PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT PROPERTIES AND PHENOLIC ACIDS DETERMINATION OF DATE PALM (*Phoenix dactylifera* L.) LEAVES AND ROOTS EXTRACTS

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ABSTRACT

The date palm (*Phoenix dactylifera L.*) is a vital phytogenetic resource in arid regions. The roots and leaves are traditionally used for various purposes, including medicine. Despite extensive research on date palm fruits, the biochemical properties of its by-products remain underexplored. This study investigates the bioactive compounds in the leaves and roots of date palm cultivars. A reflux apparatus was used to extract total phenolic content (TPC), measured using the Singleton and Rossi method, while total flavonoid content (TFC) was determined using aluminum trichloride. Antioxidant activity was assessed using the DPPH assay, and HPLC-DAD analysis identified and characterized phenolic acids. The results revealed very high TPC levels in both leaves and roots supporting their traditional uses, reaching up to 825.63 ± 0,1 mg GAE/100 g dry weight in TAD leaves. TFC ranged from 18.81±0,15 to 117.85±0,2 mg EQ/100 g dry weight and correlated significantly with TPC. The extracts demonstrated significant antiradical efficacy compared to vitamin C, suggesting that all parts of the date palm are excellent sources of natural antioxidants. Twelve cell wall-bound and soluble phenolic acids were identified, several of which were previously unreported in this plant. Leaves predominantly contained ferulic acid, p-coumaric acid, and sinapic acid, while roots were rich in p-hydroxybenzoic acid. Additionally, several hydroxybenzoic and hydroxycinnamic acids implicated in plant defense mechanisms were identified. These findings highlight the dual importance of conserving local plant biodiversity and exploring date palm phenolics for applications in food, pharmaceuticals, and organic agriculture.

Keywords: *Phoenix dactylifera* L.; Phenolic compounds; Flavonoids; DPPH; HPLC-DAD.

1. INTRODUCTION

Plants are fundamental in medicine, providing a wide range of bioactive compounds crucial for therapies due to their rich biodiversity and diverse chemical structures. Plant-based medicines not only offer cost-effectiveness, accessibility, and perceived safety over synthetic alternatives but also hold promise for discovering new therapeutic bioactive compounds [1]. Currently, there is also a rising consumer preference for minimally processed foods that do not contain synthetic chemical preservatives. As a result, the food industry faces the challenge of developing natural antioxidants and antimicrobial agent to reduce dependence on synthetic additives.

Phenolic compounds, characterized by the presence of one or more hydroxyl groups attached to an aromatic ring, represent a diverse class of natural compounds abundant in plants and fruits [2]. These compounds play crucial roles in plant defense mechanisms against pathogens, UV radiation, and environmental stressors, while also contributing to the color, flavor, and nutritional properties of foods [3]. In addition to their physiological functions, phenolics have garnered significant interest across various industries due to their versatile applications. In the food industry, phenolic compounds act as natural antioxidants, preserving the quality and extending the shelf life of food products [4]. In pharmaceuticals and cosmetics, phenolics exhibit potential health benefits, including antioxidant, anti-inflammatory, and antimicrobial properties, making them valuable ingredients in supplements, skincare products, and herbal remedies [5]. Phenolic compounds play a pivotal role in organic agriculture by serving as natural defense mechanisms in plants, thereby reducing the dependence on synthetic pesticides and fertilizers [6].

The date palm (*Phoenix dactylifera* L.) is a crucial species for the arid regions. Date palm leaves and roots have long been revered in traditional medicine for their diverse medicinal properties. Leaves are traditionally used for their diuretic, antimicrobial, and digestive aid properties, while roots are employed for treating gastrointestinal disorders, diabetes, and inflammation. At present, there is insufficient published data concerning the characterization of phenolic compounds found in both the leaves and roots of the date palm, with particular emphasis placed on the most widely commercialized cultivars. Our study seeks to address this gap. The aim of this study was to characterize and identify the bioactive compounds present in two endemic palm date cultivars from the North Saharan region. The initial phase focused on quantifying the phenolic and flavonoid content, as well as assessing the antioxidant activity of the extracts. Following this, the study proceeded with the objective of identifying the phenolic acids through HPLC analysis.

