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Comparative Investigation Of Phytochemical Composition And Antioxidant Activities In Various Parts Of Ziziphus Jujuba Mill

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Abstract
Ziziphus jujuba (jujube) is extensively recognized for its nutritional and medicinal properties. This study aimed to compare the biochemical composition and antioxidant activity of different plant organs to establish correlations between secondary metabolites and antioxidant potential. Quantitative analysis revealed that the leaves contained the highest levels of total polyphenols (0.470 ± 0.021 mg·g⁻¹) and flavonoids, including anthocyanidins (11.16 ± 0.11 mg·g⁻¹) and flavonoid aglycones (2.46 ± 0.13 mg·g⁻¹), followed by the fruit pulp and seeds. GC-MS analysis of organic extracts highlighted distinct chemical profiles among the organs. The leaves were rich in α-pyrone (59.26%) and α-hydroxyhydrocinnamic acid; the pulp was dominated by levulinic acid methyl ester (82.14%) and oleic acid, while the seeds were enriched in saturated fatty acids, olealdehyde, and butylated hydroxytoluene (BHT). Antioxidant activity, as assessed by DPPH IC_{50}, confirmed the superior activity of leaf extracts (IC50=7.60 ± 0.46 mg·mL⁻¹) compared to the pulp and seeds. Z. zizyphus represents a promising source of natural antioxidant compounds, and the differential composition of its plant organs underscores their potential for developing functional foods, nutraceuticals, and therapeutic products.
Keywords: *Zizyphus jujuba; antioxidant activity; polyphenols, flavonoids, GC-MS, antioxidant activity.*

INTRODUCTION

Medicinal plants are an important source of bioactive secondary metabolites with applications in food, cosmetics, and pharmacology Globally, nearly a quarter of recorded plant species have therapeutic uses, and approximately 80% of the population still relies on traditional medicine according to the WHO, highlighting the growing interest in exploring new plant-derived molecules[1-2]. Attention has been focused on the genus Ziziphus, particularly Ziziphus jujuba (Ziziphus zizyphus (L.) H.Karst), which is widely used in traditional medicine. Z. jujuba is now distributed across various regions of the world, including North Africa and Algeria, where it is traditionally cultivated in the Tell region[3-5].

Recent studies have highlighted its richness in bioactive compounds such as polysaccharides, flavonoids, alkaloids, vitamins, amino acids, and minerals, which explains its nutritional and therapeutic interest[6-8]. The entire plant, including leaves, fruits, seeds, and kernel oil, contains a variety of biochemical compounds (saponins, triterpenic acids, polyphenols, sterols) associated with multiple biological effects, including antioxidant, anti-inflammatory, antimicrobial, cardioprotective, immunomodulatory, and anxiolytic activities [6,9-10].

The fruits, consumed fresh or dried, are also an important source of vitamin C and amino acids, and hold a notable place in traditional medicine, particularly in China, where jujube has been considered a medicinal food since ancient times[4,7].

Although numerous studies have highlighted the nutritional and pharmacological richness of Ziziphus jujuba, detailed information regarding the chemical composition of its organic fractions remains limited, particularly in a comparative perspective across different plant organs. Most published studies focus on total or hydrophilic extracts, whereas organic phases that may contain lipophilic metabolites of interest remain largely unexplored. Accordingly, the present study focuses on the targeted analysis of organic constituents from the leaves, pulp, and kernels of Z. jujuba, aiming to highlight their biochemical specificities and their potential contribution to the antioxidant activity of the species. This comparative characterization provides new insights for the enhanced valorization of this plant resource.

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EXPERIMENTAL

MATERIALS AND METHODS

Vegetal Material

Our study used leaves, pulp and pits of Ziziphus jujuba, gathered in the Médéa area, northern Algeria (36°13′51″N, 3°18′38″E, altitude 581 m). The plant samples were thoroughly washed, then air-dried at ambient temperature in the shade. Once fully dry, they were ground into a fine powder, which was stored in sealed containers until further analysis (Fig 1, 2).

