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Sttructure-Function Correlations of the Antagonist Effects of Flavonoids Present In Medicinal Plants on Oxidative Reactive Molecules

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United Arab Emirates University
Deanship of Graduate Studies
M.Sc. Program in Environmental Sciences

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A thesis
Submitted to

United Arab Emirates University
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United Arab Emirates University
2007/2008

Dedicated to my Beloved Parents

And

To the soul of my grandfather Rahsid Al Jayousi

& Dr. Hazem Kataya

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All praise be to Allah who provided me with hope, strength and insight throughout the course of my studies. All love for the holy prophet Mohammad "peace be upon him", all devotions to Islam.

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Abstract

Flavonoids are phenolic compounds with significant antioxidant properties. The propensity of a flavonoid to inhibit free-radical mediated events is governed by its chemical structure. Since these compounds are based on the flavan nucleus, the number, positions, and types of substitutions influence radical scavenging and chelating activity. The main objective of this thesis was to establish structure-activity relationships of flavonoids by means of experimental and computational techniques.

Initially, a series of dietary flavonoids belonging to the most representative families (flavonols; , flavone and flavanones; naringenin) were studied during the reaction with DPPH radical following addition of the flavonoid by UV-Vis spectrophotometry; they distinctive steps of reaction, a first rapid and a second slower. DPPH scavenging followed a second order kinetics during the rapid step; constants as well as antiradical activities were determined. The DPPH radical allowed good discrimination between the flavonoids, as demonstrated by the relatively large ranges of rate constants (k = 10-10,000 M^{-} antiradical activities (1-84%).

Since the oxidizability of flavonoids reflects their ability to scavenge free radicals, the electrochemical oxidations of the 8 flavonoids were measured in different pH solutions using cyclic voltammetry. Flavone with no hydroxyl groups showed no oxidation potentials. Myricetin, quercetin, morin and kaempferol had the lowest oxidation potentials. This is in good agreement with the DPPH radical scavenging activities. Oxidation of flavonoids appeared to be pH dependent.

Experimental studies revealed that the catechol structure in quercetin scavenged the highest number of DPPH radicals (4.44 ± 0.24) and exhibited the highest antiradical activity (84%). On the other hand, pyrogallol structure in myricetin had the lowest oxidation potential.

A series of density functional theory calculations using Gaussian program for 28 flavonoids belonging to the major flavonoids' families were carried out to establish the structural requirements of flavonoids for appreciable radical-scavenging activity. Energy of the same number and type of nuclei were compared. On the other hand, the dipole moments were compared for flavonoids of similar structures but different substituents i.e OCH_3 and/or OH. Methoxy groups introduced unfavorable steric effects and therefore decreased the dipole moments of the studied flavonoids. Calculations of HOMO-LUMO gaps were performed to give insights of flavonoids' reactivity. Flavonols exhibited the lowest HOMO-LUMO gap among all other classes in this study.

Since chemical potential properties of flavonoids measure their tendency to give or capture electrons and therefore their antioxidant potential, these properties which include: electronic affinity (EA), ionization potential (IP), chemical potential (μ), electronegativity (χ), hardness (η) and electrophilicity (ω) were computed for all flavonoids in each class. Again, flavonols showed the lowest values among all classes which is another proof of their antioxidant ability.

Structure-activity relationships are well established from density functional calculations. Multiple hydroxyl groups confer upon the molecule substantial antioxidant activity. Methoxy groups introduce unfavorable steric effects. A double bond and

carbonyl function in the heterocycle of the nuclear structure increases activity by affording a more stable flavonoid radical through electron delocalization.

Key Words: Flavonoids, UV-VIS spectrophotometry, cyclic voltammetry, density functional theory.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

List of Abbreviations

ROS	:	Reactive oxygen species
$O_2^{\cdot-}$	:	Superoxide anion radical
ROO$^{\cdot}$	:	Peroxyl radical
RO$^{\cdot}$	:	Alkoxyl radical
HO$^{\cdot}$	:	Hydroxyl radical
NO$^{\cdot}$	:	Nitric oxide
ABTS	:	2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)
DPPH	:	1,1--diphenyl-2-picrylhydrazyl
ESR	:	Electron spin resonance
IC_{50}	:	Micro-molar concentration of flavonoid required to inhibit DPPH$^{\cdot}$ formation by 50%
AE	:	Antiradical efficiency
T_{EC50}	:	Time needed to reach the steady state with IC_{50}
MM	:	Molecular Mechanics
HF-SCF	:	Hartree-Fock Self Consistent Field
DFT	:	Density functional theory
LDA	:	Local Density Approximation
GGA	:	Generalized gradient approximation
B3LYP	:	Beck's three parameter Lee-Yang-Parr correlation functional
ZPE	:	Zero point energy
RSF	:	Rapid stoichiometric factor
TSF	:	Total stoichiometric factor
k	:	Rate constant
AR%	:	Antiradical activity

CHAPTER I

INTRODUCTION

1. Introduction

1.1 Flavonoids

A diet rich in vegetables and fruit has long been recognized to protect against chronic diseases such as cardiovascular disease and cancer.[1] Lifestyle factors, such as sufficient physical activity, abstinence from smoking, and a low-energy diet, probably explain a large part of this protection. Some components of the diet or plants may also play a role. Until recently, nutritional research mainly focused on fats, carbohydrates, proteins, vitamins and minerals. The existence of secondary plant metabolites, often present in high quantities in the fiber of plants, was largely ignored. Today, however, many of these compounds, although not essential for maintaining life, are being recognized as potentially beneficial for coronary heart diseases and cancers mainly due to their antioxidant and chelating abilities.[1,2] The efficiency of these natural phenolic phytochemicals as antioxidant compounds greatly depends on their chemical structure.

1.1.1 Chemical Structure, Occurrence & Classification

The term flavonoids is a collective noun for plant pigments, mostly derived from benzo-γ-pyrone, which is synonymous with chromone. They are a large group of low molecular weight compounds that exists in plants as secondary polyphenolics.[1,4] They can be found in a wide range of fruits, vegetables, seeds, nuts, grains, spices and tea as well as different medicinal plants.[1-6] Flavonoids occur in plants as aglycones (without sugar moieties) and glycosides (with sugar moieties).[3,5] All flavonoids consist of 15 carbon atoms arranged in 3 phenolic rings;[2,6] a benzene ring (A) condensed with a six membered ring (C), which

carries a phenyl group (B) as a substituent in the 2-position[2,5] **(Figure 1.1)**. C-Ring is either a heterocyclic pyran, which gives (flavonols, flavones and isoflavonoids)[2,4,6] or its dihydro derivative, which yields (flavonols and flavanones).[2,6]

To date, over 8000 flavonoids have been identified in plants.[3] The large number is a result of the many possible combinations of flavonoid hydroxylation, methoxylation and glycosylation patterns.[3,8]

The classification of Flavonoids is based on the level of oxidation and pattern of substitution of the C-ring, i.e. the 2,3-double bond, the 3-OH and the 4-keto group,[3,6-9] while classification within each class of flavonoids is based on the number and substitution pattern of the hydroxyl, methoxy, and glycosidic side groups.[1,3,9] There are many classes of flavonoids; those of particular interest to this research are: flavonols, flavones, flavanones and isoflavonoids.

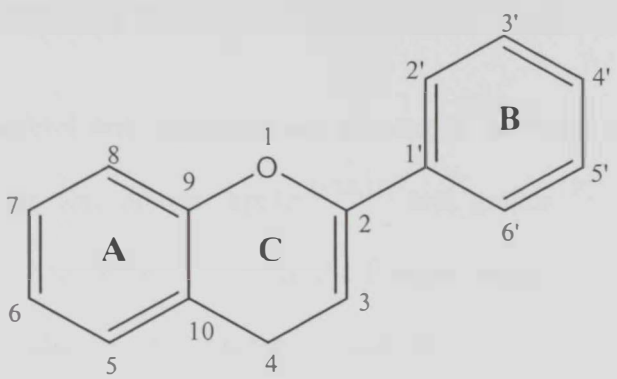


Figure 1.1: Nuclear structure of flavonoids.

1.1.1.1 Flavonols

Flavonols or 2-phenyl-3-hydroxy-chromones represent a class of flavonoids that vary in color from white to yellow.[9] In leafy vegetables and fruits, flavonols are almost present as glycosides. Flavonol glycosides are located mainly in the leaves, flowers and outer parts of plants such as skin, while very little amount is found in parts below soil except for onions.[3,6]

Quercetin, kaempferol and myricetin are the major flavonols in diet,[3,6,10-11] and their main food sources are tea, onions, apples[1,3,6,11] and grapes.[10] High concentrations of quercetin can be found in onions,[1,3,6,11] in the form of glycosides.[1,6] Kaempferol is most common in berries, herbs, legume, broccoli, grapefruit and root vegetable.[1,5] Myricetin is found in berries, tea,[5] as well as grapes.[1]

The C-ring in flavonols are characterized by the presence of 3- hydroxyl group and as well as conjugation which is provided by the 2,3 double bond with 4-oxo group[1-3] **(Table 1.1)**.

1.1.1.2 Flavones

Another important class of flavonoids is flavones. Flavones or 2-phenyl-chromones are the yellow pigments of flowers and they are not frequently found in fruit but are found in grains and herbs.[5] Common flavones are apigenin and luteolin.[1,3,5-6] Apigenin and its glycosides are present in cereal grains, some herbs and some vegetables.[1,3,5] Luteolin is found mainly in cereals, herbs[3,5] and red pepper.[1] Unlike flavonols, flavones lack the 3-hydroxy group and therefore the C- ring is a pyrone ring **(Table 1.2)**.

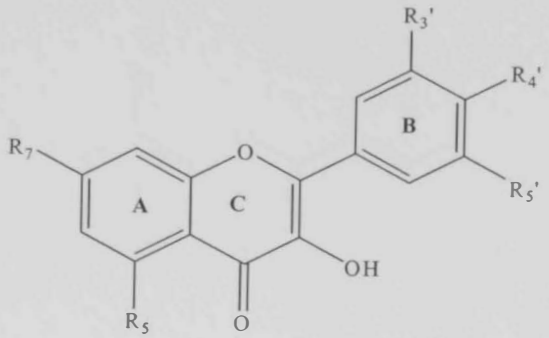
1.1.1.3 Flavanones

Flavanones, the hydrogenated analogues of flavones,[1,3,5] occur almost exclusively in citrus fruits. The highest concentrations of flavones are found in solid tissues, but several hundred mg per liter are present in the juice as well.[1,3] Hesperetin and naringenin are the main flavones in this class. Flavone glycosides like hesperidin (Hesperetin- 7- rutinoside) and narirutin (naringenin-7- rutinoside) are the major constituents of oranges and mandarins.[1,3,5] Tomatoes, especially tomato skin, have considerable amounts of naringenin.[1,12] Unlike flavones, flavanones lack the unsaturated 2,3 double bond. That's why flavanones contribute to the flavor of citrus. The main structures of flavones and some of its compounds are shown in **Table 1.3**.

1.1.1.4 Isoflavonoids

Isoflavonoids are another class of flavonoids but they differ structurally from common flavonoids in B-ring orientation.[1,3] Isoflavonoids are very similar to flavones, except the B ring is attached to position 3 of the C ring, rather than to position 2 as in the flavones **(Table 1.4)**. They have different subclasses; isoflavanones, isoflavones, isoflavonols.[5,7] The best known isoflavonoids are daidzein and genistein from the subclass isoflavones.[1,3] Isoflavones in general are found most often in legumes including soy beans, black beans and green beans. Soy beans are the major source of daidzein and Genistein.[1,5]

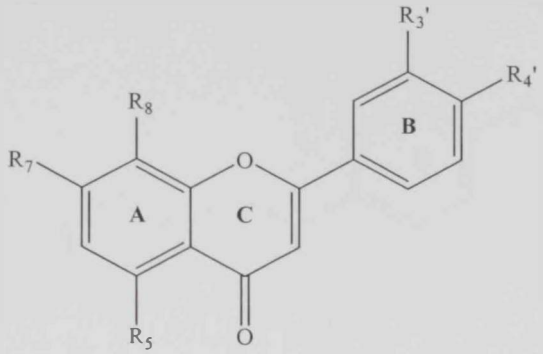
Table 1.1: Structures of flavonols



Flavonols

Compound	Code	Substituents					
		R_3	R_5	R_7	$R_{3'}$	$R_{4'}$	$R_{5'}$
Quercetin	qu	OH	OH	OH	OH	OH	H
Morin	mo	OH	OH	OH	OH	H	OH
Robinetin	ro	OH	H	OH	OH	OH	OH
Myricetin	my	OH	OH	OH	OH	OH	OH
3,5,7,3,4,5-hexamethoxy flavone	hm	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3
3,5,7,3,4-pentamethoxy flavone	pm	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OH
Laricytrin	la	OH	OH	OH	OH	OH	OCH_3
Fisetin	fi	OH	H	OH	OH	OH	H
Kaempferol	kl	OH	OH	OH	H	OH	H
Galangin	gl	OH	OH	OH	H	H	H
Kaempferide	kd	OH	OH	OH	H	OCH_3	H
3-Hydroxyflavone	h3	OH	H	H	H	H	H

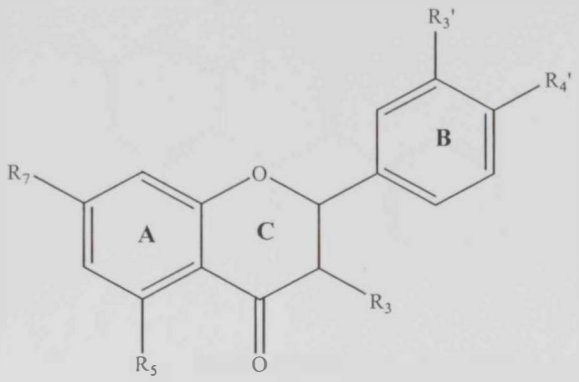
Table 1.2: Structures of flavones



Flavones

Compound	Code	Substituents				
		R_5	R_7	R_8	$R_{3'}$	$R_{4'}$
Flavone	fl	H	H	H	H	H
5-hydroxy flavone	h5	OH	H	H	H	H
7-hydroxy flavone	h7	H	OH	H	H	H
Chrysin	cr	OH	OH	H	H	H
8-methoxy flavone	m8	H	H	OCH_3	H	H
Apigenin	ap	OH	OH	H	H	OH
Luteolin	lu	OH	OH	H	OH	OH

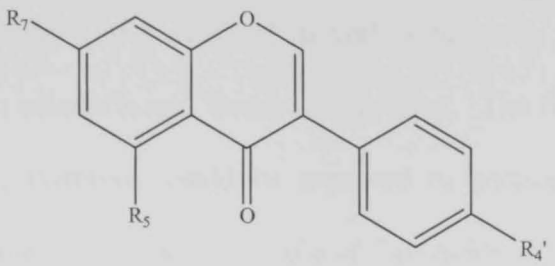
Table 1.3: Structures of flavanones



Flavanones

Compound	Code	Substituents				
		R_3	R_5	R_7	$R_{3'}$	$R_{4'}$
Flavanone	fn	H	H	H	H	H
naringenin	na	H	OH	OH	H	OH
Hesperitin	he	H	OH	OH	OH	OCH_3
Fustin	fu	OH	H	OH	OH	OH
Taxifolin	ta	OH	OH	OH	OH	OH

Table 1.4: Structures of Isoflavones



Isoflavones

Compound	Code	Substituents		
		R_5	R_7	$R_{4'}$
Daidzein	da	H	OH	OH
Formononetin	fm	H	OH	OCH_3
Genistein	ge	OH	OH	OH
Biochanin A	bi	OH	OH	OCH_3

1.1.2 Significance of Flavonoids

Flavonoids are members of a class of natural compounds that recently has been the subject of considerable scientific and therapeutic interest. The flavonoids are ubiquitous to green plant cells and, therefore, could be expected to participate in the photosynthetic process.[14] Also, detailed evidence of the role of flavonoids in gene regulation and growth metabolism is known.[3,14]

Flavonoids have shown potential health benefits arising from their anti-oxidative properties which are attributed to the phenolic hydroxyl groups attached to flavonoid structure. Flavonoids as powerful radical scavengers can be a cure against free radical mediated disease.[7

1.1.2.1 Environmental significance of flavonoids in plants

Flavonoids act as pigments in fruits and flowers which are responsible for the color of yellow, orange, and red in flowering plants.[1

petal pigments, these compounds owe important physiological qualities to their electronic properties. In this case, light absorption is linked to stimulation by nervous perception, whereas in another well-known example of a link between electronic properties and physiological function, the hemoproteins, light absorption is connected with the transport of substrates and metabolites (O_2, CO_2, 2,3-diphosphoglycerate, nitric oxide [NO], CO, C1-fragments, etc.).[

animals in order to pollinate flowers.[

scavengers in plant cells by scavenging reactive oxygen species (ROS) produced by the

electron transport system.[8,

used for light screening, which protect plants from the sun UV-radiation and scavenge UV-generated ROS.[1,3] Flavonoids play an important role in the nitrogen metabolism of nitrogen-fixating plants, because they induce the nodulation of their roots.[14] Flavonoids are also essential factors in plant sexual reproduction by promoting the pollen tube development.[7] Flavonoids also have apparent roles in plant stress defense, such as in protection against damage caused by pathogen attack, in wounding or in excess of UV-light. The low availability of nitrogen or phosphorus, and low temperatures affect flavonoid levels in plants.[7]

Flavonoids composition in plants is strongly influenced by different factors such as variation in plant type and growth, genetic factors, season, climate, degree of ripeness, food preparation, and processing.[6-7,10]

1.1.2.2 Biological significance of flavonoids

Free radicals which are very reactive oxidative molecules are of two types: Endogenous and Exogenous. Endogenous free radicals are continuously generated as by products of biological redox reactions. While exogenous free radicals come from cigarette smoke, pollutants, UV radiation, etc.[3,9] Examples of free radicals, known as reactive oxygen species (ROS), include superoxide ($O_2^{\cdot-}$), peroxyl ($ROO^{\cdot}$), alkoxyl ($RO^{\cdot}$), hydroxyl ($HO^{\cdot}$), and nitric oxide ($NO^{\cdot}$) radicals.[3,9]

Free radicals can rapidly attack molecules in nearby cells. They are capable of oxidizing lipids in cell membranes, proteins in tissues or enzymes, carbohydrates and DNA and can

cause serious damage. Oxidative stress, an imbalance between reactive oxygen species and defense and repair antioxidant systems, has been shown to be a major cause in cellular aging and other diseases associated with it, such as coronary heart diseases, cataracts, cognitive dysfunction, mutagenesis, carcinogenesis, diabetes [1,3,9,11,15] and neurological diseases such as Parkinson's and Alzheimer's diseases.[4,

destroyed by specialized enzymes such as superoxide dismutase, catalase and peroxidase, as well as some nonenzymatic counterparts such as glutathione, ascorbic acid and α-tocopherol. However, in case of excessive free radical production or decreased enzyme activities, these reactive species are capable of inducing oxidative stress and are associated with genetic mutations as well as chronic diseases.[3,9,14]

Because of the increasing effects of oxidative damage, dietary antioxidants are an important health protecting factor due to their ability to trap free radicals. Many polyphenols, such as flavonoids, have antioxidant powers that can be extremely important in inhibiting oxidative mechanisms that lead to degenerative diseases.[

proved that those flavonoids are much stronger antioxidants than vitamin C and E.[3,

flavonoids are one of the important antioxidants present in diet.

Flavonoids can act as antioxidants by inhibiting biomolecules from undergoing oxidative damage through free radicals mediated reactions[1-2,10] They can act in several ways which can include (1) direct quenching of reactive oxygen species, where flavonoids are oxidized by the radicals, resulting in a more stable, less-reactive radical; (2) inhibition of enzymes responsible for free radical production, such as protein kinase, NADH oxidase, glutathione S-transferase and lipoxygenase; (3) chelation of free metal ions (Fe^{+3}, Cu^{+}) that can promote

the formation of highly reactive ($HO^{\cdot}$) radicals; and (4) regeneration of membrane-bound antioxidants such as α-tocopherol.[1-

There are two proposed mechanisms by which antioxidants in general and flavonoids in particular can play their antioxidative role; the H-atom transfer and the one-electron transfer mechanism.[1-2,4,17-22]

In the H-atom transfer, a free radical $R^{\cdot}$ removes a hydrogen atom from the Flavonoid (FlOH):

$$R^{\bullet} + FlOH \Rightarrow RH + FlO^{\bullet} \qquad 1.1$$

The efficiency of the Flavonoid (FlOH) depends on the stability of the radical $FlO^{\cdot}$, which in turn is determined by the number of hydrogen bonds, conjugation, and resonance structure.

In the one-electron transfer mechanism, the Flavonoid can give one electron to the free radical ($R^{\cdot}$):

$$R^{\bullet} + FlOH \Rightarrow R^{-} + FlOH^{\bullet +} \qquad 1.2$$

Here, the radical cation ($FlOH^{\bullet +}$) should be stable enough, so it does not react with the substrate molecules.

A diet rich in flavonoids has been shown to be inversely correlated with the risk of cancer, coronary heart disease and cancer due to the flavonoids' antioxidant effect.[3,15] For example, high flavonoid intake study showed predicted lower mortality from coronary heart diseases and lower incidence of myocardial infarction in older men[23] and reduced the risk of

coronary heart disease by 38% in postmenopausal women.[24] The Zutphen Elderly Study demonstrated an inverse relationship between consumption of catechin, and ischemic heart disease mortality in a cohort of 806 men.[

was observed by Knekt and co-workers, in the largest prospective cohort study conducted in the United States. Only a weak but non significant inverse correlation was observed for flavonoid consumption and coronary mortality.[26]

1.1.2.3 Other biological properties of flavonoids

In addition to the ability of flavonoids to prevent diseases, they have also exhibited other medicinal properties, including antiinflammatory, antiallergic, antiviral, antibacterial, anticancer,[2.

antithrombogenic, and antiosteoporotic effects.[18] Flavonoids have been reported to display a variety of biochemical properties including inhibition of tyrosine kinases and induction of phase II metabolizing enzymes both in vivo and in vitro. These biochemical interferences elicited by flavonoids in some cell systems have been associated with their capacity to control cell growth or destroy pathogen organisms such as fungi and viruses.[7] One of the most interesting biological properties of flavonoids is their ability to inhibit human immunodeficiency virus (HIV) transcriptase and HIV replication at the level of entry.[27]

1.2 Experimental Approach

1.2.1 Analytical Methods for the Determination of the Antioxidant Activity of Flavonoids

1.2.1.1 Measurement of free radical scavenging

Different strategies have been developed for measuring the antioxidant activity of flavonoids as the ability to scavenge free radicals in aqueous and lipophilic phases.[28] The ability to scavenge specific radicals may be targeted as, for example, hydroxyl radical, peroxyl radical,[29,30] superoxide radical[30] or nitric oxide radical.[15-16,31]. One approach involves the generation of a free radical species and direct measurement of its inhibition due to addition of flavonoids.[28]

The radical that is generated varies and different systems have been described using 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS)[3,28,32-35] and 1,1–diphenyl-2-picrylhydrazyl (DPPH) radical.[8,19,33-37] Other systems may include; horseradish peroxidase-H_2O_2,[28,38] copper(II)-cumene hydroperoxide and trichloromethyl peroxyl radical.[28] The end point detection also varies and has been based on measurement of fluorescence inhibition,[28] chemiluminescence,[28,38] and absorbance.[32-37]

1.1.3.1.1 ABTS radical cation scavenging of flavonoids

ABTS can donate an electron to generate a relatively long-lived radical cation ($ABTS^{\cdot+}$), **(Figure 1.2)**.[32-33,35] $ABTS^{\cdot+}$ can be generated by either chemical reaction [i.e., manganese dioxide, ABAP [2,2'-azobis (2-amidinopropane) dihydrochloride],[28] Potassium persulfate][32] or enzyme reactions [i.e., metmyoglobin,[32] hemoglobin, or horseradish

peroxidase.[28] Generally, chemical generation requires long time (i.e., up to 16 hr for potassium persulfate generation)[32] or high temperatures (i.e., 60°C for ABAP generation), whereas enzyme generation is faster and the reaction conditions are milder.[32]

After generation of $ABTS^{\cdot+}$, the flavonoid under investigation is exposed to $ABTS^{\cdot+}$ for a period of time and then the degree of radical quenching is done by spectroscopic methods.[32-35] In general $ABTS^{\cdot+}$ reacts rapidly with antioxidants, typically within 30 min. It can be used over a wide range of pH (4.5- 9.5).[39] In addition, $ABTS^{\cdot+}$ is soluble in both aqueous and organic solvents.[3,33]

1.1.3.1.2 DPPH radical scavenging activity of flavonoids

The most commonly used method for determining antioxidant activity of flavonoids is by the measurement of their inhibitory activity against the generation of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.[36] DPPH is one of the few stable organic nitrogen radicals, which has a deep purple color. DPPH is not a naturally occurring radical and doesn't have to be generated before assay like $ABTS^{\cdot+}$.[34-35] This assay is based on the measurement of the reducing ability of antioxidants toward $DPPH^{\cdot}$.[33]

$DPPH^{\cdot}$ has been widely used to test the free radical scavenging ability of flavonoids as shown in **Figure 1.3**.[9,19]

Several studies measure the antioxidant activity of different classes of flavonoids by the inhibition of $DPPH^{\cdot}$ formation. The reducing ability of this radical can be evaluated by electron spin resonance (ESR)[33,36] or by measuring the decrease of its absorbance at 515 nm after reaction with flavonoids.[33-35,40] Experimentally, inhibition of $DPPH^{\cdot}$ generation is

performed by adding a fixed concentration of a flavonoid to an alcoholic solution (i.e.; ethanolic,[36] methanolic[34-35, 37]) or non-alcoholic such as ethyl acetate[8, 19] solution of DPPH in various concentrations. According to Tsimogiannis et al,[19] the reactivity of the DPPH radical is enhanced in the presence of methanol, due to the H-bond radical complex of DPPH and methanol, as opposed to ethyl acetate in which there is no such phenomenon.[19]

The antioxidant activity of flavonoids was indicated as the micro-molar concentration of flavonoid is required to inhibit DPPH$^{\cdot}$ formation by 50% (IC_{50}) by a spectrophotometer since DPPH$^{\cdot}$ absorbs strongly at 515 nm, whereas the yellowish reduction product does not [34-35, 37] or by ESR.[36] The IC_{50}, is widely used to measure the antioxidant activity of antioxidants in general, but it doesn't take into account the reaction time.[41]

Sanchez-Moreno and co-workers[41] introduced another parameter to express antioxidant power, called "antiradical efficiency (AE)". It is defined as:

$$AE = 1/IC_{50}T_{EC50} \qquad 1.3$$

Where T_{EC50} is the time needed to reach the steady state with IC_{50}. AE is more useful than IC_{50} because it takes into account the reaction time.[41] Yet, the use of AE has been criticized as it does not take into account the various kinetic behaviors.[42]

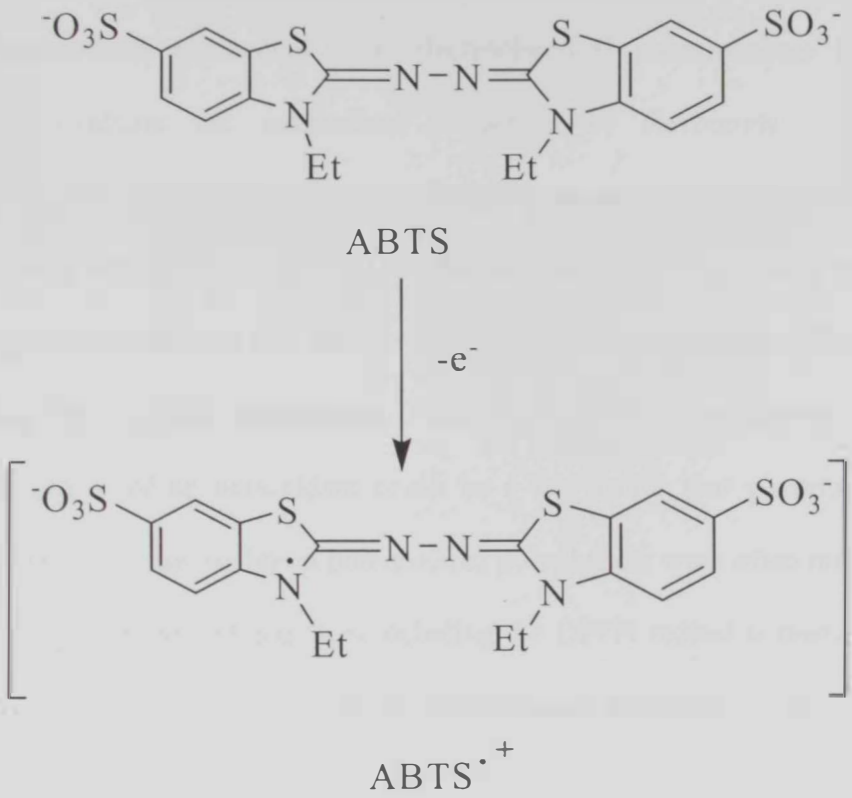


Figure 1.2: Formation of ABTS radical cation.

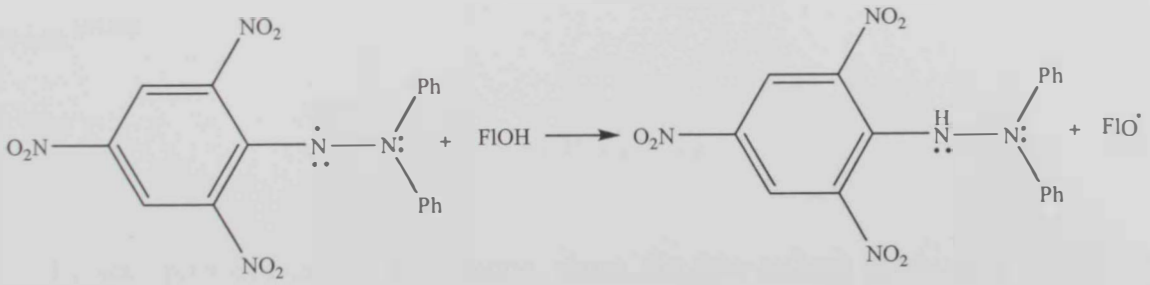


Figure 1.3: Reaction of DPPH radical with flavonoids.

1.2.1.2 Cyclic voltammetry

More fundamental approaches with electrochemical measurements have also been employed to evaluate the antioxidant capacities of flavonoids. Electrochemical measurements may help to obtain physiochemical parameters of flavonoids (i.e., redox potential, number of electrons, electron-transfer rate constant, etc.). These parameters seem to possess great potentialities not only for evaluating the antioxidant abilities but also for understanding their reaction mechanisms. Among these parameters, the redox potential, i.e., the reducing power of an antioxidant could be a key factor that governs its antioxidant activity.[43] Therefore, the oxidation potentials of polyphenols were often measured and then compared to their antioxidant activities including the DPPH radical scavenging activity and the inhibition activity of lipid peroxidation, which is more similar to biological systems.[43,44]

Cyclic voltammetry (CV) is an important analytical technique, used for studying the redox properties of chemicals. The oxidizability of flavonoids reflects their ability to scavenge free radicals through measuring an anodic potential (E_a) in CV. This technique indicates the ability of the flavonoids to donate electrons around the potential of the anodic wave.[44,45]

$$FlOH \rightarrow FlO^{\bullet} + H^{+} + e^{-} \qquad 1.4$$

E_a also provides useful information about the free radical scavenging of flavonoids because of the similarities between the H-atom transfer mechanism reaction and the oxidation reaction.[44,45]

This three electrode-method uses a reference electrode, working electrode, and counter electrode. In typical CV, a solution component is electrolyzed (oxidized or reduced) by placing the solution in contact with an electrode surface, and then making that surface sufficiently positive or negative in voltage to force electron transfer. In simple cases, the surface is started at a particular voltage with respect to a reference half-cell such as calomel or Ag/AgCl, the electrode voltage is changed to a higher or lower voltage at a linear rate, and finally, the voltage is changed back to the original value at the same linear rate. When the surface becomes sufficiently negative or positive, a solution species may gain electrons from the surface or transfer electrons to the surface. This results in a measurable current in the electrode circuitry. When the voltage cycle is reversed, it is often the case that electron transfer between electrode and chemical species will also be reversed, leading to an "inverse" current peak.[46]

Yang. B. et al,[47] developed a simple electrochemical method for estimating the antioxidant activity of flavonoids. The proposed method is based on the measurement of the half-wave potential ($E_{1/2}$) of the first oxidation wave of flavonoids by flow-through column electrolysis.[47]

Some cyclic voltammetry studies measured the oxidation potentials of flavonoids and compared it to other assays like ferric reducing antioxidant power (FRAP) assay, a simple and reliable colorimetric method based on the ability of the antioxidants to reduce Fe^{3+} to Fe^{2+}. A good correlation was observed between the FRAP assay and the electrochemical results.[44]

Furuno. K. and co-workers,[48] used the electrochemical oxidizability of flavonoids as a model for $O_2^{\cdot-}$ scavenging ability. This scavenging ability was measured on the basis of electrochemical redox potential and the reducing ability of the Cu^{+2} ion. Results suggested that the ability of the flavonoids to scavenge $O_2^{\cdot-}$ radicals was in a better correlation with their Cu^{+2}-reducing ability than their redox potential.[48]

P. Janeiro, A.M Oliveira Brett[49] investigated the electrochemical oxidation of one flavonoid; (+)catechin, over a wide range of conditions (i.e., pH, Concentrations, scan rates, etc), using cyclic, differential and square wave voltammetry.[49]

1.3 Computational Approach

1.3.1 Introduction

Computational chemistry, alternatively sometimes called theoretical chemistry or molecular modeling, deals with computations that are used to either enhance the understanding of chemical processes and properties of molecules and solids such as structure (i.e. the expected positions of the constituent atoms), absolute and relative (interaction) energies, electronic charge distributions, dipoles and higher multipole moments, vibrational frequencies, reactivity or other spectroscopic quantities, and cross sections for collision with other particles.[50-51] While its results normally complement the information obtained by chemical experiments, it can in some cases predict up till now unobserved chemical phenomena. It is widely used in the design of new drugs and materials.[51]

There are two broad areas within computational chemistry devoted to the structure of molecules and their reactivity: molecular mechanics and electronic structure methods (also referred to as quantum mechanics).[50, 52] The basic types of calculations they both perform include computing the energy, or properties related to the energy, like; performing geometry optimizations of a particular molecular structure; and, computing the vibrational frequencies of molecules resulting from interatomic motion within the molecule.[51] This section will explore these methods and their limitations to highlight the choices made for the methods employed in this research.

1.3.2 Molecular Mechanics

Molecular mechanics (MM) simulations apply the classical laws of physics to predict the structures and properties of molecules. These methods are characterized by their particular force fields representing the interactions between atomic species i.e. Amber. Molecular mechanics calculations are performed based on nuclei interactions and do not treat the electrons in a molecular system explicitly.[50,52] However, electron effects are included implicitly in the force fields through parameterizations.[51]

The approximations in molecular mechanical calculations make the computations quite inexpensive and fairly fast, which allows the methods to be used for very large systems containing thousands of atoms like proteins and other large biological molecules. However, there are two major drawbacks of these methods. Firstly, each force field achieves good results only for a limited class of molecules for which the force field is parameterized. Parameterization is crucial to the success of molecular mechanics calculations and would be expected to only have any relevance when describing molecules of the same class to each other. Secondly, molecular mechanics methods make no reference to electrons, and so cannot study electronic properties like charge distributions or nucleophilic and electrophilic behavior.[50-51]

1.3.3 Quantum Mechanics

Electronic structure theory based on quantum mechanics is one of the most fundamental tools for molecular and material modeling, and applies the laws of quantum mechanics rather than classical physics as the basis for the calculations.[53-54] Quantum mechanics enables

scientists to calculate the structure, energy and properties of a molecule or an assembly of molecules.[55] The energies and structures of molecules are obtained through the solution of the Schrödinger equation, which is one of the fundamental equations of modern physics and describes, among other things, how the electrons in a molecule behave.[50] This equation can be written as:

$$\hat{H}\Psi = E\Psi \qquad 1.5$$

E in the Schrödinger equation stands for the energy of the system, which is also the eigenvalue solution of the equation. Ψ is the wave function that determines the electron density and various properties, such as dipole moments and electrostatic potentials. $\hat{H}$ in the Schrödinger equation is named the Hamiltonian,[51,53] and it represents the sum of the total potential and kinetic energies of the system.[51] These terms can be written as:

$$\hat{H} = -\frac{\hbar^2}{2}\sum_i \frac{1}{m_i}\left(\frac{\partial^2}{\partial x_i^2} + \frac{\partial^2}{\partial y_i^2} + \frac{\partial}{\partial z_i^2}\right) + \sum_{i<j}\sum\left(\frac{e_i e_j}{r_{ij}}\right) \qquad 1.6$$

The first term in **Equation 1.6** accounts for the kinetic energies, and the second term accounts for the potential energies, including attractions or repulsions between particles. **Equations 1.5** and **1.6** are the time-independent Schrödinger equation because time-derivatives and time-dependent terms have been eliminated. The time-dependent form is usually used when one is concerned with transient phenomena such as rapidly oscillating electric fields or scattering.[51] This research does not concern these phenomena so **Equations 1.5** and **1.6** are acceptable here.

Exact solutions to the Schrödinger equation cannot be computed to any but the smallest systems.[51] Various approximate methods for solving the Schrödinger equation are available, from molecular modeling, to semi-empirical methods, to *ab initio* methods, and density functional theory (DFT) methods.

1.3.3.1 Semi-empirical methods

Semi-empirical methods use parameters derived from experimental data to simplify the computations. The Schrödinger equation is solved approximately, and it depends on the availability of appropriate parameters for the chemical system of interest.[51-53] These inexpensive methods have been used to calculate nonlinear optical properties of large molecules i.e. metal phthalocyanines, in particular for industrial applications.[55] Some of the recent developments for semi-empirical methods are intended for applications to even larger molecules. Applications so far include polypeptides consisting of 248 amino acid residues.[53,55] Some applications consist of 1960 atoms with 140 residues.[55]

The biggest merit of semi-empirical methods is definitely low computational cost. They are more expensive than the molecular mechanics methods, but allow breaking of bonds and take electronic effects explicitly into account, which molecular mechanics.[50,55] Important shortcomings of semi-empirical methods are low reliability (qualitative at best, and particularly poor for transition states) for the energetic results and the lack of reliable parameters for transition metals.[51,55]

1.3.3.2 Ab initio methods

In the *ab initio* methods, the Schrödinger equation is solved "from the beginning" (which is not "from first principles", as *ab intio* is frequently translated).[50,53,55] Unlike molecular mechanics or semi-empirical methods, *ab initio* methods do not apply any experimental parameters in their calculations. They are based on the laws of quantum mechanics only and on the values of a small number of physical constants like the speed of light, the masses and charges of electrons and nuclei, Planck's constant, etc. These methods compute solutions to the Schrödinger equation through a series of rigorous mathematical approximations.[52] *Ab initio* methods have long been applied as a major tool for investigating the structure, stability, reaction kinetics and mechanisms of different molecular systems.[55,56]

The Schrödinger equation is very difficult to solve despite its simple appearance. As a result, approximations have to be made in order to simplify the solution. One of the most important simplifications is the Born-Oppenheimer approximation. The electrons move much faster than the nuclei, which make the nuclei look stationary from the viewpoint of the electronic configuration.[50,53]

Different methods have currently been used in practice to solve the Schrödinger equation. The simplest and least inexpensive *ab initio* method in common usage is the Hartree-Fock Self Consistent Field (HF-SCF) method.[50] It introduces the average potential of electron-electron interaction to the Schrödinger equation. HF-SCF has the advantage of being the best approximation that can be achieved without taking electron correlation into consideration, and is also reasonably inexpensive to execute. This approximation appears to be reasonable for different applications such as computing equilibrium molecular geometries and

frequencies of stable molecules. But it dramatically fails for chemical processes like bond dissociation where electron effects predominate.[51,53]

Because of the "averaged field" assumption in the scheme, HF-SCF theory provides an inadequate treatment of the correlation between the motions of the electrons within a molecular system, especially those arising between electrons of opposite spin.[52] Thus, electron correlation methods or post-Hartree-Fock methods were developed in order to treat the electron correlations properly.[50,55] One of these approaches is Moller-Plesset (MP) perturbation which treats the electron correlation energy which is defined as the difference between the "exact" HF energy and the energy from the exact solution.[52] However, it has been shown that the MPn series expansion yields poor results for many heavy element systems.[51]

The difference between semi-empirical and *ab initio* methods lies in the trade-off between computational cost and the accuracy of results.[52] With the availability of good parameters, semi-empirical calculations are relatively inexpensive and provide fairly accurate energies and structures. *Ab initio* methods, in contrast, provide highly accurate predictions for a broad range of systems.[52,55] However, due to the high computational cost, the chemical systems of interest are restricted up to few hundred atoms.[55,57]

The *ab initio* methods are the ultimate theoretical methods for electronic structure calculations, applicable to any atoms or molecules in both ground and excited states.[51,55] The approximations can be systematically improved using better basis sets and better wave functions. The disadvantage of *ab initio* methods is their computational cost, much more demanding than molecular mechanics, semi-empirical and density functional theory (DFT)

methods.[52] The *ab initio* methods have been used in industrial applications when the accuracy is needed or when inexpensive alternative methods, such as semi-empirical or DFT, do not work.[55,57]

1.3.4 Density Functional Theory Methods

Density functional theory (DFT) calculations which are simple in theory and powerful in practice are based on the fact that the properties of a molecule in a ground electronic state are determined by the ground state electron density.[55] At a very basic level, density functional methods are similar to some *ab initio* methods in many ways. DFT calculations require the same computational resources as HF theory, the least expensive *ab initio* method, but include the effects of electron correlation, which is the fact that electrons react to each other's motion.[52,55] Thus, DFT methods can provide the benefits of some more expensive *ab initio* methods at essentially HF cost.[52] DFT are good candidates for calculations involving open-shell systems because they seem to suffer to a lesser extent from spin contamination. They are also more efficient for large systems in terms of computer CPU compared to post-HF methods.[58-59] On the other hand, DFT methods use the exact observable and traceable electron density instead of the complicated and unobservable wave function to calculate molecular properties, differing totally from traditional *ab initio* methods.[50,53,58-59] This was following the Hohenberg-Kohn theorem, which confirms that the electron density of a ground state determines uniquely the energy of that electronic state.

