

Determination of Coumarin in *Mikania glomerata* Infusions by Square-Wave Voltammetry Using a Boron-Doped Diamond Electrode

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Infusions of *Mikania glomerata* Sprengel (guaco) leaves are used in Brazil for the treatment of respiratory diseases. Coumarin is the chemical marker of guaco for quality control purposes. This work proposes a voltammetric methodology to determine coumarin by using a boron-doped diamond electrode. At pH 10 (0.1 mol L⁻¹ Britton-Robinson buffer solution), coumarin is irreversibly oxidized around +1.77 V in a process predominantly controlled by diffusion. The methodology described here presented a linear range from 1.54 to 15.3 µg mL⁻¹ with limits of detection and quantification of 0.20 and 0.62 µg mL⁻¹, respectively. Analysis of variance confirmed the significance of the regression and the absence of lack-of-fit at the confidence level of 95%. Infusion of commercial guaco leaves was directly analyzed, and results were in agreement with high-performance liquid chromatography after applying the paired *t*-test at a confidence level of 95%.

Keywords: coumarin, square wave voltammetry, boron-doped diamond electrode

Introduction

Ever since ancient times, human groups have looked to nature for drugs aiming to treat or cure their diseases.¹ The discovery of healing properties of some plants is part of ethnobotanical knowledge disseminated throughout generations. Only in 1987, however, the use of herbal medicines was recognized as a traditional practice by the Alma-Ata declaration,² which recommended the implementation of traditional, complementary, and alternative medicine in the national health care systems of all members of the United Nations.

Following World Health Organization guidelines in the 1980s, medicinal plants and herbal medicines began to be part of the national policy on the use of medicinal plants and herbal medicines (PMPHM) in Brazil.³ Additionally, regulations were established for their safe access and rational use in the Unified Health System (UHS), especially in Primary Health Care (PHC).⁴ Since Brazil has the largest

biodiversity in the world, accounting for more than 15% of all living species,⁵ the use of medicinal plants supported by wide knowledge of their potential among traditional users was encouraged for primary health caretakers in the public systems.⁶

Among the Brazilian diversity of vegetable species, one is the *Mikania glomerata* Spreng., popularly known as guaco, which is marketed in pharmaceutical forms of syrup and oral solutions. Its main use, however, has been with the infusion of its leaves with hot water, as a tea, because of its availability, ease of use, and affordability. Medicinal records and preclinical evidence have shown the efficiency of guaco leaf infusions for the treatment of respiratory diseases because of its expectorant and bronchodilation properties. These benefits have been associated with the presence of coumarin (2*H*-1-benzopyran-2-one), which is the major secondary metabolite defined as chemical marker.⁷

Regarding the literature, although a number of beneficial health effects have been reported, an excess intake of coumarin in guaco leaf infusions also can cause toxicological effects, such as vomiting, diarrhea, hypertension, possible interference in blood coagulation, as well as hepatotoxicity.⁸⁻¹⁰

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This makes it necessary to develop accurate and precise analytical methodologies to quantify this organic compound in medicinal plants and herbal medicines. To determine coumarin in syrups, oral solutions, and infusions of guaco, chromatographic,¹¹⁻¹⁷ spectrophotometric,^{18,19} digital images²⁰ and electroanalytical²¹ techniques have been employed. The Brazilian pharmacopeia⁹ recommends the use of high-performance liquid chromatography (HPLC) for the quantification of coumarin in guaco-based pharmaceutical formulations. Although this technique has remarkable accuracy and precision, it requires sophisticated instruments to detect analytes, involves a high cost of acquisition/maintenance, is time-consuming, and uses large amounts of organic solvents, which generates waste with potential risk to human health and the environment.

On the other hand, electroanalytical techniques, compared to HPLC, are interesting alternatives for determining coumarin in infusions of guaco leaves. This is because the instrument acquisition/maintenance is more inexpensive, these techniques usually do not use organic solvents, and they enable direct and rapid sample analysis. For this, the boron-doped diamond (BDD) electrode has been highlighted as the working electrode in the development of voltammetric methodologies due to its attractive characteristics when compared with conventional electrodes. The BDD has an especially wide working potential window, low and stable background current, chemical and mechanical long-term response stability, and resistance to passivation.¹⁰

This work proposes a BDD electrode-based voltammetric methodology for the determination of coumarin in infusions of guaco leaves without any sample pre-treatment, other than the preparation of the infusions as usually ingested by the consumers. As far as we know, the oxidation of coumarin on the surface of the BDD electrode and its electrochemical determination has not been reported in the literature up to the date of our study. Only Miyano *et al.*²¹ have reported the determination of coumarin in guaco leaf infusions by using the electrochemical reduction technique. Their measurements were subjected to chemical interferences by many metal ions that could have been reduced in the cathodic processes, affecting the accuracy of the analytical method.

