



Male date palm flowers: Valuable nutritional food ingredients and alternative antioxidant source and antimicrobial agent

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ABSTRACT

The aim of this study was to assess the nutritional properties of male date palm flower (MDPF) via determining its mineral, amino acid and bioactive compounds profiles and to investigate its antioxidant and antibacterial activities in order to prove its possible use as a healthy and potential natural source in human dietary diets. Results showed that MDPF is an excellent source of minerals especially regarding its high potassium and phosphorus contents (2684.23 and 318.66 mg/100 g, respectively). Moreover, the amino acids profile of MDPF showed considerable amounts supplying the human requirements. The phytochemical compounds analysis showed high content of polyphenol and flavonoids (3829.22 mg EAG/100 g and 168.31 mg EQ/100 g, respectively) promoting high antioxidant activity. The quantitative and qualitative analysis (LC-ESI-MS) of phenolic acids and flavonoids led to the identification of 18 phenolic compounds that were dominated by quinic acid (84.52%). The MDPF extract showed a potential antioxidant activity determined by different tests, namely DPPH radicals scavenging activity, ferric reducing power assay, ferrous ion chelating capacity and β -carotene bleaching assay. The MDPF also exhibited significant antibacterial activity against some tested bacteria. In conclusion, owing to its nutritional profile and biological activities, MDPF could be considered as potential functional-ingredient for food applications.

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1. Introduction

To guarantee the nutritional values as well as the biological activities of foods, this latter should be analyzed in order to ensure consumer satisfaction and on the other hand the correct functions of the body. Among the essential elements required for the good performance of the human body, there are minerals and amino acids. Indeed, deficit intakes of essential elements caused deficiency syndromes whereas excessive intake could produce a negative impact on health (Goldhaber, 2003) which make the determination of essential elements profiles in food a primordial step to judge their quality. In addition, the presence of polyphenolic compounds in diets is with great interest as they were found to exhibit a wide range of biological activities, e.g.: Antioxidant, anti-inflammatory, anti-cancer and antimicrobial activity (Andriani et al., 2019) and to be an effective natural food preservatives, impeding microbial contamination and oxidative

deterioration (Oniszczyk et al., 2019). According to the literature, some bacterial strains have dangerous effects on the human body such as *Listeria monocytogenes* and *Staphylococcus aureus*. In fact, *L. monocytogenes* was known as a harmful bacterium for foetuses able to cause several infections by crossing the placenta and *S. aureus* was known for the production of several types of enterotoxins leading to gastroenteritis and for its resistance to a number of phytochemical compounds (Halpin-Dohnalek and Marth, 1989; Makhoulouf-Gafsi et al., 2018). Nevertheless, the plants are a source of many substances improving the functioning of the human organism and its well-being (Costa et al., 2013).

The date palm (*Phoenix dactylifera* L.) could be defined as a dioecious species with separate male and female trees from the Arecaceae family. It constitutes a principal source of food and a vital supply for people living in the arid and semiarid regions of the world. *Phoenix dactylifera* date palm was widely used in traditional medicine to treat different disorders which include inflammation, memory disturbances, loss of consciousness, paralysis and nerve disorders and was commonly utilized to increase fertility (Chandrasekaran and Bahkali, 2013; Hamed et al., 2017; Wan Ismail and Mohd Radzi, 2013). In fact, the date palm did not only afford dates, but also other derivatives

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that can be used for various traditional and industrial activities. Among these derivatives the male date palm flowers (MDPF) which are widely used in the Arabian region for production of a tonic drink with anti-diabetic and antioxidant effects as reported by Hamed et al. (2017).

In Tunisia, the male date palms are able to provide about 1575 tonnes/year of flowers (Karra et al., 2019) which constitutes an available agro-resources that need to be valorized. To our knowledge, no study has identified the nutritional elements in MDPF of *Phoenix dactylifera* L. making this research a true reflection of this by-product importance.

The current work aimed to provide a value-addition to male date palm by valorizing its pollen-free flowers since they could be a potential source of nutritional elements and therefore represents an alternative for the farmer to well benefit from the male date palm. This work is the first study to assess the mineral and amino acid profile of MDPF, to evaluate and identify its bioactive compounds and to deal with their antioxidant and antibacterial properties.

2. Material and methods

2.1. Raw materials

Male date palm flowers (MPDF) used in this work were handily collected in April 2015 from the region of Sfax, Tunisia. Fresh plants were shaken to eliminate pollen then the flowers were separated manually from spikelet. MDPF were then dried in a ventilate oven for 24 h at 40 °C to protect them from microbial alterations and milled using a mechanical miller (Retsch GM200, Germany) with a rate of 8000 rpm for 2 min. The obtained homogeneous powder was stored at 25 °C prior to chemical and physical determinations.

2.2. Chemical analysis

2.2.1. Mineral composition

To determine the mineral composition of MDPF, ashes were taken after incineration at 550 °C for 8 h then dissolved in HCl 12N. The mineral elements (K, Ca, Na, Mg, Pb, Fe, Zn and Cu) were analyzed separately using an atomic absorption spectrophotometer (ICE3000, Thermo scientific, USA).

2.2.2. Amino acid analysis

Amino acids profile was determined according to Bouaziz et al. (2008). Sample was hydrolyzed with 6N hydrochloric acid at 110 °C for 24 h then analyzed by an amino acid analyzer (BioChrom 20 Plus, Cambridge, UK). Amino acids results were expressed as g/100 g sample and g/100 g protein.

2.3. Phytochemical analyses

2.3.1. Preparation of phenolic extracts

1 g of MDPF powder was extracted twice with 20 mL acetone–water (1:1, v/v), at room temperature 25 °C for 2 h using an orbital shaker. The extract was then centrifuged at 3000 g, for 20 min and the volume of the supernatant was used as phenolic extract.

