

**BIOACTIVE COMPOUNDS AND PHARMACOLOGICAL PROPERTIES
OF SOME DATE CULTIVARS (*PHOENIX DACTYLIFERA* L.)**

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Abstract

In this study, five Algerian date cultivars (*Phoenix dactylifera* L.) were investigated for their antioxidant contents, phenolic profiles, and some pharmacological properties (anti-inflammatory, anti-diabetic, and antioxidant activities). The results showed that the Tanslit cultivar was the most concentrated in total phenolic compounds and flavonoids with 433.03 ± 0.47 96 mg QE/100g DM and 102.5 ± 3.96 mg QE/100g DM, respectively. The Tamdjohert cultivar was the richest on anthocyanins (9.83 ± 0.07 mg CE/100g DM). HPLC analysis indicated that the Tamdjohert cultivar contained a high flavonoids concentration (8.22 ± 0.53 mg/100g DM), and the Takarmoust cultivar displayed the highest concentration of phenolic acids (15.64 ± 0.41 mg/100g DM). The results indicated that the Litim cultivar exhibited strong hydroxyl radical inhibition (134.95 ± 18.07 mg GAE/100g DM), and also potently inhibited α -amylase activity ($35.19 \pm 4.98\%$). The Tamdjohert cultivar exerted the highest reduction of inflammatory mediators (255.87 ± 18.47 mg GAE/100g DM). This study demonstrated that date fruit was a good source of bioactive compounds with important pharmacological properties and can be considered as functional food.

Keywords: *Phoenix dactylifera*, bioactive compounds, phenolic profile, pharmacological properties

Introduction

Production of free radicals in the organism was an inevitable phenomenon. However, unregulated, and prolonged imbalance between the production and their elimination by protective mechanisms (antioxidants) leads to damage of important biomolecules and cells. They interfere with the pro-inflammatory genes with potential impact on the whole organism causing many chronic diseases (Reyes-Gordillo *et al.*, 2017).

Some studies have demonstrated that antioxidants can inhibit the generation of free radicals and repress oxidative damage, thereby preventing against diseases caused by them (Gholamian-Dahkordi *et al.*, 2017). Antioxidant properties were generally attributed to phenolic compounds, they are the group of the largest and most widespread metabolites of the plant reign. These compounds possess various biological properties due to their ability to quench free radicals or inhibit enzymes implicated in diseases such as inflammatory, diabetes, cancer, and cardiovascular disorders (Balasundram *et al.*, 2006).

Fruits and vegetables are the main sources of bioactive compounds, its polyphenols are considered as the most interesting group of natural antioxidants. Biological properties of fruits vary depending on their content of antioxidants (Goni *et al.*, 2006). Date fruits are important not only for their economic impact, but also for their high contents of carbohydrates, minerals, and dietary fibres (Al-shahib and Marshall, 2003). Several researchers reported that date fruits are also considered to be an excellent source of phenolic compounds and they exert many biological activities (Vayalil, 2005; Gao *et al.*, 2014; Osman *et al.*, 2014).

To the best of our knowledge, no literature reported about α -amylase inhibitory activity and little works have been carried out related to anthocyanin contents in date fruit. Consequently, this study aimed to investigate anthocyanin, total phenolic, and flavonoid contents and the determination of phenolic profile using HPLC-DAD of five date cultivars. Pharmacological properties were evaluated through antioxidant activity, estimated using three methods (reducing power, DPPH and hydroxyl radical scavenging activities). Antidiabetic activity was assessed by the inhibition of α -amylase, and anti-inflammatory activity was measured using NO° radical scavenging assay.

Materials and methods

Chemicals

Folin-Ciocalteu and ammonium-iron (III) sulfate reagents were from Biochem, Chemopharma (Montreal, Quebec), 1,1-diphenyl-2-picrylhydrazyl radical was from Sigma Aldrich (Sternhein, Germany), gallic acid and quercetin were from Sigma Aldrich co (St. Louise, MO, USA), sodium carbonate, aluminum chloride, sodium nitrite were from Biochem Chemopharma (Georgia, USA), methanol (99.7%) was from Prolabo VWR (Fontenay-sous-Bois, France). Hydrogen peroxide (30%) was from Biochem Chemopharma (Road Town, Tortola, United Kingdom). Porcine pancreatic amylase was from Biochem Chemopharma (Georgia, USA).

Sampling and sample preparation

Five date cultivars, namely *Tamdjohert*, *Tafzouine*, *Tanslit*, *Litim* and *Takarmoust*, were studied in this investigation. The fruits were harvested in M'zab oasis of Ghardaia department (Algeria) in October 2014. After harvest, about 5 kg of each cultivar were transported to the laboratory. Mature fruits, with uniform size, free of physical damage, insect injury, and fungal infection were selected. The samples were crushed using a manual grinder, and the resulting date pastes were stored at 4°C.

Preparation of date extract

An aliquot of date paste was mixed firstly using ultra-turax with 20 mL of 65% methanol, then the extraction was followed by a sonication equipped with an ultrasonic probe (Sonics vibracell. VCX 130 PB, USA), at a frequency of 20 KHz. Extraction was performed in an ice bath to keep a low temperature. Subsequently, the mixture was centrifuged at 5000 rpm (NF 200, Nuve, Turkey) for 5 min, and the resulted extract was then filtered. (Benkerrou *et al.*, 2018).

Phytochemical analysis

Total phenolic contents (TPC)

TPC were measured according to Singleton and Rossi (1965). Two hundred microliters of extract were mixed with 750 µL of Folin-Ciocalteu reagent. A volume of 400 µL of sodium carbonate (5%) was added after 5 min and the mixture was incubated for 60 min. Absorbance was measured in a UV-vis spectrophotometer (UViline 9400, Secomam, Ales France) at 720 nm. TPC was expressed as milligrams of gallic acid equivalent per 100g dry matter of date fruit (mg GAE/100g DM).

Total flavonoids

Flavonoids content was estimated according to Kim *et al.* (2003). Five hundred microliters of the extract were added to 150 µL sodium nitrite solution (5%) followed by 300 µL aluminium chloride (10%). After incubation at room temperature for 5 min, 1mL of 1M sodium hydroxide was added to the mixture. The absorbance was determined at 510 nm after 30 min, and the results were expressed as milligrams of quercetin equivalent per 100g DM of date fruit (mg QE/100g DM).

