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Determination of phytoconstituents of leaf extracts of jujube tree leaves pre-fruit and post-harvest

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Research Article

ABSTRACT

Background and Aims *Ziziphus jujuba* Mill. leaves have long been utilized in traditional medicine; however, information regarding seasonal variations in their phytochemical profile remains limited. The present study aimed to comparatively evaluate the phytochemical composition of jujube leaves collected during the pre-fruiting (spring) and post-harvest (autumn) periods in the Isparta region.

Methods Volatile constituents were characterized using solid-phase microextraction (SPME) followed by appropriate chromatographic analysis. Total antioxidant capacity was determined by the CUPRAC assay, while total phenolic content was quantified using the Folin-Ciocalteu method. Saponin concentrations were measured spectrophotometrically. Fatty acids were derivatized to their methyl esters and analyzed by gas chromatography coupled with flame ionization detection (GC-FID).

Results A total of 18 volatile compounds were identified in pre-fruiting leaves, whereas 14 components were detected in post-harvest samples. Saponin content increased from 2.49 mg mL^{-1} in the pre-fruiting period to 4.29 mg mL^{-1} after harvest. Antioxidant capacity and total phenolic content analyses further confirmed that jujube leaves possess a rich phytochemical composition, with marked seasonal fluctuations.

Conclusion The findings demonstrate that jujube leaves constitute a significant source of bioactive compounds and that their phytochemical profile varies substantially between growth stages. These results highlight the importance of harvest timing in optimizing the functional and industrial utilization potential of jujube leaves.

Key Words: *Ziziphus zizyphus*, jujube, leaf, volatile, saponin, phytochemical

Meyve öncesi ve hasat sonrası dönemde hünnap ağacı yapraklarının fitokimyasal bileşenlerinin belirlenmesi

ÖZ

Giriş ve Hedefler Hünnap (*Ziziphus zizyphus*) yaprakları yüzyıllardır geleneksel ve tıbbi amaçlarla kullanılmaktadır. Bununla birlikte, yaprakların fitokimyasal bileşiminin mevsimsel değişime bağlı olarak nasıl farklılaştığına ilişkin bilgiler sınırlıdır. Bu çalışmanın amacı, Isparta yöresinde yetişen hünnap ağaçlarının ilkbahar (meyve öncesi) ve sonbahar (hasat sonrası) dönemlerinde toplanan yapraklarının fitokimyasal içeriklerini karşılaştırmalı olarak incelemektir.

Yöntemler Yapraklardaki uçucu bileşenler katı faz mikroekstraksiyon (SPME) yöntemi ile analiz edilmiştir. Toplam antioksidan kapasite CUPRAC yöntemiyle, toplam fenolik madde miktarı ise Folin-Ciocalteu yöntemiyle belirlenmiştir. Saponin içeriği kantitatif olarak tayin edilmiş, yağ asitleri metil esterlerine dönüştürülerek GC-FID cihazı ile analiz edilmiştir.

Bulgular Meyve öncesi dönemde 18, hasat sonrası dönemde ise 14 uçucu bileşen tespit edilmiştir. Saponin miktarı meyve öncesinde 2,49 mg/mL iken hasat sonrasında 4,29 mg/mL olarak belirlenmiştir. Antioksidan kapasite ve toplam fenolik içerik analizleri, hünnap yapraklarının zengin bir fitokimyasal profile sahip olduğunu göstermiştir.

Sonuçlar Elde edilen bulgular, hünnap yapraklarının önemli düzeyde biyoaktif bileşen içerdiğini ve bu içeriğin mevsimsel değişimlere bağlı olarak anlamlı farklılıklar gösterdiğini ortaya koymaktadır.

Sonuçlar, hasat zamanının bitkisel hammaddenin fonksiyonel ve endüstriyel kullanım potansiyeli açısından kritik bir faktör olduğunu göstermektedir.

