

## Nanomolar Quantification of Polydatin at Boron Doped Diamond Electrode. Application in Dietary Supplements

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Received: 3 February 2019 / Accepted: 27 March 2019 / Published: 10 May 2019

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In this research we demonstrated detailed electrochemistry polydatin. Polydatin is a natural compound with expressed antioxidant activity. Electrochemical behavior of polydatin was tested in the potential range from -1.5 V to 2 V at unmodified boron doped diamond electrode in various pH of supporting electrolyte. It has been noticed that polydatin provides two oxidation peaks, one at around 0.73 V and second at around 1.3 V. In the reverse scan no reduction peak was observed. Quantification of polydatin was done based on first oxidation peak using square wave voltammetry. After optimization of the method, linear working range from  $1 \cdot 10^{-7}$  M to  $7 \cdot 10^{-5}$  M was obtained, with limit of detection and limit of quantification of  $6 \cdot 10^{-9}$  M and  $2 \cdot 10^{-8}$  M, respectively. Negligible interferences effects were noticed. Developed method shows excellent accuracy and precision toward detection of polydatin. Also, developed method was used for quantification of polydatin in dietary supplements.

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**Keywords:** boron doped diamond electrode; dietary supplements; polydatin.

### 1. INTRODUCTION

Polydatin is a natural compound, phenolic type and extracted from root of the Chinese plant *Polygonum cuspidatum* [1–7]. It is most often found in grapes skin and presents a precursor of resveratrol which is present in wines and has an antioxidant ability [5,8]. Biological effects of polydatin is widespread. Namely, a number of studies indicate that polydatin has an antioxidant, anti-cancer, antibacterial, antiallergic, anti-inflammatory effects. In addition, polydatin prevents blood clotting,

reduces levels of fat in blood, but it can also express cardioprotective effect [3–7, 9–11]. Xie et al. proved that polydatin has a positive effect in the treatment of diabetes in rats [12].

According to literature data, the electrochemical quantification of polydatin is not represented. Li et al. proposed electrochemical method based on electrochemically reduced graphene oxide modified electrode with linear working range from  $4.0 \cdot 10^{-8}$  M to  $3.5 \cdot 10^{-6}$  M and detection limit of  $4.0 \cdot 10^{-8}$  M [5]. Chen et al. proposed determination of polydatin in traditional Chinese medicine based on carbon nanotube modified glassy carbon electrode with linear range from  $7.0 \cdot 10^{-7}$  M to  $4.0 \cdot 10^{-5}$  M and detection limit of  $4.57 \cdot 10^{-7}$  M [13].

On the other hand, chromatographic techniques are more common in the determination of polydatin. Mechanism of action of polydatin has been investigated in rats using high-performance liquid chromatographic (HPLC). Gao et al. were using a reversed-phase HPLC method for determination of polydatin in biological matrices of rats [14]. Liquid chromatography tandem mass spectrometry (HPLC-MS/MS) coupled with electrospray ionization (ESI) and reversed-phase HPLC with UV detection were used for determination of *trans*-polydatin in intestines [15] and plasma of rats [16], respectively. In addition, HPLC technique was used for analysis of polydatin in wine samples, because polydatin, like resveratrol, shows significant antioxidant ability [5,8]. Reversed-phase HPLC with UV detection was used for determination of *cis* and *trans*-polydatin in Portuguese [17], Spanish [18] and French wines [19], while Vitrac et al. were using the same method determined *trans*-polydatin in Brazilian red wines [20].

Boron-doped diamond (BDD) electrode is one of the best electrodes whose surface is not modified. BDD electrode has a wide range (from -3.0 V to 3.0 V) of potentials compared to other materials (e.g. glassy-carbon electrode). In addition, the advantages of the electrode are: low value of residual current, minimal noise (high signal/noise ratio), stability in acid and base media, chemical and mechanical stability, as well as minimal absorption of substances on electrode surface due to presence of  $sp^3$  hybridized diamond carbon atom [21–25].

Based on the above, it can be noticed wide clinical and pharmacological application of polydatin. The aim of this research was to develop accurate, precise, selective, sensitive, fast and cheap electrochemical method for quantification of polydatin.

