Influence of extraction solvents on the polyphenol contents, compositions, and antioxidant capacities of fig (Ficus carica L.) seeds

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Abstract: Fig seeds are considered to be significantly responsible for the bioactive compounds of fig. In this study, the effects of six different solvents (100% acetone, 100% methanol, 100% ethanol, 50% (v/v) aqueous acetone, 50% (v/v) aqueous methanol and 50% (v/v) aqueous ethanol) with changing polarities on the polyphenol contents and antioxidant capacities of fig seed extracts were investigated. Total polyphenol contents (TPCs), total flavonoid contents (TFCs), antioxidant capacities (DPPH and FRAP assays) and polyphenol compositions of the extracts were evaluated. The results indicated that fig seeds extracted by 50% (v/v) aqueous methanol exhibited the highest TPC (714 mg GAE/kg DM), TFC (312 mg (+)-CE/kg DM), DPPH (41.6%) and FRAP (8504 mg FeSO₄/kg DM) values. Also, same extract had the maximum values of chlorogenic acid (131.9 mg/kg DM), (-)-epicatechin (166.4 mg/kg DM) and rutin (50.7 mg/kg DM) (p<0.05). The extractability of syringic acid was determined to be highest with 50% aqueous methanol (8.03 mg/kg DM) and 50% aqueous ethanol (8.13 mg/kg DM) (p>0.05). The psoralen extractability was highest in 50% aqueous acetone (53.0 mg/kg DM) and 50% aqueous ethanol (54.0 mg/kg DM) (p>0.05). High correlations among TPCs, TFCs, antioxidant capacities and individual polyphenols of fig seed extracts were also observed.

Key words: Antioxidants, extraction solvents, Ficus carica L., fig seeds, polyphenols.

INTRODUCTION

Fresh and dried fruits of the fig tree (Ficus carica L., family Moraceae) are important parts for the consumers worldwide (Rtibi et al. 2018, Rodríguez-Solana et al. 2018). Fig fruits contain carbohydrates, amino acids, minerals, vitamins, low amounts of lipids, phytosterol, organic acids, and polyphenols (Nadeem & Zeb 2018). Besides being delicious, they have been consumed for centuries because of their antioxidant, anti-herpes simplex virus (HSV), hypoglycemic, anti-diabetic, anti-hyperlipidemic, hepato-protective and immune sensitive properties (Nadeem & Zeb 2018, Rodríguez-Solana et al. 2018). These health promoting properties are mainly attributed to the fact that fig fruits contain high amounts of polyphenols with antioxidant properties that can prevent oxidative stress-related diseases (Bahrin et al. 2018).

Fig fruit consists of a fleshy hollow receptacle with tiny pedicellate druplets called fig seeds (Mars 2003). Each fig fruit has small whitish seeds with numbers ranging from 30 to 1600 (Badgujar et al. 2014). In this complex matrix, fig seeds are thought to contribute significantly to the nutrient content and health-promoting effects of fruit. Therefore, it is very important to reveal the polyphenol contents and antioxidant capacities of fig seeds.
One of the most important factors affecting the extraction efficiency of polyphenols and their associated health benefits is the extraction solvent used (Ngo et al. 2017). Polar solvents are often utilized for recovering polyphenols from plant materials (Do et al. 2014). The use of organic solvents such as ethanol, methanol, acetone and diethyl ether or their aqueous mixtures is generally preferred for this purpose (Wijekoon et al. 2011, Do et al. 2014). Aqueous acetone has been generally determined to be good for the extraction of higher molecular weight flavanols, whereas methanol is found more effective for the extraction of lower molecular weight polyphenols (Do et al. 2014). Ethanol, which is known to be safe for human consumption, is thought to be a good solvent for polyphenol extraction (Do et al. 2014). The nature of the bioactive compounds is appeared to vary depending on the plant material. Therefore, in general, it is very difficult to suggest a suitable extraction solvent for every plant material (Wijekoon et al. 2011). According to previous studies, the most suitable extraction solvent that can be used to determine the polyphenols and antioxidant capacities of various plant materials has been found as 50% (v/v) aqueous acetone for the root of Salacia chinensis L. (Ngo et al. 2017), 60% (v/v) aqueous acetone for brewer’s spent grains (Meneses et al. 2013), 100% acetone for leaves extracts of bilberries (Ceylan et al. 2017), 60% (v/v) aqueous ethanol for cinnamon (Dvorackova et al. 2015), 100% ethanol for Davidson’s plum (Chuen et al. 2016), 50% (v/v) aqueous methanol for garlic husk (Kallel et al. 2014), 80% aqueous methanol for Amomum chinense C. leaves (Butsat & Siriamornpun 2016), 90% (v/v) aqueous methanol for common sunflower (Ye et al. 2015). To the best of our knowledge, a study to determine the polyphenol content, their composition and antioxidant properties of fig seeds is not available in the literature. Furthermore, there is still no information on the effect of solvents with different polarities on extracting polyphenols from fig seeds related to their antioxidant activities.

