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Chemical profiling of male date palm (*Phoenix dactylifera* L.) leaflets in El M'Ghair region, Algeria: Insights into total phenols, flavonoids, proteins, and total sugars

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Chemical profiling of male date palm (*Phoenix dactylifera* L.) leaflets in El M'Ghair region, Algeria: Insights into total phenols, flavonoids, proteins, and total sugars

Abstract: This study extensively investigates the biochemical composition of male date palm leaflets (*Phoenix dactylifera* L.) from the El M'Ghair region in Algeria, focusing on the cultivars Deglet Nour, Degla Beida, and Ghars. The chemical analysis centers on proteins, total sugars, total phenols, and flavonoids, revealing significant differences in these compounds among the three cultivars. 'Degla Beida' exhibits the highest protein content (39.09 ± 4.58 %), contrasting with 'Ghars', which records the lowest (18.33 ± 5.35 %). Total sugars exhibit variability among cultivars, with 'Ghars' displaying the highest levels (74.54 ± 6.92 %) and 'Degla Beida' the lowest (46.28 ± 9.11 %). The production ratios of crude methanolic aqueous extracts show consistent extraction efficiencies across cultivars. Abundant phenolic compounds, notably in 'Degla Beida', and significant variations in flavonoid content are observed. Specifically, 'Degla Beida' and 'Deglet Nour' boast the highest flavonoid levels (36.02 ± 3.79 and 30.18 ± 3.88 µg QE. mg^{-1}, respectively), whereas 'Ghars' demonstrates the lowest (22.02 ± 2.31 µg QE. mg^{-1}). The correlation matrix reveals positive associations among proteins, total phenols, and flavonoids, contrasting with a strong negative association with total sugar content.

Key words: *Phoenix dactylifera* L., leaflets, biochemical composition, El M'Ghair region

Kemijski profil lističev moških datljevcev (Phoenix *dactylifera* L.) na območju El M'Ghair v Alžiriji: Vpogled v vsebnost celokupnih fenolov, flavonoidov, proteinov in celokupnih sladkorjev

Izvleček: V raziskavi je bila preučevana biokemična sestava lističev moških dateljevih palm (*Phoenix dactylifera* L.) z območja El M'Ghair v Alžiriji, s poudarkom na sortah Deglet Nour, Degla Beida in Ghars. Kemijske analize so bile osredotočene na vsebnosti beljakovin, celokupnih sladkorjev, celokupnih fenolov in flavonoidov. Analiza je odkrila značilne razlike v vsebnostih teh spojin med tremi sortami. 'Degla Beida' je imela največjo vsebnost beljakovin (39,09 ± 4,58 %), v nasprotju s 'Ghars', ki jih je vsebovala najman (18,33 ± 5,35 %). Vsebnost celokupnih sladkorjev se je med sortami razlikovala, pri čemer je imela 'Ghars' največjo vsebnost (74,54 ± 6,92 %) in 'Degla Beida' najmanjšo (46,28 ± 9,11 %). Ekstrakcije preučevanih sestavin z metanolom in vodo se je izkazala za učinkovito pri vseh sortah. Pri 'Degla Beida'je bila ugotovljena velika vsebnost celokupnih fenolov, vsebnost flavonoidov je med sortami značilno variirala. 'Degla Beida' in 'Deglet Nour' sta imeli največjo vsebnost flavonoidov (36,02 ± 3,79 in 30,18 ± 3,88 µg QE. mg^{-1}), 'Ghars' je imela najmanjšo (22,02 ± 2,31 µg QE. mg^{-1}). Korelacija je odkrila pozitivne povezave v vsebnosti beljakovin, celokupnih fenolov in flavonoidov, a zelo negativno povezavo z vsebnostjo celokupnih sladkorjev.