Experimental

Instrumentation, reagents, solutions, and samples

The experimental measurements were performed using a potentiostat Eco Chemie μ Autolab, Type II, Metrohm 663 VA Stand (Metrohm Autolab, Herisau, Switzerland), coupled with a three-electrode system controlled by NOVA 2.1.3 software. The voltammetric studies were performed in

a one-compartment electrochemical cell (20 mL) composed of a BDD electrode as the working electrode, (geometric surface area of 0.36 cm²; 8000 ppm boron film from the Centre Suisse d'Electronique et de Microtechnique SA (CSEM), Neuchâtel, Switzerland), an Ag/AgCl (KCl, 3.0 mol L⁻¹), and a platinum wire as the reference and auxiliary electrodes, respectively.

All reagents used in this work were of analytical grade. The solutions were prepared with ultrapure water (conductivity < 0.1 μ S cm⁻¹) obtained from a Direct-Q 5 UV purifier (Millipore, USA).

The supporting electrolyte was a Britton-Robinson (BR) buffer solution (0.1 mol L⁻¹), which consists of a mixture of acids: acetic (Vetec, Brazil), phosphoric (Hexis Científica, Brazil), and boric (Hexis Científica, Brazil). The pH was adjusted with the addition of a 1.0 mol L⁻¹ sodium hydroxide (Hexis Científica, Brazil) solution. For polarization of the BDD electrode, a 0.5 mol L⁻¹ sulfuric acid (Hexis Científica, Brazil) solution was used.

A 1.46×10^3 μ g mL⁻¹ (1.0×10^{-2} mol L⁻¹) coumarin (Sigma-Aldrich, USA) stock solution was prepared in ultrapure water by using a USC 1400 ultrasonic bath (Unique, Brazil) at 40 °C for 30 min in order to increase its solubility in this solvent. The calibration set was comprised of nine concentration levels ranging from 1.54 to 15.30 μ g mL⁻¹, prepared by successive additions of the stock solution to the electrochemical cell containing the BR buffer solution (pH 10).

Five commercial samples of guaco leaves (packs of 20 g) from different brands and batches were purchased from natural product stores in the cities of João Pessoa and Campina Grande, Paraíba state, Brazil.

Sample preparation

Infusions were prepared by using 1.0 g guaco leaves in 50 mL of boiling ultrapure water for 5 min, and then left to cool down to room temperature. Following this, the infusion was filtered through a Whatman qualitative filter paper No. 1 and the final volume was made up to 50 mL in a volumetric flask. Finally, the infusion was stored in an amber glass bottle at room temperature.

The samples were analyzed by direct dilution transferred into the electrochemical cell containing 20 mL of the BR buffer solution (pH 10) to interpolate the analytical signals in the linear working range.

Electrode preparation, electroanalytical studies and analytical procedure

At the beginning of each working day, the BDD

electrode underwent the recommended electrochemical pre-treatment, as described elsewhere,¹⁰ in order to guarantee reproducibility of the results. A glassy carbon (GC) electrode was also used, but with no pre-treatment.

The first study was on the electrochemical behavior of coumarin ($7.69 \mu\text{g mL}^{-1}$ in 0.1 mol L^{-1} BR buffer solution) on the surface of the BDD and GC electrodes. This study was carried out by means of square wave voltammetry (SWV) with the following operational parameters: frequency (f) of 50 Hz, potential window (ΔE_w) from -2.0 to $+2.5$ V, scan increment (ΔE_s) of 2 mV, and pulse amplitude (ΔE_p) of 50 mV.

The second study was on the influence of pH (from 1.8 to 10) on the electrochemical behavior of coumarin on the BDD electrode (peak current). This study was carried out by means of differential pulse voltammetry (DPV) with the following operational parameters: potential window (ΔE_w) from $+1.5$ to $+2.2$ V, pulse amplitude (ΔE_p) of 50 mV, and modulation time (ΔE_t) of 50 ms.

