Commagene Journal of Biology

Guenane et al., (2025) *Comm. J. Biol.* 9(2), 137-144.
DOI: 10.31594/commagene.1654147

e-ISSN 2602-456X

Research Article/Araştırma Makalesi

LC–MS/MS Profiling of Algerian *Zizyphus lotus* Extracts and their *in vitro* Antioxidant Activity: A Comparative Study

Hadjira GUENANE[1], Boulanouar BAKCHICHE*[1], Ramazan ERENLER[2], Ilyas YILDIZ[2], Mosad A. GHAREEB[3]

[1]*Laboratory of Biological and Agricultural Sciences (LBAS), Amar Telidji University, Laghouat 03000, ALGERIA*
[2]*Department of Chemistry, Faculty of Arts and Sciences, Tokat, Gaziosmanpasa University, 60240 Tokat, TÜRKİYE*
[3]*Medicinal Chemistry Department, Theodor Bilharz Research Institute, Kornaish El-Nile, Warrak El-Hadar, Imbaba (P.O. 30), Giza 12411, EGYPT*
ORCID ID: Hadjira GUENANE: https://orcid.org/0000-0002-3598-5343; Boulanouar BAKCHICHE: https://orcid.org/0000-0002-3124-5153; Ramazan ERENLER: https://orcid.org/0000-0002-0505-3190 ; Ilyas YILDIZ : https://orcid.org/0000-0003-1254-1069 ; Mosad A. GHAREEB : https://orcid.org/0000-0002-8398-1937

Received: 10.03.2025 **Accepted:** 24.06.2025 **Published online:** 18.09.2025 **Issue published:** 18.12.2025

Abstract: In this study, it was aimed to determine antioxidant activities and chemical composition of leaf and fruit extracts of *Zizyphus lotus* which has been used traditionally in folk medicine of Algeria. Total phenolic content (TPC) and total flavonoid content (TFC) were determined by the colorimetric assay, respectively. The extracts were analyzed by LC–MS/MS. Furthermore, the antioxidant potential of various extracts was evaluated using different antioxidant assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, ferric reducing antioxidant power (FRAP) assay, 2,2-azinobis-ethylbenzothiozoline-6-sulphonic acid (ABTS), and Phenanthroline activity using UV spectrophotometer. The quantitative analysis of polyphenols and flavonoids content showed that the leaves have the highest amount (279.07±18.84 mg GAE/g E and 132.74±27.74 mg ER/g E, respectively). The highest antioxidant activity was observed in the leaves with the value of 24.10±3.49 µg/mL, 9.365 ± 0.532 µg/mL, 0.19±0.093, 0.636±0.033 mg AAE/g, and 12.11±0.81 µg/mL for DPPH, ABTS, total antioxidant capacity, FRAP and Phenanthroline activity, respectively. The LC–MS/MS analysis of leaves reveals the presence the major compound, rutin (24392.59 mg/g), when fruits contain hesperidin (311.70 mg/g) as a major compound.

This research suggested that these medicinal plants possess a significant antioxidant potential, are an important source of natural antioxidants, and can be effectively used in treating oxidative stress disorders.

Keywords: *Zizyphus lotus*, total phenolics, antioxidant potential, LC–MS/MS analysis.

Cezayir *Zizyphus lotus* Özütlerinin LC–MS/MS Profillemesi ve Bunların *in vitro* Antioksidan Aktivitesi: Karşılaştırmalı Bir Çalışma

Öz: Bu çalışmada, Cezayir'de halk tıbbında geleneksel olarak kullanılan *Zizyphus lotus*'un yaprak ve meyve ekstraktlarının antioksidan aktivitelerinin ve kimyasal bileşiminin belirlenmesi amaçlanmıştır. Toplam fenolik içeriği (TPC) ve toplam flavonoid içeriği (TFC) sırasıyla kolorimetrik test ile belirlenmiştir. Ekstraktlar LC-MS/MS ile analiz edilmiştir. Ayrıca, çeşitli ekstraktların antioksidan potansiyeli, 2,2-difenil-1-pikrilhidrazil (DPPH) deneyi, ferrik indirgeyici antioksidan güç (FRAP) deneyi ve 2,2-azinobis-etilbenzotiyozolin-6-sülfonik asit (ABTS) ve Fenantrolin aktivitesi gibi farklı antioksidan deneyler kullanılarak UV spektrofotometresi kullanılarak değerlendirilmiştir. Polifenol ve flavonoid içeriğinin kantitatif analizi yaprakların en yüksek miktarda olduğunu göstermiştir (sırasıyla 279.07±18.84 mg GAE/g E ve 132.74±27.74 mg ER/g E). En yüksek antioksidan aktivite 24.10±3.49 µg/mL, 9.365 ± 0.532 µg/mL, 0.19±0.093, 0.636±0.033 mg AAE/g ve 12.11±0. değeri ile yapraklarda gözlenmiştir. DPPH, ABTS, Toplam antioksidan kapasite, FRAP ve Fenantrolin aktivitesi için sırasıyla 81 µg /mL. Yaprakların LC-MS/MS analizi, meyveler ana bileşik olarak hesperidin (311.70 mg/g) içerirken, ana bileşik olan rutinin (24392.59 mg/g) varlığını ortaya koymaktadır.

Bu araştırma, bu tıbbi bitkilerin önemli bir antioksidan potansiyele sahip olduğunu ve önemli bir doğal antioksidan kaynağı olduğunu ve etkili bir şekilde kullanılabileceğini göstermiştir.

Anahtar kelimeler: *Zizyphus lotus*, toplam fenoliks, antioksidan potansiyel, LC-MS/MS analizi.

1. Introduction

The shrub *Zizyphus lotus*, commonly named 'Sedra', is the subject of many current laboratory investigations. This plant belongs to the Rhamnaceae family which includes around 550 species spread over around 45 genera (Ghedira, 2013; San et al., 2009). Being both a tropical and a subtropical plant, *Z. lotus* is commonly present in arid and semi-arid regions (Maraghni et al., 2011). In Algeria, this xerophytic plant can be found in the sand dunes of Saharan regions as well as in the arid and semi-arid zones where it occupies different types of soils (Maraghni et al., 2011; Guirado et al., 2017). *Z. lotus* is dormant from October to March and its fruits are harvested during the summer (Maraghni et al. 2010). It forms clumps of a few meters in diameter and 2 to 5 m in height. Its thorny stems possess small deciduous leaves and tasty fruits called 'Nbeg' (Ghedira, 2013; Maraghni et al., 2011; Hammi et al., 2015).

