Electrochemical Detection of Antioxidant Activities of 1,4-Dihydropyridine Derivatives

1,4-Dihidropiridin Türevlerinin Antioksidan Aktivitelerinin Elektrokimyasal Tayini

Research Article

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ABSTRACT

Antioxidant is a molecule which retards the autooxidation of fats and oils. Antioxidants block the oxidation process which can cause damage to many cells in body helping to prevent diseases. Therefore, the importance of antioxidant is in increasing demand. So far many attempts have been carried out for developing to detect antioxidant activities. Among various analytical techniques, electrochemistry has been a more appropriate way to determine antioxidant activity due to its ease of use and short-lasting application. In this study, it was aimed to determine antioxidant activities of recently synthesized condensed 1,4-dihydropyridine derivatives using disposable pencil graphite electrode. Cyclic voltammetry technique was used to determine oxidation potential value, and also differential pulse voltammetry technique was used to determine reduction potential values of the derivatives. To verify our results, nifedipine was used as a reference drug and conventional antioxidant activities were also used as a basis for comparison.

Key Words
1,4-Dihydropyridines, antioxidant activity, electrochemical methods, cyclic voltammetry, differential pulse voltammetry.

ÖZ

Antioksidan katı ve sıvı yağların otoksidasyonunu geciktiren bir moleküldür. Antioksidanlar, hastalıkları engellemeye yardımcı olarak vücutta pekçok hücreye zarar verebilen oksidasyon sürecini bloke ederler. Bu yüzden, antioksidanın önemine olan talep giderek artmaktadır. Bugüne kadar, pek çok teşebbüs antioksidan aktivite tayininin geliştirilmesi için gerçekleştirilmiştir. Çok çeşitli analitik tekniklerin arasında, elektrokimya kullanım kolaylığına ve kısa sürede uygulanabilmesine bağlı olarak antioksidan aktivitesinin belirlenmesi için daha uygun bir yoldur. Bu çalışmada, yeni sentezlenen kondanse 1,4-dihidropiridin türevlerinin antioksidan aktivitelerinin atılabilir kalem grafit elektrot kullanılarak belirlenmesi amaçlanmıştır. Dönüşümlü voltametri tekniği türevlerin oksidasyon potansiyel değerinin ve aynı zamanda diferansiyel puls voltametri tekniği redüksiyon potansiyel değerinin belirlenmesi için kullanılmıştır. Sonuçlarımızın değerlendirilmesi için, nifedipin bir referans ilaç olarak ve konvensiyonel antioksidan aktiviteleri aynı zamanda karşılaştırma için bir temel olarak kullanılmıştır.

Anahtar Kelimeler
1,4-Dihidropiridinler, antioksidan aktivitesi, elektrokimyasal yöntemler, dönüşümlü voltametri, diferansiyel puls voltametrisi.

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INTRODUCTION

Oxidation is one of the most important chemical processes in food and chemicals. Free radicals can oxidize nucleic acids, proteins and lipids by initiating degenerative diseases [1-3]. Oxidative stress has become the center of attention particularly since the end of the 20th century. It results from an imbalance between the production of oxidizing chemical species and their effective removal by protective antioxidant molecules and scavenger enzymes. It is associated to the massive generation of reactive oxygen (ROS) and nitrogen (RNS) species which induce fast oxidative reactions in chain [4]. It is now clearly accepted that numerous pathologies and clinical disorders, i.e. aging, cancers, atherosclerosis, degenerative diseases etc. are directly or indirectly consecutive with continuous or repetitive exposure to oxidative stress [5]. To prevent or reduce oxidative stress, reasonable supplementations of antioxidants are widely practiced [6,7].

1,4-Dihydropyridines (1,4-DHPs) are an important class of bioactive molecules that is, well known for their role as calcium channel modulators and used extensively for the treatment of hypertension [8-10]. The derivatives of 1,4-DHPs have shown a variety of biological activities such as vasodilator, bronchodilator, antitumor, hepatoprotective and geroprotective [11,12]. It has been also reported that similar types of 1,4-DHP derivatives have anti-cancer, anti-radiation activity [13], skin regenerating and radioprotection properties. Some of these types of compounds act as antioxidants [14]. Nifedipine, which is a dihydropyridine calcium channel blocker is a prototype of this group of drugs.

Numerous chemical or biochemical protocols are developed to evaluate the oxidative damages and antioxidant ability [15]. Several studies showed the evidence of antioxidant synergy and regeneration effect in antioxidant mixtures in various application fields such as clinical biology, pharmaceuticals, cosmetics and food. In most studies, analytical methods such as ultraviolet (UV) spectrophotometry, infrared (IR) spectroscopy, oximetry (electron spin resonance), high performance liquid chromatography (HPLC) or laser flash photolysis involve complex protocols and expensive materials. In sophisticated HPLC-UV-solid phase extraction (SPE)-nuclear magnetic resonance (NMR) technique which was done by chromatographic seperation to study radical scavenging activity, some of the chromatographic peaks had to be trapped and serious analysis and equipment were necessary. Thus it was a time consuming technique [16]. By combining liquid chromatography-ion trap mass spectrometry with LC-UV-diode array dedection (DAD), antioxidant constituent was first investigated, but this technique has been still performed on interfaces equipped with different spectrometer [17]. In the literature, cupric ion reducing antioxidant capacity (CUPRAC) assay was introduced by measuring the difference in absorbance of the probe in the absence and presence of the scavenger, but lengthy calculations were necessary because of the competition kinetics [18]. Cellular-based assays were developed to evaluate antioxidant activity, but they were still oriented natural or known antioxidants, did not improve a single analytical method to detect recently synthesized antioxidant derivatives [19]. Comparatively, electrochemistry appears as an appropriate way with its ease of use and low-cost to determine the antioxidant status of a complex medium. Electrochemical techniques such as four electrode system, three phase electrode, thin layer voltammetry and scanning electrochemical microscopy have been extensively used to study ion or charge transfer reactions at the interface between two immiscible electrolyte solutions [20-22]. Some of them have been applied to determine kinetic parameters associated with the oxidation of antioxidants at liquid/liquid interface [23-25].

Cyclic Voltammetry, CV is a unique technique for the electrochemical characterization of compounds by providing data about their oxidation/reduction potentials. Besides simplicity and rapidness, this technique is based on the chemico-physical properties of the molecules and can be widely used in evaluating antioxidant activities in oils and foodstuffs [26]. The literature review shows that there is a good correlation between the oxidation potentials of various antioxidants and their antioxidant efficiencies [27].