Anthocyanin contents

Anthocyanins were extracted according to the procedure described by Mélo *et al.* (2006). One gram of fruit was mixed with 10 mL of ethanolic solution (ethanol/1.5 M HCl; 85/15, v/v) and incubated in the dark at 4°C overnight. After filtration, the absorbance was measured at 530 nm. The anthocyanins concentration was calculated using a molar extinction coefficient of cyanidin 3-glucoside ($\epsilon = 38.000$ L/mol cm).

HPLC-DAD analysis of phenolic compounds

Methanolic extracts were concentrated under reduced pressure at 40°C using a rotary evaporator (Rotavapor R-200/205, Buchi, Flawil, Switzerland) and the remaining water was freeze-dried. Lyophilized extracts (20 mg) were dissolved in 2 mL of 80% methanol and filtered through a 0.45 µm cellulose acetate filter (Millipore). Phenolic compounds were analyzed using a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan) consisting of two LC-10AD pumps, SCTL 10A system controller, and SPD-M 10A photodiode array detector. The chromatography was carried out using

a pre-packed Luna C18-column (4 250 mm, 5-mm; Phenomenex). The elution was 50 min using a gradient system of 5-40% acetonitrile in water adjusted to pH 2.5 with TFA. The detector was set at 320 and 350 nm, with an injection volume of 20 μ L, and a flow rate of 1 mL/min. The compounds were identified by comparing them with the standards for each compound, using retention time and UV spectra, as well as by running the samples after the addition of pure standards. Phenolic acids and flavonoids were quantified using calibration curves obtained from the corresponding standards. The results were expressed as milligrams per 100g (mg/100 g DM).

Pharmacological properties

Antioxidant activities

Ferric reducing power

Reducing power was estimated according to Vijayalakshmi and Ruckmani (2016). Briefly, 500 μ L of extract were added to 1250 μ L of phosphate buffer (0.2M, pH 6.6) and 1250 μ L of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min, and then 1250 μ L of trichloroacetic acid solution (10%) was added. The mixture (1250 μ L) was combined with 1250 μ L of distilled water and 250 μ L of iron (III) chloride (1%). The absorbance was recorded at 700 nm. The results were expressed as milligrams gallic acid equivalent per 100 g of date fruit (mg GAE/100g DM).

DPPH radical scavenging activity

The antiradical activity was evaluated according to Molyneux (2004). The extract (100 μ L) was added to 1mL of methanol solution of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. After 30 min, the absorbance was measured at 515 nm. The scavenging activity of the date extract was calculated using a calibration curve of gallic acid and expressed as mg GAE/100g DM of date fruit.

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging assay was determined using the method described by De Avellar *et al.* (2004). Five hundred microliters of 1,10 phenanthroline (0.75mM) and 500 μ L of FeSO₄ (0.75mM) were prepared in a phosphate buffer (pH 7.4). Five hundred microliters of H₂O₂ (0.01%) and 500 μ L of extracts were added to the mixtures. After incubation at 37°C for 60 min, the absorbance was measured at 536 nm and expressed as mg GAE/100g DM of date fruit.

Anti-inflammatory activity

The ability of date extract to scavenge NO radical was used to evaluate the anti-inflammatory activity of date extracts. It was determined using the colorimetric method adapted from Gorinstein *et al.* (2004). A volume of 500 μ L of sodium nitroprusside (10mM) prepared in a saline phosphate buffer (20mM, pH 7.4) was added to 500 μ L of extract, the mixture was set at 25°C for 15 min., 1mL of Griess reagent (sulfanilamide solution (1%) prepared in phosphoric acid (2%) and n-1-naphthyl-ethylene- diamine dihydrochloride (NEDD) solution (0.1%)) was added and incubated for 30 min. The absorbance was measured at 532 nm, and the results were expressed as mg GAE/100g DM of date fruit.

Anti-diabetic activity

The method of Conforti *et al.* (2005) was adopted to determine the α -amylase inhibitory activity of the crude date extracts. 1% starch solution was prepared in a 20mM sodium phosphate buffer and 6.7mM sodium chloride at pH 6.9. The solution was heated at 100°C for 15 min and then cooled to room temperature. The volume was brought to 100mL with distilled water. The α -amylase solution was prepared as 10 unit/mL. One hundred microliters of sample were added to 1900 μ L of starch solution. As a control, methanol (1000 μ L) was added instead of the extracts. The tubes were incubated at 20°C for 10 min, and subsequently, 1mL of α -amylase solution was added. The tubes were incubated at 37°C for 5 min, and then at 100°C for 5 min. LiquickCor-glucose reagent (2250 μ L) was added to 200 μ L of mixture. The tubes were incubated at 37°C for 15 min, and the absorbance was measured at 500 nm. The α -amylase inhibition was expressed as a percentage of inhibition and was calculated with the help of the following equation:

$$\text{Enzyme inhibition (\%)} = ((Ac-As)/Ac)*100 \quad (1)$$

where Ac is the absorbance of the control and As is the absorbance of the sample.

Statistical analysis

All tests were done in triplicate, and the data were averaged. Statistica 5.5 was used to compare the different results using one-way analysis of variance (ANOVA, LSD). Differences were considered statistically significant at $P \leq 0.05$.

Results and discussion

Phytochemical composition

Results of antioxidant contents (phenolic, flavonoid, and anthocyanin contents) of the analyzed date cultivars were illustrated in Figure 1.

Total phenolic contents

Extraction of phenolic compounds from plants is difficult because of their structural diversity, difference of polarity, and matrix complexity. Furthermore, the solubility of polyphenols mainly depends on the number of hydroxyl groups, the molecular weight, and the length of the carbon chain of the basic chemical structure (Mohammedi and Atik, 2011). In this study, the choice of the extraction protocol is based on our previously published study about the optimized extraction of the phenolic compounds from date (Benkerrou *et al.*, 2018).