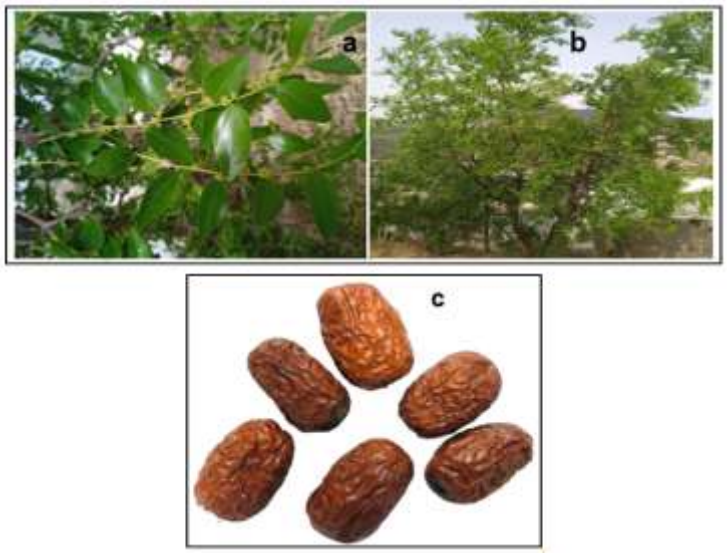

Figure 1. Morphological structure of Ziziphus jujuba and description of the samples analyzed
Note : **(a)** General morphology of the Zizyphus zizyphus tree; **(b)** Branches with leaves; **(c)** Dried fruits.

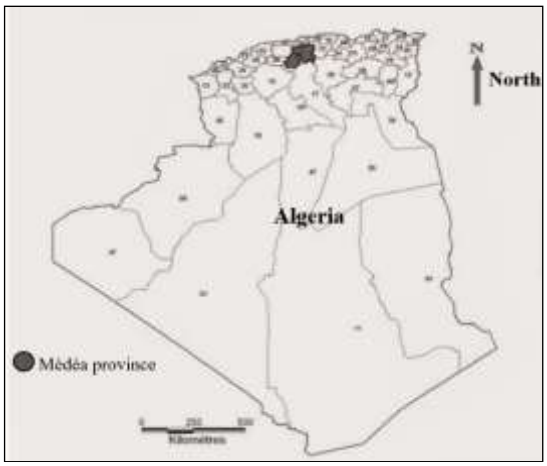

Figure 2. Geographical location of the study area in Médéa Province

Isolation and Measurement of Total Polyphenols

Five grams of powdered dried leaves, fruit pulps, and seeds were macerated in 100 mL of methanol for 24 h with continuous stirring. The filtrate was concentrated under reduced pressure at 40 °C and stored at 4 °C. Total polyphenols were quantified using the Folin–Ciocalteu method, with absorbance measured at 765 nm. Results were expressed as µg gallic acid equivalents (GAE) per gram of dried plant material (DPM)[11].

Preparation of Extracts

Two grams of dried plant material were hydrolyzed in 80 mL of 2 N HCl at 100 °C for 40 min with periodic oxygenation. The hydrolysate was sequentially extracted to separate the organic and aqueous fractions[12-14]. The aqueous fraction was used for anthocyanidin quantification, while the organic extract was employed for flavonoid

and phenolic analyses. Dissolving the residues in methanol produced the methanolic extract, used for GC-MS profiling of fatty compounds, polyphenols, thermostable molecules[15], and for antioxidant activity assessment.

Flavonoid Quantification

The anthocyanidin and flavonic aglycones were quantified using a UV-Vis spectrophotometer (Junway 7300)[14]. Diluted aqueous extracts were measured at 520 nm for anthocyanidins, and their content, expressed as mg of cyanidin per g of dried plant material (DPM), was calculated using the formula:

T (mg·g-1) = 5.2 x 10-2 x (Abs x V x d) / P

where Abs is the absorbance at 520 nm, V is the volume of the aqueous phase, d is the dilution factor, and P is the dry weight of hydrolyzed plant material.