The third study was on the kind of electroactive material transport to the BDD electrode. This study was carried out by means of cyclic voltammetry (CV) in order to verify the relationship between the peak currents (I_p) and scan rate (v) in the range from 25 to 200 mV s^{-1} .

The fourth study was on existence of memory effect on the surface of the BDD electrode. This study was carried out by means of SWV ($7.69 \mu\text{g mL}^{-1}$ in 0.1 mol L^{-1} BR buffer solution) over the surface of the BDD electrode. This study was carried out by means of SWV with the following operational parameters: frequency (f) of 50 Hz, potential window (ΔE_w) from -2.0 to $+2.5$ V, scan increment (ΔE_s) of 2 mV, and pulse amplitude (ΔE_p) of 50 mV.

For the construction of the analytical curve and analysis of the infusions of commercial guaco leaves, SWV was used with the following operational parameters: $f = 75$ Hz, $\Delta E_s = 5$ mV, and $\Delta E_p = 50$ mV.

Reference method

The Brazilian Pharmacopeia⁹ recommends the use of HPLC for quantification of coumarin. Chromatographic runs were performed using an Ultimate 3000 Dionex chromatograph (Thermo Fisher Scientific, Waltham, MA, USA), consisting of a quaternary pump, a manual injector fitted with a $20 \mu\text{L}$ fixed loop, and a UV-Vis diode array detector. A Dionex Acclaim 120 C18 column of $150 \text{ mm} \times 4.6 \text{ mm}$, $5 \mu\text{m}$ particle sizes, and 120 \AA pore size, acetonitrile:water (40:60 v/v) mobile phase, flow rate of 1.0 mL min^{-1} , temperature of $40 \text{ }^\circ\text{C}$, and detection at 275 nm were employed.

Results and Discussion

Choice of the working electrode

The electroactivity of a $7.69 \mu\text{g mL}^{-1}$ coumarin in 0.1 mol L^{-1} BR buffer solution (pH 10) was evaluated by SWV (Figure 1). According to Figure 1a, the GC electrode did not present any peak potential in the oxidation region, whereas the BDD electrode presented an anodic peak potential in (E_{pa}) ca. $+1.77 \text{ V}$ (oxidation) vs. Ag/AgCl referring to the oxidation of coumarin and a cathodic peak potential in (E_{pc}) ca. -1.81 V (reduction) vs. Ag/AgCl referent to the reduction of coumarin (Figure 1b).

Therefore, the BDD electrode was chosen as the working electrode for the determination of coumarin in guaco leaf infusions. When the current intensity of the anodic and cathodic peaks were compared, the analytical response was more intense using the oxidation process.

Influence of scan rate

To assess which kind of electroactive material transport to the BDD electrode surface occurs, CV was used to verify

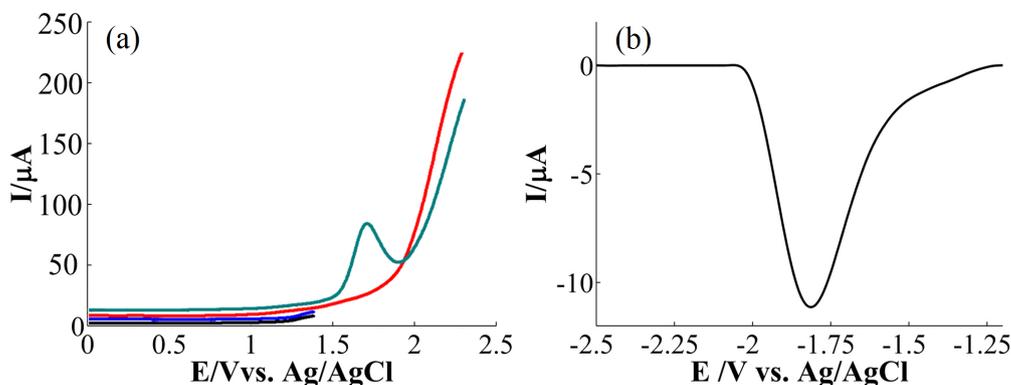


Figure 1. Square wave voltammograms of a $7.69 \mu\text{g mL}^{-1}$ coumarin solution in 0.1 mol L^{-1} BR buffer solution (pH 10). (a) The current intensity of GC support electrolyte in the absence (—) and presence of coumarin (---), and of BDD support electrolyte in the absence (—) and presence of coumarin (---) in the region of oxidation potentials. (b) The current intensity of the BDD support electrolyte in the presence of coumarin in the region of reduction potentials.

