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AN INVESTIGATION OF THE ACE INHIBITORY ACTIVITY, ANTIOXIDANT CAPACITY, AND PHYTOCHEMICAL CONSTITUENTS OF POLAR AND NON-POLAR EXTRACTS OF *ZIZIPHUS JUJUBA* FRUIT: STATISTICAL SCREENING THE MAIN COMPONENTS RESPONSIBLE FOR BIOACTIVITY

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ABSTRACT

Herein, the angiotensin I-converting enzyme (ACE) inhibitory activity, antioxidant capacity, total polyphenol contents (TPC), and phytochemical profiles of polar and non-polar extracts of dried *Ziziphus jujuba* fruits were investigated, along with the statistical determination of the main components responsible for ACE inhibitory activity. The non-polar extract expressed the strongest ACE inhibitory activity (99.81%) among the extracts. The non-polar extract also exhibited the highest DPPH scavenging activity (IC_{50} of 30.63), linoleic acid/β-carotene bleaching capacity (89.31%), and TPC (59.47 mg GAE/g). The phenolic profiles of the extracts were identified by LC-MS/MS, and the presence of seven triterpenoid species in the extracts was examined using GC-MS techniques. The principal constituents included 19 phenolics, 2 organic acids, and 4 triterpenoids. A Pearson correlation and principal component analysis were conducted to find the correlation between individual phenolic compounds and ACE inhibitory activity.

Keywords: *Ziziphus jujuba*, ACE inhibitory activity, antioxidant capacity, phytochemical profile, Pearson correlation, principal component analysis

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ZIZIPHUS JUJUBA (HÜNNAP) MEYVESİNİN POLAR VE APOLAR EKSTRAKLARININ ACE İNHİBİTÖR AKTİVİTESİ, ANTİOKSİDAN KAPASİTESİ VE FİTOKİMYASAL BİLEŞENLERİNİN İNCELENMESİ: BİYOAKTİVİTEDEN SORUMLU ANA BİLEŞENLERİN İSTATİSTİKSEL İNCELENMESİ

ÖZ

Bu çalışmada *Ziziphus jujuba*'nın kurutulmuş meyvelerinin polar ve apolar ekstraktlarının anjiyotensin I-dönüştürücü enzim (ACE) inhibitör aktivitesi, antioksidan kapasitesi, toplam polifenol içerikleri (TPC) ve fitokimyasal profilleri araştırılmıştır ve ACE inhibitör aktivitesinden sorumlu temel bileşenler istatistiksel olarak analiz edilmiştir. En yüksek ACE inhibitör aktivitesi (%99.81) meyvenin apolar ekstraktında tespit edilmiştir. Apolar ekstrakt ayrıca en yüksek DPPH radikal süpürme aktivitesi (IC_{50} : 30,63), linoleik asit/β-karoten ağartma kapasitesi (%89.31) ve TPC'yi (59.47 mg GAE/g) sergilemiştir. Ekstraktların fenolik profilleri LC-MS/MS ile tanımlanmış ve ekstraktlardaki yedi triterpenoid türünün varlığı GC-MS teknikleri kullanılarak incelenmiş ve 19 fenolik, 2 organik asit ve 4 triterpenoid tanımlanmıştır. ACE inhibisyon aktivitesinden sorumlu fenolik bileşenlerin belirlenmesi amacı ile Pearson korelasyonu ve temel bileşen analizi kullanılmıştır.

Anahtar kelimeler: *Ziziphus jujuba*, ACE inhibitör aktivitesi, antioksidan kapasitesi, fitokimyasal profil, Pearson korelasyon analizi, temel bileşen analizi

INTRODUCTION

Hypertension is the most prevalent risk factor for morbidity and mortality in cardiovascular disease, affecting one to three adults worldwide. This common disease leads to stroke, arteriosclerosis, myocardial infarction, renal disease, and numerous other health problems. Angiotensin I-converting enzyme (ACE), a key element in the renin-angiotensin aldosterone system (RAAS), is crucial in managing hypertension. ACE converts inactive angiotensin I to angiotensin II, an effector molecule that narrows blood vessels and inactivates bradykinin, the vasodilator, causing high blood pressure. A group of ACE-inhibiting drugs is considered in the management of hypertension, such as captopril, enalapril, lisinopril, etc. These ACE inhibitors reduce blood pressure by blocking the production of angiotensin II and preventing the constriction of blood vessels. However, these medications have side effects such as extremely low blood pressure, coughing, poor taste, and allergic reactions, while being prescribed frequently because of their well-known function and ease of availability. On the other hand, natural hypertension regulators work well in moderate settings and have few adverse effects, making them a respectable substitute for synthetic medications. Therefore, the search for natural ACE inhibitors has intensified because of these compounds' cost-effectiveness, safety record, and possible positive effects (Memarpoor-Yazdi et al., 2020; Paiva et al., 2023; Zheng et al., 2017).