Differential Pulse Voltammetry, DPV has been often used to make electrochemical measurements as a sensitive method for determination of low levels of organic molecules and also for drugs [28].

Pencil graphite electrodes are relatively new type of carbon electrode, it has been successfully applied to electrochemistry by superiority of its high electrochemical reactivity, disposability, low cost and technology [29-31]. Disposable pencil graphite electrodes as an alternative to high tech carbon electrodes, are extremely inexpensive and provides low tech ability [32].

Recent biochemical investigations have confirmed that the mechanism of electrochemical reduction of oxygen at the electrode occured on four stages in an electrochemical cell. Superoxide (O_2^{-}) is formed by one-electron reduction as a by-product of the oxidation metabolism [33]. The highly reactive superoxide can be oxidized (back to oxygen) or it can be reduced further to form hydrogen peroxide (H_2O_2). During normal biological processes, superoxide and hydrogen peroxide are formed in small quantities and there are natural defense mechanisms, such as superoxide dismutase which effectively remove superoxide and other active oxygen species. However, under certain conditions, such as intake of drugs, UV-radiation or metabolic disfunction, these reactive oxygen species can be generated in sufficient quantity to exceed the normal defense capabilities of the body. This can result in deleterious effects on tissues [34].

Antioxidant activity is determined by recording the current of the electrochemical oxygen reduction by using DPV in the field of electrochemical reduction of oxygen. The process proceeds at the cathode in several stages with formation of the active anion-radical of oxygen, superoxide (O_2^{-}) [35]:

$$O_2 + e^- \overset{k}{\rightleftarrows} O_2^{\cdot -} \quad (1)$$

$$O_2^{\cdot -} + H^+ \rightleftarrows HO_2^{\cdot} \quad (2)$$

$$HO_2^{\cdot} + H^+ + e^- \rightleftarrows H_2O_2 \quad (3)$$

$$H_2O_2 + 2H^+ + 2e^- \rightleftarrows 2H_2O \quad (4)$$

There are a great number of studies to determine antioxidant activities using electrochemical techniques. However, in this study recently synthesized derivatives of 1,4-DHP and nifedipine were used in order to monitor their antioxidant activities electrochemically with pencil graphite electrode (PGE) for the first time. CV technique was used to determine the antioxidant activities from anodic peak values [36] and measurements were taken in various scan rates and concentrations from +1.3 V to -1.3 V vs. saturated calomel electrode (SCE). In DPV technique, the effects of some antioxidants and drug were examined using electrochemical reduction of oxygen [37]. The voltammograms of cathodic reduction of oxygen were recorded in the conditions of potential range from E = 0.0 V to -2.0 V vs. SCE.

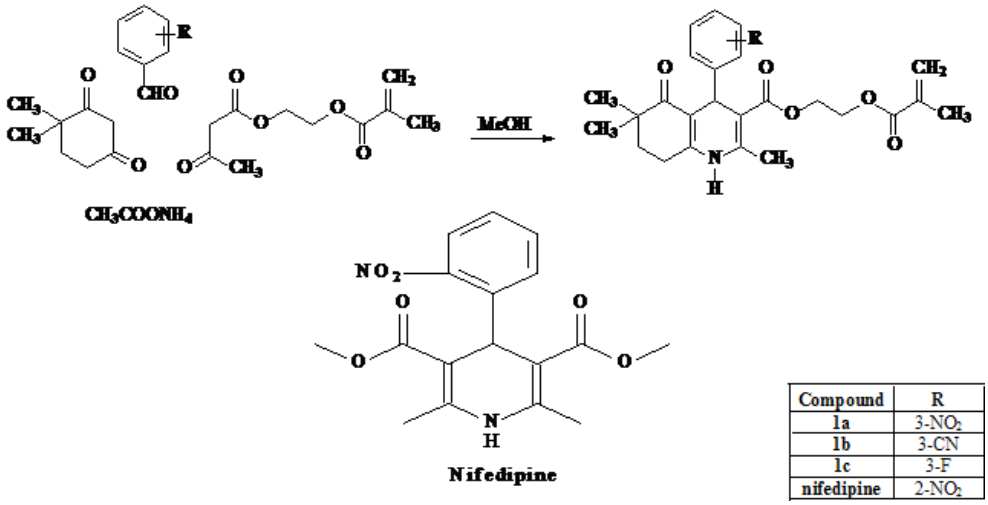
MATERIALS and METHODS

Chemicals and Preparation of Solutions

Tetrabutylammonium perchlorate salt was purchased from Aldrich and prepared by dissolving in dichloromethane solvent from Prolabo. Nifedipine was purchased from Sigma. In all studies, 0.1 mol.L^{-1} of tetrabutylammonium perchlorate (TBAP) salt in 5 mL of dichloromethane solvent was used as a support electrolyte. Concentration amounts of all derivatives and nifedipine were used between 0.2 mg.mL^{-1} and 1.0 mg.mL^{-1}. Other reagents were of analytical grade. In CV and DPV analyses, pure N_2 gas was passed from all of the prepared solutions for sufficient period of time to extract oxygen.

Instrumentation

The compounds were synthesized under a *CEM Cooperation Discover SP* microwave synthesis system. Melting points were determined on a *Thomas Hoover* Capillary Melting Point Apparatus and uncorrected values. Infrared spectra were recorded on a *Perkin Elmer Spectrum* BX (n, cm^{-1}). ^{1}H-NMR and 13C-NMR were obtained from *Bruker 400 MHz Ultra Shield Spectrophotometer* (DMSO-d6; tetramethylsilane as internal standard). Mass spectra were obtained on a *Waters 2996 Photoiodide array dedector*. Microanalysis was obtained on a Leco CHNS-932 *Elemental Analyzer* and the results were within ± 0.4% of the theoretical values.



Compound	R
1a	3-NO_2
1b	3-CN
1c	3-F
nifedipine	2-NO_2

Figure 1. Schematic of general synthesis of 2-(methacryloyloxy)ethyl 4-aryl-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate and structure of 3,5-dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (nifedipine).

