştırmaya ihtiyaç vardır.

Anahtar Kelimeler: Antioksidan, kronik hastalıklar, ekstrakt, etanol, portakal kabuğu, metanol.

Publication Date
Geliş Tarihi/Received 23.01.2024
Kabul Tarihi/Accepted 26.02.2024
Yayın Tarihi/Publication 28.02.2024

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Cite this article: Duysak, L., & Kılıç, AF. (2024). Oxidative Stress in Chronic Diseases: An Overview of Orange Peel Extracts. *Current Research in Health Sciences*, 1(1): 25-33.

Introduction

Oxidative stress is a physiological condition that arises when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize and eliminate them. ROS are highly reactive molecules that can cause damage to cells and tissues if their levels become excessive. This oxidative damage has been implicated in the development and progression of various chronic diseases, including cardiovascular diseases, neurodegenerative disorders, cancer, diabetes, and inflammatory conditions (Liguori et al., 2018).

Citrus fruits are among the fruits that attract great attention in Türkiye both in terms of production and consumption. Citrus fruits are a group of fruits belonging to the Rutaceae family that originated in tropical and subtropical areas in southeast Asia (Sawalha, Arráez-Román, Segura-Carretero, & Fernández-Gutiérrez, 2009). There are numerous natural and hybrid products available, such as oranges, grapefruits, lemons and some tangerines (Güzel & Akpınar, 2017). Except for the edible part of citrus fruits, their waste constitutes one-fourth of the whole fruit weight. Orange peel waste contains a large amount of moisture (80-90% w/w water content) and has a large organic load (Rezzadori, Benedetti, & Amante, 2012). Essential oil obtained from citrus fruits has excellent antimicrobial properties and is used in the cosmetic industry (Caccioni, Guizzardi, Biondi, Renda, & Ruberto, 1998; De la Torre et al., 2019). In addition, the waste part of citrus fruits, mostly consisting of peels and seeds, is used in the treatment of various diseases (such as diabetes, high blood pressure) among the people (Ahmad, Ansari, Alam, & Khan, 2013; G. Oboh & A. Ademosun, 2012).

Orange (Citrus sinensis L.) peels, like other citrus fruit peels, contain a rich array of bioactive compounds, including flavonoids, phenolic acids, carotenoids, and vitamin C, among others. These compounds possess potent antioxidant properties and have been studied for their potential health benefits (X.-M. Chen, Tait, & Kitts, 2017).

Several studies have investigated the potential of orange peel extracts in combating oxidative stress and its associated chronic diseases (Anagnostopoulou, Kefalas, Papageorgiou, Assimopoulou, & Boskou, 2006; X.-M. Chen et al., 2017; Hegazy & Ibrahium, 2012; Shehata et al., 2021). These extracts have shown promising antioxidant activity, helping to scavenge ROS and protect cells from oxidative damage. By reducing oxidative stress, orange peel extracts may contribute to the prevention and management of various chronic conditions (Z. T. Chen, Chu, Chyau, Chu, & Duh, 2012).

Cardiovascular diseases: Oxidative stress plays a critical role in the development of cardiovascular diseases, such as atherosclerosis and hypertension. Orange peel extracts have demonstrated antioxidant effects that may help protect against oxidative damage in blood vessels, reduce inflammation, and improve cardiovascular health (Khosravi, Poursaleh, Ghasempour, Farhad, & Najafi, 2019).

Neurodegenerative disorders: Oxidative stress is closely linked to the pathogenesis of neurodegenerative disorders, including Alzheimer's and Parkinson's diseases (Teleanu et al., 2022). Orange peel extracts have been shown to possess neuroprotective properties, which may help in reducing oxidative damage and preserving brain health (Abd El-Aziz et al., 2022).

Cancer: Chronic oxidative stress can promote DNA damage, leading to the development of cancer (Jelic, Mandic, Maricic, & Srdjenovic, 2021). Orange peel extracts contain bioactive compounds that exhibit anti-cancer properties, including antioxidant and anti-inflammatory effects. They may help in reducing oxidative stress and inhibiting the growth of cancer cells (Iannazzo et al., 2022; Tajaldini, Samadi, Khosravi, Ghasemnejad, & Asadi, 2020).