2.3.2. Total phenolic content

The total phenolic content was determined according to the adapted Folin–Ciocalteu method (Singleton Vernon et al., 1999). 500 µL of the extract was mixed with 2.5 mL of Folin–Ciocalteu reagent 1/10 in water and allowed to stand for 8 min. 2 mL of aqueous sodium bicarbonate (75 g/L) was then added, and the mixture was vortexed and allowed to stand in the dark at room temperature. After 15 min, the absorbance was measured at 765 nm using an UV spectrophotometer (Shimadzu, China). The total phenol concentration was expressed as mg of Gallic acid equivalent (mg GAE) per 100 g of dry weight of MDPF.

2.3.3. Total flavonoid content

Total flavonoid content of MDPF was determined according to (Zhishen et al., 1999). 250 µL of the extract was added to 1 mL of distilled water and 150 µL of sodium nitrite (150 g/L). The mixture was vortexed and allowed to stand for 6 min at room temperature. Then, 75 µL of aluminium chloride (100 g/L) was added. After 5 min, 1 mL of 1 M sodium hydroxide was added and the total was made up to 2.5 mL with distilled water. 15 min later, the solution absorbance was measured at 510 nm using a spectrophotometer (Shimadzu, Kyoto, Japan). The total flavonoid concentration was expressed as mg of quercetin equivalent (mg QE) per 100 g of dry weight of MDPF.

2.3.4. Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS) analysis

The MDPF extract was dissolved in methanol and the resulted solution was filtered through a 0.45 µm membrane filter before injection into the HPLC system. The method and the materials used for LC-ESI-MS analysis was performed identically as described by Jdir et al. (2017).

2.4. Antioxidant activity

2.4.1. Preparation of MDPF extract

For the antioxidant activity, 300 mg of MDPF powder were dissolved in 10 mL of distilled water using a vortex-mixer, the solution was then centrifuged for 20 min at 3000 g and filtered. Serial dilutions from the filtered supernatant of the aqueous extract were prepared to test the antioxidant activity at different concentrations.

2.4.2. DPPH radical-scavenging capacity

Free radical scavenging activity of MDPF was estimated using the DPPH (2,2-diphenyl-1-picrylhydrazyl free radical) assay according to the method of (Bersuder et al., 1998). For each concentration, a volume of 500 µL of the extract was added to 375 µL of 99% ethanol and 125 µL of DPPH solution (0.02% in ethanol) as free radical source. Absorbance at 517 nm was determined after 60 min at 25 °C in the dark. BHA was used as positive control. The radical scavenging activity was calculated as follows:

$$\% \text{ inhibition} = (A_{\text{control}} + A_{\text{blank}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

A_{control} : is the absorbance of the control reaction (containing all reagents except the sample);

A_{blank} : is the absorbance of blank containing the sample and ethanol;

A_{sample} : is the absorbance of sample (with the DPPH solution).

2.4.3. Ferric reducing power assay

The ferric reducing antioxidant power (FRAP) of MDPF extract was determined as described by (Yıldırım et al., 2001). For each concentration, an aliquot of 1 mL sample was mixed with 2.5 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C. After 30 min of incubation, 2.5 mL of 10% trichloro acetic acid (TCA) were added to the mixture. 2.5 mL of solution were mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride (FeCl_3), then left for 10 min. The absorbance was measured at 700 nm against a blank consisted of 1 mL distilled water instead of the sample. BHA was used as reference.

2.4.4. Ferrous ion chelating capacity

The ferrous ion chelating capacity of MDPF was measured as described by (Dinis et al., 1994). A volume of 500 µL of the extract at different concentrations was mixed with 50 µL of FeCl_2 (2 mM) and 1600 µL of distilled water. The mixture was left at room temperature for 15 min. Then 100 µL of ferrozine (5 mM) were added, mixed and left for 10 min. The absorbance was measured at 562 nm against a control containing distilled water instead of the sample. EDTA was

used as reference. The chelating antioxidant activity for Fe^{2+} was calculated as follows:

$$\text{Chelating effect (\%)} = ((A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}) * 100$$

2.4.5. β -carotene bleaching assay

The aptitude of MDPF extract to inhibit the bleaching of β -carotene was determined according to (Koleva et al., 2002). Briefly, 1 mg of β -carotene, 20 μL of linoleic acid and 200 μL of Tween 40 were dissolved in 1 mL chloroform. Then, the solvent was completely evaporated under vacuum at 40 °C. The resulting mixture was topped up to 50 mL with ultrapure water and mixed. The obtained β -carotene solution was freshly prepared before each experiment. For the bleaching assay, 0.5 mL of the extract at different concentration was added to 2.5 mL of the β -carotene–linoleic acid emulsion. The control tube consisted of 0.5 mL distilled water instead of sample. The tubes were placed in the dark in water bath and incubated at 50 °C for 2 h. The absorbance was carried out at 470 nm. Each sample was measured at zero time and continued until the disappearance of β -carotene color in the control (180 min). Distilled water served as blank and BHA was used as positive standard. The antioxidant activity (A) was expressed in terms of the bleaching of β -carotene as follow:

$$A = (1 - (A_{\text{samplet } 0 \text{ min}} - A_{\text{samplet } 180 \text{ min}}) / (A_{\text{controlt, } 0 \text{ min}} - A_{\text{controlt, } 180 \text{ min}})) * 100$$

$A_{\text{control, } 0 \text{ min}}$ and $A_{\text{control, } 180 \text{ min}}$: is the absorbance of the control reaction at 0 min and 180 min, respectively.

$A_{\text{sample, } 0 \text{ min}}$ and $A_{\text{sample, } 180 \text{ min}}$: is the absorbance of sample at 0 min and 180 min, respectively.

2.5. Antimicrobial activity

Antimicrobial activity was performed following the method of Vanden and Vlietinck (1991). The MDPF extract was tested against 8 strains of bacteria including 4 g-negative (*Pseudomonas savastoni*, *Escherichia coli* ATCC 10536, *Salmonella enterica* CIP 80.39, *Agrobacterium tumefaciens*) and 4 g-positive (*Bacillus subtilis* ATCC 6633, *S. aureus* ATCC 9144, *Micrococcus luteus*, *L. monocytogenes*). In fact, the MDPF powder was dissolved in 100% dimethylsulfoxide (DMSO) and the supernatant was sterilized by filtration through 0.22 μm membrane filter. The culture suspension (200 μL) of the tested bacterial cells (estimated by absorbance at 600 nm) were spread on Luria-Bertani (LB) Agar medium. Then, wells with 3 mm of depth and 4 mm of diameter were fulfilled with 50 μl of sample. The well fulfilled with only 50 μL of DMSO was used as a negative control. The Petri dishes were incubated firstly for 4 h at 4 °C then for 24 h at 30 °C. Antibacterial activity was assessed by measuring the diameter of the growth inhibition zones in millimeters (including well diameter of 4 mm). The antibacterial activities of MDPF extract for each strain were replicated three times and the results of diameter measurements of each assay were averaged.