Further work by Kohn and Sham,[52] in an attempt to find a practical method of calculating the electron density, led to the current DFT method, which introduces electronic energy as below:

$$E = E^T + E^V + E^J + E^{XC} \qquad 1.7$$

Where E^T is the kinetic energy of electrons, E^V includes terms describing the potential energy of the nuclear electron attraction and of the repulsion between pairs of nuclei, E^J is the electron-electron repulsion term, and E^{xc} is the exchange-correlation term and includes the remaining part of the electron-electron interactions.[52]

Several exchange-correlation potentials are currently available. The simplest of all is the Local Density Approximation (LDA) that results directly from the description of the uniform electron gas. However, LDA uses only the local density and as such underestimates the interactions due to other atoms. The generalized gradient approximations (GGA) have been introduced.[53] They give a better description for a wide range of phenomena than LDA. The most popular pure DFT potential is probably the combination of the Becke exchange and the Perdew correlation potentials, which has successfully been applied to predicting geometries and other properties.[50,53]

DFT may be used not only to calculate molecular, potential energy surfaces and the course of a given reaction, but also are very useful tools to obtain conceptual information about chemical reactivity as well as to treat qualitative concepts such as hardness and electro-negativity.[58-59]

Ab initio and the faster DFT enable novel molecules of theoretical interest to be studied, provided they are not too big. Semi-empirical methods, which are much faster than *ab initio* or even DFT, can be applied to fairly large molecules (e.g. cholesterol, $C_{27}H_{46}O$), while MM

will calculate geometries and energies of very large molecules such as proteins and nucleic acids; however, MM does not give information on electronic properties.[50]

1.2.4.1 Hybrid functional

The hybrid approach to density functionals was first introduced by Axel Becke in 1993.[60] Hybridization with Hartree-Fock exchange provides a simple scheme for improving many molecular properties, such as atomization energies, bond lengths and vibration frequencies, which tend to be poorly described with simple *ab initio* functionals.[61]

The exchange-correlation functional for a hybrid is usually a linear combination of the Hartree-Fock exchange and some other one or combination of exchange and correlation functionals.[53] Recently Becke has formulated a functional which include a mixture of HF and DFT exchange along with DFT correlation, conceptually defining E^{XC} as:

$$E_{XC,hybrid} = c_{HF} E_{X,HF} + c_{DFT} E_{XC,DFT} \qquad 1.8$$

Where the c's are constants.[52]

The most useful and well-tested DFT-potential is Beck's three parameter Density Functional Theory (using the Lee-Yang-Parr correlation functional- B3LYP). It combines pure DFT potentials like BLYP with a portion of exact HF exchange, where the amount of mixing is based on empirical grounds.[50,52] B3LYP exchange-correlation functional which is used in this research may be defined as:

$$E_{XC,B3LYP} = E_{X,LDA} + c_0(E_{X,HF} - E_{X,LDA}) + c_X \Delta E_{X,B88} + E_{C,VWN3} + c_C(E_{C,LYP} - E_{VWN3}) \qquad 1.9$$

Where VWN is the Vosko, Wilk, Nusair functional, and LYP is the Lee, Yang, and Parr functional. Here, the parameter c_0 allows any mixture of HF and LDA local exchange to be used. In addition, Becke's gradient correction to LDA exchange is also included, scaled by the parameter c_x. Similarly, the VWN3 local correlation functional is used, and it maybe optionally correlated by the LYP correlation correction via the parameter c_c. In the B3LYP hybrid functional method, the parameters are determined by fitting data from calculation to experimental for the atomization energies. Ionization potentials, proton affinities and first-row atomic energies in the G1 molecule set with c_0=0.20, c_x=0.72, and c_c= 0.81.[52]

1.3.5 Basis Set

The basis set can be interpreted as restricting each electron to a particular region of space.[54] For the *ab initio* molecular orbital approach, one generally considers the molecular orbitals to be linear combination of the atomic orbitals:

$$\Psi_i = \sum_{\mu=1}^{n} c_{\mu i} \varphi_\mu \qquad 1.10$$

Ψ_i is the i-th molecular orbital, $c_{\mu i}$ are the coefficients of the linear combination, ϕ_μ is the μ-the atomic orbital, and n is the number of atomic orbitals.[51] There are two types of basis functions (also referred to as atomic orbitals, AO), which are Slater Type Orbitals (STO) and Gaussian Type Orbitals (GTO). In the software used in this research, Gaussian 98, Gaussian-type orbitals are used in the calculations. A Gaussian-type orbital has the following form:

$$X_{\xi,a,b,c}(x,y,z) = N_{a,b,c,\xi} x^a y^b z^c e^{-\xi r^2} \qquad 1.11$$

Where N is the normalization constant, a, b, and c are quantum numbers describing the angular shape and direction of the orbital (for example, a + b + c = 1 is a p-orbital), and exponents ξ which apply to the radial size of the electron orbital. In general a basis set is a linear combination of these Gaussian Type Orbitals.[52]

In general, large basis sets impose fewer constraints on electrons and lead the solution to be closer to the "exact" solution. However, larger basis sets require more computational resources so there is always a trade-off in choosing the right basis set for a particular application.[54]

1.4 Objectives

The objectives of the present work are:

1. Measuring the rate constants of scavenging reaction (k), of different classes of flavonoids to investigate the effect of structural features of flavonoids on their antioxidant activities.
2. Studying the (DPPH) free radical scavenging by the flavonoids under investigation.
3. Measuring the cyclic voltammetry response of the tested flavonoids as a function of pH.
4. Using Density Functional Theory (DFT) for the determination of energy and chemical potential properties, as well as HOMO-LUMO distributions for obtaining a better knowledge of the properties of flavonoids related to their antioxidant activity.

CHAPTER II

MATERIALS AND METHODS

2. Materials and Methods

2.1 Materials and Reagents

Quercetin dihydrate (minimum 98% HPLC), Morin, Myricetin (approx. 85%), Kaempferol (minimum 90% HPLC), 3-hydroxyflavone, flavone and (±)-naringenin (approx. 95%) were all purchased from Sigma-Aldrich and used without further purification. 2,2-Diphenyl-1-picryhydrazyl free radical was obtained from Sigma-Aldrich and used as received. Solvents including methanol and ethanol were obtained from Sigma-Aldrich with the highest purity commercially available and were used without further purification. Phosphate buffer was prepared using deionized water.

2.2 Apparatus

2.2.1 DPPH free radical scavenging

All the DPPH scavenging measurements were held on a UV-Visible spectrophotometer (Cary, 50 Conc., from Varian). Quartz cell which has a path length of 1 cm was used at 515 nm.

2.2.2 Cyclic Voltammetry

Cyclic voltammetry (CV) experiments were carried out using a Princeton Applied Research Scanning Potentiostat/Galvanostat (EG&G Model 362). The potential and current analog outputs of the potentiostat were recorded using an ADC 16 data acquisition interface card (Pico Technology, UK) connected to a PC installed with PicoLog software (Pico Technology, UK) for data display and storage. The pH measurements were made using a

combination glass electrode and a Thermo-Orion pH/mV meter under room temperature. All experiments were carried out in a 10-mL double-jacket thermostated cell, using three-electrode electrochemical cell configuration. Glassy carbon electrode, saturated calomel electrode (SCE) and platinum (Pt) wire were used as working, reference and counter electrodes, respectively.

2.3 Softwares

ChemOffice 2004 was purchased from (CambridgeSoft, USA) and has been employed for drawing the flavonoids' structures using ChemDraw Ultra 8.0. Gaussian 98 and Gaussian 03W suite of programs were purchased from (Gaussian, USA). All computational studies were carried out using the DFT methods implemented in Gaussian 98 or Gaussian 03W suite of programs.

2.4 DPPH free radical scavenging measurements

A stock solution of DPPH was daily prepared at a concentration of 10^{-3} M in methanol. This solution was further diluted to obtain a concentration of 6×10^{-5} M. Stock solutions of flavonoids in methanol were prepared at a concentration of 10^{-3} M. The flavonoids' solutions were further diluted to obtain three other concentrations ranging from $1\text{-}3\times10^{-4}$ M. A reference mixture of 2.9 mL DPPH solution and 0.1 ml methanol was used. Then 2.9 mL of DPPH solution was placed in a cuvette and 0.1 mL methanolic solution of each flavonoid was added. The absorbance of the mixture was being recorded at 515 nm. The same procedure was followed for different concentrations of each flavonoid. Spectra were recorded every 1 second until reaction reached plateau.

2.5 Cyclic voltammetry measurements

This study was carried out using glassy carbon electrode (3 mm in diameter) as working electrode. Before use in electrochemical experiments, in order to avoid contamination of oxidation products and to obtain a clean renewed electrode surface, the surface of the glassy carbon electrode was hand-polished with alumina-water slurry using a polishing cloth and rinsed with deionized water and sonicated in distilled for 5 minutes. A stock solution of 10mM of each flavonoid was prepared in 99.9% ethanol. All experiments were carried out at room temperature and in the presence of dissolved oxygen. Solutions of phosphate buffer with a pH ranging from (6 – 8) were used in all experiments. The flavonoid under investigation was added to the buffer solution at a final concentration of 0.1 mM. Cyclic voltammograms were preformed at a scan rate of 20 mV/s and in the potential range -0.4 to +1 V versus calomel electrode.

2.6 Theoretical calculations

Flavonoids and their corresponding Z-matrices were obtained using ChemDraw Ultra 8.0. A series of density functional theory calculations were performed to find out both the structure and the stability of the flavonoids. The optimal structures of the species were determined by using the density functional B3LYP/6-31G(d) method. Vibrational frequencies were computed also with this method and then scaled by a factor of 0.989 to obtain the (scaled) zero-point energies (ZPE) and vibrational contributions. Single-point energy calculations were then carried out at the B3LYP optimal structures, using the B3LYP density functional and the 6-311+G(2d,P) basis set. Orbitals from this step were used as

input to a final B3LYP/6-31G(d) frequency calculation. The total energies of the species at 298.150 K are then the thermal corrections to the energy (including the translational and rotational corrections) plus the B3LYP electronic energy of the final step; these energies can be labeled in standard notation as B3LYP/6-311+G(2d,P)//B3LYP/6-31G(d). All the calculations were performed in gas phase with the purpose of obtaining the intrinsic properties of the flavonoids studied, free of any interaction.

CHAPTER III

RESULTS AND DISCUSSION

3. Results and Discussion

3.1 Kinetic Analysis

The reaction between different flavonoids from different classes and DPPH was monitored by UV/Vis spectroscopy by recording the decay of DPPH visible absorbance that follows the addition of the flavonoids to the DPPH solution. The DPPH radical has deep purple color and strongly absorbs at 515 nm, while the yellowish reduction product, $DPPH_2$, does not. A reference curve of absorbance (A) against DPPH concentration in methanol ([DPPH], M) was constructed and used for the calculation of DPPH concentration at various reaction times. The calibration reference curve is expressed by the equation: $A = 14164 \cdot [DPPH] - 0.0299$ ($R^2 = 0.9999$). The DPPH reaction with flavonoids could be separated into two steps:[8,62-63] A first rapid one followed by a much slower one. The duration of the fast step of most flavonoids lasted for 1-2 min. This step is followed by a much slower decrease in the visible absorbance which lasted from 15 to 80 min depending on the affinity of the flavonoid being used for radical scavenging. The data of the fast step of the DPPH scavenging were subjected to kinetic analysis for all concentrations ranging from 1-3 $\times$ 10^{-4} M. However, slow step reactions were used for the determination of the total stoichiometric factor (TSF), which represents the total number of scavenged molecules of DPPH per molecule of flavonoid after the completion of the reaction. Slow steps can also be used to determine the antiradical activities (AR %) of the flavonoids. The stoichiometries of the fast and slow step are essential for the explanation of the antiradical activity, because they could reveal the contribution of the different functional groups to scavenging reactions. The reaction between the flavonoid and DPPH takes place to produce less active quinones which

scavenge DPPH leading to a complex mixture of degradation products according to the following reaction scheme:

$$Flavonoid + nDPPH \leftrightarrow Quinone + nDPPH_2 \qquad 3.1$$

$$Quinone + mDPPH \leftrightarrow \text{Pr}oducts + mDPPH_2 \qquad 3.2$$

All flavonoids studied followed a second order kinetics[8,62-64] according to the following equations:

$$A = \varepsilon[DPPH] \qquad 3.3$$

$$R = -\frac{d}{dt}[FlOH] = -\frac{d}{dt}[DPPH] = k[FlOH][DPPH] \qquad 3.4$$

Integration of **Equation 3.4** will result in **Equation 3.5**:[8]

$$\underbrace{\frac{1}{[FlOH]_0 - \frac{1}{n}[DPPH]_0} \cdot \left\{ \ln\left[\frac{1}{[DPPH]} \cdot \left\{ [FlOH]_0 - \frac{1}{n}\left[[DPPH]_0 - [DPPH] \right] \right\} \right] - \ln \frac{[FlOH]_0}{[[DPPH]_0} \right\}}_{\alpha} = -kt \qquad 3.5$$

Where,

$$\alpha = \frac{1}{[FlOH]_0 - \frac{1}{n}[DPPH]_0} \cdot \left\{ \ln\left[\frac{1}{[DPPH]} \cdot \left\{ [FlOH]_0 - \frac{1}{n}\left[[DPPH]_0 - [DPPH] \right] \right\} \right] - \ln \frac{[FlOH]_0}{[[DPPH]_0} \right\} \qquad 3.6$$

n in **Equation 3.6** is the rapid stoichiometric factor (RSF) which represents the number of scavenged molecules of DPPH per molecule of flavonoid at the end of the fast step and is calculated according to:[62]

$$n = RSF = \frac{A_0 - A_f}{\varepsilon c} \qquad 3.7$$

(A_0, initial visible absorbance; A_f, final visible absorbance at the end of fast step; ϵ, absorption coefficient of DPPH; c, initial flavonoid concentration). The plot of the left term of **Equation 3.5** (α) as a function of time is linear with zero intercept over the duration of the rapid step. The slope of the line gives access to the rate constant k. **Equation 3.7** can also be used for estimating the total stoichiometry factor (TSF), with A_f now standing for the visible absorbance at the end of the overall reaction. The antiradical activity (AR%) is calculated by the following formula:

$$AR\% = 100 * \left[\frac{A_c - A_s}{A_c} \right] \qquad 3.8$$

Where A_c is the visible absorbance in absence of flavonoids, A_s is the visible absorbance in presence of flavonoids.

3.1.1 Explanation of rapid stoichiometric factor (RSF) and total stoichiometric factor (TSF)

3.1.1.1 Quercetin

The kinetics of DPPH being scavenged by different concentrations of quercetin is shown in **Figure 3.1** below; where the highest concentration of quercetin (1.00E-05) M scavenged most of the DPPH. The solution turned from a deep purple color, DPPH, to a yellowish product, $DPPH_2$.

The duration of the fast steps in the three different concentrations of quercetin is determined by visual inspection and lasted for at least 75 seconds as shown below in **Figure 3.2** while the slow reactions lasted for at least 50 minutes.

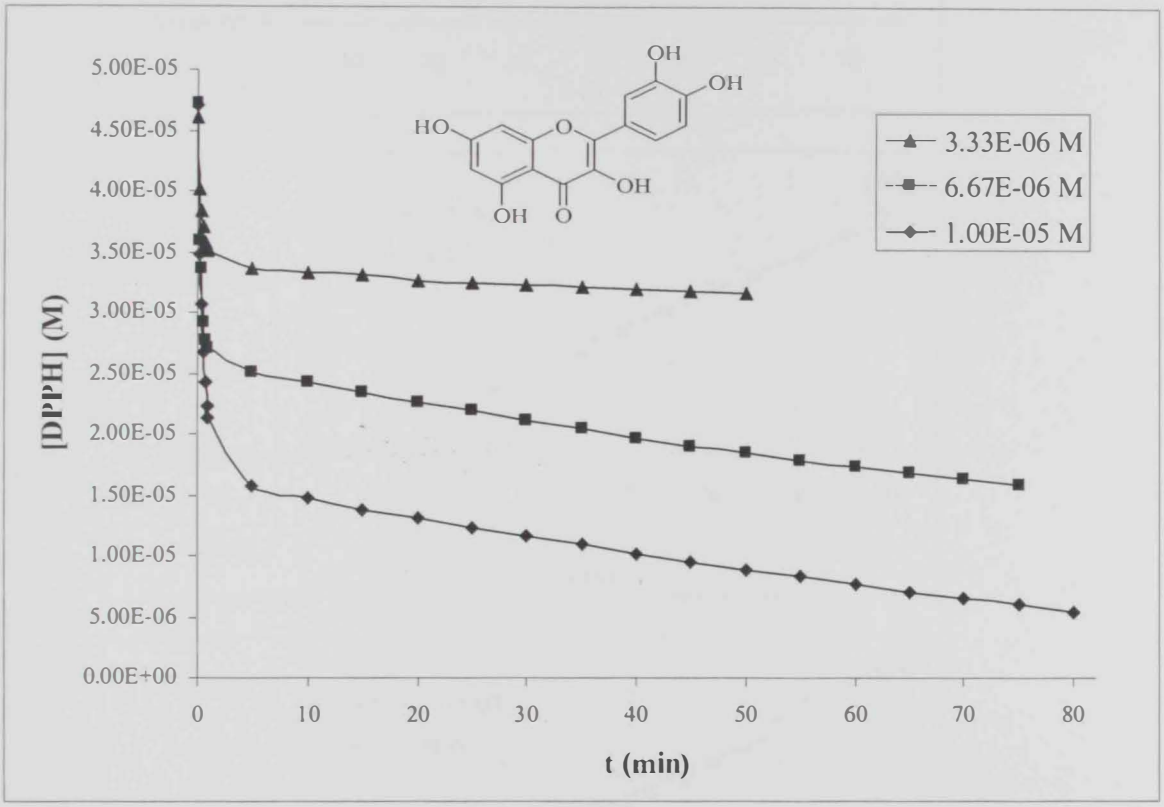


Figure 3.1: Scavenging of DPPH radical by different concentrations of quercetin.

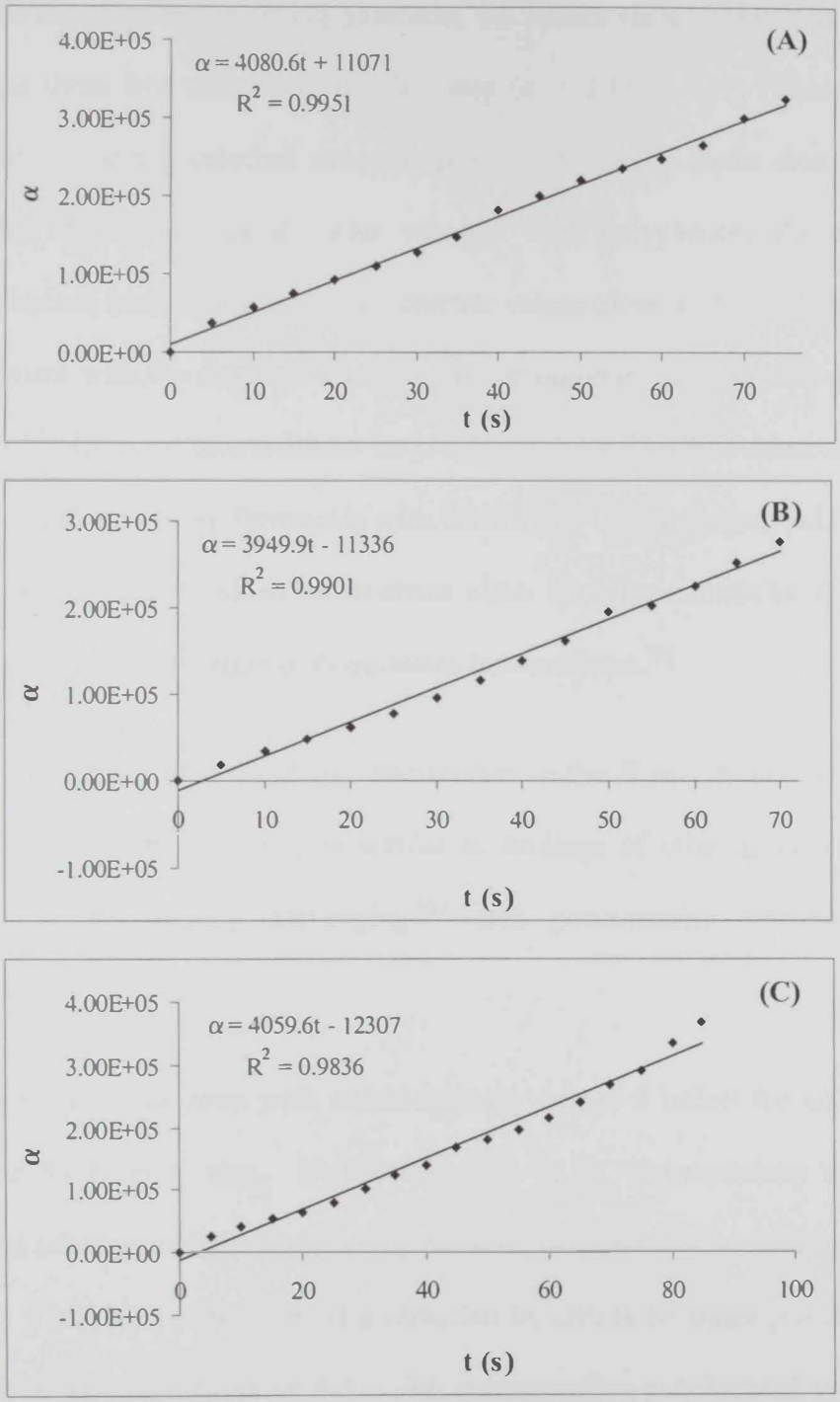


Figure 3.2: The correlation of α with time of quercetin reaction with $[DPPH]_0$ = 5.00E-05; **(A)** $[Quercetin]_0$ = 3.33E-06 M, n = 3.23, **(B)** $[Quercetin]_0$ = 6.67E-06 M, n = 3.23. **(C)** $[Quercetin]_0$ = 1.00E-05 M, n = 2.95.

In the experiment concerning quercetin, the results showed that 1 molecule of quercetin scavenges three free radicals in the fast step (n = 3.18 ± 0.17) **(Table 3.1)**. In general, flavonoids displaying catechol structure give stoichiometric factor close to 2. This is well established by Goupy et al. who reported that polyphenols displaying a free 1,2-hydroxybenzen (catechol) gave stoichiometric values close to 2 per catechol group which is in agreement with the stepwise formation of semiquinone radicals and quinones during the fast step.[62] Quinone intermediates have actually been clearly evidenced in the reaction of different 3', 4'-dihydroxy flavonoids with DPPH.[63,65] Furthermore, D.I. Tsimogiannnis and V.Oreopoulou determined stoichiometries close to 2 for catecholic flavonoids, which is consistent with the formation of *O*-quinones intermediates.[8]

The importance of *o*-dihydroxy substitution in the B ring to the antioxidant activity of flavonoids found in this study is similar to findings of other groups, which studied the peroxyl radical ($ROO^{\cdot}$) scavenging[66] and peroxynitrite scavenging activities of flavonoids.[67]

In our study, quercetin with stoichiometries around 3 reflect the additional H-donating ability of 3-OH in C ring. Furthermore, the 3, 3', 4'-trihydrdoxy substitution has the additional advantage of allowing the regeneration of a catechol nucleus upon solvent addition at C(2). Therefore, subsequent H-abstraction by DPPH becomes possible, thus leading to higher total stoichiometries of 4-5. The corresponding *p*-quinonoid solvent adducts is in complete agreement with the finding of D.I. Tsimogiannnis and V.Oreopoulou[8] and Dangless et al.[63] **(Figure 3.3)**.

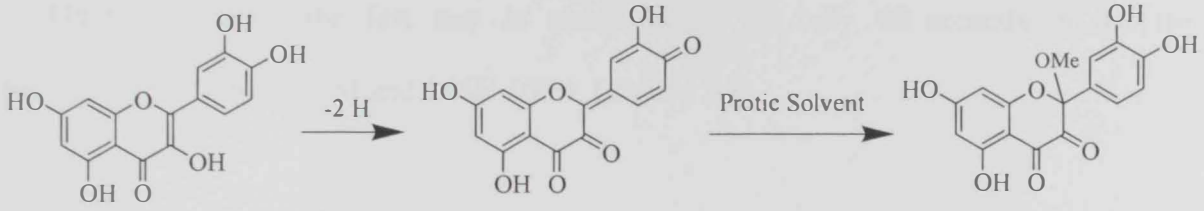


Figure 3.3: Proposed pathways of oxidative degradation of quercetin during radical capture in methanol.

3.1.1.2 Morin

Quercetin and Morin have the same number of hydroxyl groups, yet the substitution pattern in ring B in both Flavonoids is different. The slight change in the position of the two hydroxyl groups resulted in a change in their scavenging activity. **Figure 3.4** shows the kinetics of the different concentrations of Morin.

Unlike quercetin, the fast step in morin lasted for only 40 seconds in the two concentrations; 6.67E-05 M and 1.00E-05 M, **(Figure 3.5)**.

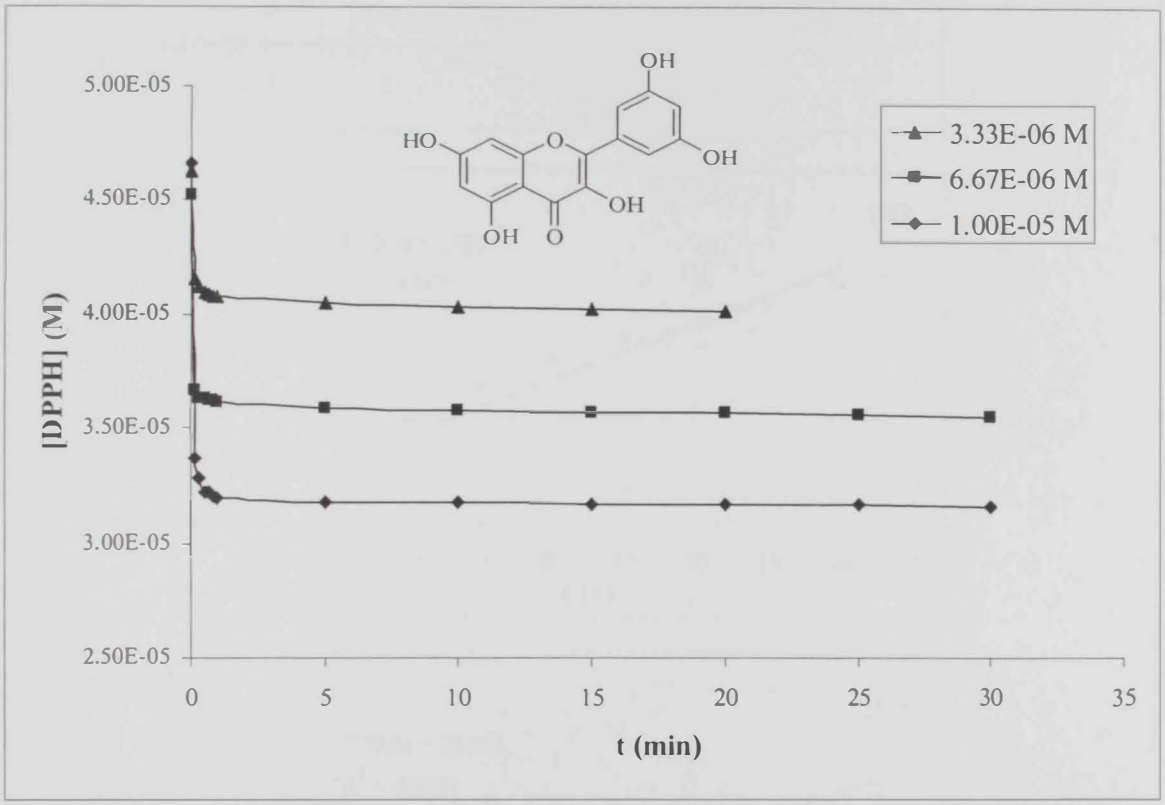


Figure 3.4: Scavenging of DPPH radical by different concentrations of morin.

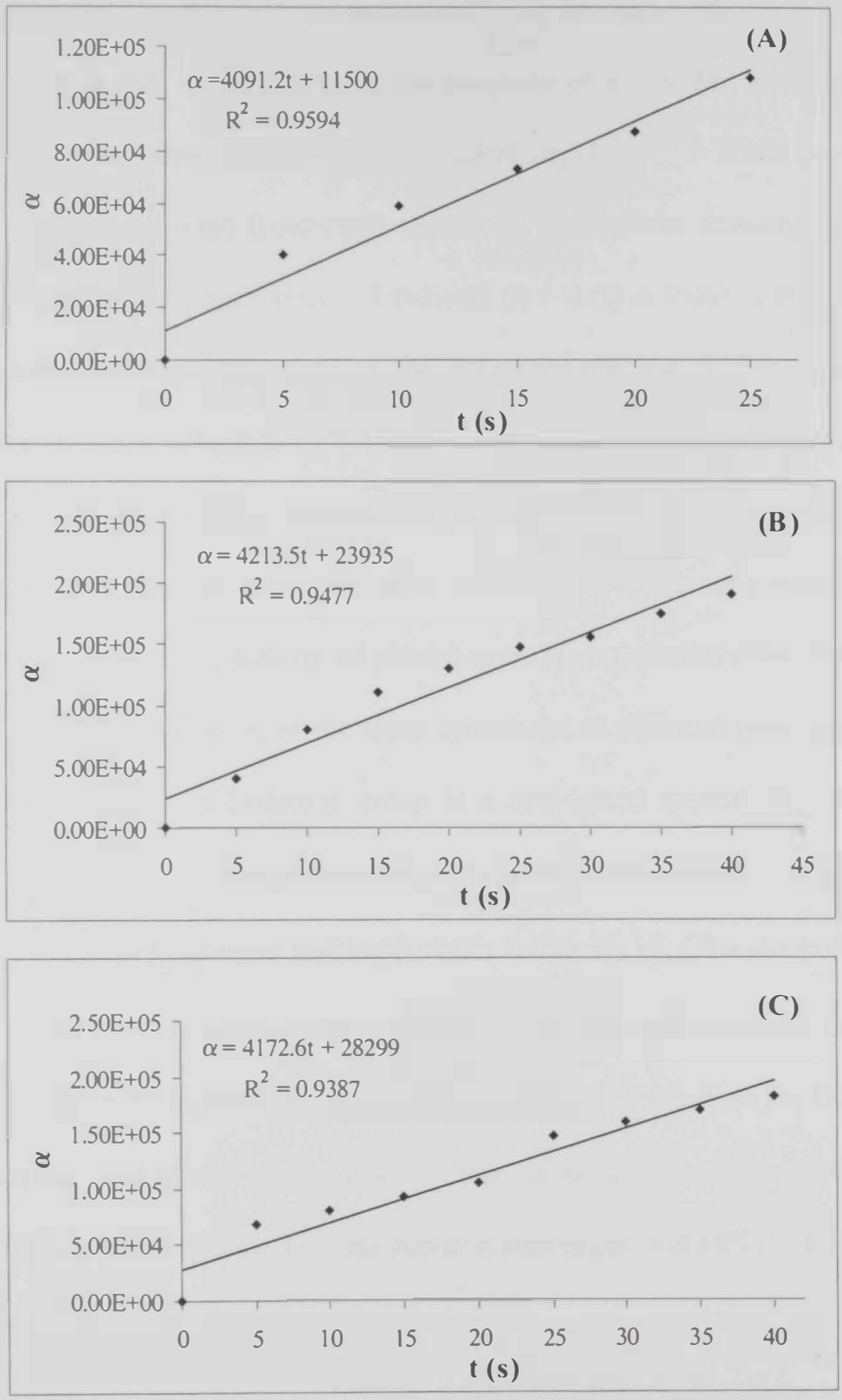


Figure 3.5: The correlation of α with time of morin reaction with $[DPPH]_0$ = 5.00E-05; **(A)** $[Morin]_0$ = 3.33E-06 M, n = 1.72, **(B)** $[Morin]_0$ = 6.67E-06 M, n = 1.39. **(C)** $[Morin]_0$ = 1.00E-05 M, n = 1.48.

In quercetin, the catecholic structure in B-ring is responsible for scavenging two DPPH molecules. It is well established that the presence of a catechol group in ring B, shows a better electron donating property and is a radical target.[3,9,31,42] While in morin the influence of *meta* hydroxyl groups (resorcinol structure) on electron donating property is weaker. Therefore, morin scavenged only 1.5 radicals (n = 1.53 ± 0.04) in the fast step. The total stoichiometries were slightly higher at the end of the reaction (TSF = 1.6± 0.19) **(Table 3.1)**. It is clear that the influence of the OH group in flavonoids is highly dependent on the position of OH substitution. According to C.G.M Heijnen et al., An additional OH group at the 2 position (catechol structure) or 4 position (hydroquinone structure) increases the peroxynitrite scavenging activity of phenol more than substitution at 3 position (resorcinol structure).[31] The influence of the same substituent at different positions was explained by an electronic effect; A hydroxyl group in a conjugated system has an electron donating effect to the ring. This electron donation from the substituent to the oxygen of the active OH group weakens the O-H bond making it easier to release $H^{\cdot}$. The electron donating effect of the OH group depends on the relative position of the OH substitution at the ring compared to the active center. The maximal donating effect is observed when the OH is at the ortho or para position. An electron withdrawing effect is seen at the meta position.[31] Therefore, morin with hydroxyl groups in meta position scavenges less DPPH molecules compared to quercetin.

3.1.1.3 Myricetin

Although myricetin has three hydroxyl groups in B-ring (pyrogallol group) yet, DPPH scavenging lasted for less than 20 minutes before the reaction is over. **Figure 3.6** shows the reaction between DPPH and increasing concentrations of myricetin.

The fast kinetics of myricetin, with three hydroxyl groups in B-ring (pyrogallol group) lasted for at least 45 seconds while the slow step lasted for the remaining 30 minutes **(Figure 3.7)**.

Myricetin, with three hydroxyl groups in B-ring (pyrogallol group) didn't increase the number of scavenged free radicals (n = 2.41 ± 0.06) compared to Quercetin with two hydroxyl groups in B-ring (catechol structure) (n = 3.18 ± 0.17) **(Table 3.1)**.

It is generally believed that an increase in the number of OH groups enhances the number of free radicals scavenged.[66] Yet, myricetin which has the highest number of OH groups among flavonols under study is less active than quercetin. This may be explained by the fact that in quercetin a nucleophilic attack may regenerate the catechol moiety and render it available for further oxidation, while this phenomenon is less probable in the presence of pyrogallol, which hinders this reaction due to both steric and electronic effects.[44]

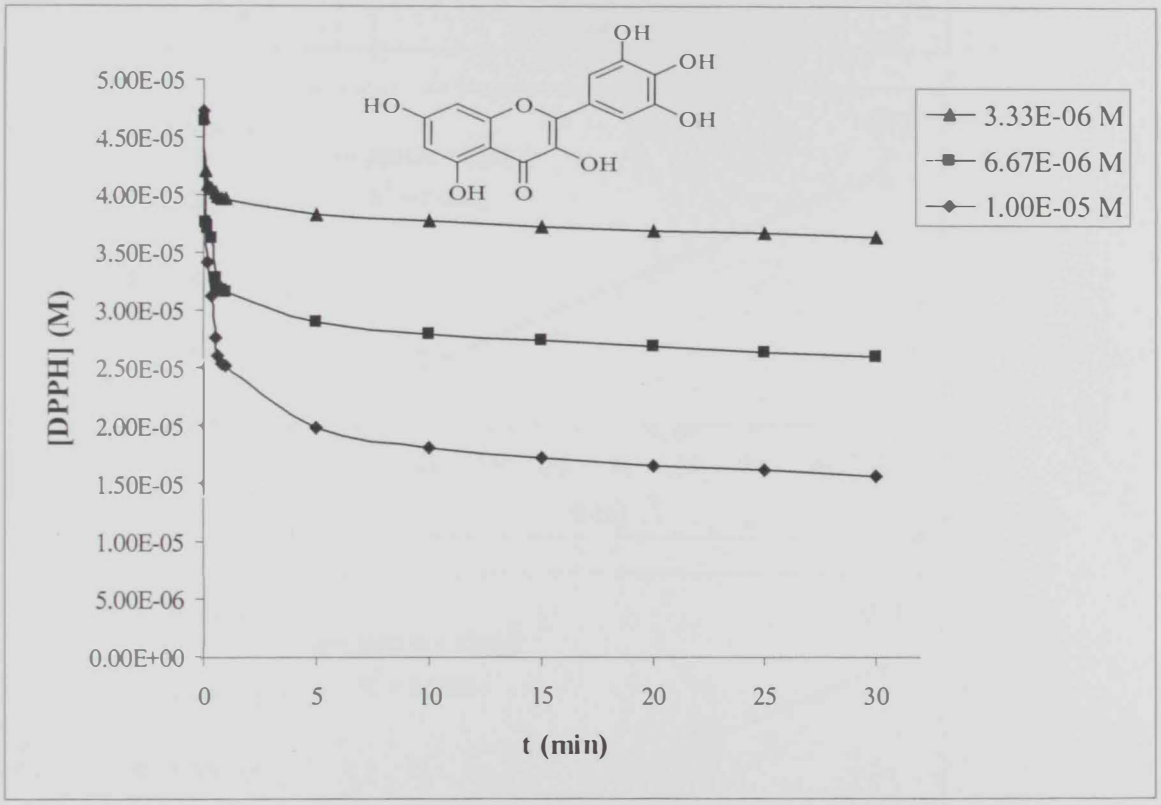


Figure 3.6: Scavenging of DPPH radical by different concentrations of myricetin.

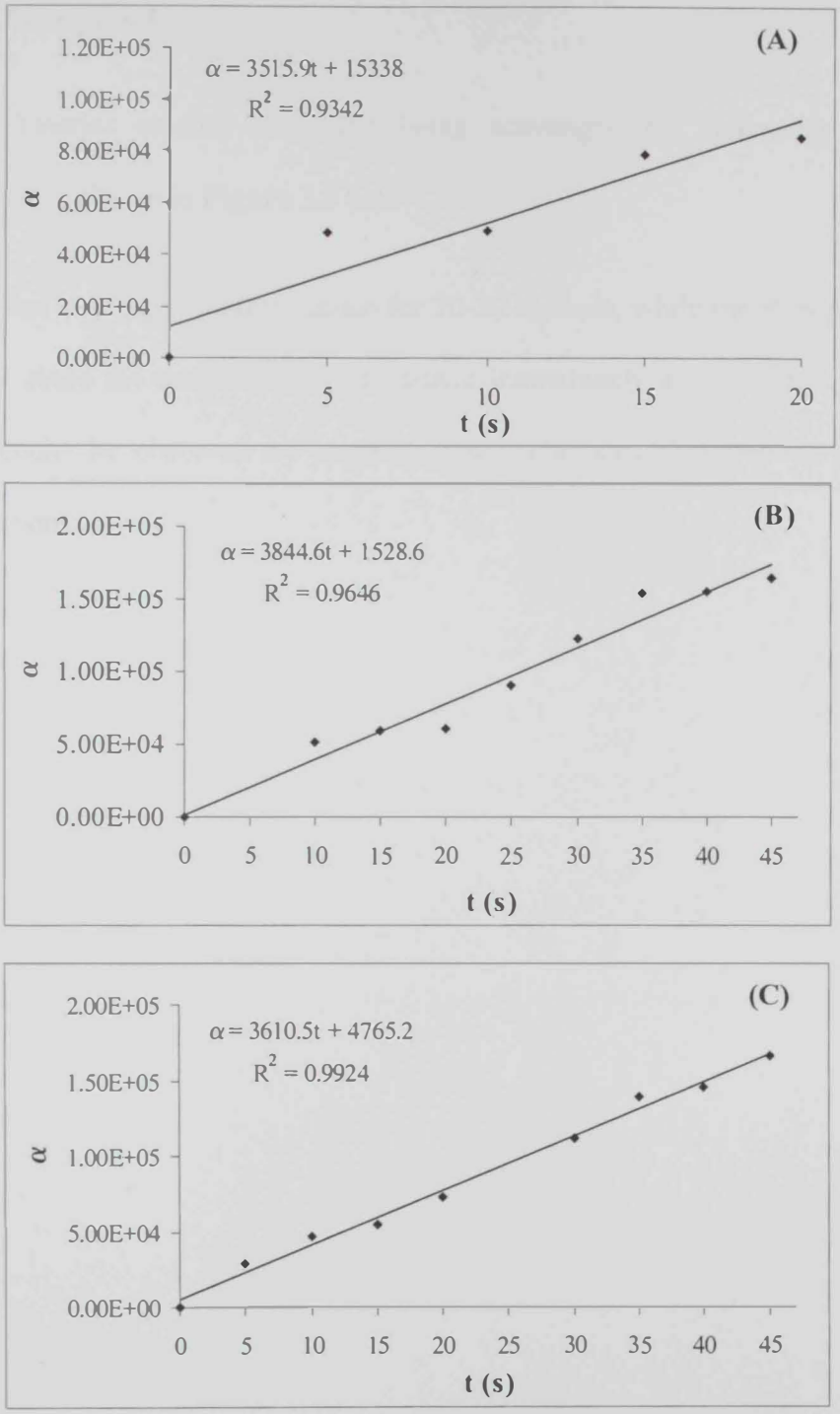


Figure 3.7: The correlation of α with time of myricetin reaction with $[DPPH]_0$ = 5.00E-05; **(A)** $[Myricetin]_0$ = 3.33E-06 M, n = 2.40, **(B)** $[Myricetin]_0$ = 6.67E-06 M, n = 2.39. **(C)** $[Myricetin]_0$ = 1.00E-05 M, n = 2.49.

3.1.1.4 Kaempferol

The kinetics studies of DPPH being scavenged by increasing concentrations of kaempferol is shown in **Figure 3.8** below.

The fast step in kaempferol lasted for 20-30 seconds, while the slow step was not clearly observed since the reaction reached plateau immediately after the fast step **(Figure 3.9)**. Which could be observed by stopped flow techniques that will be reported in future investigations.

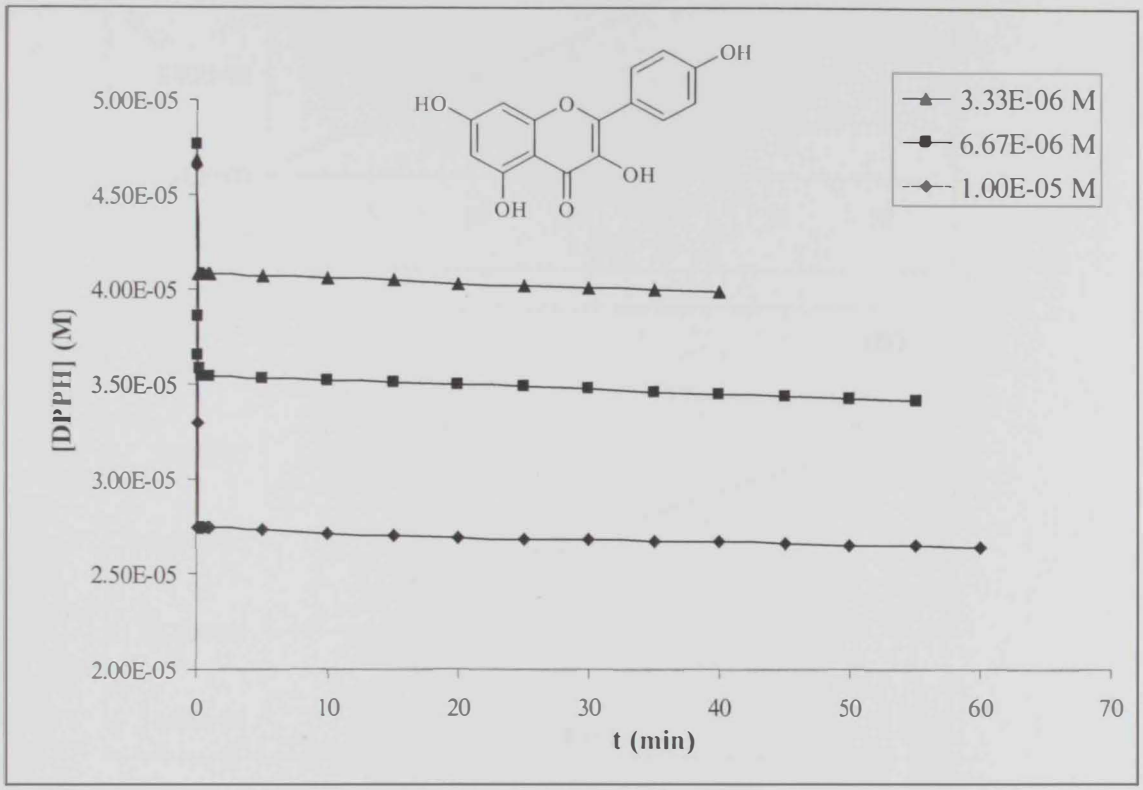


Figure 3.8: Scavenging of DPPH radical by different concentrations of kaempferol.

