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Investigating the antioxidant properties of olive leaf extracts extracted by the subcritical water

Shohreh Mallakian
Department of Food Science and Technology, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran
Email: sh.mallakian@gmail.com

Abstract
In recent years, extensive research has been conducted on the properties of various plant extracts and methods of extracting these extracts simultaneously. In this research, in addition to achieving the antioxidant properties of plant extracts, optimal methods for extraction have been used to replace the previous methods. According to the results of a study, one of the rich sources of bioactive compounds that have been extracted in various ways so far, is olive leaf extract, which due to its phenolic compounds, has significant antioxidant and antimicrobial effects. In this study, the antioxidant properties of phenolic compounds extracted from olive leaves were investigated using the method of extraction of subcritical water. The extraction process was carried out at temperatures of 120 to 180$^{\circ C}$, under pressure corresponding to the extraction temperature and the mixing ratio of 1:20, and physicochemical properties (efficiency, turbidity and brix), antioxidant properties (total phenolic compounds by Folin-Ciocalteu method and amount). Free radical inhibition by DPPH method was investigated in the extract. The results showed that the highest amount of phenolic compounds was obtained from the extract of the extracurricular liquid extraction method at a temperature of 180$^{\circ C}$, 8.08 mg per 100 g of dry matter in terms of gallic acid. The free radical inhibition power increased from 120 to 180$^{\circ C}$, and the results showed that radical receptor activity was also higher in the extracted extract at 180$^{\circ C}$. Overall, the results of the mean comparison showed that the efficacy, brix, phenolic compounds, and free radical inhibitory power of extractive extracts by the subcritical water method were higher than the extracts obtained by the extract maceration (P >0.05).

Keywords: Antioxidants, Olive leaves, Subcritical water, Phenolic compounds

Introduction

A free radical is an atom or molecule with an unpaired electron, anionic, cationic, or non-ionic, that can exist independently. Free radicals are very active and short-lived in terms of unstable energy, so by removing or pairing the electrons around them, a radical can stabilize. The separation of electrons from various substrates leads to the creation of new free radicals in the environment. Free radicals may be involved in some diseases and tissue damage of the lungs, heart-cardiovascular system, kidneys, liver, eyes, skin, muscles, brain, and the aging process of the cell. Oxidizing agents and radicals are known to be mediators of these disorders, but are generally neutralized by antioxidants in healthy individuals. However, with age and in people with certain diseases, internal antioxidants need external help to maintain the health of cell membranes, which is provided by food antioxidants (Prior and Cao, 2002). Antioxidants are compounds that significantly delay or prevent substrate oxidation. Food manufacturers use authorized antioxidants, which are mainly phenolic in nature, to prevent product degradation and protect their nutritional value. On the other hand, the safety of

chemical preservatives has been questioned, and many microbial strains have become resistant to antibiotics. Therefore, the use of plant extracts as a natural preservative has received a lot of attention (Korukluoglu et al., 2008).

In recent years, with the increase in awareness of the benefits of consuming compounds with antioxidant properties and the desire of producers and consumers to natural products, a lot of research has been done to find resources rich in natural antioxidants. Many of the antioxidant and antimicrobial properties of plant extracts are due to the presence of substances such as phenol and flavonoids and similar compounds (Mohamed et al., 2007). The term natural antioxidants refers to compounds that are naturally present in animal or plant tissues or that are formed during cooking or processing of food and can be extracted. Natural antioxidants are found in almost all plants, microorganisms, fungi, and even animal tissues. The main group of natural antioxidants are phenolic compounds, the most important of which are tocopherols, flavonoids and phenolic acids (Prior and Cao, 2002).

One of the products rich in phenolic compounds is olive leaf extract. So far, many studies have been done on different parts of the olive plant, including its roots, stems, leaves, fruits, and kernels. Olive leaves are known as agricultural by-products and waste of olive processing plants, the collection and processing of which is in the interest of the scientific and industrial community around the world, due to the promotion of health. Research on olive leaves has shown that the health benefits of this product are due to a group of secondary metabolites, biofenols. Olive leaves are one of the medicinal plants that are known for their antioxidant properties. Among the various olive-growing areas, olive leaves are the richest source of phenolic compounds, and oleuropein is the most abundant phenolic compound in leaves. Therefore, these leaves are useful and inexpensive sources of oleuropein (Lujan et al., 2006; Mohamed et al., 2007). Now that olive leaves have always been readily available as a evergreen leaf all year round and are a cheap raw material rich in phenolic compounds, the extract can be extracted using one of the extraction methods.

