MONASH University

Electrochemical Studies of Antioxidants and Its Application

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Abstract

Detection of antioxidants using various techniques had continuously received attention until today. In this research, electrochemistry was employed to detect antioxidants. The selected antioxidants (gallic acid, quercetin and rutin) were studied and characterized electrochemically using two electrochemical techniques, including cyclic voltammetry (CV) and square wave voltammetry (SWV). Gallic acid, quercetin and rutin are electroactive species. Quercetin was found to be more easily oxidised (Epa = 179 mV) compared to gallic acid (Epa = 256 mV) and rutin (Epa = 282 mV) in mixture of 0.1 M phosphate-buffered saline with ethanol (1:1 v/v) as supporting electrolyte. A series of chemically modified electrodes with different modifiers (multiwall carbon nanotubes (MWCNTs), bismuth(III) oxide (Bi_2O_3), bismuth(III) tungstate (Bi_2WO_3), bismuth oxybromide (BiOBr), fluorine doped tin oxide (FTO), and indium doped tin oxide (ITO)) were used to detect the selected antioxidants. The most effective modified electrode for detection of gallic acid was a FTO electrode, while the detection for quercetin was optimum using a Bi_2O_3 modified glassy carbon electrode (GCE), with increment in current peak of 17.4 % and 35.6 %, respectively as compared to GCE. Interestingly, the presence of gallic acid increased the peak current of bismuth ion, indicating that gallic acid might be one of the catalytic modifiers for bismuth ion oxidation peak. Other than electrochemical studies, high performance liquid chromatography (HPLC) analysis was also used to study the antioxidants content in selected commercially available supplements. In these application studies, analysis of grape seeds using both techniques revealed the presence of gallic acid and quercetin. Similarly, both electrochemical and HPLC analysis of Mindatulus's Bromo-Q showed the presence of quercetin. In comparison, electrochemical technique was complementary to analytical HPLC technique for detection of the studied antioxidants in selected supplements.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Signature: 

Name: Yaw Chong Wey

Date: 2nd February 2018

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A	Electrode’s area
Bi	Bismuth
Bi_2O_3	Bismuth (III) oxide
Bi_2WO_6	Bismuth tungstate
BiOBr	Bismuth oxybromide
c	Concentration
C	Capacitance
CE	Counter electrode
CV	Cyclic voltammetry
D	Diffusion coefficient
DAD	Diode array detection
DPV	Difference pulse voltammetry
E°	Formal potential
ΔEp	Separation between Epa and Epc
Epa	Potential peak (anode)
Epc	Potential peak (cathode)
F	Faraday’s constant
FTO	Fluorine tin oxide
GCE	Glassy carbon electrode
HPLC	High performance liquid chromatography

Hz	Hertz
I	Current
Ipa	Current peak (anode)
Ipc	Current peak (cathode)
ITO	Indium doped fluorine tin oxide
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
KOH	Potassium hydroxide
LoD	Limit of detection
M	Molar
mV	Milivoltage
MWCNTs	Multi wall carbon nanotubes
n	Number of electron
N	Pristine
O	Oxygen
OV	Oxygen vacancy
PBS	Phosphate-buffered saline
PTFE	Polytetrafluoroethylene
RE	Reference electrode
s	Second
SWV	Square wave voltammetry

TLC	Thin layer chromatography
µA	Microampere
UT	Ultrathin
V	Voltage
v/v	Volume to volume ratio
WE	Working electrode

1 Introduction

1.1. Electrochemistry

Electrochemistry is the study of chemical processes that cause electrons to move. This movement of electrons is called electricity, which can be generated by movements of electrons from one element to another in a reaction known as an oxidation-reduction ("redox") reaction.

An electrochemical cell is a device where a redox reaction spontaneously takes place to produce an electrical current, or external energy is supplied to drive a non-spontaneous redox reaction. Accordingly, electrochemical cells are classified as a galvanic cell or an electrolytic cell. Galvanic cells are those whose reactions are spontaneous when the electrodes are connected via a conductor (i.e., a copper wire), while an electrolytic cell requires application of a potential in excess of its open circuit potential in order to drive an electrochemical process.

During most electrochemical experiments, only the reaction occurring at a working electrode (WE), is of interest. In these cases, an ideal polarized electrode is paired with an electrode whose behaviour approaches that of an ideal non-polarizable electrode (e.g. a reference electrode (RE)) (Ciobanu et al., 2007, Smith and Stevenson, 2007). When the potential is measured between a WE and RE, there will be a voltage drop as a result of a current (I) flowing through a cell containing an electrolyte with a certain resistance (R), as given by Ohm's law (Ciobanu et al., 2007, Keyes and Forster, 2007, Shao, 2007):

$$V = IR = IR_s \qquad \text{Eq 1}$$

For systems where IRs is small (< 1–2 mV), a two-electrode system may be used without suffering a significant voltage drop. In the presence of a more resistive electrolyte, I must be small if a two-electrode cell is to be used. For cases where *IR*s cannot be kept below 2 mV, a three-electrode cell consisting of a counter electrode (CE), in addition to a WE and an RE, must be used. A two-electrode cell is usually used in potentiometric experiments, whereas a three-electrode cell is used in voltammetric and amperometric experiments (Ciobanu et al., 2007, Abdel-Hamid and Newair, 2011). The third electrode is a CE and is used as a current sink for the cell. In a three-electrode cell, majority of the current passes between the WE and the CE, which lowers the potential drop resulting from IR_s measured between the RE and the WE (Schulze, 1988).

Cyclic voltammetry (CV) and square wave voltammetry (SWV) are two fundamental techniques widely used in electrochemical studies. Both techniques can be used to follow a redox reaction. A difference between both CV and SWV is that CV will show the results of both an oxidation and a reduction reaction at the same time, while SWV will only show the results of either an oxidation or a reduction reaction at one time.

1.1.1. Cyclic Voltammetry (CV)

CV is commonly used as a preliminary electrochemical technique to characterise a redox system. The potentials of a redox reaction are readily determined and mechanistic information can be deduced using the results of this technique. CV can also be used to determine the formal potential (E°') and the number of electrons (n) involved in a redox reaction. In some cases, n cannot be easily determined for a reaction with a slow electron transfer rate, although useful estimates of E°' can still be made (Brownson and Banks, 2014).

A redox couple in which both species are stable and rapidly exchange electrons at the working electrode is termed an electrochemically reversible couple. The formal potential (E°') for a reversible couple is centered between anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}):

$$E^{\circ\prime} = \frac{\left(E_{pa} + E_{pc}\right)}{2} \qquad \text{Eq 2}$$

For a reversible couple, n (number of electrons transferred) can be determined from the separation between the anodic and cathodic peak potentials from:

$$\Delta E_p = E_{pa} - E_{pc} = \frac{0.058}{n} \qquad \text{Eq 3}$$

For example, CV was used to evaluate the antioxidant capacity of blood plasma (Filipe et al., 2001, Duthie and Crozier, 2000), tissue homogenates (Duthie and Crozier, 2000) and edible plants (Dai and Mumper, 2010, Gupta and Sharma, 2006, Katalinic et al., 2006). Analysis of CV traces has yielded the values of (i) the biological oxidation potential, E and $E_{1/2}$, which relate to

the nature of the specific molecule(s); (ii) the intensity (I_{pa}) of the anodic current; and (iii) the area of the anodic wave (S) (Chevion et al., 2000).

CV is very similar to linear sweep voltammetry (Kelly, 2009, Pisoschi et al., 2009, Riber et al., 2000), except that the scan is swept from an initial potential to a final potential and then back to the initial potential in CV. CV is capable of generating a new species during the forward scan and then probing its fate on the reverse and subsequent scans. The Randles-Sevcik equation (Eq 4) also holds for CV.

$$\text{Ip} = 0.4463\ \text{nFAC}\left(\frac{nF\nu D}{RT}\right)^{\frac{1}{2}} \qquad \text{Eq 4}$$

where;

Ip = current maximum in amps

n = number of electrons transferred in the redox event (usually 1)

A = electrode area in cm^2

F = Faraday's constant in C/mol

D = diffusion coefficient in cm^2/s

C = concentration in mol/cm^3

ν = scan rate in V/s

R = Gas constant in J/(K mol)

T = temperature in K

The characterisation of diffusion in electrolyte solutions is important both for fundamental reasons helping us understand the nature of the structure of aqueous electrolytes, and for practical application in fields such as biological environment (Ribeiro et al., 2010). Therefore, accurate knowledge of the diffusion coefficient (D) is required for most of the electrochemical studies.

Electrochemical experiments are often designed so that diffusion is the sole form of mass transport. Even in cases where convection and migration occur, diffusion can rarely be ignored as a significant contributor to the overall mass transport. Therefore, theoretical treatments of electrochemical systems that involve mass transport normally require that the diffusion coefficient of the redox species of interest, D, is to be known (Baur, 2007).

Yet despite its importance, values of *D* are not readily found. This is because the diffusion coefficient of a species in solution depends upon several factors (e.g. temperature, viscosity, electrolyte, *etc*.), so comprehensive tabulation is impractical. When the diffusion coefficient must be known for a new compound or a new set of conditions, the usual approach is to measure *D* for the particular species of interest under specified experimental conditions. An accurate measurement requires careful characterisation and calibration of the measurement system using a species with a known diffusion coefficient under well-defined conditions (Baur, 2007).

Techniques based upon the application of a large amplitude potential step are probably the simplest and most widely applied methods for measuring D. In using this technique, it is of vital importance that the potential is stepped from a value where the species of interest is not reduced (or oxidised) to a value where the electrolytic current is diffusion-controlled . Furthermore, the step potential must be selected so that only the redox species is electrolyzed. An advantage of chronoamperometric techniques is that species with slow heterogeneous kinetics are amenable to the determination of *D*, as long as the potential can be stepped to a value where the rate of the electron transfer reaction is large and therefore the process is under diffusion control (Baur, 2007).

1.1.2. Square Wave Voltammetry

Square wave voltammetry (SWV) is an important electroanalytical technique with a broader dynamic range and lower limit of detection compared to CV (Mann et al., 2014). Nowadays it is considered as one of the most common voltammetric techniques, which unifies the advantages of pulse techniques (enhanced sensitivity), CV (insight into the electrode mechanism) and impedance techniques (kinetic information of very fast electrode processes) (Mirceski et al., 2013).

The primary advantage of the pulse voltammetric techniques, such as normal pulse or differential pulse voltammetry (DPV), is their ability to discriminate against charging (capacitance) current. As a result, these pulse techniques are more sensitive to oxidation or reduction currents (faradaic currents) than conventional direct current (dc) voltammetry. DPV yields peaks for faradaic currents rather than the sigmoidal waveform obtained with dc or normal pulse techniques. This results in improved resolution for multiple analyte systems and more convenient quantitation (Research, 2017).

The square wave voltammetric waveform consists of a square wave superimposed on a staircase. The currents at the end of the forward and reverse pulses are both registered as a function of staircase potential. The difference between them, the net current, is larger than either of its two component parts in the region of the peak which is centred on the half-wave potential. Capacitative contributions can be effectively discriminated against before they decay away because, over a small potential range between forward and reverse pulses, the capacitance is constant and is thus annulled by subtraction. In this way the pulses can be shorter than in DPV and the square wave frequency can be higher. Instead of the effective sweep rates of 1 to10 mV/s of DPV, scan rates of 1 V/s can be employed (Brownson and Banks, 2014).

Typical potential modulation used in SWV consists of a staircase potential ramp modified with square-shaped potential pulses (Figure 1A). At each step of the staircase ramp, two equal in height and oppositely directed potential pulses are imposed. The latter two potential pulses complete a single potential cycle in SWV (Figure 1B) (Mirceski et al., 2013).

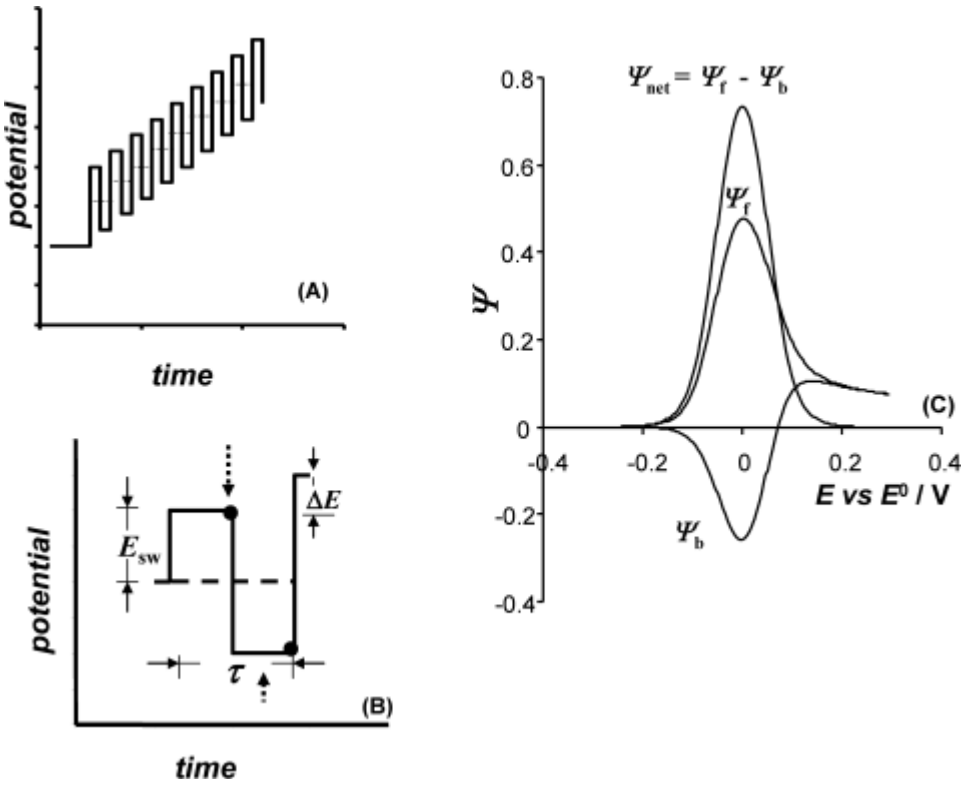


Figure 1 Potential waveform (A), one potential cycle (B), and typical voltammogram in square-wave voltammetry (C). The response consists of a forward (anodic, Ψ_f), backward (cathodic, Ψ_b) and net (Ψ_{net}) component (Mirceski et al., 2013).

Modern SWV, incorporated in digital electrochemical instruments, utilizes a combination of a staircase potential modulation and periodic square-shaped potential function, applied at a stationary electrode (Mirceski et al., 2013).

1.1.3. Chemically Modified Electrodes

Chemically modified electrodes (CMEs) comprise a relatively modern approach to electrode systems that find in applications a wide spectrum of basic electrochemical investigations, including the relationship of heterogeneous electron transfer and chemical reactivity in electrode surface chemistry, electrostatic phenomena on electrode surfaces, electron and ionic transport phenomena in polymers, as well as the design of electrochemical devices and systems for applications in chemical sensing, energy conversion and storage, molecular electronics, electrochromic displays, corrosion protection, and electro-organic synthesis (Durst, 1997). One of the ways to prepare CMEs is using mechanical attachment technique. This technique is based on the mechanical immobilization of solid particles at the surface of carbon or metal electrodes in the absence of binders. This technique has widened the possibility of solid-state electroanalysis due to its broad applicability (Janeiro and Brett, 2005).

Importantly, these CMEs, which are made by incorporating specific chemical groupings or "microstructures" on conventional electrode surfaces, are of interest because their responses have two completely separate components: the usual electrochemical component determined by the potential at which the electrode is maintained instrumentally and an additional chemical component determined by the reactivity of the attached group (Anton Alexandru, 2014). Consequently, CMEs offer not only easily variable redox characteristics but also the possibility of adjustable physical and chemical properties (such as charge, polarity, chirality, permeability) (Baldwin and Thomsen, 1991). The ability to manipulate the molecular architecture of the bulk matrix of an electrode and its surface in particular has led to a wide range of analytical applications of CMEs and created powerful opportunities for electroanalysis (Anton Alexandru, 2014). Typically, the reaction under investigation would either generate a measurable current (amperometric), a measurable potential or charge accumulation (potentiometric) or measurably alter conductive properties of a medium between electrodes (conductometric) (Chaubey and Malhotra, 2002).

The developments of CMEs are still of interest among many research groups and these electrodes have been widely applied to different fields. Piovesan’s group has modified a glassy

carbon electrode (GCE) with gold nanoperticles (AuNPs) and carboxymethylcellulose and applied the electrode to the determination of the antioxidant catechin extracted from the dietary fibre, Acacia, with LoD of 0.274 μM (Piovesan et al., 2017).

There are many different applications of CMEs, depending on the targeted objectives. Some of them focused on the detection of metal ions (Cazula and Lazarin, 2017, Macías-García et al., 2017, Rana et al., 2017, Alizadeh et al., 2017), biosensors (Dinesh et al., 2017, Mayuri et al., 2017, Palanisamy et al., 2017), nucleic acids (Makra et al., 2017, Honarvarfard et al., 2017), water analysis (Ali et al., 2017, Zhu et al., 2017b, Abrego et al., 2017), organic reagents (da Silva et al., 2017, Zhao et al., 2017, Cheng et al., 2017, Hsieh and Whang, 2017, Zhu et al., 2017b, Zhu et al., 2017a, Palakollu et al., 2017), inorganic compounds (Mohammadi et al., 2017, Kesavan et al., 2017, Movlaee et al., 2017), cell viability (Hassan et al., 2017, Sedki et al., 2017), and *etc*. Most of them used common electrodes, such as GCEs, graphene, tin oxide and gold electrode, which can be easily modified. As pertaining those aforementioned, new modifiers were also being developed and applied on conventional electrodes .

Theoretically, the current generated by an analyte will be increased if the CMEs used are compatible with the analyte. Compatible means that the CMEs will be shifting the equilibrium toward the reactant (oxidation process) where more analytes will be oxidised. Furthermore, the concentration of the analyte is directly proportional to the current generated as shown in Cottrell equation (Eq. 5). Thus, current generated by an analyte will be increased. Therefore, electrochemists often focus on the current produced by an analyte. The current response is described by the Cottrell equation (Eq. 1) for a planar electrode. Current (I) is directly proportional to concentration (C) in which that the other parameters are constant (according to Eq. 1).

$$I = \frac{nFAD^{\frac{1}{2}}C}{\prod^{\frac{1}{2}} t^{\frac{1}{2}}} \qquad \text{Eq 5}$$

There are many approaches on detecting the presence of antioxidants using electrochemical methods, but to date there is no standard procedure being developed. Therefore in this study, we are developing a chemical sensor using electrochemical approach for determination of the presence of the antioxidant in a compound.

1.2. High Performance Liquid Chromatography Analysis

The principle underlying high performance liquid chromatography (HPLC) is that the studied components are separated between a stationary phase and a mobile phase under high pressure by elution with different solvents. In this study, the detection of individual compounds is achieved by the use of diode array detection, which allows the collection of spectra and simultaneous quantification by several wavelengths (Mattila et al., 2000). One main advantage of HPLC is that better separation can be achieved compared to thin layer chromatography. This can be achieved through manipulation of various parameters such as pressure, temperature and gradient of solvents.

1.3. Antioxidants

Plants contain high concentrations of numerous redox-active antioxidants, such as polyphenols, carotenoids, tocopherols, glutathione, and ascorbic acid as well as enzymes with antioxidant activity, which fight against hazardous oxidative damage of plant cellular components (Pisoschi et al., 2009). In animal cells, antioxidant production is much more limited and oxidative damage is involved in aging and pathogenesis of most of the chronic degenerative diseases or clinical conditions, including cancer and heart diseases (Pisoschi et al., 2009, Duthie and Crozier, 2000). Antioxidants are compounds that inhibit or delay the oxidation process by blocking the initiation or propagation of an oxidising chain reaction, which leads to oxidative damage (Apetrei et al., 2011). In general, plant-sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytates and phytoestrogenes have been recognized as having the potential to reduce risk of disease occurrence (Pisoschi et al., 2009). Phenolic compounds exist in plants ubiquitously, and they have attracted attention as potential agents for preventing and treating many oxidative stress-related diseases. It has been reported that polyphenolic compounds have a great deal of benefits to human health due to their pharmacological properties of anti-inflammatory, anti-oxidative, anti-mutagenic, anti-carcinogenic and antihistaminic (Abdel-Hamid and Newair, 2011).

Analysis of antioxidants often involves methods such as HPLC, mass spectrometry, capillary zone electrophoresis, and spectrophotometry which usually need expensive equipment, complicated operation and a large amount of toxic organic solvents (Beitollahi and Sheikhshoaie,

2011, Baghayeri and Namadchian, 2013, Baghayeri et al., 2014). In contrast, the electrochemical detection methods involving CMEs have received increasing interests in environmental, clinical and food analysis owing to their intrinsic advantages of high sensitivity, good selectivity, simplicity and low-cost (Gao et al., 2015, Yang et al., 2010).

Flavonoid is a generic name for a large group of plant metabolites, which are mostly derived from the biosynthetic route of shikimic acid (Gil and Cout, 2013). They have a C_6-C_3-C_6 general core (Figure 2), which is represented by three units, A, B and C. Units A and B are essentially aromatic rings of phenolic nature, whilst unit C is an oxygen containing hetereocycle, benzo-γ-pirone, whose level of oxidation, namely the presence or absence of: C_2-C_3 double bond; C_3-OH hydroxyl group; and C_4-keto group defines their subclasses and also the controversial nomenclature (Tsao, 2010). The electroactivity of flavonoids resides on the phenolic groups and the electron donor ability is mainly governed by B-ring chemistry (Gil and Cout, 2013) as shown in Figure 2.

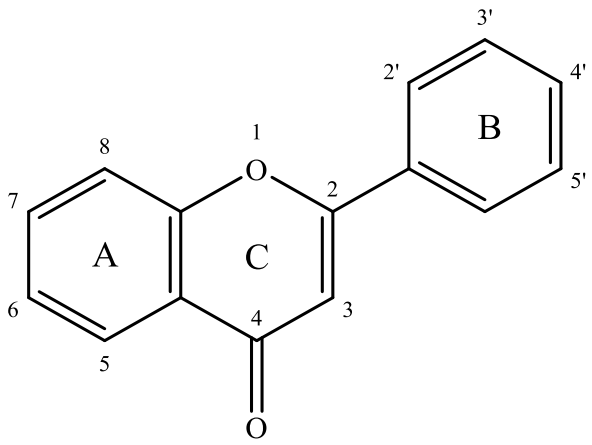


Figure 2 A basic chemical structure of flavone (Filipiak, 2002).

A total of three antioxidants including gallic acid (Abdel-Hamid and Newair, 2011, Barroso et al., 2012, Barros et al., 2011), quercetin (Jørgensen et al., 1998, Knekt et al., 2002, Yao et al., 2014) and rutin (Hu et al., 2012, Medvidović-Kosanović et al., 2010, Zare et al., 2009) has been studied. Their corresponding chemical structures are shown in Figure 3, Figure 4 and Figure 5.

Many antioxidants exhibit inherent electroactivity, acting as reducing agents in solutions (Kilmartin et al., 2001). Therefore, employing electrochemical methods could be a viable approach for evaluating the overall reducing power of antioxidant compounds within a fresh produce matrix without the need for added reactive species (Blasco et al., 2005).

1.3.1. Gallic Acid

Gallic acid (3,4,5-trihydroxybenzoic acid) can been found in fruits, vegetables and drinks (Ghoreishi et al., 2011). Gallic acid is one of the main polyphenolic compounds (Wang et al., 2007). Notably, gallic acid is often used as an electrochemical standard in the estimation of the total polyphenol content or antioxidant capacity index in foods (Gao et al., 2015).

There has been a lot of on-going research on the analytical detection of gallic acid using different modified electrodes. For example, gallic acid was detected at a boron-doped diamond film electrode in 0.1 M perchloric acid as a supporting electrolyte (Panizza and Cerisola, 2009). Panizza and Cerisola (2009) concluded that the degradation of gallic acid evidenced a pseudo first-order kinetics and the rate constant increased with applied current**.** Similarly, Abdel-Hamid and Newair (2013) reported a 2-fold improved detection of gallic acid at a polypinephrine-modified GCE as compared to bare GCE. By using a thionine and nickel hexacyanoferrate modified graphite electrode to detect gallic acid, Sangeetha and Narayanan achieved a limit of detection of 1.66 μM (2014). Liang *et al.* (2016) took advantage of the unique plane structure of sulfonated graphene has facilitated the immobilisation of a gold microcluster, which was then immobilised on a GCE and exhibited lowest LoD value (10.7 nM).

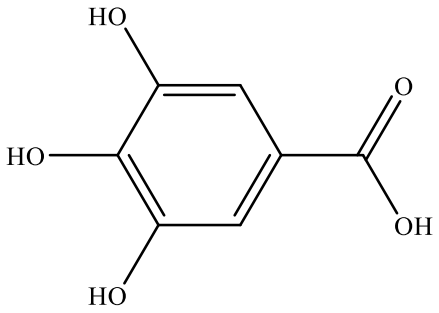


Figure 3 Molecular structure of gallic acid.

1.3.2. Quercetin

Quercetin is known to exhibit anti-cancer, anti-allergic, anti-inflammatory and antiviral activities. The chemical structure of quercetin, depicted in Figure 4 , shows that it consists of a resorcinol ring (ring A), a catechol ring (ring B) and a hydroxyl-bearing ring at position 3 (ring C) (Reddaiah et al., 2012). As an electrochemically active species, electrochemical detection of quercetin is highly preferable due to intrinsic advantages of higher sensitivity, low cost and less interferences from non-electroactive substances (Sun et al., 2013).

There have been many studies focussing on the detection of quercetin using different modified electrodes including activated silica gel-modified carbon paste electrodes (Chen et al., 2012), carbon nanotube paste electrodes (CNTPE) (He et al., 2005, Lin et al., 2006), copper microparticle modified carbon nanotube paste electrodes (Oliveira and Mascaro, 2011), Co_3O_4 nanoparticle-modified GCEs (Wang et al., 2011) and a carbon nanotube/nafion composite modified GCEs (Li and Huang, 2015).

In addition, by detecting quercetin at a platinum nanoparticle/poly(hrdroxymethylated-3,4-ethylanedioxylthiophene) nanocomposite-(PtNP/PEDOT-MeOH) modified GCE in 0.1 M phosphate buffered saline (PBS), an LoD of 5.2 nM was achieved (Yao et al., 2014). These authors attributed the sensitive detection of quercetin at the PtNP/PEDOT-MeOH/GCE to the synergic effects of the electrocatalytic activity and strong adsorption ability of PtNP together with the good water solubility and high conductivity of PEDOT-MeOH. In another study, an LoD of 27.6 ÅM quertin in green tea was reported at a siloxane-polyester/poly-L-lysine nanocomposite modified GCE in a supporting electrolyte of Britton-Robinson buffer 0.04 M with ethanol (1:1 v/v) (Pereira *et al.*, 2016). The authors highlighted the application of a hybrid of organic (poly-L-lysine) and inorganic (siloxane-polyester) material has aided in achieving the low LoD.

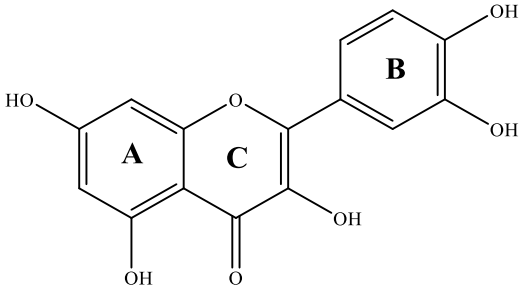


Figure 4 Molecular structure of quercetin.

1.3.3. Rutin

Rutin (vitamin P) is a therapeutically drug with physiological functions such as anti-flammatory, antitumor and anticancer. As rutin (the chemical structure is shown in Figure 5) is flavonoid glycoside compound with electrochemical activity, electroanalytical technique has been used for the direct detection of rutin, which shows many advantages including high sensitivity, simple and speedy procedure, low cost equipment with wider dynamic range (Hu et al., 2012).

A Ag nanoparticle/poly(methylene blue) functionalized graphene (Ag/NP/PMB-GR) composite film-modified Au electrode was firstly constructed via a facile electrochemical method (Yang et al., 2014). The electrochemical behaviour of rutin was investigated at the modified electrode, and the results demonstrated that the AgNP/PMB–GR could remarkably increase the redox currents of rutin. These results might be attributed to the synergistic effect of AgNP, PMB and GR on the AgNP/PMB– GR/Au electrode. PMB, as an electroactive conjugated cationic dye, always acts as a redox indicator. Moreover, PMB can provide with a p-conjugative structure. This unique property would allow the organic conjugated compound of rutin to interact with PMB through p–p electronic interactions, leading to accumulation of more rutin molecule at the electrode surface. In this way, an increased redox current of rutin was expected. Based on differential pulse voltammetry (DPV), three separate linear calibration ranges between 0.01 and 0.1 μM, 0.1 and 2.0 μM, and 2.0 and 10.0 μM rutin, and a limit of detection of 0.01 M were reported (Yang et al., 2014).

A chemically cross-linked copper-complexed chitosan/multiwalled carbon nanotube-modified GCE (Cu-CS/MWCNT/GCE) was constructed and used to detect rutin in 0.04 M Britton-Robinson buffer (pH 3) (Gholivand et al., 2016). An LoD of 0.01 μM was estimated. The Cu–CS/MWCNT GCE has also shown good selectivity, stability, and reproducibility.

In addition, electropolymerisation of pyrrole on a graphene-multiwall carbon nanotube composite (G-MWCNT) coated GCE was developed and applied to the detection of rutin (Yang et al., 2016). The netlike G-MWCNT composite, prepared by an in situ hydrothermal process, offers high conductivity, electrocatalytic activity, and an LoD of 5.0 nM was estimated.

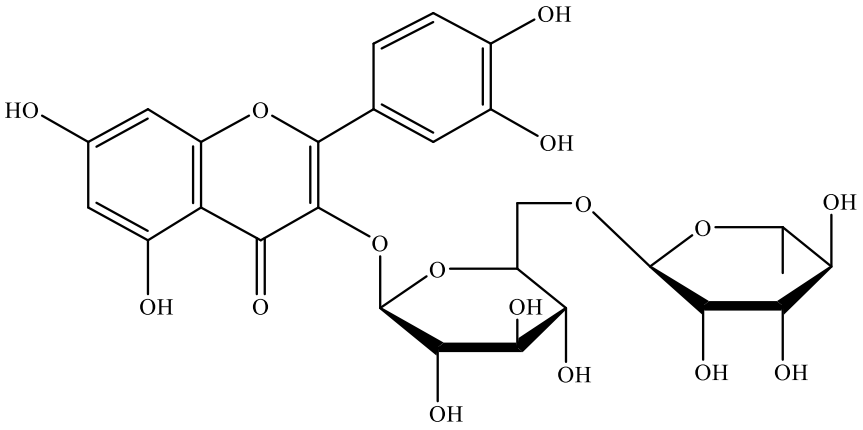


Figure 5 Molecular structure of rutin.

2 Research Background

Detection of antioxidants can be done with various methods as mentioned in Chapter 1. As compared to other methods, electrochemical study is rather fast and easy to identify the targeted antioxidants. Furthermore, electrochemical study is cheaper because the usage of solvent or electrolyte is much lesser than other detection methods. Thus, electrochemical study is good at benchmarking specific antioxidant for initial detection stage in a research. Electrochemistry can be used to quantify and qualify an antioxidant of interest through a series of electrochemical studies, including CV and SWV. The main technique utilised in this research is electrochemistry. HPLC will be used as a supporting technique as HPLC is well known for its high accuracy and its ability to identify an analyte.

Although a lot of research had focused on the detection of antioxidants, there was no universal detector for the presence of antioxidants. Therefore, the objective of this project was to develop a universal antioxidants detector. A universal detector is capable of identifying the specific antioxidant(s) in a sample, both quantitatively and qualitatively. It will be a huge breakthrough in electrochemistry if a universal detector is developed. In contrast, if that developed detector cannot be used universally, then individual detector will be developed to detect the desired or targeted antioxidant(s).

As aforementioned, electrochemists have been focusing on using different type of modifiers onto different type of working electrodes for detecting the presence of antioxidants. Another main criterion in selection of modified electrode is the LoD. LoD is referring to the ability of a detector (working electrode) in detecting the analyte(s) at its (the analyte) lowest concentration. An electrode with a lower LoD will be chosen as the working electrode (Armbruster and Pry, 2008). Thus, lower value of LoD of an electrode will be chosen as the working electrode. Every researcher had been struggling to develop a modified electrode with low LoD value and yet it is hard to achieve. In order to become a good modified electrode, the modifier must have high conductivity and yet undetectable under the studied range of potential in a voltammogram. Other than that, the modifier must not dissolve in the supporting electrolyte.

A number of antioxidants, which abundantly present in plants, will be selected in the first stage of the research. Antioxidants of interest are of plant origin and belonged to the phenolic and polyphenolic class of compounds as well as carotenoids and antioxidant vitamins, among others (Shahidi, 2015). The plant of interest is originated from Leguminosae family. Many plants from

the Leguminosae family are medicinal herbs that can be easily found in Malaysia. They can be seen growing as weeds (i.e. Mimosa pudica), woody shrubs (Peltophorum pterocarpum), crops (Arachis hypogaea) and vines (Bauhinia kockiana). Some of these plants are edible, and hence they are utilised for various purposes in food, beverages, and as food colouring agent (Chew et al., 2009). Extraction of antioxidants from the plant is not carried out in this study, where the extracted compound need to be characterised and identified. The plants from this family were extensively studied, thereby the selection of antioxidants of interest will be carried out according to published literature (Chew et al., 2009, de Rijke et al., 2004). Lastly, commercially available antioxidants will be purchased according to the list of selected antioxidants.

3 Objectives

This research is aimed at identifying the best modified electrode in detecting the presence of selected antioxidants (gallic acid, quercetin and rutin), either in bulk or individually. The antioxidants will be characterised using electrochemical techniques including CV and SWV. A series of CMEs with different modifiers (MWCNT, bismuth(III) oxide (Bi_2O_3), bismuth(III) tungstate (Bi_2WO_3), bismuth oxybromide (BiOBr), fluorine doped tin oxide (FTO), and indium doped tin oxide (ITO)) were designed and used to detect the selected antioxidants. Even though the selected antioxidants have been studied by other researchers, characterisations of antioxidants and CMEs are required as the experimental parameters may varies from case-to-case. This is then followed by optimisation of CMEs, which includes the optimum amount of modifier that needed to be attached onto GCE, selection of modifier that compatible with the targeted antioxidants, and stability of the modifier within the supporting electrolyte. Lastly, the characterised and optimised CMEs will be used as a detector to detect antioxidants within the commercially available supplements, both qualitatively and quantitatively.

4 Experimental

4.1. Electrochemical Analysis

All chemicals and antioxidants used in this study were purchased from Sigma-Aldrich. The antioxidant compounds (gallic acid, quercetin and rutin) were studied electrochemically using a three-electrode system (Figure 6) including a 3-mm diameter glassy carbon working electrode (WE), a NaCl saturated Ag|AgCl, reference electrode (RE), and a 1-mm diameter platinum counter electrode (CE).

The antioxidant compound (1 mM final concentration) was dissolved in 10 mL of 0.1 M of supporting electrolyte and placed into an electrochemical cell as shown in Figure 6. The solution was degassed using nitrogen to remove dissolved oxygen and then blanketed with nitrogen to prevent oxygen from dissolving in the solution. After that the three electrodes were immersed in the solution and a voltammogram was recorded using the Epsilon electrochemical workstation. Unless otherwise mentioned, the temperature was (25 ± 2) ºC.

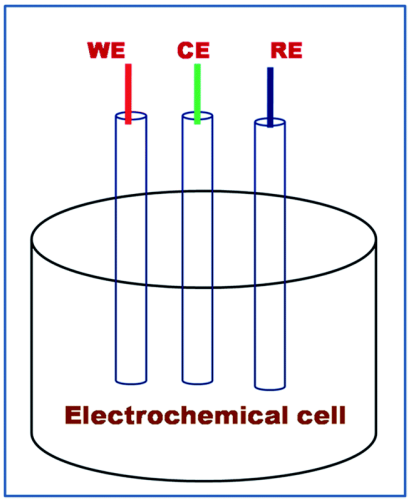


Figure 6 Electrochemical cell – three electrode system.

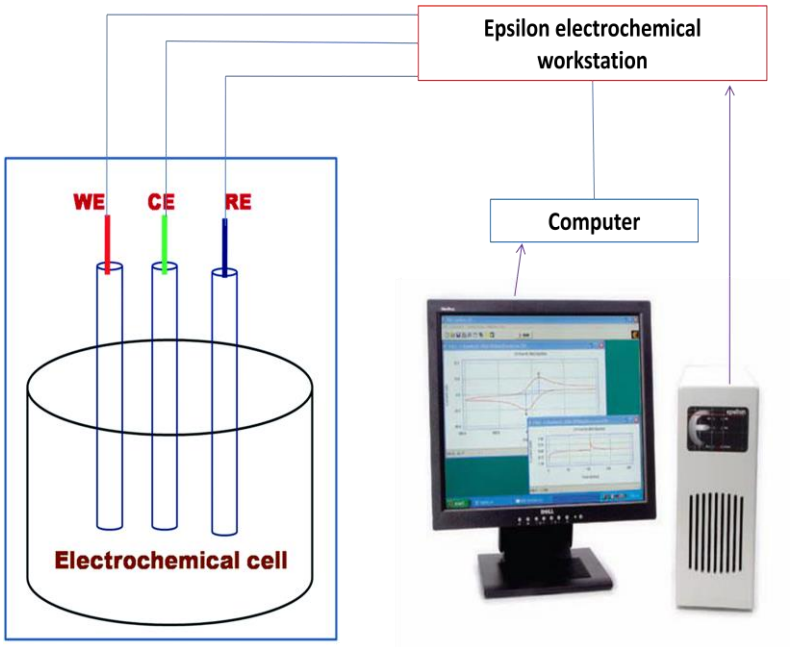


Figure 7 Epsilon electrochemical workstation.