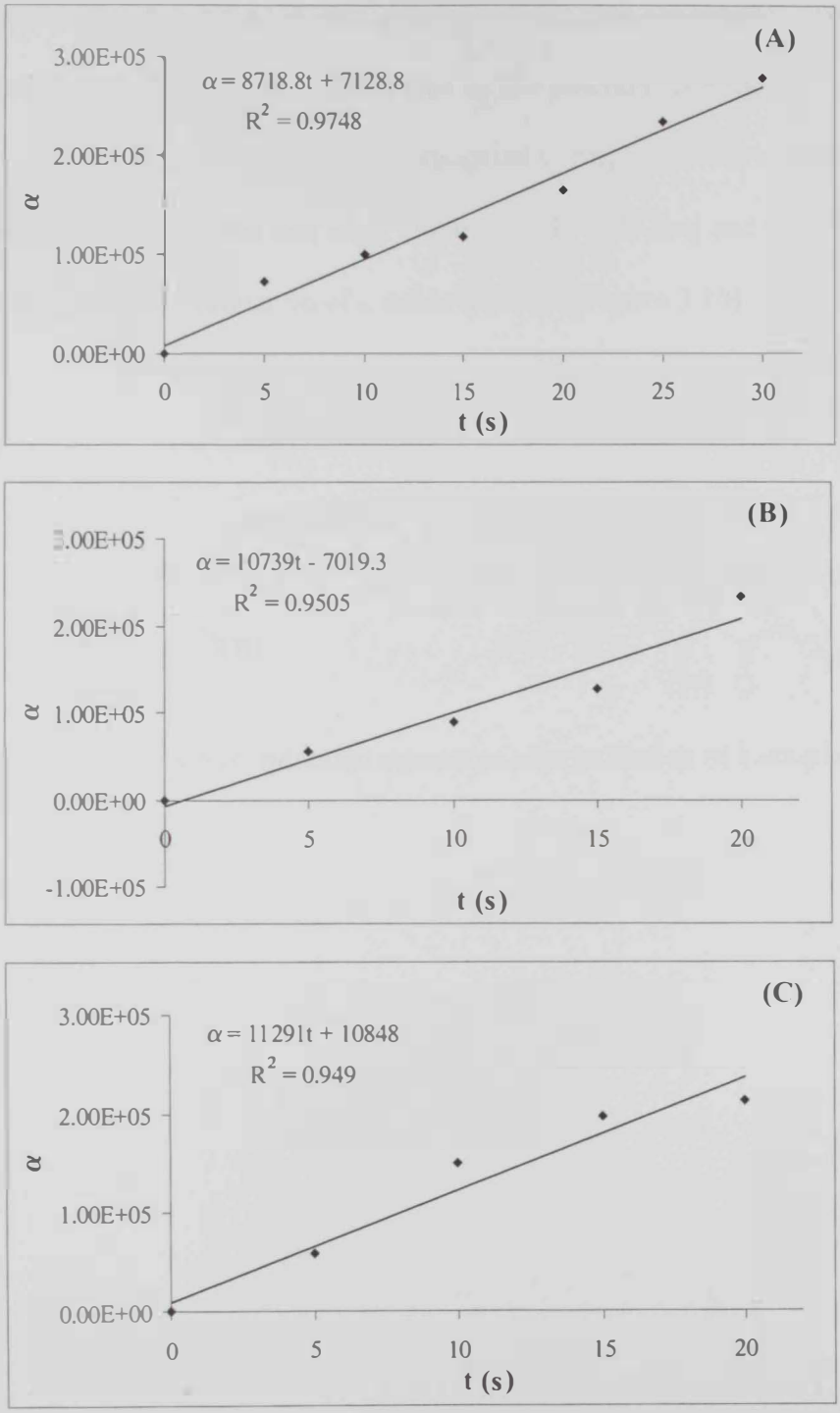


Figure 3.9: The correlation of α with time of kaempferol reaction with $[DPPH]_0$ = 5.00E-05; **(A)** $[Kaempferol]_0$ = 3.33E-06 M, n = 2.22, **(B)** $[Kaempferol]_0$ = 6.67E-06 M, n = 1.99. **(C)** $[Kaempferol]_0$ = 1.00E-05 M, n = 2.05.

Kaempferol with a single 4'-OH group in the B-ring scavenged 2 radicals (n = 2.08 ± 0.10) **(Table 3.1)**. This is most likely due to the potential conjugation between the 4'-OH group and the 3-OH group through the conjugated C-ring. Therefore, kaempferol was able to scavenge two radicals in the fast step; one by 4'-OH in B-ring and the other by 3-OH in C-ring which leads to the formation of a stable quinone **(Figure 3.10)**.

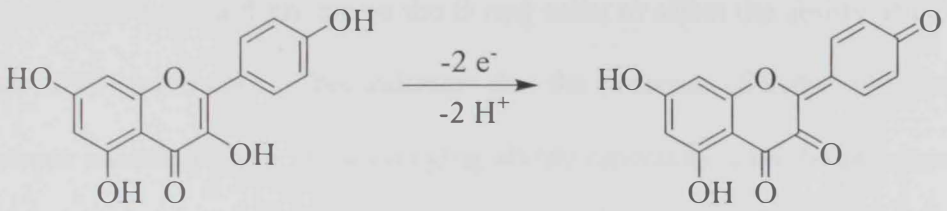


Figure 3.10: Generation of quinoinic structures after oxidation of kaempferol.

3.1.1.5 3-Hydroxy flavone

3-hydroxy flavone showed a weak scavenging activity as shown in **Figure 3.11**. The fast step lasted for 20 seconds and the scavenging of DPPH was very weak **(Figure 3.12)**.

3-hydroxy flavone, with only a 3-OH group in C-ring displayed the least stoichiometry and total stoichiometry among flavonols (RSF = 0.06 ± 0.02, TSF= 0.16 ± 0.04) respectively **(Table 3.1)**. Although 3-hydroxy flavone posses 3-OH, 2,3-double bond and 4-oxo function in C ring, lack of hydroxyl groups on the B ring seem to affect the ability of this flavonol to scavenge DPPH molecules. This indicates that the presence of hydroxyl groups in B ring give a major contribution to the scavenging ability especially with the presence of catechol group in B ring.

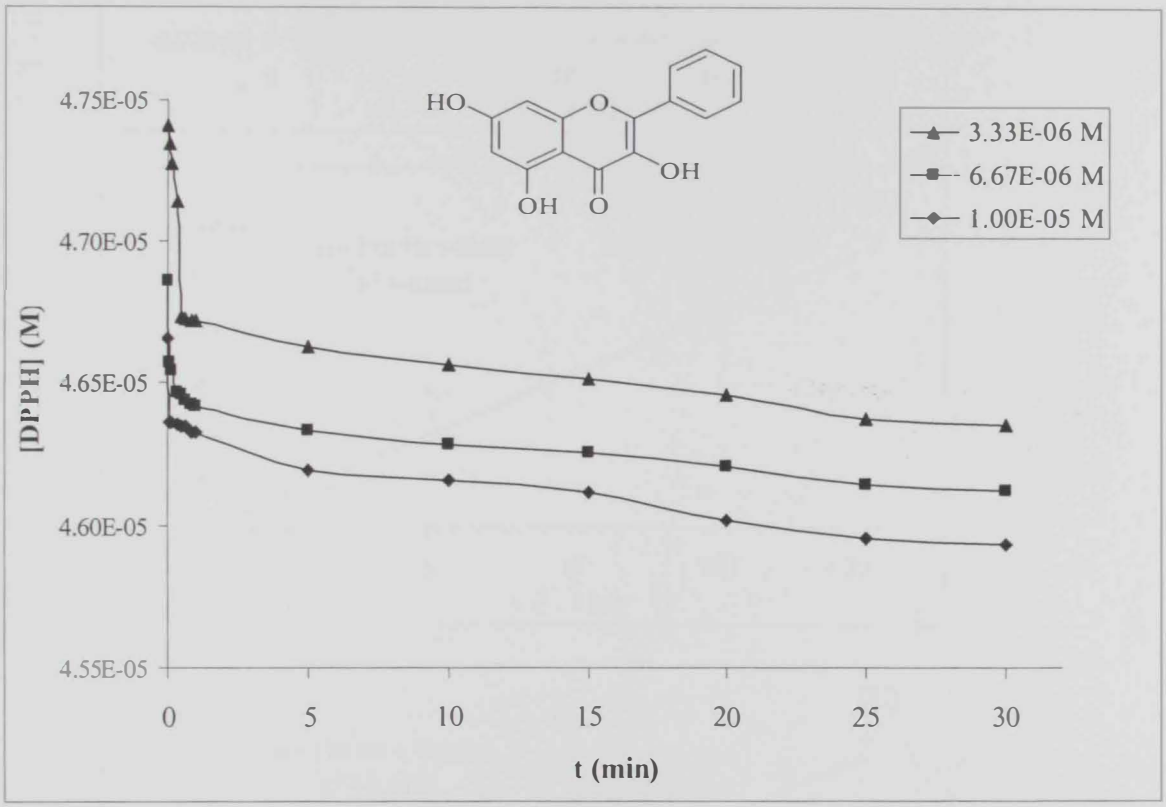


Figure 3.11: Scavenging of DPPH radical by different concentrations of 3-hydroxy flavone.

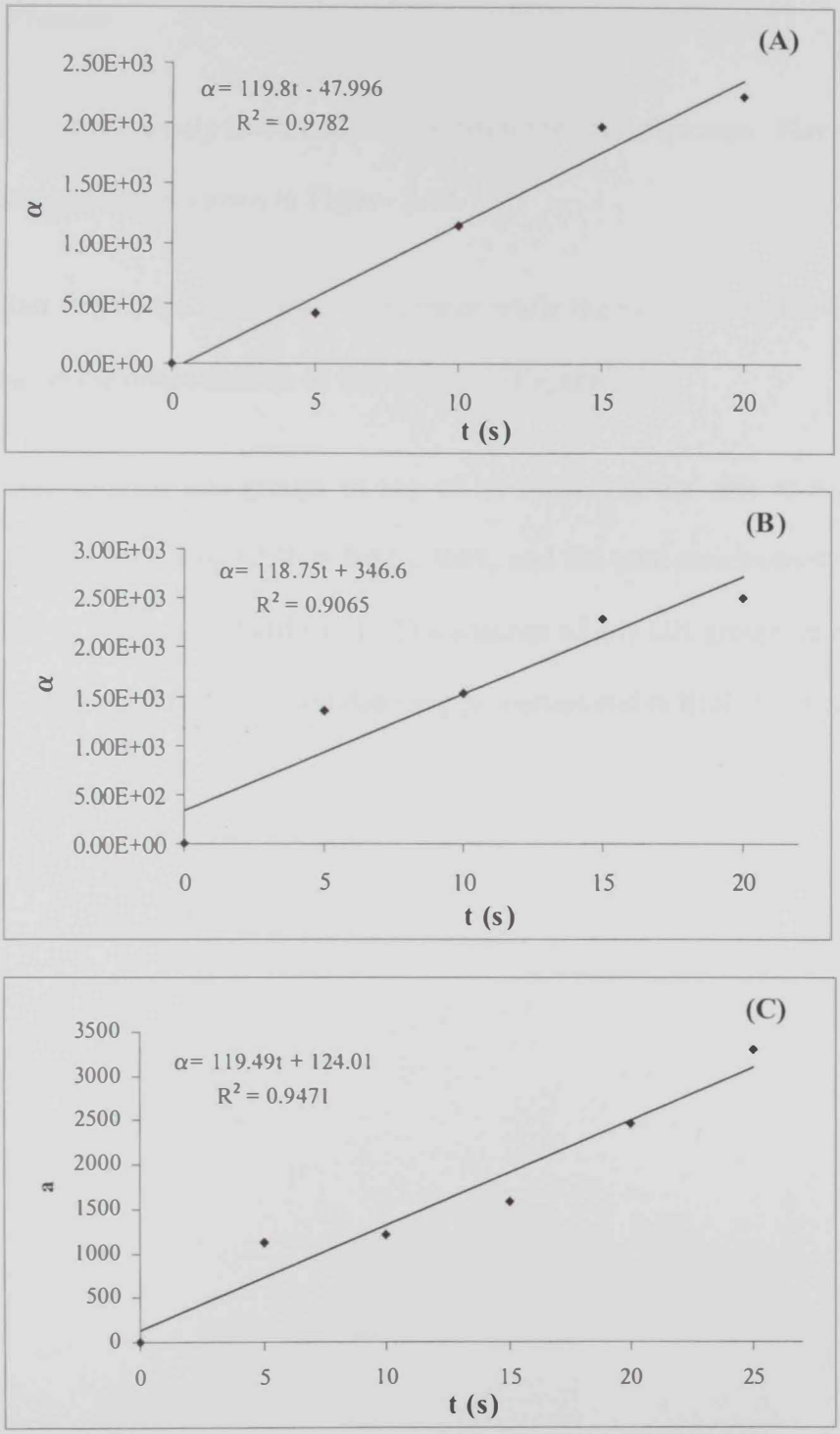


Figure 3.12: The correlation of α with time of 3-hydroxy flavone reaction with $[DPPH]_0$ = 5.00E-05; **(A)** $[3\text{-hydroxy flavone}]_0$ = 3.33E-06 M, n = 0.08, **(B)** $[3\text{-hydroxy flavone}]_0$ = 6.67E-06 M, n = 0.07. **(C)** $[3\text{-hydroxy flavone}]_0$ = 1.00E-05 M, n = 0.05.

3.1.1.6 Flavone

Flavone was the only flavonoid studied from the class flavones. Flavone showed a weak scavenging activity as shown in **Figure 3.13**.

The fast step lasted for at least 30 seconds while the slow step lasted from 15-30 minutes depending on the concentration of flavone used **(Figure 3.14)**.

Flavone, with no OH groups in any of its rings was not able to scavenge any DPPH molecules in the fast step (RSF = 0.02 ± 0.01) and the total stoichiometry was very poor as well (TSF= 0.07 ± 0.01) **(Table 3.1)**. The absence of any OH groups in any of its rings and hence the absence of their electron donating properties and radical target caused flavone to be inactive.

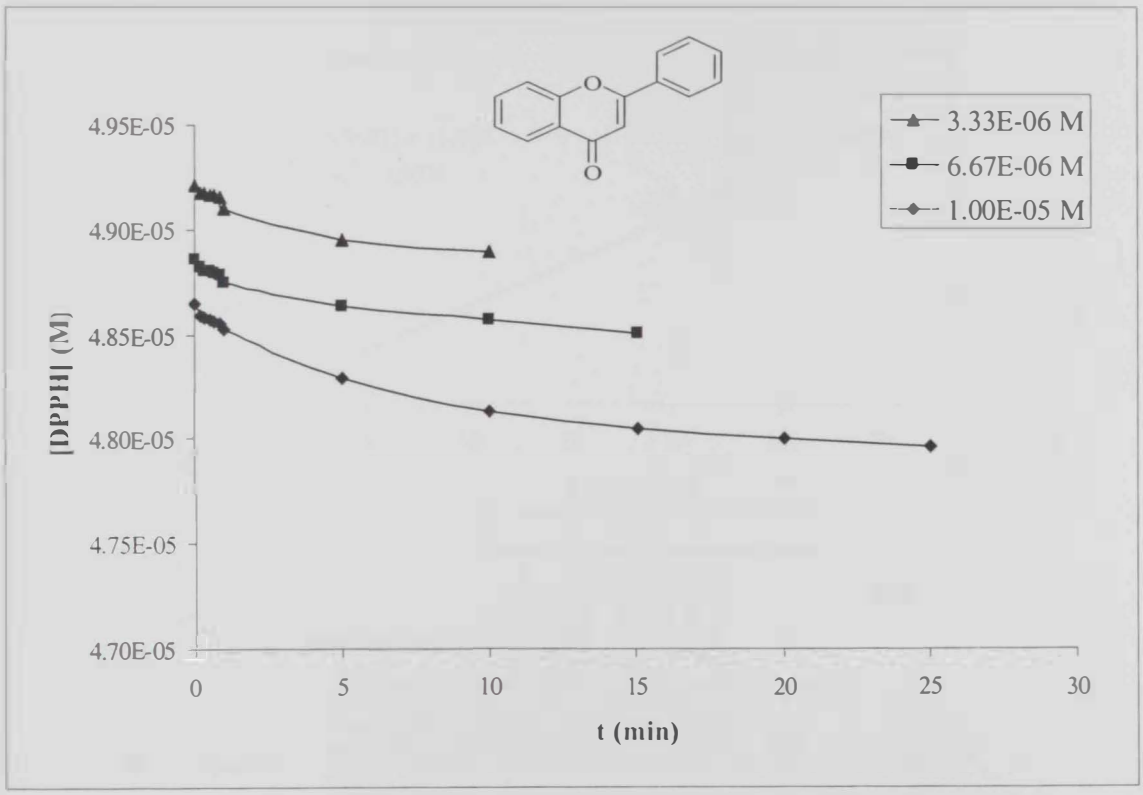


Figure 3.13: Scavenging of DPPH radical by different concentrations of flavone.

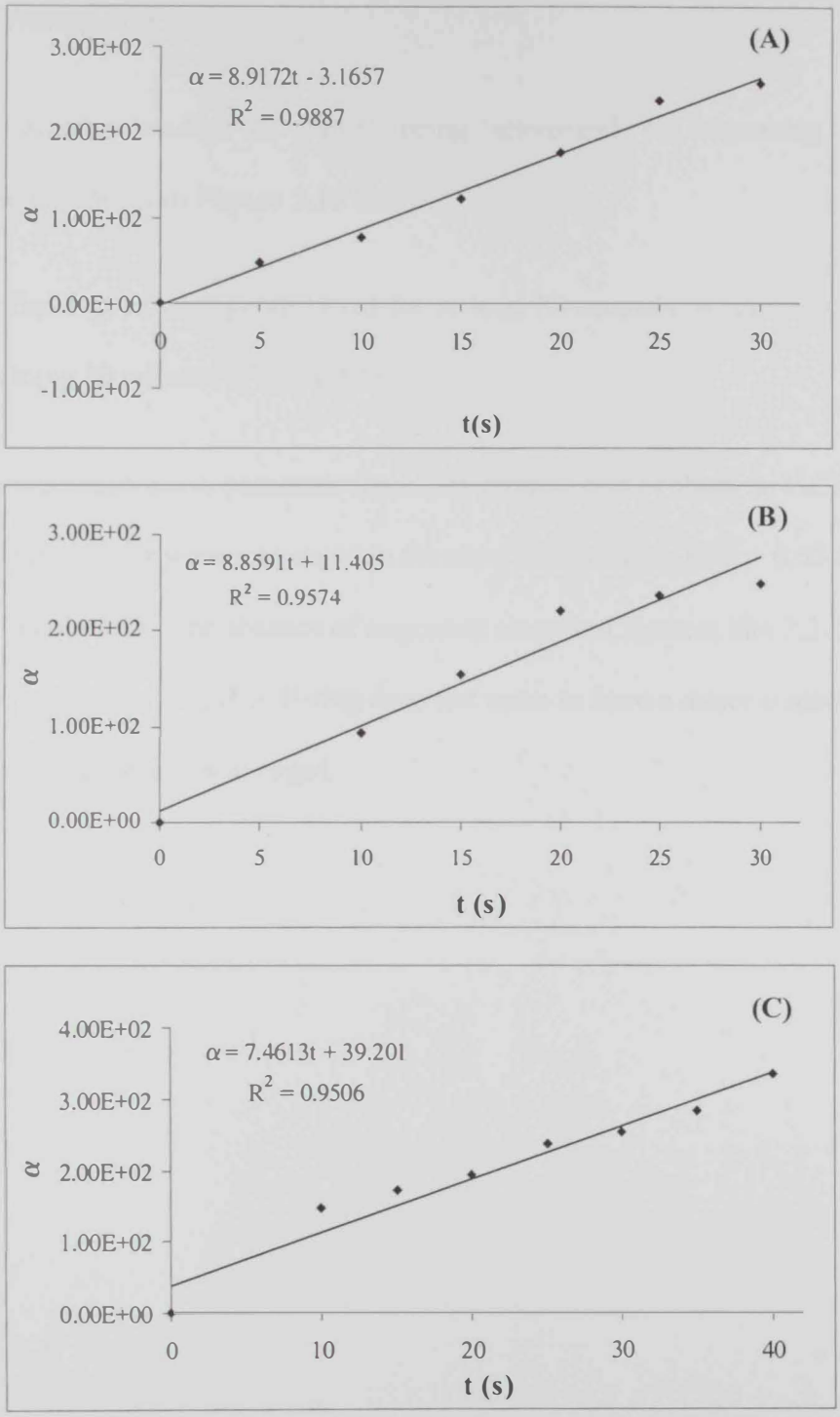


Figure 3.14: The correlation of α with time of flavone reaction with $[DPPH]_0$ = 5.00E-05; **(A)** $[flavone]_0$ = 3.33E-06 M, n = 0.03, **(B)** $[flavone]_0$ = 6.67E-06 M, n = 0.02. **(C)** $[flavone]_0$ = 1.00E-05 M, n = 0.01.

3.1.3.7 Naringenin

The kinetics studies of DPPH being scavenged by increasing concentrations of naringenin is shown in **Figure 3.15** below.

The fast step of naringenin lasted for at least 30 seconds, while the slow step lasted for the remaining 30 minutes **(Figure 3.16)**.

Although naringenin possesses three OH groups, one of them is 4'-OH group in B-ring, no DPPH molecules were scavenged in the slow or fast steps (RSF = 0.03 ± 0.00, TSF = 0.07 ± 0.01) **(Table 3.1)**. The absence of important structural features like 2,3-double bond and 3-OH, the presence of 4'-OH in B-ring does not seem to have a major impact on the number of DPPH molecules being scavenged.

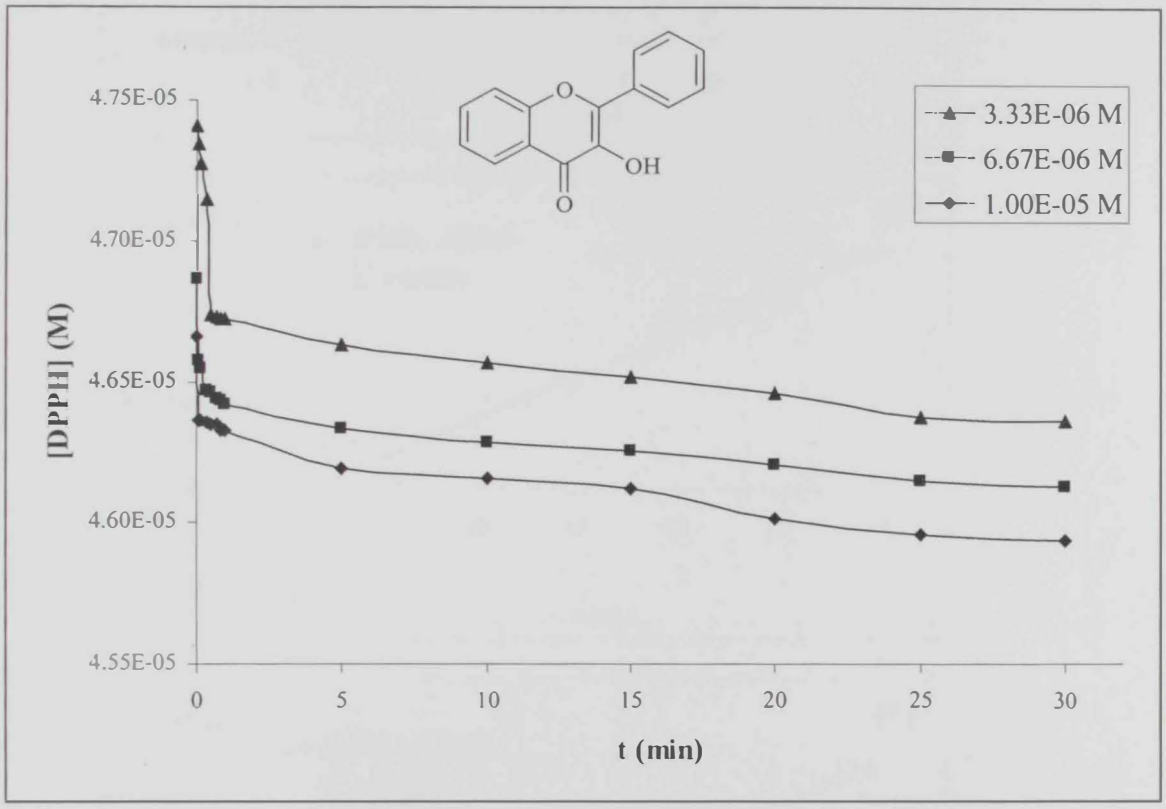


Figure 3.15: Scavenging of DPPH radical by different concentrations of naringenin.

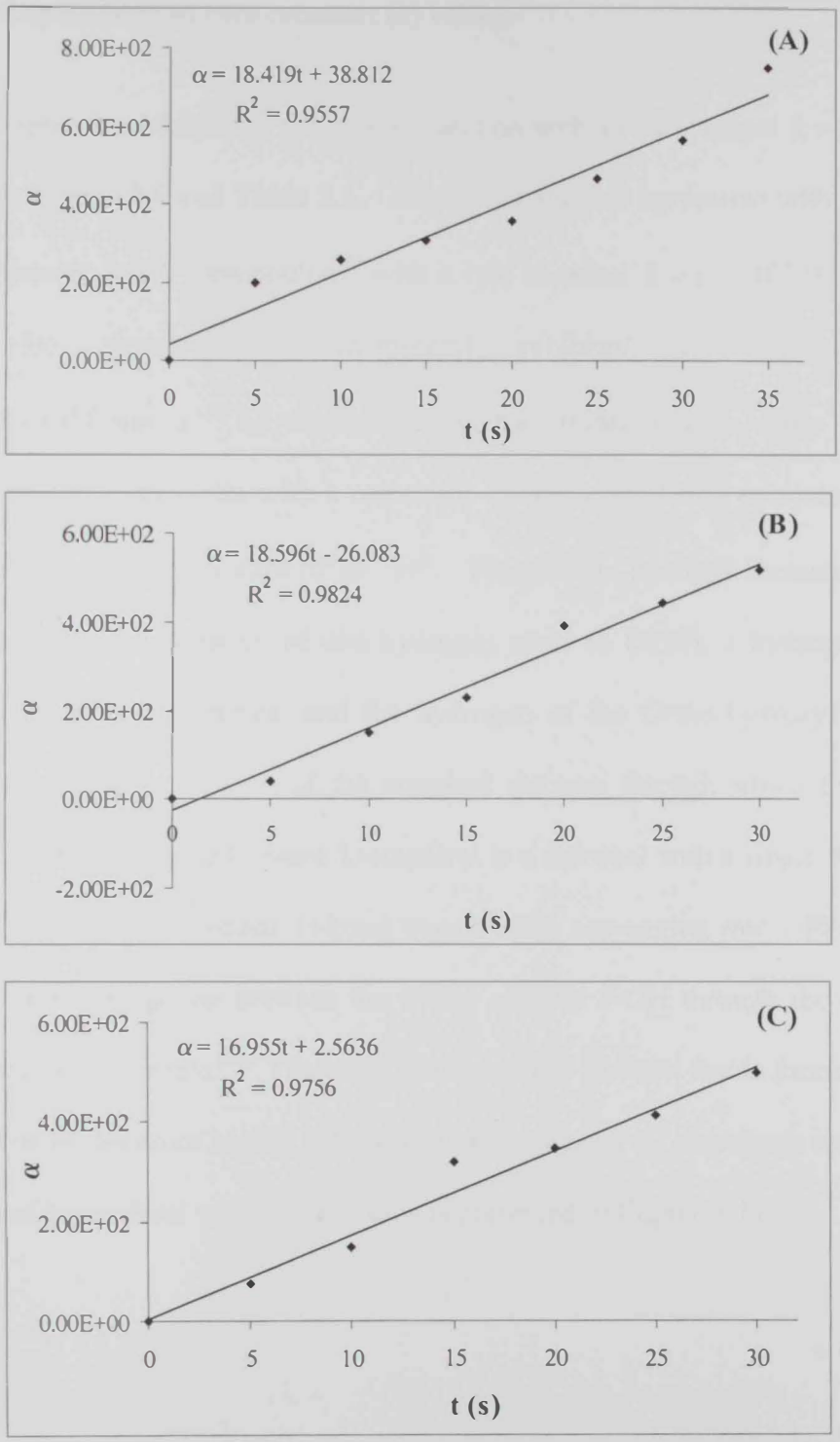


Figure 3.16: The correlation of α with time of naringenin reaction with $[DPPH]_0$ = 5.00E-05; **(A)** $[naringenin]_0$ = 3.33E-06 M, n = 0.03, **(B)** $[naringenin]_0$ = 6.67E-06 M, n = 0.02. **(C)** $[naringenin]_0$ = 1.00E-05 M, n = 0.03.

3.1.2 Explanation of rate constant (k) values

Kaempferol exhibited the most rapid reaction with a rate constant $k = 1.0 \times 10^4 M^{-1}s^{-1}$ as shown in **Figure 3.8** and **Table 3.1.** This is in complete agreement with the finding of D.I. Tsimogiannnis and V.Oreopoulou[8] with a rate constant $k = 1.1 \times 10^4 M^{-1}s^{-1}$. Butkovic et al.[42] also reported that kaempferol exhibited the highest rate constant $k = 2.38 \times 10^3 L.mol^{-1}s^{-1}$ by spectrophotometric titration and under pseudo-first-order conditions. Yet, quercetin with a catecholic structure in B-ring presents a slower reaction with a rate constant $k = 4.0 \times 10^3 M^{-1}s^{-1}$. This fact is justified because at the 3', 4'-OH members, after the donation of one hydrogen atom to DPPH, a hydrogen bond is settled between the phenoxyl radical and the hydrogen of the *Ortho*-hydroxyl.[68] This H-bond suppresses the delocalization of the unpaired electron through which the donation of the second H can be achieved. Since kaempferol is a flavonol with a single 4'-OH group in the B-ring and no intermolecular H-bond occurs after scavenging one DPPH molecule. The potential for conjugation between the 4'-OH and the 3-OH through the conjugated C-ring becomes more favorable.[32] Therefore, The unpaired electron that is formed after abstraction of the first H, becomes highly delocalized and produces 10 resonance structures during the reaction of kaempferol with DPPH which is presented in **Figure 3.17**.

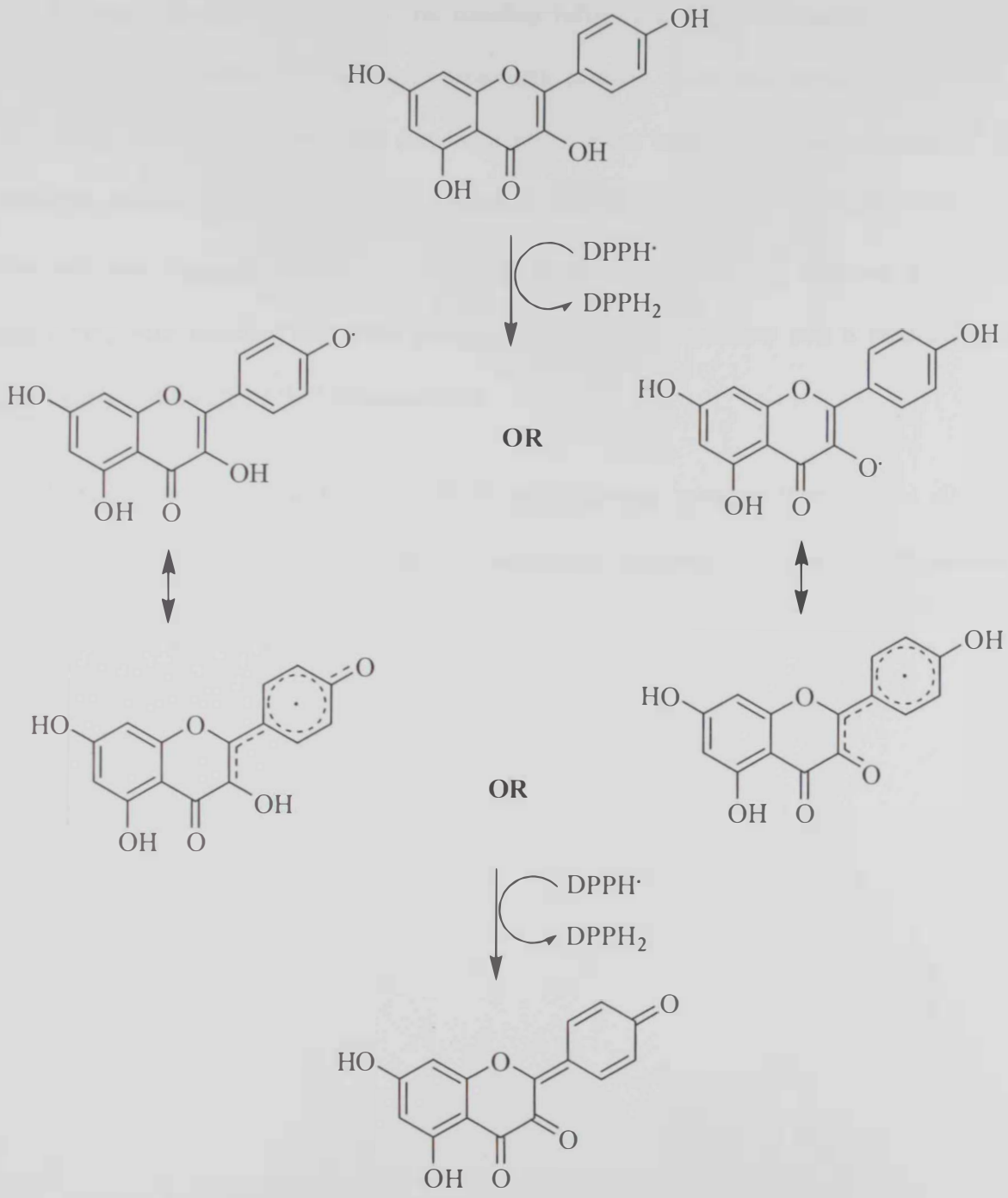


Figure 3.17: Proposed mechanism of DPPH radical scavenging by kaempferol

All other flavonols have lower rate constant values compared to kaempferol. This could be due to the number of resonance structures present, since the higher the number of resonance structures, the lower the demand energy for the formation of the free radical.[8] For example, morin produces 8 resonance structures after the donation of the first H to DPPH and the total rate constant for the primary stage is $k = 4.2 \times 10^3 M^{-1} s^{-1}$ **(Figure 3.18)** while quercetin, with catechol structure, presents 6 resonance structures and a respective rate constant $k = 4.0 \times 10^3 M^{-1} s^{-1}$ **(Figure 3.19)**.

Although myricetin, with three OH groups in B-ring, presents 9 resonance structures **(Figure 3.20)**, it reacted slower than both morin and quercetin due to steric and electronic effects of the pyrogallol group ($k = 3.7 \times 10^3 M^{-1} s^{-1}$).

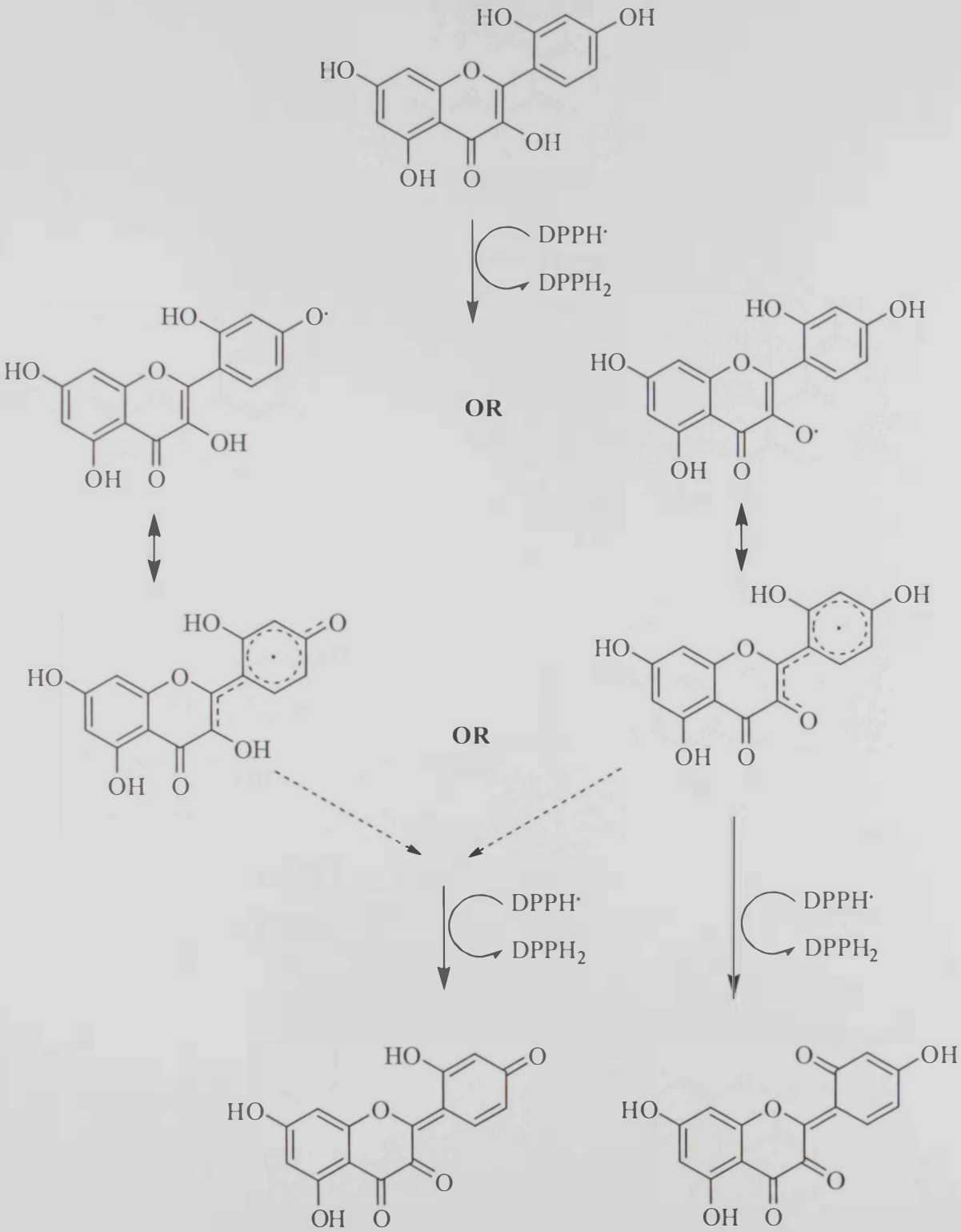


Figure 3.18: Proposed mechanism of DPPH radical scavenging by morin.

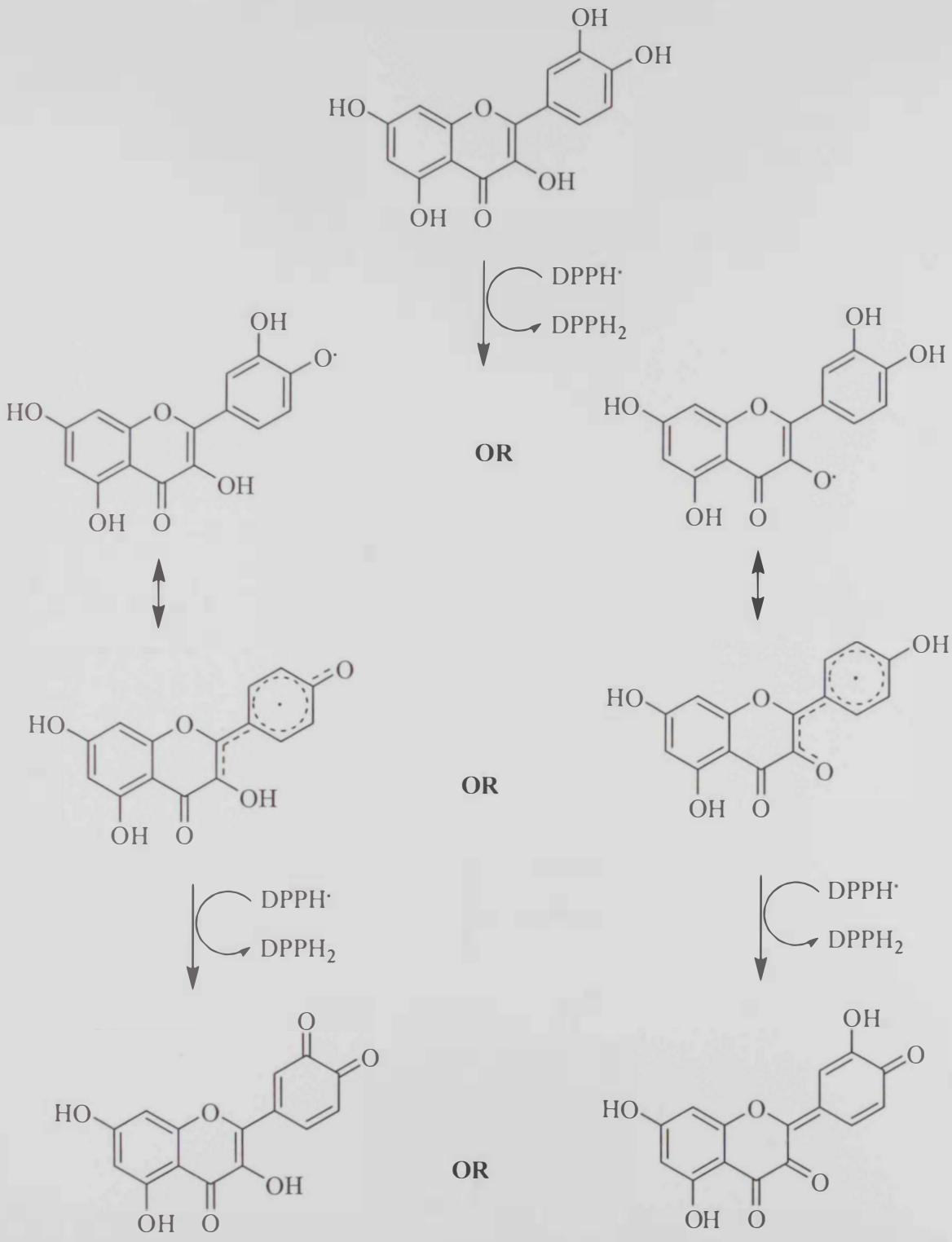


Figure 3.19: Proposed mechanism of DPPH radical scavenging by quercetin.