Extraction is the first major step in the research and application of various medicinal plants (Jalali et al., 2014). In the past, solvent-based plant extraction techniques were the most common extraction methods. This method was often costly, time consuming, with high solvent consumption with low extraction coefficient and adverse and harmful effects on the extracted compounds and products. For this reason, in recent years, the use of various methods to accelerate and facilitate the extraction of phenolic compounds has received much attention (Mohamadi et al., 2012).

One of the new methods of extraction, known as green technology, is extraction by subcritical water (SCW), Which is very effective in extracting the active ingredients of plants. In this method, with increasing temperature and pressure, the physicochemical properties of water change and water becomes a fluid with a wide range of extraction of compounds with different polarities (Mohamadi et al., 2012). The purpose of this study was to extract phenolic compounds of olive leaves under the conditions of extraction of subcritical water (temperature 120 to 180°C , pressure 10 to 50 bar and mixing ratio 1:20), and measure the physicochemical properties, the amount of phenolic compounds and the antioxidant power of the extracts obtained from the extracts.

Materials and Methods

Olive leaves (yellow variety) were harvested from Minoodasht (Country: Iran) olive groves as the main raw material for extracting the extract. After being transferred to the laboratory in a dry shade, it was passed through a powder mill (IKA, Model: A11 basic) and sieve with mesh 40.

Extracted by maceration

To do this, the sample was first placed in a fixed laboratory ratio of 1:20 (one unit of olive leaf powder against 20 units of distilled water) in a suitable laboratory container, and under constant conditions (on a mixer at a speed of 1000 rpm, at room temperature, away from light for 18 hours) extraction was performed. Then the suspension extracted by ordinary filter paper was smoothed. The filtered extract was poured into the bottom of 15 cm plates and dried in a vacuum oven (HEARUS) at 45$^{\circ C}$ until it reached a constant weight. The dried extract was scraped off the floor of the plates and kept in the refrigerator in McCarthy glass doors, which were protected by aluminum foil against light penetration (Mohamadi et al., 2012).

Extraction of subcritical water method

In this study, water was used to extract the extract from the subcritical water device. The prepared sample was placed in a 1: 20 constant mixing ratio (7 gr olive leaf powder against 140 ml of distilled water) in the tank of the device. The required volume of solvent (water) was poured into the balance tank according to the mixing ratio and the heater of the device was turned on to provide the required temperature. At the same time, the high pressure pump was applied and adjusted to the required pressure. The extraction process took place at a temperature range of 120 to 180$^{\circ C}$. After extraction, the prepared extract was passed through a filter paper. The filtered extract was poured into the bottom of 15 cm plates and dried in a vacuum oven at 45$^{\circ C}$ until it reached a constant weight. Finally, the dried extract was scraped off the floor of the plates and stored in the McCarthy glass doors, which were protected by aluminum foil against light penetration, until refrigerated (Mohamadi et al., 2012).

Measurement of physicochemical properties of extract

In the evaluation of the efficiency value, the amount of 10 ml of the extract was poured into a plate with fixed weight. It was then placed at a temperature of 45$^{\circ C}$. After re-weighing the plates, the extraction efficiency was measured with an accuracy of 0.01 gr using the following formula.

$$\text{Efficiency} = \frac{m_2 - m_0}{m_1 - m_0} \times 100 \qquad (1)$$

m1: The weight of the plate with the extract before the oven
m2: The weight of the plate with the fence after the oven
m0: Empty plate weight

The soluble solids in the extracts extracted by the digital refractometer (ATAGO, Model: Rx-5000α) were measured and declared as the total solids (brix).

