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# Study of Antioxidant Properties of Five Algerian Date (*Phoenix dactylifera* L) Cultivars by Cyclic Voltammetric Technique

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*Phoenix dactylifera* L. (date palm) is well known for its innumerable health benefits and nutritional values. Therefore, the present work aimed to determine the total phenolic content in five date palm fruit (DPF) varieties, Degla Baidha (DB), Deglet Nour (DN), Ghars (Gh), Tamjhourt (Tam), and Tafezauine (Taf), from the Ouargla region (Algeria), extracted with aqueous methanol (80%) and to evaluate in vitro their antioxidative properties by cyclic voltammetry technique (CV) in aprotic media. Consequently, the total polyphenol contents (TPC) of these extracts will be measured using Folin Ciocalteu spectrophotometric method. Total phenolic content ranged from 9.5 to 23.05 mg gallic acid equivalents (GAE/100g). Thereafter, the voltammetric response of the electrochemically gener- ated superoxide anion radical in DMF was monitored in the absence and presence of the DPF extracts. The decrease in the current was interpreted in terms of antiradical activity of the added extract**.** Effective scavenging concentration ($IC_{50}$) on superoxide anion radical ranged from 33.17-85.23mg/l, the $IC_{50}$ values decreased in the order of Taf>Gh>DB>Tam>DN. Finally, the use of DPF extracts for protection against oxidative stress has been recommended.

**Keywords:** Algeria date palm fruit; Total phenolic content; Antioxidant capacity; superoxide anion radical; cyclic voltammetry.

## 1. INTRODUCTION

In the last few years, the metabolism of oxygen in humans has been investigated by biochemists all over the world: it is well known that an excess of oxygen leads to the formation of reactive oxygen species, which contain free radicals responsible for oxidative stress in the tissues–leading to cellular death and contributing to faster aging and causing diseases, like cancer, atherosclerosis, infl-

ammatory injury, ageing, diabetes, hypertension, atherogenesis, Alzheimers, Parkinsonsand cardiovascular disease [1-6].

Reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet-}$) and hydroxyl ($^{\bullet}OH$) radical and hydrogen peroxide ($H_2O_2$) are often generated as by products of biological reactions or from exogenous factors [2,7].

Superoxide is an anionic free radical with the chemical formula $O_2^{\bullet-}$. It is formed by one-electron reduction of dioxygen ($O_2$) [8], which is present abundantly in nature. Super oxide anion is the most dangerous radical among all oxygen radicals because it has a longer half life and thus can move to a longer distance [9]. The compounds that protect cells against the damaging effects of ROS are known as antioxidants. They result in a decrease of its toxic action and stop the radical-chain processes [10]. Antioxidants play a major role in protecting biological systems against many incurable diseases. Antioxidants have been widely used in different fields of industry and medicine as substances which interrupt radical-chain oxidation processes, improve general health, help cell rejuvenation, and prevent cancer [11].

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 [12]. There are over 58 different varieties of dates in region of Ouargla [13].

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 [14-27].

Numerous analytical techniques are available to evaluate oxidative stress namely, electron spin resonance, chromatography, spectroscopy or mass spectrometry [28-31]. All these techniques need expensive materials, involve complex protocols. Electrochemical methods or techniques, for example, polarographic and voltammetric techniques (differential pulse polarography, absorptive voltammetry and linear sweep voltammetry, etc.), generally possess high sensitivity and are extensively used in analytical chemistry [32-35].

Electrochemistry deals with electron transfer phenomena between an electrode and oxidized or reduced molecules. It therefore represents a suitable methodology to study the redox properties of solid, liquid or gaseous media and to detect oxidant and antioxidant species [36]. One of the most recent methods used to measure antioxidant activity of phenolic compounds is selective electro-oxidation by cyclic voltammetry [37-39]. It tested to evaluate the global antioxidant capacity of real samples like wine, biological fluids or tissues [40-45].