## 2. MATERIALS AND METHODS

### 2.1. Instrumentation

Electrochemical measurements were performed on potentiostat/galvanostat CHI 760b (Chinstruments, USA) utilizing conventional three electrode cell. As a working electrode boron doped diamond electrode was used (BDD, 3 mm diameter, 1000 ppm of boron doping level, Windsor Scientific, UK),  $Ag_{(s)}/AgCl_{(s)}, Cl^-_{(aq)}$ , saturated KCl (Gamry Instruments, USA) as reference electrode, and platinum wire as auxiliary electrode (Sigma Aldrich, Germany) was used. pH meter model Orion 1230 equipped with combined glass electrode model Orion 9165BNWP (USA) was used for pH measurements.

## 2.2. Reagents

All reagents used in this study were analytical grade. Polydatin was supplied by Merck (Germany). Standard solution of polydatin ( $1 \cdot 10^{-3}$  M) was prepared with mixture water:methanol (80:20, v/v).

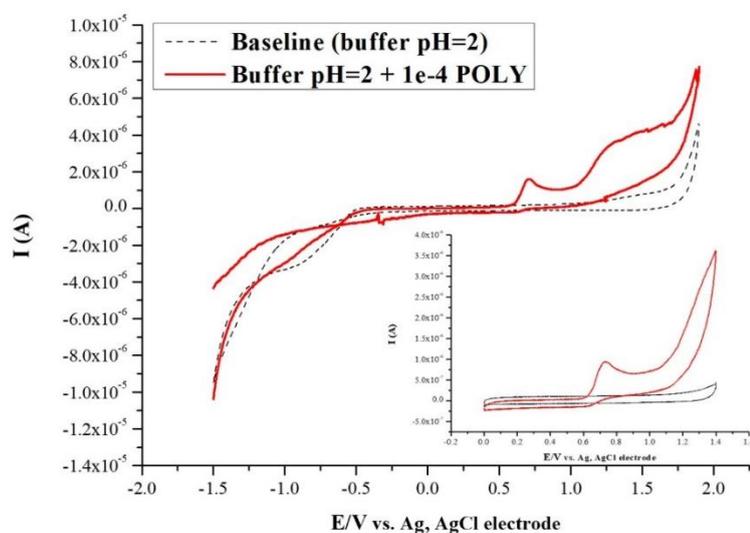
Britton-Robinson buffer solution (BRBS) was used as supporting electrolyte. BRBS was prepared with 0.04 M sulfuric acid, 0.04 M boric acid and 0.04 M phosphoric acid. For determination at pH=1, 0.1 M hydrochloric acid was used. For adjusted higher pH of BRBS 0.2 M sodium hydroxide was used. Polidal Ghimas (GHIMAS S.p.A, Italy) dietary supplement pills based on polydatin were purchased in a local pharmacy shop. According to the manufacturer's declaration each pill contains 40 mg of polydatin.

## 2.3. Preparation of samples

Polidal Ghimas pills were crushed and around 100 mg of powder were dissolved in a mixture methanol:water (20:80, v/v). Stock solution of samples were diluted with supporting electrolyte and directly analyzed in triplicate. Polydatin concentrations were calculated from the calibration curve. In addition, the influence of the matrix effect on the determination of polydatin was examined.