Diabetes: Oxidative stress plays a significant role in the complications associated with diabetes (Darenskaya, Kolesnikova, & Kolesnikov, 2021). Orange peel extracts have been studied for their potential to alleviate oxidative stress in diabetes and its related complications by reducing lipid peroxidation, enhancing antioxidant defenses, and improving insulin sensitivity (Gosslau, Zachariah, Li, & Ho, 2018; Zhang et al., 2022).

Inflammatory conditions: Oxidative stress and inflammation are closely interconnected (Hussain et al., 2016). Orange peel extracts have shown anti-inflammatory effects by modulating various inflammatory pathways and reducing oxidative stress markers, thus potentially benefiting individuals with chronic inflammatory conditions (Gosslau, Chen, Ho, & Li, 2014).

Our aim in this study is to determine the total phenolic content and in vitro antioxidant properties of the waste-generating peel part of the orange, which is cultivated in Türkiye and used as an important raw material in the food and beverage industry, in different extracts, and to determine their therapeutic potential.

Methods

Materials

Orange fruits were collected from Finike district of Antalya province in September 2022. Trolox was purchased from Fluka Chemica (Switzerland) and NH4Ac from Riedel De Haen (Germany). Neocuproine (Nc), TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) and DPPH (1,1-diphenyl-2-picrylhydrazil) from Sigma Chemicals Co. (St. Louis, USA) provided. FeCl3.6H2O was purchased from Merck (Germany). Millipore (Direct-Q® 3UV, USA) was used to obtain ultrapure water.

Preparation of Plant Extracts

The orange peels were dried at room temperature and then powdered with liquid nitrogen with the help of a pestle. To prepare ethanol and methanol extract, 50 g of dried orange peel powder was extracted in 250 mL solvent (methanol or ethanol) by filtration every 24 hours for a total of 72 hours using a horizontal shaking water bath at 50 oC. The filtrates were collected and the solvents were removed with the aid of an evaporator. The extract was protected from light and stored in an airtight bottle in the refrigerator (2-8 °C) for further studies.

Determination of Total Phenolic Compounds

The amounts of total phenolic compounds of orange peel ethanol and methanol extracts were determined by using the modified version of the method developed by Slinkard and Singleton (Slinkard & Singleton, 1977).

First, 50 mL of 7.5% Na_2CO_3 was prepared. Then, after weighing 25 mg of gallic acid for the standard, it was completed with methanol to 25 mL in a test tube. Finally, Folin & Ciocalteu reagent was taken into beaker for phenolic compound determination. Stock solutions were prepared and necessary dilutions were made. First, 40 µL of sample and 200 µL of Folin & Ciocalteu reagent were added to the plates and incubated for 5 minutes. Finally, 160 µL of Na_2CO_3 was added and incubated again for 30 minutes. After incubation, absorbance was measured at 765 nm. Using the standard graph prepared using gallic acid, the results were given as mg gallic acid equivalent (GAE)/g.

Determination of Antioxidant Capacity

DPPH Radical Scavenging Capacity Assay

The DPPH radical scavenging capacities of ethanol and methanol extracts obtained from orange peel were determined according to the Brand Williams method (Brand-Williams, Cuvelier, & Berset, 1995). Inhibitory response of samples to DPPH radical is measured spectrophotometrically to determine antioxidant capacity. The DPPH solution loses its color during the reduction reaction in the presence of an antioxidant, and the decrease in color intensity makes it easier to measure in the spectrophotometer. After preparing DPPH solution, 210 µL of extract sample was pipetted into the plate wells, and then 70 µL of DPPH solution was added to each well. The plate was mixed with a stirrer for 1 minute and incubated for 30 minutes in the dark. Trolox was used as the standard antioxidant for the control sample. Then absorbance was measured at 517 nm and the results were calculated as percent inhibition.