2. MATERIAL AND METHODS

2.1 Plant material

We examined two cultivars of date palm (DP), Tadala (TAD) and Tizzaout (TIZ), recognized for cultural desirable traits (productivity). These cultivars are endemic to the Laghouat region in Algeria. We collected several leaves (l) and roots (r) samples from different female plants during the spring of 2020.

2.2 Chemicals and reagents

All reagents and solvents used in the experiments were of the highest purity. Petroleum ether, Na2CO3, phenolic acids standard, ethanol, ethyl acetate, NaOH, HCl, n-hexane, acetonitrile, acetic acid, methanol, Folin–Ciocalteu reagent, gallic acid, rutine.

2.3 Sample preparation and extraction

The plant material was dried under ambient conditions, shielded from light, and kept at room temperature. It was then ground. Several grams of the resulting powder underwent reflux extraction in a mixture of ethanol and water (80:20, v/v) for 6 hours. The crude preparation was filtered, and the alcohol was removed using a rotary evaporator at 45°C. The aqueous phases were treated with petroleum ether to remove pigments. Phenolic compounds were extracted twice with 20 ml of ethyl acetate each time. Residual water in the ethyl acetate extract was removed using anhydrous sodium sulfate and filtered through filter paper. The extract was then evaporated to dryness using a rotary evaporator. The extracted phenolics were dissolved in 10 ml of methanol. After extracting soluble phenolics, bound phenolics were extracted from the residue using 2.5 ml of 2 M NaOH. Alkaline hydrolysis was performed at 90°C for 2 hours. Following hydrolysis, the sample was acidified with 2.5 ml of 2 M HCl to achieve a pH of 2. Lipids were removed by adding 4.0 ml of n-hexane. Bound phenolics were recovered by extracting the mixture three times with 4.0 ml of ethyl acetate. The collected ethyl acetate layer was evaporated to dryness and resuspended in 3 ml of methanol, which was then combined with the first extract. The methanolic solutions containing the phenolic compounds were stored at -18°C until analysis.

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2.4 Determination of TPC

The total phenolic content (TPC) in the plant extracts was assessed using the Folin–Ciocalteu reagent and the colorimetric method developed by Singleton and Rossi [7]. Specifically, the reaction mixture included 100 µl of the methanolic extract, 500 µl of freshly prepared diluted (1:10) Folin–Ciocalteu reagent, and 2 ml of a 20% w/v Na2CO3 solution. The mixtures were shaken and allowed to stand at room temperature for 30 minutes, after which the absorbance was measured at 760 nm using a UV-Visible spectrophotometer (Shimadzu UV-1601). TPC was determined using a standard curve of gallic acid and expressed as milligrams of gallic acid equivalents per 100 grams of dry weight plant material (mg GAE/100 g dw).

2.5 Determination of TFC

The total flavonoid content (TFC) was measured using the aluminum chloride colorimetric method outlined by Chang et al. [8] with slight modifications. This method involves quantifying the yellow color that results from the interaction between flavonoids and the AlCl3 reagent. For each sample, 1 ml was mixed with 1 ml of a 2% (w/v) AlCl3 in methanol solution and incubated in the dark at room temperature for 20 minutes. The absorbance of all samples was then measured at 409 nm using a UV-Visible spectrophotometer (Shimadzu UV-1601). TFC was calculated using a standard rutine curve and expressed as milligrams of rutine equivalents per 100 grams of dry weight plant material (mg RU/100 g dw).

2.6 DPPH radical scavenging activity

The radical scavenging activity of the extracts against stable DPPH (2,2-diphenyl-1-picrylhydrazyl) was assessed using the method described by Brand-Williams et al. [9]. The absorbance changes were monitored at 517 nm using a UV-Visible spectrophotometer (Shimadzu UV-1601). A solution of DPPH in methanol was freshly prepared before conducting measurements. For each sample, 1 ml of diluted sample solution in methanol was added to 1 ml of the DPPH radical solution. The mixture was vigorously shaken and left to stand at room temperature in the dark for 30 minutes. The decrease in absorbance was then measured at 517 nm. A blank sample, containing the same amount of methanol and DPPH solution, was also prepared and measured. The antioxidant activity of the extract was quantified as an IC50 value, which represents the concentration (mg/l) of the extract necessary to inhibit the formation of DPPH radicals by 50%, and was compared to the IC50 of vitamin C. The antiradical efficiency (EA), denoted as 1/IC50, was calculated accordingly. The vitamin C curve was established using solutions ranging from 0.0025 to 0.0225 mg/ml, derived from a 0.025 g/l stock solution. The percentage of DPPH scavenging effect was determined using the following equation: DPPH scavenging effect (%) = [(A0 – A1)/A0) × 100]. Where: A0=The absorbance of control. A1=The absorbance of standard.

