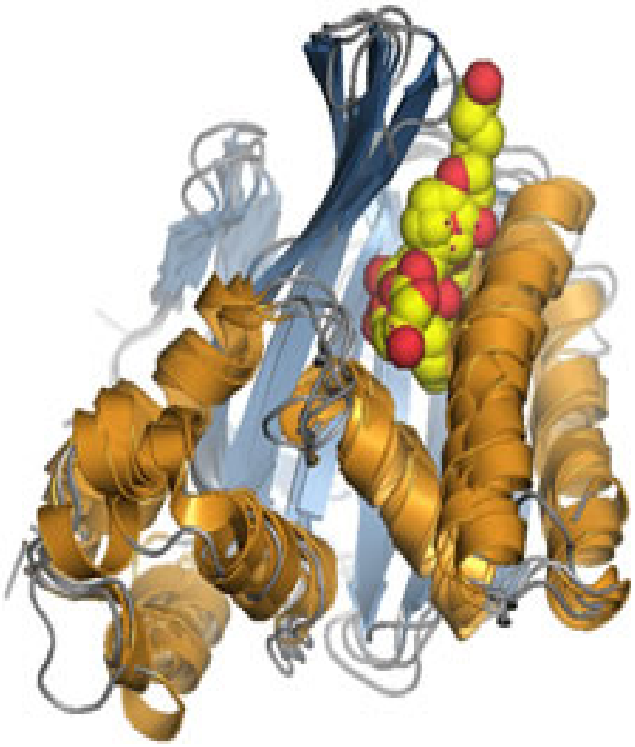
PhytoChem & BioSub Journal

Peer-reviewed research journal on Phytochemistry & Bioactives Substances

ISSN 2170 - 1768



PCBS Journal

Volume 8 N° 1, 2 & 3 2014

PhytoChem & BioSub Journal

Peer-reviewed research journal on Phytochemistry & Bioactives Substances

ISSN 2170 - 1768

PCBS Journal

PCBS Journal

Volume 8 N° 2 2014

Edition LPSO

Phytochemistry & Organic Synthesis Laboratory

http://www.pcbsj.webs.com , Email: phytochem07@yahoo.fr

PhytoChem & BioSub Journal Vol. 8(2) 2014
ISSN 2170-1768
CAS-CODEN:PBJHB3

PCBS Journal
ISSN 2170-1768

PhytoChem & BioSub Journal

2014
Vol. 8 No. 2

Total phenolic contents, radical scavenging and cyclic voltammetry of three native Date (*Phoenix Dactylifera* L.) varieties grown in Ouargla

Zineb Ghiaba[a,*], Mohamed Yousfi [b], Mokhtar Saidi[a], Mohamed Hadjadj [a] & Messaouda Dakmouche [a]

[a] *Laboratoire Valorisation et Promotion des Ressources Sahariennes, Université Kasdi Merbah, BP 511 route de Ghardaia.30000 Ouargla, Algérie.*
[b] *Laboratoire des Sciences Fondamentales, Université Amar Telidji de Laghouat, route de Ghardaia, BP 37G, Laghouat 03000, Algérie.*

Received: December 24, 2013; Accepted: April 20, 2014
Corresponding author Email *ghiaba.zi@univ-ouargla.dz*

DOI:10.163.pcbsj/2014.8.2.70

Abstract. *Fruits of date palm (Phoenix dactylifera L.) are consumed throughout the world and are a vital component of the diet in most Arabian countries. This study has been carried out to evaluate the total phenolic content and the antioxidant activity of three date palm fruit varieties grown in Ouargla (Algeria): Degla Baidha (DB), Tamjhourt (Tam), and Tefezauine (Tef). Consequently, the total phenolic contents (TPC) of these extracts will be measured using Folin Ciocalteu spectrophotometric method. TPC ranged from 9.496 to 23.045mg gallic acid equivalents (GAE/100g). Thereafter, the antioxidant properties of these polyphenols were evaluated by chemical and electrochemical assays. Hydroxyl radical (·OH) scavenging activity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity were the chemical assays, whereas, cyclic voltammetry technique (CV) in aprotic media was used as the electrochemical assay. This study suggest that Algerian date palm fruit may serve as a good source of natural antioxidants, may be used in the prevention of various free radicals related diseases.*