The Ferric Reducing Antioxidant Power (FRAP) Assay

The method of determination of antioxidant capacity of extracts obtained from orange peel based on electron transfer was applied by Huang et al (Huang, Ou, & Prior, 2005). First, 300 mmol/L acetate buffer (pH=3.6) was prepared. 10 mM TPTZ was taken into a 100 mL flask, 40 mM HCl was added and the final volume was made up to 100 mL. Finally, 20 mmol/L FeCl3 solution was prepared. A total of 30 mL of FRAP solution was obtained by taking 2.5 mL of TPTZ, 2.5 mL of $FeCl_3$ and 25 mL of acetate buffer from these prepared solutions. 10 µL of the extract sample and 200 µL of FRAP solution were pipetted into the plate wells and allowed to incubate for 30 minutes, and then the absorbance was measured at 593 nm.

Cupric İons (Cu^{2+}) Reducing-CUPRAC Assay

This method used by Apak et al. is based on the conversion of Cu(II) Neocuproin complex to Cu(I) Neocuproin by means of antioxidant compounds in the environment and the absorbance of this complex at 450 nm wavelength (Apak, Guclu, Ozyurek, & Karademir, 2004). To prepare the CUPRAC reagent, 0.4262 g $CuCl_2 \bullet 2H_2O$ was weighed and dissolved in 250 mL of distilled water (10 mM). To prepare the acetate buffer, 19.27 g of NH_4Ac was dissolved in 250 mL of water. 7.5 mM neocuproin solution was obtained by preparing 0.039 g Neocuproin compound with 96% pure ethanol in a 25 mL flask. Afterwards, solutions consisting of 60 µL $CuCl_2$, 60 µL acetate buffer, 60 µL neocuproin solution and 66 µL extracts were mixed and after 30 minutes of incubation, absorbances were measured at 450 nm wavelength. The standard antioxidant Trolox was used as a control sample. Calibration curves of the working range of 1-100 µg/mL, where the plot of absorbance versus concentration is linear, were derived.

Results

Findings of Total Phenolic Compound Quantification

Total phenolic compound amounts of ethanol and methanol extracts prepared from orange peel were determined by Folin-Ciocalteu Reagent (FCR). Gallic acid was used as the standard

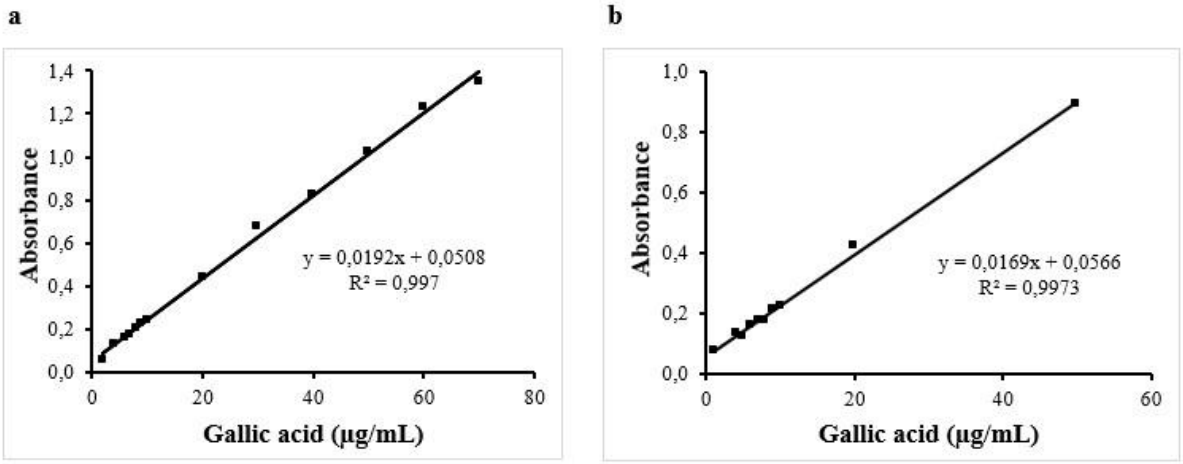

Figure 1. Calibration curves of gallic acid in different solvents (a. Ethanol b. Methanol)

phenolic compound and was calculated as gallic acid equivalent from the equations obtained from the calibration curves of gallic acid (Figure 1). Triplicate analyzes were performed and then mean and standard deviation values were given.