2.7 Determination of phenolic acids with HPLC-DAD method

The samples underwent analysis using reversed-phase High-Performance Liquid Chromatography (HPLC) employing a Shimadzu SCL-6B system equipped with a diode array detector (SPD-M20A) and an Ultra C18 column (250 × 4.6 mm, Kanto Chemical Co., Tokyo, Japan). The analytical approach followed the protocol outlined by González et *al.* [10], with specific adjustments. The analysis was conducted at a temperature of 30°C with a flow rate of 1 mL/min. The mobile phase consisted of solvent A (1% acetic acid in ultra-pure water) and solvent B (acetonitrile), utilizing a gradient starting at 10% B, reaching 100% B at 55 minutes, and returning to 10% B by 65 minutes. The PDA detector operated over a wavelength range from 190 to 800 nm, with specific monitoring at 250 nm and a slit width of 1.2 nm for enhanced sensitivity. Phenolic acids were identified by measuring absorbance at 254 nm and their retention times were compared against a mixture of reference compounds. The analysis focused on detecting 17 specific phenolic acids present in the samples.

2.8 Statistical analysis

A one-way analysis of variance (ANOVA) was performed to evaluate the significance of the data. The standard deviation (±SD) was calculated based on four replicates (n=4). Mean values were compared using Tukey's Honestly Significant Difference (HSD) test, with a significance level set at $P < 0.05$. Correlations among different parameters were also explored. The IC50 values were determined using Origin orglab 9.4 software (OriginLab Corporation, Northampton, MA, USA).

3. RESULTS AND DISCUSSION

3.1 Total phenolic content

The study revealed significant levels of total phenolic compounds (TPCs) in both date palm leaves and roots, with higher concentrations observed in the leaves (Fig. 1). Among the date palm extracts analyzed, TPC content varied notably, ranging from 332.9 ± 0,1 to 825.63 ± 0,3 mg GAE/100 g dw (Tab.1). Particularly noteworthy was the exceptionally high TPC content in the leaves of the TIZ cultivar (825.63± 0,3 mg GAE/100 g dw), while the lowest TPC content was detected in the roots of the TAD cultivar (332.9 ± 0,1 mg GAE/100 g dw). The order of TPC content in the date palm extracts was as follows: roots of TAD < roots of TIZ < leaves of TAD < leaves of TIZ.

All extracts of date palm of the experience contained a remarkable quantity of TPC. The obtained TPC levels prove to be higher compared to those found for tea (100-500 mg EAG/gdw), coffee (500-800 mg EAG/gdw), lemon (100-300 mg EAG/gdw), and apple (100-300 mg EAG/gdw) [11].The TPC results obtained from the leaves of our study are notably higher compared to those reported in Algerian Oued-Souf cultivars, with a maximum of 215.24 mg GAE/100 g dw observed in the "Ghars" cultivar [12]. Moreover, the TPC levels in our experiment surpass those found leaves of Saudi Arabian cultivars, where the maximum reported was 106 ± 1.76 mg GAE/100 g dw [13]. The TPC obtained from the roots are significantly superior to those found in the roots of cultivars from Oman [14] and cultivars from Morocco [15] .These comparisons highlight the higher TPC content in the leaves and roots of the date palm cultivars investigated in our study and the efficiency of the extraction methodology used.

