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Comparative study of phenolic profile and antioxidant activities of different parts of *Ziziphus lotus* using aqueous, hydroalcoholic and pulsed electric field extractions

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ABSTRACT

Ziziphus lotus (Z. lotus) is a deciduous plant widespread in Tunisia, traditionally used as an infusion or powder for its richness in phenolic compounds, carbohydrates, proteins, and flavonoids. However, the composition varies across different plant parts. In this study, flowers, leaves, stems, roots, and fruits of *Z. lotus* were analyzed to assess the recovery of antioxidant compounds, with a focus on phenolic profiles, carbohydrates, and proteins, using three extraction methods: pulsed electric field (PEF) extraction, conventional aqueous extraction, and conventional hydroalcoholic extraction (ethanol:water, 80:20 *v*/v). For the PEF extraction method, 4 g of each plant part were treated at 3 kV/cm and 100 kJ/kg, followed by 1 h stirring. The conventional extractions were performed with water or hydroalcoholic solution under the same stirring conditions. Chemical analyses were conducted by spectrophotometry and Triple TOF-LC-MS/MS for detailed phenolic compound profiling. Statistically significant differences were observed between plant parts and extraction methods. Flowers and leaves showed the highest antioxidant capacity and the richest content in carbohydrates, proteins, and phenolics. Quercetin, a common flavonoid, was highlighted across all parts, while ferulic acid was mainly found in stems and roots. Overall, PEF extraction proved more effective in recovering bioactive compounds compared to conventional methods, highlighting its potential as a green extraction technology aligned with Sustainable Development Goals.

1. Introduction

Since ancient times, herbal medicine has been used to prevent and treat various diseases, including chronic inflammation, cancer, and bacterial infections. Medicinal plants are harnessed for their ability to relieve inflammation, infections, and pain, thus helping to reduce the potential side effects associated with drug administration [1]. One of the traditional and medicinal species is Wild jujube (*Z. lotus* L.), a xerophytic shrub belonging to the *Rhamnaceae* family., which is generally considered safe and non-toxic for human and animal consumption.

The *Rhamnaceae* family is distributed worldwide, with a particular prevalence in subtropical and tropical regions [2]. According to Judd et al. [3], this family consists mainly of **trees and shrubs**, which are often thorny and bear simple, alternate leaves.

These leaves are alternate or, less often, opposite or palmate, with a tertiary network that is often latticed and relief-like. This shrub requires a warmer climate to flower and bear fruit, which is why the climate in tropical Asia and America is suitable for it [4,5].

The *Rhamnaceae* family includes *Z. lotus,* which is widespread in North Africa, particularly in Tunisia, Libya, Algeria and Morocco [6]. This specie is known in Tunisia as “Sedra” and is characterized by a high environmental tolerance. Alkaloids, flavonoids, vitamins, fatty acids, carbohydrates, fiber, proteins, proanthocyanidins, minerals and saponins were among the various substances discovered in the different parts of the plant. Due to these properties, *Z. lotus* is used in health, nutrition, and cosmetics in varied forms such as tea, honey, juice, jam, oil, cakes,

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and bread. In addition, in both North America and the Middle East traditional medicine, numerous parts of *Z. lotus* are used to treat a variety of diseases and conditions, such as inflammatory diseases, obesity, fever, skin diseases, diabetes, pneumonia, diarrhea, bronchitis, insomnia, sedatives, abscesses, and hypoglycemia [7].

On the other hand, this shrub produces a savory fruit called jujube, which is eaten by locals in considerable quantities fresh, dried, and processed as food and is used to treat throat and bronchial irritations [8,9]. The powder from the dried leaves and fruits is also used in the treatment of boils [10]. Furthermore, the bark and roots of *Z. lotus* are utilized in traditional medicine for the treatment of diabetes [11]. This traditional use has been further supported by recent studies highlighting the antidiabetic potential of *Z. lotus* extracts. In particular, Boutaj et al. [12] demonstrated the efficacy of root and bark extracts in reducing blood glucose levels, while Zanzabil et al. [13] provided mechanistic insights into their therapeutic action in diabetic models. Due to its therapeutic and nutritional profile, several researchers have explored various techniques to extract bioactive compounds from *Z. lotus*, including conventional methods that rely on different solvents, which are often toxic, organic, and environmentally unsustainable. In addition, these extraction processes generally require high temperatures and prolonged durations, which can degrade thermolabile compounds such as polyphenols [14]. For this reason, the efficient recovery of high-quality compounds has driven research toward innovative and sustainable alternatives such as PEF technology. PEF promotes cell membrane permeabilization, allowing for enhanced release of intracellular compounds while preserving their integrity, particularly thermosensitive ones like phenolics [14].

PEF extraction is based on the application of electric pulses to the food matrix to generate cell membrane electroporation, allowing the flow of the compounds of interest into the external medium using water as solvent [15,16,17]. In this way, the use of high temperatures and toxic solvents is avoided, as the generation of electrical pulses is used to form cavities in the cell membrane rather than to generate temperature, with electric field (kV) and specific energy (kJ/kg) being two of the most important parameters. In addition, the energy consumption and total extraction time are significantly reduced compared to conventional and traditional methods such as maceration ([18]; Jun et al. [5], Peng et al. [9], Rossi et al. [16], and Zhu et al. [19].

For the aforementioned reasons, the aim of the present study is the characterization of the phenolic profile and quantification of the total antioxidant capacity and macronutrients of extracts obtained by PEF extraction compared to conventional aqueous and ethanolic extraction from leaves, flowers, roots, stem and fruits of *Z. lotus*.