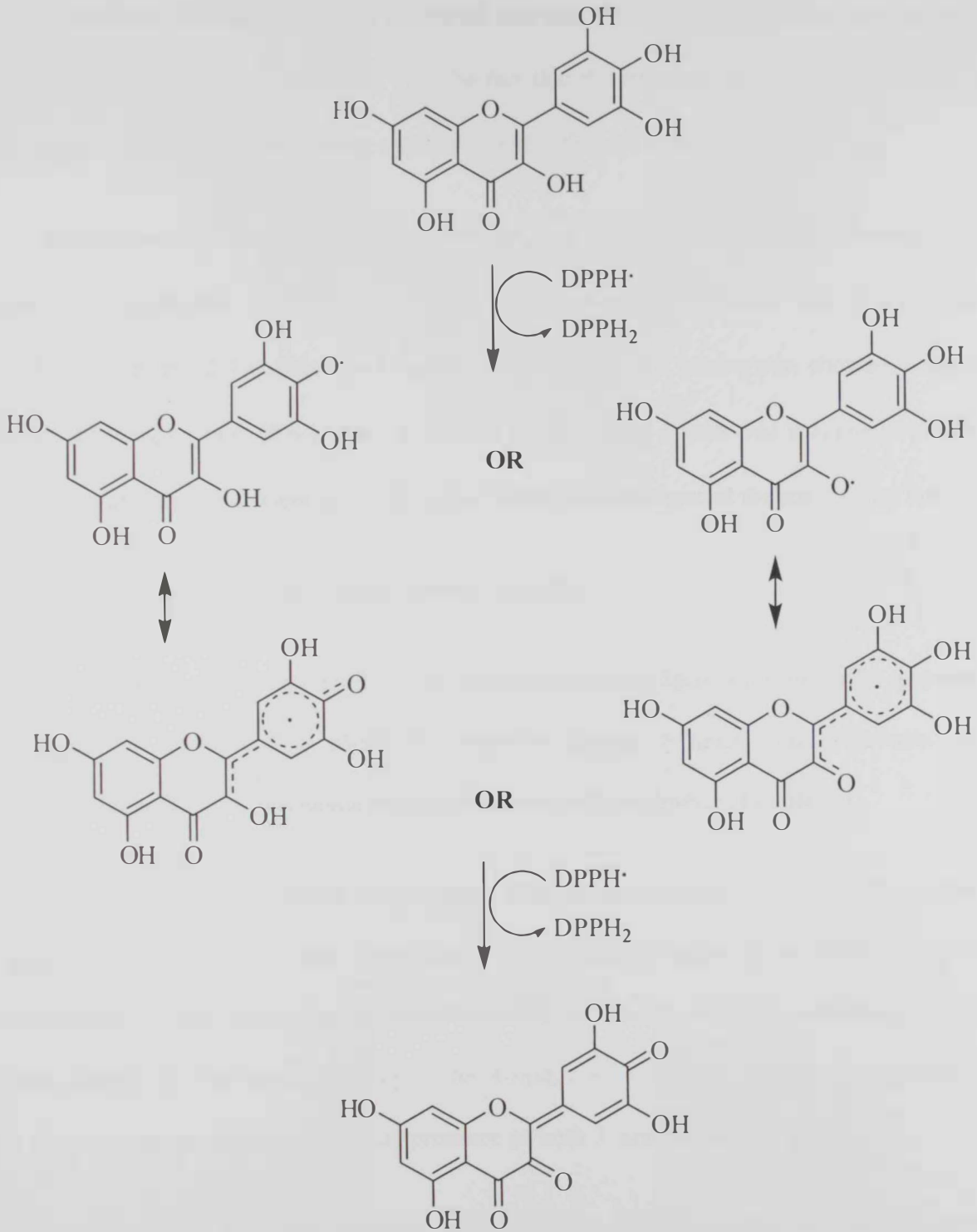


Figure 3.20: Proposed mechanism of DPPH radical scavenging by myricetin

3-hyrdorxy flavone exhibited the slowest reaction among flavonols tested here in with a rate constant of ($k = 1.2 \times 10^2 M^{-1}s^{-1}$). The fact that the structure of 3-hyrdorxy flavone has only one 3-OH group in the C-ring appears to affect the rate of the reaction.

Flavone which lacks one structural element, i.e. 3-OH group in B-ring showed a very poor scavenging rate ($k = 8.4 M^{-1}s^{-1}$). On the other hand, flavanones lack two structural elements, i.e. the 2,3 double bond and the 3-OH group yet, naringenin showed a slightly better scavenging rate ($k = 17.9 M^{-1}s^{-1}$). This is due to the reason that naringenin posses 3 hydroxyl groups one of them is 4'-OH in the B-ring which enhanced the scavenging rate.

3.1.3 Explanation of Antiradical Activity (AR %)

The antiradical activity (AR %) of the flavonoids tested decrease in the order quercetin> Myricetin > Kaempferol > Morin > 3-hydroxy flavone > naringenin > flavone. The Antiradical activity of flavonoids strongly depends on their structure **(Table 3.1)**.

The most active compound is quercetin. This flavonol presents four phenolic hydroxyl groups (in the 5, 7, 3', and 4' positions) and a vinylic hydroxyl in position 3. This substitution pattern represents the three structural groups required for exhibiting this very high activity: (1) The catechol group in the B-ring, the 2,3- double bond in conjugation with a 4-oxo functional group, (2) and the presence of both 3- and 5-hydroxyl groups. [69]

Although it is generally believed that an increase in the number of hydroxyl groups enhances the antioxidant activity of the flavonoids,[3,31,42,62,66] the antiradical activity of

myricetin is less than quercetin. Again, this is due to the steric and electronic effect of the pyrogallol moiety as explained earlier.

Kaempferol, a flavonoid where the catechol present in quercetin is replaced by a single OH group, has shown lower antiradical activity confirming the importance of the catechol presence. Morin, on the other hand showed even less antiradical activity although it possesses 2 OH groups on B-ring. This also proves that *meta* hydroxyl groups (resorcinol structure) is weaker than that of a catechol structure.

3-hydroxy flavone, naringenin and flavone showed almost no antiradical activity. The loss of structural elements in both B and C rings caused the dramatic decrease in their antiradical activity.

Table 3.1: Moles DPPH scavenged per one mole of each flavonoid during the rapid kinetics (RSF) and the total stoichiometric factor (TSF), Rate constants (k) of rapid kinetics, and the antiradical activity (AR%) of each flavonoid.

Flavonoid tested	Class	RSF (mol)	TSF (mol)	k (M^{-1} s^{-1})	AR%
Quercetin	Flavonol	3.18 ± 0.17	4.44 ± 0.24	4006 ± 73	84.2
Morin	Flavonol	1.53 ± 0.04	1.60 ± 0.19	4173 ± 32	30.6
Kaempferol	Flavonol	2.08 ± 0.10	2.11 ± 0.08	10249 ± 1105	41.9
Myricetin	Flavonol	2.41 ± 0.06	3.17 ± 0.04	3657 ±138	64.0
3-hydroxy flavone	Flavonol	0.06 ± 0.02	0.16 ± 0.04	116 ± 7	1.6
Flavone	Flavone	0.02 ± 0.01	0.11 ± 0.01	8.4 ± 0.7	0.7
Naringenin	Flavanone	0.03 ± 0.00	0.07 ± 0.01	17.9 ± 0.6	1.1

3.2 Electrochemical Analysis

Flavonoids contain hydroxyl groups attached to their ring structures that can be electrochemically oxidized. Cyclic voltammetry experiments of some flavonoids of different classes; (quercetin, morin, kaempferol, myricetin, 3-hydroxyflavone, flavone and naringenin) were performed to analyze the oxidizability of these molecules.

Some information on the mechanism of flavonoid oxidation could be provided by comparing oxidation potentials at different pH. The cyclic voltammetry response of the tested flavonoids at low scan rate (20 mV/s) was measured as a function of pH. Determinations were performed in the range of pH 6 – 8 using phosphate buffer. Oxidation of polyphenols in phosphate buffer assimilates the measurements with physiological conditions.

3.2.1 Flavonols

3.2.1.1 Quercetin

The structure of quercetin has functional OH groups attached to ring structures that can be electrochemically oxidized. Electrochemical studies reveal general trends in the electron donating abilities of flavonoids. It was demonstrated that the catechol B-ring is more easily oxidizable than the resorcinol A-ring, and on the B-ring, the most oxidizable phenolic function is the more basic site.[70]

Cyclic voltammograms of a solution of quercetin at (pH 7), showed two oxidation peaks (1, 2), **(Figure 3.21),** occurring at the anodic peak potentials, E_a, of + 0.085 and + 0.211 V. In general, it has been proposed that the charge transfer process at peak 1 corresponds to the oxidation of the 3', 4'-dihydroxy substitute (catechol) on B-ring of

quercetin, while peak 2 comprise oxidation reaction involving the hydroxyl group at 3-position.[71] On the reverse scan the counterpart of peak 1 potential appeared at a cathodic peak, E_c, value of +0.0618 V (peak 1') could be seen corresponding to reduction of the oxidation products formed in oxidation peak. This indicates the reversibility of the oxidation process of quercetin. The reversibility of peak 1 was detected over the whole pH range. The oxidation which occurs in peak 2 was always irreversible for all pH range studied. Quercetin also adsorbs strongly on the electrode surface and the final oxidation product blocks the electrode surface, as demonstrated by the rapid decrease of oxidation peak 1 on repeated cycling **(Figure 3.21)**. Blockage of electrode surface was also observed by A. M. Oliveira Brett and M.-E. Ghica.[72]

Effect of different pH on oxidation potential is shown in **Table 3.2** and **Appendix 1 (Figure 1,2 & 3)**. It was observed that oxidation potentials of quercetin were shifting towards lower values when pH was increasing. This indicates that relation between the oxidation potential and pH is proportional with a slope of -0.114 V/pH which is evidenced on **Figure 3.22.**

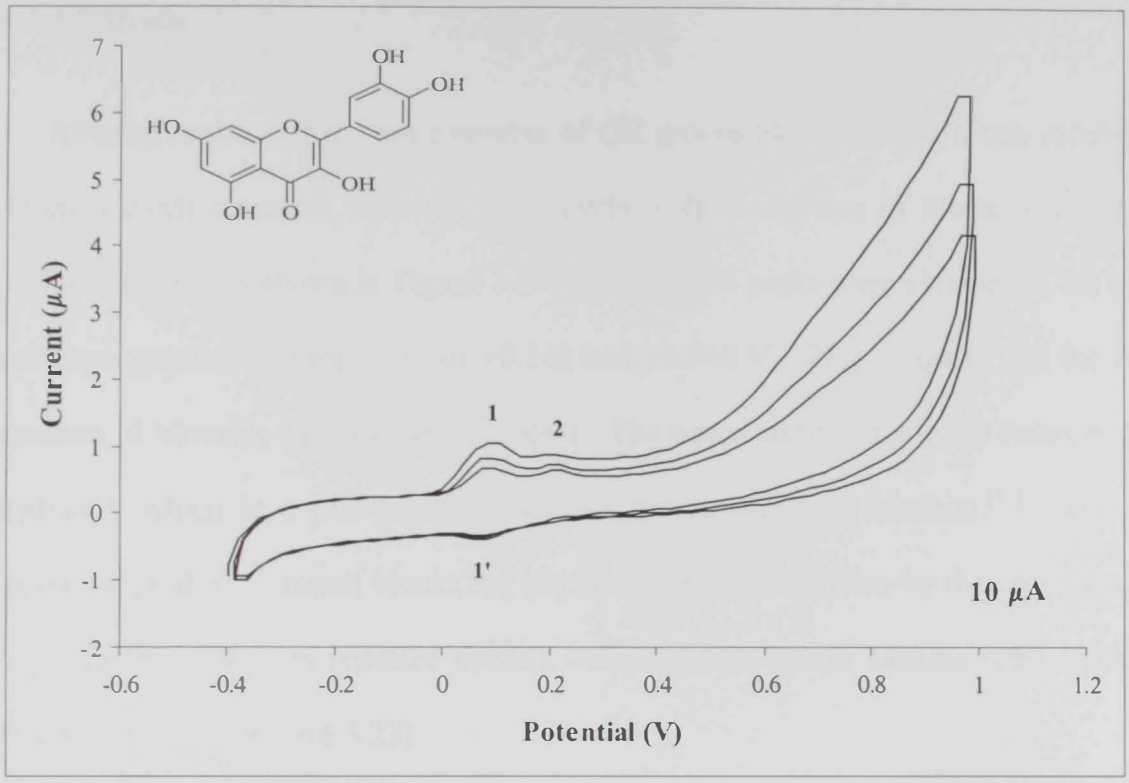


Figure 3.21: Cyclic voltammogram of 1 mM quercetin (Scan rate 20 mV s^{-1}) in pH 7 phosphate buffer.

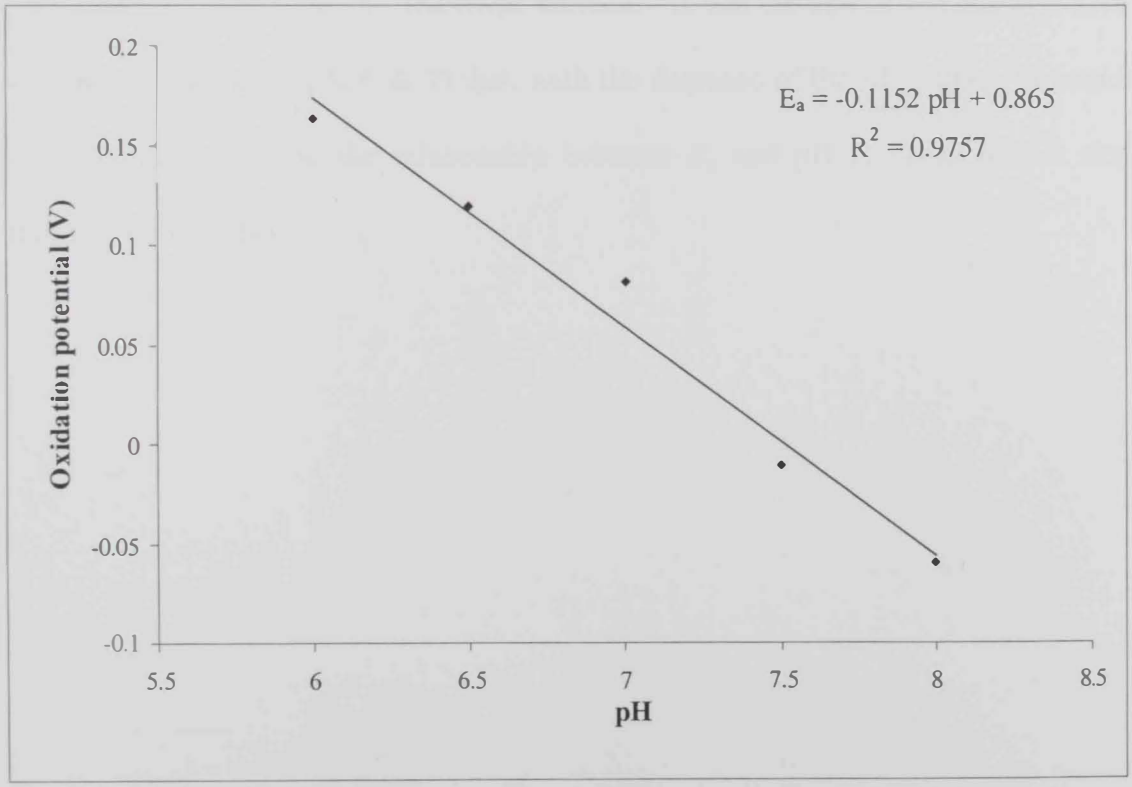


Figure 3.22: Effect of pH on CV anodic potential E_a for 1 mM quercetin at glassy carbon electrode in phosphate buffer solution.

3.2.1.2 Morin

Although morin has the same number of OH groups like quercetin, morin exhibited a different electrochemical behavior. The cyclic voltammograms of Morin in phosphate buffer with pH 7 is shown in **Figure 3.23**. Only anodic peaks were obtained in the cyclic voltammograms occurring at E_a of +0.148 and +0.540 V. This suggests that the redox reaction of Morin is an irreversible process. The peaks correspond to the redox of 2', 4' hydroxyl, which is a two-electron and two proton electrode reaction.[73] The final oxidation product of morin blocks the electrode surface, as shown by the rapid decrease of oxidation peak 1 on repeated cycling indicating that morin adsorbs strongly on the electrode surface **(Figure 3.23)**.

The variation of the oxidation potential, E_a, with pH is another proof for the redox mechanism of morin on the electrode surface. It can be seen from **Figure 3.24** and **Appendix 1 (Figure 4, 5, 6 & 7)** that, with the decrease of the pH values, the oxidation potential increases, and the relationship between E_a and pH is linear with a slope of -0.061 **(Figure 3.24)**.

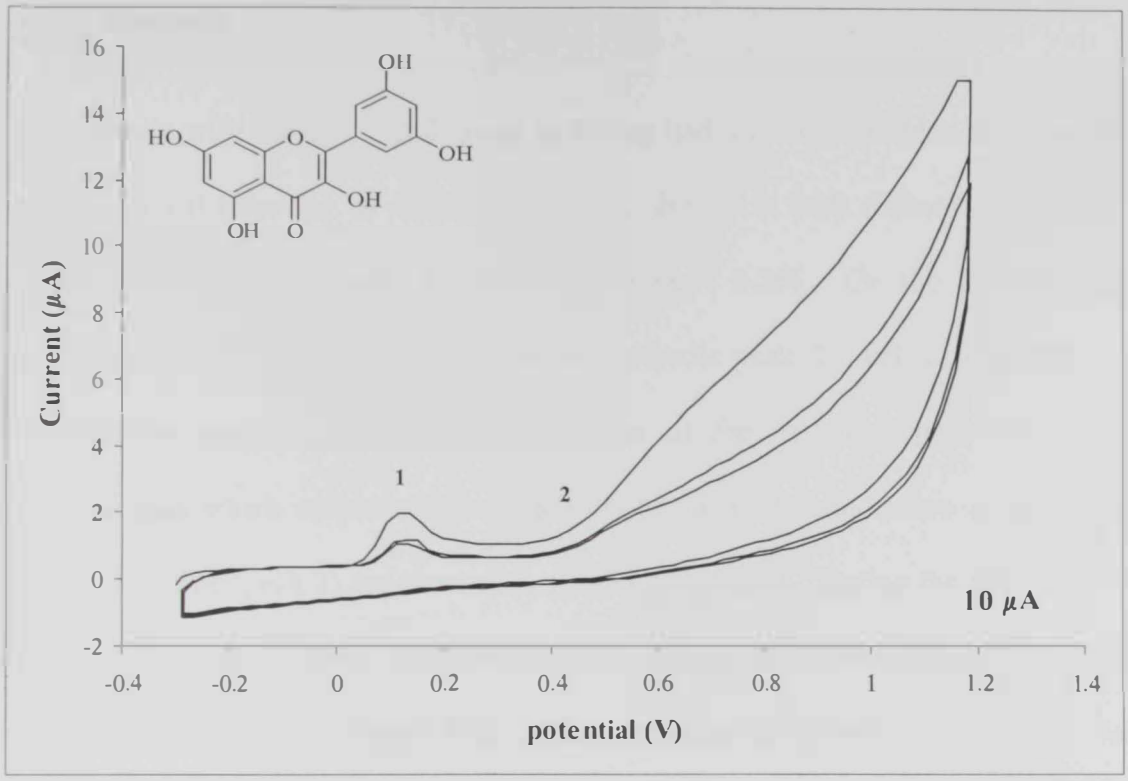


Figure 3.23: Cyclic voltammogram of 1 mM morin (Scan rate 20 mV s^{-1}) in pH 7 phosphate buffer.

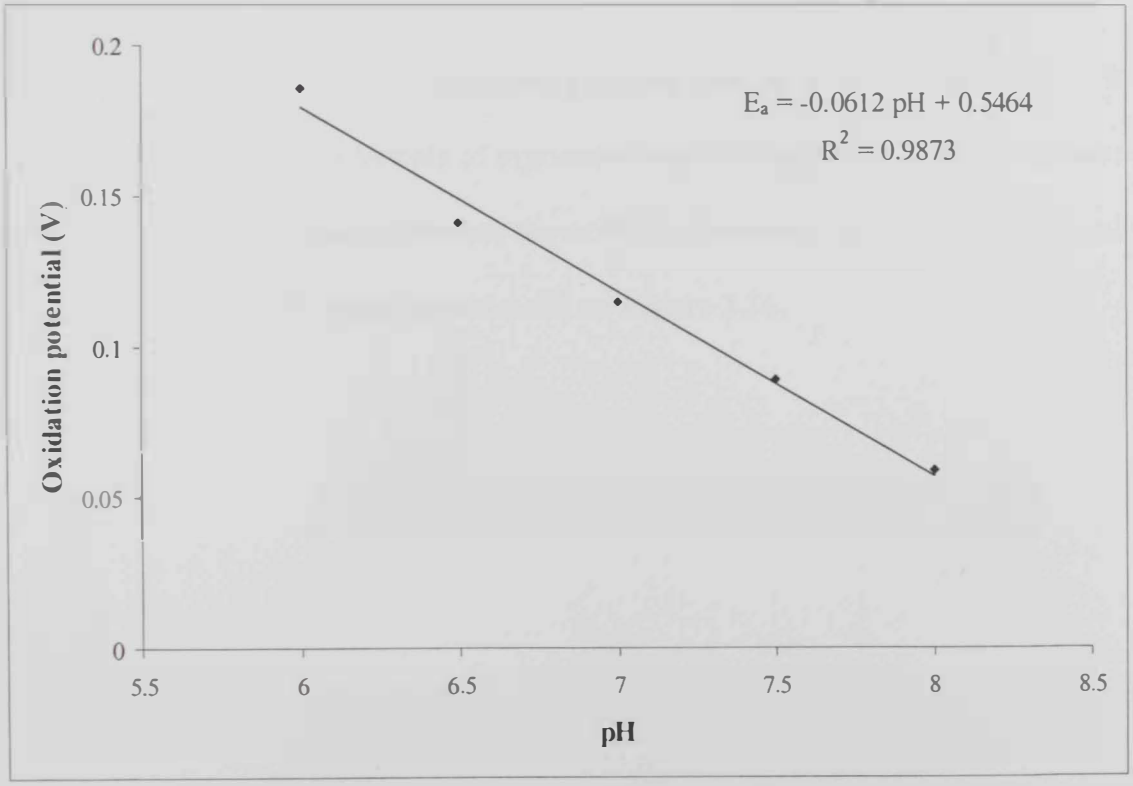


Figure 3.24: Effect of pH on CV anodic potential E_a for 1 mM morin at glassy carbon electrode in phosphate buffer solution.

3.2.1.3 Myricetin

Myricetin with the pyrogallol group in B-ring had the lowest oxidation potential. The electrochemical behavior of myricetin (pH 7), showed a well defined quasi-reversible anodic peak 1 with E_a value of -0.060 V **(Figure 3.25)**. On the reverse scan the counterpart of peak 1 potential appeared at a cathodic peak, E_c, value of -0.020 V (peak 1') could be seen corresponding to reduction of the oxidation products formed in oxidation peak which indicates the reversibility of the oxidation process of myricetin. A second oxidation (peak 2) appeared at E_a value of +0.637 V. During the second and third oxidation wave scans, there was a reduction in the current signals detected at each peak potential for myricetin **(Figure 3.25)**. The reduction is a result of myricetin having adsorbed to the electrode surface and subsequently blocking the surface after each wave scan.

Effect of different pH on oxidation potential can be seen in **Table 3.2**. It was observed that oxidation potentials of myricetin were shifting towards higher values when pH was decreasing. Relation between the oxidation potential and pH is proportional with a slope of 0.0616 V/pH which is evidenced on **Figure 3.26.**

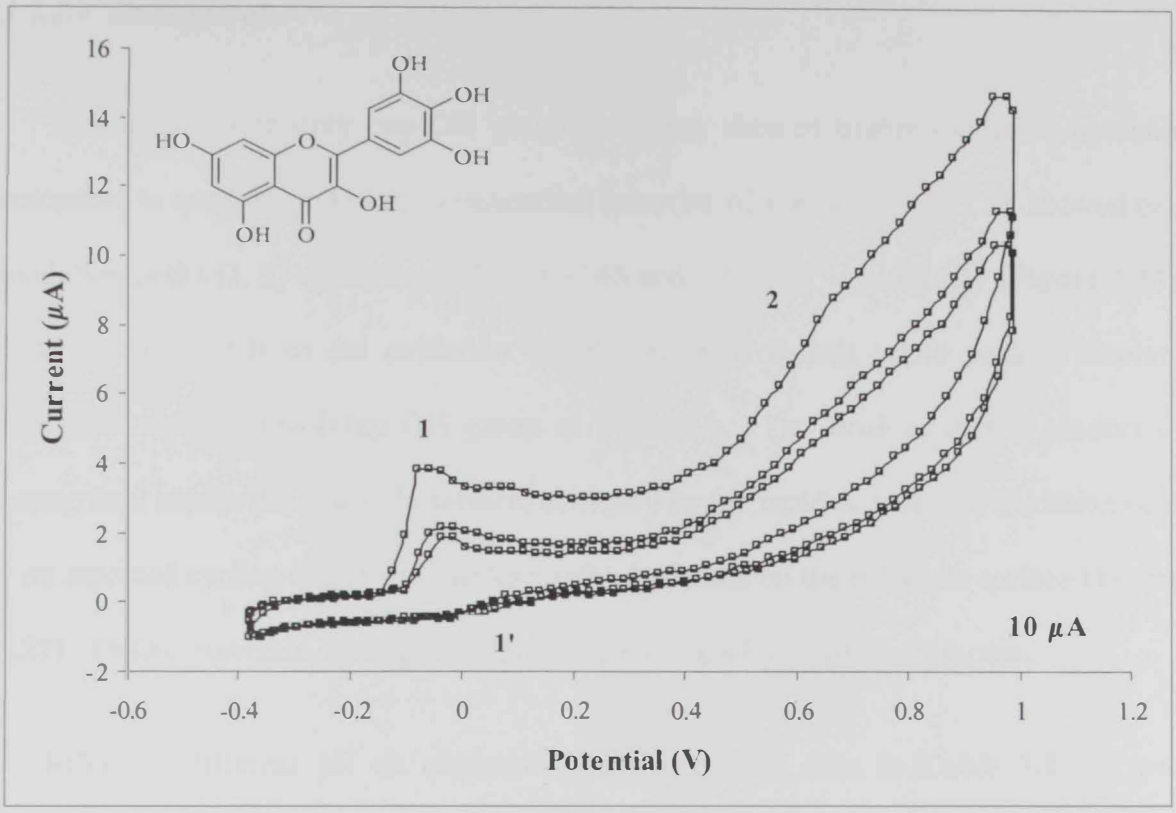


Figure 3.25: Cyclic voltammogram of 1 mM myricetin (Scan rate 20 mV s^{-1}) in pH 7 phosphate buffer.

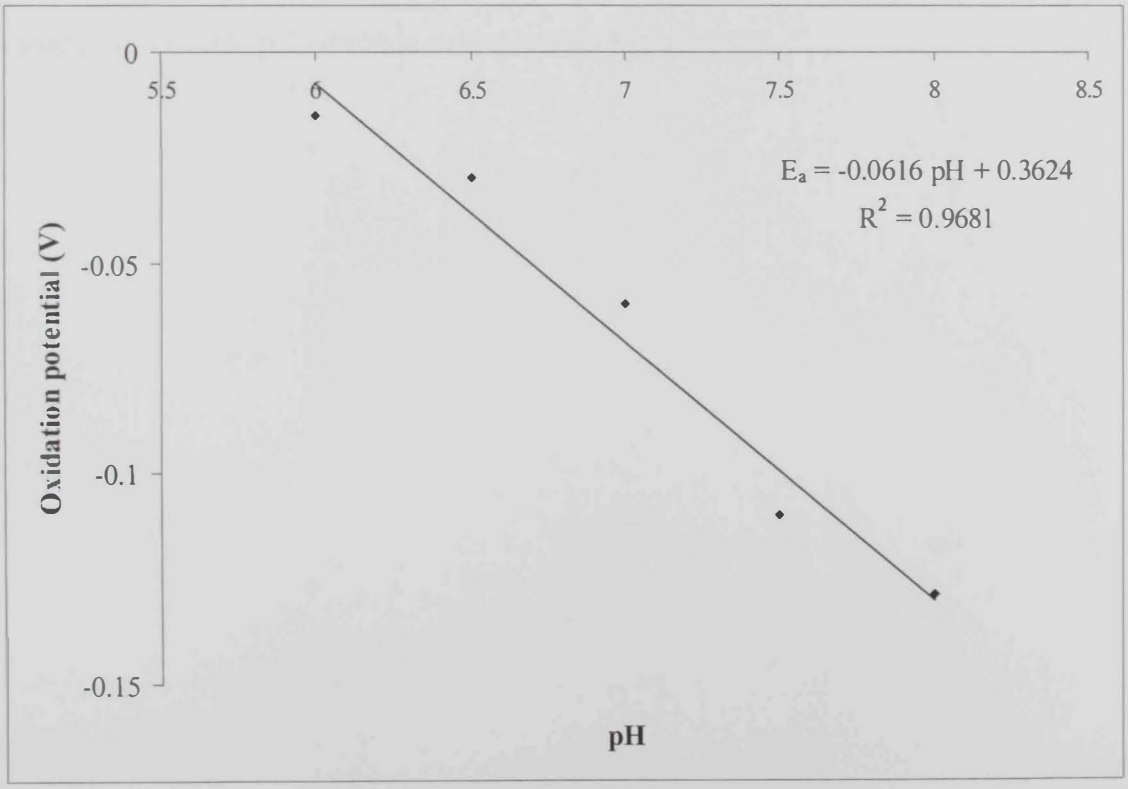


Figure 3.26: Effect of pH on CV anodic potential E_a for 1 mM myricetin at glassy carbon electrode in phosphate buffer solution.

3.2.1.4 Kaempferol

Kaempferol with only one OH group in B-ring showed higher oxidation potential compared to quercetin. The electrochemical behavior of kaempferol (pH 7), showed two oxidation peaks (1, 2) appearing at E_a = +0.148 and +0.644 V respectively **(Figure 3.27)**. Peak 1 corresponds to the oxidation of OH group in B-ring, while peak 2 involves oxidation reaction involving OH group at 3-postion. The final oxidation product of kaempferol blocks the electrode surface, as shown by the rapid decrease of oxidation peak 1 on repeated cycling indicating that kaempferol adsorbs on the electrode surface **(Figure 3.27)**. Unlike quercetin, kaempferol showed an irreversible oxidation process.

Effect of different pH on oxidation potential can be seen in **Table 3.2**. It was observed that oxidation potentials of kaempferol were shifting towards lower values when pH was increasing. Relation between the oxidation potential and pH is proportional with a slope of -0.060 V/pH which is evidenced on **Figure 3.28.**

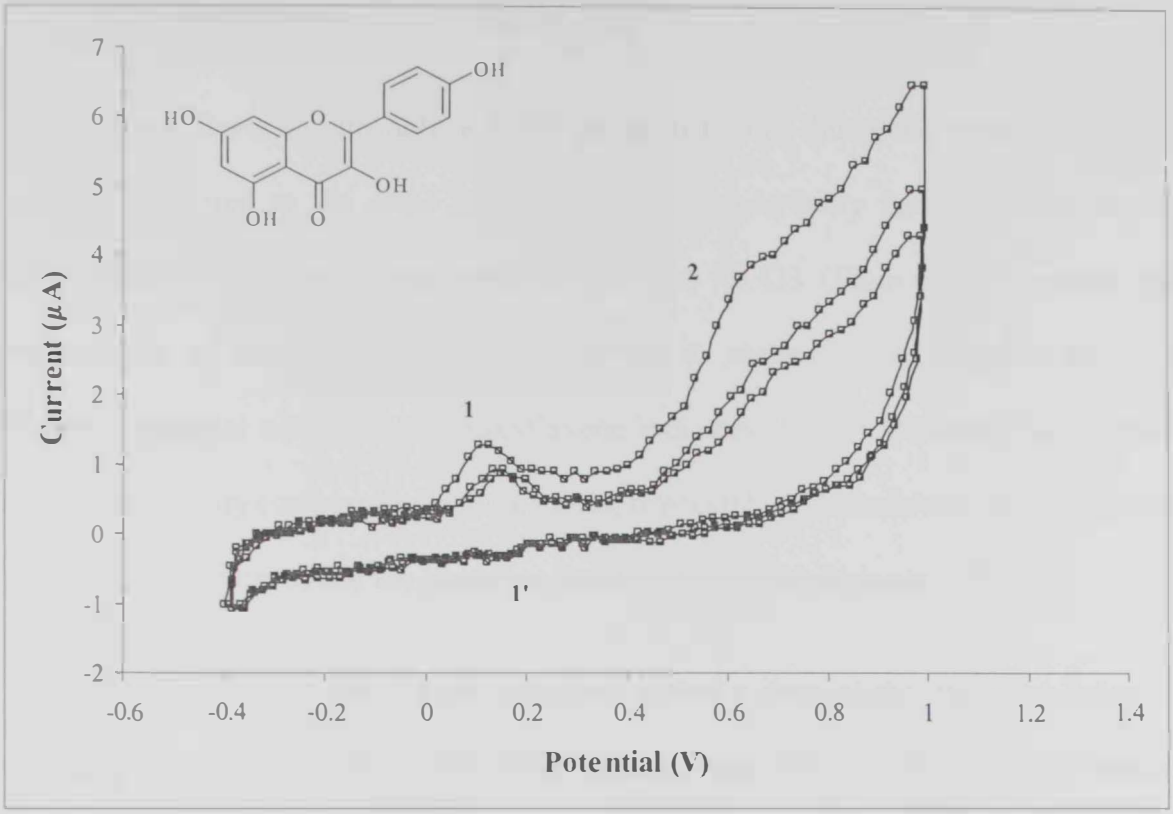


Figure 3.27: Cyclic voltammogram of 1 mM kaempferol (Scan rate 20 mV s^{-1}) in pH 7 phosphate buffer.

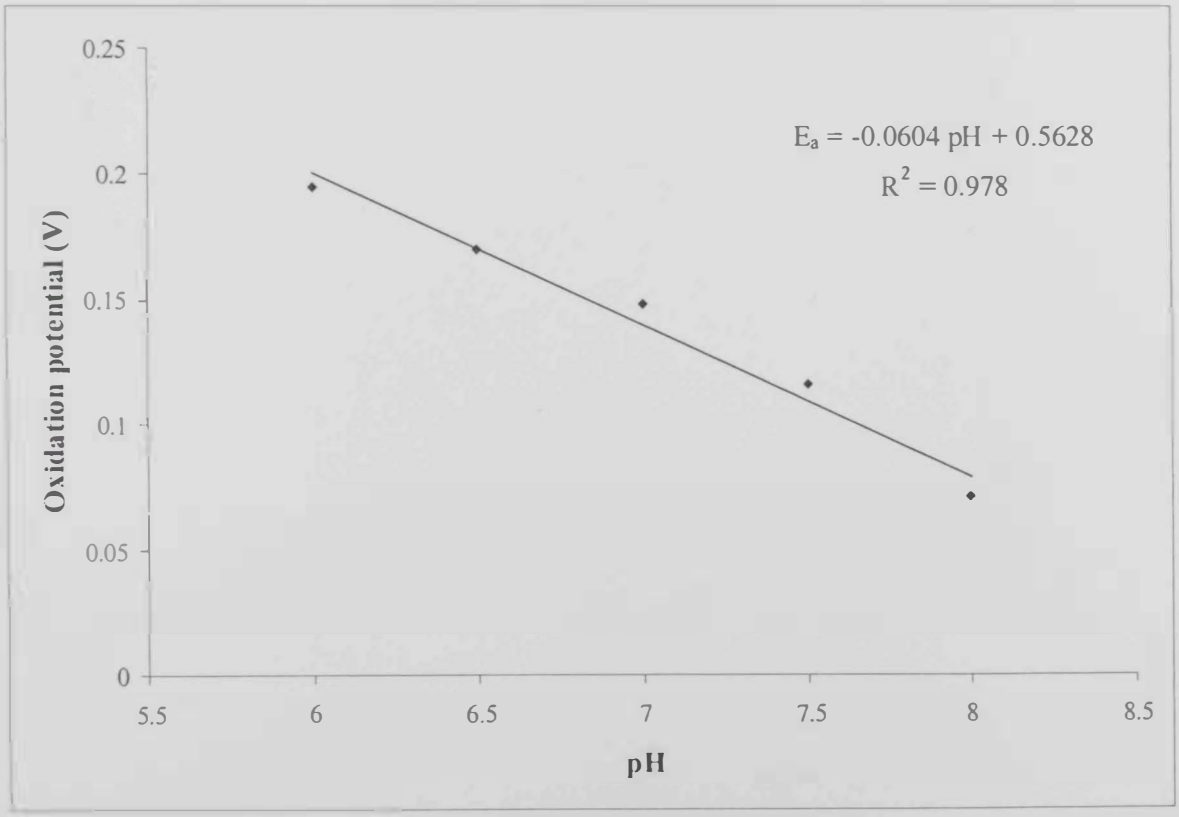


Figure 3.28: Effect of pH on CV anodic potential E_a for 1 mM kaempferol at glassy carbon electrode in phosphate buffer solution.

3.2.1.5 3-Hydroxyflavone

3-hydroxy flavone, with only a 3-OH group in C-ring shows the highest oxidation potential compared to the other studied flavonols. 3-hydroxy flavone shows a well defined irreversible anodic peak with E_a value of +0.433 **(Figure 3.29)**. Since the oxidizabiltity of flavonoids reflects their ability to scavenge free radicals, the high oxidation potential value of 3-hydroxyflavone indicates that this flavonol has a lower antioxidant activity compared quercetin, morin, myricetin and kaempferol. An adsorption process is also observed and the oxidation products block the electrode surface.

Like other flavonols, 3-hydroxyflavone shows a drop of the oxidation potential associated with the increase of pH. This indicates that relation between the oxidation potential and pH is proportional with a slope of -0.0738 V/pH which is evidenced on **Figure 3.30**.

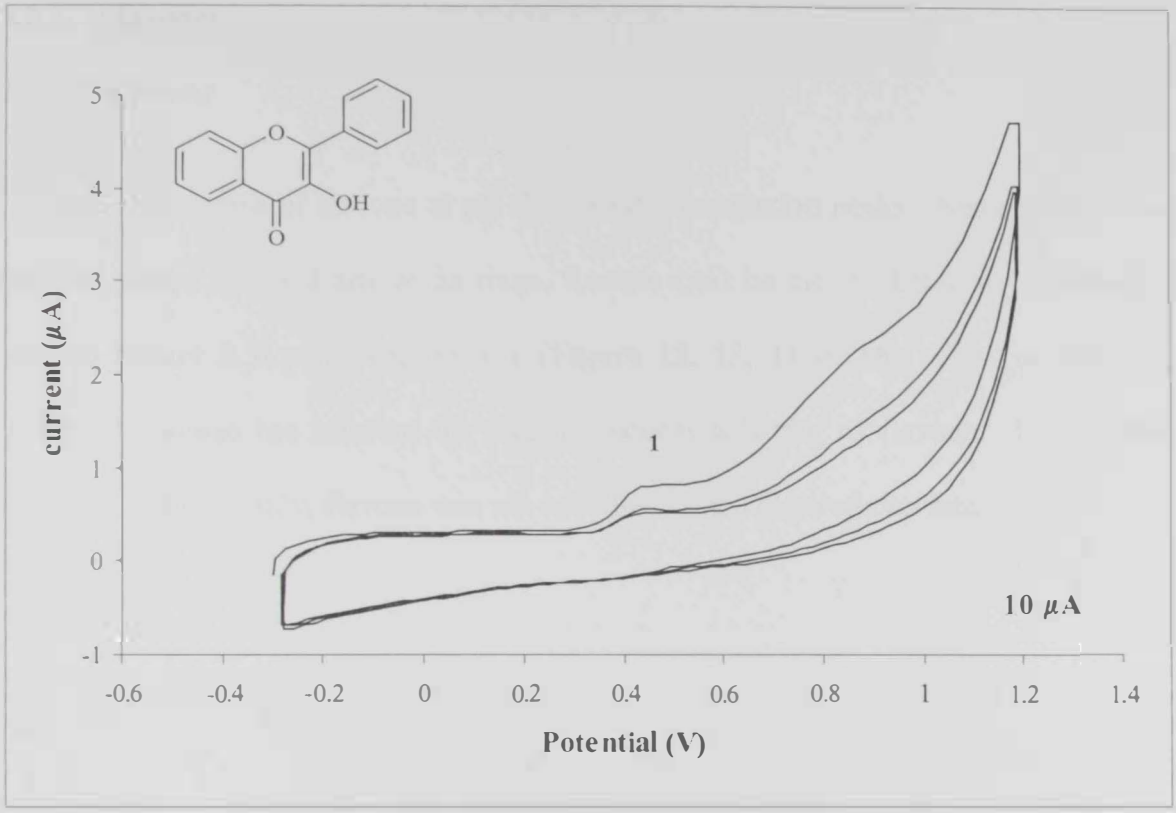


Figure 3.29: Cyclic voltammogram of 1 mM 3-hydroxyflavone (Scan rate 20 mV s^{-1}) in pH 7 phosphate buffer

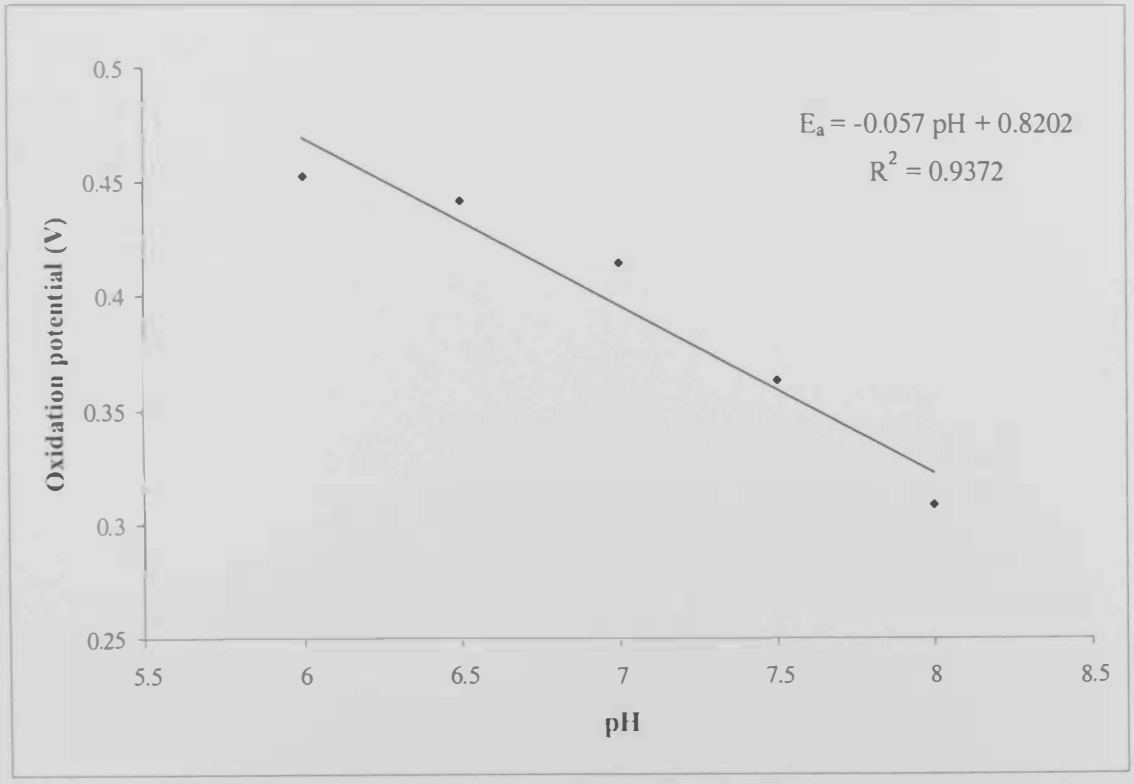


Figure 3.30: Effect of pH on CV anodic potential E_a for 1 mM 3-hydroxyflavone at glassy carbon electrode in phosphate buffer solution.

