

EVALUATION OF THE ANTIOXIDANT ACTIVITY OF POTATOES BY RADICAL SCAVENGING ACTIVITY USING $O_2^{\bullet-}$ AND DPPH

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ABSTRACT

This work aimed to evaluate the antioxidant capacity (AC), total phenolic (TPC) and flavonoids content (TFC) of the ethanolic extracts four varieties of *Solanum tuberosum* L (potato), TPC of whole roots shows values ranged from 19.21 to 08.88 mg AG/g of extract and TFC varied from 25.03 to 10.91 mg rutin/g of extract. However, this phytochemical content of sample extracts had an effect on the AC, measured with scavenging radical activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide anion radicals ($O_2^{\bullet-}$). The IC_{50} values measured using superoxide anion radical ($O_2^{\bullet-}$) and DPPH ranged from 1.03 to 1.81mg/ml and from 1.21 to 3.86 mg/ml respectively. Free binding energies and binding constants of ethanolic extracts contents and $O_2^{\bullet-}$ were also determined.

Keywords: antioxidant; flavonoids; phenolic; potatoes; DPPH; Cyclic voltametry; superoxide anion radical.

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1. INTRODUCTION

A free radical is defined as an atom or molecule containing one or more unpaired electrons that are capable of free existence [1]. Free radicals are generated during normal respiration and cellular functions. Under normal physiological conditions, approximately 2% of oxygen consumed by the human body during respiration is converted into superoxide anion free radical, which is negatively charged ($O_2^{\bullet-}$) [2]. To survive in an unfriendly oxygen



environment, living organisms generate water- and lipid-soluble antioxidants that can neutralize these highly reactive free radicals [3]. If the body's antioxidant mechanism does not operate optimally, excess free radicals can damage various biomolecules, including lipids, proteins, carbohydrates, and nucleic acids. A variety of free radical scavenging antioxidants is found in dietary sources like fruits and vegetables which were known as phytochemicals compounds. These chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophylls etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from amino acids), terpenes (a group of lipids) and phenolics (derived from carbohydrates) [4]. Consequently, characterization of polyphenols as antioxidants is essential for both plant biology and human nutrition. Flavonoids, as well as many other plant polyphenols, possess a chemical structure ideal for free radical scavenging [5]. Their antioxidant properties include reactivity to a variety of reactive oxygen species [6–7]. There are two mechanisms for the radical scavenging reactions of phenolic antioxidants: a hydrogen atom transfer from the phenolic OH group; and an electron transfer followed by a proton transfer. The most frequently used free radicals are 2,2-diphenyl-1-picrylhydrazyl (DPPH [8]) and the cation radical of 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulfonate (ABTS) of the Trolox Equivalent Antioxidant Capacity (TEAC) assay [9].

The objective of the current study was to evaluate the antioxidant property of phenolics extracted from the vegetable tissues of varieties of potatoes by scavenging activity of DPPH (diphenyl-picryl-hydrazyl) and the inhibitory effect of superoxide anion radicals.

2. MATERIALS AND METHODS

2.1. Chemical and reagents

Folin-Ciocalteu's reagent (solution 2M) was purchased from Sigma-Aldrich (Finland), gallic acid (99%), rutin (97%), Hydrochloric acid was provided by Rathburn Chemical (Walkerburn, Peebleshire, UK). Ethanol and di-sodium hydrogen phosphate were purchased from Fisher Scientific (Loughborough, UK), N, N-dimethylformamide (DMF) (analytical grade from PROLABO) was used as solvent without any purification, tetrabutylammonium tetrafluoroborate (Bu_4NBF_4) (electrochemical grade (99%) Sigma-Aldrich) was used as supporting electrolyte and its concentration was kept 0.1 M.

2.2. Instrument

UV-Vis experiments were performed using a UV-1800 UV-VIS spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan).

Voltammetric measurements were carried out using voltalab40 (radiometer analytical SAS) in a double-walled electrochemical cell of 25 mL, and a conventional three-electrode system was employed. A glassy carbon (GC) working electrode having area 0.013 cm², a platinum wire counter electrode and an Hg/Hg₂Cl reference electrode (3.0 M KCl) were used. The scan rate was kept at 100 mV/s, and the potential window was from -1.4 to 0.0 V. The studied extract samples were added to the in situ generated superoxide anion radical O₂^{•-}, and the cyclic voltammograms were recorded.

WUC-D06H, Daihan Scientific, Korea) was used for ultrasound-assisted extraction.

