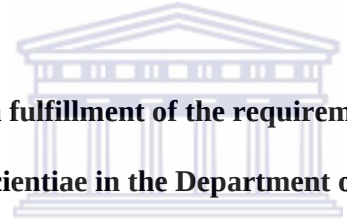
The Isolation and Electrochemical Studies of Flavanoids from *Galenia africana* and *Elytropapus rhinocerotis* from the North Western Cape

By

Khumo Gwendoline Maiko



A thesis submitted in fulfillment of the requirements for the degree of

Magister Scientiae in the Department of Chemistry

University of the Western Cape

Cape Town/South Africa

Principal supervisor: Prof. Ivan R. Green

Co-supervisor: Prof. Emmanuel I. Iwuoha

December 2010.

KEY WORDS

Flavanoids

Glassy carbon electrode

Antioxidants

Oxidation potentials

Radicals

Preparative layer chromatography

Column chromatography

Purification

Nuclear magnetic resonance

Cyclic voltammetry

Square wave voltammetry

ABSTRACT

In this study two medicinal plant species, namely *Galenia africana* and *Elytropapus rhinocerotis*, the former belonging to the family Aizoceae and the latter belonging to the family Asteraceae, have been investigated and different compounds isolated and characterized. Both species are important plants used in traditional medicine in Africa and particularly in South Africa. Flavanoids are secondary metabolites found in plants. They have a protective function against UV radiation and have a defence against invading illnesses due to their important antioxidant activity. Much of the food we eat and some beverages we drink contain flavonoids. The aim of this study was to investigate the electrochemistry of flavanoids isolated from these species. *Elytropapus rhinocerotis* contained two compounds viz., 5-hydroxy-7-methoxy-2-(4'-hydroxyphenyl chromone and 5-hydroxy-7-methoxy-2-(4'-hydroxy-3'-methoxyphenyl chromone, were isolated. From Galenia africana four known compounds, dihydroxychalcone, dihydroxy-dihydrochalcone, pinocembrin and isoliquiritigenin, were isolated. The flavanoids were isolated from the plants using column chromatography, thin layer chromatography and preparative layer chromatography in an array of solvents. The structures of the flavanoids were determined using Proton (^{1}H) and Carbon (^{13}C) Nuclear Magnetic Resonance (NMR). Electrochemical analyses on both the natural and synthetic flavanoids were performed by Cyclic Voltammetry (CV) and Square Wave Voltammetry (SWV) to give information on the accessible redox couples identified by their oxidation potentials.

DECLARATION

"I declare that **The Isolation and Electrochemical Studies of Flavonoids from *Galenia africana* and *Elytropapus rhinocerotis* from the North Western Cape** is completely my work and that all information obtained from external sources has been indicated and acknowledged by means of references".

Khumo Gwendoline Maiko

Signed: …… Date: ……..

ACKNOWLEDGEMENTS

Firstly, I would like to thank the most important presence in my life, God for all the blessings, strength, perseverance, security his has given me throughout this year. I would like to also thank my supervisors, Professor Emmanuel Iwuoha and Professor Ivan Green, who gave me advice, direction and support from the beginning of my project right until the end. Rasaq Oluwu and Dr. Waryo Tesfaye are sincerely acknowledged for assisting and guiding me throughout my project. All my sensor lab colleagues, and all friends, your support, contributions and caring attitude made feel at home in a place which was too far to be home.

To my mother, Miss L.G. Maiko, my grandmother, Mrs. S.M. Maiko, the greatest women in my life, I would like to thank you for your prayers, support and love. I would also like to acknowledge Mr. K.S. Maiko (my uncle), Mr. I.G. Maiko (cousin) and Mr. T.K. Maiko (brother) for the encouragement and support.

I would also like to thank the National Research Foundation (NRF) of South Africa, for the funding for completion of this study.

ABBREVIATIONS

AcO	**Acetate**
Ag/AgCl	**Silver/Silver chloride**
$CHCl_3$	**Chloroform**
CV	**Cyclic voltammetry**
DNA	**Deoxyribonucleic acid**
EtOAc	**Ethyl acetate**
GCE	**Glassy carbon electrode**
LDL	**Low density lipoprotein**
LPO	**Lipid peroxidation**
MeOH	**Methanol**
NMR	**Nuclear magnetic resonance**
PLC	**Preparative layer chromatography**
PG	**prostaglandins**
R_f	**Retardation factor**
TLC	**Thin layer chromatography**
SWV	**Square wave voltammetry**
UV	**Ultraviolet**

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SCHEMES

LIST OF TABLES

CHAPTER 1

1.1.General Introduction

Flavonoids constitute one of the most characteristic classes of compounds in higher plants. Many flavonoids are easily recognized as flower pigments in most angiosperm families. Their occurrence is not restricted to flowers but include all parts of the plant. In addition to various fruits and vegetables, flavonoids are found in seeds, nuts, grains, spices and different medicinal plants as well as in beverages such as wine, tea and beer [1-4]. Therapeutic effects of many traditional medicines maybe due to the presence of flavonoids [5]. Flavonoids are polyphenolic compounds possessing fifteen carbon atoms, and comprise of two benzene rings joined by a linear three carbon chain [6]. Flavonoids differ from flavonoids in that the bond between C2-C3 is saturated in the former and unsaturated in the latter as shown in figure 2.

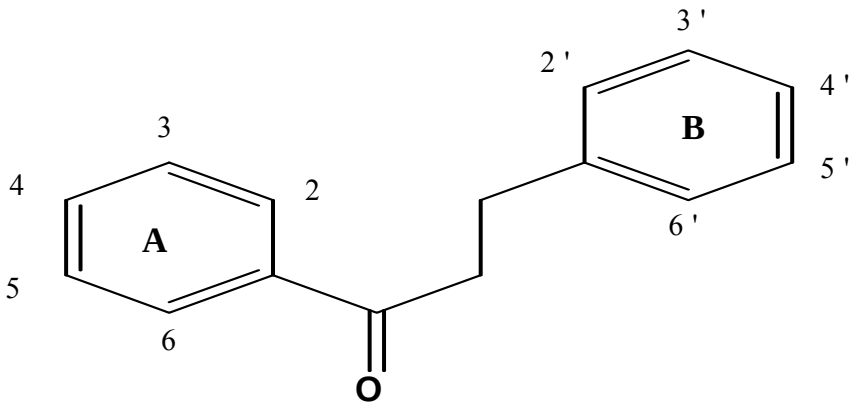
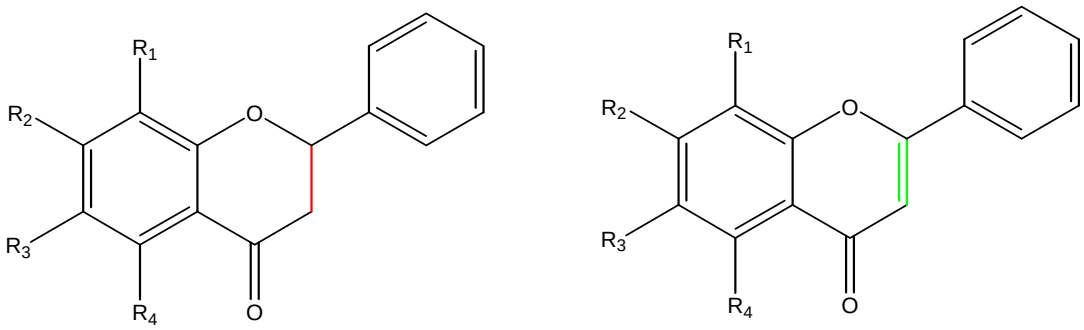


Figure 1: Flavonoid base structure.



Flavanoid **Flavonoid**

Figure 2: Illustration to show the difference between a flavonoid and a flavanoid.

Flavonoids have gained interest because of their broad pharmacological activity and their possible application in the field of nutrition. They effectively suppress lipid peroxidation (LPO) in biological tissues and sub-cellular fractions such as mitochondria, microsomes, low density lipoprotein (LDL) and erythrocyte membrane. They also have significant antioxidant, anti-inflammatory, anti-allergic, anti-platelet, anti-osteoporotic, anti-hepatotoxic, anti-viral and anti-diabetic effects and the prevention of cardio vascular disease [7-10].

Antioxidants are of great interest because of their involvement in important biological and industrial processes. It is accepted worldwide that a natural product is healthier than that of a synthetic origin. This question is still open for discussion and many studies on this subject are being carried out [11-12].

1.2. Rationale of project

To be able to understand the importance of flavonoids in our bodies we have to first understand the effect of free radicals which have a negative effect on our body.

1.2.1. Radicals

Radicals are atoms, molecules, or ions with unpaired electrons with an open shell configuration. Free radicals may have positive, negative or zero charge. Even though they have unpaired electrons, by convention, metals and their ions or complexes with unpaired electrons are not radicals. With some exceptions, the unpaired electrons cause radicals to be highly chemically reactive [13]. Free radicals play an important role in a number of biological processes, some of which are necessary for life, such as the intracellular killing of bacteria by phagocytic cells such as granulocytes and macrophages. The two most important oxygen-centered free radicals are super oxide and hydroxyl radical. They are derived from molecular oxygen after reduction processes. Free radicals because of their high reactivity can participate in unwanted side reactions resulting in cell damage. Excessive amounts of these free radicals can lead to cell injury and death, which results in many diseases such as cancer, stroke, myocardial infarction, diabetes and major disorders. Many forms of cancer are believed to be the result of reactions between free radicals and DNA, resulting in mutations that can affect the cell cycle and potentially lead to malignancy. Some of the symptoms of aging such as atherosclerosis are also attributed to free-radical induced oxidation of many of the chemicals making up the body [13].

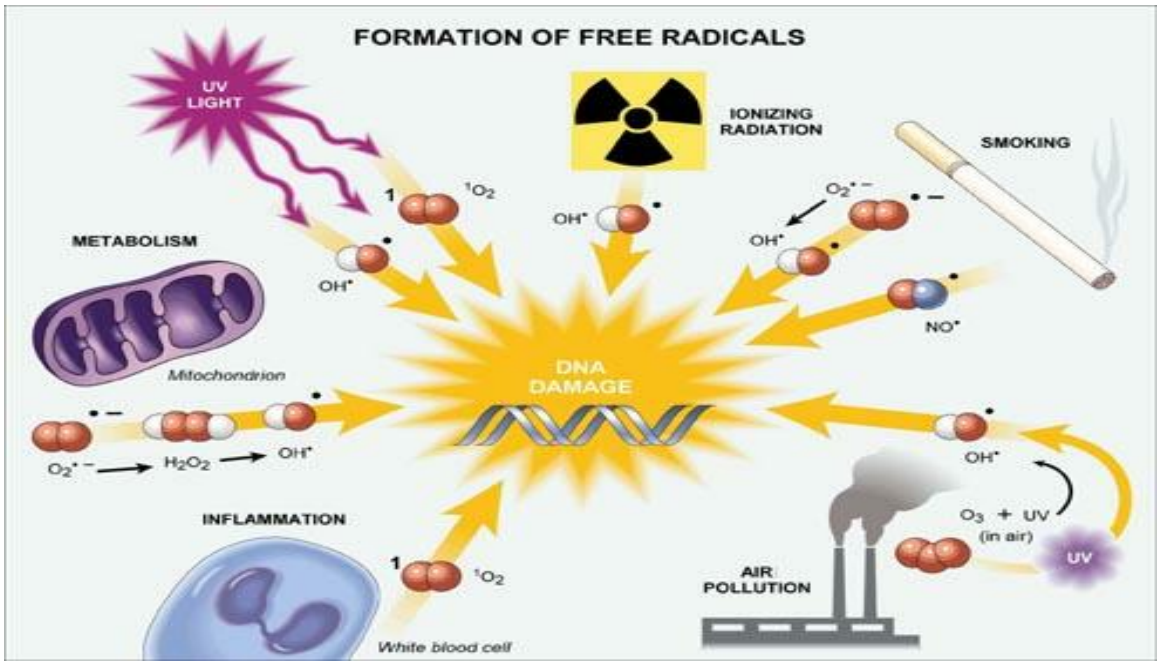


Figure 3: Free Radical Damage in the body [14]

Free radicals are necessary for life and the body has a number of mechanisms to minimize free radical induced damage and to repair damage that is caused by them, such as the enzymes super oxide dismutase, catalase, glutathione-peroxidase and glutathione reductase. Antioxidants play a key role in these defence mechanisms. The three vitamins, vitamin A, vitamin C and vitamin E and polyphenol antioxidants which include flavonoids are believed to behave as antioxidants [13].

1.2.2. Antioxidants

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrite which are collectively known as radicals. An imbalance between antioxidants and reactive oxygen species (ROS) results in oxidative stress

and this then leads to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, inflammation and neurodegenerative diseases like Parkinson's and Alzheimer's. Flavonoids help provide protection against these diseases by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defence system of the human body. Epidemiological studies have shown that flavonoid intake is inversely related to mortality from coronary heart disease and to the incidence of heart attacks [15-17].

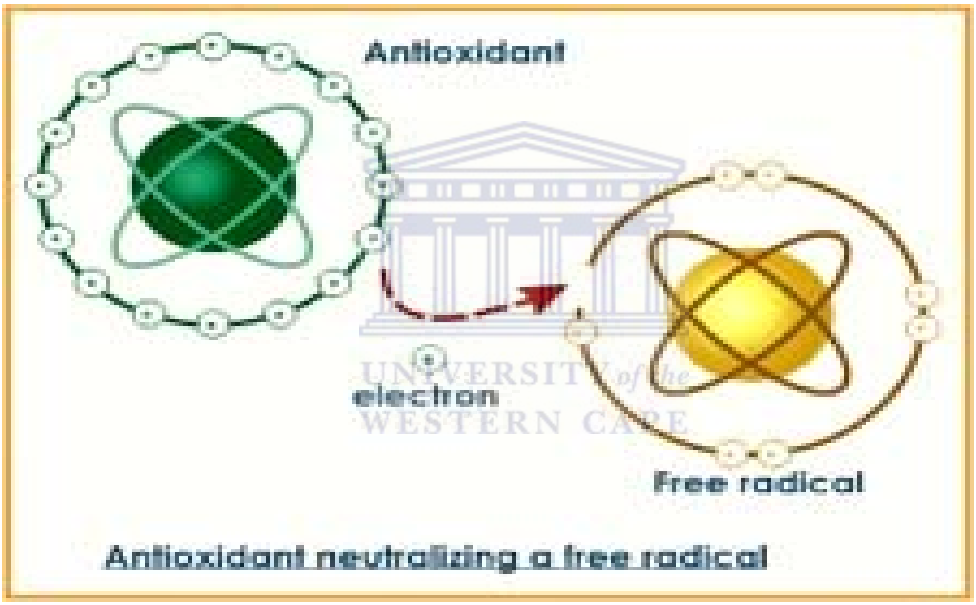


Figure 4: Antioxidant behaviour [18].

1.2.3. Flavonoids as antioxidants

Flavonoids have been shown to act as scavengers of various oxidizing, species i.e. super oxide anion, hydroxyl radical or peroxyl radicals. They may also act as quenchers of singlet oxygen. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other

features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity [19]. The chemical properties of polyphenols in terms of the availability of phenolic hydrogen's as hydrogen – donating radical scavengers suggests they will have antioxidant activity. To be defined as an antioxidant a polyphenol must satisfy two basic conditions [20]:

i. When present in low concentration relative to substrate to be oxidized, it should prevent auto – oxidation or free radical mediated oxidation [21].

ii. Resulting radical formed after scavenging must be stable through intermolecular hydrogen bonding [21].

Das and Pereira (1990) have shown that a carbonyl group at C-4 and a double bond between C-2 and C-3 are important features for high antioxidant activity in flavonoids [22].

The reactive structural groups for flavonoids are:

- The pyrogallol group.
- The catechol group.
- The 2,3 double bond in conjugation with the 4 – oxo and 3 – hydroxyl group.
- Additional resonance effect substituents [1, 20].

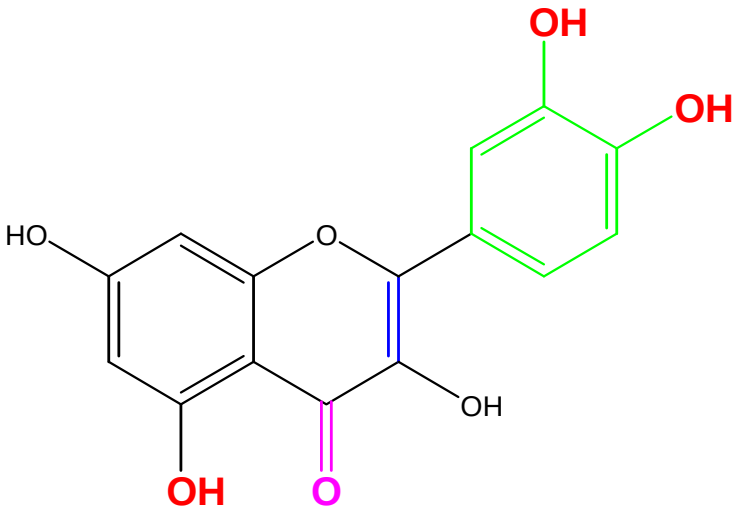
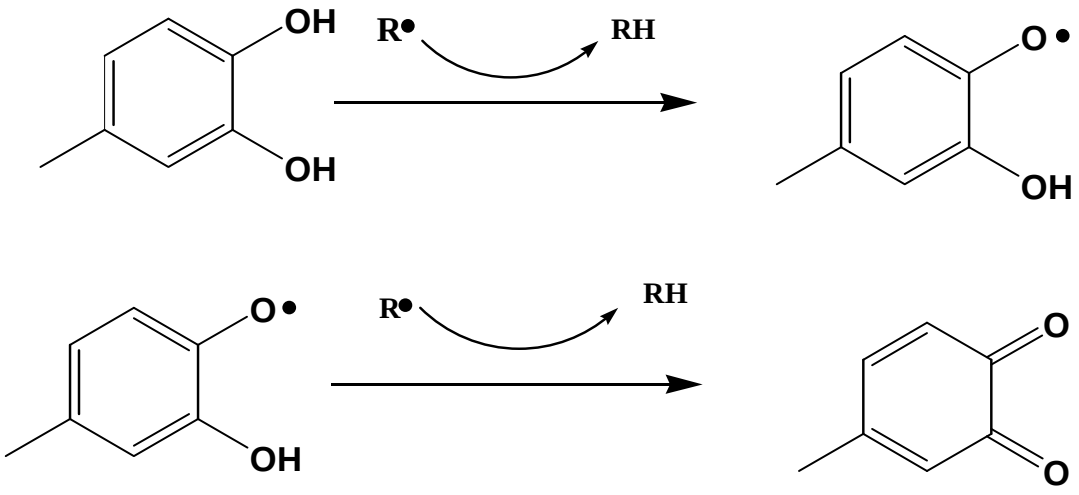


Figure 5 : Structure of the flavonol, quercetin showing features important in defining the classical antioxidant potential of flavonoids. The most important of these is the catechol (dihydroxylated B-ring – shaded blue). Other important features include the presence of unsaturation in the C-ring (shaded red) and the presence of a 4-oxo function in the C ring (shaded purple) [23]



Scheme 1: Mechanism of reaction of flavonoids with radicals (R·)

Flavonoids always present in all plants, and are very diverse as well as relatively easy to separate with modern chromatographic techniques. The increased interest in flavonoids is because the pharmaceutical industry is always on the lookout for new and novel medicinal herbs in which the functional compounds can serve as a starting point for the development of series of both analogues and derivatives. Another reason for their study is due the growing activity in the field of flavonoid biochemistry with numerous claims by many practitioners of the beneficial effects of treatment with natural products, which proved to be rich in flavonoids [24]. Studying the electrochemistry of flavonoids by recording their oxidation potentials will allow us to be able to tell which flavonoids have more health benefits. In the food industry it is important to be able to determine the concentrations of and the identities of the flavonoids a certain plant or fruit may contain. The antioxidant activity of phenolic compounds is inversely proportional to their oxidation potential, the lower the potential the higher the ability of an antioxidant compound to donate an electron [25].

1.3. Objectives

1.3.1. Main objective

The aim of the project is to develop a rapid electrochemical procedure to determine flavanoid contents of plants from a single extraction by their oxidation potentials.

1.3.2. Objectives of the project

i. To isolate flavanoids using column chromatography, thin layer chromatography and preparative layer chromatography.
ii. To synthesize other analogues from the already isolated compounds.
iii. To elucidate the structures of the flavanoids using proton and carbon nuclear magnetic resonance
iv. To do electrochemical evaluations of both the natural and synthesized flavanoids using cyclic voltammetry and square wave voltammetry.

Chapter 2

Literature review

2.1. Medicinal uses of plants

Many plants produce substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives. Many are secondary metabolites, of which thousands have been isolated. In many cases, substances such as alkaloids serve as plant defence mechanisms against predation by micro-organisms, insects, and herbivores. Many of the herbs and spices used by humans to season food yield useful medicinal compounds [26]. Many drugs listed as conventional medication were originally derived from plants [27]. Morphine, the most important alkaloid of the opium poppy, remains the standard against which new synthetic analgesic drugs are measured [28]. The use of bee products such as honey, wax and pollen in treatment of human diseases is very old. This is due to a high concentration of flavonoids found in these products. Our ancestors discovered that inflammation of the skin and throat healed rapidly when treated with honey. Early evidence in the use of honey as a healing agent was discovered by archaeologists who found resins containing flavonoids at ancient burial sites and fire places. These residues with high flavonoid content where also found in Egypt on tombs of pharaohs and are also believed to have been used for balsaming mummies. Due to oxidation of the flavonoids, these mummies acquire a black colour [29-30]. Other drugs include betulinic acid isolated from *Betula alba* which has an anti-tumor activity; caffeine extracted from *Camelia sinensis* which acts as central nervous system stimulant; digitalin, digitoxin and digoxin all isolated from *Digitalis purpurea* which have cardiotonic action [31].

Many South African plants have ethno-botanical uses for the treatment of tuberculosis and related symptoms such as coughing, respiratory ailments and fever. Extracts prepared from some of these plants as well as others selected on a random basis have been screened by South African researchers for activity against several *Mycobacterium* species using various methods [32].

2.1. Namaqualand

The Namaqualand desert falls within the succulent Karoo biome which is recognized as one of the only two biodiversity hotspots within the semi – arid areas. The winter rainfall is typically predictable while the summer rainfall is less predictable [33]. The lowland Namaqualand, in the north-western cape, South Africa has spectacular botanical diversity and because the land is used primarily as natural rangeland most of it is relatively un-reformed. It is specifically renowned for mass floral displays in winter and spring and for incredibly high number and abundance of succulent plants. More than 6300 vascular plant species occur in the succulent Karoo biome [34]. Lowland Namaqualand covers predominantly flat sandy plains from the Oliphant's river in the south to the orange river in the north which is approximately 400 km and from the Atlantic coastline to the west to the base of the escarpment in the east which is approximately 50 km. Mean annual rainfall decreases from 145mm in the south near Vredendal to 40 mm in the north at Alexandra bay [35]. The flat sandy plain consists of complex sequence of marine deposits. Generally, these sands change colour from pale-grey to yellow to red with increasing age and distance from the coast [36]. The vegetation is dominated by erect and scrambling dwarf leaf-succulent shrubs. *Mesembryanthemaceae/Aizoceae* and *Asteraceae* are the most abundant families but during winter annuals like geophytes are also common [37]. The plants

that were involved in my project, i.e. *Galenia africana* and *Elytropapus rhinocerotis* were isolated from the lowland Namaqualand.

Figure 6*: Illustration showing the boundaries of Namaqualand [38]*

2.1.1. Galenia africana

Galenia africana is belonging to the family *Aizoceae*. It is a dominant plant throughout Namaqualand, South Africa. *Galenia africana* is an aromatic perennial sub-shrub growing 0.5 – 1 m high with oppositely arranged leaves which are linearly lanceolate, attenuated at the base. The flowers are sessile, disposed in a terminal and large panicle. The branches are woody, greyish and erect [39]. In South Africa, *Galenia africana* has a wide distribution on dry flats and lower slopes from the Northern Cape and Namaqualand to Uniondale, the Karoo and Eastern Cape

Province; often on disturbed ground and road verges. In Lowland namaqualand, it is found in fields and among shrubs in the Cape flats, Hottentottsholland, Breede-rivierspoort in Wupperthal [39].

Figure 7*: Illustration of the aerial parts of Galenia Africana [40]*

Vries and Green (2001) extracted dried samples of *Galenia africana* using various solvents. The ethanol extracts showed antifungal activity with agar diffusion method. Ethanol extracts were then purified and four of the extracts showed anti fungal activity showed by clear zone inhibition. One of the fractions inhibited growth of *Alterna sp., Fusarium equiseti, Fusarium graminearum, Fusarium verticilliodes* and *Phaeomomella chlamydospoia* as the most sensitive. Spectroscopic analysis of bioactive component of *Galenia africana* resulted in identification of flavanoid structures as major components of full fraction [41].

2.1.2. Elytropapus rhinocerotis

Elytropapus rhinocerotis belongs to the family Asteraceae. *Elytropapus rhinocerotis* is a single-stemmed, usually smallish shrub up to about 2 m high. The very old branches are gnarled and the bark is smooth and grayish. Older branches are bare of leaves but bear many thin, whip-like twigs which are held erect and covered with tiny, triangular leaves pressed tightly to the stem [42]. *Elytropapus rhinocerotis* is very branched grey to grey-green aromatic shrub 0,6 - 2,5m in height with young stems densely woolly; leaves minute, numerous, pressed onto the stem, usually woolly on both surfaces; it blooms between March and September with a inconspicuous, yellow, tubular, capitula of mostly 3 florets, pappus well developed; fruit an achene with prominent longitudinal ribs [43]. Each capitulum contains several tiny purple flowers called florets. Seeds are tiny and are wind-dispersed by means of a feathery pappus. The natural range of *Elytropapus rhinocerotis* is widespread in the Cape Floristic Region and occurs throughout the Namaqualand and as far north as the Richtersveld. It is also found as far east as the great escarpment around Molteno in the Eastern Cape, and in the southern part of the Eastern Cape to East London [42].

Figure 8: ***Illustrations of Elytropapus rhinocerotis*** **[44].**

2.2. Chalcones and dihydrochalcones

The phenyl group plays an important role in the antioxidant activity of certain flavonoids. A flavonoid chalcone (chalconaringenin) and a flavanone (naringenin) with no phenyl groups act as pro-oxidants, i.e. they promote rather than limit the oxidation of LDL by copper. However, adding a phenyl group to these flavonoid molecules counteracted their pro-oxidant activities [45]. Butein and other 3,4-dihydroxychalcones are more active than analogous flavones because of their ability to achieve greater electron delocalization [46].

2.2.1. Natural abundance and structural variation

Most chalcones are mono-phenylated compounds with simple dimethylallyl, dimethylchromeno, and furano or geranyl substitutions. Others consist of di- and/or tri-phenyl derivatives. Both the dimethylchromeno and furano derivatives represent 50% of the chalcone derivatives, which are substituted at almost all positions of ring A, whereas the frequency of other phenylations involves positions 3', 5', 5, 3, 2.

About one-third of the iso-phenylated chalcones are mono-methylated at positions 2', 4', 6', 4 with few di-methyl derivatives. By contrast with the chalcones, the iso-phenylated dihydro chalcones are much less abundant, although they share most of the characteristics of phenylated chalcones. Both groups of compounds occur mostly in the *Leguminosae* but are also represented in the *Moraceae, Asteraceae, Lauraceae* and *Platanaceae* [47].

2.2.2. Applications of chalcones

Chalcones are important intermediates in the metabolic pathway of converting tyrosine to flavonoids. Chalcones have the general structure consisting of two phenyl groups both with hydroxyl group(s), connected by a C – 3 bridge [- C=C – (CO)-]. Chalcones are polyphenols so are expected to act as antioxidants. Compounds with a chalcone based structure have shown anti-protozoal, antifungal, antibacterial, anti-inflammatory, xanthine oxidase and tyrosinase inhibitory, phytoestrogenic, antipyretic, analgesic, cyto-protective, antioxidant and anti cancer pharmacological properties [48-55].

Chalcones are expected to have both antioxidant and photo-oxidant activity due to the presence of both p-coumaric acid and 2-hydroxybenzoyl moieties. The 2-hydroxyl group does not act as an antioxidant but rather as a photo-antioxidant. The 4-hydroxyl group at the para position to the carbonyl group, does not show any peroxy radical trapping activity because it is generally accepted in antioxidant chemistry, of the decrease in electron density on the hydroxyl group as a result of the electron withdrawing carbonyl group at the para position. Results showed that a chalcone with hydroxyl group(s) on the A ring cannot act as a radical trapping compound but as a

UV absorber but a chalcone with hydroxyl group(s) on the B ring especially on positions 2 and 4 had an increased anti-oxidant activity [56]. Zampini and colleagues determined the genotoxicity and anti-genotoxicity of *Zuccagnia punctata* extracts and 2,4-dihydroxychalcone and they determined that the chalcones has a higher genotoxicity activity than the *Zuccagnia punctata* extracts [57]. 2,4-dihydroxy dihydrochalcone (**1**), 2,4-dihydroxy chalcone (**5**) and 7-hydroxyl flavanones (**7**) have been analyzed in extract of *Ixeridium gracile* by Micellar Electrokinetic Chromatography (MEKC) which shows that it is a suitable and powerful technique to study flavonoid components in extracts of medicinal plants [58-59].

2.3.Flavonoids

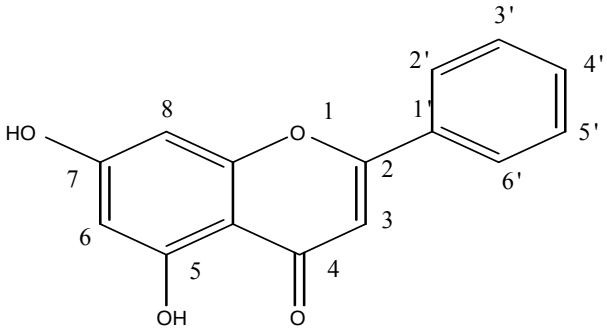
2.3.1. Natural abundance and structural variation

Flavanones represent the most abundant class of iso-phenylated flavonoids with a rich variety of structures, almost half of which occur in the (**2S**) configuration. Although a number of phenyl flavanones have been reported as natural products; it was only recently that complete proof of their identification has been provided. For example, dehydroisoderricin, which was first characterized in 1980 [60], has been fully identified, including its stereochemistry at C-2, four years later [61] . As with chalcones, iso-phenylated flavanones occur mostly in the *Leguminosae, Moraceae,* and *Asteraceae* and to a lesser extent in the *Rutaceae, Euphorbiaceae* and *Scrophulariaceae*. Flavones are of common occurrence in the *Moraceae* and *Leguminosae*, but are also represented in the *Berberidaceae, Cannabinaceae* and *Rutaceae* to a much lower extent [47].

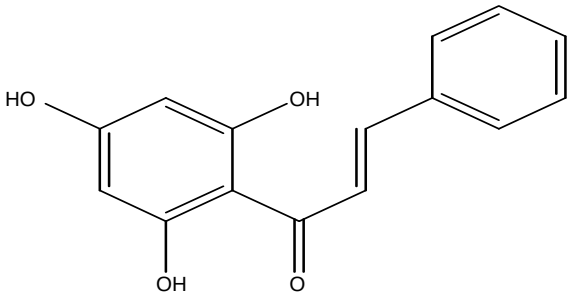
2.3.2. Chemistry of flavonoids

The existence of a great variety of structurally related flavonoids suggests that their reduction and oxidation potentials would differ. Since a large number of flavanoids and flavonoids co-exist in plant cells, in the transport system of the plant sap, and in plant products, a spectrum of electron transfer catalysts would be expected, which would accelerate physiological oxidation systems. This might reflect on an important physiological function that flavonoids might exhibit and would thus confirm their claimed importance of the well documented proven beneficial influence on our health [62].