the scan rate (ν) in the range from 25 to 200 mV s^{-1} and how it influenced the potential and current peaks (Figure 2a). Figure 2b also shows the linear relationship between the peak currents (I_p) and $\nu^{1/2}$, indicating that the oxidation of coumarin on the BDD electrode is predominantly controlled by diffusion.

Influence of pH

SWV was the technique chosen for the determination of coumarin in infusions of commercial guaco leaves. In order to assess how potential peak depends on pH, DPV measurements were carried out as DPV is faster and more sensitive to electro-oxidative processes.²² This technique makes it possible to determine the number of electrons involved in the process. For this purpose, the peak potential at half height ($W_{1/2}$) value is used, as in equation 1:

$$W_{1/2} = 3.52RT/nF \quad (1)$$

where R is the gas constant, T is the temperature, n is the number of electrons and F is the Faraday constant.

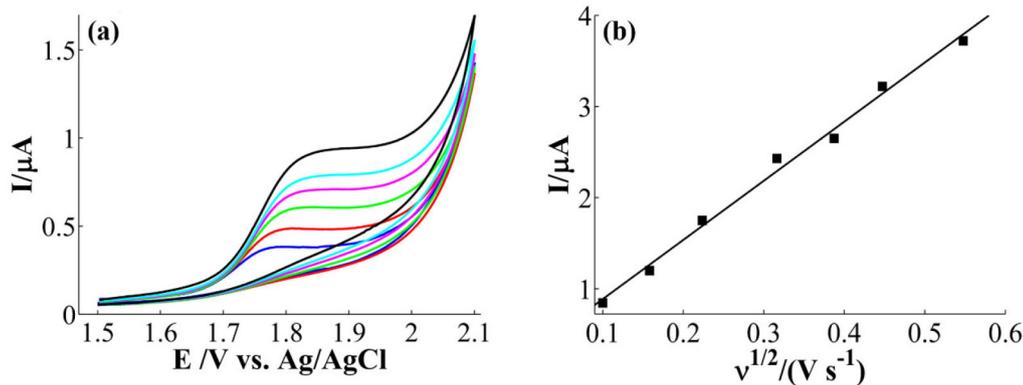


Figure 2. Electrochemical behavior of the $7.69 \mu\text{g mL}^{-1}$ coumarin solution in 0.1 mol L^{-1} BR buffer solution at pH 10. (a) Cyclic voltammograms obtained for different scan rates in the potential range of 1.5 to 2.1 V. (b) Influence of the scan rate on the peak current.

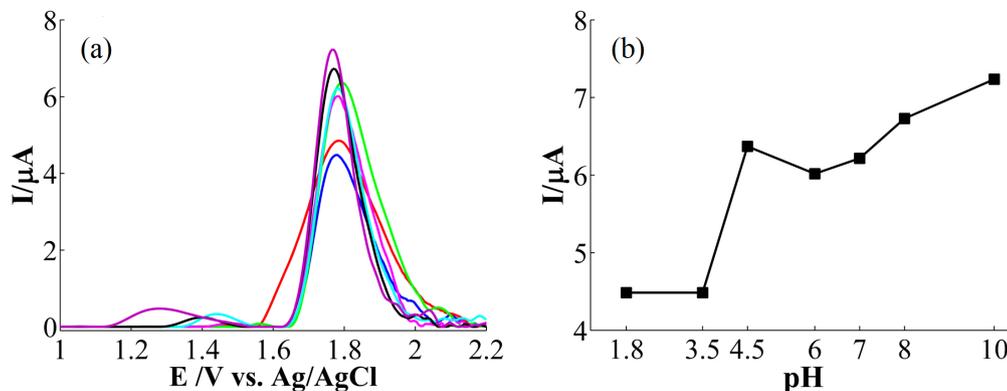


Figure 3. Influence of pH on the voltammetric response of coumarin. (a) Differential pulse voltammetry for $7.69 \mu\text{g mL}^{-1}$ of coumarin in 0.1 mol L^{-1} BR buffer at different pH values: (—) 1.8, (—) 3.5, (—) 4.5, (—) 6.0, (—) 7.0, (—) 8.0, and (—) 10. (b) Current peaks obtained for each pH.