In this work, we have applied an effective and convenient approach for the determination, for the first time, of the antioxidant activity of the investigated extracts of five varieties of date palm fruit (DPF) by recording the current of the electrochemical oxygen reduction at a glassy carbon electrode. For the purpose superoxide anion radical ($O_2^{\bullet-}$) was selected as target radical because of its presence in

human body and perilous nature; as it has longer half life and is capable of generating other harmful radicals such as hydroxyl radical. The method used to determine antioxidant activity is advantageous as it also furnishes mechanistic insight about the phenomenon under investigation. Total polyphenols contents of DPF extracts were determined by standard colorimetric 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 hexafluorophosphate $Bu_4NPF_6$ of electrochemical grade were purchased from Fluka. Folin-Ciocalteus's phenol reagent, sodium carbonate, Gallic acid (GA),Ascorbic acid (AA), All other chemicals and solvents were of the highest commercial grade and used without further purification.

### *2.2.Plant material*

Five different Algerian ripe date palm fruit (DPF) varieties, Degla Baidha variety (DB), Deglet Nour variety (DN), Ghars variety (Gh), Tamjhourt variety (Tam), Tafezauine variety (Taf), 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 [46]. 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 3ml of 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 [47]. 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: $y = 3.08x$, $R^2 = 0.993$, where x was the absorbance.

### *2.5. Electrochemical experiments*

#### *2.5.1. Instrumentation*

Cyclic voltammetric measurements were performed using a Voltalab 40 model PGZ301 (Radiometer Analytical) potentiostat/galvanostat driven by a personal computer with VoltaMaster 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.

#### *2.5.2. Procedures*

##### *2.5.2.1. Pretreatment of the electrode*

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.

##### *2.5.2.2. Cyclic voltammetry of oxygen*

10ml of an extra-dry DMF solution containing the supporting electrolyte 0.1M $Bu_4NPF_6$ was saturated by dry air during10 min. In these conditions the solubility of oxygen was assumed to be $0.94\times10^{-3}$ M, this value corresponding to a partial pressure of 0.2 bar [48]. The cyclic voltammogramm (CV) of the oxygen reduction was then recorded at a scan rate $0.1Vs^{-1}$.The applied potential range was from -0.2 V to -1.2V versus SCE.

##### *2.5.2.3. Cyclic voltammetry of oxygen in the presence of antioxidant substrate*

Measurement of superoxide radical scavenging activity was based on the method of Bourvellec, et al with slight modification [49]. 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:

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 9.5 to 23.05 mg GAE/100g DW (Table1.). Among extracts, extremely high TPC was detected in Tam variety (23.05 mg GAE/100g DW). The lowest TPC was detected in Taf variety (9.5mg GAE/100g DW). The amount of TPC in the other varieties extracts of DPF were ranged from 11.97 to 14.82mg GAE/100g DW. The order of TPC in DPF extracts is: Tam>DB>DN>Gh>Taf. The concentration of polyphenols in this study was lower compared to study of Ghiaba et al. [14] 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. [23] 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. [27] 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<br>Gallic acid eq. (mg/100 g DW) | $IC_{50}$<br>$O_2^{\bullet -}$ (mg/l) |
|---|---|---|
| DB | 14.81 | 66.33 |
| DN | 13.42 | 85.23 |
| Gh | 11.97 | 58.98 |
| Tam | 23.05 | 70.62 |
| Taf | 9.5 | 33.17 |
| GA | - | 119.21 |
| AA | - | 102.10 |

*3.1. Voltammetric behaviour of superoxide anion radical*

The superoxide anion radical was generated by one electron reduction of the atmospheric molecular oxygen ($O_2$) dissolved in DMF at room temperature (28˚C) and the resultant CV response. The presence of the radical $O_2^{\bullet-}$ is easily detected by its anodic oxidation current measured at the same electrode during the reverse scan is presented in Figure 1.

The reduction of $O_2$ is a reversible reaction and it is known that $O_2^{\bullet-}$ radical is stable in aprotic media and dismutation does not occur during the time scale of the cyclic voltammetry in DMF solution [50-52]. Consequently, it is a convenient way to generate $O_2^{\bullet-}$without enzyme systems and to study its reaction with a molecule or an extract provided the substrate is not active in the potential range of the reduction of oxygen.