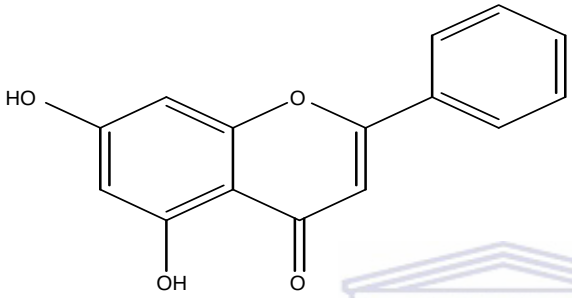
Flavonoids are divided into various sub-groups which include flavanones, flavones; flavans, flavanols, flavonols and their iso-flavonoid counter parts including other closely related compounds which include anthocyanidines, coumarins and chalcones. The heterocyclic moiety attached to the benzene ring on the left hand side occurs either as the unsaturated pyrone or its dihydro analogue and is described as having a C_6-C_3-C_6 skeleton. The position of attachment of the second phenyl rings separates the flavonoid class into the flavonoids (2-phenyl) and the iso-flavonoid (3-phenyl). Flavonols on the other hand differ from flavones by a hydroxyl group which is almost always in the 3-position and having the C2-C3 double bond. Chalcones are closely related to flavonoids and differ in that the pyran ring exists in the open chain form. However the biological properties are more often than not very similar and are in some cases even much better. Flavonoids are often hydroxylated in positions 3, 5, 7, 3', 4' and 5' and either mono, di, tri and tetrahydroxylated [63].



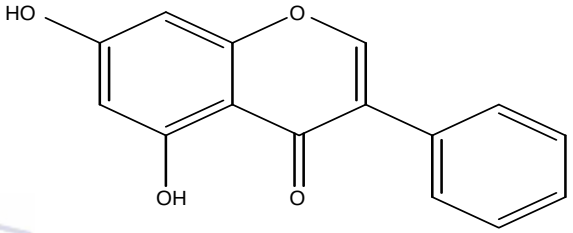
Benzopyran



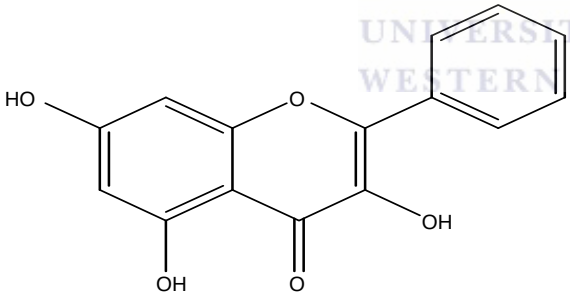
Chalchones



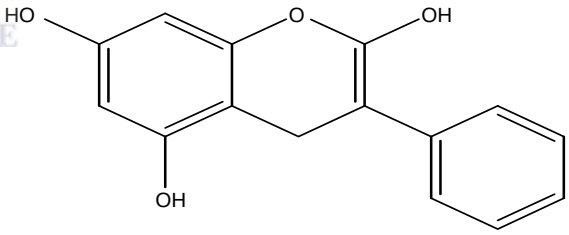
Flavone,
5,7-dihydroxy-2-phenyl-2,3-dihydro-4H-chromen-4-one



Iso-flavone,
5,7-dihydroxy-3-phenyl-2,3-dihydo-4H-chromen-4-one

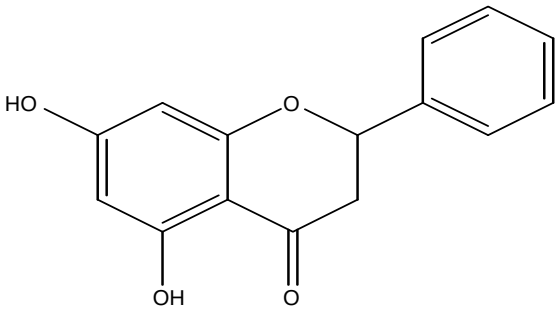


Flavonol,
3,5,7-trihydroxy-2-phenyl-4H-1-benzopyran-4-one

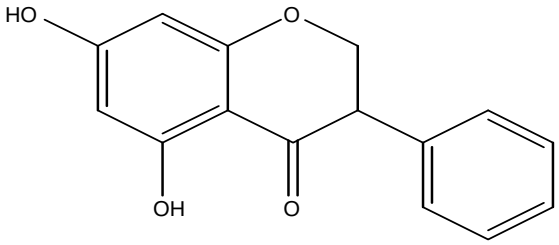


Iso-flavonol,
2,5,7-trihydroxy-3-phenyl-4H-1enzopyran-4-one

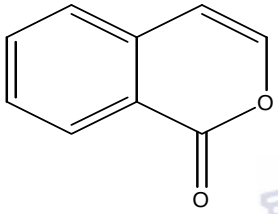
Figure 9*: Main classes of flavonoids and related compounds.*



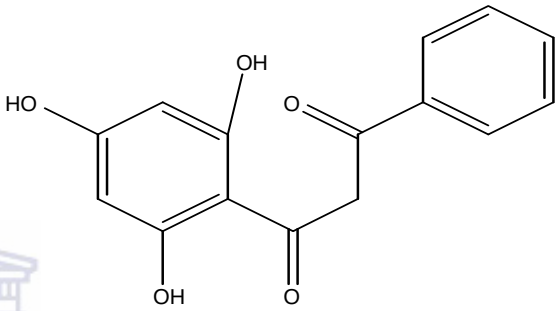
Flavanone
5,7-dihydro-2,3-dihydroxy-2-phenyl -4H-1-Benzopyran-4-one



Isoflavanone
5,7-dihydro-2,3-dihydroxy-3-phenyl-4H-1-Benzopyran-4-one



Coumarin
2H-chromen-2-one



Anthocyanin

Figure 10*: Continuation of main classes of flavonoids and related compounds*

2.3.3. Flavonoids and plants

2.3.3.1. Flavonoids as colorant

The blue flower colour is due to the presence of anthocyanins in delphinidin but since most delphinidin glycosides are mauve in colour the shift to the blue colour is most likely due to the flavone co-pigments. This blue colour has been demonstrated to be the preferred attractant of bee pollinators [19]. Many more primitive families have floral anthocyanins in the red to magenta range. Commelinin, a blue pigment from the blue flowers of *Commelina communis* , it was shown to be a complex composed of a reddish purple anthocyanin, a pale yellow flavone and magnesium [64-65]. The X-ray

crystal structure of natural commelinin demonstrate that commelinin is a tetra nuclear (4 X Mg^{2+}) metal complex, in which two magnesium ions chelate to six anthocyanin molecules, while the other two magnesium ions bind to six flavone molecules, stabilizing the commelinin complex, a new type of supramolecular complex [65-66].

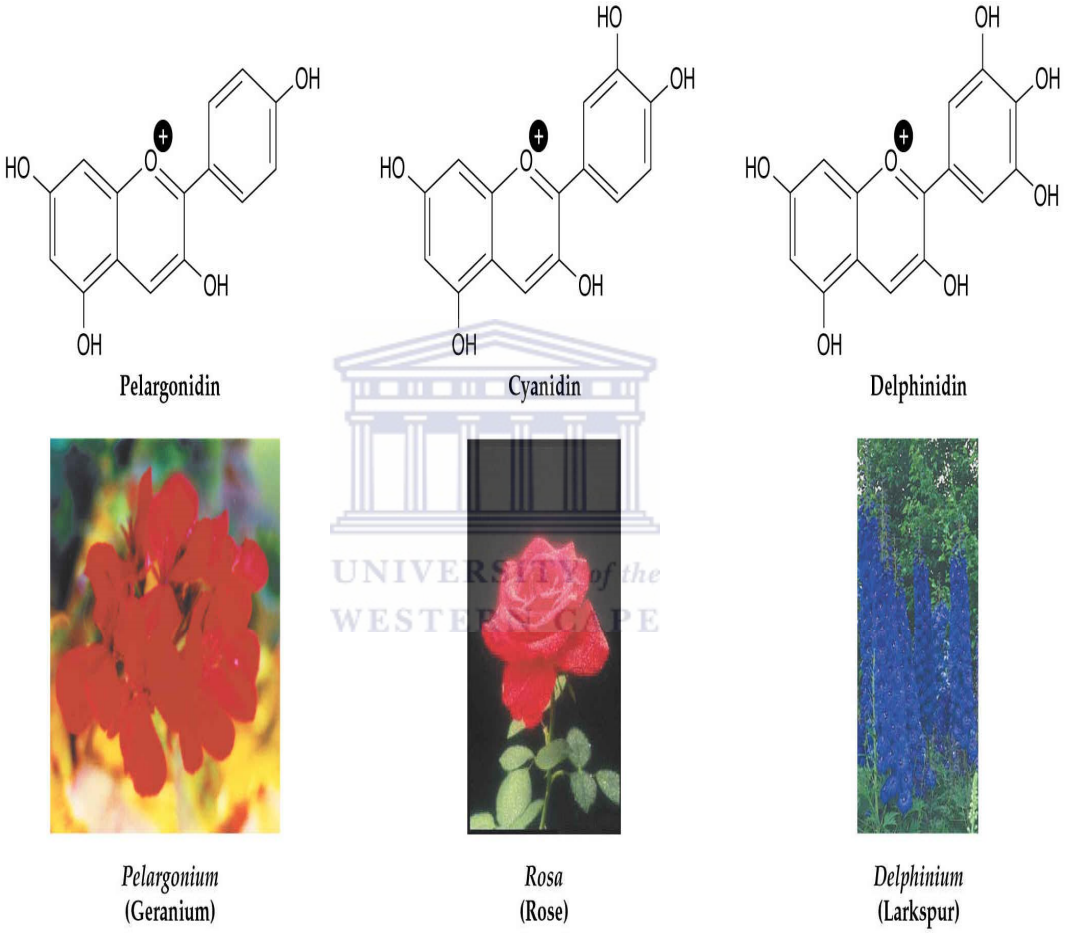


Figure 11*: Flavonoids co-pigments that influence the colour of flower species*

Table 1: ***Flavonoid complexes found in different plant species that influence colour [67] [68]***

Plant species	**Pigment, co-pigment**
1. Compositae	
a. *Centaurea cyanus*	Succinylcyanin, apigenin 7-glucutonide-4'-malonyl glucoside
2. *Ranunculaceae*	
a. *Aconitum chinense*	Delphinidin, 3-rutinoside-7-(di-p-coumaryl diglucoside)
b. *Delphinium hybridum*	Delphinidin, 3-rutinoside-7-(tetra-p-hydrobenzoyl pentaglucoside)
3.*Ponetederiaceae*	
a.*Eichhomia crassipes*	Delphinidin, 3-gentiobroside, apienin-7-malonylglucoside
4. *Leguminosae*	
a. *Lupinus cv.*	Delphinidin, 3-malonylglucoside, apigenin-7-malonylglucoside

2.3.3.2.Flavonoids and UV protection in plants

Ultraviolet light (UV) is electromagnetic radiation with a wavelength shorter than that of visible light but than that of X-rays in the range 10nm to 400nm UV light consists of electromagnetic waves with frequencies higher than those humans can identify as colour. UV spectrum has many effect many beneficial and others damaging [69]. The sun emits radiation in the UV-A, UV-B and UV-C range. Each UV band has different energy and ecological significance. The three bands are UV-C at 100nm to 280 nm,

UV-B at 280nm to 315 and UV-A at 315nm to 400nm [70]. UV-C rays have the highest energy, and hence the most dangerous type of UV light. Little attention has been given to UV-C rays because they are filtered out by the atmosphere. UV-A rays were considered less harmful but today they are known to contribute to skin cancer by indirect DNA damage through free radicals and reactive oxygen species. UV-B rays can penetrate the ozone layer and cause damage to plants. Most plants have a built-in UV-B resistance due in large part to the presence of flavonoid pigments which act as UV absorption filters. Flavonoids which are present in green leaves have been shown to absorb in the 280 – 315 nm region of the UV spectrum and consequently act as powerful UV filters, therefore preventing photosynthetic tissue damage [71-75].

Table 2: ***Plant species in which UV protective flavonoids have been identified [71-75]***

Plant species	**Flavonoid location**	**Protective flavonoid**
1. *Arabidopsis thaliana*	Epidermal cells	Kaempferol, 3-gentiobioside-7-rhamnoside
	Epidermal cells	3,7-dirhamnoside
2.*Zea mays* (Corn)	Epidermal cells	Anthocyanin
3.*Sinapis alba* (Mustard)	Epidermal cells	anthocyanin, quercetin glycoside
4.*Gnaphaliumvira-vira*	Leaf wax	7-O-methylaraneol
5. *Brassica oleracea* (cabbage)	Epidermal cells	Cyanin glycosides, sinapyl ester

2.3.4. Flavonoids and humans

Antioxidant flavonoids are naturally present in fruits, vegetables, tea and wine. Antioxidants help organisms to manage and deal with oxidative stress which is mainly caused by free radical damage. Free radicals are chemical species, which contain one or more than one unpaired electron which causes them to be highly unstable and this in turn inevitably causes damage to tissue and DNA by extracting electrons from their own molecular bonds order to attain stability [76]. Flavonoids have been shown to act as powerful scavengers of various oxidizing agents i.e. hydroxyl and peroxide radicals. The carbonyl functional groups at C-4 in conjunction with the C2-C3 double bond are among the most essential functionalities governing their high antioxidant activity [77]. The major flavonoid component in red wine is catechin, and other phenolic constituents include gallic acid, epicatechin, malvidin 3-glucoside, rutin, myricetin, quercetin, caffeic acid and resveratol. It is resveratol, a non-alcoholic component of wine which acts as a LDL oxidant inhibitor. However, quercetin and epicatechin have twice the antioxidant activity of resveratol [78].

Table 3: ***Applications of flavonoids in human systems [24].***

Disease	**Target**	**Flavonoids**	**Results proven**
Inflammation	eicosanoid synthesis	Quercetin	Local pain relieved, Body temperature normalized
Diabetes mellitus	Aldose reductase	Quercetin	Pressure in eye reduced
		Rutin, Citrin	Bleeding ceased
Allergy	H-ATPase of mast cells	Disodium chromoglycate	Secretion of histamine prevented
		Quercetin	Symptoms disappeared
Headache	eicosanoids synthesis	Quercetin	Pain relief
Virus infection	H-ATPase of lysosome	Quercetin	Coat removal prevented
Bee sting	eicosanoid synthesis	Quercetin	Local pain relieved
Oral surgery	eicosanoid synthesis	Quercetin	Local pain relieved
Stomach/ duodenal ulcer	eicosanoid synthesis	Quercetin	Bleeding ceased, Pain relieved
Cancer	(Na-K) ATPase	Quercetin	Cells normalized (tissue culture tested)

2.3.4.1.Inflammation

The treatment of sore throats and fevers by an ethanol extract of propolis plant family is an example of a quick treatment by flavonoids. This is an example is the therapeutic application of flavonoids as an anti-inflammatory agent [29-30]. Essentially inflammation is the integrated response of many defence systems of the body to the invasion of a foreign body of any kind such as bacteria, viruses and even wooden splinters. The release and oxygenation of arachidonic acid is a critical event in regulating key processes in host defence and inflammation [79-80].Oxygenation of arachidonic acid catalyzed by either cyclo-oxygenase (COX) or lipoxygenase (LO) initiates the biosynthesis of potent bioactive mediators: i.e., prostaglandins, thromboxanes, prostacyclins, mono and dihydroxy acids, leukotrienes and lipoxins, collectively known eicosanoids [81]. Eicosanoids are able to be synthesized by all cells forming part of the immune system, and especially by monocytes and macrophages which are the first line of defence against infection. Macrophages contain both COX and LO activities and are capable of generating large amounts of prostanoids, leukotrienes and different hydroxyl fatty acids [82]. The generation of eicosanoids by macrophages is relevant to their activity in, and regulation of, the immune and inflammatory responses. The eicosanoids are important intercellular signalling agents which affect cell behaviour and cell-to-cell interactions and they are said to be the key mediators of inflammation [80, 83]. Since eicosanoids possess important biological activities, the control or inhibition of their biosynthesis has been an area of intense investigation and could open new approaches to the treatment of a variety of human diseases [84-85].

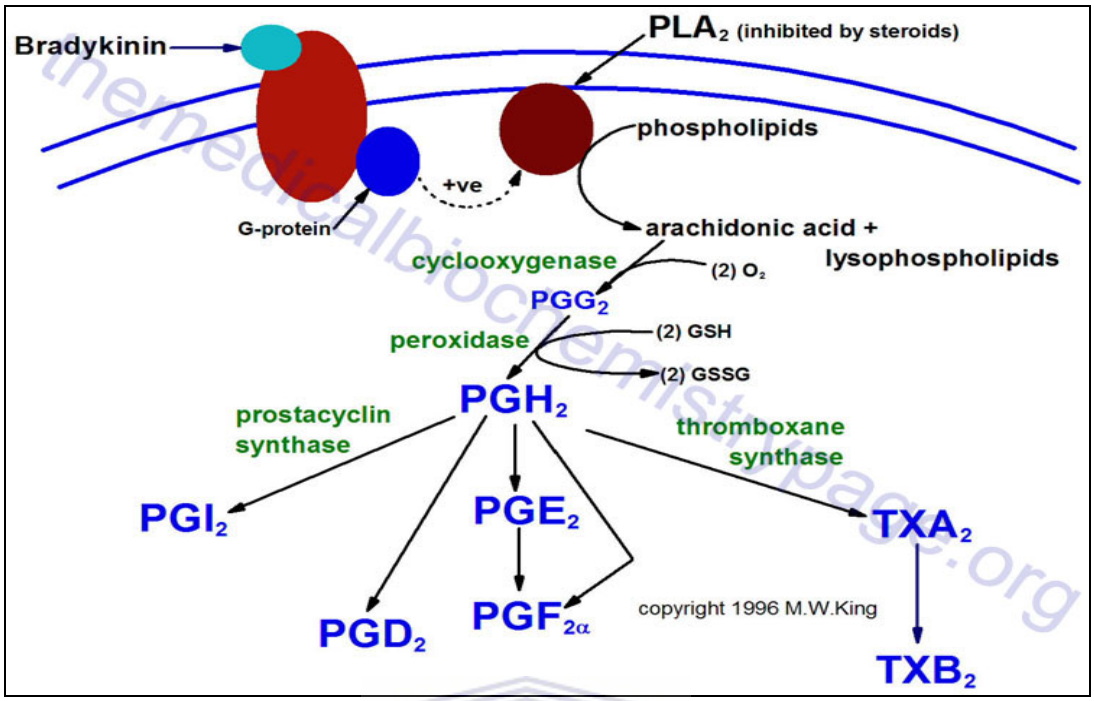


Figure 12: ***Illustration of synthesis of prostaglandins [86]***

The eicosanoids' activity is able to be inhibited by flavonoids. The PG's are transported in the blood to the brain where neurons that have receptors in the mid-brain allow interneuron communication to occur. A granular containing pain substance together with bradykinin are released which then diffuse to the neurons which are responsible for pain. The subsequent swelling of tissue is due to the process of osmosis. In normal circumstances plasma membranes are water resistant but when there is tissue damage, water retention occurs. All these processes that lead up to pain and inflammation can easily be stopped by inhibition of the key enzyme by flavonoids [79].

2.3.4.2.Anti-bacterial, anti-fungal and antimicrobial activity

Flavonoids are also efficiently used to address bacterial, protozoan and fungal infections, by killing the bacterial and fungal cells and also counteracting the effects of bacterial toxins. Apart from the active role that flavonoids play in the eradication of infectants, they additionally strengthen loose connective tissue by inhibiting some of the enzymes that are able to hydrolyze their proteoglycan and protein meshwork whose primary task is to sterically hinder the diffusion of infectants through the tissue. One such an example is the inhibition of hyaluronidase by flavonoids and in this way contributes to the immobilization and encapsulation of the infectants which are then gradually decomposed by the scavenging and repair processes [87].

Alcarad et al (2000) proved that flavonoids showing the highest anti-Methicillin-resistance to *Staphylococcus aureus* activity belong to the structural pattern of chalcones. Chalcones are open-chain flavonoids whose basic structure includes two aromatic rings bound by a α, β -unsaturated carbonyl group. In chalcones, the unsaturated carbonyl group linking aromatic rings A and B favours the delocalization of π electrons. For this reason, the carbonyl group looses part of its individual character and partially integrates into the adjacent olefinic group. This phenomenon originates transference of electronic deficiency from the carbonyl carbon atom to the C-β, which is increased in 2' (OH)-chalcones. These structural characteristics are similar to those exhibited by other antioxidant flavonoids [88-89].

Reverse transcriptase is a polymerase that forms a DNA complementary to RNA. When this polymerase is inhibited, replication is inhibited and thus the virus cannot

multiply. Flavonoids are also believed to have anti-viral properties e.g. Quercetin has been shown to be an inhibitor of the reverse transcriptase of RNA viruses [90]. Another process that occurs once the virus particle penetrates the plasma membrane is that it becomes fused with the lysosome to allow the proteases in the lysosome to hydrolyze the protein capsid around the viral genome because the viral genome encapsulated in the protein capsid is inactive. Flavonoids and related polymers can have different types of interaction with membranes. The more hydrophobic flavonoids can partition the hydrophobic core of membranes while the hydrophilic flavonoids can interact by hydrogen bonding with the polar head groups of the lipid-water interface of membranes. It is this ability of flavonoids to bind with membrane that prevents the fusion between the lysosome and virus particle hence preventing the protein capsid from being removed and rendering the virus particle inactive [91-92].

The antifungal and antimicrobial effect of flavonoids is mainly attributed to the presence of phenolic hydroxyl groups which have high affinity for proteins and, therefore, act as inhibitors of microbial enzymes [93]. It is generally agreed that at least one phenolic hydroxyl group and a certain degree of lipophilicity are required for the biocidal activity of flavonoid compounds [94].

2.3.4.3.Gastro protection

Ares and Outt (1996) raised the question as to whether other types of flavonoids might possess gastro-protective properties and what types of structural changes can be tolerated on the core flavone structure without loss of the gastro-protective activity. They found that in the rat ethanol-induced gastric damage model, flavone gastro-

protection requires a C2 – C3 double bond and an intact C ring. The phenyl ring at the 2 – position cannot be moved to the 3 – position but can be replaced by a variety of smaller and larger substituents [95].

2.3.4.4.Anti-Cancer activity

Consumption of a diet rich in soy containing foods is known to be rich in phyto-oestrogen such as iso-flavones has been proposed as a chemo preventive factor against breast cancer in asian populations [62]. Loa, Chow and Zhang (2009) analyzed the structure–activity relationships of flavonoids and chalcones and the following were found to be required for their inhibitory potencies:

i. Of the six sub-classes of the polyphenols tested, the unique backbone structure of chalcones with a open C-ring,
ii. Within the chalcone group, hydroxyl substitution at 2-carbon of B-ring,
iii. Hydroxyl substitution at 3-carbon in B-ring of Xavones.

This data is valuable for development of plant polyphenols as possible anti-proliferation agents of cancer cells and for design and modification for new anticancer agents as well. In other studies, the most potent chalcone, 2,2-dihydroxychalcone was found to induce apoptosis of the cancer cells. These processes may be involved in their inhibitory effects on the cancer cells [96].

2.3.4.5.Flavonoid inhibition of enzymes

In a number of structure-activity studies, flavonoids have been tested for their ability to inhibit key enzymes in mitochondrial respiration. It was found that a C2,3- double bond, a C4-keto group and a 3', 4', 5'-trihydroxy B-ring are significant features of those flavonoids which show strong inhibition of NADH-oxidase. The order of potency for inhibition of NADH-oxidase activity was robinetin, rhamnetin, eupatorin, baicalein, 7,8-dihydroxyflavone and norwogonin with IC_{50} values of 19,42, 43, 77, 277 and 340 nmol/mg protein, respectively [97].

2.3.5. Electrochemistry of flavonoids

Electrochemical detection is an attractive alternative method for electrochemically active species detection, because of its inherent advantages of simplicity, ease of miniaturization, high sensitivity and relatively low cost. Electrochemical methods such as differential pulse, cyclic and square wave voltammetry have been intensively utilizing for analysis of certain flavonoids [17, 98-101]. Cyclic voltammetry at an inert glassy carbon electrode has been successfully applied to analyze antioxidants in wine [102-105]. Several papers, where the authors utilized glassy carbon electrode for determination of phenolic compounds, were published [20, 106-115]. Zou, Kilmartin, Inglis and Frost (2002) employed cyclic voltammetry as a qualitative tool to measure the level of galloyl and catechol groups present in wine, which correlated with other total phenol measures. The appearance of individual phenolics at electrode potentials expected for each one of them, was matched with peaks in cyclic voltammograms of wine samples [116].

Volikakis and Efstathiou (2005) published a novel approach for a fast screening of total flavonoids (quercetin, kaemferol, myricetin) in wines, tea – infusions and tomato juice using adsorptive stripping voltammetry in a flow injection system. The proposed method was based on the property of flavonols to be pre – concentrated on carbon paste electrode where diphenylether was used as pasting liquid [116]. Hodek and Hanustiak (2006) studied the toxicological effect of flavonoids during interaction with nucleic acids using carbon paste electrode (CPE). The use of carbon paste electrode compared to other analytical techniques gives great simplification of the analysis. This method can be used for designing a simple DNA sensor. The *in vitro* study of DNA and flavonoid compound (rutin, quercetin) interaction by using square wave voltammetry revealed that ingestion of flavonoids should be taken with caution since they can interact with DNA [117]. Korbut et al (2001) carried out voltammetric investigation of selected flavonoids (quercetin, rutin, epigallocatechingallate and catechin) and their antioxidative properties using an electrically heated carbon paste electrode with the surface confined DNA. The system of a copper(II) complex with 1,10-phenanthroline, $[Cu(phen)_2]^{2+}$, in the presence of hydrogen peroxide and ascorbic acid was used as the cleavage mixture producing reactive oxygen species (ROS). A portion of original double stranded DNA on the sensor surface was quantified using the $[Co(phen)_3]^{3+}$ complex as DNA redox indicator. The evaluation of temperature effect on the flavonoid anodic signal and DNA degradation and protection by flavonoids was the aim of the study. Flavonoids under study were found to associate with double stranded DNA confined to the CPE surface. A concentration dependent antioxidative effect of flavonoids in the DNA cleavage mixture was detected using the DNA/CPE sensor [118].

Maoela et al (2009) identified catechin in *C. mellei* and *C. Quadrifidus.* They also determined mechanism of reaction of catechin using square wave and cyclic voltammetry. Other extracts that were isolated from *C. mellei* and *C. Quadrifidus* were also studied in a similar manner as for catechin. Cyclic voltammetric results of catechin showed two oxidation peaks, occurring at the potentials of +197.0 mV and +612.7 mV. These oxidation peaks are associated with oxidation of the hydroxyl groups. A reduction peak at +70.7 mV corresponds to the reduction of oxidation products formed in the first peak [119].

Vaya et al (2003) selected flavonoid compounds of five different subclasses and the relationship of their structure to the inhibition of LDL oxidation in vitro was investigated. The most effective inhibitors, by either copper ion or 2,20-azobis(2-amidino-propane) dihydrochloride (AAPH) induction, were flavonols and/or flavonoids with two adjacent hydroxyl groups at ring B. In the presence of the later catechol group, the contribution of the double bond and the carbonyl group at ring C was negligible. Iso-flavonoids were more effective inhibitors than other flavonoid subclasses with similar structure. Substituting ring B with hydroxyl group(s) at 2' position resulted in a significantly higher inhibitory effect than by substituting ring A or ring B at other positions. Position 3 was favoured only in the presence of conjugated double bond between rings A and B. This study makes it possible to assign the contribution of different functional groups among the flavonoid subclasses to in vitro inhibition of LDL oxidation [120].

Cyclic voltammetry has been used for the evaluation of antioxidant capacity for several polyphenols and their mixtures [20, 121-122]. The redox properties of

polyphenols have been used as a measure of antioxidant properties of wines on the basis of measurement of oxidation current at constant potential by HPLC with electrochemical detection [20].

2.4. Review of natural and synthesized flavonoids and chalcones

2.4.1. 1-(2',4'-dihydroxyphenyl)-3-phenyl propan-1-one (1)

The above mentioned chalcones is found in the leaves of *Ceratiola ericoides* and in black crowberry scientifically known as *Empetrum rigrum L*, Empetraceae which is widely distributed in the northern hemisphere and in the Russian federation [123]. It was synthesized as an intermediate in a reaction to form tetrahydroflemichapparin-A using Adam's catalyst and ethanol with a boiling point of approximately 91 ºC [124].

2.4.2. 2,3-dihydro-5,7-dihydroxy-2-phenyl-4H-1-benzopyran-4-one (2)

The above mentioned compound is known as pinocembrin. The UV – Vis spectra of this flavonoid as studied by Gregon and Stevanato (2010) showed an absorption peak at $275 < \lambda < 300$ nm [125]. Pinocembrin has been fractionated from Portuguese propolis high performance liquid chromatography and identification was done using electrospray mass spectrometry in negative mode and it was found to have a retention time of 40.8 min with fragmentation pattern with peaks at 213, 211, 151 [67, 126].

2.4.3. 2,3-dihydro-5,7-dihydroxy-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (3)

Only three references were found which were inaccessible. This compound has not been extensively studied.

2.4.4. 1-(2',4'-diacetoxyphenyl)-3-phenyl-2-propen-1-one (4)

The only accessible information in literature mentions this compound as an intermediate in the reaction to form phenylated flavanones using BF_3-OEt to regioselectively de protect the acetyl group on C – 2 of the A ring [127].

2.4.5. 1-(2',4'-dihydroxyphenyl)-3-phenyl-2-propen-1-one (5)

The above mentioned compound is also known as isoliquiritigenin. The 2,4-dihydroxy chalcone was reported as one of the major constituents isolated from the leaf resin of *Zuccagnia punctata* and *Flemingia chappar* [124]. It showed protective effects on the ethanol induced gastro-duodenal tract injury in rats [128], antimicrobial activity against multi – resistant gram-negative bacteria [54]. It was isolated as one of the extracts *of Ononis natrix* sub species *ramosissima* [129]. The above 2,4-dihydroxychalcone was previously isolated from the leaves of *M. calabura* collected in Thailand and was found to be cytotoxic against a small panel of human tumour cell lines [130].

2.4.6. 5-acetoxy-2,3-dihydro-7-hydroxy-2-(2'-methoxyphenyl)-4H-1-Benzopyran-4-one (6)

There is no information in literature on this compound.

2.4.7. 2,3-dihydro-7-hydroxy-2-phenyl-4H-1-Benzopyran-4-one (7)

This compound has been isolated from *Clerodendron phlomidis* [131], the roots of *Virolasurinamensis* and was found to have antifungal activity [132]. It was also isolated from the aerial parts of *Ceratiola ericoides* [123], *the* wood of *Platymiscium praecox* Mart [133]. Yang et al (2008) found that 7-hydroxyflavanone, 5,6,7-trihydroxyflavanone, and 4',5,7-trihydroxyflavanone could be applicable to be a potential anti-metastatic agent of SCC-4 cancer cells [134].

2.4.8. 5,7-(diacetoxy)-2,3-dihydro-2-phenyl-4H-1-benzopyran-4-one (8)

This compound is also known as acetylpinocembrin. It was studied as one of the thirty five plant-derived secondary metabolites against one sensitive and three multidrug-resistant clinical isolates of *Mycobacterium tuberculosis* are reported [135].

2.4.9. 5,7-(diacetoxy)-2,3-dihydro-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (9)

No information was available for this compound.

