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Qualitative and Quantitative Analysis of *Phoenix dactilifera* L. Seeds in Morocco with Antioxidant Activities Using Chemometrics

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ABSTRACT

The phytochemical compounds and the antioxidant activity of ten varieties of *Phoenix dactilifera L.* seeds in Morocco were examined. The phytochemical screening was used to reveal the presence of some important compounds, such as polyphenols, flavonoids, catechic tannins, terpenoids, leucoanthocyanins, carotenoids, alkaloids, saponins as well as the absence of Gallic tanins and cardiac glycosids. The total phenolic content was ranged between 381.29 ± 2.31 and 138.92 ± 1.75 mg Gallic acid equivalent/g of extract, the total flavonoid content was between 163.53 ± 1.82 and 46.74 ± 1.26 mg quercetin equivalent/g extract, and tannins were found from 95.87 ± 2.08 to 24.20 ± 1.45 mg catechin equivalent/g extract. Indeed, the scavenging ability with DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assays recorded a low inhibitory concentration (IC_{50}) ranged from 1.52 to 4.78 µg/mL and from 18.90 to 46.62 µg/mL respectively. Likewise, the Ferric Reducing Antioxidant Potential (FRAP) assay showed a good reduction capacity of ferric ions ranged from 69.25 to 161.26 µg/mL.

Keywords: date seed, phytochemical screening, antioxidant activity, bioactive compounds, chemometric.

INTRODUCTION

The date palmetto *(Phoenix dactylifera L.)* is very popular in Morocco, it is considered the staple food for its high energy content, dietary value, sugar content but also for its many therapeutic and medicinal properties. The fruit contains a fleshy pericarp and endocarp enfolds seed. The date seed constitutes between 5.6% and 14.2% of date fruit weight (Al-shahib, Marshall, 2003) pertaining to the variety and maturity. According to the Ministry of Agriculture, Morocco maintains its 12th place in the world ranking of date producers, and reinforces its position with a record projected production of 143,000 tons for the 2019-2020 campaign. Date seeds contain a high amount of dietary fiber, carbohydrates, fat, low protein (Adeosun et al., 2016a), presence of vital mineral ions Na^+, Mg^{2+}, K^+ and Ca^{2+} with high concentration of potassium. Otherwise, the microelements detected were Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, with Fe^{2+} was the predominant (Ali-Mohamed, Khamis, 2004). In south Moroccan folklore, date seeds are frequently used to make non-caffeinated coffee or to make an eyeliner kohl and hair coloring by women. They can also be used as an alternative

feed ingredient for cattle to increase their weight (Elgasim et al., 1995). A study of Al-Suwaiegh (2016) suggested that adding date seed up to 20% of concentrated feed for lactating goats does not negatively affect their dairy production yield, but according to Al-Dobaib et al. (2009), their milk contained higher casein nitrogen and non-casein nitrogen. Many studies reported several health benefits of date seed extract (DSE). According to a recent studies, DSE has shown a protective effect against early diabetic (Abdelaziz et al., 2015) and chemical induced toxicity (Ahmed et al., 2015) of both liver and kidney. DSE has antiviral action against various human pathogenic viruses, such as inhibition of phage infectiveness of pseudomonas ATCC 14209-B1 and prevention of bacterial lysis (Jassim and Naji, 2010). The purpose of this research study was to determinate the chemical constitution of ten seeds of most popular Moroccan date from their typical regions. The antioxidant activity was also evaluated using a radical scavenging as DPPH and ABTS and testing capacity reduction test with the ferric reducing antioxidant potency test. Then, chemometrics tools were used to underlie the relationships and structure of the studied system.

EXPERIMENTAL PROCEDURE

Instruments and chemicals

For analytical grade, all organic solvents were acquired from Sigma-Aldrich. Gallic acid (99%), quercetin (95%), catechin (98%), ascorbic acid (99%), Trolox (98%), DPPH (90%), ferric chloride (97%), and potassium ferricyanide (99%), were the chemical reagents used in this study, and they were acquired from Sigma-Aldrich. Thermo Fisher Scientific supplied the ABTS (98%) and potassium persulfate (99%). The colorimetric examination was carried out using the LLG-uniSPEC UV/VIS spectrophotometer.

Preparation of plant extracts

The date fruit variants were all collected during the Tamr stage in diverse and unique regions of Morocco: Sous-Massa, Daraa Tafilalet, and Oriental (Table 1). The seeds were separated from the fruits, steeped in distilled water, then thoroughly rinsed to remove any remaining date fruit. Then, they were dried overnight at 50°C, crushed in a swing-type electric grains miller, and stored in a freezer (-16°C) until extractions or analyses. The extraction was performed using the soxhlet technique, first with an apolar solvent to remove the fat from the sample, then with methanol as a polar solvent to obtain a crude form of methanol extract.