Nature is a source of an almost limitless variety of molecular entities that can be used to produce new effective medications for a wide range of illnesses, and other valuable bioactive compounds. Although natural products have long been part of conventional medical systems since ancient times, the characterization of compounds extracted from plants and their usage in modern pharmaceuticals only began in the mid-nineteenth century. Since then, 30% of the secondary metabolites of plants have been isolated and their biological activities have been revealed (Wink, 2010). Natural product-derived compounds have emerged as crucial contributors to modern drug development, particularly in antioxidant, antibacterial, and antitumor agents (Hui et al., 2024; Oliveira et al., 2023). Also, it has been well documented that plant-based products act as enzyme inhibitors that bind to enzymes and decrease or block their bioactivity. Many natural products constitute dynamic fields of pharmacology and biochemistry due to the discovery and development of enzyme inhibitors that can be used in the treatment of metabolic disorders (Saleem et al., 2023).

Fruits, representing a wide and diverse spectrum of crops of plant origin, are considered a reservoir of natural bioactive substances with promising health benefits. Many fruits contain a high concentration of anthocyanins, flavonoids, phenolic acids, vitamins, saponins, carotenoids, terpenes, sugars, proteins, capsaicinoids, fatty acids, and alkaloids. Numerous studies have proven that fruit extracts and their active components have different bioactivities (Ruiz Rodríguez et al., 2021). The bioactivity of the plant extract depends on various factors, such as time, temperature, solvent concentration, and solvent polarity. Since no one solvent is likely to reliably extract every phytochemical contained in the plant material, using solvents with different polarities may result in extracting distinct phytochemicals depending on the chemical nature of the compounds. To assure the extraction of a wide range of compounds with varied polarities, the extraction method should involve different solvents of increasing polarity, from non-polar solvents (n-hexane) to more polar solvents (water) (Gil-Martín et al., 2022).

Ziziphus jujuba (*Z. Jujuba*), also called jujuba, is one of the 130–170 species belonging to the Rhamnaceae family, which is distributed throughout Asia and the Mediterranean. *Z. jujuba* is a small tree or shrub that grows in hot and subtropical regions globally and produces a bright red fruit that is utilized as food as well as traditional medicine. The fruit of *Z. jujuba* is rich in fiber, minerals, proteins, sugars, phenolic acids, carotenoids, vitamins (especially vitamin C), flavonoids, cerebroside organic acids, and volatile compounds that provide a pleasant characteristic aroma (Hernández et al., 2016). Various reports have been published on the biological activities of *Z. jujuba* such as the antioxidant, anticancer, antifungal, anti-inflammatory, immuno-stimulant, hepatoprotective, antiobesity, and gastrointestinal protective activities (Li et al., 2020; Zhu et al., 2024)

A limited number of studies have proven that extracts from various parts of the *Z. jujuba* plant exhibit ACE inhibitory activities, and these inhibitory activities are mainly attributed to the presence of phytochemicals in the extracts (Kamkar-Del et al., 2020; Memarpoor-Yazdi et al., 2020; Yücetepe et al., 2023). However, the components responsible for the actual activity have not yet been elucidated completely. Herein, as an initial step in identifying the compounds that may be responsible for the inhibitory activity of *Z. jujuba* for the treatment of hypertension, a chemoinformatic profile was produced employing solvents with varying polarity. The presence of 53 phenolic compounds in dried fruit extracts was comprehensively investigated qualitatively and quantitatively by LC-MS/MS techniques. The presence of seven triterpenoid species in the extracts was examined using GC-MS techniques. The inhibition activities of the extracts against the ACE were investigated. The antioxidant activity of the extracts was determined using DPPH radical-scavenging activity and linoleic acid/β-carotene bleaching assay. The TPC of extracts was also examined using the Folin-Ciocalteu method. A Pearson correlation analysis was conducted to find the correlation between individual phenolic compounds and ACE inhibitory activities. Principal component analysis (PCA) plots were created to show the variance among the different extracts.

MATERIAL AND METHODS

Material

The fresh wild fruits of *Z. jujuba* were purchased from a local market in Mersin, Türkiye. The surface contaminants of the fruits were sorted and washed with sterile distilled water. The seeded fruits were freeze-dried and stored without exposure to light at -80 °C until use.

Moisture content of fruits

The moisture content of fruits was measured using the gravimetric method (Ng et al., 2022). The results were given as percentages (%) as follows (1):

Moisture (%) = (fresh weight -dry weight) / fresh weight x 100 (1)

The final moisture content of the freeze-dried fruits was 13.72±2.59%.