The obtained results indicated that TPC varied between cultivars, with contents ranging from 256.00 \pm 8.03 (Litim cultivar) to 433.03 \pm 0.47 mg GAE/100g DM (Tanslit cultivar). These results were higher than those reported by Khanavi *et al.* (2009), Singh *et al.* (2012), and Louaileche *et al.* (2015). However, they were lower than those found by Benmeddour *et al.* (2013) and Bouhlali *et al.* (2016). The observed differences may mainly be attributed to genetic differences of cultivars, extraction conditions, and geographical region. Indeed, TPC of Tamdjohert and Tafzouine cultivars obtained in this work were higher than the same cultivars of Ouargla region with 23.05 and 9.50mg GAE/100g DM, respectively (Ghiaba *et al.*, 2014). Results of this study showed that date presented a higher content of phenolic

compounds compared to other fruits such as cantaloupe (Ismail *et al.*, 2010), apricots (Vijaya Kumar Reddy *et al.*, 2010), and apple (Wang *et al.*, 2015). These results showed that the date fruit might be considered a good source of phenolics.

Total flavonoids

The flavonoid contents of the five date cultivars were ranged from 40.47 ± 3.67 to 102.5 ± 3.96 mg QE/100g DM. Statistical analysis showed that the Tanslit and Litim cultivars had the highest flavonoid content, with similar levels, while the Tafzouine cultivar was the least concentrated. These results were higher than those reported by Alhaider *et al.* (2017) and Mehmood *et al.* (2016), but lower than some cultivars studied by Benmeddour *et al.* (2013). These differences can be explained not only by the extraction method and the quantification protocol that were used, but also by the varietal and geographical variations. According to the flavonoid results, the studied date fruit cultivars were more concentrated than other dried fruits usually consumed such as prunes, raisins, and figs (Ouchmoukh *et al.*, 2012), and slightly higher than the light figs studied by Bachir bey and Louaileche (2015).

Anthocyanins

Several studies have indicated that anthocyanins are among the functional polyphenols which have significant antioxidant and anti-inflammatory properties (Rechner and Kroner 2005; Wang and Stoner 2008).

The anthocyanin concentrations of the five date cultivars were presented in Figure 1. Statistical analyses showed that there wasn't a significant difference between Takarmoust and Litim, compared to other cultivars. The concentrations varied between 5.22 ± 0.16 mg CE/100g DM (Takarmoust cultivar) and 9.83 ± 0.07 mg CE/100g DM (Tamdjohert cultivar). These results were significantly higher than those found by Al-Farsi *et al.* (2005). Compared to other fruits like prunes, apricots, and figs, date may be considered to be a good source of anthocyanins (Ouchmoukh *et al.*, 2012).

HPLC-DAD analysis

Phenolic compounds identified using HPLC-DAD of the five date cultivars were listed in Table 1. Statistical analysis on flavonoid contents indicated that all cultivars were significantly different ($P \leq 0.05$). Globally, five flavonoids were identified (quercetin 3- glucoside, luteolin 4'-O-glucoside, catechin, quercetin-7-O-hexoside-3-O-hyxoside, and apigenin-7-O-glycosyl-2''-acetate). Catechin was the most abundant compound in all cultivars, except for Takarmoust, as opposed to luteolin 4'- O- glucoside, which was the less abundant. It was found in all cultivars except Takarmoust, which was the only cultivar where flavonoids were not detected. The same flavonoids were identified by Benmeddour *et al.* (2013), except rutin, which was substituted by apigenin-7-O-glycosyl-2''-acetate. Flavonoids contents were averaged between 0 (Takarmoust cultivar) and 8.22 ± 0.53 mg/100g. (Tamdjohert cultivar). These results overly concord with those reported by Benmeddour *et al.* (2013) and Hamad (2014) who obtained 0.89-7.36 and 1.4-3.94 mg/100g DM, respectively.