Qualitative analysis of phytochemical compounds – the phytochemical screenings

The date seed powder and extract were analyzed to detect the presence of polyphenols, flavonoids, tannins, terpenoids, leucoanthocyanins, carotenoids, alkaloids, saponins and cardiac glycosides in accordance with standard methods (Surendra et al., 2016; Tepal, 2016; Busa, Getalado, 2019; Kerrouri et al., 2016; Bouyahya et al., 2017).

Table 1. Geographic information on fruit harvesting sites date

Variety	Locality	High ground (m)	Köppen-Geiger classification	Average temperature (°C)	Rainfall (mm)
Mejhoul (DN1)	Chtouka Ait Baha	559	BSh	18.1	229
Khalt (DN2)	Errachidia	1033	BWh	19.2	127
Boufkouss (DN3)	Errachidia	1033	BWh	19.2	127
Iklan (DN4)	Zagora	735	BWh	22.9	61
Bouslikhen (DN5)	Arfoud	803	BWh	20.8	80
Bousthami (DN6)	Errachidia	1033	BWh	19.2	127
Ablouh (DN7)	Aoufouss	904	BWh	20.1	94
Jihel (DN8)	Zagora	735	BWh	22.9	61
Aziza (DN9)	Figuig	899	BWh	19.3	137
Lkenz (DN10)	Errachidia	1033	BWh	19.2	127

Note: BSh – hot semi-arid climate; BWh – hot desert climate.

Test for phenols [ferric chloride test]

First, 1 mL of methanolic extract added 2 mL of distilled water, followed by several drops of aqueous ferric chloride solution at 10%. The blue or green formation was indicative of phenols.

Test for flavonoids

To 0.5 mL of methanolic extract from the samples, 5 to 10 drops of diluted HCl and a small quantity of Mg were added, and the solution was boiled for a several minutes. Flavonoids were indicated by a reddish rose or brown color.

Test for tannins

In this test, 0.5 g of extract has been boiled in 10 mL of water and then filtered. The addition of a few drops of 0.1% ferric chloride reveals a brown-green or dark blue coloration.

Test for terpenoids (Salkowski test)

In this test, 0.5 g of extract was added within 2 mL of chloroform. Then, 3 mL of H_2SO_4 concentrate was added with great care to form a layer. The reddish-brown appearance of the interface indicates that terpenoids are present.

Test for Leucoanthocyanins

To 2 ml of methanolic extract, 2 ml of concentrated HCl were added. The mixture was placed in a boiling water bath for about twenty minutes. The appearance of a red coloration demonstrates the presence of leucoanthocyanins.

Test for carotenoids

A mixture of 20 g of powder and 150 mL of distillate water was filtered. Then 3 mL of filtrate and 3 mL of HCl were added, followed by 3 mL of H_2SO_4. The aspect of a blue-green coloration reveals the presence of carotenoids.

Test for alkaloids – alkaloidal precipitants

Wagner reagent – some drops of Wagner reagent were added to 3 mL of sample. Brown precipitate appears to indicate the presence of alkaloids. Mayer reagent: Around 3 mL of an extract, some drops of Mayer reagent were added. When a precipitate is formed, alkaloids are present.

Test for saponins

In this test, 5 mL of boiled distilled water was added to 2.5 g of extract contained in a test tube. The solution was shaken in a vigorous manner. The formation of a stable foam that lasts for half an hour indicates the abundance of saponins.

Test for Cardiac glycosides [Keller killiani's test]:

About 0.1g of methanolic extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. Then, 1ml of concentrated H_2SO_4 was added. The presence of a deoxysugar characteristic of cardenolides was detected by a brown ring at the interface.

Quantitative analysis of phytochemical compounds

Total phenolic content

The total phenolic content of DSE was determined by Folin-Ciocalteu method, as described by Ozderin (2024) with acid gallic as a standard. Under alkaline conditions, the phenolic compounds reduce the folin-Ciocalteu reagent (phosphomolybdic and phosphotungstic acid) by generating a blue color from white to dark blue. The absorbance was read at 765 nm and the content were expressed as mg Gallic Acid Equivalent/g of sample.

Total flavonoid content

The total flavonoid content was diagnosed by Wambui et al. (2024). This method is based on the formation of a very stable complex, between the chloride and the oxygen atoms present on the carbons 4 and 5 of the flavonoids. The absorbance was taken at 510 nm. The total flavonoid content was converted as mg Quercetin Equivalents/g of sample.