Electrochemical experiments were performed on *CH Instruments CHI660C* model potentiostat with a conventional three electrode system which consisted of pencil graphite working electrode, saturated calomel reference electrode and platinum counter electrode. PGE was prepared using mechanical pencil *Model T 0.5* (Rotring, Germany) as a holder for pencil lead (Tombo, Japan) which was purchased from a local bookstore and a metallic wire was wrapped around the metallic part of the pencil to provide electrical contact to the lead. All leads had a total length of 60 mm and a diameter of 0.5 mm. A total of 10 mm of lead was immersed in solution per measurement. Surface area of PGE in such a length was 15.9 mm^2. Gold and platinum working electrodes with 0.0314 cm^2 area were used to compare with PGE.

Synthesis of 1,4-DHP Derivatives and Structure of Nifedipine

4,4-dimethylcyclohexane-1,3-dione (1 mol), 2-(methacryloyloxy)ethyl acetoacetate (1 mol), substituted benzaldehyde (1 mol) and ammonium acetate (4 mol) were dissolved in methanol and subjected to microwave irradiation for 15 min. The reaction progress was monitored by thin layer chromatography (TLC). After completion of the reaction, the mixture was dried under reduced pressure; the resulting precipitate was recrystallized from dichloromethane/ether mixture (Figure 1). The structure of 3,5-dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (nifedi-pine) was also represented in Figure 1.

2-(methacryloyloxy)ethyl 2,6,6-trimethyl-4-(3-nitrophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (1a)

M.p. 132-134. IR (cm^{-1}) 3303, 3082, 2930, 1709. 1H NMR δ 0.8-0.9 (6H; s; CH_3), 1.6-1.7 (4H; m; quinoline H7,8), 1.8 (3H; s; methacryloxy CCH3), 2.3 (3H; s; CH3), 4.2 (4H; m; $COOCH_2CH_2OCO$), 4.9 (1H; s; quinoline H4), 5.6-5.8 (2H; s; methacryloxy C=CH_2), 7.4-7.9 (4H; m; aromatic), 9.3 (1H; s; NH), 13C NMR 18.3, 18.8, 23.3, 24.5, 25.3, 34.4, 36.8, 61.5, 63, 102, 109.2, 121.3, 122.2, 126.2,129.8, 134.6, 135.9, 147.3, 147.8, 150.2, 150.6, 166.6, 166.7, 200. Anal. for $C_{25}H_{28}N_2O_7$ (MW: 468) calcd.: C, 64.09; H, 6.02; N, 5.98 found: C, 64.13; H, 5.72; N, 6.08.

2-(methacryloyloxy)ethyl 4-(3-cyanophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (1b)

M.p. 147-149. IR (cm^{-1}) 3293, 3074, 2961, 2229, 1709. 1H NMR δ 0.8-0.9 (6H; s; CH_3), 1.6-1.7 (4H; m; quinoline H7,8), 1.8 (3H; s; methacryloxy CCH_3), 2.3 (3H; s; CH_3), 4.2 (4H; m; $COOCH_2CH_2OCO$), 4.8 (1H; s; quinoline H4), 5.6-5.9 (2H; s; methacryloxy C=CH_2), 7.3-7.6 (4H; m; aromatic), 9.2 (1H; s; NH), 13C NMR 18.3, 18.8, 23.3, 24.5, 25.3, 34.4, 36.7, 61.5, 63, 101.9, 109.1, 111.1, 119.5, 126.4, 129.6, 130.1, 131.1, 132.8, 136, 147.2, 149.5, 150.5, 166.7, 166.7, 199.9. Anal. for $C_{26}H_{28}N_2O_5$ (MW: 448) calcd.: C, 69.63; H, 6.29; N, 6.25 found: C, 69.78; H, 6.29; N, 6.42.

2-(methacryloyloxy)ethyl 4-(3-fluorophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (1c)

M.p. 145-147. IR (cm^{-1}) 3321, 3078, 2930, 1712. 1H NMR δ 0.8-1 (6H; s; CH_3), 1.6-1.7 (4H; m; quinoline H7,8), 1.8 (3H; s; methacryloxy CCH_3), 2.3 (3H; s; CH_3), 4.2 (4H; m; $COOCH_2CH_2OCO$), 4.8 (1H; s; quinoline H4), 5.6-5.9 (2H; s; methacryloxy $C=CH_2$), 6.8-7.2 (4H; m; aromatic), 9.2 (1H; s; NH), 13C NMR δ 18.3, 18.8, 23.3, 24.5, 25.5, 34.5, 36.2, 61.5, 63.1, 102.4, 109.3,113, 114.3, 123.6, 126.4, 130.1, 136, 146.7, 150.3, 150.9, 161.2, 166.8, 167, 199.9. Anal. for $C_{25}H_{28}FNO_5$ (MW: 441) calcd.: C, 68.01; H, 6.39; N, 3.17 found: C, 67.92; H, 6.57; N, 3.

1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-dicarboxylic acid dimethyl ester (nifedipine)

Reactive profile of nifedipine represents sensitivity to light. The distribution coefficient in an octanol-water system is about 10,000:1. The UV (Absorption maxima at 235 nm and around 340 in methanolic solution, and at 238 nm and around 340 nm in alkaline and acid solutions respectively.), IR (3331, 3102, 2931-2842, 1689-1679, 1625, 1574-1530-1433, 1380-1227 and 1121 cm-1 frequencies for N-H stretching vibrations, CH-aromatic, CH-aliphatic, C=O ester, -C=C-aromatic, NO_2, -C-CH_3, -C-O- ester assignments respectively.), CNMR (19.10, 34.51, 50.87, 103.24, 123.72, 126.93, 130.95, 132.69, 142.16, 145.23, 147.74 and 167.62 ppm chemical shifts for C 1-12) [37] and mass spectra (346, 329, 315, 298, 284, 270, 268, 254, 224, 192 mass numbers for molecular peak M = $C_{17}H_{18}N_2O_6$, M-OH, M-OCH_3, M-OCH_3.OH, M-OCH_3.OCH_3, M-CO_2CH_3.OH, M-OCH_3.NO_2.H, M-OCH_3.OCH_3.NO, M-$C_6H_4NO_2$ and M-$C_6H_4NO_2$.$OHCH_3$ structural assighments.) have been reported. Nifedipine is soluble (gL^{-1}, at 20°C) in the methylene chloride, 160 [38].