These outcomes underscore the significant influence of both cultivar type and plant organ on the accumulation of phenolic compounds within the date palm. The substantial concentration of phenolic compounds, particularly in the leaves of the TIZ cultivar, suggests a potential role for these compounds in the physiological and biochemical processes of the date palm. The differences in TPC content among cultivars and plant organs could be attributed to genetic variations, environmental factors, and growth conditions, all of which can influence the biosynthesis and accumulation of phenolic compounds. The findings align with previous research emphasizing the diversity of phenolic compound distribution within different plant parts and cultivars [13, 16].

Phenolic compounds are involved in plant defense against non-biotic environmental stresses such as UV radiation and salinity [17, 18]. Furthermore, most phenolic compounds are known to be involved in the defense of plants. This concerns hydroxybenzoic acids, hydroxycinnamic acids, coumarins, flavonoids and stilbenes [19]. Bayoud disease, caused by *Fusarium oxysporum f. sp. albedinis* (*Foa*), has been identified as the primary disease affecting date palms [20, 21]. The pathogen enters the plant through the roots resulting in leaf withering and eventual death of the date palm tree [22, 23]. Developing or identifying genetically resistant clones is considered the most effective approach to protect palm plantations from Bayoud disease [24]. Quantitative differences in bioactive compounds between cultivars resistant and susceptible to *Fusarium oxysporum f. sp. albedinis* were observed by Ziouti and *al.* [15]. The TPC obtained from the roots in the examined cultivars are significantly superior to those found in the roots of Salt-tolerant cultivars from Oman [14] and *Foa* resistant cultivars from Morocco [15].The exceptionally high levels of Total Phenolic Content (TPC) observed suggest their potential resistance to *Foa* infection and other biotic and non-biotic stress.

3.2 Total flavonoid content

The findings revealed significant levels of total flavonoid compounds (TFCs) in both the leaves and roots of date palm, with higher concentrations observed in the leaves (Fig. 1). There were notable variations in TFC content among the date palm extracts, ranging from 18,81 ± 0,6 to 117,85 ± 0,62 mg RU/100g dw. Particularly, the TFC content was highest in the leaves of the TAD cultivar (117,85 ± 0,62 mg RU/100g dw). The sequence of TFC content in the date palm extracts was as follows: roots of TAD < roots of TIZ < leaves of TIZ < leaves of TAD.

The correlation analysis revealed a direct association between the concentration of phenolics and flavonoids in *P. dactylifera* extracts (0,73) as reported in John et *al.* [13] and Saeed et *al.* [25]. The wide range of TFC content, from 18.81 to 117.85 mg RU/100g dw, highlights the influence of cultivar and environmental factors on phenolic accumulation, a phenomenon also acknowledged by Kriaa et *al.* [26] in their study. According to research published in the journal "Molecules," flavonoids exhibit broad-spectrum antimicrobial activity against various bacteria, fungi, and viruses, underscoring their potential as natural antimicrobial agents [27]. Beyond their antimicrobial role, flavonoids also exhibit significant anti-inflammatory properties, as demonstrated in research indicating their potential therapeutic applications in inflammatory conditions [27, 28]. Moreover, their agronomic importance lies in enhancing plant resilience and productivity, contributing to sustainable agriculture practices and improving food quality.

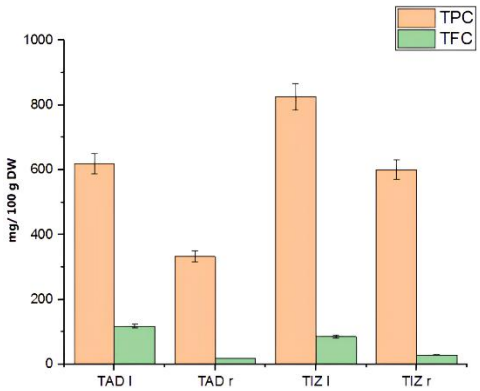

Figure 1. Phenolic content and flavonoids content in the exacts of leaves and roots of the cultivars of *P.dactylifera*. The phenolic content is estimated in mg of gallic acid equivalent in 100 g of dry weigh. The content is estimated in mg of rutine equivalent in 100 g of dry weigh.

