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Phytochemical profile, antioxidant, antibacterial, antidiabetic and anti-obesity activities of fruits and pits from date palm (*Phoenix dactylifera* L.) grown in south of Tunisia

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Abstract
The aim of the present study is to determine polyphenolic composition and related biological activities of four Tunisian date palm varieties (Deglet Nour, Ruchdi, Ftimi, Kentichi). The highest total phenolic content values were obtained in methanol extracts of date pits. The potent antiradical activity was recorded in the methanol pits extracts which varied in the following order: Kentichi > Ruchdi > Ftimi > Deglet Nour. The antibacterial activity test demonstrated that date palm methanol extracts of both fruits and pits were effective in inhibiting growth of bacteria. In addition, our study demonstrated for the first time the potent inhibitory effect of pits from various date palm, especially Kentichi variety, against key enzymes related to diabetes and obesity. Hence, date palm pits can be considered as a potent natural source for high added value products which can be applied in agro-food, cosmetic and pharmaceutical industries as alternative to undesirable synthetic additives.

Keywords: date palm, antioxidant, antibacterial, antidiabetic, anti-obesity

1. Introduction
The date palm (*Phoenix dactylifera* L.) is usually considered as the most important source of food for human in most of the arid and semi-arid regions of the world. This plant species is a monocotyledonous, perennial tree that belongs to the Arecaceae family. Its culture is of high economic importance for almost all the arid and warm regions in the Middle East and North Africa [1]. The date palm is cultivated for its edible sweet fruit. It is a medium-sized plant, 15–25 m tall, growing singly or forming a clump with several stems from a single root system. The leaves are 4–6 m long, with spines on the petiole, and pinnate, with about 150 leaflets. The leaflets are 30 cm long and 2 cm wide. The full spans of the crown ranges from 6 to 10 m.
Date palm fruit contains 20-70 calories, depending on size and species [2] and a high percentage of carbohydrates (70%), most of them are in the form of sugars reaching 88% in some varieties [3, 4]. Date fruits are also rich in mineral salts and vitamins [5]. In addition they contain tocopherols, carotenoids and polyphenols such as flavonoids and tannins [6]. These compounds exhibit some biological properties especially antibacterial and antioxidant activities. Moreover, phenolic compounds can reduce the oxidative stress attributed to chronic, heart and neurodegenerative diseases, as well as cancers and diabetes [7, 8].
Date palm pits contain 3, 82% of non-reducing sugar, 1, 68% glucose and 1,53% fructose [9]. They are rich in protein (5,1g/100g), fat (9,0g/100g), dietary fiber (73,1g/100g) and phenolics (3942mg/100g) [3].
Recently, several research efforts, including those conducted on date palm, are underway on the utilization of natural source of bioactive compounds for the dietary management of certain diseases [10] essentially for the reason that many of the today's diseases are caused by the oxidative stress.
The antioxidant capacity and phenolic content of three varieties of date palm leaves, were studied by Kriaa *et al.* [11]. Their results showed that all the extracts exhibited antioxidant and radical-scavenging activities at different magnitudes and potency.
It was also established that date palm fruits and pits are endowed with several biological activities. Antioxidant activity of date fruit was demonstrated by Vayalil [12] on aqueous

extract, and also by Chaira *et al.* [13] who suggested that the high level of flavonoids in the Tunisian korkobbi variety was responsible for its high antiradical effect. Recently, antioxidant activity was also determined using different *in vitro* antioxidant essay system of date varieties from Sultanate of Oman [14] and from Iran [15].

Other studies have shown that date fruit extracts have an antifungal activity against *Candida albicans* [16, 17] and *Streptococcus pyogenes* [18]. Further research demonstrated that date fruit extract (pulp and pits) possess anti-inflammatory activity in different models study [19-23]. Date fruit extracts were also found to possess a gastrointestinal protective activity against different ulcerogens [24, 25]. A hapatoprotective activity [26] and a nephroprotective activity [27] have also been observed using date fruit extracts. Moreover, it was reported that date extract have an antiurolithiatic activity using in vivo preclinical experiments [28] and also an antidiarrheal activity in Wistar Rats [29].

