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ELECTROCHEMICAL BEHAVIOUR AND VOLTAMMETRIC DETERMINATION OF *GESHOIDIN* AND ITS SPECTROPHOTOMETRIC AND ANTIOXIDANT PROPERTIES IN AQUEOUS BUFFER SOLUTIONS

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ABSTRACT. The electrochemical behaviour of *Geshoidin* was investigated at a glassy carbon electrode in mixtures of citric acid and di-sodium hydrogen orthophosphate aqueous buffer system over a wide pH range (pH 2-11) using cyclic voltammetry. Chemically irreversible single oxidation and reduction peaks were obtained in the potential and pH range investigated. Variations in the peak potential and peak current of the oxidation peak have been observed as function of pH. The wave characteristics, the reversibility of the reactions, the diffusion coefficient and the number of electrons transferred have been studied. Linear sweep voltammetry was applied for the voltammetric determination of *Geshoidin* and a linear calibration curve over the range 1.00×10^{-6} - 1.00×10^{-4} M *Geshoidin* was achieved. The detection limit was found to be 5.00×10^{-7} M *Geshoidin*. For eight successive determinations of 1×10^{-5} M *Geshoidin*, a relative standard deviation (RSD) of 3.2% was obtained. The voltammetric method was applied to the direct determination of *Geshoidin* in *Gesho*. The absorption spectra of *Geshoidin* are interpreted in terms of structural changes caused by protonation and deprotonation of the molecule as a result of changes in pH. The pK_a values of the compound have been determined from the voltammetry and spectrophotometry measurements. The superoxide anion scavenging ability of *Geshoidin* was examined by differential pulse voltammetry and its antioxidant activity has been compared with natural antioxidants.

KEY WORDS: *Gesho*, *Geshoidin*, *Rhamnus prionoides*, Electrochemical behaviour, Voltammetric determination, Spectrophotometry, Antioxidant activity

INTRODUCTION

Rhamnus prinoides, common name dogwood, Amharic name *Gesho*, family Rhamnaceae, is a widespread plant species in East and South African countries [1]. The plant is used as traditional medicine. A decoction of the root is taken as a blood purifier, to treat pneumonia, gonorrhoea, rheumatism and stomach-ache and as a gargle. The leaves are applied as liniment to simple sprains. Leaf decoction mixed with the bark of *Erythrina abyssinica* is used to alleviate stomach pain. In Southern Africa, the chief use of the tree is for magic; it is widely used as a protective charm toward off lightning and evil influences from homes and crops, and to bring luck in hunting [2, 3].

Gesho is used in Ethiopia in the preparation of domestic beverages such as *Tella* and *Tej* [4]. The leaves and steams of *Gesho* are indispensable ingredients in the making of these traditional fermented beverages. It has been reported that the plant regulates the microflora responsible for the fermentation process [5, 6]. It is believed that *Gesho* can serve as a commercial hopping agent in the brewery industries. Hops add bitterness *via* alpha acids being isomerised into more stable and soluble iso-alpha acids, assist in the production of tannins that combine with

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unwanted proteins, add to beer stability due to their antibacterial properties, and impart characteristic aroma to beer through their essential oils [7, 8]. It has been speculated that the role of *Gesho* in *Tella* should be similar to that of hops in beer. It plays a major role to suppress certain bacteria during the fermentation process. *Gesho* is certainly the main agent that imparts the desirable bitter test to *Tella*. Its contribution to the typical aroma of these beverages is yet to be realized. It is interesting to know that unlike hopes, *Gesho* does not contain essential oils.

A number of compounds have been isolated from the leaves and steams of *Gesho* [4, 9-12]. Previously unknown naphthalenic compound *β-sorigenin-8-O-β-D-glucoside*, (Figure 1) was isolated from the leaves and fully characterised. The name *Geshoidin* was proposed for this novel glucoside. The discovery of *Geshoidin* is very significant in the study of the chemistry of *Gesho*, because the characteristic bitter taste of this plant was ascribed by organoleptic evaluation to *Geshoidin*, one of the major constituents of the plant [4].

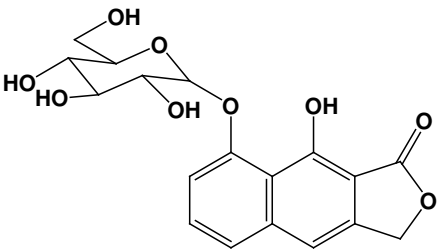


Figure 1. Structure of *Geshoidin* (*β-sorigenin-8-O-β-D-glucoside*).

There is increased evidence that reactive oxygen species and their promoted oxidative damage are involved in a large number of pathologies as well as in the aging process [13, 14]. Under normal metabolism, the active oxygen radicals produced by cells and scavenged by cells themselves are in stable equilibrium. If they are maladjusted, many kinds of diseases occur due to overabundance of free radicals in *vivo*. Therefore, how to prevent radicals and active oxygen from the harm to organism tissue has become a very interesting area of investigation for many researchers. Polyphenols are characterised by the presence of one ore more hydroxylated benzene rings and are known for their radical scavenging ability [15]. Owing to the phenolic nature of *Geshoidin*, its ability of scavenging active oxygen radicals or its chemical antioxidant activity needs to be a subject of study.

In the present work, the electrochemical, spectrophotometric, and antioxidant properties of *Geshoidin* have been investigated and are described for the first time. The electrochemical behaviour was investigated at a glassy carbon electrode and in different pH solutions. A simple voltammetric method has been demonstrated for its determination in the plant's leaf and in *Tella*. To the best of our knowledge, there are no previous reports on the above properties of *Geshoidin* in the literature.

EXPERIMENTAL

Apparatus

A model BAS 100B electrochemical analyser (Bioanalytical Systems) was used for cyclic, linear sweep and differential pulse voltammetry measurements, with a three-electrode system consisting of a glassy carbon disk working electrode (BAS MF-2012) with an active surface area

of 0.06 cm^2, an Ag/AgCl (3 M NaCl) reference electrode (BAS MF-2052) and a platinum wire auxiliary electrode (BAS MW-1032). For the measurements of the antioxidant activity, a gold disk working electrode (BAS MF-2014) of active surface area 0.02 cm^2 was employed. Before each experiment the glassy carbon electrode was polished manually with alumina (ϕ: 0.01 μm) on a micro-cloth pad and rinsed with distilled and de-ionized water. All potentials are reported with respect to Ag/AgCl (3 M NaCl) reference electrode.

