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Electrochemical Studies of Polyphenols, Anthocyanins, and Flavonoids Extracted from Blueberry Fruit

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This work was carried out for extraction and electrochemical studies of polyphenols, anthocyanins, and flavonoids in blueberry fruit. The conventional extraction (solid–liquid) with hydro-organic solvent system was used to obtain polyphenol compounds extracts from blueberries pomace. The electrochemical and high-performance liquid chromatography (HPLC) techniques were used for identification and determination of the polyphenols, anthocyanins, and flavonoids content in blueberries pomace. For electrochemical analyses, the functionalized CNTs were electrodeposited on glassy carbon electrode (f-CNTs/GCE) and used as a working electrode for study of the polyphenols substances in blueberries pomace. Results of structural and morphological analyses of electrodeposited nanostructure using SEM and XRD demonstrated that f-CNTs on the surface of GCE created a net-like film with porous reticular structure that can facilitate the electron transfer rate. Both electrochemical and HPLC methods showed that quercetin, rutin, delphinidin, cyanidin and gallic acid were observed in extracted polyphenolic compounds. The sensing properties of f-CNTs/GCE as a sensor of polyphenolic compounds were determined by DPV measurements and compared with the HPLC sensing properties in this work and other reported sensors in literature. Results of analytical studies indicated good ranges of recovery (97.05% to 99.10%) for DPV technique and (95.68% to 98.55%) for HPLC technique, and acceptable ranges of RSD (2.12% to 3.20%) for DPV technique and (2.78% to 4.29%) for HPLC technique. The better results for DPV technique using f-CNTs/GCE was implied to appropriate accuracy of proposed polyphenolic compounds sensor for analyses of fruit and food samples.

Keywords: Quercetin; Rutin; Delphinidin; Cyanidin; Gallic acid; Blueberry fruit; Functionalized CNTs; HPLC; Differential pulse voltammetry

1. INTRODUCTION

Today, one of the principle recommendations dietary guidelines is based on consuming foods rich in phytochemicals such as polyphenols, curcumin, carotenoids, flavonoid, anthocyanins, alkaloids, isothiocyanates, glycosides, phenolic acids, saponins, isoflavones and terpene [1]. The phytochemicals as bioactive non-nutrient plant compounds promote health benefits and are able to prevent the onset of chronic and degenerative disorders, such as cancer, osteoarthritis, osteoporosis, and Alzheimer disease [2]. These compounds are found in plant-based foods such as fruits, substance getable, whole grains, spices, nuts, seeds and legumes, and give plants their color, flavor, smell, and texture [3].

Blueberry and its products have high concentrations of bioactive compounds such as flavonoids and anthocyanins. The blue color of blueberry can indicate some phytochemicals substances which can promote good health and lower disease risk [4, 5]. For several decades, extraction of anthocyanin, flavonoids, and phenolics content of blueberry have been investigated with different methods, and many studies have been performed to characterize the enzymatic activity, antioxidant capacity and physicochemical properties of blueberry [6, 7].

Between these phytochemicals, gallic acid (3,4,5-trihydroxybenzoic acid) as a natural antioxidant in blueberries is basically a secondary polyphenolic metabolite [8]. Moreover, anti-inflammatory, and antineoplastic properties also have been reported as beneficial effects of gallic acid which have therapeutic activities in gastrointestinal, neuropsychological, metabolic, and cardiovascular disorders [9, 10]. Delphinidin (2-(3,4,5-trihydroxyphenyl)chromenylium-3,5,7-triol) is an anthocyanidin abundantly identified in blueberry that it is characterized by interesting antioxidant and can be useful for the prevention of RANKL-mediated bone loss such as postmenopausal osteoporosis [11]. Rutin (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one) is another polyphenol which predominant flavonol in blueberry fruits. It has been used in alternative medicine as an antioxidant to treat osteoarthritis and other inflammatory conditions, to support blood circulation and a healthy heart, and enhance the action of vitamin C [12, 13]. Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one) is widely distributed in blueberry fruits. It improves mental/physical performance and has a variety of health benefits due to its antioxidant properties [14]. Cyanidin (2-(3,4-dihydroxyphenyl)chromenylium-3,5,7-triol) is another polyphenol in blueberry fruits that its consumption can reduce risk of arthritis, cancer and diabetes [15].

Therefore, extraction and identification of polyphenolic compounds in fruit is important and many researches have been conducted on determination of polyphenols, anthocyanins, and flavonoids in fruit extracts through UV–VIS–NIR spectroscopy and chemometrics [16],mid-infrared spectroscopy [17], NMR spectroscopy [18], spectrophotometric, chromatographic electrochemical methods [19-21]. Among these methods, electrochemical techniques show favorable sensing properties because of modification of the sensor surface with nanostructures and a wide range of chemical composites [22]. Few studies have been performed for simultaneous extraction and electrochemical determination of polyphenols, anthocyanins, and flavonoids from fruits [20, 23, 24], and the comparison between the HPLC and electrochemical techniques have not been perfectly studied. Hence, this work was carried

out for extraction and electrochemical studies of polyphenols, anthocyanins, and flavonoids in blueberry fruit using the HPLC and electrochemical techniques.