On the other hand, pit acetone extract was found to have an antiviral activity against lytic *Pseudomonas* phage ATTC14209-B1 [30]. Date pits were also reported to possess a hepatoprotective activity when feeding rats with aqueous pits extract which significantly reduced CCL4-induced elevation in plasma enzyme and bilirubin concentration [31]. Pits extract also showed a cerebroprotective effect on focal cerebral Ischemia in male rats [32].

Accordingly, the objectives of this study were to investigate the phytochemical compositions of different organs (fruits and pits) of four Tunisian date palm varieties, as well as to evaluate their antioxidant, antibacterial, antidiabetic and anti-obesity activities.

2. Material and methods

2.1. Plant material

In this work, fruits and pits from four date palm varieties (Ruchdi, Deglet Nour, Kentichi and Ftimi) grown in the south of Tunisia (Tozeur) were studied. All these date palm organs were collected in the period from October to March.

2.2. Extraction

The different organs of the studied varieties were extracted three times by maceration with ethyl acetate and methanol at room temperature. The combined extracts were concentrated in vacu at 40 °C.

2.3. Phytochemical tests

Phytochemical tests for sterols and triterpens were carried out as described by Trease and Evans [33] and Sofowora [34]; each test was qualitatively expressed as negative (-) or positive (+).

2.4. Determination of total phenolic content

Total phenolic contents were assayed using the Folin-Ciocalteu reagent and gallic acid as a standard following Singleton's method slightly modified by Oktay *et al.* [35]. Folin-Ciocalteu reagent (0.5 ml) was added to a solution containing 1 mL of extract, with a concentration of 1 mg/ml and 3 ml of distilled water. The solution was mixed and after 3 min, 0.5 ml of 2% sodium carbonate solution was added. The mixture was left to incubate for 90 min, and the absorbance was measured at 760 nm. The total phenolic content was calculated by a standard gallic acid graph, and the results were expressed in mg of gallic acid equivalents per g of dry weight of extract (mg GAE/g). The assay was performed in triplicate for each extract.

2.5. Determination of total flavonoid content

The total flavonoid contents in the various extracts were determined according to Akrout *et al.* [36] using a method based on the formation of a complex flavonoid-aluminium, having the maximum absorbance at 430 nm. Quercetine was used to make the calibration curve. About 1 mL of diluted sample was mixed with 1 ml of 2% aluminium trichloride ($AlCl_3$) methanol solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm with a Shimadzu UVmin-1240 UV-Vis spectrophotometer and the total flavonoid content was expressed in mg quercetine equivalent (QE) per g of extract (mg QE/ g).

2.6. Antioxidant activity (DPPH radical scavenging method)

The DPPH radical scavenging effect was evaluated following the procedure described in a previous study [37]. Fifty microliters of various concentrations of the different extracts from date palm organs dissolved in methanol were added to 5 ml of a 0.004% methanol solution of 1.1-diphenyl 2-picrylhydrazyl (DPPH). After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical, DPPH, in percent (I%) was calculated in the following way:

$$I\% = (A_{blank}-A_{sample})/A_{blank} \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test extract), and A_{sample} is the absorbance of the test extract. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration [38]. The synthetic antioxidant reagent butylate hydroxytoluene (BHT) was used as positive control and all tests were carried out in triplicate.

2.7. Antibacterial activity

Test organisms: The strains used for the present study were *Pseudomonas aeroginosa*, *Bacillus cereus, Salmonella typhimurium, Klepsella pneumoni, Escherichia coli, Staphyloccocus aureus, Enterococcus faecalis* and *Micrococcus luteus.*

Agar disc diffusion method: The method of Brantner and Grein [39] and Ali-Shtayeh *et al.* [40] was adopted to determine the antibacterial activity of date palm fruits and pits extracts against the test organisms. The Muller Hinton Agar (MAA) was weighed and dissolved in 100 ml of distilled water (3.1 g/100 ml) in a sterile conical flask. The medium was sterilized by autoclaving and was cooled at room temperature.