2.4.10. 2,3-dihydro-5-hydroxy-2-(4'-hydroxy-3'-methoxyphenyl)-7-methoxy-4H-1-benzopyran-4-one (10)

This compound is also known as 5,4'-dihydroxy-7,3'-dimethoxyflavanone (DDF), it was detected with three other flavonoids in extracts of the flowers of *Paulownia tomentosa* using high performance liquid chromatographic (HPLC) technique coupled with photodiode array (PDA) detection was developed for the simultaneous determination of the four flavonoids [136]. DDF was also isolated in *Mariscus psilostachys* together with five flavans and two flavanones. Antimicrobial activity against *Candida albicans* and *Cladosporium cucumerinum* were determined for all compounds. DDF was found to inhibit the growth of and *Cladosporium cucumerinum* only at concentration higher than 5µg but was found to be inactive on the growth of *Candida albicans [137]*. The above compound was isolated as one of the six flavanones in chaparral sub shrub, *Mimulus aurantiacus* [138]. It was also isolated in the crude methanolic extract of the aerial parts of *Tillandsia streptocarpa* was investigated for their acute toxicity and anti-oedematogenic, antioxidant and antimicrobial activities [139].

2.4.11. 2,3-dihydro-5-hydroxy-2-(4'-hydroxyphenyl)-7-methoxy-4H-1-benzopyran-4-one (11)

This compound was prepared as one of the O-methyl analogues of naringenin using narinenin as a starting material [140]. It was present as an intermediate in the synthesis of flavocommelin, a component of the blue supramolecular pigment, commelinin, from *Commelina communis* [141].

2.5. Characterization techniques

2.5.1. Electrochemical characterization

2.5.1.1. Voltammetric techniques

The more attractive features of voltammetric methods are high sensitivity, low cost, simplicity and relatively short measurement time [116, 142] [143].

Limitations include that the sample must be dissolved, it provides little information on species identification, detection limits vary from parts per thousand to parts per trillion. Advantages include the uses of wide range of solvents and electrolytes, temperatures, has rapid analysis time, allows simultaneous determination of various analytes, has ability to determine kinetic and mechanical parameters, allows adsorption processes on surfaces and transport, speciation and thermodynamic properties of solvated species.

2.5.1.1.1. Instrumentation

Basic components in electrochemical system are a potentiostat, computer and an electrochemical cell.

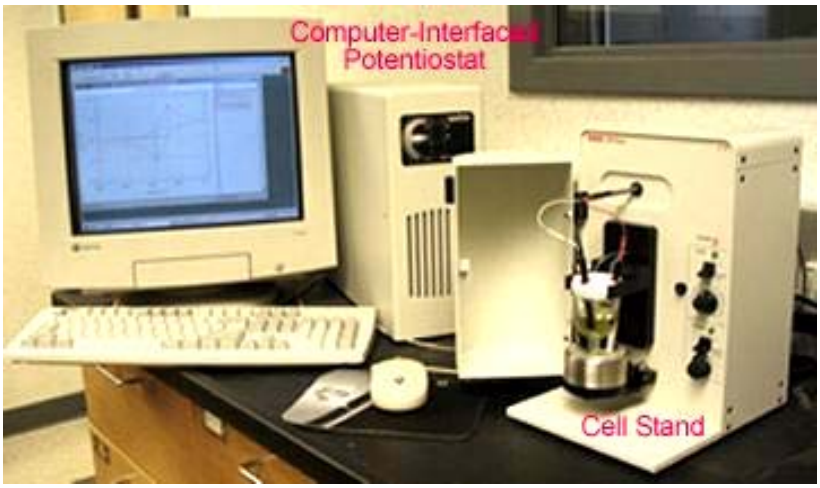


Figure 13*: Illustration of Bas Epsilon electrochemical analyzer*

Potentiostat

The potentiostat functions by maintaining the potential of the working electrode at a constant level with respect to the reference electrode by adjusting the current at an counter/auxiliary electrode [144].

Electrode and cell

The normal material for cell construction is Pyrex glass for reasons both of visibility and general chemical inertness. The photograph below (**Figure 14**) shows a conventional three electrode cell showing the working electrode (left, glassy carbon), reference electrode (middle, Ag/AgCl) and auxiliary electrode (right, Pt-disk electrode). A gas line for purging the solution with nitrogen is also evident [145].

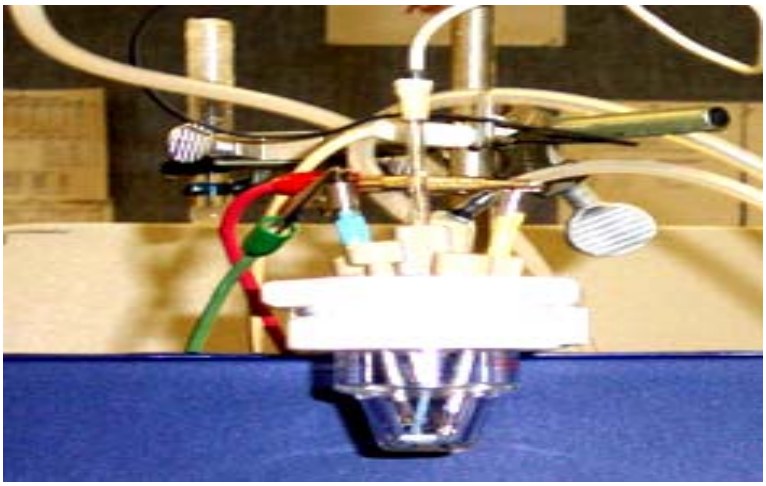


Figure 14*: Illustration of setup of a three electrode system*

Working electrode

The working electrode has various geometries and materials ranging from mercury drops to platinum disks. Others include gold, platinum and glassy carbon electrodes.

Glassy carbon electrode

Glassy carbon, as shown below (**Figure 15**) also called vitreous carbon, can be fabricated as different shapes, sizes and sections. It is a non-graphitizing carbon which combines glassy and ceramic properties with those of graphite. The most important properties are high temperature resistance, hardness (7 Mohs), low density, low electrical resistance, low friction, low thermal resistance and impermeable to gases and liquids [146-147].

Figure 15*: Illustration of the different types and sizes of glassy carbon electrodes (GCE) [146]*

Reference electrode

This is an electrode which has a stable and well-known electrode potential. The high stability of the electrode potential is usually reached by employing a redox system with constant (buffered or saturated) concentrations of each participants of the redox reaction. The major requirements of a reference electrode are that it be easy to assemble and maintain, provide a reversible half reaction with Nernstian behaviour and that its potential be stable [148].

Silver-silver chloride electrode

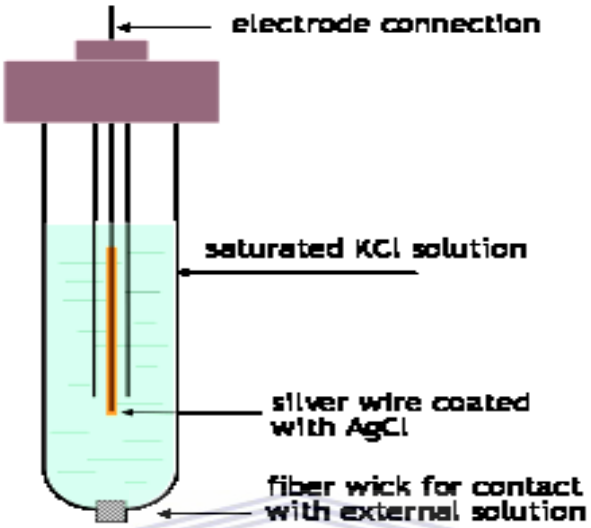


Figure 16 ***: Schematic representation of the silver-silver chloride electrode [149]***

The overall reaction can be written:

$Ag^0(s) + Cl^- \leftrightarrow AgCl(s) + e^-$

This electrode usually takes the form of a piece of silver wire coated with silver chloride. The coating is done by making the silver the anode in an electrolytic cell containing hydrochloric acid ; the silver ions combine with chloride ions as fast as they are formed at the silver surface. This reaction is characterized by fast electrode kinetics, meaning that a sufficiently high current can be passed through the electrode with the 100% efficiency of the redox reaction (dissolution of the metal or cathodic deposition of the silver-ions). The reaction has been proved to obey these equations in solutions with pH's of between 0 and 13.5 [150].

Auxiliary/counter electrode

The only condition of a counter electrode is, that is must not dissolve in the electrolyte. Only noble metals and carbon fulfil this condition perfectly. The above mentioned electrode consists of a thin platinum, gold wire or graphite [31].

2.5.1.1.2. Cyclic voltammetry

Cyclic voltammetry is used for study of redox processes rather than quantification, to understand reaction intermediates and obtaining stability of reaction products. The current at the working electrode is plotted versus the voltage. During the experiment the working potential is ramped linearly versus time, when the cyclic voltammogram reaches a set potential, the working electrode ramp is inverted. This technique is based on varying potential at the working electrode in both the forward and reversible directions at some scan rate while monitoring current. The important parameters are the peak potentials (E_{pa}, E_{pc}) and peak currents (I_{pa},I_{pc}) of the anodic and cathodic peaks [148].

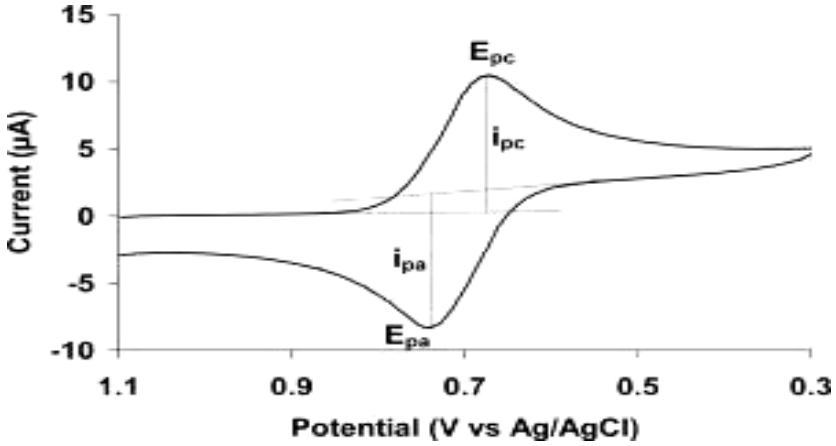


Figure 17*: Typical cyclic voltammogram where i_{pc} and i_{pa} show the peak cathodic and anodic current respectively for a reversible reaction [151]*

$$\Delta Ep = E_{pa} - E_{pc} = 2.303\ RT/nF$$

For a reversible reaction at 25º C with n electrons ΔE_p should be 0.0592/nV or 60 mV for one electron. Irreversibility due to slow electron transfer rate $\Delta E_p > 0.0592/nV$ or greater than 70 mV for one electron.

The formal potential for reversible couple is $E_f^{\circ} = (E_{pc} + E_{pa})/2$. And for an irreversible reaction, the peak current is:

$$I_p = 2.686 * 10^5\ n^{3/2}\ A.\ C^0.D^{1/2}.\upsilon^{1/2}$$

Where, I_p – peak current (Amps), A – area of electrode (cm^2), D – diffusion coefficient (cm^2s^{-1}), C^0 – concentration ($mol.cm^{-3}$), υ- scan rate ($V.s^{-1}$).

Experimental method

As the electrode potential ramps linearly versus time, this known as the scan rate ($V.s^{-1}$). The potential is measured between the reference and working electrodes and the current is measured between the working and reference electrodes and data is plotted as current (I_p) versus potential (V). The current will increase as the potential reaches the reduction or oxidation potential of the analyte and start falling as the concentration of the analyte is depleted close to the electrode surface. If redox couple is reversible, when applied potential is reversed it will reach a potential that will re oxidize the product formed in reduction reaction. Current peak will be proportional to the square root of the scan rate, if electronic transfer is fast and current is limited by diffusion of species on the electrode surface [151].

Experimental setup

A reference electrode, working electrode and counter electrode are used in what is known as a three electrode setup. Electrolyte is added to test solution to ensure conductivity. Working electrode material and solvent determines range of potential. Electrodes sit in unstirred solution during measurement. Still solution allows for diffusion controlled peaks. Stirring solution between measurements supplies the electrode with fresh analyte for each new experiment. Sometimes analyte precipitates on the electrode surface because the cyclic voltammetry alters the charge of the analyte. The working electrode is enclosed in a rod of inert insulator with disk exposed at one end. The counter electrode is any material which conducts readily and does not react with solution [151].

2.5.1.1.3. Square wave voltammetry

The excitation signal in square wave voltammetry consists of symmetrical square wave of amplitude (E_{sw}). The net current, I_{net} is obtained by taking the difference between the forward and reverse currents and is centred on the redox potential. The peak height is directly proportional to the concentration of the electro active species and the detection limits are as low as 10^{-8} M. Advantages of square wave voltammetry include speed, excellent sensitivity, reflection of background currents and allows repetitive scanning and increased signal to noise ratio [152-154]. SWV has shorter analysis time, decrease in potential associated with fouling of the electrode surface and increased sensitivity when compared to CV [106].

2.5.2. Nuclear magnetic resonance

2.5.2.1. Fundamental concepts of NMR spectroscopy

2.5.2.1.1. Spin of the nucleus

The atomic nucleus can be viewed as a positively charged sphere that is spinning on its axis. The spin is an inherent property of the nucleus and because charge is being moved it creates a small magnetic field aligned with the axis of spinning. The resonance frequency (γ) is proportional to the magnetic field strength (B_0). This relationship is the basis of nearly every phenomena in NMR [155].

$$\omega_0 = 2\pi \upsilon_0 = \gamma B_0$$

2.5.2.1.2. Chemical shift

At any given field strength each nucleus has a characteristic resonant frequency do we can choose the nucleus we are interested in observing. The nucleus is located at the centre of a cloud of electrons, and we know that the electrons are easily pulled or pushed away toward an atom changing the electron density around that nucleus. The nucleus can perceive a slight difference in field depending on its position within a molecule [155]. The shielding constant reflects the extent to which electrons cloud around the nucleus and shield it from the external magnetic field. These differences are what we call chemical shifts.

$B_{eff} = B_0 (1 - \sigma)$

$V_0 = \gamma B_{eff} = \gamma B_0 (1 - \sigma)$ where, σ is the shielding constant in parts per million (ppm),

2.5.2.1.3. Continuous wave spectrometer

The technique where radio frequency is applied continuously as frequency is gradually changed is known as continuous wave spectrometry. The continuous wave spectrometer consists of a transmitter with weak radio frequency (RF) energy; the frequency is gradually decreased with constant magnetic field (B_0). A detector then monitors the amount of RF energy absorbed and this signal is recorded.

The result is an NMR spectrum which is a graph of RF energy versus frequency, where the position of the absorption peak on the spectrum tells us about the chemical environment of the spin within the molecule [155].

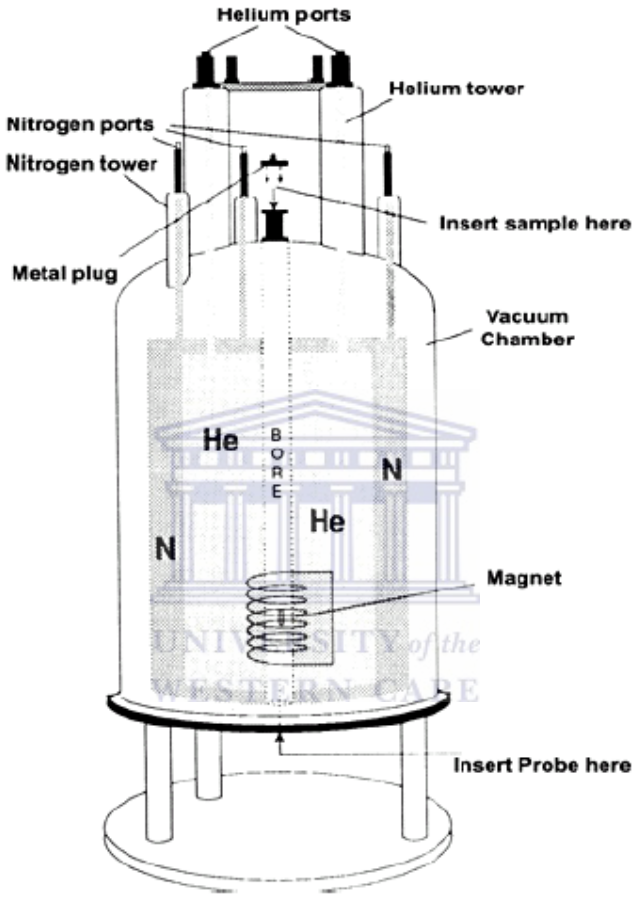


Figure 18: Illustration of a general NMR spectrometer [156]

2.5.2.1.4. Pulsed fourier transform

A very brief pulse of high-power RF energy is used to excite all the nuclei in the sample. Immediately after the pulse is over, the nuclei are organized in such a way that their preceding magnets sum together to form a net magnetization of the sample, which rotates at Larmor frequency. The coil

used to transmit RF is now used as a receiver, and the frequency is observed at the precise Larmor frequency, υ_0. This signal which oscillates in time at Larmor frequency is recorded by the computer and a mathematical calculation called Fourier transform measures the frequency of oscillation of the signal [155].

2.5.2.2. Proton nuclear magnetic resonance (NMR)

Each resonance in the ^{1}H spectrum is paired with a hydrogen or group of equivalent hydrogens. A resonance is a single chemical shift position in the spectrum and maybe be a single peak or maybe split into a complex pattern of peaks. To uniquely assign peaks we need to correlate protons to other protons using their coupling constants (*J*) by through-bond relationship. The magnitude of the coupling constants tells us about the geometrical relationship of the bonds connecting the atoms. Three bond (vicinal) relationships are the most useful because the coupling constant is related to the dihedral angle between the bonds attached to the protons. *J* coupling is transmitted through bonds and more specifically through electron in bonding orbitals. The magnitude of the coupling constant depends on orbital overlap. Minimum *J* values are observed for dihedral angles near 90º. Two or more protons may have the same chemical equivalence but may not have the same coupling constant. The relationship between the dihedral angle and coupling constant is represented by a mathematical equation:

$J = 7 - \cos\varphi + 5\cos(2\varphi)$ where φ is the dihedral angle [155]

Chapter 3

Experimental

3.1. Apparatus and reagents

Galenia africana and *Elytropapus rhinocerotis* extracts were sourced from Prof Green's laboratory as 20% solutions of the total masticated plant in ethanol. The standards benzene, anisole, resorcinol, phenol, 2-butanone, 4*H*-pyran-4-one, 1,3-methoxyphenol, 1,2-methoxyphenol and 2-penten-2-one were purchased from Sigma Aldrich (Cape Town, South Africa). All reagents are of analytical grade. All aqueous solutions were made up with ultra pure water from a Millipore Milli-Q system (South Africa). The pH of the phosphate buffer was measured using a pH meter (Hanna instrument, HI 221 Calibration Check Microprocessor pH meter with accuracy of ± 0.05). Thin layer chromatography (TLC) was performed on smaller plates cut from 20x20cm aluminium plates coated with Merck Silica gel 60 F254 with a thickness of 0.1 mm. Preparative layer chromatography (PLC) was performed on 20x20cm glass plates coated with Merck silica gel 60 F254 with thickness of 1 mm. The silica gel used to pre absorb the sample was silica gel 60 (35 – 70 mesh) with a particles size of 0.2 – 0.5 mm purchased from Kimix (Cape town, South Africa) while the silica gel used to pack the column was silica gel 60 (70 – 230 mesh) with a particle size of 0.063 – 0.200 mm) purchased from Merck (Cape town, South Africa). Aluminium oxide Fluka type 504C was also used as stationary phase (Merck). All materials were weighed using Mettler Toledo AB104 analytical scale. The NMR spectra were recorded on a GEMINI 200MHz NMR spectrometer. The spectra were recorded using 5 mm tubes using $CHCl_3$ as internal standard and deutereochloroform as solvent

unless otherwise stated. The chemical shifts were recorded in parts per million (ppm) downfield from TMS. An Epsilon electrochemical analyzer (Bioanalytical Systems, West Lafayette, Indiana) was used for cyclic and square wave voltammetry measurements.

3.2. Organic experimental

3.2.1. Isolation

The 20% ethanol extracts of *Galenia africana and Elytropapus rhinocerotis* which were known to exhibit a very high degree of biological activity (private communication from Prof Green) were the primary focus of this project. The ethanol extracts were obtained as pre-columned olive-yellow solids. The following discussion applies to the *G. Africana* solid material. The crude material (400 mg) was dissolved in acetone and spotted for thin layer chromatography (TLC) analysis in an eluent of ethyl acetate: hexane (1:4). This indicated approximately six different components were present which could be clearly seen either according to differences in colour or retardation factor (R_f) after being sprayed with a 1% solution of vanillin in sulphuric acid according to standard procedures of heating the plate afterwards. The crude solid material ~ 1g was then pre-absorbed on silica gel (particle size 0.2 – 0.5 mm) using a small amount of acetone to dissolve the sample. The column (1m x 20 mm) was then packed with silica gel (particle size 0.063 – 0.200 mm) with the sample plug on top and eluted with EtOAc: hexane (1:4). Various fractions were collected in tubes using a fraction collector. Fractions were analyzed by TLC and were grouped together into sub fractions according to behavioural similarities on TLC plates as visualized under UV light and vanillin spray giving a yellow to red spot for the flavanoids.

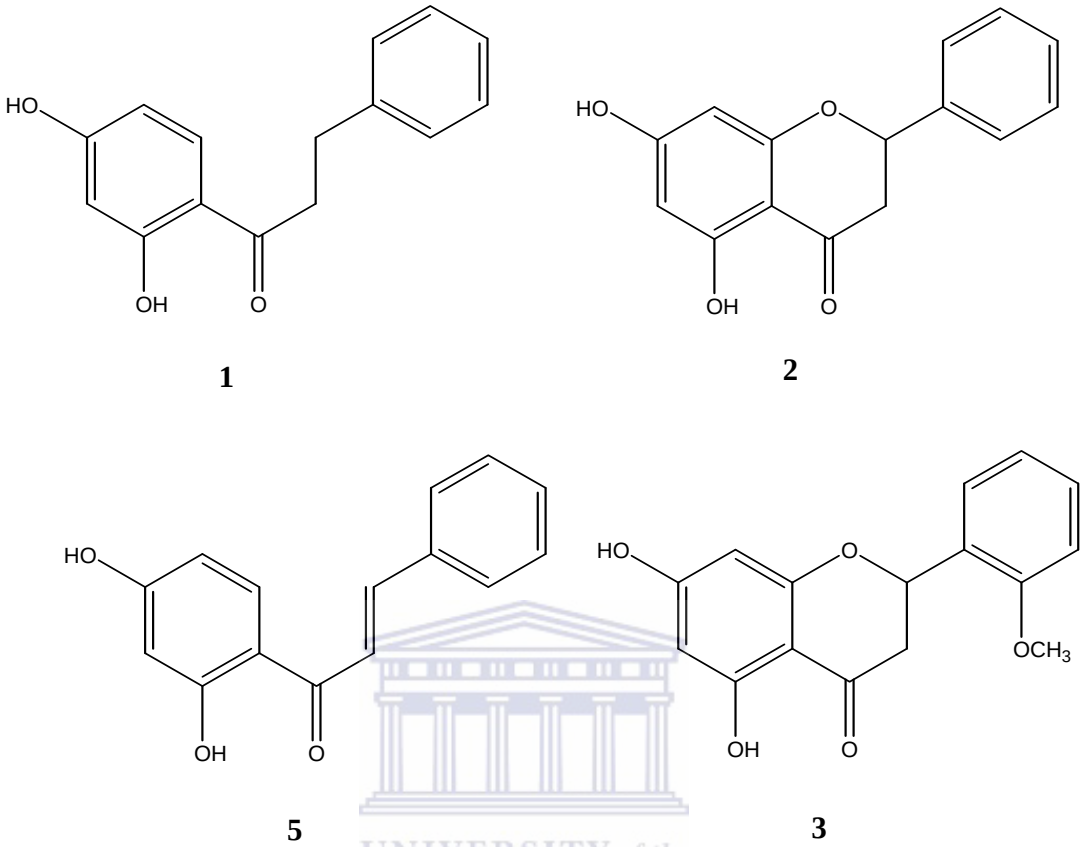


Figure 19*: Flavanoids isolated from Galenia Africana.*

Table 4: The six major fractions isolated from first separation.

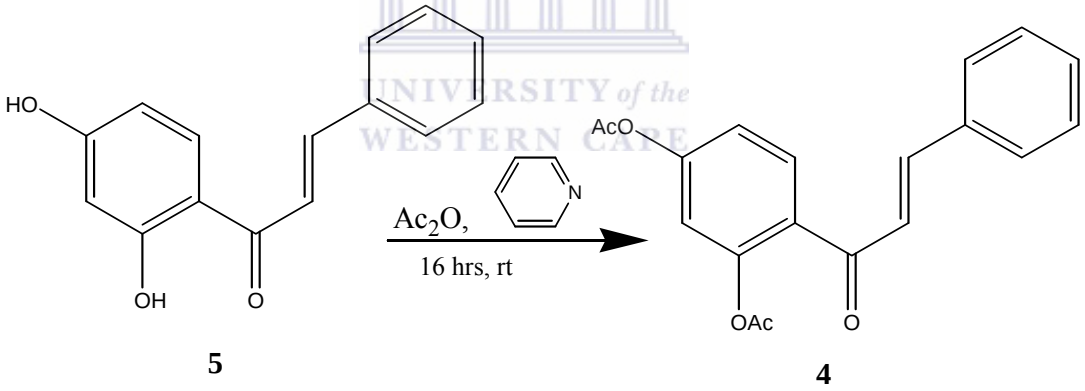
Fraction	Compounds			
	1	2	5	3
1	+	-	-	-
2	+	+	-	-
3	+	+	-	-
4	-	+	+	+
5	-	-	+	+
6	-	-	+	+
7	-	-	+	+

+ shows presence of flavanoids in fraction; - shows absence of flavanoids in fraction

The first fraction contained compound **1** and some impurities as shown by the ^{1}H NMR data. This fraction showed a single spot with a retardation factor of 0.56 and when sprayed with vanillin spray a red spot was observed. The first fraction was then separated on a preparative plate using EtOAc: hexane (1:4) as solvent. Three sub-fractions were obtained the first containing oil and solvent, the second containing the pure compound **1** and the third containing impure compound **1**. The third sub fraction was re-plated in the same solvent system and further pure compound **1** was obtained (20 mg). Fractions 2 and 3 contained similar compounds. However in the second fraction compound **1** made up 60% of the mixture while compound **5** made up 40% of the mixture and in the third fraction compound **1** made up 33% of the mixture while compound **2** made 66% of the mixture as shown and calculated using ^{1}H NMR spectroscopy. The two fractions showed two red spots when sprayed with vanillin with retardation factors of approximately 0.55 and 0.47. Fractions 2 and 3 were separately re-chromatographed on a column using EtOAc: hexane (1:4) as a solvent. For each of the fractions 2 and 3 sub-fractions were obtained and using PLC were purified to obtain pure compounds **1** (12 mg) and **2** (22 mg). Fraction 4 which contained compounds **2**, **3** and **5** was re-chromatographed on a column and eluted with EtOAc: hexane (3:7). Fractions collected were submitted to TLC and the first to elute when sprayed with vanillin indicated two spots, the one spot was red in colour with a retardation factor of 0.58 and the other yellow in colour with a retardation factor of 0.52. Compound **2** was separated successfully in later fractions (13 mg) while compound **3** and **5** were obtained as a mixture. The remaining fractions **5**, **6** and **7** all consisted of the same mixture of compound **3** and **5** which was very difficult to separate using the same solvent system as above even when using PLC. Separation was thus attempted using acidic aluminium oxide as a stationary phase, and as a

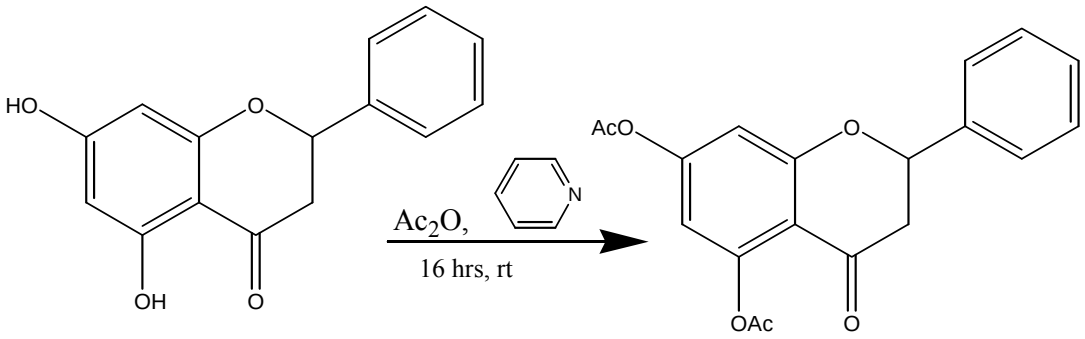
mobile phase the following combinations were used EtOAc: hexane (1:4), EtOAc: hexane (3:7), MeOH: CH_2Cl_2 (9:1) and 100% MeOH but the crude sample to be separated remained adsorbed on the aluminium oxide regardless of solvent used. The separation was then attempted using basic aluminium oxide as a stationary phase and MeOH: CH_2Cl_2 (9:1) and 100% ethyl acetate and still no movements were observed. A mixture of EtOAc: hexane: CH_2Cl_2 was then tried with the following ratios 70:30:5, 70:30:10, 70:30:20, 70:30:30, and finally 70:30:45 which managed to separate compound **5** (21 mg) and **3** (12 mg) after multiple re-runs on preparative layer plate on silica gel backed plates.