2. Materials and methods

2.1. Chemicals and reagents

2, 2′-Azinobis (3- ethylbenzothiazoline-6-sulfonic acid) (ABTS, ≥98 %), 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2- carboxylic acid (Trolox, ≥98 %), gallic acid ($C_7H_6O_5$, ACS reagent, ≥98 %), potassium persulfate ($K_2S_2O_8$, ACS reagent, ≥99 %), Folin–Ciocalteu reagent (2 M) were purchased from Sigma-Aldrich (Steinheim, Baden-Wuerttemberg, Germany). Ethanol (99 %), glucose ($C_6H_{12}O_6$, 98 %) sulfuric acid (H_2SO_4, ≥95 %) and phenol (C_6H_6O, 99 %) and Pierce BCA Protein Assay Kit were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Ultrapure water was obtained from Milli-Q SP Reagent Water System (Millipore Corporation, Bedford, MA, USA).

2.2. Sample preparation

The leaves, flowers, fruits, roots, and stem of *Z. lotus* were collected in May from Amiret El Hojjej (Al Adhyef), Monastir, Tunisia, and a voucher specimen (Voucher No. ZL2023–01) was deposited at the Herbarium of the Faculty of pharmacy, University of Monastir. The fruits were harvested in September from the same region. All parts of the plant were dried for 8 days by sunlight and then ground in a mortar and mixed to obtain a fine powder from each part. These powders were stored in plastic containers at room temperature in the dark for later chemical analysis.

2.3. Pulsed electric field (PEF)-assisted extraction and conventional extraction conditions

A PEF-Cellcrack III equipment was used for the extraction process. In this study, PEF extraction method was selected to explore its potential compared to conventional methods and to evaluate its efficacy on the different parts of the *Z. lotus* plant. This equipment was located at the Faculty of Pharmacy and Food Science of the University of Valencia (Comunitat Valenciana, Spain). For each plant part, 4 g of dry powder sample were placed in contact with 200 mL of distilled water. The conductivity reached approximately 700 to 800 μS/cm.

The extraction process was carried out in a 900 mL extraction chamber with an electrode distance of 10 cm, a pulse duration of 100 μs, and a frequency of 2.00 Hz. These parameters were selected based on previously optimized conditions reported by Martí-Quijal et al. [20] and Calleja-Gómez et al. [21], using an electric field strength of 3 kV/cm, a specific energy of 100 kJ/kg, and a total extraction time of 1 h for each plant part.

After PEF extraction, the obtained extracts were stirred for 1 h at 20 °C. Subsequently, the extracts centrifuged at 4000 rpm, 15 min and stored at −20 °C until used in chemical analysis. Conventional extracts were also prepared with aqueous and hydroalcoholic (80:20 *v*/v ethanol: water) solvent and then preserved in the same conditions.

Conventional extracts were prepared with aqueous and hydroalcoholic solvents. For the aqueous extraction, 4 g of powder from each plant part was dissolved in 200 mL of distilled water and stirred for 1 h. The mixture was then filtered and centrifuged at 4000 rpm for 15 min to remove all solid residues. For hydroalcoholic extraction, 4 g of powder from each plant part was dissolved in 200 mL of 80:20 v/v ethanol:water mixture. The mixture was then stirred for 1 h, filtered and centrifuged at 4000 rpm for 15 min. The conventional extracts were stored under the same conditions as the PEF extracted samples.

Thus, the effect of PEF extraction on the extraction of phenolic compounds in a 1-h maceration and the effect of the two solvents on the recovery is studied.

2.4. Chemical analysis

2.4.1. Total carbohydrate content

The total carbohydrate content was determined using the phenol-sulfuric method described by [22], based on the acid catalysis of the extract sugars. This process involves the addition of sulfuric acid, which produces furfural and hydroxymethyl-furfural. These compounds condense with phenols and produce yellow-orange-colored products in relation to the total carbohydrate concentration. D-glucose solutions (10 to 100 mg/L) were used as standards for this analysis. One milliliter of the sample was taken with a 1/10 dilution. To this, 0.5 mL of a 5 % phenol solution and 2.5 mL of sulfuric acid were added. Then, the mixes were incubated for 30 min at 25 °C. The absorbance was then measured at 490 nm. The results were expressed in milligrams of glucose per gram of dry matter (mg glu/g DM).

2.4.2. Total protein content

The bicinchoninic acid assay method was performed using the BCA Protein Assay Kit to evaluate the protein content in various plant parts: Leaves, fruits, stems, roots and flowers. Bovine serum albumin was used as a standard in concentrations ranging from 0 to 2000 mg/L. For analysis, 25 μL of the sample or standard was mixed with 200 μL of the BCA solution and the mixture was incubated 30 min at 37 °C. Then, absorbance was measured at 562 nm. Finally, the results were expressed

in milligrams of bovine serum albumin per gram of dry matter (mg BSA/g DM).

However, no prior treatment such as trichloroacetic acid (TCA) precipitation, acetone washing, or the use of matrix blanks was applied before the analysis. Therefore, the potential interference of natural reducing substances presents in the plant extracts, such as polyphenols and pigments, cannot be excluded. These interferences may influence the colorimetric response of the BCA assay and lead to an overestimation of protein levels. Consequently, the results should be interpreted with caution and considered as indicative values.