For flavonic aglycones, ethanolic extracts were reacted with a 1% alcoholic solution of $AlCl_3$ for 15 min, and absorbance measured at 420 nm. The content, expressed as mg of quercetin per g of DPM, was calculated from the differential absorbance (ΔAbs) using:

$T (mg \cdot g^{-1}) = 1.3 \times 10^{-2} \times (\Delta Abs \times V \times d) / P$

Where ΔAbs is the differential absorbance at 420 nm, V is the volume of the ethanolic solution, d is the dilution factor, and P is the dry weight of the hydrolyzed plant material[16].

GC-MS Profiling of Organic Extracts

Methanolic organic extracts of Ziziphus jujuba were analyzed by GC–MS using a Hewlett Packard 6800 Plus system coupled to an HP 5973 mass selective detector operating in EI mode (70 eV). Separation was achieved on an HP-5MS capillary column with helium as the carrier gas (0.8 mL min^{-1}).

The oven temperature was programmed from 60 °C to 290 °C, and samples (1 µL) were injected in splitless mode. Injector, transfer line, and ion source temperatures were set according to standard conditions. Compound identification was based on retention times, mass spectra, and retention indices compared with reference standards and spectral libraries.

In-vitro Antioxidant Assessment

The antioxidant activity of the organic extracts was examined using the DPPH radical-scavenging method as described by Brand-Williams et al[17]. Extract dilutions were mixed with a methanolic DPPH solution, incubated for 30 min in the dark, and the absorbance measured at 517 nm. Radical-scavenging percentages were calculated and compared to ascorbic and ferulic acids. Measurements were carried out in triplicate, and IC_{50} values were obtained from inhibition curves, corresponding to the extract concentration able to neutralize 50 % of the DPPH radical[18].

DPPH inhibition (%) = (AD - AE / AD) x 100

Where AD is the absorbance of the DPPH solution alone and AE is the absorbance in the presence of the extract.

Statistical analysis

Statistical analyses were carried out using GraphPad Prism (v.8.0). Differences between groups were evaluated by one-way ANOVA followed by Tukey's post-hoc test. Results were expressed as mean ± standard error of the mean (m ± SEM) from independent experiments, and statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Total Polyphenolic Content

Leaves of the jujube tree showed the highest polyphenolic content (0.4703 ± 0.021 $mg \cdot g^{-1}$) among the analyzed organs. Slight differences were observed between leaves and fruit pulp or pits, while no significant difference existed between pulp and pits (Figure 3).

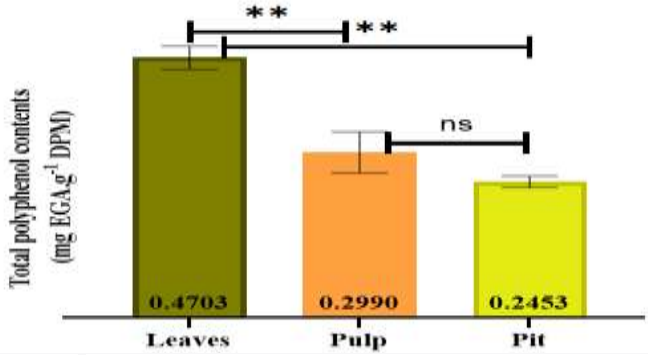

Figure 3. Total Polyphenolics Across Z. jujuba Organs

Note: ns: no significant and ****: significant

Comparative Analysis of Flavonoid Profiles

The extracts of Z. jujuba contain two major types of flavonoids: anthocyanidins (in the aqueous fraction) and flavonic aglycones (in the organic/ethanolic fraction). The leaves show the highest levels for both families (11.16 ± 0.11 mg·g^{-1} for anthocyanidins and 2.46 ± 0.13 mg·g^{-1} for aglycones), with statistically significant differences compared to the pulp and the pits. No difference is observed between pulp and pits for aglycones.

