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SCAVENGING POTENTIAL OF ASCORBIC ACID, GALLIC ACID AND α-TOCOPHEROL TOWARDS ELECTROCHEMICALLY GENERATED SUPEROXIDE ANION RADICAL AND EVALUATION OF THEIR INTERACTION USING CYCLIC VOLTAMMETRY

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Abstract
The trapping potential of the electrochemically generated superoxide anion radical by three natural phenolic compounds, ascorbic acid (AA), gallic acid (GA) and α-tocopherol (α-T) was successfully evaluated by cyclic voltammetry. This reaction has been studied in an aprotic organic medium, dimethylformamide. The decrease in the anodic current with the progressive increase of AA, GA and α-T amounts is rationalized as free radical consumption by the added antioxidant. Quantification of the antioxidant activity of AA, GA and α-T towards superoxide anion radical is made in terms of antioxidant capacity index (IC_{50} of AA=5.83 µg/ml, IC_{50} of GA= 3.45µg/ml, IC_{50} of α-T =22.03 µg/ml), antioxidant activity coefficient K_a (AA=1.48×10^{-2} M^{-1}, GA= 2.29×10^{-2} M^{-1} α-T=0.93×10^{-2} M^{-1}), binding constant K_b (AA= 21.72 M^{-1}, GA=42.56 M^{-1}, α-T= 7.18 M^{-1}) and binding free energy (AA= -7.88 KJ/mol, GA= -9.39 KJ/mol, α-T = -4.93 KJ/mol).The obtained results confirm the potential antioxidant of the three compounds and the antiradical activity was found higher for Gallic acid followed by Ascorbic acid, then α-Tocopherol.

Key words: Ascorbic acid, Gallic acid, α-Tocopherol, Antioxidant activity, Superoxide, Cyclic voltammetry

1. Introduction

A very important species of the reactive oxygen species family is the radical anion superoxide, this entity sometimes results from the oxygen necessary for the organism, and this oxygen can react with several molecules in the body-adrenaline, dopamine, tetrahydrofolates and certain components of the mitochondrial electron transport chain to produce superoxide. In addition, the superoxide is generated by the phagocytes during their destruction of foreign bodies. In

addition, superoxide can be directly produced by several oxidase enzymes, including monoamine and amino acid oxidases **(i, ii)**

A biological antioxidant is any compound capable of delaying or preventing the oxidation of a substrate. The first type of antioxidant developed against oxidative damage is those which prevent the appearance of reactive oxygen species and those which block and capture the radicals that form. Another important antioxidant is that represented by a repair process which removes damaged biomolecules before their aggregation allows for modification of cellular metabolism **(iii)**

When endogenous factors such as uric acid, bilirubin, albumin, metallothioneins, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase cannot scavenge reactive oxygenated species and protect the organism, intervention of exogenous antioxidants such as vitamin C, vitamin E, flavonoids, carotenoids, gallic acid ... etc is necessary, as food consumption, as a nutritional supplement or as a pharmaceutical product **(iv)**

Vitamin C, a water-soluble vitamin is isolated and identified in 1932. It is also called L-ascorbic acid or L-ascorbate in reference to its anti-scorbutic properties **(v, vi)**

Ascorbic acid acts as a potent reducing and antioxidant agent; its biomedical uses reside in fighting bacterial infections, in detoxifying reactions, and in the formation of collagen in fibrous tissue, teeth, bones, connective tissue, skin, and capillaries (vii).

Ascorbic acid can be found in many food sources like Broccoli, cabbage, cantaloupe, citrus fruits, guava, kiwifruit, leafy greens, peppers, pineapples, potato, strawberry, tomato, watermelon **(viii).**

Gallic acid (3, 4, 5-trihydroxybenzoic acid) is a secondary metabolite present in most plants, it is considered as one of the major phenolic acids, acid gallic or gallate is also a benzoic acid of great importance **(ix)**

Natural gallic acid found abundantly in grapes, strawberries, pineapples, bananas, lemons, gallnuts, sumac, witch hazel, tea leaves, oak bark, and apple peels **(x)**

GA, strong chelating agent, protects human cells or tissues against oxidative stress, by its biological activities, including anticancer, anti-oxidant, anti-microbial, anti-inflammatory, anti-HIV and antibacterial effects **(xi, xii).**

α-Tocopherol was proposed by Olcott and Mattil, soon after its discovery to act as an antioxidant **(xii)**, it constitutes the most important form of tocopherols in terms of abundance in the human diet, tissue levels and antioxidant activities **(xiv)**. It has been shown that tocopherol can stop the radical chain by forming a derivative with low reactivity incapable of attacking lipid substrates, therefore tocopherol can fight against lipid peroxidation of cell membranes and inflammation **(iii, xiii)**

The main sources of tocopherols in our diet: corn oil, soybeans, canola and other vegetables and nuts (xiv).