DPV measurements were carried out in a pH range from 1.8 to 10.0 (0.1 mol L^{-1} BR buffer solution).

As can be seen in Figure 3a, there were no significant potential shifts regarding the pH range in this study. The $W_{1/2}$ obtained experimentally, $W_{1/2} = 72 \text{ mV}$, indicates that the oxidation of coumarin involved one electron only. A report in the literature registered values in the order of 90 and 45 mV for the transfer of one and two electrons, respectively.²² In terms of sensitivity, Figure 3b shows that the highest value of current intensity was obtained at $\text{pH} = 10$.

In an aqueous medium, coumarin undergoes hydrolysis to produce *o*-coumaric acid, according to Figure 4a. *O*-Coumaric acid (H_2A) has two ionizable hydrogens with the following ionization constants: $\text{p}K_{a1} = 4.11$ (carboxylic) and $\text{p}K_{a2} = 9.60$ (phenol).²³

At $\text{pH} = 10.0$, *o*-coumaric acid is completely neutralized to produce its corresponding salt ($\text{H}_2\text{A} \leftrightarrow \text{H}^+ + \text{HA}^-$ at $\text{pH} = 4.11$ and $\text{HA}^- \leftrightarrow \text{H}^+ + \text{A}^{2-}$ at $\text{pH} = 9.60$), as shown in Figure 4b.

According to Stradins *et al.*,²⁴ the ionization of the phenolic hydrogen ($\text{p}K_{a2}$) facilitates the oxidation of phenols. In this sense, the phenolic group is oxidized

to a carbonyl group (ketone) which needs only one electron. The proposed mechanism for this step is shown in Figure 4c.

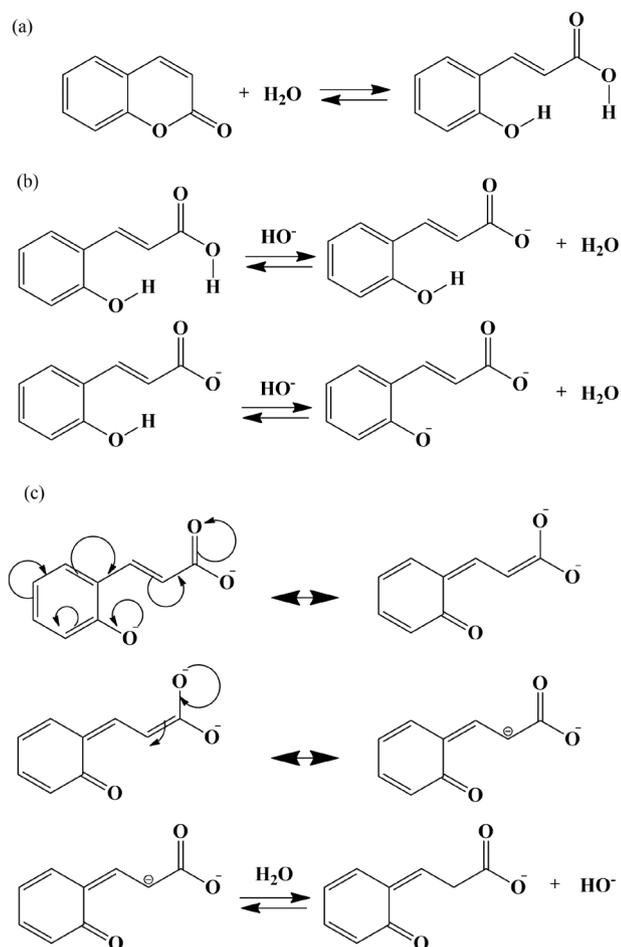


Figure 4. Reactions of coumarin in an aqueous solution: (a) hydrolysis, (b) ionization and (c) expected oxidation product.