The total amount of phenolic compounds in the samples calculated according to the regression equations of the curves was determined as GAE/g for ethanol and methanol extracts (Table 1). According to the results of the research, it was determined that the highest total amount of phenolic substance was found in the ethanol extract at a concentration of 1000 (µg/mL) and the results changed slightly depending on the solvent difference.

Table 1. Total phenolic compound amounts of orange peel extracts

Total Phenolic Compound (µg GAE/mg extract)		
Concentration (µg/mL)	**Ethanol extract**	**Methanol extract**
250	3,45 ± 0,01	3,33 ± 0,009
500	6,91±0,03	6,55 ±0,011
1000	10,64± 0,23	8,89 ± 0,26

Antioxidant Capacity Findings

Findings from DPPH Radical Scavenging Studies

DPPH radical scavenging activities of standard antioxidant compounds of ethanol and methanol extracts prepared from orange peel were determined according to the Brand Williams method (Brand-Williams et al., 1995). The analyzed concentration range (1-100 µg/mL) was determined as a result of studies on standard antioxidant compounds. Triplicate analyzes were performed and then mean and standard deviation values were given. The DPPH radical scavenging activity of trolox as a standard antioxidant reached its highest value at a concentration of 30 ug/mL for ethanol and 90 ug/mL for methanol (Figure 2).

Table 2. Comparison of DPPH free radical scavenging capacities of extracts at different concentrations

% Inhibition (Trolox (Eq µg/mL))		
Concentration (µg/mL)	**Ethanol extract**	**Methanol extract**
250	5,00 ± 0,01	12,07 ± 1,95
500	7,39 ± 0,71	21,02 ± 1,35
1000	17,25 ± 0,011	48,96± 1,74

The DPPH radical scavenging capacities of ethanol and methanol extracts prepared from orange peel and standard antioxidant compounds at 250, 500 and 1000 µg/mL concentrations are shown as % inhibition (Table 2). It was determined that the extract with the highest DPPH free radical scavenging capacity among the ethanol and methanol extracts prepared from orange peel was the methanol extract and it was at a concentration of 1000 µg/mL.

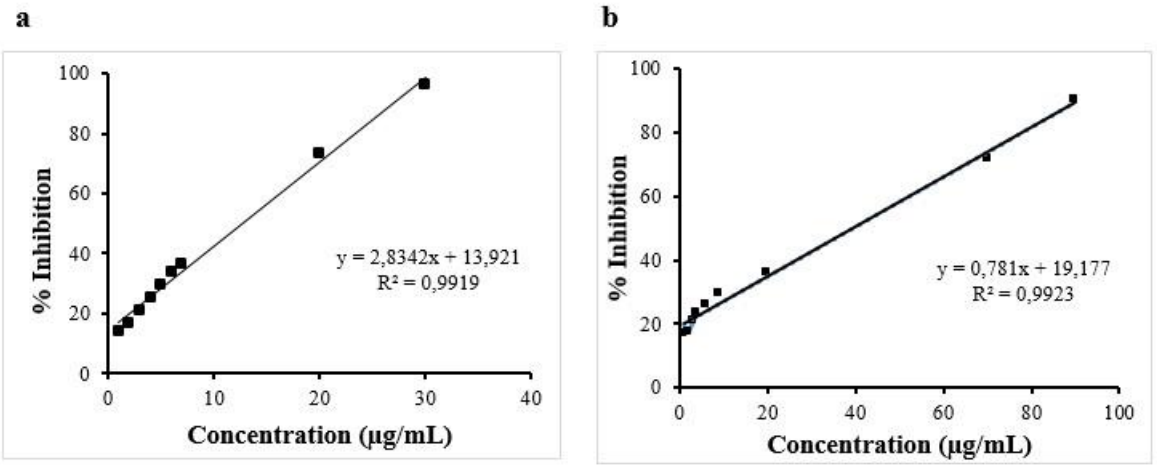

Figure 2. Concentration-% Inhibition graph of Trolox (a.Ethanol b. Methanol)