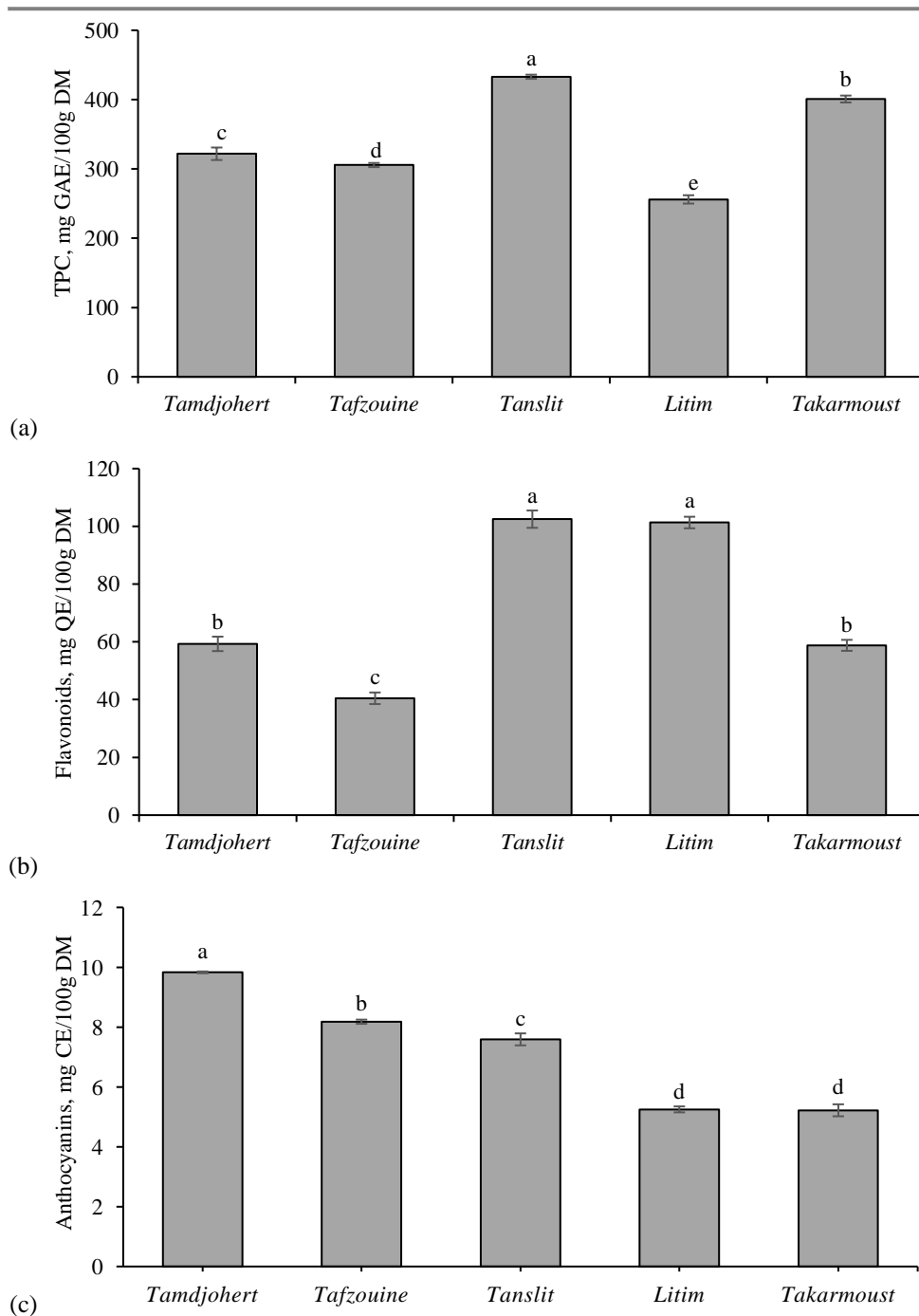


Figure 1. Total phenolic (a), flavonoids (b), and anthocyanins (c) contents of the investigated date cultivars. For each parameter, results with different letters are statistically different (ANOVA-LSD test, $P \leq 0.05$, $a > b > c > d > e$).

Four phenolic acids were globally determined in date extracts (gallic, caffeic, *P*-coumaric, and hydroxycinnamic acids). The statistical analysis showed a significant difference between cultivars, except for the Tamdjohert and Tanslit cultivars. Nevertheless, the content and composition of these acids varied from one cultivar to another. Caffeic acid is the only phenolic acid detected on the Tanslit and Tafzouine cultivars, hydroxycinnamic acid is the sole phenolic acid of the Litim cultivar, and three acids were identified in the two other cultivars (Takarmoust and Tamdjohert). The results of phenolic acids were averaged between 0.87 (Litim cultivar) and 15.64 ± 0.41 mg/100g DM (Takarmoust cultivar). Caffeic acid was the most prevalent compound of most studied cultivars. Comparing these with the results reported by Kchaou *et al.* (2016), *P*-coumaric acid was considered the most prevalent phenolic acid in the Tunisian date, in comparison to the Moroccan and Saudian date palm, where gallic acid was the most abundant (Bouhlali *et al.*, 2016; Hamad, 2014).

Table 1. Flavonoids and phenolic acids identified in date extracts (mg/100g DM).

Phenolic compound	<i>Tamdjohert</i>	<i>Tafzouine</i>	<i>Tanslit</i>	<i>Litim</i>	<i>Takarmoust</i>
Flavonoids					
Quercetin 3-glucoside	2.09±0.08	ND	ND	0.82±0.03	ND
Luteolin4'-O-glucoside	2.22±0.30	0.73±0.03	0.80±0.03	0.50±0.01	ND
Catechin	3.31±0.13	2.59±0.10	2.94±0.21	1.69±0.22	ND
Quercetin-7-O-hexoside-3-O-hyxoside	ND	1.66±0.06	3.36±0.13	ND	ND
Apigenin-7-O-glycosyl-2''-acetate	0.59±0.02	ND	ND	ND	ND
Total flavonoids	8.22±0.53 ^a	4.88±0.19 ^c	7.10±0.47 ^b	3.02±0.26 ^d	ND
Phenolic acids					
Gallic acid	ND	ND	ND	ND	2.82±0.11
Caffeic acid	0.41±0.02	1.40±0.04	3.13±0.12	ND	4.33±0.088
<i>P</i> -Coumaric acid	2.40±0.09	ND	ND	ND	8.48±0.33
Hydroxycinnamic acid	0.27±0.01	ND	ND	0.87±0.03	ND
Total phenolic acids	3.08±0.12 ^b	1.40±0.04 ^c	3.13±0.12 ^b	0.87±0.03 ^d	15.64±0.41 ^a

ND, not detected; Results of total flavonoids or total phenolic acids with different letters are statistically different (ANOVA-LSD test, $P \leq 0.05$, $a > b > c > d$).

Pharmacological properties

The results of the pharmacological properties (antioxidant, anti-inflammatory, and anti-diabetic activities) of the analyzed date cultivars were illustrated in Figure 2.

Antioxidant capacities

Evidence is accumulating, showing that most degenerative diseases that afflict humanity have their origin in deleterious free radical reactions (Florence, 1995), therefore a matrix with good antioxidant activity can prevent these diseases.

Ferric reducing power

Iron was important for the transport of oxygen and for the activity of certain enzymes. However, it is considered an initiator of lipids, proteins and other cellular components oxidations. Consequently, the reduction of Fe^{+3} was often used to study

the capacity of substances to transfer electrons. This property constitutes an important mechanism of antioxidant activity.

The results in Figure 2a showed that all extracts were active, with significant differences, except for the case of Litim and Takarmoust ($P \leq 0.05$) that displayed similar contents. The reducing power of different cultivars ranged from 43.76 to 113.52 ± 0.07 mg GAE/100g DM, and the highest reducing power was recorded for the Tanslit cultivar.

To the best of our knowledge, there is only Benmeddour *et al.* (2013), to whom we can compare our results, because they used the same unit of measurement and the same standard. The authors studied the reducing power of 10 different cultivars, including those we examined, and found that the reducing power varied from 272 to 1175 mg GAE/100g DM. These results are significantly higher than what we obtained. This can be explained by the qualitative and quantitative difference in the bioactive compounds of the different cultivars. Differences between cultivars could be due to the natures and concentrations of the extracted antioxidants.

DPPH scavenging activity

The scavenging of the DPPH radical is one of the most used methods for estimating the antioxidant activity of an extract. It was used to evaluate the ability of samples to donate hydrogen, and it was widely used to evaluate the antioxidant activity of phenolic compounds extracted from fruit, vegetables, and cereals.

Results in Figure 2b showed that the extracts from different cultivars exhibited interesting antiradical activities, with significant differences ($P \leq 0.05$) between the cultivars. The antiradical activities for all extracts were ranged from 9.70 to 40.96 ± 1.04 mg GAE/100g DM, the highest antioxidant capacity was recorded for *Takarmoust* cultivar.