Preparation of polar and non-polar extracts of dried *Z. jujuba*'s fruits

Lyophilized *Z. jujuba* fruits (25 g) were ground to powder in a laboratory blender (Waring Commercial Blender, USA). The yielded powder (3 g) was extracted using 20 mL of either deionized water or a mixture of ethanol and water (50:50, v:v) with the help of sonication in an ultrasonic water bath (SK06GT Kudos ultrasonic water bath, Korea) for 30 minutes. During sonication, the temperature of the ultrasonic bath was kept in the range of 30–40 °C with the addition of ice. The extract was centrifuged at 8,000xg (Hanil Science Industrial Combi 514R, Korea) for 15 minutes. The supernatant was taken into a different tube. 20 mL of ethanol-water (50:50, v:v) or deionized water mixture was added to the pellet and sonicated again. After repeating this process three times, the three supernatants were combined. The extracts were stored at -80 °C until analysis (Meng et al., 2011).

Determination of ACE inhibitory activity of the dried fruit extracts

The ACE inhibition assay was carried out by adapting the method reported by Kwon et al. (2006). 50 μL of the extracts (50 mg dry weight (DW)/mL) were added to 200 μL of NaCl-borate buffer (0.3 M, pH 8.3) containing 2.0 mU ACE-I solution and pre-incubated at 25 ºC for 10 minutes. 100 μL of hippuryl-histidyl-leucine (5.0 mM) solution was added to the reaction mixture and incubated at 37 ºC for one hour. 150 μL of 0.5 N HCl was used to stop the reaction. Lisinopril was used as the standard. The formation of hippuric acid was monitored using HPLC with a UV detector at 228 nm (Kwon et al., 2006). The inhibition percentage was calculated with the following equation:

$$\text{Inhibition\%} = [\text{Area}_{\text{control}} - (\text{Area}_{\text{sample}} - \text{Area}_{\text{blank}})]/(\text{Area}_{\text{control}} - \text{Area}_{\text{blank}}) \times 100 \quad (2)$$

Determination of the antioxidant activity of the dried fruit extracts

The DPPH free radical scavenging and the linoleic acid/β-carotene bleaching assay were used to determine the antioxidant properties of the extracts (Ciniviz & Yildiz, 2020). Each experiment was performed in triplicate. BHA (butylated hydroxyanisole) and BHT (Butylated Hydroxytoluene) were used as standard references.

Total polyphenols content (TPC) of the dried fruit extracts

The TPC of the fruit extracts was assessed by the Folin-Ciocalteu method (Ciniviz & Yildiz, 2020), using gallic acid as a reference compound. The results were expressed as micrograms of gallic acid equivalents per liter of the fruit extracts.

Qualitative and quantitative analyses of phytochemical content of the dried fruit extracts

Determination of the phenolic components using LC-MS/MS

The phenolic composition of the dried fruit extracts was analyzed according to the method developed and validated by Yilmaz et al. (2018) and (Yilmaz, 2020) using a Shimadzu-Neexera model UHPLC (ultra-high performance liquid chromatograph) combined with a Shimadzu-LCMS 8040 model triple quadrupole mass spectrometer. The liquid chromatography system included an analytical column (Inertsil ODS-4 model C18, 100 mm×2,1 mm, 2μm), an autosampler (SIL-30AC model), binary pumps (LC-30 AD model), a degasser (DGU-20A3R model), and a column oven (CTO-10ASvp model). The final concentrations of the extracts were 250 mg/L, and all samples were filtered before the injection. The solvent flow rate was 0.5 mL/min, and the injection volume was 5 mL. The eluent A contained water, 5 mM ammonium formate, and 0.1% formic acid; and the eluent B contained methanol, 5 mM ammonium formate, and 0.1% formic acid. The gradient elution profile was as follows: 20–100% B (0–25 min), 100% B (25–35 min), and 20% B (35–45 min) (Yilmaz et al., 2018).

Determination of the triterpenoid contents using GC–MS

The presence of seven triterpenoids in the dried fruit extracts was assessed using an Agilent 7890A model gas chromatography and an Agilent 5977B model mass spectroscopy system. A HP-5MS column (30 m × 0.25 mm × 0.25 μm film) was used for chromatographic separation. Fixed

helium gas (1 mL/min, 20 psi) was installed as the carrier gas. The GC oven was preheated to 80 °C for 2 minutes, then raised to 300 °C at a rate of 5 °C per minute, where it remained for 14 minutes. The volume of injection was set to 1.0 μL, and the split ratio was 1:10. The ionization energy of the electron ionization/mass spectrometer (EI/MS) was adjusted to 70 eV. MS data were collected by setting the complete scan mode and scan m/z to a density of 50-800 atomic mass units (amu). N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane was used for the derivatization of samples. The concentration of the samples was 1000 mg/L (Bakir et al., 2020).