Findings of İron İon Reducing Antioxidant Power (FRAP)

The absorbance values corresponding to the iron (III) reducing/antioxidant power at 593 nm of ethanol and methanol extracts prepared from orange peel and standard antioxidant compounds were measured spectrophotometrically. The analyzed concentration range (1-100 µg/mL) was determined as a result of studies on standard antioxidant compounds. Triplicate analyzes were performed and then mean and standard deviation values were given. As a standard antioxidant, trolox, iron (III) reducing/antioxidant potency activity reached the highest value at 100 µg/mL concentration. In line with these data, the concentration range of the extracts to be studied was determined as 1-100 µg/mL (Figure 3).

Table 3. Comparison of iron (III) reducing/antioxidant power of extracts at different concentrations in µg TEAC

Trolox (Eq µg/mL)		
Concentration (µg/mL)	**Ethanol extract**	**Methanol extract**
250	7,42 ± 0,14	8,04 ± 0,04
500	23,81 ± 0,32	14,41 ± 0,1
1000	28,65 ± 0,18	31,764 ± 0,1

The iron (III) reducing/antioxidant powers of ethanol and methanol extracts prepared from orange peel, standard antioxidant compounds and standard antioxidant compounds at 250, 500 and 1000 µg/mL concentrations were compared in terms of µg Trolox equivalent Antioxidant Capacity (TEAC) (Table 3). It was determined that the ethanol and methanol extracts prepared from the orange peel had the highest iron ion reducing antioxidant power capacity, and it was determined that the methanol extract had a concentration of 1000 µg/mL.

Findings of The Copper İon Reducing Antioxidant Capacity Determination Method (CUPRAC)

The conversion of ethanol and methanol extracts prepared from orange peel and standard antioxidant compounds of Cu(II) neocuproin complex at 450 nm to Cu(I) neocuproin by means of compounds with antioxidant effect in the medium was done by measuring the absorbance at 450 nm. Triplicate analyzes were performed and then mean and standard deviation values were given. The concentration range to be analyzed (1-100 µg/mL) was determined as a result of studies on standard antioxidant compounds (Figure 4).

Table 4. Comparison of the conversion of the extracts from Cu (II) neocuproin complex to Cu (I) neocuproin at different concentrations in terms of µg TEAC

Trolox (Eq µg/mL)		
Concentration (µg/mL)	**Ethanol extract**	**Methanol extract**
250	30,14 ± 0,11	12,17 ± 0,1
500	47,77 ± 0,97	22,02 ± 0,14
1000	86,85 ± 0,05	40,3 ± 0,07

The ethanol and methanol extracts prepared from orange peel and standard antioxidant compounds at 250, 500 and 1000 µg/mL concentrations are converted to Cu(I) neocuproin by

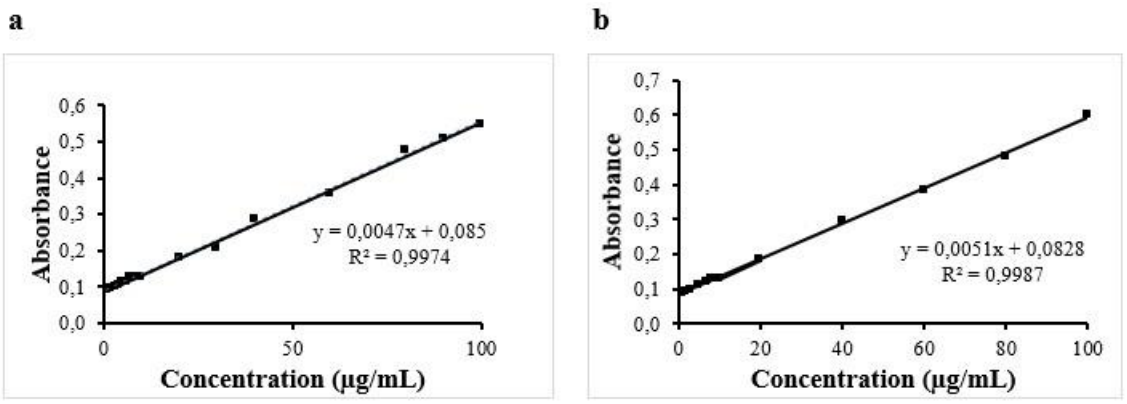

Figure 3. Trolox standard graph (a. Ethanol b. Methanol)