3.2.2 Flavones

3.2.2.1 Flavone

Voltammograms of flavone at pH 7 showed no oxidation peaks. Since flavone lack the OH groups attached any of its rings, flavone can't be electrochemically oxidized as seen in **Figure 3.31** and **Appendix 1 (Figure 12, 13, 14 & 15)**. Loss of important structural features has affected the electrochemical behavior of flavone. Unlike other flavonoids in this study, flavone was not adsorbed on the electrode surface.

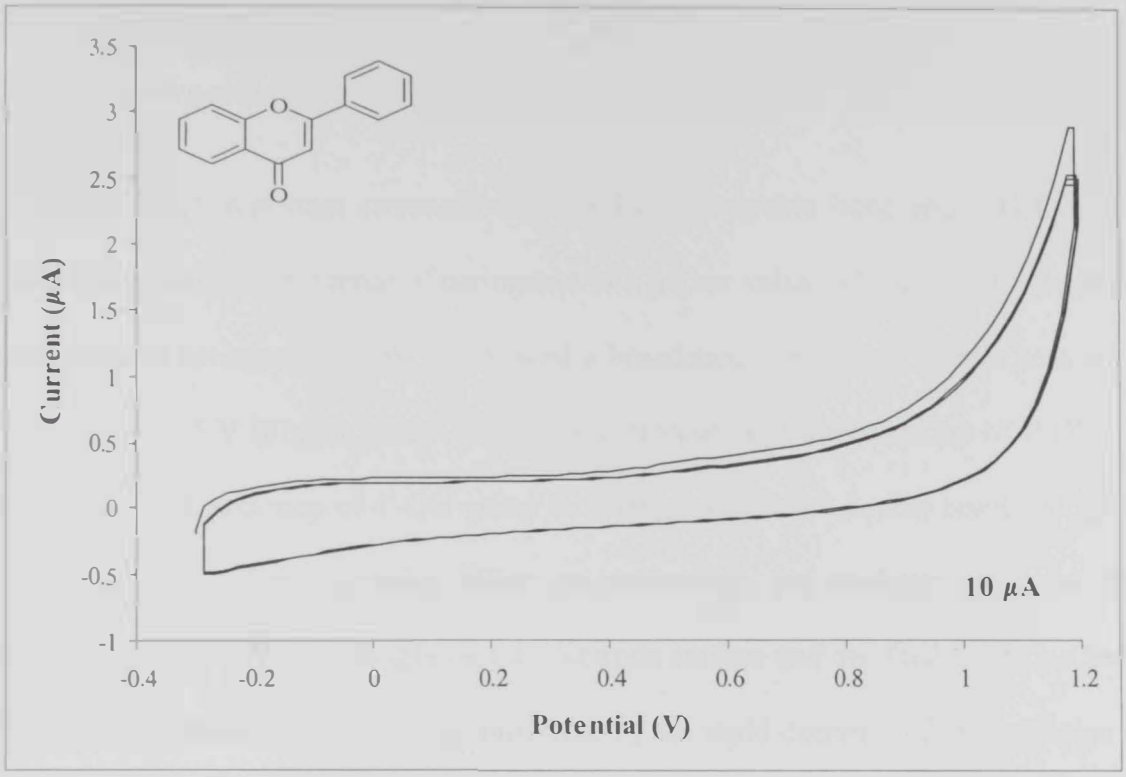


Figure 3.31: Cyclic voltammogram of 1 mM flavone (Scan rate 20 mV s^{-1}) in pH 7 phosphate buffer

3.2.3 Flavanones

3.2.3.1 Naringenin

The loss of important structural features like 2,3-double bond and 3-OH in C-ring shifted the oxidation potential of naringenin to a higher value. Cyclic voltammograms of a solution of naringenin at (pH 7), showed a broadened irreversible anodic peak with E_a value of +0.485 V **(Figure 3.32)**. This peak corresponds to the oxidation of 4'-OH group in B-ring. The presence of 4'-OH group in B-ring without 2,3-double bond and 3-OH in C-ring does not have a major effect on decreasing the oxidation potential value. Naringenin also adsorbs strongly on the electrode surface and the final oxidation product blocks the electrode surface, as demonstrated by the rapid decrease of the oxidation peak on repeated cycling **(Figure 3.32)**.

Effect of different pH on oxidation potential is shown in **Table 3.2** and **Appendix 1 (Figure 16, 17, 18 & 19)**. It was observed that oxidation potentials of naringenin were shifting towards lower values when pH was increasing. This indicates that relation between the oxidation potential and pH is proportional with a slope of -0.0616 V/pH which is evidenced on **Figure 3.33.**

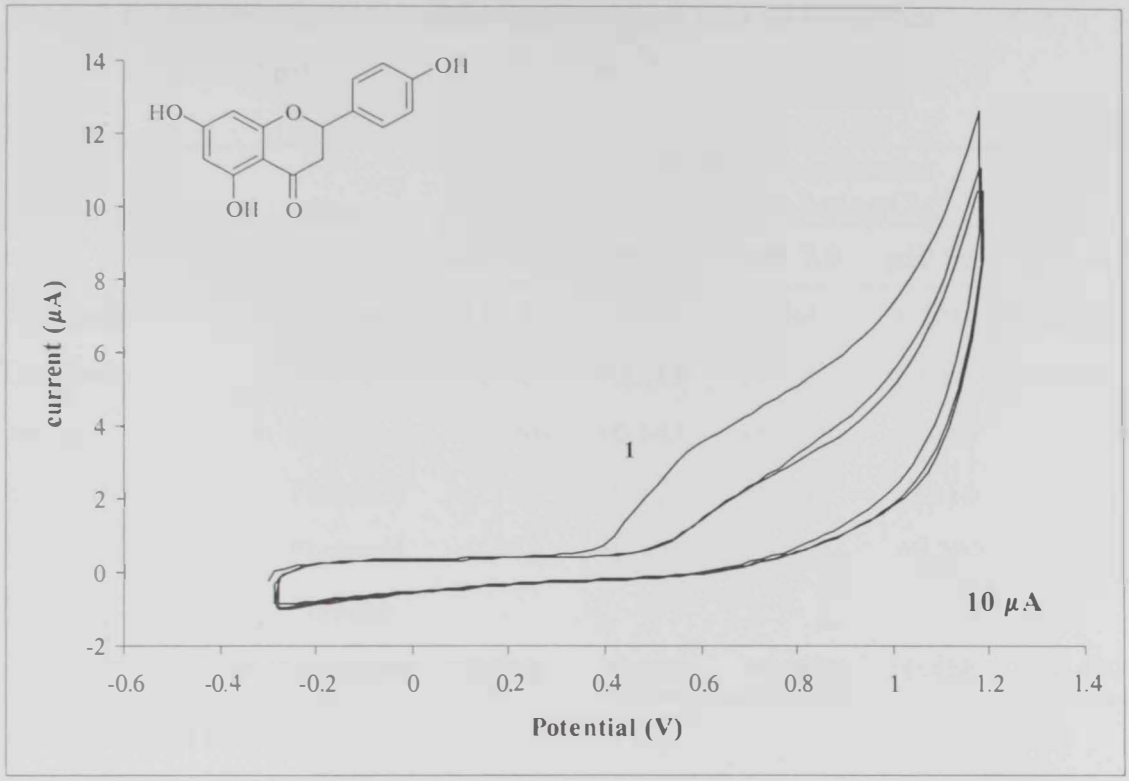


Figure 3.32: Cyclic voltammogram of 1 mM naringenin (Scan rate 20 mV s^{-1}) in pH 7 phosphate buffer

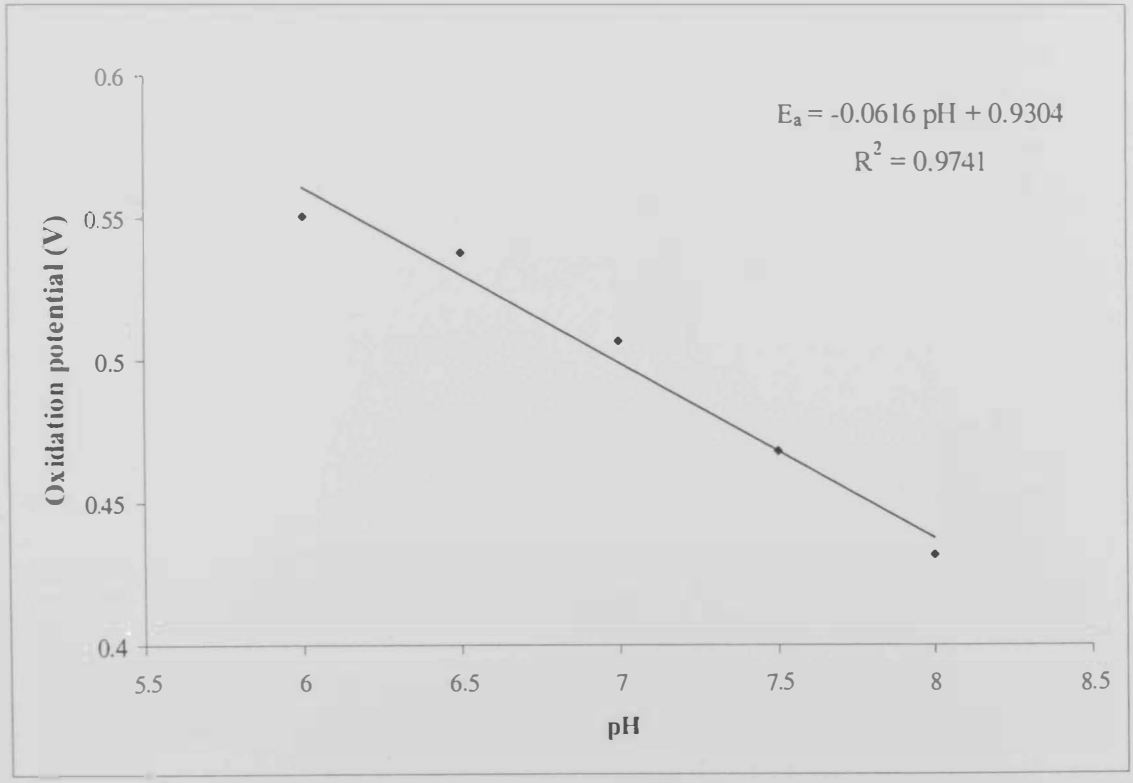


Figure 3.33: Effect of pH on CV anodic potential E_a for 1 mM naringenin at glassy carbon electrode in phosphate buffer solution.

Table 3.2: Oxidation potential of the first oxidation peak of flavonoids tested at different pH

Flavonoid	Class	Oxidation potential E_a (V) *vs.* $Ag/AgCl_2$				
		pH 6.0	**pH 6.5**	**pH 7.0**	**pH 7.5**	**pH 8.0**
Myricetin	Flavonol	-0.015	-0.03	-0.060	-0.110	-0.129
Quercetin	Flavonol	+0.142	+0.119	+0.085	-0.011	-0.060
Morin	Flavonol	+0.186	+0.141	+0.115	+0.089	+0.059
Kaempferol	Flavonol	+0.195	+0.17	+0.148	+0.116	+0.071
3-hydroxyflavone	Flavonol	+0.453	+0.442	+0.433	+0.363	+0.308
Flavone	Flavone	-	-	-	-	-
naringenin	flavanone	+0.551	+0.538	+0.507	+0.468	+0.432

3.3 Theoretical Analysis

To have some insights into the observed behavior of each phenolic acid, a series of density functional theory calculations were performed to find out both the structure and the stability of 28 flavonoids from 4 different classes.

Geometry optimizations were performed to find the lowest energy each flavonoid possessed. The geometry optimizations depend primarily on the gradient of the energy- the first derivative of the energy with respect to atomic positions.

3.3.1 Flavonols

3.3.1.1 Energy and dipole moment properties

Energies of different systems can be compared only when the number and type of nuclei are the same.[52] Thus, we could compare the energies of the alternate forms of flavonols only when the total number of nuclei of each type is the same.

Although robinetin has 3 hydroxyl groups in B-ring, it shares with quercetin and morin the same total number of OH groups (five OH groups) as well as the type of nuclei (a three-ringed molecule) **(Figure 1.1)**. According to single point energy values, robinetin with three hydroxyl groups in B-ring (pyrogallol group) is more active than quercetin with two hydroxyl groups (catechol structure) and morin with *meta* hydroxyl groups (resorcinol structure) (robinetin = -6.92942E+05 > quercetin = -6.92950E+05 > morin = -6.92954E+05 Kcal/mol) as shown in **Table 3.2**. It is well established that phenolics with three adjacent hydroxyl groups in B- ring are more active than their dihydroxyl counter parts.[32] i.e. robinetin is more active than quercetin and morin.

The influence of an OH group is dependent on the position of substitution.[31] The energy difference between the catecholic and resorcinol structure is about 0.04 cal/mol. This dissimilarity in influence of the same substituent at different positions in B-ring can be explained by an electronic effect. An OH group in a conjugated system has an electron donating effect to the ring. The electron donation from the substituent to the oxygen of the active OH group weakens the O-H bond making it easier to release an $H^{\cdot}$.[31,74] Studies involving Hammett σ calculations gives the electron donating (negative value) or withdrawing (positive value) effect of a substituent. The Hammett σ of the OH group depends on the relative position of the OH substitution at the ring compared to the active centre. The maximal electron donating effect, i.e. the most negative σ, is observed when the OH is at the ortho or para position. An electron withdrawing effect is seen at the meta position.[31] This nicely fits with the rank-order of potency observed for the catechol and resorcinol, i.e. (-6.92950E+05, -6.92954E+05 Kcal/mol) respectively.

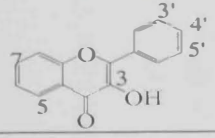
Kaempferol and Fisetin, possessing 4 OH groups in their systems show different single point energies. With 0.08 cal/mol energy difference, fisetin with catecholic structure in its B-ring is more active than kaempferol with mono-hydroxyl group in the same ring. i.e. (-6.45725E+05, -6.45733E+05 Kcal/mol) respectively. It is well established that catecholic structures in B-ring are more active than their monohydroxyl counterparts.[3] This small but significant difference in energy proves that as well.

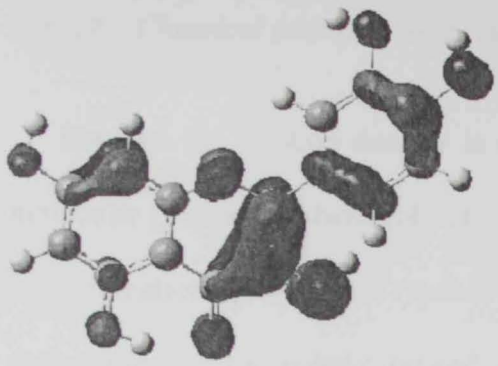
The four flavonols; myricetin, laricytrin, 3,5,7,3,4-pentamethoxy flavone and 3,5,7,3,4,5-hexamethoxy flavone have similar structures but different type of substituents i.e. OCH_3 and/or OH. Although the energies are very different, comparing them directly is of little value. It is well accepted that energies for two systems can be compared only when the number and type of nuclei are the same.[52] However, we can compare their

dipole moments. The dipole moment represents a generalized measurement of the charge density in a molecule. Therefore, it constitutes an index of reactivity, which is considered very important to define the biological properties, especially those related to the interaction with enzyme active sites.[75] In this case, we note that the OCH_3 groups in flavonols have the effect of decreasing the magnitude of the dipole moment. Myricetin with six OH groups, three of them in B-ring (pyrogallol group) has the highest dipole moment. Introducing one OCH_3 group in B-ring into the system as in laricytrin slightly decreases the magnitude of dipole moment. While further substitution with 5-6 OCH_3 groups decreases the dipole momentum more i.e. (myricetin = 9.185 > laricytrin = 8.437 > 3,5,7,3,4-pentamethoxy flavone = 5.244 > 3,5,7,3,4,5-hexamethoxy flavone = 4.363 Debye). This means that the centers of positive and negative charges are farther apart in myricetin than they are in laricytrin, 3,5,7,3,4-pentamethoxy flavone and 3,5,7,3,4,5-hexamethoxy flavone.

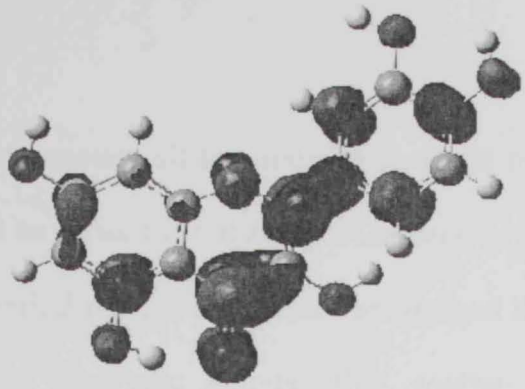
In previous studies, large energy difference between HOMO and LUMO, corresponds to stable and little reactive systems, whereas in the opposite case the systems are little stable and highly reactive.[9,50] The value of the gap, establishes that flavonols are reactive systems, since the energy difference is not large. The low energy of the LUMO in flavonols is an indication that they can behave as soft electrophiles[76] **(Table 3.3, Figure 3.34** and **Appendix II).**

Table 3.3: Energy properties and dipole moments of flavonols

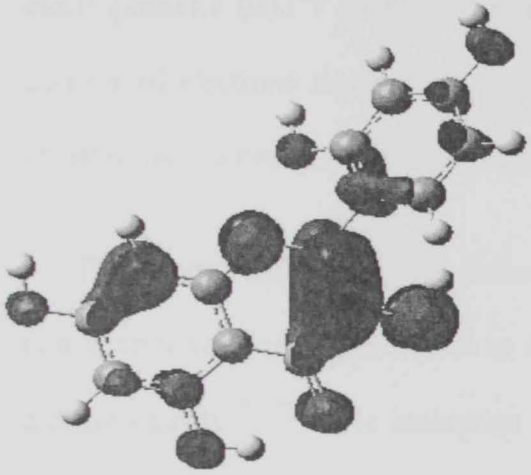
Flavonols 	Energy (Kcal/mol)	HOMO (Kcal/mol)	LUMO (Kcal/mol)	Gap ($\Delta E = E_{LUMO}-E_{HOMO}$) (Kcal/mol)	Dipole Moment (Debye)			
					X	Y	Z	Total
Quercetin (3,5,7,3',4'-OH)	-6.92950E+05	-7.19	-4.93	2.26	-4.9519	6.1200	0.8087	7.914
Morin (3,5,7,2',4'-OH)	-6.92954E+05	-7.26	-4.82	2.44	4.9966	-2.0677	1.7071	5.671
Robinetin (3,7,3',4',5'-OH)	-6.92942E+05	-7.17	-5.05	2.12	-3.3475	-7.8734	-0.6361	8.579
Myrceiten (3,5,7,3',4',5'-OH)	-7.40167E+05	-7.13	-4.91	2.21	5.6609	7.1849	-0.8381	9.185
3,5,7,3',4'-pentamethoxy flavone	-8.63396E+05	-7.40	-5.07	2.33	0.0621	5.0192	1.5173	5.244
3,5,7,3',4',5'-hexamethoxy flavone	-8.88040E+05	-7.40	-5.10	2.31	-1.0111	4.2404	-0.1832	4.363
Laricytrin (3,5,7,3',4'-OH)(5-OCH_3)	-7.64812E+05	-7.15	-4.93	2.21	5.0508	6.5818	-1.5344	8.437
Fisetin (3,7,3',3',4'-OH)	-6.45725E+05	-7.22	-5.05	2.17	-2.7090	-6.8740	-0.6833	7.420
Kaempferol (3,5,7,4'-OH)	-6.45733E+05	-7.24	-4.93	2.31	-5.7682	-5.9397	-0.8619	8.324
Galangin (3,5,7-OH)	-5.98516E+05	-7.29	-5.10	2.19	-5.1698	-4.9990	-0.0890	7.192
Kaempferide (3,5,7-OH)(4'-OCH_3)	-6.70382E+05	-7.22	-4.93	2.28	-6.9421	-3.9252	0.4185	7.986
3-hydroxy flavone (3-OH)	-5.04074E+05	-7.38	-5.28	2.10	-2.8271	-4.2686	-0.0042	5.120



Quercetin- HOMO



Quercetin- LUMO



Morin-HOMO

Morin-LUMO

3,5,7,3',4'-Pentamethoxy flavone- HOMO

3,5,7,3',4'-Pentamethoxy flavone- LUMO

Figure 3.34: Charge distribution of the HOMO-LUMO (isovalue of 0.04) in some optimized flavonols.

3.3.1.2 Chemical potential properties

Because the electron density is considered to contain all the information about the molecular properties, chemical reactivity should be reflected in the molecular sensitivity to perturbations of different types.[9,10] The chemical potential properties are defined by different variables tightly related among them: electronic affinity (EA), ionization potential (IP), chemical potential (μ), electronegativity (χ), hardness (η) and electrophilicity (ω).[75] If the electronic energy is considered to be a functional of the number of electrons and external potential, $E[N, \upsilon(\mathbf{r})]$, then these perturbations can be obtained by a series of derivatives of the energy.[9,10]

The electron affinity (EA) is defined as the energy released when an electron is added to a neutral molecule. A molecule or atom with a greater electronic affinity tends to take electrons easily.[50,75] The ionization potential (IP) is defined as the amount of energy required to remove an electron of a molecule. Therefore, a high ionization potential indicates that the systems do not lose electrons easily.[2,22,50,75]

Chemical potential is a global property that measures the escaping tendency of an electronic cloud:[9]

$$\mu = \left(\frac{\delta E[N, \upsilon(r)]}{\delta N} \right)_{\upsilon(r)} \qquad 3.9$$

In the finite difference approximation, this is equivalent to the negative of the average of the vertical ionization potential and electron affinity

$$\mu = \left(\frac{-(I + A)}{2} \right) \qquad 3.10$$

Electronegativity (χ) is a measure of the tendency to attract electrons in a chemical bond,[77] as is defined as the negative of the chemical potential in DFT:[77]

$$\chi = -\mu \qquad 3.11$$

Hardness is a global property described as the resistance to change in the electron distribution[9] or charge transference[77] that determines the stability of a molecule

$$\eta = \frac{\delta\mu}{\delta N} = \left(\frac{\delta^2 E[N, v(r)]}{\delta N^2} \right)_{v(r)} \qquad 3.12$$

In the finite difference approximation, this is equivalent to

$$\eta \approx \frac{(I - A)}{2} \qquad 3.13$$

And for closed-shell molecules, it can be further approximated as the HOMO-LUMO energy gap. According to the maximum hardness principle, molecules arrange themselves to be as hard as possible:[78]

$$\eta = \frac{(LUMO - HOMO)}{2} \qquad 3.14$$

Finally, the Electrophilicity index (ω) determines the affinity by electrons and measures the decay of binding energy due to a maximum electron flow between a donor and an acceptor.[78,79]

$$\omega = \Delta E(\Delta N^*) \qquad 3.15$$

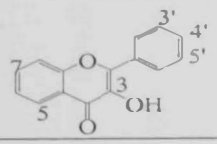
It may be recast into the more familiar form:[78,79]

$$\omega = \frac{\mu^2}{2\eta} \qquad 3.16$$

The above chemical variables have different meanings. Nevertheless, as a group they measure the tendency to give or capture electrons, that is they are an index of the antioxidant potential.[80,81] As the antioxidant potential or antioxidant activity results from the ability to give electrons.[80,3]

The values for all the variables associated with the chemical potential for flavonols are low (**Table 3.4**). For this reason, it is concluded that flavonols in general have a tendency to give electrons instead of capturing them. Low reduction potential is another sign of their good antioxidant ability.

Table 3.4: Properties related with the chemical potential in the optimized structures of flavonols

Flavonol 	Electronic Affinity (eV)	Ionization potential (eV)	Hardness (eV)	Electronegativity (eV)	Electrophilicity index (eV)
Quercetin (3,5,7,3',4'-OH)	0.214	0.312	0.049	0.263	0.706
Morin (3,5,7,2',4'-OH)	0.209	0.315	0.053	0.262	0.648
Robinetin (3,7,3',4',5'-OH)	0.219	0.311	0.056	0.265	0.763
Myreciten (3,5,7,3',4',5'-OH)	0.213	0.309	0.048	0.261	0.710
3,5,7,3',4'-pentamethoxy flavone	0.22	0.321	0.051	0.271	0.724
3,5,7,3',4',5'-hexamethoxy flavone	0.221	0.321	0.050	0.271	0.734
Laricytrin (3,5,7,3',4'-OH)(5-OCH_3)	0.214	0.310	0.048	0.262	0.715
Fisetin (3,7,3',3',4'-OH)	0.219	0.310	0.047	0.266	0.753
Kaempferol (3,5,7,4'-OH)	0.214	0.314	0.05	0.264	0.697
Galangin (3,5,7-OH)	0.221	0.316	0.048	0.269	0.759
Kaempferide (3,5,7-OH)(4'-OCH_3)	0.214	0.313	0.050	0.264	0.701
3-hydroxy flavone (3-OH)	0.229	0.320	0.046	0.275	0.828

3.3.2 Flavones

3.3.2.1 Energy and Dipole moment properties

5-hydroxy flavone and 7-hydroxy flavone are two flavones that posses one OH group in their A-ring. Yet their energies differ slightly, i.e. (7-hydroxy flavone = -5.04083E+05 > 5-hydroxy flavone = -5.04091E+05 Kcal/mol). Comparing those values with 3-hydroxy flavone (-5.04074E+05 Kcal/mol) from the class flavonols which has one OH group as well but in C-ring, shows that the position of OH group affects the stability and therefore activity of the flavonoid. It could be stated that the presence of 3-OH group in C-ring enhances the activity of flavonoids more than 5-OH or 7-OH group in A-ring.

Substituting the 7-OH group in ring A with OCH_3 group affects the dipole momentum as discussed earlier. i.e. (7-hyrdroxy flavone = 5.3922 > 8-methoxy flavone = 3.7450 Debye). This means that the centers of positive and negative charges are farther apart in 7-hydroxy flavone than in the 8-methoxy flavone.

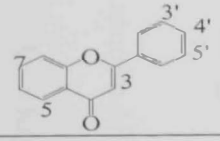
Apigenin, a flavone with three hydroxyl groups, can be compared with galangin, a flavonol with the same number and type of nuclei. (Galangin = -5.98516E+05 > apigenin = -5.98525E+05 Kcal/mol). Both flavonoids possess two OH groups in A-ring but differ with the position of the third OH group. Galangin with 3-OH in C-ring is more active than apigenin with 4'-OH in B-ring. This is in agreement with Heijnen et al,[31] as the activity of an OH group can be positively influenced by other electron donating groups when there is an even number of C-atoms between the active and stimulating group. The 3-OH group is stimulated by the OH groups at the 5 and 7 position and also by the oxygen atoms at position 1 and 4. The OH groups at position 5 and 7 are only stimulated by the 3-OH group.[31] This further emphasize the importance of 3-OH group in C-ring,

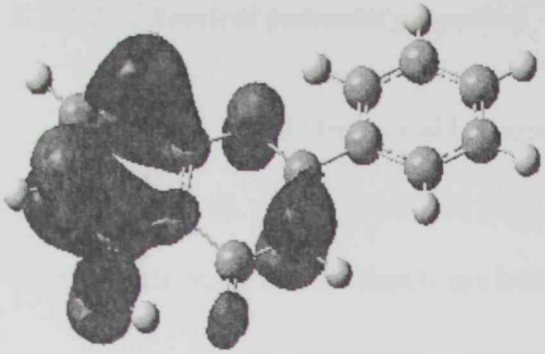
as the activity of the 5 or 7-OH is only positively influenced by a substituent at the 3 position.

Luteolin, a flavone with four OH groups, can be contemplated with both kaempferol and fisetin from the flavonols class. (Fisetin = -6.45725E+05 > kaempferol = -6.45733E+05 > luteolin = -6.45742E+05 Kcal/mol). Although both fisetin and luteolin possess a catechol structure in B-ring, yet, the absence of 3-OH in luteolin appears to affect negatively its activity. Therefore, we can state that the activity of flavonoids is enhanced by the presence of both; catechol structure in B-ring and 3-OH in C-ring.

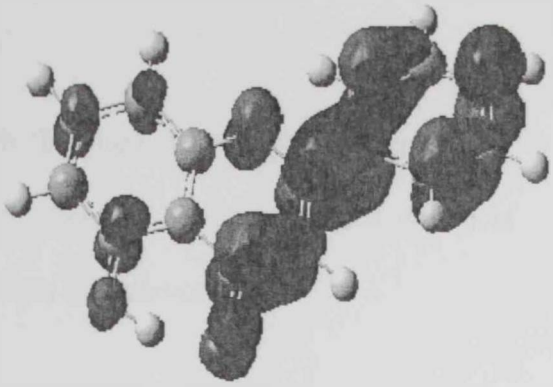
The HOMO-LUMO gap in flavones is a bit higher than flavonols. This indicates that flavonols are more reactive systems than flavones **(Table 3.5, Figure 3.35** and **Appendix II).**

Table 3.5: Energy properties and dipole moments of flavones

Flavones 	Energy (Kcal/mol)	HOMO (Kcal/mol)	LUMO (Kcal/mol)	Gap ($\Delta E = E_{LUMO} - E_{HOMO}$) (Kcal/mol)	Dipole Moment (Debye)			
					X	Y	Z	Total
Flavone	-4.56865E+05	-8.14	-5.37	2.77	-2.2675	-3.9104	0.2581	4.5276
5-hydroxy flavone (5-OH)	-5.04091E+05	-7.84	-5.30	2.54	4.1176	2.8470	0.1640	5.0087
7-hyrdroxy flavone (5-OH)	-5.04083E+05	-8.07	-5.30	2.77	-2.3515	-4.8451	0.2663	5.3922
8-methoxy flavone (8-OCH_3)	-5.28732E+05	-7.93	-5.05	2.88	0.5105	-3.7066	0.1599	3.7450
Apigenin (5,7,4'-OH)	-5.98525E+05	-7.75	-5.33	2.42	-5.2346	-5.4426	-0.1423	7.5527
Luteolin (5,7,3',4'-OH)	-6.45742E+05	-7.54	-5.05	2.49	-4.3310	5.6899	0.1526	7.1524

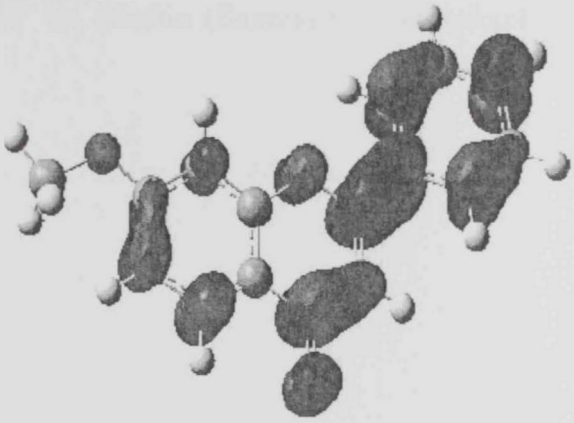


5-Hydroxy flavone-HOMO

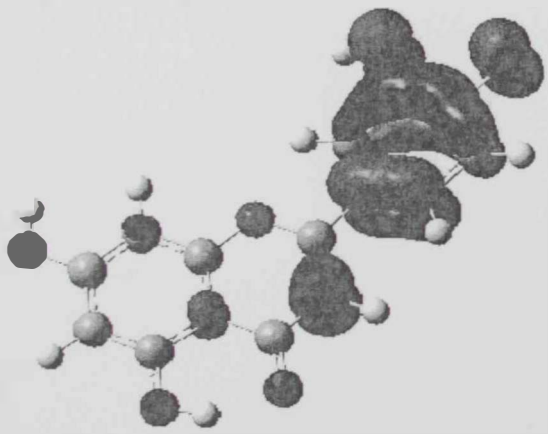


5-Hydroxy flavone-LUMO

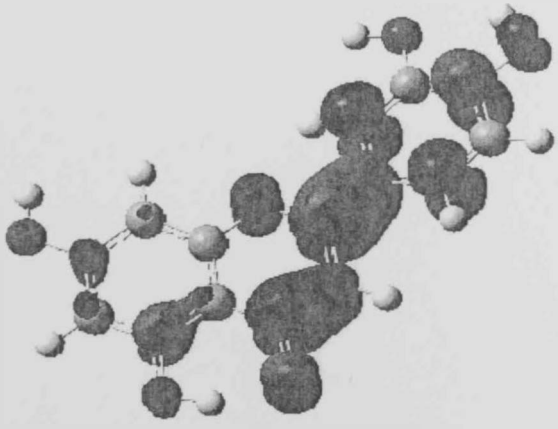
8-methoxy flavone-HOMO



8-methoxy flavone-LUMO



Luteolin-HOMO



Luteolin-LUMO

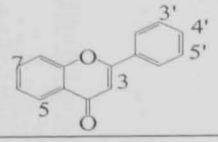
Figure 3.35: Charge distribution of the HOMO-LUMO (isovalue of 0.04) in some optimized flavones.

3.2.2.2 Chemical potential properties

The values for all the variables associated with the chemical potential are relatively low (**Table 3.6**). Yet, flavonols have even lower chemical potential values which is another indication that flavonols are better antioxidants than flavones.

According to the chemicals properties of flavonols and flavones; Flavonols with a catechol structure or even a monohydroxyl group in C-ring show better chemical properties than flavones with a catechol structure. i.e. [fisetin (flavonol) > kaempferol (flavonol) > luteolin (flavone)].

Table 3.6: Properties related with the chemical potential in the optimized structures of flavones

Flavones 	Electronic Affinity (eV)	Ionization potential (eV)	Hardness (eV)	Electronegativity (eV)	Electrophilicity index (eV)
Flavone	0.233	0.353	0.06	0.293	0.715
5-hydroxy flavone (5-OH)	0.23	0.34	0.055	0.285	0.738
7-hyrdroxy flavone (5-OH)	0.23	0.35	0.06	0.29	0.701
8-methoxy flavone (8-OCH_3)	0.219	0.344	0.063	0.282	0.634
Apigenin (5,7,4'-OH)	0.231	0.336	0.053	0.284	0.765
Luteolin (5,7,3',4'-OH)	0.219	0.327	0.054	0.273	0.690

3.3.3 Flavanones

3.3.3.1 Energy and Dipole moment properties

Flavanones lack the conjugation provided by the 2,3-double bond with the 4-oxo group.[1,3] Fustin, a flavanone with a catecholic structure in B-ring and two OH groups at 5 and 7 position, can be compared to hesperetin, a flavanone from the same class and the same total number of substituents, yet, has OCH_3 group at 4' position in B-ring instead of OH group. The dipole moments of these two flavanones are as follows; (fustin = 4.924 > hesperetin = 4.778 Debye) as seen in **Table 3.7**. In the above classes of flavonoids, the center of positive and negative charges are farther apart in fustin with four OH groups than they are with hesperetin with three OH groups and one OCH_3.

The other flavanones can be compared with their counterparts in other classes. Flavanone, from the class flavanones can be compared with flavone, a flavonoid from the class flavones. Both flavonoids lack OH groups. With 7.41 cal/mol difference in energy, flavone is more active than flavanone which lacks an unsaturated 2-3 bond. i.e. (flavone = -4.56865E+05 > flavanone = -4.57606E+05 Kcal/mol) **(Table 3.7)**.

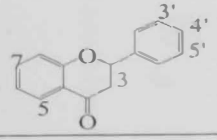
Taxifolin, a flavanone, with a catechol group in B-ring can be set against its counterpart flavonol. i.e. (quercetin = -6.92950E+05 > Taxifolin = -6.93691E+05). Naringenin, flavanone, and apigenin, flavone, with three OH groups in their system also confirm that the lack of 2-3 double bond affect the activity of flavanones. With the same energy difference (7.41 cal/mol), apigenin is more active than naringenin. i.e. (apigenin = -5.98525E+05 > naringenin = -5.99266E+05 Kcal/mol).

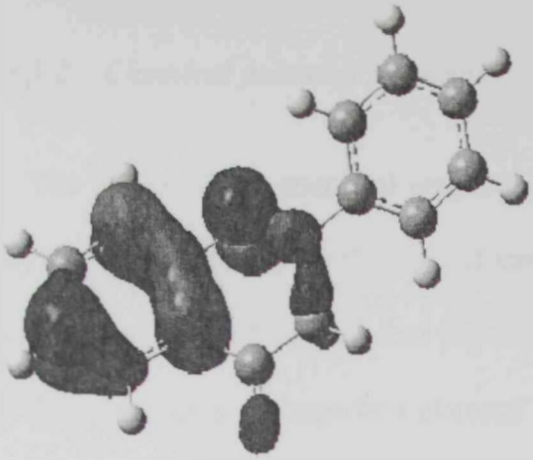
The effect of loss of structural features in flavonoids can be seen in naringenin, a flavanone, apigenin, a flavone, and galangin, a flavonol. i.e. (galangin = -5.98516E+05 >

apigenin = -5.98525E+05 > naringenin = -5.99266E+05 Kcal/mol) **(Table 3.7)**. From the single point energy values, the conjugation provided by the 2,3-double bond with the 4-oxo group is more substantial to the activity of flavonoids than 3-OH group.

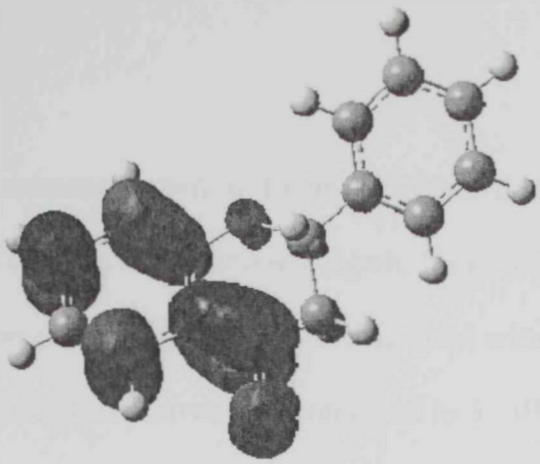
The HOMO-LUMO gap in flavanones is close to flavones and a bit higher than flavonols. This indicates that flavonols are more reactive systems than flavones **(Table 3.7, Figure 3.36** and **Appendix II)**.

Table 3.7: Energy properties and dipole moments of flavanones

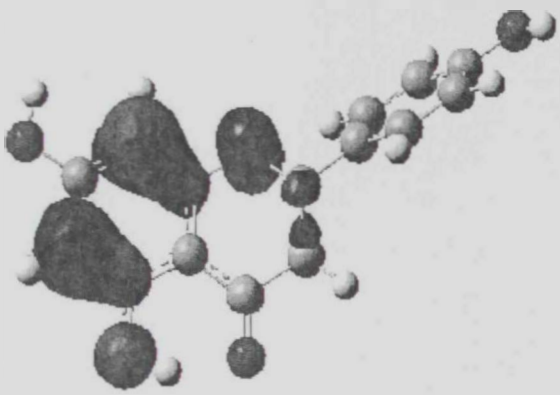
Flavanones 	Energy (Kcal/mol)	HOMO (Kcal/mol)	LUMO (Kcal/mol)	Gap ($\Delta E = E_{LUMO} - E_{HOMO}$) (Kcal/mol)	Dipole Moment (Debye)			
					X	Y	Z	Total
Flavanone	-4.57606E+05	-8.03	-5.23	2.79	-1.5195	-2.2330	-0.0217	2.701
Naringenin (5,7,4'-OH)	-5.99266E+05	-7.86	-4.80	3.07	-4.2398	-2.4115	1.5571	5.120
Hesperitin (5,7,3'-OH)(4'-OCH_3)	-6.71127E+05	-7.77	-4.80	2.97	-2.7256	3.8884	0.5260	4.778
Fustin (3,7,3',4'-OH)	-6.46467E+05	-7.33	-5.03	2.31	1.7377	-4.6059	-0.1211	4.924
Taxifolin (3,5,7,3',4'-OH)	-6.93691E+05	-7.56	-4.89	2.68	-3.4320	4.0510	-0.0865	5.310



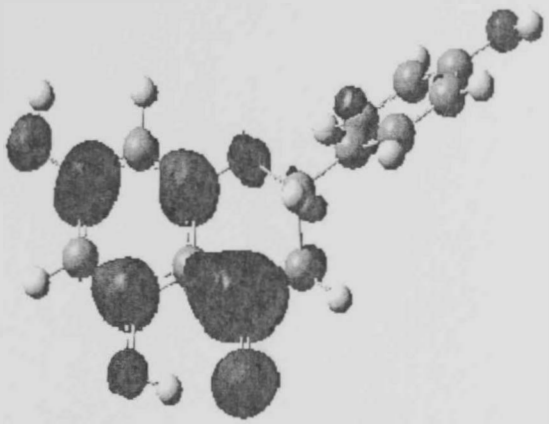
Flavanone- HOMO



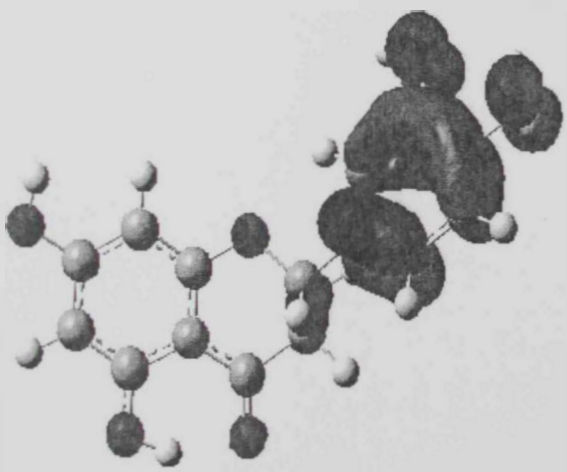
Flavanone- LUMO



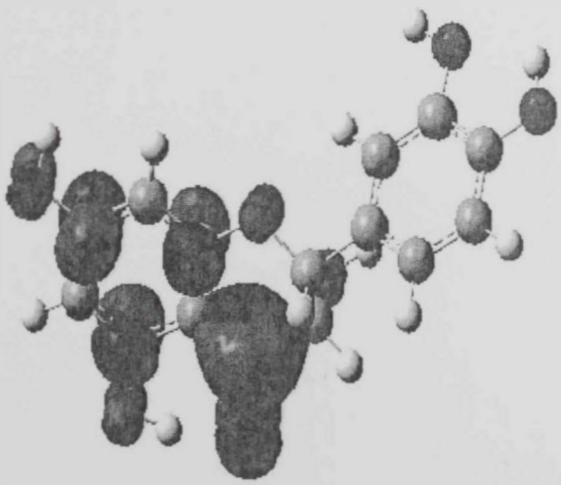
Naringenin- HOMO



Naringenin- LUMO



Taxifolin- HOMO



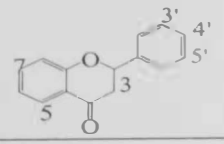
Taxifolin- LUMO

Figure 3.36: Charge distribution of the HOMO-LUMO (isovalue of 0.04) in some optimized flavanones.