Several studies have highlighted a marked variability in the total polyphenol and flavonoid contents of Ziziphus jujuba extracts, depending on cultivar, plant tissue, and extraction solvent. Adjdir et al. (2019)[19] reported that aqueous, hydromethanolic, and hydroacetonic extracts, as well as their ethyl acetate and n-butanol fractions, exhibited polyphenol contents ranging from 42.84 to 94.70 mg gallic acid equivalents per gram of extract and flavonoid contents from 47.02 to 427.33 mg catechin equivalents per gram of extract. In addition, Xue et al. (2021)[20] measured total flavonoid levels between 359.38 and 1041.33 µg/g fresh weight across different cultivars, developmental stages, and tissues, identifying major compounds such as quercetin, rutin, and (+)-catechin in leaves, stems, and fruits. Furthermore, analyses of Spanish jujube fruits revealed high total polyphenol contents ranging from 1442 to 3432 mg/100 g dry matter, emphasizing the strong phytochemical potential of this species[21].

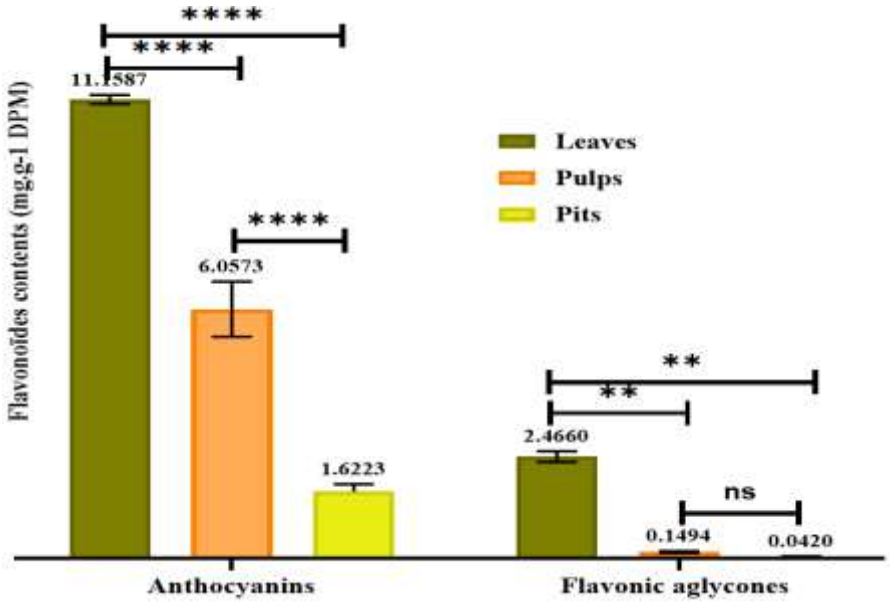

Figure 4. Comparison of flavonoid concentrations in the main organs of Z. jujuba
Note: ns: no significant; ****: Significant

Plant polyphenols, particularly flavonoids, are secondary metabolites known for their wide range of biological activities, including antioxidant, anti-inflammatory, antimicrobial effects, as well as a protective role against cancer and cardiovascular diseases[22]. In Ziziphus species, flavonoids exhibit notable health-promoting properties, with their total content strongly influenced by the extraction method; chloroform generally yields higher concentrations, whereas hexane or methanol result in lower recoveries. The plant contains various quercetin and kaempferol glycosides, along with flavanols such as catechin, epicatechin, and procyanidin B2[23]. Moreover, jujube fruits are particularly rich in flavonoids and jujubosides, bioactive compounds associated with the stimulation of erythropoietin (EPO), enhancement of red blood cell production, and anti-inflammatory activity. This phytochemical profile highlights Ziziphus fruits and extracts as promising natural sources for anemia prevention and the modulation of diverse biological responses[24].

The variations observed in flavonoid and phenolic compound contents of Z. jujuba appear to result from the interaction of environmental and biological factors, such as climate, altitude, soil type, and plant age, which influence the biosynthesis of secondary metabolites. In particular, abiotic stresses, including high altitude, have been shown to promote the accumulation of flavonoids, polyphenols, anthocyanins, and saponins[23, 25-26].

GC-MS Chemical Profile of Zizyphus jujuba Organic Extracts

GC-MS analysis of leaves, pulp, and seed extracts of Zizyphus jujuba identified 19 compounds, reflecting the complex chemical composition across different plant parts (Figure 5, Table 1).

