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SIMULTANEOUS DETERMINATION OF QUERCETIN AND LUTEOLIN IN MATE AND WHITE TEA SAMPLES BY VOLTAMMETRY

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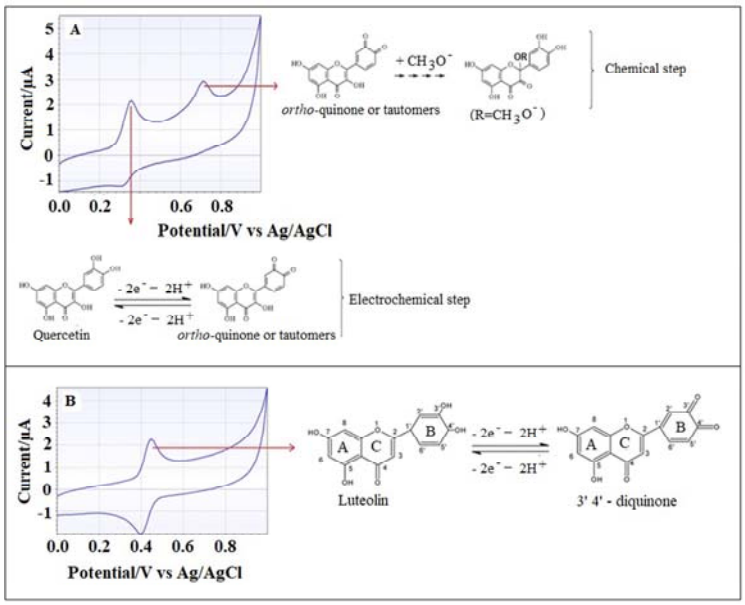
The chemical structures of quercetin and luteolin are quite similar; as a result, their oxidation peaks potential are close and interfere each other. To solve this problem, in this study, the simultaneous determination method of quercetin and luteolin has been developed on glassy carbon electrode (GCE) by using differential pulse voltammetry (DPV). 75% methanol (hydro-alcoholic) support electrolyte was used for this purpose. The peak potential difference between the quercetin and luteolin was at about 110 mV which was useful for the simultaneous electrochemical analysis of both species. The experimental parameters were optimized. Under optimized conditions, linearity was obtained in the ranges of 0.079 - 39.60 × 10^{-7} M and 39.60 - 148.50 × 10^{-7} M for quercetin, and 0.065 - 32.60 × 10^{-7} M and 32.60 - 122.50 × 10^{-7} for luteolin. The detection limits for quercetin and luteolin were 0.022 × 10^{-7} M and 0.018 × 10^{-7} M, respectively. Finally, the present method was employed for the simultaneous determination of quercetin and luteolin in the ethanol and methanol extracts of Mate and White tea samples, and the obtained results were verified by high performance liquid chromatography as a confirmatory method.



INTRODUCTION

Quercetin (Qu) (3,5,7,3′,4′-pentahydroxyflavone) is a member of polyphenol. It is one of the most common flavonoids in the daily diet. Quercetin is known as an effective antioxidant ingredient and possesses different bioactive effects such as anticancer, anti-inflammatory activities and prevention of retinal degeneration.[1-4] Luteolin (Lu) (3′,4′,5,7-tetra- hydroxyflavone) is one of the most abundant occurring flavonoids in variety of vegetables, spices, and medical plants. Luteolin has several pharmacological benefits, such as anticancer, anti-inflammatory, antioxidant and antiviral effects.[5,6] Also, Kwon (2017) reported that for neurodegenerative diseases preventive

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treatment of luteolin might have a positive effect due to its anti-inflammatory activity and other biological functions including antioxidant activity.[7]

Because of Qu and Lu have beneficial effects for human health; the determination of these flavonoids has been an interesting research area for a long time. Various analytical methods have been developed for the determination of Qu and Lu, such as HPLC-UV, HPLC-DAD, HPLC-QTOF-MS, HPLC-DAD-ESI/MS, UFLC-MS/MS, capillary electrophoresis, isocratic LC–DAD–FLD.[8-15] These methods may be highly selective but they require expensive and complex equipment, time for analysis and excessive use of chemicals. Voltammetric techniques and equipment are suitable for fieldwork, because they do not require ponderous items and electroanalytical techniques are low cost and they offer easy operation, high simplicity and short analysis time. Recently, some electrochemical determination methods and electrodes have been developed for Qu and Lu.[16-25] Oxidation peaks of Qu and Lu are overlapped because of their similar structure. Thus, it is very difficult to simultaneous determination of Qu and Lu. In the literature, there was only one study about the simultaneous electrochemical determination of Qu and Lu.[26] Chamizo-Gonzalez, Monago-Marana and Galeano-Diaz (2017) reported DPV and partial least squares.

Qu and Lu are the most common flavonoids in plants, because of this, co-evaluation of them is very important. The aim of this study is to develop a simple and sensitive DPV method for the simultaneous determination of Qu and Lu, for this reason, hydro-alcohol support electrolyte (75% methanol) and non-modified GCE were used for the simultaneous determination of both analytes. Qu and Lu have been determinated successfully in Mate and White tea extract.