Absorption spectra were obtained with Shimadzu (Kyoto, Japan) UV-1201 spectrophotometer connected to a computer working with the PC-1201 personal spectrophotometer software. The spectra were recorded from 250 to 600 nm using 1 cm quartz cuvette.

The pH of the buffer solution was measured with Hanna instruments digital pH meter with a glass combination electrode and with accuracy of ± 0.05 pH.

Reagents

Citric acid and di-sodium hydrogen phosphate from Saarchem (South Africa), ascorbic acid, citric acid, glucose and sodium perchlorate from Riedel-de Haen (Germany), and sodium hydroxide from ACE (South Africa) were used as received. Distilled, de-ionized water was used throughout.

Geshoidin was isolated from the leaves of *Rhamnus prinoides* following the procedures described elsewhere [4] and was identified and characterized by spectroscopic methods. The purity of the compound was confirmed by TLC and melting point measurement.

A 1 x 10^{-2} M aqueous standard stock solution of *Geshoidin* was prepared and stored in the dark to protect it from light. The required concentration was then prepared from the stock standard solution daily. Citric acid/di-sodium hydrogen phosphate buffer system in the pH range 2-11 were prepared from mixture of 0.1 M citric acid and 0.2 M di-sodium hydrogen phosphate aqueous solutions. The pH of the solutions for higher values was adjusted by adding drops of 1 M sodium hydroxide solution. Home brewed *Tella* was used to see the voltammetric response of *Geshoidin* in *Tella*.

Procedure

Cyclic voltammetric measurements were run from -1.200 to +1.200 V and back at a glassy carbon electrode with a scan rate of 100 mV s^{-1}. The scan rate was varied from 0.005 to 0.2 V s^{-1} to study the dependence of peak current and peak potential on the scan rate.

Controlled potential electrolysis of *Geshoidin* was carried out at a glassy carbon electrode of large surface area (0.79 cm^2) in citric acid/di-sodium hydrogen phosphate buffer for three concentrations of *Geshoidin* (c = 1 x 10^{-5}, 2 x 10^{-5}, 4 x 10^{-5} M). Solutions were stirred during electrolysis using a magnetic stirring bar. The electrolysis was terminated when the electrolytic current decreases to the residual current value measured in the supporting electrolyte prior to the addition of the analyte.

Twenty mL supporting electrolyte was placed in the electrochemical cell and the required volume of standard *Geshoidin* solution was spiked into the cell by micro-pipette. The same procedure was followed for sample analysis. The solution was deaerated with pure nitrogen (99.999%, Air Products SA).

For the voltammetric determination of *Geshoidin* from the leaves of the plant, 5 g of dry powdered leaves were added to each of 200 mL of water, methanol and ethanol. Each of the mixture was shaken for 6 h and filtered. Two hundred μL of the extract was spiked into the electrochemical cell that contained 10 mL of buffer solution and linear scan voltammograms were recorded. The standard addition method was applied adding successive aliquots of 20 μL

of 1 x 10^{-2} M *Geshoidin* standard solution to the electrochemical cell. Linear scan voltammograms were recorded by scanning anodically from 0.000 to 0.800 V at scan rate of 20 mV s^{-1}. The peak current of the oxidation wave at about 0.550 V was measured. After each experimental run, the solution was stirred for 10 s prior to the next measurement. The voltammetric response of *Geshoidin* in *Tella* was carried out by dissolving the supporting electrolytes in *Tella* without dilution.

The pK_as' of *Geshoidin* in aqueous buffer solutions of different pH (containing 1.4 x 10^{-4} M *Geshoidin* in mixtures of 1 x 10^{-1} M KH_2PO4 and 1 x 10^{-1} M Na_2HPO_4) were determined following the spectrophotometric method of Albert and Sergeant [16].

Free radical scavenging activity determination was carried out based on the electrochemical reduction of oxygen [17]. Differential pulse voltammetry (DPV) was used under the following conditions: scan rate 5 mV s^{-1}, pulse amplitude 50 mV, initial potential 200 mV and final potential -600 mV. The electrochemical cell containing 5 mL 0.9% NaCl (aq) supporting electrolyte was similar to the above cell and consisted of a gold disk working electrode in place of the glassy carbon working electrode. The supporting electrolyte was first saturated with oxygen by bubbling oxygen gas through the solution for 5 minutes. The potential of the working electrode was then set at 200 mV for 30 s while stirring the solution with a magnetic stirrer. After the stirring was stopped, the potential was scanned in the negative direction and differential pulse voltammogram was recorded that gave a peak current proportional to the amount of oxygen in the solution. This was then followed by adding a known concentration of *Geshoidin* solution to the electrochemical cell under the same condition. The proportional decrease of the oxygen peak current corresponding to concentration of the added *Geshoidin* was measured. These experiments were repeated for aqueous solutions of glucose, citric acid and ascorbic acid. All measurements were carried out at room temperature (22 ± 2 ^{o}C).

RESULTS AND DISCUSION

Cyclic voltammetry of geshoidin

The electrochemical responses of *Geshoidin* were investigated using cyclic voltammetry. Figure 2 shows the cyclic voltammogram (CV) of 5 x 10^{-5} M *Geshoidin* at a glassy carbon electrode in pH 7.0 citric acid/di-sodium hydrogen phosphate buffer. The CV shows the current potential profile of *Geshoidin* after subtracting the CV of the base electrolyte. During the first positive potential scan, an irreversible oxidation peak appeared at a potential of 0.522 V followed by a hump at 0.675 V. During the reverse negative potential scan, an irreversible reduction peak was observed at -0.690 V. The reduction peak was not seen when the starting potential of the CV was set first at +1.200 V and scanned in the negative direction up to a switching potential of -1.200 V. This implies that the reduction peak is attributed to the reduction of the oxidised species of *Geshoidin*. When repetitive cycles were run at low scan rates, no change was observed in the shape and height of both the oxidation and reduction peaks indicating the absence of electrode surface fouling due to strong adsorption or polymerisation of the electro reactive species. However, the hump that followed the oxidation peak and observed at 0.675 V during the first scan of the voltammogram is an indication of the existence of weak adsorption of the oxidation product [18]. The peak disappeared when the experiment was run for repetitive cycles or at different scan rates.

