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INVESTIGATION OF CHANGES IN SOME BIOACTIVE PROPERTIES OF PHENOLIC EXTRACTS FROM PULP AND SEED TISSUES OF *ZIZIPHUS JUJUBA* DURING IN VITRO DIGESTION

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ABSTRACT

In this study, changes in angiotensin-I converting enzyme (ACE) inhibitory, α-amylase inhibitory and antioxidant activities, total phenolic content (TPC), total monomeric anthocyanin content (TMAC) of ultrasonic phenolic extracts from pulp and seed of *Ziziphus jujuba* were investigated during in vitro digestion. Bioaccessible fractions of phenolics in seed and pulp extracts were calculated as 23.24±4.46% and 9.43±0.24%, respectively. Moreover, bioaccessibility for TMAC in seed extracts (147.83±9.20%) was higher than pulp (15.76±3.89%) (P<0.05). A decrease in the antioxidant activity of the extracts occurred after in vitro digestion (P<0.05). The ACE inhibitory activity of undigested extracts from seed (86.04±0.00%) was higher than that of the undigested pulp extract (42.74±8.57%) (P<0.05). The α-amylase inhibitory activity of seed and pulp extracts was determined as 49.18±0.35% and 36.07±5.83%, respectively. The results of the study showed that ACE inhibitory activity and α-amylase inhibitory activity of the polyphenolics from pulp increased after in vitro digestion.

Keywords: *Ziziphus jujuba*, antioxidant activity, ACE inhibitory activity, α-amylase inhibitory activity, anthocyanin

ZIZIPHUS JUJUBE (HÜNNAP) İÇ VE ÇEKİRDEK DOKULARINDAN FENOLİKLERİN IN VITRO SİNDİRİM SIRASINDA BAZI BİYOAKTİF ÖZELLİKLERİNDEKİ DEĞİŞİMİN ARAŞTIRILMASI

ÖZ

Bu çalışmada, *Ziziphus jujuba* iç ve çekirdeklerinden elde edilen ultrasonik fenolik ekstraktların in vitro sindirim sırasında anjiyotensin-I dönüştürücü enzim (ADE) inhibisyon, α-amilaz inhibisyon ve

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antioksidan aktivitesi ile toplam fenolik madde (TFM) ve toplam monomerik antosiyanin miktarı (TMAM) üzerindeki değişiklikler incelenmiştir. In vitro sindirim sonrasında, çekirdek ve iç ekstraktlarındaki biyoerişilebilir fenolik fraksiyonlar sırasıyla %23.24±4.46 ve %9.43±0.24 olarak hesaplanmıştır. Ayrıca, çekirdek ekstraktlarında toplam monomerik antosiyaninlerin biyoerişilebilirliği (147.83±9.20%) iç ekstraktların toplam monomerik antosiyaninlerinin biyoerişilebilirliğinden (15.76±3.89%) daha yüksek bulunmuştur (P<0.05). In vitro sindirim sonrasında ekstraktların antioksidan aktivitesinde azalma meydana gelmiştir (P<0.05). Çekirdek ekstraktlarının sindirim öncesi ADE inhibisyon aktivitesi (%86.04±0.00), sindirim öncesi iç ekstraktın ADE inhibisyon aktivitesinden (%42.74±8.57) daha yüksektir (P<0.05). Çekirdek ve iç ekstraktların α-amilaz inhibisyon aktivitesi sırasıyla %49.18±0.35 ve %36.07±5.83 olarak belirlenmiştir. Çalışmanın sonuçlarına göre iç fraksiyonundan elde edilen ekstraktların ADE inhibisyon ve α-amilaz inhibisyon aktivitesi in vitro sindirimden sonra artmıştır.
Anahtar kelimeler: *Ziziphus jujuba*, antioksidan aktivite, ADE inhibisyon aktivite, α-amilaz inhibisyon aktivite, antosiyanin