Therefore, the study aimed to determine the contents and compositions of polyphenols and their antioxidant capacities of seeds, and to evaluate the effects of different extraction solvents (100% acetone, 100% methanol, 100% ethanol, 50% (v/v) aqueous acetone, 50% (v/v) aqueous methanol and 50% (v/v) aqueous ethanol) on total polyphenol, total flavonoid and individual polyphenol contents and antioxidant capacities (as DPPH radical scavenging activity and FRAP assay) of the fig seeds.

MATERIALS AND METHODS

Materials

Samples

Fresh fruits of the Sarilop variety were purchased from local market in Izmir (Turkey) at the beginning of July 2018. Fig seeds were extracted after chopping the fresh fruits into a water bath. The seeds washed several times with water and allowed to dry at room temperature for one week. All the materials were kept at 4°C prior to analyses. Before extraction, fig seeds were crushed with a coffee grinder to make them homogeneous.

Methods

Extraction process

Solid–liquid extraction system was used for the extraction of antioxidant compounds from fig seeds. Six different solvents including 100% acetone, 100% methanol, 100% ethanol, 50% (v/v) aqueous acetone, 50% (v/v) aqueous
methanol and 50% (v/v) aqueous ethanol were utilized in the extractions. The sample was extracted in these solvents using a fixed solid/liquid ratio of 1:5 (w/v), for example 3 g of sample by mixing with 15 mL of solvent. The mixture was then stirred at 50°C for 90 min in a shaking water bath (Memmert WB10, Schwabach, Germany). It was centrifuged (10,000 rpm, 4°C) for 20 min and filtered using a 0.45 μm PTFE membrane filter (Sartorius, Germany). The volume of aliquot extract was completed to 15 mL. Liquid extracts were immediately analyzed for the determination of polyphenol content and antioxidant capacities of fig seeds.

**Spectrophotometric determination of polyphenols and antioxidant capacity**

Total polyphenol content (TPC) of the extracts was quantified by a spectrophotometric method (Xu & Chang 2007) using Folin–Ciocalteu reagent. The amount of total polyphenols were expressed as mg gallic acid equivalents (GAE) per kg of dry matter (DM) (y = 0.0021x + 0.0367). Total flavonoid content (TFC) of the extracts was determined according to the aluminum chloride colorimetric method described by Heimler et al. (2005) and calibrated against (+)-catechin as the reference standard. The TFC were evaluated as mg (+)-catechin equivalents ((+)-CE) per kg of DM (y = 0.0013x + 0.0059). The capacity of the extracts to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was assessed according to a modified method described by Chu et al. (2000) and Cheung et al. (2003). 6-Hydroxy-2,5,7,8- tetramethylchromane-2-carboxylic acid (trolox) was used as a reference antioxidant and the concentration was 15 μM. It was expressed as percentage inhibition of DPPH (%). The ferric reducing ability power (FRAP) of the extracts was determined following the modified methods described by Guo et al. (2003) and Xu et al. (2004). Butylated hydroxytoluene (BHT) was used as a reference antioxidant and the concentration was 10 mM. The results were expressed as mg reduced iron equivalents (FeSO₄) per kg DM (y = 0.0626x + 0.0163).