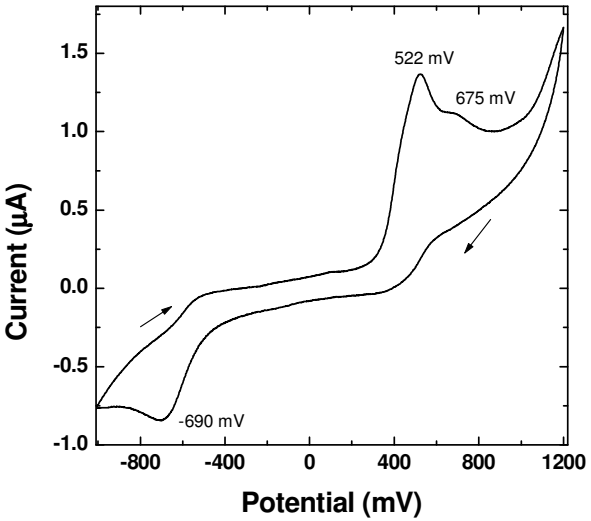


Figure 2. Cyclic voltammogram of 5 x 10^{-5} M *Geshoidin* at a glassy carbon electrode in a mixture of 0.1 M citric acid and 0.2 M di-sodium hydrogen phosphate buffer (pH 7) after subtraction of base electrolytes' voltammogram at a scan rate of 60 mV s^{-1}.

The oxidation and reduction of *Geshoidin* at glassy carbon electrode gave rise to chemically irreversible processes over the scan rate range of 5 mV s^{-1} to 5 V s^{-1}. Figure 3 shows the cyclic voltammograms for the oxidation of 5 x 10^{-5} M *Geshoidin* solution at different scan rates. The peak potential for the process became more positive as the scan rate increased while the peak currents were proportional to the square root of the scan rate, for the scan rate up to 200 mV s^{-1}, as expected when the mass transport process is diffusion controlled [18, 19]. At scan rates greater than 200 mV s^{-1}, the oxidation process lost the characteristic diffusion controlled peak shape and became broad and sigmoidal implying that surface based process becomes dominant at high scan rates [20]. The effect of the potential scan rate, ν on the oxidation peak current of *Geshoidin* was studied and the oxidation peak current was proportional to the square root of scan rate, $\nu^{1/2}$ in the entire pH range studied as predicted for a diffusion controlled regime. Plot of the peak current as a function of the square root of the scan rate for Figure 3 is described by the following equation.

$$i_p/\mu A = 0.13/\mu A + 4.32\nu^{1/2} \qquad r^2 = 0.996 \quad (\text{for } n = 8) \tag{1}$$

The dependence of the oxidation peak potential of *Geshoidin* on the logarithm of the potential scan rate for Figure 3 was evaluated and the peak potential was directly proportional to the logarithm of the scan rate and the linear plot is expressed as follows.

$$E_p/V = 0.667 + 0.01284\ln\nu \qquad r^2 = 0.998 \qquad (\text{for } n = 8) \tag{2}$$

Constant potential electrolysis of *Geshoidin* was carried out at 0.700 V for three concentrations of *Geshoidin*, (c = 1 x 10^{-5}, 2 x 10^{-5}, 4 x 10^{-5} M) to determine the number of electrons transferred in the process. From the electrolysis results, the average number of electrons n transferred per molecule was found to be 2.1 ± 0.2. For a totally irreversible oxidation reaction the peak current at 25 ^{o}C is given by:

$$i = (2.99 \times 10^5)n[(1-\alpha)n_\alpha]^{1/2}Ac_bD^{1/2}\nu^{1/2} \quad (3)$$

where A in cm^2, D in $cm^2\ s^{-1}$, C_b in M, ν in V s^{-1} and n_α is the number of electrons transferred up to, and including the rate determining step [18-21]. The peak potential is related to the scan rate ν with the following relation.

$$E_p = K + [RT/2(1-\alpha)\ n_\alpha F]\ ln\nu \quad (4)$$

where $K = E^o + [RT/(1-\alpha)\ n_\alpha F][0.78 + (1/2)ln\ [(1-\alpha)\ n_\alpha F\ D/k^{o2}RT]$

From equation (4) and t = 25 ^oC, the value of $(1 - \alpha)\ n_\alpha$ was determined from the slope of E_p versus lnν of equations (2) as 0.984. The electron transfer coefficient α for the oxidation of *Geshoidin* was determined (α = 0.49) from the Tafel slope of a linear scan voltammogram recorded at low scan rate (5 mV s^{-1}) [22]. Hence, the value of n_α was estimated to be 1.96 which is very close to 2. The $(1 - \alpha)\ n_\alpha$ value was then inserted into equation (3) and the diffusion coefficient was determined for 5 x 10^{-5} M *Geshoidin* to be 5.80 x 10^{-6} $cm^2\ s^{-1}$.

The variation of scan rate for the reduction peak (figure not shown) showed a shift in the peak potential towards negative values with increasing scan rate. The peak currents were also proportional to the square root of the scan rate. The peaks for the reduction, however, became ill-defined at high scan rates due to overlaps with the peak of the supporting electrolyte.

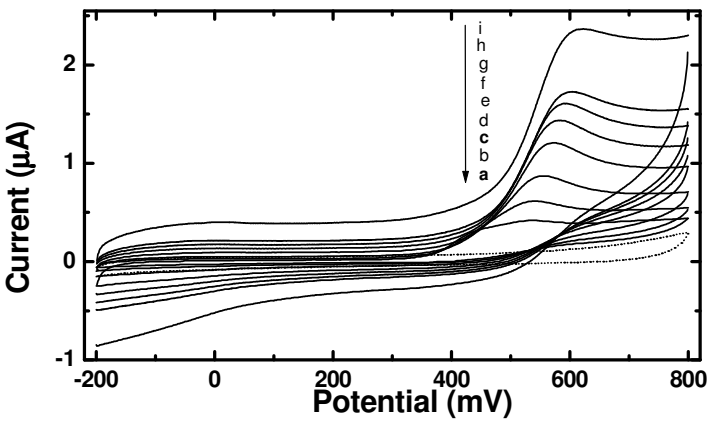


Figure 3. Cyclic voltammograms of 5 x 10^{-5} M *Geshoidin* at a glassy carbon electrode in a mixture of 0.1 M citric acid and 0.2 M di-sodium hydrogen phosphate buffer (pH 7) at different scan rates: (a) base electrolyte; (b) 5; (c) 10; (d) 20; (e) 40; (f) 60; (g) 80; (h) 100 and (i) 200 mV s^{-1}.