Statistical analysis

The results were displayed as mean values from the three replications with standard errors (S.E). A one-way analysis of variance (ANOVA) was used to assess the statistical differences between the extracts. Both Levene's test for homogeneity of variances and the Shapiro-Wilk test for normality were conducted. These tests are essential to ensure the validity of our analysis. The results of these tests influenced our approach to the Duncan analysis by providing insights into the distribution and variability of the data among groups. The Duncan multiple comparison test was used to compare mean values, and variations between mean values were defined as significant when $P < 0.05$. SPSS statistics software, version 22.00 (SPSS Inc., Chicago, IL, USA), was used for the statistical analysis. The Pearson correlation coefficient was calculated for correlation analysis ($P < 0.05$) (IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp). Significance was based on a confidence level of 95% ($P < 0.05$). Principal component analysis (PCA) condenses or summarizes the interactions between a large number of variables that are assumed to be connected to a smaller number of fundamental dimensions to visualize and explain the data. PCA was used to correlate the data obtained from phenolic profiles and ACE inhibition. The software R package version 4.2.0 was used to define PCA plots and analyses with the libraries "factoextra", "FactoMineR", "ggcorrplot", <https://CRAN.R-project.org/package=here>.

RESULTS AND DISCUSSION

ACE inhibitory activities of the dried fruit extracts

The inhibition of ACE, a Zn-dependent peptidase that regulates blood pressure, is a crucial therapeutic strategy used in the treatment of hypertension, which is a serious disease that causes cardiovascular, brain, and kidney damage (Wu et al., 2022). The inhibition activities of polar and non-polar extracts against the ACE were determined as 71.84±1.04% and 99.81±2.01%, respectively (Fig.1). The inhibition activity of non-polar extract was as high as the standard, lisinopril, a commercial ACE inhibitor. At the same time, when compared with the literature, the ACE inhibition activity of the whole fruit (99.81±2.01%) was found to be higher than the ACE inhibition activity of methanol extracts obtained from the seeds (86.04±0.00%) and pulp (42.74±8.57%) of the fruit by ultrasound-assisted extraction method (Şensu et al., 2023). The results of the present study showed that the dried fruit extracts of *Z. jujuba* are a promising source to be used as a dietary supplement or a pharmaceutical reagent for the treatment of hypertension.

Antioxidant activity and total polyphenol contents of the dried fruit extracts

The DPPH radical scavenging activity and linoleic acid/β-carotene bleaching assay were used as tools to compare the antioxidative activities of the polar and non-polar extracts. BHA and BHT were used as standards. The antioxidant activity results of the extracts are presented in Fig.2A. The antioxidant activity of non-polar extracts was by far higher than that of polar extracts. This difference detected between extracts could be due to the type of polyphenols released into the non-polar solvent during extraction. Additionally, the activity of the non-polar extract may be attributed to other phenolic compounds that cannot be screened due to the lack of standards.

The results showed that the radical scavenging capacities of the polar and non-polar extracts were 170.30 ± 2.88 and 30.64 ± 1.37 μg/mL (DPPH, IC_{50}) and 12.08 ± 0.97% and 89.31 ± 1.59% (β-carotene). In a study, the antioxidant activities of the polar and non-polar extracts of *Z.*

jujube furit (without seed) were reported to be 400 µg/mL and 300 µg/mL (DPPH, IC_{50}) (Lin et al., 2020). Since the antioxidant activity of a fruit is affected by many factors such as variety, soil, water, altitude, extraction method and antioxidant assays, it is difficult to directly compare activities between studies (Zargoosh et al., 2019; Zhu et al., 2024).

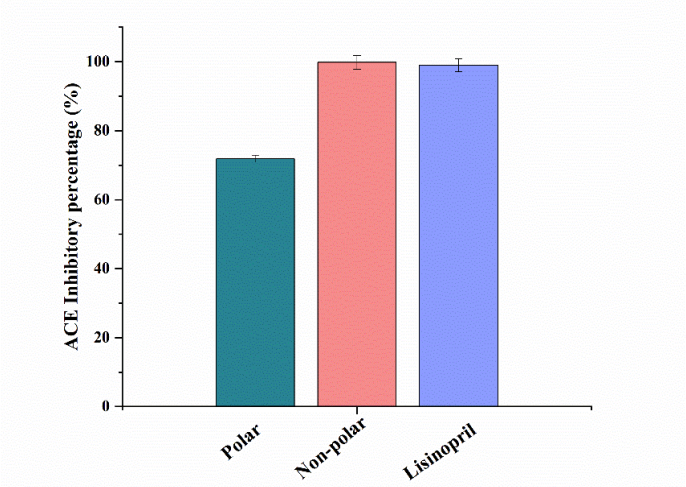

Figure 1. ACE inhibition activity of the dried fruit extracts of *Z. jujuba*. Results are expressed as mean ± standard deviation (n = 3)