Measurement of Antioxidant Activities of 1,4-DHP Derivatives and Nifedipine with CV and DPV

In CV studies, PGE was immersed into TBAP-dichloromethane solution containing 0.2 $mg.mL^{-1}$ of 1,4-DHP derivatives and Nifedipine and scanned between +1.3 V and -1.3 V vs. SCE. This was repeated between 0.4 $mg.mL^{-1}$ and 1.0 $mg.mL^{-1}$ at various scan rates. Additionally, 0.2 $mg.mL^{-1}$ of compound 1-a was used on gold and platinum surfaces repeating CV study. In DPV studies, PGE was immersed into TBAP-dichloromethane solution and scanned to obtain the original limiting current (I_{or}) of bare graphite electrode between 0.0 V and -2.0 V vs. SCE. N_2 gas was passed from the solution to extract oxygen and PGE was scanned to obtain the residual current value (I_{res}). Concentration amounts of compound 1-a, compound 1-b and nifedipine between 0.2 $mg.mL^{-1}$ and 0.6 $mg.mL^{-1}$, and compound 1-c between 0.2 $mg.mL^{-1}$ and 1.0 $mg.mL^{-1}$ were added to the solution one by one to observe the proportional decrease of the oxygen current. Voltammograms of the electrochemical reduction current (I_{red}) were taken corresponding to the concentration of the added antioxidant at constant potential.

RESULTS AND DISCUSSION

Cyclic Voltammograms of 1,4-DHP Derivatives and Nifedipine

Electrochemical behaviours of 1,4-DHP derivatives and nifedipine were investigated using CV technique. Non-aqueous 0.1 $mol.L^{-1}$ TBAP-dichloromethane solution was preferred to dissolve 1,4-DHP derivatives and nifedipine. Because, dichloromethane was one of the most widely used chlorinated solvents. It was a very weak coordinating solvent, but dissolved many organic and organometallic compounds. Dichloromethane had been used as a "nonbonding" solvent for electrochemical studies. Its nonbonding nature and lack of Lewis base properties made dichloromethane an appropriate solvent to study inorganic and organometallic complexes when it was aimed to minimize the effect of solvent on electrochemical reactivity. It had a working potential range of -1.7 V to +1.8 V vs. SCE using tetrabutylammonium perchlorate as supporting electrolyte [39] which represented excellent stability of cations, limited negative potential range, easily purified and quite resistive remarks in dichloromethane solvent. Thus, this solvent was ideal for studying both reductions and oxidations of 1,4-DHP derivatives and nifedipine. The resistivity of dichloromethane was fairly high, 7.25 ohm.m with TBAP which was used 0.1 mol/L as supporting electrolyte [40]. There had been much debate over the use of aqueous reference electrodes like saturated calomel

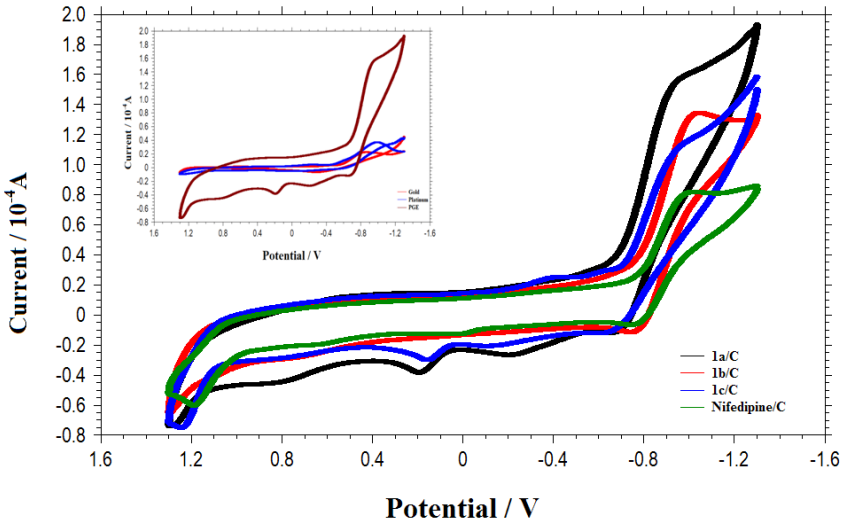


Figure 2. Cyclic voltammograms of compound 1a/C, compound 1b/C, compound 1c/C and nifedipine/C electrode between -1.3 V and +1.3 V vs. SCE in 0.1 mol.L^{-1} TBAP-dichloromethane solution. Scan rate: 100 mV.s^{-1}. Inset: Cyclic voltammograms of compound 1-a on gold, platinum and PGE surfaces.

electrode with non-aqueous solvent systems like dichloromethane. One area of concern was the junction potentials which led to a potential difference across the interface of the two solutions due to unequal rates of diffusion of the constituent ions and these potentials could range from tens to hundreds of millivolts. However, the junction potential could generally be ignored for a given solvent system provided it was constant and reproducible [41].

Cyclic voltammograms of compound 1a-c and nifedipine on PGE in 0.1 mol.L^{-1} TBAP-dichloromethane solution were compared in Figure 2 and they were denoted as compound 1a/C, compound 1b/C, compound 1c/C and nifedipine/C respectively. Cyclic voltammograms of compound 1-a on various surfaces as gold, platinum and PGE were also given in Figure 2, inset. Two anodic peaks at +0.15 V and -0.15 V vs. SCE for compound 1-c, and three anodic peaks at +0.80 V, +0.20 V and -0.20 V vs. SCE for compound 1-a were seen from the voltammograms in Figure 2. When PGE surface was compared with gold and platinum surfaces, it could be distinguished from Figure 2, inset, PGE represented highest current response of compound 1-a and it gained the upper hand over gold and platinum surfaces.

