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Research Article

FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITYN OF DIFFERENT ORGANIC CRUDE EXTRACTS OF THE STEM OF SOLANUM NIGRUM L. BY ELECTROCHEMICAL METHOD

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ABSTRACT

The present research aimed to quantify the total flavonoid content and antioxidant properties in the different crude extract of the stem of *Solanum nigrum* that grow in southern Algeria. The results showed that EtOAc extract showed the highest flavonoid content (18.41 ± 0.023 mg QE/g). The electrochemical behavior of the chloroform, ethyl acetate and n-butanol extracts were studied and indicated that all extracts were electroactive and possess antioxidant activity, chloroform extract showed the highest capacity (9.58 ± 0.010 mg/g). it has been shown that these samples showed scavenging ability on $O_2^{\cdot-}$ superoxide anion produced by electrochemical reduction of oxygen. The chloroform extract was the most efficient extract presenting the lowest IC_{50} values (0.664 mg/mL). The binding constant (K_b) of the reaction and the change in free energy (ΔG) were calculated.

Keywords: *Solanum nigrum* L.; stem; crude extract; total flavonoids; antioxidant activity; cyclic voltammetry.

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1. INTRODUCTION

Solanaceae family consists of about 2700 species including fruits and vegetables such as pepper, tomato, and potato, medicinal plants such as *Solanum trilobatum*, *Datura stramonium,* and *Hyoscyamus aureus*, and ornamental plants such as Lycianthes, Browallia, and Petunia [1]. *Solanum nigrum* (black nightshade) is a relatively fast-growing annual medicinal plant belonging to family *Solanaceae* [2]. Its great extent use found in common drug to deal different illnesses such aspirin, inflammation and fever. The growth of *Solanum nigrum* is fast, this plant found in various types of habitats, such as grasses in different crops and also grown in several countries [3]. It has been used ethnobotanically as hepatoprotective, antipyretic, anti inflammatory, antioxidant, diuretic, anticancer and microbial, activities. Previous phytochemical studies reveal that these medicinal activities are probably due to its flavonoid and steroidal glycoalkaloid content [4]. *S. nigrum* serves mainly as vegetables for soup preparation in different parts of the world. Several studies have investigated the nutritive value of this plant, which put forward evidence that this species constitutes a nutritious vegetable. This plant was chosen not only for being nutritive, but also for their folkloric reports of medicinal properties [5]. Antioxidants are compounds that delay autoxidation by inhibiting formation of free radicals or by interrupting propagation of free radical by one (or more) of several mechanisms: scavenging species that initiate peroxidation; chelating metal ions such that they are unable to generate reactive species or decompose lipid peroxides; quenching O^{2-} and preventing formation of peroxides; breaking the autoxidative chain reaction and/or reducing localized O^{2-} concentration [6]. Natural antioxidants, especially phenolics and flavonoids, are safe and also bioactive. Therefore, in recent years, considerable attention has been directed towards identification of plants with antioxidant ability that may be used for human consumption [7]. The objectives of the present study were to investigate the total flavonoid contents of stems of *Solanum nigrum* and the evaluation of the antioxidant activity by cyclic voltametry assay of different crude extract, the electrochemical behaviour was investigated at a glassy carbon electrode.

2. MATERIALS AND METHODS

2.1. Chemicals

Ethanol (EtOH), chloroform ($CHCl_3$), ethyl acetate (EtOAc), n-butanol (n-BuOH), quercetin dihydrate, aluminium chloride ($AlCl_3$), sodium sulfate (Na_2SO_4), methanol (MeOH), phosphate buffer, Dimethylformamide (DMF), tetrabutylammonium tetrafluoroborate (Bu_4NBF_4). All reagents used were for analysis.

2.2. Plant Material

Solanum Nigrum L**.** were collected from Debila, El Oued in October 2017. The samples of the plant were then washed with running tap water to remove the adhering dust and dirt. They were divided into small pieces and dried under room temperature for several days. Dehydrated stems of the plant were ground to a fine powder with the help of a mechanical grinder and the powder was stored in a suitable container.