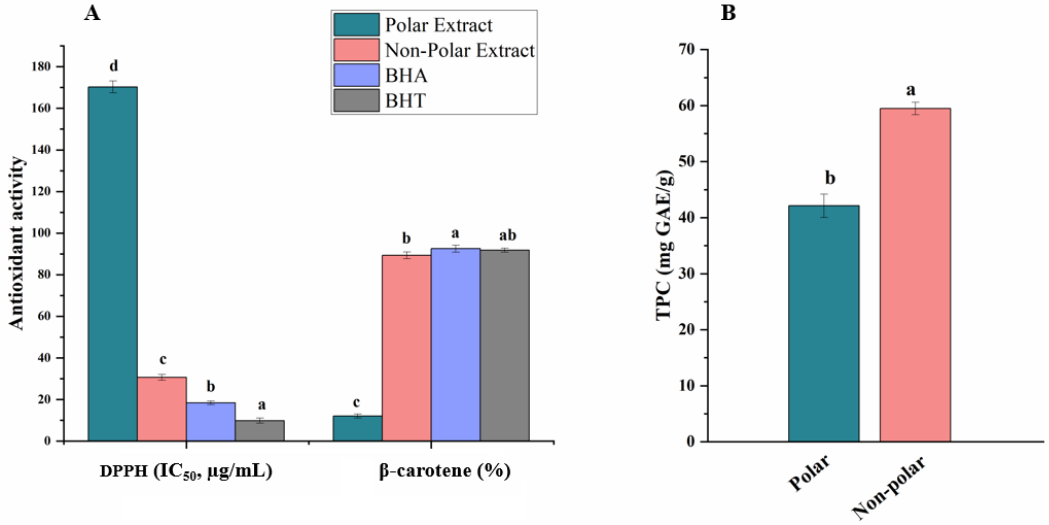

Figure 2. Antioxidant activity (A) and total phenolic content (B) of the dried fruit extracts of *Z. jujuba*. Results are expressed as mean ± standard deviation (n = 3). Different lowercase letters denote a significant difference between samples in the same group ($P < .001$)

The TPC of the extracts is shown in Fig. 2B. The TPC of the non-polar extract (59.47±1.12 mg GAE/g) was higher than that of the polar extract (42.13±2.08 mg GAE/g) ($P < 0.05$). The effects of solvent polarity on TPC showed results similar to those of this parameter's influence on the samples' antioxidant activity. Al-saeedi et al. (2016) reported that the TPC of fruit extracts obtained from *Z. jujuba* using different solvents (methanol, hexane, ethyl acetate, chloroform, and water) varied between 16.93 - 187.51 mg GAE/g and the TPC of water extract was 18.17 mg

GAE/g. Similar to our results, the amount of TPC was found to be higher in the nonpolar extracts than in the polar extract. Due to the different solvents used, a complete comparison could not be made, but the TPC of the water extract was reported to be much lower than in the present study. (Al-Saeedi et al., 2016).

Phenolic profiles of the dried fruit extracts

To obtain a more detailed phenolic profile of the fruit of *Z. jujuba*, two solvents with different polarities were used for the extraction process, and the phenolic profile of the extracts was analyzed by LC-MS/MS in comparison with 53 reference compounds. 21 of the phenolic species were discovered in at least one of the extracts at different levels. Phenolic compounds found in the extracts are listed in Table 1. The non-polar extract was found to have a higher diversity of phenolic compounds (eighteen distinct species) than the polar extract (thirteen distinct species). On the other hand, when the concentration of species detected in the two extracts was compared, the polar extract generally possessed amounts of phenolics that were far higher than the non-polar extract. The major components detected in the extracts are as follows: Polar extract: Quinic acid (84.16 mg/100 g DW), epicatechin (21.26 mg/100 g DW), fumaric acid (10.36 mg/100 g DW), catechin (5.90 mg/100 g DW), aconitic acid (4.04 mg/100 g DW), rutin (2.18 mg/100 g DW); Non-polar extract: Quinic acid (36.88 mg/100 g DW), protocatechuic acid (2.90 mg/100 g DW). Among the hydroxycinnamic acids investigated, quinic acid was the most abundant in the polar extract, with a level of 84.26 mg/100 g DW. In contrast, caffeic acid and p-coumaric acid were only detected in the non-polar extract. Three hydroxybenzoic acids (gallic acid, protocatechuic acid, and salicylic acid) are primarily found in non-polar extracts. Furthermore, epicatechin, catechin, and rutin were the most abundant flavonoids in the polar extract, with levels of 21.26, 5.90, and 2.18 mg/100 g DW, respectively. Remarkably, the lower values for all organic acids (fumaric acid and aconitic acid) were obtained in the non-polar extract. Wang et al. (2016) investigated the changes in phenolic compounds of *Z. jujube* fruits at three different edible maturity stages, using 12 standard phenolics. The results showed that the phenolic compounds varied with the ripening stage, and the most dominant flavonoid among the phenolics examined was rutin, and the most dominant phenolic acid was caffeic acid, followed by gallic acid, chlorogenic acid, and *p*-coumaric acid (Wang et al., 2016). In a study conducted by Yan et al. (2022), catechin, epicatechin and rutin were reported as the dominant species in *Z. jujube* fruit among 15 phenolic compounds (Yan et al., 2022). Quinic acid, which was found to be the most dominant species in the present study, was not used as a standard in either study.