Influence of pH of the supporting electrolyte

The influence of pH on the oxidation peak current and oxidation peak potential of the cyclic voltammogram of *Geshoidin* was investigated over the range of pH 2-11. Figure 4 shows the dependence of the peak current on pH for three different concentrations of *Geshoidin*. The magnitude of the peak current for the respective concentration remained constant in the pH range 2-6 and then decreased until a minimum value of about pH 9. Beyond this pH, the current increased sharply. Since the magnitude of current is directly proportional to the rate of the

electrochemical reaction [22, 23], it is apparent to conclude that rate of oxidation of *Geshoidin* is very high at higher pH.

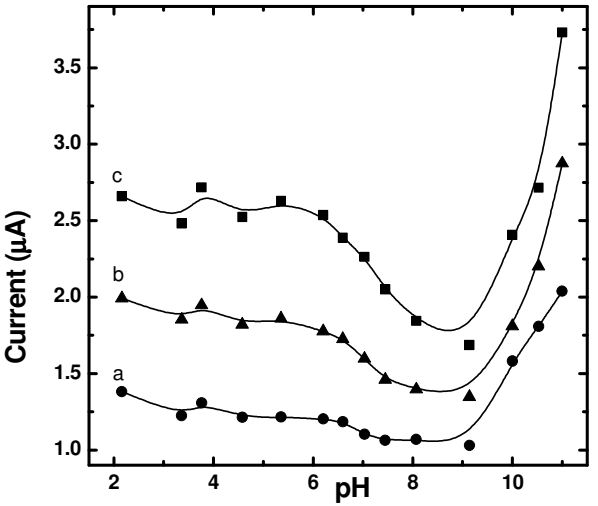


Figure 4. Plots of peak current as a function of pH for different concentrations of *Geshoidin*: (a) 5 x 10^{-5}; (b) 8 x 10^{-5}; (c) 1.0 x 10^{-4} M at a scan rate of 20 mV s^{-1}.

The shift in the oxidation peak potential as a function of pH was studied and linear dependence was observed. When the pH of the supporting electrolyte was increased the peak of the voltammograms was shifted to a more negative potential. Figure 5 shows the dependence of the oxidation peak potential on pH for the cyclic voltammetry measurements. Two regions of linear dependences were observed. This indicates that the H^+ ion takes part in the electrode reaction. According to the literature [24], $E_p = K - (0.059y/n)pH$, where y is the number of H^+ ions that take part in the electrode reaction and n is the number of electrons. As can be seen from Figure 5, there are two linear ranges, which are described by the following equations:

$$E_P/V = 0.061pH + 0.990 \qquad r^2 = 0.990 \quad (pH\ 2.0 - 7.0) \quad (for\ n = 7) \tag{5}$$

$$E_P/V = 0.015pH + 0.683 \qquad r^2 = 0.999 \quad (pH\ 7.2 - 9.2) \quad (for\ n = 4) \tag{6}$$

The peak potential is independent of pH and remains constant above pH 9.2. The dependence of the peak potential on pH has slopes of 61 and 15 mV per unit pH, respectively. This implies that the ratio of the number of protons involved changes from two (for n = 2) to zero as the pH becomes very high. Electrode processes involving a weak acid or weak base have a potential-pH variations which show a change in slope at pH = pK_a [24]. The oxidation behaviour of *Geshoidin* is strongly pH dependent and from the intersections of the linear parts of the plots of Figure 5, the pK_a of *Geshoidin* was estimated as $pK_{a1} = 6.81$ and $pK_{a2} = 9.27$.

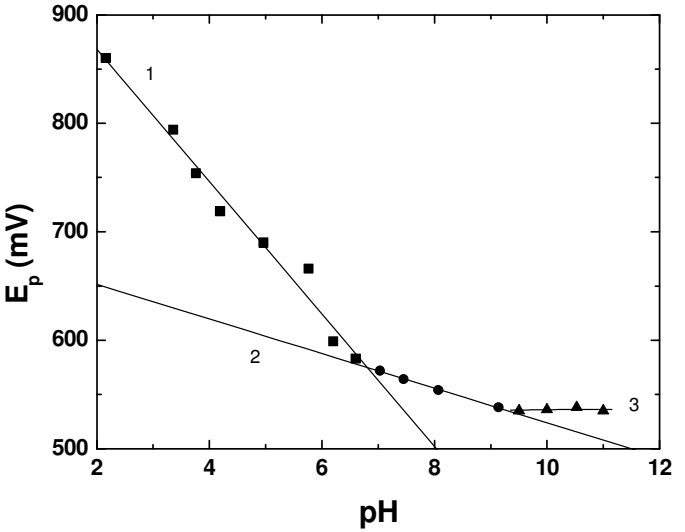
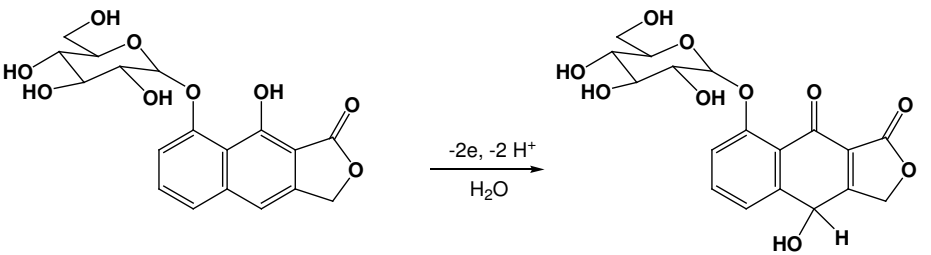


Figure 5. Plot of peak potential as a function of pH for the cyclic voltammetric oxidation peak of 5 x 10^{-5} M *Geshoidin* at a scan rate of 20 mV s^{-1}.

Organic compounds whose oxidation potentials are pH dependent undergo deprotonation reaction during oxidation [19]. The products obtained via phenol oxidation involve the formation of phenoxonium ions as intermediates. Phenoxonium ions are highly electrophilic species that react with a nucleophile solvent such as water to give quinones and substituted quinones [25]. Below pH 7.2 it is apparent that two H^+ ions are removed from a molecule of *Geshoidin*. The fact that two electron oxidation wave is obtained and two hydrogen ions are involved in the electrode reaction in neutral and acidic solutions leads to the proposal of the following oxidation mechanism (Scheme 1) for *Geshoidin* at the electrode.