We were unable to compare our results with those of other studies due to variations in the units of measurement and standards employed. Unlike most studies that estimate the inhibitory activity using the DPPH radical as a percentage or in Trolox equivalents, our investigation employed gallic acid as the standard. The observed differences in antioxidant capacity among cultivars can be attributed to multiple factors. A primary factor is the variation in the antioxidant content of different varieties; for instance, a higher content of phenolic compounds can enhance antioxidant effectiveness. Additionally, the concentration of DPPH used in the tests is crucial. An appropriate concentration of DPPH is essential for accurately measuring the antioxidants' ability to neutralize free radicals, which explains the variations observed in the results of antioxidant activity across different studies.

Hydroxyl radical scavenging activity

Hydroxyl radical is the most reactive free radical. It can be formed from superoxide anion and hydrogen peroxide in the presence of metal-ions such as copper or iron. The resulting radical can undergo further reactions, such as a reaction with oxygen to give peroxy radical or it can decompose to phenoxyl type radicals, through water elimination (Lee *et al.*, 2004). The extracts were analyzed for hydroxyl radical scavenging activity to better examine their antioxidant properties. Results in Figure 2c showed that all extracts were active against the radical OH, with significant

differences between all cultivars ($P \leq 0.05$). The antiradical activity of all extracts ranged from 17.65 to 110.29 ± 17.73 mg GAE/100g DM, where Litim cultivar exerted the highest antioxidant activity.

Antioxidant activity could be influenced by the difference of the antioxidant concentrations, which, in turn, was influenced by the varietal difference (Tomas-Barneran and Espin, 2001). In addition, generally, the antioxidant activity of phenolic compounds was affected by their chemical structures and the number and position of hydroxyl groups in the benzene rings (Moure *et al.*, 2001). Global antioxidant activity can be attributed to several compounds other than polyphenols, such as carotenoids, ascorbic acid, and vitamins. Due to differences in extraction conditions, analytical methods, and units of measurement, comparing results from different studies proves challenging.

Anti-inflammatory activity

Inflammation is a complex host response to injury. However, prolonged inflammatory causes several diseases, including cancer, arthritis, diabetes, and atherosclerosis (Ham and Moon 2013 and Woo *et al.*, 2014). NO^o (nitric oxide) is considered a mediator of inflammation; it is secreted to stimulate inflammatory cells (Labow *et al.*, 2001). Reducing the production of these inflammatory mediators is considered as an alternative to prevent or treat complications associated with diseases related to inflammatory processes (Castro *et al.*, 2014).

The anti-inflammatory properties of the extracts were determined by measuring the inhibition of nitric oxide, which was produced from sodium-nitroprusside. It interacts with oxygen to produce nitrite ion that can be estimated using Griess reagent. Scavengers of radical NO• compete with oxygen, leading to a reduction in the production of nitrite ions (Kumaran and Joel, 2006).

Results in Figure 2d showed that all extracts were active against the radical NO^o. Levels varied significantly ($P \leq 0.05$) between cultivars, with the anti-inflammatory activity of extracts ranging from 7.08 to 255.87 mg GAE/100g DM, and the highest inhibition being recorded in the Tamdjohert cultivar. This variation could be explained by the complex nature and varying amounts of compounds extracted from one cultivar to another.