Means and standard deviations for all experiments were obtained using Microsoft Excel 2016, but no comparisons between samples were done as only a single sample was used.

3. Results and discussion

3.1. Chemical analysis

3.1.1. Mineral composition

The mineral composition of MDPF was shown in Table 1. As could be seen the MDPF are an excellent source of minerals, especially of potassium and phosphorus and magnesium (2684.23, 318.66 and 140.72 mg/100 g, respectively). Regarding the macroelements content in MDPF, the obtained results were higher than that of saffron elucidated by Serrano-Díaz et al. (2013) except for Ca, whereas, the

Table 1
Mineral composition of male date palm flowers.

Minerals	mg/100 g dwb*
Macro-elements	
K	2684.23 ± 135.71
P	318.66 ± 0.01
Mg	140.72 ± 12.77
Ca	14.25 ± 0.93
Na	24.52 ± 0.95
Micro-elements	
Cu	1.35 ± 0.22
Fe	3.37 ± 0.83
Zn	2.60 ± 0.64

* dwb: dry weight basis.

microelements were higher than that determined for 12 edible flowers in the work of Rop et al. (2012). Fernandes et al. (2017) reported that a potassium-rich diet was recommended for the prevention of cardiovascular diseases. Furthermore, Rop et al. (2012) reported that a phosphorus-equilibrated diet contributes to the constitution of a strong and healthy human skeleton and to nucleic acids formation.

Moreover, the mineral elements are indispensable for human biological processes, such they contribute to the constitution of the human skeleton and of enzyme systems and to the regulation of osmotic pressure (Rop et al., 2012). Considering the daily rations of mineral recommended by the Dietary Reference Intakes (DRIs) for an adult which were 2000 mg/day for potassium, 700 mg/day for Phosphorus and 375 mg/day for magnesium (Regulation(EU)No1169/2011), the consumption of MDPF could help to supply the body requirements for these minerals by 134.21%, 45.52% and 37.53%, respectively. This confirm that the MDPF could be considered as a potential source of minerals.

3.1.2. Amino acids analysis

The proteins present the second main component in MDPF as demonstrated in a previous study (Karra et al., 2019). To assess the nutritional quality of these proteins, the amino acid profile was analysed and presented in Table 2. As can be seen, seventeen types of amino acids were detected and identified. Glutamic (Glu) and aspartic acid (Asp) showed the largest amount with 10.66 and 9.63 g/100 g of protein, respectively, followed by leucine (Leu), lysine (Lys), alanine (Ala),

Table 2
amino acid composition of male date palm flowers.

AA	g/100 g Protein	WHO/FAO/UNU (2007) g/100 g protein
Essential AA		
Thr	3.33	2.3
Val	4.37	3.5
Met	1.32	1.6
Ile	3.58	3
Leu	6.50	5.9
Phe	3.14	3
His	1.81	1.5
Lys	5.30	4.5
Total EAA	29.35	27.7
Non essential AA		
Asp	9.63	
Ser	4.08	
Glu	10.66	
Pro	3.12	
Gly	4.19	
Ala	4.88	
Cys	0.62	0.6
Tyr	2.56	
Arg	3.91	
Total NEAA	43.66	

valine (Val), glycine (Gly), serine (Ser), arginine (Arg), isoleucine (Ile), threonine (Thr), phenylalanine (Phe), proline (Pro), tyrosine (Tyr) histidine (His), methionine (Met) and cysteine (Cys). The results showed that savoury amino acids percentages (Glu and Asp) dominated the amino acid profile of MDPF and accounted for almost 28% of the total amino acids which was in accordance with findings of [Ananthan et al. \(2018\)](#) for different Naga king chilli fruit components ranging from 33 to 37%. In fact, high content of free glutamate contributes to add umami taste to foods ([Lölinger, 2000](#); [Zhang et al., 2013](#)), which opens the horizon to replace chemical additives such as monosodium glutamate (E621) by glutamate extracted from natural resources. The presence of sulfur-containing amino acid (Met and Cys) contribute to mechanisms detoxification and the presence of Arg and Tyr is considered indispensable for growing children ([Mba et al., 2017](#)). Moreover, as could be seen from [Table 2](#), the essential amino acids were present in the MDPF except for tryptophan. In fact, it is important to note that during acid hydrolysis, Tryptophan is completely destroyed, Cystine is partially destroyed (about 30%) and Methionine can be partially destroyed which leads to an underestimation of the total content of amino acids. It was obvious that essential amino acid supplied by MDPF were found higher than the reference value for adult's amino acid requirements ([WHO/FAO/UNU, 2007](#)). In addition, compared to soy protein, which is considered a mineral-rich plant source, the MDPF have higher values of most of the essential amino acids ([Lecerf and Fressin, 1995](#)). So, the MDPF proteins are of a relatively important biological value in the human diet, considering their wealth in essential amino acids.

3.2. Phytochemical analysis

Polyphenols and flavonoids are plant secondary metabolites and classified as antioxidant compounds. No studies have been published on the bioactive compounds of MDPF, thus, this study presents new data that could be used as a basis for the elaboration of functional food data charts.

Total phenolics (TPC) and total flavonoids (TFC) contents of dried MDPF powder were presented in [Table 3](#). The TPC of MDPF was relatively high (3829.22 mg EAG/100 g). It was similar to that of artichoke flower extracted with ethanol by decoction (3870 mg EAG/100 g) ([Mahmoudi et al., 2013](#)), and much higher than 12 species of edible flowers reported by [Rop et al. \(2012\)](#) (253–528 mg EAG/100 g). In comparison with olive leaf phenolics content (259–515 mg EAG/100 g) ([Aouidi and Hamdi, 2016](#)) which are well known by their active constituents, especially phenolic compounds, MDPF showed a significantly higher amount of polyphenols. Thus, it can be considered as a good source of total phenolics promoting high antioxidant capacities.