2.4.3. Total antioxidant capacity

The Trolox equivalent antioxidant capacity (TEAC) assay method was employed to evaluate the neutralization capacity of the samples in relation with $ABTS^{+}$ radical. For the assay, a mixture of ABTS (7 mM) and $K_2S_2O_8$ (140 mM) was prepared and preserved for 16 h at 20 °C in the dark to generate the $ABTS^{+}$ radical solution. This solution was then mixed with 96 % ethanol (1:100). Subsequently, 2 mL of the ethanol solution was combined with 100 μL of the extracts and the absorbance was measured at 734 nm. The reference was a standard Trolox curve at different concentrations (from 0 to 250 μM). The results were expressed in micromoles of Trolox equivalents per gram of dry matter (μmol TE/g DM).

2.4.4. Total phenolic content

The total phenolic content (TPC) was quantified using the Folin-Ciocalteu method described by [23]. Folin-Ciocalteu reagent at a concentration of 50 % *v*/v was mixed with Na_2CO_3 solution and sample or gallic acid standards. Then, 100 μL of standard/sample, 3 mL of Na_2CO_3, and 100 μL of Folin-Ciocalteu reagent were mixed. The samples were incubated for 60 min in darkness and then measured at 750 nm. The results were expressed in milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM).

2.5. Phenolic profile characterization

The phenolic profile was assessed through triple-TOF-LC-MS-MS characterization using the methodology outlined by [24]. In this assay, samples were diluted to a concentration of 10 mg/mL in methanol and filtered through a 0.22 μm filter before direct injection into the equipment. Identification of phenolic compounds was performed using the TripleTOF 5600 LC/MS/MS system coupled to the Agilent 1260 Infinity. For compounds separation, a Waters UPLC C18 column (1.7 μm, 2.1 × 50 mm) was used. The liquid chromatography method used a mobile phase consisting of water with 0.1 % formic acid (component A) and methanol with 0.1 % formic acid (component B). The elution program was as follows: 90 % A and 10 % B from 0 to 13 min, transitioning to 100 % B from 13 to 15 min, and returning to 90 % A and 10 % B from 15.1 to 22 min. This gradient program and chromatographic conditions were validated and previously applied in polyphenol profiling studies [24]. The flow rate was maintained at 0.4 mL per minute, with an injection volume of 5 μL. Mass spectrometry data were collected across the *m/z* range of 80 to 1200.

Calibration of the instrument was carried out using an external calibration system with a standard calibration solution provided by the instrument manufacturer (SCIEX). The mass spectrometer operated under an information-dependent acquisition (IDA) approach (Fig. S1), utilizing a TOF-MS survey scan and a product ion scan for dependent analysis, with a collision energy set at −50 V. The operational settings included a dust removal potential of 90 V, an ion spray voltage of −4500 V, a source temperature of 400 °C, and a curtain gas pressure of 25 psi. Both ion source gases (1 and 2) were maintained at 50 psi. IDA MS/MS was conducted under these conditions: a mass tolerance of 50 MDa, ion intensities greater than 100 CPS, a collision energy of 25 V, and background dynamic subtraction enabled.

2.6. Statistical analysis

All information were treated by variance analysis (ANOVA) to indicate significant differences between the compared variables (treatment and part of *Z. lotus*) with a significant difference < 0.05. All analyses were repeated in triplicate considering a 5 % significance level and analyzed using GraphPad Prism 8.0.2 software. Error bars in figures show the standard deviation. Lowercase letters ($p < 0.05$) show significant differences between apparent plant parts using the same extraction method. Capital letters show significant differences ($p < 0.05$) between methodologies in the same plant part.

3. Results

3.1. Recovery of phenolic compounds following PEF extraction and conventional extraction

3.1.1. Protein and carbohydrate content

The total macronutrient content of the conventional and PEF extracts of the individual parts of *Z. lotus* is shown in Fig. 1, revealing large differences between the different plant parts, as well as the levels of proteins and carbohydrates depending on the extraction method.

Protein recovery in the flower was remarkable, with 1206.03 ± 115.63 mg BSA/g DM for conventional extraction and 2298.95 ± 71.93 mg BSA/g DM for PEF extraction, representing a 1.9-fold increase compared to maceration and showing a significant difference ($p < 0.05$) between the two methods. Similarly, protein content in the stem ranged from 255.32 ± 27.19 mg BSA/g DM (maceration) to 325.90 ± 17.64 mg BSA/g DM (PEF), corresponding to a 1.27-fold increase. In fruits, PEF-assisted extraction yielded 1050.74 ± 38.47 mg BSA/g DM, compared to 693.39 ± 35.05 mg BSA/g DM with conventional extraction, i.e. a