In this study, the behavior of the electrochemical-generated superoxide anion radical in the presence of ascorbic acid, gallic acid and α-tocopherol has been investigated. The radical scavenging ability of ascorbic acid, gallic acid and α-tocopherol translated in terms of effective antioxidant activity index (IC_{50}%), antioxidant activity coefficient (K_a), binding constant (K_b**)** and binding free energy (ΔG).

2. Experimental

2.1. Chemicals

Dimethylformamide (DMF) 99.9% from Biochem Chemopharma (Canada), tetrabutylammonium tetrafluoroborate Bu_4NBF_4 (99%) from Sigma-Aldrich, ascorbic acid (99%), gallic acid (99%) and α-tocopherol (97%) were from Alfa Aeser.

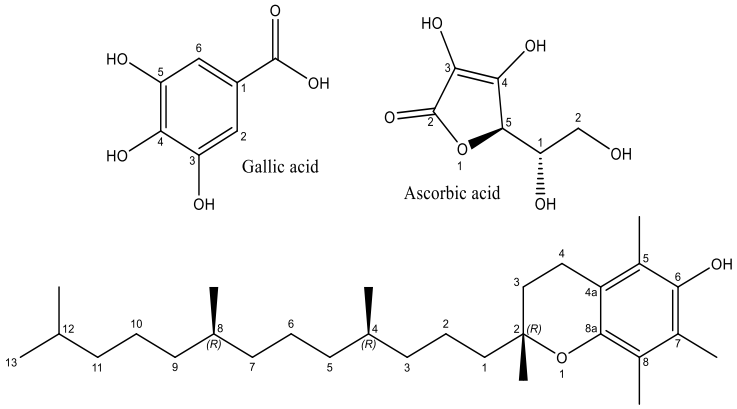


Fig.1. AA, GA and α-Tocopherol's structures.

2.2. Instruments

Electrochemical measurements were carried using Voltalab 40 model PGZ301 potentiostat / galvanostat in an electrochemical cell of volume V=25ml. All the measurements were made using conventional three electrode; glassy carbon (0.03 cm^2) served as the working electrode, a saturated calomel electrode (SCE) functioned as a reference and platinum wire functioned as a counter-electrode. All experimental data were recorded at 28 ± 1°C.

2.3. Procedure

The inhibitory activity of the superoxide radical anion is determined by cyclic voltammetry, this test is based on the Bourvellec et al method with some modifications **(xv).**

Prior to use, the working electrode is polished, rinsed with distilled water, and dried with paper towels. This cleaning procedure is always applied before each electrochemical measurement. The superoxide radical anion is generated by commercial molecular oxygen dissolved in DMF which contains 0.02 M Bu_4NBF_4 at room temperature (28±1) °C. The scanning rate is maintained at 100mV/s. The applied potential range was -1.6V to 0.0V vs SCE. AA, GA and α-T were added to the superoxide radical dissolved in the DMF and the voltammograms have been **(xvi).**

2.3.1. Quenching of $O_2^{\bullet-}$ and antioxidant capacity index (IC_{50})

The inhibition percentage of superoxide ($O_2^{\bullet-}$) caused by the addition of AA, GA is calculated by the following formula:

$$\textit{Superoxide inhibition I(\%)} = \frac{Ip0 - Ips}{Ip0} \times 100 \qquad \textit{(01)}$$

Where;

Ip_0 : anodic current density of superoxide without substrate (additive)

Ip_s : anodic current density of superoxide with substrate (additive)

The antioxidant capacity index (IC_{50}) was calculated from the graph plotted between percentage of inhibition and concentration of the sample.

2.3.2. Antioxidant Activity coefficient (K_a)

The capacity of AA, GA and α-T to trap the superoxide radical was expressed by a coefficient called the coefficient of antioxidant activity (K_a). The constant K_a is defined as **(xvii)**:

$$Ka = \frac{\Delta J}{(J0 - Jres)\,\Delta C} \qquad \textit{(02)}$$

Where;

ΔJ: change in anodic current density of superoxide caused by addition of substrate.

J0: limiting anodic current density of superoxide without substrate in the solution.
Jres: residual current density of superoxide
ΔC: change in the concentration of substrate (mol/l).

2.3.3. Binding constant (K_b)

The interaction strength between the superoxide radical and the antioxidant was quantified by a parameter named k_b binding constant, this constant was calculated using the following equation **(xvii, xviii)**

$$Log\frac{1}{[AO]} = log\,Kb + log\frac{Ip}{Ip-Ip0} \qquad (03)$$

Where;
Ipo, Ip: anodic current densities of superoxide without and with additives, respectively,
[AO] : concentration of antioxidant

2.3.4. Binding free energy (ΔG)

The binding free energy ΔG was calculated using equation (04). **(ixx)**

$$\Delta G = -RTlnK \qquad (04)$$

Where;
ΔG: binding free energy, $KJ.mol^{-1}$
R: gas constant; 8.32 $J.mol^{-1}.K^{-1}$
T: absolute temperature equal to 298K.