Measurement of phenolic compounds by Folin-Ciocalteu method

To measure the phenolic composition of the extracts, the method (Zhuang et al., 2010) was used with slight changes. To plot the calibration curve using gallic acid, concentrations of 10, 50, 100, and 150 ppm of gallic acid in distilled water were first prepared. Then, 0.5 ml of each concentration was transferred to the test tubes, and 2.5 ml of the Folin-Ciocalteu reagent diluted ten times in distilled water was transferred to the tubes, and finally, 2 ml of 7.5% sodium carbonate was added and kept at room temperature for 30 minutes. At the end of the sample absorption, the 1 cm cells were read by a spectrophotometer (Shimadzu model UV-160 A, made in Japan) at a wavelength of 65 nm. For example, 0.5 ml of distilled water was used as a control. The test was performed with three repetitions for each point. Then, the total amount of phenolic compounds in the extracts was calculated using the standard graph drawn for gallic acid. A good concentration of the extract was obtained by using the extract powder in distilled water, so that its absorption after testing the folin, is within the standard range of gallic acid. Finally, the total amount of phenolic compounds in milligrams of gallic acid per 100 gr of dried olive leaf was calculated (Mohamadi et al., 2012).

Investigating the radical receipt power of extract

Radical potency of the extracted extracts was measured by a solution containing DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals (0.1 mM in methanol). For this purpose, 0.1 ml of each extracted extract (with a concentration of 100 ppm) was mixed with 3.9 ml of DPPH solution in test tubes with lids and screw foil, mixed and after 30 minutes, The final solution adsorption rate was 517 nm. For example, 0.1 ml of methanol was used. The results were converted to the percentage of radical scavenging ability (RSA%) using the following formula and compared (Erel, 2004).

$$RSA\% = \frac{Abs_{control} - Abs_{sample}}{Abs\ control} \times 100 \qquad (2)$$

Statistical analysis

Statistical analysis to extract the extracts used two methods of maceration and subcritical water, the subcritical water method had three different temperature levels. So a total of four experimental treatments were formed. In order to optimize the extraction process of olive leaf extracts by subcritical fluid method and to obtain extracts with the best conditions in terms of physicochemical properties and antioxidant power of Duncan multi-domain test using SPSS software at the level of probability 95 percent was used for statistical analysis.

Results and discussion

Investigating the optimal temperature conditions of extraction on the physicochemical properties of olive leaf extract

The results showed that the extract extracted by the subcritical water method at a temperature of 180°C has the highest efficiency of extraction compared to the extracted extracts at temperatures of 120 150°C and and the extract was from maceration, which had a significant difference in 95% probability (P <0.05) (Figure 1). According to Figure (1), it can be stated that with increasing temperature in the subcritical water extraction method, the efficiency of extraction increased and compared to the extraction method, maceration was more efficient.

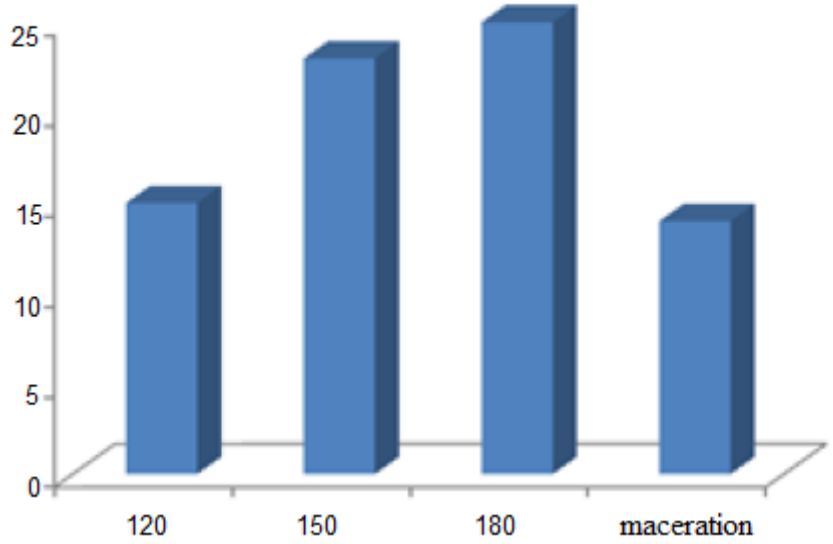

Figure 1. The effect of extraction method on the extraction efficiency of extracts

According to the results, the highest amount of turbidity and brix was related to the extract extracted by the infrared water method at 180$^{\circ C}$ and the lowest amount of turbidity and brix was related to the extract extracted by the maceration method (Table 1).