**Determination of individual polyphenols using HPLC-DAD detection**

The method described by Çam et al. (2014) was adapted with slight modifications for the qualitative and quantitative determinations of individual polyphenols. The polyphenol standards were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). The contents of individual polyphenols in the extracts were measured using a Agilent 1200 LC system (Agilent Technologies, Palo Alto, CA, USA) using DAD detection at 272 nm for gallic acid, 275 nm for (-)-epicatechin, 279 nm for chlorogenic acid, syringic acid and psoralen and 356 nm for rutin, on a Hichrom C₁₈ column (250 mm × 4.6 mm i.d. with 5μm particle diameter, Hichrom Ltd., Reading, Berkshire, UK) which was maintained at 40°C. The mobile phases were composed of A and B solvent systems; solvent A was water/acetic acid (98:2, v/v); solvent B was 100% methanol. An used elution gradient was as follows: a linear gradient from 95% A to 50% A for 10 min, from 50% A to 30% A for 5 min. The flow rate was 1 mL/min and the injection volume was 20 μL. Quantitative determinations were carried out using the external standard method (y = 0.5522x + 15.9797 for gallic acid, y = 0.0783x + 3.1251 for (-)-epicatechin, y = 0.1666x + 4.1505 for chlorogenic acid, y = 0.3481x + 6.4216 for psoralen and y = 0.2212x + 5.0548 for rutin). Qualitative determinations were performed by comparing the retention times and spectra of the samples and standards, as well as the use of standard addition method. The HPLC method used was validated by determining LOD, LOQ, and recovery values.
Statistical analysis

The results were given as mean value ± standard deviation for triplicate determinations. ANOVA and Duncan multiple range test were used to determine the differences between values by SPSS ver.20.0 (SPSS Inc., Chicago, USA) at a significance level of \( p<0.05 \). Also, Pearson correlation test was used to determine the correlation among variables.

RESULTS AND DISCUSSION

Total polyphenol contents, total flavonoid contents and antioxidant capacities of the fig seed extracts

In this study, the polyphenols and antioxidant compounds of fig seeds were extracted with various solvents having different polarities. As indicated in Figure 1a, b, total polyphenol and flavonoid contents varied in the different extracts \( (p<0.05) \). TPC increased in the following order: 100% acetone<100% ethanol<100% methanol<50% (v/v) aqueous acetone<50% (v/v) aqueous ethanol<50% (v/v) aqueous methanol \( (p<0.05) \). TFCs of the extracts were in the order of 100% acetone=100% ethanol<100% methanol.<100% methanol<50% (v/v) aqueous acetone<50% (v/v) aqueous ethanol<50% (v/v) aqueous methanol \( (p<0.05) \). TFCs of the extracts were in the order of 100% acetone=100% ethanol<100% methanol.
ethanol=100% methanol<50% (v/v) aqueous acetone<50% (v/v) aqueous ethanol<50% (v/v) aqueous methanol (p<0.05). It can be said that the changes in TPC and TFC in extracts showed approximately the same tendency. TPC of fig seeds ranged from 447 mg GAE/kg DM (in 100% acetone) to 714 mg GAE/kg DM (in 50% (v/v) aqueous methanol). The lowest TFC was observed in fig seeds extracted by 100% acetone and 100% ethanol (192 mg (+)-CE/kg DM and 195 mg (+)-CE/kg DM), whereas the fig seeds extracted by 50% (v/v) aqueous methanol had the highest flavonoid content (312 mg (+)-CE/kg DM).

The results of the DPPH and FRAP assays showed almost the same trends (Figure 1c, d). Among all the solvent types studied, the fig seeds extracted by 50% (v/v) aqueous methanol had the highest antioxidant activities from DPPH (41.6%) and FRAP (8504 mg FeSO₄/kg DM) values (p<0.05). The solvents with the lowest both DPPH and FRAP values were 100% ethanol (22.5% and 4354 mg FeSO₄/kg DM, respectively) and 100% acetone (23.6% and 4511 mg FeSO₄/kg DM, respectively) (p<0.05). There was no statistical difference between 100% ethanol and 100% acetone extracts in terms of the determined antioxidant capacities of the fig seeds (p>0.05).