The medium was poured into the sterile Petri plate. The disc was saturated with 20 µl of the extract and was allowed to dry. The disc was placed on MAA plate swabbed with the microorganisms culture. The plate was incubated overnight at 37 °C. The microbial growth was determined by measuring the diameters of inhibition zone. For each bacterial strain, controls were maintained where pure solvents were used instead of date extracts.

2.8. Alpha -amylase inhibition assay by CNPG3 method

The *in vitro* α-amylase inhibition activity of all date palm pits and fruits extracts was determined based on the spectrophotometric assay using acarbose as the reference

compound [8]. The sample was dissolved in Dimethyl sulfoxide (DMSO) to give three different concentrations (50, 100 and 200 µg/ml). The enzyme α-amylase solution was prepared by mixing of α-amylase in 100 ml of 40 mM phosphate buffer, pH 6.9. Positive control, acarbose was obtained by dissolving in phosphate buffer. The Assays were conducted by mixing 80 µl of the tested extract, 20 µl of α-amylase solution and 1ml of 2-chloro-4-nitrophenol-α-D-maltotrioside (CNPG3). The mixture was incubated at 37 °C for 5 minutes. The absorbance was measured at 405 nm spectrophotometrically (Jenway 6405 UV/Visible, Great Britain). Similarly, a control reaction was carried out without the extract/acarbose. Percentage inhibition (PI) was calculated by the expression:

$$PI = [(Absorbance_{Control} - Absorbance_{Test}) / Absorbance_{Control}] \times 100$$

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration. All tests were carried out in triplicate.

2.9. Pancreatic lipase *in vitro* assay

The method was modified from the assay reported by Nakai *et al*. [41], in which 4- methylumbelliferyl oleate (4-MU oleate) was used as a substrate to measure the pancreatic lipase inhibitory activity of all the date palm extracts. The sample was dissolved in DMSO to give three different concentrations (50, 100 and 200 µg/ml). The assay was conducted by mixing 50 µl of the pancreatic lipase solution (2 unit/ml) in a buffer consisting of 50 mmol/l Tris HCl (pH 8.0), 100 µL of diluted sample solutions and 50 µl of 0.5 mmol/l 4-MU solution dissolved in the above buffer in the well of a 96 micro well plate to start the enzyme reaction. The plate was immediately placed in the 37 °C pre-heating FL×800 micro plate fluorescence reader (Bio-Tek® Instruments, Inc, Winooski, VT) to measure the amount of 4-methylumbelliferone released by lipase every minute for 30 min at an excitation wavelength of 360 nm with a tolerance of ±40 nm and an emission wavelength of 455 nm with a tolerance of ±20 nm. The lipase inhibitive activity was determined by measuring the effect on the enzyme reaction rate after adding date palm extracts, compared with the control. Orlistat was used as positive control. Percentage inhibition (PI) was calculated by the expression:

$$PI = [(Absorbance_{Control} - Absorbance_{Test}) / Absorbance_{Control}] \times 100.$$

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration. All tests were carried out in triplicate.

2.10. Statistical analysis

All analyses were performed in triplicate and data were reported as means ± SD. Differences between experiments were analyzed using Student's t-test in Microsoft Excel 2000 (Microsoft Corporation, USA). The confidence limits used in this study were based on 95% (P <0.05).

3. Results and discussion

3.1. Phytochemical tests

Results of the phytochemical screening tests to sterols or triterpens on *Phoenix dactylifera* from 16 different extracts are shown in Table I. The sterols or triterpens test was found to be positive for all the tested extracts. High and medium contents were observed in ethyl acetate extracts of pits and fruits, respectively. However, low contents were observed in all methanol extracts. This result is obvious, since ethyl acetate is less polar than methanol and consequently it extracts the moderately polar products such as sterols and triterpens. In a previous study, it was reported that date palm fruits and pits contain sterols such as cholesterol, campesterol, stigmasterol, β-sitosterol and isofocusterolc [42].