Low oxidation potentials at +0.20 V and -0.20 V for compound 1-a and at +0.15 and -0.15 V for compound 1-c were obtained resulting from the formation of an intermediate radical which was formed by removal of H atom from pyridine ring by the attack of electron on the surface of PGE and another removal of H atom from intermediate radical, and resulted with the resonance structure of pyridine radical and were different from compound 1-b and nifedipine according to the CV [42]. The composed resonance stability determined the activities of antioxidant and the different structures of 1,4-DHPs and nifedipine represented different antioxidant abilities. Weak deactivating groups like compound 1-c direct electrophiles and attack the benzene molecule at the ortho- and para- positions while stronger groups like nitro and moderately strong groups like cyano direct electrophilic attacks to the meta-position. Therefore, compound 1-a should have the highest antioxidant activity in comparison with other derivatives due to its highest anodic current and low oxidation potential value. An extra high oxidation potential at +0.80 V vs. SCE for compound 1-a referred to stronger meta-directing nature of nitro group and did not participate to oxidation of pyridine ring. However, halides are ortho- para- directing groups but unlike most ortho- para- directors, halides tend to deactivate benzene. This unusual behavior can be explained by two ways: 1. The halogens are very electronegative and they cause inductive withdrawal (withdrawal of electrons from the carbon atom of benzene). 2. The halogens have non-bonding electrons and they can donate

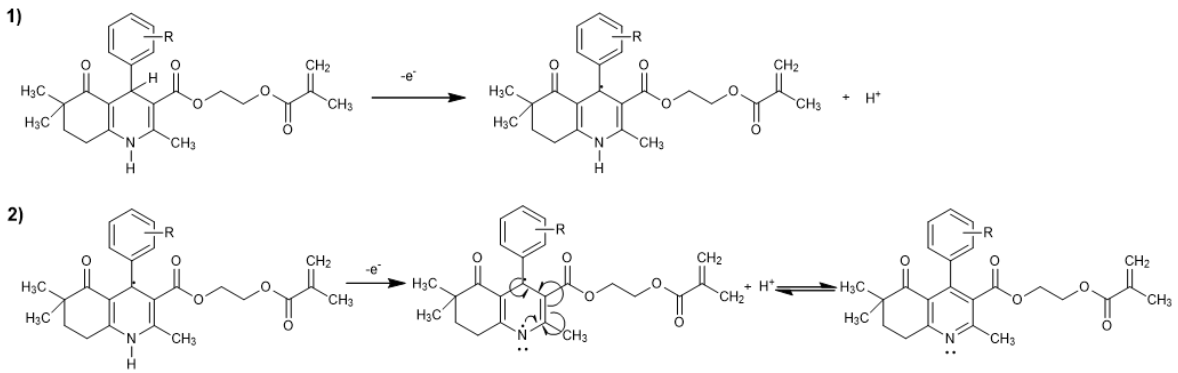


Figure 3. Schematics of two oxidation mechanism occuring on PGE.

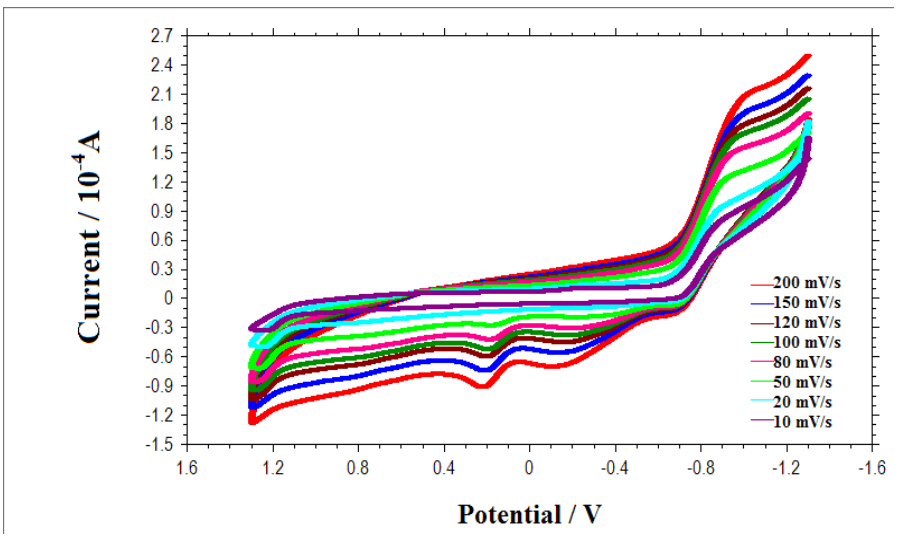


Figure 4. Cyclic voltammogram of compound 1-a in various scan rates between -1.3 V and +1.3 V vs. SCE in 0.1 mol.L^{-1} TBAP-dichloromethane solution.

electron density through δ bonding (resonance donation). The inductive and resonance properties compete with each other but finally the inductive wins this competition and fluorine is directed to the para- position. However, the ring that is substituted with the most electronegative halogen is the most reactive ring (less deactivating substituent). When it is compared with moderately deactivating cyano group, fluorine becomes dominant with its highest electronegativity and reactivity. Therefore, compound 1-c showed higher antioxidant activity than compound 1-b. When the nifedipine was also taken into account, almost no oxidative or reductive current or potential were observed because of the ortho- position of nitro group which was contrary to meta- deactivating property of nitro group. This situation caused lower antioxidant activity of nifedipine than all antioxidant derivatives. This mechanism was presumed to proceed in two oxidation steps on PGE (Figure 3) [43].

Effect of Scan Rate on the Peak Current and the Peak Potential of 1,4-DHP Derivatives and Nifedipine

To gain a better understanding of antioxidant activity, cyclic voltammograms of 1,4-DHP derivatives and nifedipine in various scan rates were recorded and cyclic voltammogram of compound 1-a was obtained in various scan rates, 10, 20, 50, 80, 100, 120, 150 and 200 mV.s^{-1}, in Figure 4.

In Table 1, cathodic and anodic peak potential and current changes of compound 1a-c and nifedipine with various scan rates were

Table 1. Anodic and cathodic peak potentials and currents vs. scan rates.