2.3. Plant material

Four potatoes cultivars '*Bartina*, *Kuroda*, *Kondor* and *Spunta*' were collected in March 2015 from different areas located in the region of El-Oued south-east of Algeria, were grown for three months before being used.

2.4. Preparation of extracts

The ultrasound assisted extraction procedure was used for the extraction of potato. Thus 120 ml of solvent ethanol were added to 20 g of fresh potatoes cutting into 5 mm thick slices, the mixture was sonicated in an ultrasonic bath for 30 min. then kept macerating for 2 hours. The extract was filtered through to remove all of the potato particles and the filtrate was concentrated under reduced pressure at 60 °C using rotary evaporator and stored in a refrigerator.

3. PHYTOCHEMICAL INVESTIGATION

3.1. Total Polyphenol content

TPC was determined with the Folin-Ciocalteu reagent following the method described by Singleton-Ross [10]. Briefly, 100 µL of suitably diluted extract was added to 0.5 mL of newly diluted 10-fold Folin-Ciocalteu reagent. Then 2 mL of 20 % aqueous Na₂CO₃ solution was added. The mixture was stand in the dark for half an hour, and then the absorbance was measured at 760 nm against a blank solution. Gallic acid was used as a reference and the results were presented as gallic acid equivalents/g of extract.

3.2. Total flavonoids content

TFC was determined as described by Zou [11]. A mixture of 1 ml of extract, 2 ml of distilled water and 0.15 ml of 5% NaNO₂ was prepared and allowed to react for 6 min. then 0.15 ml of

10% AlCl_3 solution was added and mixed stirred thoroughly. After 6 min, 2 ml of 4% NaOH solution was added and allowed to stand for another 15 min. The absorbance of the mixture was measured at 510 nm versus prepared blank solution. Rutin was used as a standard for the quantification of TFC. Results were expressed in mg of rutin equivalents/g of extract.

4. ANTIOXIDANT ACTIVITY

4.1. DPPH assay

The scavenging activity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method according to Ohinishi and others [12]. with some modifications, a 1 ml of diluted extract and 1 ml of DPPH (0.25 mM) were mixed, and after 30 min the absorbance was measured at 517 nm. The scavenging activity was measured as the decrease in absorbance of the samples versus DPPH standard solution. Results were expressed as radical scavenging activity percentage (%) of the DPPH according to the formula:

$$I\% = \frac{A_0 - A_s}{A_0} \times 100 \quad (1)$$

where: A_0 : absorbance of the control and A_s : absorbance of the sample.

4.2. Scavenging activity of superoxide anion $\text{O}_2^{\bullet-}$ radicals

Superoxide anion radical was generated electrochemically by the reduction of commercial dioxygen in ACN containing 0.1 M TBFB at 28 ± 1 °C. The studied extract samples were added to this in situ generated $\text{O}_2^{\bullet-}$, and the cyclic voltammograms were recorded following the method of Le Bourvellec [13].

The ability of the test sample to quench $\text{O}_2^{\bullet-}$ (% inhibition of $\text{O}_2^{\bullet-}$) was calculated using the following equation (2) [13,14],

$$I\% = \frac{i_0 - i_s}{i_0} \times 100 \quad (2)$$

where : i_0 and i_s are respectively the anodic peak current density of $\text{O}_2^{\bullet-}$ in the absence and in the presence of sample extract.

4.3. Determination of IC_{50} values

On the basis of screening results of triplicate measurement of the extract, the inhibition concentration values IC_{50} was determined from extrapolating the graph of scavenging activity versus the concentration of extract using linear regression analysis[15].

4.4. Statistical analysis

All the experiments were performed in triplicate, and the results were expressed as mean $\pm$ SD (standard deviation). Statistical analysis was performed using Minitab version 14 and Excel 2007 the results were significantly ($P \leq 0.005$).