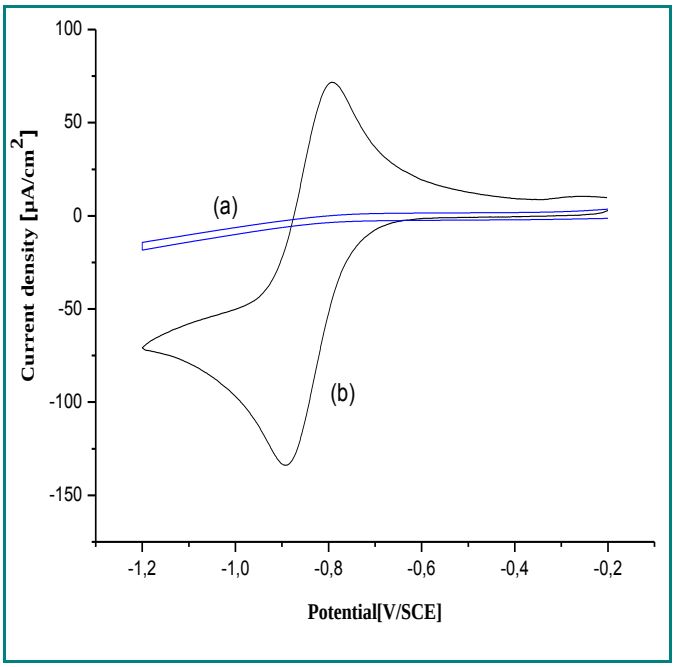



**Figure 1.** Cyclic voltammogram of: (a) medium (DMF + $Bu_4NPF_6$), (b) $O_2^{\bullet-}$in DMF + 0.1M $Bu_4NPF_6$, On the glassy-carbon as working electrode vs. ECS as reference at 28°C with scan rate of $0.1Vs^{-1}$.

*3.2. Voltammetric behaviour of the extracts*

The observed behaviour of the DPF extracts shows the electro-inactivity of the extracts in the selected potential range (-2.0 V to +2.0 V). All extracts including these exceptions were inactive in the potential window of super oxide radical (-1.0 V to 0.0 V), voltammetric behaviour of superoxide radical in the presence of the DPF extracts. The voltammograms of the DPF extract showed two peaks; one in negative potential region i.e. reduction (Epc=-0.779) and one in positive region i.e. oxidation (Epa=1.083V) (figure 2). Most of the flavonoids; phenolic acids and poly phenols oxidize or reduce in negative region [53].