3.3.3.2 Chemical potential properties

The values of the chemical properties of flavanones shown in **Table 3.8** reveal that they are being affected by the loss of structural features in flavanones. Again, From the values above it can be stated that the conjugation provided by the 2,3-double bond with the 4-oxo group is an important element to the activity of flavonoids compared to 3-OH in C-ring.

Table 3.8: properties related with the chemical potential in the optimized structures of flavanones.

Flavanones 	Electronic Affinity (eV)	Ionization potential (eV)	Hardness (eV)	Electronegativity (eV)	Electrophilicity index (eV)
Flavanone	0.227	0.348	0.061	0.288	0.683
Naringenin (5,7,4'-OH)	0.208	0.341	0.067	0.275	0.567
Hesperitin (5,7,3'-OH)(4'-OCH_3)	0.208	0.337	0.065	0.273	0.576
Fustin (3,7,3',4'-OH)	0.218	0.318	0.050	0.268	0.718
Taxifolin (3,5,7,3',4'-OH)	0.212	0.328	0.058	0.270	0.628

3.3.4 Isoflavones

3.3.4.1 Energy and Dipole moment properties

Isoflavones are a subclass of isoflavonoids. They differ structurally from common flavonoids in B-ring orientation. Daidzein with 7-OH and 4'-OH group has a dipole moment of 5.070 Debye. Substituting formononetin with one OCH_3 group in position 4' decreases the dipole momentum by 2.084 Debye. The same pattern applies to both genistein and biochanin A. i.e (genistein = 5.651 > biochanin A = 3.743 Debye) **(Table 3.9)**.

In all the studied flavonoids, substituting a high electron donating group i.e. OH with a slightly weak electron donating one i.e. OCH_3 decreases the dipole moment. In other words, the center of negative and positive charges becomes closer.

Genistein, isoflavone with three OH groups, can be compared to galangin, a flavonol, apigenin, a flavone, and naringenin, a flavanone, with the same number of hydroxyl groups. i.e. (galangin = -5.98516E+05 > genistein = -5.98330+05 > apigenin = -5.98525E+05 > naringenin = -5.99266E+05 Kcal/mol). The energy values as well as HOMO-LUMO gap and chemical properties values reveal that the losses of structural features in flavonoids affect negatively their activity **(Table 3.9** and **3.10)**. The change of B- ring orientation, loss of 3-OH group and loss of conjugation are all factors that affect the activity of flavonoids.

Table 3.9: Energy properties and dipole moments of isoflavones.

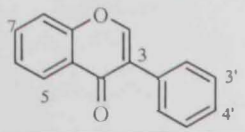
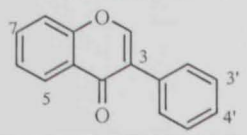
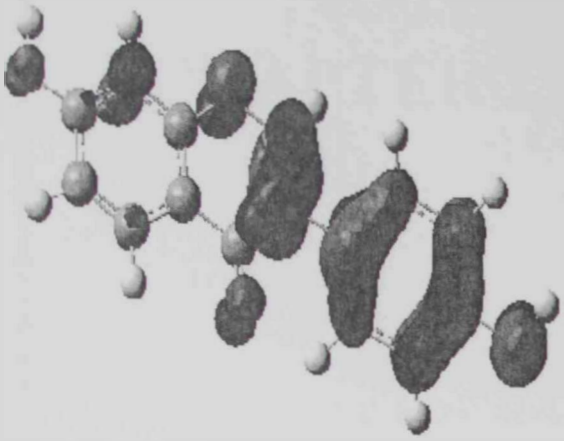
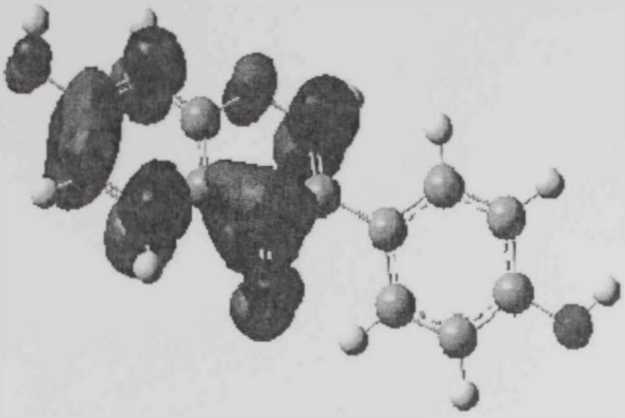
Isoflavones 	Energy (Kcal/mol)	HOMO (eV)	LUMO (eV)	Gap ($\Delta E = E_{LUMO}-E_{HOMO}$) (eV)	Dipole Moment (Debye) X	Y	Z	Total
Daidzein (7,4'-OH)	-5.51295E+05	-7.68	-4.91	2.77	1.373	-4.881	0.020	5.070
Formononetin (7-OH)(4'-OCH_3)	-5.75945E+05	-7.61	-4.91	2.70	-1.033	-2.534	1.194	2.986
Genistein (5,7,4'-OH)	-5.98330+05	-7.56	-4.68	2.88	-0.573	-5.621	0.098	5.651
Biochanin A (5,7-OH)(4'-OCH_3)	-6.23169E+05	-7.59	-4.68	2.91	-0.827	-3.417	-1.282	3.743

Table 3.10: properties related with the chemical potential in the optimized molecules of isoflavones.

Isoflavones 	**Electronic Affinity (eV)**	**Ionization potential (eV)**	**Hardness (eV)**	**Electronegativity (eV)**	**Electrophilicity index (eV)**
Daidzein (7,4'-OH)	0.227	0.348	0.06	0.273	0.621
Formononetin (7-OH)(4'-OCH_3)	0.208	0.341	0.059	0.272	0.630
Genistein (5,7,4'-OH)	0.208	0.337	0.063	0.266	0.564
Biochanin A (5,7-OH)(4'-OCH_3)	0.218	0.318	0.063	0.266	0.562

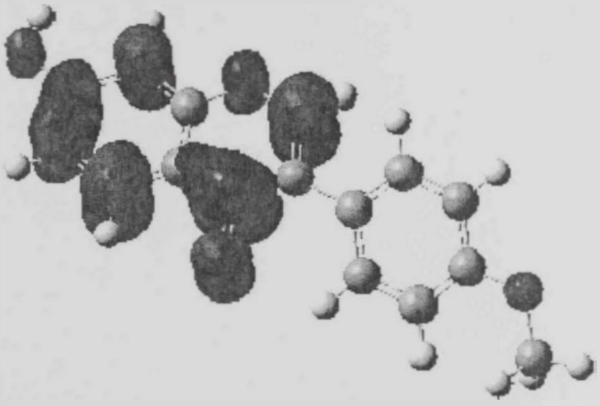


Diadzein- HOMO



Diadzein- LUMO

Formononetin- HOMO



Formononetin- HOMO

Figure 3.37: Charge distribution of the HOMO-LUMO (isovalue of 0.04) in some optimized isoflavones.

CHAPTER IV

CONCLUSIONS

4. Conclusions

The present study documents results obtained from complementary approaches in order to establish a structure-function relationship of selected flavonoids. It was shown from the kinetic studies which were carried out on 7 flavonoids from 3 different classes that the rate constants (k) of the flavonoids under investigations are dependent upon the number of resonance structures formed by each flavonoid upon hydrogen extraction with the following order; kaempferol > morin > quercetin > myricetin > 3-hydroxyflavone > naringenin > flavone. Flavonols mainly with 2,3-double bond, 3-hydroxyl group and 4-keto group exhibited the highest rate constants compared to flavones lacking one structural feature (3-hydroxyl group) and flavanones lacking both (3-hydroxyl group and 2,3-double bond).

Kinetic studies also showed that the number of moles of DPPH scavenged per one mole of flavonoid, as well as their antiradical activity is affected by the number and pattern of hydroxyl substitution on the B-ring as shown in the following trend; quercetin > myricetin > kaempferol > morin. Myricetin which has the highest number of OH groups among flavonols is less active than quercetin due to both steric and electronic effects. Yet, the oxidation potential values obtained from cyclic voltammetry analysis of these flavonoids showed that myricetin had the highest oxidation potential in different pH values; Myricetin > quercetin > morin > kaempferol > 3-hydroxyflavone > naringenin.

A series of density functional theory calculations were performed for 28 flavonoids from 4 different classes to give a clearer picture of structure activity relationship of flavonoids. Energy and dipole moment properties as well as chemical potential properties for the different classes of flavonoids showed that the activity is in the following order:

flavonols > flavones > flavanones > isoflavones. The loss of structural features in flavonoids affect their activity i.e. 3-OH group, 2,3-double bond in conjugation with a 4-keto function, which are responsible for electron delocalization, as well as orientation of the B-ring. Among each group of flavonoids the key factor of determining the activity of flavonoids is the number and pattern of hydroxyl/methoxy substitution. It was observed that multiple hydroxyl groups confer upon the molecule substantial activity, whereas, methoxy groups introduce unfavorable steric effects.

In summary, flavonols have the highest activity compared to the other classes of flavonoids due to the presence of the some structural features in their rings as shown experimentally as well as by density functional calculations.

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APPENDICIES

Appendix I

Sample of the cyclic voltammograms

Some cyclic voltammograms for different flavonoids at different pH values are shown in this section.

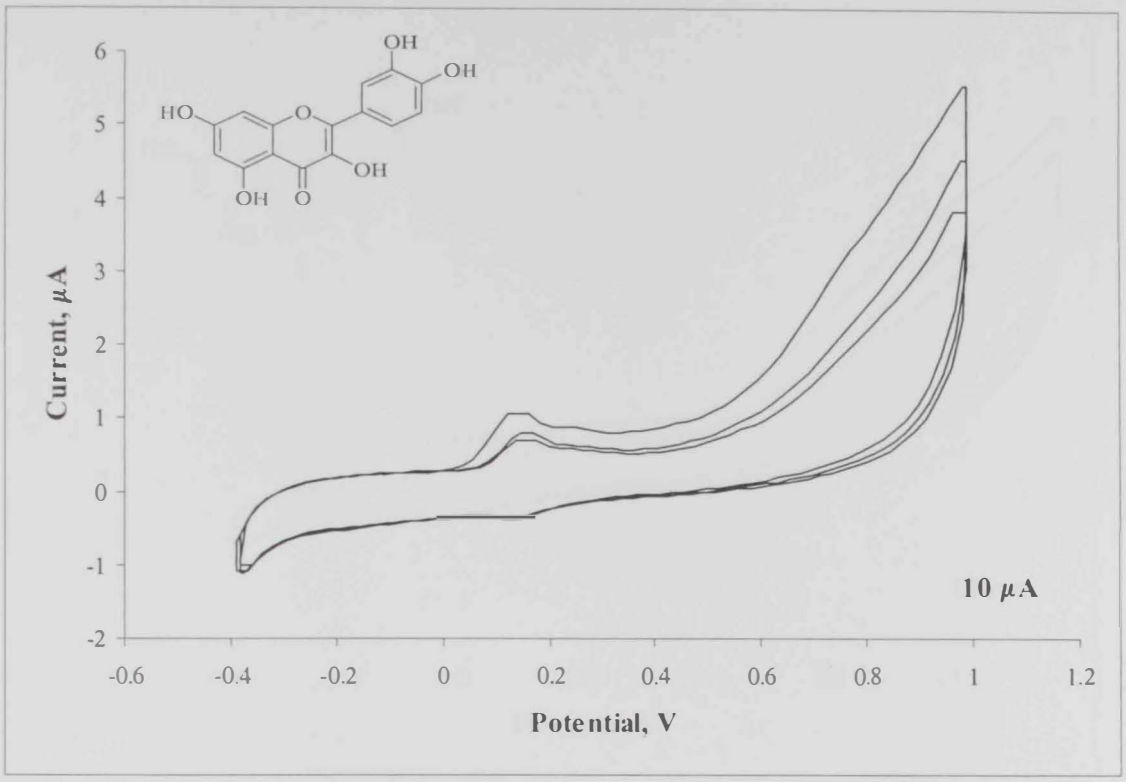


Figure 1: Cyclic voltammogram of 1 mM quercetin (Scan rate 20 mV s^{-1}) in pH 6 phosphate buffer.

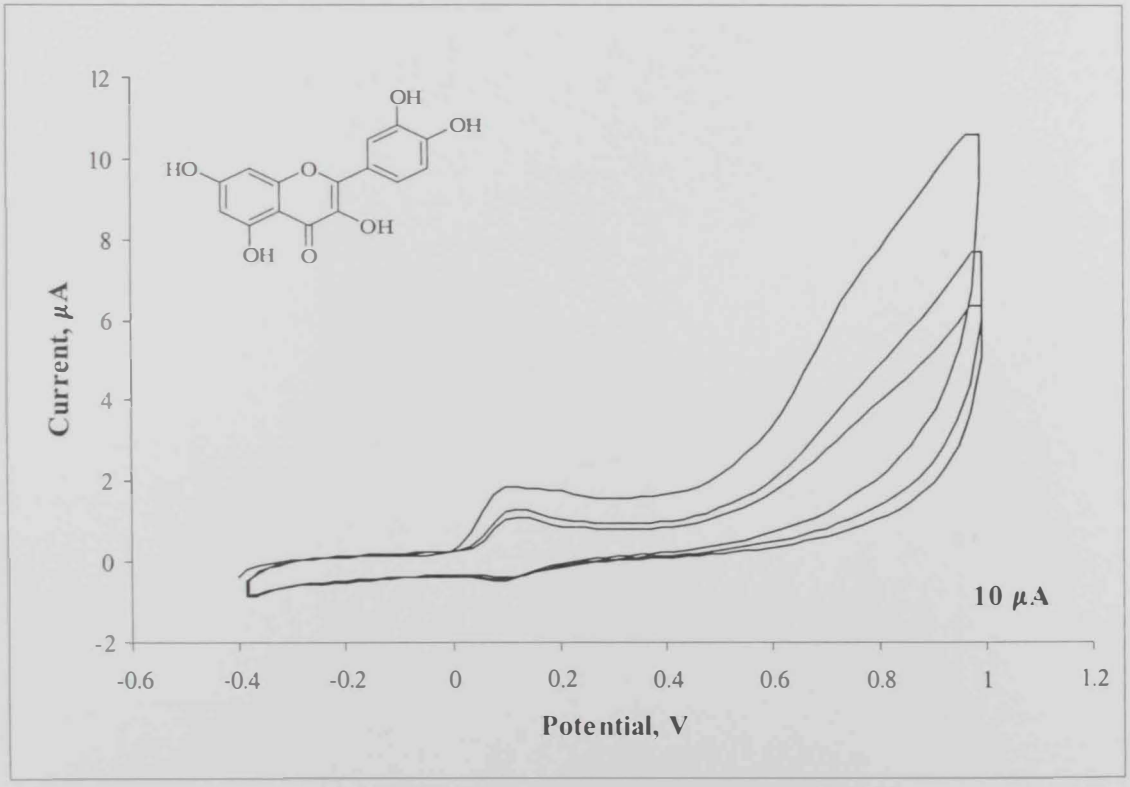


Figure 2: Cyclic voltammogram of 1 mM quercetin (Scan rate 20 mV s^{-1}) in pH 6.5 phosphate buffer.

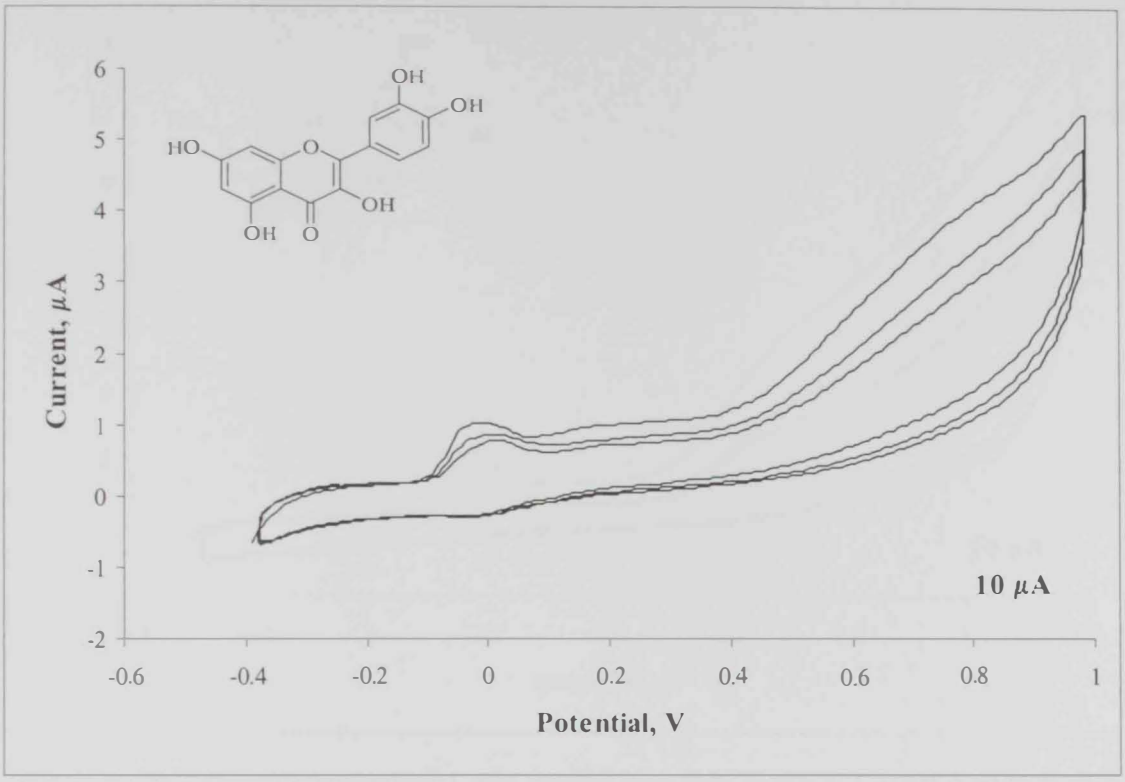


Figure 3: Cyclic voltammogram of 1 mM quercetin (Scan rate 20 mV s^{-1}) in pH 7.5 phosphate buffer.

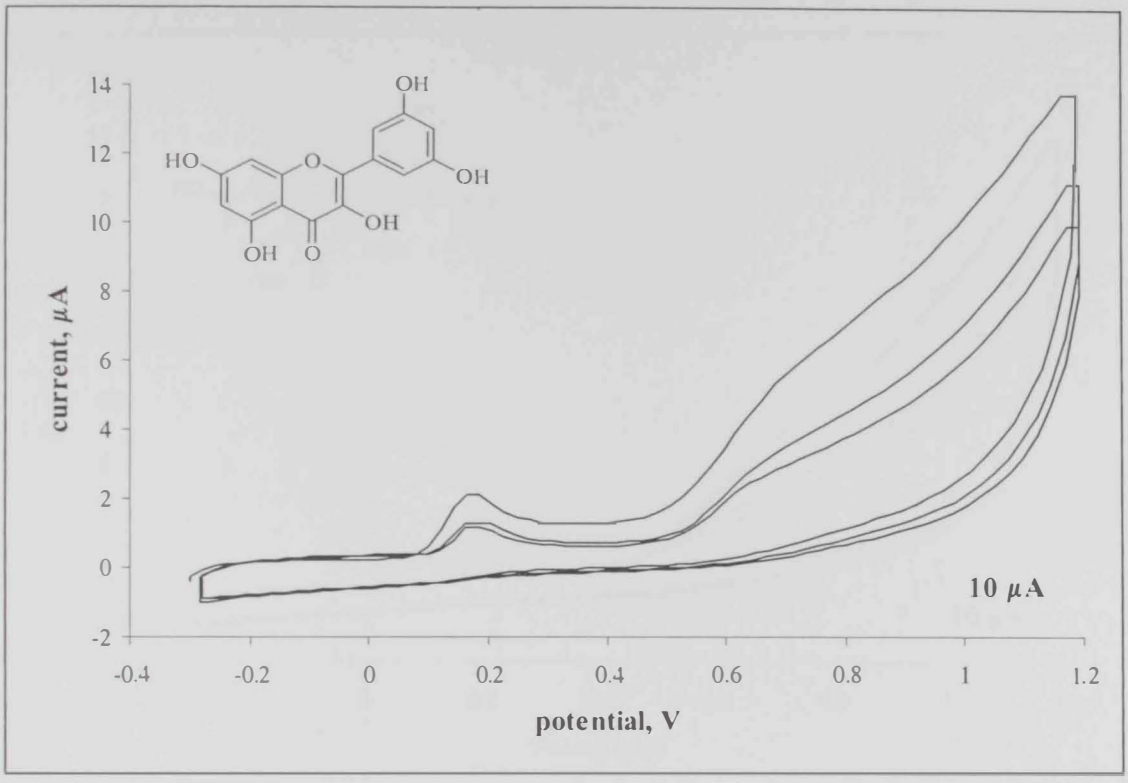


Figure 4: Cyclic voltammogram of 1 mM morin (Scan rate 20 mV s^{-1}) in pH 6 phosphate buffer.

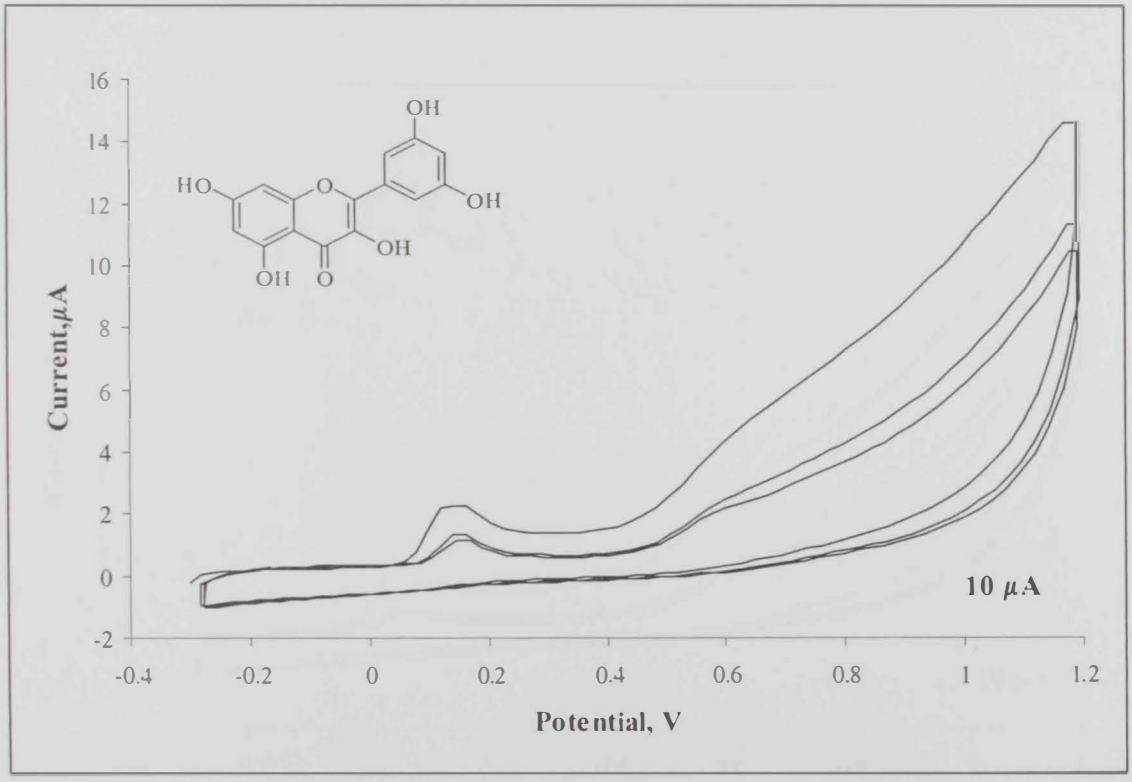


Figure 5: Cyclic voltammogram of 1 mM morin (Scan rate 20 mV s^{-1}) in pH 6.5 phosphate buffer.

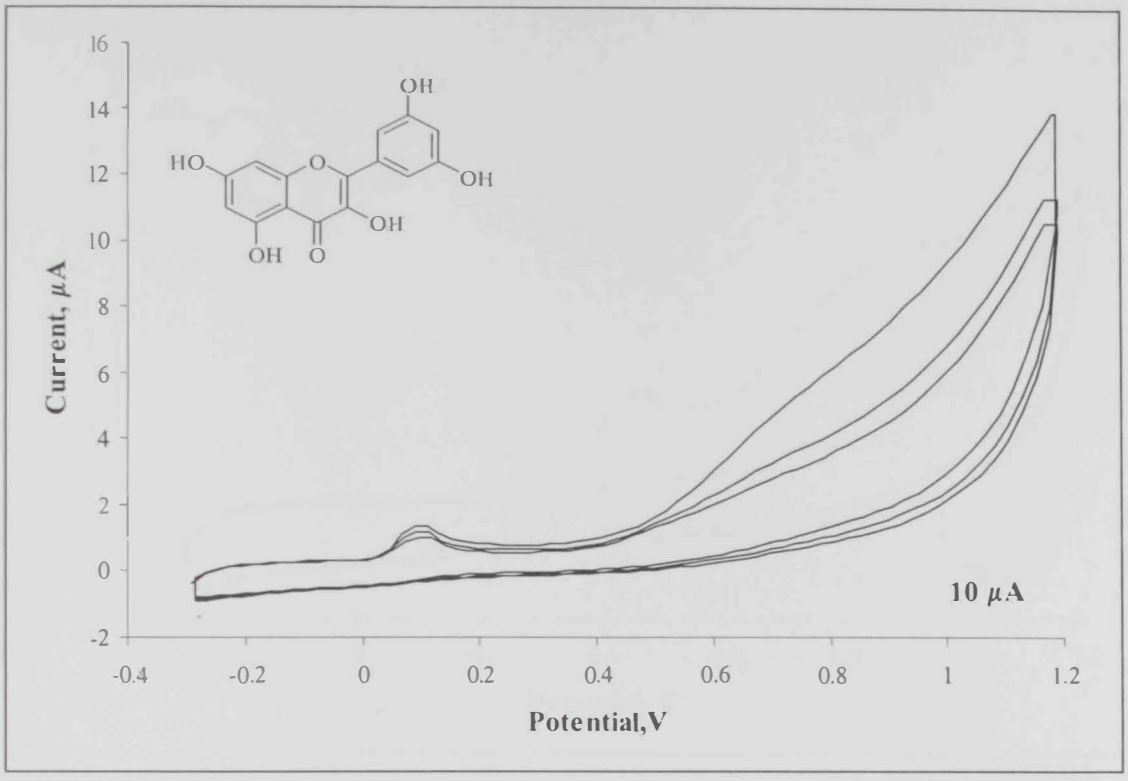


Figure 6: Cyclic voltammogram of 1 mM morin (Scan rate 20 mV s^{-1}) in pH 7.5 phosphate buffer.

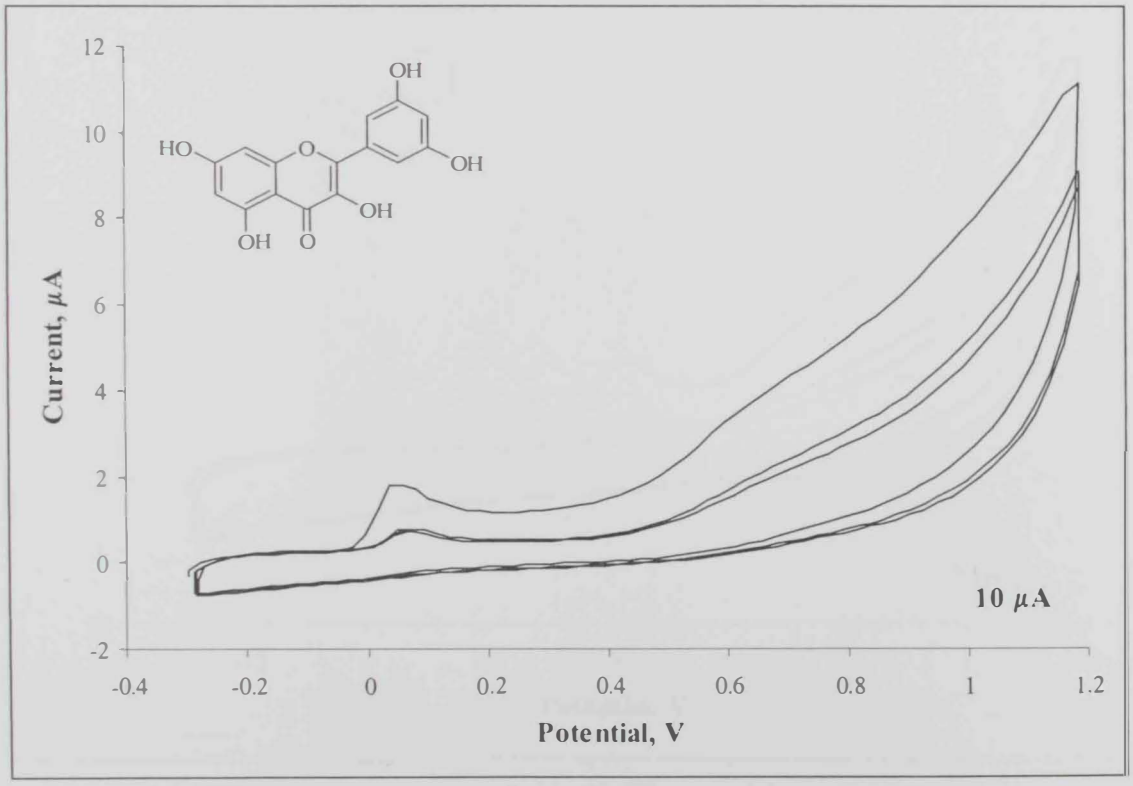


Figure 7: Cyclic voltammogram of 1 mM morin (Scan rate 20 mV s^{-1}) in pH 8 phosphate buffer.

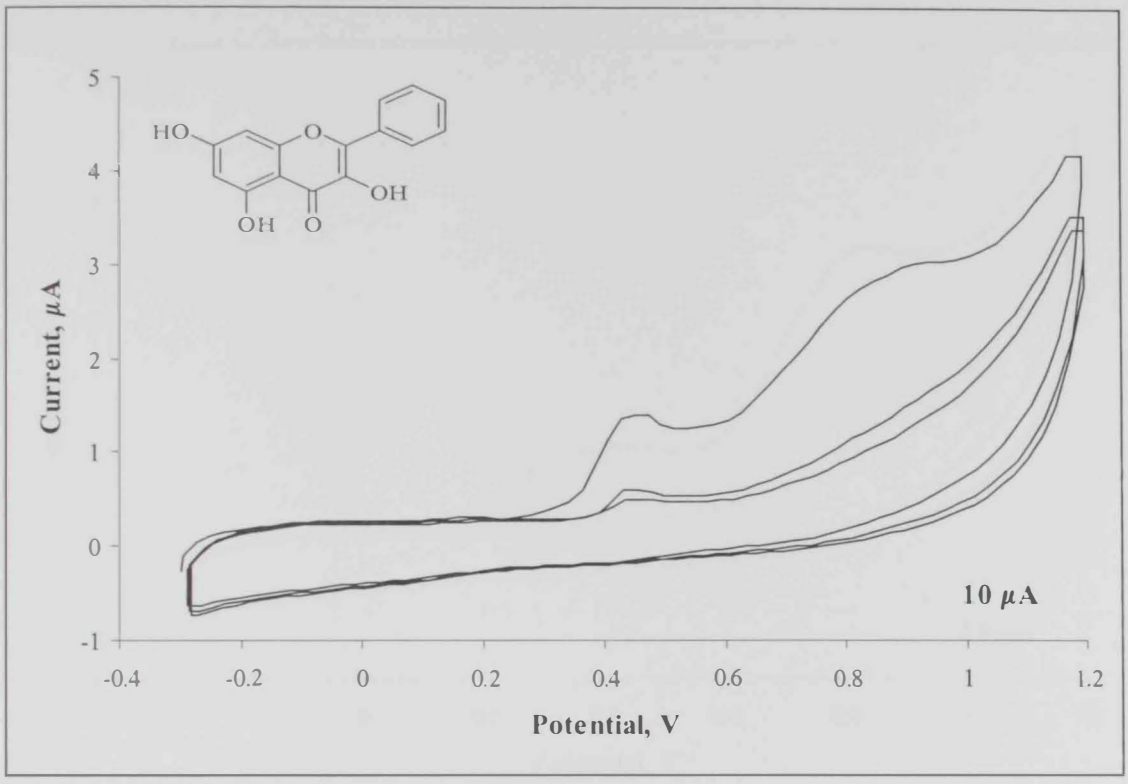


Figure 8: Cyclic voltammogram of 1 mM 3-hydroxy flavone (Scan rate 20 mV s^{-1}) in pH 6 phosphate buffer.

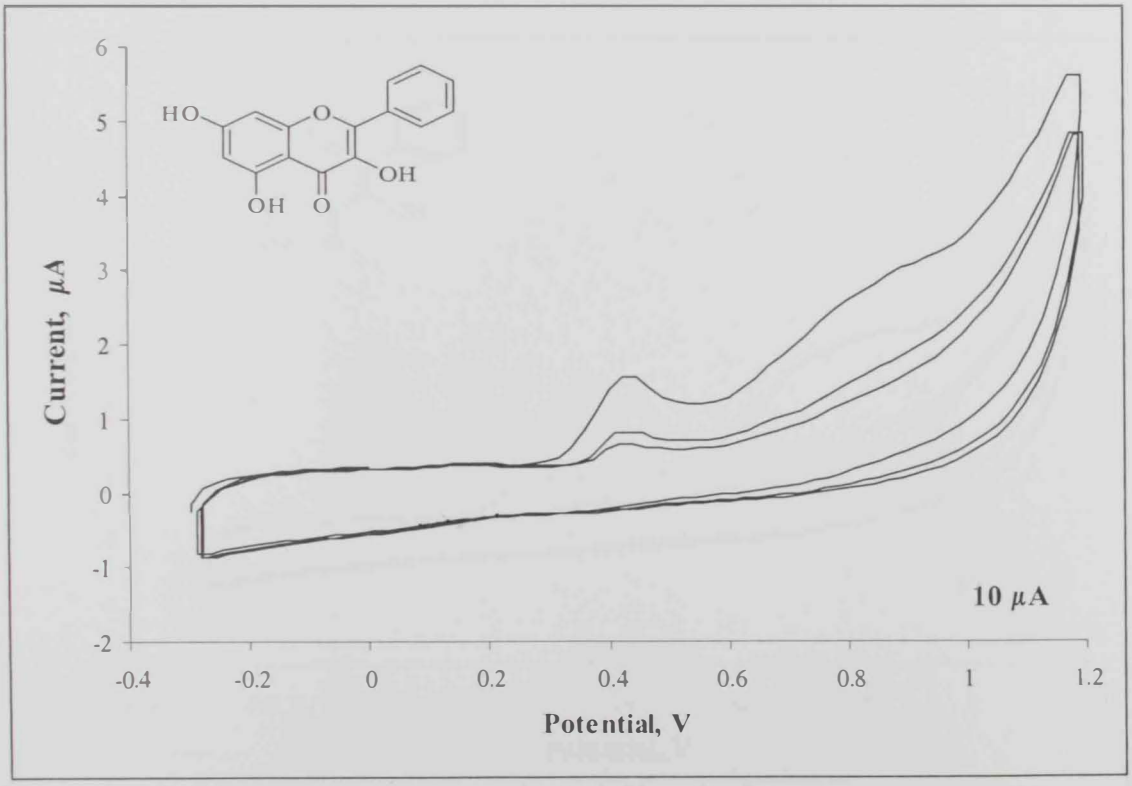


Figure 9: Cyclic voltammogram of 1 mM 3-hydroxy flavone (Scan rate 20 mV s^{-1}) in pH 6.5 phosphate buffer.

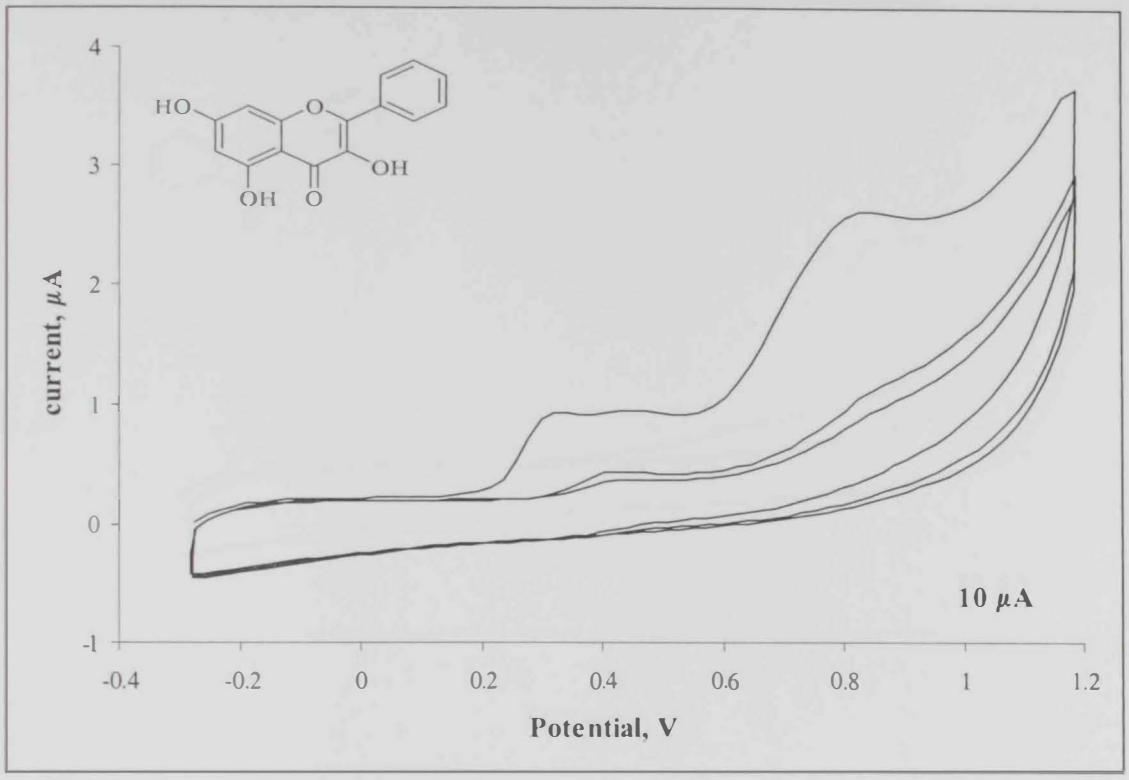


Figure 10: Cyclic voltammogram of 1 mM 3-hydroxy flavone (Scan rate 20 mV s^{-1}) in pH 7.5 phosphate buffer.

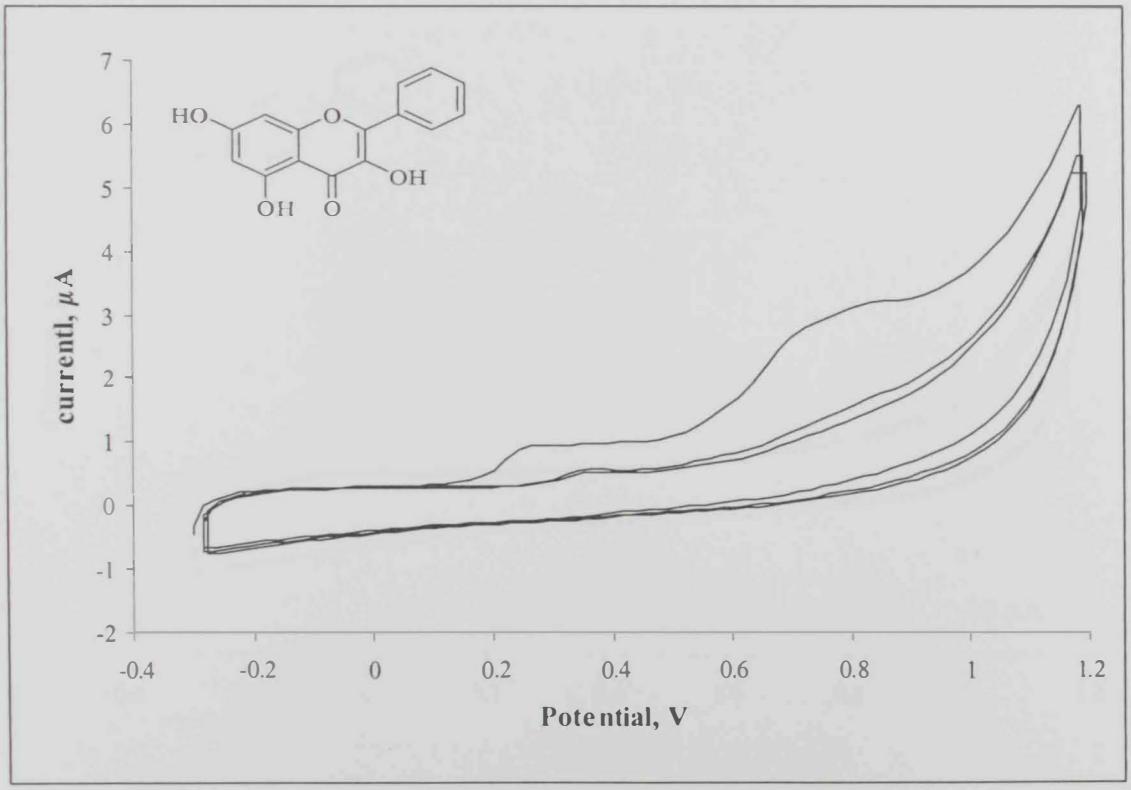


Figure 11: Cyclic voltammogram of 1 mM 3-hydroxy flavone (Scan rate 20 mV s^{-1}) in pH 8 phosphate buffer.