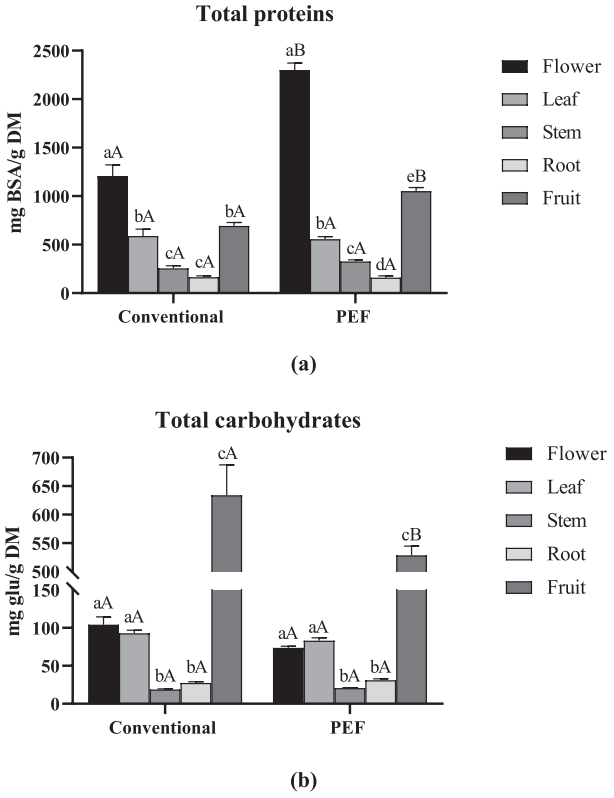

Fig. 1. Total protein content (mg bovine serum albumin (BSA)/g dry matter (DM)) (**a**) and total carbohydrate content (mg glucose (glu)/g dry matter (DM) (**b**) obtained though maceration and pulsed electric field (PEF)-assisted extraction of various parts of *Z. lotus* (flowers, fruits, leaves, stem, and root).

1.5-fold increase ($p < 0.05$).

In contrast, for leaves and roots, PEF extraction showed no significant improvement: 587.64 ± 71.71 mg BSA/g DM (conventional) vs. 556.16 ± 26.59 mg BSA/g DM (PEF) in leaves, and 162.57 ± 16.47 mg BSA/g DM vs. 159.48 ± 17.93 mg BSA/g DM in roots.

These results suggest that PEF extraction notably enhances protein recovery in *Z. lotus* flowers, fruits, and stems.

Nevertheless, it is important to acknowledge a methodological limitation. The BCA assay, while widely used for protein quantification, is susceptible to interference from reducing compounds and pigments commonly present in plant matrices, such as polyphenols. In this study, no deproteinization steps such as TCA precipitation, acetone washing, or matrix blank correction were performed prior to analysis. Therefore, potential overestimation due to these interferences cannot be excluded. The presented values should thus be interpreted as indicative rather than absolute.

From a functional point of view, the high protein levels detected particularly in flowers and fruits may provide significant benefits. As Montiel-Herrera et al. [25] reported, amino acids serve as essential precursors for protein synthesis involved in tissue repair and replacement. Moreover, several in vitro and in vivo studies have highlighted the phytochemical and pharmacological activities of *Z. lotus*, which vary with the plant part used (root, leaf, seed, pulp, or fruit). These effects include antifungal, antibacterial, anti-ulcer, anti-inflammatory, antioxidant, and immunostimulatory activities. The presence of flavonoids, polysaccharides, proteins, and triterpenic acids has been cited as a major contributor to these biological properties [26].

In addition, extraction yields were measured for each plant part to better interpret the macronutrient data:

- Flowers: 28.7 % (PEF), 19.3 % (aqueous maceration), 23.8 % (ethanolic)
- Leaves: 24.2 % (PEF), 20.5 % (aqueous), 22.1 % (ethanolic)
- Fruits: 30.1 % (PEF), 22.7 % (aqueous), 25.4 % (ethanolic)
- Stems: 18.9 % (PEF), 14.8 % (aqueous), 17.2 % (ethanolic)
- Roots: 16.3 % (PEF), 15.9 % (aqueous), 16.0 % (ethanolic)

These findings confirm that PEF technology not only improves the recovery of proteins and other macronutrients but also enhances the overall extraction yield. This dual improvement has significant implications for the efficiency and composition of botanical extracts.

In terms of carbohydrate content, an increased carbohydrate yield was observed in the fruits, which varied between 633.87 ± 53.41 mg glu/g DM in conventional extraction and 528.46 ± 53.41 mg glu/g DM after application of the PEF technology under favorable conditions, showing a statistical difference ($p < 0.05$) between the two methodologies. In flowers, carbohydrate contents ranged from 104.07 ± 10.46 mg glu/g DM for conventional extraction to 73.61 ± 2.25 mg glu/g DM for PEF extraction. Regarding the leaves, the carbohydrate content varied between 92.89 ± 3.88 mg glu/g DM for conventional extraction and 83.109 ± 3.70 mg glu/g DM for PEF extraction. The stem also contained a high carbohydrate content, which varied between 18.67 ± 0.83 mg glu/g DM and 20.609 ± 0.72 mg glu/g DM for conventional extraction and the application of PEF extraction, respectively. Therefore, the carbohydrate content is slightly increased after extraction with the conventional method compared to the pulsed electric field method, but the variation between the two methods was still not significant ($p > 0.05$), except for the fruits.

These results are in accordance with the studies of [27], who reported that the highest carbohydrate content was found in the fruit and pulp of *Z. lotus* (83 %). Moreover, a considerable amount of carbohydrates was observed in various parts of the plant, including fruits, flowers, leaves, roots and stems. The high carbohydrate content in the fruits and other parts of the plant suggests that their flours could serve as valuable energy sources and could be important as industrial raw materials for the formulation of commodities [28].

In addition, [29], investigated the nutritional composition, physical properties and sensory evaluation of cookies made from jujubes (fruits of *Z. lotus*.), further emphasizing the potential use of *Z. lotus* as a valuable food source.

3.1.2. Total antioxidant capacity and phenolic compounds

The comparison of the total phenolic content (TPC) between the different parts of *Z. lotus* and the extraction method (conventional/PEF) and solvent (water/ethanol) is shown in Fig. 2.