Total condensed tannins

The total condensed tannins were assayed by Pascal et al. (2023). This method depends on the reaction of acidified vanillin with condensed tannins to form a red colored complex. The absorbance was measured at 500 nm and the total condensed tannins were conveyed as mg Catechin Equivalent/g of sample.

The antioxidant activity

DPPH assay

The DPPH radical-scavenging activity was assayed as stated by Oubihi et al. (2020). A DPPH

solution (0.070 mg mL^{-1}) was added to a sample solution at different concentrations between 0.2 and 15.0 µg mL^{-1} and kept for a half hour at room temperature. The Abs of negative control was measured by holding a methanol and DPPH solution under the same conditions. Absorbance was determined at 517 nm with the ascorbic acid (0.5–20.0 µg mL^{-1}) used as standards. The percentage of inhibition of samples was obtained from the measured absorbance and the equation:

$$\%\,\text{Inhibition}_{(\text{DPPH})}=\left(\frac{\text{Abs}_{\text{ngative control}}-\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{ngative control}}}\right)\times 100 \quad (1)$$

The IC_{50} is referring to the sample concentration that reduces DPPH radical at 50% of absorbance; it can be calculated from the equation below.

ABTS assay

The ABTS radical scavenging was determined by using the method of Elouafy et al. (2023). An aqueous solution of ABTS (7 mM) with an aqueous solution of potassium persulfate (2.45 mM) was reacted to produce the ABTS radical cations ($ABTS^{+}$). After stirring for 12–16 hours in a darkroom at ambient temperature, methanol was progressively added to achieve an absorbance value of 0.700 (±0.03) at a wavelength of 734 nm. Then, it was added to a sample solution at different concentrations between 2.5 and 80 µg mL^{-1} and stood for 30 min. The percentage inhibition of absorbance at 734 nm is calculated and plotted as a function of concentration of antioxidants and of Trolox for the standard reference data.

$$\%\,\text{Inhibition}_{(\text{ABTS})}=\left(\frac{\text{Abs}_{\text{ngative control}}-\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{ngative control}}}\right)\times 100 \quad (2)$$

Iron reducing power assay

The total inhibitor activity of date seeds extract was measured by the ferric inhibitor power (FRAP), it was assessed by using the strategy of Afrokh et al. (2023). The FRAP assay uses antioxidants as reductants in an exceedingly redox-linked colorimetric method. The reduction of potassium ferricyanide will drive the ferrous (Fe III to Fe II) ion formation associate an intense blue color, this transformation of coloration can be measured in absorption at 700 nm. The different concentrations of sample ranged between 50 and 500 µg mL^{-1}. The modification in absorbance is directly related to the combined or "total" reducing power of the antioxidants (electron donating) present in the reaction mixture.

Statistical analysis

Data analysis

The Pearson correlation, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were done by XLSTAT 2014 software (Zielinski et al., 2014). Analysis of variance was performed for the checking of the statistical significance by Tukey tests at a confidence level of 95.00%. The data were expressed as mean ± standard error and were performed using IBM SPSS Statistics 21 software.

Principal component analysis and hierarchical cluster analysis

The purpose of PCA in this study was to show that there is a link between phytochemical compounds (TPC, TFC, and TTC) and antioxidant activity (DPPH, ABTS, and FRAP). Furthermore, this approach allows investigating the underlying factors that contribute to the difference in phytochemical component quality based on ten date seed samples. HCA was used to investigate the interrelatedness of all samples using a cluster characteristic. The Ward cluster technique dendrogram and the squared Euclidean distance were also used to calculate a similarity coefficient.

Correlation matrix

PCA was carried out on a matrix that summarized the experimental result of antioxidant compounds and antioxidant properties. Ten date seed samples were used to represent each individual.

RESULTS AND DISCUSSION

Phytochemical screenings

In the present study, the preliminary phytochemical analysis indicated the presence of polyphenols, flavonoids, tannins, terpenoids, leucoanthocyanins, carotenoids, alkaloids, saponins and cardiac glycosides in *Phoenix dactilifera* L. seeds powder and extract (Table 2). The various phytochemical compounds detected are known to possess useful importance in medical science. For instance, in keeping with Khurana et al. (2013), the studied polyphenols defend the vascular system and performance as antioxidants. They prevent substances from being regenerated to harmful change of chemical states, including low-density