As regards to flavonoid contents, TFC value of MDPF (168.31 mg EQ/100 g) was higher than the value of Deglet Nour (33 mg EQ/100 g) ([Benmeddour et al., 2013](#)). [Rop et al. \(2012\)](#) have studied 12 species of edible flowers and demonstrated that they contain high amounts of

Table 3
Phytochemical compounds and IC₅₀ of male date palm flowers.

Parameter	Value
TPC ^a	3829.22 ± 251.33
TFC ^b	168.31 ± 5.052
DPPH (IC ₅₀) ^c	0.29
Reducing power (IC ₅₀) ^d	5
Metal chelation (IC ₅₀) ^c	4.6
β-bleaching assay (IC ₅₀) ^c	0.27

^a mg GAE (Gallic Acid Equivalent)/100 g dwb.

^b mg QE (Quercetin Equivalent)/100 g dwb.

^c IC₅₀ (mg/ml): concentration for a 50% inhibition or chelating effect.

^d IC₅₀ (mg/ml): concentration at which the absorbance is 0.5.

TFC (123–227 mg EC/100 g), which signifies that MDPF contains a considerable quantity compared to some flowers. Indeed, an important content of flavonoid could lead to a high antioxidant activity due to positive correlation between these two parameters ([Mato et al., 2000](#)).

Both phenolic compounds and flavonoids are very important because of their free-radical scavenging activity and protection against oxidative stress ([Xu and Chang, 2007](#)). In addition, the high phenolic content could have a beneficial effect on human health that may prevent some diseases such as cancer and heart coronary diseases ([Besbes et al., 2009](#)).

The high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS) analysis of MDPF extract has led to the identification of 18 phenolic compounds by comparing retention times and mass spectra with those of the authentic standards. So far, there are no studies about the identification of phenolic compounds in MDPF. As illustrated in [Table 4](#), the identified compounds were essentially 8 phenolic acids and 10 flavonoids. Phenolic acid constitutes the major group accounting for 90.65% among the total identified compounds with domination of the quinic acid (93.24% of the total phenolic acids and 84.52% of the total phenolic compounds). In fact, it was reported that quinic acid has an important role in the biosynthesis of aromatic compounds in the plants ([Hulme, 1958](#)). The quinic acid was found to be able of conversion to tryptophan and nicotinamide via the gastrointestinal tract microflora which provides an in situ physiological source of these essential metabolic ingredients to humans ([Pero et al., 2009](#)). In addition, [Cinkilic et al. \(2013\)](#) have reported that quinic acid, as well as several of its derivatives, have been shown to protect human lymphocytes from damage induced by X-ray and to prevent the DNA damage. It is to note that the esterification of caffeic acid, a phenolic compound present in MDPF, with quinic acid leads to the formation of chlorogenic acid. The latter was known to be associated with several health benefits such as reduction of the relative risks of cardiovascular disease, diabetes type 2, Alzheimer's disease, and contributing to antibacterial and anti-inflammatory effects ([Farah et al., 2008](#)). Comparing to the literature many of the identified compounds had potent antioxidant potential with IC₅₀ values less than 20 μg/ml ([Jdir et al., 2017](#)) which emphasizes the consumption of food products supplemented with MDPF powder to obtain a balanced and healthy diet.

Table 4
LC-ESI-MS analysis of the male date palm flowers extract.

No ^a	Compound ^b	m/z	Retention time (min)	Content (μg/g extract)
1	Quinic acid	191	6.491	99.720
2	Gallic acid	169	7.846	0.576
3	Protocatechuic acid	153	11.227	2.682
4	Catechin	289	16.250	2.807
5	Caffeic acid	179	21.328	0.286
6	Syringic acid	197	23.879	0.185
7	Epicatechin	289	26.794	2.323
8	p-Coumaric acid	163	33.526	1.625
9	trans-Ferulic acid	193	42.445	1.194
10	Rutin	609	59.291	0.906
11	Lutolein-7-O-glucoside	447	61.288	0.432
12	Quercitrin (Quercetin-3-O-rhamnoside)	447	67.446	2.224
13	trans-Cinnamic acid	147	79.144	0.677
14	Quercetin	301	80.458	1.449
15	Kaempferol	285	81.083	0.238
16	Naringenin	271	87.159	0.067
17	Apigenin	269	89.501	0.009
18	Luteolin	285	90.699	0.581

^a The numbering refers to elution order of compounds from an Aquasil C18 column.

^b Identification was confirmed using 33 authentic commercial standards.

3.3. Antioxidant activity

Vegetables contain different antioxidant compounds and the determination of the antioxidant capacity of each compound individually is very difficult. That is why, it seems necessary to combine more than one method in order to determine in vitro, the antioxidant capacity of foodstuffs (Pérez-Jiménez et al., 2008). Hence, DPPH radical scavenging, the ferric reducing antioxidant power (FRAP), the ferrous ion chelating capacity and β -carotene bleaching assays were performed to estimate in vitro antioxidant activities of MDPF.

3.3.1. DPPH radical-scavenging capacity

DPPH is a stable organic free radical which is commonly used for evaluation of antioxidant activity and free radical scavenging ability of plant extracts. In the DPPH test, the antioxidants reduce the violet-colored DPPH radical (diphenylpicrylhydrazyl) to a yellow-colored compound (diphenylpicrylhydrazine) and therefore induce the decrease in absorbance at 517 nm. Fig. 1.A showed that the inhibition of DPPH radicals by MDPF powder happened in a concentration-dependent manner. The scavenging activity was ranged from 30% to 90%. From the concentration of 0.8 mg of MDPF /ml the results of DPPH inhibition were close to those obtained by BHA. The IC₅₀ of MDPF (concentration that inhibits 50% of the DPPH radical) (0.29 mg/mL) was mentioned in Table 3. Compared with other flowers, it was lower than that of extracts from various colored cosmos (*Cosmos bipinnatus*) flowers reported by Jang et al. (2008) (0.29 mg/mL against 0.61–1.65 mg/mL) and higher than fertilized *Rosa micrantha* flower elucidated by Guimarães et al. (2010) (0.29 mg/ml against 0.11 mg/mL). In fact, it is well known that the lower the value of IC₅₀, the better the activity.