Carryover study

The existence of the memory effect on the surface of the BDD electrode can be assessed by SWV measurements. For this, consecutive SWV scans were recorded by using two coumarin solutions at 3.85 and 7.69 $\mu\text{g mL}^{-1}$. Figure 5 shows the reproducibility of peak currents obtained for the different concentrations of coumarin, confirming that there was no significant memory effect on the BDD electrode. The rapid regeneration of the BDD electrode surface, due to its high stability, enabled successive measurements without the need for chemical/electrochemical cleaning or polishing. This is an important feature of the proposed method since the insertion of cleaning steps in an experimental procedure may decrease the throughput rate and compromise the reproducibility of the measurements.

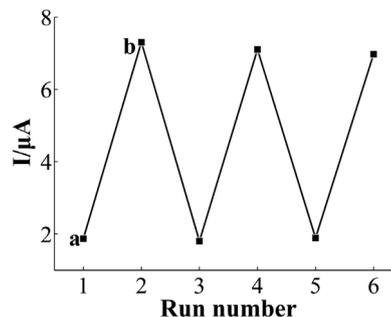


Figure 5. Carry-over experiment showing the consecutive square wave scans and peak currents obtained for concentrations of coumarin: (a) 3.85 $\mu\text{g mL}^{-1}$ and (b) 7.69 $\mu\text{g mL}^{-1}$. SW voltammograms obtained using the BDD electrode in 0.1 mol L^{-1} BR buffer, pH 10.

Optimization of SWV parameters

In SWV measurements, the sensitivity (current intensity) and selectivity (peak width at half height) are directly influenced by the frequency (f), pulse amplitude (ΔE_p), and potential increment (ΔE_s). An optimization step by using the univariate approach was performed in the ranges of 15-150 Hz, 10-100 mV, and 1-10 mV for f , ΔE_p and ΔE_s , respectively. The optimized operational parameters were $f = 75$ Hz, $\Delta E_p = 50$ mV and $\Delta E_s = 5$ mV. These were used as the definitive operational conditions in SWV measurements of blank, standard solutions and samples, respectively.

SWV measurements of the standard solutions in nine concentration levels ranging from 1.54 to 15.3 $\mu\text{g mL}^{-1}$ are presented in Figure 6a. The current intensity of each SWV measurement was used for the construction of the analytical curve, as shown in Figure 6b.

Analytical curve and figures of merit

SWV measurements at nine concentration levels of coumarin solutions in three authentic replicates were carried out under the best pH and operational parameters (described in "Influence of pH" and "Optimization of SWV parameters" sub-sections, respectively). Before the construction of the analytical curve, the homoscedasticity of data was evaluated using the Cochran's C test. The calculated C value was 0.371 against 0.478 from critical C , revealing that a hypothesis of equivalence of the variances of the residuals could not be rejected, at a 95% confidence level. In other words, the analytical responses (current intensity) presented homogeneous variance regardless of the concentration level of the coumarin. Since the obtained data are homoscedastic, the ordinary least squares (OLS) method had to be used in the construction of the analytical curve.²⁵

Using the proposed SWV method, the data fitted by OLS was estimated as $R = 0.702 (\pm 0.010) + 0.730 (\pm 0.001) \cdot C$,

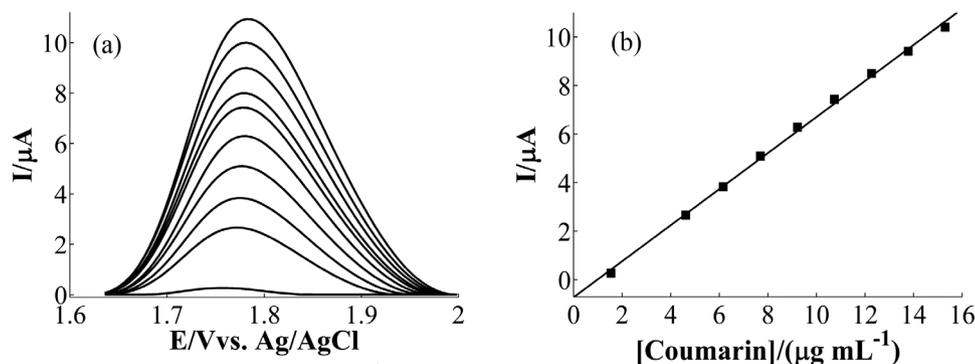


Figure 6. (a) SWV measurements obtained under optimized operational conditions in 0.1 mol L⁻¹ BR buffer, pH 10 corresponding to 1.54, 3.26, 4.98, 6.70, 8.42, 10.1, 11.9, 13.6 and 15.3 μg mL⁻¹ of coumarin; (b) analytical curve.