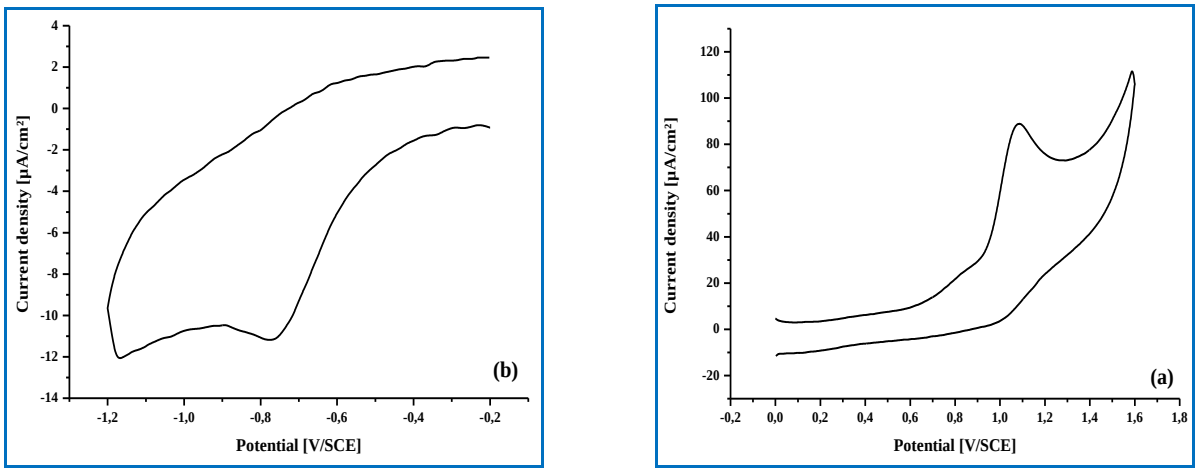
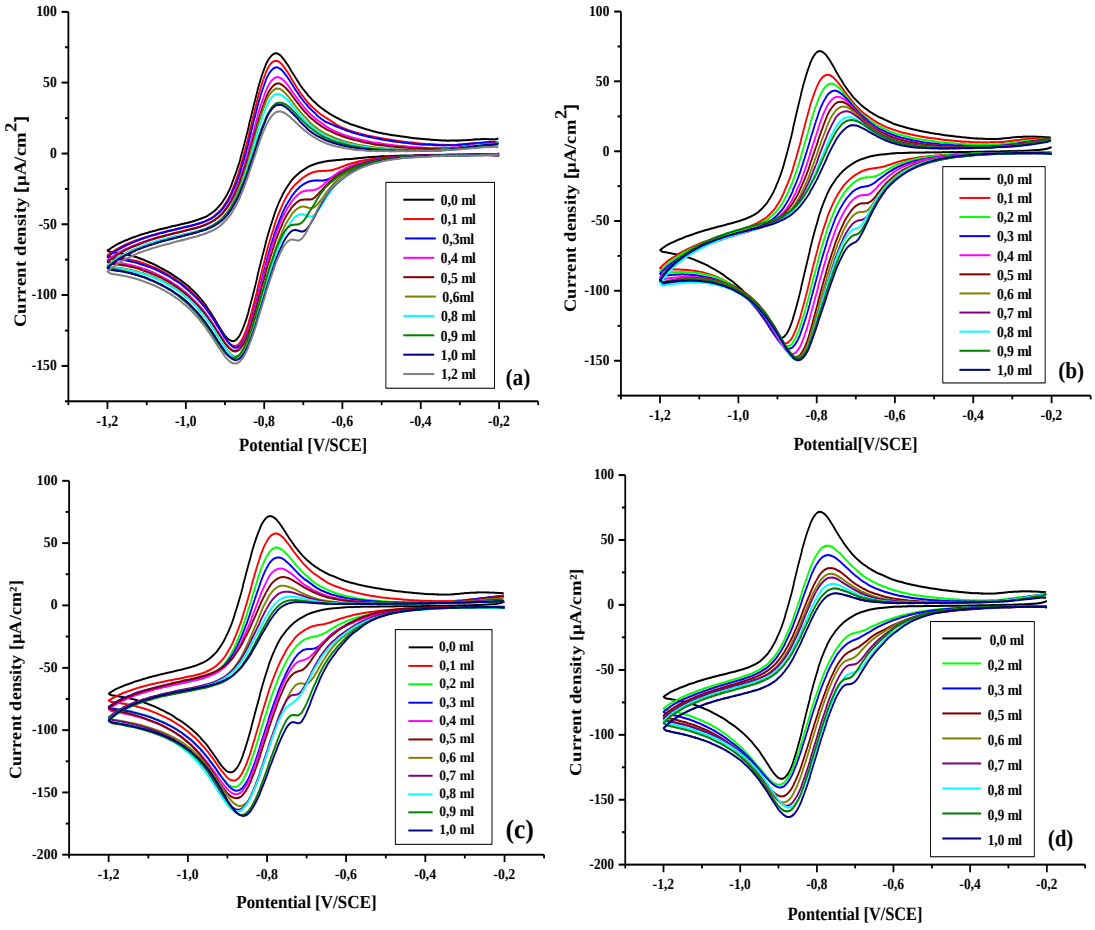



**Figure 2**. Cyclic voltammograms of 1ml extract of DN in DMF + 0.1M $Bu_4NPF_6$ on GC as working electrode vs. SCE at 28°C with scan rate of 0.1 V/s for; (a) positive region, (b) negative region**.**

*3.3. Voltammetric behaviour of superoxide radical in the presence of the extracts*

The obtained results (figure 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 (figure 3a.).



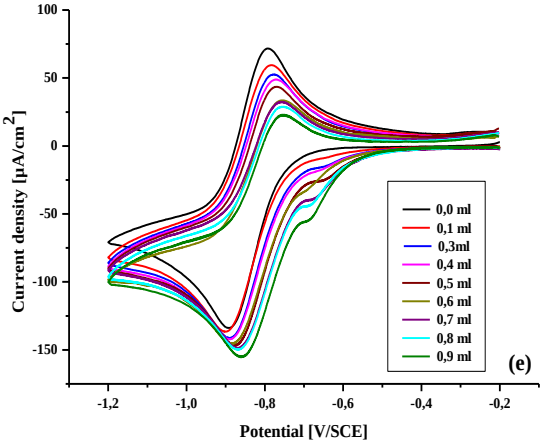



**Figure 3.** Cyclic voltammograms of $O_2^{\bullet-}$ in the presence of different concentration of DB (a), DN (b), Gh (c), Tam (d), Tef (e), in DMF + 0.1 M $Bu_4NPF_6$ on GC as working electrode vs. SCE at 28°C with scan rate of 0.1 V/s.