3. Results and Discussion

3.1. Voltammetric behavior of superoxide anion radical

In the laboratory, $O_2^{\bullet-}$ can be generated via several approaches, including enzymatic, chemical, electrochemical, photochemical and photocatalytic methods. However, electrochemical techniques in general and cyclic voltammetry in particular are operationally useful because $O_2^{\bullet-}$ can be generated electrochemically in very precise amounts, under mild conditions and with little or no by-products in dry aprotic solutions **(xx, xxi)**

In the used system, $O_2^{\bullet-}$ was generated electrochemically in the DMF solution from commercial oxygen. The cyclic voltammetry technique is used to generate the superoxide radical in the diffusion layer of the glassy carbon electrode by the one-electron reduction of molecular oxygen in DMF at room temperature **(xxii, xxiii, xvii).**

The presence of the $O_2^{\bullet-}$ radical in the electrochemical cell is easily detected by its anodic current measured at the same electrode during the return scan. The reduction of $O_2^{\bullet-}$ is a reversible reaction and this radical is known to be stable in aprotic medium and disproportionation does not occur during cyclic voltammetric measurements in a DMF solution **(xxiv, xxv)**. It is for this reason that cyclic voltammetry is a convenient way to generate $O_2^{\bullet-}$ without enzymatic systems and the study of its reaction with any molecule provided the substrate is inactive within the potential reduction range of oxygen **(xvii)**. The cyclic voltammogram of the superoxide anion radical is shown in Fig.2.

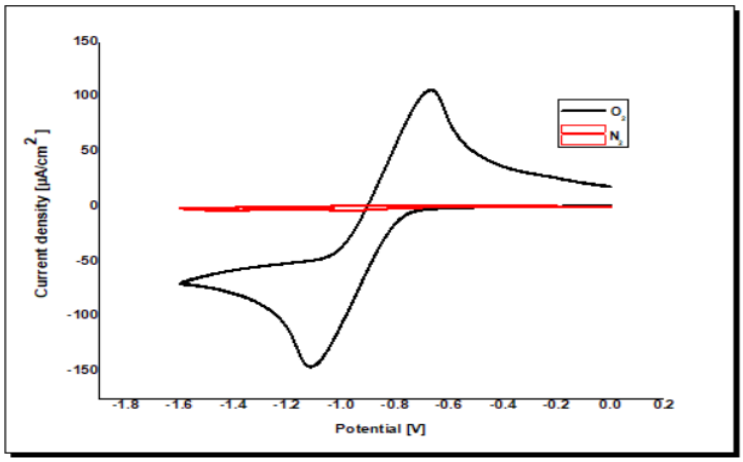


Fig.2. Cyclic voltammograms of (a) medium (DMF+0.02 M Bu_4NBF_4) and (b) O_2^- in the same medium on GC as working electrode, SCE as reference electrode at 28°C with scan rate of 100mV/s.

The cyclic voltammogram of superoxide anion radical showed one electron reversible process (fig.2), having well developed and clear oxidation and reduction peaks. The height of the voltammogram corresponds to the solubility (concentration) of the oxygen. $O_2^{\bullet-}$ is stabilized by the ion pair formation and solvation process with tetra butyl ammonium tetra fluroborate cation. Here, the superoxide can act as a strong bronsted base, a nucleophile and as a one electron donor **(xxvi, xxvii)**

3.2. Behavior of superoxide in presence of AA

The addition of the commercial antioxidant causes a proportional decrease in anodic current while the effect on the cathodic current appears to be negligible. For AA, the addition of 0.1ml to 1ml decreases the anodic peak from 139.9 to 94.24 µA/cm^2 (Fig.3)

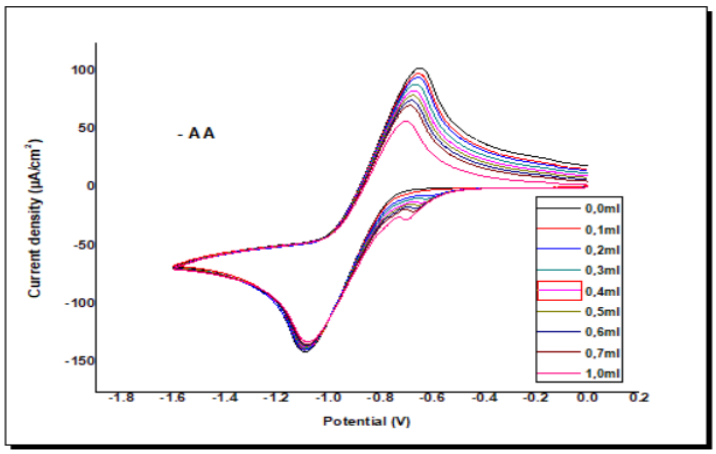


Fig.3. Voltammograms of $O_2^{\bullet-}$ in the presence of different concentrations of ascorbic acid in DMF+0.02 M Bu_4NBF_4 on CV as working electrode at 28 °C.

