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Original Article

The Impact of Drying Method on Bioactive Compounds and Microstructure of *Jujube Zizyphus Lotus* Leaves

Mourad Ouhammou[1*], Bouchra Nabil[1], Rachida Ouaabou[1], Sara Elgadi[2], Nadia Hidar[1], Yasamine Jaouad[3], Abderrahim Jaouad[1], Mostafa Mahrouz[1]

[1]Materials Science and Process Optimization Laboratory SCIMTOP, Department of Chemistry, Faculty of Sciences Semlalia, Cadi Ayyad University, Morocco
[2]Laboratory of Microbial Biotechnology, Agrosciences and Environment, Faculty of Sciences- Semlalia, Cadi Ayyad University, Morocco
[3]The Higher School of Technology of Kelâa des Sraghna, Cadi Ayyad University, Morocco

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Abstract

Background and Aim: The leaves of *Jujube Zizyphus lotus* are widely used as medicine for their health benefits, particularly in China, America and the Middle East. To determine the benefits of Jujube leaves, the leaves were analysed to distinguish certain chemical compounds, examine their antioxidant activity and study their structural morphology.

Materials and Methods: Conventional drying by ovens is a method of preserving nutritious foods. The drying temperature gradient of 10°C affects certain food compositions. In the case of the Jujube leaf, the increase in temperature revealed an increased extraction. Moreover, an increase in the release of polyphenols analyzed by reactive Folin method (2.5-3 mgEqAG/100g), and a small variation in antioxidant activity tested by 2,2-diphenyl-1-picrylhydrazyl method of about 80% were registered.

Results: We also noticed a stability of about 3 mg/100g for flavonoids, a very low concentration 0.5 mg/g for total carotenoids, a variation between 12-45 mg/g for phyophytins, and a degradation of chlorophyll a (between 3-18) and chlorophyll b (2-8 mg/g).

Conclusion: High performance liquid chromatography (HPLC) analysis showed that the Jujube tree, *Zizyphus lotus*, is very rich in phenolic compounds, above all Naringin, Furelique, Rutin, Quercetin, and Kaempferol.

Keywords: Jujube, Ziziphus lotus, Valorization, Drying, Biochemical, Antioxidant activity

***Corresponding Author:** Mourad Ouhammou, Materials Science and Process Optimization Laboratory SCIMTOP, Department of Chemistry, Faculty of Sciences Semlalia, Cadi Ayyad University, BP 2390, Marrakech 40000, Morocco. Email: ouhamoumourad@hotmail.com.

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Introduction

The jujube tree is a frutescent tree of the Rhamnaceae family, native to China 4000 years ago, with more than 700 subspecies used during more than thousands of years of cultivation in temperate and subtropical regions of the Northern Hemisphere (1). This Mediterranean species is not abundantly found in the northern Sahara, i.e. Morocco, Algeria, Tunisia, and Libya.

In Morocco, the jujube tree is abundant in the regions of the Center, particularly bordering the Middle Atlas and the highlands of Moulouya towards the mountains. The most abundant species is *Zizyphus lotus* whose valuation is devoted to the fruit as food or fruit flour as a potential nutritional ingredient for the development of commercial food products (2). Jujube fruit, which is very rich in carbohydrates, vitamin C, soluble and insoluble fiber, protein, mineral and phenolic compounds (3), has significant edible and medicinal values (1, 4).

Since the use of the leaves of the jujube tree as the main by-product of the jujube tree is very limited, they are widely used in traditional medicine against certain diseases such as smallpox and measles. They are also widely used for hair care (5). In southern Morocco, this species is indicated against boils and abscesses. Moreover, wood ash, with the addition of vinegar, constitutes a local treatment of snakebites (6). Dry leaves sprayed with mortar are used by the Malikites, because of their properties. They are also used for hair care.

Our objective is to determine certain physicochemical and biochemical characteristics of Jujube leaves of the species *Zizyphus lotus* with a study of the activity and antioxidant activities as a function of the drying temperature from 30 °C to 90 °C. Jujube leaves are very rich in total polyphenols, especially flavonoids, already analysed by "high performance liquid chromatography-photodiode array-mass spectrometry/ evaporative light scattering detector" (HPLC–PDA –MS/ELSD). Some previous analyses of the leaves of the *Ziziphus* species revealed a concentration of 29.052 ± 0.38 mg/g of flavonoids, with an abundance of eight flovonoid compounds, i.e. quercetin 3-O-robinobioside, rutin, hyperoside (quercetin-3-O-D-galactoside), quercetin-3-O-D-glucoside, kaempferol-3-O-robinobioside, kaempferol-3-O-glucoside, quercetin-3-O-β-L-arabinosyl-(1→2)-α-L-rhamnoside, quercetin-3-O-β-D-xylosyl-(1→2)-α-L-rhamnoside and Quercetin-3-O-β-L-arabinosyl-(1→2)-α-L-rhamnoside (8).

Moreover, a reliable procedure based on HPLC coupled to PDA and electrospray ionization tandem mass spectrometry (HPLC-PDA-ESI-MS) has been proposed for the investigation of the profiles of the chemicals of these jujube leaves. The results of the analysis revealed that fourteen components, including three flavonoids, two saponins, and nine triterpene acids were identified, and using HPLC coupled with an ELSD detection method, the chromatography allowed detected twelve polyphenols, e.g. quercetin-3-O-rutinoside, zizyphus saponins I and II, ceanothic acid, alphitolic acid, maslinic acid, 2αacid-hydroxyursolic, zizyberanalic acid, epiceanothic acid, ceanothenic acid, betulinic acid and oleanolic acid (9).