According to the results of this study, the TPC (447-714 mg GAE/kg DM) and TFC (192-312 mg (+)-CE/kg DM) values determined were lower than those of Sarilop type fresh figs (1988-3076 mg GAE/kg DM for TPC and 673-1475 mg RE/kg DM) found by Nakilcicoglu & Hisil (2013). The DPPH radical scavenging effect of fig seeds is almost half of that of the 15 μM trolox standard (56.3 %). Also, fig seeds had higher efficacy to scavenge DPPH radicals (22.5-41.6 %) than that of the fig fruits (20.5 %) reported by Amessis-Ouchemoukh et al. (2017). The FRAP value of 10 mM BHT (97587 mg FeSO₄/kg) is 10-20 times that of fig seeds. The FRAP values of fig seed extracts (4511-8504 mg FeSO₄/kg DM) were similar with the results obtained by Nakilcicoglu & Hisil (2013) who reported that the FRAP values of Sarilop type fresh figs changed between 5534 and 8717 mg FeSO₄/kg DM. These findings showed that TPC and TFC of fig seeds were lower than that of fresh figs while their antioxidant capacities could be determined as similar or better depending on the method used in the analysis.

In this study, the extraction efficiency of polyphenols and antioxidant compounds was found to be dependent on the polarity of the solvent used (Wijekoon et al. 2011). The best solvent for extracting polyphenols and other antioxidant compounds of fig seeds was found to be methanol. Similar findings were obtained for the extraction of polyphenols and antioxidant compounds from different raw materials such as garlic husk (Kallel et al. 2014), ray florets and disc florets of sunflower (Ye et al. 2015), Amomum chinense C. leaves (Butsat & Siriamornpun 2016), dried mushroom (Celebi Sezer et al. 2017). The use of aqueous methanol further increased the extractability of these compounds. The presence of the highest TPC, TFC and antioxidant capacities in aqueous solutions could be attributed to the increase in the polarity of the solvents by the addition of water (Kallel et al. 2014). It could be explained that the polarity of the polyphenols and other antioxidant compounds in the fig seeds was closer to that of 50% (v/v) aqueous methanol and these compounds were more soluble in this solvent. Similar results were reported earlier by Xu & Chang (2007), Wijekoon et al. (2011), Meneses et al. (2013) and Kallel et al. (2014). In this study, 50% (v/v) aqueous methanol was found to be the most effective solvent for the polyphenol and antioxidant compound extraction from the fig seeds. This result was in agreement with the observation made by Kallel et al. (2014) who reported that 50% methanol was the most
effective solvent for extraction of polyphenols in the garlic husk. In addition, the results of the Ye et al. (2015) found that the extracts of sunflower disc florets revealed the highest TPC values were 90% methanol and 50% ethanol extracts as well as 50% methanol extract, supported the findings of this study. The study reported by Wijekoon et al. (2011), which the 50% methanol extract of bunga kantan inflorescence had one of the highest percent inhibition of DPPH. Similar results have been recorded in the present study.

**Polyphenol compositions of fig seed extracts**

Individual polyphenols in the fig seed extracts were determined qualitatively and quantitatively by using the calibration curves plotted with six different standards in the concentration range of 0.834 - 83.34 mg/L ($R^2>$0.99) (Figure 2.). The LOD, LOQ and recovery values for each polyphenol are given in Table I.

The content of five different polyphenols (chlorogenic acid, (-)-epicatechin, syringic acid, rutin and psoralen) was determined in fig seeds (Figure 3). Gallic acid found by Nakilcioğlu & Hisıl (2013) in Sarilop type fresh figs could not be detected in the fig seeds. It was observed that the fig seeds had higher psoralen and chlorogenic acid content, however (-)-epicatechin, syringic acid and rutin contents were lower compared to the Sarilop type fresh fig analyzed by Nakilcióğlu & Hisıl (2013). In the study, it was observed that the polyphenol content of the fig seeds were lower than that of the fresh figs, and this result was consistent with the TPC and TFC results of the fig seeds.