Ključne besede: *Phoenix dactylifera* L., lističi, biokemična sestava, območje El M'Ghair

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1 INTRODUCTION

Phoenix dactylifera L., commonly known as the date palm, is of enormous importance both globally and specifically in Algeria. Its cultivation plays an important role in supporting the Algerian Sahara population, significantly contributing to food security and rural livelihoods (Mansouri et al., 2005). Moreover, the palm has a rich cultural and historical significance, deeply rooted in the traditions of different societies. This woody perennial plant, which belongs to the Arecaceae family, is recognized as one of the oldest and most widely cultivated fruit trees in arid and semi-arid regions, particularly in the Gulf countries of the Middle East (Al Harthi et al., 2015). Globally, Algeria ranks fourth among the largest palm producers and ninth in palm fruit exports (Benouamane et al., 2022). The date palm is also considered one of the important plants that can tolerate salinity in its final stages. Dates are considered one of the most important crops and non-traditional commodities that can be exported and consumed locally with good financial returns. The area of date palm cultivation in the world is increasing daily, especially in desert areas with sandy soil (Shareef et al., 2023).

Date palm fruits are known for their numerous health and nutritional benefits attributed to their rich phenolic content and strong antioxidant capacity (Daoud et al., 2019). Many studies are available on the phenolic composition of certain cultivars of date palm seeds and fruits (Abu-Reidah et al., 2017; Bentrad et al., 2017; Habib et al., 2014; Hilary et al., 2020; Jenny & Fereidoon, 2019; Ma et al., 2019; Mansouri et al., 2005; Messaoudi et al., 2013), and these by-products have shown promising results in pharmaceutical formulations, as they exhibit antifungal, antioxidant, and anti-inflammatory activities and anti-diabetic. The nutritional value of these fruits has been explored, leading to the identification of their various benefits when incorporated into animal diets (Al-Shahib & Marshall, 2003). However, it enjoys scarce studies on the leaves of *Phoenix dactylifera* L., specifically in the phytochemical and nutritional fields.

Determining the content of total phenolic compounds and flavonoids in phytochemical studies provides valuable insights into plant extracts' antioxidant potential and bioactive properties.

Phenolic compounds, among them flavonoids are known for their significant antioxidant activity, which can help neutralize harmful free radicals in the body and reduce oxidative stress, potentially leading to various health benefits. These compounds have been associated with anti-inflammatory, anti-cancer, and cardiovascular protective effects, making them essential components in assessing the health-promoting properties of plant materials (Djeridane et al., 2006; Martínez et al., 2022; Tungmunnithum et al., 2018).

On the other hand, determining the protein and total sugars content in proximate composition provides crucial information about the nutritional value of the analyzed samples. Proteins are vital for building and repairing tissues in the body, supporting the immune system, and serving as enzymes and hormones. Assessing protein content is essential for evaluating the nutritional quality of food and feed materials. Similarly, the determination of total sugars, which includes both simple sugars like glucose and fructose and polysaccharide like starch, is significant in understanding the energy content and sweetness of the analyzed samples. This information is critical for evaluating the nutritional value and potential applications of plant-based products in both human and animal diets (Akintimehin et al., 2022; Radha et al., 2021; Zhou et al., 2022).

The biological dimension of this study aims to explore the chemical content of male date palm leaves from three cultivars (Deglet Nour, Degla Beida, and Ghars) grown in the El M'Ghair region of Algeria. The study focuses on analyzing the levels of total protein, total sugars, totalphenolic, and flavonoids in these leaves. The objective is to comprehend the changes in these substances among the various cultivars, which may impact their therapeutic and nutritional qualities.

2 MATERIALS AND METHODS

2.1 CHEMICALS

Sigma-Aldrich (St. Louis, USA) provided the following chemicals for the study: trichloroacetic acid, chloroform, methanol, sodium hydroxide, phenol, sulfuric acid, vanillin, orthophosphoric acid, Coomassie brilliant blue, hexane, Folin-Ciocalteu, sodium carbonate, and aluminum trichloride.

2.2 PLANT MATERIAL

In January 2020, leaflets from three male cultivars of the El M'Ghair region were collected, situated at coordinates 33°57'02.4"N 5°55'27.3"E (Figure 1). The leaflets were taken from the middle crown of date palms. Following collection, the samples were washed with distilled water and dried in an oven at 40 °C for 24 hours. Subsequently, an electric blender was used to grind the dried leaflets.