INTRODUCTION

Ziziphus jujuba, also known as jujube, is an orangish-burgundy, brown fruit with aromatic flavor which grows mainly in warm and subtropical regions such as Southeast Asia, China and the Mediterranean (Hoshyar et al., 2015). Jujube, which has approximately 900 species, is grown intensively in the western and southern parts of Anatolia in Türkiye (Imamoglu, 2016; Naik et al., 2013). It has been used in the treatment of intestinal diseases and anxiety in traditional medicine for a long time (Imamoglu, 2016; Choi et al., 2012). The recent studies have also shown that jujube has various bioactive properties such as antioxidant (Wang et al., 2012; Wu et al., 2012; Zhang et al., 2010), antiallergic (Naik et al., 2013), antimicrobial (Hamedi et al., 2015), anti-inflammatory (Goyal et al., 2011; Kumar et al., 2004), immunostimulant (Ganachari et al., 2004), antidiabetic (Hemmati et al., 2015b; Shirdel et al., 2009), antiobesity (Hemmati et al., 2015a), sedative, hypnotic (Jiang et al., 2007) and hypoglycemic activities (Shirdel et al., 2009). Anthocyanins are pigments attached to the flavonoid group found in many plants and fruits. They are natural colorants with antioxidant properties that are easily soluble in water and give color in a wide area between orange, red, purple, and blue (Shi et al., 2018; Castañeda-Ovando et al., 2009).

There is a widespread understanding that plants with greater polyphenolics have better antioxidant effects (Chel-Guerrero et al., 2018). Antioxidants are various natural metabolites synthesized by plants against free radicals to protect themselves (Angerhofer et al., 2009). Free radicals cause many degenerative diseases such as diabetes, cardiovascular diseases, and cancer (Sarmadi and İsmail, 2010). The jujube is a good source of polyphenolics and anthocyanins which responsible antioxidant activity (Li et al., 2005; Koley et al., 2016; Liu et al., 2020).

Hypertension is an important risk factor for cardiovascular diseases. Angiotensin-I-converting enzyme (ACE) plays a key role in regulating blood pressure and inhibition of this enzyme is required to treat hypertension. The ACE inhibitors are widely used to control hypertension. Synthetic drugs used for this purpose have some side effects such as dry cough and skin rash (Daskaya-Dikmen et al., 2017). Therefore, researchers have investigated the effect of natural sources like plant extracts on hypertension in recent years (Şensu et al., 2021; Rawat et al., 2016). For instance, Kumar et al. (2011) reported that vanillic acid, a phenolic compound, exhibited antihypertensive effect in hypertensive mice.

Diabetes, which is one of degenerative diseases, causes various damages including retinopathy, nephropathy, and neuropathy on the related organs due to the inability to regulate the sugar rate (glycemia) in the blood (Al-Azzawie and Alhamdanii,2006). According to a report in 2019, approximately 463 million people in the world are known to have diabetes and it is estimated that this number will reach 700 million by 2045 (Senevirathne et al., 2021). Adefegha et al. (2015)

showed that phenolic extracts obtained from *Annona muricata* have antidiabetic and antihypertensive effects. It has been also reported that the jujube fruit extracts exhibited antidiabetic effect exerting inhibitory activity against α-amylase by Mourya et al., 2017; Hemmati et al., 2015a; Marmouzi et al., 2019.

There are a lot of studies about antioxidant, antihypertensive and antidiabetic activities of whole jujube fruit in the literature. However, to our knowledge, there is no study on the changes in angiotensin-I converting enzyme inhibitory, α-amylase inhibitory and antioxidant activities and total phenolic content and total monomeric anthocyanin content of seed and pulp fractions from jujube without peel tissue during in vitro digestion. Therefore, in the present study, changes in these bioactive properties were investigated during in vitro digestion. Moreover, seed and pulp fractions were compared in terms of angiotensin-I converting enzyme inhibitory, α-amylase inhibitory and antioxidant activities and total phenolic content and total monomeric anthocyanin content. Thus, it was aimed to reveal the bioactive potential of seed and pulp fractions from jujube grown in Türkiye.

MATERIALS AND METHOD

Materials

The jujube fruit was collected from an orchard, which is located at coordinates of 38˚15'56" N and 34˚04'00" E, in Aksaray province in Türkiye. The surface contaminants of the fruits were removed by washing under tap water, pulp and seed fractions were carefully separated by hand using a knife. Then, they were freeze-dried and the lyophilized samples were stored without exposure to light and oxygen at -20 °C until the extraction process. The visual appearance of the lyophilized pulp and seed fractions was given in Figure 1.