Many research findings in the literature confirmed these traditional uses. Borgi et al. (2007) outlined the anti-inflammatory, analgesic, and antispasmodic activities of *Z. lotus* extracts. The aqueous extracts obtained from different

Corresponding author: b.bakchiche@lagh-univ.dz

parts of *Z. lotus* proved their effects as cytotoxic agents against T-cells, the major cause of autoimmune diseases (Benammar et al., 2010). Furthermore, *Z. lotus* extracts obtained by different solvents showed excellent antifungal activity against nine strains of pathogenic fungi (Lahlou et al., 2002). Such endowments reflect the richness of *Z. lotus* in many active compounds, notably flavonoids and tannins (Borgi et al., 2007), cyclopeptide alkaloids such as lotusine A and lotusine D (Ghedira et al., 1993) and vitamins (A, C, E) (Benammar et al., 2010).

Therefore, the current work aimed to perform a comparative study between the leaf and fruit extracts of *Zizyphus lotus* based on their antioxidant activities, TPCs, TFCs, and chemical composition using LC–MS/MS analysis.

2. Materials and Method

2.1. Plant Material

The plant material used was fruits and leaves of *Ziziphus lotus* L. (Desf.) These parts were collected in June 2022 in area "Laghouat". After separation of the cores, the pulp was grounded by using an electric grinder.

2.2. Preparation of Methanolic Extract

The methanolic maceration was carried out on 50 g of powder (leaves or fruits) of *Z. lotus* with 100 mL of methanol, and then the mixture was placed under agitation during 24h. The extracts were filtered using Whatman paper and concentrated under vacuum with rotary evaporator to 40°C. Then sterilized using a 0.22 µm filter and conserved at +4°C until use.

2.3. Total Phenolic Content (TPC)

To determine phenolic content, the Folin-Ciocalteu's method was used (Kuhkheil et al., 2022). A total of 100 µL of the diluted extract in methanol was mixed with 200 µL of Folin Ciocalteu's reagent and 2 mL of water. The mixture was kept in obscurity for 5 min at room temperature. Then, 1mL of sodium carbonate solution (7.5 g/L, in water) was added; and after 30 min of incubation in the darkness, the absorbance was measured at 765 nm. To plot a calibration curve, the gallic acid was used as standard and the amounts of phenolic content were expressed in milligrams of Gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g DW). The measurements were carried out in triplicate.

2.4. Total Flavonoids Content (TFC)

The flavonoid content was determined spectrophotometrically, using a method based on the formation of a complex flavonoid–aluminium, having the absorptivity maximum at 430 nm. Rutin was used to make the calibration curve. 1 mL of sample was separately mixed with 1 mL of 2% aluminium chloride methanolic solution. After incubation at room temperature for 10 min, the absorbance of the reaction mixture was measured at 430 nm and The results were expressed as mg rutin equivalents per g of extract (mg RE/g) (Guenane et al., 2024).

2.5. Antioxidant Activity

2.5.1. DPPH• radical scavenging activity

The free radical scavenging ability was established according to the method recorded by Sharifi-Rad and Pohl (2020). 1800 µL of the DPPH solution (0.1 mM) was added to 200 µl of each sample at various concentrations. After 30 min of incubation at room temperature in the obscurity, the absorbance was read at 517 nm using a multimode plate reader. Butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), and vitamin C were considered as antioxidant standards. The inhibition percent was calculated as follows:

Inhibition (%) = [(Abs control - Abs sample)/ Abs control] x 100

The results were represented as IC_{50} values (µg/mL) which corresponds to the concentration of 50% inhibition.

2.5.2. ABTS (2,2'-azinobis (3-thylbenzothiazoline)6-sulfonic) radical trapping activity

The anti-radical activity of extract against $ABTS^{\bullet+}$radical was realized by the method of Saeed et al. (2012). The $ABTS^{\bullet+}$ solution was obtained by combining 7 mM of ABTS in water with 2.45 mM of potassium persulfate, stored in the obscurity at room temperature for 16h. The $ABTS^{\bullet+}$ solution was diluted in water to an absorbance of 0.7 at 734 nm. 1900 µL of this solution was added to 100 µL of each sample in methanol at various concentrations. After 10 min of incubation, the absorbance was recorded at 734 nm. The inhibition percent was calculated by applying the formula above (1). BHA and BHT were used as antioxidant standards. The inhibition percent was calculated as follows:

Inhibition (%) = [(Abs control - Abs sample)/ Abs control] x 100 (1)

The results were represented as IC_{50} values (µg/mL) which corresponds to the concentration of 50% inhibition.

2.5.3. Evaluation of total antioxidant capacity by phosphomolybdenum method

The antioxidant activity of the extract was evaluated by the phosphomolybdenum method according to the procedure described by Saravanakumar et al. (2021). The assay is based on the reduction of $Mo^{(VI)}$–$Mo^{(V)}$ by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. A 0.3 mL extract was combined with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). In case of blank, 0.3 mL of methanol was used in place of extracts. The tubes containing the reaction solution were capped and incubated in a boiling water bath at 95°C for 90 min. After cooling at room temperature, the absorbance of the solution was measured at 695 nm using a spectrophotometer.

2.5.4. Reducing power assay (iron reducing activity)

The reducing power of extracts was determined according to the method previously described by El Atki et al. (2019). Different concentrations of the tested extracts (0-1 mg) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured

at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Phosphate buffer (pH 6.6) was used as blank solution. All analyses were run in triplicate and results averaged.

2.5.5. Phenanthroline activity

The phenanthroline assay was evaluated by the method of Szydlowska-Czerniak et al. (2008). In brief, 30 µL O-phenanthroline (0.5% in methanol), 50 µL $FeCl_3$ (0.2%), and 110 µL of methanol were added to 10 µL of extract at various concentrations. After 20 min of incubation at 30°C in the obscurity, the absorbance was read at 510 nm. BHA and BHT were used as antioxidant standards.

2.6. LC-ESI-MS/MS Analysis

The 1260 infinity HPLC LC-MS/MS system coupled with an Agilent 6460 triple quadrupole mass spectrometer (USA) was used. The separation process utilized an Agilent Poroshell 120 SB-C18 column (3 × 100 mm, 2.7 µm), a type of reversed-phase column. The LC separation was performed using gradient elution with a mobile phase consisting of water (A) and methanol (B), both containing 0.1% formic acid and 5 mM of ammonium formate. The gradient elution profile was as follows: at 3 min, A= 75%, B= 25%; at 12 min, A= 50%, B= 50%; at 16 min, A= 10%, B= 90%; at 21 min, A= 10%, B= 90%; and at 24 min, A= 97.5%, B= 2.5%. The injection volume was 5.12 µL, the flow rate was 0.4 mL/min, and the temperature was maintained at 40°C. The separated compounds were introduced into a mass spectrometer which detects and identifies the target compounds based on their mass-to-charge ratios (*m/z*) and fragmentation patterns. LC-MS/MS systems often use electrospray ionization (ESI) as an ionization method employing both positive and negative ionization techniques. The nebulizer gas N2 flow was 8 L/min, the source voltage was 4000V, and the capillary temperature was 300°C during the LC-MS/MS analysis (Atalar et al., 2023). The multiple reactions monitoring (MRM) mode of the mass spectrometer was used to quantify the analyses.