Table 2. Phytochemical screenings of *Phoenix dactilifera* L. seeds

Secondary metabolites	*Phoenix dactilifera* L. seeds
Polyphenols	+++
Flavonoids	+++
Tanins Catechic	++
Tanins Gallic	-
Terpenoids	+
Leucoanthocyanins	+++
Carotenoids	+
Alkaloids	+++
Saponins	++
Cardiac glycosids	-

Note: (-) – negative interaction; (+) – low interaction; (++) – moderate interaction; (+++) – strong interaction.

lipoprotein (LDL), additionally referred to as bad cholesterol. The tannins have high-temperature stability. They can decrease protein digestibility in man and animals, by inducing proteins to be partly unprocurable or inhibiting digestive enzymes and increasing fecal nitrogen (Bressani et al., 1982a). Saponins show numerous of useful effects in humans. In keeping with revealed studies (Bressani et al., 1982b; Li et al., 2017), flavonoids have inhibitor activity and therefore the ability to scavenge free radicals. They play a role within the hindrance of coronary cardiopathy and anti-cancer activity, even having the potential for human immunological disorder virus functions.

Quantitative analysis – total phenolic, flavonoid and tannin contents

Table 3 summarizes the results of the quantitative analysis of phytochemical compounds of DSE. The total phenolic content (TPC) examined in different varieties of DSE is presented in Table 3. These results can be divided into three groups depending on the quantity of DSE in phenolic compounds. The first group contains the Jihel and Aziza varieties with more than 300 mg GAE/g E (milligrams of gallic acid equivalents per gram of extract). Next comes the second group, ranging from 200 to 300 mg GAE/g E including Boustthammi, Iklan, Ablouh, and Boufkouss. The third group is characterized by a content lower than 200 mg GAE/g E and comprises Lkhalt, Bouslikhen, and Mejhoul. According to the findings, the difference observed in TPC depends on the seed variety and crop area (Li et al., 2017).

The results were reported as the average of three individual repetitions (n = 3e ± SEM), with comparable letters appearing in the same column showing no significant difference ($P < 0.05$). According to the obtained results and findings from other studies, the TPC in kernel fruit or drupes such as apricot pit, cherry pit, mango pit, olive pit and date pit, showed an important value, but date seed exhibited a higher value for the same measuring method and experimental conditions (Table 4).

Flavonoids are the widest class of polyphenols; they share the structure of diphenylpropanes, which contain two aromatic rings joined by three carbons. Flavonoids protect against numerous diseases, particularly cardiovascular disease, because of their potential to scavenge free radicals, chelate metal catalysts, activate antioxidant enzymes, decrease alpha-tocopherol radicals, and inhibit oxidases (Grotewold, 2006). The total flavonoid content (TFC) in the ten studied varieties of DSE is shown in Table 3. The predominant variety was Jihel (163.53 mg QE/g E), followed by Aziza (116.52 mg QE/g E),

Table 3. Total phenolic, flavonoid and tannin contents

Varieties	Total phenolic content mg GAE/g E	Total flavonoid content mg eq QE/g E	Total condensed tannins mg eq CE/g E
Mejhoul (DN1)	138.92±1.75^{a}	46.74±1.26^{a}	24.20±1.45^{a}
Khalt (DN2)	184.93±1.89^{b}	57.69±1.23^{b}	40.31±1.64^{b}
Boufkouss (DN3)	237.82±1.95^{c}	94.18±1.35^{c}	76.42±1.90^{c}
Iklan (DN4)	252.69±1.96^{d}	98.38±1.68^{cd}	90.75±2.04^{d}
Bouslikhen (DN5)	166.29±1.89^{e}	51.28±1.27^{ab}	29.64±1.49^{a}
Bousthami (DN6)	269.84±2.20^{f}	105.61±1.54^{d}	91.87±2.03^{d}
Ablouh (DN7)	248.48±1.97^{d}	97.91±1.55^{c}	87.64±1.94^{d}
Jihel (DN8)	381.29±2.31^{g}	163.53±1.82^{e}	95.87±2.08^{d}
Aziza (DN9)	305.46±2.28^{h}	116.52±1.68^{f}	94.87±2.07^{d}
Lkenz (DN10)	207.87±1.91^{i}	82.15±1.41^{g}	67.2±1.83^{c}

Table 4. Total phenolic content of five stone fruit

Stone fruit Name	Total phenol content
Apricot pit (Korekar et al., 2011)	128.5 mg GAE/100g E
Cherry pit (Oliveira et al., 2014)	1.68 mg GAE/100g E
Olive pit (Alu'datt et al., 2011)	2.2 mg GAE/g E
Mango pit (Soong, Barlow, 2004)	117 mg GAE/g E
Date pit (Djaoudene et al., 2019a)	476 mg GAE/g E