2. MATERIALS AND METHOD

2.1 Extraction

The fresh blueberries were provided from a local market. After juice production, the resulting blueberry pomace was Lyophilisated (Alpha LSCplus, Christ, Osterode am Harz, Germany) and freeze-dried at a temperature range from −75 to 20 °C under 0.175 mbar. Subsequently, the freeze-dried blueberries pomace was pulverised in a laboratory mill (CM 290 Cemotec™, Gerber Instruments AG, ZÜRICH) and stored in amber bottles in the refrigerator at 4 °C until electrochemical analyses (7–10 days).

The conventional extraction (solid–liquid) with hydro-organic solvent system was used to obtain polyphenol compounds extracts from blueberries pomace [25]. First, 10g of prepared samples were passed through with ∼10g of macroporous resin column (Diaion$^{@}$ HP-20, Tokyo, Japan) a flow rate of 4 mL/min. in next step, the resin column was washed with of deionized water, subsequently, the polyphenols were desorbed with 50 ml ethanol (>99%, IndiaMART, India) as solvent with a flow rate of 4 ml/min under applying nitrogen at dynamic pressure of 10 atm. Then, the resin column was washed with deionized water, and regenerated with equal volume ratio of 2M HCl (37%, Merck, Germany) and 1M NaOH (99%, Merck, Germany). The extracts were frozen at -10 °C until electrochemical analyses.

2.2 HPLC detector

An HPLC (Model 1100, Agilent Technologies, Waldbronn, Germany) equipped with a photodiode array detector (PDA, M30A, Shimadzu Nexera X2, USA), 20 µL stainless steel sample loop for Rheodyne Injector (models 7125/7010, PerkinElmer, USA) and a quaternary pump (PU-4180, Jasco Inc., USA) were utilized for the separation and identification of polyphenolic compounds in blueberries pomace extracts. The separation process was conducted on a C18 column (Kinetex, 150 × 4.5 mm, 2.6 µm) at 45°C. The gradient mobile phase system was consisted of acetonitrile (A; 99.8%, Sigma-Aldrich), and 1mM phosphoric acid (B, 99%, Sigma-Aldrich) as HPLC-grade solvents which were used as follows: 15 minutes, linear gradient from 95% A to 85%; 10 minutes, linear gradient from 85 to 65%; 10 minutes, linear gradient from 65 to 45%; 10 minutes, linear gradient from 45 to 0%; 10 minutes isocratic. The post time of 10 minutes was applied for the column equilibration. The injection volume was 10 µL at flow rate of 1 ml/min. Data was collected in the range from 190 to 600 nm. The identity of compounds was established by comparison of retention times and UV spectra with the corresponding standards. Anthocyanins (cyanidin and delphinidin), flavonoids (quercitrin and rutin) and gallic acid were monitored using PDA with a wavelength range of 210 to 800 nm. Gallic acid absorbed at 271 nm, anthocyanins absorbed at 520 nm, and rutin and quercetin absorbed at 206 and 260 nm, respectively. Quantitative analysis of polyphenolic compounds was carried out on an external standard method. Quantitative analysis was performed using the calibration curves which

were constructed with 6 points for each phenolic compound, using the internal standard method and reference compound solutions prepared in methanol at mean working concentration of 0.05 g/l.

2.3 Electrochemical analyses

Differential pulse voltammetry (DPV) analyses and electrodeposition were performed using a potentiostat/galvanostat electrochemical workstation (Model No. CHI660, USA) at scan rate of 10 mV/s in conventional three-electrode cell, containing bare or modified GCE as working electrode, Ag|AgCl|KCl (3M) as reference electrode and platinum plate as auxiliary electrode. 0.1 M phosphate buffer solution (PBS) (pH 7.0) was utilized as electrolyte in electrochemical investigations which prepared by equal volumes of 0.1M Na_2HPO_4 (99%, Sigma-Aldrich) and 0.1M NaH_2PO_4 (99%, Sigma-Aldrich).

For modification of the working electrode, the f-CNTs were electrodeposited on GCE surface. In order to prepare the f-CNTs, the CNTs (≥90%, Shandong Gelon Lib Co., Ltd., China) was functionalized with acid treatment in sulfuric/nitric acid (≥99%, Sigma-Aldrich) mixture in volume ratio of 3:1 at 70 °C for 4 hours [26]. Then, f-CNTs were filtered and washed with deionized water until the pH reached 7. After that, f-CNTs were dispersed in ethanol and dried at room temperature. Before the electrodeposition, GCE surface was polished to a mirror-like finish using alumina powders (0.3 and 0.05 μm, (99%, Sigma-Aldrich) and then sonicated in water and ethanol for 10 minutes, respectively. The electrodeposition of f-CNTs on the surface of GCE was performed in 2.0 g/l f-CNTs dispersed in 0.1M PBS (pH 7.0) at a potential range of -1.2 V to +0.6 V at a scan rate of 10 mV/s for 30 cycles. For analyses of the extracted polyphenols, the prepared extracts were added to 0.1M PBS (pH 7.0) in equal volume ratio. The quercetin, rutin, delphinidin, cyanidin and gallic acid were purchased from Sigma-Aldrich.

2.4 Structural and morphological *analyses*

The structural and morphological characterizations of electrodeposited f-CNTs were performed using X-ray diffractometer (XRD, D8 Advance model, Bruker, Germany) and field emission scanning electron microscopy (FESEM), respectively.