1-a					1-c				
ν(mV.s^{-1})	E_{pa}(V)	I_{pa}(10^{-4}A)	Epc(V)	I_{pc}(10^{-4}A)	ν(mV.s^{-1})	E_{pa}(V)	I_{pa}(10^{-4}A)	E_{pc}(V)	I_{pc}(10^{-4}A)
10	0.05	-0.05	-0.87	0.80	10	0.01	-0.05	-0.80	0.5
20	0.10	-0.15	-0.90	0.95	20	0.05	-0.15	-0.85	0.7
50	0.18	-0.30	-0.92	1.25	50	0.15	-0.40	-0.90	1.0
80	0.19	-0.45	-0.95	1.50	80	0.16	-0.70	-0.95	1.2
100	0.20	-0.55	-0.97	1.70	100	0.17	-0.90	-0.97	1.3
120	0.20	-0.60	-1.00	1.80	120	0.18	-1.05	-1.00	1.4
150	0.21	-0.75	-1.05	1.90	150	0.20	-1.20	-1.05	1.5
200	0.22	-0.90	-1.10	2.20	200	0.22	-1.55	-1.10	1.8
1-b					nifedipine				
ν(mV.s^{-1})	E_{pa}(V)	I_{pa}(10^{-4}A)	E_{pc}(V)	I_{pc}(10^{-4}A)	ν(mV.s^{-1})	E_{pa}(V)	I_{pa}(10^{-4}A)	E_{pc}(V)	I_{pc}(10^{-4}A)
10	0.05	-0.05	-0.95	0.85	10	0.001	-0.05	-0.88	0.50
20	0.10	-0.10	-0.97	0.90	20	0.002	-0.10	-0.90	0.60
50	0.13	-0.13	-0.98	1.10	50	0.005	-0.15	-0.95	0.75
80	0.15	-0.15	-1.00	1.30	80	0.010	-0.20	-0.97	0.85
100	0.15	-0.20	-1.00	1.35	100	0.020	-0.25	-0.98	0.95
120	0.17	-0.25	-1.02	1.53	120	0.030	-0.30	-1.00	1.00
150	0.18	-0.30	-1.03	1.55	150	0.040	-0.35	-1.02	1.10
200	0.20	-0.40	-1.05	1.70	200	0.050	-0.40	-1.05	1.20

represented. It was observed that the cathodic peak potential, E_{pc} shifted slightly to the more negative potentials, but the anodic peak potential, E_{pa} shifted slightly to the more positive potentials as the scan rate, ν increased. E_{pa} at +0.2 V vs. SCE for compound 1a-c and E_{pc} at -1.0 V vs. SCE for compound 1a-c and nifedipine remained almost constant with the scan rate of 100 mV.s^{-1} because of the transformation of antioxidants to another form until the necessary electron transportation materialized in low scan rates. However, remarkable anodic and cathodic current changes were obvious from the results that cathodic peak current (I_{pc}) and absolute anodic peak current (I_{pa}) rising with the increase of scan rate and they were directly proportional to the square root of scan rate. Therefore, the results of scan rates of 1,4-DHP derivatives and nifedipine represented reversible process with stability of peak potential and anodic and cathodic peak observation. The faster changing of scan rate resulted in the faster the rate of electrolysis and more current. Oxidation of antioxidants was realized on the surface of PGE. Because antioxidants were strong reducing agents and their positive oxidation potentials were low [44].

The linearity of reaction was investigated as anodic (I_a) and cathodic (I_c) currents (μA) versus square root of scan rates [$\nu^{1/2}$ (mV.s^{-1})$^{1/2}$] in Figure 5. The linear relationship which comes from Randles-Sevcik equation between peak currents and the square root of scan rates and R squared values which was near to 1 represented the electrochemical reaction was controlled by diffusion. The peak current, I_p is defined as the maximum current obtained during the forward linear potential scan. The reaction could be considered as reversible because I_p increased with the square root of scan rate [45].

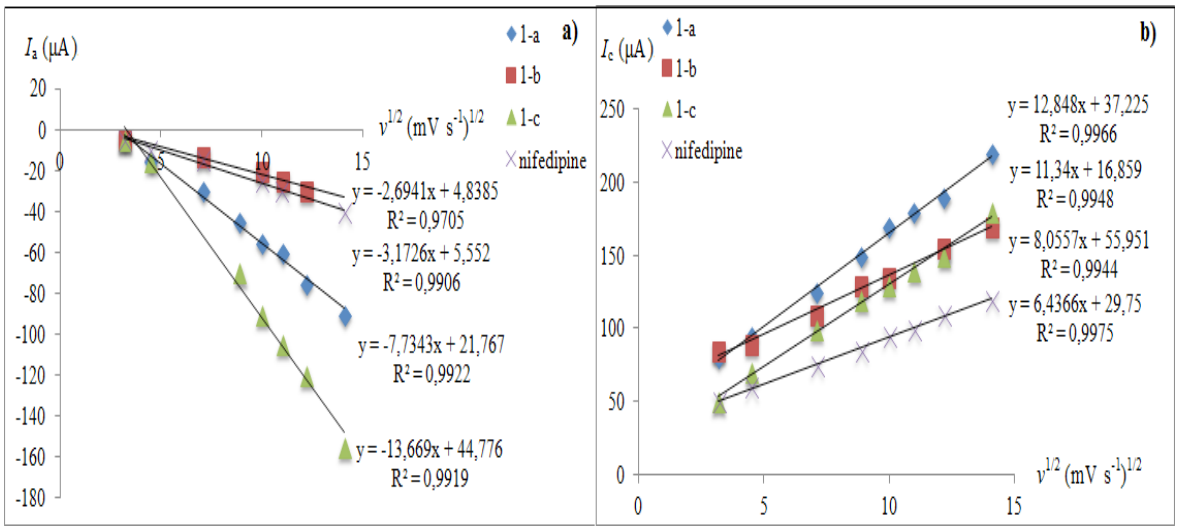


Figure 5. Graphical representation of a) I_a (µA) and b) I_c (µA) versus $v^{1/2}$ (mV.s^{-1})$^{1/2}$.

$$i_p = 0.4463\ nFAC\left(\frac{nFvD}{RT}\right)^{\frac{1}{2}}$$

n: number of electrons, δ: scan rate (V/s), F: Faraday's constant (96485 C/mol), A: electrode area (cm^2), R is the universal gas constant (8.314 J/mol.K), T is the absolute temperature (K), and D is the analyte's diffusion coefficient (cm^2/s).

Effect of Concentration on the Peak Current of 1,4-DHP Derivatives and Nifedipine

The linear relationship between peak current and concentration was also evaluated. The effects of concentration of compound 1-a and nifedipine on peak currents were investigated between 0.2 mg.mL^{-1} and 1.0 mg.mL^{-1} in 0.1 mol.L^{-1} TBAP-dichloromethane solution and it was represented in Figure 6. The oxidation current at +0.1 V vs. SCE increased for compound 1-a, but decreased for nifedipine by increase of concentration. Similarly, as it was represented in Figure 6, inset, the effect of concentration on peak current remained constant with 0.6 mg.mL^{-1} concentrations of compound 1-a and nifedipine and the linearity of curves changed as R squared value deviated from 1. Optimum operating condition was provided in 0.6 mg.mL^{-1} concentration of compound 1-a and 0.2 mg.mL^{-1} concentration of nifedipine because of the high anodic current and the low anodic potential which approached to zero potential. This represented the increasing of suitability of electroactive species on the surface of PGE by increase of concentration up to 0.6 mg.mL^{-1} for compound 1-a and other derivatives [46].