4.2. High Performance Liquid Chromatography Analysis

For each studied commercially available supplements, one tablet of the supplement was dissolved in 50 mL of ethanol and water mixture (1:1 v/v), and filtered with 0.22 µm syringe filter (PTFE). Reverse phase HPLC was carried out by using a C18 HPLC column. The flow rate and the temperature were set at 1 mL/min and 25 °C, respectively. The mobile phases utilized were HPLC grade methanol and water. A linear gradient from 5 % methanol to 100 % methanol for the first 45 min, followed by 15 min of 100 % methanol (isocratic) was used to elute the samples. Elution profile of the studied supplements was monitored at 210 nm. In addition to those aforementioned, spiking technique was used where the respective supplements was premixed with gallic acid or quercetin accordingly at 5 mg/mL before subjected to HPLC analysis.

5 Results and Discussion

5.1. Gallic acid

5.1.1. Selection of Supporting Electrolyte

The fundamental criterion to be a good supporting electrolyte is that it should not contain any peak(s) in redox reaction. Besides that, being a good supporting electrolyte, it should not have high capacitance value (area under its graph), as it will be hindering the expression (current peak) of an analyte in a voltammogram. In the end, the current peak of an analyte will not be observed if supporting electrolyte with high capacitance was used.

In this work, it is important to identify a suitable supporting electrolyte for use in the cyclic voltammetry of gallic acid. Therefore, cyclic voltammetry of a series of supporting electrolytes including 0.1 M potassium hydroxide (KOH), 0.1 M potassium chloride (KCl), 0.1 M potassium dihydrogen phosphate (KH_2PO_4), 95% ethanol, and 0.1 M phosphate-buffered saline (PBS), a mixture of 0.1 M PBS and ethanol (1:1 v/v) at GCE was conducted and the results obtained are shown in **Error! Reference source not found.**. In these voltammograms, there were no observable oxidation and reduction peaks within the potential range, except those obtained in the presence of 0.1 M KOH, 0.1 M KH_2PO_4 and 0.1 M PBS with ethanol (1:1 v/v).

Based on the Figure 8**Error! Reference source not found.**, redox peaks (compared at the same range of current in y-axis) were absence for most of the supporting electrolytes. Four out of six listed supporting electrolytes (KOH, KCl, PBS, and KH_2PO_4) had shown potential window (the rising current on the right and reducing current on the left in a voltammogram) that might hinder the current peak of an analyte. Thus, ethanol and mixture of 0.1 M PBS with ethanol was the best selection as supporting electrolyte in this research.

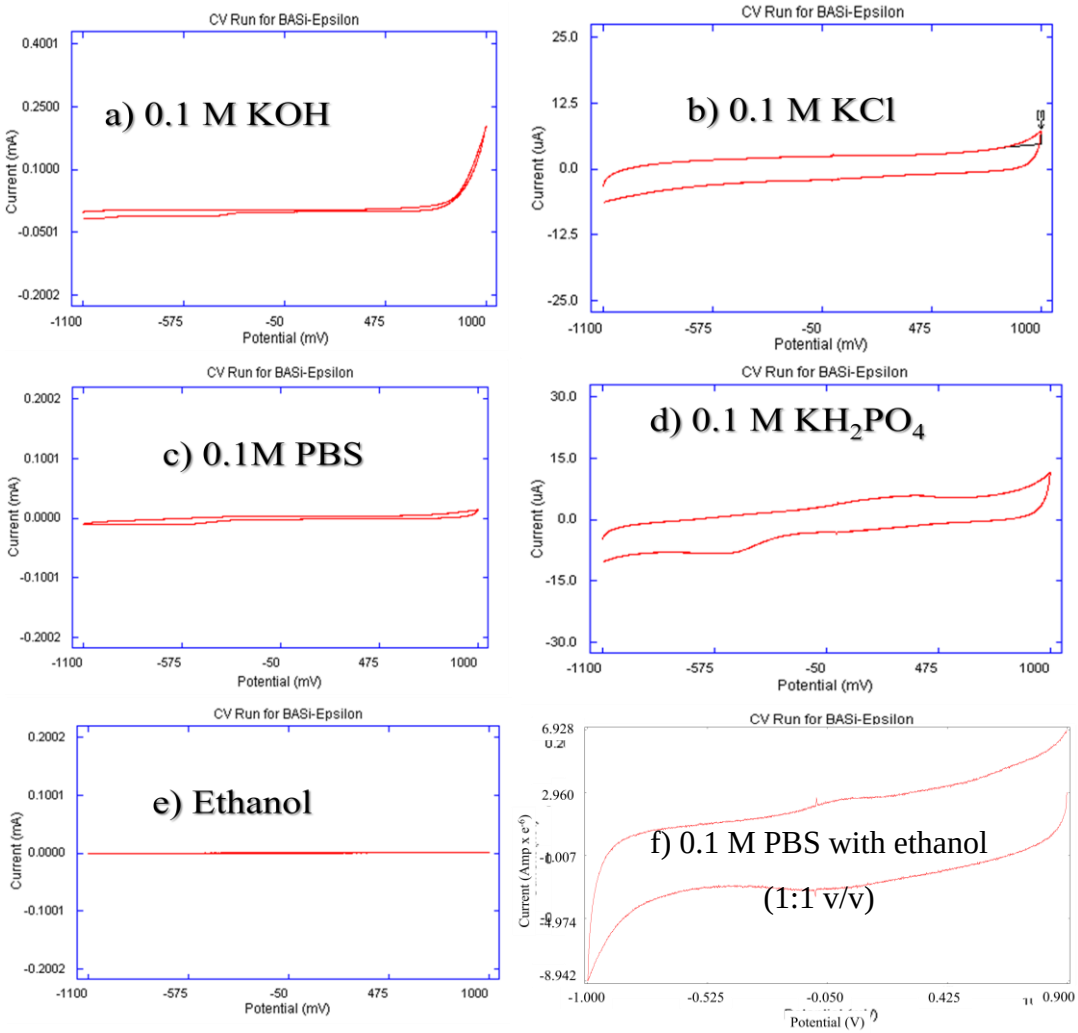


Figure 8 Cyclic voltammograms of different supporting electrolytes **a) 0.1 M KOH, b) 0.1 M KCl, c) 0.1 M PBS, d) 0.1 KH_2PO_4, e) ethanol, and f) mixture of ethanol and 0.1 M PBS (1:1 v/v) without any analyte at scan rate of 100 mV/s.**

Gallic acid exhibited two oxidation peaks at 429 mV (Ep_a I) and 671 mV (Ep_a II) and current peaks (intensity) of 28.6 µA (Ip_a I) and 1.00 µA (Ip_a II) respectively in Figure 9. In this mixture of electrolytes, gallic acid did not exhibit nice and strong antioxidant properties as shown in Figure 9. Therefore, we have pursued in using the CMEs to develop a suitable electrochemical sensor for gallic acid which could increase the sensitivity by observing the current peak.

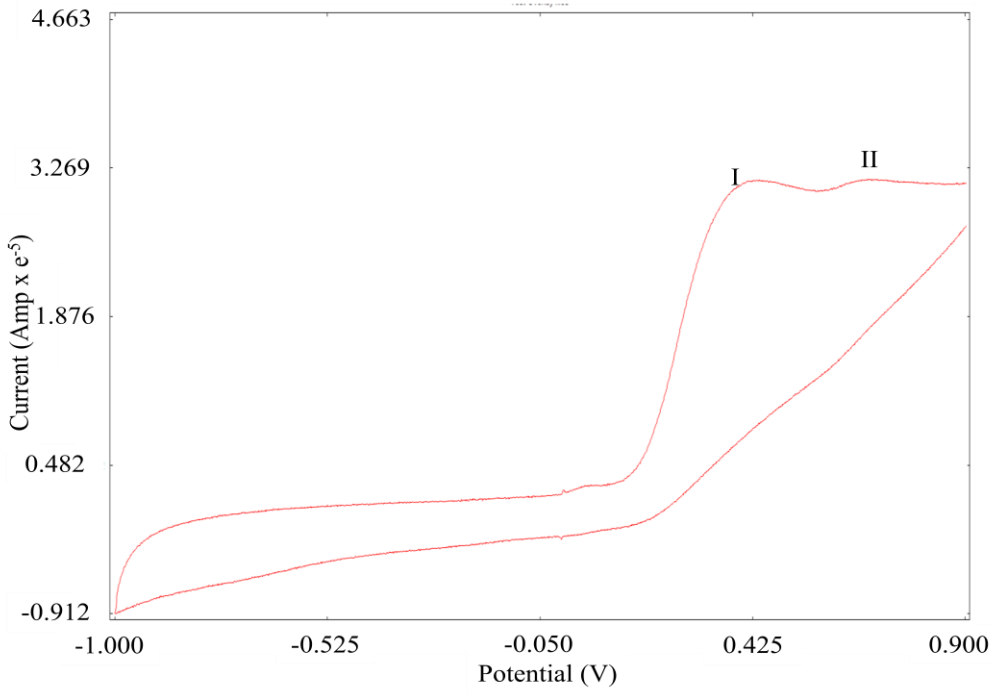


Figure 9 Cyclic voltammogram of gallic acid at bare GCE in 0.1 M PBS-ethanol (1:1 v/v) at scan rate of 100 mV/s.

5.1.2. Selection of Modified Electrodes

We have used several materials including multi-wall carbon nanotubes (MWCNTs), bismuth oxide (Bi_2O_3), bismuth oxybromide (BiOBr) in 3 different states (pristine, oxygen vacancy and bismuth and oxygen in excess), and bismuth tungstate (Bi_2WO_6) in 3 different states (pristine, oxygen vacancy and ultrathin) to modify GCEs. All materials were applied to a GCE surface using mechanical attachment. Overlaid cyclic voltammograms of gallic acid obtained at a MWCNT-modified GCE (trace a), a GCE (trace b), and a Bi_2O_3-modified GCE (trace c) in 0.1 M PBS with ethanol (1:1 v/v) are shown in Figure 10. In these voltammograms, there is a 48.9% increase and 52.4% decrease in the peak current in trace a and trace c, respectively, compared to trace b.

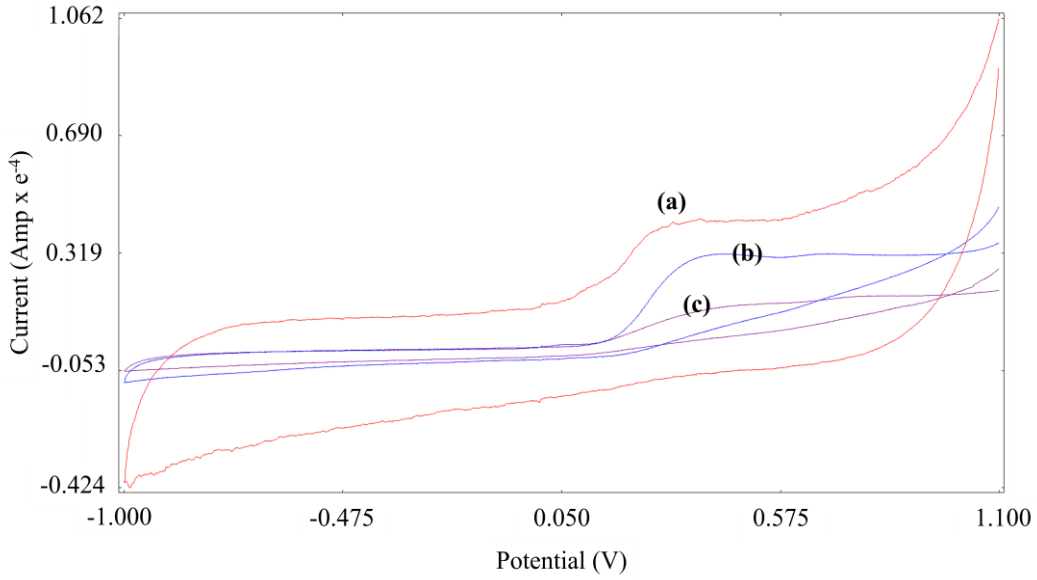


Figure 10 Overlaid cyclic voltammograms of gallic acid in different type of modified electrodes; a) GCE modified with MWCNTs (GCE-MWCNTs), b) GCE and, c) GCE modified with bismuth (III) oxide (GCE-Bi_2O_3), in 0.1 M PBS-ethanol (1:1 v/v) at scan rate of 100 mV/s.

Other than the mentioned CMEs, we have used other types of working electrodes such as indium tin oxide (ITO) electrode and a fluorine doped tin oxide (FTO) electrode. ITO films have shown good efficiency for hole injection into organic materials (Kim et al., 1999). Fluorine doped tin oxide has large surface area and good electrical conductivity, good electrocatalytic properties, mechanical stability, high selectivity, availability, low cost and environmentally friendly (Miranda et al., 2012). The effect of the fluorine content on the electrical properties of FTO was investigated by Elangovan and Ramamurthi (2005) who reported that degradation in the electrical properties is probably due to the solubility limit of fluorine content in the thin film. As reported by Banyamin et al. (2014) electrical properties of the thin film as function of the fluorine doping level revealed that a resistivity as low as 6.71 mΩ•cm was obtained.

According to Figure 11, gallic acid was detected at FTO and ITO, respectively. Peak current generated by gallic acid was quite high where 497 µA (at ITO) and 108 µA (at FTO). This value cannot be compared to the peak current of gallic acid at GCE as the base of the mentioned electrodes were different. GCE is carbon-based while ITO and FTO are glass-based electrodes. Based on the Figure 12, Figure 13, and Figure 14, there was a significant difference between the presence and absence of gallic acid. GCE modified with MWCNTs was the least favoured as the capacitance (area under the graph) generated in MWCNTs was higher as compared to ITO and FTO. The "real" peak current generated by the gallic acid might be masked under the capacitance generated by MWCNTs. Therefore, ITO and FTO were preferred over GCE modified with MWCNTs.

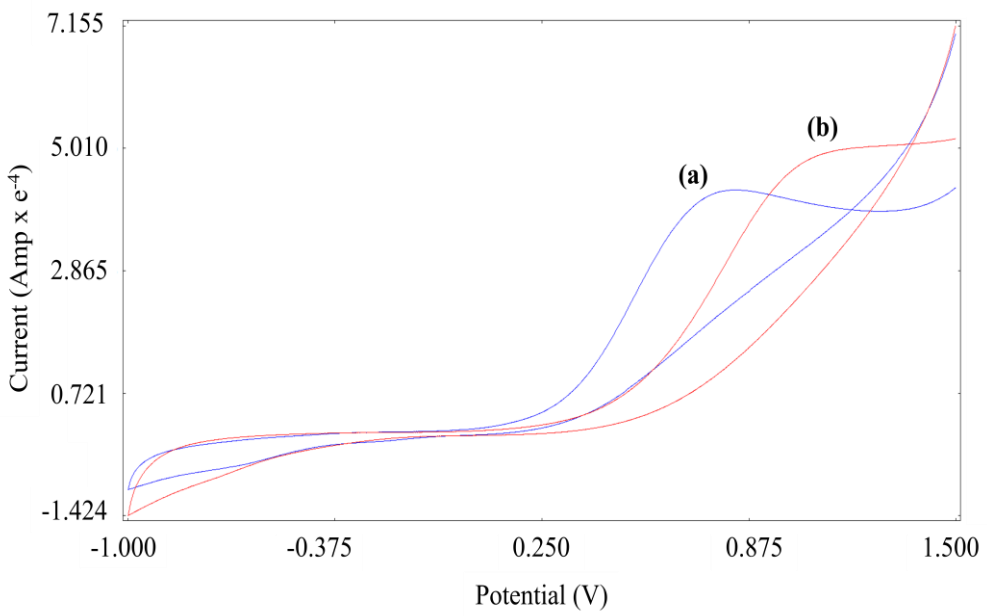


Figure 11 Cyclic voltammogram of gallic acid in a) ITO and b) FTO working electrodes in 0.1 M PBS-ethanol (1:1 v/v) at scan rate of 100 mV/s.

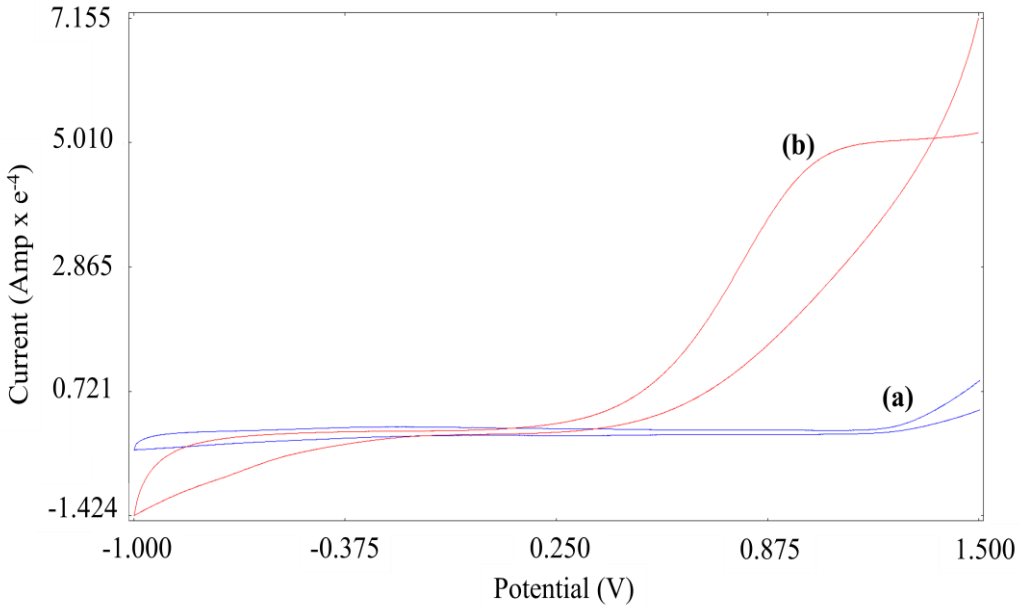


Figure 12 Cyclic voltammogram at a) ITO with absence of gallic acid, and b) ITO with presence of gallic acid in 0.1 M PBS-ethanol (1:1 v/v) at scan rate of 100 mV/s.

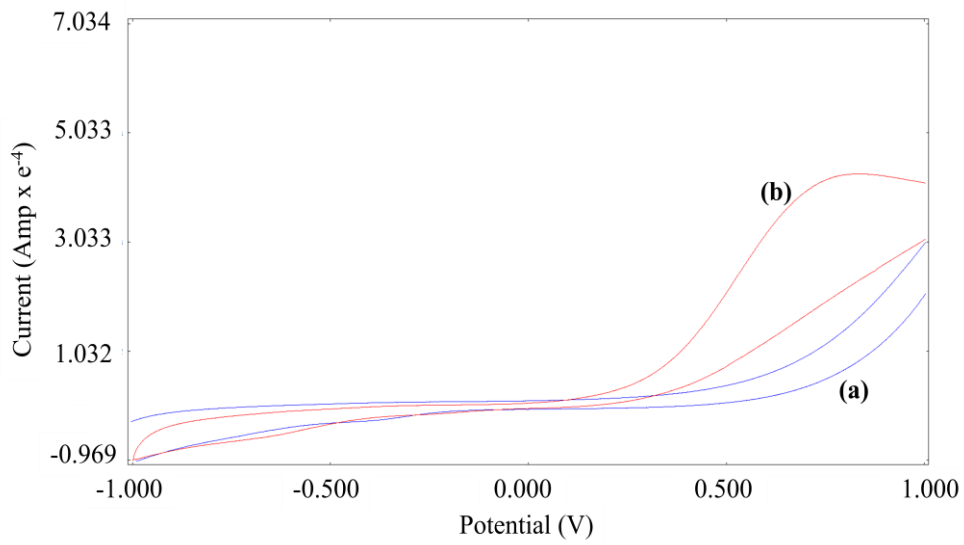


Figure 13 Cyclic voltammogram at a) FTO with absence of gallic acid, and b) FTO with presence of gallic acid 0.1 M PBS-ethanol (1:1 v/v) at scan rate of 100 mV/s.

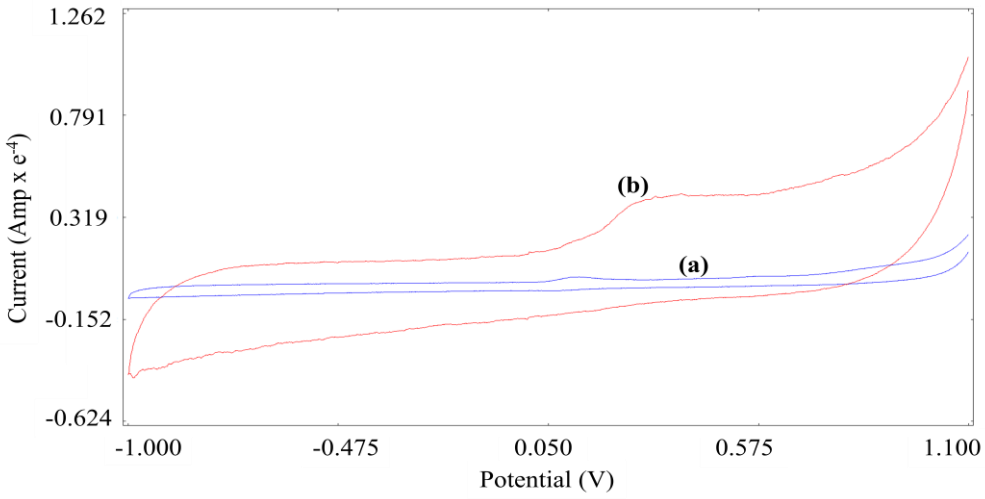


Figure 14 Cyclic voltammogram at a) GCE modified with MWCNTs with absence of gallic acid, and b) GCE modified with MWCNTs with presence of gallic acid 0.1 M PBS-ethanol (1:1 v/v) at scan rate of 100 mV/s.

5.1.3. Square Wave Voltammetry of Gallic Acid

Square wave voltammetry (SWV) is well known in its higher sensitivity, rejection of background currents, and its speed in increasing the signal-to-voice ratio if coupled with computer control and signal averaging (Kounaves, 1997) as compared to CV. SWV can only generate result pertaining either oxidation or reduction process in a voltammogram at one time. Besides that, antioxidant properties can be observed in oxidation process only. Thus, SWV was used to focus on the oxidation process of an antioxidant.

A series of SWV traces for the oxidation of gallic acid at different electrodes is shown in Figure 15. The detection strength is same as in CV results where ITO is the best detector for gallic acid among those tested electrodes. The mentioned detection strength was the ability of producing the same results and yielding the same outcome as compared to CV. As compared to CV voltammogram, gallic acid oxidation peak in SWV voltammogram exhibited a higher, sharper, better resolution, and significant oxidation peak. Therefore, SWV is chosen as the main electrochemical technique in detecting the presence of gallic acid.

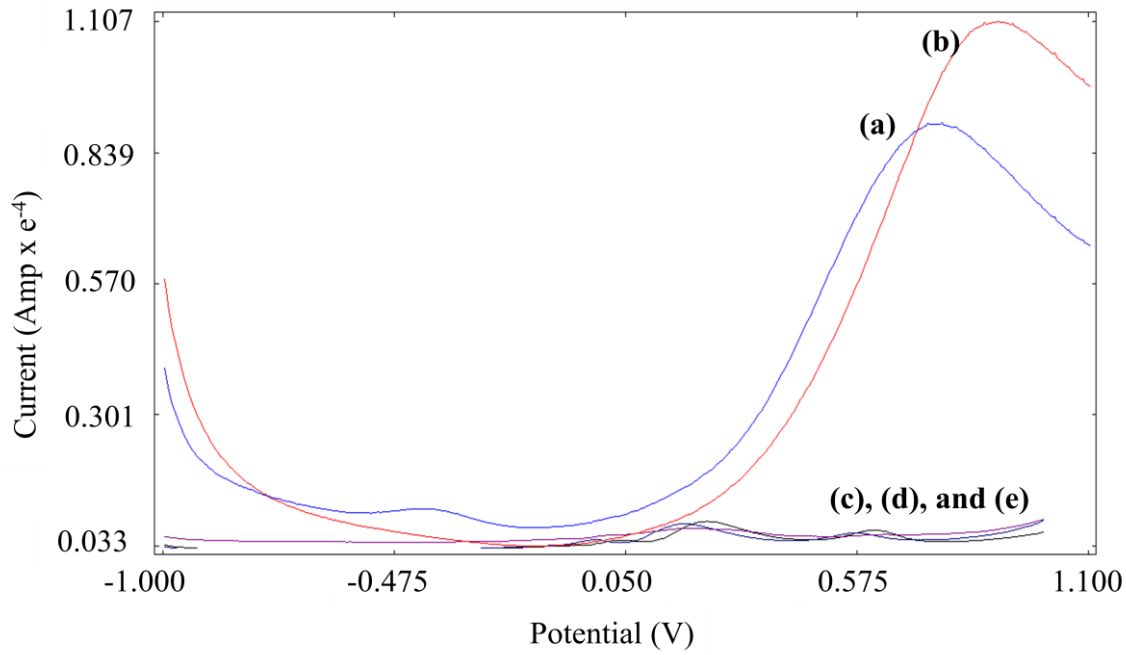


Figure 15 Square wave voltmmogram of gallic acid in different types of working electrodes; a) ITO, b) FTO, c) CNT-GCE, d) Bi_2O_3-GCE, and e) GCE in 0.1 M PBS-ethanol (1:1 v/v) at scan rate of 15 Hz.

5.2. Quercetin

5.2.1. Selection of Supporting Electrolyte

Quercetin (3,3´,4´,5,7-pentahydroxyflavone) is found predominantly in vegetables and fruits (Abdel-Hamid et al., 2012). Quercetin is also a natural pentahydroxyflavone which is believed to exhibit estrogenic and anticancer activity by acting as an effective radical-scavenger against oxidative cell damage (Zhou et al., 2007). Various epidemiological studies have shown that quercetin and related isoflavonoids were able to suppress cancerous tumor growth in vivo and in vitro(Zhou et al., 2007).

A preliminary test was conducted on the solubility of quercetin in different potassium salt based solutions, a fundamental inelectrochemical medium selection, as the supporting electrolytes. The tested supporting electrolytes were 0.1 M potassium hydroxide (KOH), 0.1 M potassium chloride (KCl), 0.1 M potassium dihydrogen phosphate (KH_2PO_4), 95% ethanol, 0.1 M phosphate-buffered saline (PBS), and mixture of 0.1 M PBS and ethanol (1:1 v/v). Among all those stated supporting electrolytes, mixture of PBS with ethanol (1:1 v/v) was the best as shown in Figure 16.

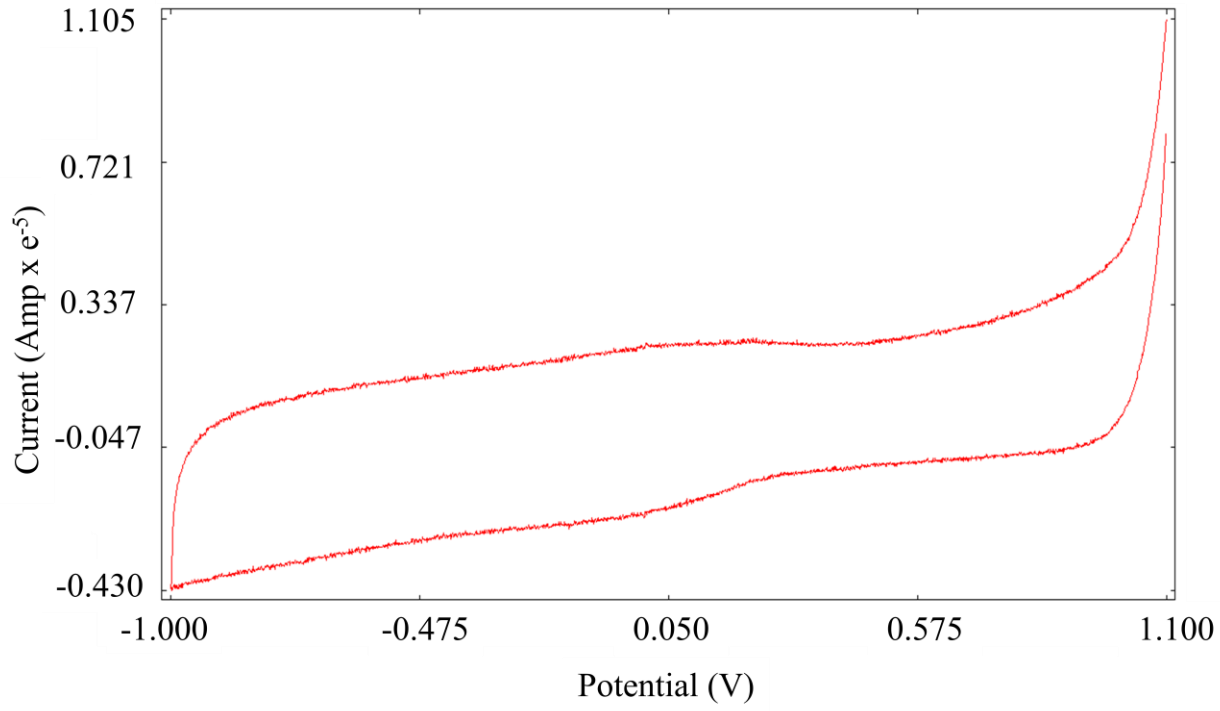
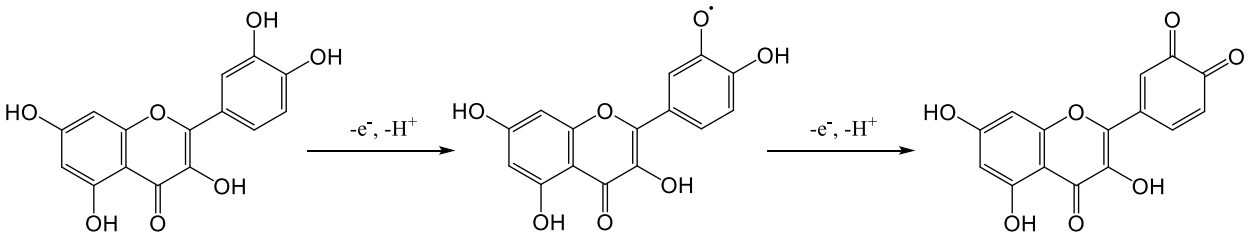


Figure 16 Cyclic voltammogram of mixture of 0.1 M PBS with ethanol (1:1 v/v) without quercetin at scan rate of 100 mV/s.

5.2.2. Cyclic Voltammetry of Quercetin

Among all the tested supporting electrolytes, mixture of 0.1 M PBS-ethanol (1:1 v/v) was the best for quercetin in exhibition of the redox reaction. Quercetin demonstrated a nice redox reaction as shown in Figure 17. There are two oxidation peaks (Ep_a) and one reduction peak (Ep_c) in the cyclic voltammogram. Quercetin oxidized twice at 179 mV (Ep_a I) and 653 mV (Ep_a II) and reduced at 124 mV (Ep_c I). Current peak of quercetin in oxidation reactions were 3.5 µA (Ip_a I) and 2.6 µA (Ip_a II) while in reduction reaction was 1.1 µA (Ip_c I). This was corresponded to 1.0 mM (resultant concentration) quercetin in the 10.0 mL of the supporting electrolyte.

According to previous literature, the number of electrons involved in oxidation process is depending on the solvent (Sokolova et al., 2012). There are two conditions where quercetin can be oxidised; in either two electrons or one electron oxidation. Most of the oxidation processes happened on the ring B as shown in Figure 4 (Abdel-Hamid et al., 2012). Sokolova et al. (2012) that a two-electron oxidation of quercetin was observed in acidic solution while a one-electron occured in both aqueous and non-aqueous solution. Oliveira and Mariana-Emilia (2003) reported that the backbone chemical structure in quercetin, catechol 3′,4′-dihydroxyl electron-donating groups oxidised at very low positive potential first, followed by hydroxyl group. The reported mechanism was complementing to the results reported in Figure 16. As there are two oxidation peaks observed, as shown in Figure 16, our results are consistent to the previous literature. In addition, a pH meter had been used to further justify the theory stated by the previous literature (Sokolova et al., 2012) where the tested pH value was 6.8.



Scheme 1 Half-cell equation of quercetin (oxidation).

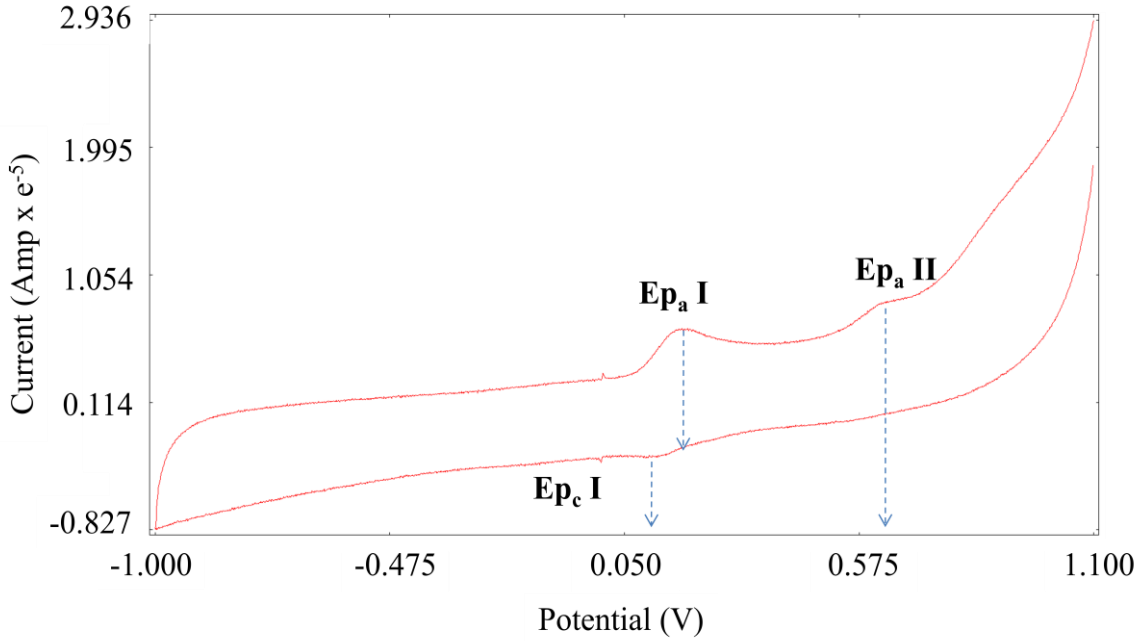


Figure 17 Cyclic voltammogram of 1.0 mM quercetin in 0.1 M PBS-ethanol (1:1 v/v) at scan rate of 100 mV/s.

5.2.3. Square Wave Voltammetry (SWV) of Quercetin

Figure 18 shows a square wave voltammogram of 1.0 mM quercetin at 15 Hz (scan rate). Two oxidation peaks were observed at 160 mV (Epa I) and 608 mV (Epa II) (Figure 18). Their peak current was measured to be 612 µA (Ipa I) and 0.76 µA (Ipa II), respectively**.** As compared to the CV results, SWV exhibited higher sensitivity with lower potential value and higher current peak value as observed in the voltammogram. Therefore, SWV was chosen as the main electrochemical technique in detecting the presence of the quercetin.

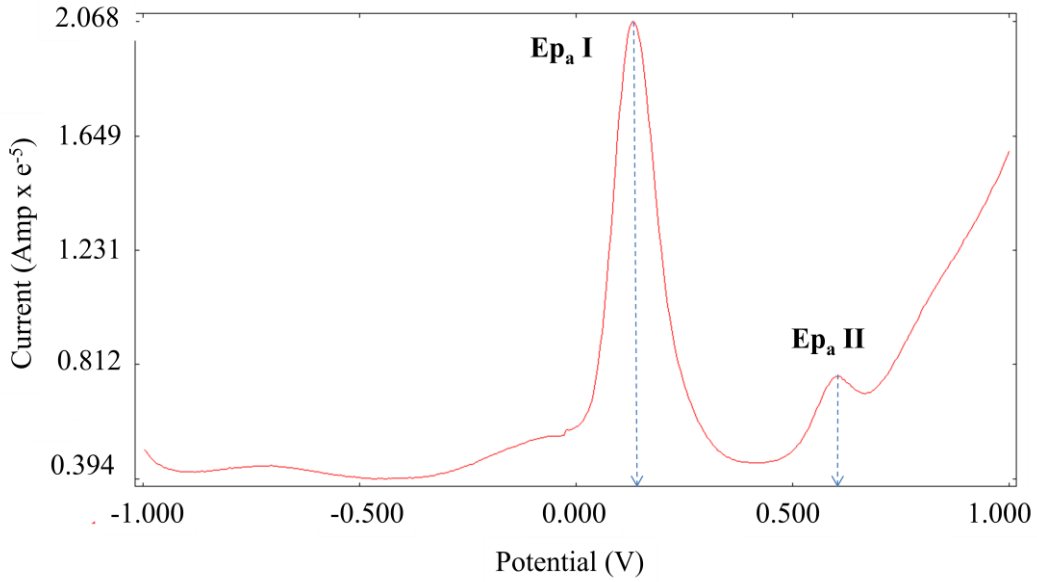


Figure 18 Square wave voltammogram of quercetin in 0.1 M PBS-ethanol (1:1 v/v) at scan rate of 15 Hz.

5.3. Rutin

5.3.1. Cyclic Voltammetry of Rutin

The electrochemical behaviour of rutin was studied in this work. At first, a few supporting electrolytes were tested. They were 0.1 M potassium chloride (KCl), 0.1 M potassium hydrogen 0.1 M orthophosphate (KH_2PO_4), 0.1 M potassium hydroxide (KOH), 0.1 M phosphate-buffered saline (PBS), and ethanol. Unfortunately, all the isolated supporting electrolytes were not good enough to showcase rutin electrochemically. Among the tested supporting electrolytes, the best supporting electrolyte is mixture 0.1 M PBS with ethanol in 1 to 1 ratio in volume.