The polarities of polyphenols in the food matrix may be different from each other. This

| Polyphenols  | LOD (mg/L) | LOQ (mg/L) | Recovery (%) |
|--------------|------------|------------|--------------|
| Chlorogenic acid | 0.3 | 0.99 | 97.52 |
| (-)-Epicatechin   | 0.26 | 0.85 | 98.20 |
| Syringic acid     | 0.3 | 1 | 94.61 |
| Rutin            | 0.39 | 1.29 | 94.83 |
| Psoralen         | 0.32 | 1.08 | 95.17 |

*Table I. LOD, LOQ values and recovery of the polyphenols from the fig seeds studied by HPLC (n = 3).*

*Figure 2. HPLC chromatograms of polyphenol standards at 279 nm (a gallic acid, b chlorogenic acid, c (-)-epicatechin, d syringic acid, e rutin and f psoralen).*
leads to difficulties in selecting the solvent that can extract all the polyphenols in the matrix with the best efficiency (Ye et al. 2015). As shown in Table II, total five polyphenols were identified and quantified in all fig seed extracts. The fig seed extract with 50% (v/v) aqueous methanol had the highest chlorogenic acid, (-)-epicatechin, syringic acid and rutin contents (p<0.05) (Figure 3). It was also statistically determined that the use of 50% (v/v) aqueous ethanol in syringic acid extraction was as efficient as 50% (v/v) aqueous methanol (p>0.05). The highest psoralen content was observed in the fig seeds extracted by 50% (v/v) aqueous acetone (53.0 mg/kg DM) and 50% (v/v) aqueous ethanol (54.0 mg/kg DM) and no significant difference was found between them (p>0.05). The 100% acetone extract of fig seeds contained the lowest amounts of chlorogenic acid, (-)-epicatechin, syringic acid and rutin compared to other solvents used (p<0.05). In addition, no statistically significant difference was observed among the absolute solvents used for syringic acid extraction (p>0.05). The lowest amount of psoralen was also found in 100% methanol extract of fig seeds (p<0.05).

These findings were consistent with the results of TPC and TFC and also showed that aqueous solvents, in particular 50% (v/v) aqueous methanol, were more successful than the other solvents used to extract polyphenols from fig seeds. These results correlated with Butsat & Siriamornpun (2016) who reported that the aqueous methanol extract of *Amomum chinense* leaves had high contents of catechin, rutin and chlorogenic acid compared to other aqueous solvents.

**Correlation analysis among polyphenols and antioxidant capacities**

Correlation analyses were conducted among polyphenols and antioxidant capacities of fig seed extracts (Table III). Significant linear correlations were observed between the TPC values and the values of TFC, FRAP, DPPH and individual polyphenols (r = 0.469-0.991) (p<0.05). Since flavonoids are a subgroup of polyphenol, there is a high correlation between TPC and TFC (r = 0.991). TPC values were highly positively correlated with the values of DPPH (r = 0.989) and FRAP (r = 0.961) (p<0.01). This situation showed that the polyphenols contributed to the antioxidant capacities of the extracts samples. It is an indication that most of the fig seed polyphenols are capable of reducing H\(^+\) and Fe\(^{3+}\) ions. Moreover, the correlation between DPPH and TPC is higher than that of FRAP because