5. RESULTS AND DISCUSSION

5.1. Phytochemical contents

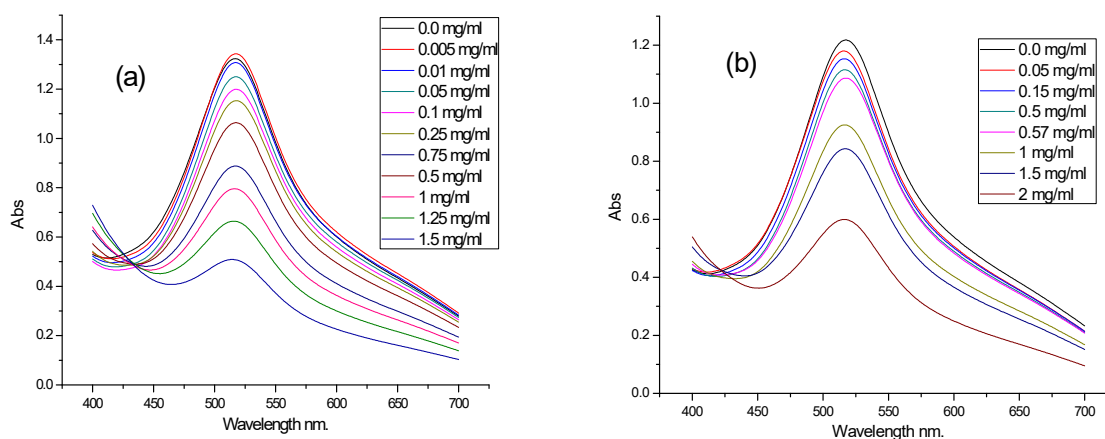
Table 1 shows TPC and TFC of potato samples. The amount of TPC from tuber ranged from 19.2144 to 08.8853 mg GA/g of extract, the results of different extracts showed that the amount of TFC varied considerably from 10.9129 to 25.0350 mg rutin/g of extract. Overall the results indicate that all of the ethanolic extract of four kinds has amount of phenolics and flavonoids compound but their content in ethanolic extract increasing in order was: Kondor > Bartina > Spunta > Kuroda.

Table 1. Determination of total Phenolics contents and total flavonoids of four kinds of potatoes

Sample	TPC (mg GA*/g of extract)	TFC (mg rutin/g of extract)
Kondor*	19.2144 ± 0.096	25.0350 ± 0.125
Bartina*	18.9596 ± 0.094	23.5592 ± 0.235
Spunta**	12.4628 ± 0.124	21.8057 ± 0.218
kuroda*	08.8853 ± 0.088	10.9129 ± 0.076

* = red tuber, ** = yellow tuber

5.2. Total antioxidant activity



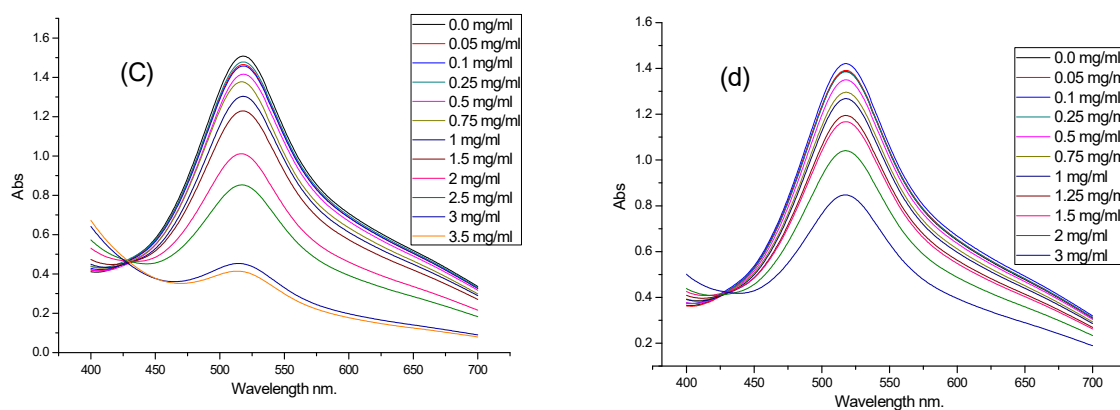


Fig.1. Electronic absorption spectra of 0.25 mM of DPPH interaction with (a) Kondor, (b) Bartina, (c) Spunta, (d) Kuroda

The antioxidant capacities of potato extracts in term of IC_{50} were measured using DPPH and $O_2^{\bullet-}$ radical scavenging activity figure 1 and 3. The equation obtained from the linear calibration graph in the studied concentration range of sample extract is presented in table 2 where y stand for the value of absorbance DPPH or the oxidation peak current density of $O_2^{\bullet-}$ and x represents the value of the concentration of ethanolic extracts, expressed as mg/mL. Both methods show that the Kondor extracts have lower IC_{50} values of 1.21 and 1.03 mg/mL for DPPH and $O_2^{\bullet-}$ respectively, whereas Kuroda sample has 3.867 mg/mL (DPPH) and 1.819 mg/mL for ($O_2^{\bullet-}$) radical scavenging activity. All the IC_{50} values were considered as very statistically significant with $p < 0.005$.