Bousthammi (105.61 mg QE/g E), while the lowest amount was Khalt (57.69 mg QE/g E), Bouslikhen (51.28 mg QE/g E) and Mejhoul variety (46.74 mg QE/g E). Djaoudene et al. (Djaoudene et al., 2019b) and Adeosun et al. (Adeosun et al., 2016b) found that the total quantity of flavonoid in DSE was lower than the obtained results, with 6.52 mg QE/g E and 24.71 mg QE/g E, respectively. Tannins are polyphenols the molecular weight of which ranges from 500 to 3000 Da. They can occur in complexes with proteins, alkaloids, and polysaccharides with a molecular weight of up to 20.000 Da. Tannins are commonly classified as hydrolyzable and nonhydrolyzable tannins (condensed tannins). Condensed tannins are the most abundant in nature, while hydrolyzable ones can be found in a few dicotyledon species or in combination with condensed tannins in different proportions (Fraga-Corral et al., 2020).

In the presented study, the determination of total condensed tannin content (TTC) in DSE showed a significant value (Table 3). Indeed, there is a widely similar value between varieties, such as DN6, DN9 and DN4 whose values ranged between 95.87 and 87.64 mg CE/g E, followed by DN3 and DN10 with 76.42 and 67.2 mg CE/g E, while the lowest one was DN1 with 24.20 mg CE/g E. According to the results of Adeosun et al. (2016c), DSE contains a very low amount of tannins with 133.2 mg CE/g E. Likewise, Thouri et al. (2017a) found that the quantity of tannin in DSE was greater than that in Mejhoul, Bouslikhen with more than 30 mg CE/g E.

Antioxidant activities

The in vitro assay for antioxidant activity was conducted using the FRAP, ABTS, and DPPH assays. The FRAP is based on reducing the ferrictripyridyl triazine complex to ferrous ions (Fe III to Fe II), with dark blue revealing the presence of antioxidants. The ABTS and DPPH assays are based on the scavenging ability of antioxidants using 2,20-azino-bis (ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+}$) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) as radicals. The results was reported as the average of three individual repetitions (n = 3e ± SEM), with comparable letters appearing in the same column showing no significant difference ($P < 0.05$). The antioxidant capacity of DSE was assessed by donating a hydrogen atom or an electron to free radicals through the formation of a stable intermediate. The antioxidant activities of various DSE are given in Table 5. All varieties showed good scavenging activity via the DPPH and ABTS of both radicals.

Therefore, there is a significant difference in result from one DSE to another. Likewise, Jihel and Aziza seeds registered a better neutralization of the DPPH radical with IC_{50} = 1.13 µg/

Table 5. Antioxidant activity of date seeds varieties

Varieties	DPPH IC_{50} µg/mL	ABTS IC_{50} µg/mL	FRAP EC_{50} µg/mL
Mejhoul (DN1)	4.78±0.17^{a}	46.62±1.56^{a}	161.26±1.66^{a}
Khalt (DN2)	4.5±0.13^{a}	34.92±1.38^{be}	131.82±1.38^{b}
Boufkouss (DN3)	3.8±0.1^{c}	27.38±1.17^{cf}	116.95±1.02^{c}
Iklan (DN4)	2.44±0.49^{d}	26.62±0.80^{cf}	104.29±0.79^{d}
Bouslikhen (DN5)	4.72±1.62^{a}	39.51±1.46^{b}	148.26±1.56^{e}
Bousthami (DN6)	1.9±0.04^{e}	24.25±0.75^{cd}	97.51±0.69^{f}
Ablouh (DN7)	3.01±0.07^{f}	27.31±0.91^{cf}	107.45±0.82^{d}
Jihel (DN8)	1.13±0.02^{g}	18.90±0.63^{d}	69.25±0.5^{g}
Aziza (DN9)	1.52±0.03^{eg}	22.13±0.74^{cd}	88.98±0.62^{h}
Lkenz (DN10)	4.29±0.11^{ac}	31.44±1.26^{ef}	127.30±1.27^{b}
Ascorbic acid	1.91	-	45.8
Trolox	-	29.01	-

mL and IC_{50} = 1.52 µg/mL while the lowest was shown in Bouslikhen and Mejhoul seeds with IC_{50} = 4.72 µg/mL and IC_{50} = 4.78 µg/mL. ABTS assay results showed also a strong inhibition for DSE of Jihel with IC_{50}=18.9 µg/mL, and a similar inhibition for DSE of Aziza, Bousthami, Iklan, Ablouh, and Boufkouss ranged from 22.13 µg/mL to 27.38 µg/mL. Although Khalt, Bouslikhen, and Mejhoul had the lowest activity, it varied from 34.92 to 46.62 (µg/mL). The antioxidant activity of DSE had the same range as reported by Djaoudene et al. (2019) from eight different date seed varieties, the IC_{50} varied from 13.89 to 32.31 µg/mL.