Table 1. Total phenolic content (TPC), total flavonoid content (TFC), and IC50 in the different samples

	TPC Gallic acid eq. (mg/100 g)	TFC Rutine eq. (mg/100 g)	IC50 DPPH (mg/l)
TAD l	619,99 ± 0, 1 [b]	117,85 ± 0,62 [a]	0,089
TAD r	332,90 ± 0,1 [c]	18,81 ± 0,6 [b]	0,161
TIZ l	825,63 ± 0,3[a]	85,9 ± 0,74 [a]	0,069
TIZ r	600,43 ± 0,1[b]	27,07 ± 0,24 [b]	0,036

Values are presented as mean ± SD (n=4). Means ± SD followed by the same letter within a column are not significantly different ($p > 0.05$)

3.3 Antioxidant activity

Curves of the test samples were prepared using percent inhibition of DPPH values plotted against the concentrations (Fig.2).

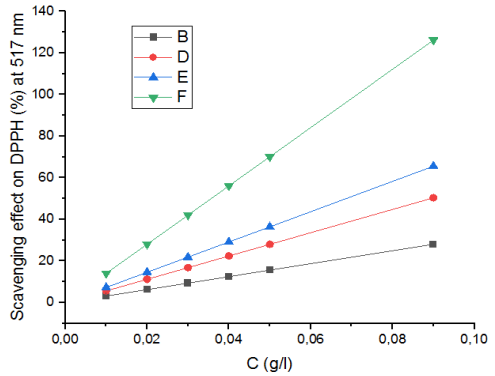

Figure 2. Scavenging effect on DPPH (%) of the extract of *P.dactylifera* Leaves and Roots

The study assessed the ability of methanolic extracts from date palm (DP) to scavenge DPPH radicals by determining their IC50 values, which represent the concentration required to decrease the absorbance at 517 nm of the DPPH radical solution by half. The results were compared with ascorbic acid (vitamin C), a potent antioxidant.

The methanolic extracts exhibited notable anti-free radical efficacy (see Table 1) compared to vitamin C (IC50= 0,016 mg/ml). The IC50 values of DP extracts ranged from 0.036 to 0.161 mg/l. The lowest IC50 value, indicating the highest antioxidant activity, was observed in TIZ r, whereas the highest IC50 value was found in TAD r. Antioxidant efficiency (AE=1/IC50) in the methanolic extracts of DP (Fig.3) followed the order TIZ r < TIZ l < TAD l < TAD r, indicating varying levels of antioxidant potency among the cultivars studied.

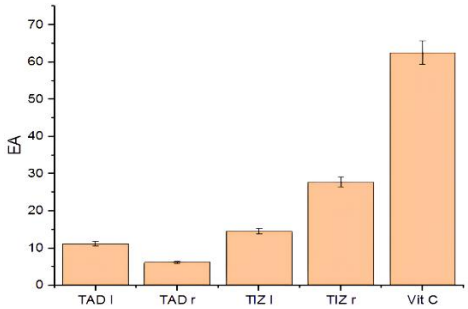

Figure 3. Antiradical efficiency (EA) of the exacts of leaves and roots of the cultivars of *P.dactylifera* using the DPPH method for the estimation of antioxidant activity.

The correlation analysis revealed a direct association between the antioxidant strength, capacity for scavenging free radicals, and the concentration of phenolics (0,63) in *P. dactylifera* extracts as reported in John et *al.* [13] and Saeed et *al.* [25]. This activity is likely attributable to the properties of phenolics, which enhance their ability to transfer electrons or donate hydrogen [29]. Antioxidant activity plays a crucial role in mitigating oxidative damage by scavenging free radicals and protecting cellular components from oxidative stress [30]. This process is essential for maintaining cellular homeostasis and overall health. Antioxidant activity is crucial too in the food industry for preserving the quality and extending the shelf life of food products. Antioxidants help prevent oxidative degradation of fats, oils, and other food components, thereby maintaining flavor, color, and nutritional value [31]. Moreover, investigations into the defense mechanisms of date palm against (*Foa*) have emphasized the significance antioxidant capacity in conferring tolerance to the pathogen [32, 33].

3.4 Identification of soluble and cell-wall phenolics acids

The identification of the phenolic compounds was done by comparing the chromatograms of the samples with those of the authentic standards, ensuring a match in terms of retention time and chromatographic behavior. This verification process provided confidence in the accurate identification of the phenolic compounds present in the samples.