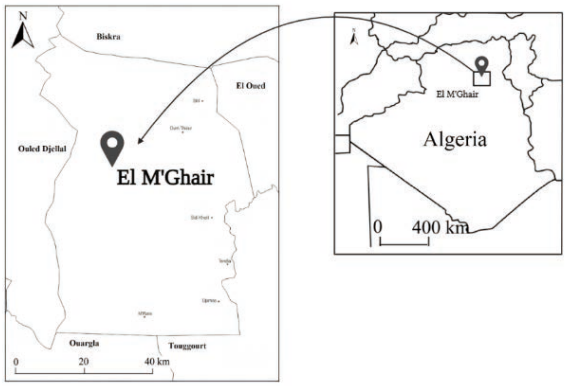

Figure 1: Sketch map of collection sites for study samples (El M'Ghair)

2.3 CLIMATIC DATA

To analyze the climatic conditions of El M'Ghair region over the period from 2008 to 2019, monthly averages of temperature and precipitation were computed. The x-axis of the graph corresponds to the months of the year, while the y-axis denotes the temperature (°C) on the left side and precipitation (mm) on the right side. The data for the climate map was sourced from both the World Weather and Local Weather Forecast (Tutiempo Network, 2023) for the specified time frame (2008-2019).

2.4 EXTRACTION AND ESTIMATION OF SUGARS

10 g of fresh leaflets (ground) were peeled in 50 ml of distilled water then complete the volume up to 100 ml, agitate for 15 min, left in contact for 24 hours, and agitate for 15 min. We obtain the extract. In each test tube, place 2 ml of the carbohydrate extract diluted 1/100, previously prepared. Then, 2 drops of phenol at (5 %) and 3 ml of concentrated sulfuric acid are added; then, the tubes are allowed to cool for 3 min in the dark. Place the tubes in a water bath at (30 °C) for 20 minutes (appearance of the yellow-red color), and stop the reaction with a stream of cold water. The absorbances were read at a wavelength of 490 nm by a UV-visible spectrophotometer Dubois et al. (1951). The concentration of total sugars was determined using a calibration curve constructed using glucose ($y = 0.0216x + 0.1471$, $R^2 = 0.9881$) and expressed in percentage (%).

2.5 EXTRACTION AND ESTIMATION OF PROTEIN

The proteins are extracted by primary hydrolysis (Snyder & Desborough, 1978). We took 0.15 g of leaflets into a beaker. Add 5 ml of (5 %) NaOH solution, stir the mixture with a vortex, then let stand for 30 hours. Then, the mixture was put into a cold centrifuge at a speed of 5000 rpm. Finally, the supernatant was obtained and stored at (-20 °C) until used for the assay.

To determine the total proteins, the method of Bradford (1976) was used. 100 µl of the supernatant was taken and 4 ml of BBC was added, which was prepared by dissolving 30 mg of BBC in 15 ml of ethanol (95 %), to which 30 ml of phosphoric acid (85 %) was added. Then complete the volume up to 300 ml with distilled water. The absorbance was read at a wavelength of 595 nm by a UV-vis spectrophotometer. Protein concentration was performed using a calibration curve generated with BSA ($y = 0.0096x + 0.0114$, $R^2 = 0.9967$) and expressed as percentage (%).

2.6 PREPARATION OF EXTRACTS

The ground leaflets (10 g) were soaked with 100 ml of methanol/water (80/20 %) and left to rest with stirring at a cold temperature for 48 hours. The mixture was filtered using a clean cloth and then filter paper to obtain the first filtrate, which was kept at (4 °C). Then the extraction was refined, and the two obtained filtrates were evaporated at (40 °C) using a rotating steam (Heidolphe) to remove most of the solvent. Then, the crude extract was hexane-extracted in a separating funnel to remove lipids and chlorophyll. The aqueous phase was evaporated, oven-dried at (40 °C) and scraped with a scalpel to obtain a powder. The powder was stored at (4 °C), and the following equation determined the extraction yield:

$$R\ (\%) = (M_2 - M_1) / M_0 \times 100$$

R (%): Yield expressed in (%).
M_0: Mass in grams of the sample.
M_1: Mass in grams of empty petrie dish.
M_2: Mass in grams of petrie dish full by the extract.