Scheme 1

Voltammetric analysis, linear range and detection limit of Geshoidin

Although the optimum oxidation current response of *Geshoidin* is achieved at very high pH (pH >10), performing electrochemical measurements at high pH is not desirable and hence a neutral pH was chosen for the analysis of Geshoidin in *Tella* and in plant extracts. Using pH 7 and linear scan voltammetry the peak current was linearly dependent on *Geshoidin* concentration. Linear scan voltammograms at different concentrations of *Geshoidin* are shown in Figure 6. The

dependence of the peak current as a function of concentration of *Geshoidin* is also shown in Figure 6 as insert. Each data point in Figure 6 of the insert is the mean value of the peak currents obtained from three linear scan voltammetry runs. The peak current increased with increasing concentration of *Geshoidin*. The response was found to be linear in the concentration range 1.00 x 10^{-6} - 1.00 x 10^{-4} M *Geshoidin* and the correlation coefficient was r^2 = 0.999. At higher concentrations (≥ 3.00 x 10^{-4} M) deviation from linearity occurred due to saturation of the electrode surface. The detection limit (three times signal-to-noise ratio) was found to be 5.00 x 10^{-7} M *Geshoidin*. For eight successive determinations of 1 x 10^{-5} M *Geshoidin*, a relative standard deviation (RSD) of 3.2% was obtained.

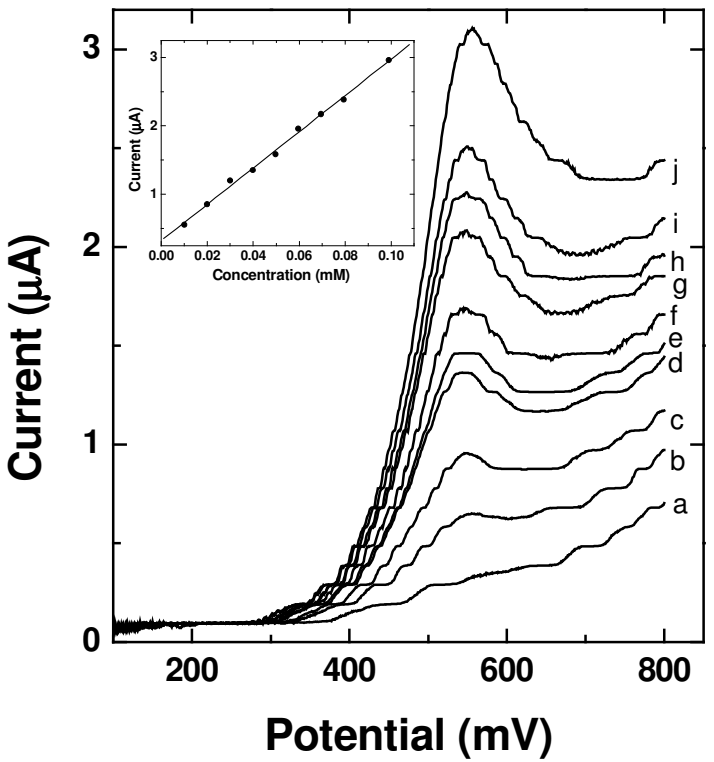


Figure 6. Selected linear scan voltammograms of *Geshoidin* for different concentrations of *Geshoidin*: (a) 0.1 M citric acid and 0.2 M di-sodium hydrogen phosphate buffer (pH 7); (b) 1 x 10^{-5}; (c) 2 x 10^{-5}; (d) 3 x 10^{-5}; (e) 4 x 10^{-5}; (f) 5 x 10^{-5}; (g) 6 x 10^{-5}; (h) 7 x 10^{-5}; (i) 8 x 10^{-5}; (j) 1 x 10^{-4} M.

Analytical application

Figure 7 shows the cyclic voltammogram of *Tella*. A well defined voltammogram that had a peak similar to that of pure *Geshoidin* was obtained implying the promising application of voltammetry for the direct determination of *Geshoidin* in *Tella* without the requirement of purification or additional procedure. The voltammetric method was applied to the determination of *Geshoidin* in: water, ethanol and methanol extracts from the leaf of *Gesho* powder by using

the standard additions method. Figure 8 shows the linear scan voltammograms for different concentrations of standard solutions of *Geshoidin* and for the water extract of *Geshoidin* from *Gesho* powder. Similar plots were obtained for those of ethanol and methanol extracts. Using the standard additions method, the amount of *Geshoidin* in each solvent extract was determined as a mean value of 50.4 ± 2.3, 91.4 ± 3.2 and 130.2 ± 3.1 mg of *Geshoidin* per gram of *Gesho* powder for the water, ethanol and methanol extracts, respectively, showing the ease of extracting the compound from *Gesho* powder when methanol is used as solvent. The linear scan voltammograms of *Geshoidin* for the three solvent extracts are compared in Figure 9 and it is also seen that very well defined and enhanced wave is observed for the methanol extract of *Gesho*. So far only a qualitative analysis of methanol extract of *Gesho* had been reported in the literature using electrospray liquid chromatography mass spectrometery [26]. Except this, there is no any alternative analytical method for the determination of *Geshoidin* reported in the literature.

Anthraquinone and flavonoid compounds that are found in the leaves of *Gesho* [4] did not show any peak in the potential window and hence did not affect the voltammograms of Geshoidin both in *Tella* and alcohol extracts. Some of these compounds exist in the plant in trace level and some are electrochemically inactive. The presence of ethanol and methanol in *Gesho* extracts and in *Tella* also did not show any effect on the peak of *Geshoidin* since their oxidation was not possible at bare glassy carbon electrode, at least before the onset of the base electrolyte decomposition [27].

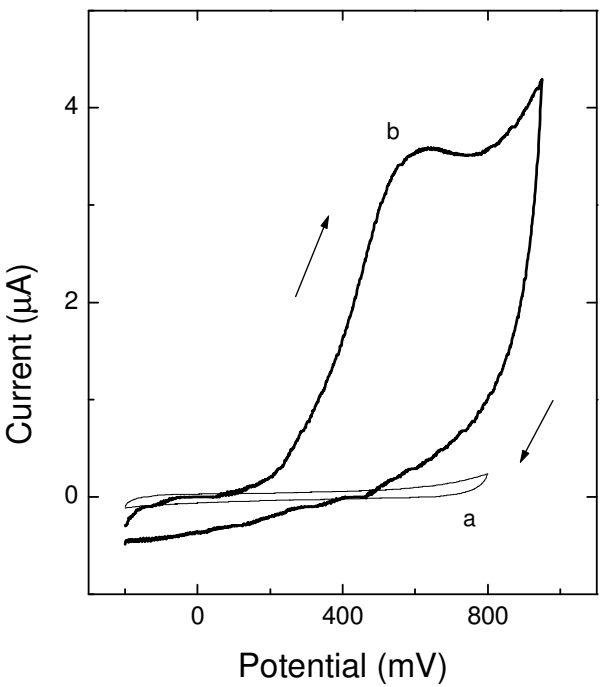


Figure 7. Cyclic voltammogram of *Tella*: (a) base electrolyte 0.1 M citric acid and 0.2 M di-sodium hydrogen phosphate buffer (pH 7); (b) *Tella*.