2.3. Extract Preparation

Dried powder (200 g) of stems of the plant was extracted for 24 h in a rotary shaker with 1200 mL EtOH/H_2O (80:20 v/v) at room temperature three times. The extract was filtered through Whatman No. 1 filter paper. The filtrates were combined and concentrated up to 55°C under reduced pressure. The residue obtained was diluted with distilled water under magnetic stirring and maintained at 4°C for one night to precipitate a maximum of chlorophylls. After filtration, the resulting solution was successively extracted several times with chloroform, ethyl acetate and n-butanol. The organic phases were dried with Na_2SO_4, filtered using filter paper and concentrated until to obtain dry extracts. The percentage yield of chloroform, ethyl acetate and n-butanol extracts were 0.15 %, 0.065% and 1.53 % respectively.

2.4. Total Flavonoid Content Assay

The total flavonoid content of *S. nigrum* L. stem extracts (chloroform, ethyl acetate and n-butanol) was measured by the aluminium chloride colorimetric assay with some modification [8]. An aliquot 1mL of the extract was added to 1 mL 2% $AlCl_3$ in a volume trick flask, the solution was incubated in darkness at room temperature for 10 minutes. The absorbance was measured against the blank at 430 nm. The standard curve was prepared using different concentrations of quercetin solutions. The total flavonoid content was expressed as

mg quercetin equivalents per gram of extracts (QE/g).

2.5. Antioxidant activity by electrochemical assay

They are several methods used to measure antioxidant activity. Antioxidant activity can be measured by spectrophotometric and electrochemical assays. Antioxidant respond to a voltammetric scan according to their redox potential [9-11]. Voltammetric experiments were carried out with a potentiostat/galvanostat PGP301 (radiometer analytical SAS). The measurements were performed in a 25 mL one-compartment electrochemical cell, employing a three-electrode system consisting of a glassy carbon electrode with a 3 mm diameter, a platinum wire and the saturated calomel $Hg/Hg_2Cl_2/KCL$ electrode, representing the working electrode, the counter electrode and the reference electrode, respectively. The experimental conditions for cyclic voltammetry were: scan rate of 100 mV s^{-1} and scan range from 0 to 1 V. The voltammetric assays were performed in 20 mL of supporting solution 0.2 M phosphate buffer solution, pH 7.0. Ascorbic acid was used as a standard in the calculation of antioxidant capacity of the studied sample extracts of stems of *Solanum Nigrum.* The antioxidant capacity was calculated using the following equation (1):

$$Antioxidant\ capacity, AC\ \left(\frac{g}{mg}\right) = \frac{C_{eq}}{C_{ext}} \dots\dots\dots\dots (1)$$

where AC: antioxidant capacity, C_{eq}: equivalent concentration of ascorbic acid and C_{ext}: concentration of the extracts in electrochemical cell.

2.6. Superoxide Scavenging Assay

The superoxide anion free radical is widely used for the determination of the antioxidant activity of potentially antioxidant compounds. The assays are based on the *in situ* electrochemical generation of the radical in aprotic solvents [12-14]. The cyclic voltammetry technique was used to generate the superoxide radical anion by the one-electron reduction of oxygen molecular saturated in DMF media [15]. A solution of 10 mL of DMF containing the supporting electrolyte Bu_4NBF_4 was saturated by molecular oxygen. The experimental conditions for cyclic voltammetry were: scan rate of 100 mV s^{-1} and scan range from -1.6 V to 0.0 V at room temperature. The ability of the extract to quench $O_2^{\cdot-}$ was calculated using the equation (2):

$$\%O_2^{\cdot-}\text{ radical scavenging activity}, \% = 100 \times \frac{i_0 - i_s}{i_0} \ldots\ldots\ldots\ldots\ldots (2)$$

The results were expressed as percent inhibition (I %), where i_0 and i_s are the anodic peak current densities of the superoxide anion radical in the absence and the presence of extract.

3. RESULTS AND DISCUSSION

The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant taxonomically distinct [16]. Several researchers have been carried out on *Solanum nigrum* L. plant by chemical and biological assay [17-23], but there are no published reports on the electrochemical study of Algerian *Solanum nigrum* L. Thus, in the present study, we examined for the first time the antioxidant activity and the interaction of superoxide anion free radical with chloroform, ethyl acetate and n-butanol crude extracts obtained from the stem of this plant using cyclic voltammetry assay.