## 3. RESULTS AND DISCUSSION

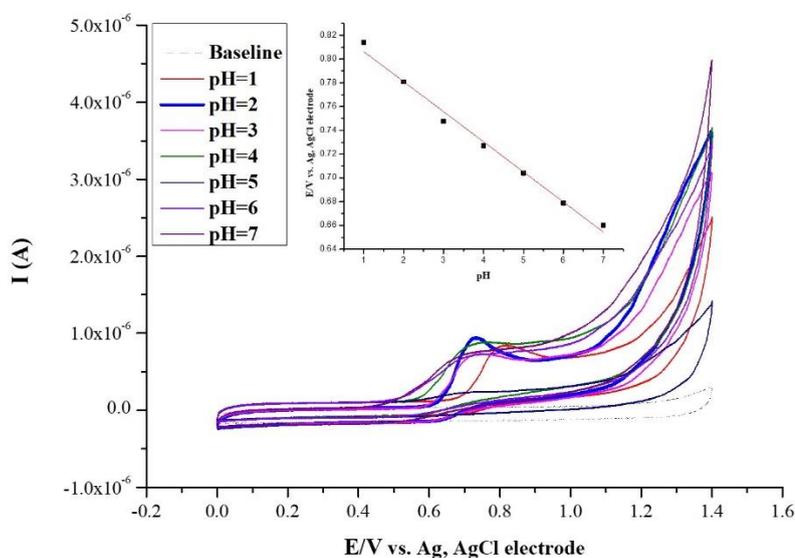
### 3.1. Electrochemistry of polydatin



**Figure 1.** Cyclic voltammogram of  $1 \cdot 10^{-4}$  M of polydatin in BRBS at pH = 2 in full potential range of BDD electrode. Scan rate of 100 mV/s. Insert of figure shows the electrochemical response of polydatin in positive potential region.

Cyclic voltammetry was used for monitoring electrochemical behavior of polydatin. In the first step, the behavior of polydatin was examined in the potential range from -1.5 V to 2 V (Figure 1). Anodic electroactivity of polydatin can be noticed due to well-defined and sharp oxidation peak at potential of 0.73 V and second wider oxidation peak at around 1.3 V. On the other hand, in reversed scan reduction peak does not occur. Based on this, it can be concluded that oxidation of polydatin in supporting electrolyte is irreversible process. Also, the electrochemical behavior of polydatin in positive potential region in BRBS at pH=2 using BDD electrode is shown in Figure 1.

Influence of different pH values on the electrochemical behavior and peak potential and current was studied in the pH range from 1 to 9. Cyclic voltammogram in the pH range from 1 to 7 is shown in Figure 2. Further increase of pH was followed with absence of peak. The best oxidation response which takes into account peak shape and peak current was observed in BRBS at pH=2. It is observed that as the pH value increases, the peak potential is reducing as shown in insert of Figure 2. This negative linear correlation between pH value and peak potential can be explained by the equation:  $E (V) = 0.83169 - 0.02533 \text{ pH}$ , where regression coefficient was  $R = -0.9954$ . This can indicate the mechanism of polydatin oxidation on the BDD electrode at BRBS pH=2 is pH dependent. However, to investigate oxidation mechanism of polydatin more detailed studies have to be done.



**Figure 2.** Cyclic voltammograms of  $1 \cdot 10^{-4}$  M of polydatin at BDD electrode at different pH values of BRBS. Scan rate of 100 mV/s. Insert figure show dependence of the peak potential vs. pH.

In order to determine the process which occurs on the surface of the electrode, electrochemical behavior of  $1 \cdot 10^{-5}$  M of polydatin in BRBS at pH=2 on different scan rates (25-200 mV) was investigated. Increase of the scan rate is followed with increase of the peak current. This dependence of current of the scan rate can be represented by equation:  $I (A) = 5.1695 \cdot 10^{-8} v^{1/2} (mV/s)^{1/2} - 1.2288 \cdot 10^{-7}$  where regression coefficient was  $R = 0.9976$ , so the linear dependence between intensity of oxidation peak current and square root of the scan rate can be noticed (Figure S1). Based on the obtained results it can be concluded that dominant process on electrode surface was diffusion controlled [22,26,27]. To confirm the diffusion, as the main process responsible for the oxidation of polydatin at BDD electrode,

it was considered dependence of log peak current ( $\log I$ ) and peak potential (E) versus log of scan rate ( $\log V$ ). In Figure S2, observing the plot  $\log I$  vs.  $\log V$ , the slope is 0.5081, indicating that the diffusion-controlled process is the dominant process at electrode surface [28]. Selva et al. state that the diffusion-controlled process can be present in three regions (kinetic, diffusion and mixed region) and based on the above dependence (E vs.  $\log V$ ) can be determined the dominant region at BDD electrode [28]. In our case, it prevails kinetic region of diffusion-controlled process, slope 29 mV (Figure S2). Also, adsorption phenomenon on electrode surface was negligible, or is completely avoided. These statements confirm advantages of selected electrode material and approve idea of this work.