Table 1. The effect of extraction method on extract and brix extracts

Characteristics tested	Maceration	Subcritical water (T=120°C)	Subcritical Water (T=150°C)	subcritical water (T=180°C)
Turbidity	0.25±0.01	0.197±0.005	0.816±0.002	2.50±0.05
Brix	1.04±0.01	1.31±0.02	1.75±0.05	1.83±0.01

The study, conducted by Jalali et al. (2012) on date syrup, found that among the independent variables that included pH, temperature, water-to-date mixing ratio and diffusion time, it was found that in all conditions applied to extract syrup from dates, the ratio of water and date mixing had the greatest effect on the mentioned process. The effect of mixing ratio on linear extraction efficiency (simple effect) was linear, while adsorption was secondarily affected by water-date mixing ratio. So that with increasing the mixing efficiency ratio, the upward trend and the rate of syrup uptake have decreased and at the optimal point, it was 62.5% and 0.28%, respectively. Saim et al. (2008) investigated the optimization of water extraction by super-critical extraction method from coriander seeds and confirmed the effect of temperature range 65 to 150$^{\circ C}$, pressure 870 to 1000 psi and time 15 min.

Investigation of antioxidant properties of extracts

Investigate the amount of phenolic compounds in the extracts

To measure the total amount of phenolic compounds in the extracts, the Folin-Ciocalteu method was used. In this method, the total amount of phenolic compounds was calculated using the standard graph drawn for gallic acid (Figure 2).

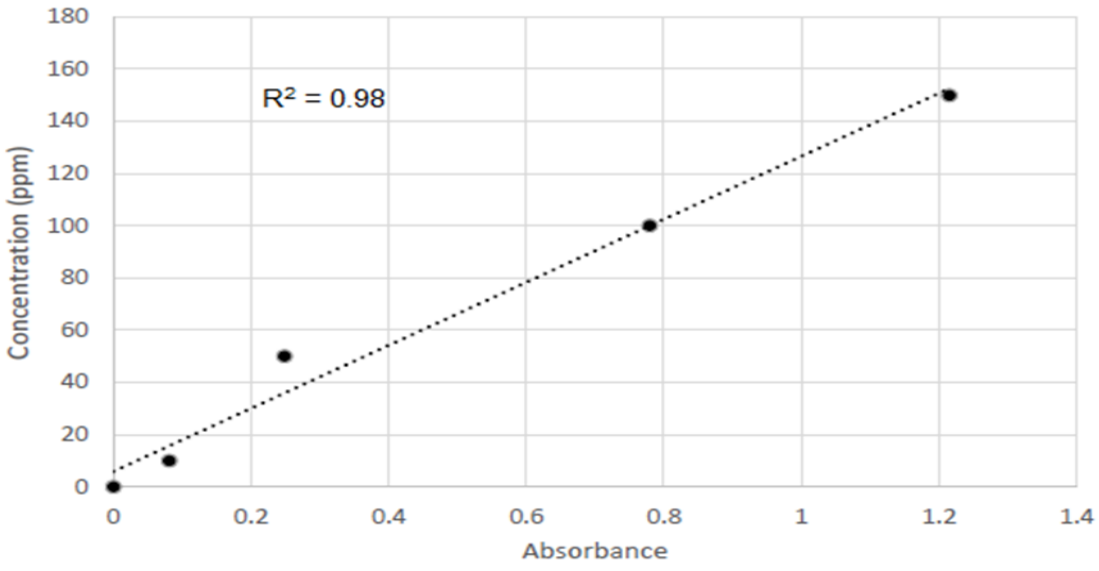

Figure 2. Standard graph of gallic acid

The results showed that increasing the temperature in the extraction method by subcritical water had a significant effect on the content of phenolic compounds in the extracted extracts (Figure 3). In other words, the extract extracted by the subcritical water method of water at 180°C had the highest phenolic composition compared to the extracts extracted at temperatures of 120 and 150°C and the extract obtained by maceration, which was the difference. Significantly, they had a 95% probability level (P <0.05) (Figure 3).