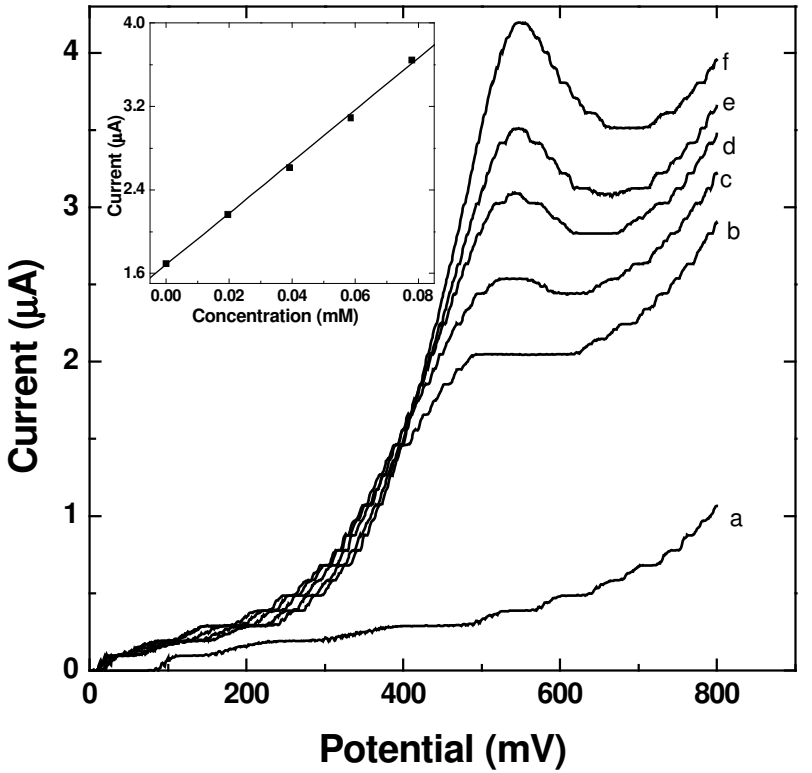


Figure 8. Linear scan voltammograms of *Geshoidin* for different standard concentrations of *Geshoidin* and *Geshoidin* from water extract of *Gesho*, (a) 0.1 M citric acid and 0.2 M di-sodium hydrogen phosphate buffer (pH 7); (b) water extract of *Gesho* (unknown concentration); (c) 1.96 x 10^{-5}; (d) 3.91 x 10^{-5}; (e) 5.85 x 10^{-5}; (f) 7.78 x 10^{-5} M standard *Geshoidin* solution.

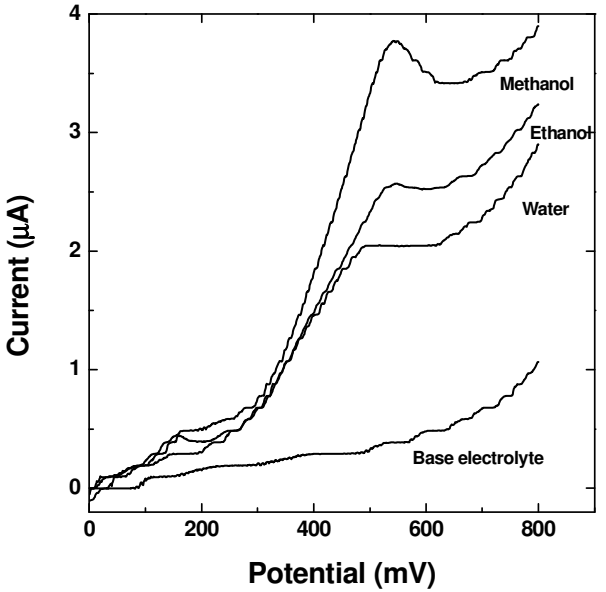
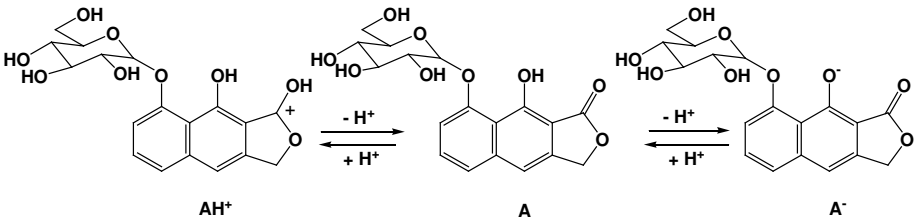


Figure 9. Comparison of linear scan voltammograms of *Geshoidin* extracted from Gesho using water, ethanol and methanol as solvents.

Spectrophotometric behaviour of Geshoidin

The spectrophotometric behaviour of *Geshoidin* was studied in the pH range 0.5 – 13.0. The electronic absorption spectra of *Geshoidin* obtained in acidic, neutral and basic media are presented in Figure 10. In acidic medium of pH 0.5, *Geshoidin* existed in its cationic form (AH^+). At that pH the spectrum showed two characteristic absorption bands for the cationic structure at λ_{max} 360 (main band) and 311 nm (small band). When the pH was increased to pH 5.0 and 6.5, respectively, the small band shifted to λ_{max} 312 nm and the intensity of the band increased with increasing pH, whereas the main band remained at λ_{max} 360 nm without significant variation in the peak's intensity implying that *Geshoidin* existed in its cationic and neutral forms. At pH 7.5, a major bathchromic shift was observed for the small band (λ_{max} 329 nm) while the main band shifted to λ_{max} 364 nm and both bands showing slightly enhanced peak intensities. This implies the transformation of *Geshoidin* from cationic (AH^+) to neutral (A) and anionic forms (A^-). A subsequent increase in pH up to 13 further enhanced the peak intensity of the small band while its λ_{max} essentially remained constant. λ_{max} values of the main band showed bathochromic shift (λ_{max} 376 nm at pH 13.0) with substantial increase in the absorbance of the band. These changes showed that the process of deprotonation of *Geshoidin* molecule had taken place resulting in the formation of the conjugate base A^- as shown in Scheme 2. The conjugate base formed possesses an additional double bond, which increased the number of π electrons relative to the cation causing an increased delocalization of the π electrons, i.e., significant bathochromic shifts of the absorption bands [28].