![Figure 3. HPLC chromatograms of 50% (v/v) aqueous methanol extract obtained from fig seeds (a chlorogenic acid, b (-)-epicatechin, c syringic acid, d rutin and e psoralen).](image-url)
most of the antioxidants in fig seeds have H+ reducing characteristics. These correlations were higher than the correlations of individual polyphenols’ values with the antioxidant capacities (p<0.05). This is an expected result because the antioxidant capacities of all bioactive compounds are determined in the DPPH and FRAP assays. This result supported the findings of Ye et al. (2015) who declared that the Pearson results of Folin-Ciocalteu method were more significant than HPLC method in the determination of polyphenols of sunflower floret extract. Statistically significant correlations were determined between TFC value with both DPPH (r = 0.982) and FRAP values (r = 0.962) (p<0.01). This proved that flavonoids significantly affected the antioxidant capacities of the extracts. Correlation analysis between the antioxidant capacities of the extracts revealed a significant linear correlation between DPPH and FRAP (r = 0.984) (p<0.01). In addition, significant linear correlations existed between both DPPH (r = 0.468-0.891) and FRAP (r = 0.700-0.852) values with individual polyphenols’ values (p<0.05). Polyphenol which contributed most to the antioxidant capacities of extracts was syringic acid (r = 0.852 and 0.891) (p<0.01), whereas the least contributor was psoralen (r = 0.468) (p<0.05). Psoralen was not correlated with FRAP values of extracts, implying that this compound was not responsible for the ferric-reducing antioxidant power of the tested extracts. Several studies in literature have also been clearly stated a close relationship between the polyphenol content and the antioxidant capacity (Xu & Chang 2007, Ye et al. 2015, Ghasemzadeh et al. 2015).

**CONCLUSIONS**

The results obtained from this study showed that fig seeds were found to be a natural source of bioactive compounds and the important parts of polyphenols and antioxidant compounds came from fresh figs. It was also found that extraction with solvents of different polarities affected the TPC, TFC, individual polyphenol content and antioxidant capacity of fig seed extract. The extractability of bioactive compounds in fig seeds was increased by addition of water to organic solvents. This proved that medium polar solvents (aqueous solution of methanol, ethanol, acetone, chloroform etc.) were more effective than solvents with low polarity (absolute organic solvents) in the extraction of

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**Table II. Effect of various solvent on the recovery of the polyphenols from the fig seeds (n = 3 ± S.D.).**

| Solvent type | Chlorogenic acid (mg/kg DM) | (-) Epicatechin (mg/kg DM) | Syringic acid (mg/kg DM) | Rutin (mg/kg DM) | Psoralen (mg/kg DM) |
|-------------|-----------------------------|---------------------------|-------------------------|-----------------|-------------------|
| Acetone     |                             |                           |                         |                 |                   |
| 100%        | 65.1 ± 2.5 e                | 106.7 ± 3.2 e             | 6.02 ± 0.15 c           | 30.1 ± 1.4 f    | 48.6 ± 1.4 b      |
| 50%         | 82.5 ± 3.1 d                | 125.2 ± 4.8 c             | 7.12 ± 0.11 b           | 35.3 ± 1.7 e    | 53.0 ± 0.7 a      |
| Methanol    |                             |                           |                         |                 |                   |
| 100%        | 101.3 ± 3.6 c               | 127.2 ± 2.1 c             | 6.05 ± 0.24 c           | 40.2 ± 0.4 c    | 25.1 ± 0.4 e      |
| 50%         | 131.9 ± 2.6 a               | 166.4 ± 2.1 a             | 8.03 ± 0.27 a           | 50.7 ± 1.2 a    | 39.4 ± 0.9 c      |
| Ethanol     |                             |                           |                         |                 |                   |
| 100%        | 79.6 ± 1.3 d                | 117.9 ± 0.7 d             | 5.98 ± 0.74 c           | 37.5 ± 1.2 d    | 29.9 ± 2.3 d      |
| 50%         | 107.3 ± 1.6 b               | 143.9 ± 2.4 b             | 8.13 ± 0.67 a           | 48.0 ± 1.0 b    | 54.0 ± 1.1 a      |

Different letters in the same column show the statistical differences at p<0.05.
polyphenols and other antioxidant compounds from the fig seeds. In this study, 50% (v/v) aqueous methanol extract of fig seeds had the highest polyphenol content and antioxidant capacity. Additionally, significant positive correlations were determined among the TPCs, TFCs, antioxidant capacities and individual polyphenol contents of fig seed extracts. Fig seed could be considered as a source of important phytochemicals with antioxidant properties. Therefore, it was significantly responsible for the health-promoting effects of figs. These results also indicated that the fig seed extract obtained using a suitable extraction solvent could have protective effects against free radical-associated oxidative damage.

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