2.7 ESTIMATION OF TOTAL PHENOLIC CONTENT

The determination of total polyphenols by the Folin-Ciocalteu reagent was described by Singleton and Rossi (1965). The protocol consisted of incubating a mixture of 200 µl of sample and 1 ml of Folin-Ciocalteu reagent (10 %) for 4 min, then 800 µl of sodium carbonate (7.5 %) was added. After incubation in the dark and at room temperature, the absorbance was read at a wavelength of

765 nm. Gallic acid was used as a standard, and a calibration curve (y = 0.0049x + 0.1279, R^2 = 0.9762) was prepared under the same conditions. The phenolic content was expressed in µg GAE. mg^{-1} of extract.

2.8 ESTIMATION OF FLAVONOID CONTENT

The aluminum trichloride method (Bahorun et al., 1996) is used to quantify flavonoids in various extracts. 1 ml of each sample or standard (Quercetin), diluted in methanol (1 mg of extract in 1 ml of methanol), was added to 1 ml of an $AlCl_3$ solution (2 % in methanol). After 10 minutes of incubation, a UV-vis spectrophotometer read the absorbance at a wavelength of 430 nm.

The flavonoid concentrations of the different extracts are derived from a calibration range (y = 0.0216x + 0.1471, R^2 = 0.9881), which was established with quercetin, and the flavonoid content is expressed as: (µg QE. mg^{-1} of extract).

2.9 STATISTICAL ANALYSIS

All estimates were performed in triplicate, and the results were expressed as the mean ± standard deviation. One-way ANOVA (Analysis of Variance) and Duncan's test in SPSS 15.0 were used to determine the significance of the results, and the correlation matrix was also determined.

3 RESULTS AND DISCUSSION

3.1 CLIMATIC DATA OF EL M'GHAIR

The ombrothermic diagram for the El M'Ghair region from 2008 to 2019 (Figure 2) shows the monthly precipitation (P in mm) and temperature (T in °C) values. It reveals the variations in weather conditions throughout the year, with different levels of precipitation and temperature for each month. In the El M'Ghair region from 2008 to 2019, the hottest months were June and July, with temperatures reaching 31.58 °C and 34.94 °C, respectively. January is the coldest month, with a temperature of 11.46 °C. The wettest month is April, with a precipitation value of 7.31 mm, while June is the driest month, with no recorded precipitation.

Based on the information provided in the ombrothermic diagram for the El M'Ghair region, it is reasonable to classify the area's climate as either desert or arid. The hot temperatures during June and July, coupled with the dry conditions in June (no recorded precipitation), suggest characteristics commonly associated with desert climates. Additionally, the low precipitation levels throughout the year, with the wettest month being April (7.31 mm), further indicate arid conditions.

3.2 BIOCHEMICAL CONTENT

Figures 3, 4, 5, and 7 show the results of the chemical content analysis of the leaflets of three male palm cultivars (Deglet Nour, Ghars, and Degla Bieda) cultivated in El M'Ghair region, Algeria. The analysis focused on determining the total protein content and total sugars from the dry biomass, as well as total phenols and flavonoids from the water-methanolic extract. Data are presented in units of µg. mg^{-1} or percentages (%). The results of analyzing the chemical content of the leaflets of three male palm cultivars, namely Deglet Nour, Ghars, and Degla Beida, showed significant differences in the levels of the different compounds.

Figure 3 shows the protein content in palm leaflets of the three cultivars, Deglet Nour, Deglet Beida, and Ghars, and the statistical study ($p < 0.05$) shows dis-