Anahtar Kelimeler: *Ziziphus zizyphus*, hünnap, yaprak, uçucu bileşenler, saponin, fitokimyasal içerik

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1. Introduction

Ziziphus zizyphus Mill. (jujube) is a thorny deciduous tree typically reaching 4-5 meters in height and flowering between April and May with fragrant yellow inflorescences. The fruits are characterized by a red-brown pericarp and a hard endocarp; wild genotypes bear smaller fruits, whereas grafted cultivars produce larger, commercially valuable drupes. Although often associated with Syria as its historical origin, jujube has been cultivated in China for more than 4000 years, where extensive domestication has resulted in over 400 reported varieties (Yılmaz, 2019). Its distribution extends across India, Russia, Southern Europe, North Africa, the Middle East, and Anatolia, and since its introduction to the United States in 1837, it has been successfully cultivated in semi-arid regions of the southwest.

Ecologically, jujube is a temperate species capable of tolerating harsh environmental conditions. It can grow at elevations up to 1700 m above sea level, withstand temperatures as low as -20 °C, and thrive in sandy-loam, neutral to slightly alkaline soils. The species exhibits notable drought tolerance and adapts to annual precipitation ranges between 120 and 2200 mm (Kavas and Dalkılıç, 2015). Such ecological plasticity suggests a well-developed secondary metabolism, as stress tolerance in woody perennials is often linked to the biosynthesis of protective phytochemicals.

Beyond its agronomic resilience, *Z. zizyphus* has long attracted attention for its multifunctional uses. Outlaw et al. (2002) emphasized that few plant species exhibit comparable versatility: its durable wood is used in artisanal and structural applications; its fruits serve nutritional and medicinal purposes; its nectar supports apiculture; and various plant organs - including leaves, bark, roots, and seeds - have been employed in traditional medicine for over three millennia. While fruit-focused research has dominated literature, increasing scientific interest has shifted toward the leaves, which represent an abundant yet underutilized biomass resource.

Phytochemical studies have confirmed that jujube leaves are rich in bioactive secondary metabolites. Elaloui et al. (2016) reported substantial levels of polyunsaturated fatty acids, phytosterols (β-sitosterol and stigmasterol), and flavonoids such as rutin and apigenin, supporting potential applications in cosmetic and pharmacological formulations. Guo et al. (2011) identified a diverse profile of triterpenic acids, saponins, and flavonoids - including quercetin-3-O-rutinoside - highlighting the leaves as a promising natural source of compounds with antioxidant and therapeutic potential. Complementing these findings, Damiano et al. (2017) demonstrated significant antioxidant and antibiofilm activity in jujube leaf infusions, proposing their incorporation into oral health products and functional beverages.

From a biochemical perspective, the production and accumulation of phenolics, saponins, terpenoids, and volatile compounds are closely associated with plant defense strategies and environmental adaptation. Theoretical frameworks such as the Growth - Differentiation Balance Hypothesis and Optimal Defense Theory propose that secondary metabolite allocation is dynamically regulated according to developmental stage and resource availability. In perennial woody species, seasonal transitions often trigger shifts in carbon partitioning and oxidative stress responses, potentially leading to enhanced synthesis of protective metabolites during later phenological stages.

Despite the recognized importance of temporal variation in secondary metabolism, systematic comparisons of pre-fruiting and post-harvest leaf chemistry in *Z. zizyphus* remain scarce, particularly under region-specific cultivation conditions. In Mediterranean agroecosystems, where seasonal drought and temperature fluctuations are pronounced, phenological stage may significantly influence metabolite allocation patterns. Building upon these theoretical and ecological considerations, we hypothesize that post-harvest (autumn) leaves accumulate higher levels of defensive secondary metabolites - such as saponins and antioxidant-related phenolics - compared to pre-fruiting (spring) leaves, reflecting resource reallocation and stress-adaptive metabolic regulation during late developmental stages.

In parallel with phytochemical interest, global environmental challenges have intensified the search for sustainable resource utilization strategies. The valorization of agricultural residues and plant-derived by-products is increasingly recognized as a key component of circular bioeconomy models and cleaner production systems (Erdoğan and Karaboyacı, 2021; Karaboyacı et al., 2017; Gülsoy et al., 2014). In jujube cultivation systems, leaves generated during seasonal pruning or post-harvest senescence are often discarded as waste, despite their potential as sources of high-value bioactive compounds. Accordingly, the present study aims to comprehensively investigate seasonal changes in the phytochemical composition of *Z. zizyphus* leaves collected during the pre-fruiting (spring) and post-harvest (autumn) stages in the Isparta region.