***Key Words*:** Algeria date palm fruit; Total phenolic content; Antioxidant activity; DPPH; Cyclic voltammetry

1. Introduction

The formation of reactive species (RS) is a natural consequence of aerobic metabolism and is associated with oxygen homeostasis, i.e. the balance between constitutive oxidants and antioxidants [1]. Reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), peroxyl radical ($ROO^{\bullet}$) and nitric oxide radical ($NO^{\bullet}$), attack biological molecules, such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury associated with aging, atherosclerosis, carcinogenesis [2] and may lead to the development of chronic diseases related to the cardio and cerebrovascular systems [3]. The term 'antioxidant' refers to the activity of numerous vitamins, minerals and phytochemicals which provide

protection against the damage caused by ROS [4]. Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors [5].
Dates, fruits of the date palm (*Phoenix dactylifera* L.) are a main source of staple food in arid and semi arid regions of North Africa, middle east and South-Asian countries. Dates have always played an important role in the economic and social lives of people of this area. Algeria is ranking the fifth world producer of date palm fruits with production about 710, 000 tons occupying an area of 170000 hectares [6] .There are over 58 different varieties of dates in region of Ouargla [7]. Moreover, recent Studies have shown that date fruits are an excellent source of phenolics and therefore possess an extremely high antioxidant capacity. Dates have potent anthocyanins, carotenoids, and phenolics compounds (protocaechuic, p. hydroxy benzoic, vanillic, syringic, caffeic, coumaric, ferulic, hydroxy benzoic, mainly cinnamic acids) and flavonoids (flavones, flavonols and flavanones), As of today, dates also have the unique distinction of being the only food to contain flavonoid sulfates, which like most other fruits, have antioxidant properties [8-20].
Although the considerable importance of antioxidants, there is not a unique method or protocol for determination of antioxidant capacity (ORAC, FRAP, TEAC. . .) [21-26]. Therefore, results obtained from all these methods are not constantly compatible and materials used for these analyses are costly (AAPH, ABTS, DPPH. . .) [27]. Electrochemistry approaches are also of special advantage in evaluation of antioxidant properties regarding the reducing capacity of a substrate by measuring the oxidation potential of the substrates, the studies on this subject has been reviewed recently [28].
In this study, we have investigated the in vitro antioxidant activity of three date palm fruit varieties grown in Ouargla (Algeria) (DB, Tam and Tef, respectively). We used the following three assay systems: (1) Hydroxyl radical (˙OH) scavenging activity , (2) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and (3) cyclic voltammetry assay. Total polyphenol contents of DPF were determined by standard colourimetric methods.

2. Material and methods

2.1. Chemicals
Commercially available chemicals were used without any further purification. The *N*,*N* dimethylformamide extra dry, and the tetrabutylammonium hexafluoropho- sphate Bu_4NPF_6 of electrochemical grade were purchased from Fluka. Folin-Ciocalteus's phenol reagent, sodium carbonate, Gallic acid (GA),Ascorbic acid (AA), DPPH, All other chemicals and solvents were of the highest commercial grade and used without further purification.

2.2. Plant material
Three different Algerian ripe date palm fruit (DPF) varieties, Degla Baidha variety(DB), Tamjhourt variety(Tam), Tefezauine variety(Tef), fruits were collected from the Ouargla region (Algeria), in autumn 2010. The fruit were segmented and their seeds were carefully removed and stored in paper bags in refrigerator. The different varieties were identified within the Agronomic National Institute of Ouargla.

2.3. Sample preparation and extraction
Forty grams of date fruit were pitted, crushed and cut to small pieces with a sharp knife and blended for 3 min. The phenolics from samples were isolated by a modified version of the method described by Djerridane et al [29]. Each sample was macerated in 100 ml methanol:water (80:20, v/v) for 48 h at room temperature. After filtration, the alcohol is

removed under vacuum at 40 °C. Then, the Phenolic compounds were extracted three times with ethyl acetate (1:1, v/v) in the presence of an aqueous solution containing 20% ammonium sulphate and 2% of ortho-phosphoric acid solution. The three organic phases were combined; the residual water in the ethyl acetate was eliminated with anhydrous sodium sulphate, and then evaporated to dryness using a rotary evaporator. The extracted phenolics were dissolved in DMF and then filtered using filter paper. The solutions of phenolic were stored inside freezer until analysis. The storage conditions (time and temperature) were the same for all types of fruit.