The observed systematic depletion in the anodic current is interpreted in terms of the scavenging of the radical who was formed while going towards negative potential. The intactness of the cathodic wave and systematic decrease in anodic current, upon further addition of AA, ensures the consumption of the $O_2^{\bullet-}$. No change in the reduction wave also imparts that there is no interaction between AA and the molecular oxygen. As for as the effect of added AA on the peak potential is concerned, a very small positive shift (towards less negative potential) was observed which is a usual observation in such type of systems **(xvii, xviii)**.

Vit C or AA can directly scavenge singlet oxygen, superoxide and hydroxyl radicals**.** According to Muhammed and al; slow interactions of ascorbic acid and superoxide anion

radical could be attributed to the high stability of the compound or conformational changes before the start of the interaction. With regard to the interaction mechanism, the behavior observed demonstrates an electron transfer process, this electron transfer, will always be followed by a proton transfer from the antioxidant to the free radical to stabilize the resulting product **(xxviii)**.

3.3. Behavior of superoxide in the presence of gallic acid

Fig.4 shows cyclic voltammograms of superoxide anion radical in the presence of gallic acid. The addition of 0.1ml to 1ml of GA decreases the anodic and cathodic currents simultaneously, and shifts both peak positions towards more positive values. Maximum effect was observed up to 0.7ml while further additions of GA had little effect on the peak potentials and peak currents. The decrease in the anodic current is attributed to the decrease of the radical concentration upon scavenging while the simultaneous decrease in the cathodic current indicates an electron transfer (ET) mechanism from the antioxidant to the superoxide radical immediately followed by a proton transfer. The slow interactions could be attributed to the high stability of the compound or conformational changes before the interaction start. As far as the mechanism of interaction is concerned, the observed behavior demonstrates an ET process. It is well established that whenever there is an ET mechanism it will always be followed by a proton transfer from the antioxidant to the free radical to stabilize the resultant product. In case of protic solvents, proton abstraction can occur from the solvent if it is a better proton donor compared to the antioxidant

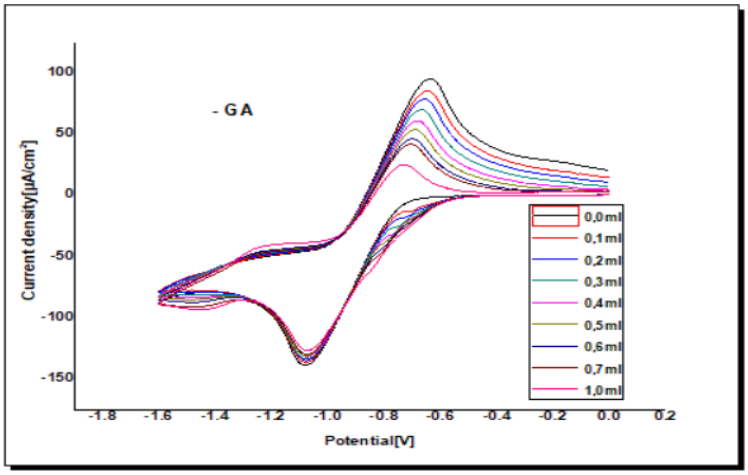


Fig. 4. Voltammograms of $O_2^{\bullet-}$ in the presence of different concentrations of gallic acid in DMF+0.02 M Bu_4NBF_4 on CV as working electrode at 28 °C

3.4. Behavior of superoxide anion radical in the presence of α-tocopherol

As shown in fig.5, the addition of α-tocopherol with increasing concentrations ranging from 0.398µg ml to 3.846µg / ml to the superoxide dissolved in DMF causes a decrease in the antodic current density of superoxide from 134.84 µA / cm^2 to 124.72µA / cm^2. This slight decrease is explained by the moderate trapping of superoxide by α-T. It is noted that the intensity of the anodic current of superoxide in the absence of α-T equal to 137.32 µA / cm^2.

We notice that the decrease in the intensity of the anodic current is practically negligible compared to that for ascorbic and gallic acid, while the intensity of the cathodic current remains stable, which allows us to deduce that the α-T has a weaker superoxide trapping power than ascorbic and gallic acid.