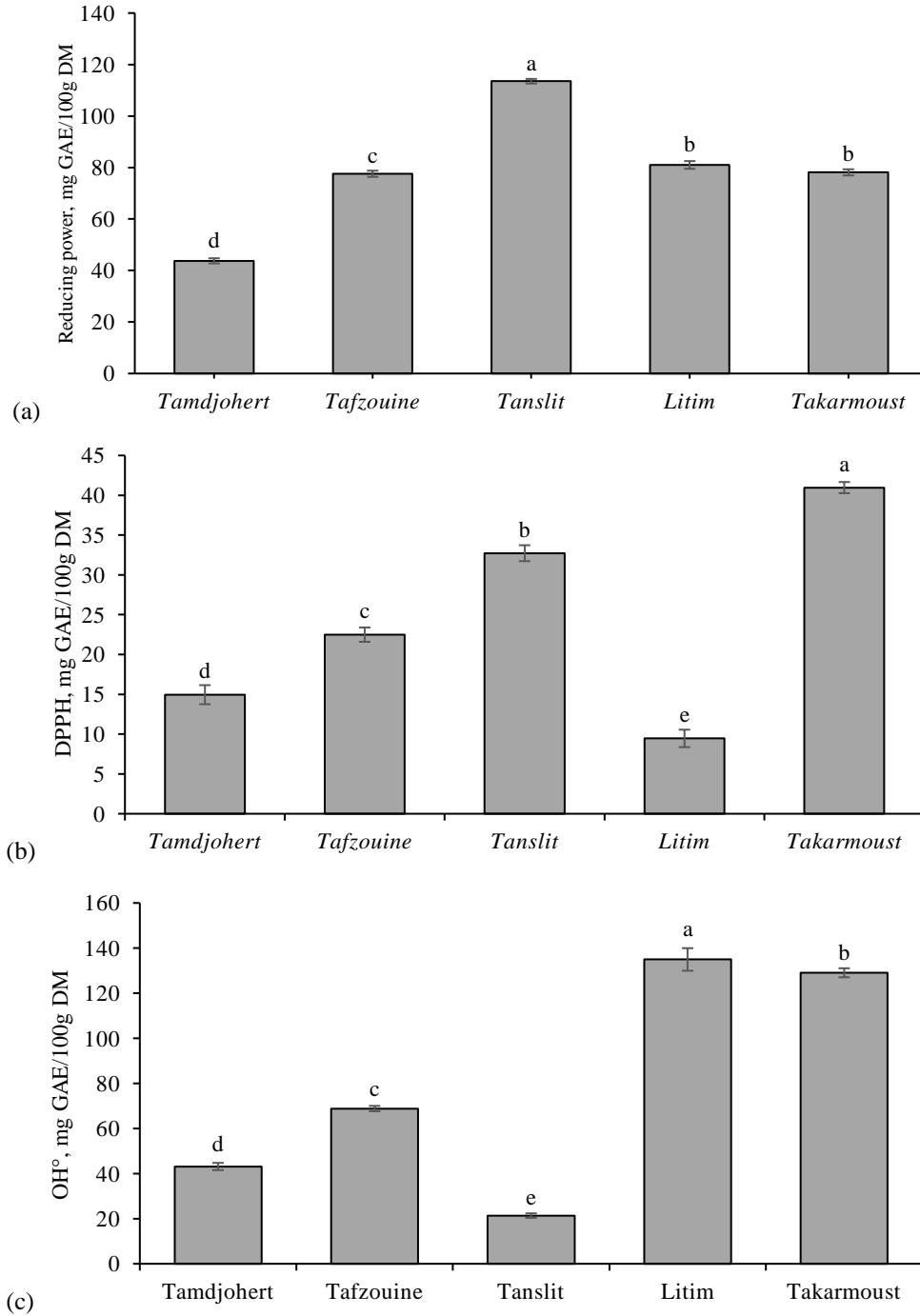
Antidiabetic activity

α -amylase is the key enzyme that hydrolyses starch to easily digestible molecules, which allows to increase the blood glucose level. It is believed that the inhibition of this enzyme can significantly decrease blood glucose level, and therefore it can be an important strategy in the management of hyperglycemia linked to type II diabetes (Kwon *et al.*, 2008).

Results in Figure 2e showed the potential of cultivars to inhibit α -amylase activity. The inhibition percentages changed between cultivars from 8.36 to 35.19%; the Litim cultivar exerts the best enzyme inhibitory activity. Statistical analysis indicated that α -amylase activities of all cultivars were different.

Flavonoid compounds present in plants were reported to inhibit α -amylase (Yao *et al.*, 2013) in particular, quercetin and its glycoside derivatives and rutin (Marrelli *et al.*, 2013; Wang *et al.*, 2010; You *et al.*, 2012). These findings reported in the

literature help explain the highest inhibition percentage for Tamdjohert cultivar, according to HPLC analysis. Differences between cultivars could be attributed to the nature and content of the extracted compounds.



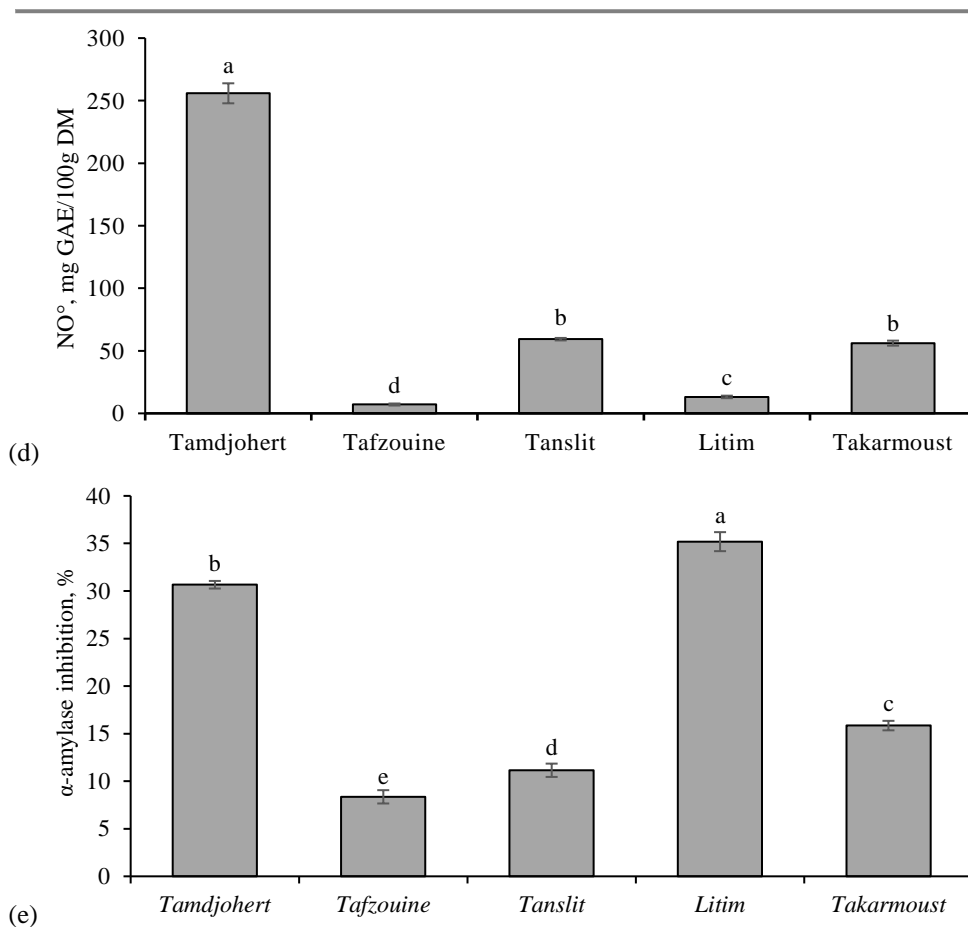


Figure 2. Antioxidant and anti-inflammatory activities of five date fruit cultivars: (a) reducing power, (b) DPPH scavenging activity, (c) hydroxyl radical scavenging (OH°), (d) anti-inflammatory activity (NO°), (e) anti-diabetic activity. For each parameter, results with different letters are statistically different (ANOVA-LSD test, $P \leq 0.05$, $a > b > c > d > e$).

A study conducted by Abdennabi *et al.* (2017) explored the inhibition of the α -amylase enzyme using saps from five different types of date palms. The promising results showed inhibition rates ranging from 35.47 to 88.72%. However, no study has specifically investigated α -amylase inhibition using the date fruit.

Conclusions

The results of the present investigation demonstrated that the five studied date cultivars can be considered to be good sources of phenolic compounds, flavonoids and anthocyanins. According to the HPLC profiles, nine compounds were identified and quantified, four phenolic acids and five flavonoids. Furthermore, the extracts were tested for pharmacological properties such as antioxidant, anti-inflammatory, and anti-diabetic properties. All extracts were found to possess potent antioxidant, anti-inflammatory and antidiabetic activities, which confirmed the functionality of

the compounds present in date fruits. So, considering that dates are relatively cheap, rich in nutrients and devoid of toxic effects, it is safe to suggest that their consumption should be recommended daily for better health, vitality and vigor.