2.7. Statistical Analysis

All experiments were performed in triplicate. The values of different parameters were expressed as the mean ± standard deviation (±SD).

3. Result

3.1. Determination of Total Phenolic and Flavonoid Contents

For the two parts of the plant studied by *Z. lotus*, we noticed variations in the contents of total phenols. The highest content is found in the extract of *Z. lotus* leaves that is around 279.07±18.84 mg GAE/g DW, followed by the fruit of *Z. lotus* which have a total polyphenol content of 61.25±4.75 mg GAE/g DE.

For the two parts of the studied plant *Z. lotus*, we noticed variations in the flavonoid contents. The highest flavonoid content is found in the extract of the Leaves methanolic of *Z. lotus*, in the order of 132.74±27.74 mg ER/g DW but a low flavonoid content in the extracts of the fruits of *Z. lotus* 31.47± 6.59 ER/g MW.

3.2. Antioxidant Activity

Variability of antioxidants and their properties requires the use of more than one method to assess the antioxidant activity of extracts. In order to study antioxidant activity of different extracts, three different techniques were used (DPPH, FRAP, and ABTS assay). DPPH assay measures the total antioxidant capacity (TAC) of compounds that are able to transfer hydrogen atoms, similar to ABTS, but it includes the action of polar and non-polar antioxidants, while FRAP assay measures the capacity to reduce iron ion Fe^{3+} to Fe^{2+}.

3.2.1. DPPH assay

The scavenging ability of the *Z. lotus* extracts samples (leaves and fruits) on DPPH free radical was shown in Table 1. The results showed a dose dependent scavenging power. As shown, the lower the IC_{50} values are the higher the antioxidant capacity of the flowers and leaves extracts become. With reference to the positive control ascorbic acid, the results revealed that both extracts have very notable antioxidant capacity. We also observed that leaves extract exhibited more potent antioxidant activity (IC_{50}= 24.10±3.49 µg/mL) than fruits extract (IC_{50}= 240.01±8.149). The results of the antioxidant capacity of the extracts tested show that all our extracts exert a less significant antioxidant powerin comparison with the antioxidant capacity of standard BHA, vitamin C, and BHT (IC_{50}= 1.12±6.23, 3.62 ±0.20, 5.40±0.10 µg/ml, respectively).

Positive correlation was observed between DPPH assay and phenolic compounds for both flowers and leaves extracts with a high level of significance ($p<0.05$). This correlation indicated that the richness in phenolic compounds enhances the antioxidant activity of the plant extract.

Table 1. Antioxidant activity of the phenolic extracts for *Z. lotus* expressed in IC_{50} (µg/mL) for DPPH and ABTS assay, VCEAC (vitamin C equivalents mmol of vitamin C/g dry weight) forPhosphomolybdate assay, mg AAE / g (mgAscorbic acid equivalent /g) for FRAP

Z. lotus extract	DPPH-IC_{50} (µg/mL)	ABTS-IC_{50} (µg/mL)	Total antioxidant capacity-VCEAC	FRAP (mg AAE/g)	Phenanthroline activity-IC_{50} (µg/mL)
Fruits methanolic	240.01±8.149	89.69±0.547	0.086±0.003	0.466±0.016	86.38±4.74
Leaves methanolic	24.10±3.49	9.365±0.532	0.19±0.093	0.636±0.033	12.11±0.81
Vitamin C	3.62 ±0.20	6.38±0.28	-	-	-
BHT	5.40±0.10	1.60±0.11	-	-	-
BHA	1.12±6.23	-	-	-	-
Trolox	-	4.25 ± 0.2	-	-	-

The IC_{50} values found for *Jujube* leaves in this study were recorded much higher than those reported by Dhibi et al. (2022) who showed that the extracts of *Jujube* leaves exhibited the activity at IC_{50} concentration (1.28±0.13 mg/mL).

3.2.2. Scavenging activity of $ABTS^{\bullet+}$ free radical

The principal objective of this test is to measure the capacity of different substances to scavenge the $ABTS^+$ radical cation. Antioxidant capacities were expressed by IC_{50} values, indicating the concentrations of extracts scavenge 50% of $ABTS^+$ radical.

As shown in Table 2, *Z. lotus* leaves showed the IC_{50} value of 9.365±0.532 µg/mL, while for fruit it was lower, 89.69±0.547 µg/mL. Comparing these values with standard (Trolox IC_{50} = 4.25±0.2 µg/mL), it is obvious that tested samples are effective to provide their capacity to scavenge the $ABTS^+$ radical cation at low concentration.

Table 2. Total phenol compounds and flavonoids content in *Z. lotus* extract

Z. lotus extract	Total phenolic content (mg GAE/g)	Total flavonoids content (mg ER/g)
Leaves methanolic	279.07±18.84	132.74±27.74
Fruits methanolic	61.25±4.75	31.47± 6.59

3.2.3. Ferric-reducing antioxidant power assay

It was suggested that the electron donating capacity, reflecting the reducing power of bioactive compounds, is associated with antioxidant activity.

Antioxidants can be reductants and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species is reduced at the expense of the oxidation of the other. The presence of reductants, such as antioxidant substances in the samples, causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. The reducing power of the extracts increased with increasing concentration which suggests that the electron donating ability of the extracts is concentration dependent. The assay for reducing power capacity involving reduction of Fe^{3+} (ferric form) of potassium ferricyanide to Fe^{2+} (ferrous form) revealed the presence of substances such as electron donors with antioxidant activity (Chanda & Dave, 2009).

The SET involves the transfer of an electron from the antioxidant compound as in the FRAP assay. The samples reduce ferricyanide complex into ferrous (Fe^{2+}) form. The FRAP value of 0.636±0.033 mg AAE/g in leaf extract was significantly higher than that of the fruit (0.466±0.016 mg AAE/g) (Table 1).

3.2.4. Phenanthroline activity

The highest Phenanthroline activity was observed in extract of *Z. lotus* leaves (The IC_{50} value is 12.11±0.81 µg/mL). However, the lower activity was detected in the fruits (86.38±4.74 µg/mL).