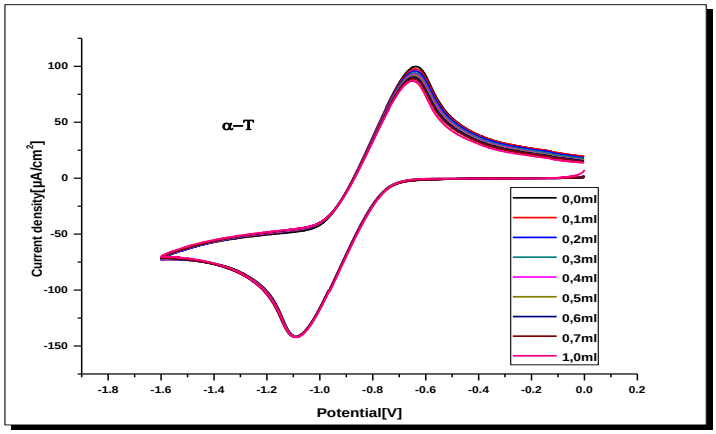


Fig.5. Voltammograms of $O_2^{\bullet-}$ in the presence of different concentrations of gallic acid in DMF+0.02 M Bu_4NBF_4 on CV as working electrode at 28 °C **(xvi)**

3.5. Quenching of superoxide anion radical by antioxidants

The methodology developped by le Bourvellec and al is based on the reaction Kinetics of the antioxidant substrate with the superoxide anion radical. A cyclic voltammetry forward scan generates $\mathbf{O_2^{\bullet-}}$ by reduction of molecular oxygen in an aprotic medium, (DMF); the consumption of the radical is directly measured at the back ward scan by the anodic current decay from its oxidation in the presence of AA,GA and α-T **(xxviv, xx).**

The evolution of the density of the anodic superoxide current as a function of the different concentrations of the ascorbic acid, gallic acid and α-tocopherol solutions introduced into the electrochemical cell is shown in fig.3, fig.4 and fig.5.

Table 1. IC_{50}, K_a values of AA, GA and α-T

Compound	**IC_{50} (μg/ml)**	**K_a (M^{-1})**
AA	**5.87**	**$1.84.\ 10^{-2}$**
GA	**3.45**	**$2.29.\ 10^{-2}$**
α-T	**22.03**	**$0.93.10^{-2}$**

The reduction of the anodic current of superoxide anion radical is attributed to the action of antioxidants which reacts with the superoxide radical and decreases its concentration at/around the surface of the electrode.

Ascorbic acid and gallic acid which have more than one -OH in their structures are powerful superoxide scavengers, the hydrogen of the -OH group is responsible for the interaction with the superoxide and it can be carried out by abstraction of the H atom of AA or GA with a superoxide radical. On the basis of this analogy, the proposed mechanism is therefore the abstraction of hydrogen atoms of the hydroxyl group by the superoxide anion radical.

On the contrary, α-tocopherol has in its structure one hydroxide, which reflects the poor trapping power of superoxide by this antioxidant compared to ascorbic acid and gallic acid

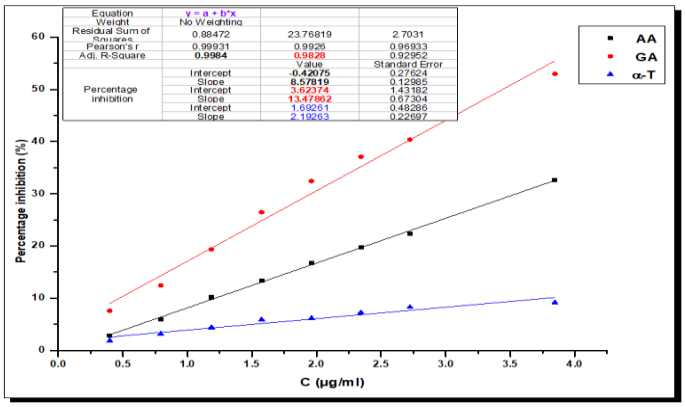


Fig.6. Plotting of scavenging of superoxide anion against the corresponding concentration of AA, GA and α-T

It is well known that the superoxide radical abstracts proton when it reacts with a week acid. By nature, antioxidants are weak acids and the -OH group containing antioxidants are capable of donating proton(s) **(xviii)**.

The antioxidant capacity index IC_{50} is the amount of antioxidant for 50% consumption of the radical concentration (xx). From the above equation (01), the percentages of superoxide inhibition caused by the addition of AA, GA and α-T were calculated

The table 1 regroups the IC_{50}, Ka_0 and K_b values; IC_{50} which is the concentration of the scavenger to cause loss of superoxide activity was calculated from the graph plotted between percentage of inhibition and concentration of the sample (I_{AA} (%) =8.5782C- 0.4207 with R^2= 0.9984, I_{GA} (%) =13.479C+3.6237 with R^2= 0.9828, $I_{\alpha\text{-}T}$ (%) =2.192x+1.692)

GA show an important scavenging effect of superoxide anion radical with IC_{50} value of 3.45µg/ml, followed by AA which show a scavenging effect towards superoxide anion radical with IC_{50}= 5.87 µg/ml, finally α-T with IC_{50}= 22.03 µg/ml.