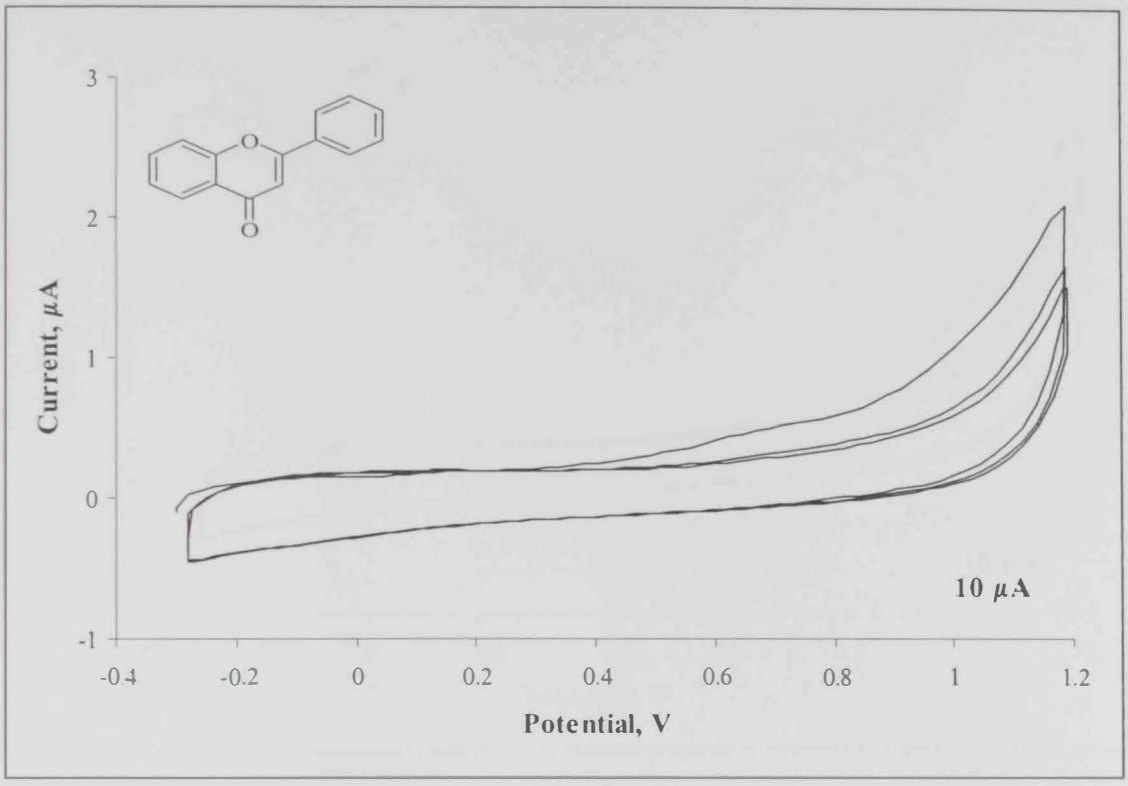


Figure 12: Cyclic voltammogram of 1 mM flavone (Scan rate 20 mV s^{-1}) in pH 6 phosphate buffer.

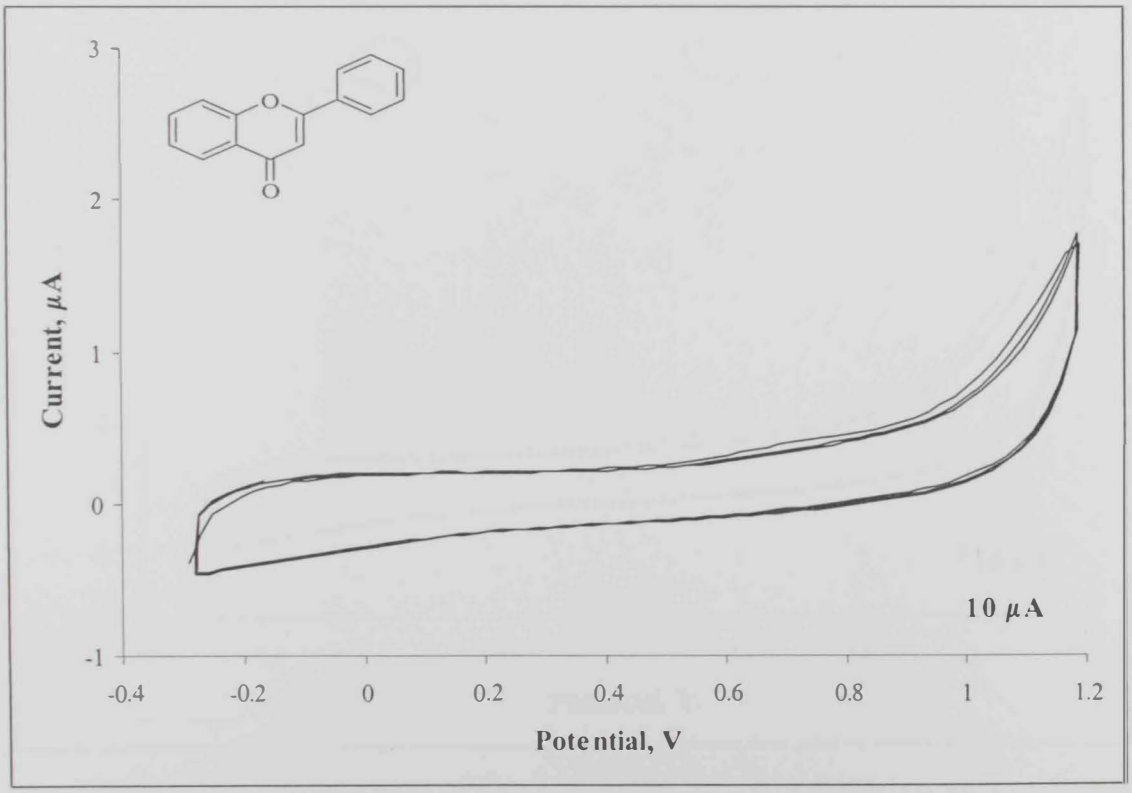


Figure 13: Cyclic voltammogram of 1 mM flavone (Scan rate 20 mV s^{-1}) in pH 6.5 phosphate buffer.

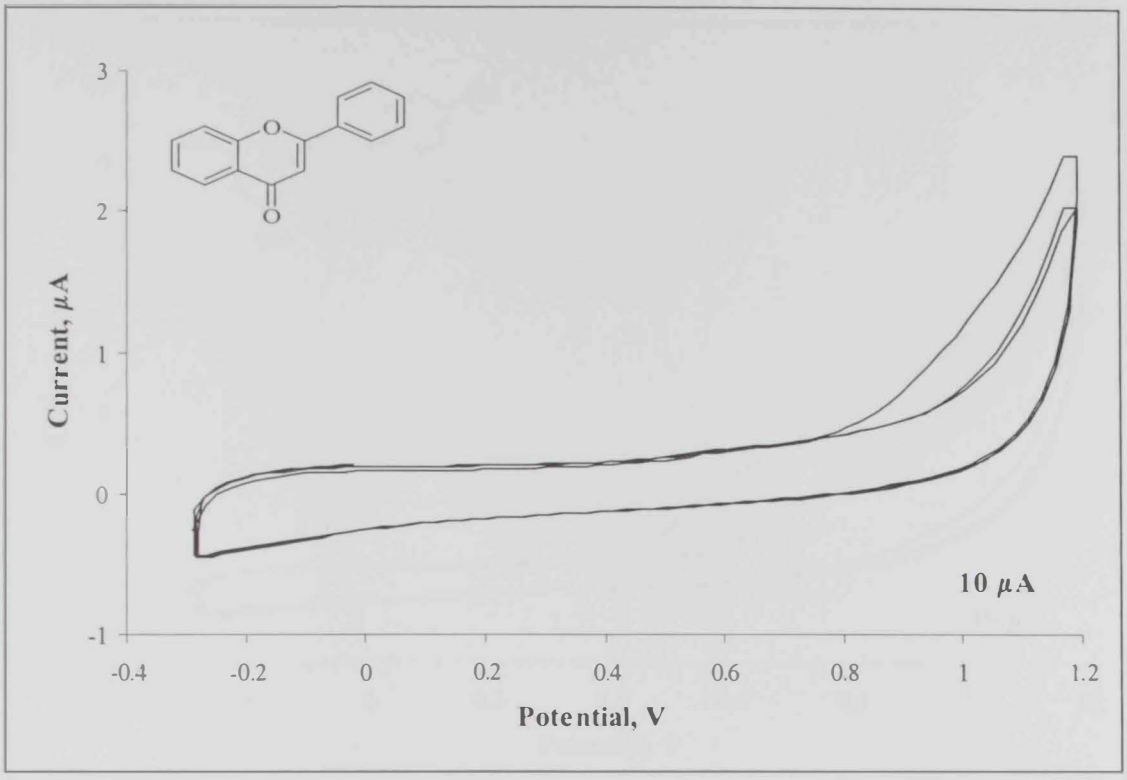


Figure 14: Cyclic voltammogram of 1 mM flavone (Scan rate 20 mV s^{-1}) in pH 7.5 phosphate buffer.

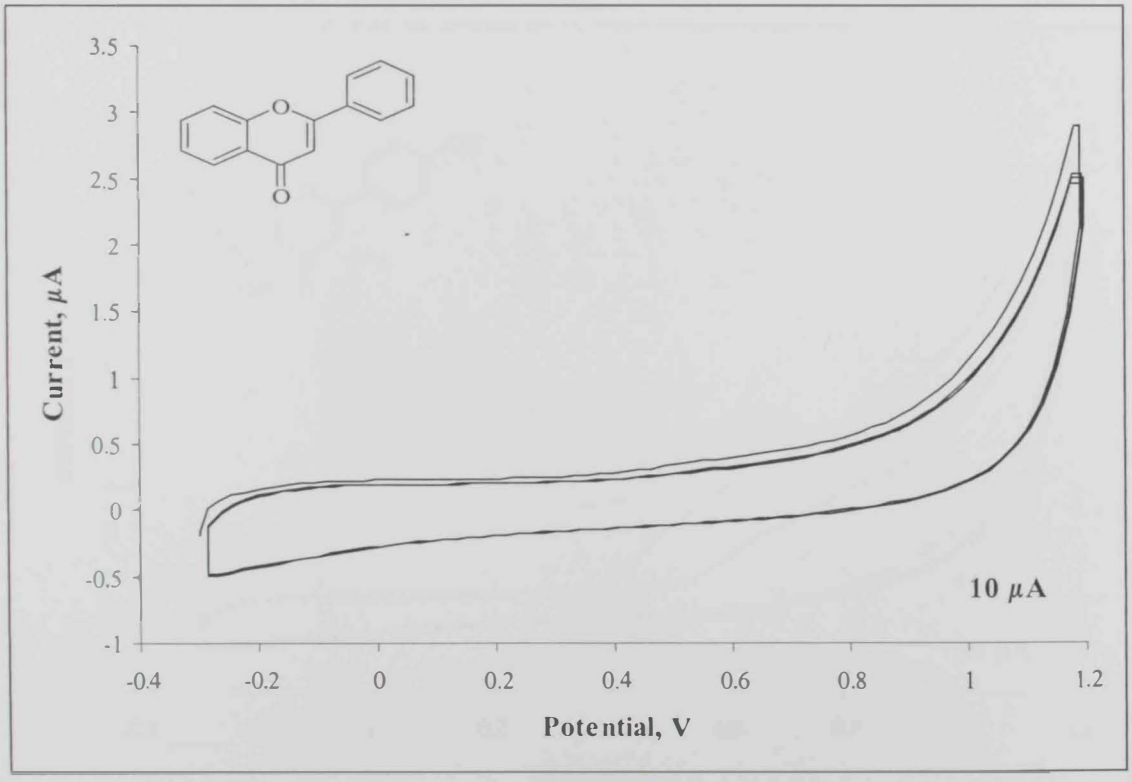


Figure 15: Cyclic voltammogram of 1 mM flavone (Scan rate 20 mV s^{-1}) in pH 8 phosphate buffer.

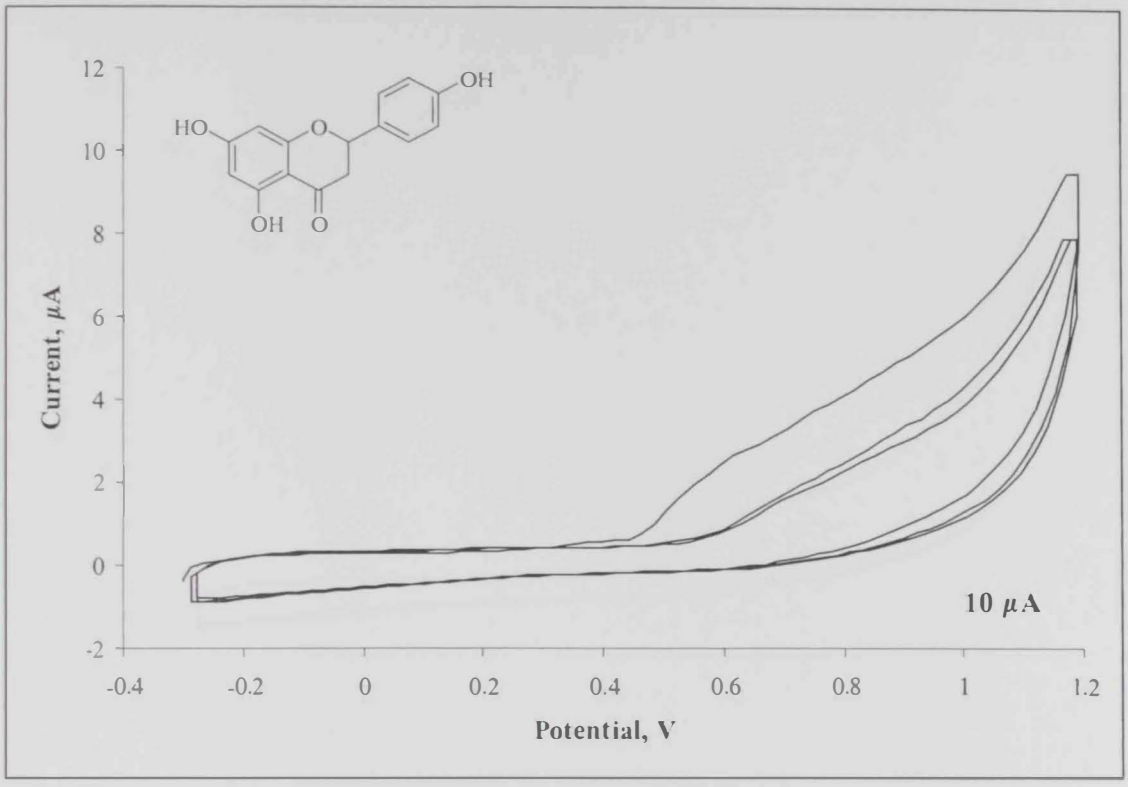


Figure 16: Cyclic voltammogram of 1 mM naringenin (Scan rate 20 mV s^{-1}) in pH 6 phosphate buffer.

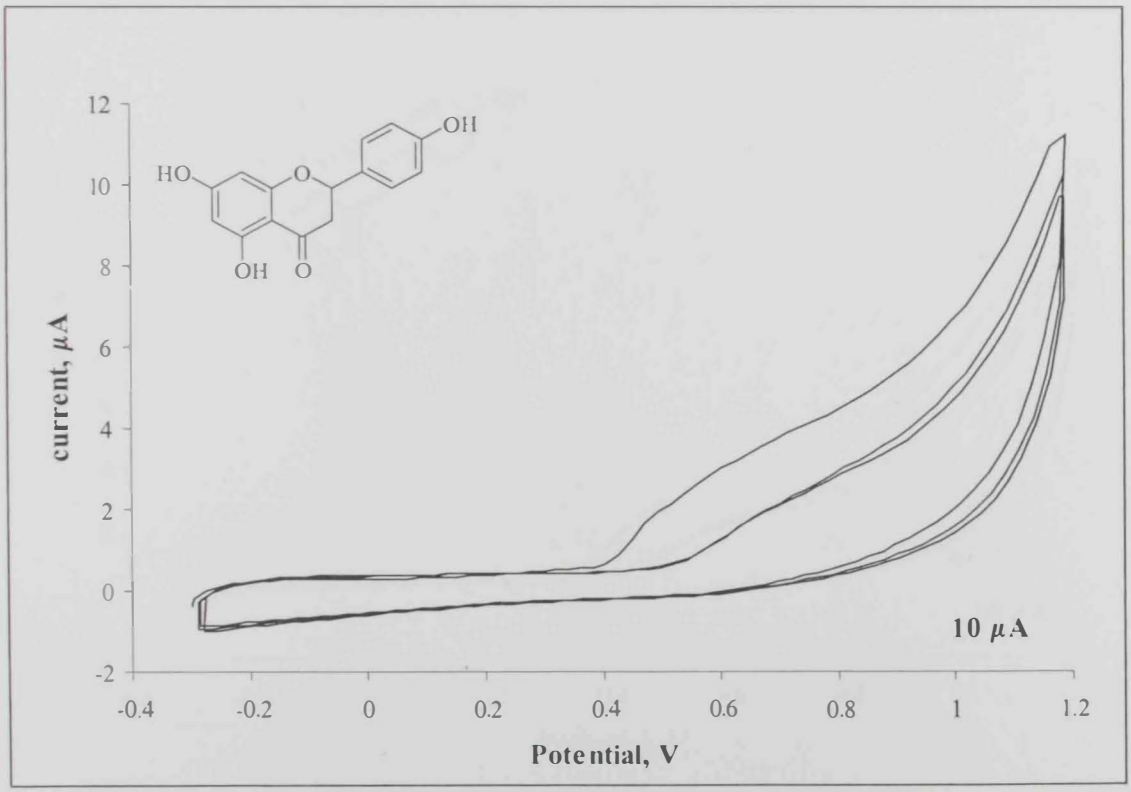


Figure 17: Cyclic voltammogram of 1 mM naringenin (Scan rate 20 mV s^{-1}) in pH 6.5 phosphate buffer.

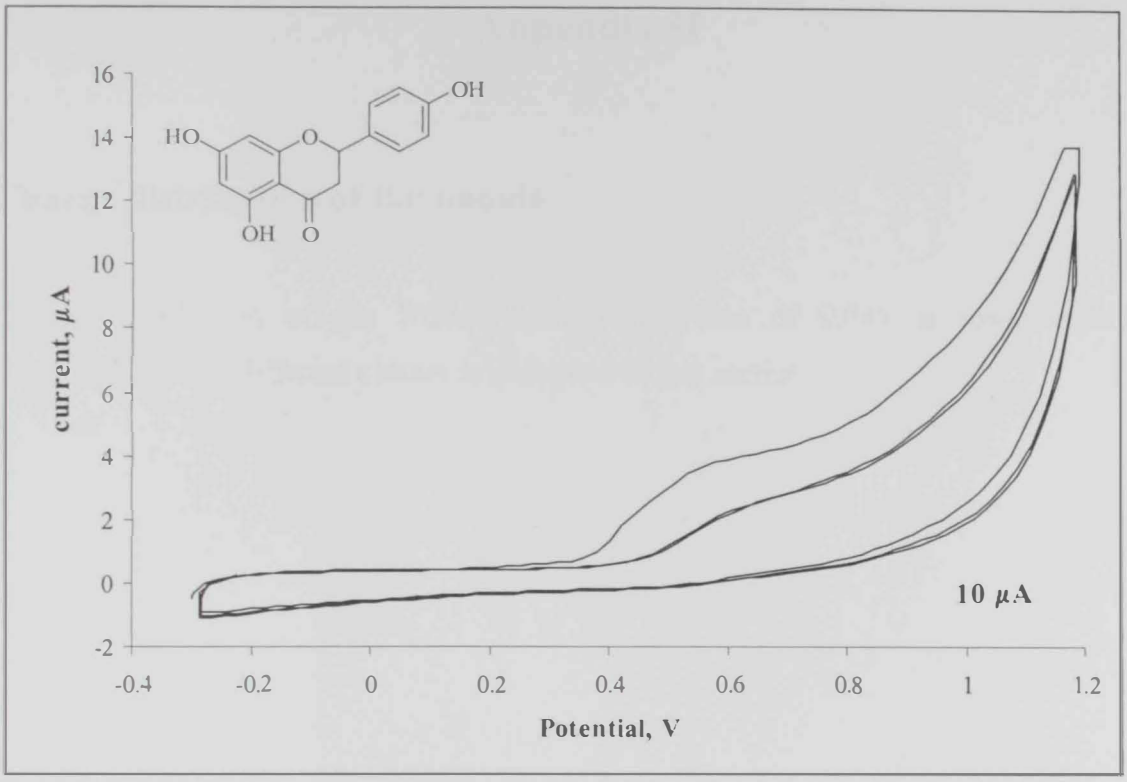


Figure 18: Cyclic voltammogram of 1 mM naringenin (Scan rate 20 mV s^{-1}) in pH 7.5 phosphate buffer.

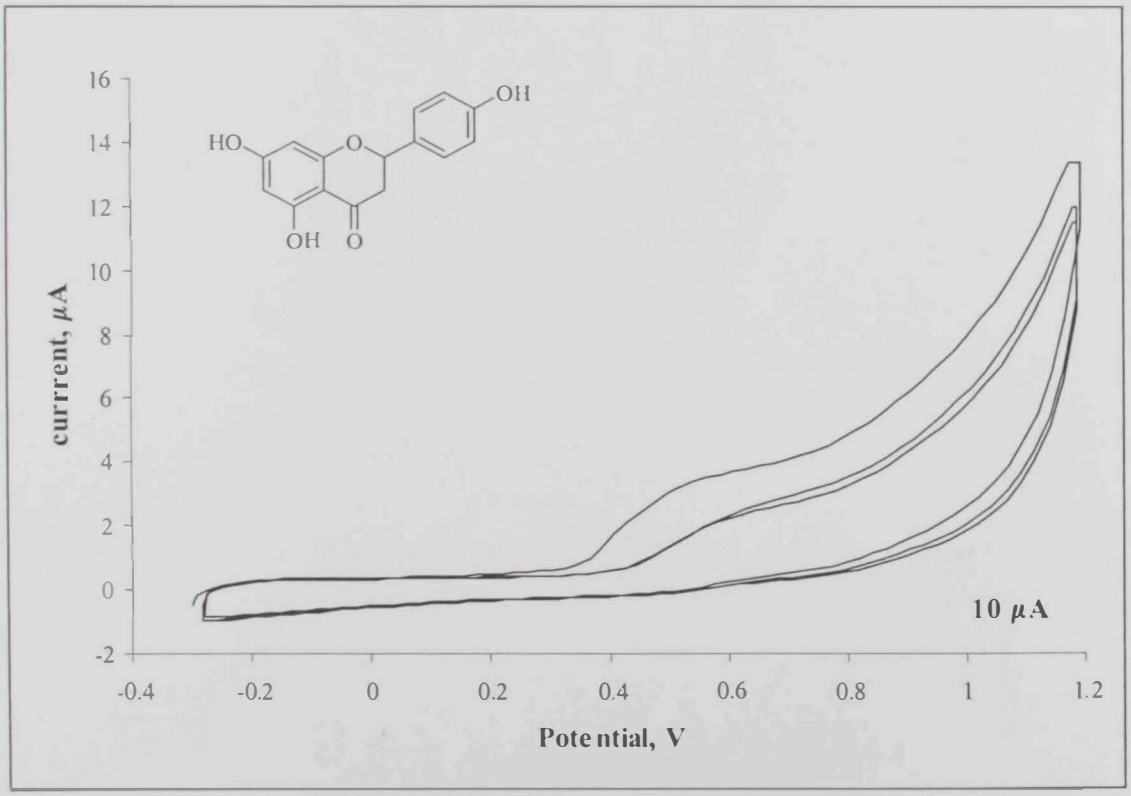


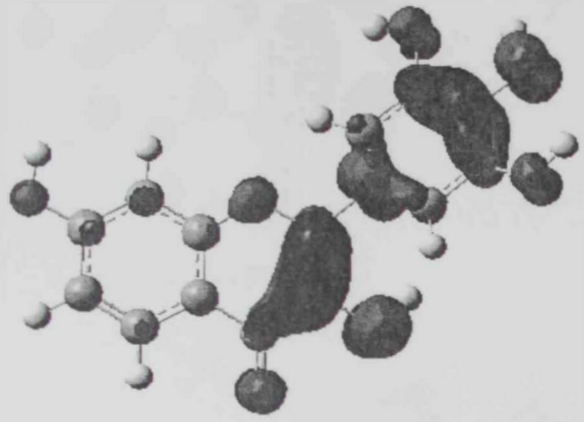
Figure 19: Cyclic voltammogram of 1 mM naringenin (Scan rate 20 mV s^{-1}) in pH 8 phosphate buffer.

Appendix II

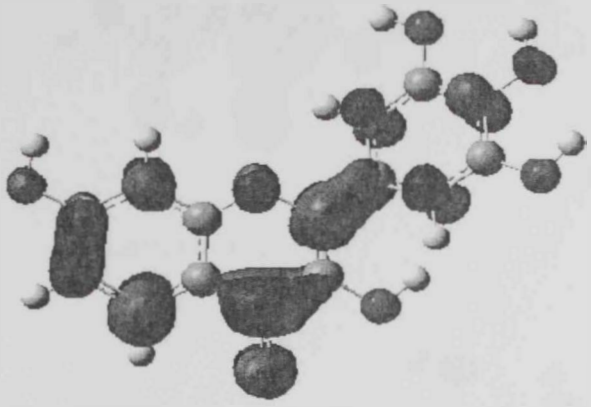
Charge distribution of flavonoids

Charge distribution of the HOMO-LUMO (isovalue of 0.04) in some optimized flavonoids from 4 different classes is presented in this section.

Charge distribution of the HOMO-LUMO (isovalue of 0.04) in some optimized flavonols.

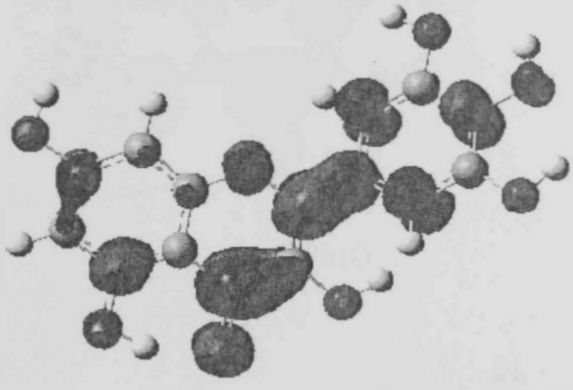


Robinetin- HOMO

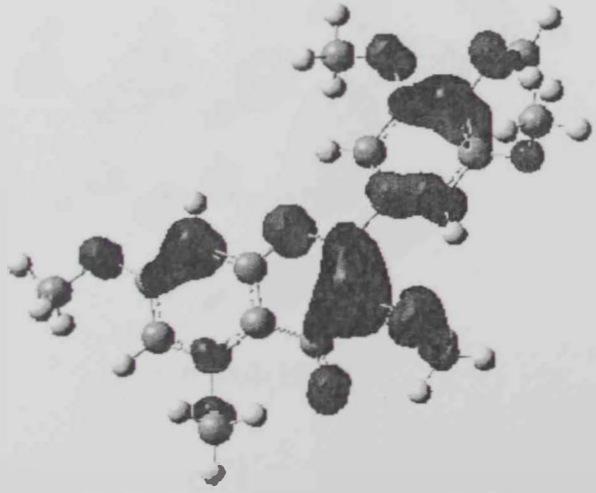


Robinetin- LUMO

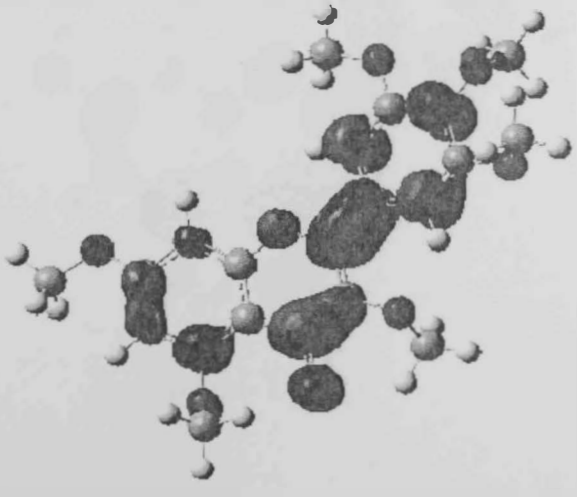
Myricetin- HOMO



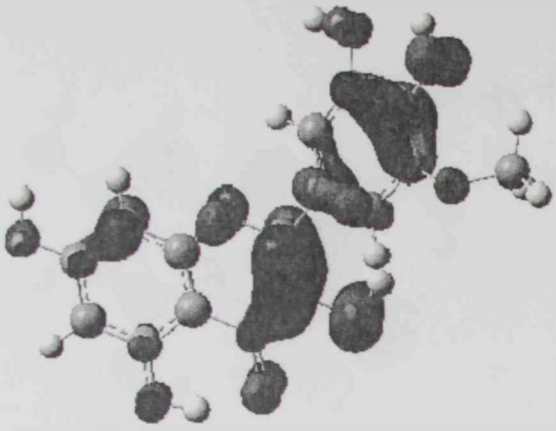
Myricetin- LUMO



3,5,7,3',4'-Hexamethoxy flavone-HOMO

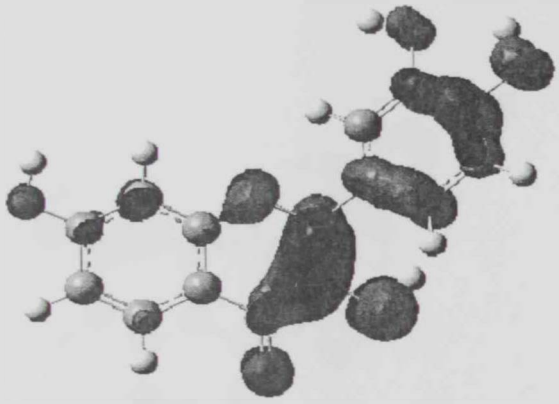


3,5,7,3',4'-Hexamethoxy flavone-LUMO

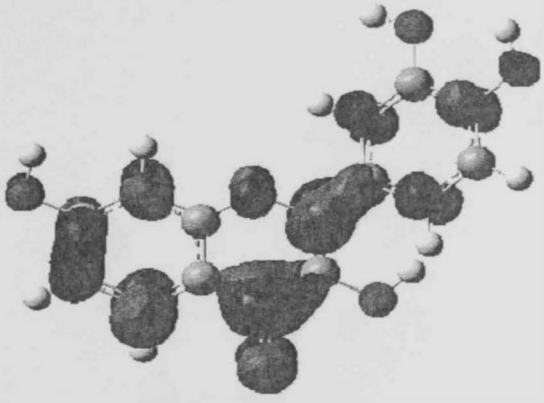


Laricytrin- HOMO

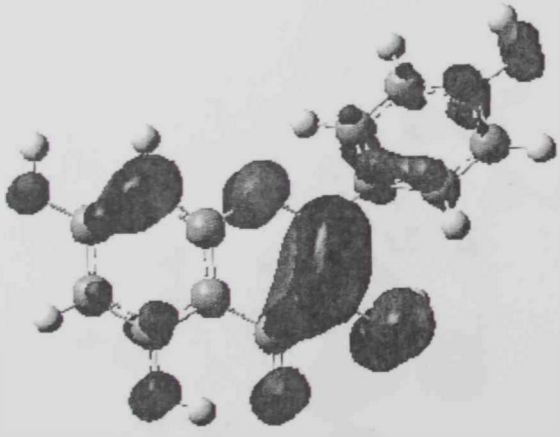
Laricytrin- LUMO



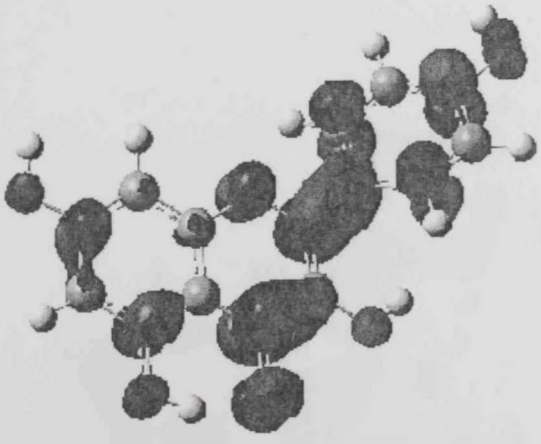
Fisetin- HOMO



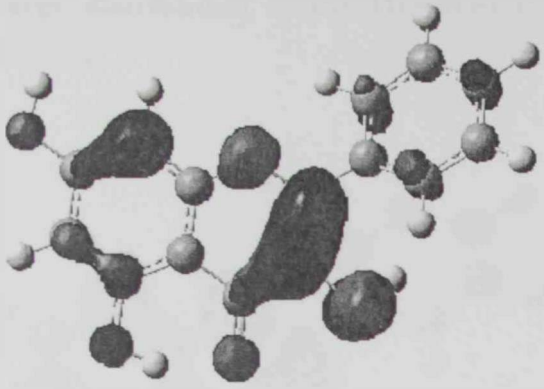
Fisetin- LUMO



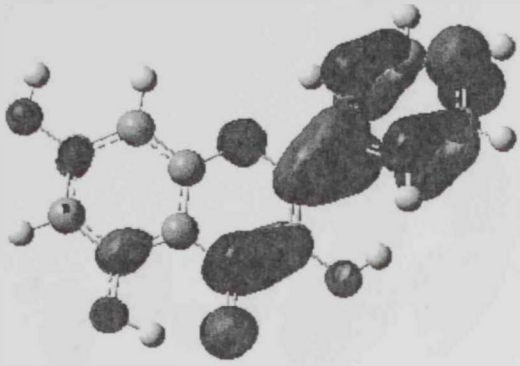
Kaempferol- HOMO



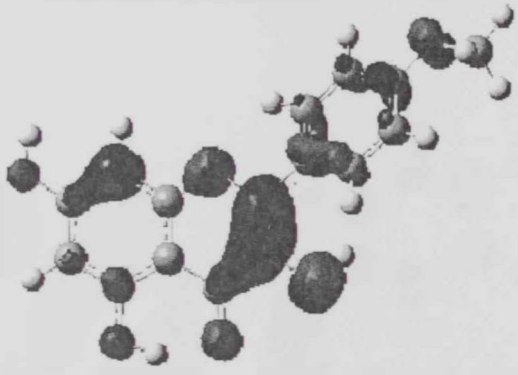
Kaempferol- LUMO



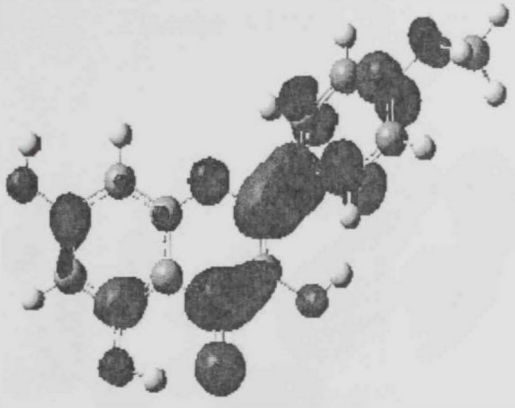
Galangin- HOMO



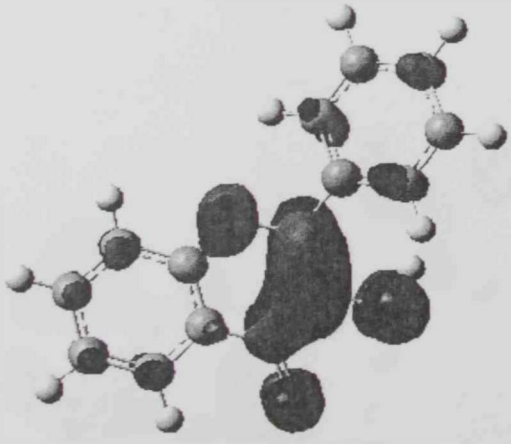
Galangin- LUMO



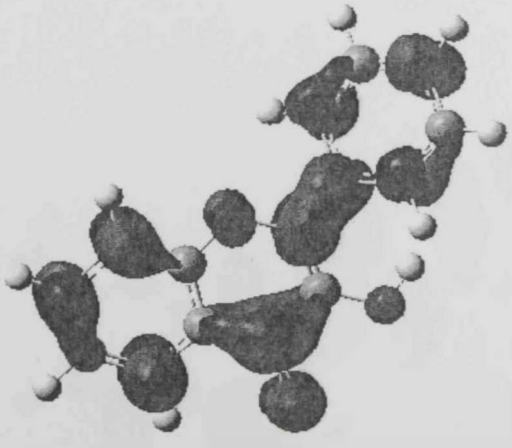
Kaempferide- HOMO



Kaempferide- LUMO

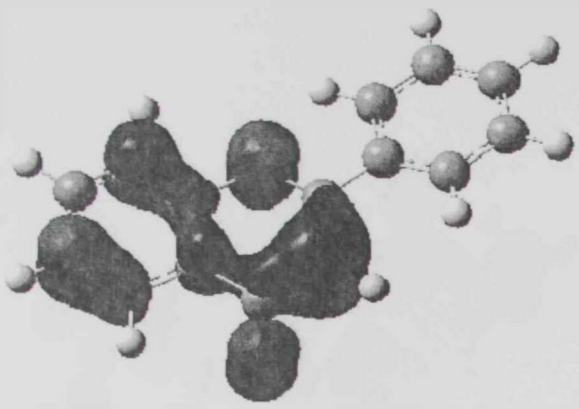


3-Hydroxy flavone- HOMO

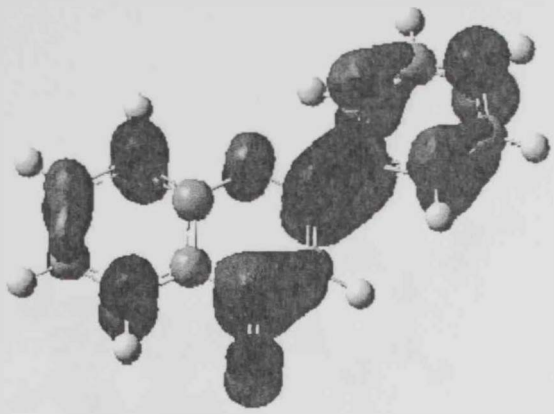


3-Hydroxy flavone- HOMO

Charge distribution of the HOMO-LUMO (isovalue of 0.04) in some optimized flavonoes.

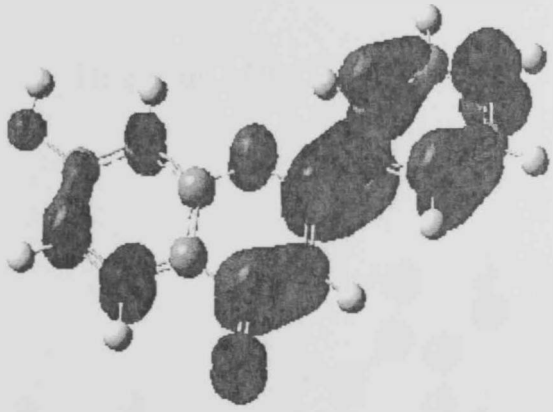


Flavone- MOMO

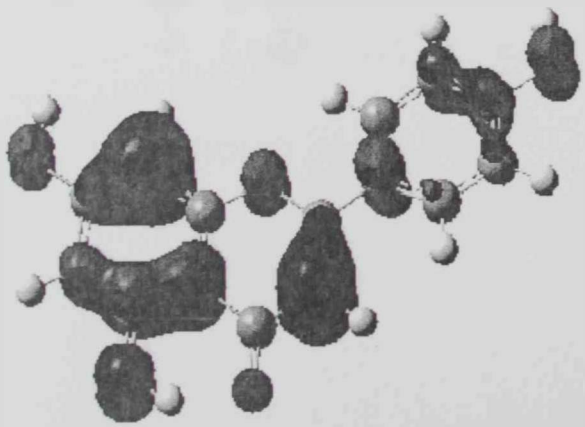


Flavone- LUMO

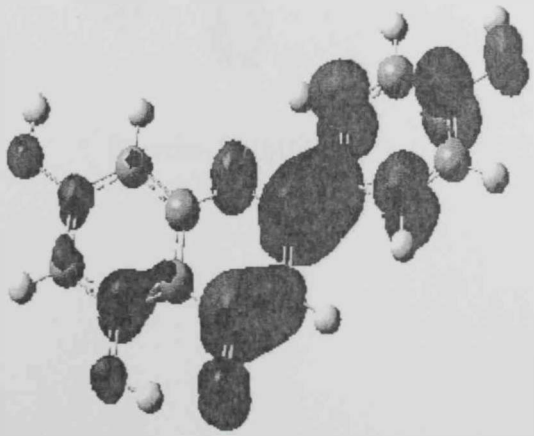
7-Hydroxy flavone- HOMO



7-Hydroxy flavone- LUMO

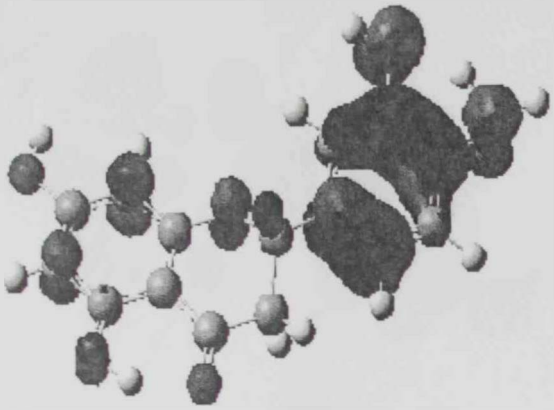


Apigenin- HOMO

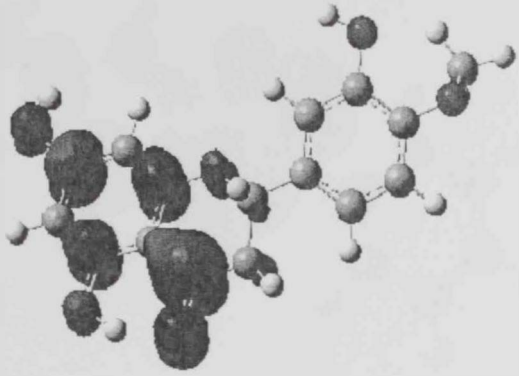


Apigenin- HOMO

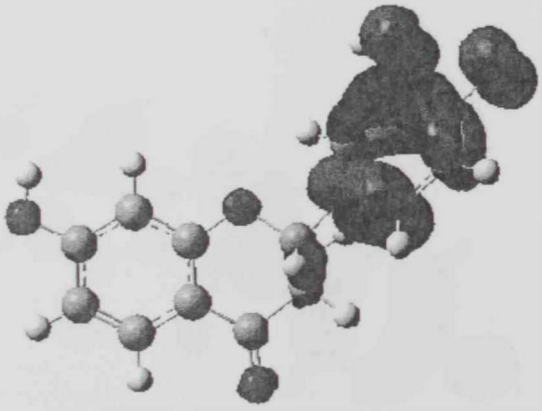
Charge distribution of the HOMO-LUMO (isovalue of 0.04) in some optimized flavanones.