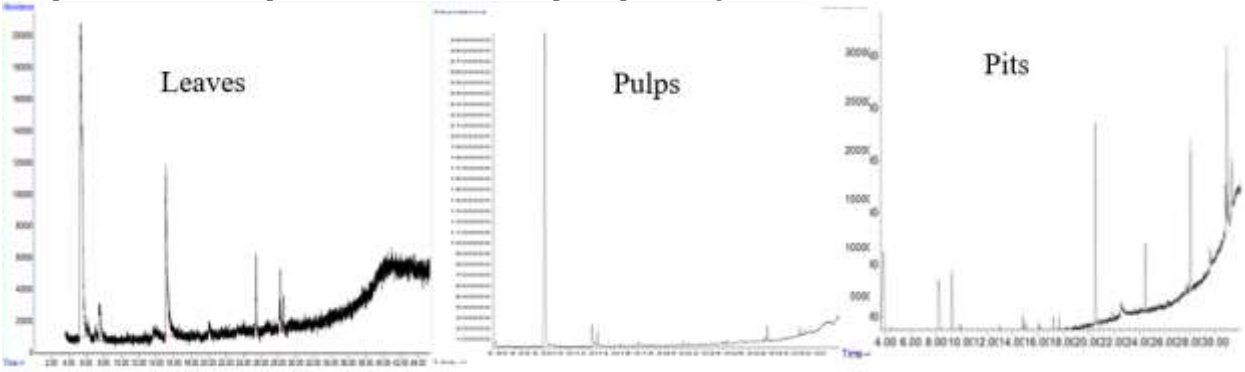

Figure 5. GC-MS Chromatograms of Organic Extracts from Leaves, Pulp, and Pits of Ziziphus jujuba

Table 2. Volatile Compound Profile of Organic Extracts by GC-MS (Area %)

N°	Groups of volatile compounds	Area (%)		
		Leaves	Pulps	Pits
1	Saturated fatty acid	5.27	2.7	21.69
2	Monounsaturated fatty acid	1.76	4.41	0.46
3	Polyunsaturated fatty acid	5.59	–	–
4	Other fatty substance	–	1.92	34.31
5	γ-Ketoacid	–	82.14	25.17
6	Phenolic compound	21.82	3.07	18.39
7	Other volatile compound	65.56	5.8	–
	Total area (%)	100	100.04	100.02

Note: –: Compound not detected

The GC-MS analysis of organic extracts from Zizyphus jujuba (Table 1) revealed a marked qualitative and quantitative variability among the different organs studied, while also highlighting the presence of certain compounds common to all fractions. The pulp showed the greatest chemical diversity, with twelve volatile compounds accounting for 100.04% of the chromatographic area. The seeds exhibited intermediate richness, characterized by eight compounds representing 100.02% of the total area, whereas the leaves were quantitatively the poorest, with only six molecules covering 100% of the detected area.

The identified compounds were classified into several major chemical classes, including saturated and unsaturated fatty acids, lipid derivatives, keto acids, phenolic compounds, and other volatile substances (Table 2). Fatty acids and their derivatives constituted a significant fraction, particularly in the seeds, where they reached 65.46%, compared to 12.62% in the leaves and 9.03% in the pulp. Keto acids were predominant in the fruit extracts, representing 82.14% in the pulp and 25.17% in the seeds, while phenolic compounds were mainly concentrated in the leaves, with a proportion of 21.82%.

Qualitatively, levulinic acid, methyl ester (peak 4) appeared as the major compound in the pulp (82.14%) and seeds (25.17%) and is widely used in medicine, particularly in phytotherapy, as well as a precursor for the production of pharmaceuticals, cosmetics, food additives, pesticides, and polymeric materials[27]. Oleic acid (peaks 15 and 17), found in higher proportion in the pulp, is recognized for its energy value and nutritional properties and contributes to skin protection as well as the prevention of cardiovascular and metabolic diseases[28-30].

The leaves were distinguished by specific compounds, mainly α-pyrone (peak 2), the dominant compound in this fraction (59.26%), and α-hydroxyhydrocinnamic acid (peak 7), the main phenolic representative, possessing antioxidant and anti-inflammatory properties that help protect cells against oxidative stress[31]. The seeds contained characteristic compounds such as olealdehyde (peak 1), butylated hydroxytoluene (BHT, peak 9), a potent antioxidant commonly used in the food and cosmetic industries, as well as a notable proportion of saturated fatty acids (peaks 11, 12, and 19), including palmitic acid, myristic acid, and methyl stearate (5.18%).