3.2.5. Total antioxidant capacity assay

The phosphomolybdate method has been used routinely to evaluate the total antioxidant capacity of plant extracts (Prieto et al., 1999; Prasad et al., 2009). In the presence of extracts, Mo(VI) is reduced to Mo(V) and forms a green colored phosphomolybdenum V complex which shows a maximum absorbance at 695 nm. Therefore, the antioxidant activity of *Z. lotus* extract leaves (VCEAC = 0.19±0.093) was higher than that of *Z. lotus* extract fruit (VCEAC = 0.086±0.003).

3.3. LC–MS/MS Analysis

The results of chromatogram profiles and phenolic compounds concentrations are shown in Figure 1 and 2 and in Table 3. Various phenolic components were identified and quantified in leaf and fruit parts of the *Z. lotus* extracts using the LC-MS-MS analysis, the same phenolic compounds were detected in different amounts in both parts of the plants. Rutin was determined as the major phenolic compound in leaves (24392.59 mg/g) and the hesperidin in fruits (311.70 mg/g). However, the amounts of hesperidin, isoquercitrin, epigallocatechin, kaempferol-3-glucoside, o-coumaric acid, and caffeine in leaves were measured as higher than those in the fruits.

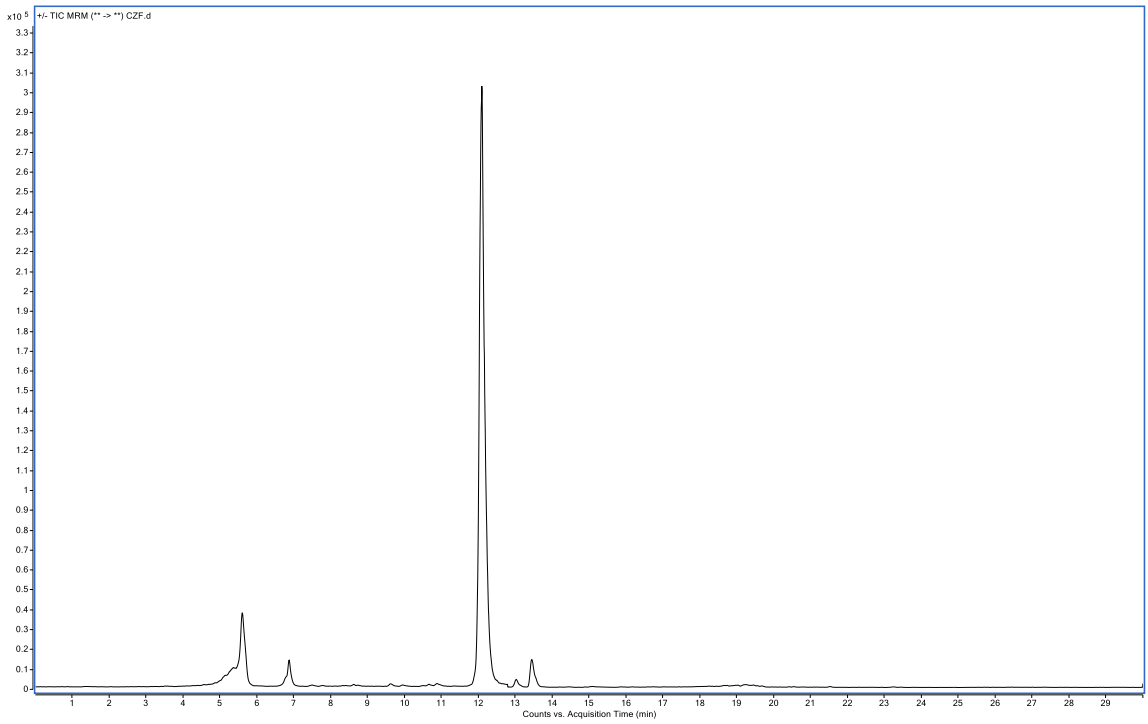

Figure 1. LC-MS/MS chromatogram of *Z. lotus* fruits

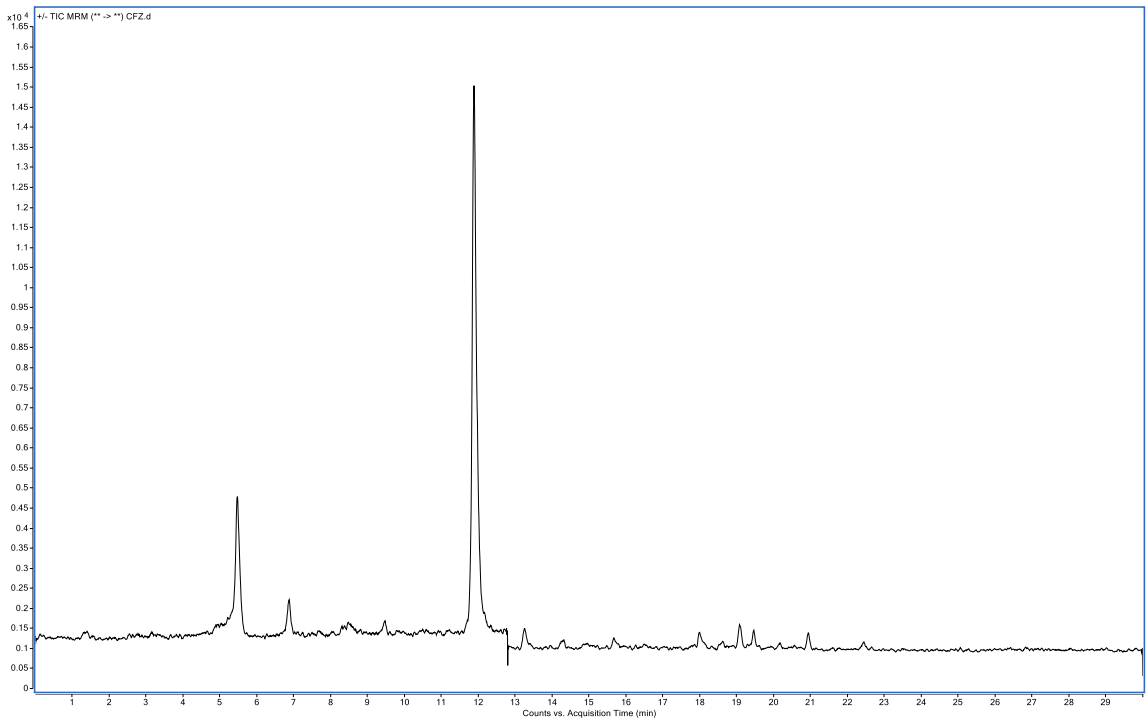

Figure 2. LC-MS/MS chromatogram of *Z. lotus* leaves

Table 3. Phenolic content of leaves and fruits *Z. lotus* using LC-MS/MS

Peak No.	Phenolic Standards (mg/g)	Rt (min)	Leaves	Fruits
1	Shikimic acid	2.2161	ND	0.2128
2	Gallic acid	3.292	ND	5.5424
3	Protocatechuic acid	5.537	ND	6.9446
4	Epigallocatechin	6.878	154.8849	1.7953
5	Chlorogenic acid	7.498	27.0084	ND
6	Hydroxybenzaldeyde	7.791	5.4149	0.0099
7	Caffein	8.404	3.6009	1.8879
8	Vanillin	8.631	9.3370	0.0070
9	Catechin	9.2987	0.0005	ND
10	O-coumaric acid	9.628	8.3504	2.7982
11	Taxifolin	9.921	13.9085	ND
12	Vanillic acid	10.4565	0.0076	ND
13	Polydatin	11.2842	0.0091	0.0062
14	*trans*-ferulic acid	11.5327	0.0108	0.0215
15	Hesperidin	12.094	7690.0572	311.7035
16	Isoquercitrin	12.110	218.1616	10.1358
17	Rutin	12.078	24392.5950	1.4801
18	Quercetin 3-xyloside	12.676	18.6118	1.3583
19	Kaempferol-3-glucoside	13.443	51.3885	2.2024
20	Fisetin	13.461	3.1832	ND
21	Morin	13.7851	0.0223	ND
22	*trans*-cinnamic acid	14.281	ND	2.7519
23	Quercetin	15.074	19.7175	0.0024
24	Naringenin	15.184	5.5983	ND
25	Silibinin	15.211	1.6991	ND
26	Diosgenin	20.5226	0.0001	0.0001

4. Discussion

The current findings revealed that the tested extracts contain high amount of phenolic compounds with TPC values of 279.07±18.84 and 61.25±4.75 mg GAE/g DW for the leaves and the fruits, respectively. According to Ait Bouzid et al. (2022), fruits of *Z. lotus* collected from Zagora presented the highest TPC in acetone extract (21.67 ± 0.85 mg GAE/g DM).

However, the fruits of Tafraout had the greatest content of TPC in both water and methanol extracts (15.29 ± 0.17 and 10.13 ± 0.02 mg GAE/g DM) and for ethanol extracts, the highest TPC was observed in Khnifra (10.35 ± 0.26 mg GAE/g DM). Fruits of *Z. lotus* from Zagora contained the highest TFC in acetone extracts (18.92 ± 0.16 mg QE/g DM), water extracted more flavonoids in fruits of Tafraout (17.56 ± 1.24 mg QE/g DM).

In ethanol extracts, fruits collected from Beni Mellal had the highest TFC (7.48 ± 0.04 mg QE/g DM).