2. Material and Methods

Z. zizyphus fresh leaves were collected from the cultivated fields of Isparta Gönen region on 10 June and 10 November. The leaves were saved in the shade at room temperature and ground for aallysis with the aid of a rondo. Powdered leaves (10.0 g) was extracted at room temperature with 100 mL distilled water for 12 hours and filtered.

2.1 Determination of total saponins

The total saponins contents of *Z. zizyphus* leaves extracts were determined by the vanillin-sulfuric acid method (Hiai et al., 1976). 0,25 mL of extracts were reacted with 0,25 mL vanillin/ethanol (8%) and 2 mL sulfuric acid (72%). Then the mixture was incubated at 60 °C for 10 min. After the incubation mixture was cooled for another 15 minutes at room temperature, followed by absorbance measurement at 538 nm. Quillaja saponin was used as a standard and the content of total saponins was expressed as Quillaja equivalents.

2.2 Determination of volatile components

GC-MS SPME technique was used in the analysis of the volatile components of the leaf extracts. The SPME technique is a simple and sensitive sample preparation method that extracts

the components in the sample to be analyzed by absorbing the fiber coated with a thin polymeric stationary phase on silica.

2.3 Preparation of ethanolic extracts for phenolic content analysis

Autumn leaf and spring leaf extracts were prepared using 96% ethanol. 5 grams dry powdered samples were soaked 50 mL in ethanol (96%) in an ultrasonic-assisted water bath for 1 hour at 40°C, with intermittent shaking. Extracts were further washed with fresh ethanol (30 mL and 20mL) and were filtered through Whatman No. 41 filter paper. Then the filtrates were collected and the final volume was made up to 100 mL. The filtrates kept in obscurity at +4°C until use.

2.4 Determination of total phenolic content (TPC)

Total phenolic content (TPC) of the spring and autumn leaf extracts was quantified using the Folin–Ciocalteu colorimetric assay, following a modified protocol based on Slinkard and Singleton (1977). Gallic acid was employed as the reference standard for calibration. Briefly, 40 µL of sample extract, gallic acid standard solution, or blank (deionized water) was transferred into a 15 mL centrifuge tube. Subsequently, 3.16 mL of distilled water and 200 µL of Folin–Ciocalteu reagent was added, and the mixture was homogenized by vortexing or gentle inversion. After an initial reaction period of 1–8 minutes, 600 µL of 20% (w/v) sodium carbonate solution was introduced. The reaction mixture was then allowed to stand for 2 hours at room temperature with intermittent shaking to ensure complete color development. Absorbance was recorded at 765 nm using a UV–Vis spectrophotometer (Shimadzu UV-1280). A calibration curve was constructed using 40 µL aliquots of gallic acid solutions at concentrations of 25, 50, 100, 200, and 400 µg mL^{-1}. Total phenolic content was expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE g^{-1}). The calibration equation was determined as: Absorbance = 0.0011 × Total phenols (mg GAE) + 0.0174 (R^2 = 0.9994). All measurements were performed in triplicate to ensure analytical reliability.

2.5 Lipid extraction

Autumn and spring leaf extracts were prepared using cyclohexane. 10 grams dry powdered samples were soaked 100 mL in cyclohexane in an ultrasonic-assisted water bath for 1 hour at 50°C, with intermittent shaking and were filtered through Whatman No. 41 filter paper. Then the filtrates were concentrated under vacuum using a rotary evaporator under reduced pressure at 55 °C. The obtained extracts were kept at 4 degrees until used in analysis.

2.6 GC analysis of fatty acids

Fatty acid methyl esters (FAMEs) were generated in accordance with the IUPAC (1987) standard protocol for fatty acid derivatization (American Oil Chemists' Society, 1997). Gas chromatographic analyses were performed following the instrumental conditions previously described by Erdoğan and Gökçe (2021). Briefly, FAMEs were analyzed using a Shimadzu GC-2010 gas chromatograph equipped with a flame ionization detector (FID) and a TR-CN100 capillary column (100 m × 0.25 mm i.d., 0.20 µm film thickness; Teknokroma). The oven temperature was initially maintained at 140°C for 10 minutes, increased to 240°C at a rate of 3°C min^{-1}, and then held at 240°C for 15 minutes. Injector and detector temperatures were set at 250°C and 260°C, respectively. Nitrogen was used as the carrier gas at a flow rate of 40 mL min^{-1}, with a split ratio of 1:20. Individual fatty acids were identified by comparing their relative retention times with those of a certified commercial FAME standard mixture (Supelco® 37 Component FAME Mix, Sigma-Aldrich). The relative composition of the detected fatty acids was expressed as percentage of total oil content, with particular emphasis on palmitic, stearic, oleic, linoleic, and linolenic acids.