Hespertin- HOMO



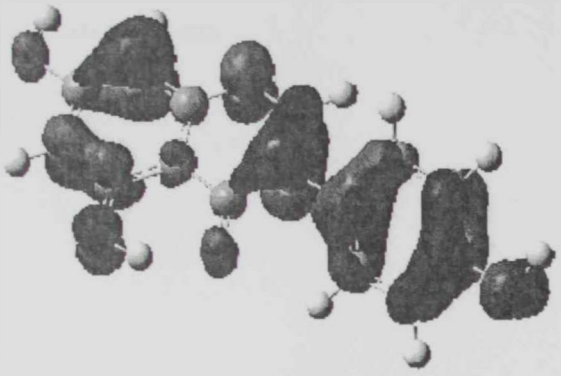
Hespertin- LUMO



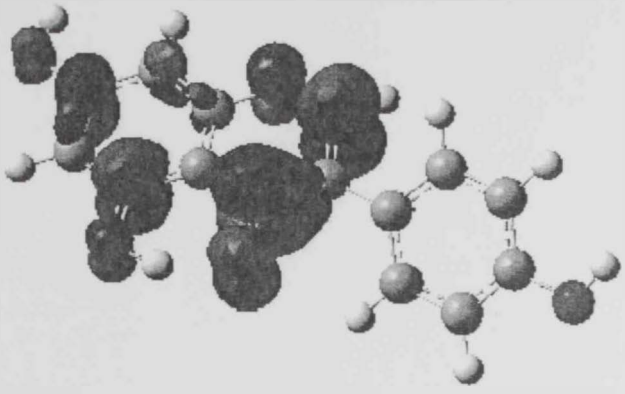
Fustin- HOMO

Fustin- LUMO

Charge distribution of the HOMO-LUMO (isovalue of 0.04) in some optimized isoflavones.

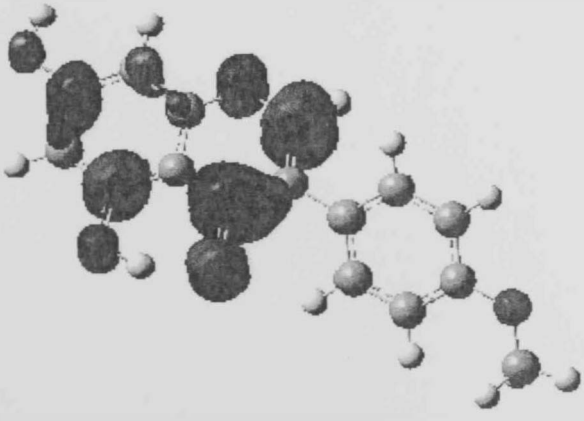


Genistein- HOMO



Genistein- LUMO

Biochanin A- HOMO



Biochanin A- HOMO

Appendix III

Sample of the calculations

Here we list calculation samples of some flavonoids from different classes conducted by Gaussian program.

Class: Flavonol, quercetin

Entering Link 1 = C:\G98W\l1.exe PID= 2144.

Carnegie Office Park, Building 6, Pittsburgh, PA 15106 USA

Warning -- This program may not be used in any manner that
competes with the business of Gaussian, Inc. or will provide
assistance to any competitor of Gaussian, Inc. The licensee
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Gaussian, Inc. access to this program. By using this program,
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Cite this work as:
Gaussian 98, Revision A.11.4,
M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria,
M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr.,
R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam,
A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi,
V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo,
S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui,
K. Morokuma, N. Rega, P. Salvador, J. J. Dannenberg, D. K. Malick,
A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski,
J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko,
P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox,
T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe,
P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres,
C. Gonzalez, M. Head-Gordon, E. S. Replogle, and J. A. Pople,
Gaussian, Inc., Pittsburgh PA, 2002.

****
Gaussian 98: x86-Win32-G98RevA.11.4 7-May-2002
14-Dec-2005
****
Default route: MaxDisk=2000MB

# B3LYP/6-311+G(2d,P)//B3LYP/6-31G(d) test scf=tight gfinput gfprint p
op=reg

1/18=20,38=1/1,3;
2/9=110,17=6,18=5,40=1/2;
3/5=1,6=6,7=1,11=2,24=11,25=1,30=1/1,2,3;
4//1;
5/5=2,32=2,38=4,42=-5/2;
6/28=1/1;
7//1,2,3,16;
1/18=20/3(1);

```
99/13=2/99(9);
2/9=110/2;
3/5=1,6=6,7=1,11=2,25=1,30=1/1,2,3;
4/5=5,16=2/1;
5/5=2,32=2,38=4,42=-5/2;
7//1,2,3,16;
1/18=20/3(-5);
2/9=110/2;
6/19=2,28=1/1;
99/9=1,13=2/99;
2/9=110/2;
3/5=4,6=6,7=112,11=2,25=1,30=1/1,2,3;
4/5=1/1;
5/5=2,32=2,42=-5/2;
6/28=1/1;
99/9=1/99;
------
QU.PDB
------
Symbolic Z-matrix:
Charge =  0 Multiplicity = 1
C
C             1  R2
C             1  R3     2  A3
O             1  R4     2  A4     3  D4     0
C             2  R5     1  A5     3  D5     0
C             3  R6     1  A6     2  D6     0
H             2  R7     1  A7     5  D7     0
H             3  R8     1  A8     6  D8     0
H             4  R9     1  A9     2  D9     0
C             5  R10    2  A10    1  D10    0
O             6  R11    3  A11    1  D11    0
O             5  R12    2  A12    10 D12    0
C             10 R13    5  A13    2  D13    0
C             11 R14    6  A14    3  D14    0
H             12 R15    5  A15    2  D15    0
C             13 R16    10 A16    5  D16    0
C             14 R17    11 A17    6  D17    0
O             13 R18    10 A18    16 D18    0
C             17 R19    14 A19    11 D19    0
C             17 R20    14 A20    19 D20    0
O             16 R21    13 A21    10 D21    0
C             19 R22    17 A22    14 D22    0
C             20 R23    17 A23    14 D23    0
H             19 R24    17 A24    22 D24    0
H             20 R25    17 A25    23 D25    0
H             21 R26    16 A26    13 D26    0
C             22 R27    19 A27    17 D27    0
O             23 R28    20 A28    17 D28    0
```

H	22	R29	19	A29	27	D29	0
O	27	R30	22	A30	19	D30	0
H	28	R31	23	A31	20	D31	0
H	30	R32	27	A32	22	D32	0

Variables:

R2	1.39532
R3	1.39772
R4	1.35995
R5	1.40067
R6	1.397
R7	1.10215
R8	1.10275
R9	0.97109
R10	1.40806
R11	1.36312
R12	1.36224
R13	1.48566
R14	1.36489
R15	0.96652
R16	1.48716
R17	1.48795
R18	1.22717
R19	1.39973
R20	1.39945
R21	1.35986
R22	1.39453
R23	1.39797
R24	1.1021
R25	1.10222
R26	0.97052
R27	1.39625
R28	1.35914
R29	1.10395
R30	1.35973
R31	0.97149
R32	0.97138
A3	117.26568
A4	121.663
A5	122.86625
A6	122.85739
A7	118.38491
A8	118.39896
A9	107.56247
A10	117.51418
A11	118.91836
A12	118.51639
A13	121.54613
A14	118.56412
A15	111.51036

A16	116.29482
A17	117.9611
A18	123.53677
A19	122.92364
A20	118.97331
A21	116.71162
A22	120.2439
A23	122.0121
A24	120.75486
A25	119.82196
A26	109.19993
A27	121.3337
A28	120.77947
A29	119.43663
A30	121.69793
A31	107.90624
A32	106.3833
D4	179.11734
D5	0.75514
D6	-0.72169
D7	-179.9624
D8	179.52109
D9	-179.17899
D10	-0.41971
D11	178.12007
D12	-179.41919
D13	178.72926
D14	165.13805
D15	175.67859
D16	-169.80393
D17	-168.39562
D18	177.83647
D19	139.5242
D20	-177.43745
D21	173.89018
D22	179.46499
D23	-179.52237
D24	179.08908
D25	178.50319
D26	171.35261
D27	1.31807
D28	178.67646
D29	179.19267
D30	179.87805
D31	0.21451
D32	-178.77515

GradGradGradGradGradGradGradGradGradGradGradGradGradGradGradGradGradGrad

! Initial Parameters !
! (Angstroms and Degrees) !

Name	Definition	Value	Derivative Info.
R1	R(1,2)	1.3953	estimate D2E/DX2
R2	R(1,3)	1.3977	estimate D2E/DX2
R3	R(1,4)	1.36	estimate D2E/DX2
R4	R(2,5)	1.4007	estimate D2E/DX2
R5	R(2,7)	1.1022	estimate D2E/DX2
R6	R(3,6)	1.397	estimate D2E/DX2
R7	R(3,8)	1.1028	estimate D2E/DX2
R8	R(4,9)	0.9711	estimate D2E/DX2
R9	R(5,10)	1.4081	estimate D2E/DX2
R10	R(5,12)	1.3622	estimate D2E/DX2
R11	R(6,10)	1.4051	estimate D2E/DX2
R12	R(6,11)	1.3631	estimate D2E/DX2
R13	R(10,13)	1.4857	estimate D2E/DX2
R14	R(11,14)	1.3649	estimate D2E/DX2
R15	R(12,15)	0.9665	estimate D2E/DX2
R16	R(13,16)	1.4872	estimate D2E/DX2
R17	R(13,18)	1.2272	estimate D2E/DX2
R18	R(14,16)	1.3528	estimate D2E/DX2
R19	R(14,17)	1.4879	estimate D2E/DX2
R20	R(16,21)	1.3599	estimate D2E/DX2
R21	R(17,19)	1.3997	estimate D2E/DX2
R22	R(17,20)	1.3994	estimate D2E/DX2
R23	R(19,22)	1.3945	estimate D2E/DX2
R24	R(19,24)	1.1021	estimate D2E/DX2
R25	R(20,23)	1.398	estimate D2E/DX2
R26	R(20,25)	1.1022	estimate D2E/DX2
R27	R(21,26)	0.9705	estimate D2E/DX2
R28	R(22,27)	1.3963	estimate D2E/DX2
R29	R(22,29)	1.104	estimate D2E/DX2
R30	R(23,27)	1.4006	estimate D2E/DX2
R31	R(23,28)	1.3591	estimate D2E/DX2
R32	R(27,30)	1.3597	estimate D2E/DX2
R33	R(28,31)	0.9715	estimate D2E/DX2
R34	R(30,32)	0.9714	estimate D2E/DX2
A1	A(2,1,3)	117.2657	estimate D2E/DX2
A2	A(2,1,4)	121.663	estimate D2E/DX2
A3	A(3,1,4)	121.0653	estimate D2E/DX2
A4	A(1,2,5)	122.8663	estimate D2E/DX2
A5	A(1,2,7)	118.3849	estimate D2E/DX2
A6	A(5,2,7)	118.7488	estimate D2E/DX2
A7	A(1,3,6)	122.8574	estimate D2E/DX2
A8	A(1,3,8)	118.399	estimate D2E/DX2
A9	A(6,3,8)	118.742	estimate D2E/DX2

```
! A10   A(1,4,9)        107.5625     estimate D2E/DX2      !
! A11   A(2,5,10)       117.5142     estimate D2E/DX2      !
! A12   A(2,5,12)       118.5164     estimate D2E/DX2      !
! A13   A(10,5,12)      123.9667     estimate D2E/DX2      !
! A14   A(3,6,10)       117.7035     estimate D2E/DX2      !
! A15   A(3,6,11)       118.9184     estimate D2E/DX2      !
! A16   A(10,6,11)      123.3377     estimate D2E/DX2      !
! A17   A(5,10,6)       121.7889     estimate D2E/DX2      !
! A18   A(5,10,13)      121.5461     estimate D2E/DX2      !
! A19   A(6,10,13)      116.6532     estimate D2E/DX2      !
! A20   A(6,11,14)      118.5641     estimate D2E/DX2      !
! A21   A(5,12,15)      111.5104     estimate D2E/DX2      !
! A22   A(10,13,16)     116.2948     estimate D2E/DX2      !
! A23   A(10,13,18)     123.5368     estimate D2E/DX2      !
! A24   A(16,13,18)     120.1331     estimate D2E/DX2      !
! A25   A(11,14,16)     123.2383     estimate D2E/DX2      !
! A26   A(11,14,17)     117.9611     estimate D2E/DX2      !
! A27   A(16,14,17)     118.4887     estimate D2E/DX2      !
! A28   A(13,16,14)     118.9766     estimate D2E/DX2      !
! A29   A(13,16,21)     116.7116     estimate D2E/DX2      !
! A30   A(14,16,21)     124.2691     estimate D2E/DX2      !
! A31   A(14,17,19)     122.9236     estimate D2E/DX2      !
! A32   A(14,17,20)     118.9733     estimate D2E/DX2      !
! A33   A(19,17,20)     118.0554     estimate D2E/DX2      !
! A34   A(17,19,22)     120.2439     estimate D2E/DX2      !
! A35   A(17,19,24)     120.7549     estimate D2E/DX2      !
! A36   A(22,19,24)     118.9951     estimate D2E/DX2      !
! A37   A(17,20,23)     122.0121     estimate D2E/DX2      !
! A38   A(17,20,25)     119.822      estimate D2E/DX2      !
! A39   A(23,20,25)     118.1496     estimate D2E/DX2      !
! A40   A(16,21,26)     109.1999     estimate D2E/DX2      !
! A41   A(19,22,27)     121.3337     estimate D2E/DX2      !
! A42   A(19,22,29)     119.4366     estimate D2E/DX2      !
! A43   A(27,22,29)     119.2248     estimate D2E/DX2      !
! A44   A(20,23,27)     119.315      estimate D2E/DX2      !
! A45   A(20,23,28)     120.7795     estimate D2E/DX2      !
! A46   A(27,23,28)     119.9043     estimate D2E/DX2      !
! A47   A(22,27,23)     118.9652     estimate D2E/DX2      !
! A48   A(22,27,30)     121.6979     estimate D2E/DX2      !
! A49   A(23,27,30)     119.3315     estimate D2E/DX2      !
! A50   A(23,28,31)     107.9062     estimate D2E/DX2      !
! A51   A(27,30,32)     106.3833     estimate D2E/DX2      !
! D1    D(3,1,2,5)        0.7551     estimate D2E/DX2      !
! D2    D(3,1,2,7)     -179.2073     estimate D2E/DX2      !
! D3    D(4,1,2,5)      179.8725     estimate D2E/DX2      !
! D4    D(4,1,2,7)       -0.0899     estimate D2E/DX2      !
! D5    D(2,1,3,6)       -0.7217     estimate D2E/DX2      !
! D6    D(2,1,3,8)      178.7994     estimate D2E/DX2      !
! D7    D(4,1,3,6)     -179.8446     estimate D2E/DX2      !
```

```
! D8   D(4,1,3,8)        -0.3235      estimate D2E/DX2      !
! D9   D(2,1,4,9)        -179.179     estimate D2E/DX2      !
! D10  D(3,1,4,9)         -0.095      estimate D2E/DX2      !
! D11  D(1,2,5,10)        -0.4197     estimate D2E/DX2      !
! D12  D(1,2,5,12)        -179.8389   estimate D2E/DX2      !
! D13  D(7,2,5,10)        179.5426    estimate D2E/DX2      !
! D14  D(7,2,5,12)        0.1234      estimate D2E/DX2      !
! D15  D(1,3,6,10)        0.3548      estimate D2E/DX2      !
! D16  D(1,3,6,11)        178.1201    estimate D2E/DX2      !
! D17  D(8,3,6,10)        -179.1647   estimate D2E/DX2      !
! D18  D(8,3,6,11)        -1.3995     estimate D2E/DX2      !
! D19  D(2,5,10,6)        0.0211      estimate D2E/DX2      !
! D20  D(2,5,10,13)       178.7293    estimate D2E/DX2      !
! D21  D(12,5,10,6)       179.4057    estimate D2E/DX2      !
! D22  D(12,5,10,13)      -1.8861     estimate D2E/DX2      !
! D23  D(2,5,12,15)       175.6786    estimate D2E/DX2      !
! D24  D(10,5,12,15)      -3.7003     estimate D2E/DX2      !
! D25  D(3,6,10,5)        0.0096      estimate D2E/DX2      !
! D26  D(3,6,10,13)       -178.7586   estimate D2E/DX2      !
! D27  D(11,6,10,5)       -177.649    estimate D2E/DX2      !
! D28  D(11,6,10,13)      3.5828      estimate D2E/DX2      !
! D29  D(3,6,11,14)       165.1381    estimate D2E/DX2      !
! D30  D(10,6,11,14)      -17.2303    estimate D2E/DX2      !
! D31  D(5,10,13,16)      -169.8039   estimate D2E/DX2      !
! D32  D(5,10,13,18)      8.0325      estimate D2E/DX2      !
! D33  D(6,10,13,16)      8.9675      estimate D2E/DX2      !
! D34  D(6,10,13,18)      -173.196    estimate D2E/DX2      !
! D35  D(6,11,14,16)      18.1248     estimate D2E/DX2      !
! D36  D(6,11,14,17)      -168.3956   estimate D2E/DX2      !
! D37  D(10,13,16,14)     -8.3826     estimate D2E/DX2      !
! D38  D(10,13,16,21)     173.8902    estimate D2E/DX2      !
! D39  D(18,13,16,14)     173.7025    estimate D2E/DX2      !
! D40  D(18,13,16,21)     -4.0247     estimate D2E/DX2      !
! D41  D(11,14,16,13)     -5.1632     estimate D2E/DX2      !
! D42  D(11,14,16,21)     172.38      estimate D2E/DX2      !
! D43  D(17,14,16,13)     -178.6103   estimate D2E/DX2      !
! D44  D(17,14,16,21)     -1.0671     estimate D2E/DX2      !
! D45  D(11,14,17,19)     139.5242    estimate D2E/DX2      !
! D46  D(11,14,17,20)     -37.9132    estimate D2E/DX2      !
! D47  D(16,14,17,19)     -46.6795    estimate D2E/DX2      !
! D48  D(16,14,17,20)     135.883     estimate D2E/DX2      !
! D49  D(13,16,21,26)     171.3526    estimate D2E/DX2      !
! D50  D(14,16,21,26)     -6.2414     estimate D2E/DX2      !
! D51  D(14,17,19,22)     179.465     estimate D2E/DX2      !
! D52  D(14,17,19,24)     -1.4459     estimate D2E/DX2      !
! D53  D(20,17,19,22)     -3.0753     estimate D2E/DX2      !
! D54  D(20,17,19,24)     176.0137    estimate D2E/DX2      !
! D55  D(14,17,20,23)     -179.5224   estimate D2E/DX2      !
! D56  D(14,17,20,25)     -1.0192     estimate D2E/DX2      !
```

```
! D57   D(19,17,20,23)      2.915       estimate D2E/DX2        !
! D58   D(19,17,20,25)   -178.5819       estimate D2E/DX2        !
! D59   D(17,19,22,27)      1.3181       estimate D2E/DX2        !
! D60   D(17,19,22,29)   -179.4893       estimate D2E/DX2        !
! D61   D(24,19,22,27)   -177.7869       estimate D2E/DX2        !
! D62   D(24,19,22,29)      1.4057       estimate D2E/DX2        !
! D63   D(17,20,23,27)     -0.9236       estimate D2E/DX2        !
! D64   D(17,20,23,28)    178.6765       estimate D2E/DX2        !
! D65   D(25,20,23,27)   -179.4508       estimate D2E/DX2        !
! D66   D(25,20,23,28)      0.1493       estimate D2E/DX2        !
! D67   D(19,22,27,23)      0.7298       estimate D2E/DX2        !
! D68   D(19,22,27,30)    179.8781       estimate D2E/DX2        !
! D69   D(29,22,27,23)   -178.4646       estimate D2E/DX2        !
! D70   D(29,22,27,30)      0.6837       estimate D2E/DX2        !
! D71   D(20,23,27,22)     -0.9259       estimate D2E/DX2        !
! D72   D(20,23,27,30)    179.9053       estimate D2E/DX2        !
! D73   D(28,23,27,22)    179.4705       estimate D2E/DX2        !
! D74   D(28,23,27,30)      0.3018       estimate D2E/DX2        !
! D75   D(20,23,28,31)      0.2145       estimate D2E/DX2        !
! D76   D(27,23,28,31)    179.8122       estimate D2E/DX2        !
! D77   D(22,27,30,32)   -178.7752       estimate D2E/DX2        !
! D78   D(23,27,30,32)      0.3701       estimate D2E/DX2        !
---------------------------------------------------------------------------
Trust Radius=3.00D-01 FncErr=1.00D-07 GrdErr=1.00D-07
Number of steps in this run= 173 maximum allowed number of steps= 192.

GradGradGradGradGradGradGradGradGradGradGradGradGradGradGradGradGrad

Total atomic charges:
                1
 1  C    0.179906
 2  C    0.196509
 3  C   -0.232307
 4  O   -0.406331
 5  C    0.070481
 6  C    0.184607
 7  H    0.110723
 8  H    0.080632
 9  H    0.274093
10  C   -0.238243
11  O   -0.494440
12  O   -0.527670
13  C    0.678207
14  C    0.014950
15  H    0.374377
16  C    0.155621
17  C    0.996578
18  O   -0.596556
19  C   -0.641775
```

20 C -0.421481
21 O -0.462082
22 C -0.163392
23 C 0.321073
24 H 0.137610
25 H 0.096468
26 H 0.289627
27 C 0.300379
28 O -0.518032
29 H 0.107829
30 O -0.461428
31 H 0.289409
32 H 0.304659
Sum of Mulliken charges= 0.00000
Atomic charges with hydrogens summed into heavy atoms:
1
1 C 0.179906
2 C 0.307232
3 C -0.151675
4 O -0.132238
5 C 0.070481
6 C 0.184607
7 H 0.000000
8 H 0.000000
9 H 0.000000
10 C -0.238243
11 O -0.494440
12 O -0.153293
13 C 0.678207
14 C 0.014950
15 H 0.000000
16 C 0.155621
17 C 0.996578
18 O -0.596556
19 C -0.504165
20 C -0.325013
21 O -0.172455
22 C -0.055563
23 C 0.321073
24 H 0.000000
25 H 0.000000
26 H 0.000000
27 C 0.300379
28 O -0.228623
29 H 0.000000
30 O -0.156770
31 H 0.000000
32 H 0.000000
Sum of Mulliken charges= 0.00000

Electronic spatial extent (au): <R**2>= 8250.5401
Charge= 0.0000 electrons
Dipole moment (Debye):
X= -4.9526 Y= 6.2130 Z= 0.6258 Tot= 7.9700
Quadrupole moment (Debye-Ang):
XX= -139.4652 YY= -119.6203 ZZ= -125.8239
XY= 5.8781 XZ= -5.4224 YZ= 5.5650
Octapole moment (Debye-Ang**2):
XXX= -75.5842 YYY= 112.3798 ZZZ= 8.2667 XYY= -44.2164
XXY= 38.0699 XXZ= 15.0192 XZZ= -9.7424 YZZ= 0.9568
YYZ= -0.3874 XYZ= -17.9841
Hexadecapole moment (Debye-Ang**3):
XXXX= -8862.2072 YYYY= -1954.2338 ZZZZ= -257.7153 XXXY= -210.2875
XXXZ= -108.7387 YYYX= 95.2761 YYYZ= 26.1339 ZZZX= -18.7392
ZZZY= 10.0378 XXYY= -1659.8043 XXZZ= -1456.5245 YYZZ= -374.1363
XXYZ= 66.3214 YYXZ= -56.2503 ZZXY= -25.4014
N-N= 1.717622352132D+03 E-N=-6.019835547531D+03 KE= 1.100199664521D+03

Test job not archived.
1|1|UNPC-UNK|SP|RB3LYP|6-31G(d)|C15H10O7|PCUSER|15-Dec-2005|0||# B3LYP
/6-311+G(2D,P)//B3LYP/6-31G(D) TEST SCF=TIGHT GFINPUT GFPRINT
POP=REG|
|QU.PDB||0,1|C,-4.1546928949,-0.4259841573,0.0274531049|C,-4.262621259
,-0.3869299221,1.4232018263|C,-2.9209522408,-0.3044282453,-0.621265009
3|O,-5.3138262025,-0.5879826201,-0.6674133334|C,-3.1184092131,-0.22008
62542,2.1966920764|C,-1.7855658697,-0.1374603359,0.1668643179|H,-5.231
6424118,-0.4849286176,1.8977356355|H,-2.8326754106,-0.3284495501,-1.70
29705749|H,-5.1191487243,-0.589959704,-1.6176353942|C,-1.8412621281,-0
.0870803031,1.5688364228|O,-0.5945724073,-0.0124941351,-0.4861954536|O
,-3.2227957184,-0.1857784512,3.5307537326|C,-0.631990064,0.0698837135,
2.3532302607|C,0.5772073178,0.1700859071,0.2086343702|H,-2.2972464572,
-0.0687013778,3.8866889767|C,0.604250929,0.1767393253,1.5745784038|C,1
.7413831514,0.2883230535,-0.682476862|O,-0.6288130203,0.1022443684,3.6
003093202|C,2.8195481185,1.1381673859,-0.379206935|C,1.7812822396,-0.4
507356884,-1.8822124589|O,1.7417260775,0.2722889904,2.3176515233|C,3.9
165908049,1.2352690559,-1.2363876098|C,2.8713741898,-0.3487756391,-2.7
304332037|H,2.7822496886,1.7709965508,0.5019974492|H,0.9511673503,-1.1
02879721,-2.1395466765|H,2.5068482873,0.0973440772,1.7435771031|C,3.95
65873984,0.4925165871,-2.4122076672|O,3.0062126242,-1.0314360062,-3.91
57373032|H,4.7453914,1.8987155224,-1.0118051684|O,5.023618349,0.585472
5541,-3.2463250698|H,2.2283327724,-1.5892189689,-4.0672334904|H,4.8579
430087,0.0015372899,-4.0069614121||Version=x86-Win32-G98RevA.11.4|HF=-
1104.530505|RMSD=3.362e-009|Dipole=0.6023198,-0.6008253,-3.0179975|PG=
C01 [X(C15H10O7)]||@

Class: Isoflavone, diadzin

Entering Link 1 = C:\G98W\l1.exe PID= 3528.

52.227-19.

Gaussian, Inc.
Carnegie Office Park, Building 6, Pittsburgh, PA 15106 USA

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A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi,
V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo,
S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui,
K. Morokuma, N. Rega, P. Salvador, J. J. Dannenberg, D. K. Malick,
A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski,
J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko,
P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox,
T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe,
P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres,
C. Gonzalez, M. Head-Gordon, E. S. Replogle, and J. A. Pople,
Gaussian, Inc., Pittsburgh PA, 2002.

****
Gaussian 98: x86-Win32-G98RevA.11.4 7-May-2002
27-Nov-2005
****
Default route: MaxDisk=2000MB

# B3LYP/6-311+G(2d,p)//B3LYP/6-31G(d) test scf=tight gfinput gfprint p
op=reg

```
---------------------------------------------------------------------
1/18=20,38=1/1,3;
2/9=110,17=6,18=5,40=1/2;
3/5=1,6=6,7=1,11=2,24=11,25=1,30=1/1,2,3;
4//1;
5/5=2,32=2,38=4,42=-5/2;
6/28=1/1;
7//1,2,3,16;
1/18=20/3(1);
99/13=2/99(9);
2/9=110/2;
3/5=1,6=6,7=1,11=2,25=1,30=1/1,2,3;
4/5=5,16=2/1;
5/5=2,32=2,38=4,42=-5/2;
7//1,2,3,16;
1/18=20/3(-5);
2/9=110/2;
6/19=2,28=1/1;
99/9=1,13=2/99;
2/9=110/2;
3/5=4,6=6,7=112,11=2,25=1,30=1/1,2,3;
4/5=1/1;
5/5=2,32=2,42=-5/2;
6/28=1/1;
99/9=1/99;
------
DA.PDB
------
Symbolic Z-matrix:
Charge =  0 Multiplicity = 1
C
C             1   R2
C             1   R3      2   A3
O             1   R4      2   A4      3   D4      0
C             2   R5      1   A5      3   D5      0
C             3   R6      1   A6      2   D6      0
H             2   R7      1   A7      5   D7      0
H             3   R8      1   A8      6   D8      0
H             4   R9      1   A9      2   D9      0
C             5   R10     2   A10     1   D10     0
O             6   R11     3   A11     1   D11     0
H             5   R12     2   A12     10  D12     0
C             10  R13     5   A13     2   D13     0
C             11  R14     6   A14     3   D14     0
C             13  R15     10  A15     5   D15     0
```

O	13	R16	10	A16	15	D16	0
H	14	R17	11	A17	6	D17	0
C	15	R18	13	A18	10	D18	0
C	18	R19	15	A19	13	D19	0
C	18	R20	15	A20	19	D20	0
C	19	R21	18	A21	15	D21	0
C	20	R22	18	A22	15	D22	0
H	19	R23	18	A23	21	D23	0
H	20	R24	18	A24	22	D24	0
C	21	R25	19	A25	18	D25	0
H	21	R26	19	A26	25	D26	0
H	22	R27	20	A27	18	D27	0
O	25	R28	21	A28	19	D28	0
H	28	R29	25	A29	21	D29	0

Variables:

R2	1.39726
R3	1.39967
R4	1.35971
R5	1.39471
R6	1.39949
R7	1.10234
R8	1.1018
R9	0.97095
R10	1.40086
R11	1.36546
R12	1.1018
R13	1.48286
R14	1.36198
R15	1.48437
R16	1.22815
R17	1.10492
R18	1.48558
R19	1.40023
R20	1.40057
R21	1.39657
R22	1.39468
R23	1.10199
R24	1.10225
R25	1.3991
R26	1.10203
R27	1.10292
R28	1.35947
R29	0.9709
A3	117.75347
A4	121.51609

A5	120.79098
A6	123.36962
A7	119.40307
A8	118.31618
A9	107.54171
A10	119.9864
A11	119.6047
A12	118.93856
A13	119.99204
A14	116.96412
A15	114.90359
A16	122.41188
A17	114.40909
A18	120.10324
A19	121.6189
A20	120.16907
A21	120.57884
A22	120.75109
A23	120.44807
A24	120.31114
A25	121.49887
A26	119.38395
A27	119.3367
A28	121.61841
A29	107.45789
D4	179.55317
D5	0.55075
D6	-0.80555
D7	179.57237
D8	-179.80915
D9	-177.64173
D10	-0.11815
D11	-179.87853
D12	179.27252
D13	178.48777
D14	172.26172
D15	-173.57309
D16	176.9365
D17	-174.06442
D18	174.91236
D19	47.22629
D20	178.6849
D21	178.90589
D22	-179.79016
D23	-177.84561

D24	-178.2409
D25	0.47064
D26	-179.48954
D27	-178.63403
D28	179.42944
D29	-177.42211

GradGradGradGradGradGradGradGradGradGradGradGradGradGradGradGradGradGr
adGrad

Total atomic charges:

		1
1	C	0.060913
2	C	0.165173
3	C	-0.092611
4	O	-0.414990
5	C	-0.703073
6	C	0.216084
7	H	0.103291
8	H	0.089705
9	H	0.276837
10	C	0.094307
11	O	-0.499266
12	H	0.134817
13	C	0.762466
14	C	0.067882
15	C	-0.254163
16	O	-0.548188
17	H	0.124452
18	C	1.070269
19	C	-0.285461
20	C	-0.446437
21	C	-0.160508
22	C	-0.283147
23	H	0.117654
24	H	0.086466
25	C	0.323368
26	H	0.096177
27	H	0.069657
28	O	-0.446895
29	H	0.275219

Sum of Mulliken charges= 0.00000

Atomic charges with hydrogens summed into heavy atoms:

		1
1	C	0.060913
2	C	0.268465
3	C	-0.002905
4	O	-0.138153
5	C	-0.568256
6	C	0.216084
7	H	0.000000
8	H	0.000000
9	H	0.000000
10	C	0.094307
11	O	-0.499266
12	H	0.000000
13	C	0.762466
14	C	0.192334
15	C	-0.254163
16	O	-0.548188
17	H	0.000000
18	C	1.070269
19	C	-0.167807
20	C	-0.359971
21	C	-0.064331
22	C	-0.213490
23	H	0.000000
24	H	0.000000
25	C	0.323368
26	H	0.000000
27	H	0.000000
28	O	-0.171676
29	H	0.000000

Sum of Mulliken charges= 0.00000
Electronic spatial extent (au): <R**2>= 6754.3713
Charge= 0.0000 electrons
Dipole moment (Debye):
X= 1.4679 Y= -4.9775 Z= 0.0237 Tot= 5.1895
Quadrupole moment (Debye-Ang):
XX= -91.5540 YY= -103.8672 ZZ= -111.4944
XY= 2.2109 XZ= -5.6451 YZ= -3.0287
Octapole moment (Debye-Ang**2):
XXX= 1.0814 YYY= -19.5037 ZZZ= -0.4129 XYY= 18.9909
XXY= -105.2294 XXZ= 27.1667 XZZ= -3.1541 YZZ= -1.2089
YYZ= -2.2079 XYZ= 21.0969
Hexadecapole moment (Debye-Ang**3):
XXXX= -7211.4629 YYYY= -928.9488 ZZZZ= -209.4058 XXXY= 25.3235
XXXZ= -296.4201 YYYX= -20.9604 YYYZ= -5.6229 ZZZX= -8.6707
ZZZY= 0.7378 XXYY= -1219.3311 XXZZ= -1373.6054 YYZZ= -189.2986
XXYZ= -37.8989 YYXZ= 2.4984 ZZXY= -8.7736
N-N= 1.287432600764D+03 E-N=-4.626574280094D+03 KE= 8.751349381887D+02

Final structure in terms of initial Z-matrix:
C
C,1,R2
C,1,R3,2,A3
O,1,R4,2,A4,3,D4,0
C,2,R5,1,A5,3,D5,0
C,3,R6,1,A6,2,D6,0
H,2,R7,1,A7,5,D7,0
H,3,R8,1,A8,6,D8,0
H,4,R9,1,A9,2,D9,0
C,5,R10,2,A10,1,D10,0
O,6,R11,3,A11,1,D11,0
H,5,R12,2,A12,10,D12,0
C,10,R13,5,A13,2,D13,0
C,11,R14,6,A14,3,D14,0
C,13,R15,10,A15,5,D15,0
O,13,R16,10,A16,15,D16,0
H,14,R17,11,A17,6,D17,0
C,15,R18,13,A18,10,D18,0
C,18,R19,15,A19,13,D19,0
C,18,R20,15,A20,19,D20,0
C,19,R21,18,A21,15,D21,0
C,20,R22,18,A22,15,D22,0
H,19,R23,18,A23,21,D23,0
H,20,R24,18,A24,22,D24,0
C,21,R25,19,A25,18,D25,0
H,21,R26,19,A26,25,D26,0
H,22,R27,20,A27,18,D27,0
O,25,R28,21,A28,19,D28,0
H,28,R29,25,A29,21,D29,0

Variables:

R2=1.41034101
R3=1.39241985
R4=1.36112845
R5=1.38060367
R6=1.39722206
R7=1.08484764
R8=1.0865048
R9=0.97020392
R10=1.40878348
R11=1.36789319
R12=1.08509258
R13=1.47509176
R14=1.35672286
R15=1.48249855
R16=1.23101198
R17=1.08377587
R18=1.4812586
R19=1.40823321

R20=1.40363568
R21=1.38927119
R22=1.39325374
R23=1.08262639
R24=1.0868486
R25=1.39929068
R26=1.08545381
R27=1.0887675
R28=1.36715213
R29=0.96984507
A3=120.62089811
A4=116.82168009
A5=119.5791068
A6=118.53556816
A7=118.64176419
A8=121.99210076
A9=109.43234304
A10=121.30710283
A11=116.58801799
A12=121.29433254
A13=121.22006595
A14=118.69175899
A15=114.43359571
A16=121.976276
A17=110.2499686
A18=121.59213136
A19=121.53862448
A20=120.67081596
A21=121.16428155
A22=121.46908692
A23=119.31163353
A24=119.68563011
A25=120.13658243
A26=120.98585863
A27=120.0243982
A28=117.56561488
A29=108.9393865
D4=179.97025339
D5=-0.04731982
D6=-0.06910534
D7=-179.92923825
D8=179.9409726
D9=-179.90269848
D10=0.12163715
D11=179.7497336
D12=179.96181458
D13=179.8280833
D14=179.28837699
D15=-178.22176325

D16=179.94856658
D17=178.89845356
D18=176.75415637
D19=38.78399674
D20=179.11518612
D21=177.91883088
D22=-177.73768064
D23=179.42014557
D24=-178.45677279
D25=0.30640574
D26=179.88519266
D27=-179.5484132
D28=179.96299142
D29=-179.42721447

Test job not archived.
1|1|UNPC-UNK|SP|RB3LYP|6-31G(d)|C15H10O4|PCUSER|28-Nov-2005|0||# B3LYP
/6-311+G(2D,P)//B3LYP/6-31G(D) TEST SCF=TIGHT GFINPUT GFPRINT
POP=REG|
|DA.PDB||0,1|C,-4.284780417,-0.3390581458,-1.5687814154|C,-4.328094846
6,-0.4275836845,-0.161888079|C,-3.0694092294,-0.197959608,-2.233459631
4|O,-5.4758270319,-0.3990405176,-2.2248917214|C,-3.1529430737,-0.37457
14892,0.560797552|C,-1.8965906498,-0.1447612811,-1.4759003917|H,-5.292
4239211,-0.5373512066,0.3227921453|H,-3.0099801007,-0.1297348373,-3.31
61905534|H,-5.3218613588,-0.3300716091,-3.1803149279|C,-1.906021157,-0
.2308137542,-0.0788823253|O,-0.7252318553,-0.0109976777,-2.1695556695|
H,-3.153787956,-0.4414033235,1.6438297258|C,-0.6519216177,-0.170623942
3,0.6953961994|C,0.4383503203,0.0598317139,-1.4754687685|C,0.567002297
1,0.0236714714,-0.1257467421|O,-0.6374612319,-0.2740541185,1.921970125
6|H,1.2769799302,0.1442413217,-2.1567510345|C,1.9036225883,0.177637110
1,0.4938239335|C,2.2836301952,-0.5746062225,1.6220282472|C,2.842734899
9,1.0723026741,-0.0426783531|C,3.554206518,-0.4551907019,2.1710730457|
C,4.1206356175,1.1964600734,0.4983490767|H,1.5686576437,-1.2505104259,
2.0737445884|H,2.5659738845,1.7031838109,-0.8832925363|C,4.4822996793,
0.4282805776,1.6088133405|H,3.8450698888,-1.0387151692,3.0388908099|H,
4.8281143474,1.9033482766,0.0680031763|O,5.7166585314,0.5045355523,2.1
916069114|H,6.2518235962,1.1553785092,1.7113973062||Version=x86-Win32-
G98RevA.11.4|HF=-878.7724239|RMSD=1.489e-009|Dipole=0.0430506,0.448404
,-1.9913759|PG=C01 [X(C15H10O4)]||@

الملخص العربي

الفلافونيدات مركبات ذات خصائص مضادة للأكسدة، ويخضع نزوعها إلى صد وكبح التأكسد إلى تركيبها الكيميائي. ولما كانت هذه المركبات تعتمد على نواة الفلافان (flavan) فإنها عدد و موقع و نوع المجموعات الجانبية يؤثر على إزالة الشوارد الحرة. تهدف هذه الأطروحة بشكل رئيس إلى إيجاد علاقات (تركيبية- وظيفية) بواسطة طرائق وتقنيات تجريبية و حسابية.

في البداية، تمت دراسة سلسلة من الفلافونيدات التي تنتمي إلى المجموعات النموذجية (فلافونولات، فلافونات، فلافانونات، أيزوفلافونات) (flavonols, flavones, flavanones, isoflavones) وذلك من خلال تفاعل هذه الفلافونيدات مع شوارد حرة (DPPH) محضرة بالمختبر ومن ثم متابعة التفاعل بواسطة مضواء طيفي (UV-Vis Spectrophotometry) حيث أظهرت الدراسة أن التفاعل يتم على مرحلتين: سريعة وتتبعها أخرى أبطأ. وتم من خلال التفاعل تحديد معدل سرعة إزالة الشوارد الحرة وتحديد النشاط المضاد للشوارد.

ولما كانت قدرة الفلافونيدات على التأكسد تعكس قدرتها على إزالة الشوارد الحرة، فقد تم قياس الأكسدة الكهروكيميائية للفلافونيدات السبعة بواسطة مقياس التيار بالتحليل الكهربائي (cyclic voltammetry) باستخدام محاليل ذات رقم هيدروجيني (pH) مختلف. ولم يُظهر مركب "فلافون" (flavone) الذي لايحتوي على أية مجموعة هيدروكسيل أي جهد أكسدة. وتوافقت نتائج هذه الدراسة مع أنشطة الإزالة للشوارد الحرة (DPPH)، وبينت أيضًا أن نشاط الفلافونيدات يعتمد على الرقم الهيدروجيني.

كما أظهرت الدراسة التجريبية أن مجموعة كاتيكول (catechol) في الكويرستين (Quercetin) لها القدرة على إزالة أكبر عدد من الشوارد الحرة، وكما أظهر أكبر نشاط مضاد للشوارد الحرة، ومن ناحية أخرى فإن مجموعة (pyrogallol) في مركب ميرستين (Myricetin) كان له أقل جهد تأكسد.

وقد تم إجراء سلسلة من الحسابات المتعلقة بنظرية الكثافة الوظيفية (Density Functional) باستخدام برنامج جاوسيان (Gaussian) على 28 فلافونيد تابعة لمجموعات الفلافونيدات الرئيسية لتحديد المتطلبات الهيكلية للفلافونيدات لمعرفة نشاط كسح الشوارد الحرة.

ولما كانت الخصائص الكيماوية الكامنة للفلافونيدات تقيس ميلها لمنح أو اصطياد الالكترونات، وبالتالي قدرتها الكامنة المضادة للأكسدة، وتشمل هذه السمات على: الميل للتفاعل الالكتروني (EA)، القدرة على التأين (IP)، القدرة على التفاعل الكيميائي (μ)، سالبية التأين (χ)، الصلابة (η)، خاصية تقبل الالكترونات (ω). فإنه تم احتساب هذه الخصائص بشأن كل فلافونيد في كل مجموعة. وقد أظهرت الفلافونولات (Flavonols) النشاط الأكبر مقارنة بالمجموعات الأخرى.

إن العلاقات التركيبية الوظيفية للفلافونيدات تم تأكيدها بحسابات الكثافة الوظيفية التي أظهرت أن مجموعات الهيدروكسيل تزيد من نشاط الفلافونيدات، في حين أن إضافة مجموعة ميثيل تقلل من نشاطها وقدرتها على إزالة الشوارد الحرة. كما أن وجود الرابطة الثنائية ومجموعة الكربونيل في مركبات الفلافونيد تزيد من نشاطها وبالتالي قدرتها على إزالة الشوارد الحرة.

جامعة الإمارات العربية المتحدة
عمادة الدراسات العليا
برنامج ماجستير علوم البيئة

العلاقة بين التركيب الكيميائي والوظيفة الحيوية للفلافونيدات كمضادات أكسدة للجزيئات المؤكسدة النشطة

رسالة مقدمة من الطالبة
ديمة خليل راشد الجيوسي

إلى
جامعة الإمارات العربية المتحدة
استكمالاً لمتطلبات الحصول على درجة الماجستير في علوم البيئة

المشرفون

د. إحسان شحادة أستاذ مساعد في الكيمياء الفيزيائية قسم الكيمياء كلية العلوم جامعة الإمارات العربية المتحدة	د. أحمد المهدي أستاذ مشارك في الكيمياء الحيوية قسم الكيمياء كلية العلوم جامعة الإمارات العربية المتحدة

2007 -2008

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