2.4. Determination of total phenolic content

Total polyphenol contents of the extracts of Algerian ripe date palm fruit were determined by Folin-Ciocalteu reagent [30]. About 0.1 ml of each extract was separately mixed with Folin-Ciocalteu reagent (0.5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (2 ml, 20%). The mixture was allowed to stand for 30 min at room temperature. The absorbance of the reaction mixture was measured at 760 nm using a UV–visible spectrophotometer (SpectroScan 80D/80DV). Total phenolic content was expressed as mg/100g gallic acid equivalent using the following equation based on the calibration curve:

$$y = 3.08x,\ R^2 = 0.993,\ \text{where x was the absorbance.}$$

2.5. Antioxidant activity assays

2.5.1. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of samples was measured according to the method of Jin et al [31]. with some modifications. In this system, hydroxyl radicals were generated by the Fenton reaction. Hydroxyl radicals could oxidize Fe^{2+} into $Fe^{3+,}$ and only Fe^{2+} could be combined with 1, 10-phenanthroline to form a red compound (1,10-phenanthroline-Fe^{2+}) with the maximum absorbance at 536 nm. The concentration of hydroxyl radical was reflected by the degree of decolourization of the reaction solution. Briefly, 1, 10-phenanthroline solution (1.0 ml, 1.865 × 10-3 mol/l), phosphate buffer saline (2.0 ml, 0.2 mol/l, pH 7.40), and samples (1.0 ml, 30 μg/ml) were added into a screw-capped tube orderly and mixed homogeneously. The $FeSO_4.7H_2O$ solution (1ml, 1.865×10^{-3} mol/l) was then pipetted into the mixture. The reaction was initiated by adding 1ml H_2O_2 (0.03% v/v). After incubation at 37°C for 60 min in a water bath, the absorbance of reaction mixture was measured at 536 nm against reagent blank. The reaction mixture without any antioxidant was used as the negative control, and without H_2O_2 was used as the blank. The hydroxyl radical scavenging activity (HRSA) was calculated by the following formula:

$$\text{HRSA (\%)} = [(\text{As-An})/(\text{Ab-An})] \times 100$$

Where As, An, and Ab were the absorbance values determined at536nm of the sample, the negative control, and the blank after reaction, respectively. AA and GA were used as positive controls.

2.5.2. DPPH radical scavenging activity assay

Radical scavenging activity of DPF extracts against stable DPPH. (2,2-diphenyl-1-picrylhydrazyl hydrate) was determined using the method of Brand-Williams et al. [32] modified by Djeridane et al. [33]. This method was determined spectrophotometrically. When DPPH_ reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The solution of DPPH in methanol (500mM) was prepared daily, before the measurements.

Various concentrations of 1ml of sample solution diluted in Tris buffer solution (100mM; pH 7.4) were added to 1ml of the DPPH$^{\bullet}$ radical solution. The mixture was then shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The decrease in absorption was measured at 517nm. Absorption of a blank sample containing the same amount of buffer and DPPH solution was prepared and measured daily. The antioxidant activity of the extract was expressed as an IC_{50} value defined as the concentration (mg/l) of the extract that inhibited the formation of DPPH radicals by 50%.

2.5.3. Cyclic voltammetry assay

Cyclic voltammetric measurements were performed using a Voltalab 40 model PGZ301 (Radiometer Analytical) potentiostat/galvanostat driven by a personal computer with Volta Master 4 software. The electrochemical cell (V=10 ml) consists of three electrodes immersed in a solution containing the analyte and an excess of supporting electrolyte. A saturated calomel electrode (SCE) was used as the reference electrode, a platinum wire as the auxiliary electrode, and a glassy carbon electrode (Ø_3.0 mm) as the working electrode respectively. Prior to use, the working electrode was polished daily with silicon carbide 4000 paper in, then rinsed with distilled water, and dried with a dry tissue paper. This cleaning procedure was applied always before any electrochemical measurements. All experiments were conducted at ambient laboratory temperature (28°C). Potentials were measured with respect to a saturated calomel electrode.

To estimate the total antioxidant capacity of the DPF extracts, cyclic voltammetry was used the method of Bourvellec, et al. [34] with slight modification. The effect of various extracts was checked by the method of the proportioned additions and the successive addition of 100µl of initial solution of extract to the 10ml oxygen solution in order to get an antioxidant substrate concentration in the range (0-0.083g/l). After each aliquot addition, CV of the oxygen solution was recorded at a scan rate $0.1Vs^{-1}$.The total antioxidant activity of DPF extracts determined in comparison with gallic acid (GA) and ascorbic acid (AA). The capability of scavenging on superoxide radical was calculated using the following equation:

$$\text{Scavenging effect } (\%) = (I^{0}_{p_a} - I^{s}_{p_a}) \times 100 / I^{0}_{p_a}$$

Where $I^{0}_{p_a}$ and $I^{s}_{p_a}$ are the anodic peak current of $O_2^{\bullet-}$ oxidation with and without the DPF extracts.

All determinations were carried out by means of software Origin Pr8 the data analysis and graphing workspace.