RESULTS AND DISCUSSION

1. Electrochemical behaviors of Quercetin and Luteolin

Electrochemical behaviors of Qu and Lu were evaluated by CV. Figure 1A shows the CV curves at GCE for increasing amounts of Qu in pH 5 phosphate buffer (0.1 M). Quercetin had two oxidation peaks and these peaks were clearly seen at +0.32 V and +0.72 V. The oxidation peaks of Qu were rather broad, which indicates a slow electron transfer kinetics. When Lu was added, the rather broad oxidation peak (+0.41 V) of Lu overlapped with the oxidation peak of Qu at +0.32 V and the Qu oxidation peak at +0.72 V decreased and disappeared. Figure 1B shows the CV curves of different concentration of Lu in pH 5 phosphate buffer (0.1 M). The oxidation peak of Lu appeared at +0.41V. By addition of Qu to the support electrolyte, the oxidation peaks of Lu and Qu were overlapped and voltammogram did not show specific peaks for each analyte, making difficult to construct individual calibration curves.

When the electrochemical behaviors of Qu and Lu at different pH values were examined, similar electrochemical behaviors were observed at pH 2 for Qu and Lu. The increasing of pH caused the shift of anodic peak potentials of Qu and Lu towards the less positive potential side and these peaks overlapped (Figure 2). Because, at pH 2 and pH 5, hydroxyl groups of Qu and Lu were active along with catechol hydroxyl group.[16]

However, there is still the overlap problem. To overcome this problem, hydro-alcoholic support electrolyte was used. In pH 5 phosphate buffer (0.1 M, 75% methanol), well discriminated oxidation peaks were obtained for both species as shown in Figure 3. Two oxidation peaks of quercetin were obtained distinctly. The oxidation peaks of Qu became more accentuated with methanol, while the addition of Lu did not affect the oxidation peak of Qu. This result showed that the methanolic medium was suitable for both analyte determinations.

The electrochemical oxidation of quercetin was related with the groups of catechol in B ring and the three hydroxyl groups of A and C rings (Figure 4A). Firstly, the two-OH catechol groups of quercetin B ring were oxidized at low positive potentials and two electrons and protons were transferred.[27] In this experimental conditions, quercetin showed two oxidation peaks at +0.319 V and +0.660 V, respectively. This result was similar to that of Timbola et al. (2006).[28] Timbola et al. (2006) studied in ethanolic medium and explained two oxidation peaks of quercetin. In pH 5 phosphate buffer (0.1 M, 75% methanol), luteolin had one oxidation peak at +0.429 V. Figure 4A. shows the suggestion of Qu oxidation processes. In the first step, which is electrochemical step, quercetin oxidizes to electroactive ortho-quinone or tautomers with electrochemically and in the chemical step, ring C deprotonates. In this study, first oxidation peak at +0.319 V was used for the determination of quercetin.

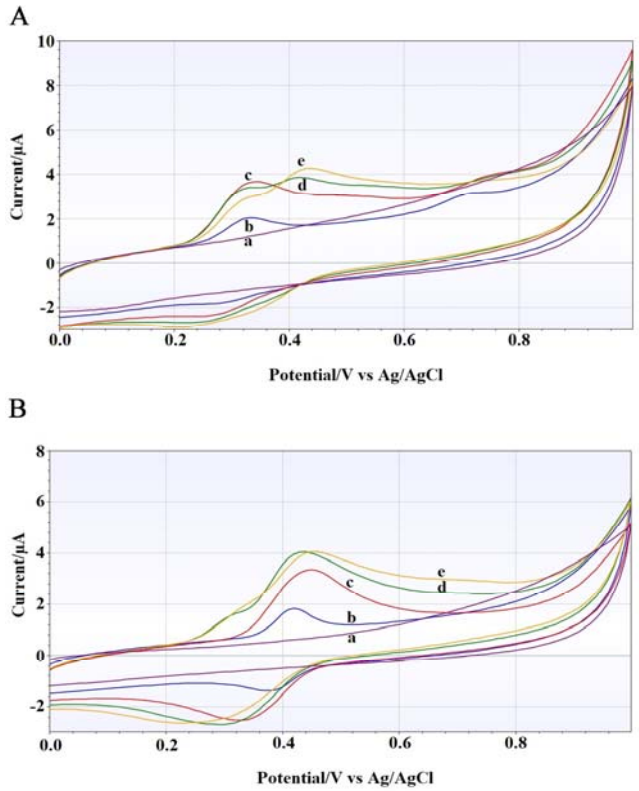

Fig. 1 – (A) Cyclic voltammograms of Qu (b: 1.92×10^{-6} M, c: 3.82×10^{-6} M) and Qu (3.82×10^{-6} M) + Lu (d: 1.92×10^{-6} M, e: 3.82×10^{-6} M) in pH 5 phosphate buffer (0.1 M); (B) Cyclic voltammograms of Lu (b: 1.92×10^{-6} M, c: 3.82×10^{-6} M) and Lu (3.82×10^{-6} M) + Qu (d: 1.92×10^{-6} M, e: 3.82×10^{-6} M) in pH 5 phosphate buffer (0.1 M); a: support electrolyte, scan rate: 100 mV/s.