Other studies have shown that jujube leaves are very rich in saponosides, flavonoids, and various compounds such as β-tocopherol (Vit E), linoleic acid (10), and supercritical extraction fluid by CO_2 (SFE-CO_2). Analysis by ultra-performance mass spectrometry and HPLC-ESI-MS showed the presence of eight flavonoid compounds based on the compounds of kaempferol glycosides and quercetin (8). Moreover, the physicochemical analysis revealed the presence of significant contents of total soluble solids, dry matter, Vitamin C, and ash that are very rich in calcium, potassium, magnesium, sodium, nitrogen, calcium, phosphorus, and proteins (11). Other studies showed antidiabetic, dermatoprotective, and antioxidant functionalities (12), as well as biological activity and associated flavonoid compositions (13). Transcriptome data indicated 20 structural flavonoid genes and three major kinds of flavonoid regulatory genes were remarkably and differentially expressed (14).

In this context and to valorize Moroccan Jujube Zizyphus lotus, this study investigates the possibility of drying plants at the exact temperature with an important bioactive compound.

Drying in the oven at different temperatures affects the increase of phenolic compounds and other bioactive agents, which are used to determine the storage conditions and a debacterization of the plant, especially at high temperatures. This axis will be the objective of the next article.

Materials and Methods

Raw Materials

Sampling was carried out in August during the harvest season of the jujube fruit, in the rural area of Tamslouhte, Marrakech, Morocco. The jujube leaves were chosen according to size and maturity. A specimen of *Ziziphus lotus jujube* was deposited and conserved under the voucher specimen code MARK-13

560 at the Regional Herbarium "MARK" of the Faculty of Sciences Semlalia, University of CadiAyyad, Marrakech, Morocco.

Drying of Jujube Leaves

The drying of the leaves of the Jujube tree was carried out using thermo-adjustable ovens at different temperatures from 30°C up to 90°C to know the impact of the drying temperatures on the bioactive agents of the matrix as a method of food preservation.

Scanning Electron Microscopy

The leaf microstructure of the Jujube species *Zizyphus lotus* at different temperatures was examined by a scanning electron microscope (SEM) (TESCAN VEGA 3).

Each sample was carbon sprayed before being scanned and photographed at distinct magnifications. We used an acceleration potential of 10 kV in the acquisition of micrograph images.

Fourier Transform Infrared Spectroscopy

The use of Fourier transform infrared spectroscopy (FTIR) allows the study of the functional groups of the leaves of the Jujube tree *Zizyphus lotus* with the identification of the consequent alterations in the drying temperature and the surface reactions. The FTIR spectra have been superimposed and presented in the following figure.

Physico-chemical and biochemical analysis of jujube leaves

The leaves of the *Jujube Zizyphus lotus* dried at different temperatures were analyzed for pH-meter (pH), soluble solids (Brix), and conductivity (Ω).

We conducted the quantification of chlorophyll a, chlorophyll b, carotenoids, and pheophytins (degradation products of chlorophylls) in a whole acetone pigment extract by UV-Vis spectroscopy. The determination of chlorophylls was based on the method described by (15). Chlorophyll a, chlorophyll b, total carotenoids, and total pheophytins were calculated using the equations given below:

$$\textbf{\textit{Chlorophyll(}}a\textbf{\textit{)}}=11{,}24*A662-2{,}04*A645 \text{ [µg/mL]}$$

$$\textbf{\textit{Chlorophyll(}}b\textbf{\textit{)}}=20{,}13*A645-4{,}19*A662 \text{ [µg/mL]}$$

$$\textbf{\textit{Totalcarotenoids}}=(1000*A470-1{,}90*Cha-63{,}14*Chb)214 \text{ [µg/mL]}$$

$$\textbf{\textit{Totalpheophytin}}s=(321{,}3*A653)-(208{,}4*A654) \text{ [µg/mL]}$$

One gram (1g) of powder was extracted with 10 mL of pure methanol in a SELECTA brand ultrasonic bath for three hours. After extraction, the extracts were filtered and then stored at 4°C until analysis.

The determination of the total phenolic content (TPC) was carried out according to a method explained by Chougui et al. on the cactus *Opuntia ficus indica*. 0.5 mL of the extract was blended with 1.5 mL of reactive Folin-Ciocalteu (diluted ten times). After 5 min, 1.5 mL of sodium carbonate (6%) was added. The incubation of the mixture was carried out in the dark for 1 h. Subsequently, the absorbance was measured at 760 nm against a blank. The value was determined using a standard curve prepared from gallic acid and expressed as mg gallic acid equivalents (mg/GAE) of sample per 100 g of dry weight.

The total flavonoid content (TFC) in the leaves of the Jujube tree *Zizyphus lotus* powder extracts were calculated based on the method described by Chougui et al. A 1.5 mL of the sample extract was blended with 1.5 mL of $AlCl_3$ reagent (2%). After 30 min of incubation, the absorbance was recorded at 430 nm against a blank. The estimation of the flavonoid content was carried out using a standard calibration curve that was prepared using catechin standard solutions. The flavonoid content was expressed in mg catechin/100 (g) on a dry weight basis.

Antioxidant Activity and Analysis by HPLC

The potential of all the extracts to have antioxidant activity was evaluated using DPPH method. Method (17) was used for the DPPH tests. One mL of DPPH solution of 100 mM concentration in methanol was blended with 1 mL of the sample extract. Incubation of the reaction mixture was carried out in the dark for 30 min, and then its optical density was recorded at 517 nm against a blank. For the control, 1 mL of methanolic solution of DPPH was mixed with 1 mL of methanol. The results obtained were expressed as a percentage inhibition of DPPH based on the following formula: Percentage of DPPH inhibition in % = (Control Abs - Sample Abs) / Control Abs * 100.

Abs Control is the absorbance of the DPPH solution without the sample extracted and Abs Sample is the absorbance of the sample with DPPH Solution.

The analysis of polyphenols by liquid chromatography was conducted at the Analysis and Characterization Center (CAC) of Cadi Ayyad University, SemLalia Faculty of Sciences, Marrakech.