Scheme 2

The change in the absorbance of the main band as a function of pH was plotted following the method of Albert and Sergeant [16]. Figure 11 shows the plot of $\Delta A/\Delta pH$ as a function of pH that was obtained by differentiation of the plot of absorbance versus pH. As can be seen, the plot gave two peak maxima at around pH 7.0 (large peak) and pH 10.0 (small peak), corresponding to the pK_{a1} and pK_{a2} values, respectively. These two values are in good agreement with the pK_{a1} and pK_{a2} values determined from the voltammetric measurements of this study.

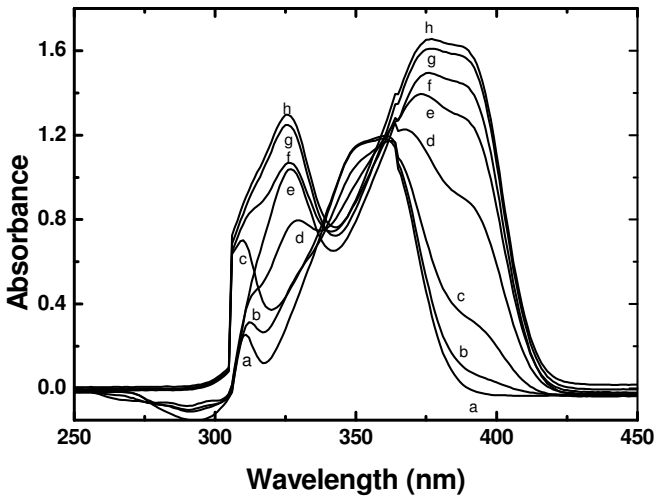


Figure 10. Variation of absorbance maxima with pH for 1.4 x 10^{-4} M *Geshoidin*. pH: (a) 0.5; (b) 5.0; (c) 6.5; (d) 7.5; (e) 8.0; (f) 9.0; (g) 11.0; (h) 13.0.

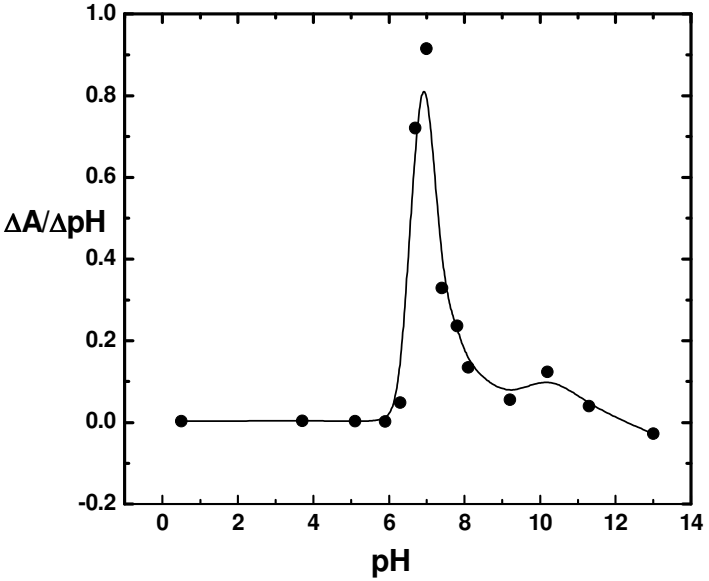


Figure 11. Plot of ΔA/ΔpH as a function of pH for 1.4 x 10^{-4} M *Geshoidin*.

Scavenging ability of Geshoidin on superoxide anion

Free radical scavenging activity determination was carried out based on the electrochemical reduction of oxygen [17]. Different electrodes including glassy carbon, carbon paste, gold and platinum were tested for the reduction of oxygen and the current response obtained with gold electrode was superior. Hence a gold disk electrode was used for the study. When oxygen is reduced it proceeds at the cathode in several stages with formation of the active anion-radical of oxygen or superoxide anion ($O_2^{\bullet-}$) as intermediate [17]. Figure 12 shows selected differential pulse voltammograms of oxygen reduction at gold disk electrode before and after the addition of different concentrations of *Geshoidin*. The reduction peak was observed at a potential of about -0.160 V. When *Geshoidin* was added the peak current decreased with increasing concentration of *Geshoidin*. It is obvious that *Geshoidin* can scavenge the active oxygen radicals yielded by the cathodic reduction of oxygen. The inhibitory rate which is defined as the percentage of the ratio of the difference of the peak current obtained due to oxygen reduction before and after adding *Geshoidin* to the peak current obtained due to oxygen reduction before adding *Geshoidin* was plotted as a function of concentration of *Geshoidin* [29, 30]. The plot (not shown) was linear and is described by the following equation.

Inhibitory rate for superoxide ($O_2^{\bullet-}$) = 23570.1c + 4.4 $\quad r^2 = 0.966$ (for n = 7) (7)

The IC_{50} value [31] of *Geshoidin*, that is the concentration of *Geshoidin* when inhibitory rate reaches 50%, was calculated from the linear curve as 1.9 x 10^{-3} M. A comparative analysis of the activity of well-known antioxidants such as ascorbic acid, citric acid and glucose was also made. Successive addition of citric acid and glucose solutions, respectively, did not show systematic decrease in the oxygen reduction peak current. While the additions of ascorbic acid solutions exhibited regular decrease in the peak current. The plot of the inhibitory rate as a function of ascorbic acid concentration gave a straight line which is given by equation (8).

Inhibitory rate for superoxide ($O_2^{\bullet-}$) = 27838.7c + 3.5 $r^2 = 0.973$ (for n = 7) (8)

Its IC_{50} value was calculated from the linear curve as 1.7 x 10^{-3} M indicating that *Geshoidin* has comparable radical scavenging activity as that of ascorbic acid. The high scavenging property may be due to the phenolic hydroxyl and glucose moieties that could provide the necessary component as a radical scavenger [30, 31].