3. Results and discussion

3.1. Total phenolic content (TPC)

The amount of TPC varied widely in the DPF extracts investigated and ranged from 9.496 to 23.0454mg GAE /100g DW (Table1.). Among extracts, extremely high TPC was detected in Tam variety (23.0454mg GAE/100g DW). The lowest TPC was detected in Tef variety (9.496 mg GAE/100g DW). The order of TPC in DPF extracts is: Tam>DB>Tef. The concentration of polyphenols in this study was lower compared to study of Ghiaba et al. [8] for the same varieties and using a similar measuring technique. They found that total phenolic content ranged from 41.80 to 84.73 mg gallic acid equivalents (GAE)/100g DW. This is possibly due to harvest season, growing conditions and environmental conditions. Respectively, the present results were much higher compared to those reported by Mansouri et al. [17] who found that TP content of methanolic extracts of seven Algerian date fruits from Ghardaia varied from

2.49 to 8.36mg GAE/100g FW. However, Zahia Benmeddour et al. [35] reported that TPC values were ranged from 226 to955mg GAE/100g DW and 167 to 709mg GAE/100g FW in ten Algerian date from Tolga (Biskra). The observed differences may mainly be attributed to the cultivars and extraction conditions such as solvent and ratio material/solvent.

Table. 1. Total phenolic content (TPC), IC_{50} values of DPF extracts against superoxide anion radical

Sample	TPC[a]	Antioxidant activity IC_{50}mg/l		
		Hydroxyl radical scavenging activity	DPPH radical scavenging activity	Superoxide anion radical scavenging
DB	14.814	0,0430	16.77	66.3296
Tam	23.0454	0,0379	14.48	70.62
Taf	9.496	0,0274	21.27	33.1677
GA	-	-	-	119.2067
AA	-	0,0305	-	102.0502

3.2. Hydroxyl radical scavenging activity

The hydroxyl radical is one of representative reactive oxygen species generated in the body. In this study, hydroxyl radical-scavenging activity was investigated on the DPF extracts using the Fenton reaction mechanism (Table 1.). In Fig. 1, all extracts exhibited good hydroxyl radical scavenging ability. Effective scavenging concentration (IC_{50}) on Hydroxyl radical decreased in the order of DB>AA>Tam>Tef. Hydroxyl radicals are known to be capable of abstracting hydrogen atoms from membranes and bring about peroxidic reactions of lipids [36]. Based on the evidence, we hypothesized the extracts from DPF would show antioxidant effects against lipid peroxidation on biomembranes and scavenge the hydroxyl radicals at the stage of initiation and termination of peroxy radicals.

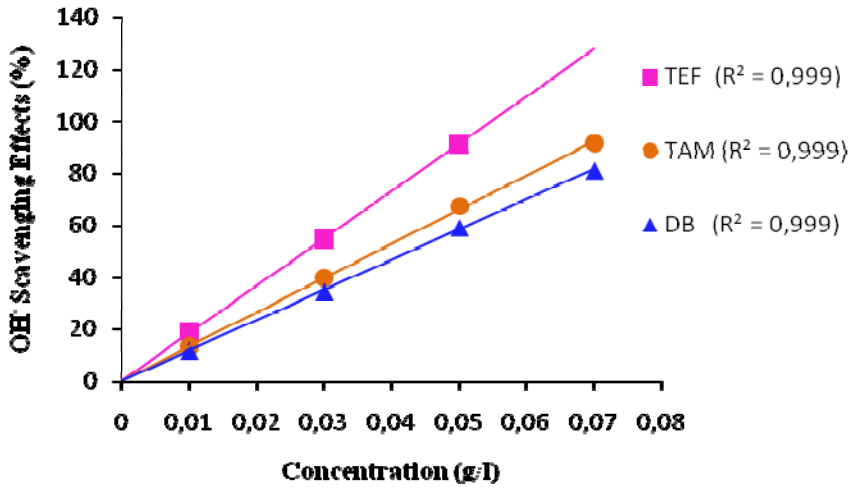


Fig. 1. Concentration–response plots for inhibition of the absorbance of hydroxyl radical at 536 nm for DPF extracts

3.3. DPPH radical scavenging activity

DPPH$^{\bullet}$ radical is a stable lipophilic free radical which has been generally used for estimating antioxidant activity of food and medicine materials [37]. DPPH$^{\bullet}$ is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [38,39]. The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radial by hydrogen donation. It is visually noticeable as a colour change from purple to yellow [40,41]. In the current study, DPPH percent scavenging activities of plant crude extracts were measured in different concentrations ranging between 0.0042 g/l and 0.0295 g/l and results are given for DPPH percent radical scavenging activity (% RSA) versus extract concentrations in g/l in Fig. 2. The scavenging effect was increased with increasing concentration. Table. 1. shows the comparative data of DPPH radical scavenging activity, as determined by the IC_{50} values (the concentration required to inhibit radical formation by 50% and was obtained from interpolation from linear regression analysis) of the different DPF. Highest activity was found in Tef, followed by DB, and the lowest activity was found in Tam.