Rutin exhibited one oxidation and one reduction process as shown in Figure 19. According to Eq 4, intensity of the peak is directly proportional to concentration of an analyte. Rutin oxidized at 318 mV while reduced at 268 mV with current peaks (I_p) of 3.8 µA and 1.70 µA, respectively. The oxidation of rutin was corresponded to the oxidation of the 3',4'-dihydroxy substituent on the ring-B of rutin (Mariana-Emilia and Oliveira, 2005). Rutin has reversible redox reaction where the difference of the potential peak (ΔE_p) is around 50 mV ($\Delta E_p > 54$ is quasi-reversible and $\Delta E_p < 54$ is reversible process). There are 2 types of reversibility; fully reversible and quasi-reversible process. It is fully reversible if the ratio of current between oxidation and reduction is equal to 1. Conversely, it is quasi-reversible process if the ratio of current between oxidation and reduction is less than 1. Therefore, rutin has quasi-reversible redox reaction.

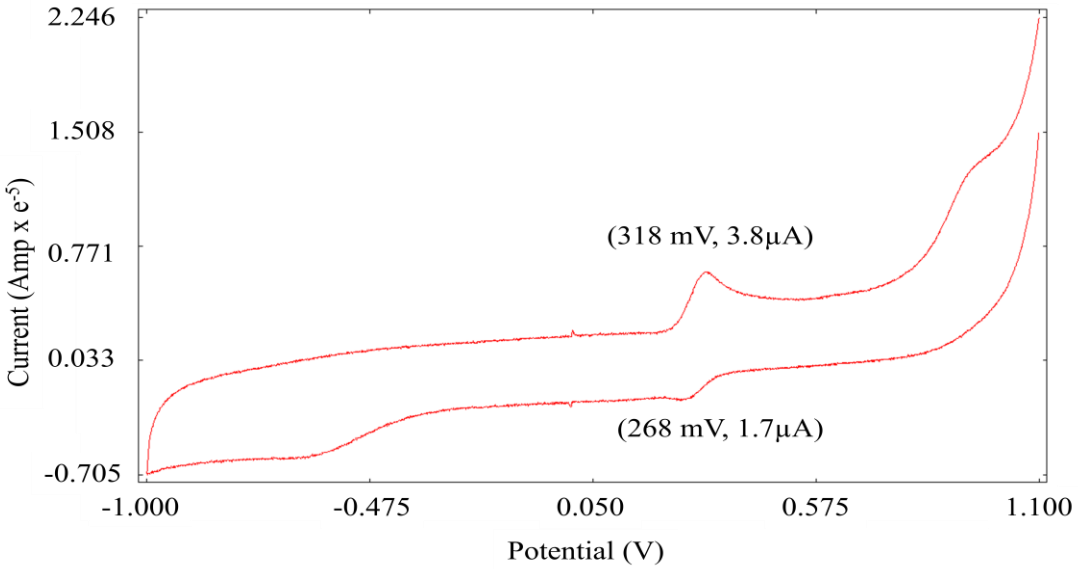


Figure 19 Cyclic voltammetry of 1 mM rutin in 0.1 M PBS with ethanol (1:1 v/v) at scan rate of 100 mV/s.

5.3.2. Multiple Cycling Studies of Rutin

Other than using CV to determine the reversibility of an analyte, CV can be used to determine the stability of a compound by using multiple scan rate technique. A total of 9 cycles of the redox reactions was conducted on rutin in 0.1 M PBS with ethanol (1:1 v/v) at scan rate of 100 mV/s. According to Figure 20, the redox reaction of rutin was not 100% reversible. It can be seen in Figure 20, the oxidation peak of rutin did not overlap on each other in each cycle. Furthermore, the current peak values given in CV also proved that the reversible rate was not 100% where the ratio of Ipa towards Ipc was not equal to 1. Besides that, the recovery rate of rutin was around 44% in one cycle or redox reaction. Therefore, reversibility of rutin in redox reaction was low.

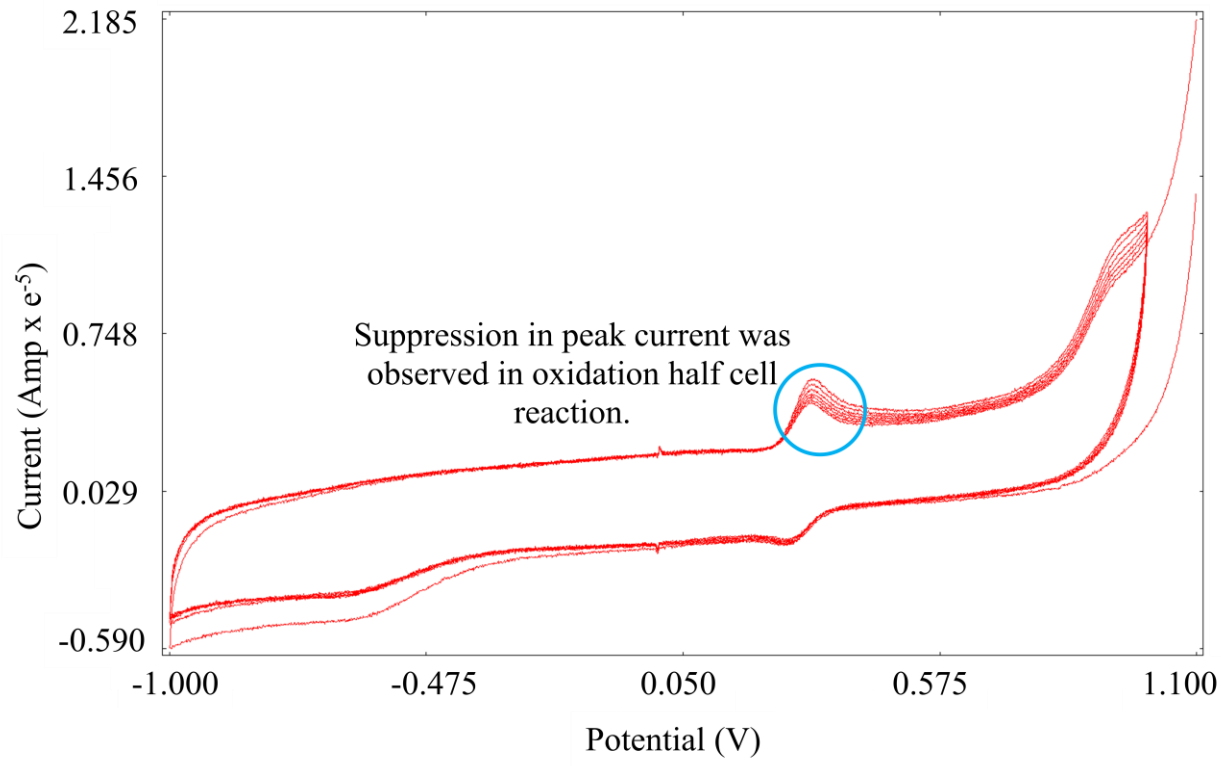


Figure 20 Multiple cycle studies of rutin in 0.1 M PBS with ethanol (1:1 v/v) in scan rate 100 mV/s.

5.3.3. Square Wave Voltammetry of Rutin

A concern in the redox reaction of an antioxidant is its oxidation potential. In general, a lower oxidation potential indicates the ease of oxidation of a particular antioxidant. Hence, electrochemical technique such as square wave voltammetry (SWV) was chosen as the main technique since SWV has higher and better sensitivity as compared to CV, as illustrated in Figure 21 (intensity = 8.08 µA) in comparison to Figure 19 (intensity = 3.80 µA) in which we observed higher intensity in current in SWV. As mentioned before, concentration of an analyte is directly proportional to current intensity. By using the same concentration of rutin and the same conditions on both CV and SWV technique modes, the generated current peak in SWV was higher than in CV. Thus, sensitivity can be explained in terms of the intensity of current peak generated on the same condition between two technique modes. In cases of an analyte can be oxidised on a lower potential value, it means that the analyte can easily release electron or oxidise. If an analyte was easily oxidised, it indicates that the analyte was ionised faster where more ions will be produced in the same amount of time. As a result, current peak of an analyte will be increased.

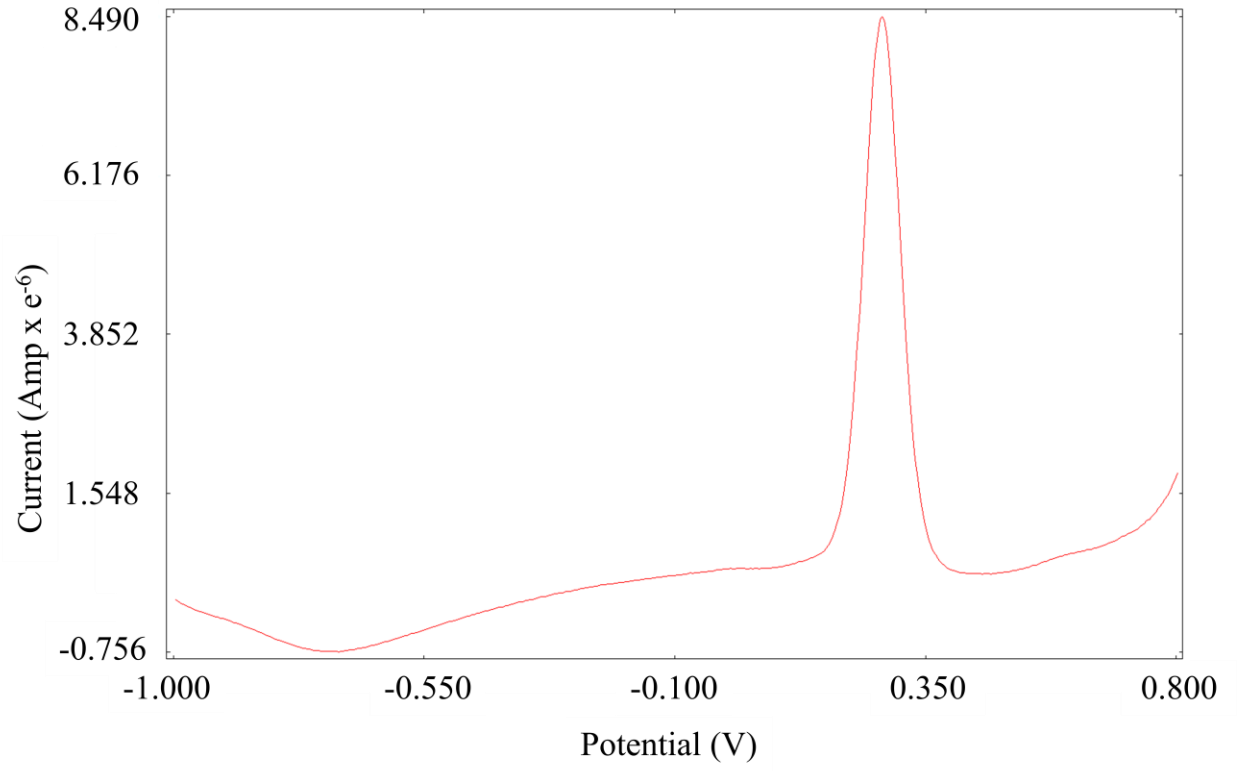


Figure 21 Square wave voltammogram of rutin in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.4. Chemically Modified Electrodes (CMEs)

All the tested antioxidants (gallic acid, quercetin, and rutin) were characterised using the CV and SWV. With the optimized supporting electrolyte (medium), mixture of 0.1 M phosphate-buffered saline (PBS) and ethanol (1:1 v/v), all the antioxidants exhibited oxidation peak(s) in CV and SWV. Gallic acid and quercetin had two oxidation peaks respectively while rutin had only one oxidation peak. Besides that, antioxidant properties can be shown in oxidation peak or oxidation process but not in reduction process. Therefore, SWV was chosen as the main electrochemical technique in detecting the presence of the antioxidants in this study.

Section 5.4.1 shows the application of modified electrodes to the oxidation of gallic acid, quercetin and rutin. Among all the test modified electrodes, FTO was the best modified electrode on detection of gallic acid, while a bismuth(III) oxide-modified GCE was the best for quercetin. However, none of the modified electrodes was found to be suitable for the detection of rutin.

Nanoscience has been rapidly developed and applied to the construction of novel electrochemical sensors because of their excellent electrical conductivity, large surface area and good biocompatibility. Similarly, owing to the microcrystalline structure, the nanomaterial, Bi_2O_3, can offer a large surface area, electrochemical stability, thermo-chemical stability (Deepi et al., 2018), pseudocapacitive behaviour, and catalysis behaviour, making it a significant contribution to the advancement of electrochemistry (Zidan et al., 2011) and supercapacitor technology (Gujar et al., 2006). Zuo et al. (2016) reported that Bi_2O_3 electrode exhibited reversible redox reaction in a list of cationic (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+} Al^{3+}) salts, which made it a versatile electrode material in developing new types of aqueous rechargeable batteries.Thus, different type of bismuth complexes was used as GCE modifier on detection of the antioxidants as shown from section 5.4.2 to section 5.4.7.

5.4.1. Modified electrodes

In this project, we used a number of modifiers on a GCE including bismuth(III) coordination compounds and multiwall carbon nanotubes. Bismuth(III) coordination compounds have been used vastly in different applications. For examples, as a photocatalyst (Bagheri et al., 2018, Zhang et al., 2018, Raza et al., 2018), as an electrocatalyst (Su et al., 2018), as a potential homo- and heterometallic precursor (Mehring, 2007), an electrochemical sensor (Mahmoud et al., 2017), as an immunosensor (Solanki et al., 2017), and *etc*. On the other hand, multiwall carbon nanotubes have been used in various applications, for instance, biomaterial coordination (Gutiérrez-Hernández et al., 2017), as a composites (Sun et al., 2017, Mustafa et al., 2017, Heeley et al., 2017), used in polymer blend (Roman et al., 2017, Cipiriano et al., 2017), used in pulmonary studies (Duke et al., 2017, Mandler et al., 2017), used in hybrid cell (Lee et al., 2017), in phytotoxicity studies (Song et al., 2017), as an adsorbent (Enayatpour et al., 2017), and *etc*. As mentioned before, bismuth(III) coordination compounds and multiwall carbon nanotubes had been vastly applied into different fields, and yet application as electrochemical sensors were lacking. Therefore, we had selected a few bismuth(III) coordination compounds and multiwall carbon nanotubes as the modifier in this project.

Indium tin oxide (ITO) has been used in a wide range of applications such as liquid crystal flat panel display and solar cell devices because ITO films have shown good efficiency for hole injection into organic materials; apart from their widespread application as the anode contact for organic light-emitting diodes (Kim *et al.,* 1999). The electrochemical activity of fluorine doped tin oxide (FTO) electrodes can be explained by the generation of a negatively charged surface provided by fluorine ions occupying interstitial space within tin oxide crystal lattice that would facilitate positive species and electrode surface interaction (Armijo *et al.,* 2010; Miranda *et al.*, 2012; Rojas *et al.,* 2011). FTO has large surface area and electrical conductivity, good electrocatalytic properties, mechanical stability, high selectivity, availability, low cost and tend to be harmless (Miranda *et al.,* 2012). Although both ITO and FTO are not modifier to GCE, they were used in this research to study the presence of the studied antioxidants (whether they will be having better results as compared to the studied modifiers).

CMEs were prepared by transferring the solid reagents, such as multiwall carbon nanotubes (MWCNTs), bismuth(III) oxide (Bi_2O_3), bismuth(III) tungstate (Bi_2WO_6) and bismuth oxybromide (BiOBr), to the GCE surface using a mechanical attachment technique**.** Mechanical

attachment technique is based on the mechanical immobilization of solid particles at the surface of carbon or metal electrodes in the absence of binders, which in turn widens the possibility of solid-state electroanalysis due to its broad applicability (Janeiro and Brett, 2005).The material was placed on a coarse grade filter paper. A clean GCE was then pressed onto the solid in order to adsorb the solid compound onto the surface of electrode. Finally, the modified GCE was cleaned after the measurement by physical removal of the coat or film, followed by polishing with 0.5 µm alumina slurry and ultrasonic cleaning for 1 minute.

The LoD is referring to the lowest concentration of an analyte that can be reliably detected by an analytical procedure but not necessarily quantitated as an exact value (Armbruster and Pry, 2008). The LoD of the antioxidants were estimated based on the standard deviation of the response and the slope of a linear calibration plot. It was expressed as three times the standard deviation of low concentration/slope of the calibration plot.

The three studied antioxidants (gallic acid, quercetin and rutin) were mixed and dissolved in a supporting electrolyte of 0.1 M PBS mixed with ethanol (1:1 v/v). The corresponding square wave voltammogram is depicted in Figure 22, which shows only two oxidation peaks.

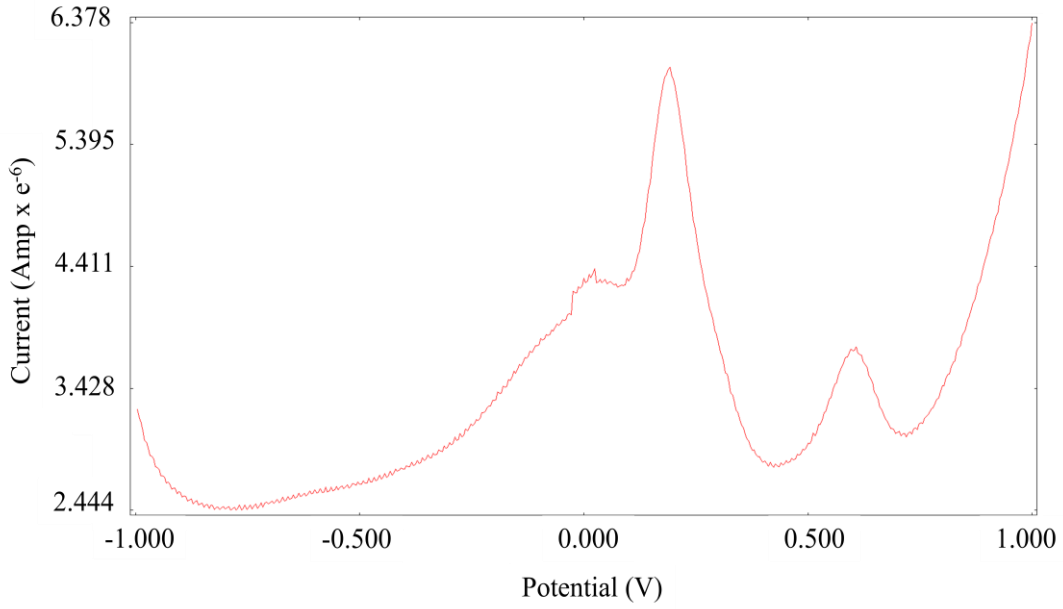


Figure 22 Square wave voltammogram of antioxidants combination (gallic acid + quercertin + rutin) in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

Evidently, the individual oxidation peaks of gallic acid, quercetin, and rutin were not resolved using the current parameters and protocols. Therefore, individual detection system had been designed to identify the current and potential of each antioxidant. Based on CMEs, this study is to develop a suitable electrochemical sensor for each antioxidant, through the increment on the intensity of the peak current . Several CMEs including MWCNT-modified GCE, Bi_2O_3-modified GCE, ITO and FTO were tested. A summary on the different electrodes with respect to different antioxidants are as shown in the following table (Table 1).

Table 1 Electrochemical sensor results on each antioxidant.

Electrode	Gallic Acid	Quercetin	Rutin
GCE	✓	✓	✓
GCE + MWCNTs	X	✓ (high capacitance)	X
GCE + Bi_2O_3	X	✓ (weaker than MWCNTs)	X
ITO	✓ (weaker than FTO)	X	X
FTO	✓	X	X

Legend: X - not suitable ✓- suitable

First of all, all the details of the CV results obtained in the detection of the gallic acid using different working electrodes are tabulated in Table 2. Selection of best CME is based on the highest increment in peak current, minimum shift in peak potential, and with a low capacitance in value. Among all those tested working electrodes, ITO was the best working electrode in detection of the presence of the gallic acid. Although only one oxidation peak was observed when using ITO as the working electrode, it showed that the oxidation peak of the gallic acid increased about 17.4 times in current peak as compared to GCE. The LoD of this ITO electrode on gallic acid was 2.61 nM and calculated using a calibration graph as shown in Figure 23.

Table 2 Details of cyclic voltammograms of gallic acid at different working electrodes.

Working Electrodes	Potential Peak (mV)		Current Peak (µA)	
	Peak 1	Peak 2	Peak 1	Peak 2
GCE	429	671	28.6	1.00
GCE + MWCNTs	318	781	14.4	9.40
GCE + Bi_2O_3	536	772	13.6	2.10
ITO	992	-	497.1	-
FTO	768	-	49.2	-

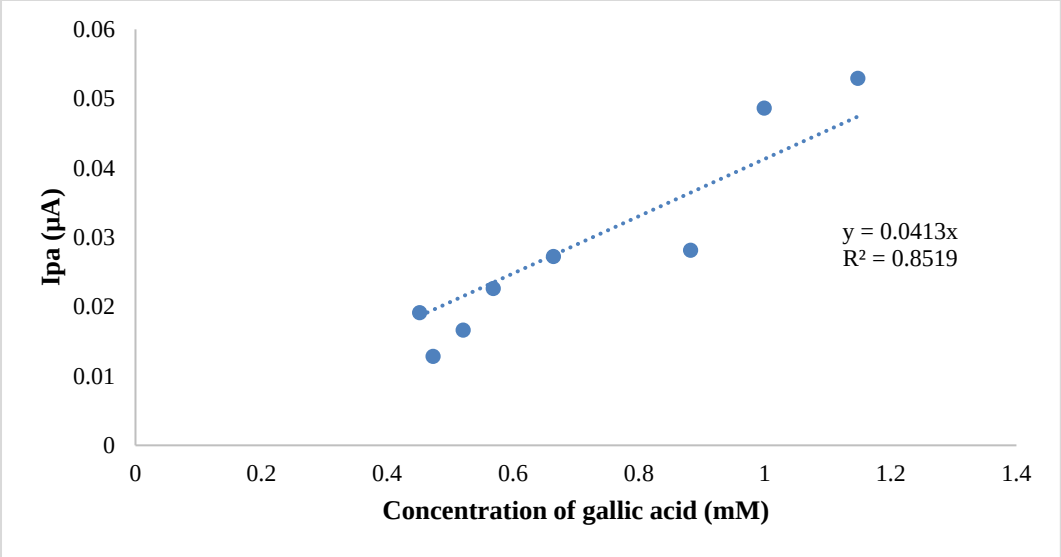


Figure 23 Calibration graph of gallic acid on FTO.

On the other hand, the best tested working electrode for detecting quercetin is a Bi_2O_3-modified GCE because of the highest peak current obtained compared to other electrodes, as shown in Table 3. Although GCE modified with MWCNTs showed quite a significant value in current peak, it also showed high capacitance (areas under the graph), therefore it was still classified as a weaker working electrode as compared to GCE modified with Bi_2O_3. Higher capacitance at a working electrode will mask the possible detection of the analyte(s) under its graph area. The LoD of this GCE modified with Bi_2O_3 electrode on quercetin was 3.64 nM and calculated using a calibration graph as shown in Figure 24.

Table 3 Details of cyclic voltammograms of quercetin at different working electrodes.

Working Electrodes	Potential Peak (mV)		Current Peak (µA)	
	Peak 1	Peak 2	Peak 1	Peak 2
GCE	160	608	6.12	0.76
GCE + MWCNTs	168	-	7.20	-
GCE + Bi_2O_3	100	568	8.30	0.78
ITO	120	-	0.80	-
FTO	-456	396	0.50	0.50

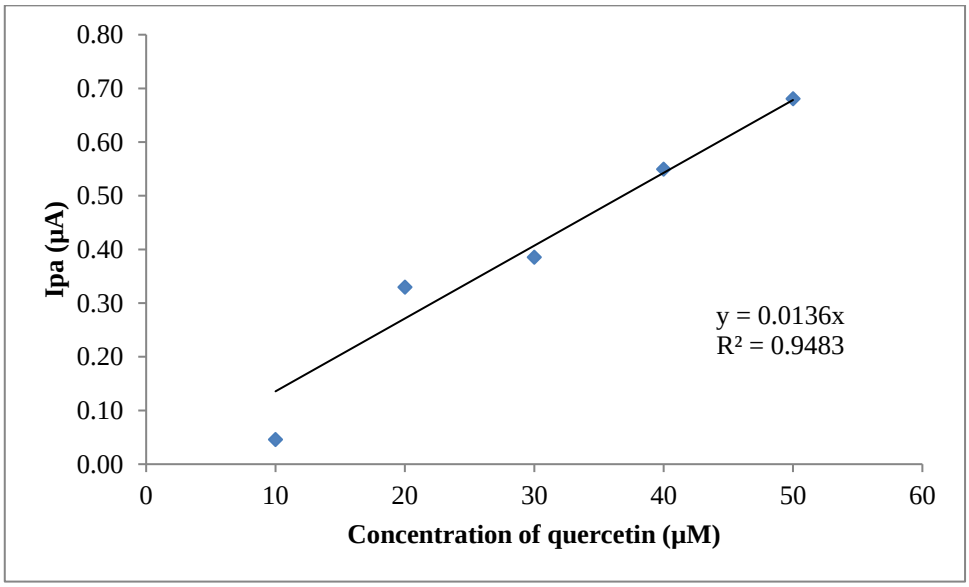


Figure 24 Calibration graph of quercetin at GCE modified with Bi_2O_3.

Besides that, currently none of the modified electrodes is better than GCE for the detection rutin (as shown in Table 4). There was only one oxidation peak (for rutin) observed at a GCE, GCE modified with MWCNTs, and GCE modified with Bi_2O_3. Rutin is undetectable when using ITO as the working electrode while FTO (another working electrode) detected three oxidation peaks for rutin. Rutin was electro-inactive while using ITO as working electrode. FTO is a modified form of ITO electrode, where fluorine was doped on the surface of ITO. At the presence of fluorine, rutin was able to become electroc-active and was oxidised. Each oxidation peak represented an oxidation state or number for rutin.

Table 4 Details of cyclic voltammograms of rutin at different working electrodes.

Working Electrodes	Potential Peak (mV)			Current Peak (µA)		
	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3
GCE	272	-	-	7.69	-	-
GCE + MWCNTs	248	-	-	3.94	-	-
GCE + Bi_2O_3	232	-	-	8.05	-	-
ITO	-	-	-	-	-	
FTO	-448	352	844	0.10	0.10	1.20

5.4.2. Bismuth tungstate (Bi_2WO_6) – Pristine (N)

Since Bi_2O_3 exhibited remarkable results as a modifier on a GCE, a new batch of bismuth coordination compounds was introduced in this project to explore the potential use of bismuth compounds in enhancing the electrochemical detection of the studied antioxidants. Two bismuth coordination compounds were tested, including bismuth tungstate (Bi_2WO_6) and bismuth oxybromide (BiOBr). Both bismuth coordination compounds were synthesized and characterized by another research group from School of Engineering, Monash University Malaysia.

Figure 25 shows that Bi_2WO_6 did not increase the intensity (or peak current) of gallic acid. In the presence of gallic acid (Figure 25(d)), the bismuth oxidation peak increased by 2.8 times compared to the voltammogram obtained in the absence of gallic acid (Figure 25(c)). According to Figure 25(a), oxidation peak was not observed in the absence of gallic acid. After Bi_2WO_6 was coated onto the GCE and with absence of gallic acid in the system, only one oxidation peak was observed, as shown in Figure 25(c). Other than that, no oxidation peak was present in the negative region when gallic acid was introduced into the system where GCE was the working electrode. Therefore, the oxidation peak observed in Figure 25(c) was meant to be bismuth(III) ion as there was no other metal to be presence in the system. Therefore, GCE modified with Bi_2WO_6 did not work effectively in detection of gallic acid.

In the presence of gallic acid, bismuth oxidation peak increased but this phenomenon did not happen in the presence of quercetin and rutin. Bismuth oxidation peak was omitted when quercetin and rutin was introduced into the respective system. Therefore, a hypothesis had been made where GCE modified with gallic acid might be able to become a better working electrode as compared to GCE in detecting the presence of bismuth ion. In this research, bismuth ion

oxidation peak was absent in voltammogram when Bi_2O_3 was used as modifier onto GCE, whereas a bismuth ion oxidation peak was observed when GCE was modified with Bi_2WO_6-N. For the bismuth ion oxidation peak to be observed in voltammogram, the bismuth ion have to diffuse out from its chemical structure to become free ion, followed by oxidation of the free ion which give rise to current peak observed in voltammogram. In this case, the bonds shared between bismuth ion and oxide ion in Bi_2O_3 and the bonds between bismuth ion and tungstate ion in Bi_2WO_6-N are different in strength, in which the linkage within Bi_2WO_6-N is weaker than linkage within Bi_2O_3. As a result, the bismuth ions can be easily diffused out of the structure of Bi_2WO_6-N, contributing to the bismuth ion oxidation peak as observed.

Other than that, there was no increment in current peak when detecting the presence of quercetin (Figure 25) and rutin (Figure 27) respectively using GCE modified with Bi_2WO_6 as working electrode. Therefore, Bi_2WO_6-N was not capable of detecting the presence of the selected antioxidants.

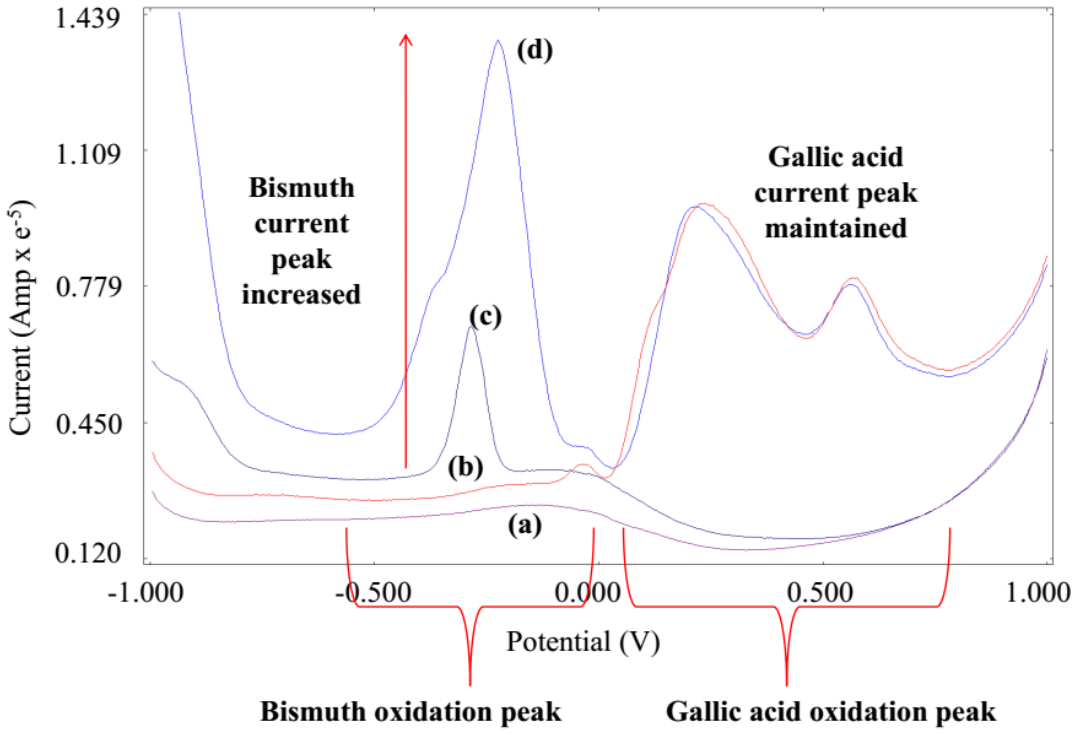


Figure 25 Square wave voltammogram of gallic acid in GCE modified with Bi_2WO_6-pristine; (a) at bare GCE without gallic acid, (b) at bare GCE in the presence of gallic acid, (c) at GCE modified with Bi_2WO_6 without gallic acid, and (d) at GCE modified with Bi_2WO_6 in the presence of gallic acid in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

Meanwhile, the oxidation peaks of quercetin at a GCE (Figure 25 (b)) and a Bi_2WO_6-modified GCE (Figure 25(d)) were compared, where suppression of 45.5 % (Ipa I) and 47.7 % (Ipa II) were observed. The current peaks of quercetin were 12.14 µA (Ipa I) and 2.00 µA (Ipa II) at GCE while the current peaks were reduced to 6.62 µA (Ipa I) and 1.05 µA (Ipa II), respectively. Apart from suppressing the current peaks of quercetin, Bi_2WO_3-N was also capable of omitting the bismuth oxidation peak, as shown in the following voltammogram (Figure 26) after Bi_2WO_3-N was used to detect the presence of quercetin. The same pattern was observed in Figure 27 where rutin oxidation peak was suppressed and bismuth oxidation peak also was inhibited after modified electrode was introduced into the system. The overall purpose of this part was to differentiate the oxidation peak of antioxidant at GCE and at CMEs. In this case, the CME was prepared by doping the solid of the modifier without any adhesive onto the surface of GCE. Therefore, this modified electrode was not suitable in detection presence of quercetin and rutin. Gallic acid had higher affinity towards to the ligand in Bi_2WO_6-N in which bismuth(III) ion was oxidised.

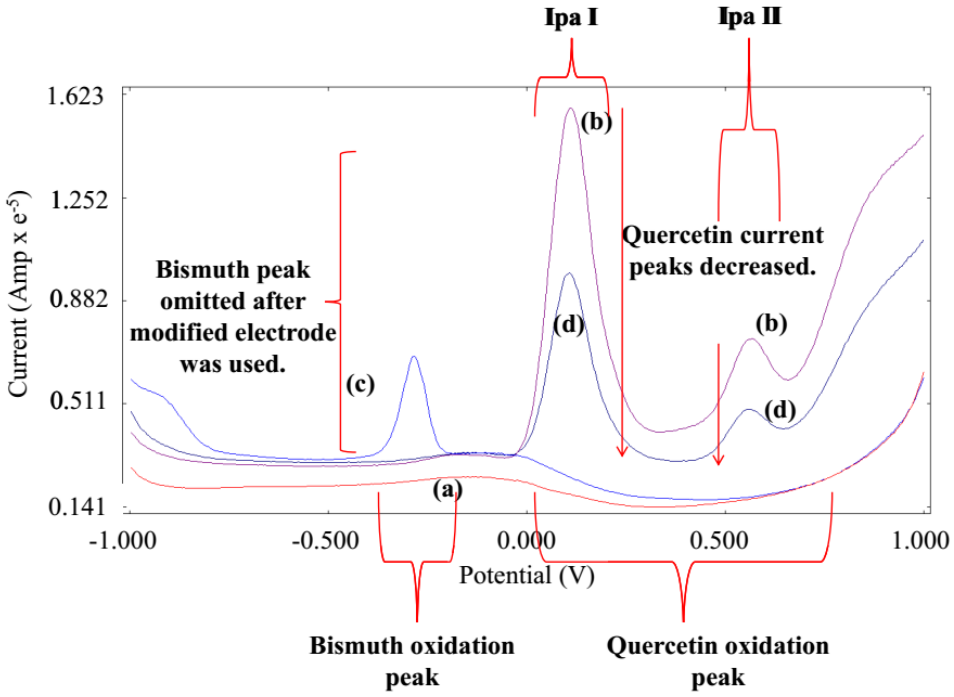


Figure 26 Square wave voltammogram of quercetin in GCE modified with Bi_2WO_6-pristine; (a) at bare GCE without quercetin, (b) at bare GCE in the presence of quercetin, (c) at GCE modified with Bi_2WO_6-pristine without quercetin, and (d) at GCE modified with Bi_2WO_6-pristine in the presence of quercetin in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

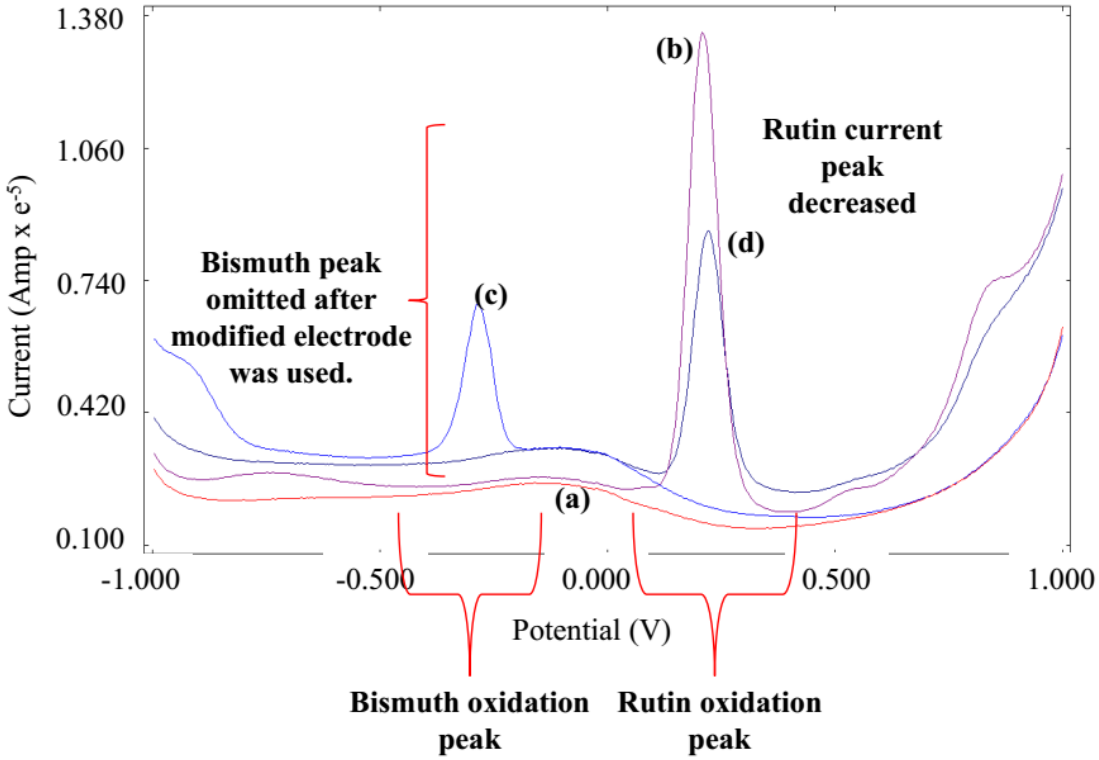


Figure 27 Square wave voltammogram of rutin in GCE modified with Bi_2WO_6-pristine; (a) at bare GCE without rutin, (b) at bare GCE in the presence of rutin, (c) at GCE modified with Bi_2WO_6-pristine without rutin, and (d) at GCE modified with Bi_2WO_6-pristine in the presence of rutin in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.4.3. Bismuth tungstate (Bi_2WO_6) – Oxygen Vacancy (OV)

Figure 28**Error! Reference source not found.** shows an overlay of multiple square wave voltammograms of rutin at different working electrodes. The oxidation of bismuth was observed in **Error! Reference source not found.**. The oxidation peak of rutin was suppressed at GCE modified with Bi2WO6-oxygen vacancy as compared to only GCE. Same pattern was observed after adding of gallic acid and quercetin into the system as shown in **Error! Reference source not found.** Figure 29 and Figure 30, respectively. Besides that, gallic acid (Figure 30) also helped in increasing current peak of bismuth (III) ion as mentioned in section 5.4.2. Since all the targeted antioxidant peaks were reduced, then this modified electrode was not appropriate as a working electrode.