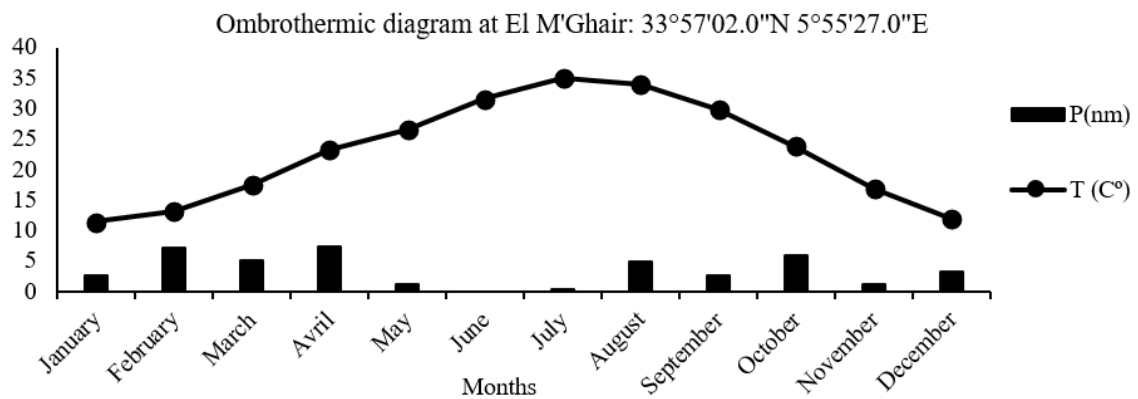

Figure 2: Ombrothermic diagram at El M'Ghair region from 2008 to 2019

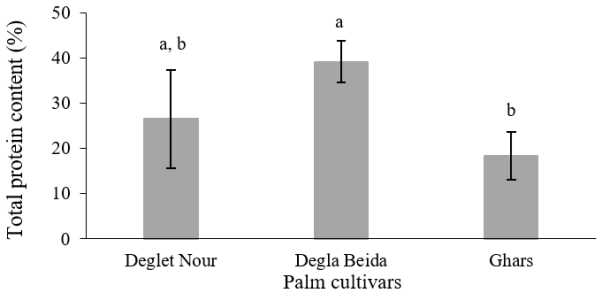

Figure 3: Protein content of date palm leaflets extracts (*Phoenix dactylifera* L.) for three Algerian cultivars (Deglet Nour, Degla Beida and Ghars). Different letters indicate significant differences between cultivars ($p < 0.05$)

tinct differences between them. Among the three cultivars, Degla Beida showed the highest protein content (39.09 ± 4.58%). It was followed by Deglet Nour (26.53 ± 10.84%). On the other hand, Ghars had the lowest protein content among the three cultivars, reaching 18.33 ± 5.35%.

Compared to the study of Harrak and Hamouda (2005), the protein content in the studied cultivars is high, as the percentage of protein in Moroccan dates ranged between 1.99 to 4.22 % (Langyan et al., 2021).

According to the results obtained from determining the content of total sugars in palm leaflets (Figure 4), it is clear that there are differences between the three cultivars, namely Deglet Nour, Degla Beida, and Ghars. The highest sugar content was observed in Ghars leaflets with a value of 74.54 ± 6.92 %. Deglet Nour leaflets showed the highest total sugar content with a value of 61.41 ± 10.68 %. On the other hand, Degla Beida leaflets showed the lowest total sugar content among the three cultivars, with a value of 74.54 ± 6.92 %.

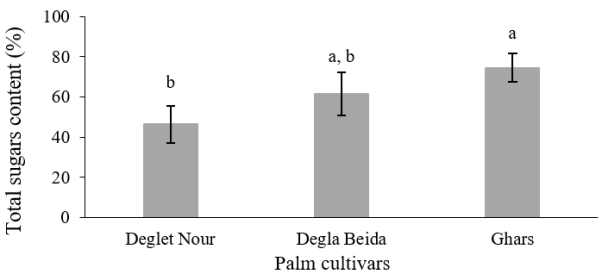

Figure 4: Total sugars content of date palm leaflets extracts (*Phoenix dactylifera* L.) for three Algerian cultivars (Deglet Nour, Degla Beida and Ghars). Values in the same column followed by different letters indicate significant differences ($p < 0.05$)

The results of this study are nearly identical to those found by Taouda et al. (2014), who reported that the content of five Moroccan date cultivars (Medjool, Khalas, Sukkari, Barhi, and Zaghloul) in total sugars ranged from 58 ± 2.76 % to 83 ± 0.26 %. It is higher than that Mehaoua (2006) obtained from date palm leaflets infected with *Parlatoria blanchardi* (Targioni Tozzetti, 1892) in the Biskra region, with content ranging between 0.02 %-0.04 %. This is because diseases affecting palm trees affect the levels of primary metabolites (Zaynab et al., 2019).