3.3.2. Ferric reducing power assay

The reducing power mechanism of antioxidant capacity is based on the analyte ability to donate electrons to reactive radical cation, reducing them to more stable and unreactive compound. In other words, it measures the conversion of a Fe³⁺ ferricyanide complex to the ferrous form. The quantification of sample reducing power is based on the principle of increase in the absorbance of the reaction mixtures. Thus, Increase in the absorbance at 700 nm indicates an increase in the antioxidant activity (Alam et al., 2013). As can be seen from Fig. 1.B, the reducing power of the aqueous extract of MDPF powder increased with increasing concentrations. BHA, used as positive standard, showed higher reducing power activities than MDPF at all tested concentrations. The IC₅₀ value of MDPF (concentration for increasing 0.5 value in optical density) was equal to 5 mg/ml (Table 3). It was close to that of *Tricholomapormentosum* (a wild edible mushroom) reported by Ferreira et al. (2007) (4.82 mg/ml) and higher than those of *Opuntia ficus-indica* flowers water extract indicated by Ammar et al. (2015) (0.20–0.31 mg/mL).

3.3.3. Ferrous ion chelating capacity

The method of metal chelating activity is an important mechanism of the antioxidant capacity which is based on the ability to chelate/deactivate transition metals, that possess the ability to catalyze hydroperoxide decomposition and Fenton-type reactions (Manian et al., 2008). In fact, the iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity, that is why this assay is of great significance as the chelation of ferrous ion is of great potential interest in the food industry. Analysis of the ferrous chelating properties was revealed in Fig. 1.C which shows that MDPF powder, all concentration tested, was capable of chelating Fe⁺² and did so in a

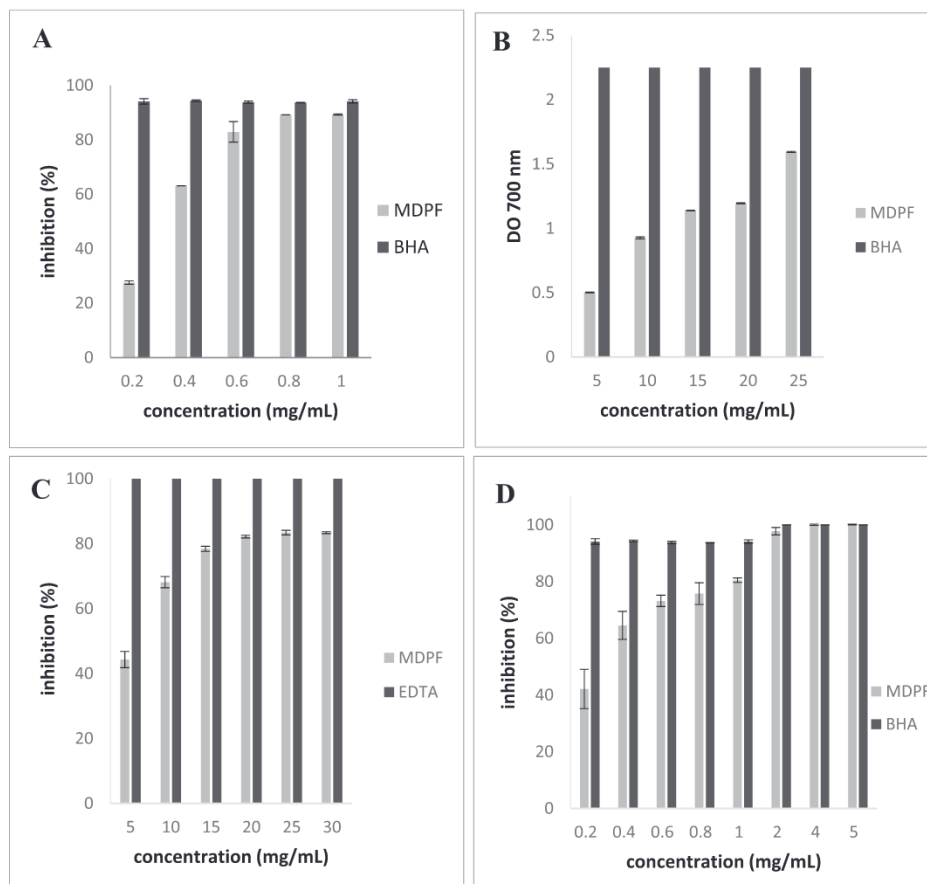


Fig. 1. Antioxidant activity of male date palm flowers. A: DPPH radical-scavenging capacity; B: Reducing power assay; C: Metal chelating assay; D: β -bleaching assay.

dose-dependent way. EDTA, a known metal ion chelator which was used as reference, exhibited the highest chelating effect whatever the concentration level. From a concentration of 20 mg of MDPF/mL, the chelating capacity was stable and around 80%. The obtained results showed that MDPF could be able to chelate Fe²⁺ known as the most powerful prooxidant among different species of metal ions, and to catalyze the formation of reactive oxygen species, such as hydroxyl radical ($\cdot\text{OH}$) (Abbès et al., 2013). The IC₅₀ value (4.60 mg/mL) illustrated in Table 3 was much lower than those of date syrups ranged between 6.12 and 36.15 mg/mL (Abbès et al., 2013) and those of fig co-products which were between 47.34 and 56.35 mg/mL (Viuda-Martos et al., 2015). Indeed, a low IC₅₀ value promote a better reducing activity which add value to MDPF extract.