3.1. Total Flavonoid Content

Flavonoid content was calculated from the regression equation (Y = 17.24 X - 0.0189, R^2 = 0.999) of the calibration curve and is expressed as Quercetin equivalents (QE). Y represents the average absorbance of the sample and X stands for the amount of quercetin in mg/mL. The flavonoid content in chloroform, ethyl acetate and n-butanol stems extracts of *S. nigrum* is given in Table 1.

Table 1. Total flavonoid content of stems of *Solanum Nigrum* chloroform, ethyl acetate, and n-butanol extracts

Stem extracts	Total flavonoid content (mg QE/g)
Chloroform	10.027 ± 0.009
Ethyl acetate	18.410 ± 0.023
n-Butanol	5.008 ± 0.009

Ethyl acetate extract had higher flavonoid contents (18.410±0.023 mg QE/g) followed by chloroform extract (10.027±0.009 mg QE/g) and the lowest (5.008±0.009 mg QE/g) was

given by n-butanol extract. Flavonoids from different species of *Solanum* have been reported and reviewed. Quercetin and its derivative glycosides make up most of the flavonoid content in *S. Nigrum* [24]. Flavonoids are large family of polyphenolic components synthesized by plants. It was found that flavonoids functioned to reduce blood-lipid and glucose and to enhance human immunity. Flavonoids were also a kind of natural antioxidant capable of scavenging free superoxide radicals, anti-aging and reducing the risk of cancer [25].

3.2. Antioxidant Activity By Electrochemical Assay

Antioxidants exert their reducing activity through electron transfer mechanisms. The cyclic voltammetry tracing provides the total reducing power of the sample without the necessity to measure the specific antioxidant capacity of each component alone [26]. Moreover, the main electrochemical parameters, namely the peak potentials (Ep) and peak currents (Ip) can be provided by cyclic voltammetry. Some features suggesting high antioxidant activity include the presence of electroactive species. On the other hand, the anodic peak current (Ipa) is higher, may be the concentration of related species is higher [27]. The equation obtained from the linear calibration of different standard concentrations of ascorbic acid, Y= 75.95 X + 3.332, R^2 = 0.988, there is an increase in peak current with the increase in ascorbic acid concentrations (Figure .1). The antioxidant capacities of different extracts of stems of *Solanum Nigrum* were exspressed in equivalent terms of ascorbic acid (AC).

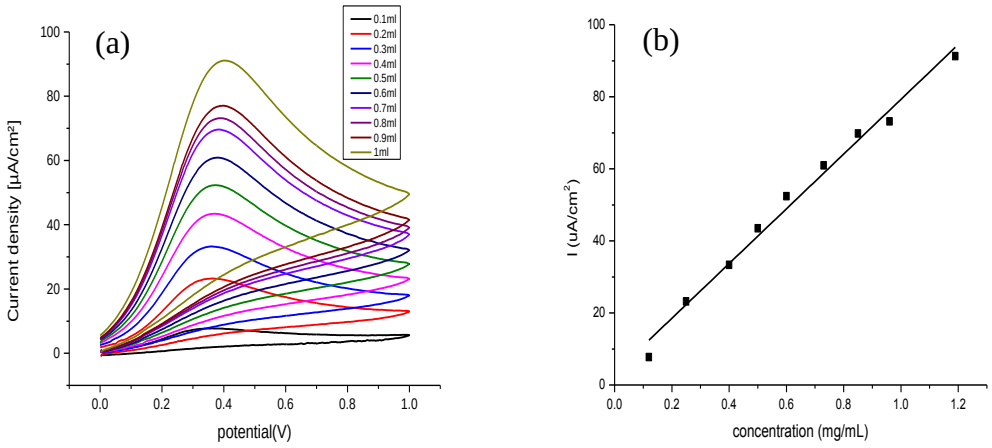


Fig.1. Cyclic voltammograms (a) and linear curves (b) referring to different ascorbic acids concentrations in pH = 7, 0.2 M phosphate buffer solution at scan rate 100 mV/s

Electrochemical data obtained from voltammograms of the stem of *Solanum Nigrum* chloroform, ethyl acetate, and n-butanol extracts are summarized in Table 2. All extracts are electroactive. We observe only one anodic peak appeared for chloroform and n-butanol extracts; ethyl acetate extract represents two anodic peaks (Figure 2). No cathodic peak was observed on inverting the scan direction indicating the irreversibility of oxidation of the three studied extracts. We can classify the peak anodic current:

ipa (chloroform) > ipa (ethyl acetate) > ipa (n-Butanol).