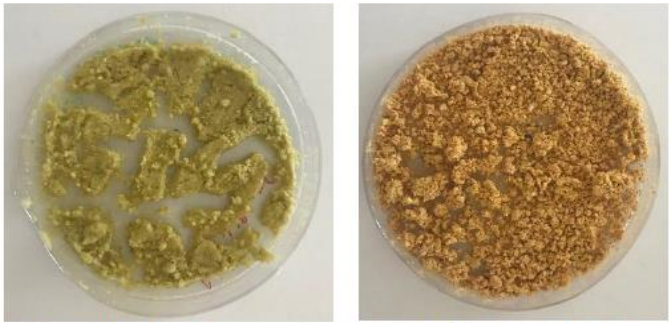

Figure 1. Visual observation of liyofilized pulp and seed fractions from *Ziziphus jujube*.

Folin-Ciocalteu's reagent and gallic acid were purchased from (Merck, Darmstadt, Germany). Sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), copper (II) chloride, Trolox, neocuprin, potassium chloride, sodium acetate, ammonium acetate, dimethyl sulfoxide (DMSO), sodium phosphate, starch, 3,5-dinitrosalicylic acid (DNS), sodium chloride, sodium hydroxide, ethyl acetate, α-amylase, hippuryl-his-leu (HHL), angiotensin-converting enzyme (ACE), methanol, pepsin, bile salts and pancreatin were purchased from Sigma-Aldrich (Sigma-Aldrich Chemie, St. Louis, Missouri, USA). All of the solvents and chemicals utilized were of the analytical grade.

Method

Ultrasound-assisted extraction

Ultrasound-assisted extraction of polyphenolics from seed and pulp fractions of jujube without peel fraction was carried out using the method of Erşan et al. (2017) with slight modification. Accordingly, 0.5 g lyophilized sample was mixed with 5 mL of extraction solvent (methanol: Milli-Q water:formic acid (80:19:1, v/v/v)). The ultrasound procedure was applied to the mixture for 20 seconds at 65% power using an ultrasound device (Sonopuls HD 2200 ultrasonic homogeniser, Bandelin Electronic GmbH & Co. KG, Berlin, Germany) with a titanium type probe (Bandelin Titanium Long Tip Probe VS 70 T, 13

mm diameter). Ultrasonication application was performed by immersing the probe to a depth of 2.5 mL into a 50 mL beaker including the sample. During the ultrasonication application, in order to prevent heating of the sample, the beaker containing the sample was placed in a beaker with a water-ice mixture, and temperature of the samples was kept between 20-25 °C. After ultrasonication, the samples were centrifuged at 6000 rpm for 5 minutes. The supernatant was discarded and the pellet was extracted two more times using the same amount of extraction solvent. The extracts were combined and evaporated by using a rotary evaporator (IKA RV10, Germany) at 40 °C. Then, the extracts were stored at -20 °C further analysis.

Total phenolic content (TPC)

The TPC of the samples was performed according to the Folin-Ciocalteu method (Toor and Savage, 2006). Briefly, 200 µL sample was mixed with 1.5 mL of 10-fold diluted Folin-Ciocalteu's reagent and 1.2 mL of sodium carbonate solution. The mixture was left to stand in the dark for 90 min. The absorbance was read at 765 nm by using a microplate reader (Synergy HT, BioTek Instruments Inc., Winooski, VT, USA). The results were expressed as mg gallic acid equivalent (GAE)/g sample in dry weight (dw).

Total monomeric anthocyanin content (TMAC)

Analysis of total monomeric anthocyanin was carried out according to the pH differential method (AOAC method 2005.02). Briefly, the samples were diluted with 0.025 M KCl (pH 1.0) and 0.4 M CH_3COONa (pH 4.5) and the absorbance was measured at 530 nm and 700 nm TMAC was determined by following formula:

$$\text{TMAC (cyanidin-3-O-glucoside equivalent, µg/g dw)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times L} \quad (1)$$

Where $A = (A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH\ 4.5}$, MW is the molecular weight of cyanidin-3-O-glucoside (449.2 g/mole), DF is the dilution factor, ε is the molar extinction coefficient of cyanidin-3-O-glucoside (26,900 L/cm.mole) and L is the path length (0.75 cm).