Our values of TFC were higher than those reported by El Maaiden et al. (2020) for *Z. lotus* fruit water extracts (5.49 ± 0.10 mg CE/g DM). They were also higher than those reviewed by Abdoul-Azize (2016) (1.22 mg/g DM). TFC values from water extracts were higher than those obtained by Dahlia et al. (2020) (0.83 ± 0.09–0.98 ± 0.01 mg QE/g DM) and Bencheikh et al. (2021) (2242.89 ± 25 μg QE/mg DM).

The quantification of the total phenolic and flavonoids contents was determined by Bencheikh et al. (2021) showed that the *ZLF* aqueous extract is rich in flavonoids (2242.89 ± 25 µg QE/mg) and to a lesser extent in total phenolic (278 ± 12 µg AGE/mg).

Samples investigated by Khouchlaa et al. (2020) showed higher values of these bioactive compounds (phenolics 285.19 mg GAE/mg, flavonoids 2.66 mg

QE/mg).

The total phenolic content in lotus leaves extract varied from 67.3 to 140.4µg GAE/mg extract depending on extraction conditions (Jae Young et al., 2019).

As reported by Elaloui et al. (2023), the richest provenance for phenolic compounds was Mahdia. The highest levels reached the values of 245.15 mg GAE/g DW and 180.23 mg QE/g DW for total phenol and flavonoid, respectively.

Our values of TFC were lower than those reported by Dhibi et al. (2022). Jujube leaves contained phenolic content (468.57±56 mg GAE/g DW) and flavonoids content (12.96 ± 1.25 CEQ/g DW) and these values were lower than those reported by Bekkar et al. (2021) who determined a total flavonoids content of 149.87 ±0.12 (mg QE/g DE).

Similarly, Bekkar et al. (2021) found the content of total flavonoids as 149.87± 0.12 (mg QE/g DE) in methanolic extract of Jujube leaves collected from western Algeria (Bekkar et al., 2021).

According to outcomes, TFC of *Z. lotus* fruits were significantly influenced by the geographical origin as well as the solvent used. TFC contributes to the nutritional and medicinal importance of *Z. lotus* fruits.

This proves the importance of *Z. lotus* fruits as source of natural antioxidants. The findings reported in this work provide scientific data about chemical composition of Moroccan jujube for different uses and also contribute to the enrichment of the database of medicinal and aromatic plants.

Total antioxidant capacity, DPPH, ABTS, and FRAP assays are four tests with good repeatability frequently used for phytoextracts. As shown in Table 1, leaves and fruits of the *Z. lotus* extracts exhibited a wide range and a potential antioxidant capacity to quench radicals. The present study also shows that *Z. lotus* exerted an antioxidant activity.

Yahia et al. (2020) found that the IC_{50} value of fruits (12.16 µg/mL) of *Z. lotus* from Bengardane is endowed with an interesting antioxidant activity.

According to Jae Young et al. (2019), the total extract of lotus leaves showed dose-dependent antioxidant activity with IC_{50} value of 100.2 µg/mL.

The maximum % inhibition of DPPH in fruits (67%) and leaves (71%) were measured for species *Z. spina-christi* reported by Umair Riaz et al. (2021).

The DPPH through the IC_{50} values (concentration required for 50 % inhibition) decreased slightly from 175 µg/mL (Sfax) to 55 µg/mL (Mahdia) (Elaloui et al., 2023).

The half-maximum inhibitory concentration of the aqueous *ZLF* extract was IC_{50} = 116±0.02 µg/mL determined by Bencheikh et al. (2021).

Both the AE and the ME of this plant part were ranked as potent extracts with an IC_{50} of 16.46±0.60 mg/L and 18.03±0.61 mg/L, respectively and the ME of the leaves expressed the highest value among this plant organ extracts (33.66±0.11 mg/L) were confirmed by Letaief et al. (2021).