2.7 Determination of total antioxidant capacity by CUPRAC method

The CUPRAC method, developed by Apak et al (2004), was used in the study. This method is a widely applicable antioxidant capacity determination method, such as plasma antioxidants, flavonoids, and food polyphenols, using a chromogenic oxidant Cu (II)-neocuproin (Nc) reagent. Appropriately positioned phenolic hydroxyls are converted into corresponding quinone structures by the CUPRAC redox reaction, and the Cu(I)-Nc chelate formed as a result of this redox reaction gives maximum absorbance at 450 nm. The resulting color is the result of a charge transfer in the metal→ligand direction.

According to this method, 1 mL of copper (II) solution, neocuproin solution and ammonium acetate buffer are added into a glass tube, respectively. 0.5 mL antioxidant solution and 0.6 mL distilled water were added, and the tubes were shaken well. The solutions prepared with a total volume of 4.1 mL were kept closed for 30 minutes at room conditions. At the end of this period, absorbance values were measured at 450 nm against the reference solution without sample. Three parallel test groups were prepared for each measurement. Measurements made against the reference solution at 450 nm were recorded.

3. Results and Discussion

Visual inspection revealed noticeable differences between seasonal extracts. Extracts obtained from spring leaves appeared lighter in color, whereas post-harvest extracts exhibited a darker tone, suggesting increased pigment and secondary metabolite accumulation. The presence of a stable foam layer in both extracts qualitatively indicated saponin content.

Quantitative analysis confirmed significant seasonal variation in total saponin concentration, which increased from 2.49 mg mL^{-1} in pre-fruiting leaves to 4.29 mg mL^{-1} in post-harvest leaves. This substantial elevation indicates that saponin biosynthesis in *Z. zizyphus* is developmentally regulated and intensifies during later phenological stages.

Saponins are widely recognized as defensive secondary metabolites involved in herbivore deterrence, pathogen resistance, and oxidative stress mitigation. Their accumulation is often associated with metabolic reallocation during senescence, when reduced photosynthetic activity allows carbon resources to be redirected toward differentiation-related pathways. In perennial woody species, late-season enhancement

of triterpenoid and saponin production has frequently been linked to increased exposure to abiotic stress factors such as temperature fluctuations and water limitation.

The elevated saponin levels observed in autumn leaves therefore likely reflect an adaptive metabolic response, consistent with theoretical frameworks such as the Growth–Differentiation Balance Hypothesis, which predicts increased allocation to secondary metabolites under reduced growth conditions. From an applied perspective, the marked seasonal enrichment of saponins underscores the potential of post-harvest leaf biomass - typically regarded as agricultural residue - as a concentrated source of bioactive triterpenoid compounds, supporting its valorization within sustainable production systems. In parallel with saponin variation, pronounced differences were observed in volatile composition. The volatile profile of post-harvest leaves is summarized in Table 1. A total of 14 compounds were detected, with hexane, benzofuran, eugenol, and dihydropseudoionone emerging as dominant constituents, collectively accounting for approximately 69.7% of the total volatile fraction. The predominance of these compounds suggests a restructuring of volatile metabolism during late developmental stages.