Triterpenoid contents of the dried fruit extracts

Triterpenoids are a diverse group of secondary metabolites found in plants. They exhibit a broad spectrum of potential pharmacological effects combined with a low toxicity profile. Triterpenoids are thought to be the primary functional constituents in *Z. jujuba* fruit. However, the content of triterpenoids varies considerably from region to region and from cultivar to cultivar (Pan et al., 2023). In the present study, seven triterpenoid species were screened in the fruit extracts using GC/MS. The results are given in Table 2. Oleanonic acid, oleanolic acid, betulinic acid, and ursolic acid were detected in the non-polar extract. Oleanonic acid was the dominant triterpenoid acid in the fruit of *Z. jujuba,* with a level of 14.116 mg/100 g DW. A lower concentration of these acids in *Z. Jujuba* fruit was previously reported by Song et al. (2020) as follows: betulinic acid (516.41–4097.96 μg/g DW), oleanolic acid (36.70–837.46 μg/g DW), ursolic acid (5.27–685.33 μg/g DW), oleanonic acid + ursonic acid (9.83–244.80 μg/g DW) (Song et al., 2020). In the present study, none of the species were detected in the polar extract. Due to their low polarity, the detected triterpeneoids are practically insoluble in water and the extraction process requires the use of organic solvents (Castellano et al., 2022).

Table 1. Phenolic profiles of polar and non-polar extracts of the dried fruit of *Z. jujuba*

Reference Phenolic Compound	M.I. (m/z)[a]	F.I. (m/z)[b]	U[c]	Quantification (mg/100 g DW)	
Simple Phenols				Polar extract	Non-polar extract
Phenolic acids					
Hydroxycinnamic acids					
Caffeic acid	179.0	134.0	0.0354	ND	0.04
p-Coumaric acid	163.0	93.0	0.0516	ND	0.36
Quinic acid	190.8	93.0	0.0082	84.26	36.88
Hydroxybenzoic acids					
Gallic acid	168.8	79.0	0.0282	0.04	0.16
Protocatechuic acid	152.8	108.0	0.0411	0.28	2.90
Salicylic acid	137.2	65.0	0.0329	ND	0.04
Coumarins					
Coumarin	146.9	103.1	0.0237	ND	0.04
Polyphenols					
Flavonoids					
Flavones					
Luteolin	284.8	151.0/175.0	0.0174	ND	0.01
Flavonols					
Kaempferol	285.0	239.0	0.0209	ND	0.14
Nicotiflorin	592.9	255.0/284.0	0.0276	1.14	0.54
Rutin	608.9	301.0	0.0159	2.18	1.22
Quercetin	301.0	272.9	0.0543	0.04	0.64
Flavanones					
Hesperidin	611.2	449.0	0.0262	1.78	1.00
Hesperetin	301.0	136.0/286.0	0.0562	ND	0.04
Naringenin	270.9	119.0	0.0521	0.004	ND
Flavanols					
Catechin	288.8	203.1	0.0221	5.90	ND
Epicatechin	289.0	203.0	0.0221	21.26	ND
Non-Flavonoids					
Tannins					
Tannic acid	182.8	78.0	0.019	ND	0.36
Hydroxybenzaldehydes					
Protocatechuic aldehyde	137.2	92.0	0.0396	0.072	1.196
Organic acids					
Fumaric acid	115.2	40.9	0.0124	10.36	1.988
Aconitic acid	172.8	129.0	0.0247	4.03	0.222
Rutin-D3-IS[d]	612.2	304.1	ND	IS	IS
Ferulic acid-D3-IS[d]	196.2	152.1	ND	IS	IS
Quercetin-D3-IS[d]	304.0	275.9	ND	IS	IS

[a] MI (m/z): Molecular ions of the standard analytes (m/z ratio).

[b] FI (m/z): Fragment ions.

[c] U (%): percent relative uncertainty at 95 % confidence level (k = 2).

ND: Not determined.

Epigallocathechin, gentisic acid, chlorogenic acid, epigallocatechingallate, 1,5-dicaffeoylquinic acid, 4-hydroxybenzoic acid, vanilic acid, syringic acid, vanillin, syringic aldehyte, daidzin, epicatechingallate, piceid, ferulic acid, sinapic acid, cynaroside, miquelianin, isoquercitrin, *o*-Coumaric acid, genistin, rosmarinic acid, elagic acid, cosmosiin, quercitrin, astragalin, fisetin, daidzein, genistein, apigenin, amentoflavone, chrysin, acacetin were not detected either of the extracts.