Results and Discussion

Physico-chemical properties of the leaves of the *Jujube tree Zizyphus lotus*

The physicochemical properties of the leaves of the Jujube tree *Zizyphus lotus* for various drying temperatures have been indicated in Table 1. The results showed that there was little variation in the pH values of all the studied samples. It was also indicated that conductivity had decreased with the increase of the temperature which meant a degradation of the matter. The ash contents are generally high in all the dried samples (1.9-2.3%). According to San et *al.* (2009), the *Zizyphus lotus* leaves ash contents are high because the

Figure 1. *Zizyphus lotus jujube* leaf sampling area.

Table 1: Physicochemical properties of leaves of the jujube tree Zizyphus lotus drying at different temperatures.

The parameters / **The sample**	pH	Brix (%)	The Conductivity (mS)	Dry matter (%)	The ashes (%)
jujube leaves	**9,34**	**0,1**	**352**	**56**	**2,8**
the dried Jujube leaves 30 °C	**9,55**	**0,1**	**106,9**	**54**	**2,3**
the dried Jujube leaves 40 °C	**9,64**	**0,1**	**157,7**	**52**	**1,9**
the dried Jujube leaves 50 °C	**9,68**	**0,1**	**153,7**	**54**	**2,0**
the dried Jujube leaves 60 °C	**9,50**	**0,1**	**145,9**	**53**	**2,2**
the dried Jujube leaves 70 °C	**9,32**	**0,1**	**154,2**	**51**	**2,1**
the dried Jujube leaves 80 °C	**9,52**	**0,1**	**193,3**	**50**	**1,9**
the dried Jujube leaves 90 °C	**9,58**	**0,1**	**110,2**	**50**	**2,1**

tree likes alkaline pH, sandy to loamy and well-drained soil (11).

Analysis by FTIR and SEM

The use of FTIR Fourier transform infrared spectroscopy allows the study of functional groups of dried leaves of *Jujube Zizyphus lotus* at different temperatures, with the identification of the resulting changes in the increase in temperature and surface reactions. The FTIR spectra of Jujube leaves have been overlaid and shown in the figure below.

Slight variations were observed for different samples in the peaks of transmission or absorption, but no variations were observed in the functional groups of the leaves of the Jujube tree. FTIR spectra show the appearance of a peak at 3778.72 cm^{-1} due to the OH bond of the alcohol or phenol, a peak between 2900 and 3420 cm^{-1} of the CH laison of the alkynes or the N─H bond of primary amines.

SEM analyses showed altered morphology of the leaves of *Jujube Zizyphus lotus* after drying treatment, and damage in leaf components due to high temperature as shown in Figure 3.

In contrast, the fractions dried at different temperatures were less porous, and identification of their structures was difficult. A greater degree of damage was observed in the fibers of the sheets due to the drying temperature. It was indicated in a study that jujube leaves sick with witches broom disease exhibited multivesicular bodies (MVBs) with vesicles and tubules in phloem parenchyma cells and sieve elements. The remarkable increase in the number of MVBs in the diseased jujube leaves could be related to endoplasmic reticulum stress-dependent exosome release (29).

The Content of Polyphenols, Flavonoids, Chlorophyll, Carotenoids, and Phyophitins

The results obtained for the total phenolic compounds, the content of flavonoids, the content of chlorophyll, the content of carotenoids, pheophytins, and the antioxidant activity of the leaves of the Jujube tree at different drying temperatures have been shown respectively in Figures 4-7.

The maximum of total phenolics was recorded for the different drying temperatures for 80°C and 90°C, approximately in 2.80 g Eq AG / 100g (Eq AG: gallic acid equivalent) dry weight. The more the temperature increases the more the polyphenol content increases in