2-Pyrones, present in the leaves, play a key role in defense mechanisms and exhibit a wide range of biological activities, including antiviral, antibacterial, antifungal, and anticancer effects. They are also effective against

amyloid-related toxicity associated with Alzheimer's disease, mainly through enzymatic inhibition, making them promising candidates for the development of new drugs[32].

Studies using gas chromatography coupled with mass spectrometry (GC-MS) on Ziziphus jujuba extracts remain very limited in the literature, particularly for leaves and pulp. El Aloui et al. (2012)[33] analyzed oils extracted from seeds collected from four regions in Tunisia by GC, identifying ten major fatty acids, including myristic, palmitic, oleic (omega-9), linoleic (omega-6), and linolenic (omega-3) acids. Mustafa, (2025)[34] conducted a chemical profiling of seed oil by GC-MS, revealing twelve main components, such as methyl heptadecanoate (51.77%), methyl tetracosanoate (8.70%), and methyl hexadecanoate (7.99%). In another study, oleic acid was identified as the main volatile compound, and triterpenic acids as well as C-glycoside flavonoids were detected, highlighting the pharmacological potential of the seeds[35]. For the leaves, GC-MS analyses revealed bioactive compounds such as octadecenoic acid, ethyl hexadecanoate, neophytadiene, and bis (2-ethylhexyl) phthalate[36], emphasizing the phytochemical richness of the organic extracts of this plant.

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Table 1. Chemical Profile of Organic Extracts from Leaves, Pulp, and Pits of Ziziphus jujuba. Determined by GC-MS (% Area)

Peak	Nomenclature IUPAC	Compounds[a]	Nature[b]	RT (min)	MF	MW ($g \cdot mol^{-1}$)	CAS# number	Area peak[c] (%)		
								Leaves	Pulps	Pits
1	9-Octadecenal, (9E)-	Olealdehyde	Other fatty substances	3.81	$C_{18}H_{34}O$	266.462	5090-41-5	–	1.52	15.36
2	2H-Pyran-2-one	alpha-Pyrone	Other volatile compound	5.32	$C_5H_4O_2$	96.08	504-31-4	59.26	–	–
3	5-Vinyl-1H-Tetrazole	1H-Tetrazole, 5-vinyl	Other volatile compound	7.50	$C3H_4N_4$	96.09	18755-47-0	6.30	–	–
4	Pentanoic acid, 4-oxo-, methyl ester	Levulinic acid, methyl ester	γ-Ketoacid	8.17	$C_6H_{10}O_3$	130.1418	624-45-3	_	82.14	25.17
5	c-2, c-3-epoxy-t-6-methylcyclohept-4-en-r-1-ol	8-Oxabicyclo[5.1.0]oct-5-en-2-ol, 4-methyl-, (1a,2b,4b,7a)-	Other volatile compound	12.37	$C_8H_{12}O_2$	140.18	139597-22-1	–	5	–
6	Benzeneacetic acid, methyl ester	Methyl 2-phenylacetate	Phenolic compound	12.86	$C_9H_{10}O_2$	150.1745	101-41-7	–	2.48	–
7 8	Benzenepropanoic acid, .alpha.-hydroxy-, methyl ester Propane, 1,1-dimethoxy-	alpha-Hydroxyhydrocinnamic acid 1,1-Dimethoxypropane	Phenolic compound Other fatty substances	15.12 16.51	$C_{10}H_{12}O_3$ $C_5H_{12}O_2$	180.20048 104.14758	13674-16-3 4744-10-9	21.82 –	– 0.80	– –
9	Phenol, 2,6-bis (1,1-dimethylethyl)-4-methyl-	Butylated hydroxytoluene	Phenolic compound	20.50	$C_{15}H_{24}O$	220.35046	128-37-0	–	0.59	18.39
10	Hexadecanoic acid (or n-Hexadecanoic acid)	Palmitic acid	Saturated fatty acid	23.75	$C_{16}H_{32}O_2$	256.42408	9006-59-1	–	0.74	–
11	Tetradecanoic acid, methyl ester	Myristic acid, methyl ester	Saturated fatty acid	24.48	$C_{15}H_{30}O_2$	242.3975	124-10-7	–	0.49	5.30
12	Hexadecanoic acid, methyl ester	Palmitic acid, methyl ester	Saturated fatty acid	25.41	$C_{15}H_{28}O_2$	240.38162	4727-18-8	5.27	1.47	11.21