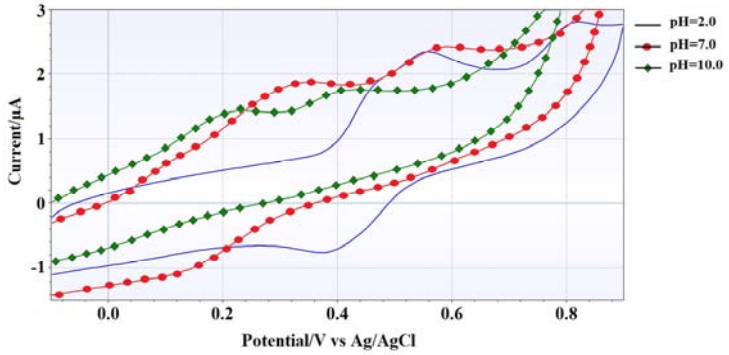

Fig. 2 – Cyclic voltammograms for 1.92×10^{-6} M Qu and Lu on GCE at different pH (pH 2, pH 7 and pH 10); scan rate: 100 mV/s.

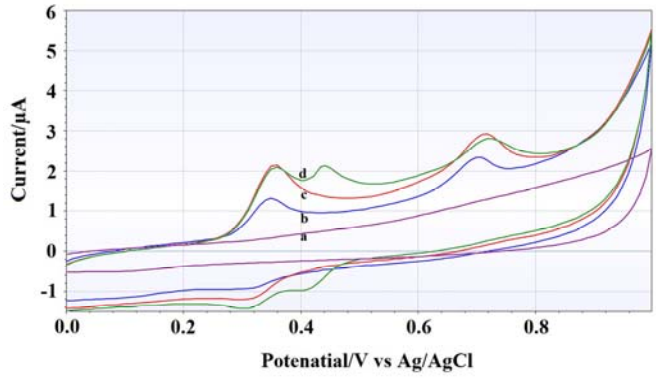

Fig. 3 – Cyclic voltammograms of Qu (b: 1.92×10^{-6} M, c: 3.82×10^{-6} M) and Qu (3.82×10^{-6} M) + Lu (1.92×10^{-6} M) (d) in pH 5 phosphate buffer, support electrolyte (pH 5 phosphate buffer (0.1 M, 75% methanol) (a).

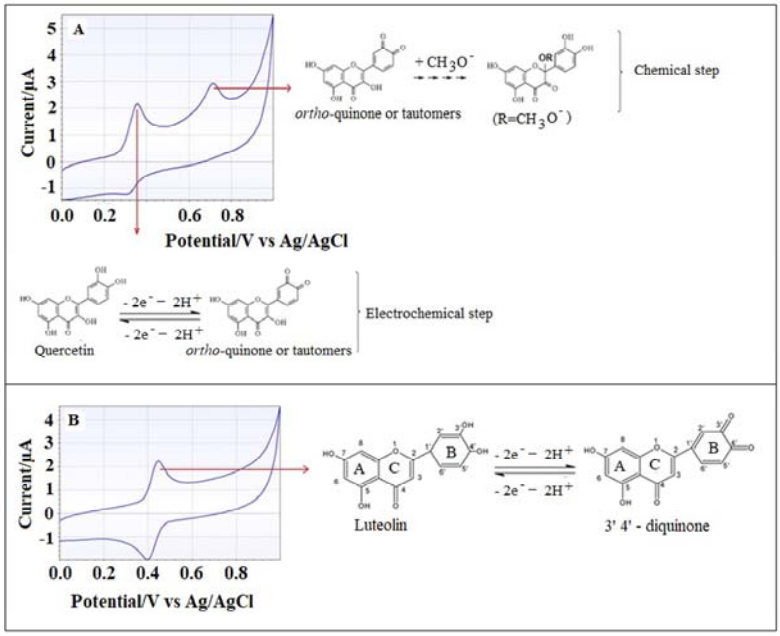

Fig. 4 – Cyclic voltammograms and oxidation reactions of quercetin (A) and luteolin (B), in pH 5 phosphate buffer (0.1 M, 75% methanol) at GCE, concentrations of quercetin and luteolin: 1.92×10^{-6} M, scan rate: 175 mV/s.

The electrooxidation mechanism of luteolin was involved in losing two protons and two electrons to give the final product of 3' 4'-diquinone.[29] A single oxidation peak was at over +0.429 V (Figure 4B).