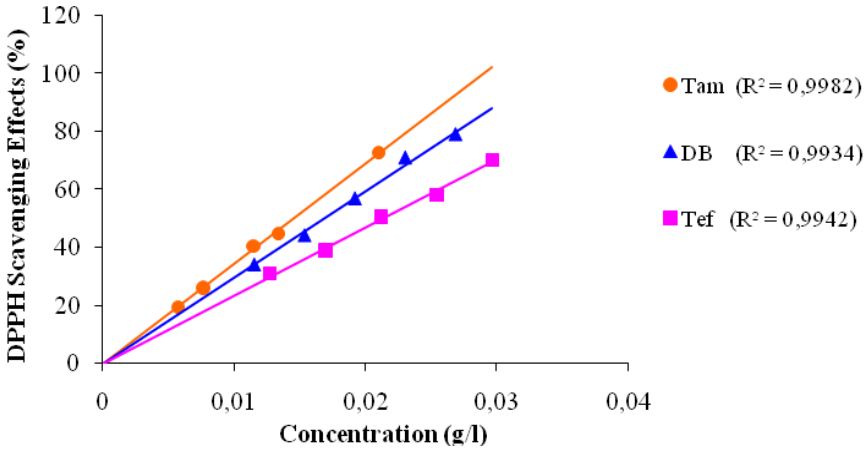


Fig. 2. Concentration–response plots for inhibition of the absorbance of DPPH radical at 517 nm for DPF extracts

These reports indicated that the radical scavenging capacity of extracts might be mostly affected by the presence and position of phenolic hydroxyl group. The radical scavenging activity of phenolic compounds depends on their molecular structure, that is, on the availability of phenolic hydrogens and on the possibility for stabilization of the resulting phenoxyl radicals formed by hydrogen donation [42,43]. From these results, DPPH radical scavenging ability of all solvent extracts from ginseng leaves were related to various amount of antioxidants.

3.3. Cyclic voltammetry assay

The obtained results (Fig. 3.) show that in all the five cases the addition of the extract causes a proportional decrease of $O_2^{\bullet -}$ anodic peak current ($I^{s}_{p_a}$) while the intensity of O_2 cathodic current appears to be negligible. in the case of DB (Fig. 3. a). The decrease of the anodic peak current of $O_2^{\bullet -}$ suggests that the polyphenol substrate reacts irreversibly with $O_2^{\bullet -}$. For each antioxidant compound, a series of $I^{s}_{p_a}$ values is determined from the CVs recorded for increasing antioxidant concentrations (Fig. 3). All antioxidant substrates exhibited a similar effect upon the O_2 reduction. The scavenging activity of the antioxidant is often evaluated

according to its IC_{50}, it is defined by the concentration inhibiting the reaction by 50%. In this system, which were calculated from the linear regression of the % antioxidant activity versus extracts concentrations. Results shown in table. 1. Lower values correspond to higher antioxidant activities. It can be seen from this table all the IC_{50} values of DPF extracts showed an antioxidant capacity higher than the corresponding gallic acid (GA) and ascorbic acid (AA). The IC_{50} values of DPF extracts ranged from 33.1677 to 70.62mg/l. The lowest value of IC_{50} (33.1677 mg/l) was detected in Tef variety and it corresponds to the highest antioxidant activity; while the highest value of IC_{50} (70.62mg/l) was detected in Tam variety. The antioxidant activity in the extracts of DPF decreases in the order Tef>DB>Tam.