Table 2. IC₅₀ values of sample extracts using DPPH and O₂^{•-} radical scavenging activity

Sample	DPPH			
	Equation	R ² value	IC ₅₀ [*]	P
Kondor	Y = 3.33 + 38.3 X	0.994	1.21	0.000
Bartina	Y = - 0.44 + 23.7 X	0.968	2.12	0.000
Spunta	Y = - 4.53 + 21.4 X	0.952	2.54	0.000
Kuroda	Y = - 5.30 + 14.3 X	0.973	3.86	0.000
	O ₂ ^{•-}			
	Equation	R ² value	IC ₅₀ [*]	P
Kondor	Y = 1.56 + 46.8 X	0.989	1.0350	0.000
Bartina	Y = - 7.52 + 50.6 X	0.923	1.1367	0.002
Spunta	Y = - 0.134 + 27.6 X	0.988	1.8164	0.000
Kuroda	Y = - 4.23 + 29.8 X	0.987	1.8197	0.000

* Inhibition expressed as mg/ml

5.3. Voltammetric Studies of O₂^{•-} – sample interaction

Figure 2 shows the CV's behavior of oxygen-saturated DMF/0.1 TBFP in the potential window of -1.4 to 0.0 V at a glassy carbon electrode in the absence and presence of 0.4 mL sample extracts. The CV technique was also used for the determination of the binding constants and mode of interaction of the extract with O₂^{•-} in the same manner, used for study of binding of drug molecules to DNA[16-17].

By adding 0.4 ml of sample extracts in DMF solution, the anodic peak potential of O₂^{•-} was shifted by ΔE, to more negative values for O₂^{•-}-Kondor and to more positive values for the other samples, these shifts were associated with the decrease in oxidation peak current densities ΔI_p% which confirms the interaction of ethanolic extracts with O₂^{•-} (table3) The significant decrease in oxidation peak current densities can be attributed to the diminution in O₂^{•-} concentration due to the formation of sample extract complex.

Table 3. Shifts in peak potential and a decrease in anodic peak current of O₂^{•-} bound forms of extract samples.

Sample	I _p (μA)	E _p (V)	E _c (V)	E _f ⁰ (V)	ΔE _f ⁰ (mV)	ΔI _p %	K _{ox} /K _{red}
O ₂ ^{•-}	134.2672	-0.7068	-0.7247	-0.7157	-	-	-
O ₂ ^{•-} -Kondor	104.2637	-0.678	-0.7566	-0.7173	-1.5574	22.3460	0.94
O ₂ ^{•-}	137.3784	-0.7251	-0.7714	-0.7482	-	-	-
O ₂ ^{•-} -Bartina	118.844	-0.7251	-0.7624	-0.7438	4.4776	13.4914	1.19
O ₂ ^{•-}	137.5848	-0.7149	-0.7438	-0.7294	-	-	-
O ₂ ^{•-} -Spunta	114.627	-0.7021	-0.7477	-0.7249	4.4776	16.6863	1.19
O ₂ ^{•-}	142.4359	-0.7613	-0.7800	-0.7706	-	-	-
O ₂ ^{•-} -Kuroda	123.7957	-0.7519	-0.7617	-0.7568	13.8221	13.0867	1.71