Naser et al. (2016) also reasoned that the high rate of highly efficient photosynthesis in the vast photosynthetic zone and the high amount of photosynthetic pigments may be directly responsible for the high content of total sugars and some bioactive components. Elevated glucose and sucrose levels available under stress are physiological characteristics associated with stress tolerance. Furthermore, accumulation of carbohydrates and amino acids is required to control osmotic activities and protect the cellular structure from stresses by maintaining membrane stability and water balance in the cell.

3.3 CONTENT OF PHYTOCHEMICALS

Figure 5 shows the yield percentages of the crude hydro-methanolic extracts obtained from leaflets of male date palms cultivars (Deglet Nour 14.67 %, Degla Beida 16 %, and Ghars 14.33 %). The results indicate no significant differences among the cultivars ($p > 0.05$), suggesting similar extraction efficiency for all three cultivars. Further exploration of the extracted compounds' chemical composition and potential applications may be warranted.

The statistical study results, expressed with a $p > 0.05$, indicate no statistically significant differences in yield percentage between the three date palm cultivars. Similar results were reported by Benouamane et al. (2022) in their study on five cultivars of date palms in Biskra, where no significant difference was observed in the yield of extraction with different solvents.

According to the results of the quantitative assessment of total phenols (Figure 6), it is clear that the three water-methanolic extracts had an abundant content of phenolic compounds, whereas the leaflets of 'Degla Beida' had a higher content (206.03 ± 30.55 µg GAE. mg^{-1} D), followed by the leaflets of 'Deglet Nour' (179.42 ± 17.27 µg GAE. mg^{-1} D), then the leaflets of 'Ghars', which had the lowest content among the samples (135.81 ± 11.38 µg GAE. mg^{-1} D).

Laouini et al. (2014) found that the hydroethano-

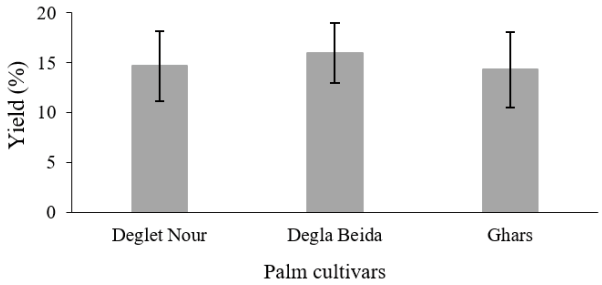

Figure 5: Yield (%) of crude hydro-methanolic extracts of leaflets of male date palms. (n = 3, One-factor ANOVA test, p > 0.05)

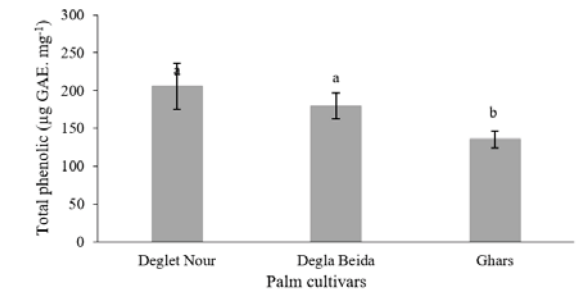

Figure 6: Total phenolic content of date palm leaflets extract (*Phoenix dactylifera* L.) for three Algerian cultivars (Deglet Nour, Degla Beida and Ghars). Different letters indicate significant differences between cultivars (p < 0.05)

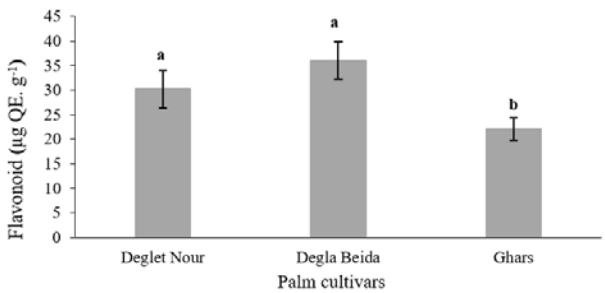

Figure 7: Flavonoid content of date palm leaflets extract (*Phoenix dactylifera* L.) for three Algerian cultivars (Deglet Nour, Deglet Beida and Ghars). Different letters indicate significant differences between cultivars (p < 0.05)

lic extract of 'Ghars' leaflets contained 342.45 ± 12.5 mg GAE. g^{-1} D of phenolic content. 'Deglet Nour' and 'Hemraya' leaflets contained phenolic compounds with a concentration of 221.75 ± 9.59 and 190.27 ± 6.55 mg GAE. g^{1}.