3. RESULTS AND DISCUSSION

3.1 Structural and morphological analyses of electrodeposited f-CNTs

Figure 1a displays the SEM image of f-CNTs/GCE which indicates the homogeneously electrodeposition of f-CNTs on the surface of GCE that creates a net-like film with porous reticular structure that can facilitate the electron transfer rate [27-29]. Figure 1b shows the XRD pattern of powder of electrodeposited f-CNTs on GCE surface. As shown in Figure 1b, two diffraction peaks are observed at 25.93° and 43.01° which arise from the graphitic structure of carbon (00-058-163) [30,

31]. The strong peak at 25.93° is attributed to the (002) plane and characteristic of graphitic materials and related to the distance between the concentric graphene sheets of f-CNTs [32]. The weak peak at 43.01° is corresponding to the (100) plane of f-CNTs.

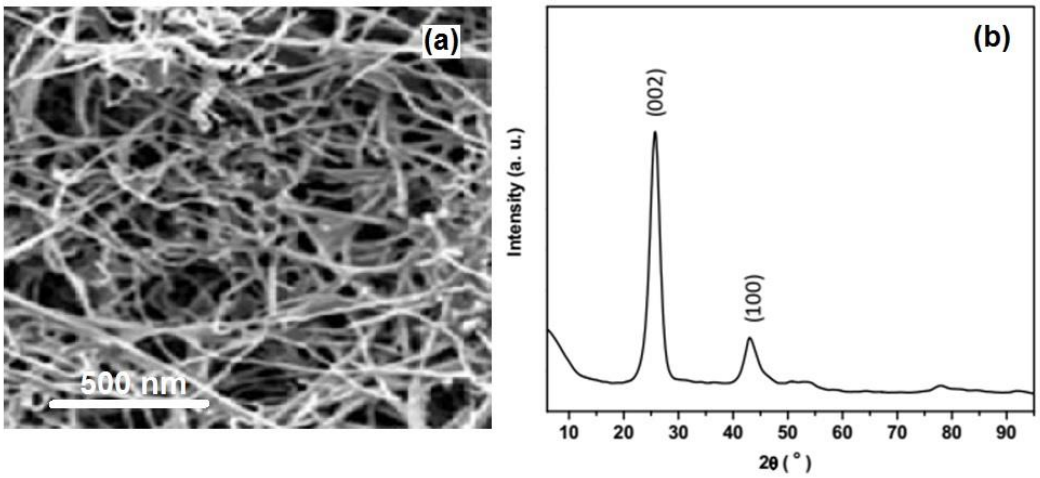

Figure 1. (a) The SEM images of f-CNTs/GCE and (b) XRD patterns of powders of electrodeposited f-CNTs.

3.2 Electrochemical analyses

Figures 2a and 2b depict the DPV responses of GCE and f-CNTs/GCE, respectively in 0.1 M PBS (pH=7.0) at a scan rate of 10mV/s. As observed, there is not any redox peak for both electrodes. Figures 2a' and 2b' show the DPV responses of GCE and f-CNTs/GCE, respectively in 0.1M PBS containing 10g/l extracted polyphenolic compounds (EPCs) at a scan rate of 10mV/s. It may be observed that both electrodes show five peaks at 0.095 V, 0.19V, 0.27V, 0.34V and 0.43V, which are related to the electrochemical behavior of polyphenolic compounds [33]. polyphenols contain several hydroxyl groups which are attached to the aromatic ring structures and its functional OH groups undergo electrochemical oxidation [20]. Moreover, the comparison between DPV responses of GCE and f-CNTs/GCE reveals that f-CNTs modified electrode shows the higher currents which related to high electrochemical performance of f-CNTs because of high conductive and porous CNTs network film [34-37].

Figure 3 shows the suggested mechanism for oxidation of the phenolic and polyphenolic compounds [38]. Polyphenolic compounds show a great structural diversity and their oxidation occurs at the same electroactive groups, predominantly occurring at the phenol moiety. The main difference in oxidation potential of compounds is related to the influence of the non-electroactive substituents and depends on its stability which causes to oxidation at less positive potentials, and a greater potential shift occurs when the substituents are linked at the ortho- and para-positions. The phenolic -OH moiety undergoes anodic oxidation that this reaction depended on the stability of the electrogenerated phenoxy radical and phenol and phenolic substituents. The phenol oxidation take place with the transfer of one electron and one proton (reaction A, Figure 3), and phenol is oxidized, in one-electron, one-proton

irreversible step to a phenoxy radical which is thermodynamically unstable and coexists in resonant forms of ortho-position (reaction B, Figure 3) and para- positions (reaction C, Figure 3), being stabilized by hydrolysis. It leads to formation of two electroactive products, ortho-quinone and para-quinone. The presence of an additional electroactive -OH group at the ortho- and para-positions leads to a two-electron and two-proton reversible process.