2. Effect of co-solvent and amount of co-solvent

Ghasemzadeh, Jaafar and Rahmat (2011) reported the methanol has better characteristics as a solvent for phenolic compounds and flavonoids than ethanol, acetone and chloroform because of its high polarity.[30] Therefore, aqueous methanol supporting electrolyte was chosen for simultaneous determination of Qu and Lu. The influence of methanol ratio to Qu and Lu oxidation peak separations and peak current values were studied and this parameter was evaluated at pH 5 phosphate buffer (% methanol: 25% → 75%). When methanol ratio in support electrolyte was decreased, the electrochemical behaviors of Qu and Lu became similar to the behaviors of non-methanol medium. With the aim of securing the separation and current value of peaks, and support electrolyte features, 75% methanol was selected for the current study.

3. Effect of pH

The electrochemical behaviors of Qu and Lu at glassy carbon electrode were affected with the pH of support electrolyte (Table 1). The peak potentials of oxidation were shifted through less potential with the increase of pH value, which was about protons' participation in the electrochemical oxidation processes of Qu and Lu.[18] From pH 2 to pH 12, the highest oxidation current values were obtained at pH 5 phosphate buffer (75% methanol) by CV. The oxidation peak current values of Qu and Lu were closed at pH 2 and pH 5, but the electrochemical oxidation of Qu and Lu at pH 5 showed less potential than pH 2. pH 5 (75% methanol) was chosen for the simultaneous determination of Qu and Lu.

4. Effect of scan rate

Figure 5 shows the cyclic voltammograms of 1.92×10^{-6} M Qu and Lu in pH 5 phosphate buffer (0.1 M, 75% methanol) with scan rates ranging from 25 to 225 mV/s at GCE. The oxidation peak currents of Qu and Lu gradually increased when increasing the scan rate from 25 to 175 mV/s. The linear regression equations of Qu and Lu were expressed as;

$$I_p = 1.182\upsilon - 0.848 \ (R^2 = 0.9994) \qquad (1)$$

and

$$I_p = 1.275\ \upsilon - 0.740 \ \ (R^2 = 0.9938) \qquad (2)$$

respectively, which suggest that the redox process of both analytes at GCE was an adsorption-controlled process.

Table 1

Effect of pH on oxidation peaks potential and current value, each analyte: 1.92×10^{-6} M (methanol: 75%)

Analyte	pH 2		pH 5		pH 7		pH 12	
	E_p (mV)	*Ip* (μA)	E_p (mV)	*Ip* (μA)	E_p (mV)	*Ip* (μA)	E_p (mV)	*Ip* (μA)
Quercetin	529	1.34	319	1.38	139	1.20	-190	0.42
Luteolin	619	1.43	429	1.48	269	0.97	99	0.17

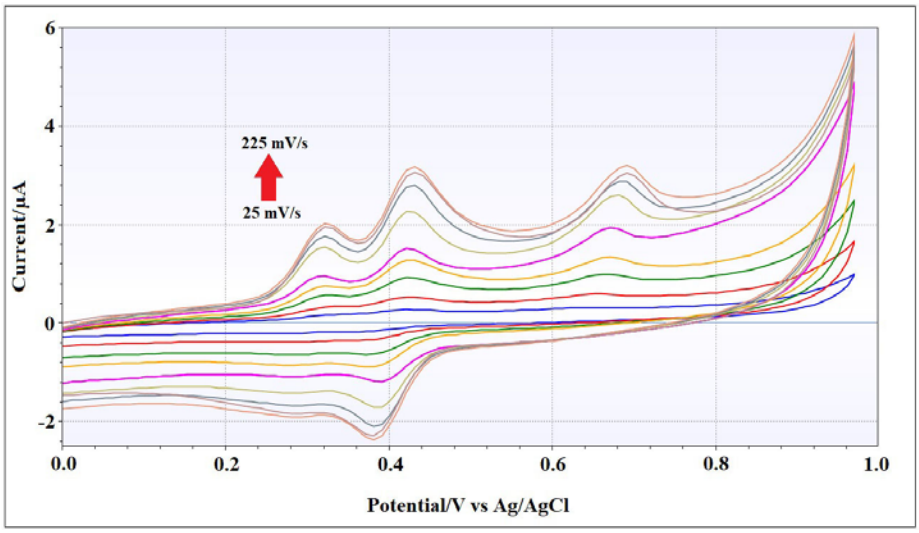

Fig. 5 – CV voltammograms of Qu and Lu with different scan rate at GCE in pH 5 phosphate buffer (0.1 M, 75% methanol). The scan rates were as follows: 25, 50, 75, 100, 125, 150, 175, 200, 225 mV/s, each analyte concentration: 1.92×10^{-6} M.

It can be seen from Figure 5 that the oxidation peak currents of Qu and Lu increased with an increase of scan rate until it reached 175 mV/s; therefore, 175 mv/s of scan rate was selected as the optimum scan rate in the subsequent process.

5. Selective determinations of Qu and Lu in the presence of fixed concentrations of Lu and Qu

Figure 6A shows the differential puls voltammogram obtained from the different concentrations of Lu in the presence of 2.49×10^{-7} M Qu. The oxidation peaks did not shift during the addition of Lu. The oxidation peak current of Lu was increased linearly (R^2: 0.9976 and the linear regression equation: $I_p = 0.4153C + 0.5808$).