spectrophotometric direction at 450 nm and Cu(II) neocuproin complex by means of compounds with antioxidant effect in the environment and this complex is µg Trolox equivalent. Comparison in terms of Antioxidant Capacity (TEAC) is shown in Table 4. It was determined that the extract with the highest copper ion reducing antioxidant capacity in ethanol and methanol extracts prepared from orange peel was the ethanol extract and it had a concentration of 1000 µg/mL.

Discussion

In this study, when all data of ethanol and methanol extracts prepared from orange peel were evaluated in terms of antioxidant activity, it was determined that ethanol extract was rich in copper ion reducing antioxidant capacity (CUPRAC) and total phenolic compounds. Methanol extract had high antioxidant activity in terms of DPPH radical scavenging activity and iron ion reducing antioxidant power (FRAP). We think that this feature is due to the compounds contained in the orange peel. It is thought that the various antioxidant activity differences observed in the extracts are due to the level of polyphenolic compounds transferred to the solvent used and the difference in their chemical structures. For this reason, the data to be obtained from in vitro studies on natural antioxidants of plant origin, which are preferred instead of synthetic antioxidants, will form the basis of in vivo studies.

Orange peels are often discarded as waste, but recent research has highlighted their potential as a valuable source of bioactive compounds with antioxidant properties. Studies have shown that the total phenolic substance, mineral substance and vitamin content of the peels is higher than the fruit and fruit juice in some citrus species (Belitz, Grosch, Belitz, & Grosch, 1999) and in orange (Gorinstein et al., 2001). Such wastes are a good source for extracting bioactive molecules such as carotenoid pigments, pectins and terpenes (essential oils). Compared to other fruits, most of the complex carotenoids are found in citrus fruits (Chedea, Kefalas, & Socaciu, 2010). Many of these bioactive substances are found in the peel rather than the inside of the orange. However, these beneficial compounds are usually destroyed by the production of citrus juices (Sawalha et al., 2009).

Orange peels are rich in various bioactive compounds such as flavonoids (e.g., hesperidin, naringin), carotenoids (e.g., beta-carotene, lutein), phenolic acids (e.g., ferulic acid, gallic acid), and ascorbic acid (vitamin C) (Mahato, Sinha, Sharma, Koteswararao, & Cho, 2019; Panwar, Saini, Panesar, & Chopra, 2021). These compounds possess strong antioxidant properties and contribute to the overall antioxidant capacity of orange peel extracts.

The extraction of the plant ingredient depends critically on the solvent. The antioxidant effects of the extracts of orange peel obtained with different organic solvents are variable. It has been shown that ethanol and methanol, which are very polar among these solvents, are effective for antioxidant activity compared to hexane, petroleum ether, and acetone. Further ethanol extract showed more antioxidant capacity compared to methanol extract (Hegazy & Ibrahium, 2012). Again, polar solvents are convenient for extracting phenolic compounds from citrus peels compared to organic solvents (Shehata et al., 2021). Therefore, we chosed ethanol and methanol as solvents in this study and compared the efficacy of the two solvents in antioxidant activity.