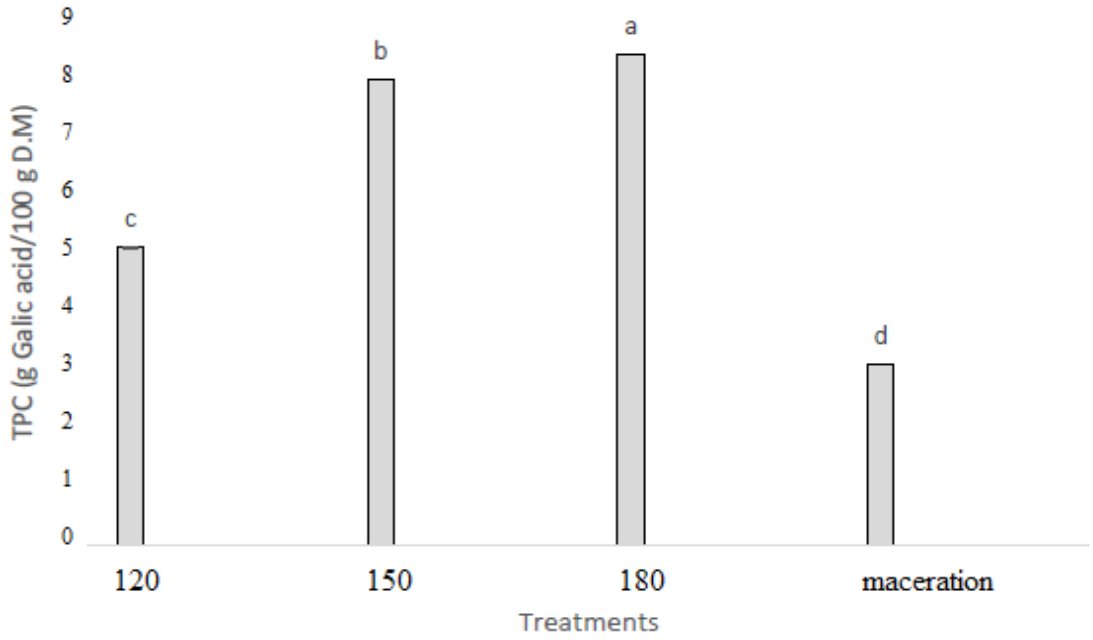

Figure 3. The effect of extraction method on the total amount of phenolic compounds

The important thing about extracting phenolic compounds using subcritical water is that extraction at high temperatures does not necessarily cause oxidation or thermal decomposition of bioactive compounds; Rather, by placing water in an subcritical state, by changing some of its physicochemical properties, water becomes a low-polarity fluid suitable for extracting non-polar or low-polar compounds, such as polyphenols.

Mohamadi et al. (2012) examined the amount of phenolic compounds extracted from seedless barberry fruit using subcritical water at temperatures of 120 to 180°C. The results of this study showed that the temperature was more effective at 150°C than at temperatures between 120 and 180°C. Also, the highest amount of phenolic compounds was reported in 2.5 g / 100 g of dry matter in terms of gallic acid at 150 °C. Comparing this amount with the maximum amount of phenolic compounds extracted in this study (8.08 g / 100 g of dry matter in terms of gallic acid), it can be stated that, the content of phenolic compounds extracted from olive leaves using the subcritical water method was higher than that of seedless barberry fruit. The results of another study by Rafiee et al. (2011) on extracts extracted from olive leaves by both immersion and microwave methods, showed that the highest amount of phenolic compounds from microwave extraction method is 0.244 g / 100 g of extract in terms of gallic acid at 40°C.

Investigating the percentage of free radical inhibition of extracts (DPPH)

In this study, the results showed that the radical reception power of extracts extracted by the subcritical water method at

temperatures of 120 and 180°C was significantly higher than the extracted extract at 150°C. It seems that the difference in the properties of the type of compounds extracted at the studied temperatures has reduced the radical power of the extract obtained at 150°C compared to 120 and 180°C ($P < 0.05$) (Figure 4). On the other hand, the extraction extracted by maceration method had the lowest level of radical receptivity compared to the extracts extracted with subcritical water at temperatures of 120, 150 and 180°C.