Similarly, the oxidation peak of Qu was evaluated at the fixed concentration of Lu. As shown in Figure 6B, the oxidation potential of Qu did not shift to any potential and the peak current value increased linearly with the increasing concentration of Qu (R^2 = 0.9982 and the linear regression equation: $Ip = 0.3633C + 1.2212$). In all of the studies, the voltammetric peak currents of fixed species remained the same.

6. Analytical application

Two well-separated oxidation peaks at about +0.319 V and +0.429 V were obtained for simultaneous determination of Qu and Lu, respectively. The peak current was linearly proportional to the concentration of Qu ranging from 0.0792 to 39.60×10^{-7} M and 39.60 to 148.50×10^{-7} M (the first linear regression equation: $Ip_1 = 4.5235C - 0.7364$ (R^2 = 0.9994) with a sensitivity of 63.71 μA μM^{-1} cm^{-2} and the second linear regression equation: $Ip_2 = 2.2126C + 91.732$ (R^2 = 0.9989) with a sensitivity of 31.16 μA μM^{-1} cm^{-2}) and, the calibration plot displayed a good linear relationship between the peak currents and the concentrations of Lu in the range from 0.0652 to

32.60 × 10^{-7} M and 32.60 to 122.50 × 10^{-7} M (the first linear regression equation: Ip_1 = 6.3996C + 1.3851 (R^2 = 0.9989) with a sensitivity of 91.13 µA $µM^{-1}$ cm^{-2} and the second linear regression equation: Ip_2 = 3.7447C + 84.087 (R^2 = 0.9996) with a sensitivity of 52.74 µA $µM^{-1}$ cm^{-2}) (Figure 7A - 7C). Two slopes were observed. The reason for these two different regions was that high concentrations of Qu and Lu caused saturation in the interface and less signals at the current value.[31]

The detection limits were estimated to be 0.0220 × 10^{-7} M (S/N=3) for Qu and 0.0182 × 10^{-7} M (S/N=3) for Lu, which were comparable with the values reported by the other researchers (Table 2).

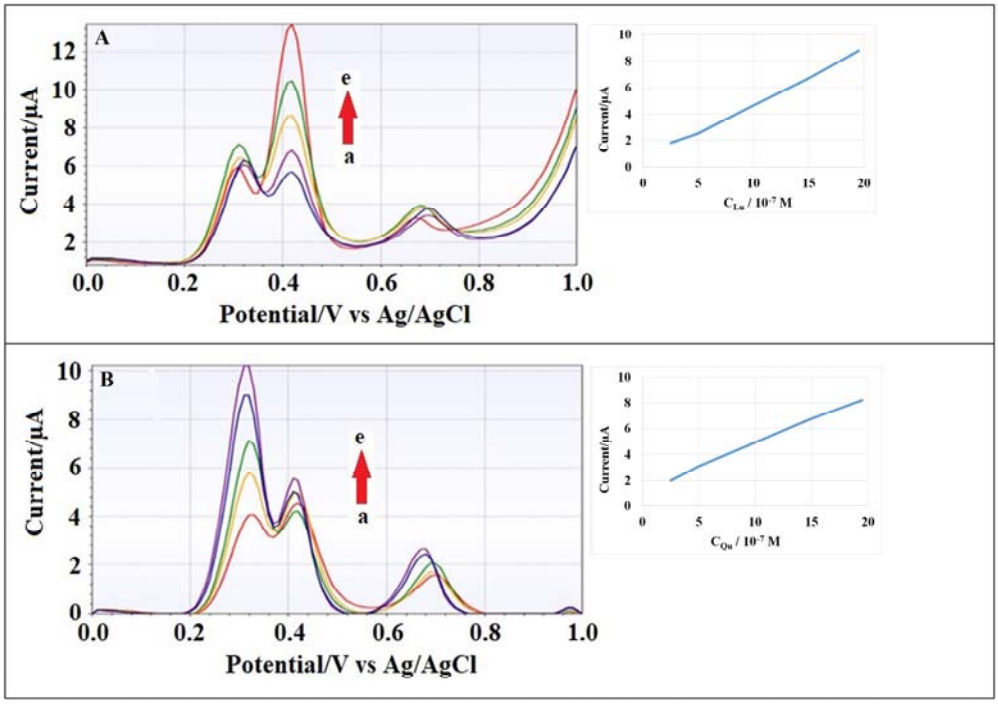

Fig. 6 – (A) Differential puls voltammograms of Lu in the presence of 2.49 × 10^{-7} M Qu at GCE in pH 5 phosphate buffer (0.1 M, 75% methanol), Lu concentration range (a → e): 2.49 × 10^{-7}, 4.95 × 10^{-7}, 9.95 × 10^{-7}, 14.70 × 10^{-7}, 19.51 × 10^{-7} M; (B) differential puls voltammograms of Qu in the presence of 2.49 × 10^{-7} M Lu at GCE in pH 5 phosphate buffer (0.1 M, 75% methanol), Qu concentration range (a → e): 2.49 × 10^{-7}, 4.95 × 10^{-7}, 9.95 × 10^{-7}, 14.70 × 10^{-7}, 19.51 × 10^{-7} M. Pulse amplitude: 50 mV, pulse width: 25 ms.