Table I: Sterols and Triterpens tests

Extracts	Ethyl acetate extracts	Methanol extracts
Deglet Nour (fruits)	+	+-
Deglet Nour (pits)	++	+-
Ruchdi (fruits)	+	+-
Ruchdi (pits)	++	+-
Ftimi (fruits)	+	+-
Ftimi (pits)	++	+-
Kentichi (fruits)	+	+-
Kentichi (pits)	++	+-

++: high content; +: medium content; +-: low content

3.2. Determination of total phenol and total flavonoid contents

The total phenol content (TPC) in date palm extracts from pits and fruits was determined using the Folin-Ciocalteu assay, expressed as gallic acid equivalents (GAE). Table II shows significant difference ($p < 0.05$) in total phenol contents which varied from 18.05 to 344.15 mg GAE/g DW in ethyl acetate and methanol extracts, respectively, from Deglet Nour pits. The highest TPC values were obtained in methanol extracts of date pits which varied as follows: Deglet Nour > Kentichi > Ftimi > Ruchdi. The rest of TPC values were in the range of 18.05 to 80.37 mg GAE/g DW. The obtained TPC values are significantly higher if compared to other medicinal plants such as *G. multifolial* 12.36 mg GAE/g DW and *G. villosa* 20.81 mg GAE/g DW [43]. The TPC values obtained in our study are different from those reported in previous investigations [7, 44]. Various factors might be responsible for the observed differences such as variety, growing condition, maturity, season, geographic origin, fertilizer, soil type, storage conditions, cultural methods, process and stabilisation conditions, climatic conditions, use of different analytical methods and use of different phenolic acid standards and extraction solvents [45, 46]. On the other hand, table II shows that for each date palm organ, the TPC in methanolic extract is higher than that measured in ethyl acetate extract. This can be explained by the high polarity of methanol when compared with that of ethyl acetate. This result is in agreement with previous investigation who reported that polar solvents are among the most employed for polyphenols extraction [47]. Moreover, in our previous study we have demonstrated that the polyphenols extraction yield increases with increasing polarity of the extractant solvent [48].

Total flavonoid content (TFC) of date palm samples was measured using aluminum chloride colorimetric methods (table II). Results indicated that TFC varied in the range of 1.18 to 26.09 in terms of quercetine equivalents/g of DW of sample. The highest TFC values were measured in methanol extracts of date fruits which varied as follows: Ruchdi (26.09 mg QE/ g DW) > Ftimi (22.12 mg QE/ g DW) > Kentichi

(17.65 mg QE/ g DW) > Deglet Nour (15.15 mg QE/ g DW). It is well known that phenolic compounds and especially flavonoids are endowed with several biological activities such as antioxidant and antibacterial properties [49].

Table II: Total phenol and flavonoid contents in ethyl acetate and methanol extracts from the studied date palm fruits and pits.

varieties	Total phenol content (mg GAE/g DW)		Total flavonoid content (mg QE/ g DW)	
	ethyl acetate extracts	methanol extracts	ethyl acetate extracts	methanol extracts
Deglet Nour (fruits)	29.89±1.22	65.18±1.11	8.02±0.40	15.15±0.50
Deglet Nour (pits)	18.05±0.41	344.15±7.60	1.18±0.02	12.11±0.81
Ruchdi (fruits)	42.40±1.60	80.37±2.02	3.00±0.07	26.09±0.41
Ruchdi (pits)	60.15±1.70	235.34±0.71	5.8±0.5	12.10±0.63
Ftimi (fruits)	26.12±0.32	73.00±1.06	3.2±0.09	22.12±1.12
Ftimi (pits)	20.44±1.13	245.54±4.21	4.2±0.4	12.70±1.12
Kentichi (fruits)	62.62±1.70	74.51±3.10	3.4±0.7	17.65±0.84
Kentichi (pits)	65.05±2.01	322.22±5.43	5.4±0.6	14.50±1.53