where R is the analytical response (current intensity in μA) and C is the concentration of coumarin in $\mu\text{g mL}^{-1}$ (Figure 5b). This model ($\hat{y} = \alpha + \beta X$) explained 99.71% of the data variance (determination coefficient (R^2) = 0.9971). The linearity of the model was verified and statistically validated by using analysis of variance (ANOVA). For this, two F -tests were carried out to evaluate the significance of regression and the absence of lack-of-fit, whose values obtained are shown in Table 1. Since the regression of the model was highly significant ($F_{\text{calculated}} (8.58 \times 10^3) > F_{\text{critical}} (4.24)$) and had no lack-of-fit ($F_{\text{calculated}} (2.56) > F_{\text{critical}} (2.58)$) at a 95% confidence level, the analytical curve could be considered validated and ready for an estimation of the coumarin content in samples of commercial guaco infusions. The limits of detection (LOD) and quantification (LOQ) were estimated from International Union of Pure and Applied Chemistry (IUPAC) recommendation by the equations 2 and 3:²⁶

$$\text{LOD} = 3s_b/\beta \quad (2)$$

$$\text{LOD} = 10s_b/\beta \quad (3)$$

where s_b is the standard deviation of the analytical blank and β is the slope of the analytical curve. Thirty independent measurements of the blank were used for this purpose, yielding LOD and LOQ values of 0.20 and 0.62 $\mu\text{g mL}^{-1}$, respectively. These values were similar to those obtained by Miyano *et al.*²¹ also using a BDD electrode but by electrochemical reduction, with LOD and LOQ of 0.22 and 0.66 $\mu\text{g mL}^{-1}$, respectively.

Analytical determinations

The validated SWV analytical curve was applied to the determination of coumarin in guaco leaf infusions of five commercial samples as shown in Table 2.

The results were compared with those obtained by the

Table 1. Analysis of variance (ANOVA) results for the analytical curve in the range from 1.54–15.3 mg L⁻¹ coumarin, considering a confidence level of 95%

Source	Square sum (SS)	Degrees of freedom	Mean square (MS)
Regression	2.59×10^{-10}	1	2.59×10^{-10}
Residuals	7.53×10^{-13}	25	3.01×10^{-14}
Lack-of-fit	3.76×10^{-13}	7	5.37×10^{-14}
Pure error	3.78×10^{-13}	18	2.10×10^{-14}
Total	2.60×10^{-10}	26	–
$MS_{\text{Regression}}/MS_{\text{Residuals}}$	8.58×10^3	$F_{\text{crit}} (1,25)$	4.24
$MS_{\text{Lack-of-fit}}/MS_{\text{Pure Error}}$	2.56	$F_{\text{crit}} (7,18)$	2.58

$MS_{\text{Regression}}/MS_{\text{Residuals}}$ ratio is compared with the point of the F -distribution (with same degrees of freedom and confidence level) to evaluate the significance of regression. $MS_{\text{Lack-of-fit}}/MS_{\text{Pure Error}}$ ratio is compared with the point of the F -distribution (with same degrees of freedom and confidence level) to evaluate the absence of lack of fit.

Table 2. Results of the determination of coumarin in *Mikania glomerata* infusions by using the proposed and reference methods

Sample	Proposed (SWV) / (mg mL ⁻¹)	Reference (HPLC) / (mg mL ⁻¹)	Relative error / %
1	24.7 ± 0.04	25.6 ± 0.60	-3.51
2	8.57 ± 0.01	7.23 ± 0.06	18.53
3	10.2 ± 0.01	12.4 ± 0.11	-17.74
4	25.3 ± 0.05	27.6 ± 0.21	-8.33
5	24.6 ± 0.09	25.6 ± 0.01	-3.91
Overall RSD / %	0.24	0.50	–

SWV: square wave voltammetry; RSD: relative standard deviation.

HPLC reference method.⁹The concentrations of coumarin in the infusions varied from 8.57 to 27.6 $\mu\text{g mL}^{-1}$.