### 3.2. Selection of the quantification method; Optimization of selected method

Square wave voltammetry (SWV) and differential pulse voltammetry were two methods used for investigation of electrochemical response of  $1 \cdot 10^{-4}$  M polydatin in BRBS pH=2. A better electrochemical response was obtained with square wave voltammetry (data not shown). In order to achieve better performance for determination of polydatin important parameters of this method such as pulse amplitude and frequency were optimized. Pulse amplitude was tested in the range of 10 mV to 80 mV at constant frequency of 15 Hz. Increase of oxidation peak current was occurring up to the pulse amplitude value of 50 mV, after which increase of pulse amplitude does not affect the characteristics of the peak. Based on these results, for further determination of polydatin optimal pulse amplitude value of 50 mV was selected. After optimizing of pulse amplitude, the frequency is optimized in the range from 10 Hz to 100 Hz (Figure S3). Similar like pulse amplitude, the frequency value was selected based on the best analytical response of polydatin at 50 mV. Optimal value of frequency was 70 Hz. All further experimental determinations were made with these optimized parameters values.

### 3.3. Calibration curve and analytical characteristics of the proposed method

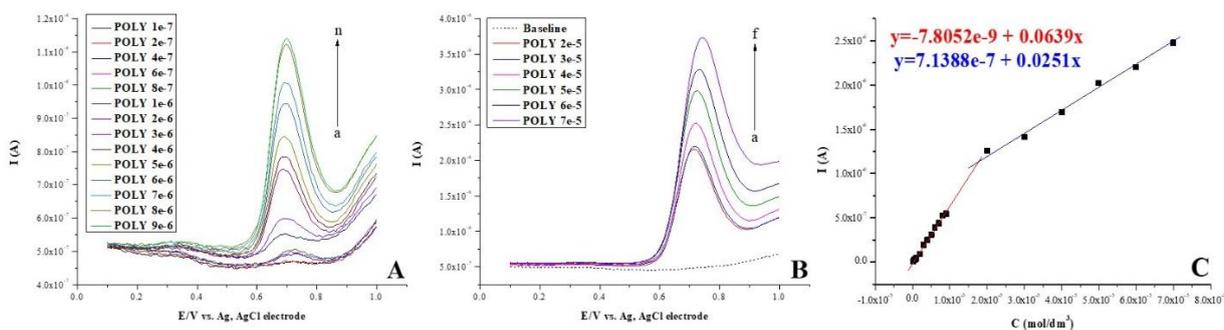
Using the optimized SWV parameters (pulse amplitude 50 mV and frequency 70 Hz) different concentration of polydatin were analyzed (from  $1 \cdot 10^{-7}$  M to  $7 \cdot 10^{-5}$  M) where current of oxidation peak was monitored (Figure 3A, 3B). A linear dependence of oxidation peak versus increase of polydatin concentration is shown in Figure 3C. However, polydatin in concentration range from  $1 \cdot 10^{-7}$  M to  $9 \cdot 10^{-6}$  M shows one type of linearity, and this dependence can be explained by the equation:  $I (A) = 0.06392 C (M) + 7.80524 \cdot 10^{-9}$ , where correlation coefficient is 0.9977. Oxidation of polydatin at the BDD electrode is probably controlled by the diffusion process in monitored concentration range [28]. Limit of detection was calculated and it is equal to  $6 \cdot 10^{-9}$  M, while limit of quantification was calculated to be  $2 \cdot 10^{-8}$  M. On the other hand, oxidation peak of polydatin is also showing linear dependence in concentration range from  $2 \cdot 10^{-5}$  M to  $7 \cdot 10^{-5}$  M, but with another slope. This may indicate that the adsorption phenomenon is responsible for the oxidation of polydatin in the higher concentrations area [28]. Calibration curve from this linearity can be explained by the equation:  $I (A) = 0.02511 C (M) + 7.13885 \cdot 10^{-7}$ , with 0.9961 as correlation coefficient. Comparison of our obtained characteristics with literature are given in Table 1. As can be seen, compared to literature data we have developed an

electrochemical method that provides the lowest limit of detection. Modified electrodes were used in noted research papers, which requires complicated and long-term preparation. Also, advantage is seen in the use of free electrochemical sensor based on an unmodified BDD electrode.