According to Letaief et al., 2021), the ME of leaves of *Z. lotus* showed the best IC_{50} value (23.48±0.63 mg/L). Consequently, *Z. lotus* extracts react better with the $ABTS^{\bullet+}$ assay which is based on rapid electron transfer reactions.

Jujube leaves extract was also more efficient in the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form with a lower IC_{50} values (2.18±0.05 mg/mL) (Dhibi et al., 2022).

Moreover, *Z. lotus* leaf extract showed the highest total antioxidant capacity with the value of 173.09±2.99 mg AAE/mg (Letaief et al., 2021).

According Yahia et al. (2020), *Z. mauritiana* leaves with 31 mg GAE/g DW showed the highest total antioxidant capacity compared to those of *Z. lotus*.

The antioxidant activity differences observed among different species of *Ziziphus* may be attributed to phenolic compounds which depend on the region (Chen et al., 2017; Tlili et al., 2014), species (Elaloui et al., 2017), genotypes (Gao et al., 2012), and/or the extraction method (Hossain et al., 2016). These data extend to confirm the presence of substantial amounts of phenolics in *Ziziphus* extracts indicating that they are a significant source of antioxidants which may provide health promoting advantages to the consumers.

Our study represents a comprehensive effort towards elucidating the chemical profile of this species and preliminary evidence supporting its potential pharmaceutical characteristics.

The results of LC-MS analysis revealed that flavonoids particularly rutin and the hesperidin were predominant in the leaves and fruits of the *Z. lotus* extract respectively. Mkadmini Hammi et al. (2017) revealed that extract of *Z. lotus*. with Quercetin was qualified as the major phenolic compound in the hydromethanolic extract (129.54 µg/g) followed by catechin (78.38 µg/g DE) and chlorogenic acid (35.38 µg/g DE).

According to Cadi et al. (2020), hydroxycinnamic acids were detected only in the EtOAc extract (84.69±0.5), while flavonols were more abundant in the MeOH-H_2O extracts (31.99±0.05 vs. 14.45±0.01).

Marmouzi et al. (2019) found that the gallic acid was the major component of fruits and leaves of *Zizyphus lotus*: 15,640 ± 310 mg/kg and 2715±112 mg/kg, respectively.

Similarly, ferulic and vanillic acids, rutin, catechin, and epicatechin were much more abundant in fruits than leaves. On the other hand, pyrogallol, naringin, chlorogenic, caffeic, syringic, p-coumaric, sinapic, salicylic, and rosmarinic acids were more elevated in leaves than fruits extracts.

The main compounds in *Jujube* leaves are fumaric acid (18%), gallic acid (40%), and vanillin (about 8-10%). Unknown peak with a higher level (around 18%) was detected in the methanolic extract of both leaves reported by Dhibi et al. (2022).

An analysis by HPLC-DAD-ESI/MS shows that aqueous *ZLF* extracts are rich in phenolic compounds such as sinapic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, *p*-coumaroyl glucose, benzoic acid, cinnamic acid derivative,

galloyl shikimic acid, (−)-catechin 3-*O*-gallate, and quercetin (Cadi et al., 2020).

Difference in phenolic profile can be due to maturation phase, pedoclimatic factors, and genetic variability which involved in modification of the biosynthesis path of secondary phenolic metabolites.

According to Yahia et al. (2020), among the identified phenolics, quinic acid (65.12–902.4 µg/g), *p*-coumaric acid, (2.58–15.67 µg/g) and rutin (9.29–4803.82 µg/g) were the only compounds shared between the different plant parts of *Ziziphus* genus.

5. Conclusion

In conclusion, the results of this study will provide new knowledge about the composition and content of phenolic compounds in leaves, fruits, and the antioxidant activity of their extracts which will give a wide range of possibilities to employ these plants as the source of phenolic compounds.

The highest total amounts of phenolic compounds and flavonoids were determined in the leaves and fruits of the *Z. lotus* (279.07±18.84 mg GAE/g DW, 61.25±4.75 GAE/g DW and 132.74±27.74 mg RE/g DW, 31.47±6.59 RE/g DW resp.). In this study, a total of polyphenolic bioactive compounds was screened and confirmed through LC-MS/MS. Rutin was determined as the major phenolic compound in leaves (24392.59 mg/g) and the hesperidin in fruits (311.70 mg/g).

The preliminary *in vitro* experiments examining the antioxidant activity of the leaf and fruit extracts by the ABTS, DPPH, Phosphomolybdate, Phenanthroline, and FRAP assays have shown that these extracts possess a strong antioxidant activity which positively correlated with the total phenolic and flavonoid contents.

Extracts of leaf and flower parts of the plant showed strong antioxidant activity. However, antioxidant activity values in the leaf were higher than the fruits.

Acknowledgement: The authors are grateful to the DGRSDT and the Ministry of Higher Education and Scientific Research of Algeria for PRFU Project (A16N01UN030120230001), and the team of Medicinal Chemistry Department, Theodor Bilharz Research Institute, Egypt.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The authors declare that there is no conflict of interest.

Author Contributions: Conception – H.G, B.B.; Design – H.G, B.B.; Supervision – B.B.; Fund – H.G.; Materials – B.B.; Data Collection and Processing – H.G., R.E., I.Y.; Analysis Interpretation – H.G., B.B., M.A.G.; Literature Review – H.G., B.B., M.A.G., R.E., I.Y.; Writing – H.G., B.B., M.A.G., R.E., I.Y.; Critical Review – H.G., B.B., M.A.G., R.E., I.Y.

References

Abdoul-Azize, S. (2016). Potential benefits of *Zizyphus lotus* L. bioactive compounds for nutrition and health. *Journal of Nutrition and Metabolism*, 2016, 2867470. https://doi.org/10.1155/2016/2867470

Ait Bouzid, H., Sakar, E.H., Bijla, L., Ibourki, M., Zeroual, A., Gagour, J., Koubachi, J., Majourhat, K., & Gharby, S. (2022). Physical fruit traits, proximate composition, antioxidant activity, and profiling of fatty acids and minerals of wild jujube (*Zizyphus lotus* L. (Desf.)) fruits from eleven Moroccan origins. *Journal of Food Quality*, 2022(1), 9362366. https://doi.org/10.1155/2022/9362366

Atalar, M.N., Erenler, R., Turkan, F., Alma, M.H., Demirtas, I., Baran, A., Irtegun Kandemir, S., Kekec, A.I., & Saltan, F.Z. (2023). Phytochemical analysis and biological activity of *Corchorus olitorius* L.: Quantitative analysis of bioactive compounds by LC–MS/MS, antibacterial, enzyme inhibition, and cytotoxic activities. *European Journal of Integrative Medicine*, 62, 102290. https://doi.org/10.1016/j.eujim.2023.102290