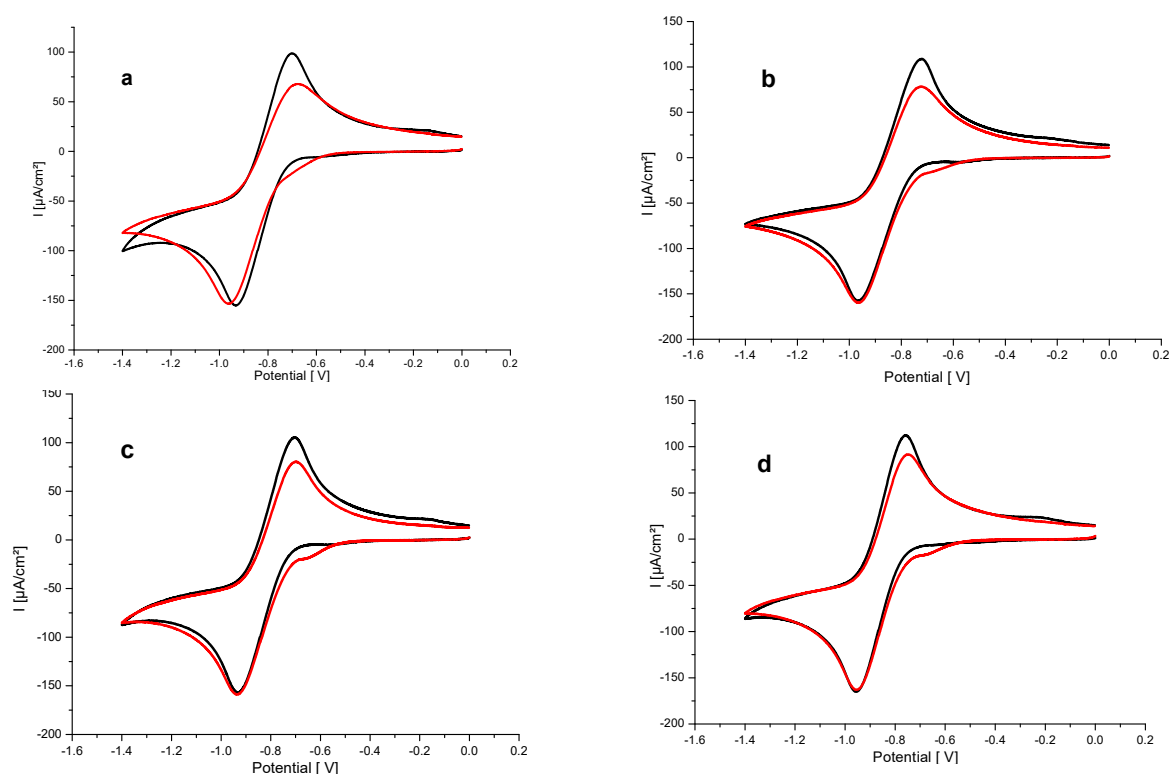
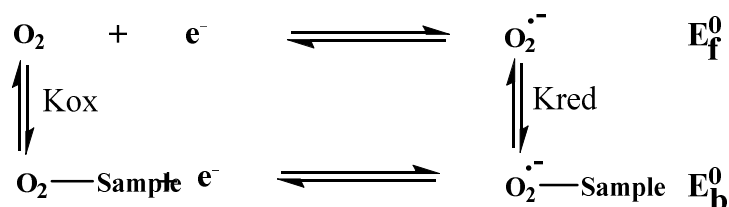


Fig.2. Cyclic voltammograms of oxygen-saturated DMF/0.1 TBFP on a GC electrode in the absence (black line) and in presence of 0.4 ml sample extract (a) Kondor, (b) Bartina, (c) Spunta, (d) Kuroda at Scan rate 100 mV.s^{-1} , $T = 28^\circ$

5.4. Ratio of binding constants (K_{ox}/K_{red})



Scheme1: The redox behavior of the free radical and its bounded forms SE

The peak potential shift values additionally pointed out that $\text{O}_2^{\cdot-}$ is easier to oxidize in the presence of ethanolic extract because its reduced form $\text{O}_2^{\cdot-}$ is more strongly bound to extract than its oxidized form. For such a system, where both forms of the $\text{O}_2/\text{O}_2^{\cdot-}$ redox couple interact with the potential antioxidant compound, scheme 2 can be applied [18]. Based on the process presented in scheme 2, the following equation is obtained [19],

$$E_b^0 - E_f^0 = 0.059 \text{ Log } \frac{K_{red}}{K_{ox}} \quad (3)$$

Where E_b^0 and E_f^0 are the formal potentials of the $O_2/O_2^{\bullet-}$ redox couple in the free and bound forms, respectively. E^0 and ΔE^0 are calculated using the following formulas (4) and (5)

$$E^0 = \left| \frac{E_{pc} + E_{pa}}{2} \right| \quad (4)$$

$$\Delta E^0 = E^0(O_2^{\bullet-} - SE) - E^0(O_2^{\bullet-}) = E_b^0 - E_f^0 \quad (5)$$