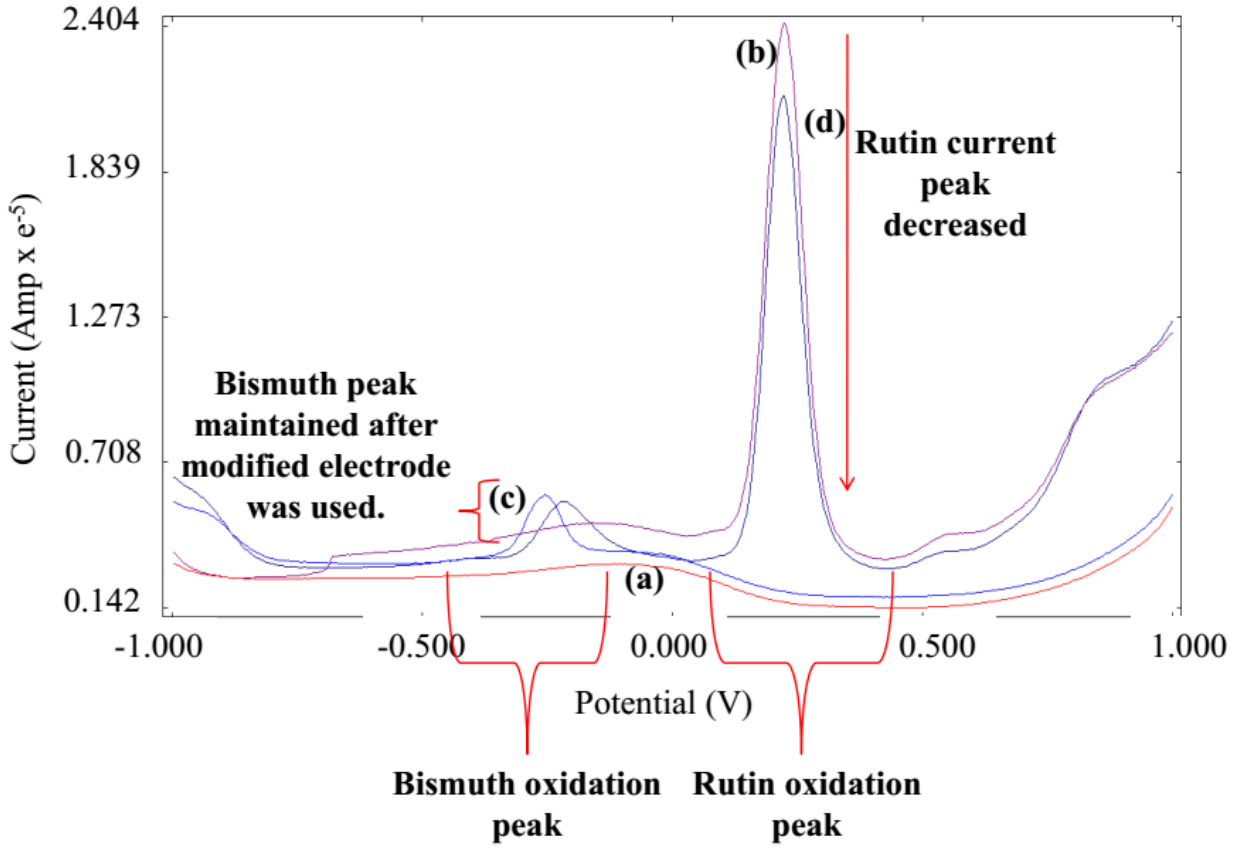


Figure 28 Square wave voltammogram of rutin in GCE modified with Bi_2WO_6-oxygen vacancy; (a) at bare GCE without rutin, (b) at bare GCE in the presence of rutin, (c) at GCE modified with Bi_2WO_6 without rutin, and (d) at GCE modified with Bi_2WO_6 in the presence of rutin in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

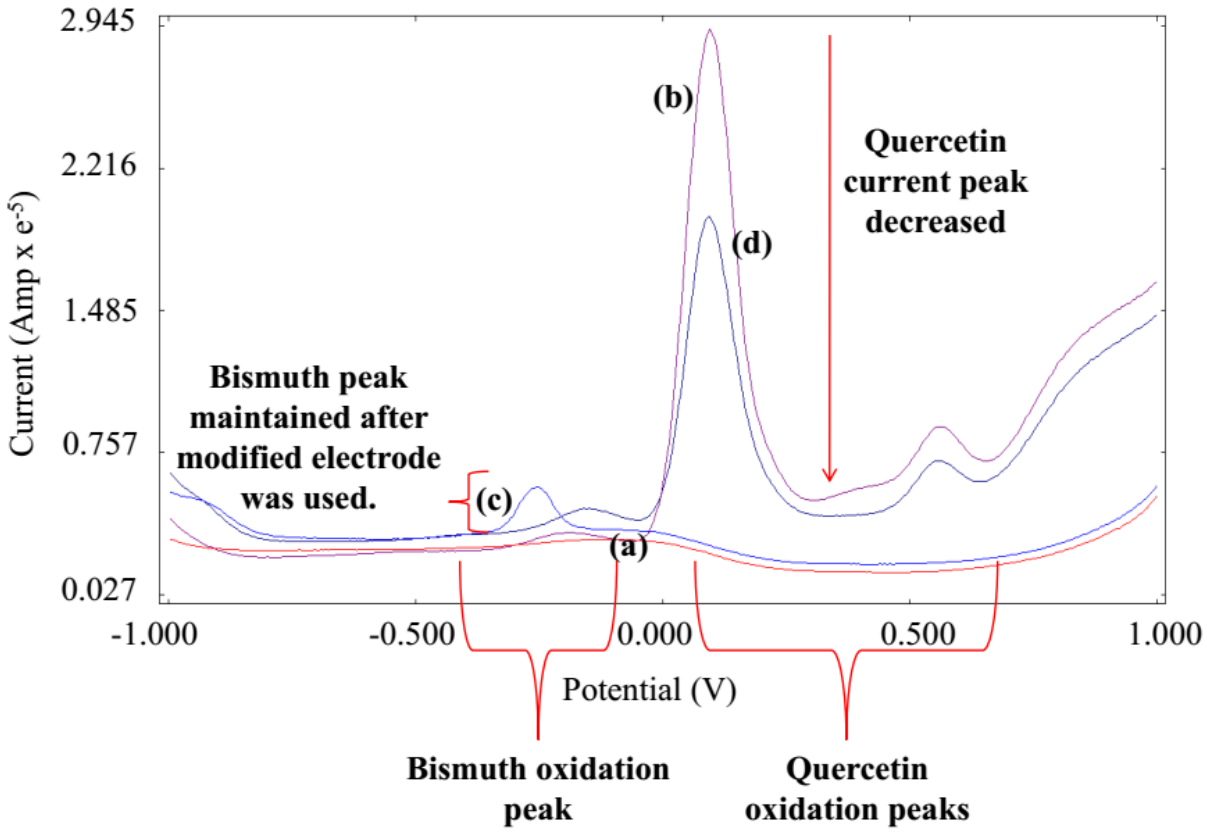


Figure 29 Square wave voltammogram of quercetin in GCE modified with Bi_2WO_6-oxygen vacancy; (a) at bare GCE without quercetin, (b) at bare GCE in the presence of rutin, (c) at GCE modified with Bi_2WO_6 without quercetin, and (d) at GCE modified with Bi_2WO_6 in the presence of quercetin in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

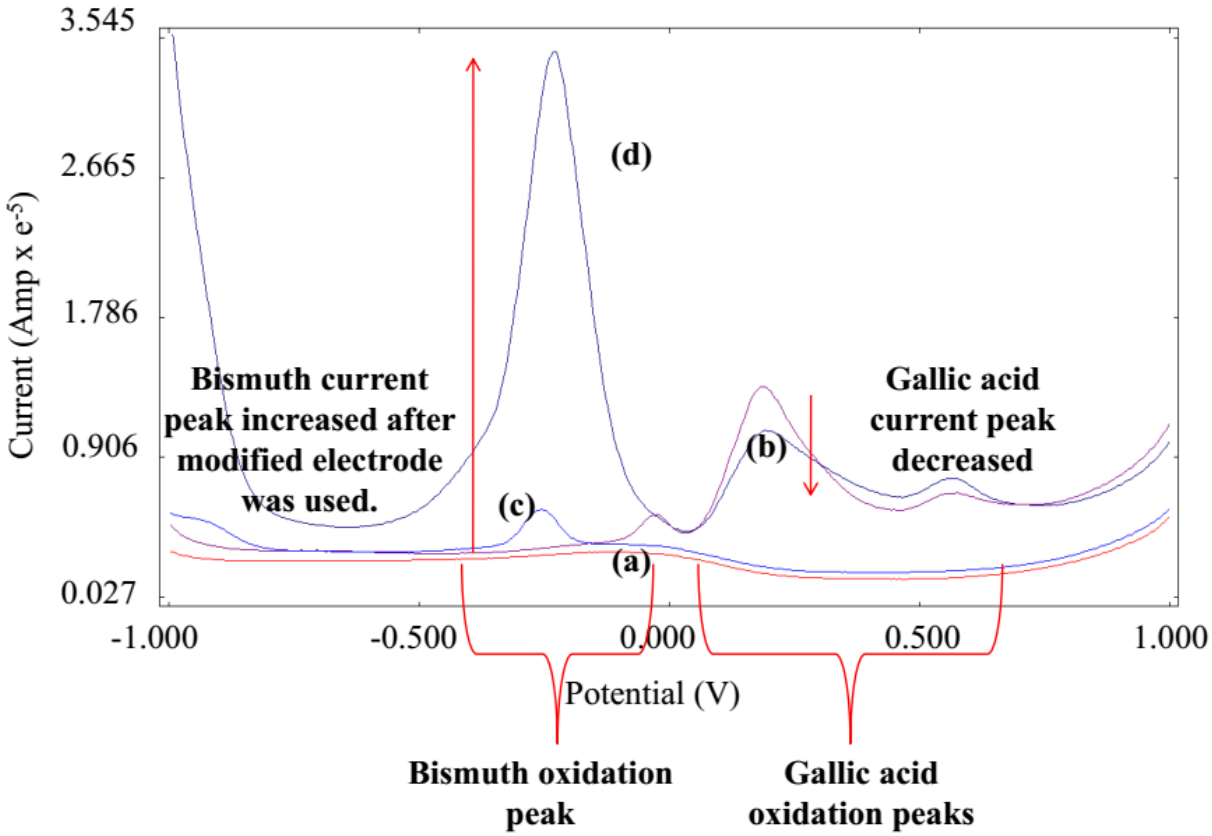


Figure 30 Square wave voltammogram of gallic acid in GCE modified with Bi_2WO_6-oxygen vacancy; (a) at bare GCE without gallic acid, (b) at bare GCE in the presence of rutin, (c) at GCE modified with Bi_2WO_6 without gallic acid, and (d) at GCE modified with Bi_2WO_6 in the presence of gallic acid in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.4.4. Bismuth tungstate (Bi_2WO_6) – Ultrathin (UT)

The pattern and explanation of the square wave voltammogram in Figure 31 were the same as in the Bi_2WO_6-(N). Gallic acid also helped in increasing the current peak of bismuth ion in Bi_2WO_6-(UT). Therefore, gallic acid might be able to help in detection of the presence of bismuth's peak in terms of electrochemistry. Unfortunately, quercetin and rutin showed negative findings when GCE modified with Bi_2WO_6-(UT) was used in both antioxidants respectively. Suppression in current peaks were observed in Figure 32 (for quercetin) while current peak of rutin was maintained when using GCE modified with Bi_2WO_6-(UT) as working electrodes as shown in Figure 33 (for rutin). Thus, ultrathin bismuth tungstate was not a suitable detector in detecting the presence of quercetin (Figure 32) and rutin (Figure 33) respectively. In Bi_2WO_6-(UT), gallic acid was not the only one that helped in increased the current peak of bismuth (III) ion but quercetin and rutin also increased it too. All three antioxidants had higher affinity towards ligand in Bi_2WO_6-(UT). That is why bismuth's current peak was increased after addition of those three antioxidants into the same system. A hypothesis can be made in which Bi_2WO_6-(UT) had the weakest linkage or strength among itself as compared to other tested bismuth tungstate.

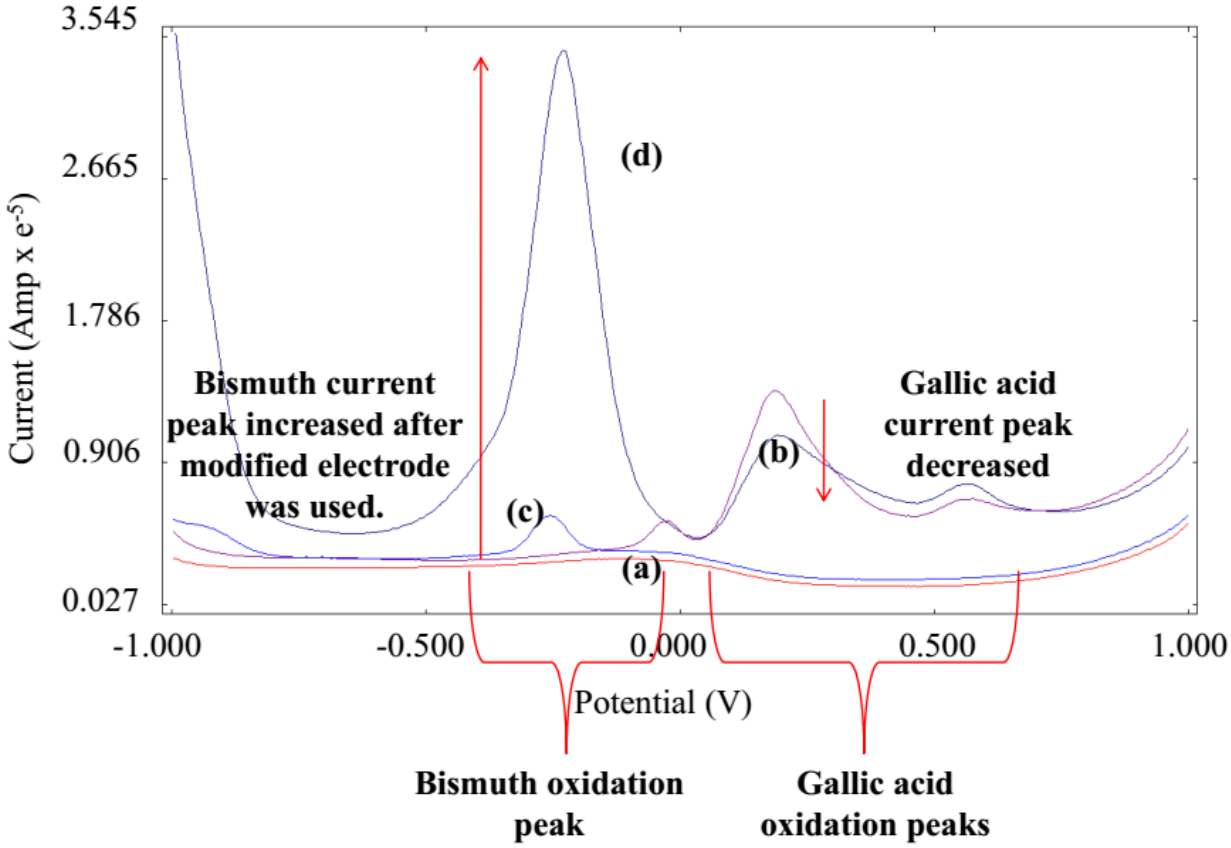


Figure 31 Square wave voltammogram of gallic acid in GCE modified with Bi_2WO_6-UT; (a) at bare GCE without gallic acid, (b) at bare GCE in the presence of gallic acid, (c) at GCE modified with Bi_2WO_6-UT without gallic acid, and (d) at GCE modified with Bi_2WO_6-UT in the presence of gallic acid in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

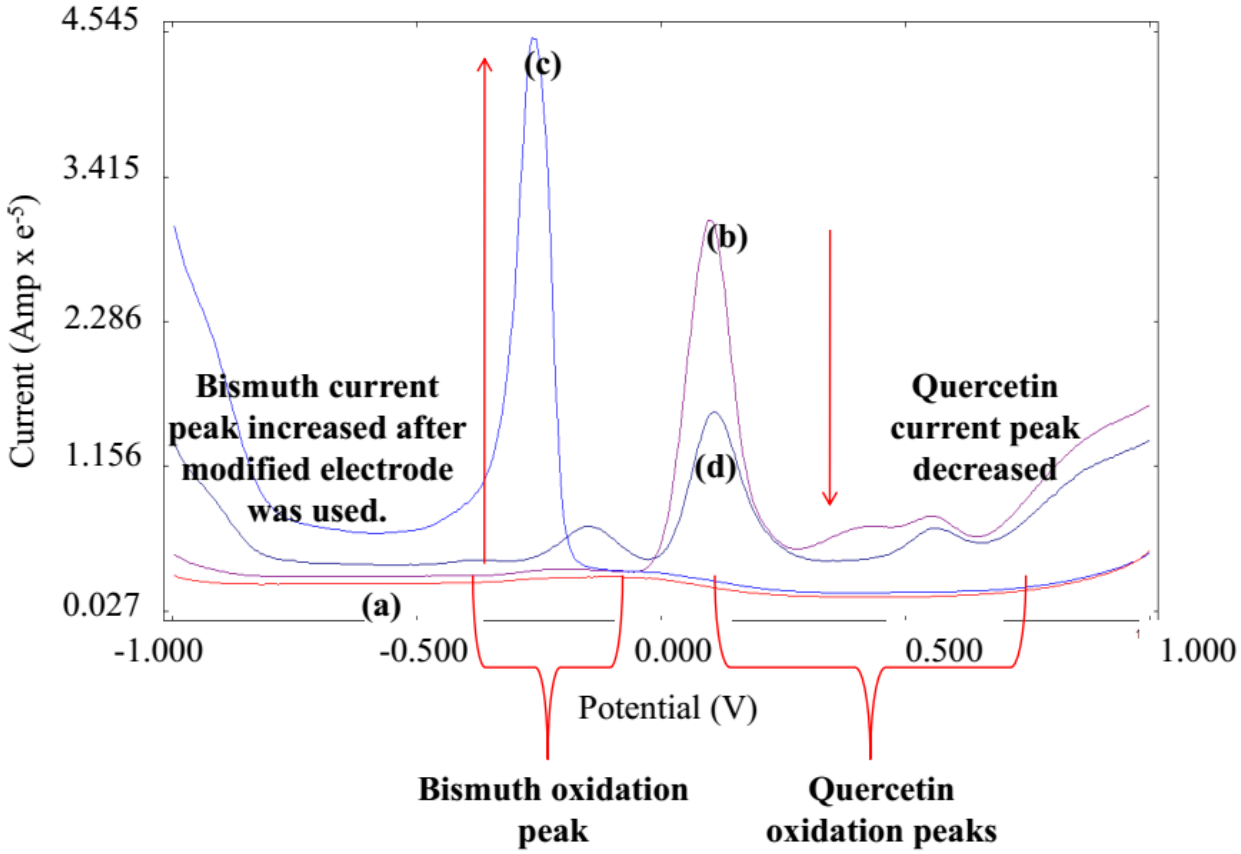


Figure 32 Square wave voltammogram of quercetin in GCE modified with Bi_2WO_6-UT; (a) at bare GCE without quercetin, (b) at bare GCE in the presence of quercetin, (c) at GCE modified with Bi_2WO_6-UT without quercetin, and (d) at GCE modified with Bi_2WO_6-UT in the presence of quercetin in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

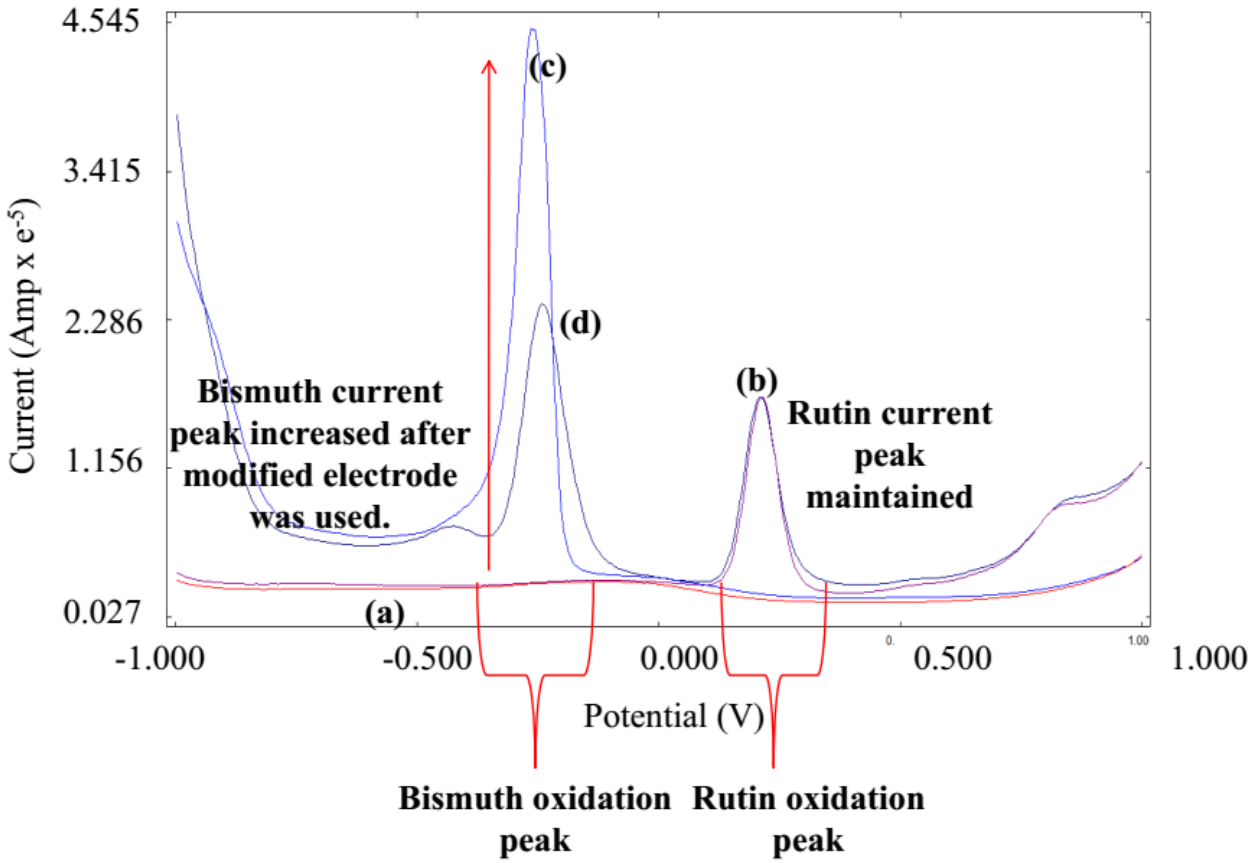


Figure 33 Square wave voltammogram of rutin in GCE modified with Bi_2WO_6-UT; (a) at bare GCE without rutin, (b) at bare GCE in the presence of rutin, (c) at GCE modified with Bi_2WO_6-UT without rutin, and (d) at GCE modified with Bi_2WO_6-UT in the presence of rutin in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.4.5. Bismuth Oxybromide (BiOBr) – Oxygen Vacancy (OV)

Figure 34**Error! Reference source not found.** shows a square wave voltammogram of 1 mM gallic acid at a BiOBr-OV-modified GCE in mixture of 0.1 M PBS and ethanol solution. About 37.1 % suppression in current peak after GCE had been modified (Figure 34**Error! Reference source not found.**). Hence, GCE modified with BiOBr-OV was a better working electrode as compared to GCE modified with Bi_2O_3. Nevertheless, GCE modified with BiOBr-OV was able to increase the current peak quercetin, around 64.1 % increment as compared to GCE as shown in Figure 35. Compression in current peak (1.1 %) was observed in the presence of rutin (Figure 36) when using GCE modified with BiOBr-OV. In short, BiOBr-OV was not capable in detecting the presence of gallic acid and rutin.

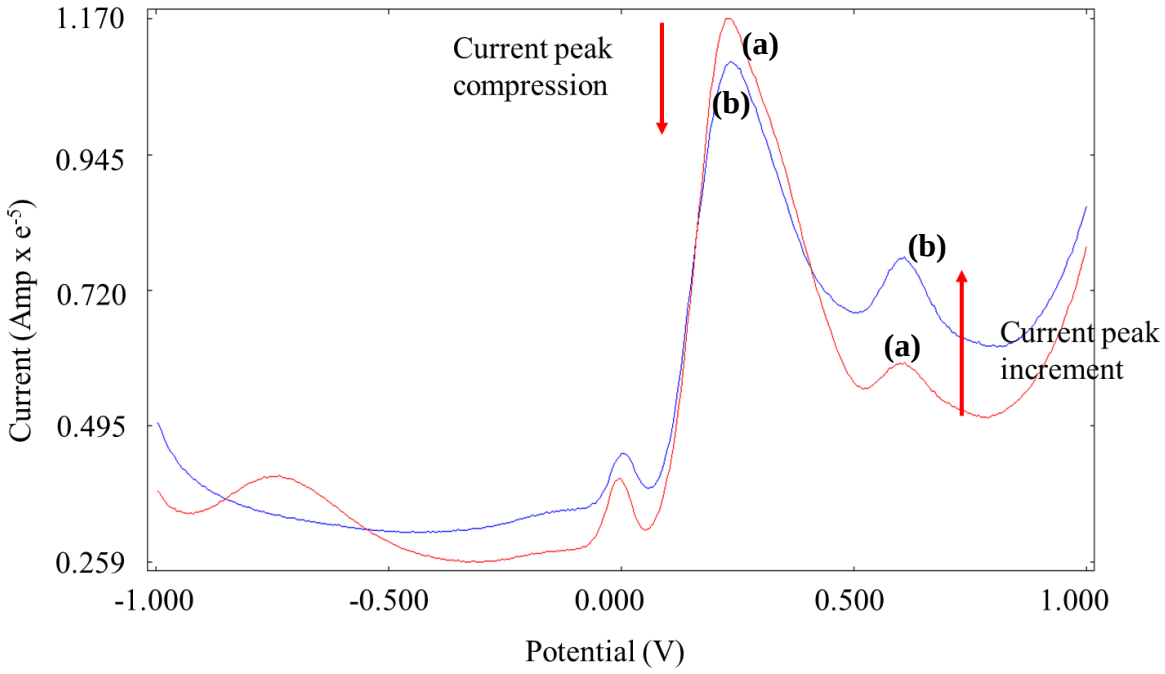


Figure 34 Square wave voltammogram of gallic acid in GCE modified with BiOBr-Oxygen Vacancy; (a) at bare GCE in the presence of gallic acid and (b) at GCE modified with BiOBr-OV in the presence of gallic acid in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

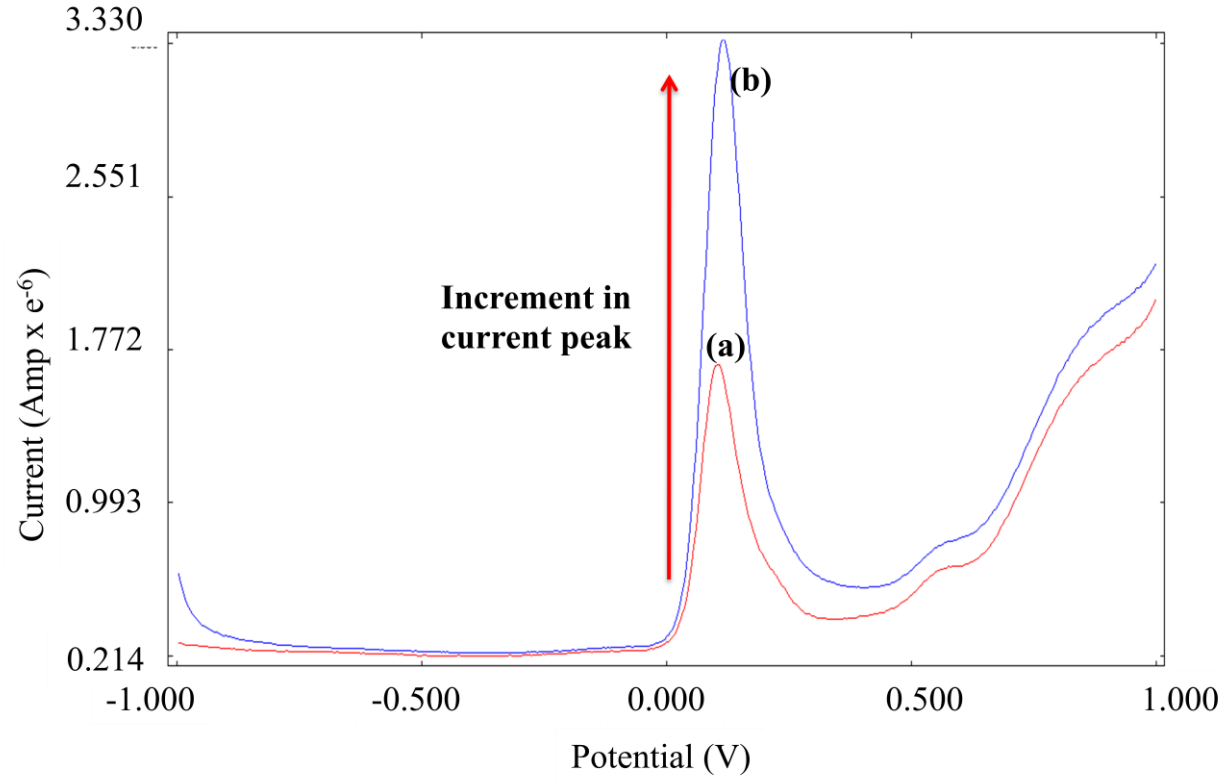


Figure 35 Square wave voltammogram of quercetin in GCE modified with BiOBr-Oxygen Vacancy; (a) at bare GCE in the presence of quercetin and (b) at GCE modified with BiOBr-OV in the presence of quercetin in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

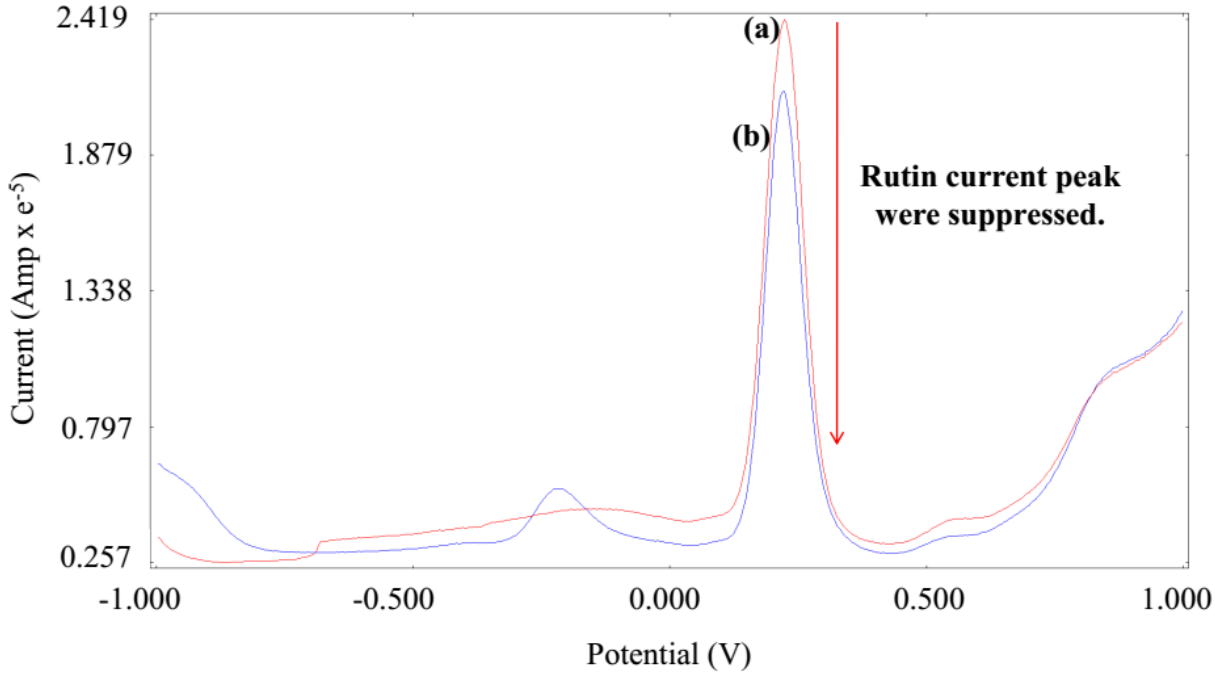


Figure 36 Square wave voltammogram of rutin in GCE modified with BiOBr-Oxygen Vacancy; (a) at bare GCE in the presence of rutin and (b) at GCE modified with BiOBr-OV in the presence of rutin in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.4.6. Bismuth Oxybromide (BiOBr) – Pristine (N)

The following figure showed that current peak was compressed when using the modified electrode (Figure 37) after addition of quercetin. About 59.8 % compression rate was observed in current peak. Therefore, this was not a good working electrode as compared to GCE. Apart from that, the other antioxidants (gallic acid and rutin) had the same pattern as shown in Figure 38 and Figure 39. So, pristine bismuth oxybromide also was not suitable in detecting the presence of the designated antioxidants.

