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 x 10^-6 - 1.00 x 10^-4 M Geshoidin was achieved. The detection limit 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. 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 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 prinoides, 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 taste to Tella. Its contribution to the typical aroma of these beverages is yet to be realized. It is interesting to know that unlike hops, Gesho does not contain essential oils.

A number of compounds have been isolated from the leaves and stems of Gesho [4, 9-12]. Previously unknown naphthalenic compound \( \beta \)-sorigenin-8-O-\( \beta \)-D-glucoside, (Figure 1) was isolated from the leaves and fully characterised. The name Geshoodin was proposed for this novel glucoside. The discovery of Geshoodin 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 Geshoodin, one of the major constituents of the plant [4].

![Figure 1. Structure of Geshoodin (\( \beta \)-sorigenin-8-O-\( \beta \)-D-glucoside).](image)

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 \( \textit{vivo} \). 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 or more hydroxylated benzene rings and are known for their radical scavenging ability [15]. Owing to the phenolic nature of Geshoodin, 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 Geshoodin 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 Geshoodin 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 ($\phi$: 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_a's 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_2PO_4 and 1 x 10^{-2} 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 °C).

RESULTS AND DISCUSSION

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.
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, \(\nu\) 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.

\[
i_{\text{p}}/\mu\text{A} = 0.13/\mu\text{A} + 4.32\nu^{1/2}
\]

\(r^2 = 0.996 \quad \text{(for } n = 8)\)  

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.

\[
E_{\text{p}}/V = 0.667 + 0.01284\ln\nu
\]

\(r^2 = 0.998 \quad \text{(for } n = 8)\)  

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

\[
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\]
where $A$ in cm$^2$, $D$ in cm$^2$s$^{-1}$, $C_b$ in M, $\nu$ in V s$^{-1}$ and $n_{\alpha}$ 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 $\nu$ with the following relation.

$$E_p = K + \frac{RT}{2(1-\alpha)n_{\alpha}F}\ln\nu$$

(4)

where $K = E^0 + \frac{RT}{(1-\alpha)n_{\alpha}F}[0.78 + \frac{1}{2}\ln\left(\frac{1-\alpha}{n_{\alpha}F}D/kD}RT\right]$.

From equation (4) and $t = 25$ °C, the value of $(1 - \alpha)n_{\alpha}$ was determined from the slope of $E_p$ versus $\ln\nu$ of equations (2) as 0.984. The electron transfer coefficient $\alpha$ for the oxidation of Geshoidin was determined ($\alpha = 0.49$) from the Tafel slope of a linear scan voltammogram recorded at low scan rate (5 mV s$^{-1}$) [22]. Hence, the value of $n_{\alpha}$ 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 $5 \times 10^{-5}$ M Geshoidin to be $5.80 \times 10^{-6}$ 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 $5 \times 10^{-5}$ 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.

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:

$$E_p/V = 0.061pH + 0.990 \quad r^2 = 0.990 \quad (pH 2.0 - 7.0) \quad (for \ n = 7) \quad (5)$$

$$E_p/V = 0.015pH + 0.683 \quad r^2 = 0.999 \quad (pH 7.2 - 9.2) \quad (for \ n = 4) \quad (6)$$

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

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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 $\text{H}^+$ 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](image)

*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 $1.00 \times 10^{-6} - 1.00 \times 10^{-4}$ M Geshoidin and the correlation coefficient was $r^2 = 0.999$. At higher concentrations ($\geq 3.00 \times 10^{-4}$ 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 $5.00 \times 10^{-7}$ M Geshoidin. For eight successive determinations of $1 \times 10^{-5}$ 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) $1 \times 10^{-5}$; (c) $2 \times 10^{-5}$; (d) $3 \times 10^{-5}$; (e) $4 \times 10^{-5}$; (f) $5 \times 10^{-5}$; (g) $6 \times 10^{-5}$; (h) $7 \times 10^{-5}$; (i) $8 \times 10^{-5}$; (j) $1 \times 10^{-4}$ M.](image)

**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 spectrometry [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) $1.96 \times 10^{-5}$; (d) $3.91 \times 10^{-5}$; (e) $5.85 \times 10^{-5}$; (f) $7.78 \times 10^{-5}$ 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 \( \lambda_{\text{max}} \) 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 \( \lambda_{\text{max}} \) 312 nm and the intensity of the band increased with increasing pH, whereas the main band remained at \( \lambda_{\text{max}} \) 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 bathochromic shift was observed for the small band (\( \lambda_{\text{max}} \) 329 nm) while the main band shifted to \( \lambda_{\text{max}} \) 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 \( \lambda_{\text{max}} \) essentially remained constant. \( \lambda_{\text{max}} \) values of the main band showed bathochromic shift (\( \lambda_{\text{max}} \) 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 \( \pi \) electrons relative to the cation causing an increased delocalization of the \( \pi \) electrons, i.e., significant bathochromic shifts of the absorption bands [28].
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 $\Delta A/\Delta \text{pH}$ 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 $1.4 \times 10^{-4}$ 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.
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$_2^-$) 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$_2^-$) = 23570.1c + 4.4 \quad r^2 = 0.966 \quad (for \ n = 7) \quad (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).

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

Its IC\textsubscript{50} value was calculated from the linear curve as $1.7 \times 10^{-3}$ 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].

![Differential pulse voltammograms for the electrochemical oxygen reduction in 0.9% NaCl supporting electrolyte at a gold disk electrode: (a) deaerated solution with N\textsubscript{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.
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