13	Glycerol-1,2-and-1,3-dioleate	Dioleoylglycerol	Other volatile compound	27.75	$C_{39}H_{72}O_5$	620.98598	2465-32-9	–	0.40	–
14	Hexadecatrienoic acid, methyl ester	Methyl hexadecatrienoate	Polyunsaturated fatty acid	28.19	$C_{17}H_{28}O_2$	264.403	37822-81-4	5.59	–	–
15	9-Octadecenoic acid (Z)-	Oleic acid (Omega-9)	Monounsaturated fatty acid	28.38	$C_{18}H_{34}O_2$	282.46136	9000-69-5	–	2.81	0.46
16 17	Octadecanoic acid, 11-methyl-, methyl ester 9-Octadecenoic acid (Z)-, methyl ester	Vaccenic acid methyl ester Oleic acid, methyl ester	Monounsaturated fatty acid Monounsaturated fatty acid	28.59 30.87	$C_{19}H_{36}O_2$ $C_{19}H_{36}O_2$	296.4879 296.48794	52380-33-3 61788-34-9	1.76 –	– 1.60	– –
18	Oleicacidtriglyceride	Trioleoylglycerol / Triolein	Other volatile compound	30.93	$C_{57}H_{104}O_6$	885.43206	41755-78-6	–	–	18.95
19	Octadecanoic acid, methyl ester	Stearic acid, methyl ester	Saturated fatty acid	31.25	$C_{19}H_{38}O_2$	298.50382	172226-14-1	–	–	5.18
	Total number of volatile compounds detected							6	12	8
	Total area (%)							100	100.04	100,02

Note: **(a)**: Compound listed in order of elution; **(b)**: Nature of compound; **(c)**: Percentage based on GC-MS peak area normalization; **IUPAC**: International Union of Pure and Applied Chemistry; **RT**: Retention time (min); **MF**: Molucular formula;
MW: Molucular weight ($g \cdot mol^{-1}$); **CAS**: Chemical Abstract Service; –: Compound not detected

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Antioxidant Activity Assessment: DPPH Radical Scavenging

The organic extracts from the leaves, pulp, and seeds of Z. jujuba exhibited a significant ability to neutralize the DPPH radical, measured spectrophotometrically at 517 nm. The leaf extract showed the highest inhibition (94.37 ± 0.68 %), while the pulp and seeds exhibited moderate values (69.60 ± 0.77 % and 65.42 ± 0.87 %, respectively).

The determination of IC50 values, corresponding to the concentration required to reduce 50% of the DPPH radical, confirmed the superiority of the leaf extract (7.60 ± 0.46 mg·mL^{-1}) compared to the pulp (14.43 ± 0.73 mg·mL^{-1}) and seeds (15.30 ± 0.45 mg·mL^{-1}), as well as to the reference standards (ascorbic acid and ferulic acid). A highly significant difference ($P < 0.05$) was observed between the antioxidant activity of the extracts and that of the standards, whereas no significant difference was found between the pulp and seed extracts (table 5, figure 7). These results indicate that the leaf extract is the most active, although it remains less potent than vitamin C and ferulic acid, two powerful reference antioxidants.