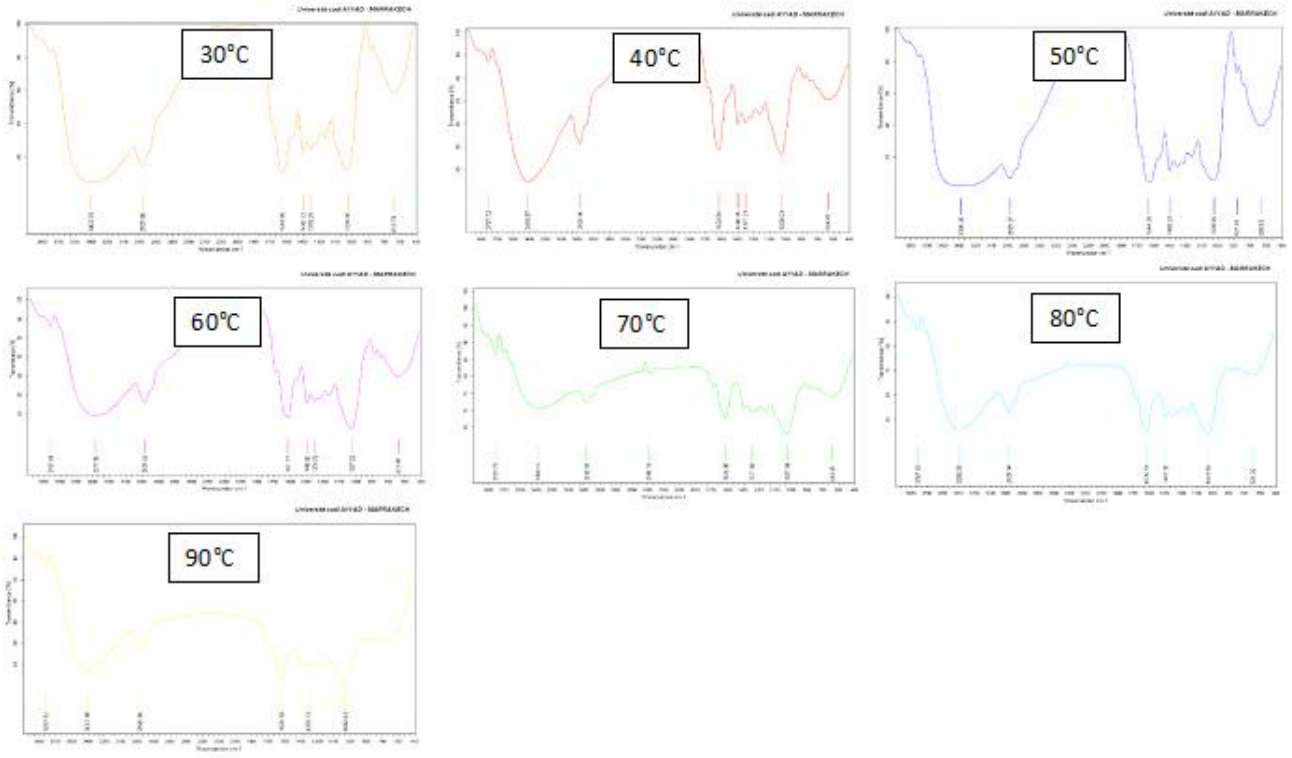

Figure 2. Infrared spectra of *jujube leaves Zizyphus lotus* dry at different temperatures.

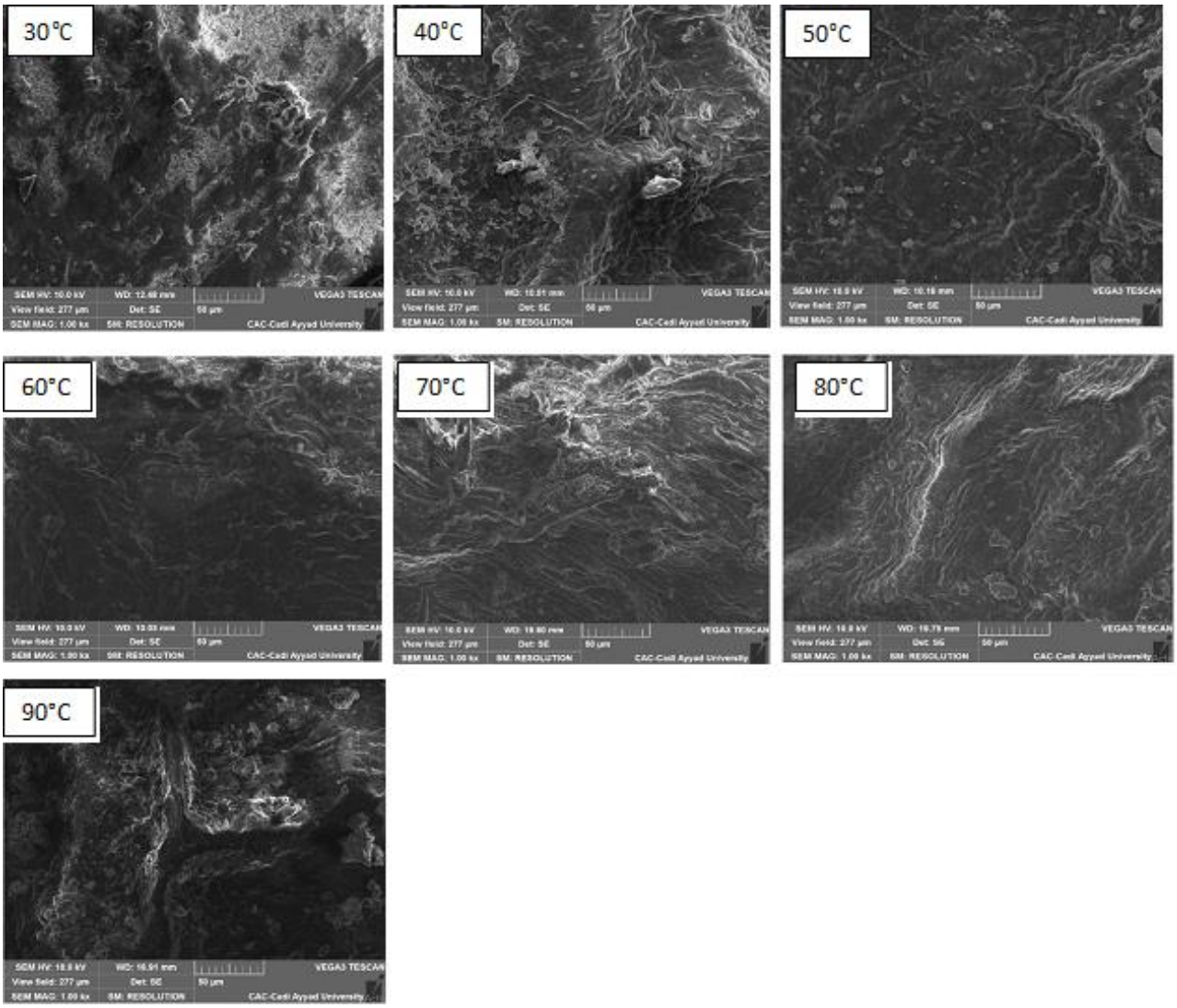

Figure 3. Morphological analysis by scanning the electron microscopy of different samples to dry them.

the literature. The results of air drying of red pepper were similar to Capsicum annuum, L. var. Hungarian (19). Moreover, in the process of food preservation by irradiation, the irradiation impact has been indicated for distinct plant matrix treated with different doses of gamma irradiation. Khattak indicated a remarkable increase in the composition of irradiated Fagonia arabica in comparison with non-irradiated cases. Similar findings were reported about the collection of phenolic materials in the irradiated strawberry in a study conducted by Cheng and Breen. Furthermore, Mahrouz et al. reported an intensification of phenolic compounds in irradiated clementine. These results are based on the hypothesis that the enzyme responsible for the synthesis of polyphenols (PAL enzyme) is activated during irradiation or high temperatures.