A significant difference between roots and leaves, concerning the relative abundance (% area) and diversity of the phenolic compounds was noted (Tab.2, Fig.4 and Fig.5). The phenolic compounds in the leaves were more abundant and diverse than those found in the roots. Quantitative and not qualitative differences were observed between the two date palm cultivars. Out of the seventeen phenolic compounds tested, twelve phenolic acids were detected in the roots and leaves of date palm. The extracts contained diverse hydroxycinnamic and hydroxybenzoic acids.

The four main peaks (3,4,5 and 6), showing maximum absorption at 254 nm in the leaves of the two cultivars of date palm (Fig. 4), corresponded to Syringic acid and cell-wall bound phenolic acids (p-Coumaric acid, Ferulic acid, Sinapic acid). These four main compounds in the leaves were accounting for 32% for Tadala leaves and 19% for Tizzaouet leaves of all compounds detected at 254 m. Others peaks corresponds to other phenolic acids (trans-Cinnamic acid, p-Hydroxybenzoic acid, Gallic acid, Salicylic acid, Vanillic acid and Hydroxycinnamic acid) and probably to flavonoids and others phenolic compounds.

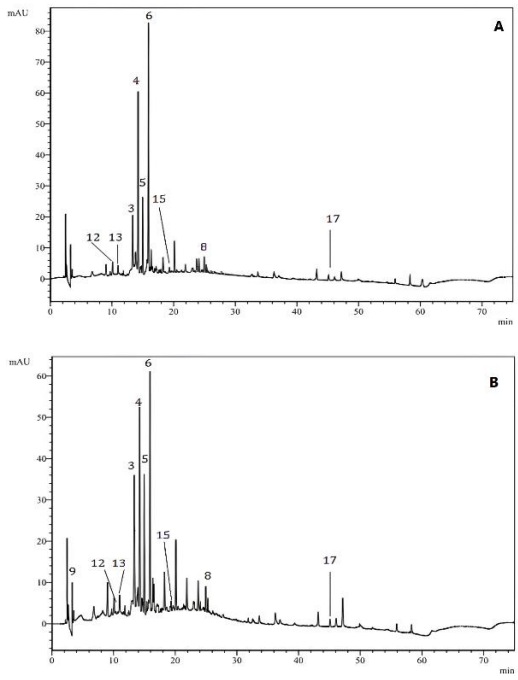

Figure 4. HPLC-DAD chromatogram of phenolic acids from methanolic extracts of leaves of the date palm cultivars *(P. dactylifera L.)* (256 nm). (A) Tadala cultivar; (B) Tizzaouet cultivar. (3) Syringic acid; (4) p-Coumaric acid; (5) Ferulic acid; (6) Sinapic acid; (8) trans-Cinnamic acid; (9) Gallic acid; (12) p-Hydrobenzoic acid; (13) Vanillic acid; (15) Salicylic acid; (17) Hydrocinnamic acid.