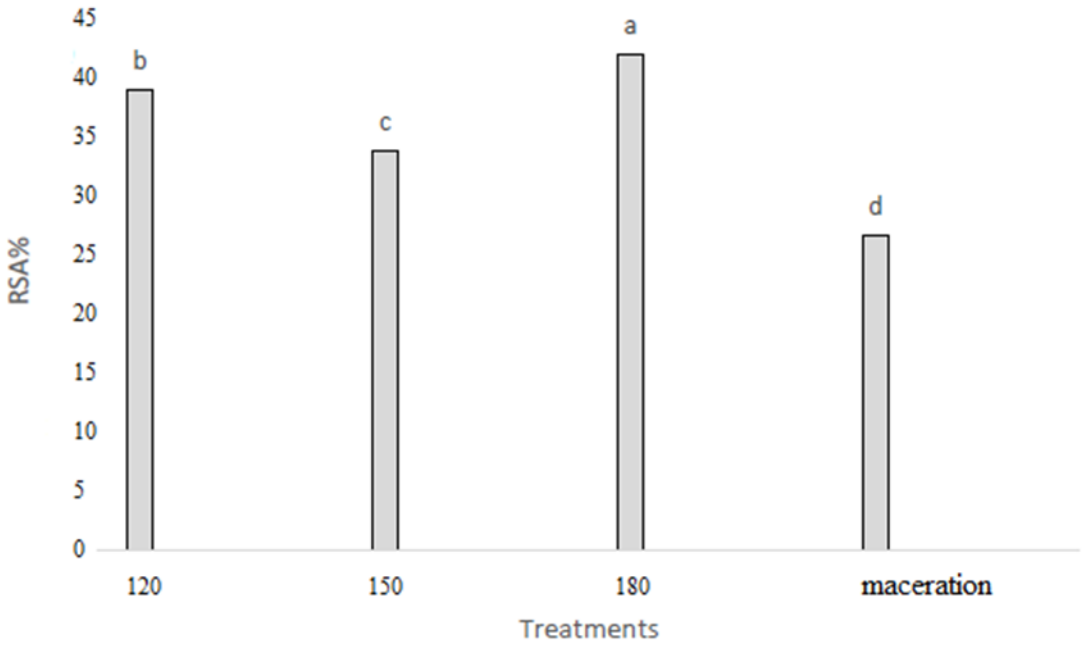

Figure 4. The effect of extracts extracted on free radical inhibition (DPPH)

An examination of the amount of phenolic compounds extracted from seedless barberry fruit using the subcritical water by Mohamadi et al. (2012) showed that, with increasing temperature, in addition to reducing the solubility of the solvent and thus increasing its strength to extract antimicrobial compounds, the ionization constant of the subcritical water increases steadily, eventually leading to an increase in the radical receptive strength of the extracts. The important point in extracting anti-oxidation extracts at high temperatures, which is used in the extraction method of water in the infrared, is that high temperatures will not necessarily reduce the anti-oxidant properties of the extracted compounds. However, the amount of phenolic compounds extracted at high temperatures may be reduced.

Conclusion

The efficiency and brix of the extract extracted by the subcritical water method at 180°C were increased compared to the extracts extracted by the subcritical water method at temperatures of 120 and 150°C and the extract obtained by the extraction method by maceration. Extraction from the extraction method by maceration had the lowest efficiency and brix. Regarding the amount of total phenolic compounds and the radical inhibitory power of DPPH, the extract extracted by the subcritical water method at 180°C compared to the extracts extracted by the subcritical water at temperatures of 120 and 150°C and extracts from the extraction method by maceration

had the highest total phenolic composition. The percent of radical inhibitory power of DPPH extracts extracted by the subcritical water method was higher than the extracted extract by the maceration method. The results showed that the radical inhibitory power of DPPH extracts extracted by the subcritical water method at temperatures of 120 and 180°C was significantly higher than the extract extracted by the subcritical water method at 150°C. It seems that the decrease in the percent of radical inhibitory power of DPPH extract extracted by the subcritical water method at a temperature of 150°C compared to the other two temperatures is due to differences in the properties of the type of compounds extracted at this temperature. Comparison of physicochemical and anti-oxidant results of extracts extracted by the two methods of subcritical water and maceration showed that increasing the temperature has a significant effect on these factors.

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