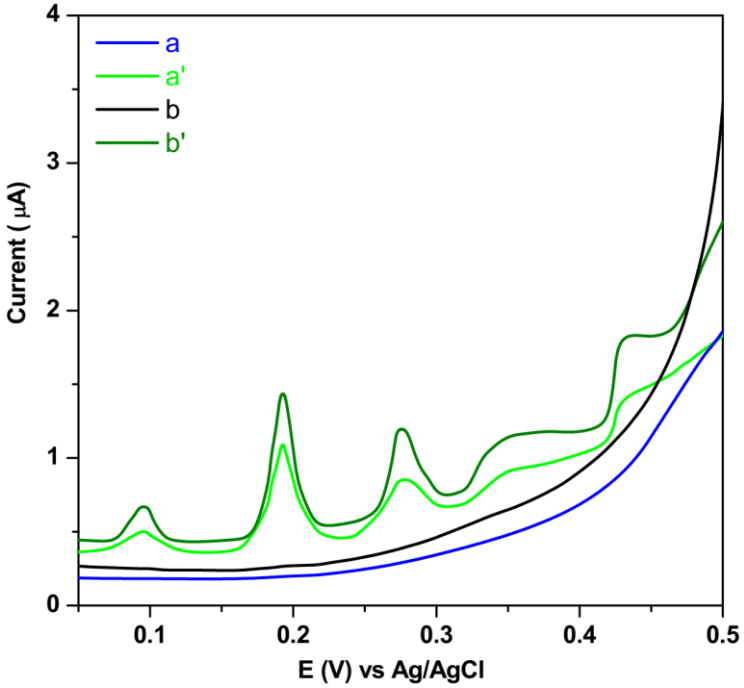

Figure 2. DPV responses of (a and a') GCE and (b and b') f-CNTs/GCE in 0.1 M PBS pH 7.0 at scan rate of 10 mV/s without (a and b) and with (a' and b') 10 g/l EPCs.

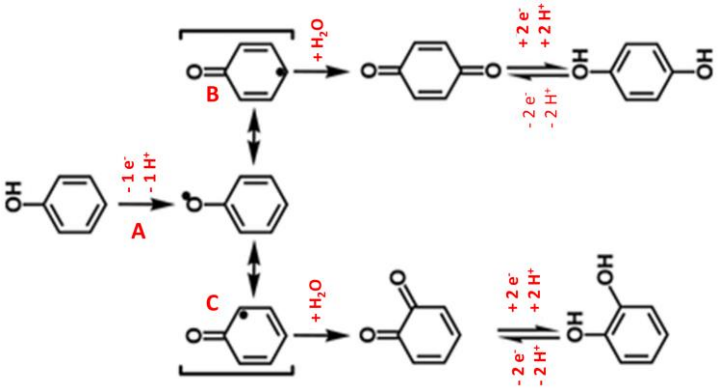

Figure 3. The suggested mechanism for oxidation the phenolic and polyphenolic compounds [38].

The stability of electrochemical responses of GCE and f-CNTs/GCE was investigated under successive DPV in 0.1 M PBS pH 7.0 containing EPCs at the scan rate of 10 mV/s. Figure 4 depicts the first and 40th DPV responses of GCE and f-CNTs/GCE in presence of 10 g/l EPCs that it is

demonstrated to 15% and 6% change, respectively, evidence to high stability of electrochemical answer of f-CNTs/GCE toward polyphenolic compounds. It is attributed to great mechanical flexibility and high chemical stability of CNTs due to highest specific modulus and highest specific strength which resulted from the strong covalent C–C bonds in electrodeposited CNTs [39]. Therefore, f-CNTs/GCE was used for following electrochemical studies due to its higher sensitivity and stability.

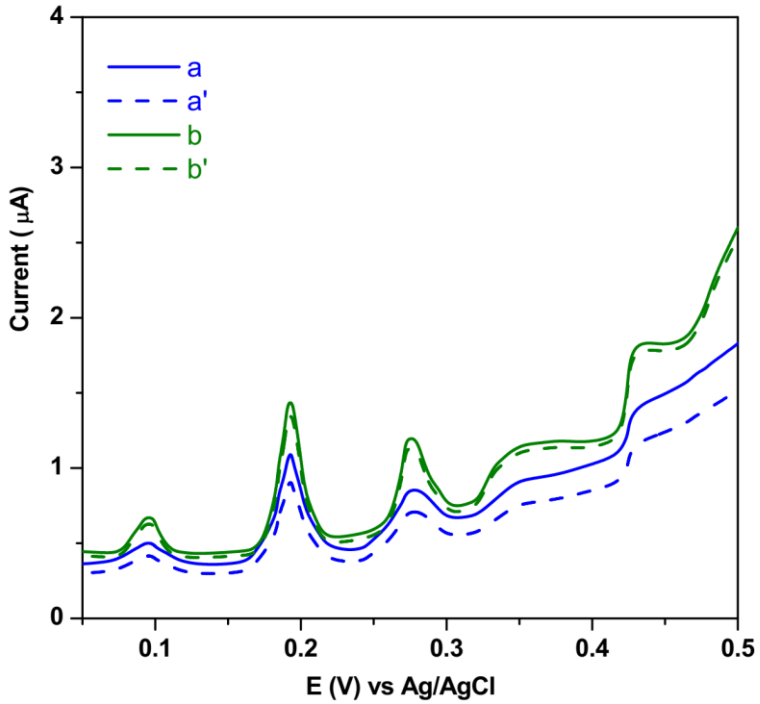

Figure 4. First (solid line) and 40th (dashed line) DPV responses of (a and a') GCE and (b and b') f-CNTs/GCE in 0.1M PBS containing 10 g/l EPCs at a scan rate of 10mV/s.