Table 5. Radical scavenging activity of Zizyphus zizyphus leaves, pulp and pits organic extracts and positive control

Extract	Compounds	Inhibition rate (%)	IC_{50} (mg·mL^{-1})
Organic extract	Leaves	94,37± 0,68	7,60 ± 0,46
	Pulps	69,60 ± 0,77	14,436 ± 0,735
	Pits	65,42 ± 0,87	15, 30 ± 0,45
Positive control	L Ascorbic acid	–	0.0025±0.53
	Ferulic acid	–	0.0049±0.17

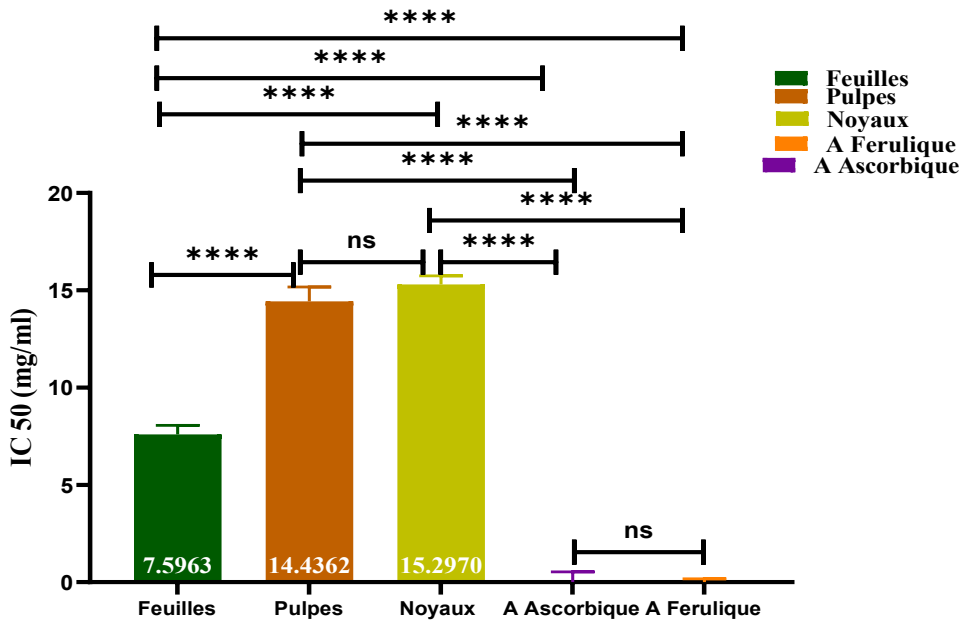

Figure 7. Antioxidant Activity of Organic Extracts from Leaves, Pulps, and Seeds of Z jujuba : IC_{50} Values Compared to Positive Controls
Note: ns: No significant and ****: Highly significant

The antioxidant activity of Z. jujuba varies among its different tissues, with leaves showing the highest radical scavenging capacity due to their richness in phenolic compounds and 2-pyrones, particularly α-pyrone and α-hydroxyhydrocinnamic acid, which effectively neutralize free radicals and protect against oxidative stress. Moderate amounts of fatty acids may further enhance this activity, whereas the pulp and seeds, dominated by levulinic acid and saturated fatty acids, exhibit lower antioxidant potential.

These findings are consistent with previous studies[21, 37], showing that antioxidant activity in jujube fruits varies according to chemical composition, particularly flavonoids, vitamin C, phenolic compounds, and proanthocyanidins, with the peel generally more active than the pulp.
This activity is influenced by the type of extract, cultivation conditions, and harvesting methods, reflecting the key role of phenolics, flavonoids, and uronic acids[19, 23]. Beyond fruits, seed oil and leaf extracts also demonstrate notable antioxidant and antimicrobial properties, linked to their high content of flavonoids and other bioactive phenolics, without cytotoxicity[38-40]. Additionally, aqueous extracts protect neuronal cells against oxidative stress in vitro, confirming the antioxidant, cytoprotective, and neuroprotective potential of Z. jujuba, mainly attributed to flavonoids and jujubosides[41]. Collectively, these findings highlight multiple parts of Z. jujuba promising sources of natural antioxidants with potential health benefits.

CONCLUSION

Ziziphus jujuba is a rich source of flavonoids, polyphenols, and fatty acids, which confer significant antioxidant activity to its fruits, seeds, and leaves. These properties make the plant a promising candidate for disease prevention and protection against oxidative stress

Declaration of interest. There is no actual or potential conflict of interest in relation to this article.

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