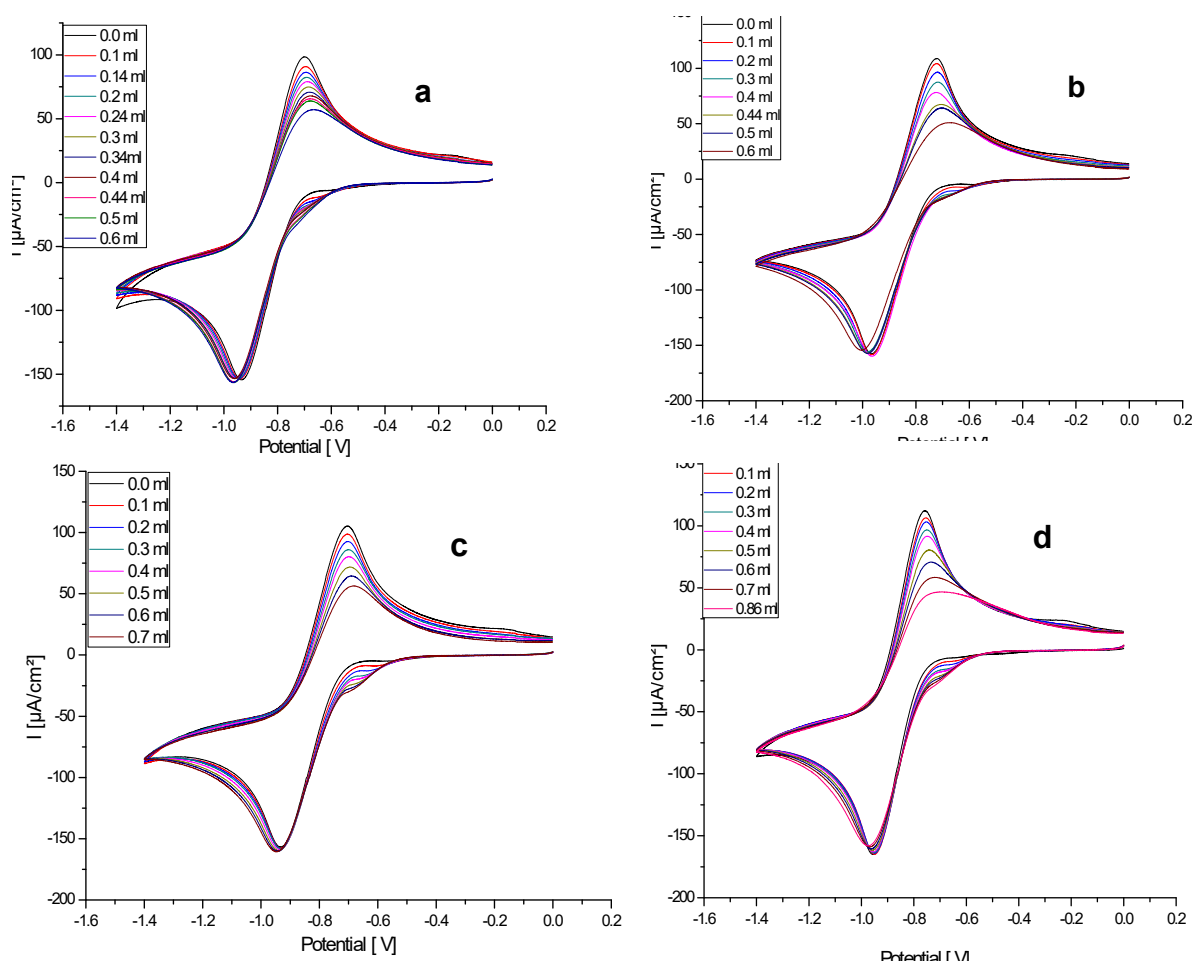


Fig.3. Cyclic voltammograms of oxygen-saturated DMF/0.1 TBFP on a GC electrode in the absence and presence of different concentration of sample extract (a) Kondor, (b) Bartina, (c) Spunta, (d) Kuroda at scan rate 100 mV.s^{-1} , $T = 28^\circ$

5.6. Thermodynamic properties

To quantify the results, the strength of interaction, between superoxide anion radical and the probable antioxidant in the extract, was estimated in terms of binding constant K_b . Based on the decrease in peak current, the binding constant (K_b) was calculated using following equation of Feng (6) [20].

$$\log \left[\frac{1}{[AO]} \right] = \log K_b + \log \left[\frac{I_p}{I_{po} - I_p} \right] \quad (6)$$

Where, I_{p_0} and I_p are the peak currents of superoxide anion radical in the absence and presence of additives, respectively, $[AO]$ is the concentration of the antioxidant. As $[AO]$ is not known, therefore, this term was replaced by the volume of the extracts (ΔV_{ext}). It is noticeable that the volume of the solution containing $O_2^{\bullet-}$ is fixed thus the addition of volume increments of the extract is proportional to the addition of more number of moles (i.e. concentration) of the compound(s) according to Safeer et al [21]. Another thermodynamic parameter, standard Gibbs free energy (ΔG°) was calculated using the measured K_b .