Determination of antioxidant activity (AOA)

2,2-diphenyl-1-picrylhydrazyl free radical scavenging (DPPH) method

The antioxidant activity of the samples was determined by DPPH method (Kumaran and Karunakaran, 2006). Briefly, 100 µL sample was diluted with 2 mL of 0.1 mM DPPH (in methanol). After shaking for 10 seconds, the sample was kept at room temperature in the dark for 30 min and absorbance was measured at 517 nm. The results are expressed in mg Trolox equivalent (TE)/g sample dw.

The cupric ion reduction antioxidant capacity determination (CUPRAC)

The antioxidant activity of the samples was determined by CUPRAC method (Apak et al., 2004). Accordingly, 100 µL sample was mixed with 1 mL of 10^{-2} mM copper (II) chloride, neocuprine (in ethanol), ammonium acetate buffer (pH=7.0) solutions and Milli-Q water. After standing for 30 min at room temperature, absorbance was recorded at 450 nm and the results were expressed as mg TE/g sample dw.

The α- amylase inhibitory activity

The α-amylase inhibition analysis was performed according to the method by Yu et al. (2012) with slight modification. Accordingly, 30 µL sample (in 10% DMSO), 120 µL Milli-Q water, 300 µL of starch solution in 20 mM sodium phosphate buffer (pH 6.9) and 150 µL α-amylase solution (1 U/mL) was pre-incubated at 25 °C for 10 min. Then, 300 µL of the DNS reagent (1% 3,5-dinitrosalicylic acid, 12% Na-K tartrate in 0.4 M NaOH) was added to terminate the reaction. Enzymatic inhibition was carried out at 85 °C for 10 min. After adding 675 µL deionized water, the mixture was cooled down to room temperature. The α-amylase activity was measured at 540 nm and calculated by the following formula:

$$\alpha - \text{amylase inhibitory activity} = \frac{(A-B)}{A} \times 100 \quad (2)$$

Where A represents the absorbance in the presence of buffer solution and B represents

absorbance in the presence of the α-amylase and sample solution.

Angiotensin-converting enzyme inhibitory activity

The ACE inhibitory analysis was performed by the method of Ahn et al. (2012). Briefly, 50 μL ACE solution (25 mU/mL) was added to 50 μL of diluted sample which was dissolved in borate buffer and pre-incubated at 37 °C for 10 min. Then, 150 μL of HHL substrate (5 mM, pH 8.3) was added to the mixture and incubated at 37 °C for 30 min. The reaction was stopped by adding 250 μL of 1.0 M HCl. To extract hippuric acid, 0.5 mL of ethyl acetate was added and the test tubes were centrifuged at 3200 rpm for 15 min. After centrifugation, 0.2 mL of the supernatant was evaporated at room temperature for 12 h. The hippuric acid was dissolved in deionized water and the ACE inhibition activity was measured at 228 nm calculated by the following formula:

$$\text{ACE inhibition activitiy (\%)} = \frac{A_{control} - A_{sample}}{A_{control} - A_{blank}} \times 100 \quad (3)$$

Where A_{blank} represents absorbance in the presence of Milli-Q water, $A_{control}$ represents absorbance in the presence of buffer solution and A_{sample} represents absorbance in the presence of the ACE and sample solution.

In vitro gastro-intestinal digestion and bioaccessibility

Bioaccessibility analysis by in vitro gastro-intestinal digestion was performed according to the INFOGEST method developed by Minekus et al. (2014) with some modifications. Briefly, 15 mL of salivary solution and 1 g of samples were mixed and the mixture was pre-incubated at 37 °C and 100 rpm for 15 min. After 15 min, 20 mL of stomach solution was added to this mixture and incubated again at 37 °C and 100 rpm for 1 h. Then, 10 mL of the mixture was collected as post-gastric (PG) digestion. Then, 5 mL of 120 mM NaCl, 5 mL of 120 mM KCl and 4.5 mL of intestinal solution were added to the remained mixture and reincubated again at 37 °C and 100 rpm for 2 h. At the end of the time, the mixture was collected as post-intestinal (PI) digestion. The PG and PI samples were centrifuged 10,000 rpm for 15 min and the supernatants were stored at -20 °C until further analysis. Bioaccessibility (%) of the samples was calculated by the following formula:

$$\text{Bioaccessibility (\%)} = \frac{\text{Bioactive content of digested samples}}{\text{Bioactive content of undigested samples}} \times 100 \quad (4)$$