**Table 1.** Comparison of our results with some literature data.

Electrode	Sample	Working range	LOD	Reference number
Electrochemically reduced graphene oxide/glassy carbon electrode	<i>P. cuspidatum</i> extract	$4.0 \cdot 10^{-8}$ M to $3.5 \cdot 10^{-6}$ M	$4.0 \cdot 10^{-8}$ M	[5]
Carbon nanotube/glassy carbon electrode	Chinese medicine	$7.0 \cdot 10^{-7}$ M to $4.0 \cdot 10^{-5}$ M	$4.57 \cdot 10^{-7}$ M	[13]
Boron doped diamond electrode	Pharmaceutical pills	$1 \cdot 10^{-7}$ M to $7 \cdot 10^{-5}$ M	$6 \cdot 10^{-9}$ M	<i>This work</i>

Three different concentrations from the calibration curve, in three consecutive measurements, were analyzed in order to determine the reproducibility of the selected method. Relative standard deviation (RSD) was calculated to be  $4 \cdot 10^{-7}$  M,  $4 \cdot 10^{-6}$  M and  $4 \cdot 10^{-5}$  M for each concentration, and equal to 2.79 %, 1.52 % and 0.46 %, respectively. From RSD values can be concluded that developed method exhibits excellent accuracy and precision.



**Figure 3.** Voltammograms of dependence oxidation peak of different concentration of polydatin in BRBS pH 2 at BDD electrode; A) from a to n:  $1 \cdot 10^{-7}$  -  $9 \cdot 10^{-6}$  M; B) from a to f:  $2 \cdot 10^{-5}$  -  $7 \cdot 10^{-5}$  M; C) corresponding calibration curves obtained for these measurements.

### 3.4. Influence of possible interfering compounds on determination of polydatin. Selectivity of the proposed method

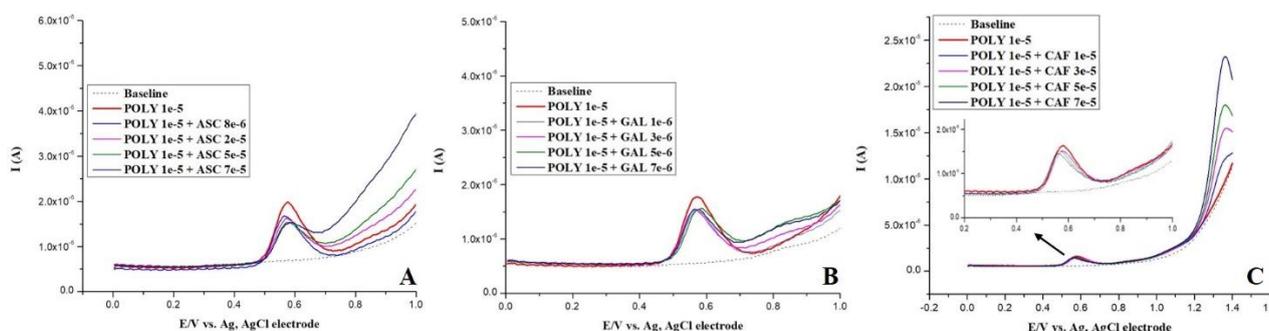
As already mentioned, polydatin is a precursor of antioxidant resveratrol. Therefore, the effect of two antioxidants on electrochemical response of polydatin was tested. Also, alkaloids are present in real samples in addition to antioxidants, so their influence was checked. In that way, the selectivity of the developed method was examined. Ascorbic acid, gallic acid and caffeine were used as interferences

and their influence was tested at concentration of  $1 \cdot 10^{-5}$  M of polydatin. The concentrations of these natural compounds have been selected based on the most common working range in which electrochemical methods provide a positive response, but also based on our experience. In addition, the applied concentrations of interfering compounds were in the area of linear working range of polydatin at BDD electrode, in BRBS pH=2.