These agricultural varieties are all feminine and growing in the valley region. In a study by Benouamane et al. (2022), the phenolic content did not exceed the value of 180 mg GAE. g^{-1} in the methanolic, ethanolic, and acetone extracts of the female palm leaflets of five cultivars: Hamray, Safray, Ghars, Horra, and Deglet Nour, grown in the Biskra region.

While the results of this study are higher than those obtained by Kriaa et al. (2012), as the content ranged from 69.06 ± 0.41 to 146.46 ± 2.61 mg GAE. g^{-1} of the methanolic extract of three Tunisian cultivars (Deglet Nour, Medjool, and Barhi).

The difference in total' phenolic content among the *Phoenix dactylifera* L. cultivars studied in the four studies can be attributed to the different geographical locations in which they were grown, which resulted in a difference in climate and soil conditions. Geographical location plays an important role in shaping the environment in which palm trees thrive. Factors such as temperature, humidity, sun exposure, precipitation patterns, and soil composition can significantly influence plants' metabolic processes, including synthesizing phenolic compounds (Zakraoui et al., 2023).

According to the results shown in Figure 7 and the statistical study, it is clear that the three methanolic extracts of the leaflets of cultivars Deglet Nour, Deglet Beida and Ghars have a highly significant difference (p < 0.01) at the level of flavonoid content. 'Deglet Beida' and 'Deglet Nour' leaflets had the highest content of 36.02 ± 3.79 and 30.18 ± 3.88 µg QE. mg^{-1}. 'Ghars' leaflets had the lowest content of 22.02 ± 2.31 µg QE. mg^{-1}.

According to the results, it is concluded that there is a difference in the content of flavonoids according to the different cultivars of palm trees, as shown in the results of the estimation of total phenols (Figure 6), and this is what was obtained in the previous studies mentioned above (Benouamane et al., 2022; Kriaa et al., 2012).

Table 1: Correlation matrix between the biochemical parameters studied

Variables	Total phenolic	Flavonoid	Protein	Total sugars
Total phenolic	1	0.829	0.781	-0.768
Flavonoid	0.829	1	0.530	-0.965
Protein	0.781	0.530	1	-0.437
Total sugars	-0.768	-0.965	-0.437	1
*Correlation is significant at the 0.05 level (2-tailed)				

3.4 CORRELATION RELATIONSHIP AMONGELEMENTS CONTENT

Table 1 presents the correlation matrix of the studied biochemical contents (total phenolic content, flavonoids, protein, total sugars) of palm leaflets in the three cultivars: Deglet Nour, Degla Beida, and Ghars. The values in the table represent correlation coefficients, which indicate the strength and direction of relationships between variables. Where it is clear that among the three variables; proteins, total phenols, and flavonoids had a positive correlation among them, with values ranging from 0.53 to 0.829. Specifically, there is a weak positive relationship between proteins and flavonoids and between them and total phenols, while total phenols and flavonoids have a strong positive relationship. This indicates that when one of these biochemical contents increases, the others also tend to increase. This positive correlation indicates these compounds' possible co-occurrence or association within the studied samples. On the other hand, there is a strong negative correlation between these three biochemical contents (proteins, total phenols, flavonoids) and total sugar content, with correlation coefficient values ranging from -0.965 to -0.437. This indicates that as the levels of proteins, total phenols, and flavonoids increase in the sample, the total sugar content tends to decrease.

4 CONCLUSIONS

This analytical study of the leaflets of different cultivars of date palm shows significant differences in their chemical content, including proteins, total sugars, total phenols, and flavonoids. Among the cultivars, Degla Beida showed the highest protein, total phenolic and flavonoids content, making it the class with the highest levels of these compounds.

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