Statistical analysis

Experimental data were given as mean ± standard deviation. Statistical analysis was carried out using a IBM SPSS software (version 22.0, SPSS, Chicago, IL, USA). The differences were compared using Tukey test and $p \leq 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Change in TPC during in vitro digestion

According to the results, the differences between TPCs of the undigested seed and pulp extracts were determined as 3.44±0.66 mg GAE/g dw and 10.72±0.48 mg GAE/g dw, respectively (P<0.05, Table 1). On the other hand, Wang et al. (2011) reported that TPCs of jujube pulp and seed extracts were 38.98 mg GAE/g dw and 30.95 mg GAE/g dw, respectively. Gao et al. (2012) determined that TPC of jujube between 27.56 mg GAE/g and 54.18 mg GAE/g fruit weight. These differences among the results can be associated with fruit variety, growing conditions, ripening level of the fruit, and the extraction conditions applied for the extraction of phenolics (Wang et al., 2011).

In the present study, TPC in the pulp extract was found to be higher than that of the seed (P< 0.05). Furthermore, the presence of phenolic compounds in different forms in jujube pulp and seed tissues may cause the differences in the phenolic levels in these tissues. For instance, the phenolic compounds in jujube seed tissues are mainly in insoluble-bound form whereas they are found in glycoside form in fruit pulp (Gao et al., 2013).

A decrease in TPCs of jujube seed and pulp fractions was observed after in vitro gastric and intestinal digestions (P<0.05, Table 1). The bioaccessibility (%) of seed and pulp extracts after

in vitro gastric digestion was determined to be 48.73±7.02% and 25.50±0.68%, respectively (P<0.05). After in vitro intestinal digestion, the bioaccessibility (%) of seed and pulp extracts was calculated as 23.24±4.46% and 9.43±0.24%, respectively. Similarly, Ma et al. (2020) reported that TPC of the soup obtained from bamboo leaves decreased approximately by 20% after in vitro digestion. According to Ma et al. (2020), this decrease may result from the loss in the stability of phenolic compounds under high pH values during intestinal digestion (Ma et al., 2020).

Table 1. Changes in total phenolic content, total monomeric anthocyanin content, antioxidant activity of jujube seed and pulp extracts during in vitro gastrointestinal digestion.

	Sample	Initial	In vitro gastric digestion	In vitro intestinal digestion
Total phenolics (mg/g)-TPC	Seed	3.44±0.66^{b,x}	1.63±0.08^{b,x}	0.77±0.00^{b,x}
	Pulp	10.72±0.48^{a,x}	2.73±0.05^{a,y}	1.01±0.02^{a,z}
Total anthocyanin content (μg cyn-3-gly/g)-TMAC	Seed	1.25±0.58^{a,y}	8.39±0.00^{a,x}	1.45±0.00^{a,y}
	Pulp	2.23±0.55^{a,x}	0.32±0.00^{b,y}	0.33±0.00^{b,y}
DPPH (mg TE/g)	Seed	4.04±0.18^{b,x}	4.72±0.48^{a,x}	0.58±0.46^{b,y}
	Pulp	7.34±0.19^{a,x}	4.80±0.21^{a,y}	4.21±0.30^{a,y}
CUPRAC (mg TE/g)	Seed	4.15±0.44^{b,x}	5.93±2.43^{a,x}	3.79±0.30^{a,x}
	Pulp	9.38±0.51^{a,x}	6.98±0.12^{a,y}	2.99±0.44^{a,z}

All the values were expressed as mean ± standard deviation. The mean is an average of three samples (n = 3) obtained from triplicated experiment. Different superscripts letters within the same line (x, y, z) and column (a, b) indicate significant difference (P< 0.05, Tukey).

Change in TMAC during in vitro digestion

The TMAC of pulp extract (2.23±0.55 μg cyn-3-gly/g dw) was higher than the TMACs of seed extract (1.25±0.58 μg cyn-3-gly/g dw) before in vitro digestion (P<0.05, Table 1). After gastric digestion, TMACs of the seed extract (8.39±0.00 μg cyn-3-gly/g dw) were higher than that of pulp extract (0.32±0.00 μg cyn-3-gly/g dw) (P<0.05). As seen in Table 1, the increase in TMAC of seed extract after in vitro gastric digestion was statistically significant (P<0.05). Pérez-Vicente et al. (2002) stated that there was a significant increase in the concentration of anthocyanin compounds in pomegranate juice after in vitro gastric digestion. According to Koh et al. (2020), anthocyanin compounds are converted from the hemiketal form to the flavylium cation, which is more stable form, during gastric digestion where the pH was 2.0.