3.2.2. Synthesis



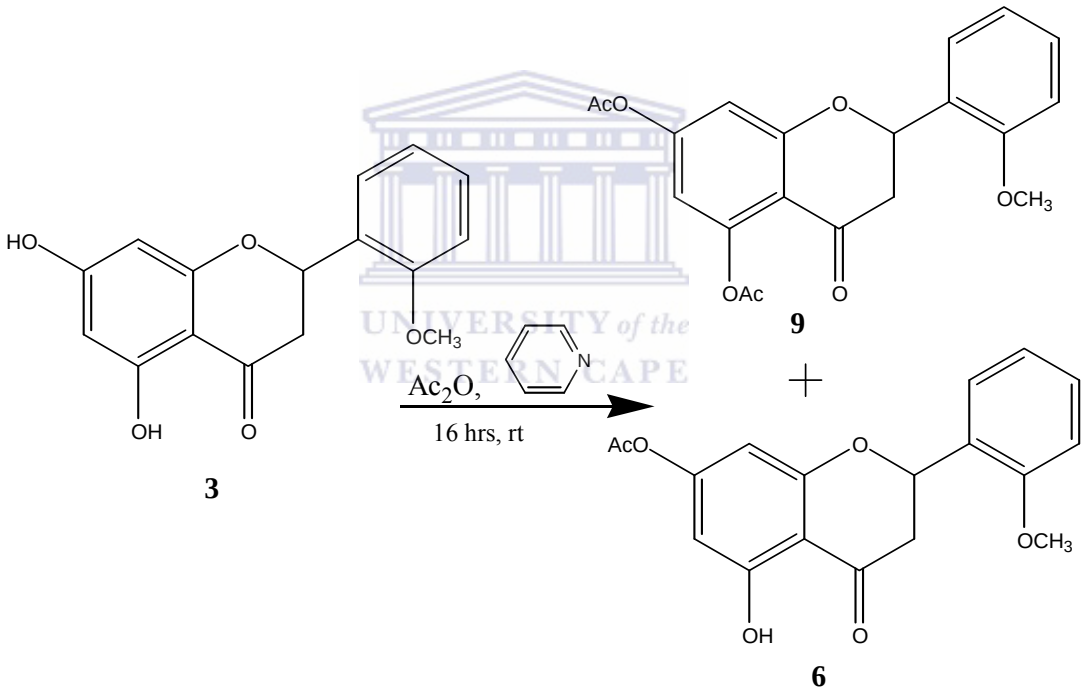
5 **4**

Scheme 2*: Acylation of 1-(2', 4'-dihydroxyphenyl)-3-phenyl-2-propen-1-one (5)*



2 **8**

Scheme 3*: Acylation of 2,3-dihydro-5,7-dihydroxy-2-phenyl-4H-1-benzopyran-4-one (2)*

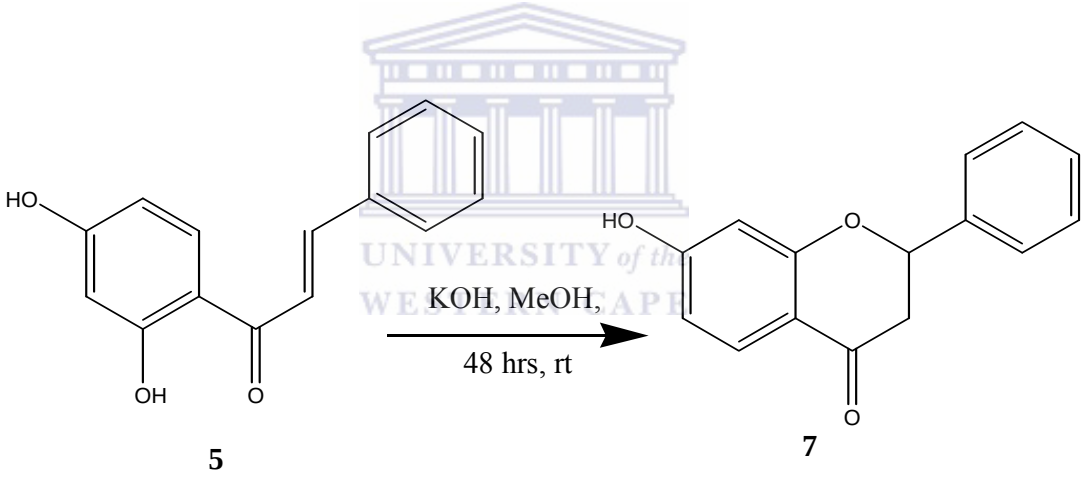


3 **9** **6**

Scheme 4*: Acylation of 2,3-dihydro-5,7-dihydroxy-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (3)*

Fraction 4 (0.2765g) containing compounds **2**,**3** and **5** was added to a solution of 10 ml glacial acetic anhydride and 3 ml pyridine and the resultant solution was stirred at room temperature for 16 hours. The basic pyridine removes the hydrogen atom from

the hydroxyl group and allows the electrophylic O-acylation with acetic anhydride to occur. After stirring, the solution was treated with ice water and stirred for an hour. A sticky slurry formed at the bottom of the beaker and the aqueous solution was then extracted with dichloromethane and rinsed with 5% $NaHCO_3$ to remove any remaining acetic acid. The dried extract (anhydrous magnesium sulphate) was filtered and evaporated to leave a residue. After spotting trials on TLC plates the spots were eventually resolved when eluted with the solvent system EtOAc: hexane (3:7). The residue was then separated by PLC using the same solvent system and pure acylated products were obtained after a few repeated elutions to afford the acetates **8**, **9** and **6** (40 mg, 45 mg and 35 mg respectively).



5 **7**

Scheme 5*: Cyclization 1-(2', 4'-dihydroxyphenyl)-3-phenyl-2-propen-1-one (5).*

Compound **5** (50 mg) was then cyclised, using a concentrated solution of 50% potassium hydroxide in methanol with stirring at room temperature for 48 hours. After stirring, mixture was poured into water and then acidified with 10% hydrochloric acid. The aqueous mixture was then extracted with EtOAc and dried with magnesium sulphate. Chromatographic purification using EtOAc: hexane (1:4) as eluent afforded the flavanoid (30 mg; 60%).

3.3. Electrochemistry

3.3.1. Preparation of solutions

Phosphate buffer solution was prepared by mixing appropriate volumes of 0.1M sodium dihydrogen phosphate (NaH_2PO_4) and 0.1M sodium hydrogen phosphate (Na_2HPO_4) with ultra pure water with a conductivity of 5.5 X 10^{-6} $S.m^{-1}$ to produce the desired pH. Ethanol was selected as the most suitable solvent, all flavonoids and their acetate derivatives were dissolved in absolute ethanol to produce various concentration ranges between 0.029 and 0.142 M.

3.3.2. Voltammetric measurements

For electrochemical measurements, a three electrode cell consisting of a 3 mm diameter glassy carbon working electrode, a platinum wire auxiliary electrode, and an Ag/AgCl (3 M NaCl) reference electrode were used. The surface of the glassy carbon electrode was freshly polished with 1.0 um, 0.05 μm and 0.3 μm alumina powder on a micro cloth pad, rinsed with distilled water and degreased with ethanol in an ultrasonic bath because of possible film formation and memory effects [5, 157]. The GCE was also electrochemically cleaned with 1M H_2SO_4 and rinsed with distilled water. After electrochemical cleaning of the GCE, the required volume of sample was added by micropipette into supporting electrolyte. The potentials were recorded against the silver-silver chloride electrode as a reference electrode used in conjunction with the platinum electrode placed in a 50 ml volume glass cell together with the glassy carbon electrode. Phosphate buffer (5ml) of pH ~ 7 was used as the supporting

electrolyte. The scan was taken in a potential range of – 1500 mV and + 1500 mV with the following scan rates 20, 50 and 100 mV/s for cyclic voltammetric measurement and the scan was taken in the same potential window with a frequency of 15 hertz for square wave measurements. Prior to each measurement, the background currents were measured in phosphate buffer alone and subtracted from the currents measured with compound added in phosphate buffer. In order to minimize adsorption onto the electrode surface, measurements were performed immediately after the addition of the compound. The sample in the electrochemical cell was de-aerated by purging with high purity argon during electrochemical measurements. All experiments were carried out at room temperature. For square wave measurements, determination of the flavonoids was carried out at a frequency of 15 Hz, step potential 20 mV/s with phosphate buffer of pH 7 as supporting electrolyte. For smoothing and baseline correction the software Origin 7.0 and Sigma plot were employed.

Chapter 4

Organic results and discussion

Fractionation of the ethanol-soluble extract of *Galenia africana* on a silica gel packed column followed by PLC led to the purification of two known flavanoids, two known chalocones. From the isolated compounds two new compounds were synthesized and the other three were known compounds. From the ethanol-soluble extract of *Elytropapus rhinocerotis* two known compounds were isolated. The structures of these flavanoids were determined by physical spectroscopic data measurements which included ^{1}H and ^{13}C NMR spectroscopy.

4.1. Flavanoids from *Galenia africana*

4.1.1 Compound **1: 1-(2',4'-dihydroxyphenyl)-3-phenyl propan-1-one (1)**

Formula: $C_{15}H_{14}O_3$

Molecular mass: 242 g/mol

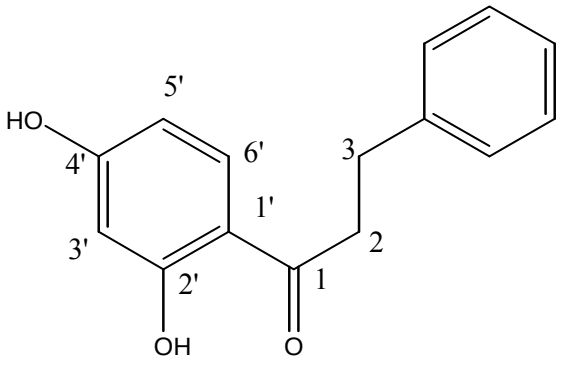


Figure 20*: Chemical structure of 1-(2',4'-dihydroxyphenyl)-3-phenyl propan-1-one (1)*

NMR: δ_H = 3.03 (t, 2H, *J* = 7.0 Hz, H - 3); 3.21 (t, 2H, *J* = 7.0 Hz, H - 2); 6.33 (m, 2H, H – 3' and H – 5'), 7.24 (m, 5H, 3 – Ar) and 7.60 (d, 1H, *J* = 9.6 Hz, H – 6').

Calculated spectrum:

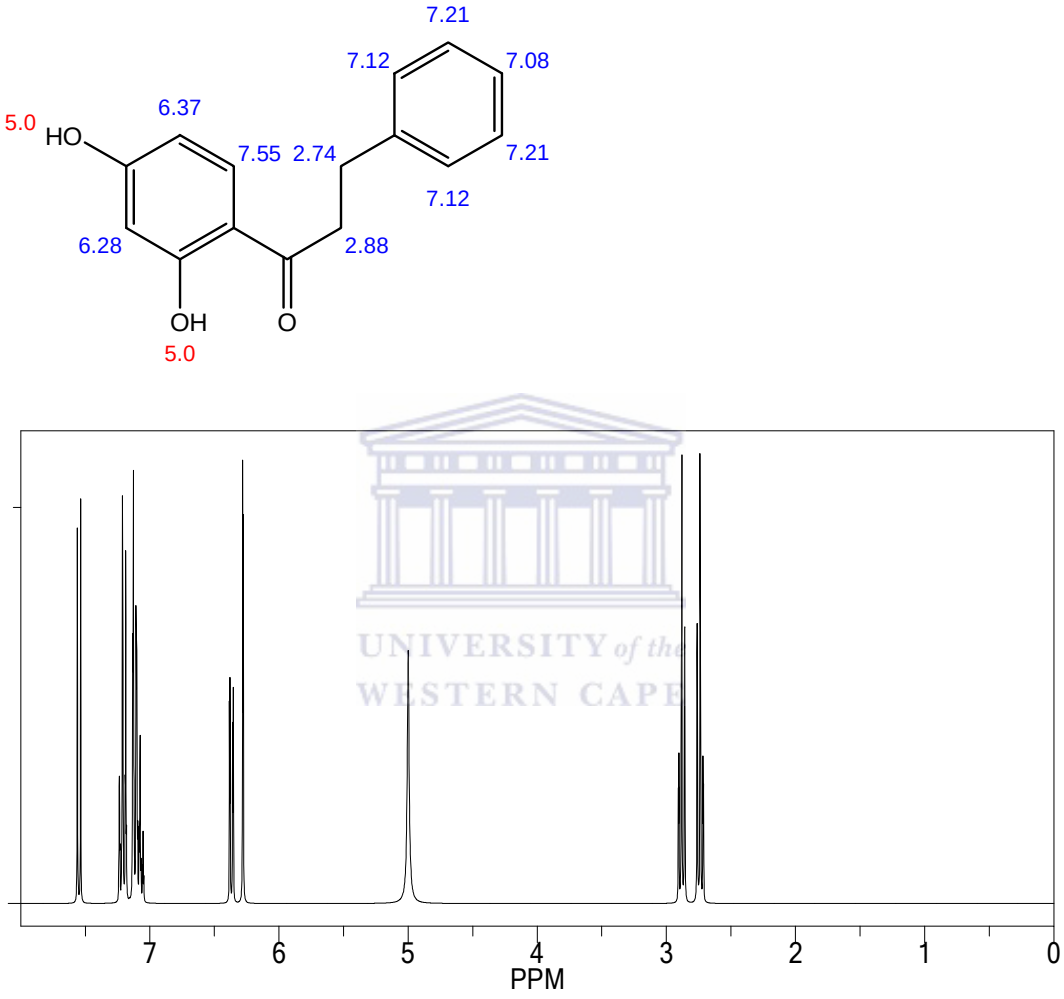


Figure 21*: ^{1}H NMR spectrum of 1-(2',4'-dihydroxyphenyl)-3-phenyl propan-1-one (1)*

The ^{1}H NMR spectra of compound **1** shows two triplets at δ of 3.03 and 3.21 belonging to protons 2 and 3 which corresponds to the values in literature of 3.02 and 3.22. A multiplet was observed for the 5 aromatic protons of ring B at 7.21 – 7.32

ppm as well as doublet at 7.60 for H – 6' which is coupled to H – 5' with a coupling constant of 9.6 Hertz which characteristic of ortho aromatic protons [130, 158].

4.1.2 Compound **2**: **<u>2,3-dihydro-5,7-dihydroxy-2-phenyl-4H-1-benzopyran-4-one (2)</u>**

Formula: $C_{15}H_{12}O_4$

Molecular mass: 256 g/mol

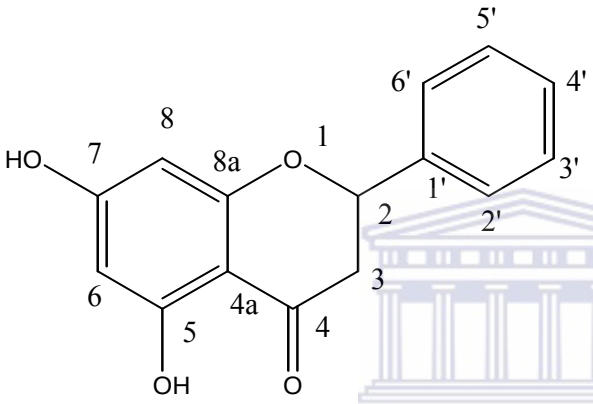


Figure 22*: Chemical structure of 2,3-dihydro-5,7-dihydroxy-2-phenyl-4H-1-benzopyran-4-one(2)*

NMR: δ_H = 2.82 (dd, 1H, *J* = 17.3, 3.1 Hz, H – 3_e), 3.09 (dd, 1H, *J* = 17.3, 13.0 Hz, H – 3_a), 5.42 (dd, 1H, *J* = 13.0, 3.1 Hz, H – 2_a), 6.00 (s, 2H, H – 6 and H – 8), 7.42 (m, 5H, Ar), 12.04 (s, 1H, 5-OH).

Calculated spectrum:

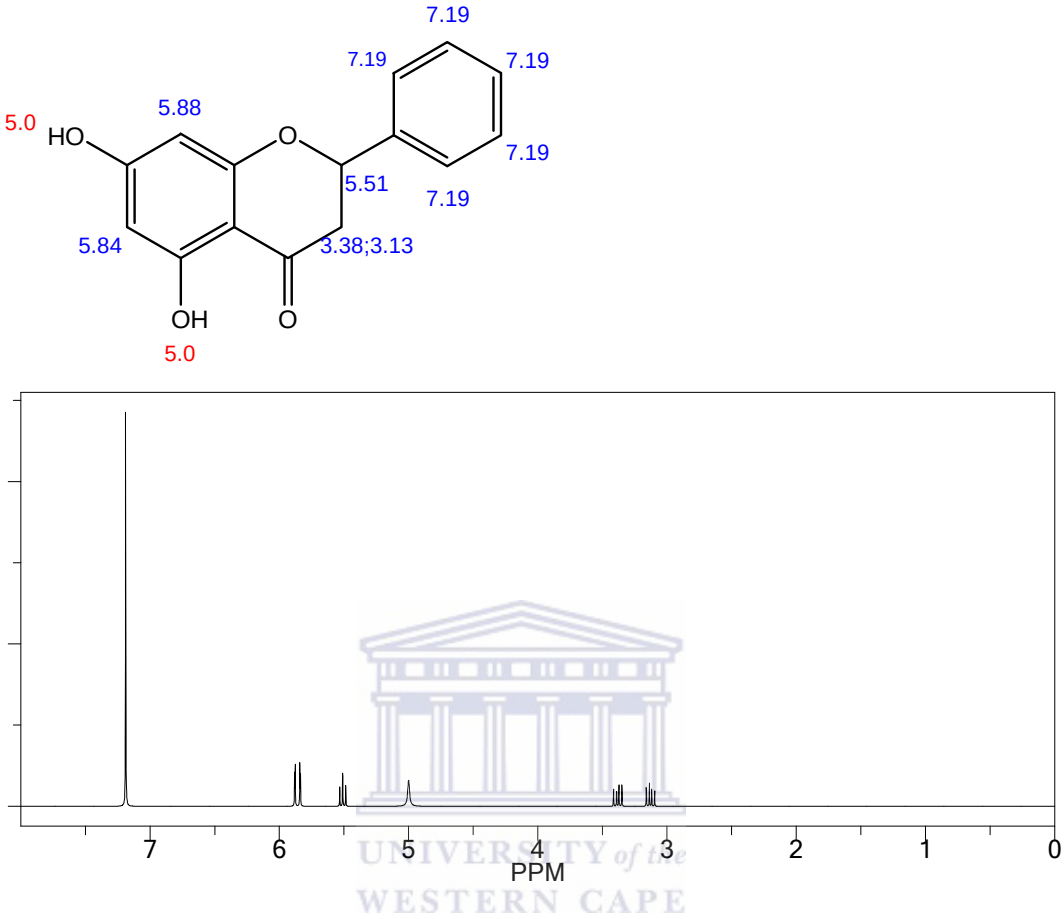


Figure 23*: ^{1}H NMR spectrum of 2,3-dihydro-5,7-dihydroxy-2-phenyl-4H-1-benzopyran-4-one(2)*

The spectrum of compound **2** displayed signals of the three oxygenated methine and methylene doublet-doublets at δ of 5.42 (dd, 1H, 13.0 and 3.1 Hz, H – 2_a), 2.82 (dd, 1H, *J* = 17.3, 3.1 Hz, H – 3_e), 3.09 (dd, 1H, *J* = 17.3, 13.0 Hz, H – 3_a), which demonstrate geminal and vicinal coupling. The geminal coupling between proton H – 3a and H – 3e is 17.3 Hz while the axial – axial vicinal coupling between H - 3a and H – 2 is 13.0 Hz. Axial – equatorial coupling constant between H – 3e and H – 2_a is 3.1 Hz due to the dihedral angle of almost 90°. A surprising finding was that H – 6

and H – 8), appeared as a singlet at δ 6.00. A 5-proton multiplet for the unsubstituted ring B appeared at δ 7.42. [158-160]. Also observed was a downfield exchangeable singlet at 12.04 (s, 1H, OH – 5) which is characteristic of the chelated OH – 5 of flavones and flavanones. According to the above spectrum compound **2** is a flavanoid with an unsubstituted B ring [159, 161].

4.1.3 Compound 3: **2,3-dihydro-5,7-dihydroxy-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (3)**

Formula: $C_{16}H_{14}O_5$

Molecular mass: 286 g/mol

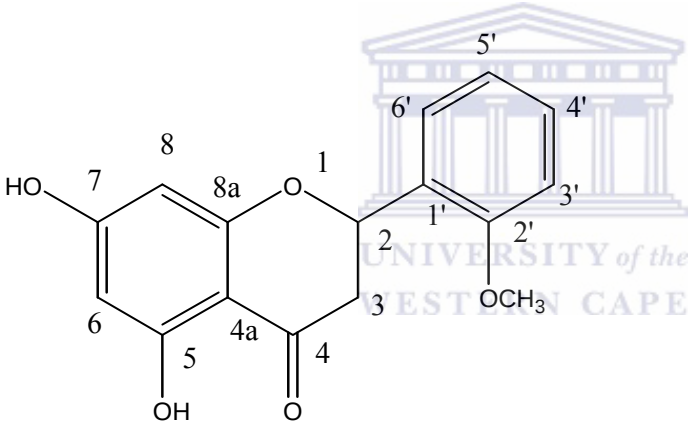


Figure 24*: Chemical structure of 2,3-dihydro-5,7-dihydroxy-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (3)*

NMR: δ_H = 2.92 (dd, 1H, *J* = 17.4 ,3.6 Hz, H – 3e), 3.15 (dd, 1H, *J* = 17.4 , 12.5,Hz, H – 3a), 3.82 (s, 3H, - OCH_3), 5.71 (dd, 1H, *J* = 12.5 , 3.6 Hz, H – 2a), 5.96 (broad s, 1H, 7 – OH), 6.09 (d, 1H, *J* = 2.2 Hz, H – 6), 6.11 (d, 1H, *J* = 2.2 Hz, H – 8), 6.89 (dd, 1H, *J* = 8.0, 2.0 Hz, H – 3'), 6.98 (dt, 1H, *J* = 8.0, 2.0 Hz, H – 5'), 7.27 (m, 1H, H – 4'), 7.33 (dd, 1H, *J* = 7.6, 1.8 Hz, H – 6'), 12.05 (s, 1H, 5 – OH).

Calculated spectrum:

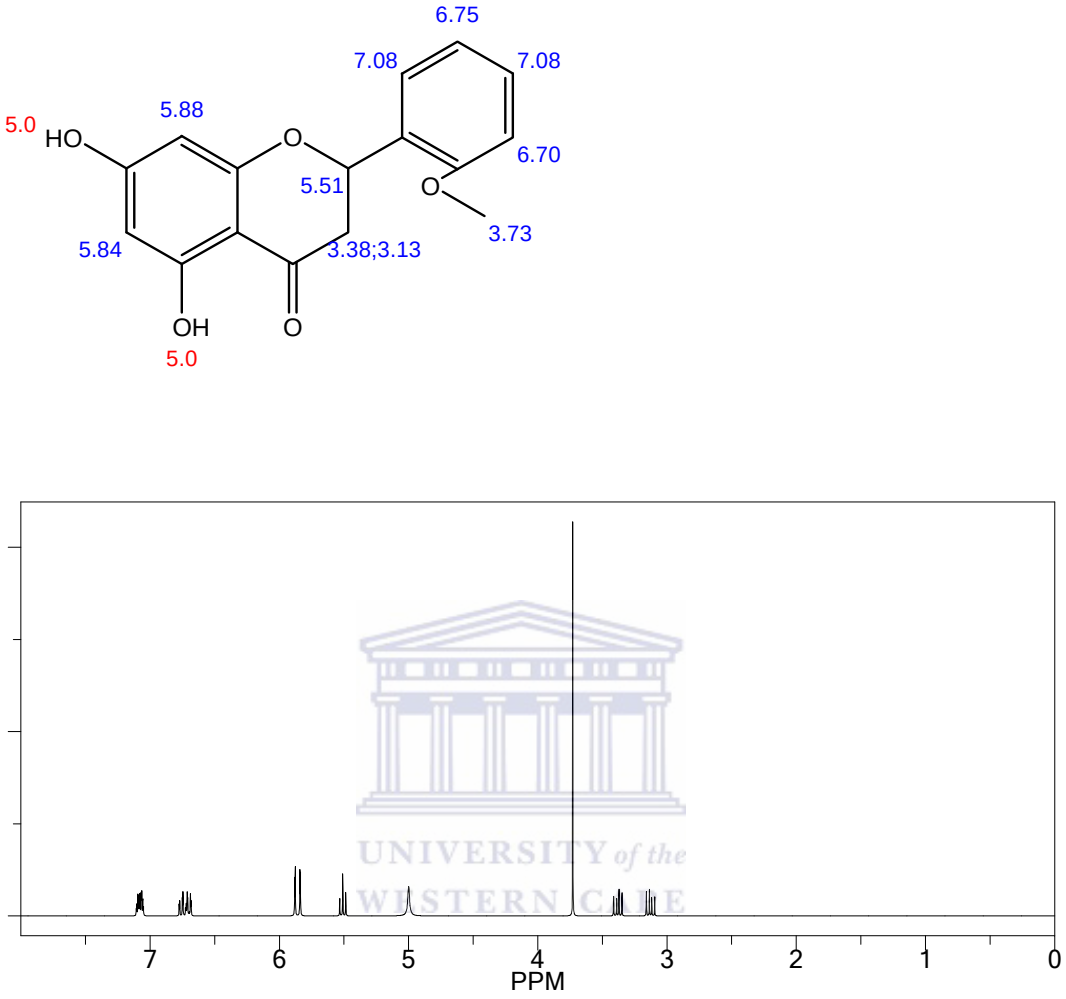


Figure 25*: ^{1}H NMR spectrum of 2,3-dihydro-5,7-dihydroxy-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (3)*

The ^{1}H NMR spectrum of compound **3** is similar to that of compound **2** because they share a similar structural backbone scaffold. There are thus similarities in δ values for H-2 and H-3 viz., δ_H 5.71 (dd, 1H, *J* = 12.5, 3.6 Hz, H – 2a), 2.92 (dd, 1H, *J* = 17.4, 12.5 Hz, H – 3e) and 3.15 (dd, 1H, *J* = 17.4, 3.6 Hz, H – 3a). The spectrum indicated the existence of two meta coupled protons at δ_H 6.09 (d, 1H, *J* = 2.2 Hz, H – 6), 6.11

(d, 1H, J = 2.2 Hz, H – 8), a methoxy group at 3.82 (s, 3H, - OCH_3) and a hydroxyl group at 12.05 (s, 1H, 5 – OH) [162].

NMR: δ_C = 43.0 (C – 3), 55.9 (- OCH_3), 77.8 (C – 2), 94.6 (C – 8), 95.6 (C – 6), 103.3 (C – 4a), 116.8 (C – 3'), 121.8 (C – 5'), 124.3 (C – 1'), 127.07 (C – 6'), 130.1 (C – 4'), 153.6 (C – 2'), 165.5 (C – 5), 165.9, (C – 8a), 168.0 (C – 7), 196.1 (C – 4).

Calculated spectrum:

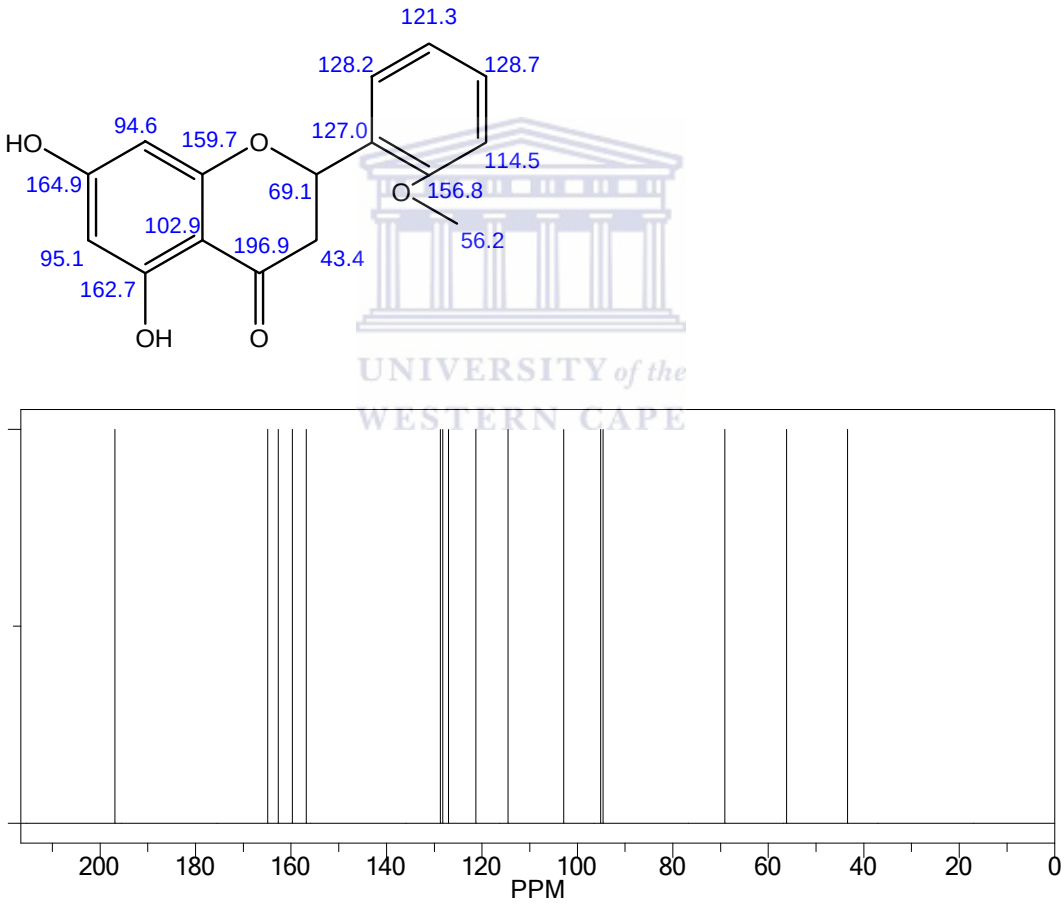


Figure 26: ***^{13}C NMR spectrum of 2,3-dihydro-5,7-dihydroxy-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (3)***

The ^{13}C NMR spectra of compound **3** on average showed a pattern comprising a minimum of 15 carbon signals for each of the compounds isolated strongly suggesting a flavanoid nucleus. The spectra further all displayed an oxygenated methines at δ_C 77.8 (C – 2) and a methylene carbon having a carbonyl functionality attached viz., δ 43.0 (C – 3). Signals for the mono-substituted aromatic ring for compound **3** are assigned as follows: δ_C 116.8 (C – 3'), 121.8 (C – 5'), 124.3 (C – 1'), 127.07 (C – 6'), 130.1 (C – 4'), 153.6 (C – 2') and an aryl- conjugated ketone at δ_C 196.1 (C – 4) [158-159].

4.1.4 Compound **4**: **<u>1-(2',4'-diacetoxyphenyl)-3-phenyl-2-propen-1-one (4)</u>**

Formula: $C_{19}H_{15}O_5$

Molecular mass: 323 g/mol

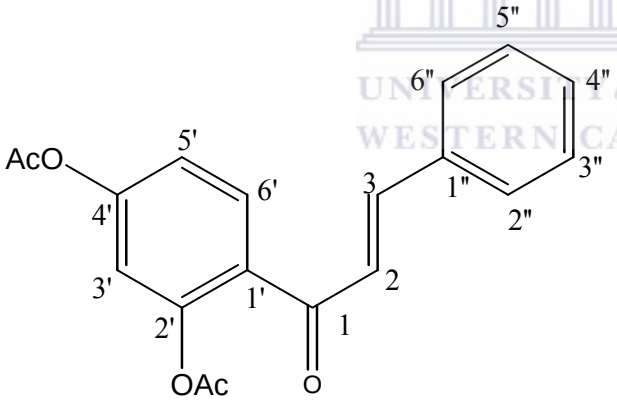


Figure 27*: Chemical structure of 1-(2',4'-diacetoxyphenyl)-3-phenyl-2-propen-1-one (4).*

NMR: δ_H = 2.24 (s, 3H, CH_3CO), 2.32 (s, 3H, CH_3CO), 7.02 (d, 1H, *J* = 2.2 Hz, H – 3'), 7.13 (dd, 1H, *J* = 8.4, 2.2 Hz, H – 5'), 7.17 (d, 1H, *J* = 16.0 Hz, H – 2), 7.42 (m, 3H, H – 3'', H – 4'' and H – 5''), 7.60 (m, 2H, H – 2'' and H – 6''), 7.63 (d, 1H, *J* = 16.0 Hz, H – 3), 7.75 (d, 1H, *J* = 8.4 Hz, H – 6').