3.3. DPPH radical-scavenging activity

1.1-diphenyl 2-picrylhydrazyl (DPPH) radical is a dye free radical. Due to its odd electron, DPPH radical gives a strong absorption band at 520 nm. The change in absorbance produced by reduced DPPH was used to evaluate the ability of fruits and pits from the different date palm varieties to act as free radical scavenger. The obtained results are shown in table III. Accordingly, all the tested date palm organs possessed radical scavenging activity on DPPH radical. Comparing with fruits extracts the highest antiradical activity indicated by lowest IC_{50} values was recorded in the pits extracts which varied in the following order: Kentichi > Ruchdi > Ftimi > Deglet Nour. Methanol extract from Kentichi pits is endowed with the powerful activity (IC_{50} = 31.05 µg/ml) which was comparable to that of BHT (IC_{50}= 12.80 µg/ml). On the other hand, for all the investigated date palm varieties, methanol extracts (IC_{50} between 31.05µg/ml and 123.41µg/ml) are more active than ethyl acetate extracts (IC_{50} between 40.21µg/ml and 184.24 µg/ml). This can be explained by the highest phenolic and flavonoid contents measured in the methanol extracts compared with the ethyl acetate ones. In previous studies, it was established that all the phenolic classes including flavonoids and ortho-diphenol compounds are endowed with interesting biological properties including free radical scavenging activity [50, 51]. The antioxidant activity of phenolics is mainly due to their redox properties which make them act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They may also have a metal chelating potential [52].

In addition, the antioxidant activity of date palm can be attributed to its antioxidant compounds, such as ascorbic acid, vitamin E, carotenoids and selenium [53, 54], and also to flavonoids, tannins and other phenolic constituents [55, 56]. Ferguson *et al.* [57] considered that the presence of diverse phenolic compounds and selenium have been responsible for the free radical scavenging and antioxidant effects.

Table III: Free radical-scavenging capacities of the date palm extracts measured in DPPH assay.

Date palm varieties	IC_{50} (µg/ml) of ethyl acetate extracts	IC_{50} (µg/ml) of methanol extracts
Deglet Nour (fruits)	130.21±0.01	107.41±0.03
Deglet Nour (pits)	85.31±0.21	68.13±0.04
Ruchdi (fruits)	142.21±0.12	123.41±0.15
Ruchdi (pits)	48.21±0.31	39.12±0.31
Ftimi (fruits)	104.31± 0.12	89.14±0.41
Ftimi (pits)	84.23±0.04	53.57±0.12
Kentichi (fruits)	184.24±0.03	123.21±0.14
Kentichi (pits)	40.21±0.03	31.05±0.34

3.4. Antibacterial activity

The results of the antibacterial assay (20 µg of crude extract per disc) against a range of microorganisms are presented in Tables IV and V. Most of the tested extracts showed variable inhibitory activities against different microorganisms.

The results obtained from Table IV showed that ethyl acetate extracts from date palm fruits didn't show any effect on inhibiting growth of all the test bacterial strains. While, ethyl acetate extracts from pits of Ftimi, Ruchdi and Deglet Nour possessed antibacterial activity against *B. cereus* (15 mm), *E. faecalis* (15 mm) and *M. leteus* (10 mm) for Ftimi variety; against *K. pneumoni* (8 mm) for Ruchdi variety and against *E. faecalis* (8 mm) for Deglet Nour variety. The antibacterial activity exhibited by Ftimi pits extracts is comparable to that of Ampicillin (AMP) against *B. cereus* (15 mm) and *E. faecalis* (16 mm). Ethyl acetate extract from kintichi variety was inactive against all tested strains.