After intestinal digestion, TMACs of the seed and pulp extracts were determined as 1.45±0.00 μg cyn-3-gly/g dw and 0.33±0.00 μg cyn-3-gly/g dw, respectively (P<0.05). Moreover, the bioaccessibility (%) value of TMAC in seed extract (147.83±9.20%) was higher compared to pulp extract (15.76±3.89%) after in vitro intestinal digestion. As seen in Table 1, there was a decrease in TMAC of pulp extracts after in vitro intestinal digestion (P<0.05). Likewise, de la Fuente et al. (2019) reported that anthocyanin compounds in broccoli and radish could not detect after in vitro digestion. In the study of Ma et al. (2020), anthocyanin compounds were degraded during in vitro digestion. Similarly, Koh et al. (2020) showed that there was a decrease in TMAC during in vitro intestinal digestion, due to the decreasing trend in all individual anthocyanin compounds. According to Pérez-Vicente et al. (2002), the decrease in TMAC is associated with conversion of flavylium cations to colorless chalcones during in vitro intestinal digestion. It has been stated that these "colorless anthocyanin pseudobases" are stable and in equilibrium with

the colored cationic forms in an acidic environment, however the anhydrobases gradually become more stable with the increase in pH after pH 5.0 (Pérez-Vicente et al. 2002).

Change in AOA during in vitro digestion

As shown in Table 1, the pulp extract exhibited higher AOA than that of the seed fraction because the pulp extract had the highest TPC than that of the seed extract (P<0.05). According to Chel-Guerrero et al. (2018), plants with higher level of polyphenolics also exhibit higher antioxidant activity. Zhang et al. (2010) found that jujube juice contained higher levels of ascorbic acid than other fractions. In the present study, the higher antioxidant activity of jujube pulp tissue may be associated with the higher concentration of ascorbic acid in jujube pulp. Also, it might be thought that the carbohydrates in the pulp fraction may have increased AOA. In the study of Li et al. (2011), polysaccharide extracts obtained from jujube fruit had antioxidant activity. Additionally, Wang et al. (2011) stated that the ripening level and variety of jujube and differences in its growing conditions may lead to discrepancy in TPC and AOA of jujube.

Antioxidant activity of the extracts decreased after in vitro digestion, in parallel with the decrease in both TPC and TMAC (P<0.05, Table 1). The bioaccessibility for AOA was determined to be as 24.65±1.76% and 91.58±2.48% for seed extract and 57.29±2.60% and 31.71±2.97% for pulp extract by DPPH and CUPRAC methods, respectively. Similarly, Vinholes et al. (2018) and Goulas and Hadjisolomou (2019) showed that antioxidant activity of some fruits and vegetables decreased significantly after in vitro digestion. This decrease was associated with the fact that antioxidant compounds such as total phenolics and total anthocyanins are less reactive at the pH value of the intestinal environment, which is about 7.4 (Puangkam et al., 2017; de la Fuente et al., 2019).

Change in ACE inhibitory activity during in vitro digestion

The ACE inhibitory activity of the seed and pulp extracts was 86.04±0.00% and 42.74±8.57%, respectively (P<0.05, Figure 2). Similary, it was shown in other studies that polyphenolics can exhibit ACE inhibitory activity. For instance, Mohebbati et al. (2017) investigated the antihypertensive activity of jujube fruit in mice with high blood pressure and reported that hydroalcoholic extracts obtained from jujube fruit exhibited an antihypertensive effect in mice. Likewise, Nho et al. (2010) showed that ACE inhibitory activity of phenolic extracts from the leaves of *Boehmeria nivea* ranged from 14.47% to 80.31%. Also, López-Fernández-Sobrino et al. (2021) reported that wine sediments had antihypertensive potential in mice due to flavonols and anthocyanins in its composition.