Table 2. Anodic current densities of AA, GA and α-T at different concentrations

C(µg/ml)			Ip (µA/cm^2)			Log (1/[AO])			Log [Ip/(Ip$_0$-Ip)]		
AA	**GA**	**α-T**	**AA**	**GA**	**α-T**	**AA**	**GA**		**AA**	**GA**	**α-T**
0	**0**	**0**	**139.9**	**132.16**	**137.32**	**-**	**-**	**-**	**/**	**/**	**/**
0.398	**0.398**	**0.398**	**135.91**	**122.14**	**134.84**	**2.63**	**2.63**	**3.03**	**1.53**	**1.085**	**1.735**
0.793	**0.793**	**0.793**	**131.5**	**115.68**	**132.99**	**2.33**	**2.33**	**2.73**	**1.19**	**0.846**	**1.487**
1.185	**1.185**	**1.185**	**125.66**	**106.58**	**131.3**	**2.15**	**2.15**	**2.56**	**0.94**	**0.619**	**1.338**
1.574	**1.574**	**1.574**	**121.21**	**97.17**	**129.24**	**2.04**	**2.04**	**2.43**	**0.81**	**0.443**	**1.204**
1.96	**1.96**	**1.96**	**116.51**	**89.27**	**128.88**	**1.94**	**1.94**	**2.34**	**0.70**	**0.318**	**1.184**
2.343	**2.343**	**2.343**	**112.29**	**83,09**	**127.39**	**1.86**	**1.86**	**2.26**	**0.61**	**0.228**	**1.108**
2.723	**2.723**	**2.723**	**108.58**	**78.82**	**125.94**	**1.79**	**1.79**	**2.20**	**0.54**	**0.169**	**1.044**
3.846	**3.846**	**3.846**	**94.24**	**62.14**	**124.72**	**1.64**	**1.64**	**2.05**	**0.31**	**0.052**	**0.995**

3.6. Antioxidant Activity coefficient (K_a)

The relative capacity of AA, GA and α-T to scavenge the target radical was determined as antioxidant activity coefficient (K_a). The values necessary to deduce K_a are collected in Table 3.

Table 3. Parameters deduced to calculate K_a

C (M) *10^{-3}			J (µA/cm^2)			J/J$_0$-J$_{res}$		
AA	**GA**	**α-T**	**AA**	**GA**	**α-T**	**AA**	**GA**	**α-T**
0	**0**	**0**	**139.9**	**132.16**	**137.32**			
2.31	**2.338**	**0.925**	**135.91**	**122.14**	**134.84**	**0.971**	**0.924**	**0.98**
4.60	**4.659**	**1.841**	**131.5**	**115.68**	**132.99**	**0.940**	**0.875**	**0.968**
6.88	**6.965**	**2.751**	**125.66**	**106.58**	**131.3**	**0.898**	**0.806**	**0.956**
9.14	**9.252**	**3.654**	**121.21**	**97.17**	**129.24**	**0.866**	**0.735**	**0.941**

11.38	**11.521**	**4.550**	**116.51**	**89.27**	**128.88**	**0.833**	**0.675**	**0.933**
13.61	**13.772**	**5.440**	**112.29**	**83.09**	**127.39**	**0.802**	**0.629**	**0.928**
15.82	**16.006**	**6.322**	**108.58**	**78.82**	**125.94**	**0.776**	**0.597**	**0.917**
22.34	**22.607**	**8.930**	**94.24**	**62.14**	**124.72**	**0.673**	**0.470**	**0.908**

Fig.7 presents the relative change of the $O_2^{\bullet-}$ current density versus change in sample concentration for anodic peak of AA,GA and α-T