Figure 5 shows the concentration effect of EPCs, and quercetin, rutin, delphinidin, cyanidin and gallic acid on DPV response of f-CNTs/GCE in 0.1 M PBS pH 7.0 containing 10 g/l EPCs at scan rate of 10mV/s. As observed from Figure 5a, the peak current of five peaks are increased with increasing the EPCs concentration in electrochemical cells. Figure 5b shows with successive injections of gallic acid in electrochemical cell, the oxidation peak (I) current at 0.095 V also is linearly increased, and other peak in DPV curve do not change which indicted to the oxidation peak current at 0.095 V is belonging to gallic acid. Further studies were conducted on successive injections of delphinidin (Figure 5c, peak II), rutin (Figure 5d, peak III), quercetin (Figure 5e, peak IV), and cyanidin (Figure 5f, peak V), and DPV response of f-CNTs/GCE exhibit as delphinidin, rutin, quercitrin, and cyanidin content increase, the oxidation peak current at 0.19V, 0.27V, 0.34V and 0.43V are linearly increased, respectively that these results in agreement with the electrochemical reports on polyphenolic compounds in [33, 40-43]. Figure 6 shows the obtained calibration plots of DPV measurements for determination of linear range, sensitivity and limits of detection (LOD) of polyphenolic compounds that the obtained calibration plots indicates that the sensitivity of f-CNTs/GCE to determination of cyanidin, quercetin, rutin, delphinidin and gallic acid are 0.0165, 0.0710, 0.0462, 0.0219 and 0.0482

$\mu A/\mu gl^{-1}$, respectively. The linear range and limits of detection (LOD) of the proposed sensor for determination of cyanidin, quercetin, rutin, delphinidin and gallic acid are presented in Table 1.

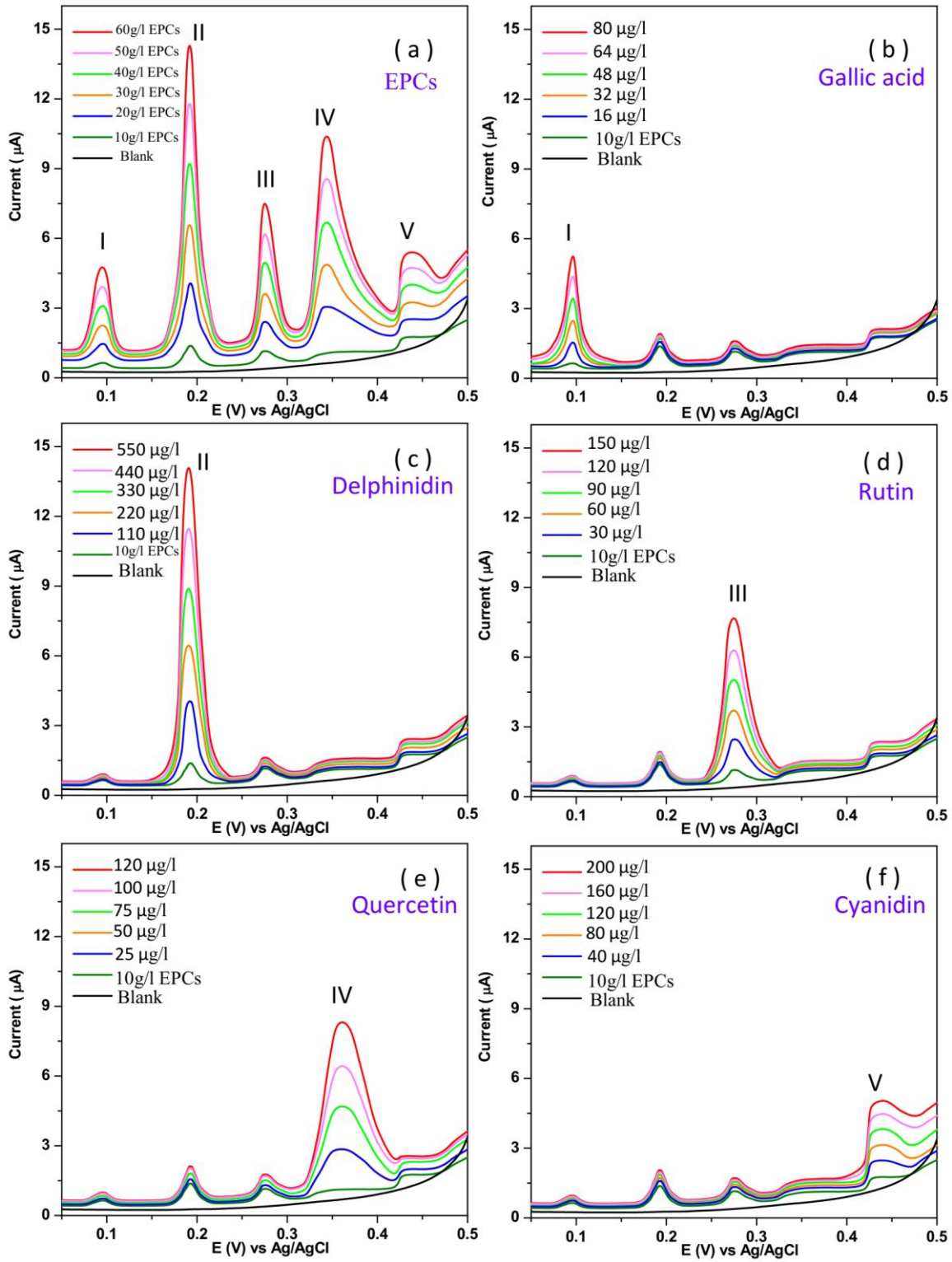

Figure 5. The DPV curves of f-CNTs/GCE for successive injections of (a) EPCs, (b) gallic acid, (c) delphinidin, (d) rutin, (e) quercetin and (f) cyanidin in 0.1 M PBS pH 7.0 containing 10g/l EPCs at scan rate of 10 mV/s.