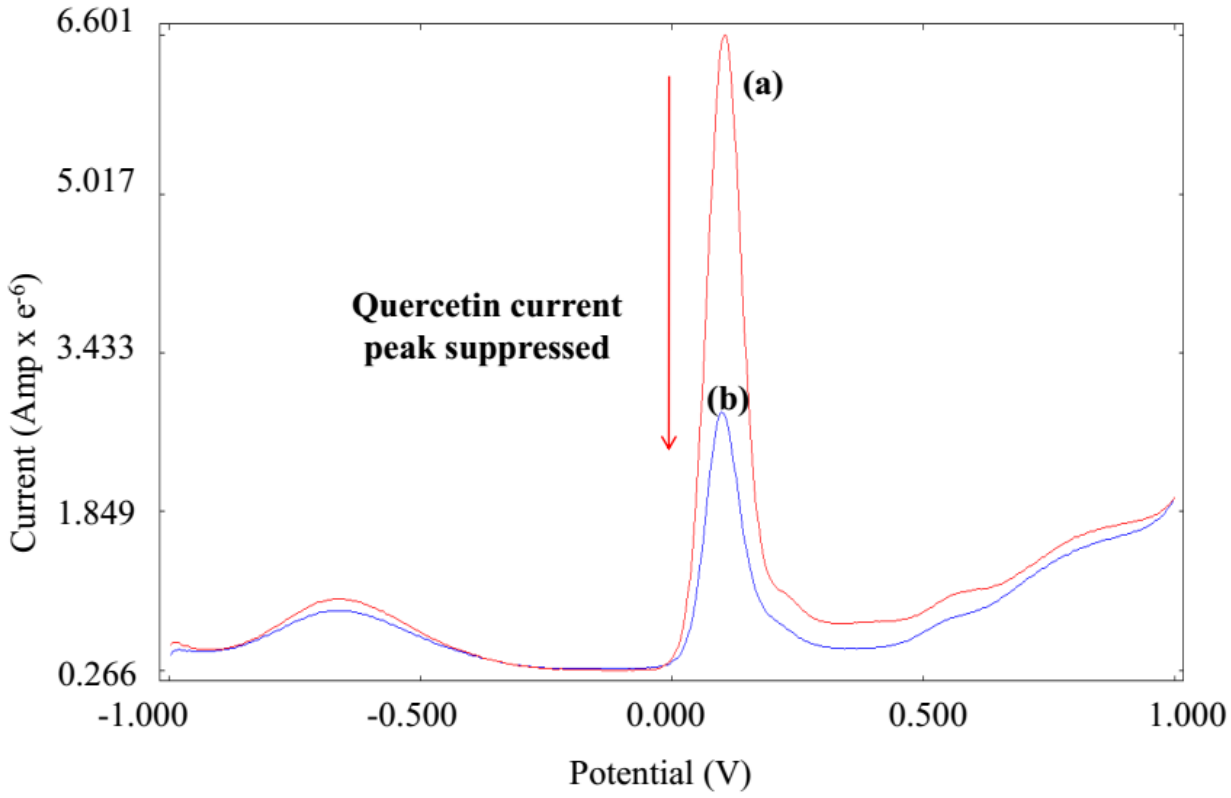


Figure 37 Square wave voltammogram of quercetin in GCE modified with BiOBr-N; (a) at bare GCE in the presence of quercetin and (b) at GCE modified with BiOBr-N in the presence of quercetin in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

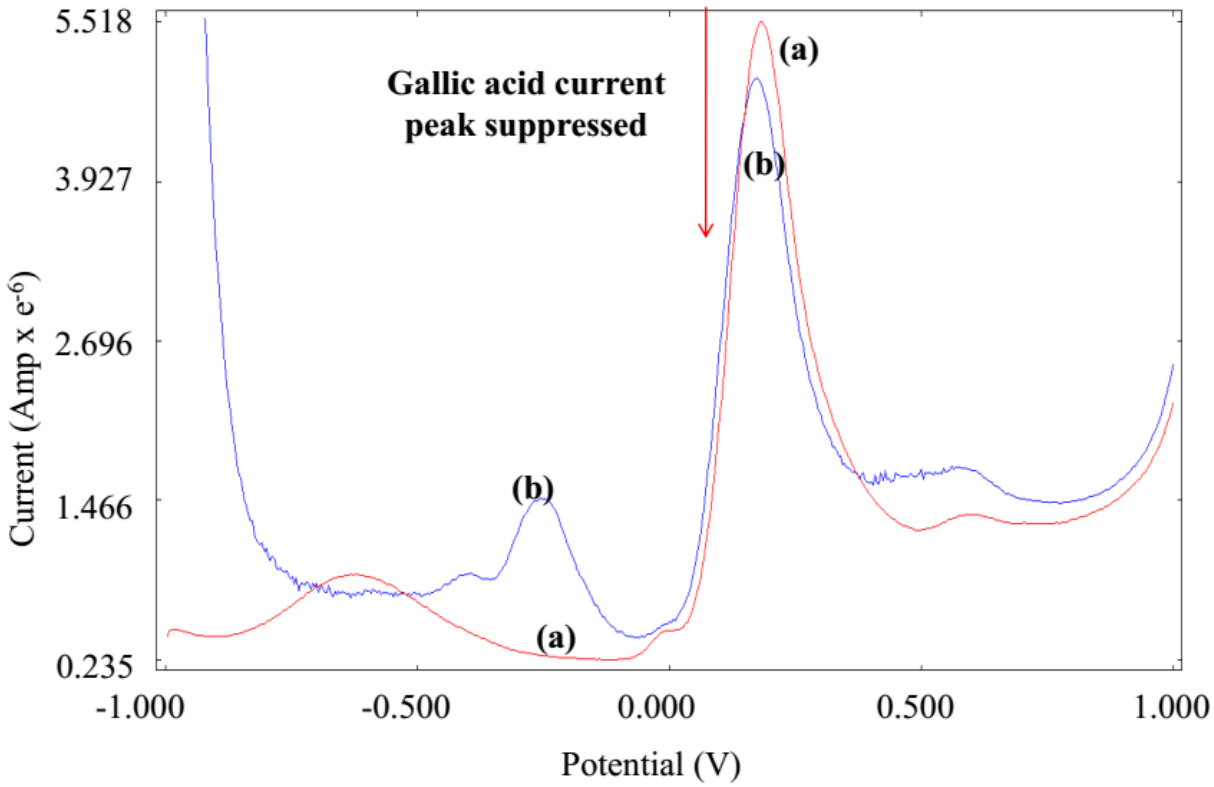


Figure 38 Square wave voltammogram of gallic acid in GCE modified with BiOBr-N; (a) at bare GCE in the presence of gallic acid and (b) at GCE modified with BiOBr-N in the presence of gallic acid in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

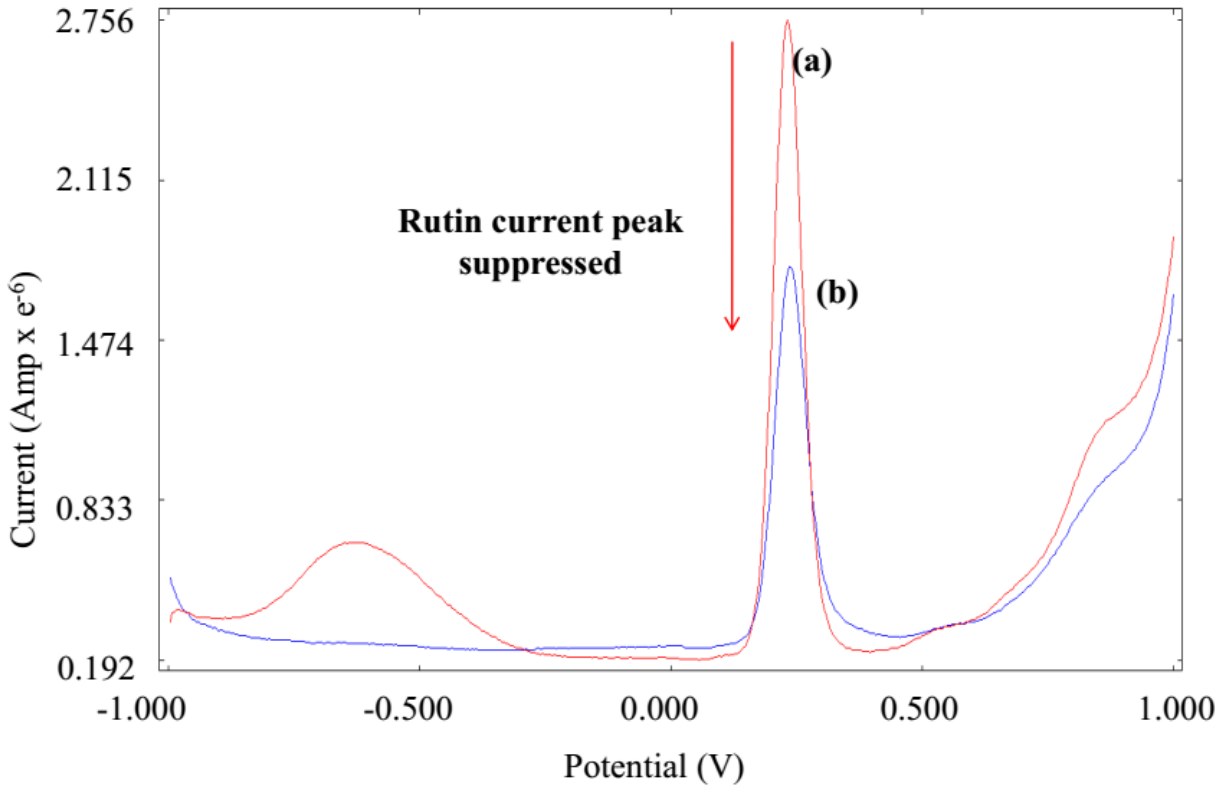


Figure 39 Square wave voltammogram of rutin in GCE modified with BiOBr-N; (a) at bare GCE in the presence of rutin and (b) at GCE modified with BiOBr-N in the presence of rutin in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.4.7. Bismuth Oxybromide (BiOBr) – Bismuth and Oxygen In Excess (Bi&O excess)

For the last modified electrode, GCE modified with BiOBr-Bi&O electrode had increased the current peak of quercetin (Figure 40). About 16.1 % compression in current peak for quercetin was observed in Figure 40. An extra oxidation peak had been observed in the negative region for both gallic acid (Figure 41) and rutin (Figure 42). Hence, further test will be done on rutin and gallic acid.

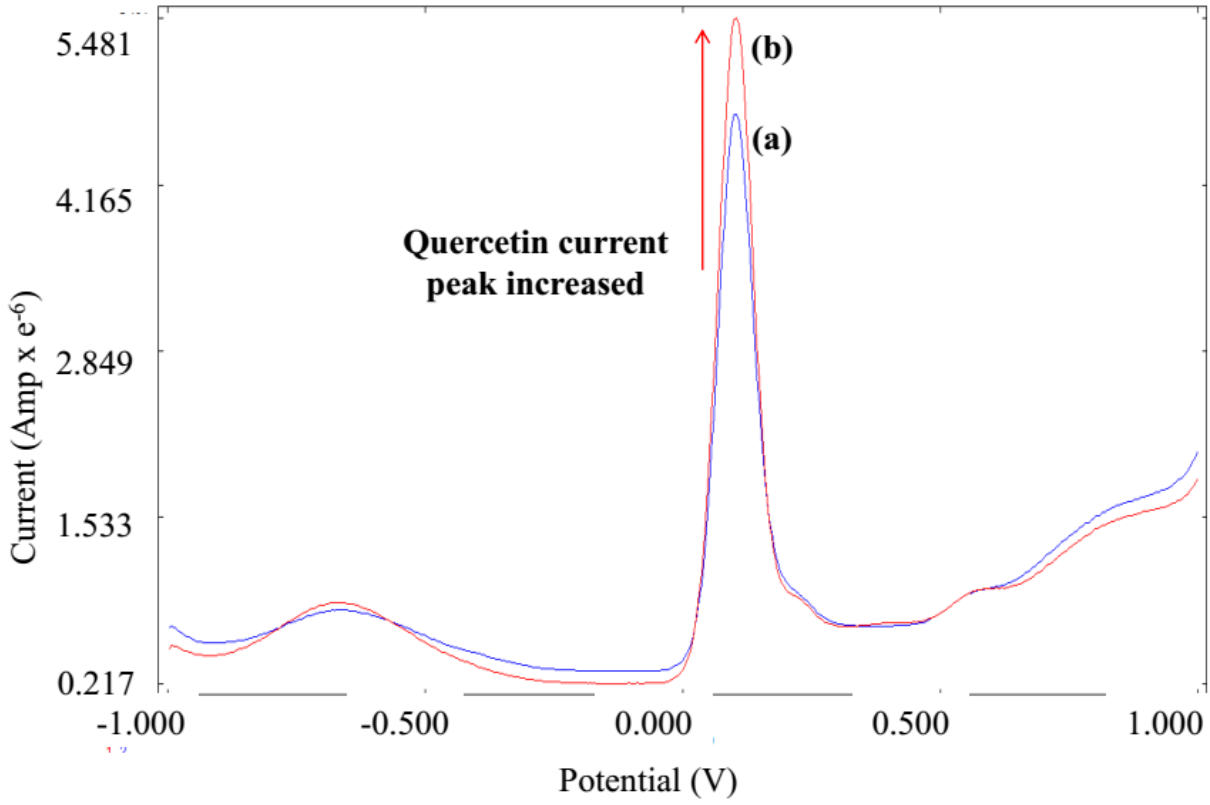


Figure 40 Square wave voltammogram of quercetin in GCE modified with BiOBr-Bi&O excess; (a) at bare GCE in the presence of quercetin and (b) at GCE modified with BiOBr-Bi&O in the presence of quercetin in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

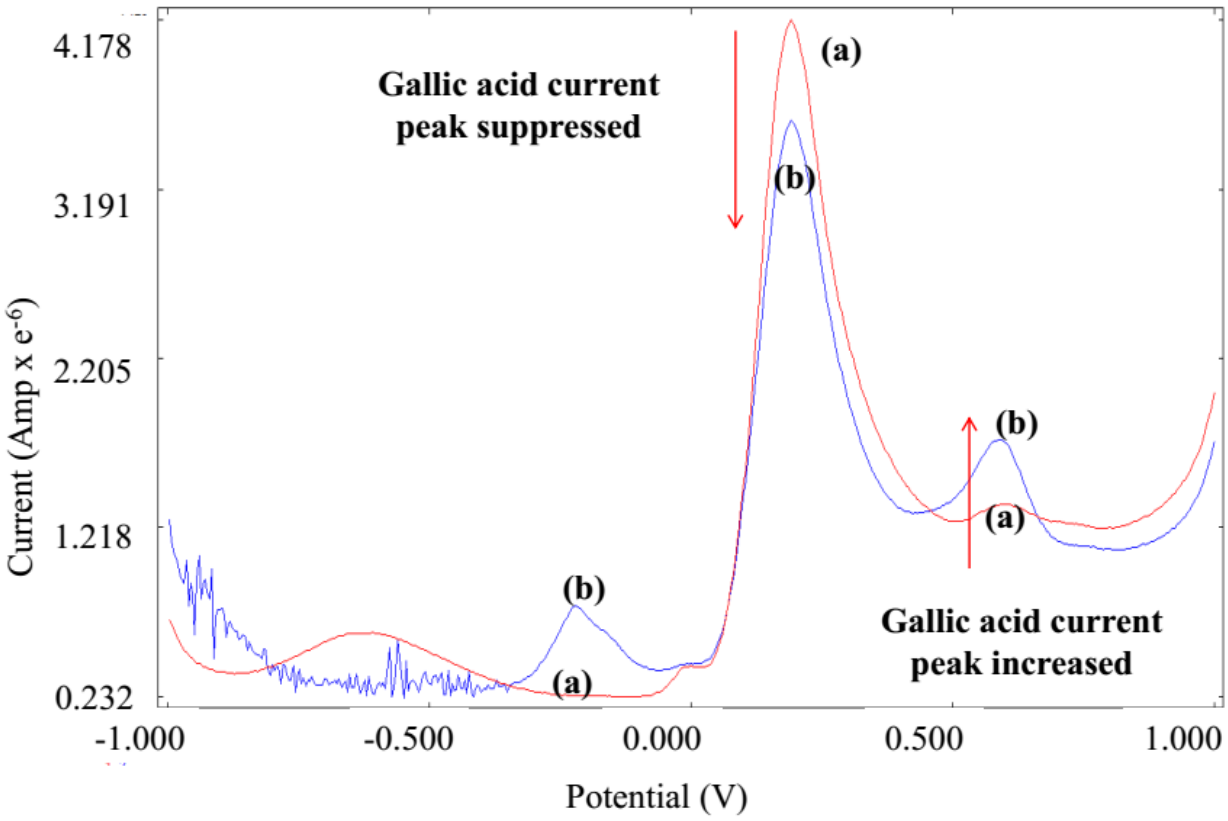


Figure 41 Square wave voltammogram of gallic acid in GCE modified with BiOBr-Bi&O excess; (a) at bare GCE in the presence of gallic acid and (b) at GCE modified with BiOBr-Bi&O excess in the presence of gallic acid in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

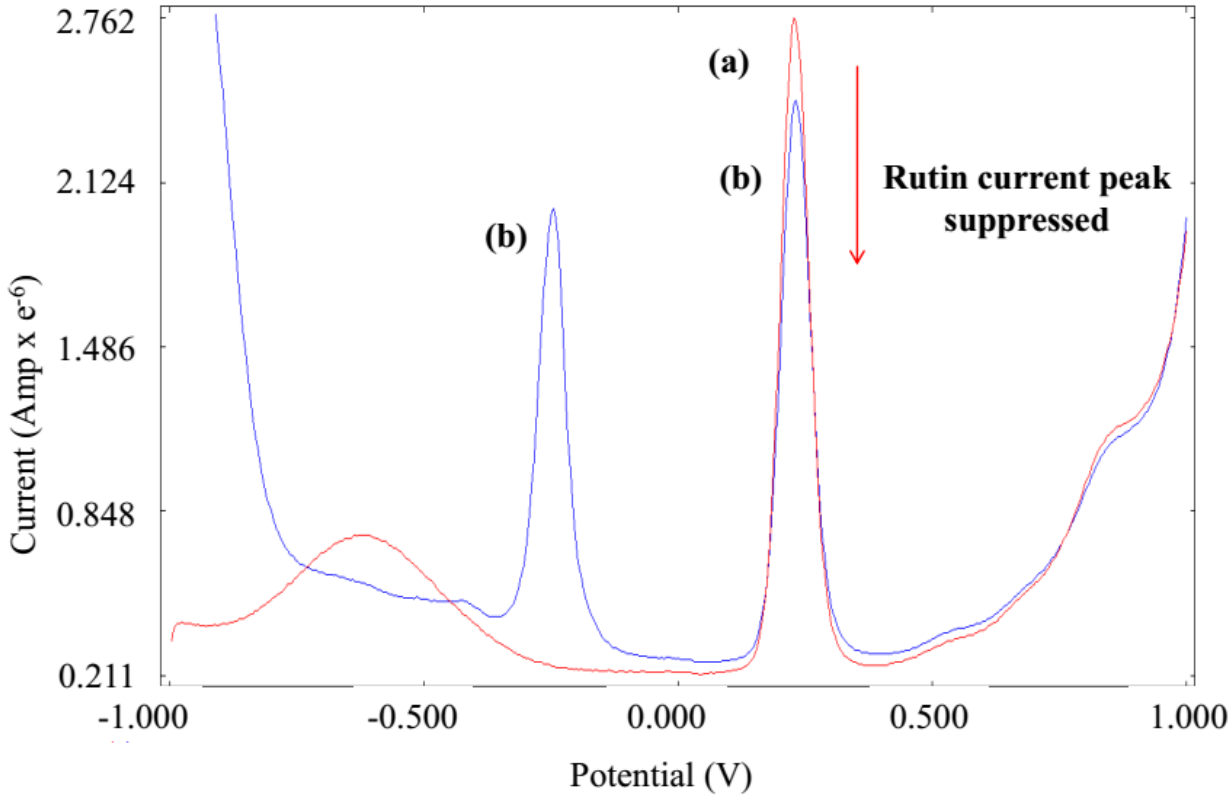


Figure 42 Square wave voltammogram of rutin in GCE modified with BiOBr-Bi&O excess; (a) at bare GCE in the presence of rutin and (b) at GCE modified with BiOBr-Bi&O in the presence of rutin in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.4.8. Summary Results of CMEs

The overall results of CMEs are summarised in Table 5. Only BiOBr-oxygen vacancy and pristine exhibited positive results, where about 176 % and 215 % increment in current peak in detection of gallic acid and quercetin respectively. On the other hand, bismuth ion current peak was increased when gallic acid was introduced into the system. According to the generated voltammograms from section 5.4.2 to 5.4.7, gallic acid might be able to become the material in modification of electrode in detecting the presence of bismuth ion as the target ion. Although there was an increment in oxidation current peak, and yet bismuth tungstate and bismuth oxybromide were not be chosen in such a study because a bismuth oxidation peak was observed in the voltammogram. Furthermore, this bismuth oxidation peak might hinder the actual antioxidant peak in the application studies.

Therefore, the best modified electrode for detection of gallic acid was FTO while the best detection for quercetin was GCE modified with Bi_2O_3. There was absence of better modified electrode for detection of rutin as shown Table 5.

Table 5 Summary of the chemical modified electrodes

Chemically Modified Electrode (CME)		Gallic Acid	Quercetin	Rutin
GCE		✓	✓	✓
GCE + MWCNTs		X	✓ high capacitance	X
GCE + Bi_2O_3		X	✓ weaker than MWCNTs	X
ITO		✓ weaker than FTO	X	X
FTO		✓	X	X
Bi_2WO_6	**Pristine**	X Enhanced bismuth peak	X	X
	Oxygen Vacancy	X Enhanced bismuth peak	X	X Inhibited oxidation of bismuth
	Ultrathin	X Enhanced bismuth peak	X	X
BiOBr	**Oxygen Vacancy**	✓ 176 % increment in current	✓ 215 % increment in current	X
	Pristine	X	X	X
	Bi and O excess	X	X	X

5.5. Application Studies

5.5.1. Electrochemical Analysis

In this section, all the supplements with oxidation peak(s) will be subjected to further test by developed CME if and only if antioxidant peak was observed. Further test was carried out by using either an FTO (detector for gallic acid) or a Bi_2O_3-modified GCE (detector for quercetin) in order to identify the presence of gallic acid or quercetin. As mentioned before, the studied antioxidant has its own potential range to be oxidised. If the targeted antioxidant is present, the antioxidant current peak(s) (the intensity) in the supplement as shown in the voltammogram will be increased. Thus, further test will be performed only if there was an oxidation peak observed and it must be on the same studied potential range as the targeted antioxidant. Unfortunately, supplements containing vitamin C were not included in further test as vitamin C is oxidised in the same potential window as that for the targeted antioxidants. As a result, vitamin C's oxidation peak had the possibility for hindering the antioxidant's oxidation peak to be seen in the voltammogram.

5.5.1.1. Herbs of Gold – Black seed with Garlic

Figure 43 shows SWV of Black Seed with Garlic in 0.1 M PBS with ethanol. There was no antioxidant peak observed at the studied antioxidants potential range (the positive value in potential range). Thus, no further test will be done on this supplement.

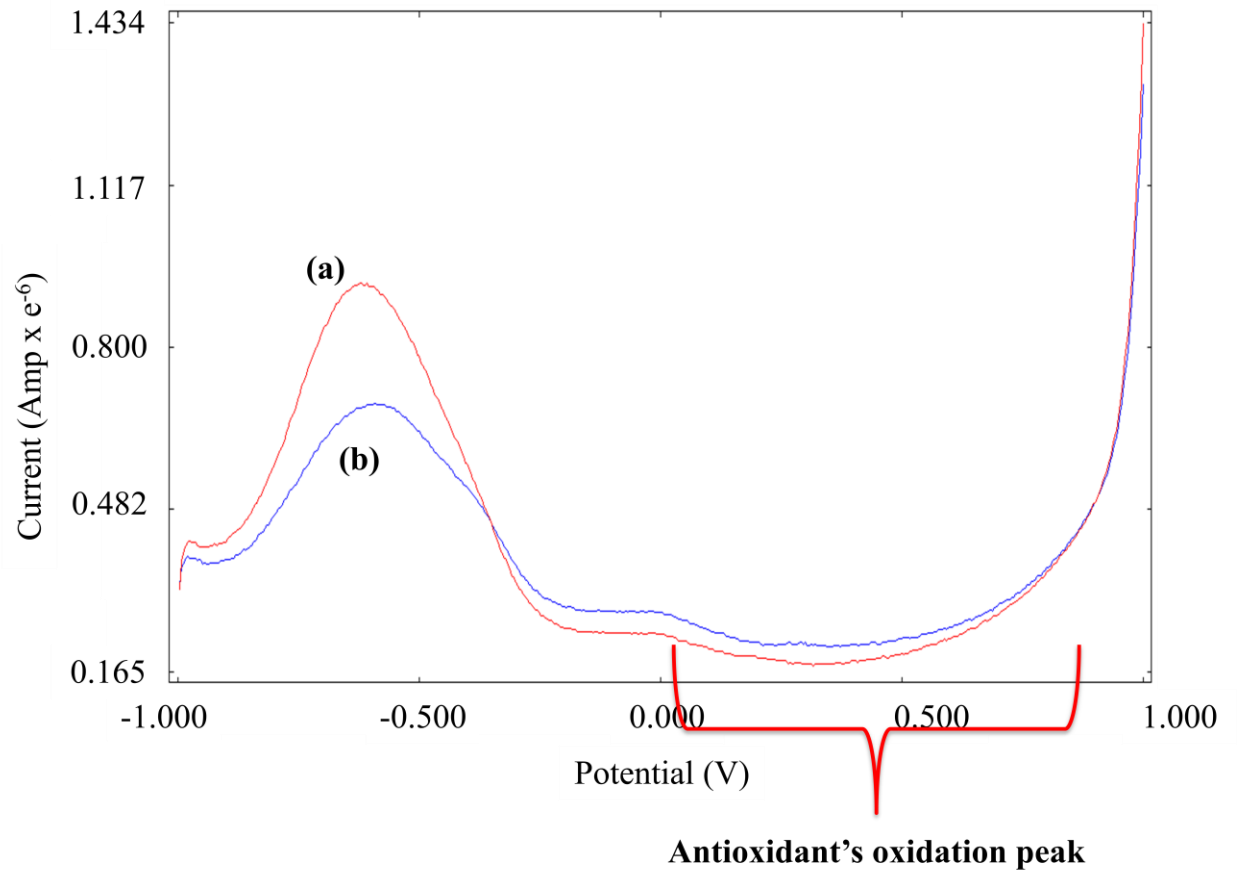


Figure 43 Square wave voltammogram of Black Seed with Garlicin (a) GCE and (b) GCE with Black Seed and Garlic in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.5.1.2. Herbs of Gold – Grape Seed 15000

Grape seed and its skin have good source of gallic acid, catechin and epitachein (Yilmaz and Toledo, 2004). An antioxidant peak was observed as shown in Figure 44. The potential peak was 184 mV with intensity of 0.211 µA. As compared to all the studied antioxidants, the expected antioxidant was gallic acid. Thus, modified electrode was needed to further prove the content of the antioxidant in grape seed. Previous sections showed that ITO and FTO were better working electrodes than GCE. The presence of gallic acid was proven in Grape Seed 15000 since there was an increment in intensity when FTO was the working electrode as shown in Figure 45**Error! Reference source not found.**. Other than that, there was also presence of quercetin in this supplement where the intensity of the SWV of Grape Seed 15000 increased when GCE was modified with Bi_2O_3 as shown in Figure 46**Error! Reference source not found.**. Therefore, there were two antioxidants (gallic acid and quercetin, corresponded to 14.7 mg, 4.77mg, respectively) that contained in the Grape Seed 15000 manufactured by Herbs of Gold.

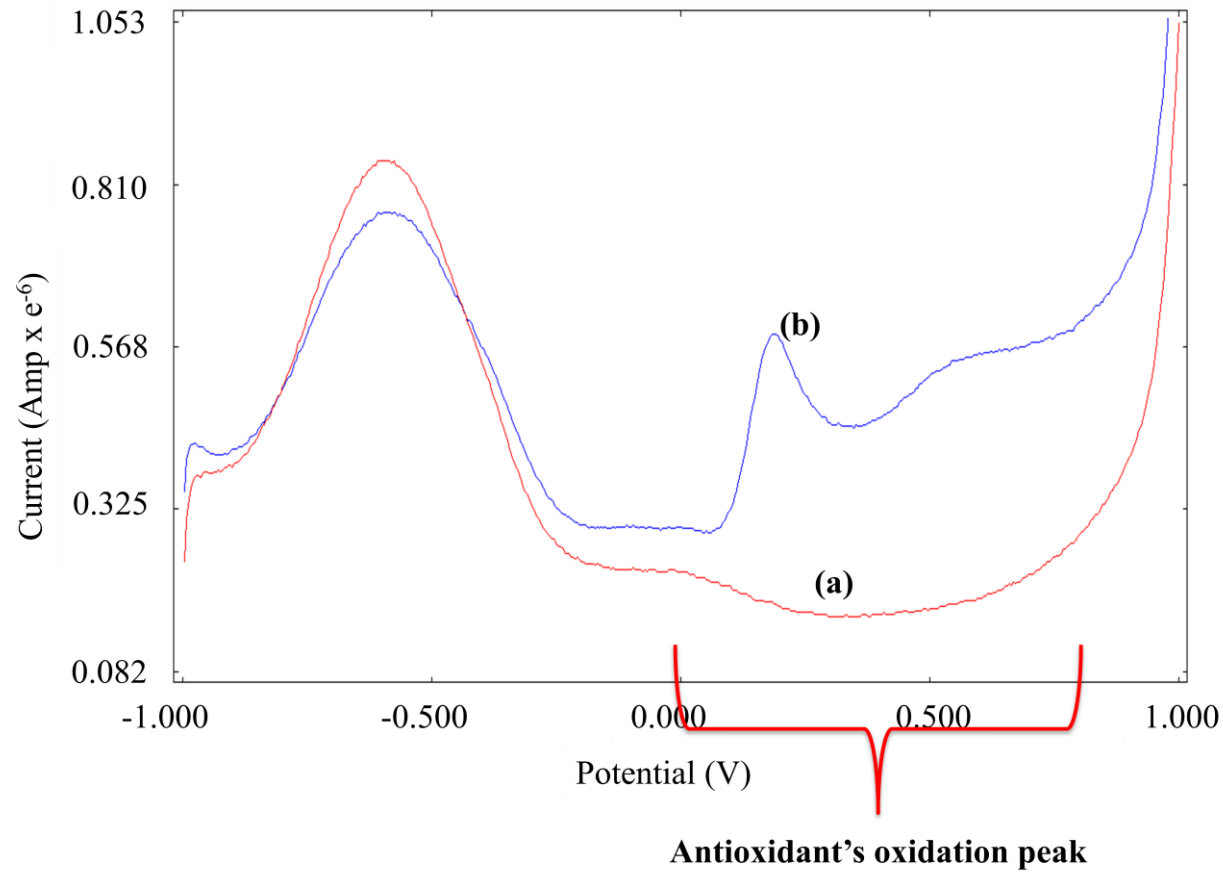


Figure 44 Square wave voltammogram of Grape Seed 15000 in (a) GCE and (b) GCE with Grape Seed 15000 in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

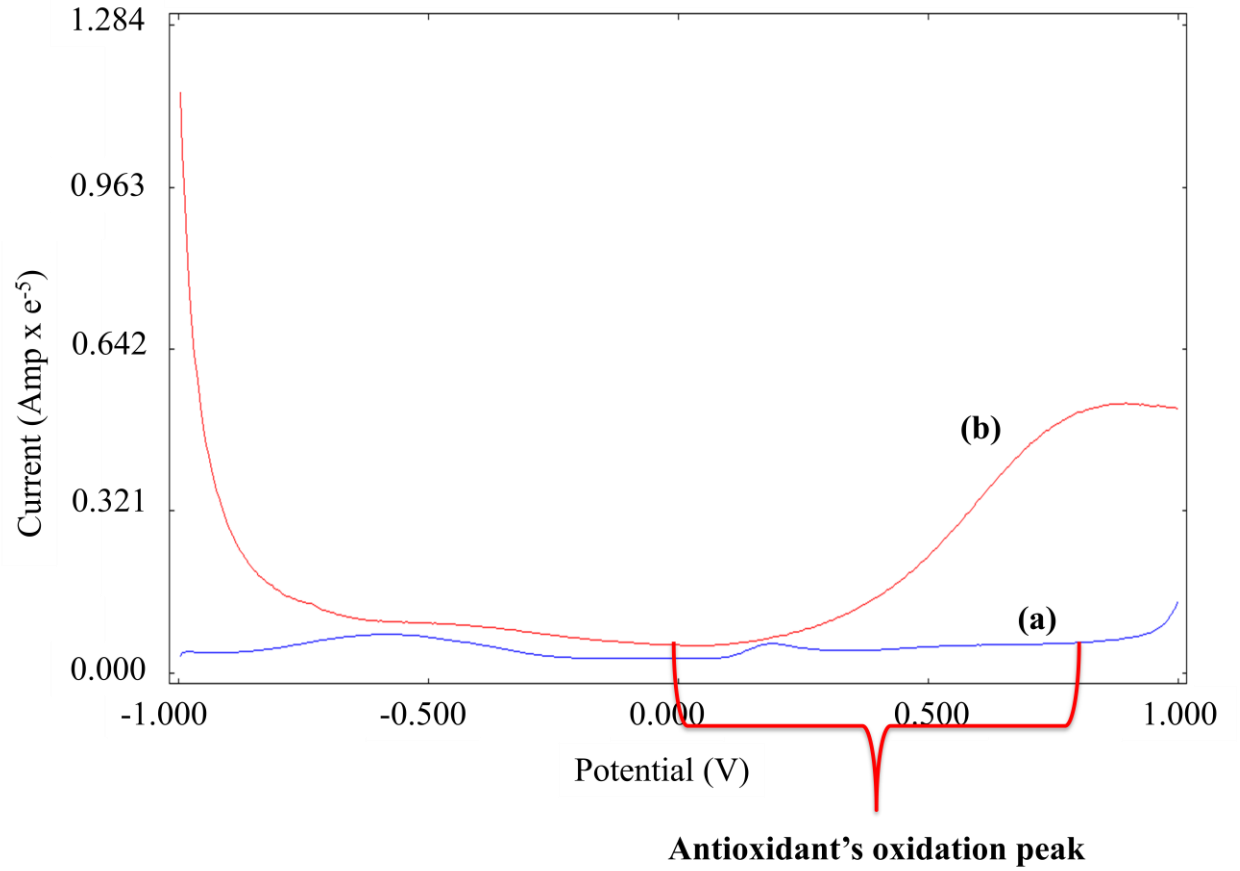


Figure 45 Square wave voltammogram of Grape Seed 15000 in (a) bare GCE and (b) FTO in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

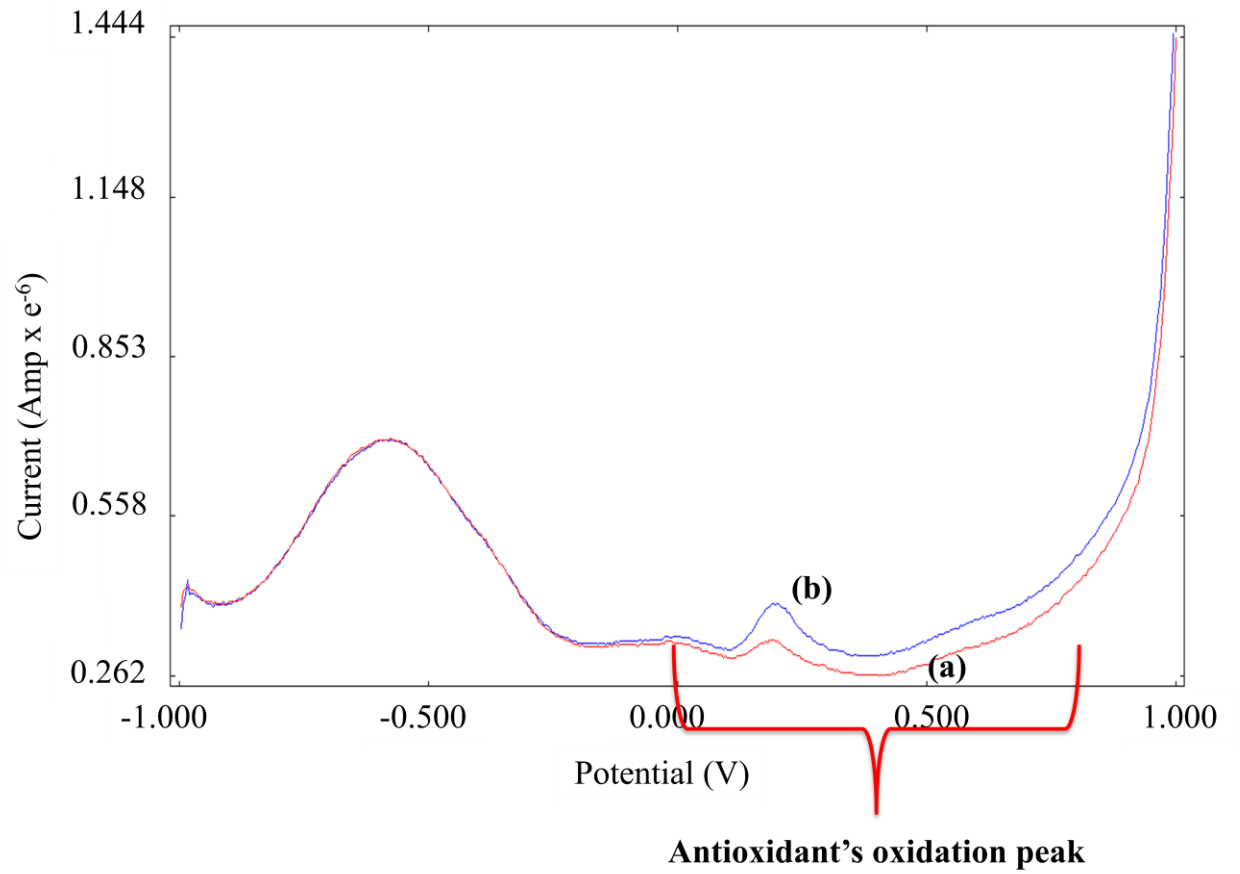


Figure 46 Square wave voltammogram of Grape Seed 15000 in (a) bare GCE and (b) GCE modified with Bi_2O_3 in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.5.1.3. Himalaya – Arjuna

The Arjuna's SWV was shown in Figure 47(a). There was an antioxidant peak observed in the following results. Modified electrodes have been tested on the Arjuna. Electrochemical analysis using glassy carbon electrode modified with bismuth oxide showed positive results (Figure 47), where increment in oxidation peak was observed. Therefore, quercetin (corresponded to 90.5mg) was present in Arjuna manufactured by Himalaya.