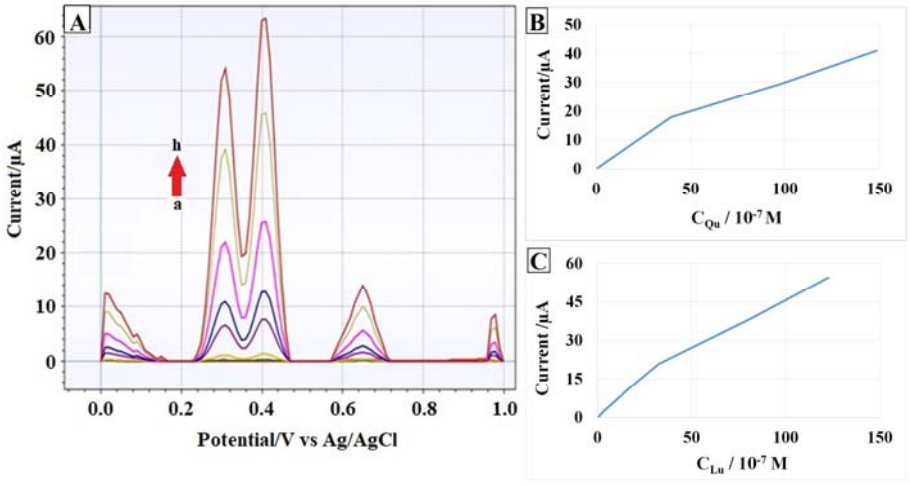

Fig. 7 – DPV curves of quercetin and luteolin at GCE in pH 5 phosphate buffer (0.1 M, 75% methanol), the concentrations of quercetin as follows (from a to h); 0.0792 × 10^{-7}, 1.98 × 10^{-7}, 3.96 × 10^{-7}, 11.88 × 10^{-7}, 19.80 × 10^{-7}, 39.60 × 10^{-7}, 99.00 × 10^{-7}, 148 × 50.10^{-7} M, and luteolin 0.0652 × 10^{-7}, 1.63 × 10^{-7}, 3.26 × 10^{-7}, 9.78 × 10^{-7}, 16.30 × 10^{-7}, 32.60 × 10^{-7}, 81.50 × 10^{-7}, 122.50 × 10^{-7} M (A); The linear relationship between the peak currents and the quercetin (B) and luteolin (C) concentrations. Pulse amplitude: 50 mV, pulse width: 25 ms.

Table 2

Comparison of different researchers for Qu and Lu determination

Electrode material	Linear Range for Qu ($\times 10^{-7}$ M)	LOD for Qu ($\times 10^{-7}$ M)	Linear Range for Lu ($\times 10^{-7}$ M)	LOD for Lu ($\times 10^{-7}$ M)	Sensitivity for Qu (μA/μM × cm^2)	Sensitivity for Lu (μA/μM × cm^2)	Reference
Pt-PDA@SiO2/GCE	0.5 – 3.83	0.16	-	-	48.28	-	16
MIP/MIL-101 (Cr)/MoS_2/GCE	1.0 to 105.0 and 105.0 to 7000	0.6	-	-	1.90		18
Pd/pGN-CNTs	0.1 – 5.0	0.050	-	-	-	-	20
GNs/HA/GCE	-	-	0.20 to 100.0	0.10	-	32.07	21
Nbim/CNT-modified glassy carbon electrode			0.05 – 3.2	0.006	-	-	24
MoS_2/GN-CNTs	-	-	0.40 to 20.0	0.090	-	381	25
GCE	16.5 – 662	16.5	8.7 - 419	14.7	-	-	26
GCE	0.079 - 39.60 and 39.60 - 148.50	0.022	0.065 - 32.60 and 32.60 - 122.50	0.018	63.71	91.00	*This work*

Several modified electrodes were fabricated and successfully used for individual determination of Qu and Lu, only one study reported simultaneous determination of Qu and Lu. In the literature, individual or simultaneously determination of these molecules were carried out after some modification procedure or different calculation method (Table 2).[16,18,20,21,24-26] In this study, the simultaneous determination of Qu and Lu was performed with high sensitivity and wide linear ranges using the unmodified GCE and calibration curve.

Under the optimum conditions, as an example of the analytical performance of the developed method for the simultaneous determination of Qu and Lu, synthetic samples in different concentrations were analyzed by DPV. Linear response of the GCE was observed by studying the increment in the peak currents for the Qu and Lu in the concentration range 2.49 – 19.23 × 10^{-7} M (Figure 8A). Figures 8B and 8C show that the peak currents and the concentrations possessed a good linearity at the optimized conditions. The linear regression equations were:

$$Ip = 3.5541C + 2.568 \ (R^2 = 0.9980) \text{ for Qu} \quad (3)$$

and

$$Ip = 5.8653C + 0.2759 \ (R^2 = 0.9995) \text{ for Lu} \quad (4)$$

The results were evaluated statistically (Table 3). The acceptable recoveries indicate the successful applicability of the developed method for simultaneous determination of Qu and Lu.