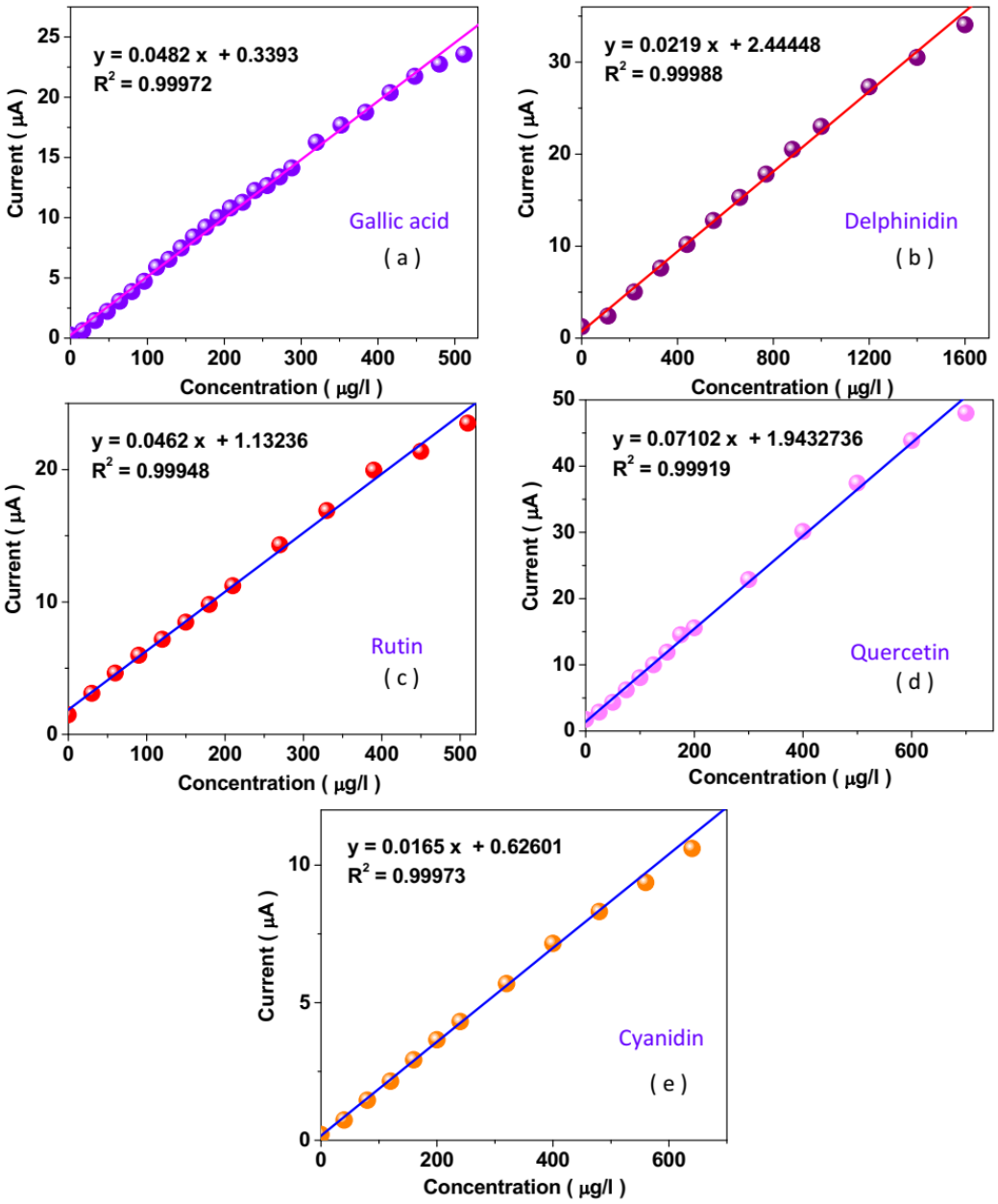

Figure 6. The obtained calibration plot of f-CNTs/GCE in 0.1M PBS containing 10g/l EPCs at 10mV/s scan rate to successive addition of (a) EPCs, (b) gallic acid, (c) delphinidin, (d) rutin, (e) quercetin and (f) cyanidin.

Furthermore, the obtained sensing properties of f-CNTs/GCE are compared by reported electrochemical sensors of polyphenolic compounds in Table 1 that it reveals the comparable or better performance of f-CNTs/GCE than other polyphenolic compounds sensors. It can be related to acid treatment of CNTs that it enhances the covalent functionalization consisting in attaching organic functionalities as free radicals and phenolic compounds exploiting the chemistry of oxygen groups [39]. Moreover, functionalization of CNTs leads to overcoming hydrophobic properties [44].