Bekkar, N.E.H., Meddah, B., Cakmak, Y.S., & Keskin, B. (2021). Phenolic composition, antioxidant, and antimicrobial activities of *Zizyphus lotus* L. and *Ruta chalepensis* L. growing in Mascara (Western Algeria). *Journal of Microbiology, Biotechnology and Food Sciences*, 10, 1–9. https://doi.org/10.15414/jmbfs.300

Benammar, C., Hichami, A., Yessoufou, A., Simonin, A.M., Belarbi, M., Allali, H., & Khan, N.A. (2010). *Zizyphus lotus* L. (Desf.) modulates antioxidant activity and human T-cell proliferation. *BMC Complementary and Alternative Medicine*, 10, 54. https://doi.org/10.1186/1472-6882-10-54

Bencheikh, N., Bouhrim, M., Alami Merrouni, I., Boutahiri, S., Kharchoufa, M., Addi, L., Tungmunnithum, D., Hano, C., Eto, B., Legssyer, A., & Elachouri, M. (2021). Antihyperlipidemic and antioxidant activities of flavonoid-rich extract of *Zizyphus lotus* (L.) Lam. fruits. *Applied Sciences*, 11, 7788. https://doi.org/10.3390/app11177788

Borgi, W., Bouraoui, A., & Chouchane, N. (2007). Antiulcerogenic activity of *Zizyphus lotus* (L.) extracts. *Journal of Ethnopharmacology*, 112, 228–231. https://doi.org/10.1016/j.jep.2007.02.024

Chanda, S., & Dave, R. (2009). In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research*, 3, 981–996.

Chen, J., Liu, X., Li, Z., Qi, A., Yao, P., Zhou, Z., ... & Tsim, K. W. (2017). A review of dietary *Ziziphus jujuba* fruit (jujube): Developing health food supplements for brain protection. *Evidence-Based Complementary and Alternative Medicine*, 24, 110. https://doi.org/10.1155/2017/3019568

Dahlia, F., Barouagui, S., Hemida, H., Bousaadia, D., & Rahmoune, B. (2020). Influence of environmental variations on anti-glycaemic, anti-cholesterolemic, antioxidant, and antimicrobial activities of natural wild fruits of *Ziziphus lotus* (L.). *South African Journal of Botany*, 132, 215–225. https://doi.org/10.1016/j.sajb.2020.04.033

Dhibi, M., Amri, Z., Mnari Bhouri, A., Hammami, S., & Hammami, M. (2022). Comparative study of the phenolic profile and antioxidant activities of *Moringa oleifera* Lam. and *Ziziphus lotus* Linn. leaf extracts and their protective effects in frying stability of corn oil. *Measurement: Food*, 7, 100045. https://doi.org/10.1016/j.meafoo.2022.100045

El Atki, Y., Aouam, I., El Kamari, F., Taroq, A., Nayme, K., Timinouni, M., Lyoussi, B., & Abdellaoui, A. (2019). Phytochemistry, antioxidant, and antibacterial activities of two Moroccan *Teucrium polium* L. subspecies: Preventive approach against nosocomial infections. *Journal of Advanced Pharmaceutical Technology & Research*, 10(2), 63–67. https://doi.org/10.1016/j.arabjc.2019.04.001

Cadi, H.E., Bouzidi, H. E., Selama, G., Cadi, A. E., Ramdan, B., Oulad El Majdoub, Y., ... & Cacciola, F. (2020). Physico-chemical and phytochemical characterization of Moroccan wild jujube *Zizyphus lotus* (L.) fruit crude extract and fractions. *Molecules*, 25, 5237. https://doi.org/10.3390/molecules25225237

El Maaiden, E., El Kharrassi, Y., Lamaoui, M., Allai, L., Essamadi, A.K., Nasser, B., & Moustaid, K. (2020). Variation in minerals, polyphenolics, and antioxidant activity of pulp, seed, and almond of different *Ziziphus* species grown in Morocco. *Brazilian Journal of Food Technology*, 23, e2019206. https://doi.org/10.1590/1981-6723.20619

Elaloui, M., Ennajah, A., Ghazghazi, H., Youssef, I.B., Othman, N.B., Hajlaoui, M.R., ... & Laamouri, A. (2017). Quantification of total phenols, flavonoids, and tannins from *Ziziphus jujuba* (Mill.) and *Ziziphus lotus* (L.) (Desf.) leaf extracts and their effects on antioxidant and antibacterial activities. *International Journal of Secondary Metabolites*, 4, 18–26. https://doi.org/10.21448/ijsm.275886

Elaloui, M., Ammari, Y., Ghazghazi, H., Ben Othmen Gorrab, A., Laamouri, A., & Driouich Chaouachi, R. (2023). Effect on *Ziziphus jujuba* Mill. fruit powders embedded on physicochemical properties, biological activities, and rheologic quality of cake. *Food Science & Nutrition*, 11, 2942–2955. https://doi.org/10.1002/fsn3.3276

Gao, Q.H., Wu, C.S., Yu, J.G., Wang, M., Ma, Y.J., & Li, C.L. (2012). Textural characteristic, antioxidant activity, sugar, organic acid, and phenolic profiles of 10 promising jujube (*Ziziphus jujuba* Mill.) selections. *Journal of Food Science*, 77, 1218–1225. https://doi.org/10.1111/j.1750-3841.2012.02946.x

Ghedira, K., Chemli, R., Richard, B., Nuzillard, J.M., Zeches, M., & Men-Olivier, L. (1993). Two cyclopeptide alkaloids from *Zizyphus lotus*. *Phytochemistry,* 32, 1591–1594.