Table 2. Electrochemical data of stem of Solanum *Nigrum* chloroform, ethyl acetate, and n-butanol extracts

Extract	Epa (V)	ipa (µA/cm^2)
Chloroform	0.438	4.727
Ethyl acetate	0.480	3.468
n-Butanol	0.536	3.403

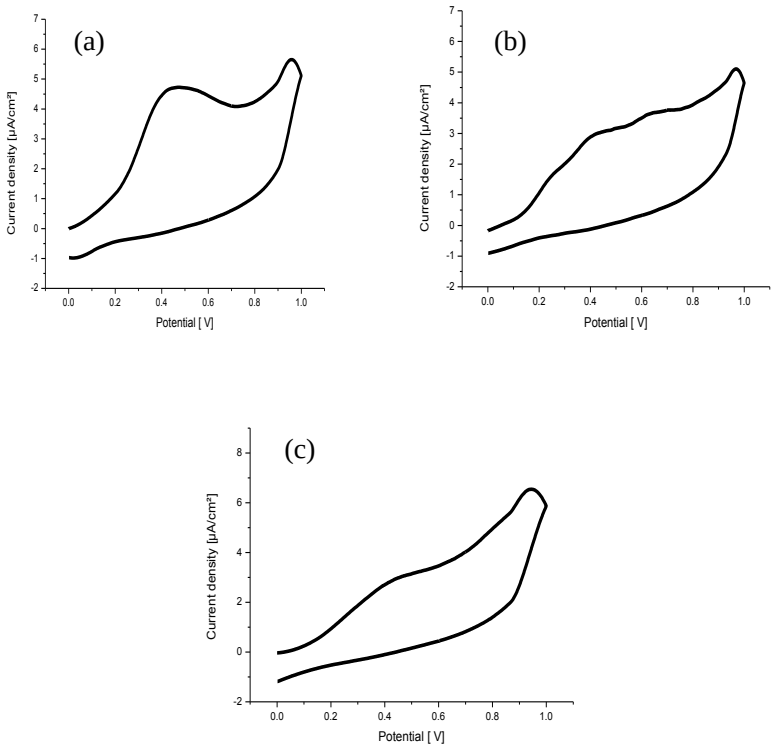


Fig.2. Cyclic voltammograms of stem of Solanum *Nigrum* chloroform (a), ethyl acetate (b), and n-butanol (c) extracts, in pH=7, 0.2 M phosphate buffer solution, at scan rate 100 mV/s

Table 3 represent the value of the antioxidant activity of the three extract, we can classify the values AC (chloroform) > AC (ethyl acetate) > AC (n-Butanol). Chloform extract showed the highest antioxidant activity by cyclic voltametry assay.

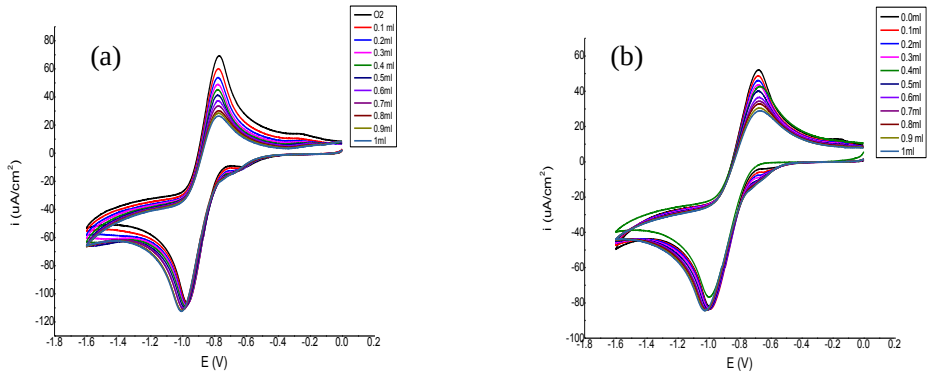
Table 3. Antioxidant capacities of the stem of Solanum *Nigrum* chloroform, ethyl acetate, and n-butanol extracts by cyclic voltametry

Extract	Ascorbic acid antioxidant capacity AC (mg/g)
Chloroform	9.58 ± 0.010
Ethyl acetate	0.83 ± 0.270
n-Butanol	0.23 ± 0.286

Electrochemical methods are based on a mechanism in which electron transfer occurs from the antioxidant molecules to the surface of the working electrode [28]. The antioxidant capacity is dependant on the phenolic composition in the extract and its concentration.