An increase in phenolic compounds was observed in the flowers, ranging from 231.02 ± 1.54 mg GAE/g DM after PEF application to 218.92 ± 2.89 mg GAE/g DM in the conventional extraction (with water as solvent) and 190.68 ± 10.34 mg GAE/g DM when ethanol was used, indicating a statistically significant difference ($p < 0.05$) between the two methods under favorable conditions in the flowers. The content of phenolic compounds in leaves varied between 171.42 ± 1.96 mg GAE/g DM in conventional extraction using ethanol as solvent to 124.71 ± 15.66 mg GAE/g DM in aqueous extraction and 126.53 ± 8.16 mg GAE/g DM in PEF extraction, indicating a significant difference ($p < 0.05$) between the two methods. In the stems, the phenolic compound content varied between 60.43 ± 0.6 mg GAE/g DM in the conventional aqueous extraction to 96.21 ± 11.8 mg GAE/g DM in the ethanolic extraction and 57.8 ± 9.62 mg GAE/g DM in the PEF extraction. The stem also contained a high content of phenolic compounds, ranging from 102.46 ± 5.43 mg GAE/g DM to 28.64 ± 0.49 mg GAE/g DM and 27.48 ± 0.49 mg GAE/g DM in conventional EtOH extraction, aqueous extraction, and PEF extraction, respectively. In the fruits, the phenolic compound content was quite high at 22.27 ± 0.39 mg GAE/g DM, 32.31 ± 0.50 mg GAE/g DM and 31.23 ± 0.25 mg GAE/g DM in conventional EtOH-extraction, conventional aqueous extraction, and PEF extraction, respectively. Therefore, the content of phenolic compounds is slightly increased ($p < 0.05$) after extraction with the conventional method, especially with ethanol as solvent, compared to the pulsed electric field method, except for flowers, where the difference between the two methods is significant ($p < 0.05$).

The antioxidant compounds recovery is shown in Fig. 3, with a comparison of the values between the five parts of the plant (flowers, leaves, fruits, stem and root) and between the two methodologies (conventional and PEF). The flowers of *Z. lotus* have the highest antioxidant activity with values ranging between 775.84 ± 24.03 μmol TE/g DM, 706.44 ± 45.98 μmol TE/g MD and 764.60 ± 20.54 μmol TE/g for conventional aqueous extraction, conventional EtOH extraction and after PEF extraction, respectively, representing a significant difference ($p < 0.05$) between the conventional EtOH and PEF methodology. For leaves, the values ranged from 366.86 ± 3.89 μmol TE/g for conventional extraction to 358.30 ± 15.39 μmol TE/g for PEF extraction. Concerning the stems, the values varied between 95.06 ± 6.54 μmol TE/g for conventional aqueous extraction, 411.74 ± 15.59 μmol TE/g for conventional EtOH-extraction and 109.93 ± 15.59 μmol TE/g for PEF extraction. Roots also contained high levels of phenolic compounds with antioxidant capacity, ranging between 75.64 ± 2.48 μmol TE/g, 274.01 ± 26.20 μmol TE/g and 75.30 ± 5.43 μmol TE/g for conventional aqueous, EtOH-extraction and PEF extracts, respectively. For fruits, the values ranged between 73.53 ± 6.94 μmol TE/g for aqueous extraction, 56.46 ± 6.94 μmol TE/g for EtOH extraction and 81.91 ± 3.82 μmol TE/g for PEF extracts.

In this sense, the flowers and leaves of *Z. lotus* showed a high content of antioxidant compounds, which is closely related to the total and specific phenolic compounds found after PEF extraction. However, the fruits, roots and stems showed the lowest antioxidant capacity. On the other hand, significant differences were found in the extraction technology, with a higher efficiency of PEF in the flowers, although the results varied greatly depending on the plant part. Noteworthy was the extraction of phenolic compounds from the roots and stems with ethanol. Therefore, the content of phenolic compounds with antioxidant capacity was slightly increased after extraction with the conventional

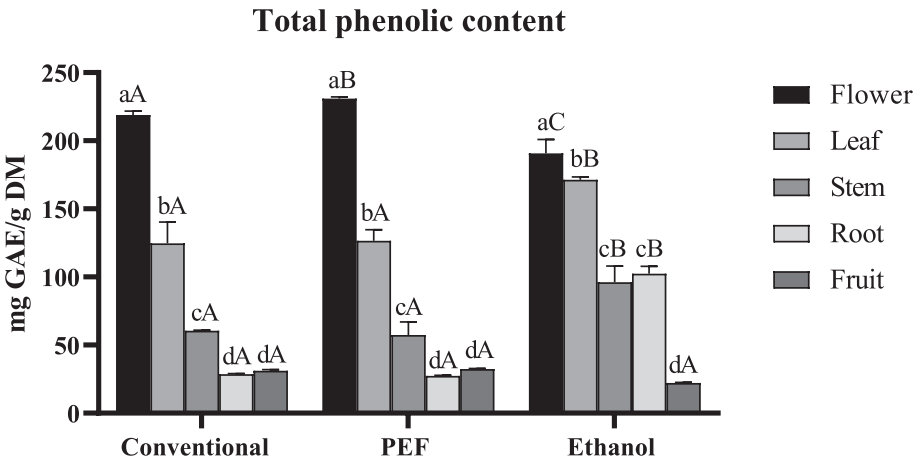

Fig. 2. Total phenolic content (TPC) (mg gallic acid equivalents (GAE)/g dry matter (DM)) in conventional (aqueous and ethanolic) and PEF -assisted extraction of different parts of *Z. lotus* (leaves, flowers, stem, root, and fruit).