In ethanol and methanol extracts of mate and white tea sample, standard addition method was used for the determination of Qu and Lu. The results were listed in Table 4. For comparison, the Qu and Lu contents in extracts were also determined by HPLC (Table 4). The calculation of statistical evaluations showed good consonance between the mean values (t-test) and precision (F-test) for the two methods and the RSD values were less than 1%. In other words, the results prove that the developed method is suitable for the

simultaneous determination of Qu and Lu in real samples with high sensitivity and precision.

7. Interference study

For evaluating the selectivity of the development method, the influence of several interfering agents such as Na^+, K^+, Cl^-, NO_3^-, CO_3^{2-}, glucose, fructose and sucrose and apigenin on the determinations of Qu and Lu at the optimal experimental conditions were investigated (each analyte (Qu and Lu) concentration: 1.92×10^{-5} M). The interference of these common inorganic ions and organic compounds were investigated for the electrochemical analysis of Qu and Lu in previous reports.[16,20,24,32–34] The tolerance limit was calculated as the maximum concentration of the interfering agent which caused an approximately ± 5% decrease in the oxidation peak currents of Qu and Lu or shift in the oxidation potential of Qu and Lu. Na^+, K^+, Cl^-, NO_3^-, CO_3^{2-} did not significantly influence the height of the oxidation peak currents of Qu and Lu. 150 fold of sugars like glucose, fructose, and sucrose and, 10 fold of ascorbic acid did not affect the detections of Qu and Lu. Besides, interference study was performed for the determinations of Qu and Lu in the presence of apigenin. Under the optimized conditions, the oxidation potential of apigenin was obtained at 0.920 V, for this reason, apigenin had no interference effect.

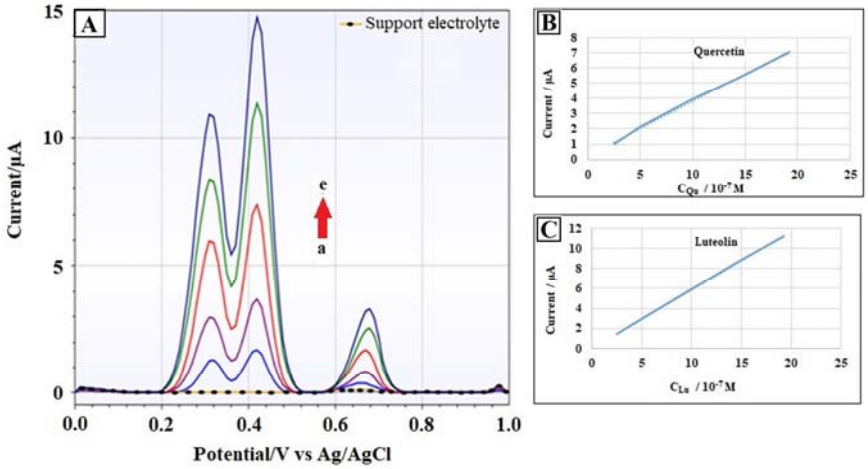

Fig. 8 – Differential puls voltammograms of quercetin and luteolin on GCE in pH 5 phosphate buffer (0.1 M, 75% methanol) (A); (B) and (C) plots of current versus concentration of Qu and Lu. Concentration of each analyte (from a to e): 2.49×10^{-7}, 4.95×10^{-7}, 9.80×10^{-7}, 14.56×10^{-7}, 19.23×10^{-7} M. Pulse amplitude: 50 mV, pulse width: 25 ms.

Table 3

The statistical evaluations of Qu and Lu at different concentrations

No	Concentration of Added ($\times 10^{-7}$ M)		Concentration of Found ($\times 10^{-7}$ M)		Recovery %		RSD %	
	Qu	Lu	Qu	Lu	Qu	Lu	Qu	Lu
1	2.98	2.98	2.93 ± 0.05	2.95 ± 0.02	98.46	99.06	1.64	0.78
2	2.98	5.93	2.94 ± 0.07	5.88 ± 0.05	98.79	99.09	2.31	0.89
3	5.93	17.37	5.97 ± 0.01	17.26 ± 0.02	100.64	99.34	0.20	0.13
4	5.93	2.98	5.99 ± 0.01	2.94 ± 0.03	101.05	98.66	0.13	1.06
5	17.37	5.93	17.26 ± 0.04	5.88 ± 0.01	99.34	99.22	0.22	0.25

*n=5

Table 4

Electrochemical determination results of Qu and Lu in ethanol and methanol extracts