3.3. Superoxide Scavenging Assay

$O_2^{\cdot-}$ radical scavenging activity was plotted against different plant extract concentrations of *Solanum nigrum* (figure .3). The antioxidant activity of the three extract and ascorbic acid as standard was calculated and expressed as IC_{50} in Table 4. The IC_{50} value was defined as the concentration (mg/mL) of samples that inhibits the formation of $O_2^{\cdot-}$ radicals by 50 %.



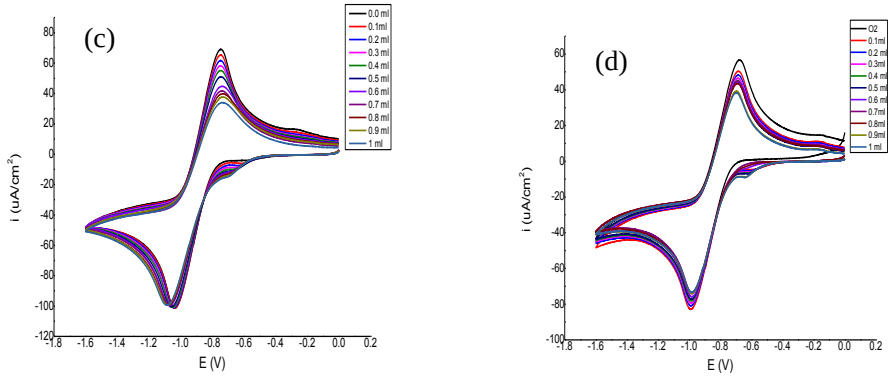


Fig.3. Cyclic voltammograms of oxygen-saturated DMF in the absence and presence of different concentrations of (a) chloform, (b) ethyl acetate, (c) n-butanol extracts of fruit of *Solanum nigrum* and (d) ascorbic acid at scan rate 100 mV/s

The equations obtained from the linear calibration graphs from the different concentrations of the chloroform, ethyl acetate and n-butanol of stem extract of the studied plant and ascorbic acid are summarized in Table 4, where y represents the value of the anodic peak current density and x represents the value of sample concentration, expressed as mg/mL.

Table 4. $O_2^{\cdot-}$ radical scavenging activities of the stem of Solanum Nigrum chloroform, ethyl acetate, and n-butanol extracts and ascorbic acid expressed as IC50

Sample	Equation	R^2	IC_{50} (mg/mL)
$CHCl_3$ extract	y = 59.22x + 10.644	0.99	0.664
ETOAc extract	y = 34.063x - 0.2382	0.99	1.330
n-BuOH extract	y = 34.063x - 0.2382	0.99	1.474
Ascorbic acid	y = 234.11x + 0.6611	0.99	0.210

All extracts showed $O_2^{\cdot-}$ radicals scavenging activities. Chloroform extract showed antioxidant activity (0.664 mg/mL) higher than ethyl acetate extract (1.330 mg/mL). The n-butanol extract was the lowest one (1.474 mg/mL). Standard antioxidant ascorbic acid was the highest one (0.210 mg/mL). The decrease in peak current can be attributed to the decrease in free superoxide anion radical concentration due to the interaction with probable antioxidant

extract compounds.