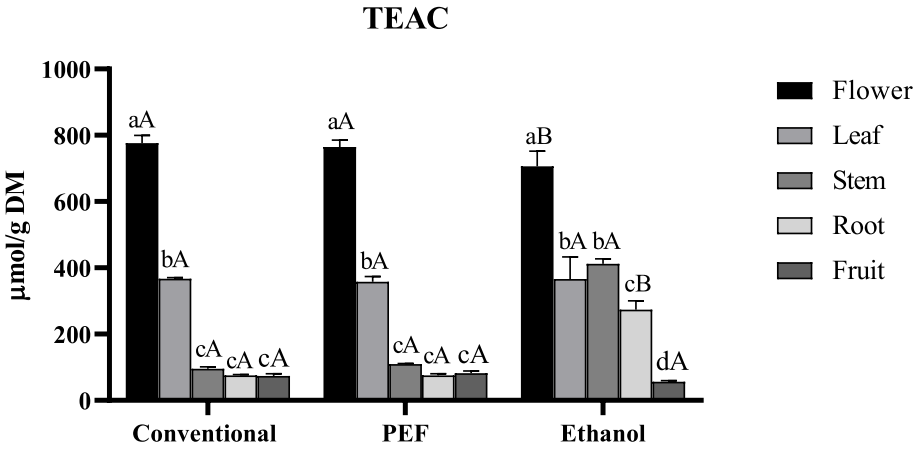

Fig. 3. Trolox equivalent antioxidant capacity (TEAC) (µmol Trolox equivalents (TE)/g dry matter (DM)) values in maceration (aqueous and ethanolic) and pulsed electric field- assisted extraction of various parts of the plant (leaves, flowers, stem, root and fruit).

method (using ethanol as a solvent), especially for the stem and roots ($p < 0.05$) compared to the PEF extraction, but the difference between the two methodologies in the case of fruits and leaves was not significant ($p > 0.05$).

In general, the results are consistent with other published studies that have shown that the application of PEF improves the overall activity of phenolic compounds and antioxidant activity [30]. However, the flower, leaves, stem, root and fruit of *Z. lotus* contain a variety of antioxidant compounds such as phenolic acids, flavonoids, alkaloids and saponins. These compounds have been found to reduce oxidative stress and inflammation by reducing levels of reactive oxygen species (ROS) [31]. Interestingly, numerous in vitro studies have demonstrated the ability of *Z. lotus* leaf constituents to eliminate free radicals and inhibit lipid peroxidation, indicating their antioxidant properties. In addition, several studies have shown that free radical scavenging activity, expressed by an equivalent amount of ascorbic acid, was significant and very high in *Z. lotus* stem extract [32].

In this context, the phenolic content in the stem extract is very high and it is known that polyphenols have an antioxidant effect, as there is a positive relationship between the antioxidant effect and the total phenolic content. In addition, research suggests that this plant can restore the antioxidant status altered by diabetes [33] and protect the human body and food from the phenomenon of oxidative stress [34]. However, phenolic and flavonoid compounds are known to have a strong antioxidant effect against various reactive oxygen and nitrogen species [35]. Therefore, the high content of polyphenols and flavonoids in the extracts of the five parts of the plant may be the cause of their antioxidant effect.

It is also remarkable that a favorable correlation between antioxidant activity and total phenol concentration were suggested [32], which is in agreement with the results observed, showing that the phenolic compounds were very high in the different parts of *Z. lotus* related to a great antioxidant capacity.

3.1.3. *Phenolic profile characterization of conventional and PEF extracts*

The phenolic profiles of the different *Z. lotus* parts extracted using conventional (aqueous and ethanolic) and PEF extraction methods are presented in Fig. 4 and summarized in Table 1. Qualitative LC-MS/MS analysis revealed the presence of diverse phenolic groups including flavonoids (e.g., kaempferol, apigenin, quercetin, chrysoeriol, luteolin, myricetin, catechin, morin, eriocitrin/neoeriocitrin, phloretin, and eriodictyol), hydroxycinnamic acid derivatives (e.g., ferulic acid), and lignan glycosides (e.g., sesaminol glycoside).

Kaempferol derivatives were detected in all parts of the plant, except in the aqueous and PEF stem extracts. Notably, the kaempferol content ranged from 41.68 % in the aqueous flower extract to 16.9 % in the aqueous fruit extract. Apigenin was also broadly distributed but particularly enriched in roots treated with PEF (41.78 %) versus aqueous extraction (22.66 %). This suggests that PEF rxtraction significantly enhances the recovery of this compound from roots. Interestingly,