A F -test was applied to confirm whether there was a significant difference between the accuracy of the two methods, with $F_{\text{calculated}} = 4.62 < F_{\text{critical}} = 6.39$. A paired t -test was then performed to confirm that the results obtained were according to the reference values. As the calculated t value was lower than the critical t value

Table 3. Analytical characteristics of the proposed voltammetric determination of coumarin and other electroanalytical methods

	This work	Miyano <i>et al.</i> ²¹	Wang and Liu ²⁸	Krishnan and Saraswathyamma ²⁹
Technique	SWV	SWV	DPV	
Electrode	BDD	BDD	Hg film	pencil graphite electrode (PGE)
Sample	infusions of commercial guaco leaves	infusions of commercial guaco leaves	essential oils and herbal medicines	raw milk
Process	oxidation	reduction	reduction	oxidation
pH	10.0	8.0	8.5	13.0
Linear range / (mg mL ⁻¹)	1.54-15.3	0.73-14.6	not reported	0.29-4.97
LOD / (mg mL ⁻¹)	0.20	0.22	not reported	0.19
LOQ / (mg mL ⁻¹)	0.62	0.66	not reported	0.62
Overall RSD / %	0.24	not reported	not reported	5.01

SWV: square wave voltammetry; DPV: differential pulse; BDD: boron-doped diamond; LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation.

($t_{\text{calculated}} = -1.52 < t_{\text{critical}} = 2.57$), the methods did not differ statistically at a confidence level of 95%.

Table 2 shows that the two samples presented relative error higher than 10%. The accuracy of the proposed method was confirmed by the elliptical joint confidence region (EJCR) test, which estimates the intercept and slope (\hat{a} and \hat{b} , respectively) from the results obtained by the SWV method and compares them with their ideal values ($a = 0$ and $b = 1$, respectively). Since the joint confidence ellipse contained the ideal theoretical point, it confirmed the absence of systematic error, as shown in Figure 7.²⁷

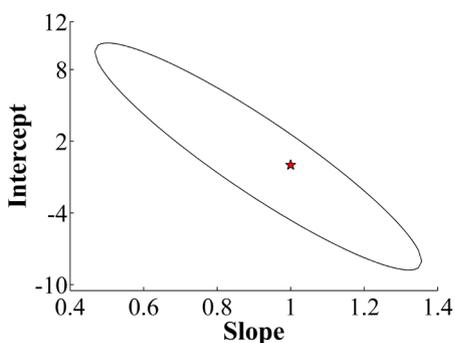


Figure 7. The joint confidence ellipse obtained for the samples of guaco leaf infusions containing the ideal theoretical point (slope = 1 and intercept = 0), indicated with a red star.

Table 3 summarizes the characteristics of the reported electrochemical determination of coumarin in different sample matrices. The analytical performance, the proposed SWV methodology, was comparable with few works described in literature.

Conclusions

The proposed methodology employing SWV and a BDD electrode demonstrated a suitable alternative for the

quantification of coumarin in infusions of *Mikania glomerata* leaves. Coumarin showed a single oxidation peak around +1.77 V, with characteristics of an irreversible process controlled predominantly by diffusion on the electrode surface. In addition, a value of $W_{1/2}$ equal to 72 mV was obtained, indicating that the coumarin reaction mechanism involved the transfer of only one electron. Optimum experimental parameters were obtained in a buffer solution BR, pH 10, with a frequency of 75 Hz, pulse amplitude of 50 mV, and potential increment of 5 mV. The SWV method was linear (validated by ANOVA), accurate, and precise, with LOD and LOQ of 0.20 and 0.62 $\mu\text{g mL}^{-1}$, respectively. The analysis of infusions of commercial guaco leaves showed coumarin contents varying in the range of approximately 8.0-28.0 $\mu\text{g mL}^{-1}$, which have no statistically significant difference from those obtained by the reference method. Moreover, this method was rapid and simple, involving a single sample preparation step by preparing the aqueous infusions as ingested by consumers.

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Author Contributions

Denise M. Nóbrega was responsible for the conceptualization, formal analysis, investigation, visualization and writing original draft; Anabel

S. Lourenço for conceptualization, formal analysis, investigation, visualization and writing original draft; David Douglas S. Fernandes for conceptualization, data curation, formal analysis, investigation, software; visualization and writing original draft; Wellington S. Lyra for data curation, writing-review and editing; Edilene D. T. Moreira for validation and formal analysis; Paulo Henrique G. D. Diniz for conceptualization, methodology, data curation and writing-review and editing; Mario C. U. Araújo for supervision and resources.

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