Differential Pulse Voltammograms of 1,4-DHP Derivatives and Nifedipine

On the basis of antioxidant activity of 1,4-DHP derivatives and nifedipine, effects of electron withdrawing and electron donating substituents on the antioxidants were considered. The differential pulse voltammograms of oxygen reduction (Figure 7) in the supporting electrolyte (0.1 mol.L^{-1} TBAP-dichloromethane solution) including 1,4-DHP derivatives and nifedipine were recorded to calculate the activities of antioxidant. As it was seen from the voltammograms, oxygen reduction currents at -0.9 V vs. SCE decreased by the increase of concentration. The reduction current at -1.6 V vs. SCE for compound 1-a and nifedipine including electrolyte represented the reduction of nitroaromatic to aniline because of the nitro functional group in the structure of compound 1-a and nifedipine and did not referred to antioxidant activity due to no regular interaction by the increase of concentration. PGE in blank electrolyte solution represented the highest cathodic current (j_{or}) because of the highest oxygen content and PGE in N_2 gas passed solution represented the lowest cathodic current (j_{res}) because of the lowest oxygen content.

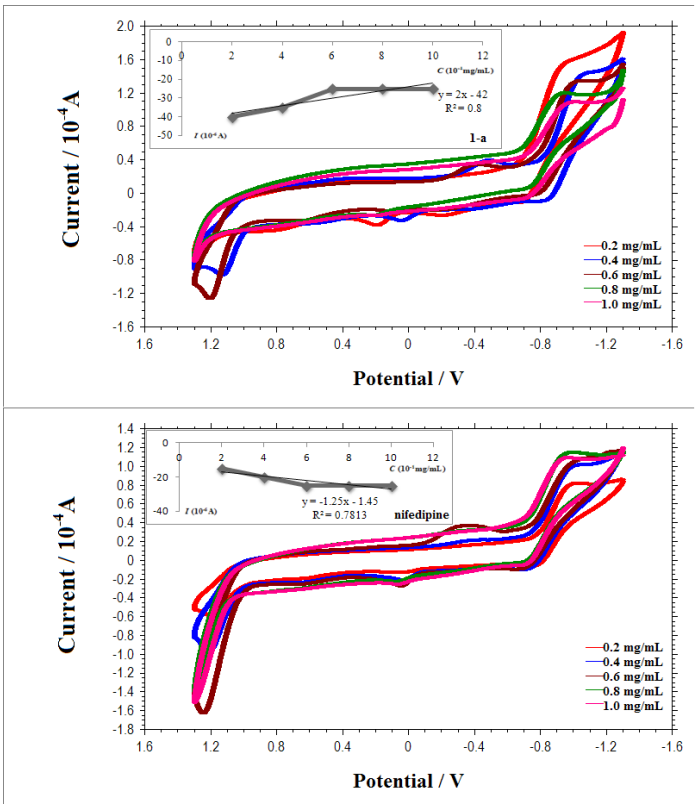


Figure 6. Cyclic voltammograms of compound 1-a and nifedipine in different concentrations between -1.3 V and +1.3 V vs. SCE in 0.1 mol.L^{-1} TBAP-dichloromethane solution. Scan rate: 100 mV.s^{-1}. Inset: variation of oxidation current with concentration.

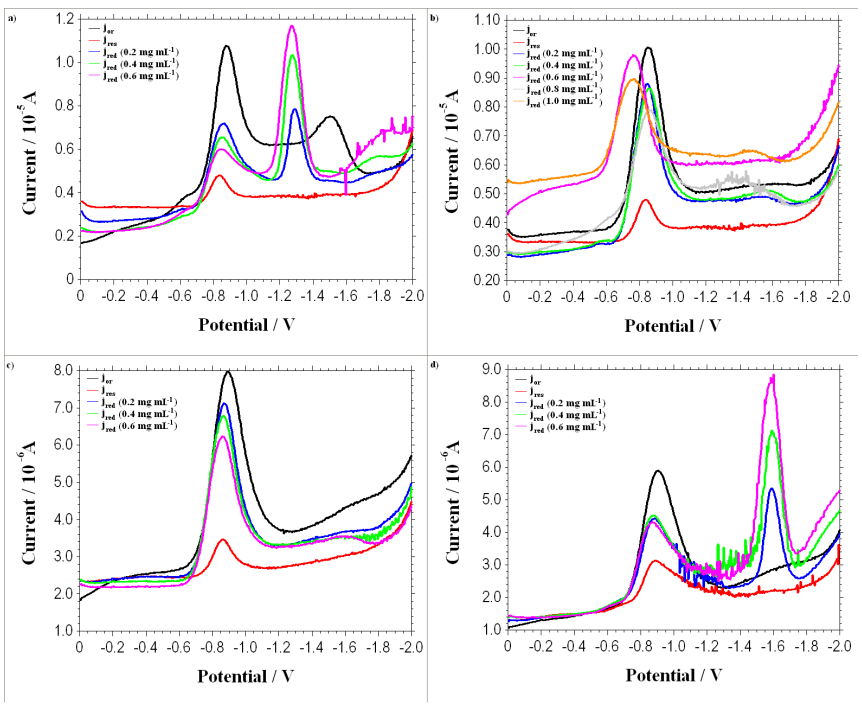


Figure 7. Differential pulse voltammograms of a) compound 1-a, b) compound 1-b, c) compound 1-c and d) nifedipine between 0.0 V and -2.0 V vs. SCE in 0.1 mol.L^{-1} TBAP-dichloromethane solution.