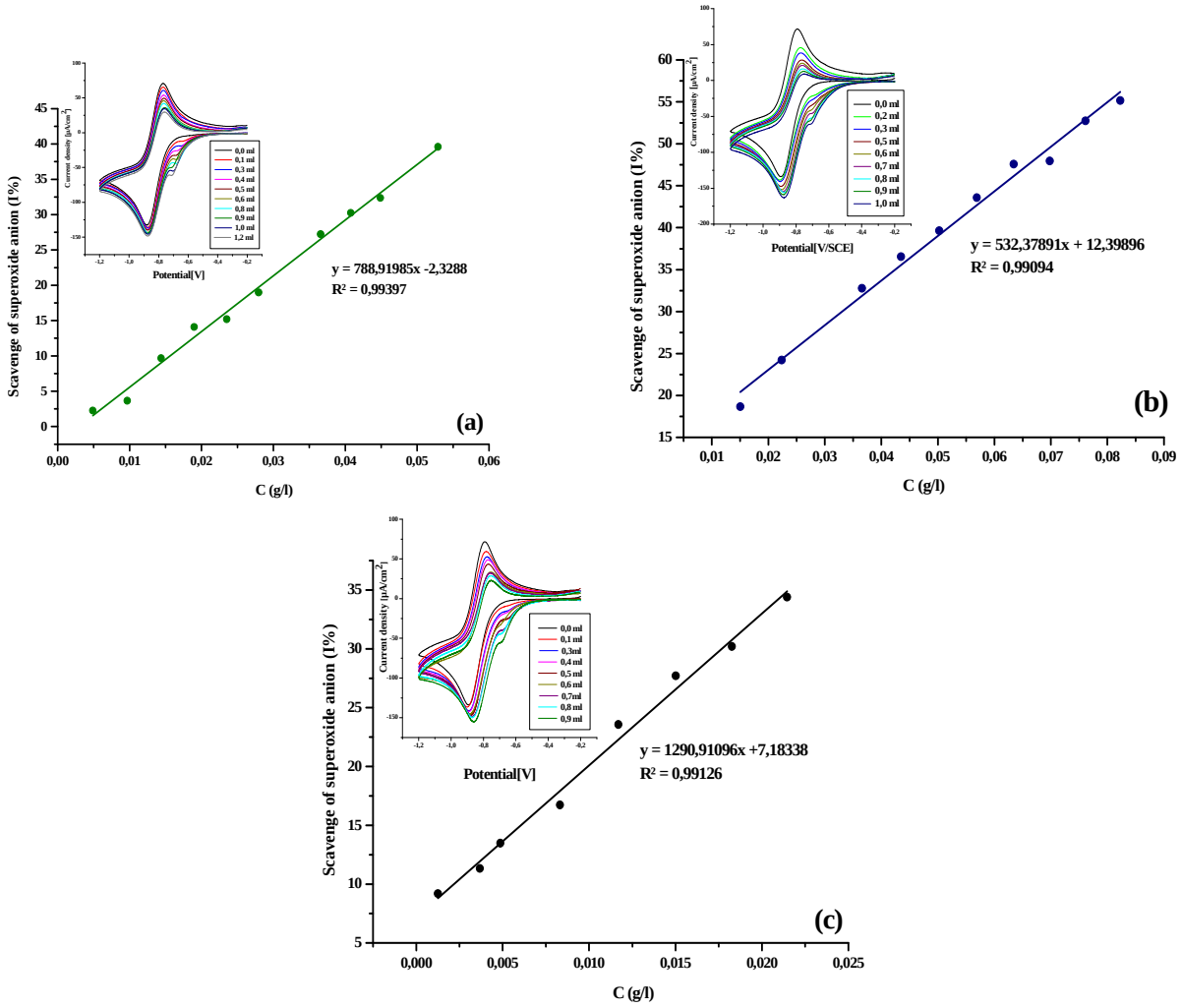


Fig. 3. Plotting of scavenging of superoxide anion of cyclic voltammogram against the corresponding concentration of DB (a), Tam (b), Tef (c). Operative condition: DMF + 0.1M Bu_4NPF_6 on GC as working electrode vs. SCE at 28°C with scan rate of 0.1 V/s 1.

4. Conclusions

All the assays confirmed the good antioxidant potential of the Three date palm fruit (DPF) varieties, Degla Baidha (DB), Tamjhourt (Tam), and Tefezauine (Tef). The proposed

electrochemical protocol is easy, fast and showed a good precision and a high sample throughout. The results suggested that the DPF has important benefits to human health, and could serve as a source of antioxidants or nutraceuticals with potential applications.

Acknowledgements
This material is based upon work supported by a grant from University Kasdi Merbah (UKM) , University Amar Telidji (UAT).