Ascorbic acid (ASK) was applied in the concentration range from  $8 \cdot 10^{-6}$  M to  $7 \cdot 10^{-5}$  M, while the influence of gallic acid (GAL) was tested in range from  $1 \cdot 10^{-6}$  M to  $7 \cdot 10^{-6}$  M. In both cases, the oxidation peak of polydatin decreases from 15 % to 23% ie. 12 % to 15%, respectively (Figure 4A, 4B). Polydatin cannot be determined in the range of investigated concentrations of ascorbic and gallic acid by selected method. Lower concentrations of ascorbic acid than  $8 \cdot 10^{-6}$  M and  $1 \cdot 10^{-6}$  M of gallic acid were not examined, so it cannot be claimed in which concentration range of this interferences polydatin can be determined at BDD electrode, in BRBS pH=2.

The influence of different concentration of caffeine on oxidation peak of polydatin was also examined. Caffeine was applied in concentration range from  $1 \cdot 10^{-5}$  M to  $7 \cdot 10^{-5}$  M. Oxidation peak of caffeine occurs at potential 1.35 V (Figure 4C). Caffeine concentration levels from  $1 \cdot 10^{-5}$  to  $5 \cdot 10^{-5}$  do not affect the determination of polydatin with selected method at BDD electrode. Concentration of  $7 \cdot 10^{-5}$  M reduces the oxidation peak of polydatin by 12 % (inset Figure 5C).

Based on reported results, it can be considered that our developed methodology is satisfactory selective and can be applied for application in real samples.



**Figure 4.** Influence of tested interferences on electrochemical response of polydatin at BDD electrode in BRBS pH 2; pulse amplitude 50 mV, frequency 70 Hz. A) ascorbic acid; B) gallic acid; C) caffeine.

### 3.5. Application of optimized method on real sample

The optimized SW method was applied for detection of polydatin in Polidal Ghimas pills. The procedure for preparing the pills for the determination using optimized method is given in the Experimental section. As already stated, the declared values of polydatin in Polidal Ghimas pills is 40 mg. Good agreement was observed between the results obtained by the optimized method and the declared value (Table 2). Also, recovery confirms good selectivity and minimal influence of the matrix effect during the determination of polydatin in pills using optimized technique.

**Table 2.** Quantification of polydatin by optimized method

Sample	Polydatin (mg)	Declared value (mg)	Recovery (%)
Pill 1	38.7	40	97
Pill 2	37.1	40	93
Pill 3	39.1	40	98

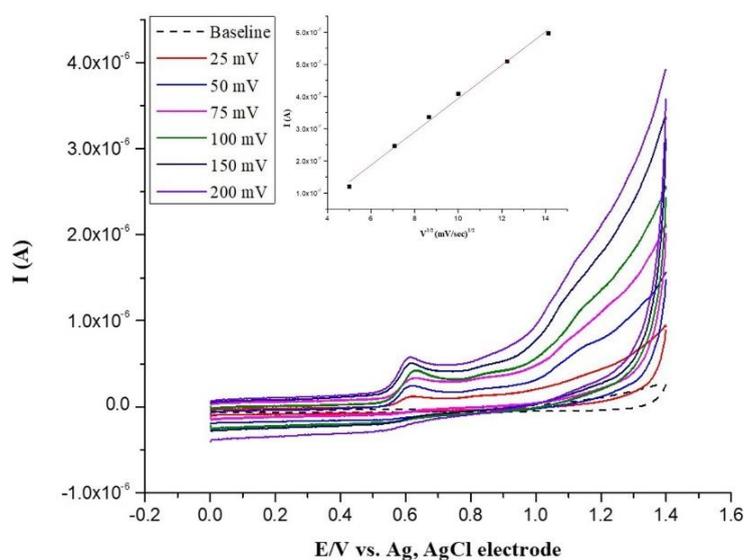
#### 4. CONCLUSIONS

In summary, electrochemical behavior of polydatin was investigated using unmodified free electrochemical sensor, boron doped diamond electrode. Quantification method was developed based on square wave voltammetry with linear range from  $1 \cdot 10^{-7}$  M to  $7 \cdot 10^{-5}$  M and LOD and LOQ of  $6 \cdot 10^{-9}$  M and  $2 \cdot 10^{-8}$  M, respectively. Applications of the method for quantification of polydatin in dietary supplements indicate good precision and reproducibility with satisfactory recovery.