The obtained values from CV's voltammograms showed in table 3 indicated that the binding constant is in the following ordre,

$$K_{O_2^{\bullet-}-Spunta} < K_{O_2^{\bullet-}-Kondor} < K_{O_2^{\bullet-}-Kuroda} < K_{O_2^{\bullet-}-Bartina}$$

The values of free energy varied in the same order as of the binding constant. The negative values of ΔG indicated the spontaneity of $O_2^{\bullet-}$ – sample interaction. The data obtained in table 3 were very statistically significant with $p < 0.005$

Table 3. Values of binding constants and binding free energies

Compound	Equation	R ²	K	ΔG	P
$O_2^{\bullet-}$ -Kondor	$y = 0.9086x + 2.8778$	0.990	7.5474×10^2	-16.42	0.000
$O_2^{\bullet-}$ -Bartina	$y = 0.4818x + 3.1119$	0.956	13.1522×10^2	-17.80	0.001
$O_2^{\bullet-}$ -Spunta	$y = 0.8952x + 2.8012$	0.986	6.3270×10^2	-15.99	0.000
$O_2^{\bullet-}$ -Kuroda	$y = 0.576x + 2.9473$	0.986	8.8572×10^2	-16.82	0.000

5.7. Diffusion coefficients

The redox process of the four sample extracts as a function to scan rates presented in Fig4. The voltammogram contained a couple of a clear stable oxidation and reduction peaks attributed to the electrochemical behaviour of the redox $O_2/O_2^{\bullet-}$ couple. In addition, demonstrate clearly the reversibility of the oxidation reaction of the redox couple $O_2/O_2^{\bullet-}$ as the oxidation peak potentials are not significantly affected by the variation in the scan rate.

The diffusion coefficients of the free radical $O_2^{\bullet-}$ and its $O_2^{\bullet-}$ bounded forms with ethanolic extract were calculated from the following Randles–Sevcik equation [22].

$$i = 2.69 \times 10^5 n^{\frac{3}{2}} S C D^{\frac{1}{2}} \nu^{\frac{1}{2}} \quad (7)$$

Where i presents the oxidation peak current density, S presents the surface of the working electrode (cm^2), C is the bulk concentration ($\text{mol} \cdot \text{cm}^{-3}$) of the electroactive species, D present

the diffusion coefficient (cm^2/s) and v is the scan rate (V/s). The linearity of the plot $i_{pa} = f(v^{\frac{1}{2}})$ of the four adducts. Further demonstrates that the kinetic of the oxidation reaction is limited by the diffusion process. The diffusion coefficients were determined from the slopes of the plot i_{pa} vs $v^{\frac{1}{2}}$. Values are given in Table 4.

Table 4 shows that the diffusion coefficient of complexes $\text{O}_2^{\bullet-} - \text{SE}$ is lower than that of the free $\text{O}_2^{\bullet-}$ radical. The diffusion coefficients of four adducts are very close because sample complexes have the same contents the slight difference can only be attributed to the amount of the phytochemical compound.