Table 2. Triterpenoid contents of the fruit extracts by GC-MS

Compounds	R_t[a]	Molecularion-m/z (relative intensity %) (m/z)[b]	% RSD[c]	Three major fragment ions m/z (relative intensity %)			Polar extract (mg/100 g DW)	Non-polar extract (mg/100 g DW)
Alphaamyrin	17.99	498 (2.5)	0.025	218(100)	203(16.6)	189(18.3)	ND[d]	ND
Moronic acid	20.71	527 (21.1)	0.029	189(100)	203(40.3)	409(24.3)	ND	ND
Oleanonic acid	20.96	527 (12.3)	0.023	203(100)	408(64.5)	189(52.6)	ND	14.116
Oleanolic acid	21.55	601 (2.3)	0.026	203(100)	189(31.5)	320(28.6)	ND	8.158
Betulinic acid	21.90	601 (4.9)	0.019	189(100)	203(34.5)	320(21.8)	ND	6.778
Ursolic acid	22.55	601 (2.3)	0.015	203(100)	189(32.9)	320(79.6)	ND	9.082
Ursonic acid	22.91	527 (9.5)	0.028	203(100)	320(60.4)	189(24.9)	ND	ND

[a]Rt: Retention time.
[b]Mother ion(m/z): Molecular ions of the standard compounds (m/z ratio).
[c]RSD: Relative standard deviation
[d]ND: Not detected

Correlation analysis

Pearson's Correlation

The potential relationships between specific phenolic components and the enzyme-inhibitory properties of the polar and non-polar extracts of *Z. jujuba* fruit were highlighted using Pearson's correlation analysis (Fig.3). The direction of the correlation is either positive (acquiring a positive relationship) or negative (acquiring a negative relationship). 21 of the phenolic compounds identified in the fruit extracts showed a high correlation with the inhibition of ACE. Caffeic acid, coumarin, gallic acid, hesperetin, kaempferol, luteolin, *p*-coumaric acid, protocatechuic acid, protocatechuic aldehyde, quercetin, salicylic acid, and tannic acid showed a high positive correlation with ACE inhibitory activity. On the other hand, aconitic acid, catechin, epicatechin, fumaric acid, hesperidin, naringenin, nicotiflorin, quinic acid, and rutin showed a high negative correlation with ACE inhibitory activity.

Several studies were conducted on the anti-hypertensive properties of extracts obtained from different parts of plants. Also, several reports investigating the ACE inhibitory activity of individual phenolics revealed that caffeic acid (Agunloye & Oboh, 2018), coumarin (Ali et al., 2019), hesperetin (Yamamoto et al., 2008), protocatechuic acid (Safaeian et al., 2018), kaempferol, luteolin, and quercetin (Guerrero et al., 2012) have ACE inhibitory activity, consistent with our results. On the other hand, it has been reported that rutin, which was found to have a negative correlation in the present study, showed a dose-dependent ACE inhibitory activity; increasing the concentration from 100 μM to 500 μM increases the inhibition activity from 36% to 87% (Guerrero et al., 2012). The positive correlation of *p*-coumaric acid, protocatechuic aldehyde, salicylic acid, and tannic acid in ACE inhibitory activity has been shown for the first time in the literature with the presented study.

Principle Component Analysis

PCA analysis evaluated the relationship between individual phenols and ACE inhibitory activities. The first principle component (Dim 1) explained 99.8% of the total variance, while the second principle component (Dim 2) explained 0.2% of the variance. Together, PCs 1 and 2 accounted for 100% of the variance. As delineated in Fig.4, both positions of each phenolic in terms of the positive and negative sides of the axis are used to visualize which phenolics are positive contributors and which are not. A closer arrow denotes a high correlation, whereas the length of the arrows shows which variable contributes the most to the principle component. As shown in the PCA diagram, along axis 1 of the PCA analysis, 12 phenolics were grouped on the positive side and strongly contributed to ACE inhibitory activity. In the negative part of axis 1, nine phenolics formed an additional group, indicating a negative correlation with the ACE inhibitory activity. Upon examination of the biplot, it is apparent that ACE demonstrates a robust positive correlation with coumarin, protocatechuic acid, hesperetin, kaempferol, luteolin, protocatechuic aldehyde,

quercetin, *p*-coumaric acid, tannic acid, gallic acid, salicylic acid, and caffeic acid. Conversely, a negative correlation exists between ACE and aconitic acid, catechin, epicatechin, fumaric acid, hesperidin, naringenin, nicotiflorin, quinic acid, and rutin. These findings provide valuable insights into the associations between ACE inhibitory activities and specific compounds present in the polar and non-polar extracts of *Z. jujuba* fruit.