Another factor for the yield of extraction and antioxidant capacity is the extraction method. A study investigating the impact of four extraction techniques used at 35°C (conventional solvent extraction, supercritical CO_2 extraction, microwave assisted extraction, and ultrasound assisted extraction) on the total phenol, total flavonoid, individual flavonoid, vitamin C, and

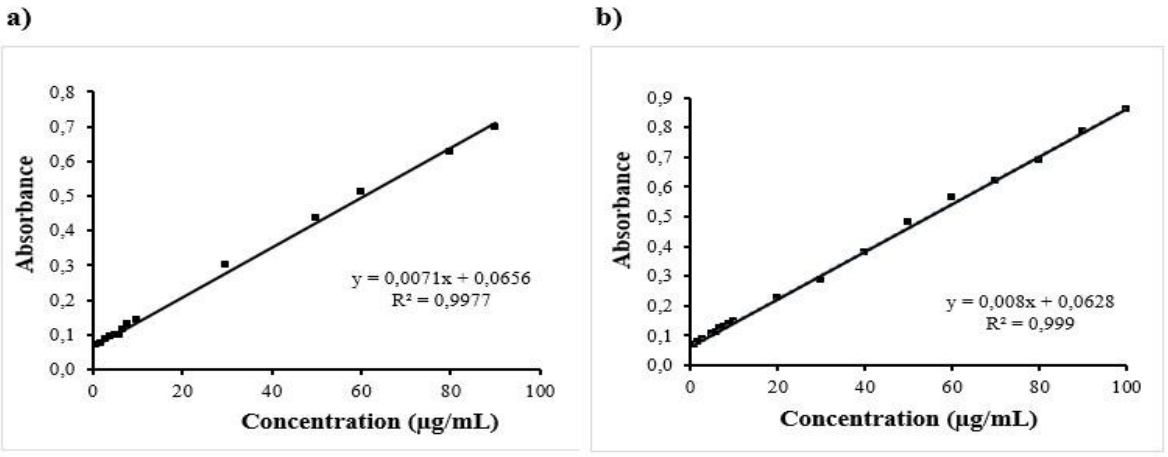

Figure 4. Trolox standard graph (a.Ethanol b.Methanol)

antioxidant activity of orange peel has demonstrated the most effective method as conventional solvent extraction (Irina, Cédric, Ghoul, & Boudhrioua, 2017). In this study we used the conventional solvent extraction method which is expected to bring high antioxidant activity.

The drying method of the plant is another determinant of phenolic content and thus antioxidant activity. Literature findings have shown that hot-air oven drying at 50 °C and 70 °C results in higher antioxidant activity compared to shade drying and microwave drying (Lai et al., 2022). In this study we used shade drying. We suppose that further studies with different drying methods, including hot-ait oven drying, shall be conducted to determine the optimum method for gaining maximum antioxidant activity from orange peel extracts.

Total phenolic content of plants is related with reducing activity, thus antioxidant activity (Hegazy & Ibrahium, 2012). UPLC-ESI-MS/MS analysis of ethanolic extract of orange peels has revealed more than 40 polyphenolic compounds, including phenolic acids and flavonoids (Shehata et al., 2021). C-glycosylated flavones, O-glycosylated flavones, O-glycosylated flavanones, flavonols, and phenolic acids and their derivatives are the main families of flavonoids identified by HPLC (Anagnostopoulou et al., 2006). We determined the phenolic content of ethanolic extract 10,6 µg GAE/mg extract and methanolic extract 8,8 µg GAE/mg extract, at 1000 µg/mL concentratin. In a study conducted in Malaysia with various Citrus sinensis extracts, total phenolic contents of different C. sinensis peel extracts ranged from 12.08 to 38.24 mg GAE/g, with 70% acetone/water extract (AEC) displaying the greatest total phenolic content (Liew, Ho, Yeap, & Sharifudin, 2018). Our results of phenolic content is lower than the aforementioned study. We think this difference may arise from mainly extraction solvent and geographical diversity.

We determined that the ethanolic extract had higher phenolic content and therefore higher antioxidant activity demostrated by the results of CUPRAC, FRAP and DPPH.

Numerous studies have shown that orange peel extracts exhibit significant radical scavenging activity against various free radicals, including superoxide anion, hydroxyl radical, and lipid peroxides (Gorinstein et al., 2001; G. Oboh & A. O. Ademosun, 2012). The radical scavenging capacity of orange peel extracts can be attributed to the presence of flavonoids and other phenolic compounds, which effectively neutralize free radicals and prevent cellular damage.