Regarding flavonoids, stability of the content of flavonoic compounds was noted with the increase in the drying temperature. Hence, there is no destruction of the flavonoids compounds, particularly the flavonoids that are well known for their chemical structure based on 15 carbon atoms. They are made of two benzene rings. Moreover, they are stable and rigid against temperature.

For chlorophylls a and b, a decrease was observed in the content of these two compounds. Given the degradation of the organic matter with the drying temperature, a small change occurred in the coloring aspect of the leaves of the Jujube tree.

Regarding total pheophytins and carotenoids, we also noticed a decrease in the content with increasing drying temperature until the disappearance of the carotenoids.

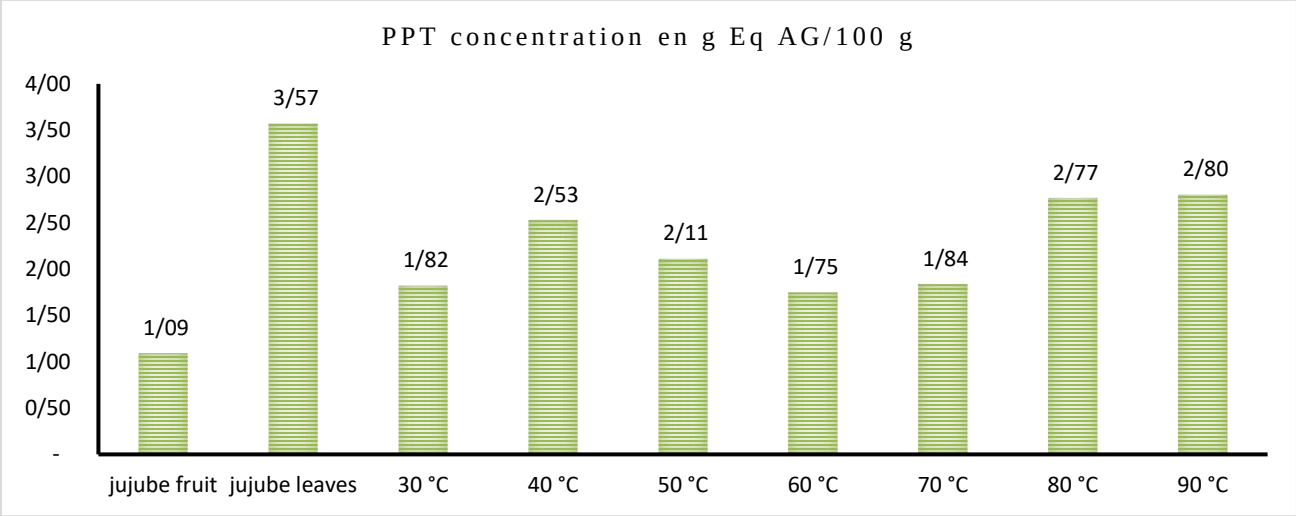

Figure 4. The concentration of total polyphenols (PPT) in the leaves of the Jujube tree *Zizyphus lotus* at different drying temperatures.

These values are almost similar to the values found for the Jujube fruit according to Rashwan et al.

Separation and Analysis by HPLC

The chromatographic separation was conducted using a Kinetex C18 reversed-phase (100×4.6 mm, 2.6 µm particles) column and gradient elution with 0.1 % formic acid aqueous solution and methanol mobile phases, as previously established by HPLC with UV absorbance detection (24). The flow rate of the mobile phase entering into the instrument was 500 µL/min. Under these conditions, an acceptable chromatographic separation of the 17 polyphenolic compounds was obtained in less than 30 min. However, several full or partial coelutions occurred. Analysis of the five samples that dried at the following temperatures: 40°C, 50°C, 60°C, 70°C, 80°C. They have been shown in Figure 9. The appearance of 14 peaks in the chromatograms was obtained by HPLC. The HPLC analysis made it possible to identify the presence of naringin, furelique, rutin, quercetin, and kaempferol, as predominant phenolic compounds. The other peaks of chromatograms were not identified given the non availability of other standards. These results are similar to the findings reported by Lijun Song et al. Moreover, the appearance of other chromatograms of the other phenolic compounds was notted with the