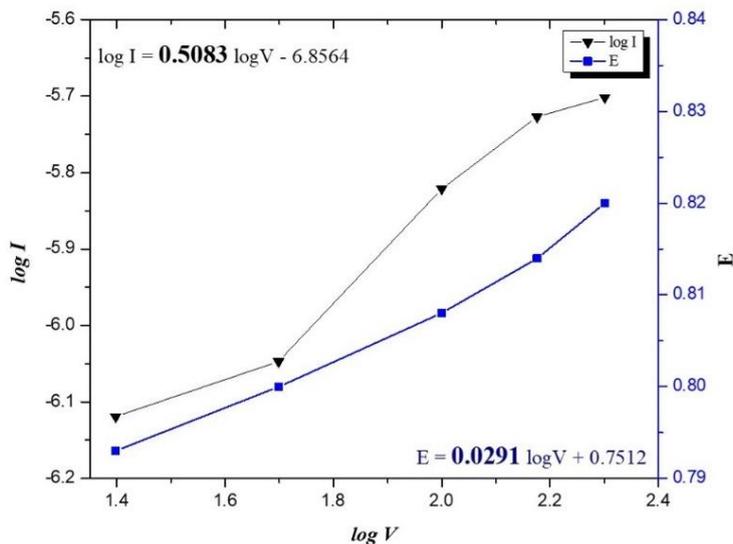
#### ACKNOWLEDGMENTS

This work was supported by MagBioVin project (FP7-ERACHairs-Pilot Call-2013, Grant agreement: 621375) and the Ministry of Education, Science and Technology, the Republic of Serbia, Project No. OI 172030. LŠ wish to acknowledge to Grant Agency of the Slovak Republic (grant No. 1/0489/16).

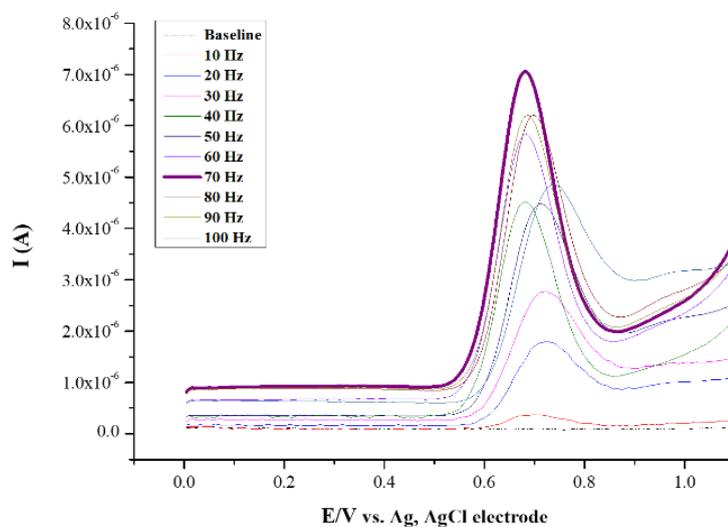
#### SUPPLEMENTARY MATERIAL



**Figure S1.** Cyclic voltammograms of  $1 \cdot 10^{-5}$  M of polydatin at BDD electrode in BRBS pH=2 at different scan rates. Inset figure represent the relationship between peak current and square root of the scan rate.



**Figure S2.** Plots of  $\log I$  vs.  $\log V$  and  $E$  vs.  $\log V$  for oxidation of polydatin at BDD electrode in BRBS pH=2.



**Figure S3.** SW voltammogram for optimization of frequency with  $1 \cdot 10^{-4}$  M polydatin at BDD electrode in BRBS at pH 2. Pulse amplitude 50 mV.

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