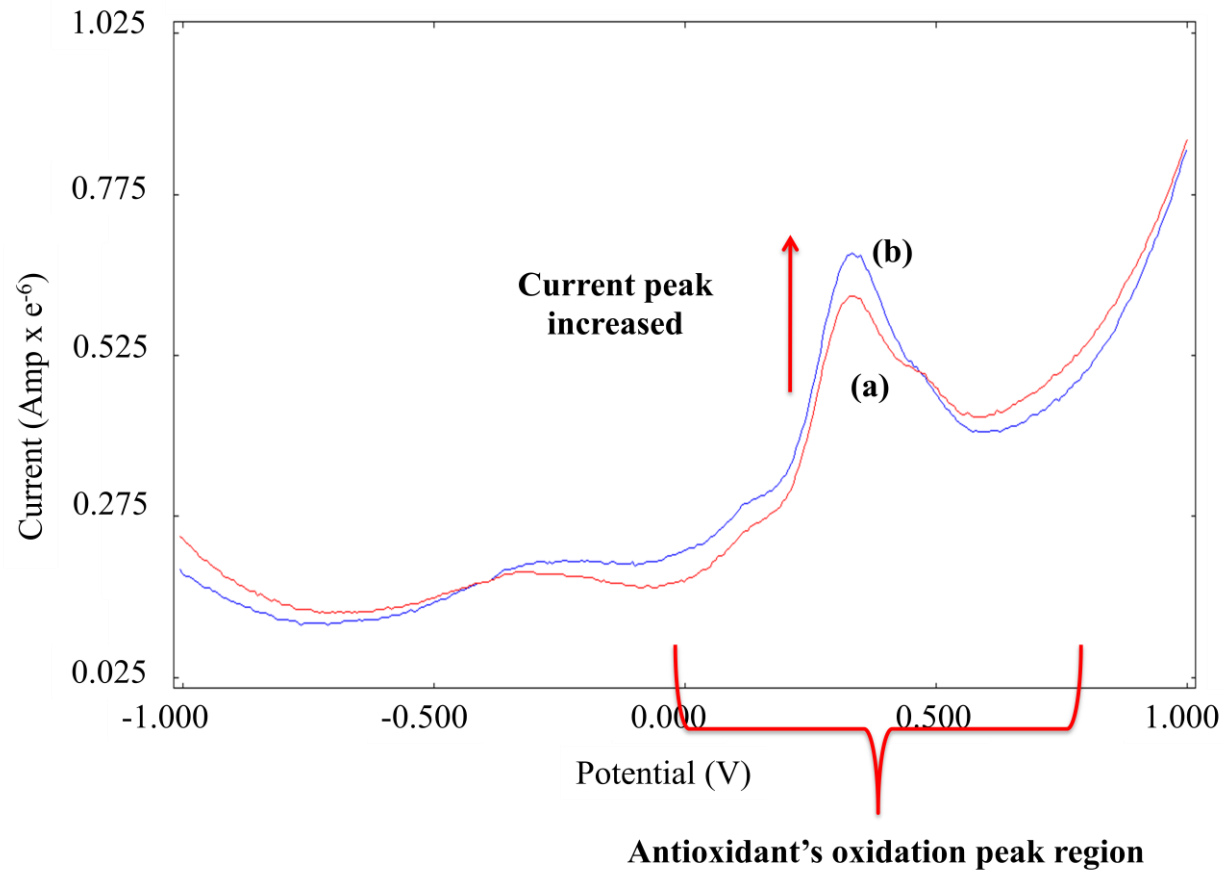


Figure 47 Square wave voltammogram of Arjuna in (a) GCE and (b) GCE modified with Bi_2O_3 in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

Figure 48 shows the voltammogram obtained at a GCE and an FTO electrode in the presence of Arjuna. GCE had detected the presence of antioxidant(s) as shown in Figure 48(a) but there was absence of antioxidant peak when FTO as working electrode Figure 48(b). Therefore, gallic acid was not present in Arjuna manufactured by Himalaya.

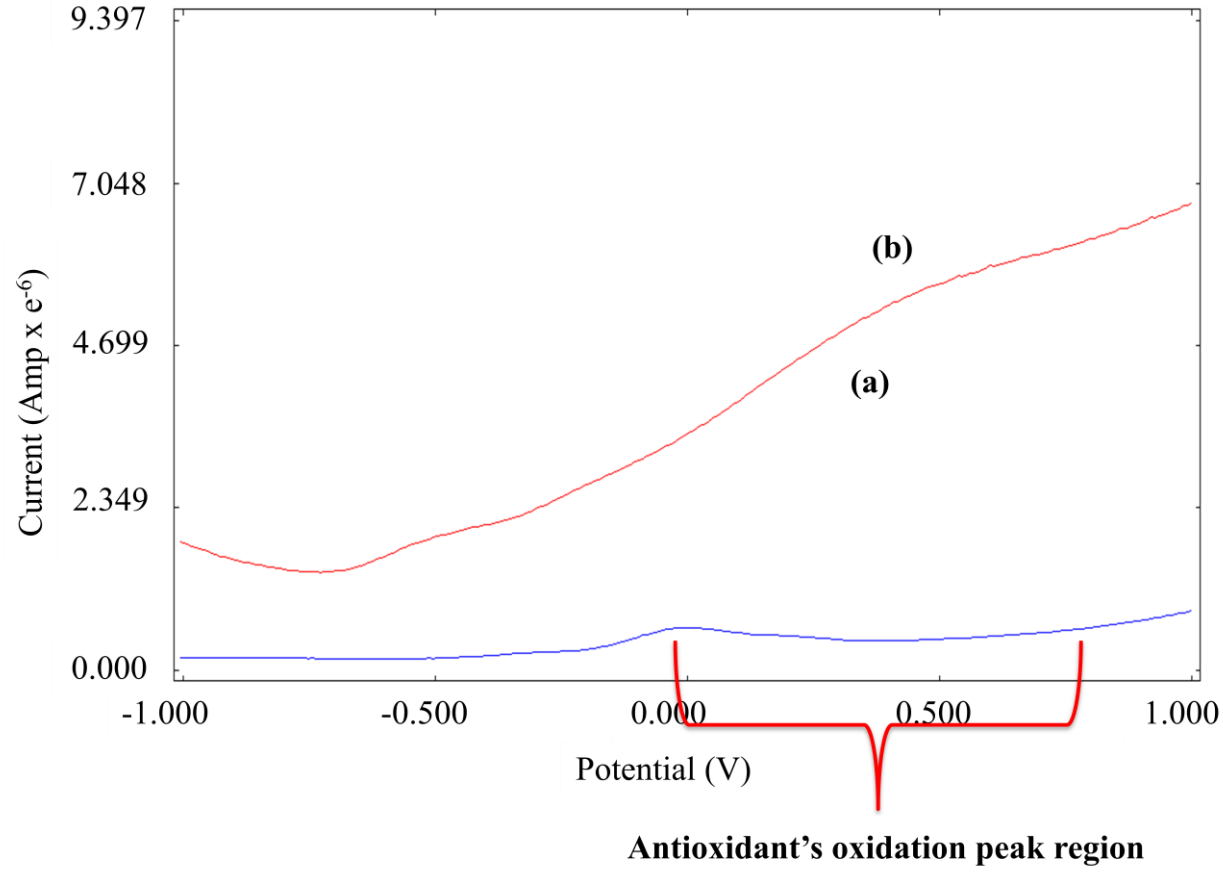


Figure 48 Square wave voltammogram of Arjuna in (a) GCE and (b) FTO in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.5.1.4. Himalaya – Amalaki

Amalaki showed two oxidation peaks in Figure 49. In order to reassure the content of the antioxidant in Amalaki, modified electrode was needed. FTO and ITO had been tested on the amalaki. The results showed that both FTO (Figure 50) and ITO (Figure 51) increased in intensity and it was proven that Amalaki contained gallic acid (corresponded to 20.2 mg) when the current peak of the antioxidant was increased.

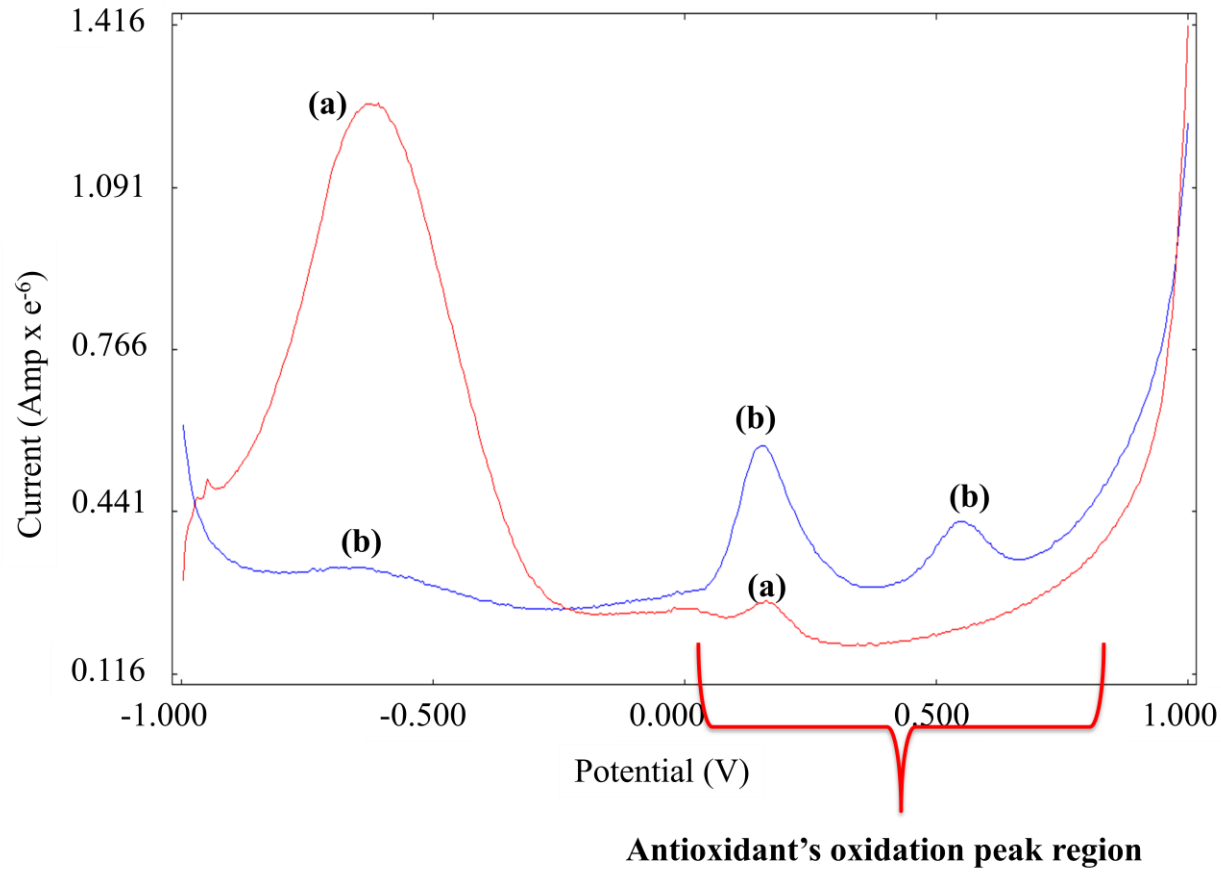


Figure 49 Square wave voltammogram of Amalaki in (a) GCE and (b) GCE with Amalaki in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

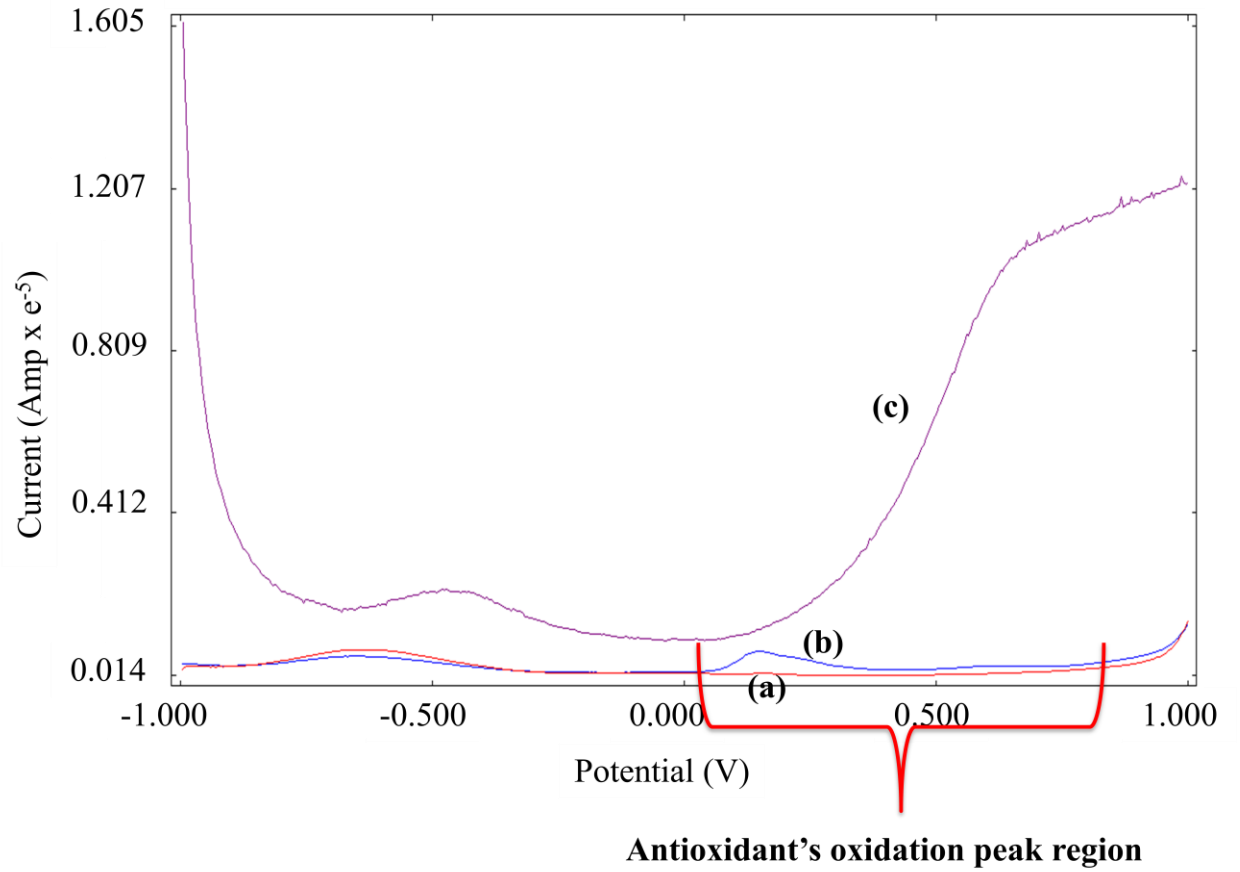


Figure 50 Square wave voltammogram of Amalaki in (a) bare GCE, (b) bare GCE with Amalaki, and (c) FTO with Amalaki in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

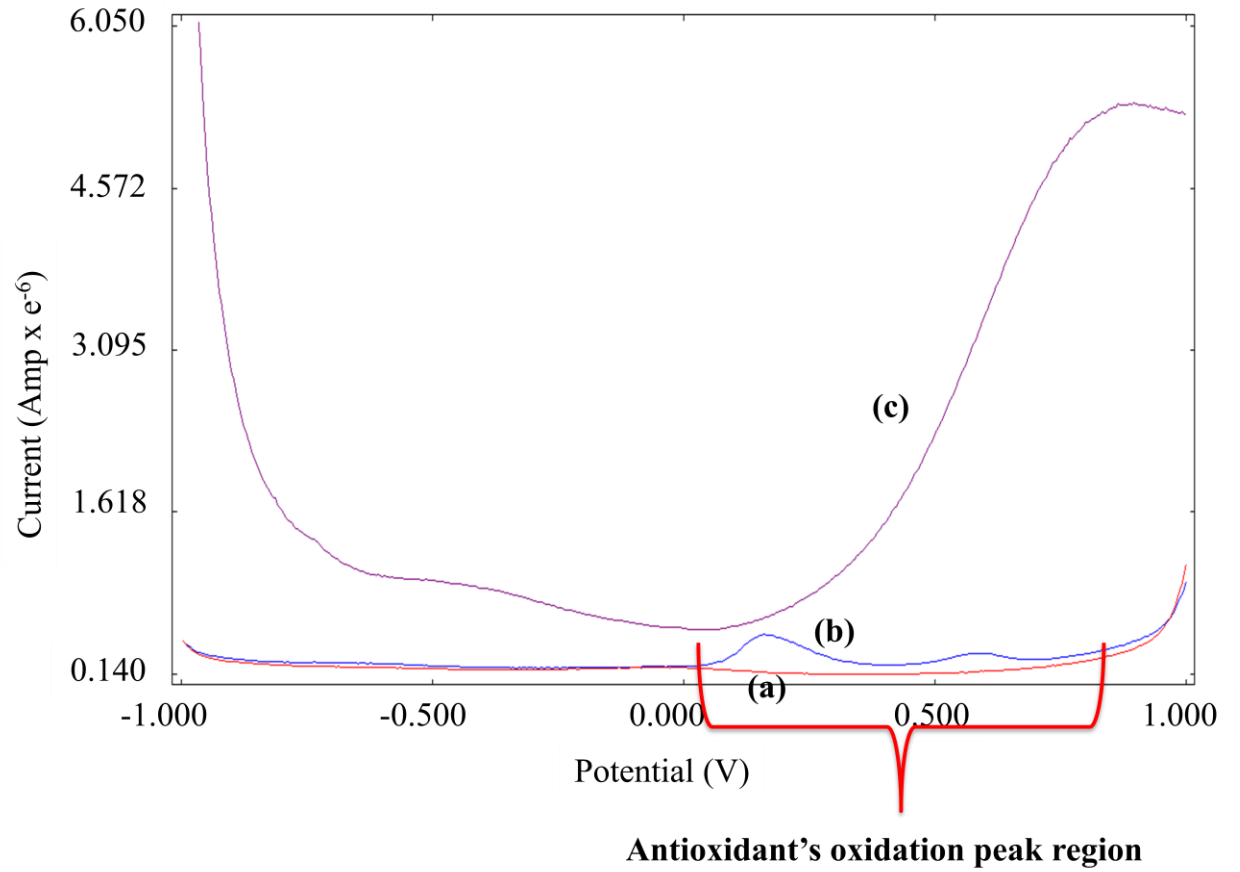


Figure 51 Square wave voltammogram of Amalaki in (a) bare GCE, (b) bare GCE with Amalaki, and (c) ITO with Amalaki in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.5.1.5. Himalaya – Mandukaparni

No oxidation peak can be observed in Figure 52. Thus, no further test will be done on this particular supplement.

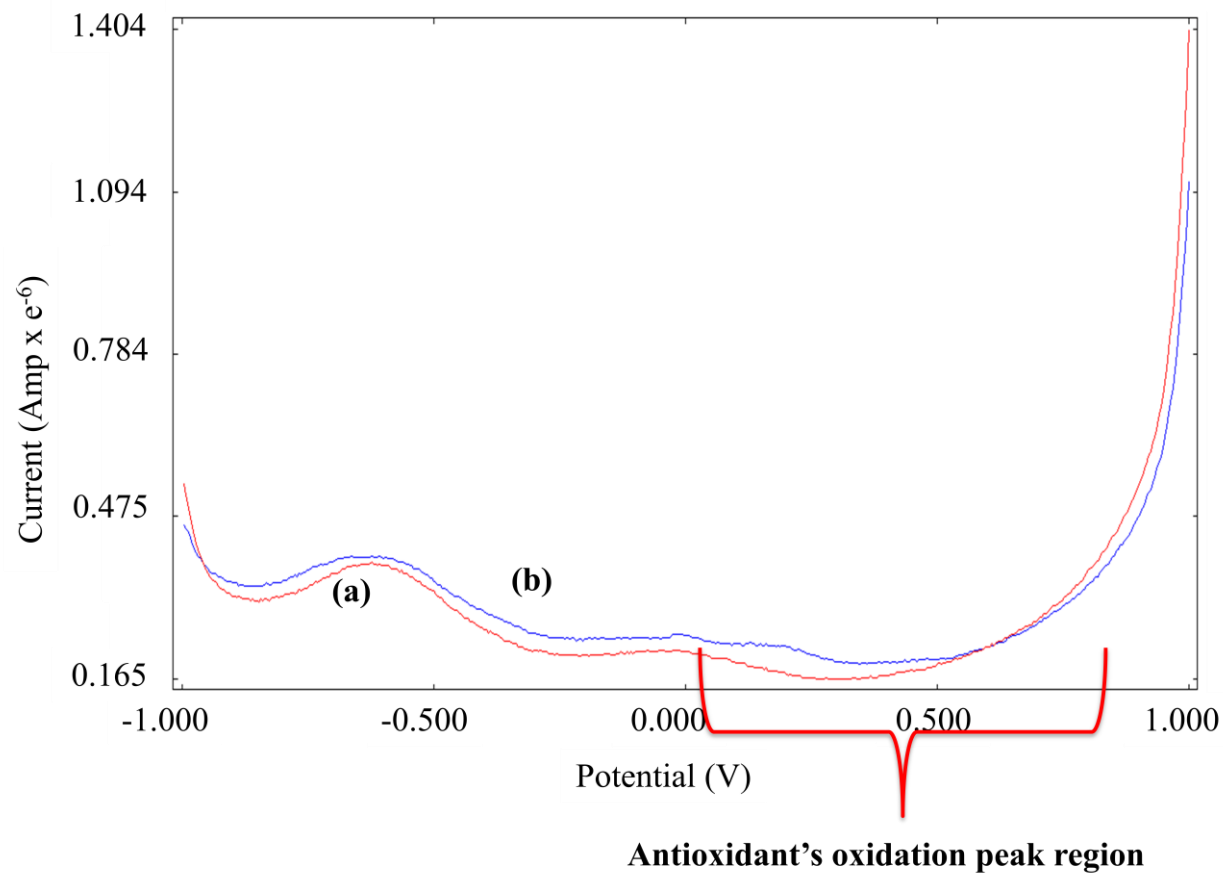


Figure 52 Square wave voltammogram of Mandukaparni in (a) GCE and (b) GCE with Mandukaparni in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.5.1.6. Himalaya – Neem

There were two oxidation peaks were observed in the following figure (Figure 53). FTO and GCE modified with Bi_2O_3 were used to study Neem, whereby only FTO showed increment in oxidation peak (Figure 54). Thus, Neem contained gallic acid and was detected electrochemically by using FTO as working electrode. This finding was not unexpected as there was a reported journal article which mentioned that Neem contained gallic acid (Upadhayay and Vigyan, 2014).

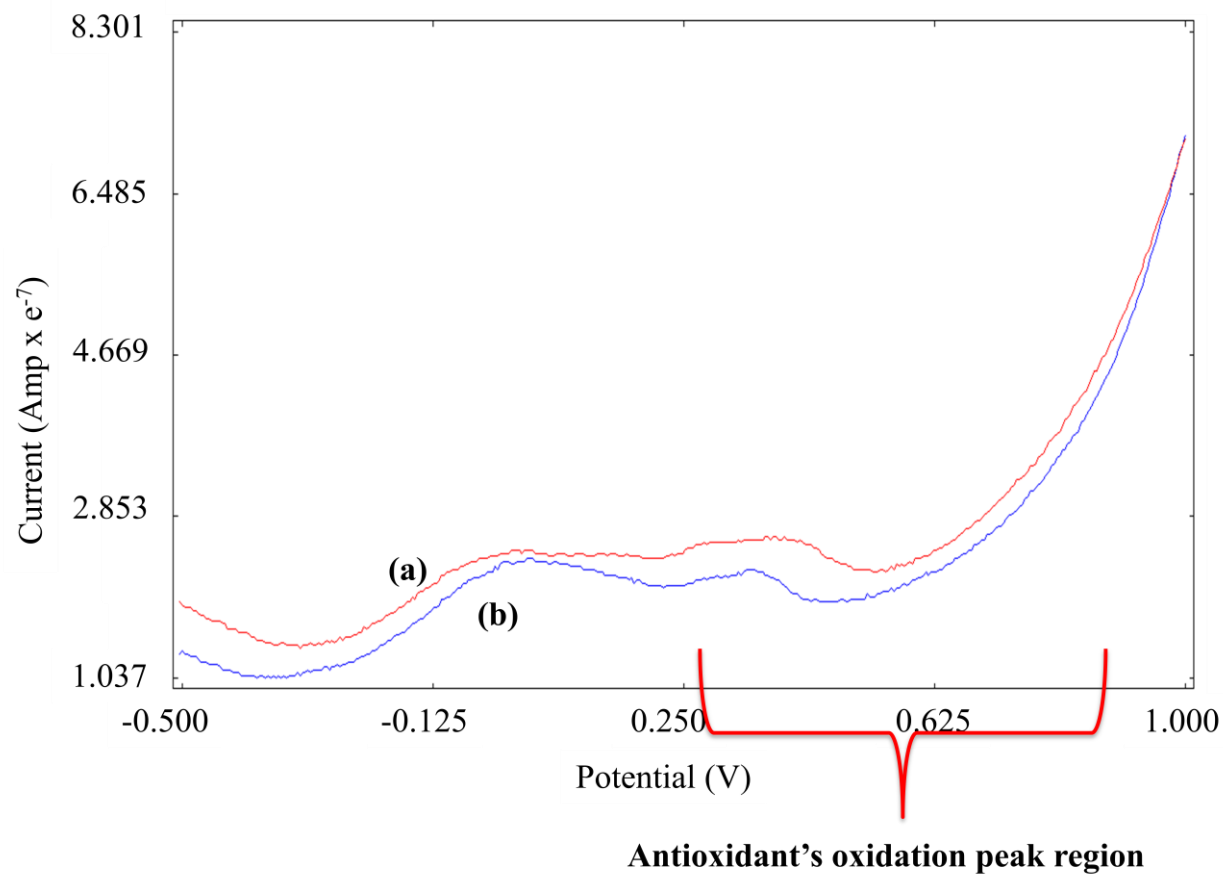


Figure 53 Square wave voltammogram of Neem in (a) GCE and (b) GCE modified with Bi_2O_3 in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

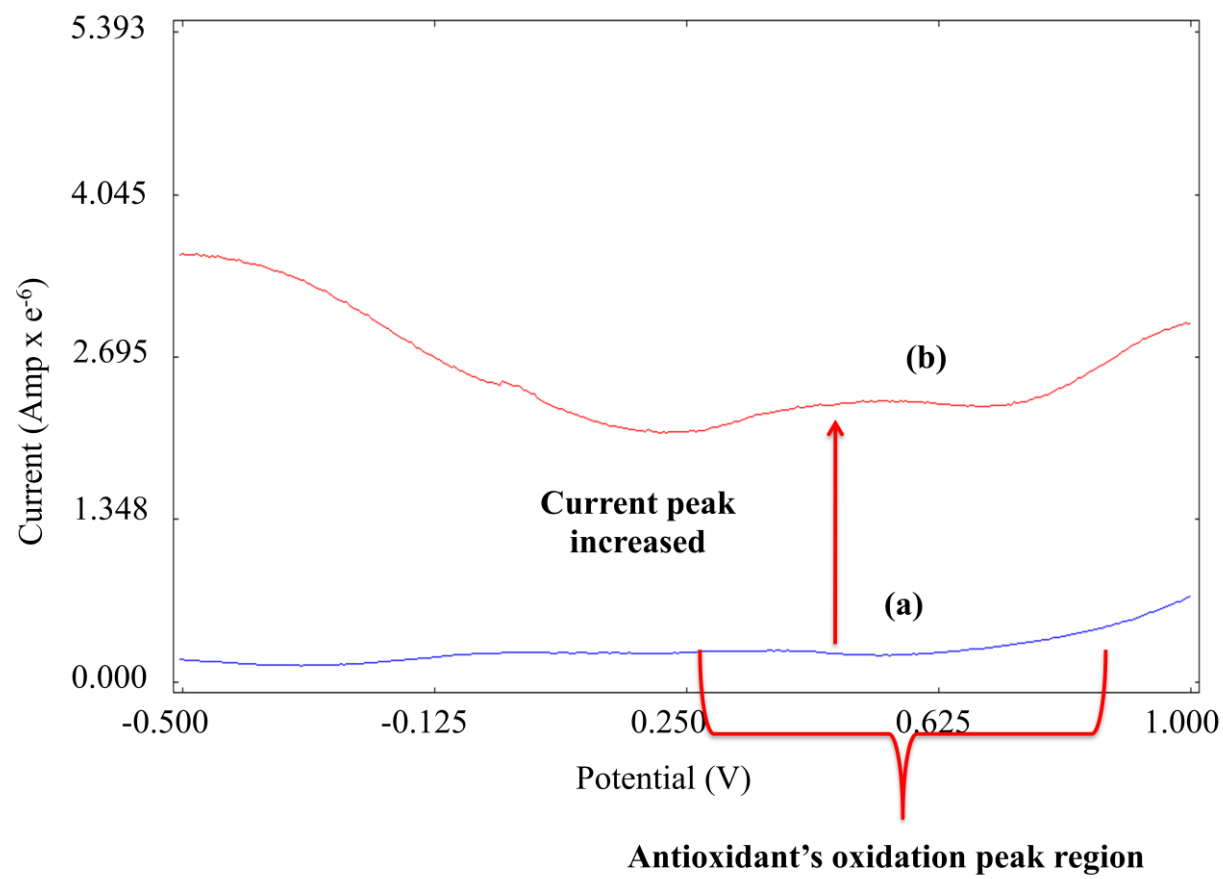


Figure 54 Square wave voltammogram of Neem in (a) GCE and (b) FTO in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.5.1.7. Himalaya – Triphala

Triphala had two oxidation peaks as shown in Figure 55. FTO and ITO had detected the presence of gallic acid (corresponded to 14.2 mg) in Triphala manufactured by Himalaya as shown in **Error! Reference source not found.** Figure 56 and Figure 57, respectively.

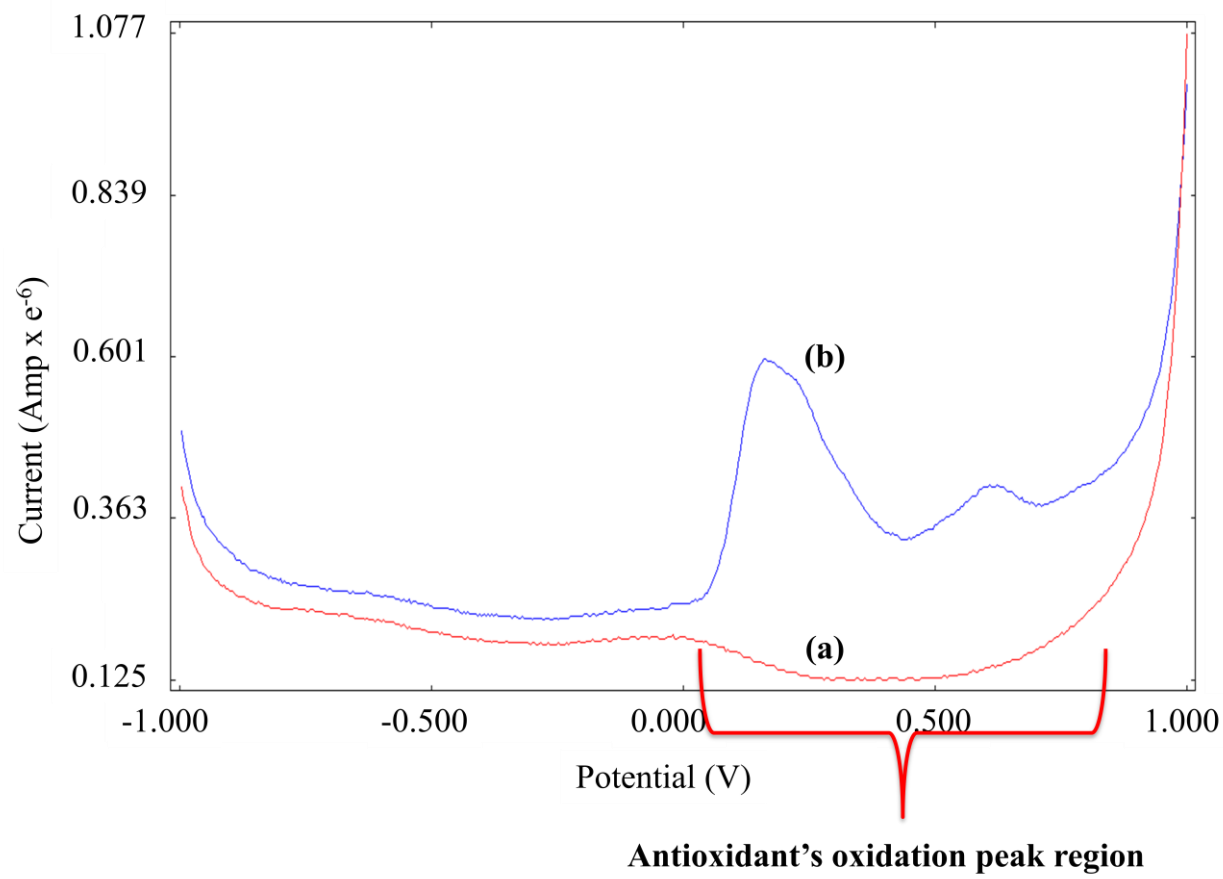


Figure 55 Square wave voltammogram of Triphala in (a) GCE and (b) GCE with Triphala in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

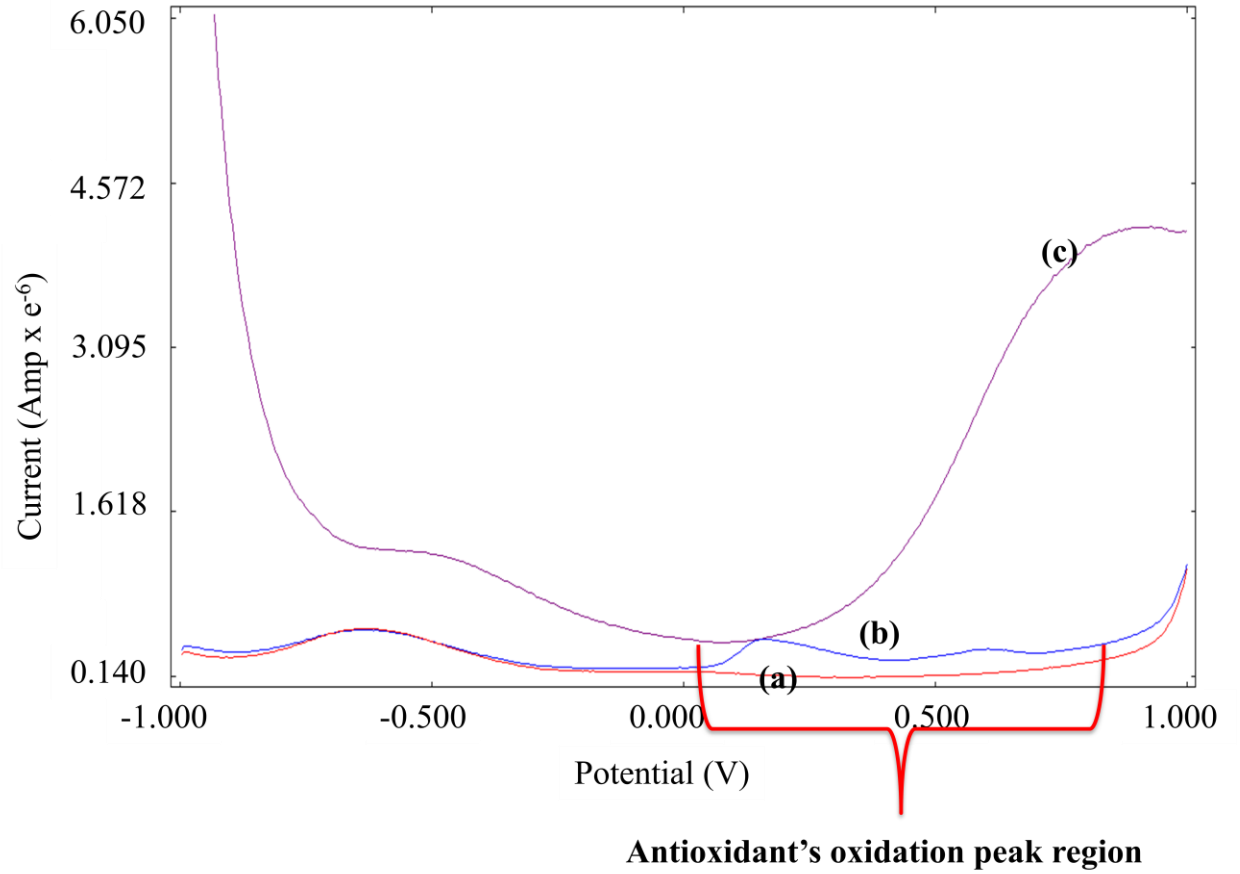


Figure 56 Square wave voltammogram of Triphala in (a) bare GCE, (b) bare GCE with Triphala, and (c) FTO with Triphala in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

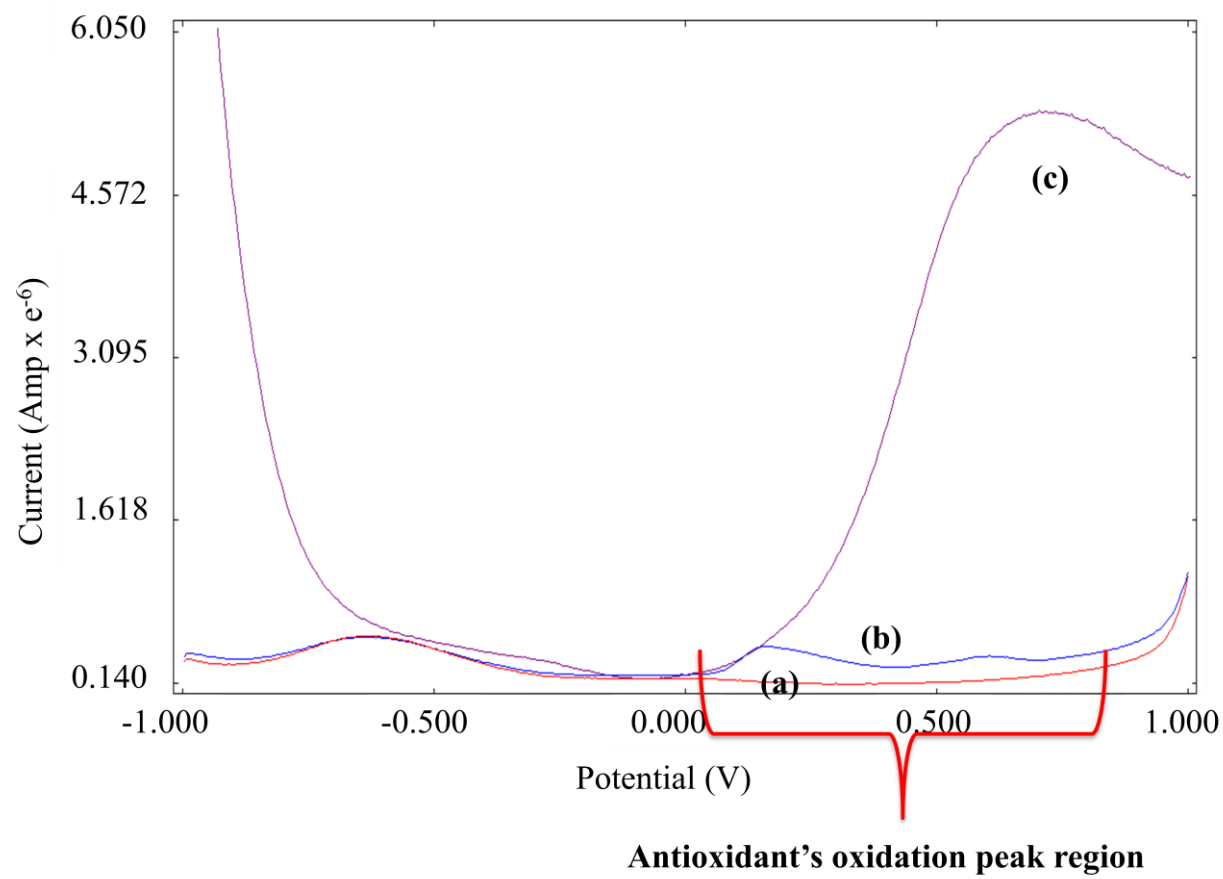


Figure 57 Square wave voltammogram of Triphala in (a) bare GCE, (b) bare GCE with Triphala, and (c) ITO with Triphala in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.5.1.8. Kordel's – Vitamin C Time

A significant oxidation peak was observed in the Figure 58. The significant peak was confirmed to be vitamin C. Thus, no further test which will be done on this supplement.