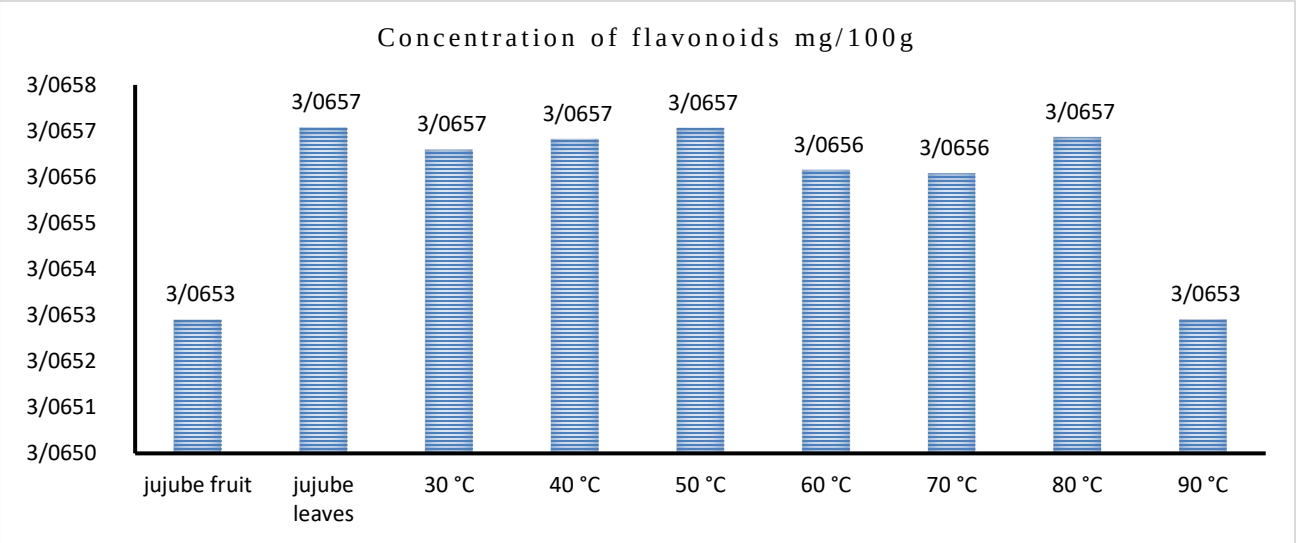

Figure 5. The flavonoid content of the leaves of the *Jujube Zizyphus lotus* at different drying temperatures.

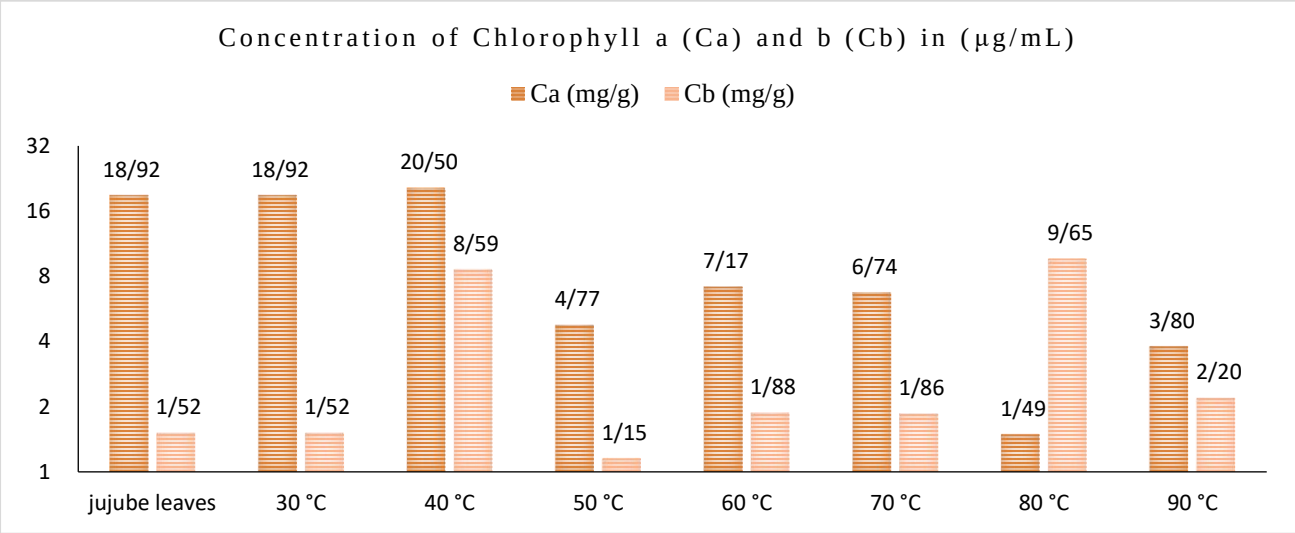

Figure 6. The content of chlorophylls a and b leaves of the *Jujube Zizyphus lotus* at different drying temperatures.

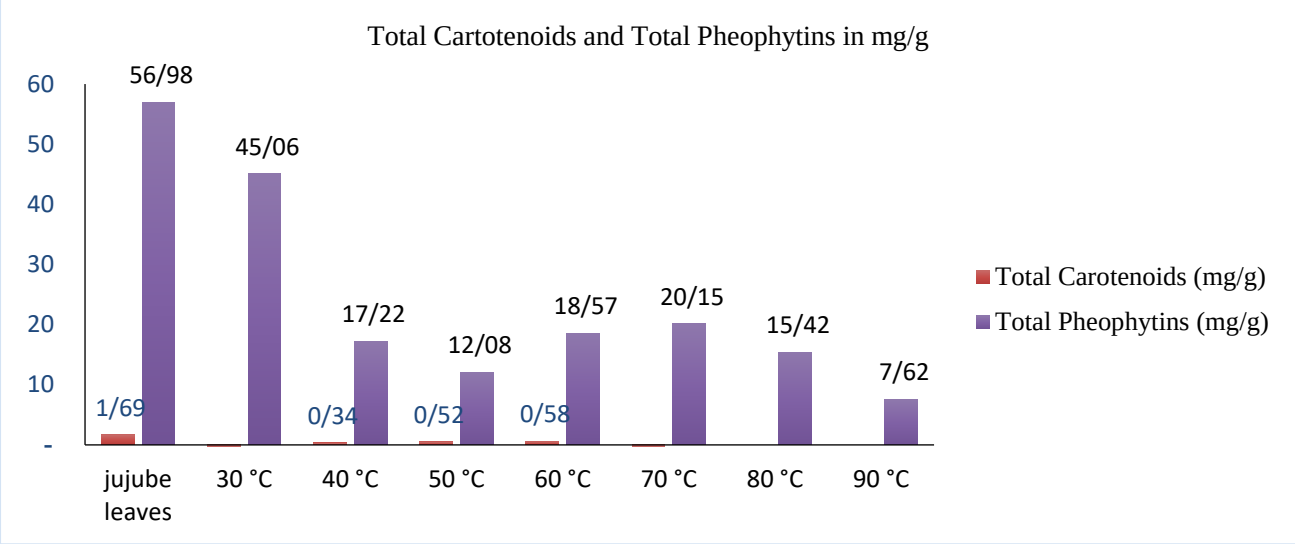

Figure 7. The content of carotenoids and total pheophytins, leaves of *Jujube Zizyphus lotus* at different drying temperatures.

increase in the drying temperature. This means that the temperature has an effect on the alteration in the composition of the polyphenols during the drying.