Acknowledgments

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References

- Abdennabi, R., Gaboriaud, N., Ahluwalia, V., Tchoumtchoua, J., Elgheryeni, A., Skaltsounis, A. L., Gharsallah, N. 2017. Microwave-assisted extraction of phenolic compounds from date palm saps (*Phoenix dactylifera* L.) and their antioxidant, antidiabetic and antibacterial activities evaluation. *Mathews Journal of Diabetes & Obesity*, **2**(2), 1-6.
- Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M., Shahidi, F. 2005. Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. *Journal of Agricultural and Food Chemistry*, **53**, 7592–7599.
- Alhaider I.A., Mohamed M.E., Ahmed K.K.M. Kumar A.H.S. 2017. Date palm (*Phoenix dactylifera*) fruits as a potential cardioprotective agent: The Role of circulating progenitor cells. *Frontiers in Pharmacology*, **8**, 592.
- Al-Shahib, W., Marshall, R. J. 2003. The fruit of the date palm: its possible use as the best food for the future? *International journal of food sciences and nutrition*, **54**(4), 247-259.
- Bachir Bey, M., Louaileche, H. 2015. A comparative study of phytochemical profile and in vitro antioxidant activities of dark and light dried fig (*Ficus carica* L.) varieties. *Journal of Phytopharmacology*, **4**(1), 41-48.
- Balasundram, N., Sundram, K., Samman, S. 2006. Phenolic compounds in plants and industrial by-products: antioxidant activity occurrence, and potential uses. *Food Chemistry*, **99**, 191-203.
- Benmeddour, Z., Mehinagic, E., Le Meurlay, D., Louaileche, H. 2013. Phenolic composition and antioxidant capacities of ten Algerian date (*Phoenix dactylifera* L.) cultivars: a comparative study. *Journal of Functional Foods*, **5**, 346-354.
- Benkerrou, F., Bachir bey, M., Amrane, M., Louaileche, H. 2018. Ultrasonic-assisted extraction of total phenolic contents from *Phoenix dactylifera* and evaluation of antioxidant activity: statistical optimization of extraction process parameters. *Journal of Food Measurement and Characterization*, **12**(3), 1910–1916.
- Bouhlali, E.D.T., Bammou, M., Sellam, K., Benlyas, M., Alem, C., Filali-Zegzouti, Y. 2016. Evaluation of antioxidant potential of six Moroccan date fruit (*Phoenix dactylifera* L.) varieties. *Journal of King Saud University*, **28**, 136-142.
- Castro, J.P., Ocampo, Y.C., Franco, L.A. 2014. *In vivo* and *in vitro* anti-inflammatory activity of *Cryptostegia grandiflora* Roxb. ex R. Br. Leaves. *Journal of Biological Research*, **47**, 1-8.
- Conforti F., Statti G., Loizzo M.R., Sacchetti G., Poli F., Menichini F. 2005. *In vitro* antioxidant effect and inhibition of alpha-amylase of two varieties of *Amaranthus caudatus* seeds, *Biological and Pharmaceutical Bulletin*, **28**(6), 1098-1102.
- De Avellar, I. G., Magalhães, M. M., Silva, A. B., Souza, L. L., Leitão, A. C., Hermes-Lima, M. 2004. Reevaluating the role of 1, 10-phenanthroline in oxidative reactions involving

- ferrous ions and DNA damage. *Biochimica et Biophysica Acta (BBA)-General Subjects*, **1675**(1-3), 46-53.
- Florence, T.M. 1995. The role of free radicals in disease. *Australian and New Zealand Journal of Ophthalmology*, **23**(1), 3-7.
- Gao, H., Cheng, N., Zhou, J., Wang, B., Deng, J., Cao, W. 2014. Antioxidant activities and phenolic compounds of date plum persimmon (*Diospyros lotus* L.) fruits. *Journal of Food Science and Technology*, **51**(5), 950-956.
- Ghiaba, Z., Yousfi, M., Hadjadj, M., Saidi, M., Dakmouche, M. 2014. Study of antioxidant properties of five Algerian date (*Phoenix dactylifera* L.) cultivars by cyclic voltammetric technique. *International Journal of Electrochemical Science*, **9**, 909-920.
- Gholamian-Dehkordi, N., Luther, T., Asadi-Samani, M., Mahmoudian-Sani, M.R. 2017. An overview on natural antioxidants for oxidative stress reduction in cancers; a systematic review. *Immunology and pathology person*, **3**(2), e12.
- Goni, I., Serrano, J., Saura-Calixto, F. 2006. Bioaccessibility of α -carotene, lutein, and lycopene from fruits and vegetables. *Journal of Agricultural and Food Chemistry*, **54**, 5382–5387.
- Gorinstein, S., Cvikrova, M., Machackova, L., Haruenkit, R., Park, Y.S., Jung, S J., Yamamoto, K., Martinez Ayala, A L., Katrich, E., Trakhtenberg, S. 2004. Characterization of antioxidant compounds in Jaffa sweeties and white grapefruits. *Food Chemistry*, **84**, 503-510.
- Ham, M., Moon, A. 2013. Inflammatory and micro environmental factors involved in breast cancer progression. *Archives of Pharmacal Research*, **36**, 1419-1431.
- Hamad, I. 2014. Phenolic profile and antioxidant activity of Saudi date palm (*Phoenix dactylifera* L.) fruit of various cultivars. *Life Sciences Journal*, **11**(10), 1268-1271.
- Ismail, H.I., Chan, K.W., Mariod, A.A., Ismail, M. 2010. Phenolic content and antioxidant activity of cantaloupe (*Cucumis melo*) methanolic extracts. *Food Chemistry*, **119**, 643-647.
- 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 Chemistry*, **194**, 1048–1055.
- Khanavi, M., Saghari, Z., Mohammadirad, A., Khademi, R., Hadjiakhoondi, A., Abdollahi, M. 2009. Comparison of antioxidant activity and total phenols of some date varieties. *DARU*, **17**, 104-108.
- Kim, D.O., Joeng, S.W., Lee, C.Y. 2003. Antioxidant capacity of phenolic phyto-chemicals from various cultivars of plums. *Food Chemistry*, **81**, 321-326.
- Kumaran A, Joel K.R. 2006. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. *Food Chemistry*, **97**, 109–114.
- Kwon, Y.I., Apostolidis, E., Shetty, K. 2008. In vitro studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. *Bioresource Technology*, **99**, 2981–2988.
- Labow, R.S., Meek, E., Santerre, J.P. 2001. Model systems to assess the destructive potential of human neutrophils and monocyte-derived macrophages during the acute and chronic phases of inflammation. *Journal of Biomedical Materials Research*, **54**, 189-97.
- Lee, J., Koo, N., Min, D.B. 2004. Reactive oxygen species, aging, and oxidative nutraceuticals. *Comp Reviews in Food Science and Food Safety*, **3**, 21- 33.
- Li, Y., Jiang, B., Zhang, T., Mu, W., Liu, J. 2008. Antioxidant and free radical-scavenging activities of chickpea protein hydrolysate (CPH). *Food Chemistry*, **106**, 444–450.
- Louaileche, H., Hammiche, D., Hamoudi, F. 2015. Total phenolic, flavonoid contents and in vitro antioxidant of Algerian date palm varieties: a comparative study. *American Journal of Food Science and Health*, **3**, 63-68.