The results of FRAP assay showed a good reducing power of ferric ions with EC_{50} = 69.25µg/mL for Jihel Followed by Aziza with EC_{50} = 88.98 µg/mL, then Bousthami, Iklan, Ablouh, Boufkouss, Lkenz and lkhalt went from EC_{50} = 97.51 µg/mL to EC_{50} = 131.82 µg/mL while the lowest reducing power was for Mejhoul with EC_{50} = 161.26 µg/mL. On the basis of the results of the reduction power of Thouri et al. (2017b), the aqueous extracts reduction capacity of korkobbi and arechti date seeds was close to Boufkouss and Mejhoul, respectively with 120 µg/mL and 190 µg/mL, whereas their methanolic extract showed a low reduction ranging from 270 µg/mL to 260 µg/mL.

Statistical analysis

Correlation matrix

The correlation coefficients are shown in Table 6, and their p-values were presented in Table 7. The antioxidant activities of DPPH, ABTS, and FRAP were represented by the inverse of IC_{50} and EC_{50}. The values in bold are different from 0 at a significance level alpha = 0.05. The values in bold are different from 0 at a significance level alpha = 0.05.

According to Tables 6 and 7, the Pearson correlations between bioactive compounds and their antioxidant activities showed that the TPC, TFC and TTC had strong positive correlation significant (p-value < 0.0001) with the antioxidant capacities by DPPH, ABTS, and FRAP assay. The correlation results of total polyphenol content (TPC) were 0.941, 0.995, and 0.991 with DPPH, ABTS and FRAP assays respectively. These results indicate that the antioxidant activity of the samples examined can be attributed to the presence of phenolic compounds. Several studies reported that there is a correlation between TPC and antioxidant activity (Amri et al., 2015; Guettaf et al., 2016). Likewise, the TFC had also a strong positive correlation between DPPH with r^2= 0.919, ABTS with r^2= 0.983, and FRAP with r^2= 0.980. Furthermore, TTC had a positive correlation

Table 6. Pearson's correlation matrix coefficient for Physico-chemical characteristics and antioxidants substances

Variables	TPC	TFC	TTC	DPPH(1/ IC_{50})	ABTS(1/ IC_{50})	FRAP(1/EC_{50})
TPC	1					
TFC	0.991	1				
TTC	0.871	0.877	1			
DPPH(1/IC_{50})	0.941	0.919	0.721	1		
ABTS(1/IC_{50})	0.995	0.983	0.901	0.923	1	
FRAP(1/EC_{50})	0.991	0.980	0.819	0.968	0.978	1

Note: the values in bold are different from 0 at a significance level alpha = 0.05

Table 7. p-values of the correlation matrix coefficient

Variables	TPC	TFC	TTC	DPPH(1/IC_{50})	ABTS(1/ IC_{50})	FRAP(1/ EC_{50})
TPC	0					
TFC	< 0.0001	0				
TTC	0.001	0.001	0			
DPPH(1/IC_{50})	< 0.0001	0.000	0.019	0		
ABTS(1/IC_{50})	< 0.0001	< 0.0001	0.000	0.000	0	
FRAP(1/EC_{50})	< 0.0001	< 0.0001	0.004	< 0.0001	< 0.0001	0

Note: the values in bold are different from 0 at a significance level alpha = 0.05.

significant (p-value < 0.05) between the antioxidant activity, but they are low compared to those of TPC and TFC. These results showed that the flavonoids and phenolic compounds contribute strongly in this antioxidant activity, more than tannins. In addition to that, all three assays of antioxidant activities were correlated (p-value < 0.05) with each other. Therefore, these high correlations indicate that the bioactive compounds, which provide free radical scavenging power by ABTS and DPPH assays, are themselves, who contribute to the reducing power of ferric ions by FRAP assay. Similarly, Thouri et al. (2017c) found a strong correlation (p < 0.01) between the bioactive compounds and the antioxidant activity by DPPH, ABTS and FRAP assay from korkobbi and arechti date seed using Pearson's correlation.

