Effects of Different Solvent Extractions on the Total Phenolic Content and Antioxidant Activity of Lemon and Orange Peels

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Abstract

In recent years, the antioxidant activity of citrus peel and their roles in the prevention of various diseases have attracted more and more human attention. Citrus peels are suggested to be a good source of antioxidants. This study focused on the effects of different solvents (ethanol, methanol, and acetone) on the total phenolic contents and antioxidant activities of lemon and orange peels. The results revealed that both orange peels extract and lemon peels extract exhibited variable antioxidant activity and total phenolic content. The ethanolic extracts of lemon and orange peels showed the highest total phenolic content value (238.1 and 387.7 mg GAE/g, respectively), while acetone extracts had the lowest values. The highest antioxidant value was determined in the ethanolic extract of orange peels as 607.67 µmol trolox/g, whereas acetone extract of lemon peels showed the lowest (55.42 µmol trolox/g) value. According to results of study, it can be concluded that solvents have a big role on the total phenolic compounds and antioxidant activity of orange and lemon peels.

Keywords: ethanol, methanol, acetone, citrus peel, phenolic compounds, antioxidant activity

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INTRODUCTION

Waste is defined as “parts of those materials which we use to meet our needs that is not currently used or disposed of after use" (Anonim, 2011; Meneguzzo et al., 2019). The amount and type of waste is increasing day by day and evaluation of different agricultural wastes is gaining importance with supporting by many studies (Ucak, 2019; Yerlikaya et al., 2017; Ucak et al., 2019; Yerlikaya et al., 2015; Ucak et al., 2018; Ucak, 2020). Therefore, solid wastes have become one of the most important environmental problems nowadays. The utilization of waste is one of the important issues, as it consists only some parts of plant grown with intensive labor and high costs while the rest is thrown away (Alkaya et al., 2010; Singh et al., 2014). Although it does not have any important economic value, some of the agricultural industrial wastes can be used as animal feed or fertilizer, while fruit and vegetable wastes such as kernel, fruit and vegetable peel, root, plant peel and leaves are mostly discarded, which creates a serious waste problem in the food and agriculture sector (Ashoush and Gadallah, 2011; Otles et al., 2015; Filimonau et al., 2019).

On the other hand, about 25% to 30% fruits yield are non-edible products such as peels and seeds (Ajila, 2010). Generally these waste by-products include high amounts of antifungal, antibacterial and antivirus compounds that can be successfully applied as a source of phytochemicals and antioxidant agents (Ayala-Zavala, 2004; Bocco et al., 1998; Singh et al., 2002; Guclu et al. 2021; Yerou et al 2017). Orange peels have been found to contain high levels of fiber, calcium, vitamin C, vitamin B6, folate and other essential nutrients in some studies (Hoffman, 1971; Economos, 1992; Okwu, 2008). These peels are rich in phytochemicals like liminoids, synephrine, hesperidin flavonoid, polyphenols and pectin.

About 170 phytonutrients can be found in just one orange and they also contain over 60 flavonoids that prevent many sicknesses and diseases (Karima, 2016; Ibri et al., 2017). Peels contain limonene which has properties that can prevent cancer. They also contain essential oils that serve as immunity boosters due to their anti-inflammatory properties (Guignard, 2000; Ibri et al., 2017). Lemon peels are also rich in fiber, vitamin C which provides 9% of daily value, minerals such as calcium, potassium and magnesium. Further to this, lemon peel has crude fiber of 15.18%, crude fat of 4.98%, protein of 9.42%, and ash content of 6.26%. Lemon juice contains 5% acid at a pH of 2 to 3 giving its sour taste (Jana, 2021). In addition, citrus fruits are the mostly growing fruit all over the world comprising valuable beneficial phytochemicals (Hou et al., 2019; Satari and Karimi, 2018; Rafiq et al., 2017). In this study, the effects of different solvents (ethanol, methanol and acetone) on the total phenolic compounds and antioxidant activity of lemon and orange peels were investigated.

MATERIALS AND METHODS

Lemon and orange peels extraction

Citrus sinensis (orange) and *Citrus limon* (lemon) fruits were purchased from a local market in Niğde, Turkey. Fruits were squeezed to extract the juice and peeled carefully using a sharp knife. Then the peels were washed by distilled water and dried at 45°C for 48 hours. Afterwards, lemon and orange peels were grounded into powder. 10 gram each of the lemon and orange peels powder was extracted in 100 mL of 70% acetone, 70% methanol, and 70% ethanol, respectively. The powder was mixed by an ultrasonic water bath for 30 min and then filtered. Ethanol, methanol and acetone were evaporated with a rotary evaporator at 50°C, 55°C and 45°C under vacuum respectively.

Analysis

Total phenolic content

The total phenolic content (TPC) was determined by using spectroscopic Folin-Ciocalteu colorimetric at 765 nm and showed as gallic acid equivalent (GAE) by using the method described by Spanos and Wrolstad (1990). The samples were put into Folin-Ciocalteu reagent and Na_2CO_3 solution and mixture was mixed by vortex and then kept in a dark site at room temperature for two hours. Total phenolic content value was determined by extrapolating calibration line which was remarked as gallic acid (mg) equivalents/mL sample (GAE/g sample). The standard curve was prepared by 160, 140, 120, 100, and 80 mg/mL solutions of gallic acid in ethanol, methanol and acetone: water with the ratio of 70v:30v.