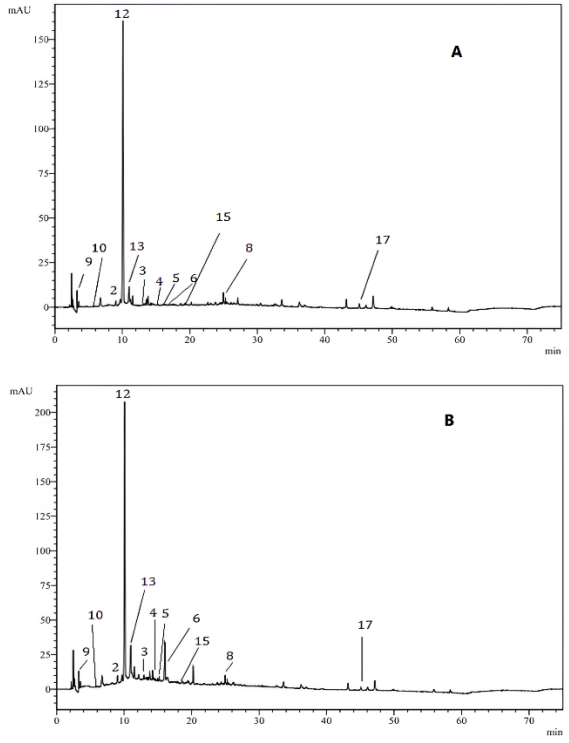

Figure 5. HPLC-DAD chromatogram of phenolic acids from methanolic extracts of roots of the date palm cultivars *(P. dactylifera L.)* (256 nm). (A) Tadala cultivar; (B) Tizzaouet cultivar.(2) 2,3-Dihydroxybenzoic acid; (3) Syringic acid; (4) p-Coumaric acid; (5) Ferulic acid; (6) Sinapic acid; (8) trans-Cinnamic acid; (9) Gallic acid; (10) 2,5-Dihydrobenzoic acid; (12) p-Hydroxybenzoic acid; (13) Vanillic acid; (15) Salicylic acid;(17) Hydroxycinnamic acid.

Table 2. HPLC identification of soluble and cell-wall bound phenolic acids of methanolic extracts of the palm date (*Phoenix dactylifera*) leaves and roots at 254 nm.

Peak	Phenolic acid	Retention time (min)	Occurrence	Area (%)
1	**Pyrogallol**	2,442	ND	-
2	**2,3-Dihydroxybenzoic acid**	9,111	Tadala roots Tizzaouet roots	1,397 0,859
3	**Syringic acid**	13,359	Tadala leaves Tadala roots Tizzaouet leaves Tizzaouet roots	4,320 0,501 4,898 1,434
4	**p-coumaric acid**	14,224	Tadala leaves Tadala roots Tizzaouet leaves Tizzaouet roots	10,914 1,825 6,089 0,511
5	**Ferulic acid**	14,978	Tadala leaves Tadala roots Tizzaouet leaves Tizzaouet roots	4,011 0,859 3,235 0,054
6	**Sinapic acid**	15,914	Tadala leaves Tadala roots Tizzaouet leaves Tizzaouet roots	12,839 3,987 5,154 0,19
7	**Resveratrol acid**	23,416	ND	-
8	**trans-Cinnamic**	25,362	Tadala leaves Tadala roots Tizzaouet leaves Tizzaouet roots	0,759 0,521 0,713 0,931
9	**Gallic acid**	3,760	Tadala roots Tizzaouet leaves Tizzaouet roots	0,131 0,350 0,238
10	**2,5-Dihydroxybenzoic acid**	5,951	Tadala roots Tizzaouet roots	0,096 0,089
11	**Tyrosol**	9,578	ND	-
12	**p-Hydroxybenzoic acid**	10,179	Tadala leaves Tadala roots Tizzaouet leaves Tizzaouet roots	1,204 48,062 1,600 23,448
13	**Vinylic acid**	11,168	Tadala leaves Tadala roots Tizzaouet leaves Tizzaouet roots	0,248 0,778 2,342 1,892
14	**4-Acetylresorcinol**	18,646	ND	-
15	**Salicylic acid**	19,652	Tadala leaves Tadala roots Tizzaouet leaves Tizzaouet roots	0,869 0,214 1,531 0,638
16	**3,5-Dimethoxyphenol**	43,144	ND	-
17	**Hydroxycinnamic acid**	45,124	Tadala leaves Tadala roots Tizzaouet leaves Tizzaouet roots	0,436 0,793 0,196 0,274

ND: Not detected.

The analysis of the soluble phenolic compounds of palm date roots tissues (Fig.5) showed a substantial amount of p-Hydroxybenzoic acid (43% in Tadala roots and 23 % in Tizzaouet roots). Second in line, was Hydroxycinnamic acid (2,6% in Tadala roots and 1% in Tizzaouet roots) and 2,3-Dihydroxybenzoic acid (1,4% in Tadala roots and 0,8% in Tizzaouet roots). 2,5-Dihydroxybenzoic acid content was the lowest (0,09% in Tadala roots and 0,08% in Tizzaouet roots) among the phenolic compounds identified in the palm date roots. The other soluble phenolic acids detected and identified at 254 nm were syringic acid, Gallic acid, trans-Cinnamic acid, Vanillic acid and Salicylic acid. The cell wall-bounds phenolics acids (Ferulic acid, p-Coumaric acid, Sinapic acid) presents in palm date roots were significantly lower than those found in the leaves. They were far from being the major compounds and only represent less than 4% of all compounds for the most abundant (sinapic acid in tadala roots).