These fluctuations in the content of phenolic compounds can be associated with their degradation under the effect of temperature and time (25). These results confirm the findings related to air drying of red pepper Capsicum annuum, L. var. Hungarian (19). Furthermore, the activation of phenolic compounds at high temperature may be due to the availability of precursors of phenolic molecules by enzymatic inter-conversion between phenolic molecules. Arslan and Özcan demonstrated the effect of drying temperature on total polyphenols depending on the drying process. Drying could accelerate the release of phenolic compounds by breaking bonds and decomposing constituents cellular.

Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was applied to determine the antioxidant activity of different drying temperatures. The maximum antioxidant activity estimated by DPPH was recorded for two classes, i.e. 91.7% and 86.9%, for the leaves of fresh jujube, and for the leaves to dry at 80°C, and 90°C respectively.

The antioxidant activities determined by DPPH under optimal conditions have been shown in Figure 10. We observed that the extracts of the jujube leaf dried at

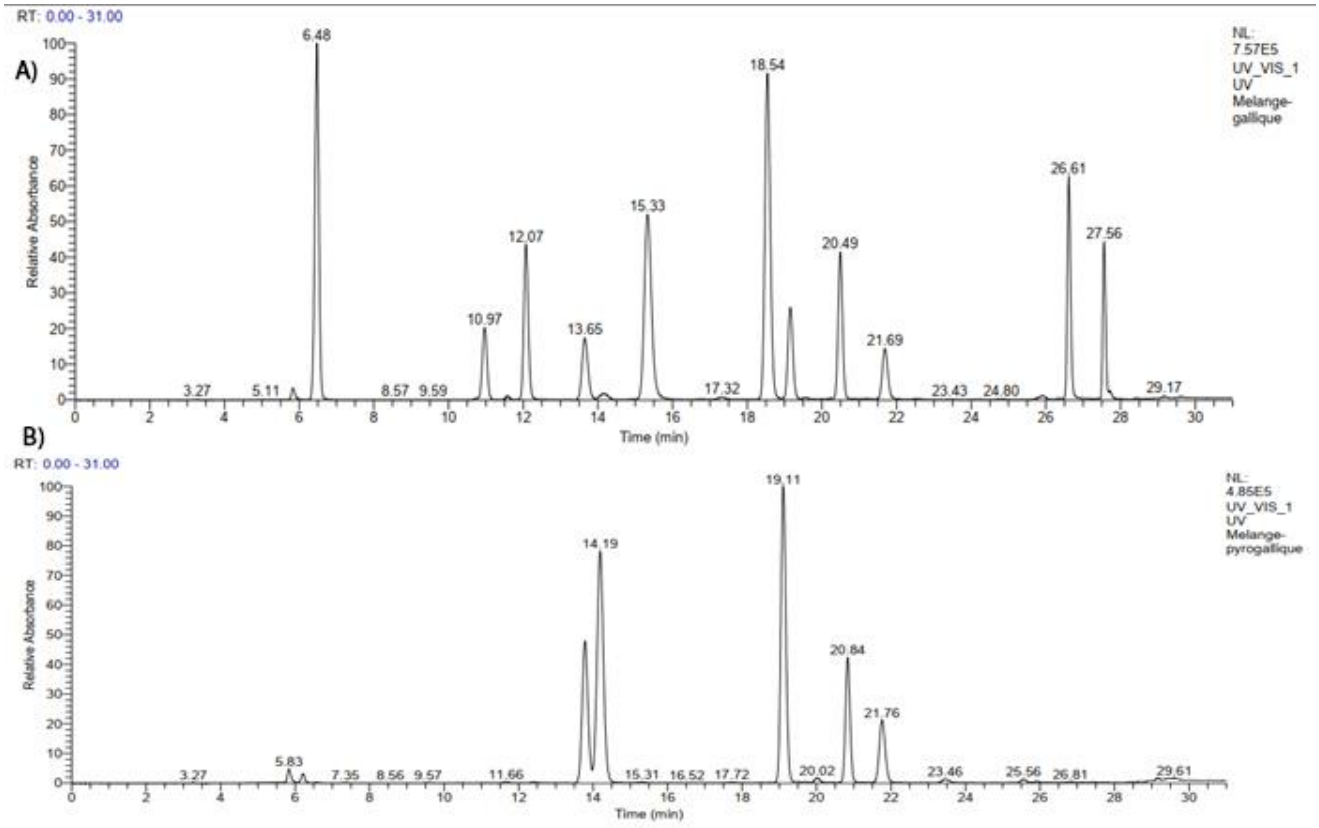

Figure 8. (A) Chromatogram peaks of Pyrogallic mixture standards, (B) Chromatograms of standards mixture.

different temperatures exhibited notable antioxidant activities. It was also noticed that the antioxidant activity of the fresh leaves was quite high, implying that our matrix was very rich in anti-radical substances, particularly flavonoids. These results are those reported by Lijun Song et al. (8), Bimakr et al. (27), and Masci et al. (28).

Conclusion

This study provided insight into the effect of drying temperature on the extraction of polyphenols from the leaves of *Jujubia Zizyphus lotus*. The study showed an increase in phenolic compounds with increasing temperature. Moreover, a highly important antioxidant activity, stability in the flavonoid content, a decrease in chlorophyll a and b, and appearance of other phenolic compounds were observed using HPLC, degradation of organic compounds by FTIR analysis, and alteration of particle morphology. Another study will be conducted for the exact identification of the flovonoids of species. Furthermore, research will be conducted on bacterial and antifungal activities.

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Conflict of Interest

The authors declare that they have no conflict of interest.