Table1. Volatile components of jujube leaves after harvest season in November

Peak	Name	Area
1	Ethyl alcohol	4.76
2	Propanenitrile, 2-hydroxy-2-methyl- (CAS) Acetone cyanohydrin	3.16
3	2,3-Butanedione (CAS) Diacetyl	2.68
4	Hexane (CAS) n-Hexane	21.38
5	1-Propene, 3-bromo- (CAS) Allyl bromide	2.33
6	2-Hexenal, (E)- (CAS) (E)-2-Hexenal	2.06
7	6-Methyl-5-hepten-2-one	3.33
8	dl-Limonene	2.05
9	6-Octen-1-ol, 3,7-dimethyl-, formate (CAS) Citronellyl formate	4.89
10	Benzofuran, 2,3-dihydro-	18.76
11	Guaiacol 4-vinyl-	5.04
12	Eugenol	19.68
13	5,9-Undecadien-2-one, 6,10-dimethyl- (CAS) Dihydropseudoionone	9.88

Eugenol, a phenylpropanoid derivative widely associated with antimicrobial and antioxidant properties, may contribute to the enhanced defensive chemical profile detected in autumn leaves. Benzofuran derivatives and ionone-related compounds have similarly been implicated in stress-mediated metabolic pathways and oxidative regulation. Their relative enrichment in post-harvest samples further supports the interpretation that senescence-associated metabolic transitions favor the accumulation of defense-oriented volatiles. In contrast, pre-fruiting leaves exhibited a greater diversity of volatile constituents (18 compounds), reflecting a broader but more evenly distributed metabolic configuration during active growth. Such seasonal reorganization of volatile profiles aligns with dynamic regulation of terpene and phenylpropanoid biosynthesis observed in other perennial species.

Taken together, these results reinforce the hypothesis that secondary metabolite allocation in *Z. zizyphus* leaves is not static but undergoes structured seasonal modulation. From a valorization standpoint, the dominance of bioactive volatiles such as eugenol enhances the industrial potential of post-harvest leaf biomass for applications in natural preservatives, functional formulations, and phytochemical extraction processes.

The volatile composition of pre-fruiting (spring) leaves is presented in Table 2. A total of 18 compounds were identified following SPME analysis, indicating a more diverse volatile profile compared to post-harvest samples. The dominant constituents included ethyl alcohol (23.63%), eugenol (14.35%), 2-hexenal, 2-pentanone, 3-octanone, and 3-methylpentane, collectively accounting for 75.51% of the total volatile fraction.

Table 2. Volatile components of jujube leaves extracted before fruit season in June

Peak	Name	Area
1	Ethanol (CAS) Ethyl alcohol	23.63
2	Propanenitrile, 2-hydroxy-2-methyl- (CAS) Acetone cyanohydrin	2.26
3	2,3-Butanedione (CAS) Diacetyl	2.90
4	Pentane, 3-methyl- (CAS) 3-Methylpentane	5.12
5	2-Pentanone (CAS) Methyl propyl ketone	9.30
6	2-Hexenal, (E)- (CAS) (E)-2-Hexenal	9.40
7	2-Hexen-1-ol, (Z)- (CAS) Cıs-Hex-2-En-1-Ol	3.47
8	2-Heptanone (CAS) Heptan-2-one	1.85
9	7-Octen-2-one	2,29
10	3-Octanone (CAS) Eak	7.65
11	2-Octanone (CAS) Octan-2-one	6.06
12	Octanal (CAS) n-Octanal	2.73
13	l-Limonene	1.85
14	Linalool	2.63
15	Phenol, 4-ethenyl-2-methoxy-	1.33
16	Eugenol	14.35
17	5,9-Undecadien-2-one, 6,10-dimethyl- (CAS) Dihydropseudoionone	1.10
18	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	2.08

The relatively high proportion of ethyl alcohol suggests enhanced primary metabolic activity during the active growth phase, potentially reflecting increased carbohydrate turnover and fermentative or oxidative metabolic pathways associated with rapid tissue development. In contrast to the more concentrated dominance pattern observed in autumn leaves, the spring profile appears metabolically broader and more evenly distributed, consistent with active biosynthetic flux during vegetative expansion.

Eugenol, present at 14.35%, represents a substantial phenylpropanoid contribution even at the pre-fruiting stage, indicating that defensive secondary metabolism is initiated early in leaf development. Meanwhile, the presence of green leaf volatiles such as 2-hexenal supports the association of spring leaves with active photosynthetic tissues and membrane-derived oxylipin pathways.

Overall, the greater compound diversity observed in pre-fruiting leaves reflects a metabolically dynamic state characterized by growth-associated volatile production. This contrasts with the more defense-oriented restructuring detected in post-harvest samples and further supports the interpretation that volatile metabolism in *Z. zizyphus* undergoes phenology-dependent modulation.