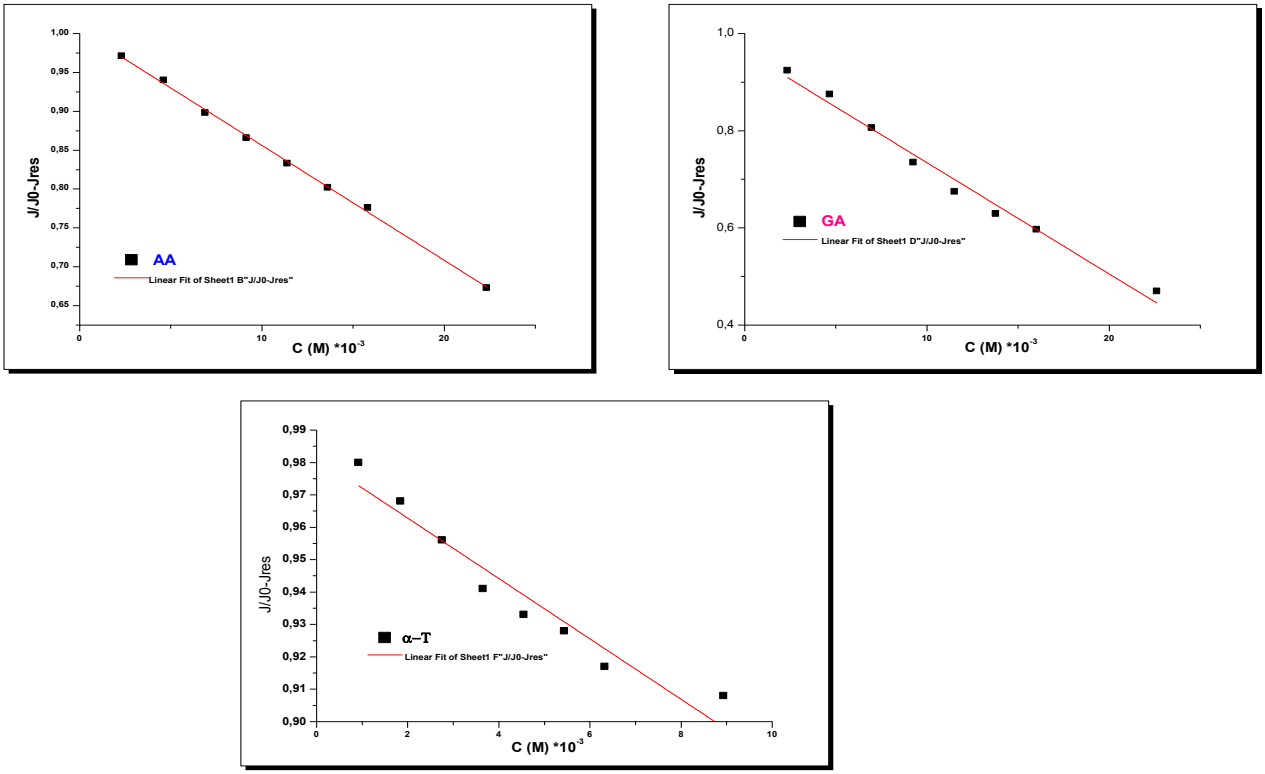


Fig.7. Relative change in $O_2^{\bullet-}$ current density versus change in sample concentration for the anode peak of AA, GA and α-T

From Table 1, the most powerful antioxidant in terms of antioxidant activity coefficient (K_a) is gallic acid with $K_a=2.29\times10^{-2}$ M^{-1} followed by ascorbic acid with $K_a=1.48\times10^{-2}$ M^{-1} then α-tocopherol with $K_a=0.93\times10^{-2}$ M^{-1}.

3.7. Binding constant (K_b)

The degree of interaction between the superoxide radical and the antioxidant was quantified by a parameter named k_b binding constant, this constant was calculated using the equation

The Fig.8 presents the plots to determine interaction constant (K_b) between the superoxide anion radical and the corresponding antioxidants: AA, GA and αT. Compounds resulting in higher K_b values show strong interaction with the free radical. As shown in table 3, the values of K_b follow the order: GA> AA> α-T