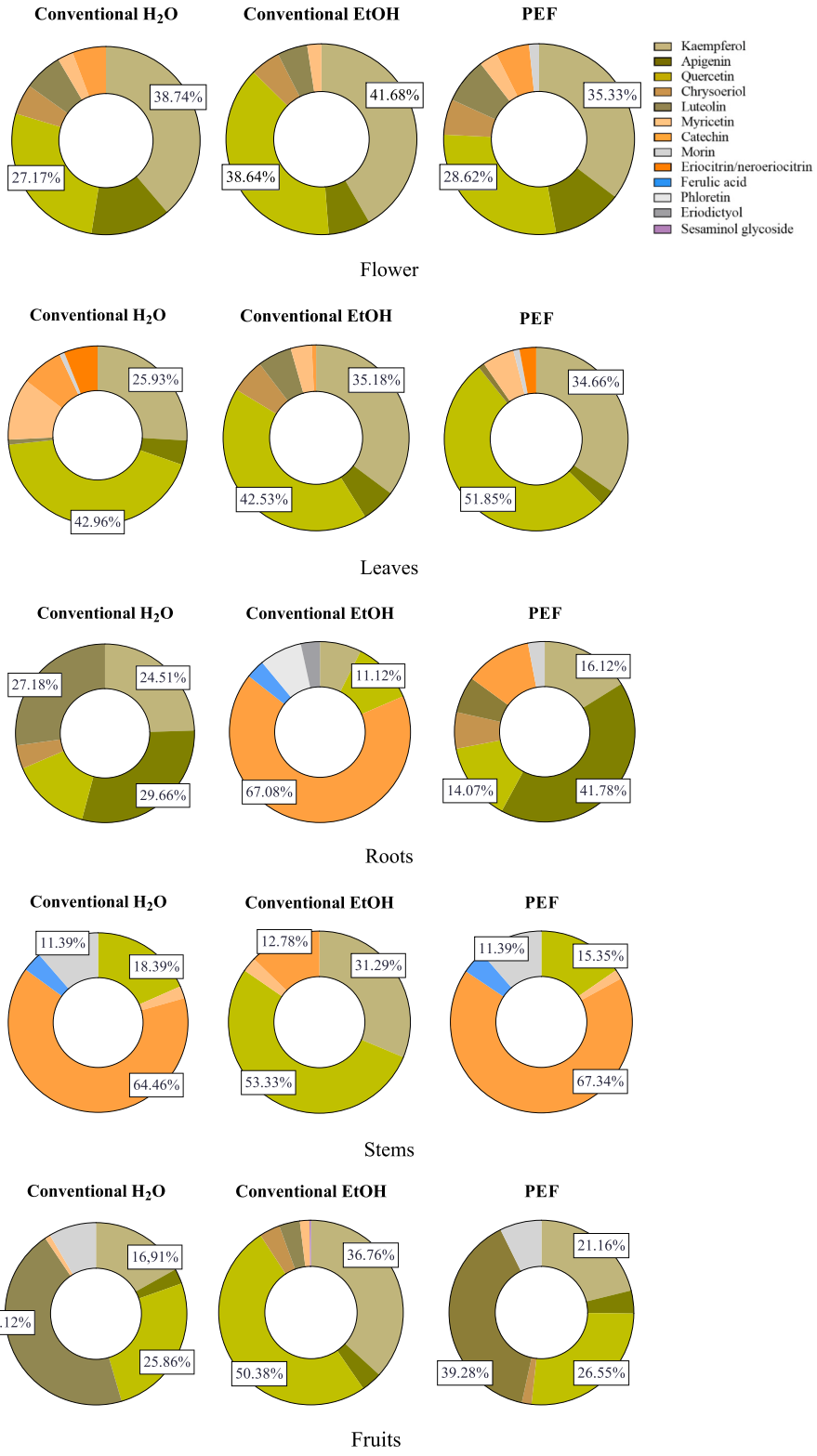

Fig. 4. Comparison of the main phenolic groups and derivatives between the different extraction methodologies: conventional aqueous and ethanolic, and extraction by pulsed electric fields (PEF), and the different parts of *Z. lotus* (flowers, leaves, roots, stems and fruits). The main derivatives observed in the different parts and treatments are shown as percentages.

apigenin derivatives were absent in the ethanolic root extracts and all three stem extracts.

Quercetin derivatives were consistently detected in all extracts and plant parts, with notable variations in concentration. The highest level (53.33 %) was observed in the ethanolic stem extract, while the lowest (11.13 %) occurred in the ethanolic root extract. PEF extraction also enhanced quercetin extraction in leaves, achieving 51.85 %.

Chrysoeriol was present in all flower extracts and was notably

Table 1
LC-MS/MS Identification of Phenolic Compounds in *Z. lotus* Extracts.

Compound Name	m/z (Parent Ion)	Fragment Ions (m/z)	Ionization Mode	Molecular Formula	Plant Part(s)	Extraction Method(s)
Kaempferol	285.04	270.02, 255.03, 151.00	Negative	C15H10O6	Flowers, Fruits, Leaves	Aqueous, Ethanolic, PEF
Apigenin	269.04	151.00, 117.02	Negative	C15H10O5	Roots, Flowers	Aqueous, PEF
Quercetin	301.04	151.00, 179.03, 121.03	Negative	C15H10O7	All parts	Aqueous, Ethanolic, PEF
Chrysoeriol	301.05	286.03, 151.00	Negative	C16H12O6	Flowers, Leaves, Fruits	Ethanolic, PEF
Luteolin	285.04	151.00, 133.03	Negative	C15H10O6	Fruits, Leaves	Aqueous, Ethanolic, PEF
Myricetin	317.04	179.03, 151.00	Negative	C15H10O8	Flowers, Leaves	Aqueous, PEF
Catechin	289.07	245.07, 205.05	Positive	C15H14O6	Roots, Stem	Ethanolic, PEF
Morin	301.04	286.03, 151.00	Negative	C15H10O7	Flowers, Leaves, Fruits	PEF
Eriocitrin/Neoeriocitrin	595.17	289.07, 301.04	Negative	C27H32O15	Leaves	Aqueous, PEF
Phloretin	273.07	167.03, 123.04	Negative	C15H14O5	Various	Various
Eriodictyol	289.07	151.00, 135.03	Negative	C15H12O6	Roots	Ethanolic
Ferulic Acid	193.05	134.04, 149.06	Negative	C10H10O4	Roots, Stem	Aqueous, Ethanolic, PEF
Sesaminol Glycoside	529.16	369.12, 151.00	Negative	C24H32O12	Fruits, Roots, Stem	Ethanolic, Aqueous, PEF

enhanced by ethanol extraction in leaves and by PEF and ethanol extraction in fruits. Conversely, this compound was undetectable in root ethanol extracts and all stem extracts. Luteolin was abundant in fruit extracts (45.17 % in aqueous and 39.28 % in PEF-extract fruits), but undetectable in root ethanol and all stem extracts.