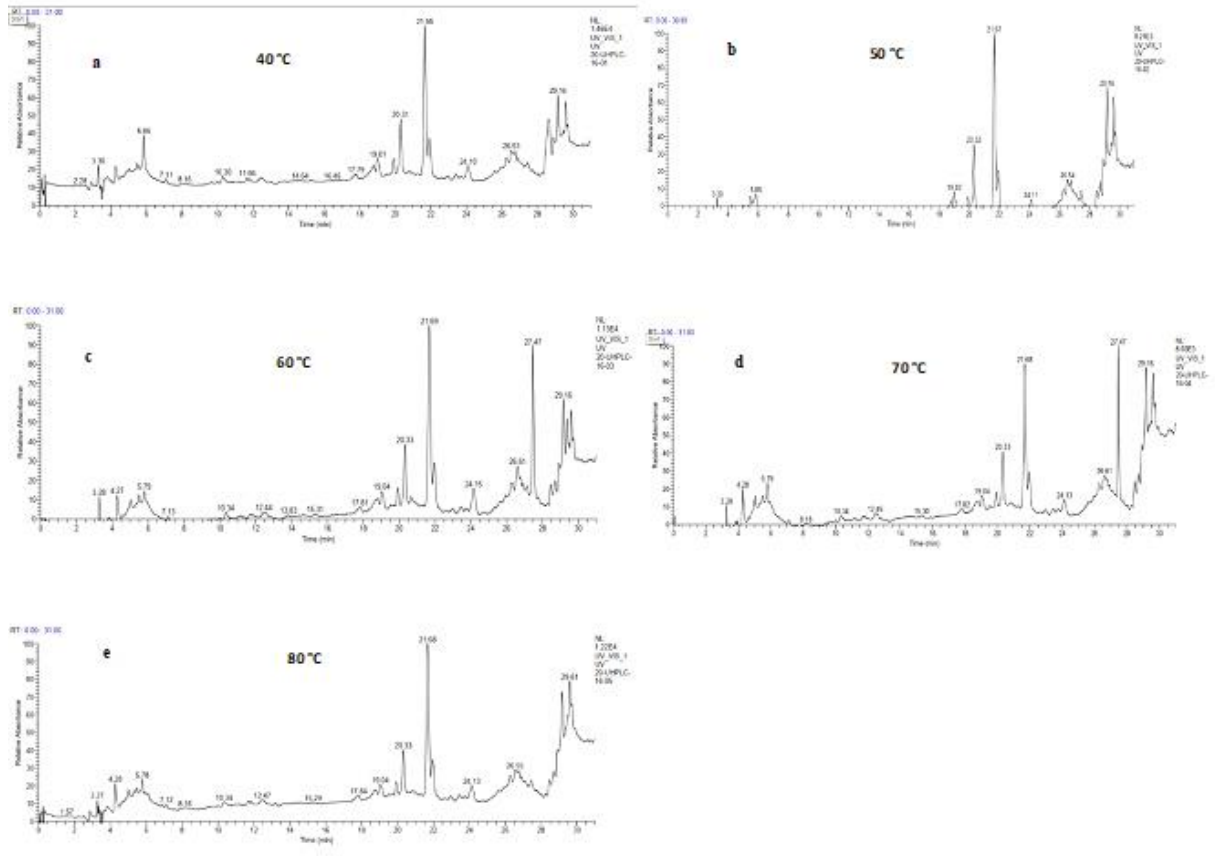

Figure 9. The peaks of phenolic compounds analysed by HPLC for the five samples dried at 40°C (a), 50°C (b), 60°C (c), 70°C (d) and 80°C (e).

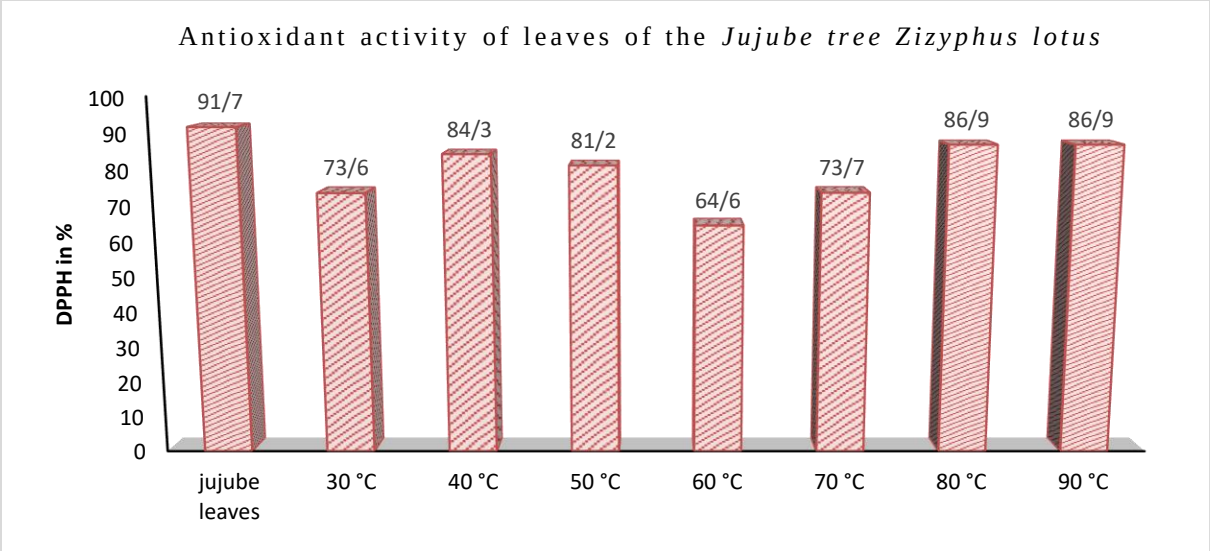

Figure 10. Antioxidant activity of jujube *leaves Zizyphus lotus*.

Funding

None.

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