3.4. Thermodynamic Parameters

To quantify the interaction between superoxide anion radical and the probable antioxidant in the extract, was estimated in terms of binding constant extract K_b based on the decrease in peak current and free energy (ΔG). The binding constant (K_b) was calculated using following equation (3) [29], and the calculated results are shown in Table 5.

$$\log \frac{1}{[\text{extract}]} = log\, K_b + \log \frac{i}{i_0 - i} \ldots\ldots\ldots (3)$$

Where i_0 and i are the anodic peak current densities of superoxide anion radical in the absence and presence of the added plant extract of the stem of *Solanum nigrum*, respectively, [extract] is the concentration of the antioxidant. As [extract] is not known, this term is replaced by the volume of the plant extract [30]. The representative plots are given in Figure 4. The extract which shows a high value of the binding constant reveals a strong interaction with the radical. From Table 5 it is apparent that the binding constant (K_b) values follow in the following order

$$K\ (O_2^{\cdot -} - \text{CHCl}_3 \text{ extract}) > K\ (O_2^{\cdot -} - \text{n-BuOH extract}) > K\ (O_2^{\cdot -} - \text{EtOAc extract})$$

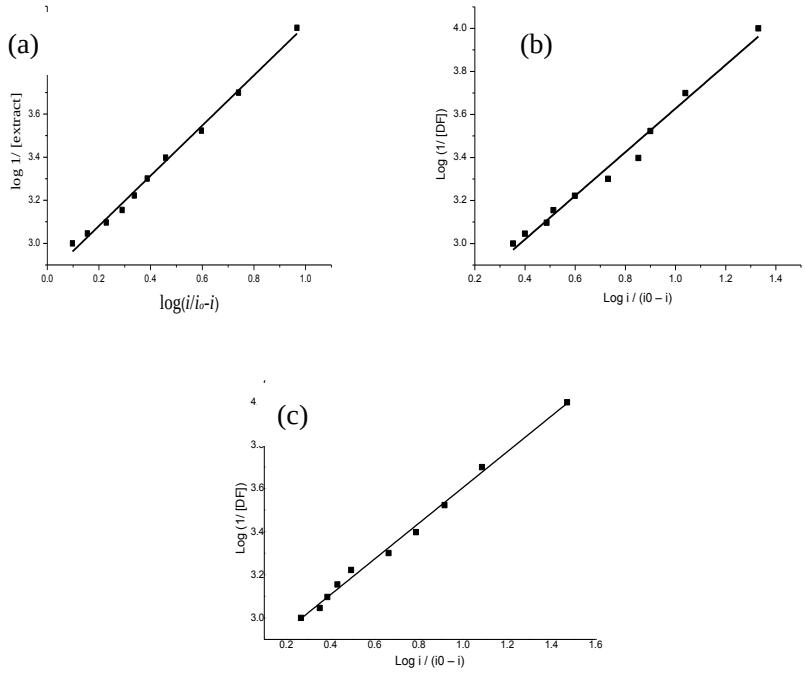


Fig.4. Plots to determine binding constant (K_b) using equation log (1/[extract]) vs log [i/(i_0 – i)] for (a) chloroform (b) ethyl acetate (c) butanol extract of the stem of *Solanum*

nigrum

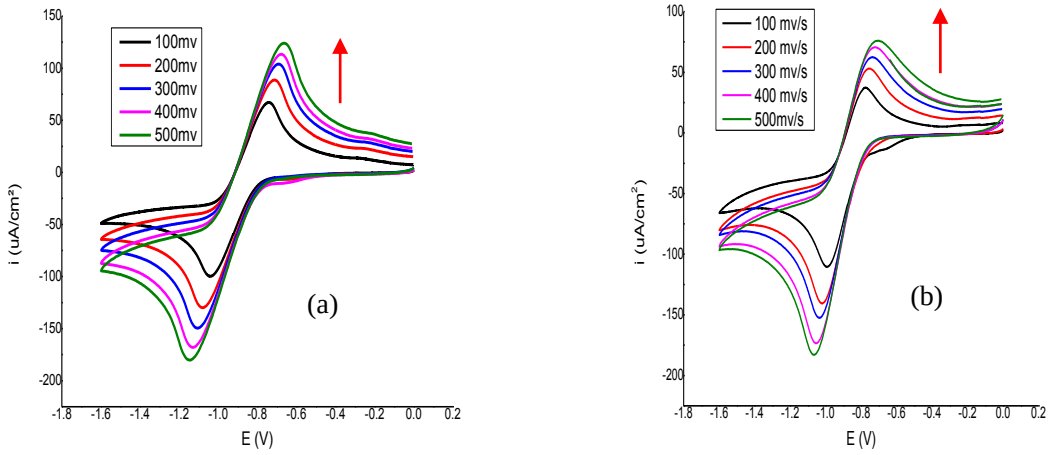
Table 5. binding constant (K_b) and change in free energy of reaction (ΔG) for the chloroform, ethyl acetate, n-butanol extract of the stem of *Solanum nigrum*