Antioxidant activity

A 7 mM ABTS solution including 2.45 mM potassium persulfate was made and the radical solution (ABTS + •) was prepared in water by storing 12-16 hours at 24°C to exclude the influence of light. To determine the antioxidant activity of the orange and lemon peel extract as a 22 trolox response, a series of extract concentrations and trolox were prepared. 10 µl of sample was added on 1 mL ABTS + and absorbance was checked for 6 minutes that how much it decreased. The slope obtained from the graphs where the percent inhibition was drawed against the concentrations (Re et al., 1999). The standard curve was prepared by using 40, 60, 80, 100,120, 160, 170 and 180 mg/ml solutions of 1 mM trolox in ethanol, methanol, and acetone: water ratio (70v:30v).

Statistical analysis

All samples were repeated three times and analysis was carried out by using the SPSS software (Statistical Analysis System, Cary, NC, USA). Variance analysis (ANOVA) was used to evaluate the data and $P<0.05$ significance level of Duncan's test was based on comparison between the mean differences of parameters.

RESULTS AND DISCUSSION

Total phenolic content of lemon and orange peel extracts

Generally phenolic compounds are known as secondary metabolites in most vegetables and fruits and other things mostly represented as a source of polyphenols like phenolic acids, flavanol, flavanones, and flavones (Singh et al., 2020; M'hiri et al., 2015). Scientists are more concerned about these compounds because of their antioxidant capacity and the aggregation between their utilization and prevention of illness and diseases.

Table 1. Changes in total phenolic content (mg GAE/g) of orange and lemon peels extracted in different solvents

	Ethanol	**Methanol**	**Acetone**
Orange peel	387.7 ± 9.14^{Aa}	70.60 ± 0.29^{Ba}	33.42 ± 2.96^{Ca}
Lemon peel	238.1 ± 0.00^{Ab}	65.24 ± 0.29^{Bb}	20.62 ± 0.28^{Cb}

Different capital letters indicate a significant difference among solvents, and different lower-cases letters indicate a significant difference between groups (P<0.05).

Table 1 shows the changes in total phenolic content (TPC), after drying and analyzing the TPC of orange and lemon peel extracted with methanol, ethanol, and acetone at 70% concentrations. It was found to be 387.7±9.14 mg GAE/g, 70.60±0.29 mg GAE/g and 33.42±2.96 mg GAE/g, for orange peel in ethanol, methanol and acetone, respectively. The total phenolic content of lemon peel was 238.1±0.00 mg GAE/g, 65.24±0.29 mg GAE/g and 20.62±0.28 mg GAE/g, respectively ($P<0.05$). While the highest TPC value was found in the 70% ethanol group, the lowest value was observed in the 70% acetone group. Among the groups, the highest TPC value was found in the orange peel group while the lowest value was observed in the lemon peel group. Anagnostopoulou et al. (2006) notified that the TPC of orange peel extract ranged between 3.0 and 105 mg GAE/g dry extract.

While Hegazy et al. (2012) found the TPC of orange peel extract using ethanol to be 169.56 mg GAE/g, methanol 165.38 and acetone 145.79 mg GAE/g dry weight. The total phenolic content in the ethanol extract of orange peel was lower than that reported by Casquete et al. (2015) (222.76 mg GAE/100 g). However, our result was higher than that of Irkin et al. (2015) (11.08± 9.55 mg GAE/100 g). Yerlikaya et al. (2017) reported the total phenolic content of bitter orange as 8.31 g GAE/100 g. In another study, Yerlikaya et al. (2015) reported the lower total phenolic content in bitter orange and grapefruit albedo and flavedo fragments. To conclude, the TPC of citrus species depends on several factors such as the origin of species, extraction temperature and time, extraction solvent and extraction method.

Antioxidant activity of lemon and orange peel extracts

Effects of various solvents on the antioxidant activity of lemon and orange peels were presented in Table 2.

Table 2. Changes in antioxidant activity (µmol trolox/g) of orange and lemon peels extracted in different solvents

	Ethanol	**Methanol**	**Acetone**
Orange	*607.67±18.92*^{Aa}	*237.44 ±4.31*^{Ba}	224.39±4.37^{Ba}
Lemon	203.22±66.95^{Ab}	132.90±11.48^{ABb}	55.42± 4.37^{Bb}

Different capital letters indicate a significant difference among solvents, and different lower-cases letters indicate a significant difference between groups (P < 0.05).

In this study, it was found that orange peels have strong antioxidant activity than lemon peel. Moreover, the ethanolic extract of orange peel showed the highest value as 607.67 µmol trolox/g. This result agrees with that of Gorinstein et al. (2001) who reported that extracts comes from peel of orange have perfect and potent antioxidant activity. Acetone extract of orange peel showed the lowest antioxidant value (224.39 µmol trolox/g). The antioxidant value of lemon peel was reported highest (203.22 µmol trolox/g) in the ethanolic extract, whereas acetone extract showed the lowest value (55.42 µmol trolox/g). Jayaprakasha et al. (2006) confirmed that the rank order of the navel orange extract by acetone, methanol:water and methanol antioxidant activity result was showed acetone is better than methanol:water and methanol. Park et al. (2014) confirmed that acetone is the best extraction solvent of antioxidant compounds from orange fruit and orange peels. However, our result showed that ethanol is the best extraction solvent of antioxidant compounds from orange peel and lemon peels. It seemed that the antioxidant capacity of the orange peel extract correspond with the quantity of phenolic compounds present in each fraction, so the extracts of the orange peel and lemon peel might be a good source of antioxidants.

CONCLUSION

In the present study the effects of different solvent extraction (ethanol, methanol, and acetone) on the total phenolic content and antioxidant activity of of lemon and orange peels were determined. Orange peels showed higher total phenolic content and antioxidant activity than the lemon peels.

Additionally, the highest antioxidant activity and total phenolic content were determined in the ethanol, methanol and acetone solvents, respectively both in the orange and lemon peels. It can be concluded that the solvent plays a vital role in the extraction of the vegetable and fruit (juice, peel, seed, and pulp) constituents.

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