Phenolic acids are known for their significant antimicrobial activity, which contributes to their role in food preservation and health maintenance. These compounds exhibit inhibitory effects against a wide range of microorganisms, including bacteria, fungi, and viruses [27]. Their presence not only supports soil health and biodiversity but also enhances crop quality and yield, making them essential components of organic farming strategies. Phenolic acids found in plants can be categorized into two primary groups: hydroxybenzoic acid and hydroxycinnamic acid derivatives.

Twelve different hydroxycinnamic and hydroxybenzoic acids were detected in the roots and leaves of date palm of the study. Hydroxycinnamic acids, such as p-Coumaric acid, have been shown to possess antioxidant activity by scavenging free radicals and protecting cells from damage [30]. Similarly, hydroxybenzoic acids, like p-hydroxybenzoic acid, exhibit anti-inflammatory effects by modulating inflammatory pathways and reducing the production of pro-inflammatory molecules [34]. Research suggests that the consumption of foods rich in these phenolic acids may contribute to a well-balanced diet that supports overall health and well-being. Furthermore, studies have indicated that these compounds might have a role in preventing cardiovascular diseases, cancer, and neurodegenerative disorders [35].

In this study, the leaves exhibited a predominance of phenolic acids linked to the cell wall, including ferulic acid, p-coumaric acid, and sinapic acid. In contrast, the roots were distinguished by the prevalence of p-hydroxybenzoic acid. These results correspond to those found in previous studies [36]. Moreover, the identification of several hydroxycinnamic and hydroxybenzoic acids further enriched the findings of this study. These hydroxycinnamic acids were found to be associated with potential resistance against pathogenic agents, adding another layer of significance to the observed bioactive compounds

No qualitative differences in phenolic acids were observed between the phenolic profiles of two date palm cultivars, but significant quantitative variations were noted (Table 2). Hydroxycinnamic acid and hydroxybenzoic acid derivatives were predominant in both cultivars, crucial for their role in defense against (*Foa*) [37]. These compounds, including p-hydroxybenzoic acid, accumulate in response to pathogen interactions [38, 39]. The significant role of p-hydroxybenzoic acid in defense mechanisms has been highlighted in numerous studies [40].Salicylic acid, although present in low amounts across all parts of the date palm, activates phenolic compound metabolism and enhances biosynthesis of hydroxycinnamic acid derivatives, particularly sinapic acid derivatives, known for inhibiting *Foa* germination and growth [41, 42].

Differential accumulation of phenolic acids between date palm leaves and roots is attributed to their histological differences. Roots, with abundant parenchyma cells, favor soluble forms of hydroxycinnamic acids, while date palm leaves accumulate p-coumaric acid in their lignified cell walls, particularly in sclerenchyma cells [36]. This histological variation affects resistance mechanisms against *Foa*, where phenolic acids reinforce cell walls and make them less susceptible to degradation by pathogens [43, 44]. Consequently, the abundance of phenols in cell walls renders polysaccharides less susceptible to the cell wall-degrading enzymes of pathogens [45].

Cell wall-bound phenolic compounds like p-hydroxybenzoic, p-coumaric, ferulic, and sinapic acids inhibit *Foa* mycelium growth and the production of cell wall-degrading enzymes. Their effectiveness depends on concentration and chemical structure, with methoxylation enhancing and hydroxylation diminishing their inhibitory effects [46, 47].

CONCLUSION

In conclusion, the significant levels of total phenolic compounds (TPC) and total flavonoid compounds (TFC), coupled with high antioxidant activity, highlight the nutritional and potential health benefits of these date palm cultivars. Furthermore, the identification of twelve beneficial phenolic acids within these plant parts suggests their potential as valuable sources of bioactive compounds with various therapeutic applications. These results not only emphasize the importance of conserving and studying local plant biodiversity but also provide a foundation for further exploration into harnessing date palm phenolics for food, pharmaceutical, and organic agricultural purposes. Future research could delve deeper into the specific health-promoting properties and mechanisms of these phenolic acids, paving the way for innovative uses in functional foods and natural medicine.

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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