Calculated spectrum:

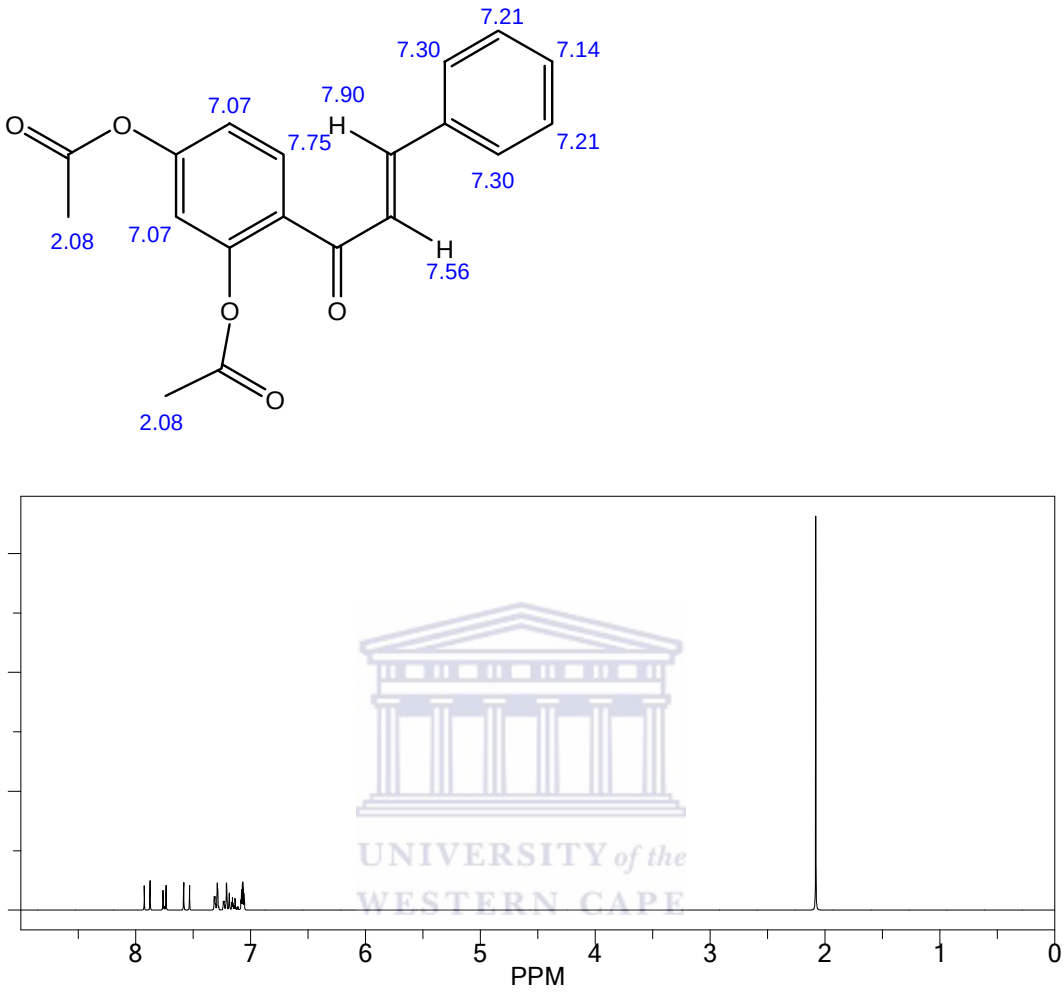


Figure 28*: ^{1}H NMR spectrum of 1-(2',4'-diacetoxyphenyl)-3-phenyl-2-propen-1-one (4)*

The proton spectrum of this compound displayed similarities to that of compound **5** viz., with a pair of *trans*-coupled doublets at δ_H 7.17 (d, 1H, J = 16.0 Hz, H – 2) and 7.63 (d, 1H, J = 16.0 Hz, H – 3). Signals observed for the aromatic protons on ring A are δ 7.02 (d, 1H, J = 2.2 Hz, H – 3'), 7.13 (dd, 1H, J = 8.4, 2.2 Hz, H – 5') and 7.75 (d, 1H, J = 8.4 Hz, H – 6'). Two multiplets were observed at δ 7.60 and 7.42 for the protons on the un-substituted phenyl ring B. Also observed in the spectrum are two

singlets at δ 2.24 (s, 3H, CH_3CO) and 2.32 (s, 3H, CH_3CO) belonging to the two acyl groups on the A ring.

4.1.5 Compound **5**: **(1-(2',4'-dihydroxyphenyl)-3-phenyl-2-propen-1-one (5)**

Formula: $C_{15}H_{12}O_3$

Molecular mass: 240 g/mol

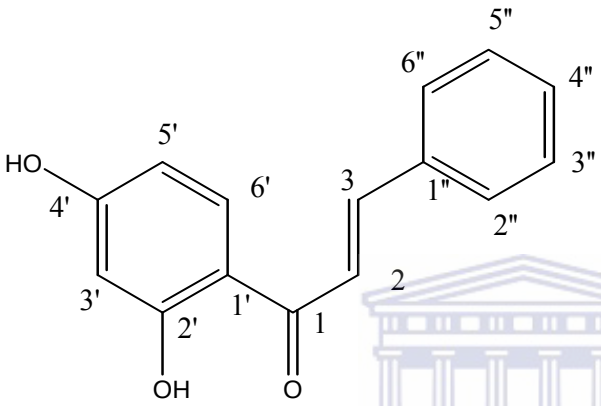


Figure 29*: Chemical structure of 1-(2',4'dihydroxyphenyl)-3-phenyl-2-propen-1-one (5)*

NMR: δ_H = 6.45 (m, 2H, H – 3' and H – 5'), 7.43 (m, 3H, H – 3'', H – 4'' and H – 5''), 7.57 (d, 1H, *J* = 15.8, H – 2), 7.65 (m, 2H, H – 2'' and H – 6''), 7.84 (d, 1H, *J* = 9.2 Hz, H – 6'), 7.89 (d, 1H, *J* = 15.8 Hz, H – 3).

Calculated spectrum:

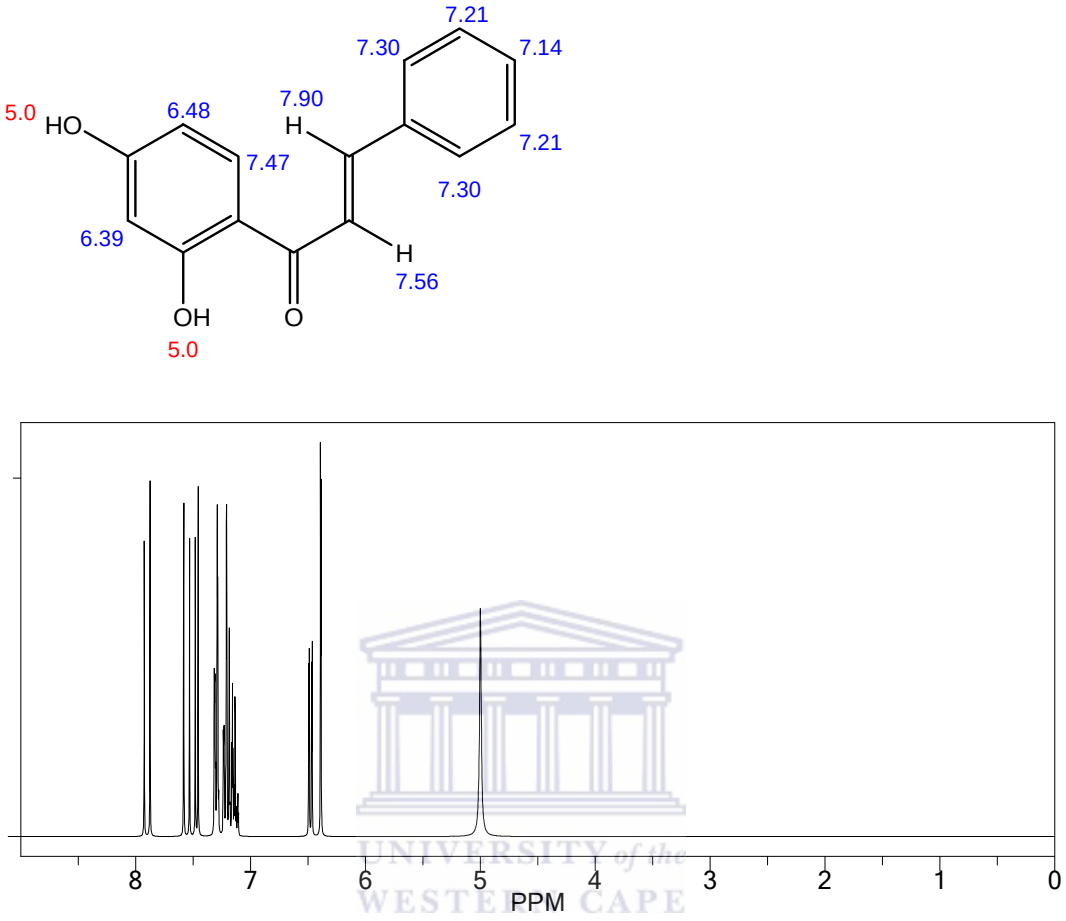


Figure 30*: ^{1}H NMR spectrum of 1-(2',4'dihydroxyphenyl)-3-phenyl-2-propen-1-one (5)*

In the proton spectrum two *trans*-coupled doublets were observed at δ 7.57 and 7.89 ppm belonging to H – 2 and H – 3 respectively, which were coupled to each other with a coupling constant of 15.8 Hz which is characteristic of *trans*-coupled alkenes. A doublet and multiplet were observed at δ 6.45 and 7.84 respectively belonging to the protons on the substituted phenyl ring A. Two multiplets were observed at δ 7.43 and 7.65 for the protons on the unsubstituted B phenyl ring. The values obtained

compared well with those given in literature [56, 123, 158]. The pattern of the peaks also corresponds with those observed in the calculated spectra as shown above.

NMR: δ_C = 103.9 (C – 3'), 107.9 (C – 5'), 114.7 (C – 1'), 120.4 (C – 3), 128.7 (C – 3'' and C – 5''), 129.1 (C – 2'' and C – 6'') , 130.8 (C – 4''), 132.1 (C – 6'), 134.9 (C – 1''), 144.7 (C – 2), 162.9 (C – 2'), 166.6 (C – 4'), 192.1 (C – 1).

Calculated spectrum:

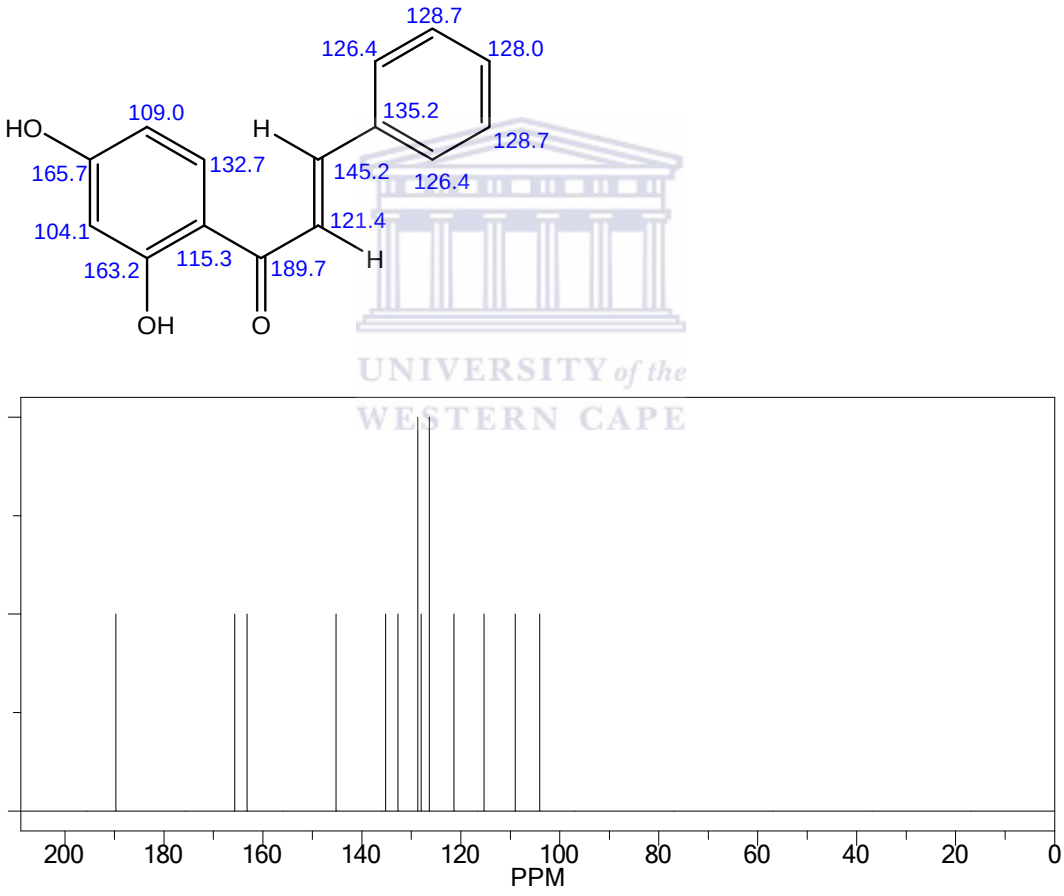


Figure 31*: ^{13}C NMR spectrum of 1-(2',4'-dihydroxyphenyl)-3-phenyl-2-propen-1-one (5)*

The signals of the unsubstituted aromatic ring are δ_C 134.9 (C – 1''), 128.7 (C – 3'' and C – 5''), 129.1 (C – 2'' and C – 6''), 130.8 (C – 4'') and those of the substituted ring A are δ_C 103.9 (C – 3'), 107.9 (C – 5'), 114.7 (C – 1'),162.9 (C – 2'), 166.6 (C – 4'). The carbonyl signal is at 192 ppm [158, 162].

4.1.6 Compound **6**: **5-acetoxy-2,3-dihydro-7-hydroxy-2-(2'-methoxyphenyl)-4H-1-Benzopyran-4-one (6)**

Formula: $C_{18}H_{16}O_6$

Molecular mass: 328 g/mol

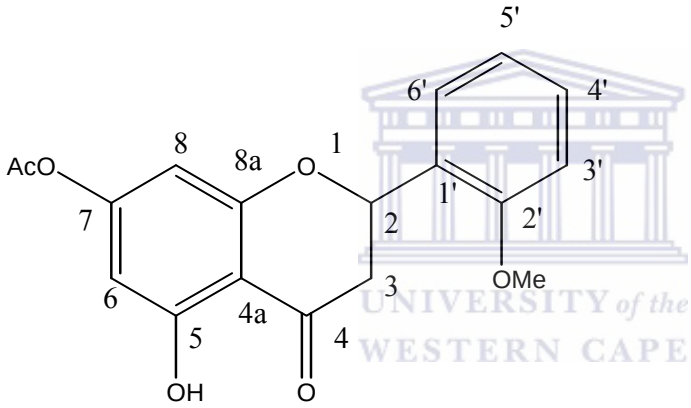


Figure 32*: Chemical structure of 5-acetoloxy-2,3-dihydro-7-hydroxy-2-(2'-methoxyphenyl)-4H-1-Benzopyran-4-one (6)*

NMR: δ_H = 2.30 (s, 3H, CH_3CO), 2.79 (dd, 1H, J = 16.9, 3.1 Hz, H – 3e), 3.08 (dd, 1H, *J* = 16.9, 13.0 Hz, H – 3a), 3.81 (s, 3H, CH_3O), 5.53 (dd, 1H, J = 13.0, 3.1 Hz, H – 2a), 6.03 (d, 1H, *J* = 2.2 Hz, H – 6), 6.09 (d, 1H, *J* = 2.2 Hz, H – 8), 7.15 (dd,1H, *J* = 8.2, 1.6 Hz, H – 3'), 7.38 (m, 2H, H – 4' and H – 5'), 7.62 (dd, 1H, *J* = 7.6, 1.8 Hz, H – 6').

Calculated spectrum:

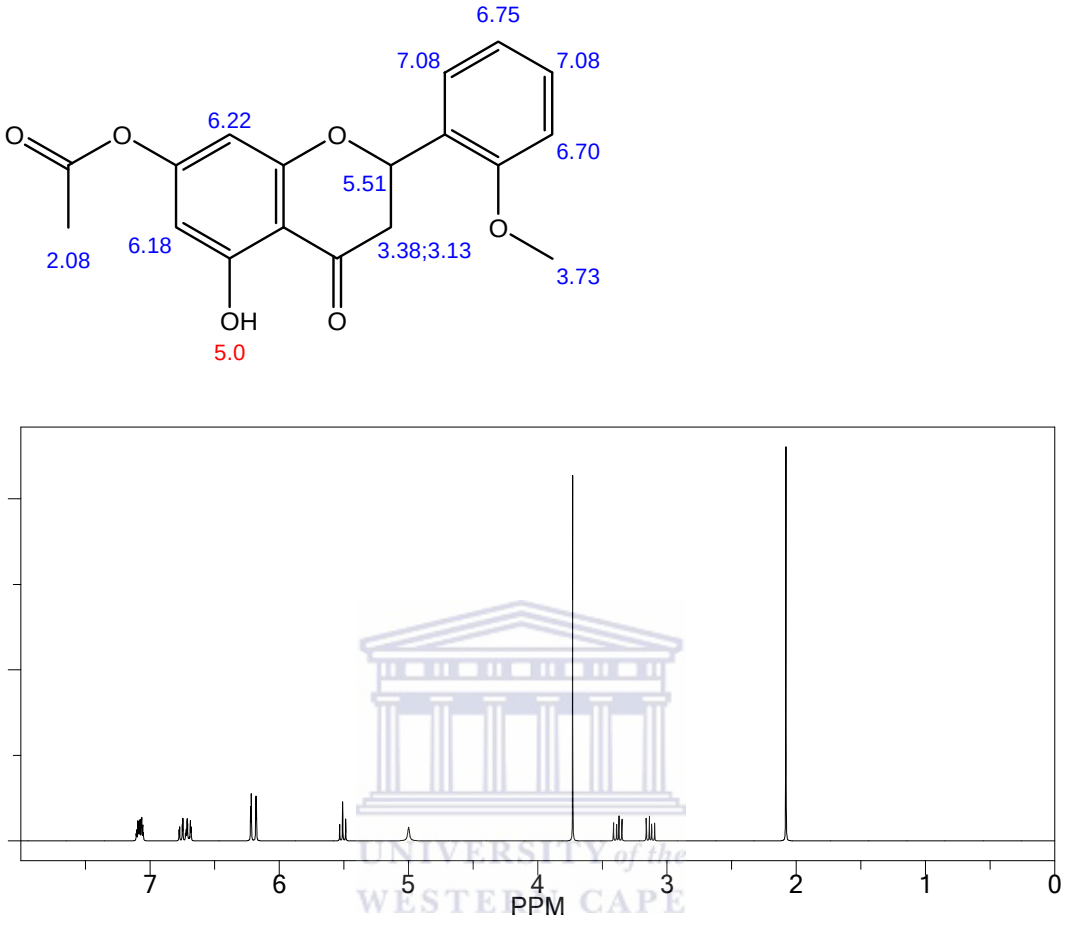


Figure 33: ***^{1}H NMR spectrum of 5-acetoxy-2,3-dihydro-7-hydroxy-2-(2'-methoxyphenyl)-4H-1-Benzopyran-4-one (6)***

The proton spectrum displays a singlet at δ 2.30 (s, 3H, CH_3CO) for the acetyl group. A methoxy signal is observed at δ 3.81 (s, 3H, CH_3O). The arrangement of the remainder of the spectrum is similar to that of compound **3**.

NMR: δ_C = 21.1 (CH_3, CH_3CO), 42.4 (C – 3), 55.8 (CH_3O), 74.6 (C – 2), 94.4 (C – 8), 95.4 (C – 6) , 103.2 (C – 4a), 123.2 (C – 3'), 126.7 (C – 5'), 127.4(C – 6'), 129.9

(C – 4'), 130.5 (C – 1'), 147.9 (C – 2'), 164.3 (C – 7 and C – 8a), 168.2 (C – 5), 169.2 (C=O, CH_3CO), 195.7 (C – 4).

Calculated spectrum:

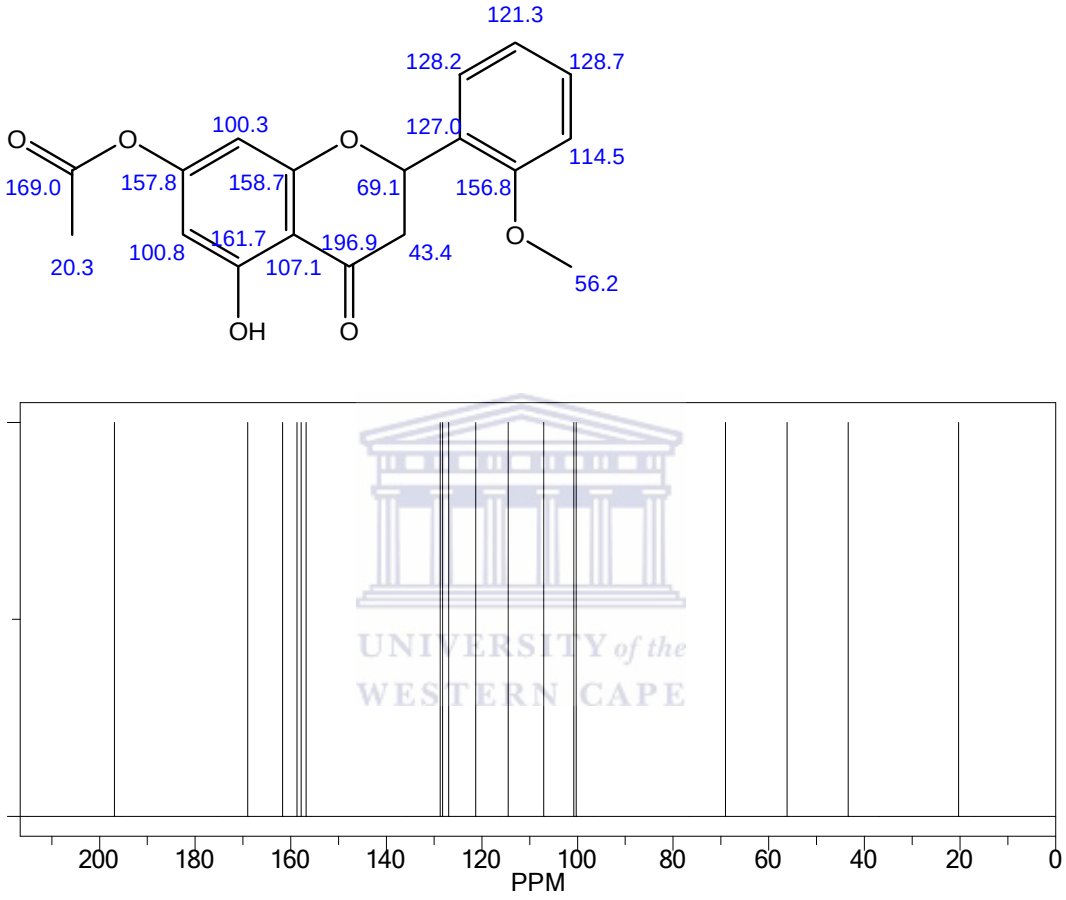


Figure 34: ***^{13}C NMR spectrum of 5-acetoloxy-2,3-dihydro-7-hydroxy-2-(2'-methoxyphenyl)-4H-1-Benzopyran-4-one (6)***

The ^{13}C NMR spectrum of compound **6** displayed 17 signals suggesting a flavanoid nucleus with two additional carbons. The typical methine signal at δ_C 74.6 (C – 2) and 42.4 (C – 3) supported the C-ring scaffold. Observed signals of the B ring are more or

less similar to those of compound **3** with a slight variations and a characteristic aryl-conjugated ketone at δ_C 195.7 (C – 4) completes the assignment. [159]

4.1.7 Compound **7**: **2,3-dihydro-7-hydroxy-2-phenyl-4H-1-Benzopyran-4-one (7)**

Formula: $C_{15}H_{12}O_3$

Molecular mass: 210 g/mol

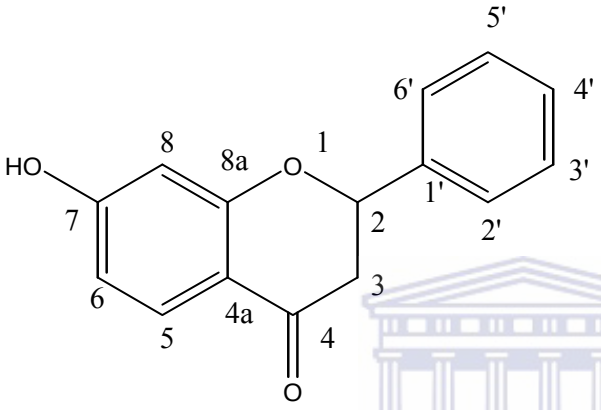


Figure 35*: Chemical structure of 2,3-dihydro-7-hydroxy-2-phenyl-4H-1-Benzopyran-4-one (7)*

NMR: δ_H = 2.84 (dd, 1H, *J* = 17.2, 3.4 Hz, H – 3e), 3.06 (dd, 1H, *J* = 17.2, 13.0 Hz, H – 3a), 5.47 (dd, 1H, *J* = 13.0, 3.1 Hz, H – 2a), 6.48 (d, 1H, *J* = 2.2 Hz, H – 8), 6.56 (dd, 1H, *J* = 8.4, 2.2 Hz, H – 6), 7.44 (m, 5H, Ar), 7.62 (bs, 1H, 7 – OH), 7.85 (d, 1H, *J* = 8.4 Hz, H – 5).

Calculated spectrum:

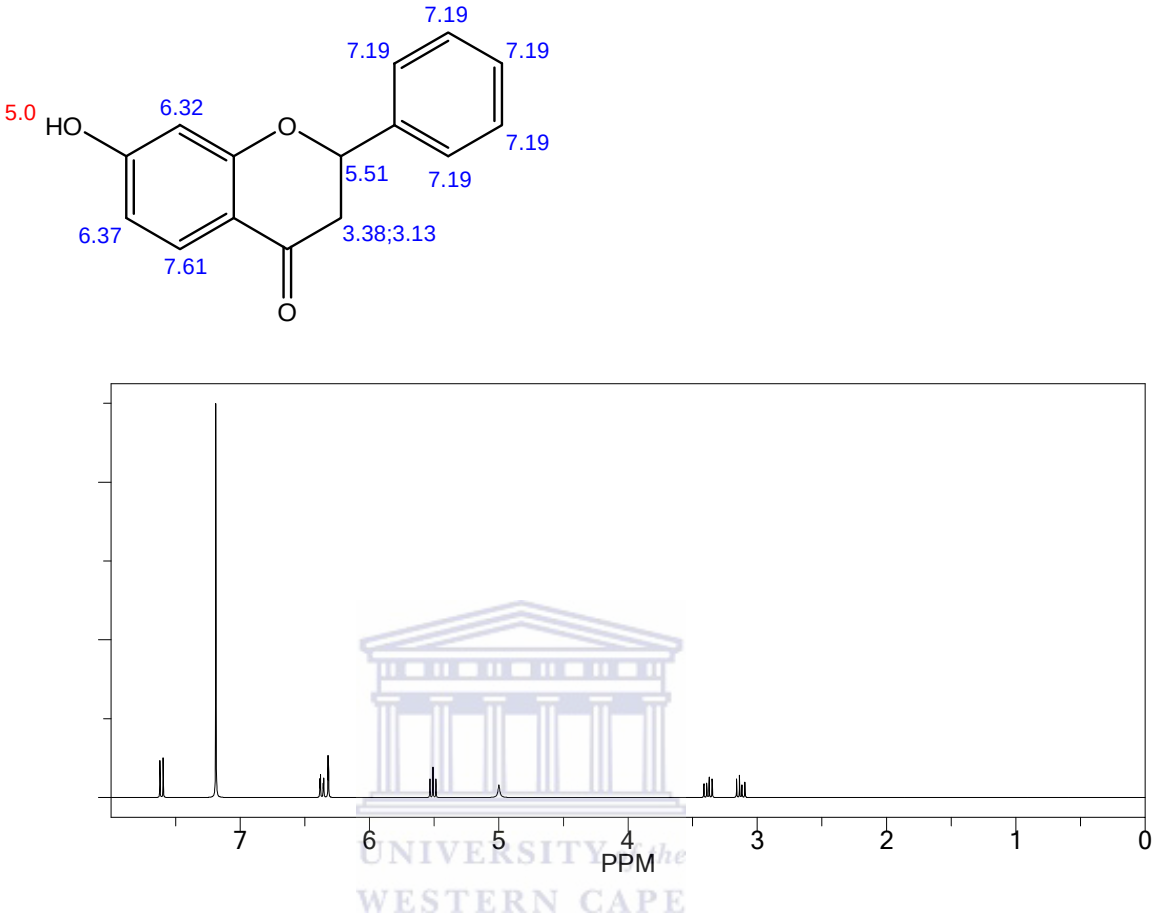


Figure 36: ***^{1}H NMR spectrum of 2,3-dihydro-7-hydroxy-2-phenyl-4H-1-Benzopyran-4-one (7)***

The proton spectrum of this compound is also similar to that of compound **2** except that in this spectra we observe a doublet at δ_H 7.85 (d, 1H, J = 8.4 Hz, H – 5) assigned to the aromatic proton at C – 5 which was not observed in compound **2** due to the presence of the hydroxyl group at position 5. In the above spectrum we managed to observe a broad singlet at δ_H 7.62 (bs, 1H, 7 – OH) for the hydroxyl on ring A at position 7 which also is supported on biosynthetic grounds [161].

NMR: δ_C = 44.4 (C – 3), 80.0 (C – 2), 103.57 (C – 8), 110.71 (C – 6) , 115.3 (C – 4a), 126.3 (C – 2' and C – 6'), 128.9 (C – 3' and C – 5'), 129.1 (C –1'), 129.5 (C – 5), 138.8 (C – 4'), 162.8 (C – 8a), 163.7(C – 7), 190.8 (C – 4) [159].

Calculated spectrum:

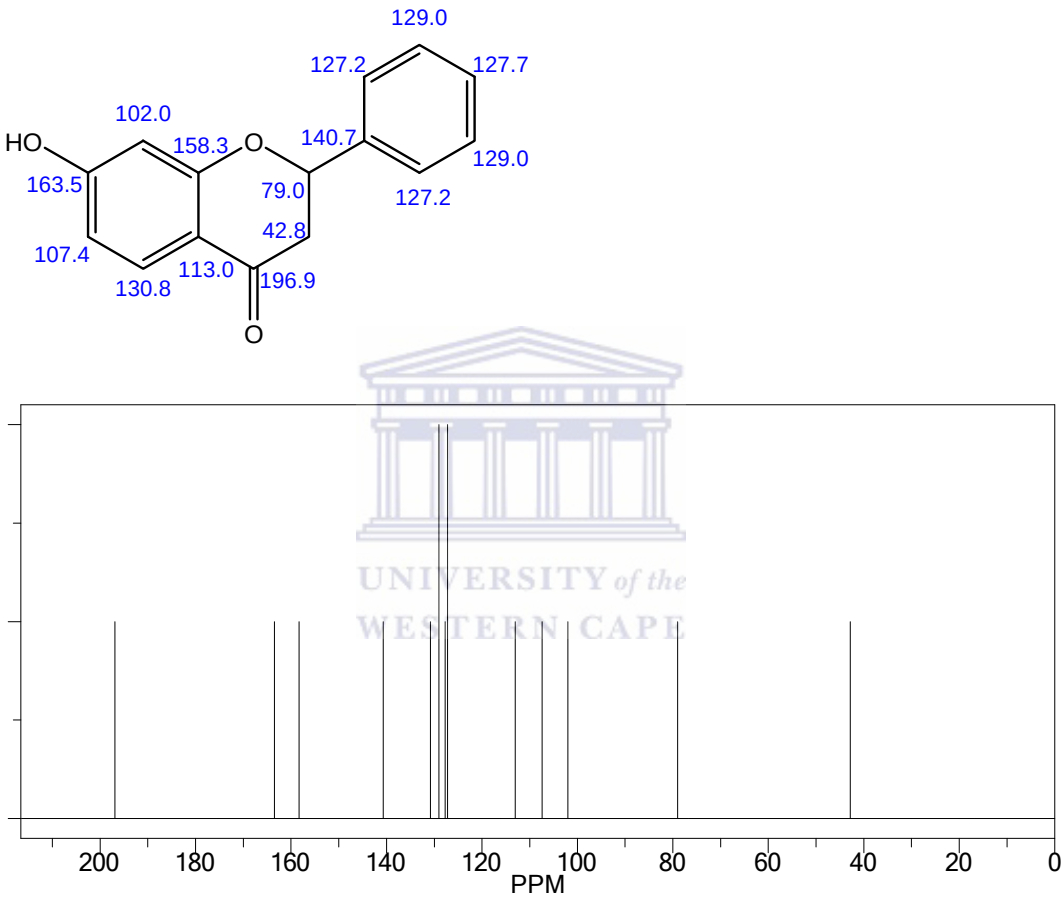


Figure 37*: ^{13}C NMR spectrum of of 2,3-dihydro-7-hydroxy-2-phenyl-4H-1-Benzopyran-4-one (7)*

In the carbon spectra we have the same number of signals as for compound **2** with a slight shift in chemical shift values [159].