3.3.4. β -carotene bleaching assay

Unlike other tests, β -carotene bleaching inhibition assay employs a biologically substrate which can be oxidized to prove the extract's protective action. The β -carotene bleaching mechanism is based on the principle that linoleic acid free radical, attacks the highly unsaturated β -carotene and thus the molecules lose their double bonds by oxidation and therefore the compound loses its characteristic orange color. However, the presence of antioxidants could prevent the extend of β -carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system leading to the decrease of the discoloration which can be measured spectrophotometrically (Pereira et al., 2007; Sowndhararajan et al., 2010). Fig. 1.D showed that MDPF soluble fraction was able to inhibit the bleaching of β -carotene in a concentration-dependent manner. The MDPF revealed a high antioxidant potential similar to BHA since the concentration of 4 mg/ml. This better lipid peroxidation inhibition might be due to the highest content and synergy between phenolic compounds and flavonoids found in the sample. The IC₅₀ value of MDPF presented in Table 3 was less important than *Rosa micrantha* IC₅₀ values (Guimarães et al., 2010) (0.27 mg/ml against 0.03–0.17 mg/mL). However, it was much more important compared to bee pollen IC₅₀ values reported by Morais et al. (2011) which means a better protective action of MDPF (0.27 mg/mL against 3.11–6.52 mg/mL).

Although the chemical standards used in these assays, such as BHA and EDTA, exhibited the highest antioxidant activity, natural antioxidants are of growing interest. Indeed, the incorporation of MDPF powder in food matrix could confer better solution to replace chemical products used as antioxidant agents.

3.4. Antimicrobial activity

The antibacterial activity of MDPF extract was evaluated against Gram-positive and Gram-negative bacteria by measuring the inhibition zone (IZ). As elucidated in Table 5, the MDPF showed varying antibacterial behaviors against the tested bacteria. For the Gram-positive bacteria, MDPF exhibited a considerable antibacterial activity expressed by a zone of inhibition greater than 10 mm except for *M. luteus*. The most susceptible Gram-negative bacterium for the MDPF extract was *E. coli* with IZ value equal to 12.1 mm whereas the other Gram-negative bacteria were insensitive to the MDPF extract. The obtained results were in accordance with previous studies showing that Gram-negative bacteria, due to their outer membrane, are more resistant to antibiotics and toxic drugs (Rurián-Henares and Morales, 2008). The antimicrobial activities of MDPF could be related to the presence of phenolic compounds which are known as antimicrobial agents against several pathogens with variable activity spectra as illustrated by Makhoulouf-Gafsi et al. (2018). In addition, flavonoids were demonstrated to have an antimicrobial effect on Gram-positive or negative bacteria as reported by Kchaou et al. (2016). Moreover, these results are of great importance as they demonstrate the role of MDPF extract as a contributor to limit the harmful effect of both *L. monocytogenes* and *S. aureus* which are known by

Table 5
Antibacterial activities of male date palm flowers extract (inhibition zone in mm).

Bacterial strains	MDPFsupernatant
Gram positive	
<i>Bacillus subtilis</i> ATCC 6633	10.5 ± 0.3
<i>Staphylococcus aureus</i> ATCC 9144	11.1 ± 0.06
<i>Micrococcus luteus</i>	0
<i>Listeria monocytogenes</i>	12.1 ± 0.1
Gram negative	
<i>Pseudomonas savastoni</i>	0
<i>Escherichia coli</i> ATCC 10536	12.1 ± 0.2
<i>Salmonella enterica</i> CIP 80.39	0
<i>Agrobacterium tumefaciens</i>	0

their ability to disturb the organism functioning (Halpin-Dohnalek and Marth, 1989; Makhoulouf-Gafsi et al., 2018). According to (Ramos et al., 2014), it was proved that the presence of phenolic compounds naturally presented in grape and grape juices, particularly polymeric phenolic compounds: caffeic acid, gallic acid and flavonoids (rutin and quercetin), may be responsible for its high antilisterial activity, which could be the same case for MDPF.

In the agri-food field, these results could be useful to replace some organic food preservatives such as benzoic acid (E210) as the gallic and the protocatechuic acids are benzoic acid's derivatives and are known for their antibacterial and antioxidant effect (Alves et al., 2013; Semaming et al., 2015)

4. Conclusion

The results of the current study showed that MDPF could be an excellent source of minerals and amino acids, given its wealth in macro and microelement satisfying the human body requirements and in essential amino acids that exceed the references values. Indeed, the MDPF was rich in glutamate. This amino acid can serve as a flavor enhancer for canned food and thus its presence in the MDPF is promising to replace some chemical additives having the same role. Moreover, MDPF has high amounts in bioactive compounds and their identification has demonstrated that quinic acid was the predominant phenolic compound giving promoting health benefits and interesting physiologic activities. Additionally, it was proved that MDPF powder revealed an important antioxidant capacity especially via the DPPH radical scavenging assay and was able to partially inhibit the proliferation of some bacterial strains. The richness of the MDPF in phenolic compounds could confer to him beside its antibacterial and antioxidant activity, a preservative function and thus the opportunity to replace certain chemical food preservatives such as benzoic acid (E210). Consequently, from the obtained data, a high nutritional value associated with a considerable antioxidant and antibacterial activity predetermine MDPF powder to be a new and promising foodstuff that could be used as raw material for the production of various food products in gastronomy.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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References