Principal component analysis

In this segment, the variables include the various phytochemical contents and antioxidant activity results of ten samples (Figure 1). PCA was used to design them based on the F1-F2 factorial plan. The first (F1) and second (F2) primary components account for 93.80% and 5.12% of the overall information. Furthermore, the cumulative proportion of these components is 98.93%, implying that the linear combination is representative of the variables due to its value up to 50%. Thusly, the first two axes are appropriate for representing the data as a whole. Figure 1 exhibits the plane generated by axes F1 and F2,

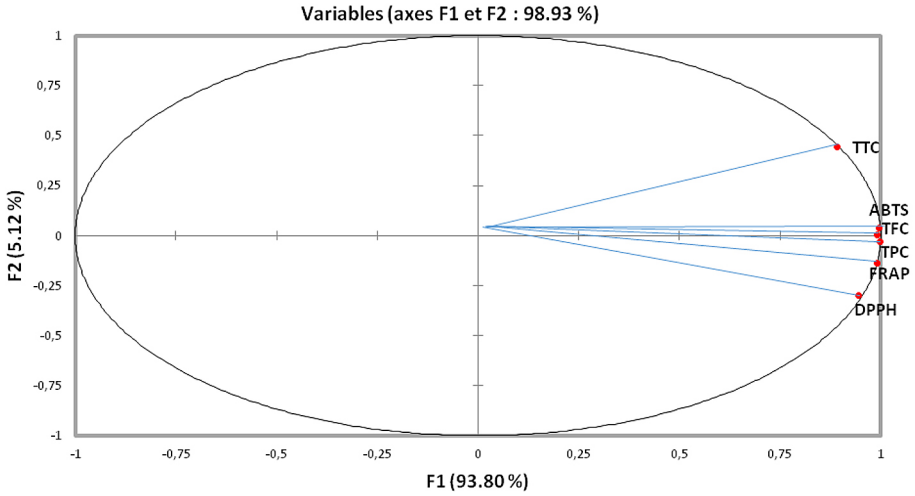

Figure 1. PCA factorial design for bioactive compounds and antioxidants activities of samples

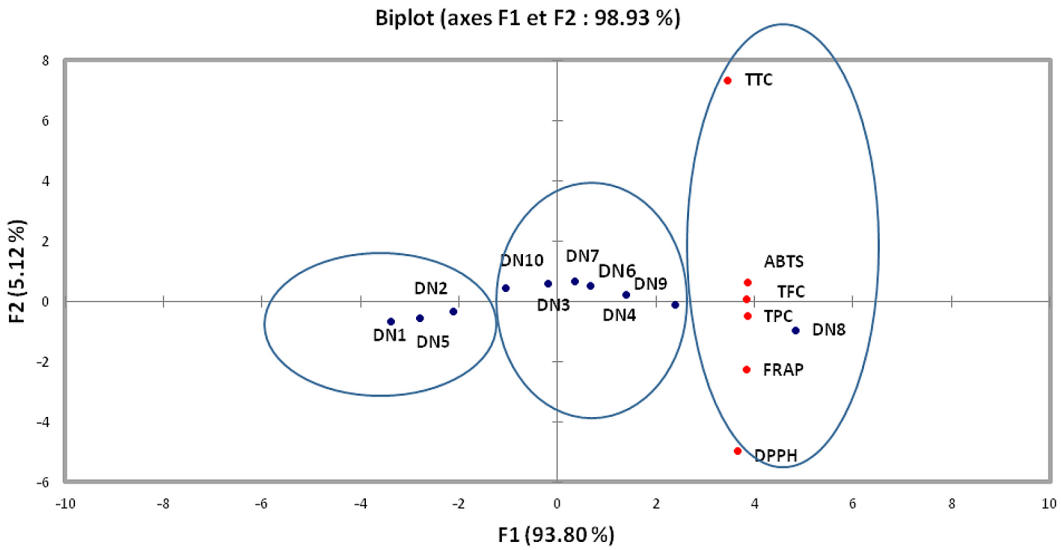

Figure 2. Projection of individuals on the factorial plan (F1×F2). GI: Group I; GII: Group II