Table 1. Comparison between obtained sensing properties of f-CNTs/GCE and other reported electrochemical sensors for determination of cyanidin, quercetin, rutin, delphinidin and gallic acid.

Electrode	**Phenolic compound**	**Technique**	**Linear Range (µg/l)**	**LOD (µg/l)**	**Ref.**
f-CNTs/GCE	Cyanidin	DPV	10 to 560	0.18	This work
f-CNTs/GCE	Quercetin	DPV	10 to 600	0.04	This work
f-CNTs/GCE	Rutin	DPV	10 to 460	0.06	This work
f-CNTs/GCE	Delphinidin	DPV	100 to 1400	0.13	This work
f-CNTs/GCE	Gallic Acid	DPV	10 to 480	0.06	This work
GCE	Cyanidin	RP-HPLC-ED	-	21.4×10^{-6}	[20]
GCE	Cyanidin	RP-HPLC-ED	28.7 to 861.7	28.7	[45]
Gromsil ODS-4 HE column	Cyanidin	HPLC-CD	-	14.36	[21]
GCE	Quercetin	RP-HPLC-ED	-	44.2×10^{-6}	[20]
Carbon disc electrode	Quercetin	CE-ED	151.1 to 302236	68	[46]
Molecularly imprinted polymer based on polypyrrole /rGO	Quercetin	DPV	181.3 to 4533.5	14.50	[43]
GCE	Rutin	RP-HPLC-ED	-	62.1×10^{-6}	[20]
carbon disc electrode	Rutin	CE-ED	4579 to 610520	265	[46]
Rigid carbon-polyurethane composite	Rutin	SWV	671 to 1892	4.3	[47]
$NiCo_2O_4$/rGO/GCE	Rutin	CV	61 to 91577	6.1	[48]
GCE	Delphinidin	RP-HPLC-ED	90 to 900	90	[45]
GCE	Delphinidin	RP-HPLC-ED	-	52.8×10^{-6}	[20]
ZrO_2/Co_3O_4/rGO/fluorine doped tin oxide	Gallic acid	DPV	1.02 to 81.14	0.26	[49]
rGO/GCE	Gallic acid	SWV	1360 to 68048	71.45	[50]
Hanging mercury drop electrode	Gallic acid	AdCSV	0.1 to 600	0.05	[51]

RP-HPLC-ED: Reverse-phase high-performance liquid chromatography with electrochemical detection; HPLC-CD: Gradient HPLC system with coulometric detection; CE-ED: Capillary electrophoresis with electrochemical detection; SWV: Square wave voltammetry; AdCSV: Cathodic adsorptive stripping voltammetry.

The interference effect and selectivity of response of f-CNTs/GCE was studied in present of some polyphenolic compounds such as p-hydroxybenzoic acid, caffeic acid, chlorogenic acid,

kaempferol, hyperoside, isoquercitrin, catechin and phloridzin as interferents. Table 2 displays the resulted electrochemical current using DPV technique on f-CNTs/GCE in 0.1M PBS at 0.43V, 0.34V, 0.27V, 0.19V and 0.095V for addition of 100 µg/l of cyanidin, quercetin, rutin, delphinidin and gallic acid, and successive additions of 200 µg/l of interferents. As seen, the proposed electrode illustrates a clear signal to injections of cyanidin at 0.43V, quercetin at 0.34V, rutin at 0.27V, delphinidin at 0.19V and gallic acid at 0.095V, and there are not remarkable signal for injections of interferents. The results prove that the presented interference in Table 2 don't interfere with DPV determination of cyanidin, quercetin, rutin, delphinidin and gallic acid at potential of 0.43V, 0.34V, 0.27V, 0.19V and 0.095V, respectively, and the -CNTs/GCE shows the selective performance for analysis cyanidin, quercetin, rutin, delphinidin and gallic acid.

Table 2. The resulted electrochemical current (EC) using DPV technique on f-CNTs/GCE in 0.1M PBS (pH=7.0) at 0.43V, 0.34V, 0.27V, 0.19V and 0.095V for addition of 100 µg/l of cyanidin, quercetin, rutin, delphinidin and gallic acid, respectively, and successive additions of 200 µg/l of interferents.

Substance	**Added (µg/ml)**	**EC (µA) at 0.43V**	**EC (µA) at 0.34V**	**EC (µA) at 0.27V**	**EC (µA) at 0.19V**	**EC (µA) at 0.095V**
Cyanidin	100	1.668±0.024	0.621±0.041	0.209±0.033	0.105±0.009	0.115±0.010
Quercetin	100	0.110±0.014	7.121±0.094	0.111±0.010	0.208±0.018	0.211±0.015
Rutin	100	0.226±0.011	0.421±0.031	4.624±0.091	0.179±0.015	0.180±0.011
Delphinidin	100	0.103±0.021	0.303±0.052	0.223±0.030	4.631±0.033	0.155±0.011
Gallic Acid	100	0.096±0.009	0.196±0.021	0.211±0.012	0.189±0.011	4.829±0.019
P-hydroxybenzoic acid	200	0.093±0.010	0.089±0.011	0.077±0.010	0.083±0.009	0.150±0.013
Caffeic acid	200	0.078±0.008	0.198±0.028	0.088±0.009	0.068±0.008	0.246±0.011
Chlorogenic acid	200	0.068±0.007	0.051±0.009	0.063±0.008	0.058±0.007	0.153±0.021
Kaempferol	200	0.082±0.008	0.077±0.009	0.033±0.007	0.096±0.006	0.196±0.011
Hyperoside	200	0.078±0.010	0.076±0.008	0.059±0.008	0.066±0.008	0.073±0.007
Isoquercitrin	200	0.095±0.009	0.100±0.010	0.088±0.009	0.101±0.010	0.088±0.008
Catechin	200	0.109±0.011	0.111±0.010	0.101±0.009	0.118±0.013	0.077±0.007
Phloridzin	200	0.087±0.009	0.087±0.008	0.079±0.009	0.099±0.010	0.060±0.008