Oxidative stress occurs when there is an imbalance between free radicals and the body's antioxidant defense mechanisms. Chronic oxidative stress has been linked to several diseases, including cardiovascular disorders, neurodegenerative conditions, and cancer (Liguori et al., 2018). Orange peel extracts' antioxidant activity has been shown to mitigate oxidative stress, thereby potentially reducing the risk of such diseases. An *in-vitro* study has demonstrated water extract of orange and its bioactive components prevented the cytotoxic effect in t-BHP-induced HepG2 cells (Z. T. Chen et al., 2012). Researchers have concluded that a positive regulation of GSH levels and antioxidant enzymes may contribute to the protective effect of orange water extract and its bioactive compounds on t-BHP-induced HepG2 cells (Z. T. Chen et al., 2012). Aforementioned study revealed that orange peel extract and its bioactive components may play a role in the improvement of chronic diseases by antioxidant mechanism.

In addition to their antioxidant properties, orange peel extracts have anti-inflammatory effects. Further, it has been demonstrated that in comparison to equal flavonoid

combinations, orange peel extract has stronger anti-inflammatory properties (X.-M. Chen et al., 2017). Chronic inflammation is closely associated with oxidative stress, and by reducing inflammation, orange peel extracts further contribute to their overall health benefits.

Studies have suggested that orange peel extracts may act synergistically with other antioxidants, enhancing their overall efficacy (Babbar, Oberoi, Uppal, & Patil, 2011). This property makes orange peel extracts valuable in formulating antioxidant-rich supplements and functional foods. As it is known, oxidative stress increases after meals. The risk of cardiovascular disease increases, especially due to increased lipemia after meals. In a study, it was shown that mixtures containing orange peel extracts reduced the risk of developing post-meal cardiovascular complications, and this effect was primarily attributed to the antioxidant properties of orange peel extracts (Papagianni et al., 2021). In addition, considering that oxygen radicals are involved in the pathophysiology of many chronic diseases such as chronic obstructive pulmonary disease (COPD), hypertension, diabetes and malignancies, it can be thought that antioxidant compounds obtained from orange peel extracts may play a role in the prevention and treatment of these diseases. Considering the literature and our findings, it can be thought that the use of antioxidant compounds containing orange peel extracts may be more beneficial, especially before tissue damage caused by oxygen radicals.

Conclusion

Ethanol extract of orange peels may be preferred instead of methanol extract to gain higher antioxidant activity. While the research on orange peel extracts and their effects on oxidative stress in chronic diseases is promising, it's important to note that most studies have been conducted in laboratory settings or animal models. Further research, including well-designed clinical trials, is needed to better understand the potential benefits and determine optimal dosages for human consumption.

Etik Komite Onayı: In vitro çalışma olduğu için etik kurul onayına ve hasta onamına gerek yoktur.
Hakem Değerlendirmesi: Dış bağımsız.
Yazar Katkıları: Fikir- LD., AFK.; Tasarım- LD.; Denetleme- LD.; Kaynaklar- LD., AFK.; Veri Toplanması ve/veya İşlemesi- LD., AFK.; Analiz ve/ veya Yorum- LD.; Literatür Taraması- LD., AFK.; Yazıyı Yazan- LD., AFK.; Eleştirel İnceleme- LD., AFK.
Çıkar Çatışması: Yazarlar, çıkar çatışması olmadığını beyan etmiştir.
Finansal Destek: Yazarlar, bu çalışma için finansal destek almadığını beyan etmiştir.

Ethics Committee Approval: Since it is an in vitro study, ethics committee approval and informed consent is not required.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept- LD., AFK.; Design- LD., AFK.; Supervision- LD.; Resources- LD., AFK.; Data Collection and/or Processing- LD., AFK.; Analysis and/or Interpretation- LD.; Literature Search- LD., AFK.; Writing Manuscript- LD., AFK.; Critical Review- LD., AFK.
Conflict of Interest: The authors have no conflicts of interest to declare.
Financial Disclosure: The authors declared that this study has received no financial support.

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