References
[1]. Seifried, D.E., Anderson, E.I., Fisher, J.A., AND Milner, A., 2007: Review of the interaction among dietary antioxidants and reactive oxygen species. *J Nutr Biochem.*18: 567–579.
[2]. Keli-chen; Geoff W Plumb; Richard N Bennett; Youngping Bao. 2005: Antioxidant activities ofextracts from five anti-viral medicinal plants. *Journal of Ethnopharmacology*. 96: 201-205.
[3]. Halliwell, B., Gutteridge, J.M.C., 1989: Free radicals in Biology and Medicine Clarendon Press,Oxford, 23-30.
[4]. Khilfi, S., Hachimi, E., Khalil, A., Es-Safi, A., Belahyam, A., Tellal, A., El Abbouyi, A., 2006: *In-vitro* antioxidant properties of *Salvia verbenaca*. L. hydromethanolic extract. *Indian Journal of Pharmacology*. 38: 276-280.
[5]. Gulcin, I., Alici, H.A., Cesur, M., 2005: Determination of *in- vitro* antioxidant and radical scavenging activities of propofol. *Pharmacology Bulletin*. 53: 281-285.
[6]. FAO. Statistical Databases (2013): www.FAO.org Accessed 20.01.2013.
[7]. Acourene, S., Allam, A., Taleb, B., Tama, M., 2007: Inventory of the different date palm (Phoenix dactylifera) cultivars in the regions of Oued-Righ and Oued-Souf (Algeria). *Sécheresse*. 18:135-142.
[8]. Ghiaba, Z., Boukouada, M., Djeridane, A., Saidi, M.; Yousfi, M., 2012: Screening of Antioxidant Activity and Phenolic Compounds of Various Date Palm (Phoenix Dactylifera) Fruits From Algeria. *Mediter J Nutr Metab*. 5: 119-126.
[9]. Ghiaba, Z., Boukouada, M., Saïdi, M., Yousfi, M., Ghiaba, N., Kendour, Z. Comparison of Antioxidant Activity and Phenolic Content of Three Varieties of Algerian Dates. 2012: *Algerian journal of arid environment*. 2: 42-48.
[10] Al-Turki, S., Shahba, M.A., and Stushnoff, C., 2010: Diversity of antioxidant properties and phenolic content of date palm (*Phoenix dactylifera L.*) fruits as affected by cultivar and location. *J. Food, Agric & Envir*. 8: 253-260.
[11]. Biglari, F., AlKarkhi, A.F.M., Mat, E.A., 2009: Cluster analysis of antioxidant compounds in dates (*Phoenix dactylifera*): effect of long-term cold storage. *Food Chem*. 112:998-1001
[12]. Allaith Abdul Ameer A., 2008: Antioxidant activity of Bahraini date palm (*Phoenix dactylifera* L.) fruit of various cultivars. *Int J Food Sci Technol*. 43:1033-1040.
[13]. Biglari, F., AlKarkh,i A.F.M., Mat, E.A., (2008): Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. *Food Chem*. 107:1636-1641.
[14]. Boudries, H., Kefalas, P., Hornero-Méndez, D., 2007: Carotenoid composition of Algerian date varieties (*Phoenix dactylifera*) at different edible maturation stages. *Food Chem*. 101:1372-1377.
[15]. Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M., Shahidi, F., 2005a: Compositional and sensory characteristics of three native sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. *J Agric Food Chem.* 53:7586-7591.
[16]. Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M., Shahidi, F., 2005b: Comparison of antioxidant activity, anthocyanins, carotenoids and phenolics of three native fresh and sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. *J Agric Food Chem*. 53:7592-7599.
[17]. Mansouri, A., Embarek, G., Kokkalou, E., Kefalas, P., 2005: Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). *Food Chem.* 89:411-420.
[18]. Vayalil, P.K., 2002: Antioxidant and antimutagenic properties of aqueous extract of date fruit. *J. Agric. Food Chem*. 50:610-617.
[19]. Chaira, N., Smaali, M. I., Martinez-Tomé, M., Mrabet, A., Murcia, M. A., & Ferchichi, A. (2009): Simple phenolic composition,flavonoid contents and antioxidant capac-ities in water–

methanol extracts of Tunisian common date cultivars (*Phoenix dactylifera* L.). *Int. J. Food Sci. Nutr*. 60:316-329.

[20]. Hong, Y. J., Tomas-Barberan, F. A., Kader, A. A., & Mitchell, A. E., 2006: Theflavonoid glycosides and procyanidin composition of Deglet Noor dates (*Phoenix dactylifera*). *J.Agric. Food Chem.*54: 2405-2411.

[21]. Benzie, I.F., & Strain, J.J., 1996: The ferric reducing ability of plasma (FRAP) as a measure of ‘‘antioxidant power’’: The frap assay. *Analytical Biochemistry*. 239: 70–76.

[22]. Cao, G., Alessio, H.M., & Culter, R.G., 1993: Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology and Medicine*. 14: 303-311.