The myricetin group was broadly present except in root and PEF fruit extracts. Catechin was most abundant in stems, reaching 67.34 % after PEF extraction, and 11.78 % in ethanol-extracted stems. This suggests PEF is the most effective technique for catechin extraction from stems, while ethanol is preferable for roots. Morin was detected in flowers, leaves, and fruits following PEF extraction but absent in other parts.

Eriocitrin appeared exclusively in the leaves, particularly in aqueous and PEF extracts. Ferulic acid was found in the roots and stems, especially in ethanolic root extracts and aqueous/PEF stem extracts. Eriodictyol was uniquely detected in the ethanolic root extract. Sesaminol glycoside was present in ethanolic fruit extracts, as well as in aqueous and ethanolic root and stem extracts, and in PEF roots extracts.

These results underscore the significant influence of both plant part and extraction method on the phytochemical profile. PEF extraction not only enhances the recovery of certain bioactive phenolics but also diversifies the compound spectrum obtained. The phenolic richness of *Z. lotus* supports its traditional use and biofunctional potential, especially due to the identified flavonoids and phenolic acids known for their antioxidant, anti-inflammatory, and antimicrobial properties.

In addition to PEF and conventional aqueous/ethanol extraction methods, several alternative techniques have been explored for the recovery of bioactive compounds. Ultrasound-assisted extraction utilizes acoustic cavitation to enhance mass transfer and cell wall disruption, offering shorter extraction times and reduced solvent consumption [36]. Microwave-assisted extraction accelerates extraction through rapid heating of the solvent and plant matrix, resulting in high efficiency and selectivity for certain phytochemicals [37]. Supercritical fluid extraction, particularly with supercritical CO_2, provides a solvent-free alternative that is environmentally friendly and suitable for thermolabile compounds; however, it may require costly equipment and is less effective for highly polar compounds [38]. These techniques differ in terms of efficiency, selectivity, environmental impact, and scalability. Compared to these methods, PEF offers the advantage of being non-thermal, energy-efficient, and capable of preserving heat-sensitive compounds, making it a valuable alternative in phytochemical extractionmatory, and antimicrobial properties.

To enhance reproducibility and transparency, LC-MS/MS chromatograms for each extract are provided in Fig. 4, and a detailed table (Table 1) includes compound names, *m/z* values, fragment ions, parent ions, ionization mode, molecular formula, and the corresponding plant part. This comprehensive data set provides a valuable reference for researchers interested in the phytochemical characterization of *Z. lotus* and the impact of extraction technologies.

Notes

- *m/z* values correspond to the parent ion detected in MS.
- Fragment ions indicate characteristic breakdown products used for compound identification.
- Ionization mode indicates whether negative or positive electrospray ionization was used.
- Molecular formulas correspond to the most common form of the compound.
- Plant parts and extraction methods indicate where and how the compound was detected.

4. Conclusion

The current study highlights the significant influence of both plant part selection and extraction methodology on the nutritional and phytochemical profiles of *Z. lotus*. Distinct profiles were observed across flowers, fruits, leaves, stems, and roots, with the flowers and fruits standing out for their high antioxidant potential and rich phenolic composition. The total antioxidant capacity (TAC) was notably higher in the flowers, while substantial variations in the phenolic profiles particularly in flavonoids such as kaempferol, quercetin, apigenin, and ferulic acid were observed, indicating that antioxidant richness alone does not fully reflect the phytochemical diversity.

Importantly, ferulic acid was absent in flowers, leaves, and fruits, underscoring the importance of root and stem extracts when targeting this specific compound. The choice of extraction technique also played a pivotal role: PEF extraction significantly enhanced the recovery of macronutrients (e.g., proteins, carbohydrates) and phenolic compounds without thermal degradation, likely due to cell membrane electroporation that facilitates compound release.

While PEF extraction showed a superior overall yield and improved extraction of various flavonoids, the selectivity of extraction remains dependent on both the plant matrix and the target compound. For example, catechin and apigenin were better extracted from specific tissues only under certain methods. Therefore, methodological optimization should not rely solely on yield or TAC, but rather on a targeted approach based on compound-specific goals.

In conclusion, an integrated understanding of the interplay between plant anatomy, compound distribution, and extraction conditions is vital for directing extraction strategies toward the recovery of desired bioactive molecules. Such insights are essential for future applications in functional food development, nutraceuticals, or phytopharmaceutical formulations derived from *Z. lotus*.

CRediT authorship contribution statement

Haifa Dhif: Writing – original draft, Methodology, Investigation, Formal analysis. **Jalila ben Salah-Abbès:** Writing – review & editing, Visualization. **Samir Abbès:** Writing – review & editing, Supervision, Resources. **Mara Calleja-Gómez:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data

curation. **Noelia Pallarés:** Writing – review & editing, Visualization, Supervision, Data curation. **Houda Berrada:** Writing – review & editing, Validation, Resources, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2025.114815.

Data availability

Data will be made available on request.

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