Sample	Equation	R^2	K_b $(L)^{-1}$	ΔG $(KJ.mol^{-1})$
$O_2^{\cdot-}$ – Chloroform extract	Y = 1.1637 X+2.848	0.994	0.704×10^3	-16.259
$O_2^{\cdot-}$ – Ethyl acetate extract	Y = 1.014 X+ 2.613	0.984	0.410×10^3	-14.917
$O_2^{\cdot-}$ – n-butanol extract	Y = 0.829 X+ 2.776	0.995	0.597×10^3	-15.84801

The values of Gibbs free energy varied in the following order:

$$\Delta G(O_2^{\cdot-} - CHCl_3 \text{ extract}) > \Delta G\ (O_2^{\cdot-} - \text{n-BuOH extract}) > \Delta G\ (O_2^{\cdot-} - \text{EtOAc extract})$$

The negative values of ΔG indicated the spontaneity of the $O_2^{\cdot-}$ – extract interaction. The electrochemical behavior of all crude extract of the stem of *Solanum nigrum* at various scan rates is shown in Figure 5. We obtained redox process for all samples.



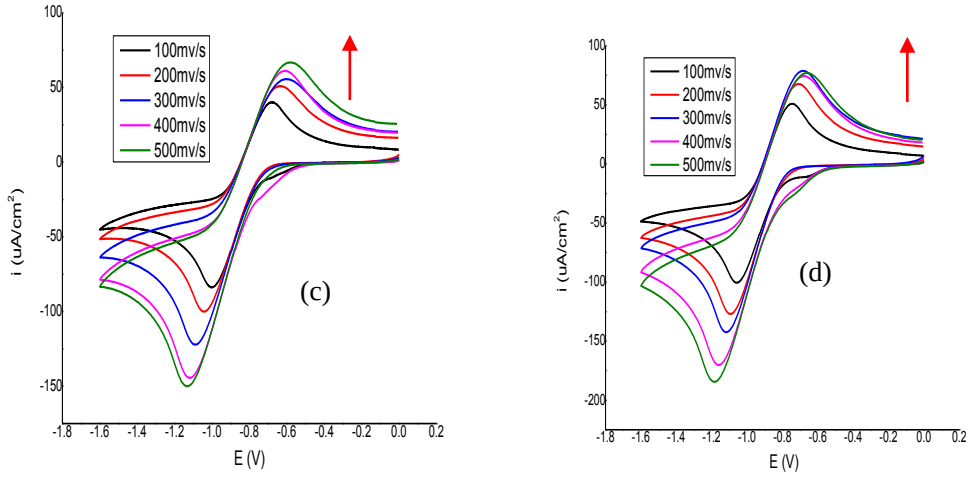


Fig.5. Succession of cyclic voltammograms at GC electrode in oxygen-saturated DMF at various scan rates the (a) absence and the presence of (b) chloroform (c) ethyl acetate (d) n-butanol extract of the stem of *Solanum nigrum*

The diffusion coefficients of the free and $O_2^{\cdot-}$ bound forms of chloroform, ethyl acetate and butanol extract of the stem of *Solanum nigrum* were determined using the following Randles–Sevcik equation (4):

$$i = 2.69 \times 10^5 \, n^{\frac{3}{2}} \, ACD^{\frac{1}{2}} \, v^{\frac{1}{2}} \text{.....(4)}$$

where *i* is the peak current in amperes (A), n is the number of electrons involved in the reaction, A is the area of the electrode (cm^2), C is the concentration in (mol/ cm^3), D is the diffusion coefficient or diffusivity in ($cm^2.s^{-1}$) and *v* is the scan rate ($V.s^{-1}$).