- Abbès, F., Kchaou, W., Blecker, C., Ongena, M., Lognay, G., Attia, H., Besbes, S., 2013. Effect of processing conditions on phenolic compounds and antioxidant properties of date syrup. *Ind. Crops Prod.* 44, 634–642.
- Alam, M.N., Bristi, N.J., Rafiquzzaman, M., 2013. Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharm. J.* 21, 143–152.
- Alves, M.J., Ferreira, I.C., Froufe, H.J., Abreu, R., Martins, A., Pintado, M., 2013. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. *J. Appl. Microbiol.* 115, 346–357.
- Ammar, I., Ennouri, M., Attia, H., 2015. Phenolic content and antioxidant activity of cactus (*Opuntia ficus-indica* L.) flowers are modified according to the extraction method. *Ind. Crops Prod.* 64, 97–104.
- Ananthan, R., Subhash, K., Longvah, T., 2018. Capsaicinoids, amino acid and fatty acid profiles in different fruit components of the world hottest Naga king chilli (*Capsicum chinense* Jacq.). *Food Chem.* 238, 51–57.
- Andriani, Y., Ramli, N.M., Syamsumir, D.F., Kassim, M.N.I., Jaafar, J., Aziz, N.A., Marlina, L., Musa, N.S., Mohamad, H., 2019. Phytochemical analysis, antioxidant, antibacterial and cytotoxicity properties of keys and cores part of *Pandanus tectorius* fruits. *Arab. J. Chem.* 12 (8), 3555–3564.
- Aouidi, F., Hamdi, M., 2016. Antioxidant capacity and phenolic content in olive leaf tisane as affected by boiling treatment. *PhytoChem BioSub. J.* 10 (2), 39–50.
- Benmeddour, Z., Mehniag, E., Le Meurlay, D., Louaileche, H., 2013. Phenolic composition and antioxidant capacities of ten Algerian date (*Phoenix dactylifera* L.) cultivars: a comparative study. *J. Funct. Foods* 5, 346–354.
- Bersuder, P., Hole, M., Smith, G., 1998. Antioxidants from a heated histidine-glucose model system. I: investigation of the antioxidant role of histidine and isolation of antioxidants by high-performance liquid chromatography. *J. Am. Oil Chem. Soc.* 75, 181–187.
- Besbes, S., Drira, L., Blecker, C., Deroanne, C., Attia, H., 2009. Adding value to hard date (*Phoenix dactylifera* L.): composition, functional and sensory characteristics of date jam. *Food Chem.* 112, 406–411.
- Bouaziz, M.A., Besbes, S., Blecker, C., Wathelet, B., Deroanne, C., Attia, H., 2008. Protein and amino acid profiles of Tunisian Deglet Nour and Allig date palm fruit seeds. *Fruits* 63, 37–43.
- Chandrasekaran, M., Bahkali, A.H., 2013. Valorization of date palm (*Phoenix dactylifera*) fruit processing by-products and wastes using bioprocess technology—Review. *Saudi J. Biol. Sci.* 20, 105–120.
- Cinkilic, N., Cetintas, S.K., Zorlu, T., Vatan, O., Yilmaz, D., Cavas, T., Tunc, S., Ozkan, L., Bilaloglu, R., 2013. Radioprotection by two phenolic compounds: chlorogenic and quinic acid, on X-ray induced DNA damage in human blood lymphocytes in vitro. *Food Chem. Toxicol.* 53, 359–363.
- Costa, A.G.V., Garcia-Diaz, D.F., Jimenez, P., Silva, P.I., 2013. Bioactive compounds and health benefits of exotic tropical red–black berries. *J. Funct. Foods* 5, 539–549.
- Dinis, T.C., Madeira, V.M., Almeida, L.M., 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys.* 315, 161–169.
- Farah, A., Monteiro, M., Donangelo, C.M., Lafay, S., 2008. Chlorogenic acids from green coffee extract are highly bioavailable in humans. *J. Nutr.* 138, 2309–2315.
- Fernandes, L., Casal, S., Pereira, J.A., Saraiva, J.A., Ramalho, E., 2017. Edible flowers: a review of the nutritional, antioxidant, antimicrobial properties and effects on human health. *J. Food Compos. Anal.* 60, 38–50.
- Ferreira, I.C., Baptista, P., Vilas-Boas, M., Barros, L., 2007. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: individual cap and stipe activity. *Food Chem.* 100, 1511–1516.
- Goldhaber, S.B., 2003. Trace element risk assessment: essentiality vs. toxicity. *Regul. Toxicol. Pharmacol.* 38, 232–242.
- Guimarães, R., Barros, L., Carvalho, A.M., Ferreira, I.C., 2010. Studies on chemical constituents and bioactivity of *Rosa micrantha*: an alternative antioxidants source for food, pharmaceutical, or cosmetic applications. *J. Agric. Food Chem.* 58, 6277–6284.
- Halpin-Dohnalek, M.I., Marth, E.H., 1989. Growth and production of enterotoxin A by *Staphylococcus aureus* in cream. *J. Dairy Sci.* 72, 2266–2275.
- Hamed, A.I., Ben Said, R., Al-Ayed, A.S., Moldoch, J., Mahaleh, U.A., Mahmoud, A.M., Elgebaly, H.A., Perez, A.J., Stochmal, A., 2017. Fingerprinting of strong spermatogenesis steroidal saponins in male flowers of *Phoenix dactylifera* (Date Palm) by LC-ESI-MS. *Nat. Prod. Res.* 31, 2024–2031.
- Hulme, A., 1958. Quinic and shikimic acids in fruits. *Qualitas Plantarum et Materiae Vegetabiles* 3, 468–473.
- Jang, I.-C., Park, J.-H., Park, E., Park, H.-R., Lee, S.-C., 2008. Antioxidative and antigenotoxic activity of extracts from cosmos (*Cosmos bipinnatus*) flowers. *Plant Foods Hum. Nutr.* 63, 205–210.
- Jdir, H., Jridi, M., Mabrouk, M., Ayadi, M., Nasri, M., Zouari, N., Fakhfakh, N., 2017. The rocket, *Diplotaxis simplex*, as a functional ingredient: LC-ESI-MS analysis and its effects on antioxidant and physical properties of bread. *J. Food Nutr. Res.* 5, 197–204.
- Karra, S., Sebbi, H., Borhani, C., Danthine, S., Blecker, C., Attia, H., Besbes, S., Bouaziz, M.A., 2019. Physico-chemical and functional properties of dried male date palm flowers. *Food Biosci.* 31, 100441.
- Kchaou, W., Abbès, F., Mansour, R.B., Blecker, C., Attia, H., Besbes, S., 2016. Phenolic profile, antibacterial and cytotoxic properties of second grade date extract from Tunisian cultivars (*Phoenix dactylifera* L.). *Food Chem.* 194, 1048–1055.