4.1.8. Compound **8**: **<u>5,7-(diacetoxy)-2,3-dihydro-2-phenyl-4H-1-benzopyran-4-one (8)</u>**

Formula: $C_{19}H_{16}O_6$

Molecular mass: 340 g/mol

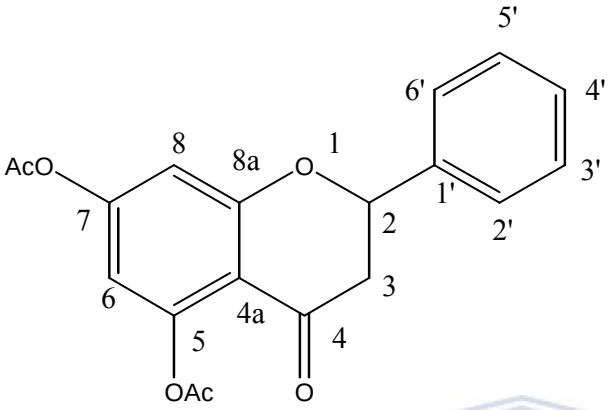
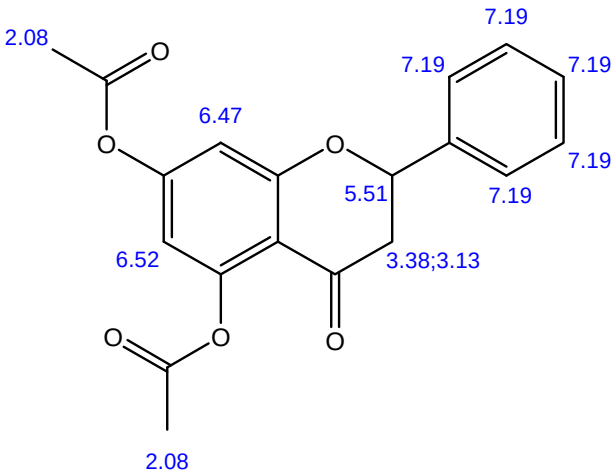


Figure 38*: Chemical structure of 5,7(diacetoxy)-2,3-dihydro-2-phenyl-4H-1-benzopyran-4-one (8)*

NMR: δ_H = 2.30 (s, 3H, CH_3CO), 2.39 (s, 3H, CH_3CO), 2.78 (dd, 1H, *J* = 16.7, 2.8 Hz, H – 3e), 3.06 (dd, 1H, *J* = 16.7, 13.2 Hz, H – 3a), 5.49 (dd, 1H, *J* = 13.2, 2.8 Hz, H – 2a), 6.54 (d, 1H, *J* = 2.2 Hz, H – 6), 6.54 (d, 1H, *J* = 2.2 Hz, H – 8), 7.43 (m, 5H, Ar).

Calculated spectrum:



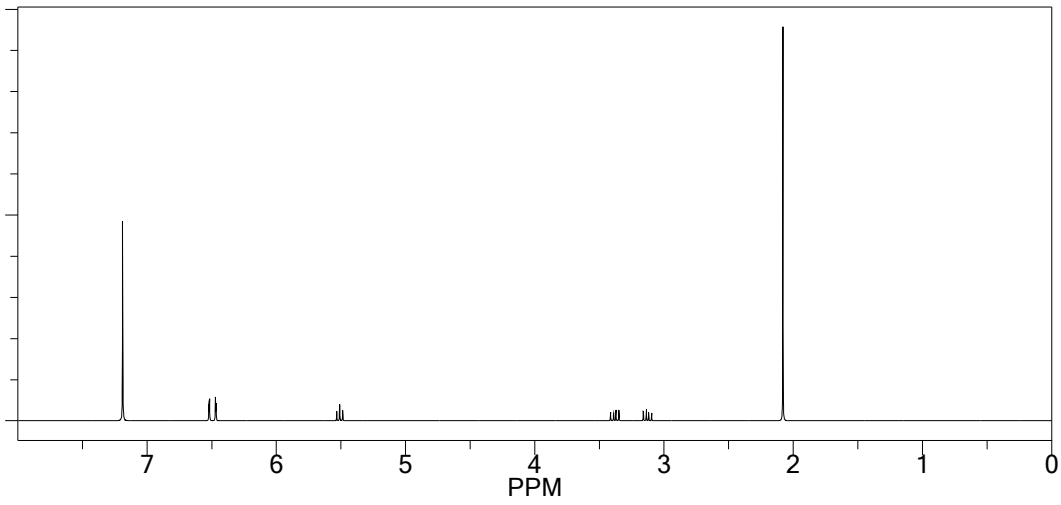


Figure 39*: ^{1}H NMR spectrum of 5,7(diacetoxy)-2,3-dihydro-2-phenyl-4H-1-benzopyran-4-one (8)*

The spectrum of compound **8** displayed signals of the three oxygenated methine and methylene doublet-doublets at δ of 5.49 (dd, 1H, J = 13.2, 2.8 Hz, H – 2a), 2.78 (dd, 1H, J = 16.7, 2.8 Hz, H – 3_e), 3.06 (dd, 1H, J = 16.7, 13.2 Hz, H – 3_a), which demonstrate geminal and vicinal coupling. The geminal coupling between proton H – 3a and H – 3e is 16.7 Hz while the axial – axial vicinal coupling between H - 3a and H – 2 is 13.2 Hz. Axial – equatorial coupling constant between H – 3e and H – 2_a is 2.8 Hz due to the dihedral angle of approximately 90^o The spectrum indicated the existence of two meta coupled protons at δ_H 6.54 (d, 1H, J = 2.2 Hz, H – 6), 6.54 (d, 1H, J = 2.2 Hz, H – 8) and two singlets at δ_H 2.30 (s, 3H, CH_3CO), 2.39 (s, 3H, CH_3CO) for the two acetyl groups on aromatic ring A.

NMR: δ_C = 21.1 (CH_3, CH_3CO), 21.2 (CH_3, CH_3CO), 45.3 (C – 3), 79.7 (C – 2), 109.2 (C – 8), 110.6 (C – 6) , 111.9 (C – 4a), 126.2 (C – 2’ and C – 6’), 129.0 (C – 3’

and C – 5'), 129.1 (C – 1'), 138.2 (C – 4'), 151.4 (C – 7),156.0 (C – 5), 163.4 (C – 8a), 168.1 (C=O, 7 – CH_3CO), 169.4 (C=O, CH_3CO), 189.3 (C – 4).

Calculated spectrum:

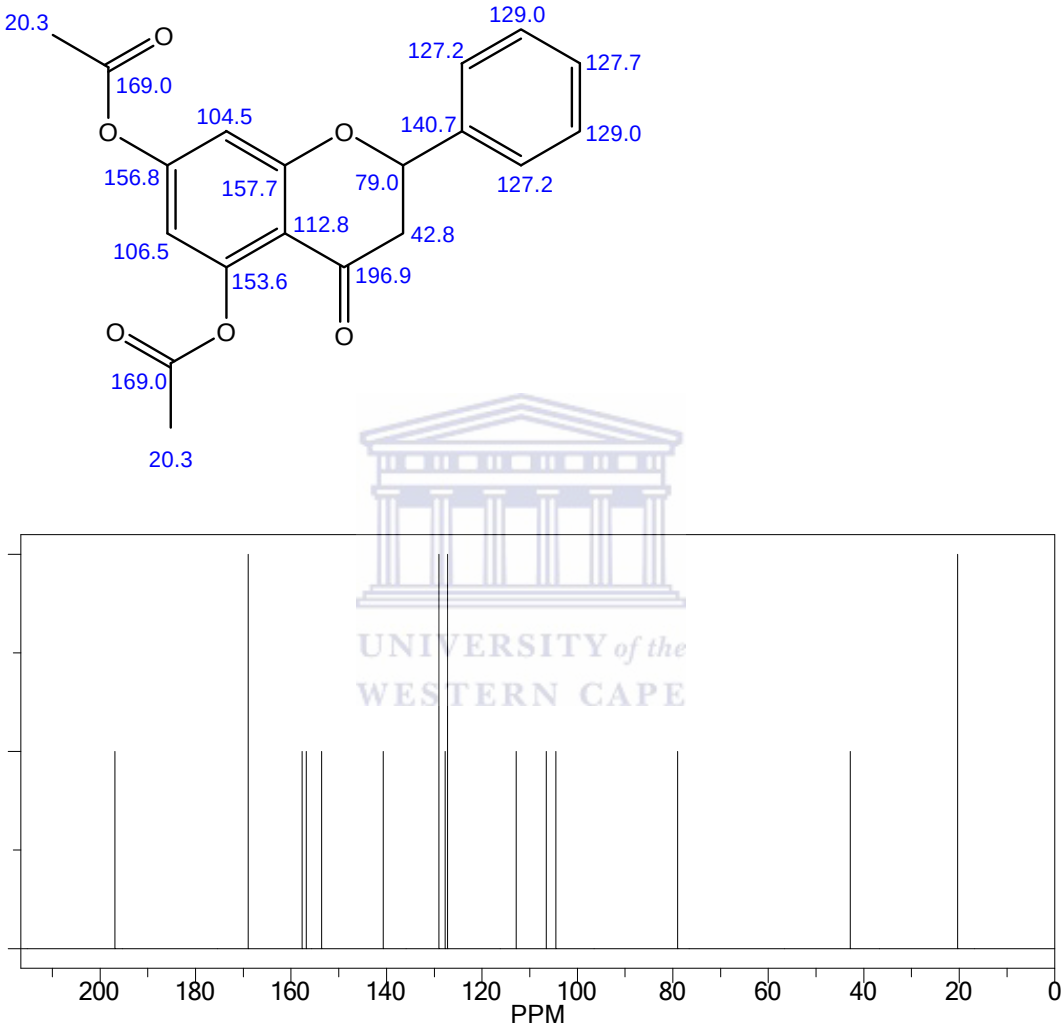


Figure 40: ***^{13}C NMR spectrum of 5,7(diacetoxy)-2,3-dihydro-2-phenyl-4H-1-benzopyran-4-one (8)***

The ^{13}C NMR spectrum of compound **8** indicated two acetyl signals at δ_C 21.1 and 21.2 (CH_3, CH_3CO) in conjunction with the corresponding two carbonyl signals at δ_C 168.1 and 169.4 (C=O, CH_3CO).

4.1.9 Compound **9**: **5,7-(diacetoxy)-2,3-dihydro-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (9)**

Formula: $C_{20}H_{19}O_7$

Molecular mass: 371 g/mol

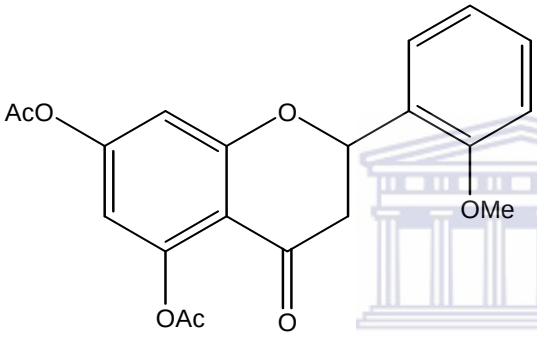


Figure 41*: Chemical structure of 5,7-(diacetoxy)-2,3-dihydro-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (9)*

NMR: δ_H = 2.29 (s, 3H, CH_3CO), 2.39 (s, 3H, CH_3CO), 2.69 (dd, 1H, *J* = 16.9, 2.9 Hz, H – 3e), 3.61 (dd, 1H, *J* = 16.9, 13.3 Hz, H – 3a), 3.82 (s, 3H, CH_3O), 5.59 (dd, 1H, *J* = 13.3, 2.9 Hz, H – 2a), 6.29 (d, 1H, *J* = 2.4 Hz, H – 6), 6.40 (d, 1H, *J* = 2.4 Hz, H – 8), 7.13 (dd, 1H, *J* = 7.8, 1.6 Hz, H – 3 '), 7.38 (m, 2H, H – 5' and H – 4'), 7.62 (dd, 1H, *J* = 7.8, 1.6 Hz, H – 6').

Calculated spectrum:

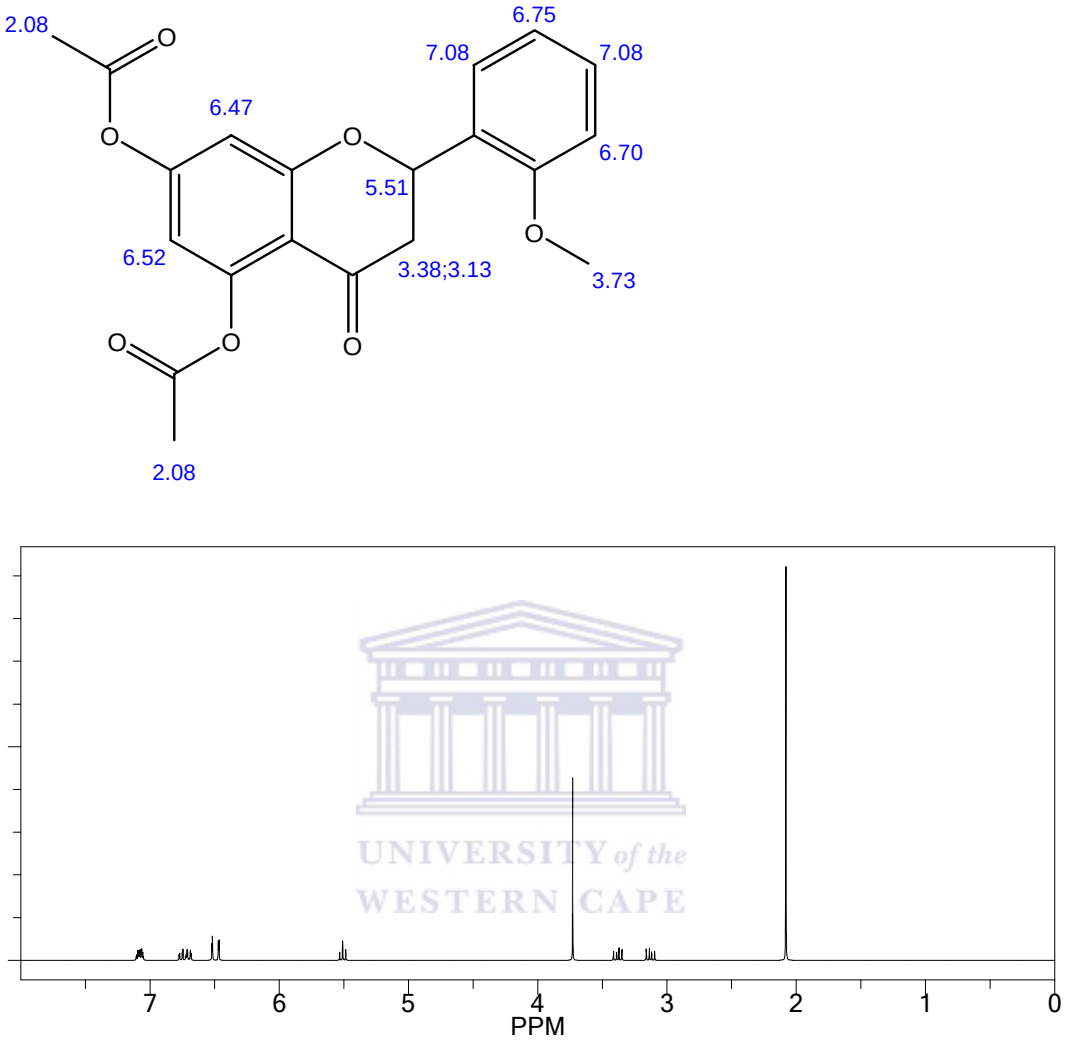


Figure 42*: ^{1}H NMR spectrum of 5,7(diacetoxy)-2,3-dihydro-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (9)*

This compound has displayed the two singlets for the acetyl groups as seen in compound **8** at δ_H 2.29 (s, 3H, CH_3CO) and 2.39 (s, 3H, CH_3CO) as well as the methoxy signal at 3.82 (s, 3H, CH_3O).

NMR: δ_C = 21.1 (CH_3, CH_3CO), 21.2 (CH_3, CH_3CO), 44.0 (C – 3), 55.9 (CH_3O), 74.7(C – 2), 99.6 (C – 8), 105.1 (C – 6), 108.1 (C – 4a), 123.1 (C – 3'), 126.6 (C – 5'), 127.3 (C – 1'), 129.8 (C – 6'), 130.5 (C – 4'), 147.9 (C – 2'), 152.1 (C – 7), 164.2 (C – 5), 165.6 (C – 8a), 169.2 (C=O, CH_3CO),169.6 (C=O, CH_3CO),188.6 (C – 4).

Calculated spectrum:

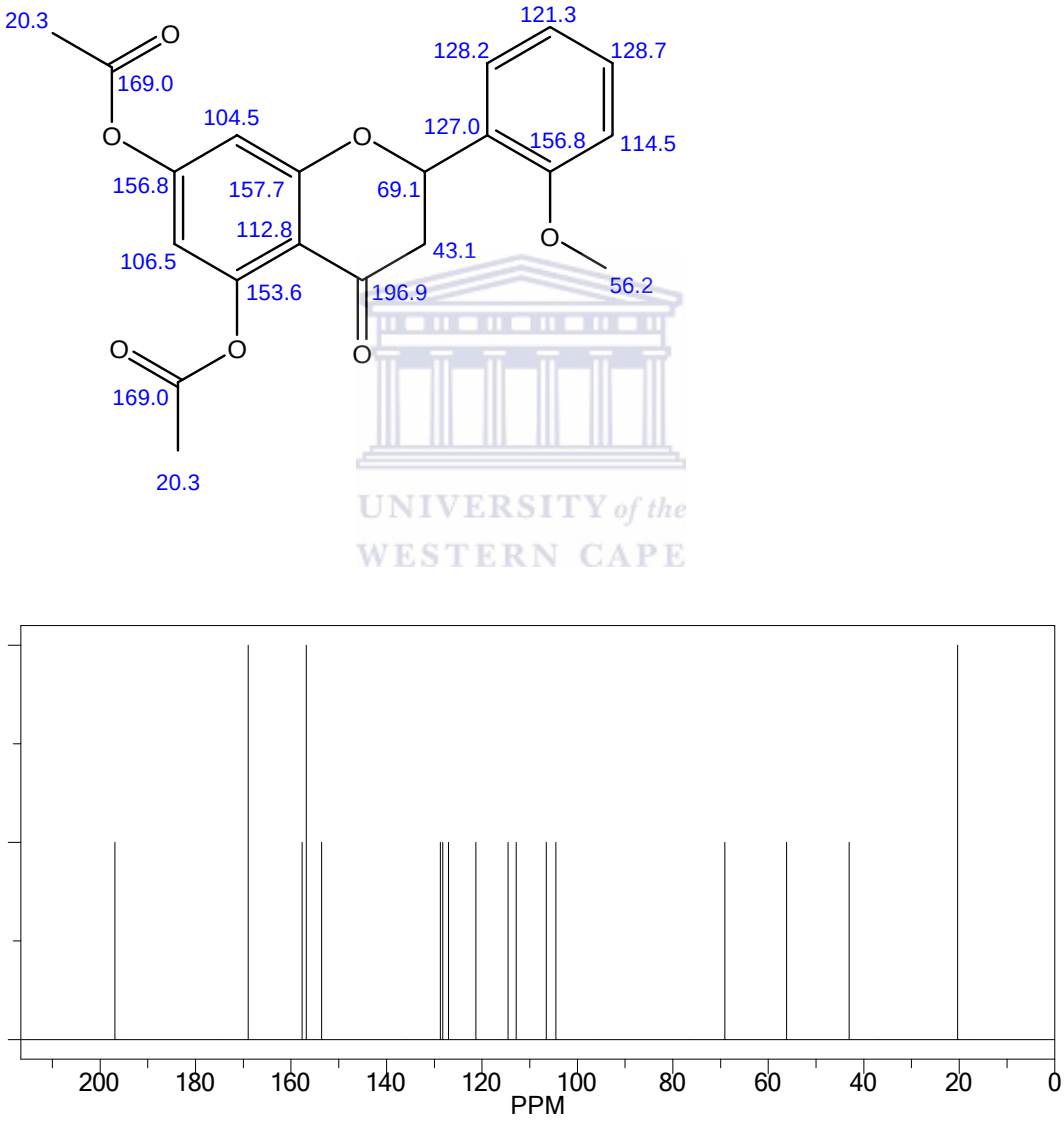


Figure 43: ***^{13}C NMR spectrum of 5,7-(diacetoxy)-2,3-dihydro-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (9)***

Similarly in this spectrum the two acyl methyl signals at δ_C 21.1 (CH_3, CH_3CO), 21.2 (CH_3, CH_3CO) and the two corresponding carbonyl signals at δ_C 169.2 and 169.6 confirm the presence of the two acetate groups in ring A.

4.2. Flavanoids of Elytropapus rhinocerotis

4.2.1. Compound **10**: 2,3-dihydro-5-hydroxy-2-(4'-hydroxy-3'-methoxyphenyl)-7-methoxy-4H-1-benzopyran-4-one (10)

Formula: $C_{17}H_{16}O_6$

Molecular mass: 316 g/mol

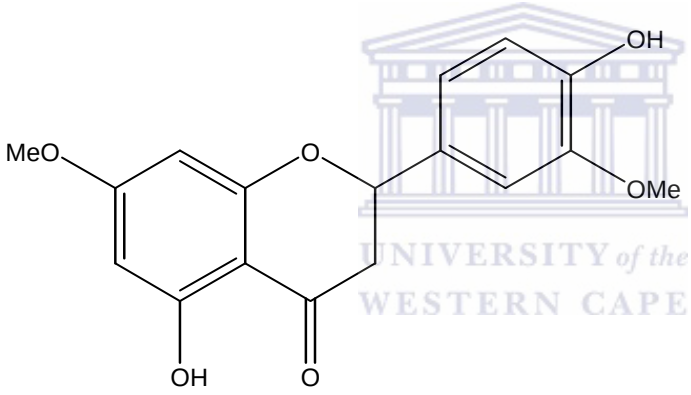


Figure 44 : Chemical structure of 2,3-dihydro-5-hydroxy-2-(4'-hydroxy-3'-methoxyphenyl)-7-methoxy-4H-1-benzopyran-4-one(10)

NMR: δ_H = 2.84 (dd, 1H, *J* = 16.8, 3.2 Hz, H – 3e), 3.08 (dd, 1H, *J* = 16.8, 12.8 Hz, H – 3a), 3.52 (s, 3H, 7-OMe), 3.79 (s, 3H, 3' – OMe), 5.48 (dd, 1H, *J* =12.8, 3.2 Hz, H – 2a), 6.17 (d, 1H, *J* = 1.2 Hz, H – 8), 6.21 (d, 1H, *J* = 1.2 Hz, H – 6), 6.79 (d, 1H, *J* = 8.8 Hz, H – 5'), 6.93 (dd, 1H, J = 8.8, 1.8 Hz, H – 6 '), 7.11 (d, 1H, J = 1.8 Hz, H – 2'), 9.50 (s, 1H, 4' - OH).

Calculated spectrum:

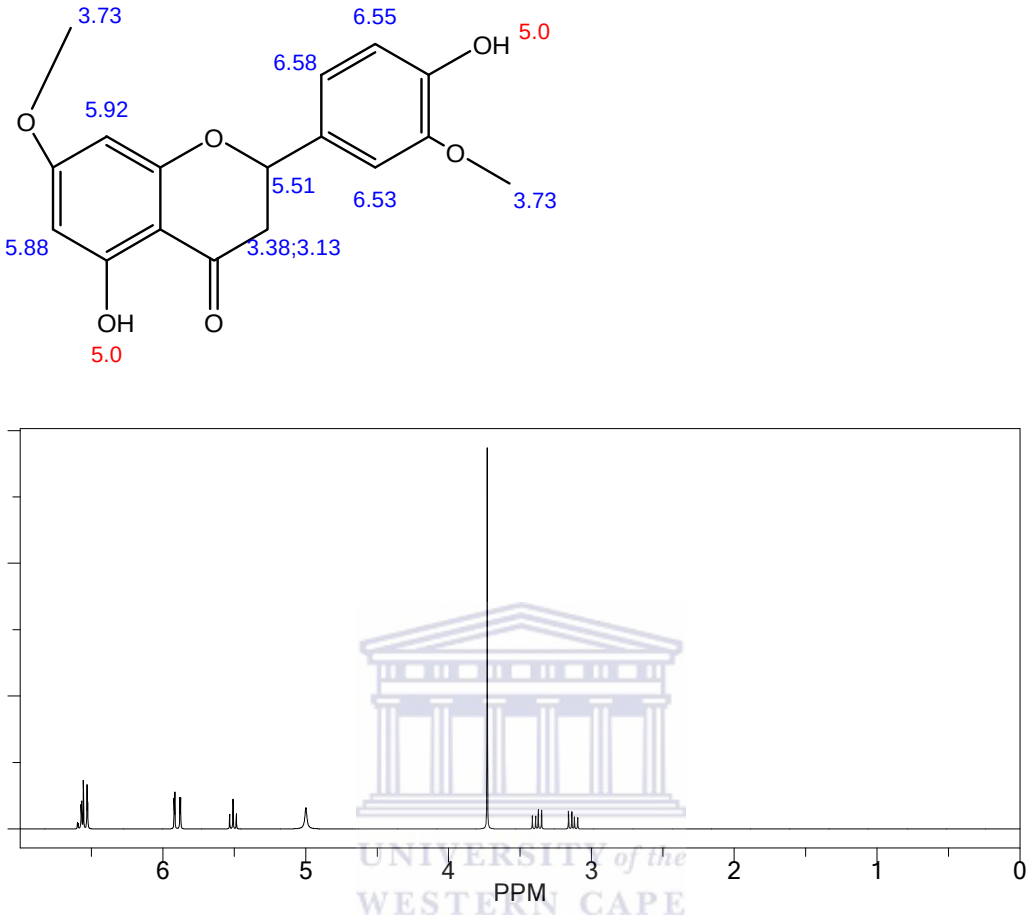


Figure 45 : ^{1}H NMR spectrum of 2,3-dihydro-5-hydroxy-2-(4'-hydroxy-3'-methoxyphenyl)-7-methoxy-4H-1-benzopyran-4-one (10)

The spectrum of compound **10** displayed signals of the three oxygenated methine and methylene doublet-doublets at δ of 5.48 (dd, 1H, *J* =12.8, 3.2 Hz, H – 2a), 2.84 (dd, 1H, *J* = 16.8, 3.2 Hz, H – 3e), 3.08 (dd, 1H, *J* = 16.8, 12.8 Hz, H – 3a), which demonstrate geminal and vicinal coupling. The geminal coupling between proton H – 3a and H – 3e is 16.8 Hz while the axial – axial vicinal coupling between H - 3a and H – 2 is 12.8 Hz. Axial – equatorial coupling constant between H – 3e and H – 2_a is 3.2 Hz . Two singlets with chemical shift of 3.52 and 3.79 where observed for the

hydrogens on methoxy groups 7 and 3' respectively. A single peak was observed for the hydroxyl proton on carbon 4' at a chemical shift of 9.50.

NMR: δ_C = 42.29 (C – 3), 55.7 (CH_3O), 78.7(C – 2), 93.7 (C – 8), 94.6 (C – 6), 102.5 (C – 4a), 111.3 (C – 2'), 115.2 (C – 5'), 119.7 (C – 6'), 129.1 (C – 1'), 147.0 (C – 4'), 147.5 (C – 3'), 163.1 (C – 8a), 163.1 (C – 5), 167.4 (C – 7), 196.6 (C – 4).

Calculated spectrum:

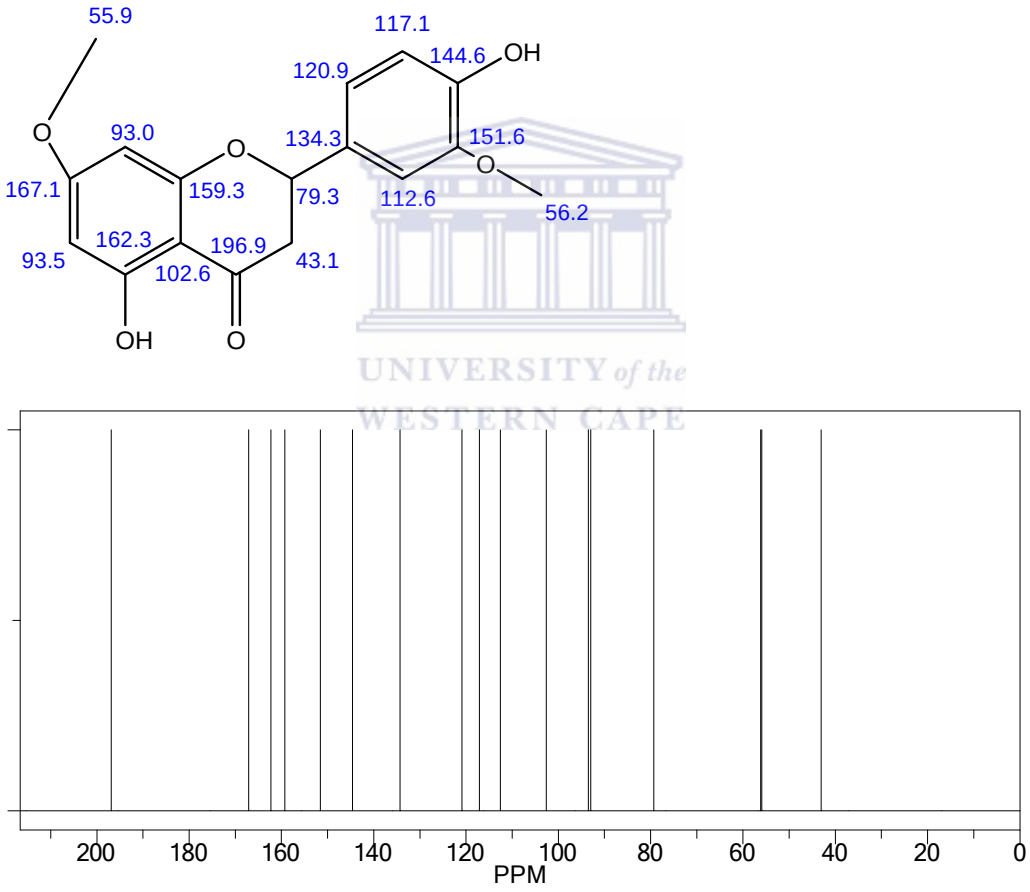


Figure 46: ^{13}C NMR spectrum 2,3-dihydro-5-hydroxy-2-(4'-hydroxy-3'-methoxyphenyl)-7-methoxy-4H-1-benzopyran-4-one (10)

The ^{13}C NMR spectra of compound **10** showed a pattern comprising a minimum of 15 carbon signals for each of the compounds isolated strongly suggesting a flavanoid nucleus. The spectra further all displayed a peak at δ_C 55.7 (C – 2) for the methoxy carbons on ring A and C.