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 $I_{Pa}^{s}$ values is determined from the CVs recorded for increasing antioxidant concentrations (Figure 3.). All antioxidant substrates exhibited a similar effect upon the $O_2$ reduction.

The CV technique was also helpful to determine the mechanism of free radical scavenging. The observed changes in the shape of the cyclic voltammograms are same, as reported data by and Bourvellec et al; Ahmed et al for some commercial flavonoids [49,54]. There it was designated as H-atom abstraction from the flavonoid by the superoxide radical. The identified compounds of the DPF have more than one –OH in their structure therefore it is assumed that the hydrogen of OH group is responsible for the interaction with superoxide. The voltammograms are identical in all the DPF extracts which indicates the presence of polyhydroxyl compounds and thus make the operating mechanism same for all the extracts. The analysis of our data and the literature data suggests that the reduction of oxygen in the presence of antioxidants proceeded by an electrochemical mechanism and that superoxide radical anion was protonated [55].

$$O_2 + e\,(\text{from electrode}) \longrightarrow O_2^{\bullet-} \qquad (1)$$

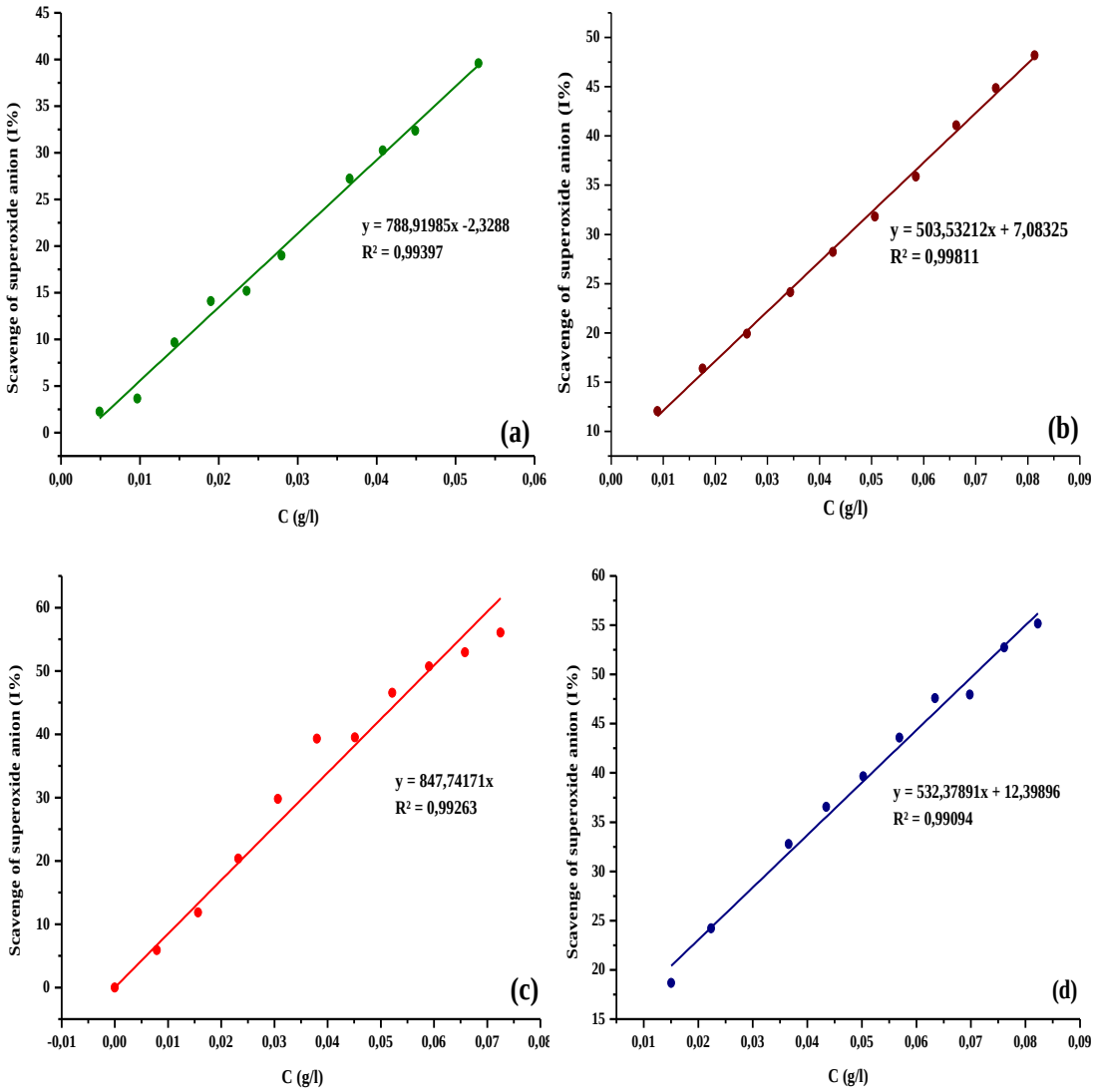
$$ROH + O_2 \longrightarrow [RO\text{--}H\text{--}\ O_2^{\bullet-}]^{\pm} \longrightarrow RO^{\bullet} + HO_2^{-} \qquad (2)$$