Phenolic compounds constitute a major class of plant secondary metabolites characterized by their redox-active hydroxyl groups, which enable electron donation and free

radical scavenging. Seasonal variation in total phenolic content (TPC) was evident between developmental stages, with spring leaves exhibiting higher levels (16.18 ± 0.84 mg GAE g^{-1}) compared to autumn leaves (13.56 ± 0.76 mg GAE g^{-1}). This pattern suggests that phenolic accumulation may be more closely associated with active growth and photosynthetically dynamic tissues, where oxidative metabolism is elevated. The measured TPC values fall within the range previously reported for *Z. zizyphus* leaf extracts. Mahmoud et al. (2022) documented a total phenolic content of 18.12 ± 1.92 mg g^{-1} in methanolic leaf extracts, indicating comparable magnitudes despite differences in extraction solvent and geographic origin. Such consistency across studies reinforces the classification of jujube leaves as a phenolic-rich biomass resource.

In contrast to total phenolics, antioxidant capacity determined by the CUPRAC assay was higher in autumn leaves (0.842 mmol Trolox equivalents g^{-1}) than in spring leaves (0.705 mmol Trolox equivalents g^{-1}). This divergence suggests that antioxidant activity is not solely dependent on total phenolic concentration but may also reflect qualitative differences in metabolite composition, including saponins, phenylpropanoids, and other redox-active constituents enriched during later developmental stages. Damiano et al. (2017) reported antioxidant activity values of 375 μmol TE g^{-1} using alternative assays, and although direct comparison is limited by methodological variation, the present findings confirm substantial antioxidant potential in *Z. zizyphus* leaves under Mediterranean growing conditions.

The fatty acid composition of post-harvest leaves is presented in Table 3. Arachidonic acid constituted the dominant fatty acid (55.47%), followed by eicosenoic and linoleic acids. The high relative abundance of polyunsaturated fatty acids in autumn leaves may reflect membrane remodeling processes associated with senescence and environmental stress adaptation. In plant tissues, unsaturated fatty acids play a central role in maintaining membrane fluidity and serve as precursors for oxylipins and signaling molecules involved in stress response pathways. Rather than serving merely as structural lipids, the elevated proportion of arachidonic acid-like polyunsaturated species in late-stage leaves may indicate enhanced lipid-mediated signaling and oxidative modulation during phenological transition. Such compositional shifts further support the broader interpretation that metabolic allocation in *Z. zizyphus* leaves undergoes coordinated seasonal reorganization across multiple biochemical pathways.

Table 3. Fatty acid composition of jujube leaves after harvest season in November

Peak	Name	%
1	Linoleic acid	13.80
2	Eicosenoic acid	30.73
3	Arachidonic acid	55.47

The fatty acid composition of pre-fruiting (spring) leaves is presented in Table 4. In contrast to post-harvest samples, spring leaves exhibited a more diverse lipid profile, with six detectable fatty acids compared to three in autumn leaves. Palmitic acid (29.39%) and elaidic acid (31.88%) emerged as the dominant constituents, together accounting for more than 61% of the total fatty acid fraction.

Table 4. Fatty acid composition of leaves extracted before fruit season in June

Peak	Name	%
1	Myristic acid	3.91
2	Palmitic acid	29.39
3	Stearic acid	10.91
4	Elaidic acid	31.88
5	Linoleic acid	15.54
6	Eicosenoic acid	8.37

The higher diversity and relative abundance of fatty acids in spring leaves likely reflect active membrane biosynthesis and cellular expansion during vegetative growth. Saturated fatty acids such as palmitic acid are essential structural components of membrane lipids and contribute to membrane stability during rapid cell division. Meanwhile, unsaturated fatty acids - including elaidic and linoleic acids - play crucial roles in maintaining membrane fluidity and modulating lipid-mediated signaling pathways.

The shift from a more diverse spring lipid composition to a polyunsaturated-dominated autumn profile suggests coordinated seasonal membrane remodeling. During active growth, lipid metabolism appears oriented toward structural assembly and cellular proliferation, whereas in later developmental stages, lipid composition may become increasingly associated with stress adaptation and oxidative regulation. Such phenology-dependent lipid reconfiguration aligns with the broader pattern of seasonal metabolic modulation observed in secondary metabolite and antioxidant pathways.