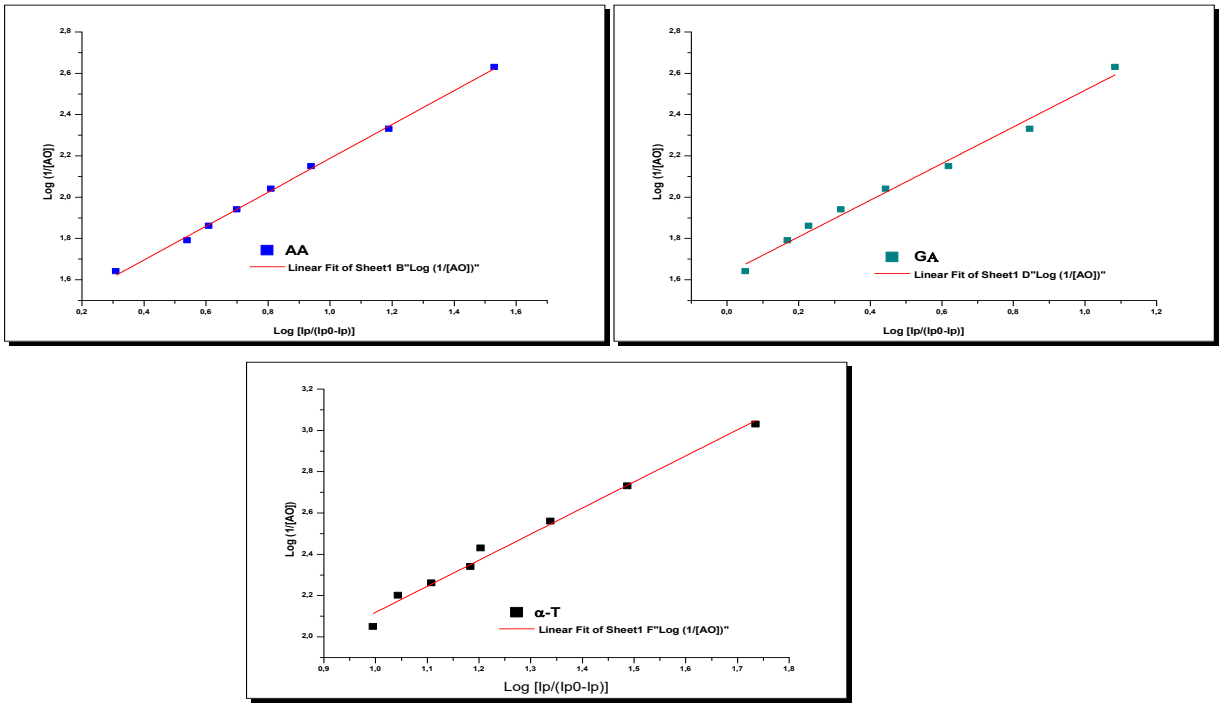


Fig. 8. Plots to determine binding constant (k_b) using equation log (1/[AO]) vs log [Ip/(Ipo–Ip)] for AA, GA. and α-T

3.8. Binding free energy (ΔG)

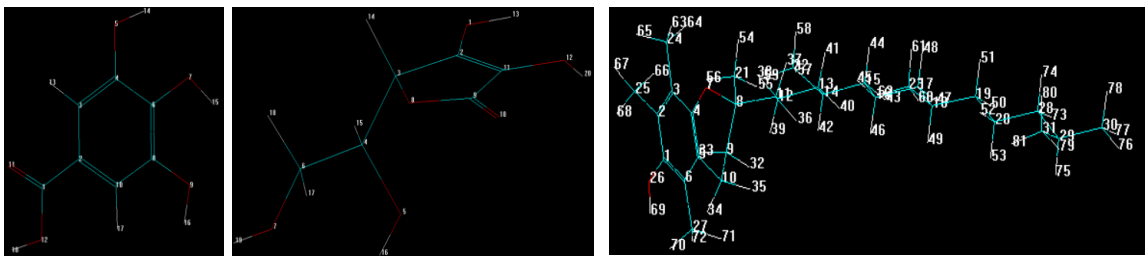
The negative values of free energy support that the binding of AA, GA and α-T to superoxide is spontaneous and exothermic **(xxx)**. The ΔG values in table 4 indicates more affinity of superoxide to GA followed by AA and at last α-T which is consistent with the above mentioned results **(xxxi)**.

Table.4. K_b and ΔG values of AA, GA and α-T

Antioxidant	Eq.	R^2	K_b	-ΔG (KJ/mol)
AA	$Log_{10}(1/[A_O])=0.8201Log_{10}(I_p/(I_{po}-I_p)+1.3678$	0.9979	23.324	7.8856
GA	$Log_{10}(1/[A_O])=0.8875Log_{10}(I_p/(I_{po}-I_p)+1.6304$	0.9868	42.697	9.3994
α-T	$Log_{10}(1/[A_O])=1.2631Log_{10}(I_p/(I_{po}-I_p)+o.8559$	0.9864	7.176	4.9343

QSAR's properties study

Total energy of three antioxidants molecules have been geometry optimization using the Molecular Mechanics (MM^+) force field included in HyperChem version 8.0.3.



Compoand	Acide acecorbique	Acide gallique	Tocopherol
Total energy	**0.771**	**-2.9154**	**24.77**

We studied 8 physico-chemical properties of series of 3 standards antioxidants (AA, AG and αT), using HyperChem software. QSAR properties such as van der Waals surface, molecular volume (MV), Surface Area Grid (SAG) and Surface Area Approx (SAA), octanol-water partition coefficient (log P), molar refractivity (MR), polarisability (Pol), molecular volume and molecular weight (MW) accessible to solvents and bound to the surface were studied and are gathered in the table 5.

Table 5. QSAR Properties

Compound	(MW) amu	(Pol) Cm^3	(MR) Cm^3	(logP)	(HE) kcal/mol	(MV) Cm^3	(SAG) Cm^2	(SAA) Cm^2
AA	**176.13**	**14.09**	**36.46**	**-2.44**	**-21.48**	**481.62**	**323.38**	**268.98**
AG	**170.12**	**14.90**	**41.77**	**-2.09**	**-24.66**	**453.96**	**300.77**	**258.50**
αT	**430.71**	**53.14**	**136.77**	**6.41**	**1.79**	**1458.14**	**820.15**	**835.26**

4. Conclusion

The current work was aimed to reinvestigate the antioxidant character of ascorbic acid, gallic acid and α-tocopherol towards electrochemically generated superoxide anion radical using the cyclic voltammetric method in DMF by monitoring the changes in the anodic currents densities of superoxide in the presence of increasing amounts of substrates.

GA was found to have a strong anti-superoxide anion radical character followed by AA then by α-T. IC_{50} values are in accordance with K_a, K_b and ΔG data. Quantification, in terms of antioxidant capacity index (IC_{50}), binding constant (K_b), coefficient of antioxidant activity (K_a), binding free energy (ΔG) perfectly reflect the ability of superoxide radical to be inhibited by ascorbic acid and gallic acid and moderately by α-tocopherol.

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