Ghedira, K. (2013). *Zizyphus lotus* (L.) Desf. (Rhamnaceae): Jujubier sauvage. *Phytothérapie,* 11, 149–153. https://doi.org/10.1007/s10298-013-0776-8

Guirado, E., Tabik, S., Alcaraz-Segura, D., Cabello, J., & Herrera, F. (2017). Deep-learning Versus OBIA for scattered shrub detection with Google Earth Imagery: *Ziziphus lotus* as case study. *Remote Sensing,* 9, 1220. https://doi.org/10.3390/rs9121220

Guenane, H., Bakchiche, B., Erenler, R., Yildiz, I., Mohamed, A.S., & El-Shazly, M.A.M. (2024). Mineral contents, antioxidant and antimicrobial activities of Algerian *Terfezia claveryi* extracts. *FABAD Journal of Pharmaceutical Sciences,* 49(1), 19–36. https://doi.org/10.55262/fabadeczacilik.1326710

Hammi, K.M., Jdey, A., Abdelly, C., Majdoub, H., & Ksouri, R. (2015). Optimization of ultrasound-assisted extraction of antioxidant compounds from Tunisian *Zizyphus lotus* fruits using response surface methodology. *Food Chemistry,* 184, 80–89. https://doi.org/10.1016/j.foodchem.2015.03.047

Hossain, A., Hamood, A., & Humaid, M.T. (2016). Comparative evaluation of total phenols, flavonoids content and antioxidant potential of leaf and fruit extracts of Omani *Ziziphus jujuba* L. *Pacific Science Review A: Natural Science and Engineering,* 18, 78–83. https://doi.org/10.1016/j.psra.2016.09.001

Jae Young, J., Jong Hoon, A., Yang Hee, J., Bang Yeon, H., & Mi Kyeong, L. (2019). Antioxidant Activity and Phenolic Content of Different Parts of *Lotus* and Optimization of Extraction Condition using Response Surface Methodology. *Natural Product Sciences,* 25(1), 44-48. https://doi.org/10.20307/nps.2019.25.1.44

Khouchlaa, A., Talbaoui, A., El Yahyaoui El Idrissi, A., Bouyahya, A., Ait Lahsen, S., Kahouadji, A., & Tijane, M. (2020). Determination of phenol content and evaluation of in vitro litholytic effects on urolithiasis of Moroccan *Zizyphus lotus* L. extract. *Phytotherapie,* 16, 1. https://doi.org/10.1007/s10298-017-1106-3

Kuhkheil, A., Naghdi Badi, H., Mehrafarin, A., & Abdossi, V. (2020). Phytochemical and morpho-physiological variations in sea buckthorn (*Hippophae rhamnoides* L.) populations of Taleghan region in Iran. *Journal of. Medicinal Plants*, 21–35. https://doi.org/10.29252/jmp.19.76.21

Lahlou, M., & Mahi, J.H. (2002). Évaluation des activités antifongique et molluscicide de *Zizyphus lotus* (L.) Desf. du Maroc. *Annales Pharmaceutiques Françaises,* 60, 410–414.

Letaief, T., Garzoli, S., Masci, V.L., Mejri, J., Abderrabba, M., Tiezzi, & Ovidi, E. (2021). Chemical composition and biological activities of Tunisian *Ziziphus lotus* extracts: Evaluation of drying effect, solvent extraction, and extracted plant parts. *Plants (Basel),* 10(12), 2651. https://doi.org/10.3390/plants10122651

Maraghni, M., Gorai, M., & Neffati, M. (2010). Seed germination at different temperatures and water stress levels, and seedling emergence from different depths of *Ziziphus lotus*. *South African Journal of Botany,* 76, 453–459.

Maraghni, M., Gorai, M., & Neffati, M. (2011). The influence of water-deficit stress on growth, water relations and solute accumulation in wild Jujube. *Journal of Ornamental and Horticultural Plants,* 1, 63–72. https://doi.org/10.1016/j.sajb.2010.02.092

Marmouzi, I., Kharbach, M., El Jemli, M., Bouyahya, A., Cherrah, Y., Bouklouze, A., Vander Heyden, Y., & Faouzi, M. (2019). Antidiabetic, dermatoprotective, antioxidant and chemical functionalities in *Zizyphus lotus* leaves and fruits. *Industrial Crops and Products,* 132, 134–139. https://doi.org/10.1016/j.indcrop.2019.02.007

Mkadmini Hammi, K., Jellouli Ennigrou, D., Majdoub, H., & Ksouri, R. (2017). Recovery of phenolic compounds and carbohydrates from hydro-ethanolic extract of *Zizyphus lotus* fruit using ultrafiltration process. *International Journal of Food Engineering,* 13, 1–13. https://doi.org/10.1515/ijfe-2017-0343

Prasad, K. N., Yang, B., Yang, S., Chen, Y., Zhao, M., Ashraf, M., & Jiang, Y. (2009). Identification of phenolic compounds and appraisal of antioxidant and antityrosinase activities from litchi (*Litchi sinensis* Sonn.) seeds. *Food Chemistry,* 116, 1–7. https://doi.org/10.1016/j.foodchem.2009.01.079

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry,* 269, 337–41. https://doi.org/10.1006/abio.1999.4019

Saeed, N., Khan, R., & Shabbir, M.M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complementary and Alternative Medicine,* 12, 221. https://doi.org/10.1186/1472-6882-12-221

San, B., Yildirim, A.N., Polat, M., & Yildirim, F. (2009). Mineral composition of leaves and fruits of some promising jujube (*Zizyphus jujuba* Miller) genotypes. *Asian Journal of Chemistry,* 21, 2898–2902.

Saravanakumar, K., Sarikurkcu, C., Sahinler, S.S., Sarikurkcu, R.B., & Wang, M. (2021). Phytochemical Composition, Antioxidant, and Enzyme Inhibition Activities of Methanolic Extracts of Two Endemic *Onosma* Species. *Plants,* 10, 1373. https://doi.org/10.3390/plants10071373

Sharifi-Rad, M., & Pohl, P. (2020). Synthesis of biogenic silver nanoparticles (AgCl-NPs) using a *Pulicaria vulgaris* Gaertn. aerial part extract and their application as antibacterial, antifungal, and antioxidant agents. *Nanomaterials,* 10, 638. https://doi.org/10.3390/nano10040638

Szydlowska-Czerniak, A., Dianoczki, C., Recseg, K., Karlovits, G., & Szlyk, E. (2008). Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods. *Talanta,* 76(4), 899–905. https://doi.org/10.1016/j.talanta.2008.04.055

Tlili, N., Tir, M., Benlajnef, H., Khemiri, S., Mejri, H., Rejeb, S., & Khaldi, A. (2014). Variation in protein and oil content and fatty acid composition of *Rhus tripartitum* fruits collected at different maturity stages in different locations. *Industrial Crops and Products,* 59, 197–201. https://doi.org/10.1016/j.indcrop.2014.05.020

Umair Riaz, M., Ali Raza, M., Saeed, A., Ahmed, M., & Tanveer, H. (2021). Variations in Morphological Characters and Antioxidant Potential of Different Plant Parts of Four *Ziziphus* Mill. Species from the Cholistan. *Plants (Basel),* 10(12), 2734. https://doi.org/10.3390/plants10122734

Yahia, Y., Benabderrahim, M.A., Tlili, N., Bagues, M., & Nagaz, K. (2020). Bioactive compounds, antioxidant and antimicrobial activities of extracts from different plant parts of two *Ziziphus* Mill. Species. *PLoS One,* 15(5), e0232599. https://doi.org/10.1371/journal.pone.0232599