On the other hand, methanol extracts exhibited the highest inhibitory activity against various microorganisms mainly Gram-positive and some Gram-negative microorganisms. Date palm methanol extracts (Table V) of both fruits and pits are the most effective in inhibiting growth of bacteria. The results indicated that methanolic extracts from fruits of Deglet Nour an Kintichi varieties revealed a strong activity against all the tested strains. However, methanol extracts of Ruchdi and Ftimi fruits didin't reveal any antibacterial activity. In addition, pits methanol extracts of all the date palm varieties studied exhibited antibacterial activity. Pits methanol extracts from Deglet Nour and Kintichi varieties were effective against all the tested microorganisms with important inhibition zone diameters. The pits methanolic extract of Ruchdi variety was effective in inhibiting growth of

S. typhimurium, K. pneumoni, S. aureus, E. faecalis and of M. lateus. However, only one of the tested strain, *E. faecalis*, was found to be a sensitive organism producing zone of growth inhibition of 12 mm using pits methanol extract of Ftimi variety.

Or findings demonstrated that the methanol extracts of date palm fruits and pits exhibited an important antibacterial activity against the tested organisms producing varying diameters of inhibition zones ranging from 11 to 17 mm which are comparable to those of Ampicillin.

Our result is in a good concordance with the study of Kahkashan *et al.* [58] who reported the antibacterial activity of *Phœnix dactylifera* pits extract against some selected Gram negative and Gram positive pathogenic bacteria. Their results revealed that methanol extracts showed good antibacterial

activity *against Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Shigella flexineri, Staphylococcus aureus* and *Staphylococcus pyogenies.* In addition, Jassim *et al*. [59] established that the date pit extracts show a strong ability to inhibit the infectivity of *Pseudomonas* phage ATCC14209-B1 and completely prevented bacterial lysis. Furthermore, other researchers demonstrated that antimicrobial activities of pits methanolic extracts of *Phœnix dactylifera* and *Annona squamosa* are quite effective as an antibacterial agent [60]. These authors precised that when both extracts were used in combination, they have shown strong synergistic effect against all the pathogens tested (*Escherichia coli, Salmonella typhi, Staphylococcus aeurus, Enterococcus faecalis, Pseudomonas aeruginosa, Salmonella paratyphi and Klebshiella pneumonia*) [60]. On the other hand, it was established that the antibacterial activity of plants methanolic extracts is due to their content in polyphénols. The antimicrobial action of phenolic compounds is well known and is related to their ability to denaturize proteins. They act by causing the leakage of cytoplasmic constituents such as proteins or minerals and testifying their ability to cross the cells wall. Polyphenols are also known to bind to the peptidoglycan leading to the breaking of the bacterial cell-wall integrity [61, 62].

Table IV: Antibacterial activity of ethyl acetate extracts: Diameter of inhibition zone (mm) of ethyl acetate extracts against the tested organisms. AMP: Ampicillin, DMSO: Dimethyl sulfoxide.

		Fruits				Pits				
	AMP	**Ruchdi**	**Deglet Nour**	**Ftimi**	**Kentichi**	**Kentichi**	**Deglet Nour**	**Ruchdi**	**Ftimi**	**DMSO**
Pseudomonas aeroginosa	20	-	-	-	-	-	-	-	-	-
Bacillus cereus	15	-	-	-	-	-	-	-	15	-
Salmonella typhimurium	16	-	-	-	-	-	-	-	-	-
Klepsella pneumoni	19	-	-	-	-	-	-	8	-	-
Escherichia. coli	15	-	-	-	-	-	-	-	-	-
Staphyloccocus aureus	10	-	-	-	-	-	-	-	-	-
Enterococcus faecalis	16	-	-	-	-	-	8	-	15	-
Micrococcus luteus	16	-	-	-	-	-	-	-	10	-

Table V: Antibacterial activity of methanol extracts: Diameter of inhibition zone (mm) of methanol extracts against the tested organisms. AMP: Ampicillin, DMSO: Dimethyl sulfoxide.