4.2.2. Compound **11**: **2,3-dihydro-5-hydroxy-2-(4'-hydroxyphenyl)-7-methoxy-4H-1-benzopyran-4-one (11)**

Formula: $C_{16}H_{14}O_5$

Molecular mass: 286 g/mol

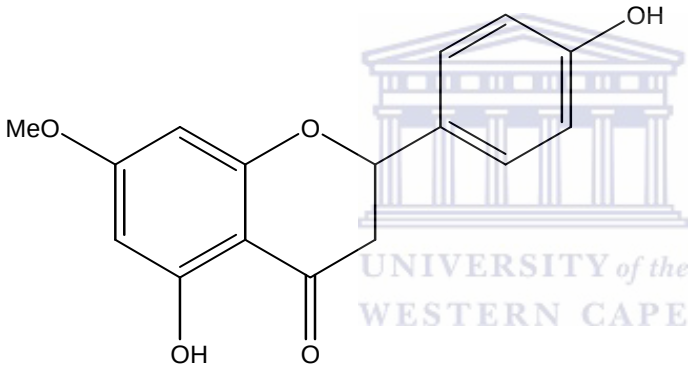


Figure 47: Chemical structure of 2,3-dihydro-5-hydroxy-2-(4'-hydroxyphenyl)-7-methoxy-4H-1-benzopyran-4-one (11)

NMR: δ_H = 2.77 (dd, 1H, *J* = 16.7, 3.2 Hz, H – 3e), 3.19 (dd, 1H, *J* = 16.7, 12.7 Hz, H – 3a), 5.50 (dd, 1H, J = 12.7, 3.2 Hz, H – 2a), 6.18 (d, 1H, *J* = 1.2 Hz, H – 8), 6.20 (d, 1H, *J* = 1.2 Hz, H – 6), 6.88 (d, 2H, J = 8.8 Hz, H – 3 ' and H – 5'), 7.37 (d, 2H, J = 8.8 Hz, H – 2' and H – 6'), 10.4 (s, 1H, 4' – OH), 12.13 (s, 1H, 5 – OH).

Calculated spectrum:

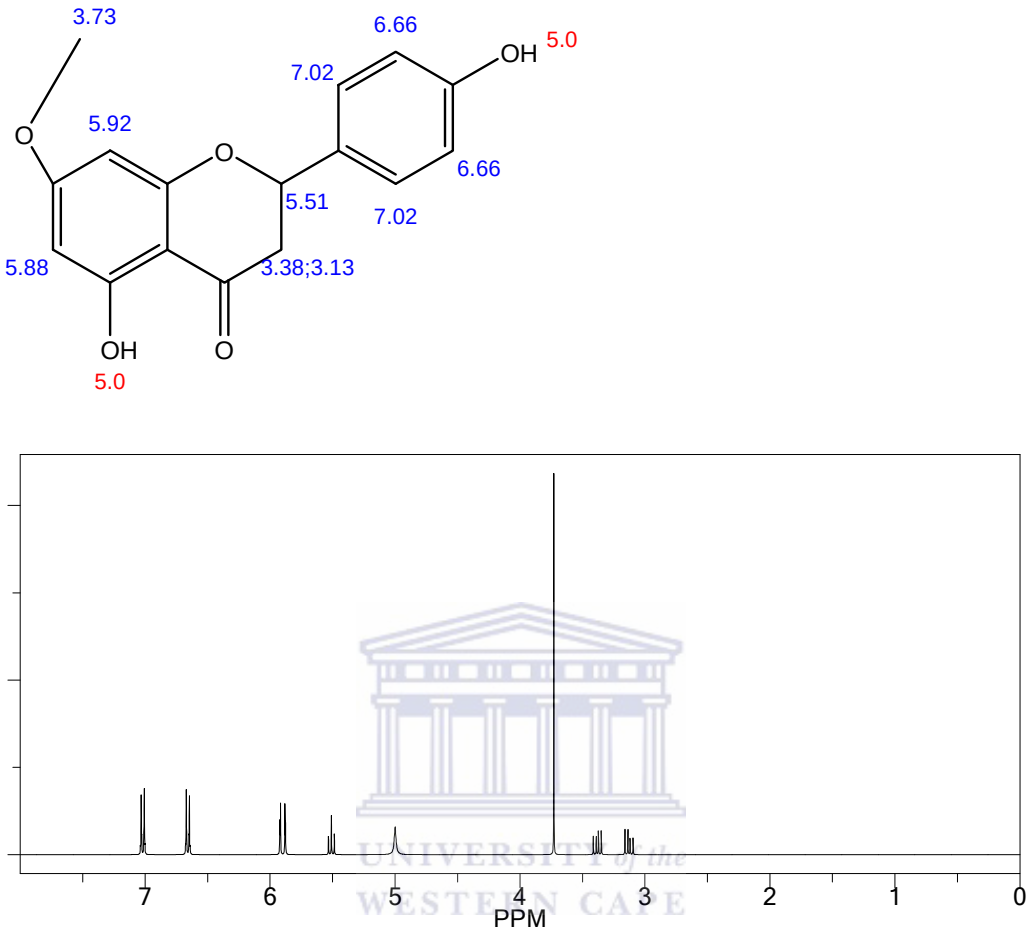


Figure 48: ^{1}H NMR spectrum of 2,3-dihydro-5-hydroxy-2-(4'-hydroxyphenyl)-7-methoxy-4H-1-benzopyran-4-one (11)

The proton spectrum of this compound displayed similarities to that of compound **10** with displayed signals of the three oxygenated methine and methylene doublet-doublets at δ of 2.77 (dd, 1H, *J* = 16.7, 3.2 Hz, H – 3e), 3.19 (dd, 1H, *J* = 16.7, 12.7 Hz, H – 3a), 5.50 (dd, 1H, J = 12.7, 3.2 Hz, H – 2a), which demonstrate geminal and vicinal coupling. The geminal coupling between proton H – 3a and H – 3e is 16.7 Hz while the axial – axial vicinal coupling between H - 3a and H – 2 is 12.7 Hz. Axial – equatorial coupling constant between H – 3e and H – 2_a is 3.2 Hz . Two singlet peaks

were observed at chemical shifts of 10.5 and 12.13 for the hydroxyl protons on carbons 4' and 5.

NMR: δ_C = 42.3 (C – 3), 55.4 (7 - CH_3O), 78.8(C – 2), 94.3 (C – 8), 95.5 (C – 6), 103.2 (C – 4a), 115.5 (C – 3' and C – 5'), 128.5 (C – 2' and C – 6'), 129.0 (C – 1'), 157.5 (C – 4'), 163.1 (C – 8a), 164.1 (C – 5), 168.1 (C – 7), 196.3 (C – 4).

Calculated spectrum:

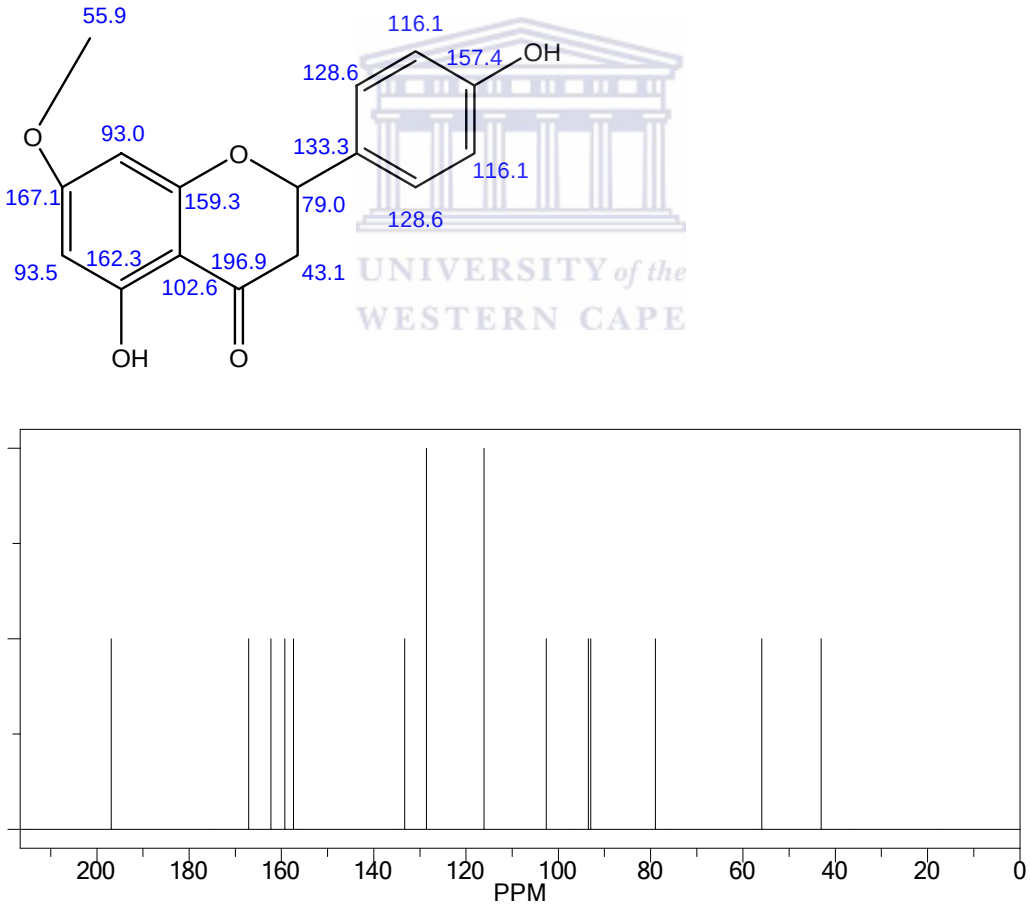


Figure 49 : ^{13}C NMR spectrum 2,3-dihydro-5-hydroxy-2-(4'-hydroxyphenyl)-7-methoxy-4H-1-benzopyran-4-one (11)

The ^{13}C NMR spectra of compound **11** showed a pattern comprising a minimum of 15 carbon signals for each of the compounds isolated strongly suggesting a flavanoid nucleus. The ^{13}C spectrum of compound **11** has similarities to the peaks for compound **10** with only a slight shift in the chemical shifts.

CHAPTER 5

Electrochemical results and discussion

Cyclic Voltammetry and square wave voltammetry were used to investigate flavanoid characteristics by the peak potential, number and position of peaks at those potentials, the current heights at each peak potential for all 11 flavanoids.

5.1. Flavanoids from *Galenia Africana*

5.1.1. Compound 1: 1-(2',4'-dihydroxyphenyl)-3-phenyl propan-1-one (1)

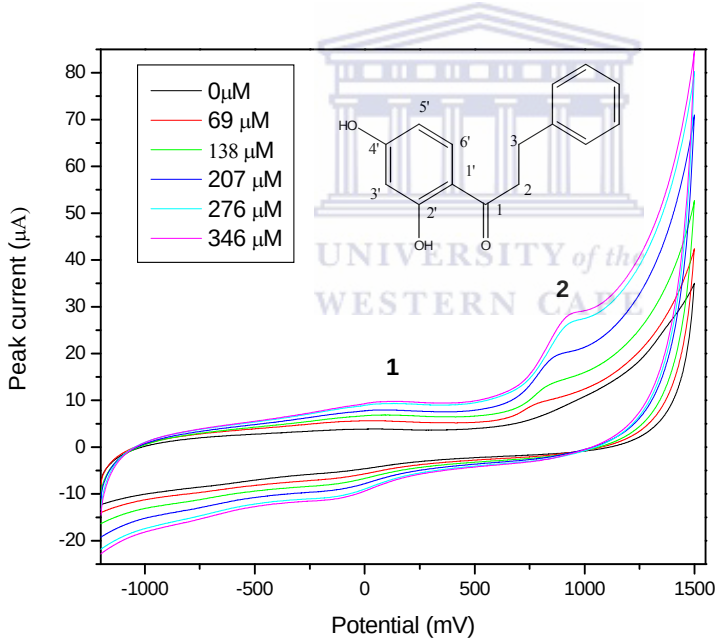


Figure 50*: Cyclic voltammogram of compound 1, scan rate 100 mV/s.*

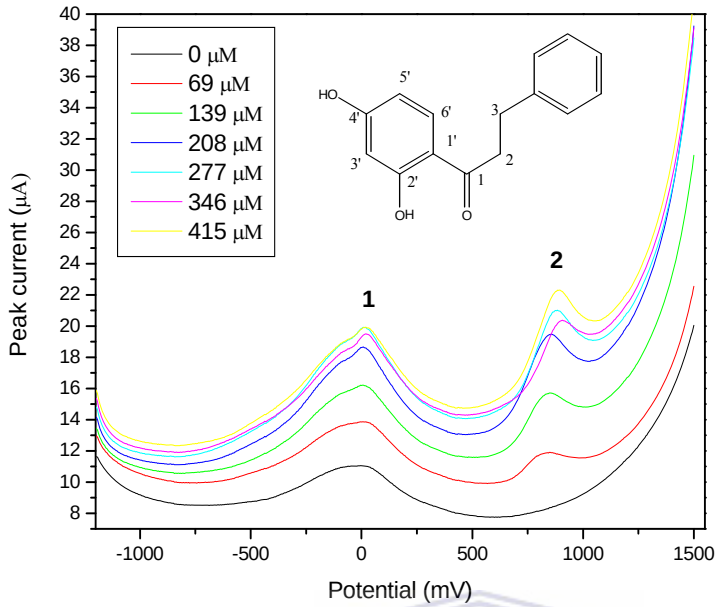


Figure 51*: Square wave voltammogram of compound 1, scan rate 20 mV/s.*

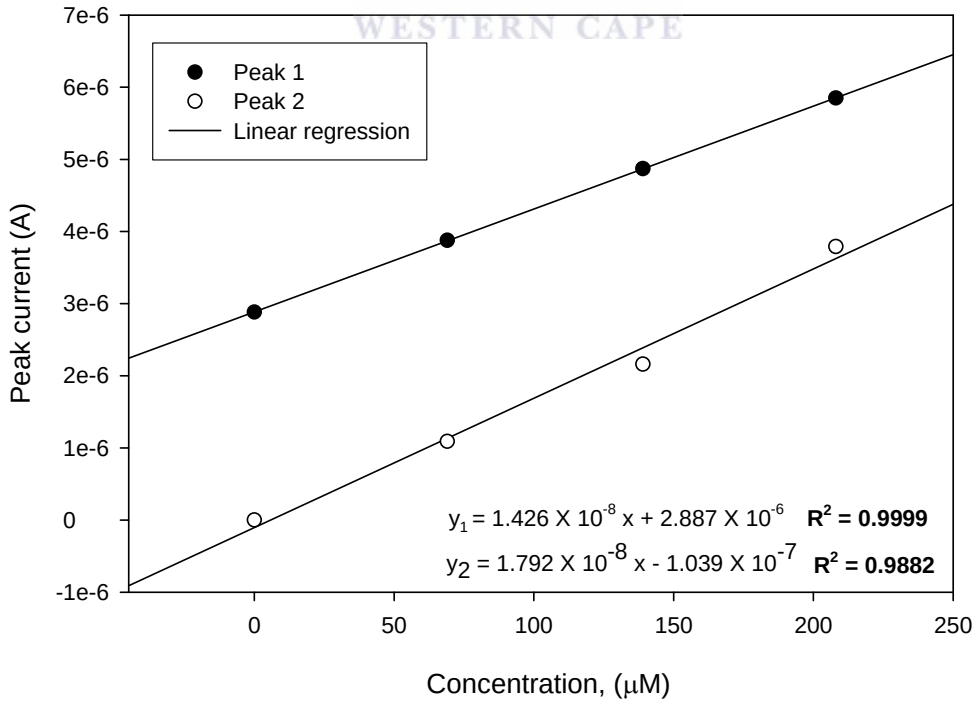


Figure 52*: Linear regression graph of compound 1.*

Figure 50 shows the cyclic voltammogram of compound **1**, recorded when potential sweep was began from -1500 mV to +1500 mV and reversed at a scan rate of 100 mV/s. The square wave voltammogram of compound **1 (Figure 51)** shows two sets of defined peaks one at 24.4 mV and the other at 843 mV. The linear regression graphs for both of both peaks were constructed by a plot of oxidative peak current (I_p) versus concentration (C^0). This showed that the I_{p1} and I_{p2} of compound **1** increase with increasing concentration and after I_p had achieved its maximum value it tends to fluctuate with further addition of the compound. The first and second oxidation peaks (I_{p1}, I_{p2}) were used as additional variables to analyze the effect of concentration by extracting the linear region between 0 and 208 µM and a multiple regression analysis was performed (**Figure 52**). The regression equation for peak 1 $I_{p1} = 1.426 \text{ X } 10^{-8} C^0 + 2.887 \text{ X } 10^{-6}$ with a correlation coefficient of 0.9999 and peak 2 has a regression equation of $I_{p2} = 1.792 \text{ X } 10^{-8} C^0 + (-1.039) \text{ X } 10^{-7}$ with a correlation coefficient of 0.9882.

Resorcinol

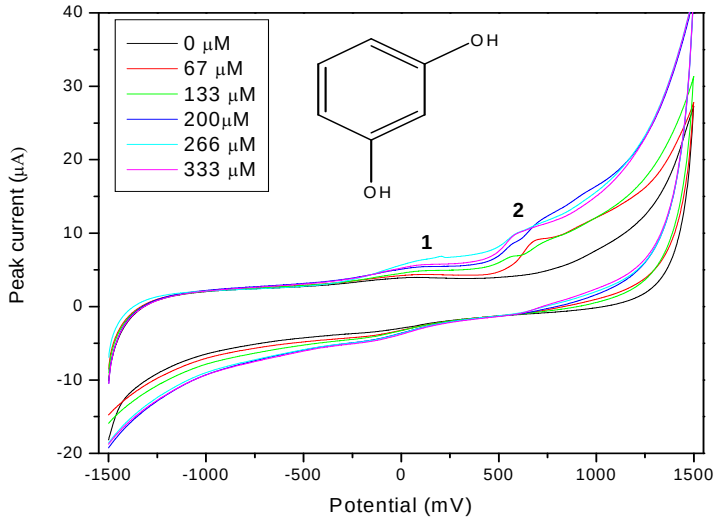


Figure 53*: Cyclic voltammogram of resorcinol, scan rate 100 mV/s.*

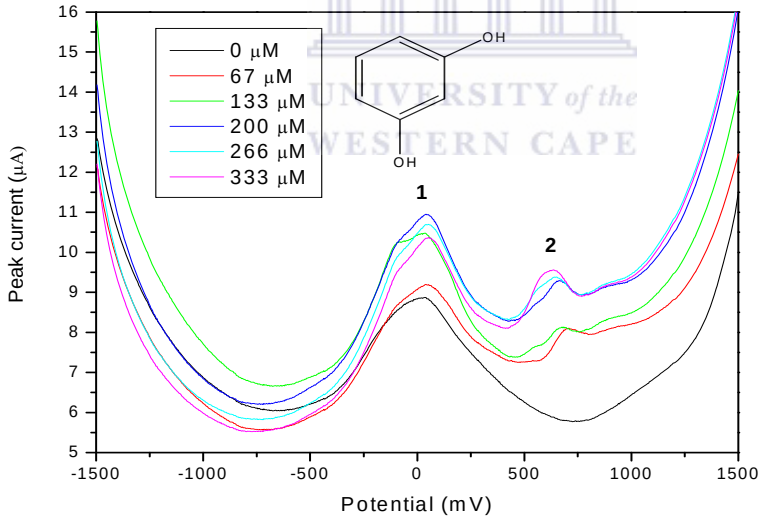


Figure 54*: Square wave voltammogram of resorcinol, scan rate 20 mV/s.*

2 - butanone

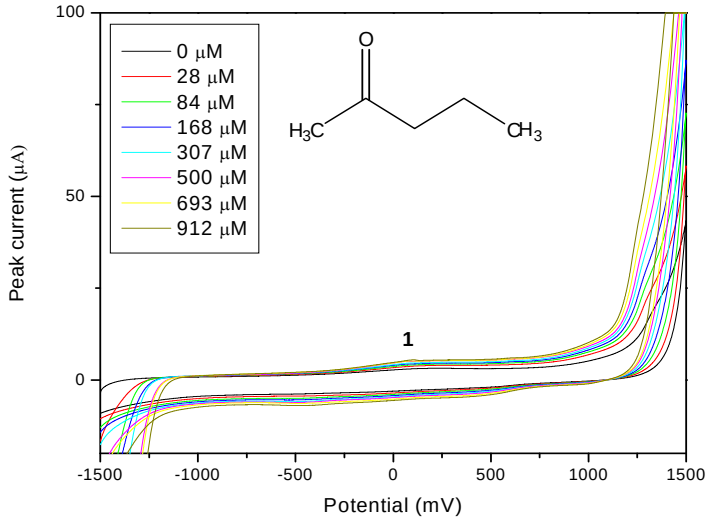


Figure 55*: Cyclic voltammogram of 2-butanone, scan rate 100 mV/s.*

Benzene

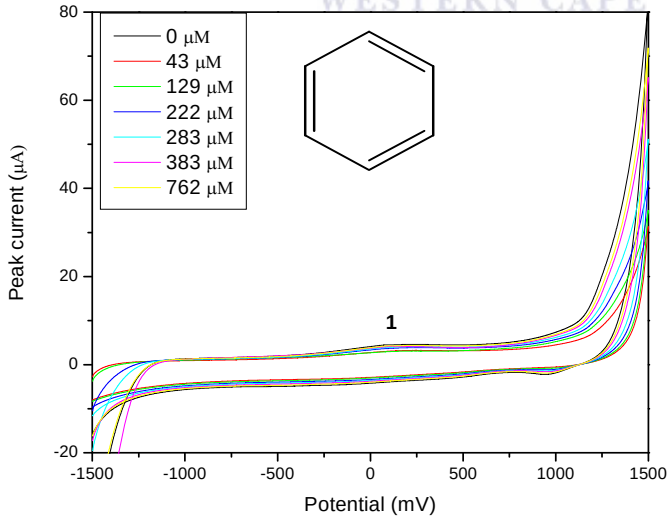


Figure 56*: Cyclic voltammogram of benzene, scan rate 100 mV/s.*

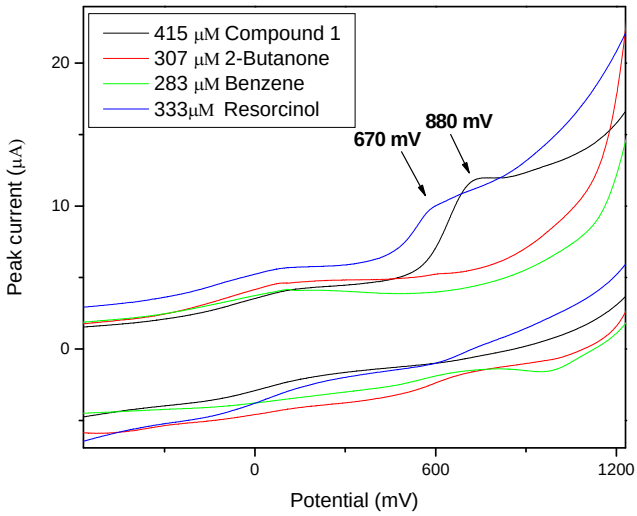


Figure 57*: Cyclic voltammograms of resorcinol, 2-butanone, benzene and compound 1.*

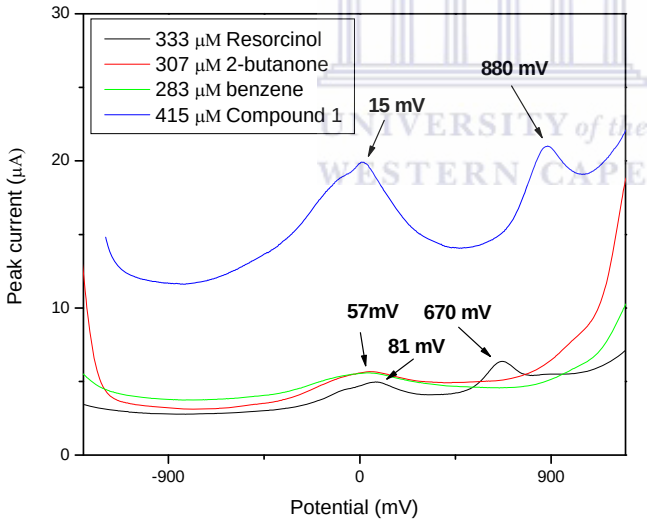


Figure 58*: Square wave voltammograms of resorcinol, 2-butanone, benzene and compound 1.*

In this analysis peak 1 will be the main focus as it is said in literature that the first anodic peak is the most important in telling us about the antioxidant activity of a

compound. They mention that the lower the oxidation potential of the first peak the greater the ability of a compound to donate an electron and hence behave as an antioxidant [17, 163]. Peak 2 for resorcinol is present at approximately 663 mV which corresponds to half peak potential found in literature at 632 mV [164]. This peak is absent for benzene and 2-butanone but is present for compound 1 but has shifted to more positive potentials at 882 mV. In literature, the higher the number of hydroxyl groups the more enhanced the flavonoid activity. This was demonstrated when the number of hydroxyl groups was compared to IC_{50} value which depicts the strength of the antioxidant activity of a compound [165]. Since benzene and 2-butanone do not possess hydroxyl groups, oxidation peak 2 was absent. In resorcinol and compound **1** hydroxyl groups were present and hence the presence of peak 2. The shift of peak 2 from 663 mV in resorcinol to 882 mV in compound **1** shows a reduction antioxidant ability of compound 1 by the substituents namely benzene and 2-butanone.

In **figure 58**, considering the first oxidation peak, benzene and tetrahydrofuran will be disregarded as the presence of a hydroxyl group is necessary assign a compound as an antioxidant [165]. Comparing the oxidation peak potential of peak 1 for resorcinol at 81 mV with that of compound **1** at 15 mV we can assume that compound 1 is a better antioxidant than resorcinol [17, 164].

5.1.2. Compound **2**: **2,3-dihydro-5,7-dihydroxy-2-phenyl-4H-1-benzopyran-4-one (2)**

Formula: $C_{15}H_{12}O_4$

Molecular mass: 256 g/mol

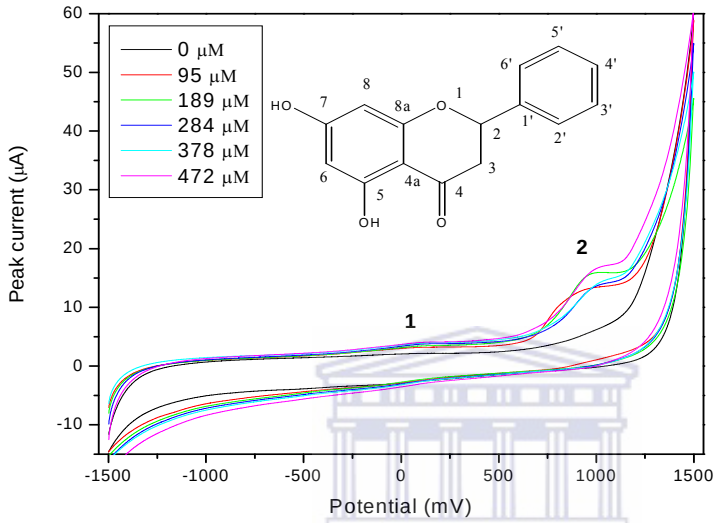


Figure 59*: Cyclic voltammogram of compound 2, scan rate of 100 mV/s.*

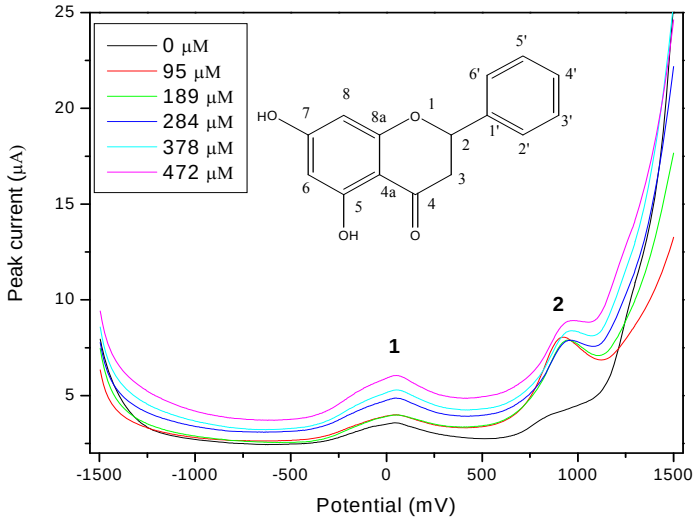


Figure 60*: Square wave voltammogram of compound 2, scan rate 20mV/s.*

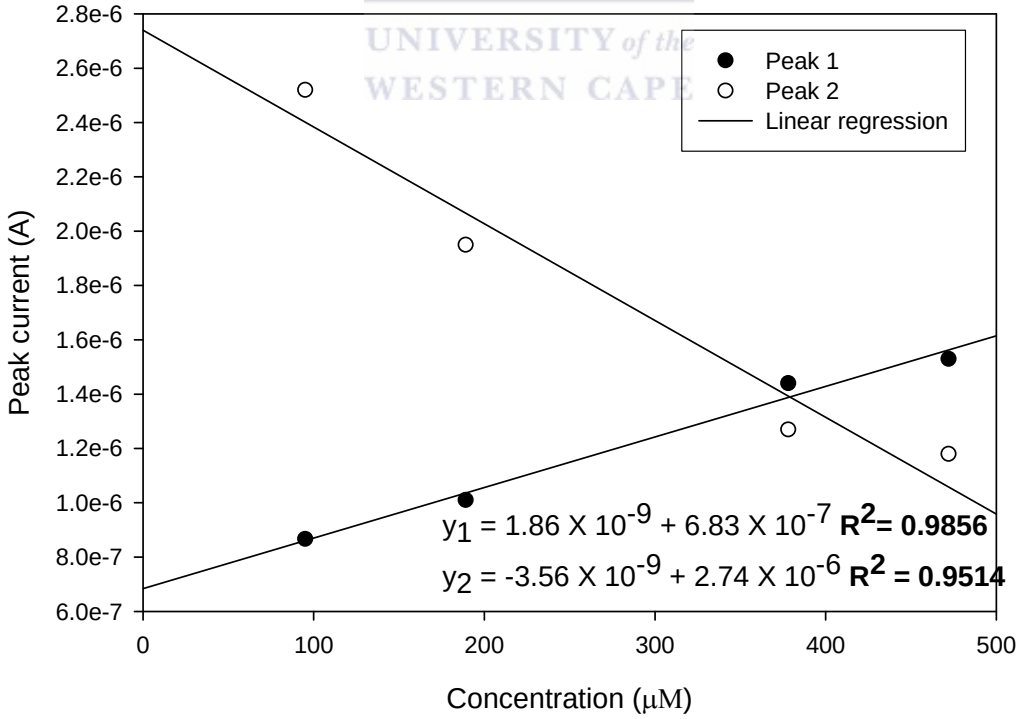


Figure 61*: Linear regression graph of compound 2.*

Figure 59 shows the cyclic voltammogram of compound **2** shows one anodic peak at 972.6 mV. When dealing with compounds that behave as antioxidants anodic peaks are assigned to individual compounds based on the oxidation potentials (E_{pa}) while their concentrations are proportional to the current density (I_{pa}) [105]. The absence of a corresponding reduction peak points to the irreversibility of the oxidation reaction product produced in this reaction. It is known that oxidation of mono phenol group occurs at high positive potentials forming a phenoxy radical or phenoxium ion and can successively under go different secondary reactions [166]. The square wave voltammogram of compound **2** shows two sets of peaks one at 49 mV and the other at 952mV. This showed that the Ip_1 was increasing while Ip_2 was decreasing with increasing concentration. The first and second oxidation peaks (I_{p1}, I_{p2}) were used as additional variables to analyze the effect of concentration by extracting the linear region between 100 and 472 μM and a multiple regression analysis was performed. The regression equation for peak 1 $I_{p1} = 1.86 \text{ X } 10^{-9} \text{ C}^0 + 6.83 \text{ X } 10^{-7}$ with a correlation coefficient of 0.9856 and peak 2 has a regression equation of $I_{p2} = -3.56 \text{ X } 10^{-9} \text{ C}^0 + 2.74 \text{ X } 10^{-6}$ with a correlation coefficient of 0.9514.