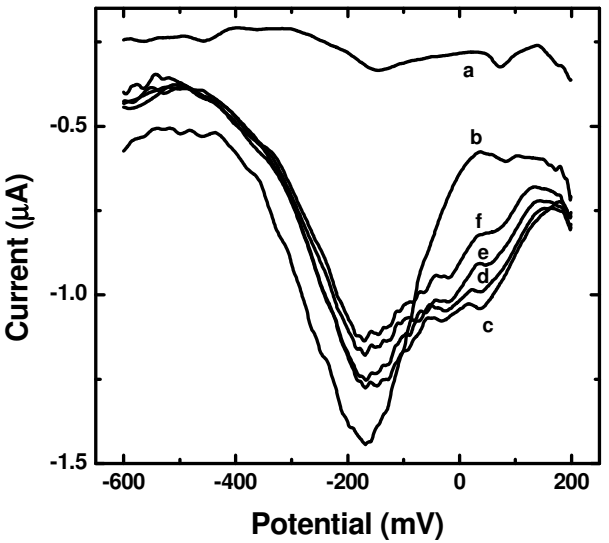


Figure 12. Differential pulse voltammograms for the electrochemical oxygen reduction in 0.9% NaCl supporting electrolyte at a gold disk electrode: (a) deaerated solution with N_2; (b) air saturated solution; (c) 4 x 10^{-4}; (d) 8 x 10^{-4}; (e) 1.1 x 10^{-3}; (f) 1.3 x 10^{-3} M *Geshoidin* spiked air saturated solution.

CONCLUSIONS

This article presents for the first time the electrochemical behaviour of *Geshoidin* and its analytical determination using voltammetric technique. The cyclic voltammetric responses obtained are chemically irreversible over the range of scan rates employed, and are consistent with electron transfer being followed by fast chemical process. The voltammetric method gives wide linear range for the determination of *Geshoidin*. Successful application of linear scan voltammetry for the determination *Geshoidin* in water, ethanol and methanol extracts from the leaf of the plant is demonstrated. The promising application of voltammetry for the direct determination of *Geshoidin* in *Tella* without the requirement of purification or additional procedure is also demonstrated. The absorption spectra reveal the structural changes of *Geshoidin* caused by changes in the pH of the medium and that could lead to changes in the electrochemical behaviour of the molecule. The radical scavenging activity of the compound exhibited the ability of *Geshoidin* to act as a chemical antioxidant.

REFERENCES

1. Watt, J.M.; Breyer-Brandwijk, M.G. *Medicinal and Poisonous Plants of South and Eastern Africa*, E & S Livingstone Ltd.: London; **1962**.
2. Palmer, E.; Pitman, N. *Trees of Southern Africa*, Volume II; Balkema Publisher: Cape Town; **1972**.
3. Coates-Palgrave, K. *Trees of Southern Africa*, C.S. Struik Publishers: Cape Town; **1988**.
4. Abegaz, B.M.; Kebede, T. *Bull. Chem. Soc. Ethiop.* **1995**, 9, 107.
5. Kleyn, J.; Hough, J. *The Microbiology of Brewing: Annual Review of Microbiology* **1971**, 25, 583.
6. Sahle, S.; Abegaz, B. *SINET: Ethiop. J. Sci.* **1991**, 14, 93.
7. Nickerson, G.; Van Engel, L. *J. Am. Soc. Brew. Chem.* **1992**, 50, 82.
8. Peacock, V.; Deinzer, M. *J. Am. Soc. Brew. Chem.* **1981**, 39, 136.
9. Abegaz, B.M.; Ngadjui, B.T.; Bezabih, M.; Mdee, L.K. *Pure Appl. Chem.* **1999**, 71, 919.
10. Abegaz, B.M; Dagne, E. *Bull. Chem. Soc. Ethiop.* **1988**, 2, 15.
11. Abegaz, B.M.; Peter, M.G. *Phytochemistry* **1995**, 39, 1411.
12. Bezabih, M.; Abegaz, B.M. *Bull. Chem. Soc. Ethiop.* **1998**, 12, 45.
13. Valdez, L.B.; Arnaiz, S.L.; Bustamante, J.; Alvarez, S.; Costa, L.E.; Boveris, A. *Biol. Res.* **2000**, 33, 65.
14. Chevion, S.; Roberts, M.A.; Chevion, M. *Free Radic. Bio. Med.* **2000**, 28, 860.
15. Brett, A.O.; Ghica, M.E. *Electroanalysis* **2003**, 15, 1745.
16. Albert, A.; Sergeant, E.P. *The Determination of Ionization Constants: A Laboratory Manual*, Chapman and Hall: New York; **1984**.
17. Korotkova, E.I.; Karbainov, Y.A.; Shevchuk, A.V. *J. Electroanal. Chem.* **2002**, 518, 56.
18. Southampton Electrochemistry Group *Instrumental Methods in Electrochemistry*, Ellis Horwood; Chichester, **1985**.
19. Brown, E.R.; Sandifer, J.R. *Physical Methods of Chemistry*, Volume II, *Electrochemical Methods*, Rossiter, W.; Hamilton, J.F. (Eds.); Wiley-Interscience: New York; **1986**.
20. Coomber, D.C.; Tucker, D.J.; Bond, A.M. *Electroanalysis* **1998**, 10, 163.
21. Golabi, S.M.; Zare, H.R.; Hamzehloo, M. *Microchem. J.* **2001**, 69, 111.
22. Bard, A.J.; Faulkner, L.R. *Electrochemical Methods Fundamentals and Applications*, John Wiley: New York; **1980**.
23. Kissinger, P.T; Bott, A.W. *Current Separation* **2002**, 20, 51.
24. Rieger, P.H. *Electrochemistry*, Prentice-Hall International: New Jersey; **1987**.
25. Morrow, G.W. *Anodic Oxidation of Oxygen-Containing Compounds, in Organic Electrochemistry,* Lund, H; Hammerich, O, (Eds.); Marcel Dekker Inc.: New York; **2001**.
26. Nindi, M.M.; Kgarebe, B.V.; Wolfender, J.L.; Abegaz, B.M. *Phythochem. Anal.* **1999**, 10, 69.
27. Ciszewski, A. *Electroanalysis* **2005**, 7, 1132.
28. Skoog, D.A.; Leary, J.J. *Principles of Instrumental Analysis*, Saunders College Publishing: New York; **1992**.
29. Halliwell, B.; Gutteridge, J.M.C. *Arch. Biochem. Biophys*. **1986**, 246, 501.
30. Zhang, X.; Zheng, J.; Gao, H. *Anal. Lett.* **2001**, 34, 1901.
31. Pourmorad, F.; Hosseinimehr, S.; Shahabimajd, N. *Afric. J. Biotechnology* **2006**, 5, 1142.