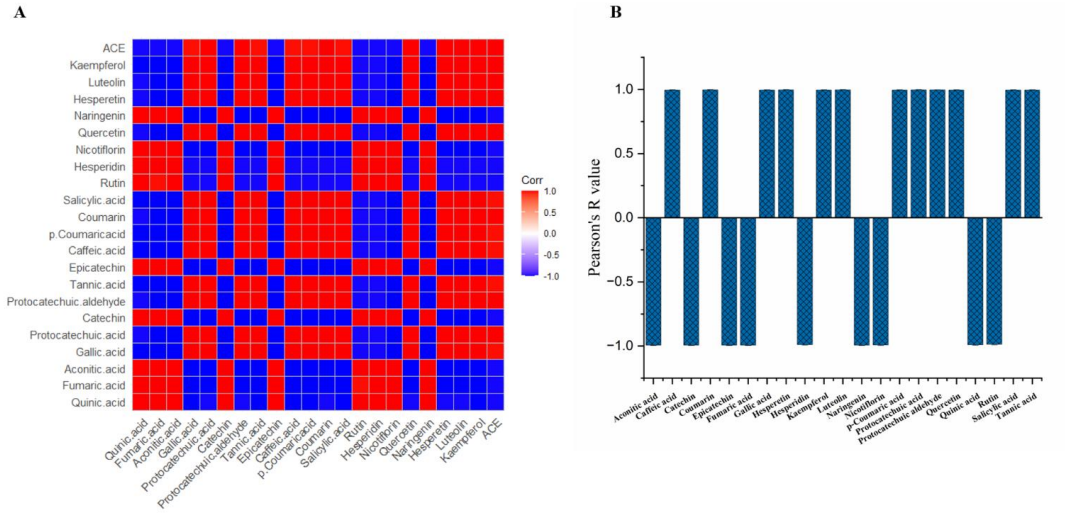

Figure 3. Pearson correlation of the phenolic compounds and ACE inhibitory activity in the dried fruit extracts of *Z. jujuba* (A) and bar graph of Pearson's correlation coefficient (B). The red and blue color's intensity represents higher to lower correlation levels. A correlation coefficient of +1 indicates a perfect positive correlation.

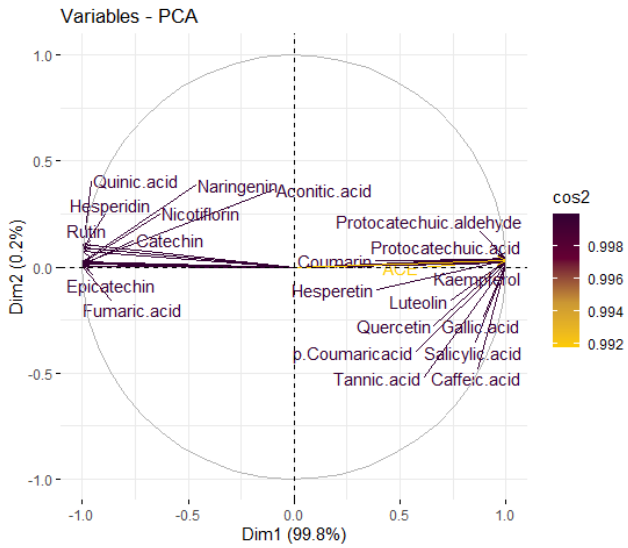

Figure 4. Principal component analysis of the phenolic compounds and ACE inhibitory activity in the dried fruit extracts of *Z. jujuba*. The figure depicts a biplot generated from data matrices representing the polar and non-polar extracts of *Z. jujuba* fruit in conjunction with ACE inhibitory activities. In a PCA biplot, the angle between the vectors representing the original variables serves as an estimate of the correlation between those variables. A slight angle signifies a positive correlation, while an angle near 180 degrees suggests a negative correlation

CONCLUSION

In the present study, ACE inhibitory activity, the phytochemical profile, radical scavenging capacity, and total phenolic content of polar and non-polar extracts obtained from the fruit of *Z. jujuba* were investigated, and the main components responsible for ACE inhibitory activity were revealed through statistical analysis. It was proven that the non-polar extract of the fruit of *Z. jujuba* exhibited excellent ACE inhibitory activity, radical scavenging capacity, and total phenolic content. The non-polar extract also contained high amounts of oleanonic acid, oleanolic acid, betulinic acid, and ursolic acid. The finding highlights the ACE inhibitory effects of individual phenolics, including caffeic acid, coumarin, gallic acid, hesperetin, kaempferol, luteolin, *p*-coumaric acid, protocatechuic acid, protocatechuic aldehyde, quercetin, salicylic acid, and tannic acid. Based on our results, the fruit of *Z. jujuba* could be a promising natural supplement for the treatment of hypertension. Both the fruit of *Z. Jujuba* and its by-products have the potential to be used in the pharmaceutical and food industries for future innovations.

CONFLICT OF INTEREST

The author(s) declares no conflict of interest.

AUTHORS' CONTRIBUTIONS

Bahar Tuba Findik: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. Hilal Yildiz: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. Esma Birisci: Methodology, Formal analysis, Data curation, Review and Editing. Serkan Yiğitkan: Methodology, Formal analysis. Pelin Köseoğlu Yılmaz: Methodology, Formal analysis. Abdulselam Ertaş: Methodology, Formal analysis, Data curation, Review and Editing. All authors approved the final manuscript and accepted to be held responsible for the content.

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