3.3 Determination of Polyphenolic compounds by HPLC detector

The quantitative analysis of polyphenolic compounds by HPLC detector is summarized in Table 3 which presents the linearity of calibration curves (Y= A + BX) and LOD where B is the slope and solely governs the sensitivity [52]. Comparison between the obtained sensing properties of f-CNTs/GCE and HPLC detector indicates that the HPLC detector shows the higher sensitivity, and proposed DPV technique shows lower LOD values. Moreover, the results of the average concentrations of cyanidin, quercetin, rutin, delphinidin and gallic acid in EPCs from blueberries using DPV and HPLC techniques are presented in Table 4. It is observed from the results that there is good

agreement between both techniques. In addition, results show the anthocyanins have the maximum concentration in blueberries pomace. The results are in agreement with the report of Loncaric et al [53].

Table 3. Calibration equations and LOD value for polyphenolic compounds obtained by the proposed HPLC method**.**

Polyphenolic compounds	**Calibration equation Y= A + BX**		**Correlation coefficient (R^2)**	**LOD (µg/l)**
	A (µA)	**B** (µA/µgl^{-1})		
Cyanidin	589.38	0.01551	0.99789	0.19
Quercetin	1689.97	0.06101	0.99902	0.06
Rutin	717.98	0.03628	0.99885	0.11
Delphinidin	3560.16	0.03199	0.99858	0.13
Gallic Acid	266.46	0.03823	0.99980	0.09

Table 4. The results of the average concentrations of cyanidin, quercetin, rutin, delphinidin and gallic acid in EPCs from blueberries pomace using DPV and HPLC techniques (n = 4).

Technique	**Cyanidin (mg/g)**	**Quercetin (mg/g)**	**Rutin (mg/g)**	**Delphinidin (mg/g)**	**Gallic Acid (mg/g)**
DPV	0.7589	0.5475	0.4902	2.2325	0.1408
HPLC	0.7601	0.5541	0.3959	2.2258	0.1395

Table 5. Results of the accuracy of the DPV and HPLC techniques (n = 4).

Technique	**Polyphenolic compounds**	**Blank (µg/l)**	**Added (µg/l)**	**Found (µg/l)**	**Recovery (%)**	**RSD (%)**
DPV	Cyanidin	37.94	50.00	87.94	97.70	3.20
	Quercetin	27.37	20.00	47.37	97.05	3.01
	Rutin	24.51	20.00	44.51	98.10	2.81
	Delphinidin	111.62	100.00	211.62	97.88	3.17
	Gallic Acid	7.04	10.00	17.04	99.10	2.12
HPLC	Cyanidin	38.00	50.00	88.00	96.30	4.29
	Quercetin	27.70	20.00	47.70	98.55	3.42
	Rutin	19.79	20.00	39.79	96.10	2.78
	Delphinidin	111.29	100.00	211.29	95.68	3.57
	Gallic Acid	6.97	10.00	16.97	96.20	3.25

Furthermore, Table 5 displays the analytical findings of standard addition technique for analysis of EPCs. It is indicated good ranges of recovery (97.05% to 99.10%) for DPV technique and (95.68% to 98.55%) for HPLC technique, and acceptable ranges of RSD (2.12% to 3.20%) for DPV technique and (2.78% to 4.29%) for HPLC technique. The better results for DPV technique using f-

CNTs/GCE is implied to appropriate accuracy of proposed polyphenolic compounds sensor for analyses of fruit and food samples.

4. CONCULUSION

This study presented extraction and electrochemical studies of polyphenols, anthocyanins, and flavonoids in blueberry fruit. Both electrochemical measurements with f-CNTs/GCE and HPLC technique showed that quercetin, rutin, delphinidin, cyanidin and gallic acid were observed in extracted polyphenolic compounds. The sensing performance of f-CNTs/GCE such as detection limit, sensitivity and linear range for detection of the polyphenolic compounds were compared with the HPLC sensing properties in this work and other reported sensors in literature. Comparison indicated comparable or better performance of f-CNTs/GCE and there was good agreement between the both of electrochemical and HPLC techniques. In addition, results showed the anthocyanins have the maximum concentration in blueberries pomace. Results of analytical studies indicated good ranges of recovery and acceptable ranges of RSD for DPV technique and HPLC technique. The better results for DPV technique using f-CNTs/GCE was implied to appropriate accuracy of proposed polyphenolic compounds sensor for analyses of fruit and food samples.

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