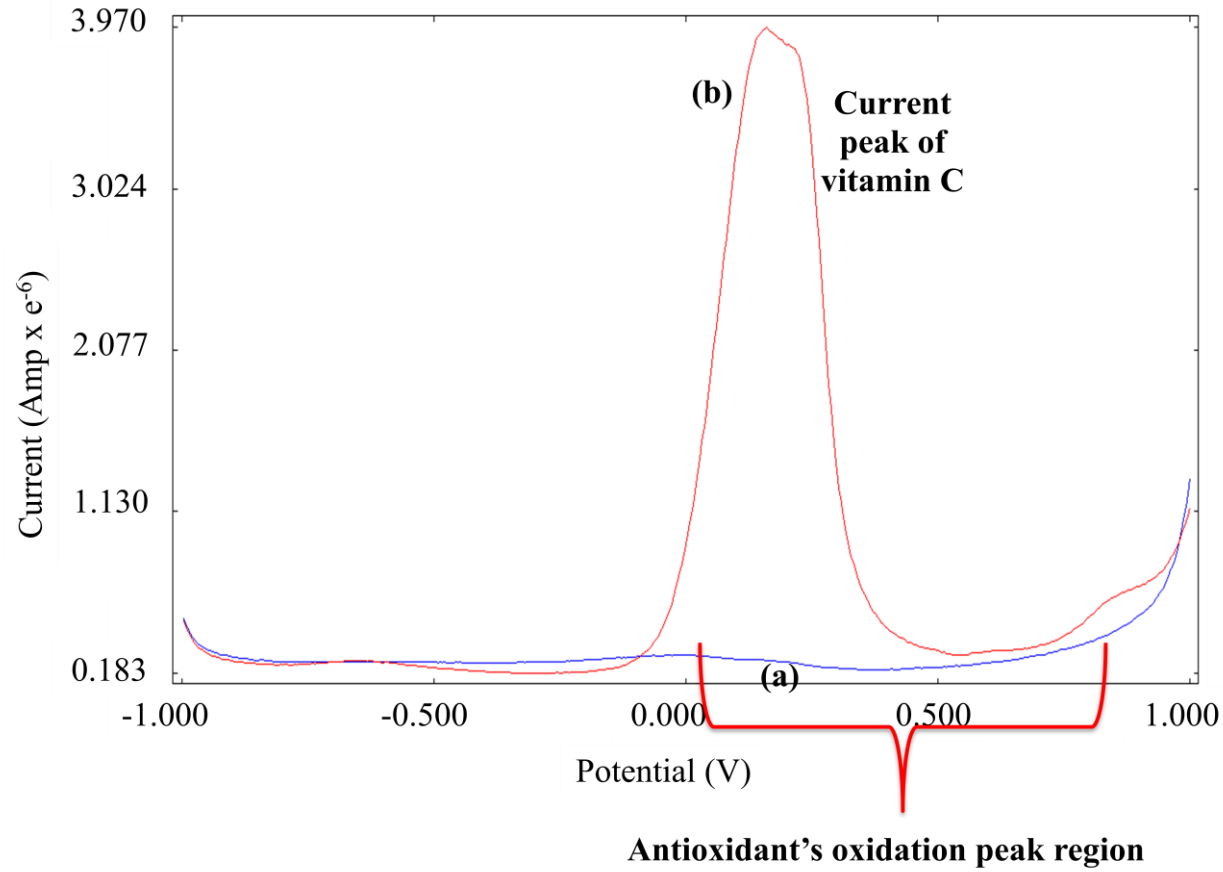


Figure 58 Square wave voltammogram of Vitamin C Time in (a) GCE and (b) GCE with Vitamin C Time in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.5.1.9. Kordel's - Bilberry

Two oxidation peaks were shown in Figure 59, where GCE was used as the working electrode. Electrochemical studies with modified electrodes showed that Bilberry contained gallic acid, which was verified by using FTO as working electrode (Figure 60). Oxidation peak increased after GCE was replaced by FTO as working electrode. Therefore, gallic acid (corresponded to 8.4 mg) was detected in Bilberry.

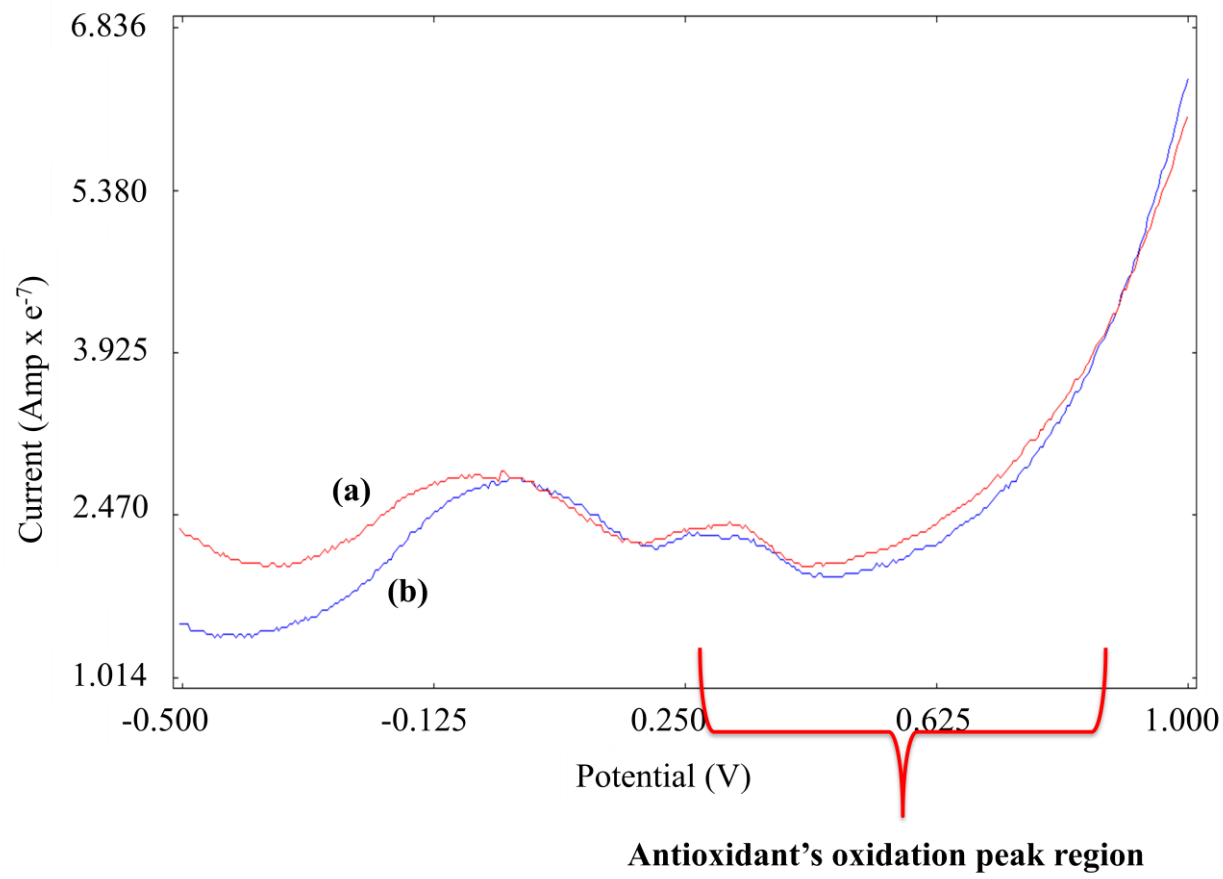


Figure 59 Square wave voltammogram of Bilberry in (a) GCE and (b) GCE modified with Bi_2O_3 in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

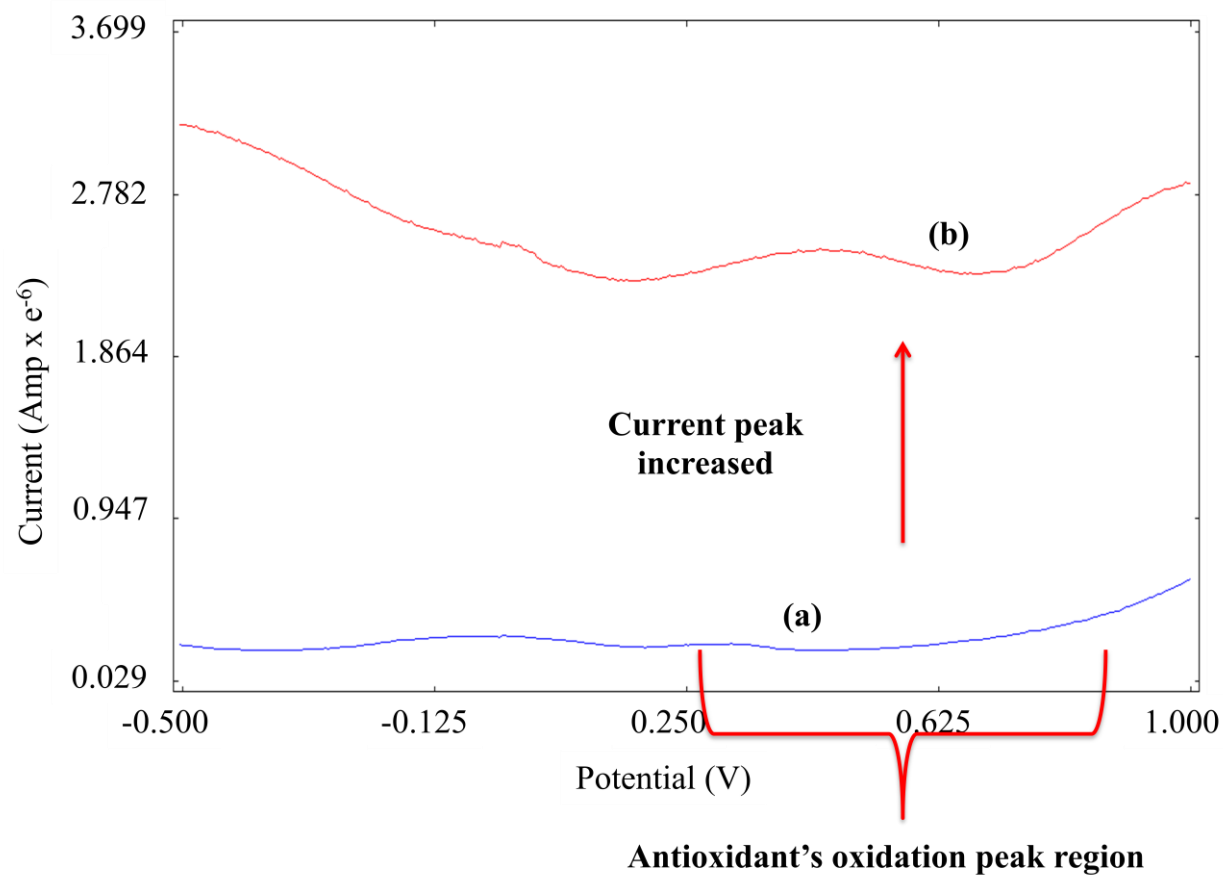


Figure 60 Square wave voltammogram of Bilberry in (a) GCE and (b) FTO in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.5.1.10. Kordel's – Grape Seed

There was one oxidation peak observed in Figure 61, where GCE was used as the working electrode. With regard to this, modified electrodes were utilized to study Kordel's Grape Seed by using electrochemical technique. Both FTO (Figure 62) and GCE modified with bismuth electrode (Figure 61) have been applied as working electrodes in two independent experiments. As mentioned before, a study had revealed that Kordel's Grape Seed contained both gallic acid and quercetin, which is in agreement with our results where oxidation peak was increased after modified electrodes were used.

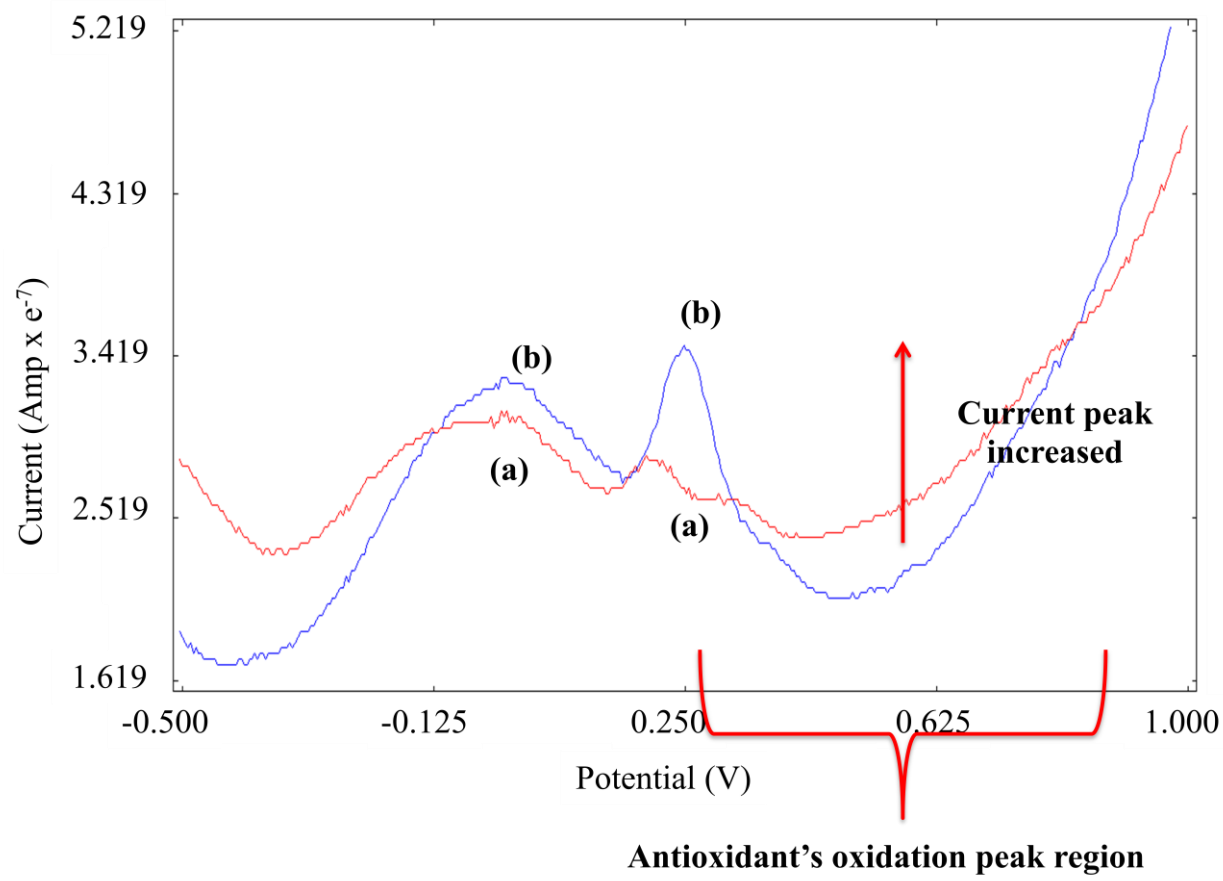


Figure 61 Square wave voltammogram of Kordel's Grape Seed in (a) GCE and (b) GCE modified with Bi_2O_3 in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

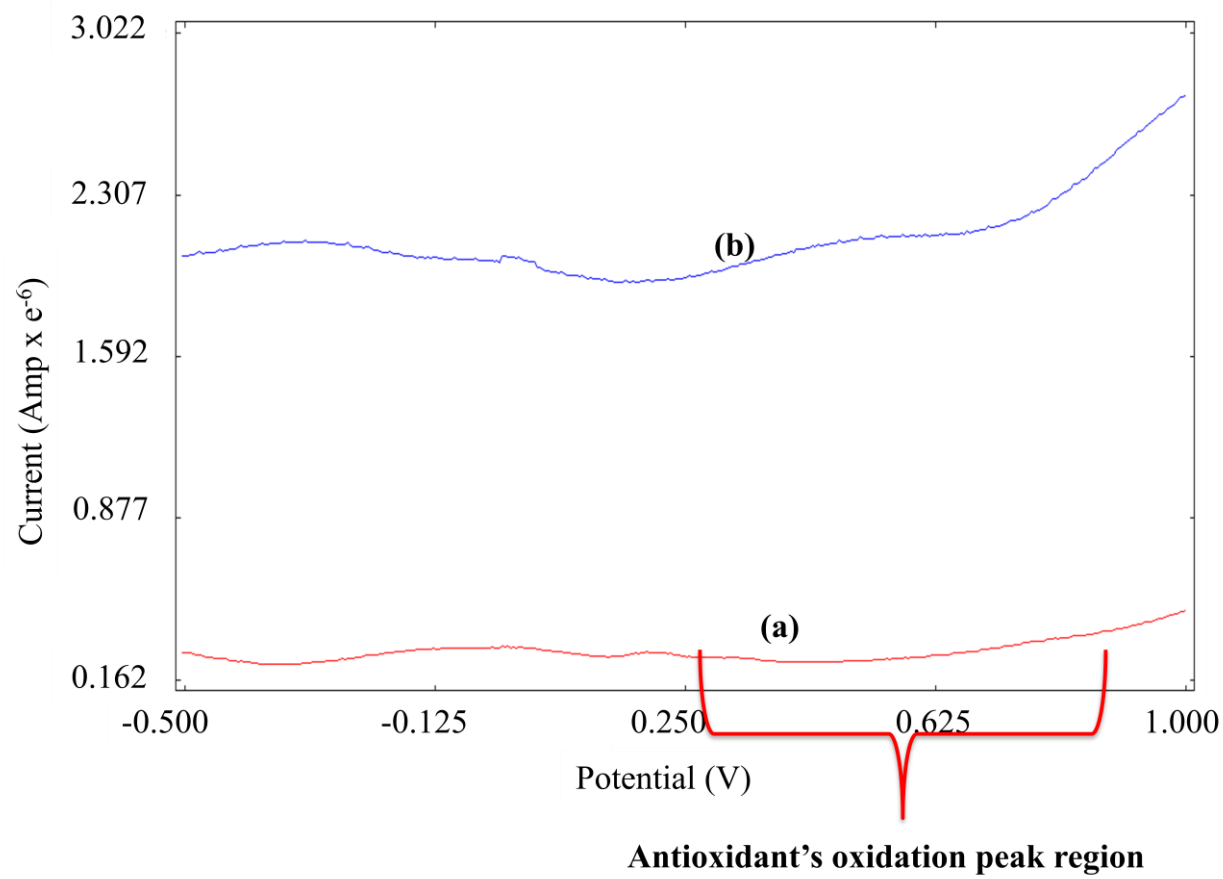


Figure 62 Square wave voltammogram of Kordel's Grape Seed in (a) GCE and (b) FTO in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.5.1.11. Mindatulus – Bromo-Q

No oxidation peak was observed in the voltammogram (Figure 63). Although the content of this supplement mentioned that it contained quercetin, but there was no oxidation peak observed in Figure 63 by using electrochemical techniques. Therefore, no further test was needed on Bromo-Q.

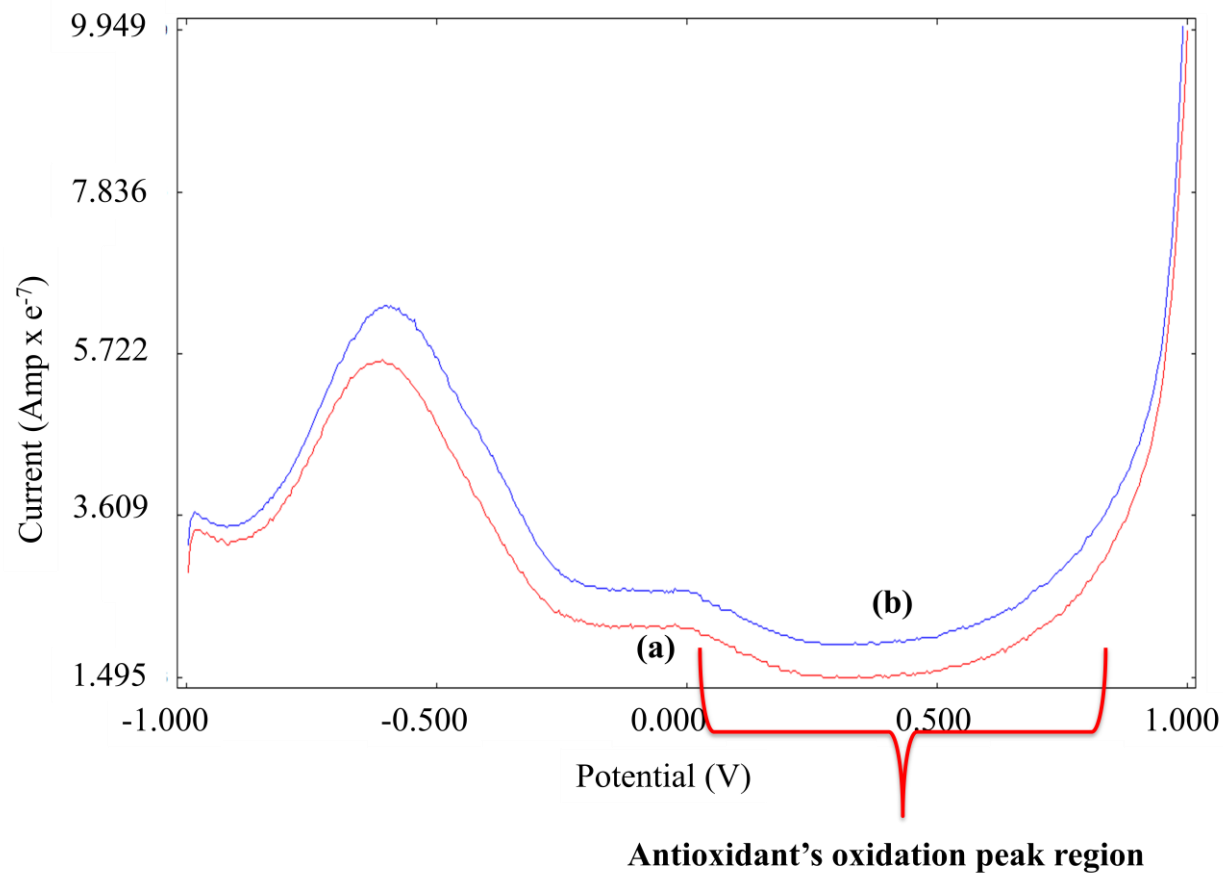


Figure 63 Square wave voltammogram of Bromo-Q in (a) GCE and (b) GCE with Bromo-Q in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.5.1.12. Solaray – CranActin (Cranberry)

There was no oxidation peak observed in the following SWV (Figure 64). Hence, no further test is required on this particular supplement.

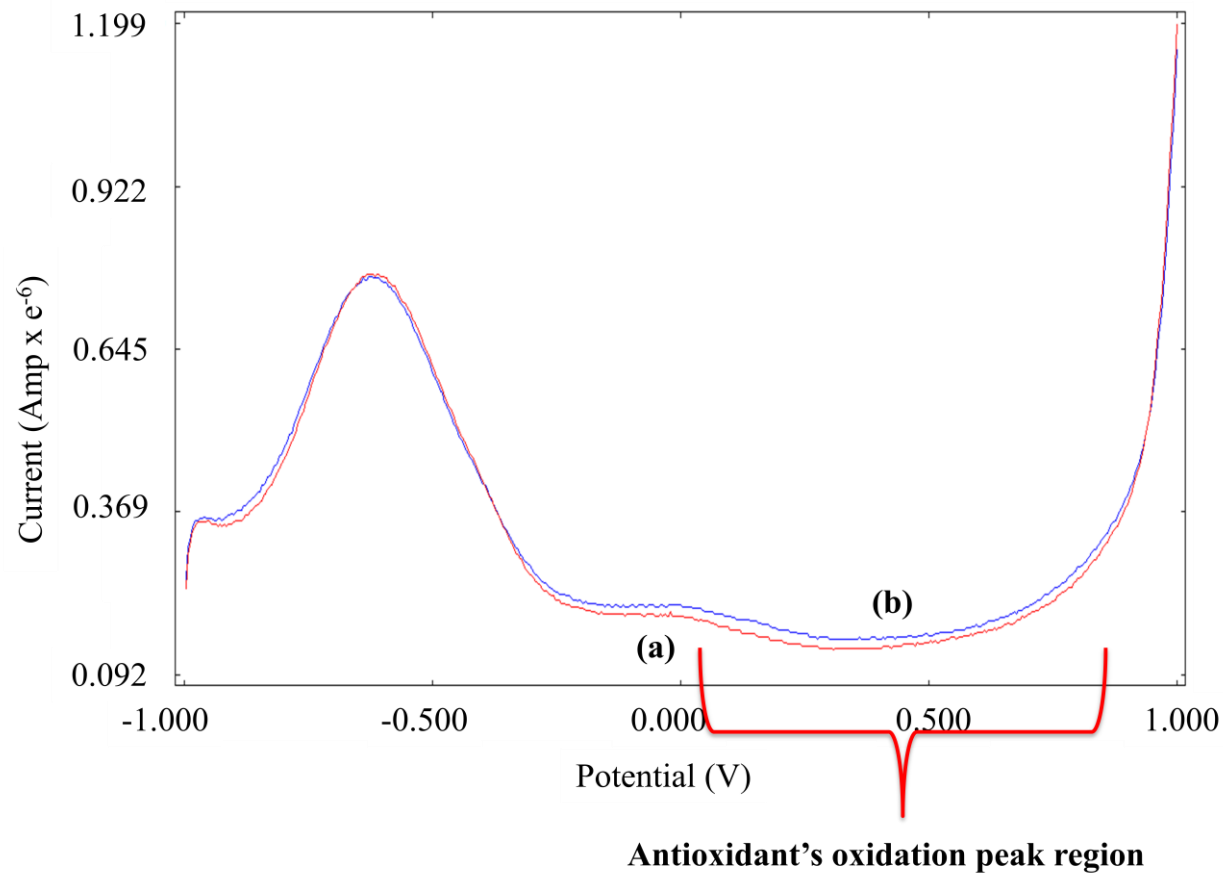


Figure 64 Square wave voltammogram of CranActin in (a) GCE and (b) GCE with CranActin in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.5.1.13. Vitahealth – Grape Seed

There was only one oxidation peaks observed in Figure 65. Regarding this, modified electrodes were then utilized to study Vitahealth's Grape Seed by using electrochemical technique. Both FTO and GCE modified with Bi_2O_3 electrode have been applied as working electrodes in two independent experiments. As aforementioned, a study had shown that grape seed contained both gallic acid and quercetin, which is same in our case where oxidation peak was increased after modified electrodes were used. Therefore, gallic acid and quercetin were detected in Grape Seed manufactured by Vitahealth.

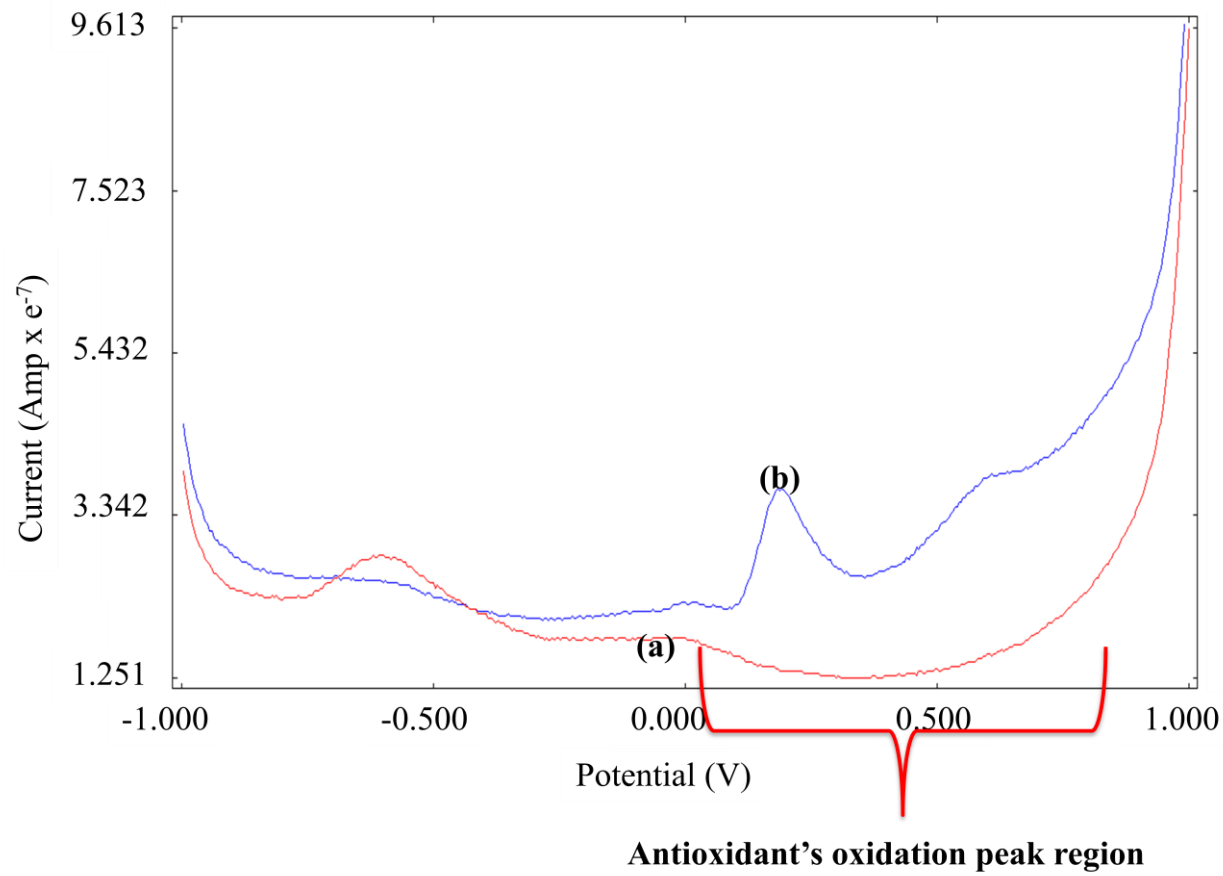


Figure 65 Square wave voltammogram of Vitahealth's Grape Seed in (a) GCE and (b) GCE with Vitahealth's Grape Seed in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.5.1.14. Vitahealth – Bioflavonoids C 1000 Plus Zinc

Similar to another vitamin C supplement result (Figure 58), the significant peak as shown in Figure 66 was vitamin C. Thus, no further test was needed for Bioflavonoids C 1000 Plus Zinc.

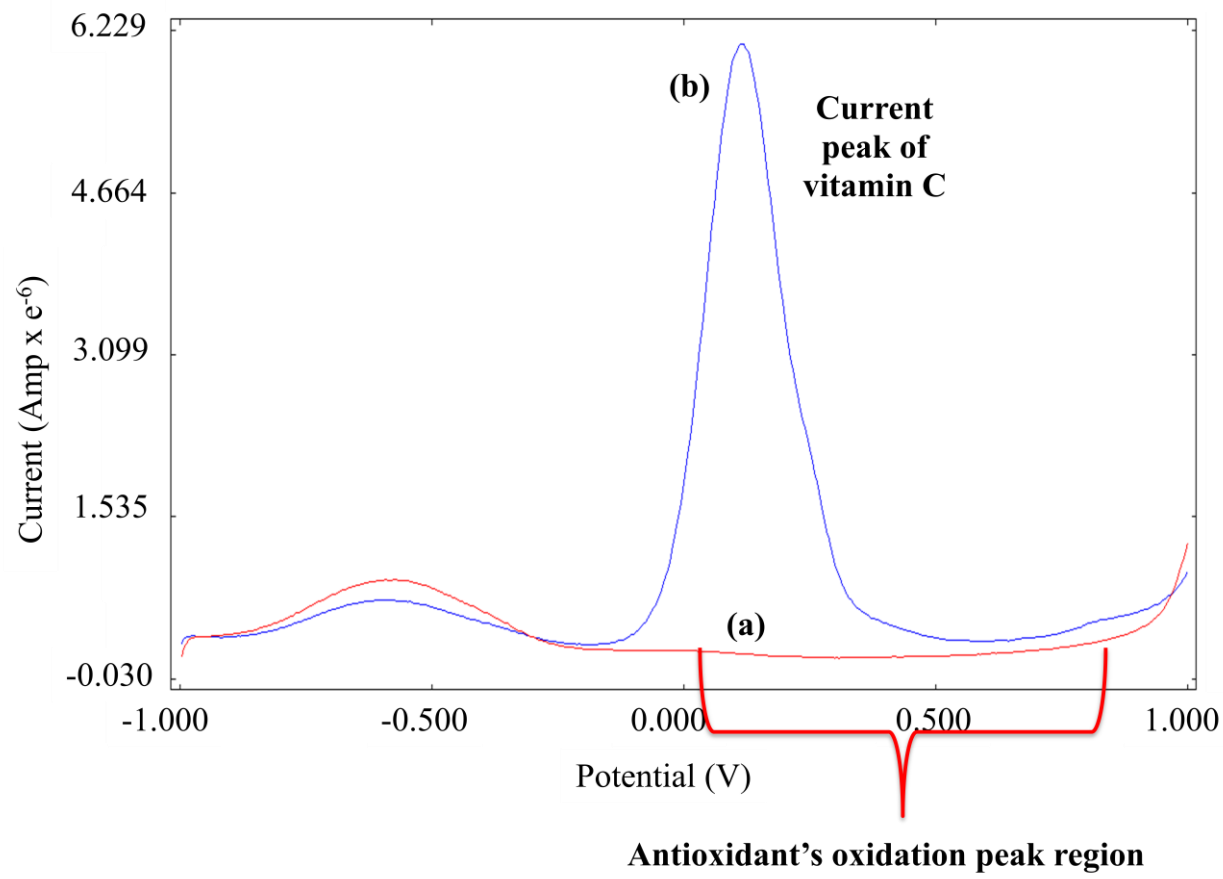


Figure 66 Square wave voltammogram of Bioflavonoids C 1000 plus zinc in (a) background and (b) background with Bioflavonoids C 1000 plus zinc in 0.1 M PBS with ethanol (1:1 v/v) at 15 Hz.

5.5.1.15. Summary of the Application Studies Using Electrochemical Analysis

The overall results of the application studies had been summarized in Table 6. Supplements containing vitamin C were not tested further because a vitamin C oxidation peak was observed in the same potential window as the antioxidant under investigation. All the grape seed extracts contain oxidation peak(s) and speculated to be gallic acid. Grape Seed 15000 manufactured by Herbs of Gold contained gallic acid and quercetin had been confirmed by using the desired modified electrodes, such as FTO and ITO for detection of gallic acid, and GCE modified with Bi_2O_3 for detection of quercetin. Other than that, Amalaki and Triphala manufactured by Himalaya contained gallic acid. Arjuna and Neem from Himalaya contain oxidation peak(s) with quercetin and gallic acid were identified, respectively. Bilberry contained gallic acid while Grape Seed extract from Kordel's contained both gallic acid and quercetin, as detected by using electrochemical technique.

Table 6 An overall results of the supplements studies.

Manufacturer	Supplements	Detection of Antioxidant(s)	
		✓ or X	Remarks
Herbs of Gold	Black Seed With Garlic	X	No antioxidant
	Grape Seed 15000	✓	Gallic acid and quercetin
Himalaya	Arjuna	✓	Quercetin
	Amalaki	✓	Gallic acid
	Mandukaparni	X	No antioxidant
	Neem	✓	Gallic acid
	Triphala	✓	Gallic acid
Kordel's	Vitamin C Time	X	Contains vitamin C
	Bilberry	✓	Gallic acid
	Grape Seed	✓	Gallic acid and quercetin
Mindatulus	Bromo-Q	X	No antioxidant
Solaray	CranActin (Cranberry)	X	No antioxidant
Vitahealth	Grape Seed	✓	Gallic acid and quercetin
	Bioflavonoids C 1000 Plus Zinc	X	Contains vitamin C

Legends:✓ = positive detection, X = negative detection

5.5.2. High Performance Liquid Chromatography Studies

HPLC analysis was carried out to identify the presence of the studied antioxidants, including gallic acid and quercetin, in commercially available supplements used in this study. HPLC was performed by using HPLC grade methanol and water as the mobile phases. The HPLC analysis was started with 5 % methanol and increased gradually to 100 % methanol in 45 min, and then followed by 100 % methanol (isocratic) for 15 min. The elution profile was monitored at 210 nm, background (as shown in Figure 67), gallic acid and quercetin (without any supplements) being detected at the retention time of 22.26 min (as shown in Figure 68) and 36.95 min (as shown in Figure 69), respectively.