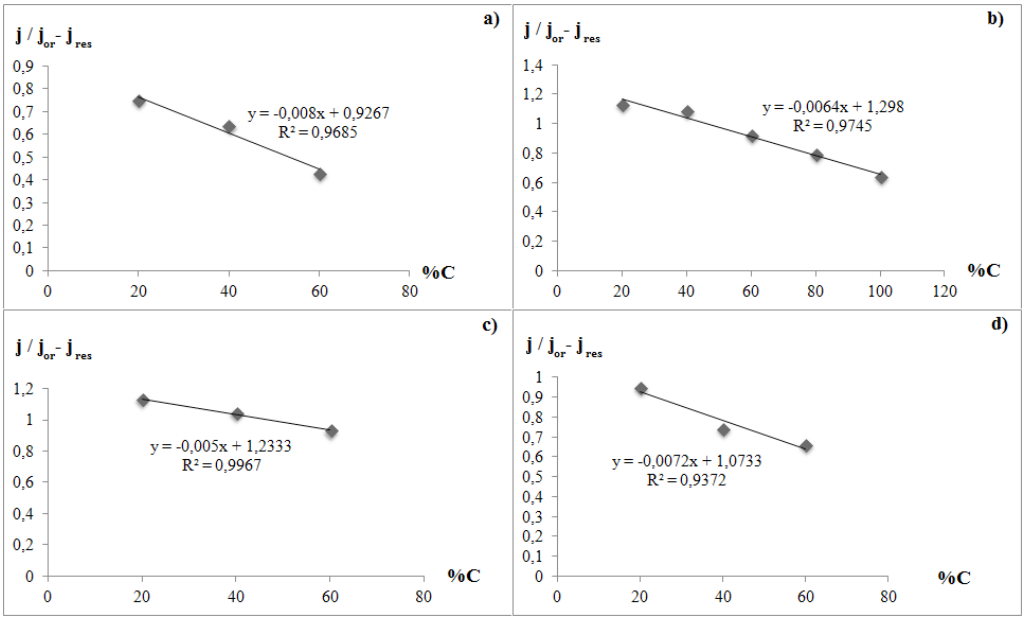


Figure 8. Cyclic voltammograms of compound 1-a and nifedipine in different concentrations between -1.3 V and +1.3 V vs. SCE in 0.1 mol.L^{-1} TBAP-dichloromethane solution. Scan rate: 100 mV.s^{-1}. Inset: variation of oxidation current with concentration.

The curves of limiting current of oxygen reduction vs. antioxidant concentration in the supporting electrolyte were investigated (Figure 8). As a result of the investigation, the curves of relative change of current density of oxygen reduction [j / (j_{or}–j_{res})] vs. antioxidant concentration [% C (mg. mL^{-1})] in supporting electrolyte were drawn.

jor and *jres* values of compound 1a-c and nifedipine were taken in various concentrations. All of the curves represented simple lines in the interval of small antioxidant concentration. The tangent of slope angle of these lines was accepted as a coefficient of antioxidant activity (K) [47].

$$K = \frac{\Delta j}{(j_{\mathrm{or}} - j_{\mathrm{res}})\Delta c}$$

As it could be seen from Table 2, the highest antioxidant activity was observed in compound 1-a due to the highest slope of graph. At the same time, all K values were near to slopes of the graphs showing a little experimental error and the results were consistent with each other. Therefore, the antioxidant activities of derivatives and nifedipine could be arranged as 1-a › nifedipine › 1-b › 1-c on the basis of reduction peak current.

Synthetic antioxidants like 1,4-DHPs were developed for the stabilization of bulk fats and oils or foods rich in lipids. If it was considered from the viewpoint of natural antioxidants, only a part of them could be absorbed and used as free-radical scavengers in vivo and they should be added to food in larger amounts than synthetic antioxidants because of the low activation. In the meanwhile, natural antioxidants were not pure substances and information about their safety was insufficient [48]. To represent the superiority of synthetic 1,4-DHP derivatives over natural antioxidants, the antioxidant activities of grains, dry beans, fresh vegetables and fruits were given in Table 3 as conventional antioxidant sources and were expressed in coefficient of antioxidant activity (K) [49]. When the antioxidant activities of derivatives were chosen from this table as a comparative basis, it was seen that compound 1-a was close to green grapes with its activity of 0.79, compound 1-b was close to broccoli flowers and spinach with its activity of 0.61, compound 1-c was almost same as wheat flour (refined) with its activity of 0.50 and nifedipine was close to broccoli flowers and spinach as in compound 1-b with its activity of 0.72.

Table 2. Antioxidant activity coefficients for compound 1a-c and nifedipine.

Compound	K	slope	δ%	R^2
1-a	0.79	0.80	1.25	0.97
1-b	0.61	0.64	4.69	0.97
1-c	0.50	0.50	0.00	0.99
Nifedipine	0.72	0.72	0.00	0.94

*σ was the experimental error; R^2 was the correlation coefficient.

Table 3. Antioxidant activities of some grains, dry beans, fresh vegetables and fruits [49].

Food	Antioxidant Activity (K)
Red Graphs	0.24
Red Gabbage	0.33
Broccoli Flowers	0.65
Spinach	0.65
Green Grapes	0.82
Tomato	1.09
Green Beans	1.89
Green Gabbage	2.17
Lima Beans	0.31
Red Beans	0.03
Blueberries	0.10
Raisins	0.06
Wheat Bran	0.07
Wheat Flour (refined)	0.55

CONCLUSION

In the present work, the antioxidant activities of condensed 1,4-DHP derivatives were determined by cyclic voltammetry technique from the oxidation potentials and also by differential pulse voltammetry technique from the reduction potentials and the results of antioxidant activities were compared with nifedipine. Generally, there was a relationship between antioxidative and peroxidative activities with oxidative potentials. The lower antioxidant potential resulted in the higher antioxidant activity. The lower oxidative potential brought the higher tendency to give electrons easily to the system which generated free radicals. The determination of antioxidant activity with a useful and sensitive voltammetric approach allowed to compare the activities of some antioxidants. Antioxidant activities of recently synthesized 1,4-DHP derivatives were determined by using electrochemical CV and DPV techniques and disposable PGE on the surface and DPV technique gave the order of antioxidant as 1-a › nifedipine › 1-b › 1-c. When the antioxidant activities of 1,4-DHP derivatives were compared with conventional antioxidants, similar activities were obtained. Therefore, synthetic antioxidants like 1,4-DHP derivatives could replace with natural antioxidants and eliminate deficiencies of natural antioxidants. Electrochemical way of determining antioxidant activities had two important advantages when compared to spectroscopic techniques. Antioxidant activities were determined both quantitatively and qualitatively, and also small amounts of antioxidants were found sufficient for analysis. It was shown

that disposable PGE electrode was suitable for studying antioxidant activities and it could be used as a sensor for detecting antioxidant activities. As a result, this work could open a way for more detailed electrochemical studies of recently synthesized 1,4-DHP derivatives which could be used as healthy antioxidants instead of conventional antioxidant sources since the method was new, fast and inexpensive.

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