Collectively, the fatty acid data further support the interpretation that *Z. zizyphus* leaves undergo integrated biochemical restructuring across developmental stages, encompassing both structural lipids and specialized secondary metabolites.

The observed seasonal restructuring of volatile compounds, saponins, phenolics, antioxidant capacity, and fatty acid profiles collectively indicates that metabolic allocation in *Z. zizyphus* leaves is tightly regulated by phenological stage. Rather than isolated biochemical changes, the coordinated shifts across multiple metabolite classes suggest an integrated reprogramming of primary and secondary metabolism during developmental transition.

The enrichment of saponins, dominant defense-related volatiles, and elevated antioxidant capacity in post-harvest leaves is consistent with enhanced differentiation-oriented metabolism and stress-responsive regulation during late growth stages. In contrast, the broader lipid diversity and higher phenolic concentration observed in spring leaves reflect a metabolically active phase characterized by growth-associated biosynthesis and membrane expansion. These contrasting biochemical configurations support theoretical models of seasonal resource partitioning in perennial plants, wherein growth and defense functions are dynamically balanced over time. From an applied perspective, the findings demonstrate that leaf biomass generated after fruit harvest - typically regarded as agricultural residue - retains substantial biochemical value. The seasonal enrichment of bioactive metabolites highlights the potential of autumn leaves as a sustainable raw material for nutraceutical, cosmetic, pharmaceutical, and functional product development. Integrating such biomass into valorization

frameworks may therefore contribute to circular bioeconomy strategies and resource-efficient production systems.

4. Conclusions

In this study, the seasonal variation in the phytochemical profile of *Z. zizyphus* leaves cultivated in the Isparta region was comprehensively evaluated. The results clearly demonstrate that both qualitative and quantitative changes occur in volatile constituents, saponin concentration, phenolic content, antioxidant capacity, and fatty acid composition between the pre-fruiting and post-harvest periods. Autumn leaves exhibited higher saponin levels and antioxidant capacity, whereas spring leaves showed a richer diversity of fatty acids and volatile constituents. These findings suggest that secondary metabolite accumulation in jujube leaves is closely linked to phenological stage and possibly to plant defense and metabolic regulation mechanisms. The observed compositional shifts indicate dynamic metabolic allocation throughout the growing season. Importantly, the data reveal that leaves collected after fruit harvest-often regarded as agricultural waste-retain considerable bioactive potential. This highlights their possible utilization as a sustainable raw material in nutraceutical, pharmaceutical, cosmetic, and functional food applications.

Overall, this study provides region-specific phytochemical data and contributes to a better understanding of seasonal metabolic variation in *Z. zizyphus* leaves. Future research should focus on elucidating the biochemical drivers underlying these seasonal changes and evaluating the biological activities of the identified compounds through in vitro and in vivo models.

References

American Oil Chemists' Society, 1997. Official Methods and Recommended Practices of the AOCS. American Oil Chemists' Society, Champaign.

Apak, R., Güçlü, K., Özyürek, M., Karademir, S.E., 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. Journal of Agricultural and Food Chemistry, 52(26), 7970-7981. https://doi.org/10.1021/jf048741x.

Aryal, S., Baniya, M.K., Danekhu, K., Kunwar, P., Gurung, R., Koirala, N., 2019. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from western Nepal. Plants, 8(4), 96. https://doi.org/10.3390/plants8040096.

Damiano, S., Forino, M., De, A., Vitali, L.A., Lupidi, G., Taglialatela-Scafati, O., 2017. Antioxidant and antibiofilm activities of secondary metabolites from *Ziziphus jujuba* leaves used for infusion preparation. Food Chemistry, 230, 24-29. https://doi.org/10.1016/j.foodchem.2017.02.141.

Elaloui, M., Laamouri, A., Ennajah, A., Cerny, M., Mathieu, C., Vilarem, G., Chaar, H., Hasnaoui, B., 2016. Phytoconstituents of leaf extracts of *Ziziphus jujuba* Mill. plants harvested in Tunisia. Industrial Crops and Products, 83, 133-139. https://doi.org/10.1016/j.indcrop.2015.11.029.