Sample	Extract	Analyte	Detected ($\times 10^{-7}$ M)	Added ($\times 10^{-7}$ M)	Found* ($\times 10^{-7}$ M)	RSD (%)	HPLC result* ($\times 10^{-7}$ M)	RSD (%)	F_{test}	F_{table} (95%)	t_{test}	t_{table} (95%)
Mate tea	Ethanol	Qu	1.03	2.00	3.08	0.49	3.09	0.48	4.00	6.39	0.56	2.78
		Lu	0.88	2.00	2.92	0.48	2.92	0.43	5.06	6.39	0.00	2.78
	Methanol	Qu	1.13	2.00	3.17	0.65	3.19	0.54	4.00	6.39	1.49	2.78
		Lu	0.94	2.00	2.97	0.62	2.98	0.54	2.97	6.39	0.75	2.78
White tea	Ethanol	Qu	0.99	2.00	3.02	0.71	2.99	0.16	2.25	6.39	1.34	2.78
		Lu	0.86	2.00	2.83	0.62	2.87	0.62	5.44	6.39	1.28	2.78
	Methanol	Qu	1.01	2.00	3.03	0.64	3.01	0.64	1.44	6.39	0.75	2.78
		Lu	0.96	2.00	2.92	0.70	2.95	0.50	1.00	6.39	1.34	2.78

* $x = \bar{x} \pm s_x$ for n = 5, s_x denotes standard deviation

EXPERİMENTAL

1. Materials and Methods

1.1. Chemicals

Phosphate buffered saline (PBS) tablets were purchased from OXOID (Hampshire, England), quercetin and luteolin and all chemicals were purchased from Sigma-Aldrich (St. Louis, MO 63103 USA). All of them were used without further purification. Mate and white tea were purchased from a herbalist in Golbası/Ankara. The support electrolyte, pH 5 phosphate buffer (0.1 M), was prepared using PBS tablet in methanol (75%) and the pH was adjusted using 0.1 M H_3PO_4 and 0.1 M NaOH. All chemicals were analytical grade. Double distilled water (18.2 MΩ cm) was used in the preparation of each solution.

1.2. Apparatus

PalmSens3 Potentiostat/Galvanostat (PalmSens BV, Netherlands) was used for cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements with a conventional three-electrode system, consisting of glassy carbon electrode (GCE) (BASi, MF-2012 (3.0 mm dia., West Lafayette, IN 47906 USA), Ag/AgCl reference electrode (BASi, MF-2052, West Lafayette, IN 47906 USA) and platinum wire auxiliary electrode, was used. Agillent 1200 series HPLC system (Agilent Technologies, Inc., Santa Clara, CA 95051 United States) was also used for Qu and Lu chromatographic analysis. Qu and Lu were measured at room temperature on a column of ACE C18 (250 × 4.6 mm) (Advanced Chromatography Technologies Ltd, Aberdeen, Scotland). The HPLC mobile phase consisted of water:methanol:acetic acid (75:20:5, *v:v:v %*) (Solvent A) and water:methanol:acetic acid (50:45:5, *v:v:v %*) (Solvent B). The gradient parameters were used as 0% B at 0 min, 100% at 30 min, 0% at 50 min. The flow rate of 1.0 mL/min was used throughout the experiment. The UV was also adjusted at 309 nm for detection.[35] A bare GCE was mechanically polished with 0.3 μm and 0.05 μm alumina slurries on a microcloth pad. Polished GCE was sonicated in ultra-pure water and then with methanol for 10 min. After these procedures, GCE was washed with ultra-pure water.

1.3. Analytical procedure

The necessary volume of standard or sample solution of Qu and Lu were pipetted to the electrochemical cell which was placed in 5 mL of pH 5 phosphate buffer (0.1 M, 75% methanol). Then the CV and DPV oxidation peaks of Qu and Lu were recorded. The cyclic voltammetry was recorded from 0.0 to over the 1.0 V at a scan rate of 175 mV/s quiet time of 4 s and the DPV was recorded from 0.0 to 1.0 V with amplitude of 50 mV, quiet time of 4 s and pulse width of 25 ms.

1.4. Preparation of extracts

Dried plant materials were powdered and were weighed thirty grams. 300 mL ethanol and methanol were used for extraction. Extraction was carried out with Soxhlet apparatus for 6 h. The extract productions were concentrated under vacuum at 80 ^oC by using a rotary evaporator and before analysis; extracts in solvent were filtered with 0.20 μm syringe filter and were stored in dark at 4 ^oC. [36]

CONCLUSIONS

In this study, the overlapping of the oxidation peaks of Qu and Lu were shown and a method for their simultaneous determination was developed. For this purpose, optimized conditions such as chemical effects and the instrument parameter were investigated for obtaining the well-separation of peaks and better oxidation current values of Qu and Lu. Methanol exhibited a significant effect for the separation of two analytes oxidation peaks on GCE. This simple and sensitive method was used for the determination of two species in real samples and voltammetric results were compared with HPLC results. Electrochemical and chromatographic results were in compliance. This method has some advantages such as short analysis time, small volume of sample and solvent, portable instrument, and cheap electrode.

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