- Marrelli, M., Loizzo, M.R., Nicoletti, M., Menichini, F., Confort, F. 2013. Inhibition of key enzymes linked to obesity by preparations from Mediterranean dietary plants: effects on α -amylase and pancreatic lipase activities. *Plant Foods for Human Nutrition*, **68**, 340–346.
- Mehmood, T., Khan, Z.A., Karim, A., Shaheen, M.A., Akram, M., Afzal, A., Siddique, F. 2016. Variation in bioactive composition, antioxidant attributes and fatty acids profile of *Phoenix dactylifera* L. fruits in relation to different extraction solvents. *Pure and Applied Biology*, **5**(4), 996-1007.
- Mélo, E.A., Lima, V.L.A., Maciel, M.I.S., Caetano, A.C.S., Leal, F.L.L. 2006. Polyphenol, ascorbic acid and total carotenoid contents in common fruits and vegetables. *Brazilian Journal of Food Technology*, **9**(2), 89-94.
- Mohammedi, Z., Atik, F. 2011. Impact of solvent extraction type on total polyphenols content and biological activity from *Tomarix aphylla* (L.) Karst. *International Journal of Pharma and Bio Sciences*, **2**, 609-615.
- Molyneux, P. 2004. The use of the stable free radical diphenyl-picryl-hydrazyl DPPH for estimating antioxidant activity, *Songklamakarn Journal of Science and Technology*, **26**, 211-219.
- Moure, A., Cruz, J.M., Franco, D., Dominguez, J.M., Sineiro, J., Dominguez, H., Nunez, M.J., Parajo, J.C. 2001. Natural antioxidants from residual sources. *Food Chemistry*, **72**, 145- 171.
- Osman Khaled, A., Al-Humaid, A.I., Al-Redhaiman, K.N., El-Mergawi, R. 2014. A Safety methods for chlorpyrifos removal from date fruits and its relation with sugars, phenolics and antioxidant capacity of fruits. *Journal of Food Science and Technology*, **51**(9), 1762–1772.
- Ouchmoukh, S., Hachoud, S., Boudraham, H., Mokrani, A., Louaileche, H. 2012. Antioxidant activities of some dried fruits consumed in Algeria. *LWT - Food Science and Technology*, **49**, 329-332.
- Rechner, A R., Kroner, C. 2005. Anthocyanins and colonic metabolites of dietary polyphenols inhibit platelet function, *Thrombosis Research*, **116**(4), 327-334.
- Reyes-Gordillo, K., Shah, R., Muriel, P. 2017. Oxidative stress and inflammation in hepatic diseases: current and future therapy. *Oxidative Medicine and Cellular Longevity*, **2017**, 3140673.
- Singh, V., Guizani, N., Essa, M.M., Hakkim, F.L., Rahman, M.S. 2012. Comparative analysis of total phenolics, flavonoid content and antioxidant profile of different date varieties (*Phoenix dactylifera* L.) from sultanate of Oman. *International Food Research Journal*, **19** (3), 1063-1070.
- Singleton, V.L., Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Journal of Enology and Viticulture*, **16**(3), 144-158.
- Tomas-Barberan, F., Espin, J.C. 2001. Phenolic compounds and related enzymes as determinants of quality of fruits and vegetables. *Journal of the Science of Food and Agriculture*, **81**, 853-876.
- Vayalil, P.K. 2005. Antioxidant and anti-mutagenic properties aqueous extract of date fruit (*Phoenix dactylifera* L. Arecaeae). *Journal of Agricultural and Food Chemistry*, **50**, 610–617.
- Vijaya Kumar Reddy, C., Sreeramulu, D., Raghunath, M. 2010. Antioxidant activity of fresh and dry fruits commonly consumed in India. *Food Research International*, **43**(1), 285–288.
- Vijayalakshmi, M., Ruckmani, K. 2016. Ferric reducing antioxidant power assay in plant extract. *Bangladesh Journal of Pharmacology*, **11**(3), 570-572.

- Wang, H., Du, Y.J., Song, H.C. 2010. α -Glucosidase and α -amylase inhibitory activities of guava leaves. *Food Chemistry*, **123**, 6–13.
- Wang, L.S., Stoner, G.D., 2008. Anthocyanins and their role in cancer prevention. *Cancer Letters*, **269**, 281-290
- Wang, X., Li, C., Liang, D., Zou, Y., Li, P., Ma, F. 2015. Phenolic compounds and antioxidant activity in red-fleshed apples. *Journal of Functional Foods*, **18**, 1086–1094.
- Woo, Y., Jeong, D., Chung, D.H., Kim, H.Y. 2014. The roles of innate lymphoid cells in the development of asthma. *Immune Network*, **14**(1), 71-81.
- Yao, X., Zhu, L., Chen, Y., Tian, J., Wang, Y. 2013. *In vivo* and *in vitro* antioxidant activity and α -glucosidase, α -amylase inhibitory effects of flavonoids from *Cichorium glandulosum* seeds. *Food Chemistry*, **139**, 59–66.
- You, Q., Chen, F., Wang, X., Jiang, Y., Lin, S. 2012. Anti-diabetic activities of phenolic compounds in muscadine against α -glucosidase and pancreatic lipase. *LWT - Food Science and Technology*, **46**, 164–168.