[23]. Guo, C.J., Yang, J.J., Wei, J.Y., Li, Y.F., Xu, J., & Jiang, Y.G., 2003: Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutrition Research. 23: 1719-1726.

[24]. Re, R., Pellegrini, N.P., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999: Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*. 26: 1231-1237.

[25]. Roginsky, V., & Lissi, E. A. 2005: Review of methods to determine chain-breaking antioxidant activity in food. Food Chemistry. 92: 235-254.

[26]. Van Den Berg, R., Haenen, G.R.M. M., Van Den Berg, H., & Bast, A. 1999: Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem*. 66: 511-517.

[27]. Keyrouz, R., Abasq, M.L., Le Bourvellec, C., Blanc, N., Audibert, L., ArGall, E., Hauchard, D., 2011: Total phenolic contents, radical scavenging and cyclic voltammetry of seaweeds from Brittany. *Food Chem*. 126: 831-836.

[28]. Blasco, A. J., Crevillen, A.G., Gonzalez, M.C., Escarpa, A., 2007: Direct Electrochemical Sensing and Detection of natural antioxidants and Antioxidant capacity in Vitro Systems. *Electroanalysis*.19: 2275-2286.

[29]. Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., Vidal, N., 2006: Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem*. 97:654-660.

[30]. Singleton, V.L., Rossi J.A., 1965: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic*. 16:144-158.

[31]. Jin, M., Cai, Y.X., Li, J.R., Zhao, H., 1996: 1, 10-Phenanthroline-Fe^{2+} oxidative assay of hydroxyl radical produced by H_2O_2/Fe^{2+}. *Prog. Biochem. Biophys*., 23: 553-555.

[32]. Brand-williams, W., Cuvelier, M.E., Berset, C., 1995: Use of a free radical method to evaluate antioxidant activity. *LWT*. 28:25-30

[33]. Djeridane, A., Yousfi, M., Nadjemi, B., Maamrim S., Djireb, F., Stocker, P., 2006: Phenolic extracts from various Algerian plants as strong inhibitors of porcine liver carboxylesterase. *J Enzym Inhib Med Chem*. 21:719-726.

[34]. Bourvellec, C.L., Hauchard, D., Darchen, A., Burgot, J.L., and Abasq, M.L., 2008: Validation of a new method using the reactivity of electrogenerated superoxide radical in the antioxidant capacity determination of flavonoids. *Talanta*. 75: 1098-1103.

[35]. 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.

[36]. Kitada, M., Igarashi, K., Hirose, S., Kitagawa, H., 1979: Inhibition by polyamines of lipid peroxide formation in rat liver microsomes. Biochem. Biophys. Res. Commun., 87: 388-394.

[37]. Li, X.-M., Shi., Y.-H., Wang., F., Wang., H.-S., Le., G.-W. 2007: In vitro free radical scavenging activities and effect of synthetic oligosaccharides on antioxidant enzymes and lipid peroxidation in aged mice .*J.Pharma. . Biomedical Anal*., 43: 364–370.

[38]. Soares, J. R., Dins, T. C. P., Cunha, A. P., & Ameida, L. M. 1997: Antioxidant activity of some extracts of Thymus zygis. *Free Radical Research*, 26, 469–478.

[39]. Yamaguchi, T., Takamura, H., Matoba, T., Terao, J., 1998: HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1,-diphenyl-2-picrylhydrazyl. Biosci. Biotechnol. Biochem. 62: 1201-1204.
[40]. Yen, W.-J., Chang, L.-W., & Duh, P.-D. 2005: Antioxidant activity of peanut seed testa and its antioxidative component, ethyl protocatechuate. *LWT*, 28: 193-200.
[41]. Siddhuraju, P., Becker, K. 2007: The antioxidant and free radical scavenging activities of processed cowpea (Vigna unguiculata (L.) Walp.) seed extracts. *Food Chemistry*, 101: 10-19.
[42]. Rice-Evans, C. A., Miller, N. M., & Paganda, G. 1996: Structureantioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine,20: 93-956.
[43]. Ramarathnam, N., Ochi, H., & Takeuchi, M. 1997: Antioxidant defense system in vegetable extracts. In F. Shahidi (Ed.), Natural antioxidants; chemistry, health effects and applications (pp. 76-87). Champaign, IIL: AOCS Press.

PhytoChem & BioSub Journal

Peer-reviewed research journal on Phytochemistry & Bioactives Substances

ISSN 2170 - 1768

PCBS Journal

PCBS Journal
ISSN 2170-1768

POSL

Edition LPSO

Phytochemistry & Organic Synthesis Laboratory
http://www.pcbsj.webs.com , Email: phytochem07@yahoo.fr