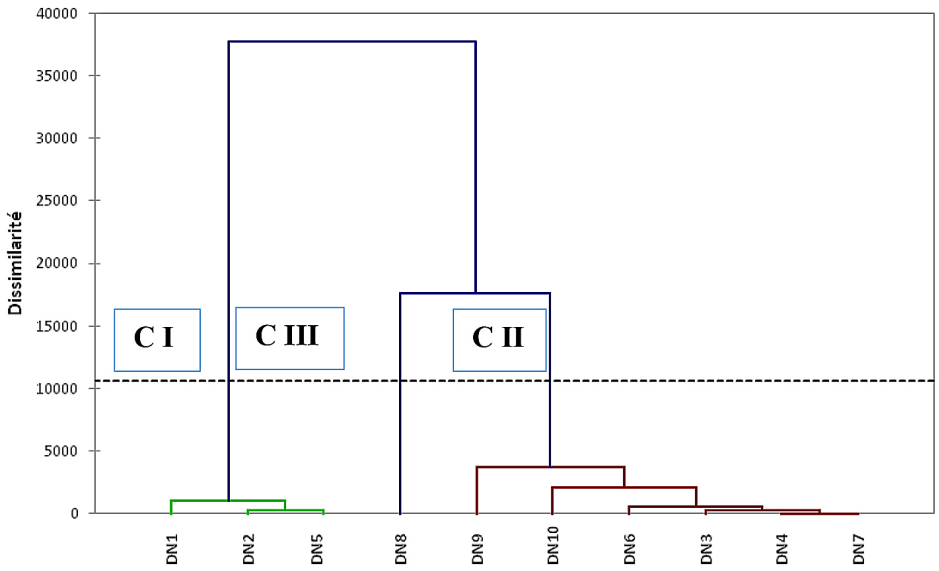

Figure 3. Dendrogram of the studied samples generated by cluster analysis (Ward and Euclidean distance)

which shows the correlation between the variables. The F1 axis is primarily defined by the positive connection between bioactive chemicals and their antioxidant properties.

As it is shown in Figure 2, the ten varieties of DSE are spread into 3 main groups (GI, GII and GIII). The GI group consists of 3 DSE (DN1, DN2, DN5) named Mejhoul, Khalt, and Bouslikhen respectively. These samples were characterized by a lower value of bioactive compounds (TPC, TFC, TTC), as well as, they had a lower antioxidant power compared to other groups. The GII group is made up of 6 samples (DN3, DN4, DN6, DN7, DN9, DN10) named Boufkouss, Iklan, Bousthami, Ablouh, Aziza, and Lkenz, respectively. They can be qualified as a medium-high value of antioxidant content, as well as, by a medium-high antioxidant activity over three assays DPPH, ABTS, and FRAP. The GIII group takes shape by one sample named Jihel (DN8). It is characterized by a high value of phytochemical contents. Likewise, it had the strongest antioxidant activity by the same assays.

Hierarchical clustering analysis (HCA)

The ten samples (varieties) of DSE were sorted out by the Ward's method and the squared Euclidean for evaluating the similarity measure. HCA was utilized to assess the connection among samples and to demonstrate their similarity on bioactive content and antioxidant activity using a dendrogram. According to the results of ten samples, they were classified into three clusters (Figure 3).

CI: Cluster I; CII: Cluster II; C III; Cluster III

The Cluster I held 3 samples of DSE (DN1, DN2, DN5) named Mejhoul, Khalt, and Bouslikhen respectively, representing 30% of the total samples. They are characterized by the lowest mean value of TPC 163.38 mg GAE/g, TFC 51.90 mg QE/g and TTC 31.38 mg CE/g, along with a lower antioxidant power compared to cluster II and III. The Cluster II was formed by 6 samples (DN3, DN4, DN6, DN7, DN9, DN10), and accounted 60% of total samples. These samples commonly held a medium mean value of TPC 253.69 mg GAE/g, TFC 99.12 mg QE/g and TTC 84.79 mg CE/g with a medium antioxidant activity. Cluster III contained one sample named Jihel (DN8), which represents approximately 10% of the total samples. This cluster is described by a higher value mean of TPC 381.29 mg GAE/g of, TFC 163.53 mg, TTC 95.87 mg CE/g. Likewise, this sample recorded the highest antioxidant power compared to cluster I and II. The overview of these results shows some affinity with those of the PCA, which the distribution of all samples on the score plot shows a similar trend. The analysis of the outcomes indicates that there is consistency between the data in the PCA score chart and the HCA scores.

CONCLUSIONS

The analysis of *Phoenix dactylifera* L. seeds showed an important value for total polyphenols, flavonoids, and tannins. It also showed a strong

inhibition of radicals (DPPH and ABTS) and a high reducing capacity of ferric ions (FRAP assay). There was also a significant difference in the phytochemical content of one DSE sample compared to another, depending on the variety of seed and the region of cultivation. The high amount of phenolic compounds in DSE, along with their antioxidant capacity, makes them a potential functional food in a daily diet. Also, as it was mentioned, they have several health benefits and are a good candidate for drug discovery, but it is necessary to conduct further studies to make correct and full use of their potential as effective natural remedies against several diseases.

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