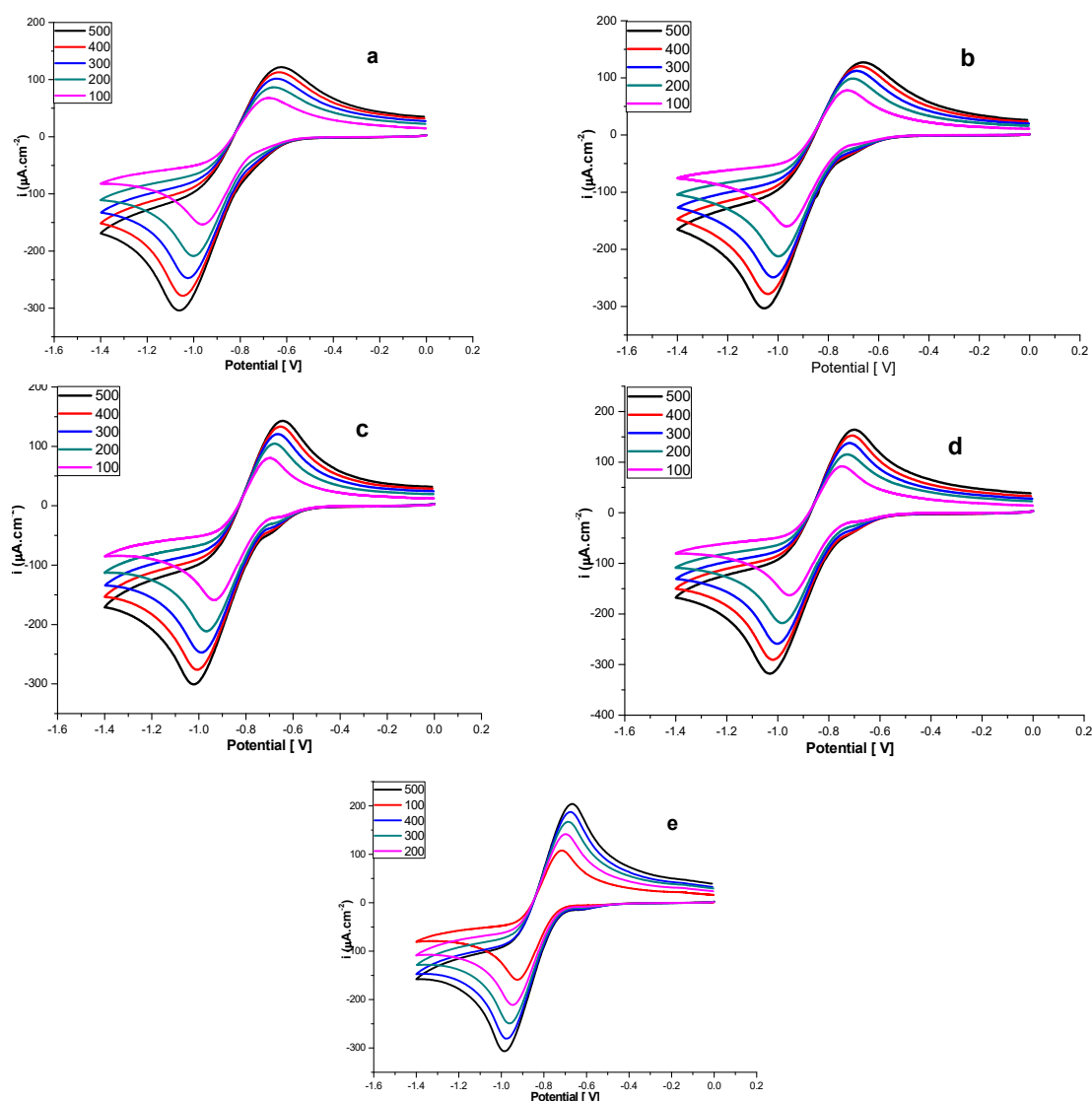


Fig.4. Succession of cyclic voltammograms at GC electrode in oxygen-saturated DMF/0.1 TBFP at different scan rates ranging from 100 to 500 mV/s ,(a) $\text{O}_2^{\bullet-}$ -Kondor, (b) $\text{O}_2^{\bullet-}$ -Bartina, (c) $\text{O}_2^{\bullet-}$ -Spunta, (d) $\text{O}_2^{\bullet-}$ -Kuroda and (e) for $\text{O}_2^{\bullet-}$

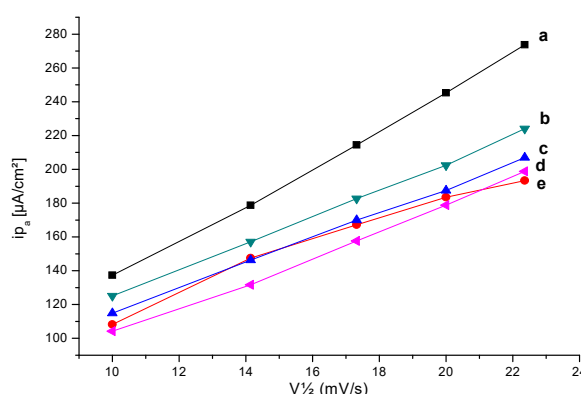


Fig.5. i_{p_a} versus $v^{1/2}$ plots of oxygen-saturated DMF in the absence (a) and in presence of 0.4 ml of the extract of kuroda (b), spunta (c), bartina (d) and kondor (e)

Table 4. Diffusion coefficient values

Sample	Equation	R ² values	D (cm ² /s)
O ₂ ^{•-}	$y = 11.046x + 24.721$	0.998	1.87×10^{-6}
O ₂ ^{•-} -Kondor	$y = 7.6929x + 25.282$	0.997	9.09×10^{-7}
O ₂ ^{•-} -Bartina	$y = 6.8567x + 44.991$	0.978	7.22×10^{-7}
O ₂ ^{•-} -Spunta	$y = 7.3768x + 41.425$	0.999	8.36×10^{-7}
O ₂ ^{•-} -Kuroda	$y = 7.941x + 45.149$	0.999	9.68×10^{-7}

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