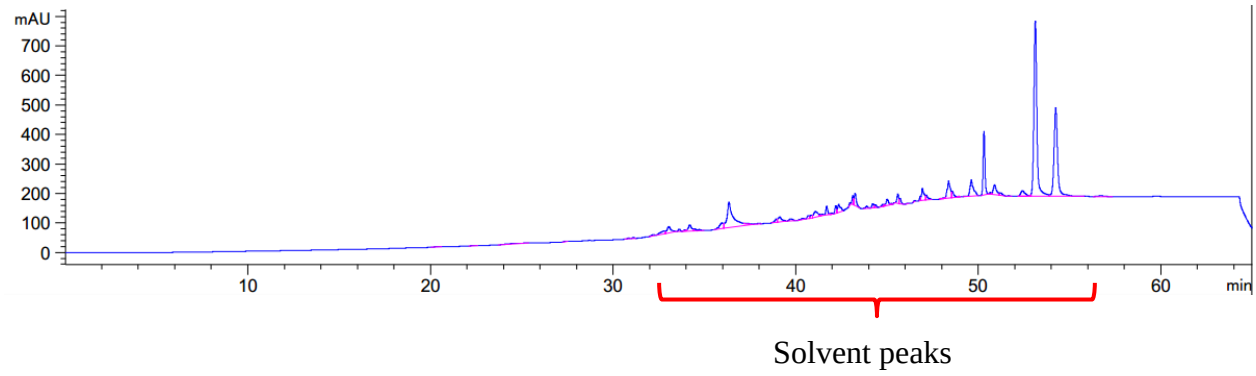


Figure 67 Elution profile of mobile phase (ethanol/water), gradient mode before 45 minutes and isocratic after 45 minutes.

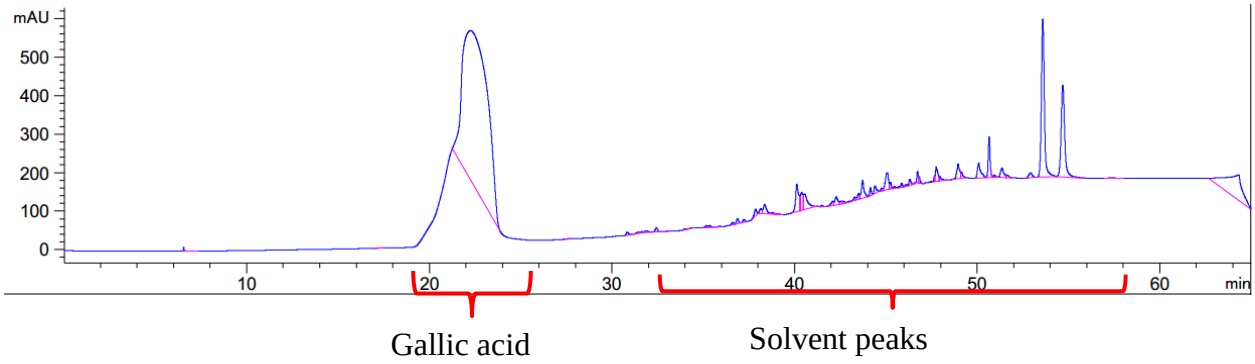


Figure 68 Elution profile of gallic acid (0.5 mg/mL), gradient mode before 45 minutes and isocratic after 45 minutes.

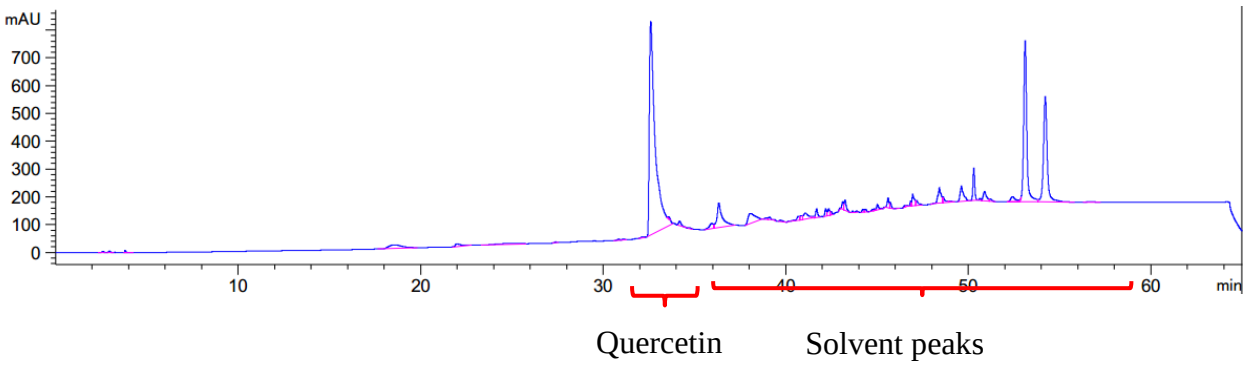


Figure 69 Elution profile of quercetin (0.5 mg/mL), gradient mode before 45 minutes and isocratic after 45 minutes.

5.5.2.1. Kordel's – Bilberry

Elution profile of Kordel's Bilberry was shown in Figure 72. Spiking addition method had been used onto Kordel's Bilberry with addition of gallic acid. Gallic acid was eluted around 12 minutes and had been identified as compared to the solvent HPLC analysis Figure 70(a).The elution time of pure gallic acid as shown in Figure 68 was different might be caused by synergy effect from other substances in the supplement. The final concentration in the HPLC vial was ranging from 0.15 mg to 2.5 mg in 2 mL of HPLC vial. Based on the calibration curve in Figure 71, the weight of gallic acid contained in 500 mg of capsulated Kordel's Bilberry was corresponded to 6.69 mg.

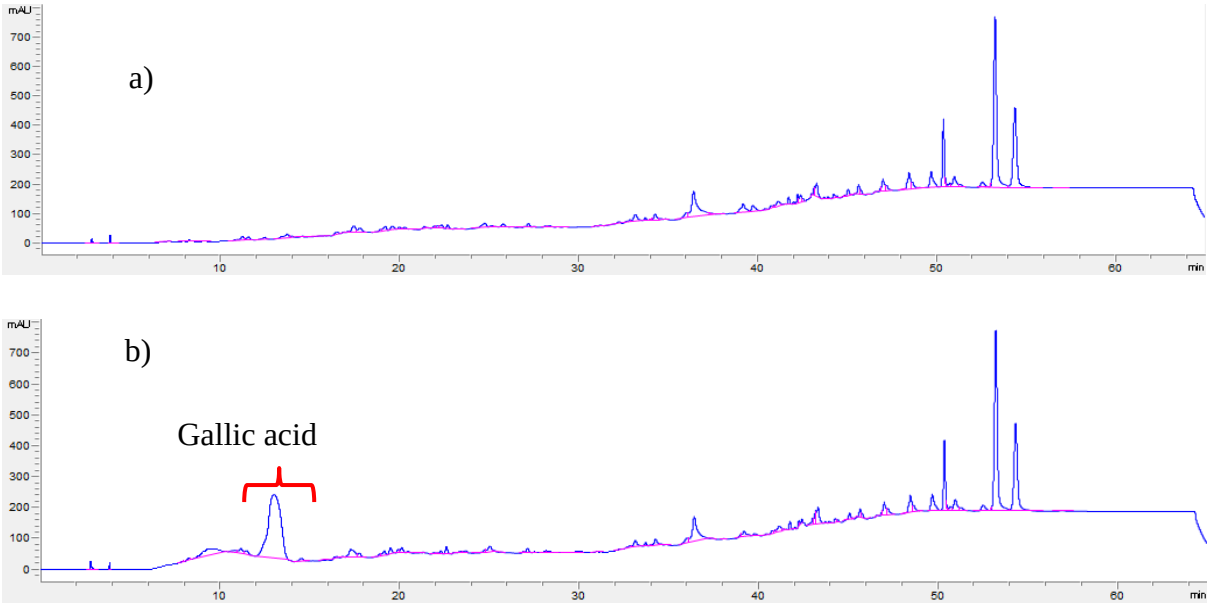


Figure 70 Elution profile of Kordel's Bilberry in a) Bilberry only and b) Bilberry with gallic acid by using spiking technique.

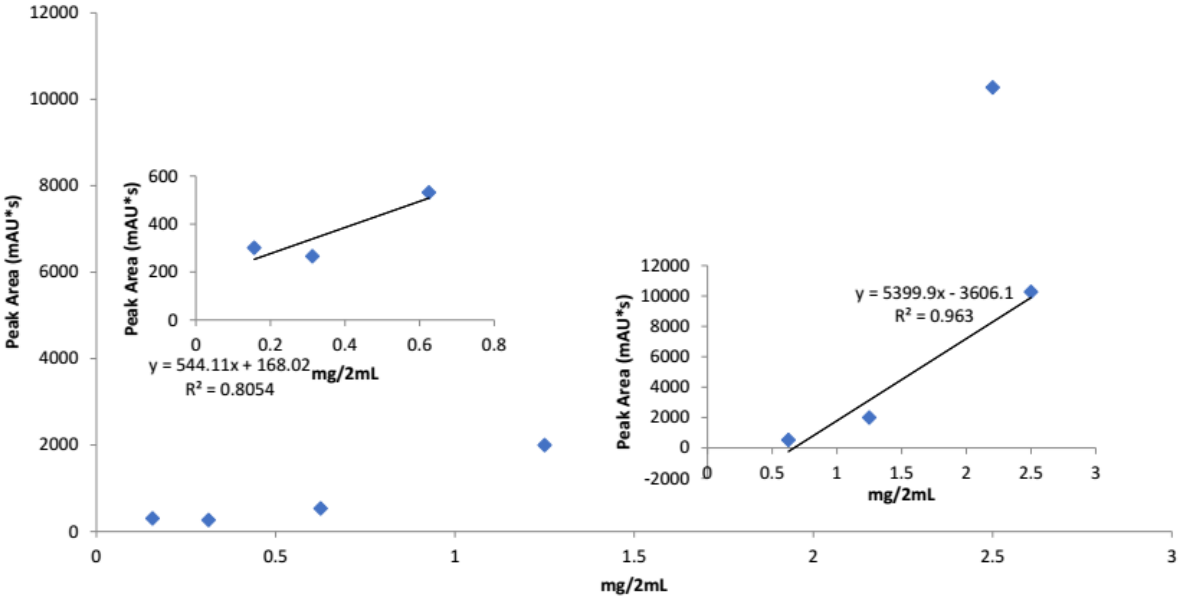


Figure 71 Standard addition curve of Kordel's Bilberry for gallic acid obtained from HPLC analysis.

According to Figure 72, quercetin was detected and identified by using spiking addition method. The retention time of quercetin in Bilberry was at around 32.63 minutes. A series of spiking addition method had been used onto Bilberry. The final concentration in the HPLC vial was ranging from 0.15 mg to 2.5 mg in 2 mL of HPLC vial. A calibration curve was constructed as shown in Figure 73. Based on the calibration curve, the weight of quercetin contained in 50 mL of stock solution was corresponded to 3.07 mg. Therefore, there was about 3.07 mg of quercetin in each 500 mg of Kordel's Bilberry capsule.

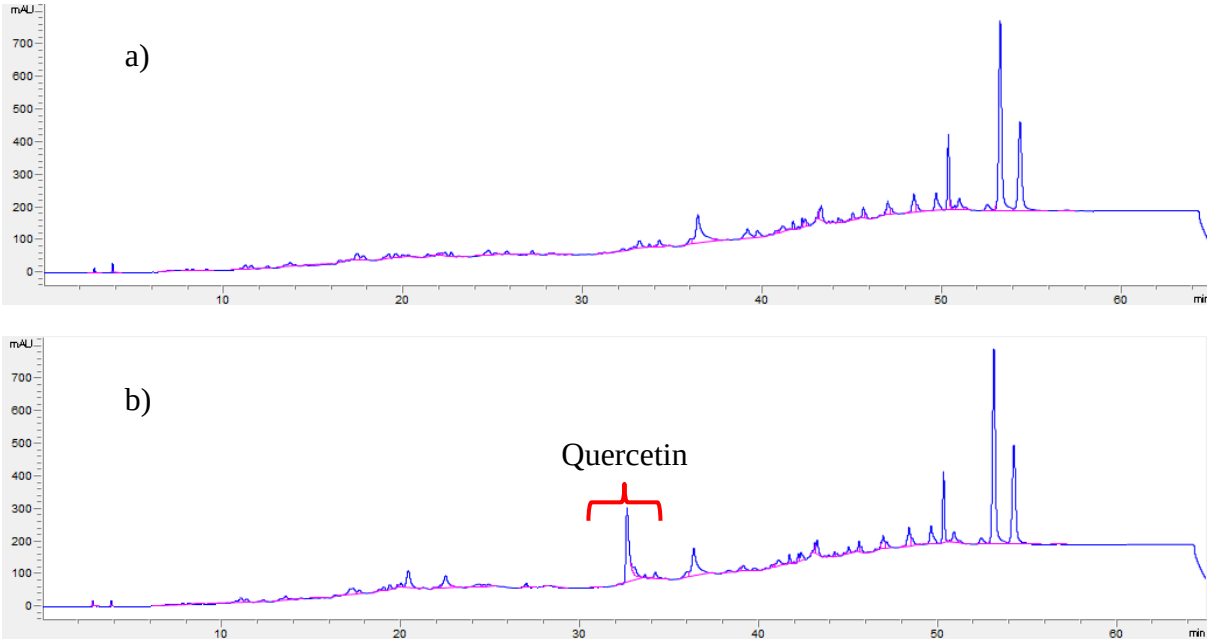


Figure 72 Elution profile of Kordel's Bilberry in a) Bilberry only and b) Bilberry with quercetin by using spiking technique.

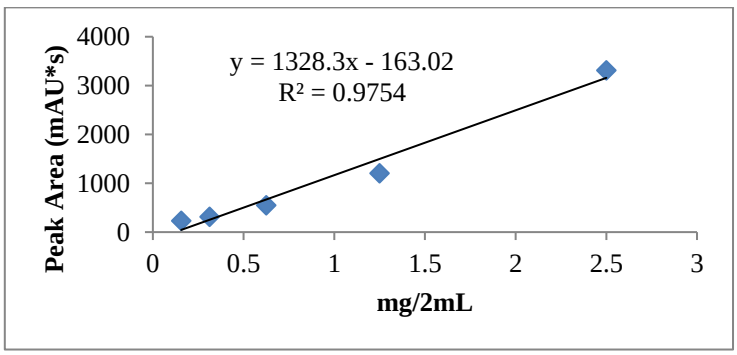


Figure 73 Standard addition curve of Kordel's Bilberry for quercetin obtained from HPLC analysis.

5.5.2.2. Kordel's – Grape Seed

Gallic acid was eluted around 12 minutes (Figure 74) and had the same retention time as discussed in 0, in which consistency was observed. Spiking addition method was used on Kordel's Grape Seed with addition of gallic acid. The final concentration in the HPLC vial was ranging from 0.15 mg to 2.5 mg in 2 mL of HPLC vial. The corresponded weight (calculation based on Figure 77) of gallic acid incapsulated Kordel's Grape Seed was 6.79 mg.

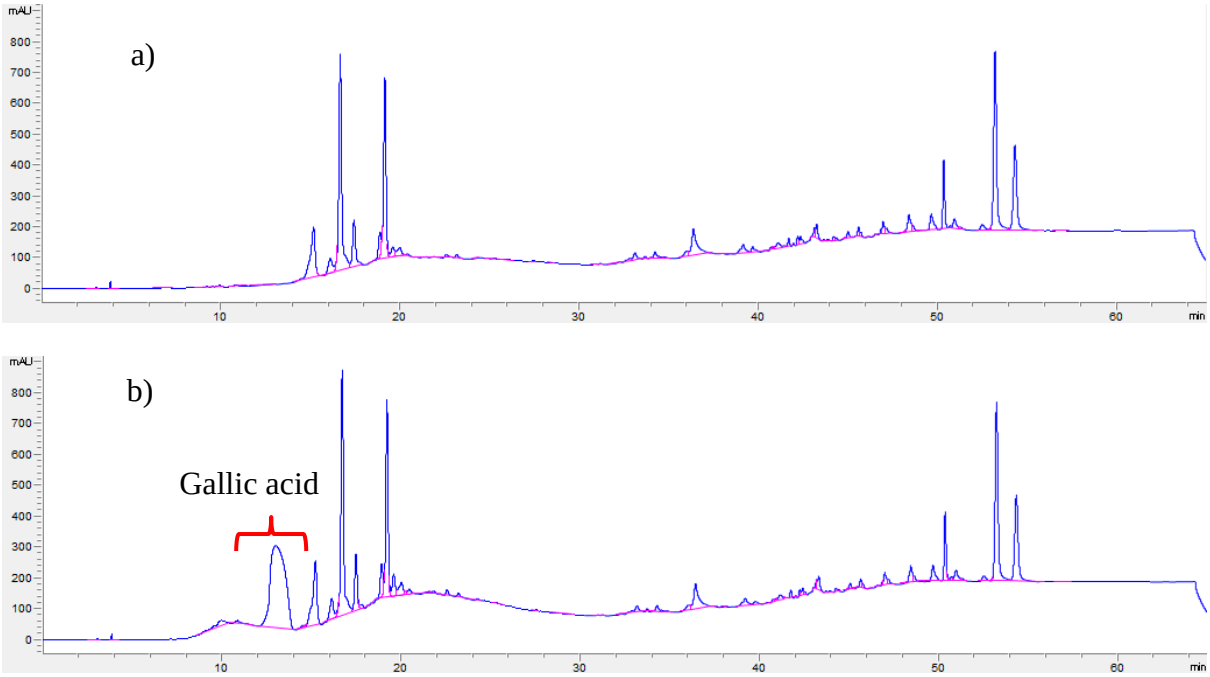


Figure 74 Elution profile of Kordel's Grape Seed in a) Grape Seed only and b) Grape seed with gallic acid by using spiking technique.

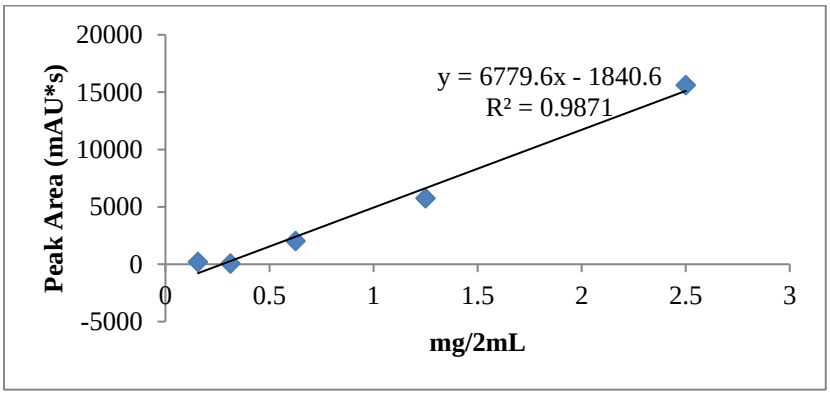


Figure 75 Standard addition curve of Kordel's Grape Seed for gallic acid obtained from HPLC analysis.

Quercetin was added into the Kordel's Grape Seed by using spiking addition method in HPLC analysis. Based on Figure 76, quercetin was identified and had retention time around 32 minutes. The final concentration in the HPLC vial was ranged from 0.15 mg to 2.5 mg in 2 mL of HPLC vial. In 500 mg of capsulated Kordel's Grape Seed, there was around 3.50 mg of quercetin by using the calibration curve in Figure 77.

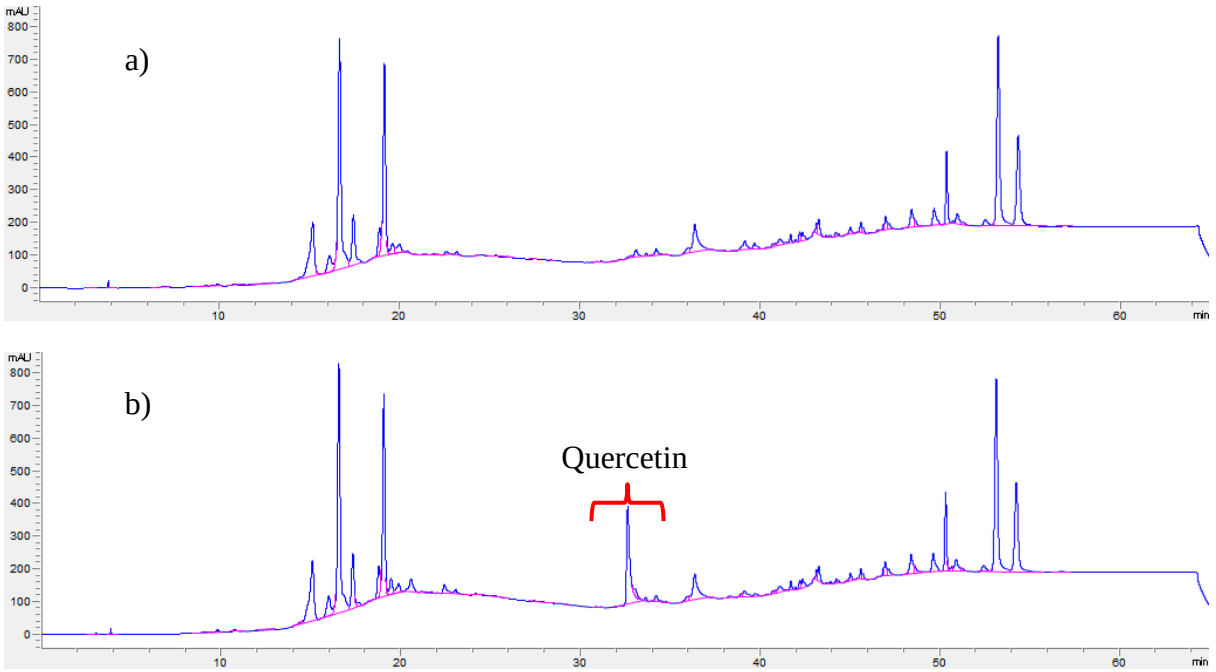


Figure 76 Elution profile of Kordel's Grape Seed in a) Grape Seed only and b) Grape Seed with quercetin by using spiking technique.

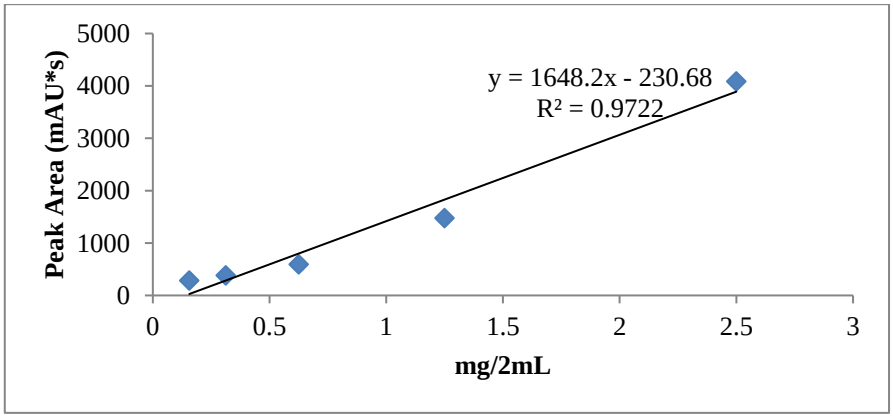


Figure 77 Standard addition curve of Kordel's Grape Seed for quercetin obtained from HPLC analysis.

5.5.2.3. Mindatulus – Bromo-Q

Bromo-Q was subjected to HPLC analysis and the elution profile was shown in Figure 78. Based on the HPLC profile, gallic acid was not detected but quercetin can be detected in Bromo-Q. Quercetin was eluted around 32 minutes. The final concentration in the HPLC vial was ranging from 0.15 mg to 2.5 mg in 2 mL of HPLC vial. According to Figure 79, the weight of quercetin in Bromo-Q was about 3.49 mg. Furthermore, the mentioned weight of quercetin was corresponded to 500 mg of capsulated Bromo-Q manufactured by Mindatulus.

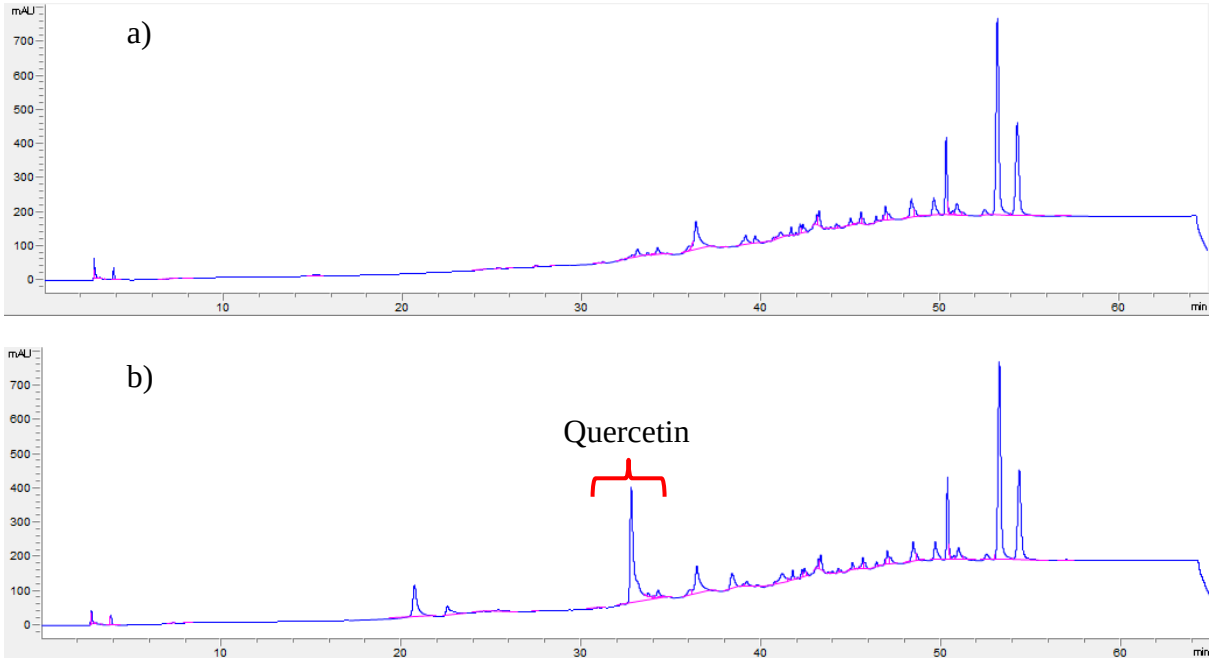


Figure 78 Elution profile of Bromo-Q in a) Bromo-Q only and b) Bromo-Q with with quercetin by using spiking technique.

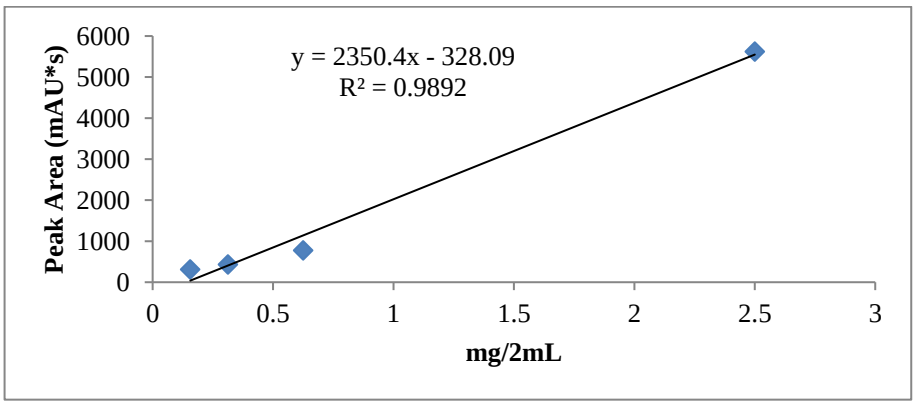


Figure 79 Standard addition curve of Bromo-Q for quercetin obtained from HPLC analysis.

5.5.2.4. Vitahealth – Grape Seed

Vitahealth's Grape Seed was analyzed by HPLC. Quercetin was detected, identified, and eluted at retention time around 37 minutes as shown in Figure 80.The final concentration in the HPLC vial was ranging from 0.15 mg to 2.5 mg in 2 mL of HPLC vial. Calibration curve generated was shown in Figure 81. The quantity of quercetin in 500 mg of capsulated Grape Seed was calculated to be 0.37 mg.

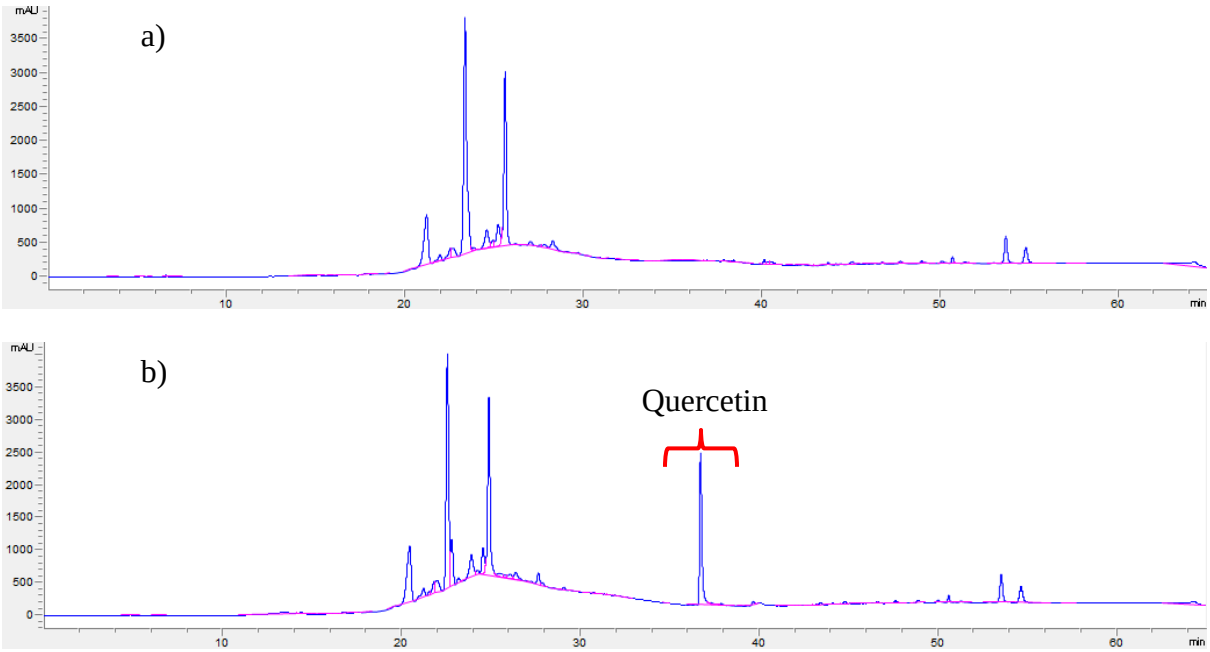


Figure 80 Elution profile of Vitahealth's Grape Seed in a) Grape Seed only and b) Grape Seed with quercetin by using spiking technique.

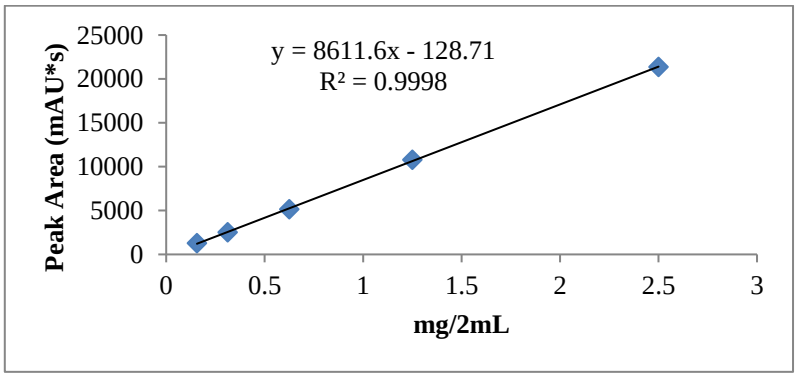


Figure 81 Standard addition curve of Vitahealth's Grape Seed for quercetin obtained from HPLC analysis.

5.5.2.5. Summary of Application Studies using HPLC Analysis

With the intention to identify the presence of either gallic acid or quercetin within the selected commercially available supplements, spiking technique was used where the respective supplements were premixed with gallic acid or quercetin correspondingly before subjected to HPLC analysis. Better accuracy in retention time of the respective antioxidant can be achieved by allowing the antioxidant to be separated in the same matrix condition as the tested supplements.

By incorporating gallic acid or quercetin into the supplement samples at a range of concentrations (0.15 mg to 2.5 mg per 2 mL), a series of standard addition method curves were plotted by using the peak area of selected peak at retention time of 12 minutes and 32 minutes which corresponded to gallic acid and quercetin that obtained from the elution profiles, respectively (Section 5.5.2.1 to 5.5.2.4). Among all those studied supplements, only Bromo-Q, Kordel's Bilberry, Kordel's Grape Seed, and Vitahealth's Grape Seed were able to exhibit positive results from HPLC analysis, which were increment in the peak area at specific retention time, indicating the presence of antioxidant(s). Out of four supplements mentioned above, HPLC analysis through the standard addition method have shown that only quercetin was presented in Vitahealth's Grape Seed, meanwhile both gallic acid and quercetin were present in three other samples.

In order to plot the standard addition calibration plot, a total of 5 different concentrations of the gallic acid or quercetin were prepared, respectively. This had been done by serial dilution to minimize the errors that might be happened. From the standard calibration plot, the concentration of the detected antioxidant(s) can be obtained. This was done by referring to the linear equation generated. Thus, the concentration of the detected antioxidant(s) was shown in Table 7. Kordel's Bilberry had the highest content of gallic acid while Bromo-Q had the highest content of quercetin. The elution profiles of the 4 supplements were shown in the following sections.

Table 7 Quantity of detected antioxidant(s) in the commercial supplements.

Commercial Supplements	Weight (mg) in 50 mL of 500 mg tablet	
	Gallic Acid	Quercetin
Bromo-Q	none	3.49
Kordel's Bilberry	6.69	3.07
Kordel's Grape Seed	6.79	3.50
Vitahealth's Grape Seed	none	0.37

5.5.3. Comparison between Electrochemical and HPLC analysis

A series of analysis had been used and applied in this project. Electrochemical studies provided fast, simplicity, low cost, and good selectivity in an initial start-up research. In contrast, HPLC needs higher expenses on equipment, time consuming, and a lot of experimental consumables, especially solvents. In this project, detection of antioxidants in supplement had been done using both electrochemical and HPLC analysis as shown in Table 8. In some cases, we have shown that electrochemical studies detected the presence of antioxidant(s) while HPLC analysis did not detect the presence of antioxidant(s), and vice versa. Therefore, comparison between electrochemical studies and HPLC analysis revealed that both techniques are complementary to each other. Consideration for choosing either of the mentioned analysis depends on the user's desire and needs.

Furthermore, the provided package information merely mentioned that the product was extracted from certain fruits or plant. There was absence of details on composition of ingredients on the packaging. Thus, it was difficult to compare the content in the supplements in terms of the studied antioxidants.

Table 8 Detection of antioxidant(s) using electrochemical and HPLC analysis.

Manufacturer	Supplements	Detection of Antioxidant(s)			
		Electrochemistry		HPLC	
		✓or X	Remarks	✓ or X	Remarks
Herbs of Gold	Black Seed With Garlic	X	No antioxidant	N/A	N/A
	Grape Seed 15000	✓	Gallic acid and quercetin	N/A	N/A
Himalaya	Arjuna	✓	Quercetin	X	No antioxidant
	Amalaki	✓	Gallic acid	X	No antioxidant
	Mandukaparni	X	No antioxidant	X	No antioxidant
	Neem	✓	Gallic acid	X	No antioxidant
	Triphala	✓	Gallic acid	X	No antioxidant
Kordel's	Vitamin C Time	X	Contained Vit C	N/A	Contained Vit C
	Bilberry	✓	Gallic acid	✓	Gallic acid and quercetin
	Grape Seed	✓	Gallic acid and quercetin	✓	Gallic acid And quercetin
Mindatulus	Bromo-Q	X	No antioxidant	✓	Quercetin
Solaray	CranActin (Cranberry)	X	No antioxidant	X	No antioxidant
Vitahealth	Grape Seed	✓	Gallic acid and quercetin	✓	Quercetin
	Bioflavonoids C 1000 Plus Zinc	X	Contained Vit C	N/A	Contained Vit C

Legends: ✓ = detected X = not detected N/A = not subjected to run

6 Conclusion

Gallic acid, quercetin, and rutin are electroactive species. Quercetin was found to be more easily oxidised (Epa = 179 mV) compared to gallic acid (Epa = 256 mV) and rutin (Epa = 282 mV). Gallic acid and quercetin had two oxidation peaks while rutin had only one oxidation peak in purged condition. Based on the potential peak value, quercetin is a better antioxidant as compared to gallic acid and rutin. There were two modified electrodes which successful in detecting the presence of gallic acid and quercetin but not rutin. ITO was the best working electrode in detecting the presence of gallic acid, while for quercetin was GCE modified with Bi_2O_3. Furthermore, an interesting discovery is that gallic acid can be used as a modifier in detecting the presence of bismuth ion in a sample, as voltammograms had shown that the presence of gallic acid was able to increase about 2.8 times in current peak of bismuth ion. In the application studies, it had been identified that gallic acid and quercetin were present in all grape seed extracts. Supplements containing vitamin C showed a significant sharp and high intensity peak in the SWV result and this has masked the presence of studied antioxidant peak(s) which oxidised at the same potential region. In comparison, electrochemical technique was complementary to analytical HPLC technique for detection of the studied antioxidants in selected supplements, as evidenced by some cases where antioxidant(s) can be detected by using electrochemical techniques but not by using HPLC analysis, and vice versa. The capability of electrochemical techniques to detect the presence of various types of antioxidants in selected supplements compared to HPLC analysis could be owing to the versatility of various types of chemically modified electrodes used in the electrochemical analysis in this project. Mechanical attachment is the first step to build a potential electrochemical sensor for detecting antioxidant(s). Further work can be performed by using covalent attachment technique (Bard, 1983), where chemical reactions can be carried out to form bonds between the substrate and a molecule of interest. It is speculated to be a better electrochemical sensor for detection of the antioxidants.

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