Erdoğan, M.Ü., Karaboyacı, M., 2021. Investigation of alternative ecologic bleaching methods for the wool fibers. Journal of Natural Fibers, 18(9), 1229-1246. https://doi.org/10.1080/15440478.2019.1689883.

Erdoğan, Ü., Gökçe, E.H., 2021. Fig seed oil-loaded nanostructured lipid carriers: evaluation of the protective effects against oxidation. Journal of Food Processing and Preservation, 45(10), e15835. https://doi.org/10.1111/jfpp.15835.

Guo, S., Duan, J.A., Tang, Y., Qian, Y., Zhao, J., Qian, D., Su, S., Shang, E., 2011. Simultaneous qualitative and quantitative analysis of triterpenic acids, saponins and flavonoids in the leaves of two *Ziziphus* species by HPLC–PDA–MS/ELSD. Journal of Pharmaceutical and Biomedical Analysis, 56(2), 264-270. https://doi.org/10.1016/j.jpba.2011.05.025.

Gülsoy, S., Süel, H., Celik, H., Özdemir, S., Özkan, K., 2014. Modelling site productivity of Anatolian black pine stands in response to site factors in Buldan District, Turkey. Pakistan Journal of Botany, 46(1), 213-220.

Hiai, S., Oura, H., Nakajima, T., 1976. Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. Planta Medica, 29, 116-122. https://doi.org/10.1055/s-0028-1097639.

Karaboyacı, M., Tama, B., Şencan, A., Kiliç, M., 2017. Recycling of rose wastes to activated carbon with ecological precursor. Bilge International Journal of Science and Technology Research, 1(1), 1-8.

Kavas, İ., Dalkiliç, Z., 2015. Bazı hünnap genotiplerinin morfolojik, fenolojik ve pomolojik özelliklerinin belirlenmesi ve melezleme olanaklarının araştırılması. Adnan Menderes Üniversitesi Ziraat Fakültesi Dergisi, 12(1), 57-72.

Mahmoud, K.B., Wasli, H., Mansour, R.B., Jemai, N., Selmi, S., Jemmali, A., Ksouri, R., 2022. Antidiabetic, antioxidant and chemical functionalities of *Ziziphus jujuba* (Mill.) and *Moringa oleifera* (Lam.) plants using multivariate data treatment. South African Journal of Botany, 144, 219-228. https://doi.org/10.1016/j.sajb.2021.08.017.

Outlaw Jr, W.H., Zhang, S., Riddle, K.A., Womble, A.K., Anderson, L.C., Outlaw, W.M., Thistle, A.B., 2002. The jujube (*Ziziphus jujuba* Mill.), a multipurpose plant. Economic Botany, 56(2), 198-200. https://doi.org/10.1663/0013-0001%282002%29056%5B0198%3ATJZJMA%5D2.0.CO%3B2.

PubChem, 2022a. Arachidonic acid. https://pubchem.ncbi.nlm.nih.gov/compound/Arachidonic-acid (accessed 18.02.2026).

PubChem, 2022b. Compound 637517. https://pubchem.ncbi.nlm.nih.gov/compound/637517 (accessed 18.02.2026).

PubChem, 2022c. Compound 985. https://pubchem.ncbi.nlm.nih.gov/compound/985 (accessed 18.02.2026).

Slinkard, K., Singleton, V.L., 1977. Total phenol analysis: automation and comparison with manual methods. American Journal of Enology and Viticulture, 28(1), 49-55. https://doi.org/10.5344/ajev.1977.28.1.49.

Ulusan, M.D., Karaboyacı, M., 2021. Determination of volatile component and saponin content of jujube tree leaves pre-fruit and post-harvest. International Conferences on Science

and Technology, Engineering Sciences and Technology, pp. 473-477. ISBN: 978-605-70965-2-4.

Yılmaz, G., 2019. Hünnap (*Ziziphus zizyphus*) ağacı yaprak ve meyve ekstraktlarının antioksidan ve antimikrobiyal özelliklerinin araştırılması. Namık Kemal Üniversitesi, Tekirdağ. https://hdl.handle.net/20.500.11776/3568.