$$HO_2 + ROH \longrightarrow H_2O_2 + RO^{\bullet} \qquad (3)$$

The $RO^{\bullet}$ produced is assumed to be of very low toxicity because of bulky groups attached and also because of high probability of the dimerization. As the whole process is taking place in the electrochemical cell, therefore, the overall mechanism can be pictured as reversible electron transfer

(formation of stable $O_2^{\bullet-}$ from molecular oxygen) followed by an irreversible coupled chemical reaction (H-atom transfer from the extract to the radical) [55].

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 concentration (Fig. 4.). Results shown in table 1. Lower values correspond to higher antioxidant activities. It can be seen from this table that 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 85.2314mg/l. The lowest value of $IC_{50}$ (33.1677mg/l) was detected in Taf variety and it corresponds to the highest antioxidant activity; while the highest value of $IC_{50}$ (85.2314mg/l) was detected in DN variety. The antioxidant activity in the DPF extracts decreases in the order Taf>Gh>DB>Tam>DN. The $IC_{50}$ values confirm the presence of antioxidants as main components of the extracts against superoxide radical. The antioxidant potential of phenolic compounds is dependent on the number and arrangement of the hydroxyl groups as well as thepresence of electron-donating substituents in the ring structure [56].



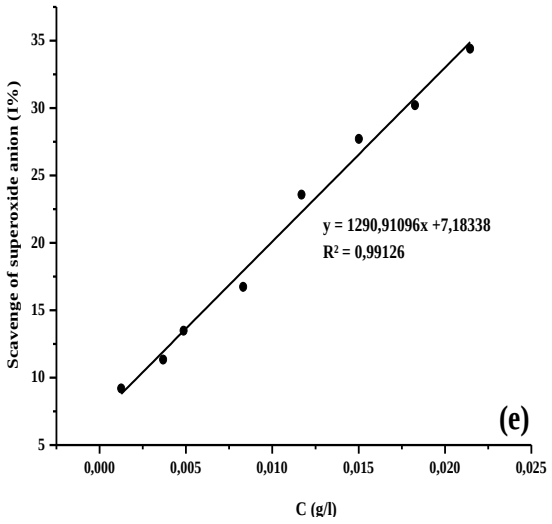



**Figure 4.** Plotting of scavenging of superoxide anion of cyclic voltammogram against the corresponding concentration of DB (a), DN (b), Gh (c), Tam (d), Tef (e).

## 4. CONCLUSIONS

Date palm (*Phoenix dactylifera* L) is an important fruit and cash crop in Algeria, it is socio-economically and traditionally important for local populations where the culture thrives. Algeria, being a vast country, has a number of varieties of dates differing. But, unfortunately, it is still to be explored. To our knowledge, this is the first report describing the antioxidant activity of Algeria Dates extracts by cyclic voltammetric technique; the method of CV is a simple and relatively reliable method, has been developed to measure antioxidant activity of five date palm fruit (DPF) varieties, Deglet Nour (DN), Degla Baidha (DB), Ghars (Gh), Tamjhourt (Tam), and Tafezauine (Taf) against superoxide anion radical. Further, the proposed mechanism indicates the presence of polyhydroxyl group containing compounds in the used extracts. The isolation and identification of such species would be an interesting future study.

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