		Fruits				Pits				
	AMP	**Ruchdi**	**Deglet Nour**	**Ftimi**	**Kentichi**	**Kentichi**	**Deglet Nour**	**Ruchdi**	**Ftimi**	**DMSO**
Pseudomonas aeroginosa	20	-	15	-	13	12	13	-	-	-
Bacillus cereus	15	-	14	-	16	15	15	-	-	-
Salmonella typhimurium	16	-	13	-	13	14	14	13	-	-
Klepsella pneumoni	19	-	16	-	14	16	15	17	-	-
Escherichia.coli	15	-	12	-	13	15	15	-	-	-
Staphyloccocus aureus	10	-	15	-	12	14	13	11	-	-
Enterococcus faecalis	16	-	14	-	13	11	12	11	12	-
Micrococcus luteus	16	-	11	-	12	12	13	14	-	-

3.5. Alpha-amylase inhibitory assay *in vitro*

This assay evaluated the ability of date palm pits and fruits extracts to inhibit the activity of α-amylase, a digestive enzyme secreted from the pancreas and salivary gland. Pancreatic α-amylase is involved in the breakdown of starch into disaccharides and oligosaccharides and finally liberating glucose which is later absorbed into the blood circulation. Inhibition of α-amylase would diminish the breakdown of starch in the gastro-intestinal tract. Therefore, the postprandial hyperglycemia level may also be reduced [63]. Diabetes is characterized by high concentrations of blood sugar which can cause serious complications in the kidneys, eyes and cardiovascular system. The treatment of diabetes therefore mainly focuses on reducing fluctuations in blood sugar and subsequent complications. The α-amylase inhibitors are currently used for diabetic treatment as oral hypoglycemic agents. Acarbose is a commercially available α-amylase inhibitor for type II diabetes. However, it is reported to cause various side effects such as abdominal distention, flatulence and possibly diarrhea. Hence, searches for safe and effective inhibitors from natural sources are of emerging interest. Table VI indicates that except ethyl acetate and methanol extracts of Ruchdi and Ftimi fruits, all the other tested extracts showed an inhibition effect on α-amylase enzyme with IC_{50} ranging from 0.072 µg/ml (methanol extract of Kentichi pits) to 150.89 µg/ml (ethyl acetate extract of Deglet Nour fruits). For all active organs, the methanol extracts activity is more important than that of ethyl acetate extracts. This result can be explained by the high phenolic and flavonoid contents as well as the antioxidant potential of the methanol extracts from date palm organs. In this way, Shobana *et al*. [64] reported that the phenolic-rich plant extracts have high ability to inhibit α-amylase enzyme. In addition, many phenolic compounds and specially flavonoids have been reported as potential antidiabetic agents because they exert a good inhibiting action of α-amylase and could have potential prevention in diabetes mellitus as part of a dietary strategy [65]. On the other hand, the activity of ethyl acetate extracts can be due to their triterpenes and sterols contents. In a previous study, it was established that terpenes such as α-pinene might inhibited key enzymes related to type 2 diabetes principally α-amylase. It was reported that administration of terpenenes to diabetic exerts blood glucose lowering effect and high antioxidant activity in alloxan-induced diabetic rat [66].

Table VI shows also that the α-amylase inhibition activity of pits extracts is more important than that of fruits extracts. Comparing with Acarbose (IC_{50} = 15.92 µg/ml), pits methanol extracts from Kentichi (IC_{50} = 0.072 µg/ml), Ruchdi (IC_{50} = 0.54 µg/ml) and Deglet Nour (IC_{50} = 0.59 µg/ml) are endowed with a powerful activity. This result indicates that biological activities especially α-amylase inhibition activity depend on the plant variety.