- Koleva, I.L., van Beek, T.A., Linsens, J.P., Groot, A.D., Evstatieva, L.N., 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem. Anal.* 13, 8–17.
- Lecerf, J.-M., Fressin, C., 1995. L'intérêt nutritionnel du soja. *Nutr. Clin. Métab.* 9, 137–144.
- Löliger, J., 2000. The use and utility of glutamates as flavoring agents in foods. *J. Nutr.* 130, 915S–920S.
- Mahmoudi, S., Khali, M., Mahmoudi, N., 2013. Etude de l'extraction des composés phénoliques de différentes parties de la fleur d'artichaut (*Cynara scolymus* L.). *Nat. Technol.* 35.
- Makhlouf-Gafsi, I., Krichen, F., Mansour, R.B., Mokni, A., Sila, A., Bougateg, A., Blecker, C., Attia, H., Besbes, S., 2018. Ultrafiltration and thermal processing effects on Maillard reaction products and biological properties of date palm sap syrups (*Phoenix dactylifera* L.). *Food Chem.* 256, 397–404.
- Manian, R., Anusuya, N., Siddhuraju, P., Manian, S., 2008. The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. *Food Chem.* 107, 1000–1007.
- Mato, M., Onozaki, T., Ozeki, Y., Higeta, D., Itoh, Y., Yoshimoto, Y., Ikeda, H., Yoshida, H., Shibata, M., 2000. Flavonoid biosynthesis in white-flowered *Sim carnations* (*Dianthus caryophyllus*). *Sci. Hortic.* 84, 333–347.
- Mba, A.R.F., Kansci, G., Viau, M., Hafmaoui, N., Meynier, A., Demmano, G., Genot, C., 2017. Lipid and amino acid profiles support the potential of *Rhynchophorus phoenicis* larvae for human nutrition. *J. Food Compos. Anal.* 60, 64–73.
- Morais, M., Moreira, L., Feás, X., Estevinho, L.M., 2011. Honeybee-collected pollen from five Portuguese natural parks: palynological origin, phenolic content, antioxidant properties and antimicrobial activity. *Food Chem. Toxicol.* 49, 1096–1101.
- Oniszczuk, A., Olech, M., Oniszczuk, T., Wojtunik-Kulesza, K., Wójtczyc, A., 2019. Extraction methods, LC-ESI-MS/MS analysis of phenolic compounds and antiradical properties of functional food enriched with elderberry flowers or fruits. *Arab. J. Chem.* 12 (8), 4719–4730.
- Pereira, J.A., Oliveira, I., Sousa, A., Valentão, P., Andrade, P.B., Ferreira, I.C., Ferreres, F., Bento, A., Seabra, R., Estevinho, L., 2007. Walnut (*Juglans regia* L.) leaves: phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. *Food Chem. Toxicol.* 45, 2287–2295.
- Pérez-Jiménez, J., Arranz, S., Taberner, M., Díaz-Rubio, M.E., Serrano, J., Goñi, I., Saura-Calixto, F., 2008. Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: extraction, measurement and expression of results. *Food Res. Int.* 41, 274–285.
- Pero, R.W., Lund, H., Leanderson, T., 2009. Antioxidant metabolism induced by quinic acid. Increased urinary excretion of tryptophan and nicotinamide. *Phytother. Res.* 23, 335–346.
- Ramos, B., Brandão, T.R., Teixeira, P., Silva, C.L., 2014. Balsamic vinegar from Modena: an easy and effective approach to reduce *Listeria monocytogenes* from lettuce. *Food Control* 42, 38–42.
- Regulation(EU)No1169/2011, of the European Parliament and of the Council of 25 October 2011, Daily Reference Intakes for vitamins and minerals (adults), Annex XIII.
- Rop, O., Mlcek, J., Jurikova, T., Neugebauerova, J., Vabkova, J., 2012. Edible flowers—a new promising source of mineral elements in human nutrition. *Molecules* 17, 6672–6683.
- Rurián-Henares, J.A., Morales, F.J., 2008. Antimicrobial activity of melanoidins against *Escherichia coli* is mediated by a membrane-damage mechanism. *J. Agric. Food Chem.* 56, 2357–2362.
- Semaming, Y., Pannengetch, P., Chattipakorn, S.C., Chattipakorn, N., 2015. Pharmacological properties of protocatechuic acid and its potential roles as complementary medicine. *Evid.-Based Complementary Altern. Med.* 2015.
- Serrano-Díaz, J., Sánchez, A.M., Martínez-Tomé, M., Winterhalter, P., Alonso, G.L., 2013. A contribution to nutritional studies on *Crocus sativus* flowers and their value as food. *J. Food Compos. Anal.* 31, 101–108.
- Singleton, V., Orthofer, R., Lamuela-Raventós, R., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* C 299, 152–178.
- Sowndhararajan, K., Joseph, J.M., Arunachalam, K., Manian, S., 2010. Evaluation of *Merremia tridentata* (L.) Hallier f. for in vitro antioxidant activity. *Food Sci. Biotechnol.* 19, 663–669.
- Vanden, B., Vlietinck, A., 1991. Screening methods for antibacterial and antiviral agents from higher plants. *Methods Plant Biochem.* 6, 47–69.
- Viuda-Martos, M., Barber, X., Perez-Alvarez, J.A., Fernandez-Lopez, J., 2015. Assessment of chemical, physico-chemical, techno-functional and antioxidant properties of fig (*Ficus carica* L.) powder co-products. *Ind. Crops Prod.* 69, 472–479.
- Wan Ismail, W., Mohd Radzi, M., 2013. Evaluation on the Benefits of Date Palm (*Phoenix dactylifera*) to the Brain. *Altern. Integr. Med.* 2, 2–3.
- WHO/FAO/UNU, 2007. Protein and Amino Acid Requirements in Human Nutrition. p. 1 World Health Organization Technical Report Series.
- Xu, B., Chang, S., 2007. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *J. Food Sci.* 72, S159–S166.
- Yıldırım, A., Mavi, A., Kara, A.A., 2001. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. *J. Agric. Food Chem.* 49, 4083–4089.
- Zhang, Y., Venkatasamy, C., Pan, Z., Wang, W., 2013. Recent developments on umami ingredients of edible mushrooms—a review. *Trends Food Sci Technol* 33, 78–92.
- Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 64, 555–559.