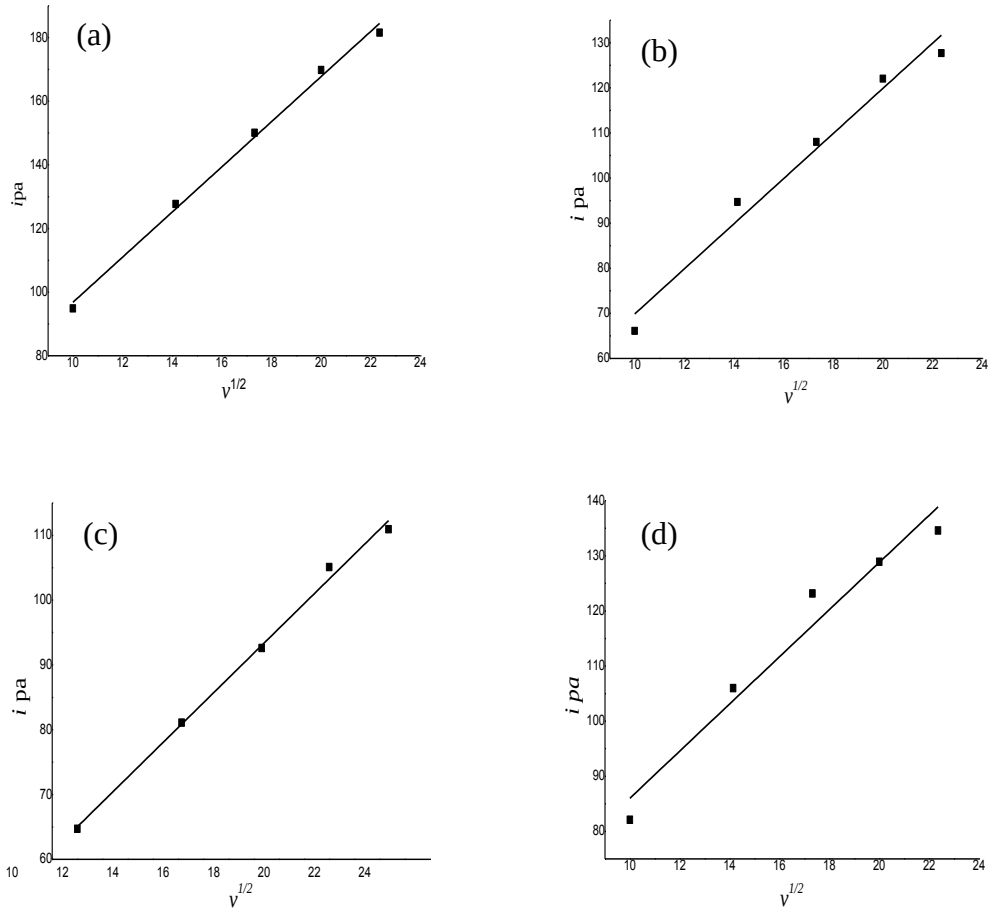


Fig.6. *i* p_a *versuss* $\sqrt{v}$ plots of oxygen-saturated DMF/0.1 Bu_4NBF_4, in the (a) absence and the presence of (b) chloroform (c) ethyl acetate (d) n-butanol extract of the stem of *Solanum nigrum*, at different scan rates

The linearity of the relation $i\ p_a = f(v^{1/2})$ suggests that the redox process is kinetically controlled by the diffusion step. Table 6 showed equations obtained from the linear calibration of the free and bounded $O_2^{\cdot-}$ extract and R^2 values.

Table 6. Equation and R^2 values obtained from from the linear calibration of the free and bounded $O_2^{\cdot-}$ extract

Sample	Equation	R^2
$O_2^{\cdot-}$	Y =7.096 X+25.83	0.995
$O_2^{\cdot-}$ – Chloroform extract	Y =5.002 X+19.83	0.977
$O_2^{\cdot-}$ – Ethyl acetate extract	Y = 3.823 X+ 26.79	0.996
$O_2^{\cdot-}$ – n-butanol extract	Y = 4.278 X+ 43.24	0.959

4. CONCLUSION

The results of the present study indicated that the three different crude extract of the stem of Solanum *nigrum* are rich in flavonoid contents and pocess an electrochemical behavior. Moreover, scavenging activities of chloroform, ethyl acetate and n-butanol extract for superoxide radicals are remarkable. This indicated that the stem of *Solanum nigrum* contained potential antioxidant bioactive compounds.

5. ACKNOWLEDGEMENTS

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