3.6. Pancreatic lipase inhibitory activity assay *in vitro*

Actually, obesity is caused by excess caloric intake [67], and this can be remedied by inhibiting pancreatic lipase activity and by inhibiting or delaying lipid absorption [68]. The inhibitory activities towards pancreatic lipase of date palm pits and fruits extracts are reported in Table VI. Except extracts from date palm fruits, all the tested pits extracts were active against pancreatic lipase. Methanol pits extracts showed a relatively high anti-lipase activity comparing with that of ethyl acetate extracts. The activity of pits methanol extracts varied in following order: Kentichi (IC_{50} = 1.21 µg/ml) > Deglet Nour (IC_{50} = 12.11 µg/ml) > Ruchdi (IC_{50} = 18.10 µg/ml) > Ftimi (IC_{50} = 96.45 µg/ml). All the active extracts were less potent than Orlistat, a well-known anti-lipase agent, in inhibiting pancreatic lipase (IC_{50} = 0.92 µg/ml). The methanol extract from Kentichi pits is endowed with the potent lipase inhibition activity which is comparable to that of Orlisat. This drug is the only pancreatic lipase inhibitor currently approved for a long-term treatment of obesity. Our results suggest that Kentichi pits could be useful for prevention or treatment of obesity. The inhibitive capacities of pits from the tested date palm varieties against lipase activity might perfectly coincident with their total phenolics compounds. In fact, various natural products containing polyphenols have been reported to have anti-obesity by inhibiting lipase activity [69, 70]. In addition, McDougall *et al*. [71] have established that the inhibitory lipase activity might be derive from the phenolic compounds found in some medicinal plants such as gallic acid, catechin, epicatechin, ellagic acid myricetin, quercetin, kaempferol, resveratrol, and anthocyanin [71].

Table VI: Alpha-amylase and pancreatic lipase inhibition activities of ethyl acetate and methanol extracts from the studied date palm fruits and pits.

varieties	Alpha-amylase inhibition activity (IC_{50} µg/ml)		Pancreatic lipase inhibition activity (IC_{50} µg/ml)	
	ethyl acetate extracts	methanol extracts	ethyl acetate extracts	Methanol extracts
Deglet Nour (fruits)	150.89±3.22	70.18±2.11	nd	nd
Deglet Nour (pits)	45.4±0.6	0.59±0.03	123.10±0.63	12.11±0.81
Ruchdi (fruits)	nd	nd	nd	nd
Ruchdi (pits)	126.44±2.13	0.54±0.02	158.35±3.51	18.10±0.63
Ftimi (fruits)	nd	nd	nd	nd
Ftimi (pits)	135.4±1.6	95.54±2.21	186.70±3.12	96.45±1.52
Kentichi (fruits)	106.44±3.83	47.51±3.10	nd	nd
Kentichi (pits)	25.4±0.6	0.072±0.003	5.4±0.6	1.21±0.23

nd: not determined

4. Conclusion

Fruits and pits from four Tunisian date palm varieties were studied here in order to investigate their phytochemical compositions, as well as to evaluate their antioxidant, antibacterial, antidiabetic and anti-obesity activities. Ethyl acetate and methanol extracts were prepared from the different samples. Our results demonstrated that all the tested date palm organs possessed radical scavenging activity on DPPH radical. Comparing with fruits extracts the highest antiradical activity indicated by the lowest IC_{50} values was recorded in the pits extracts which varied in the following order: Kentichi > Ruchdi > Ftimi > Deglet Nour. Methanol extract from Kentichi pits was endowed with the powerful activity which was comparable to that of BHT. In addition, antibacterial activity experiment demonstrated a strong activity against all the test organisms when using pits methanol extracts from all the studied date palm varieties as well as fruits methanol extracts from Deglet Nour and Kentichi varieties. On the other hand, our study demonstrated for the first time the potent inhibitory effect of pits from various date palm varieties especially Kentichi variety against key enzymes related to diabetes and obesity. Therefore, date palm pits can be considered as a potent natural source for high added value products which can be applied in agro-food, cosmetic and pharmaceutical industries as alternative to undesirable synthetic additives. Our study is in progress in order to isolate and identify some bio-products from date palm pits as well as to evaluate their *in-vitro* and *in-vivo* biological activities.

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6. References

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