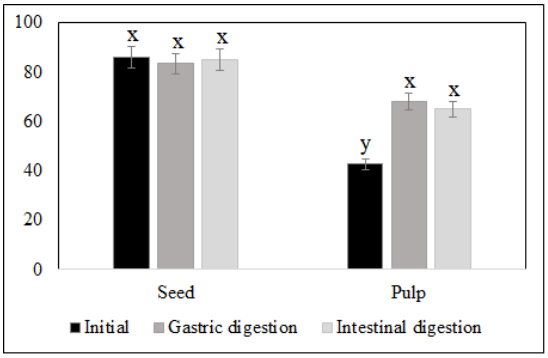

Figure 2. Changes in ACE inhibitory activity of jujube seed and pulp extracts during in vitro gastrointestinal digestion. Different letters (x, y) indicate significant difference (P< 0.05, Tukey).

The ACE inhibitory activity of bioaccessible fraction of the seed extract in the intestinal phase was 99.04±4.95%, while it was 157.82±27.90% for the pulp extracts. Similarly, Fernandez et al. (2013) reported that ACE inhibitory activity of grape extracts was >90% before in vitro digestion and was >80% after in vitro digestion. Likewise, Sensu et al. (2020) reported that red Berberis fruit had 73.84% ACE inhibitory activity before in vitro digestion and ACE inhibitory activity decreased to 65.51% after in vitro digestion.

Change in α-amylase enzyme inhibition activity during in vitro digestion

The α-amylase enzyme inhibition activity of the undigested seed and pulp extracts were found to be 49.18±0.35% and 36.07%±5.83%, respectively ($p \geq 0.05$, Figure 3). The α-amylase enzyme inhibition activity after in vitro intestinal digestion of the pulp extract was statistically higher than that of the seed extract ($P<0.05$). Zhao et al. (2014) showed that polysaccharides extracted from jujube fruit had a significant *in vivo* antidiabetic activity by reducing the plasma glucose level. Similarly, Benammar et al. (2014) investigated the antidiabetic activities of extracts from leaves, roots and seeds of *Zizyphus lotus* in diabetic mice and reported that these extracts exhibited a reducing effect on the glucose level in mice. Furthermore, other studies in the literature have reported that natural polyphenols had an inhibitory effect on α-amylase activity (Quesada et al., 1996; McDougall et al., 2005; Ghosh et al., 2012; Liu et al., 2013; Zaidan et al., 2019).

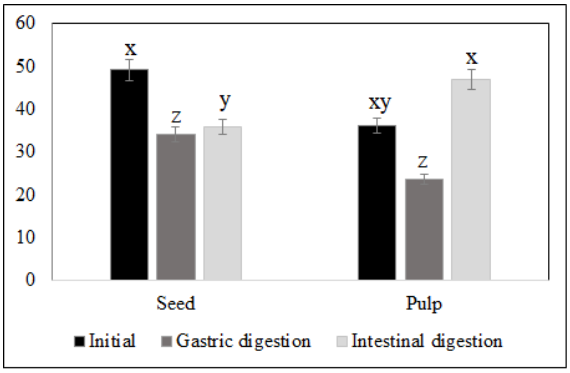

Figure 3. Changes in α-amylase enzyme inhibition activity of jujube seed and pulp extracts during in vitro gastrointestinal digestion. Different letters (x, y, z) indicate significant difference ($P< 0.05$, Tukey).

The bioaccessibility (%) value for α-amylase enzyme inhibition activity of the pulp extract increased after in vitro digestion ($P<0.05$, Figure 3). Similarly, in the study of Ng and See (2019) it was showed that in vitro digestion caused a positive effect on the α-amylase enzyme inhibition activity of plant extracts. According to Ng and See (2019), this increase in the carbohydrate hydrolyzing enzymes inhibitory activity of the digested extracts may be because of the release of aglycones phenolic from the glycosides throughout the digestion process.

CONCLUSION

In the present study, the changes in angiotensin-I converting enzyme inhibitory, α-amylase inhibitory and antioxidant activities, total phenolic content, total monomeric anthocyanin content of the extracts from the pulp and seed tissues of *Ziziphus jujuba* were investigated during in vitro gastro-intestinal digestion. It was found that, the pulp parts of the jujube fruit exhibited higher antioxidant activity compared to the seed since the total phenolic and anthocyanin contents in these plant tissues were higher than the seed part. Also, the results of the study showed that angiotensin-I converting enzyme and α-amylase inhibitory activities of the pulp extract increased after *in vitro* digestion. Consequently, it was concluded that jujube fruit can be used as a natural functional compound by food industry.

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