Tetrahydrofuran

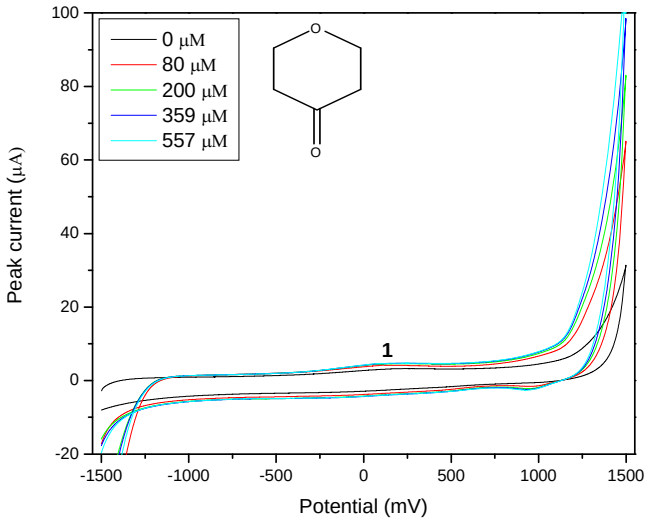


Figure 62*: Cyclic voltammogram of tetrahydrofuran, scan rate 100 mV/s.*

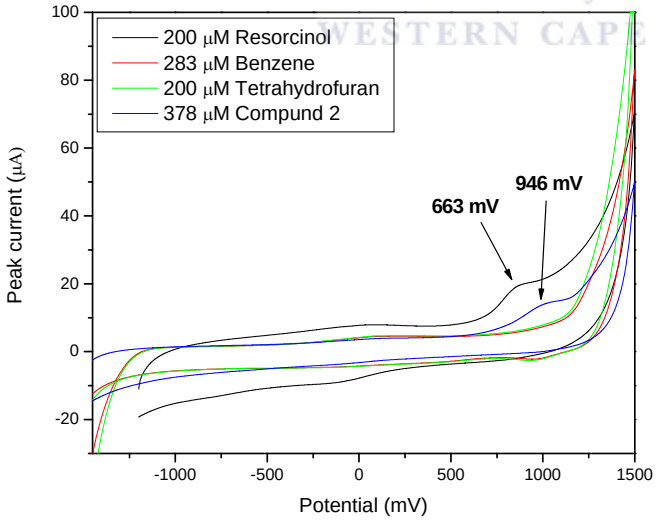


Figure 63*: Cyclic voltammograms of resorcinol, benzene, tetrahydrofuran and compound 2.*

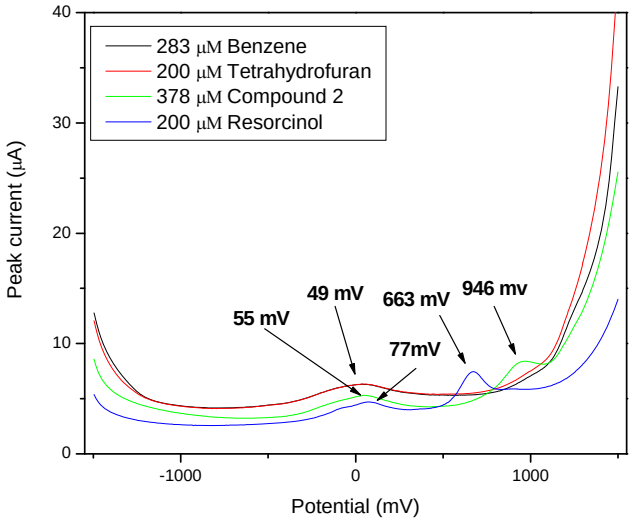


Figure 64*: Square wave voltammograms of resorcinol, benzene, tetrahydrofuran and compound 2.*

The second oxidative peak is absent for benzene and tetrahydrofuran. As discussed for compound **1** the number of hydroxyl groups the affects the activity of the compound [165]. Since tetrahydrofuran does not possess any hydroxyl groups, oxidation peak 2 was absent just as was seen for benzene and 2-butanone. There is a shift in peak potential of peak 2 from 663 mV in resorcinol to 946 mV in compound **2** due to the presence of substituents namely benzene and tetrahydrofuran [17]. Peak 1 for resorcinol has a first oxidation peak potential of 77 mV while compound **2** has an oxidation peak potential of 15 mV, making compound **2** a better antioxidant than resorcinol [99, 163].

5.1.3. Compound **3**: **2,3-dihydro-5,7-dihydroxy-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (3)**

Formula: $C_{16}H_{14}O_5$

Molecular mass: 286 g/mol

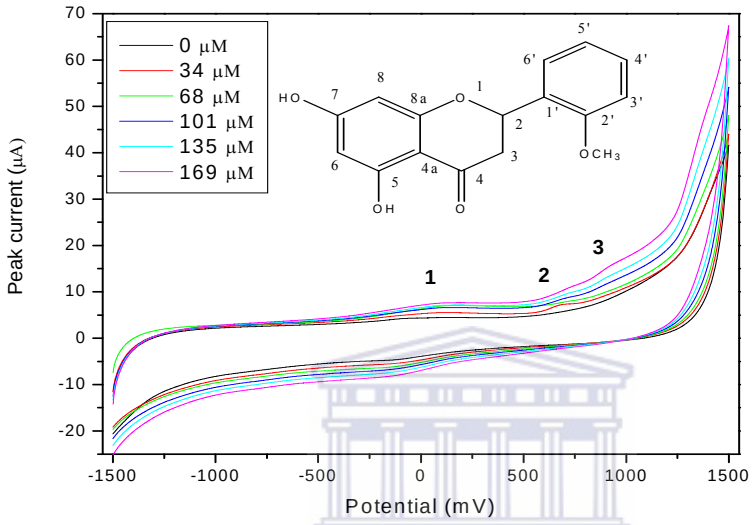


Figure 65*: Cyclic voltammogram of compound 3, scan rate 100 mV/s.*

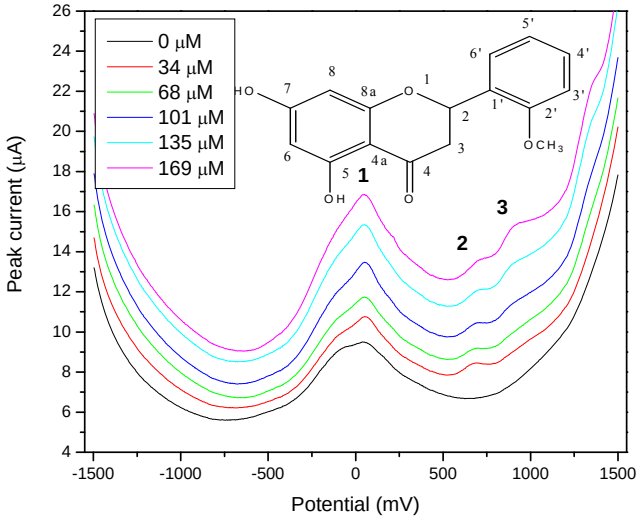


Figure 66*: Square wave voltammogram of compound 3, scan rate 20 mV/s.*

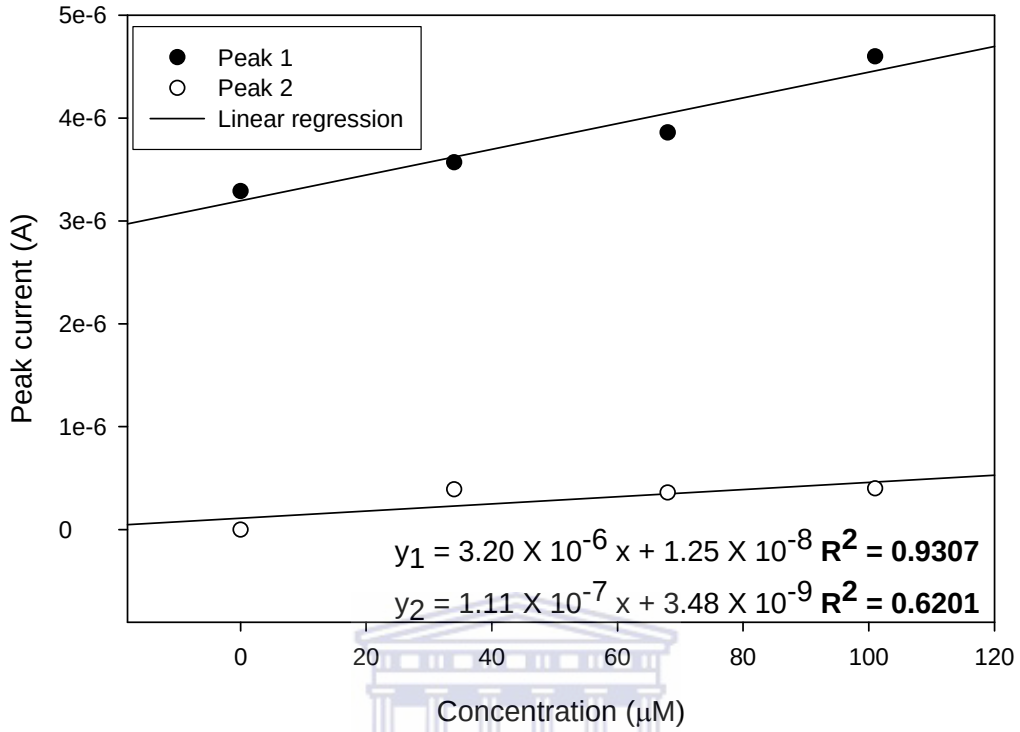


Figure 67*: Linear regression compound 3.*

The square wave voltammogram of compound **3** (**Figure 66**) shows three sets of peaks at 48 mV, 710 mV and 919 mV respectively. The faradaic current for peak 1 is larger than that of peak 2 and peak 3. The first and second oxidation peaks (Ip1, Ip2) were used to analyze the effect of concentration by extracting the linear region between 0 and 169 μM and a multiple regression analysis was performed (**Figure 67**). The regression equation for peak 1 I_{p1} = 3.20 X 10^{-6} C^0 + 1.25 X 10^{-8} with a correlation coefficient of 0.9307 and peak 2 has a regression equation of I_{p2} = -1.11 X 10^{-7} C^0 + 3.48 X 10^{-9} with a correlation coefficient of 0.6201.

Anisole

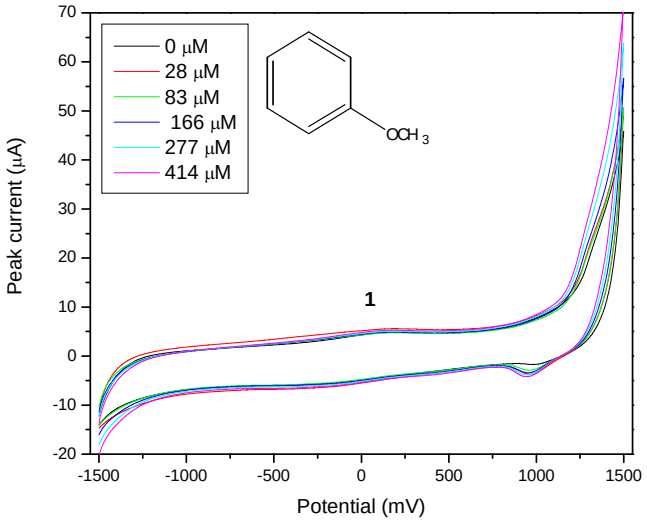


Figure 68*: Cyclic voltammogram of anisole, scan rate 100 mV/s.*

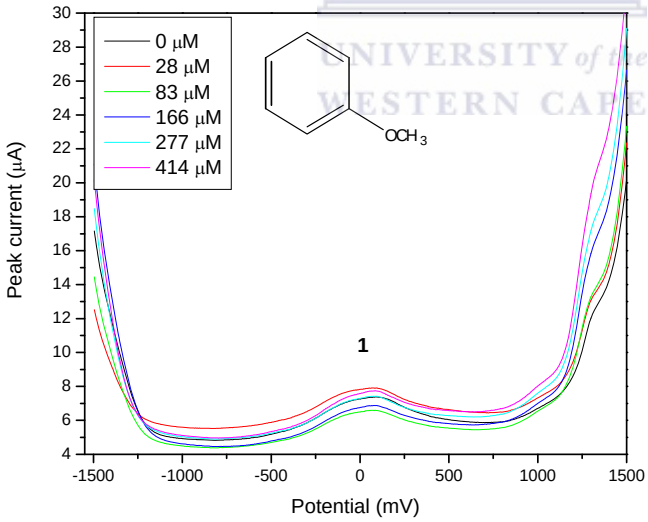


Figure 69*: Square wave voltammogram of anisole, scan rate 20 mV/s.*

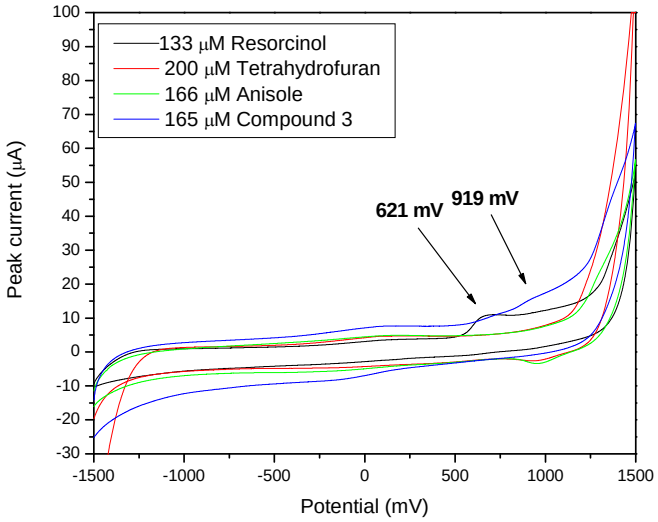


Figure 70*: Cyclic voltammograms of resorcinol, tetrahydrofuran, anisole and compound 3.*

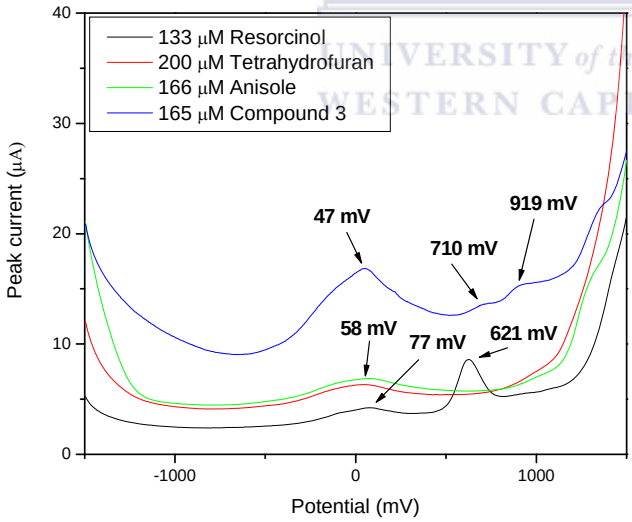


Figure 71*: Square wave voltammograms of resorcinol, tetrahydrofuran, anisole and compound 3.*

The first oxidative peak which gives us information about the antioxidant activity of the compound , we will consider compound **3** and resorcinol which both contain

hydroxyl groups which can behave as electron donors [165]. Compound **3** is a better antioxidant than resorcinol as it has a lower oxidation potential of 47 mV as compared to that of resorcinol at 77 mV for the first peak. For peak 2, resorcinol has its characteristic peak at 621 mV [99, 164]. Peak 2 for compound **3** could be attributed to the presence of the resorcinol moiety in the structure and the shift in potential could be due to the substituents namely tetrahydrofuran and anisole which do not have an antioxidant activity themselves.

5.1.4. Compound **4**: **1-(2',4'-diacetyloxyphenyl)-3-phenyl-2-propen-1-one (4)**

Formula: $C_{19}H_{15}O_5$

Molecular mass: 323 g/mol

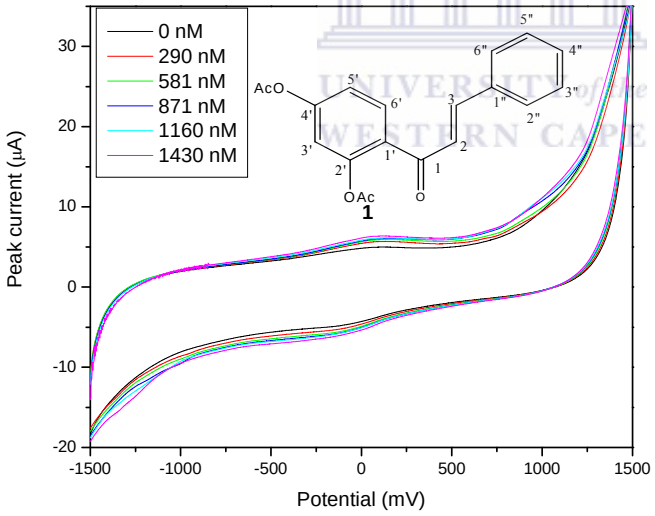


Figure 72*: Cyclic voltammogram of compound 3, scan rate 100 mV/s.*

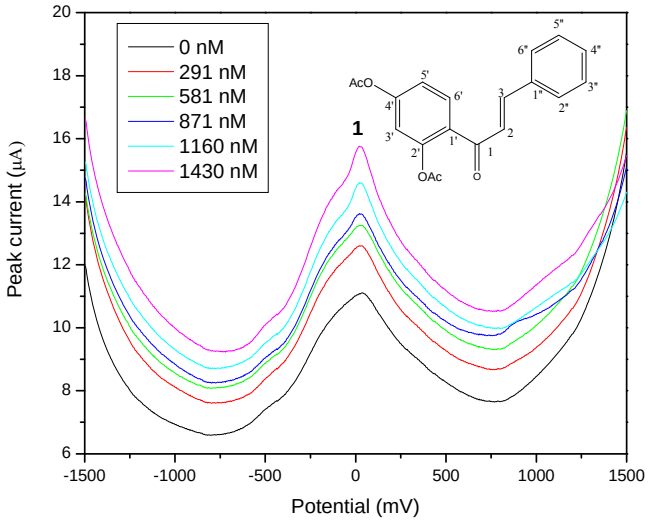


Figure 73*: Square wave voltammogram of compound 3, scan rate 20 mV/s.*

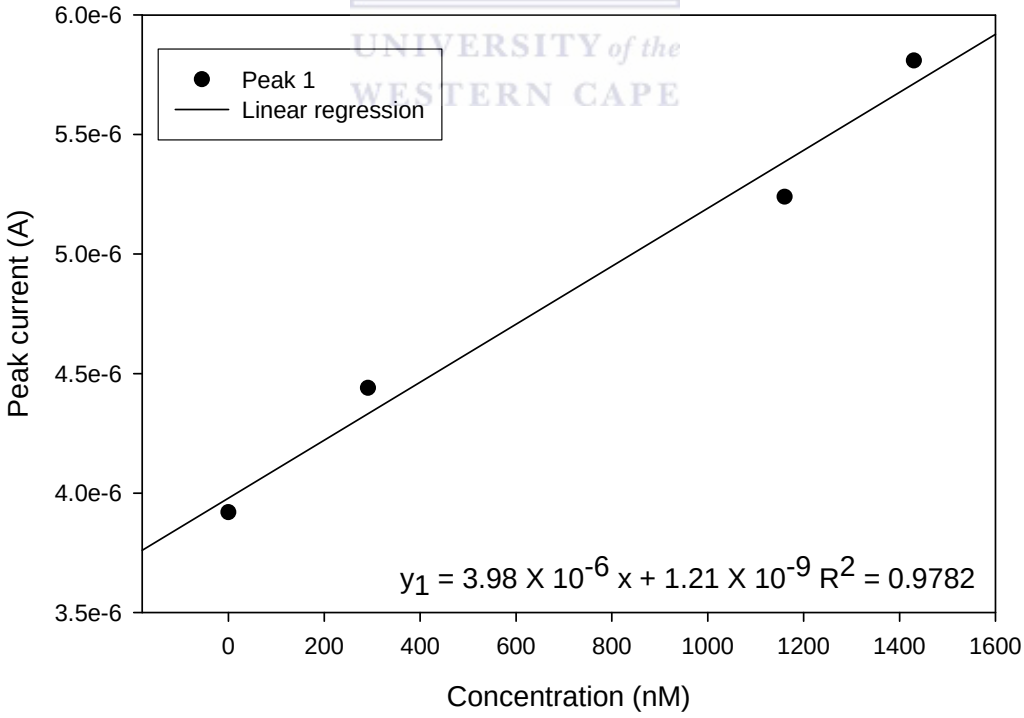


Figure 74*: Linear regression graph of compound 4.*

Figure 73 shows the square wave voltammogram of compound **4** which shows only one sets of peaks one at 26 mV. The first oxidation peak (I_{p1}) was used as additional variable to analyze the effect of concentration and a simple regression analysis was performed (**Figure 74**). The regression equation for peak 1 $I_{p1} = 3.98 \text{ X } 10^{-6} C^0 + 1.21 \text{ X } 10^{-9}$ with a correlation coefficient of 0.9782.

Diacetoxy benzene

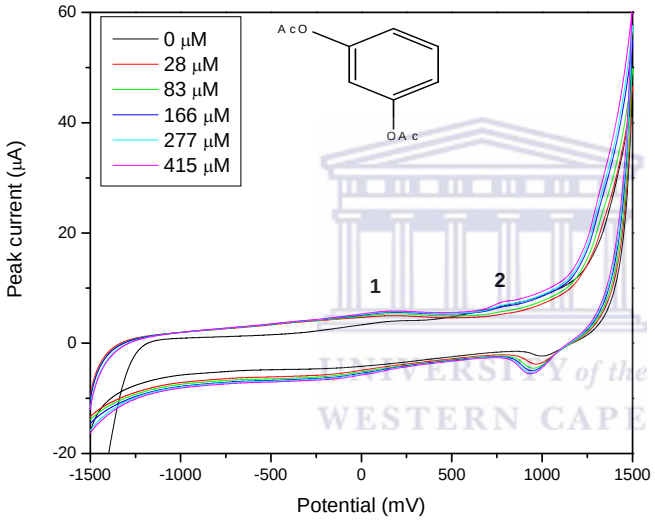


Figure 75*: Cyclic voltammogram of diacetoxybenzene, scan rate 100 mV/s*

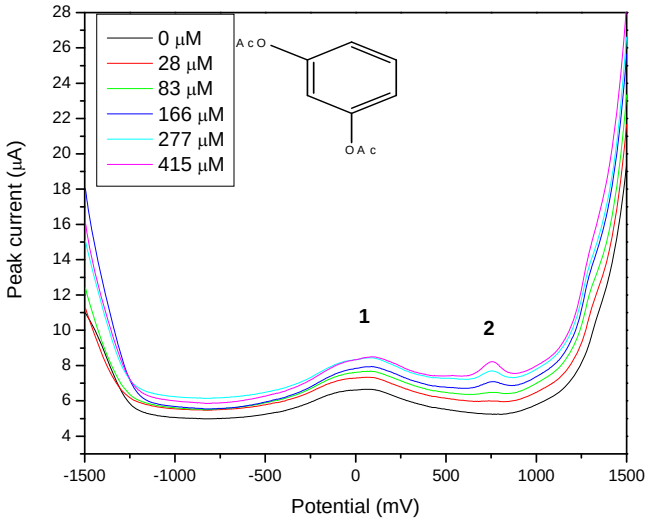


Figure 76*: Square wave voltammogram of diacetobenzene, scan rate 20mV/s.*

2-penten-1-one

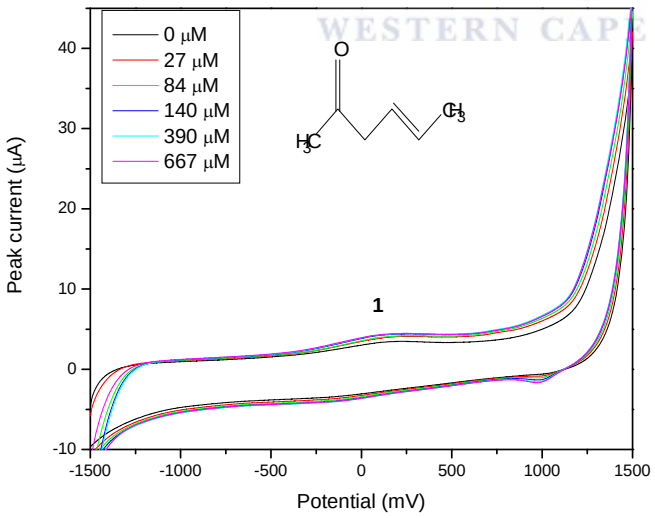


Figure 77*: Cyclic voltammogram of 2-pentenone, scan rate 100mV/s.*

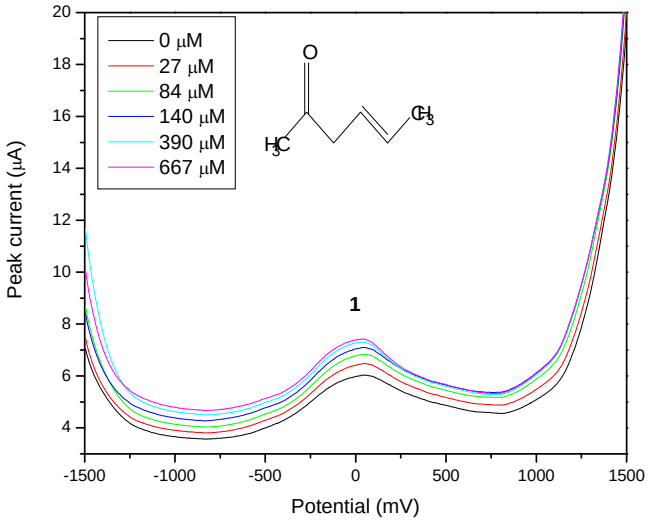


Figure 78*: Square wave voltammogram of 2-pentenone, scan rate 20mV/s.*

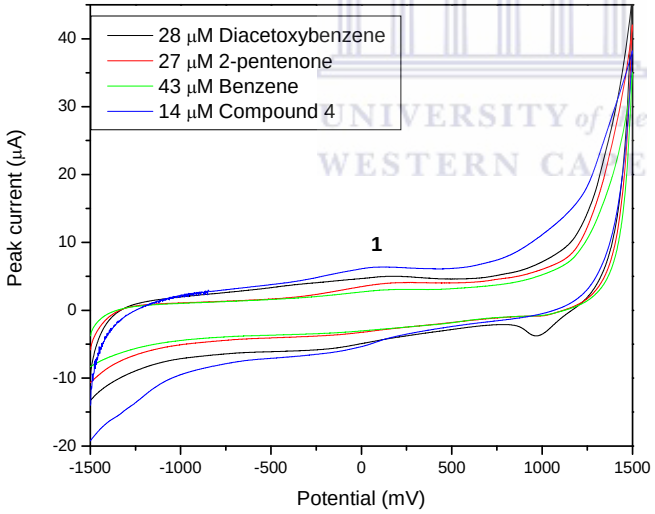


Figure 79*: Cyclic voltammogram of diacetoxybenzene, 2-pentenone, benzene and compound 4.*

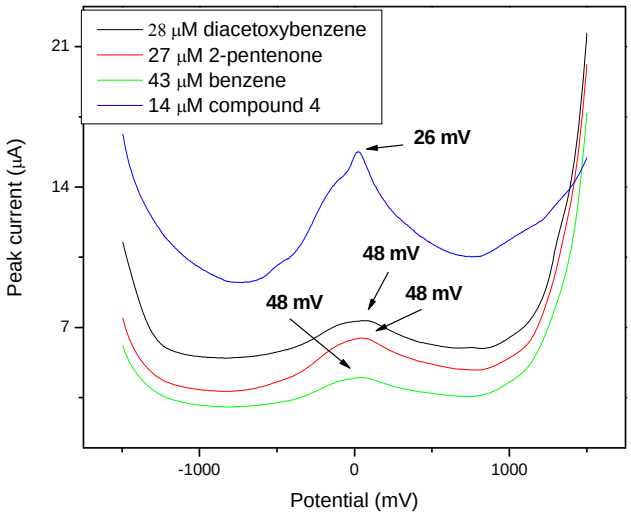


Figure 80*: Square wave voltammograms of diacetoxybenzene, 2-pentenone, benzene and compound 4.*

From the square wave overlay of compound **4** and its standards we can see that there is no evidence of a second oxidation peak at those concentrations. We can also conclude that compound **4** has a better antioxidant activity that any of its standards due to a low half peak oxidation potential [17, 164].

5.1.5. Compound 5: **1-(2',4'-dihydroxyphenyl)-3-phenyl-2-propen-1-one (5)**

Formula: $C_{15}H_{12}O_3$

Molecular mass: 240 g/mol

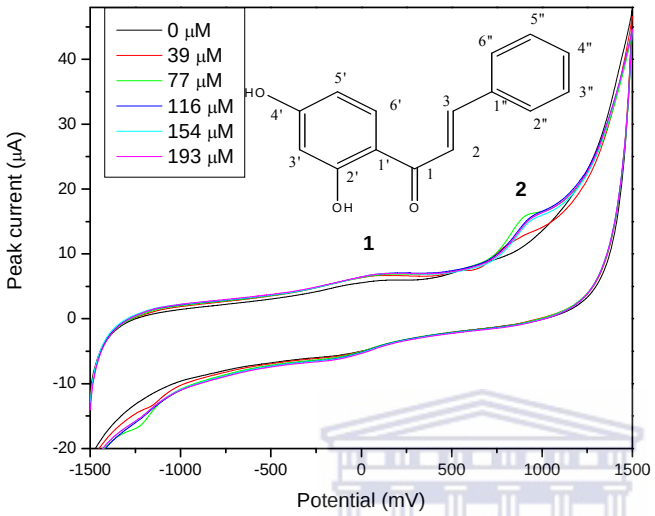


Figure 81*: Cyclic voltammogram of compound 5, scan rate 100 mV/s.*

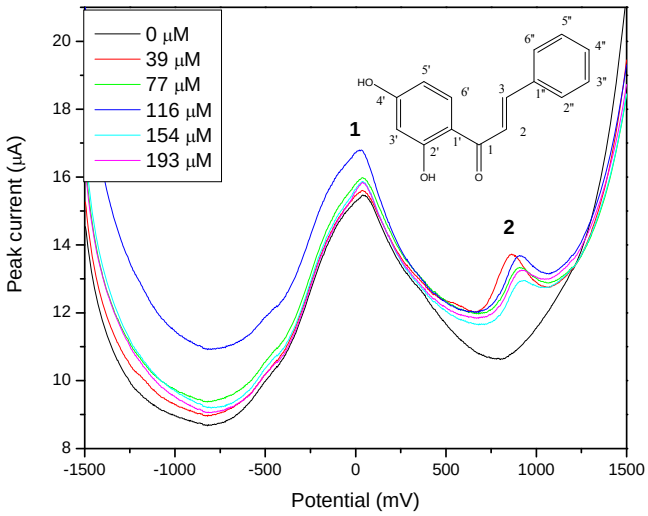


Figure 82*: Square wave voltammogram of compound 5, scan rate 20 mV/s.*

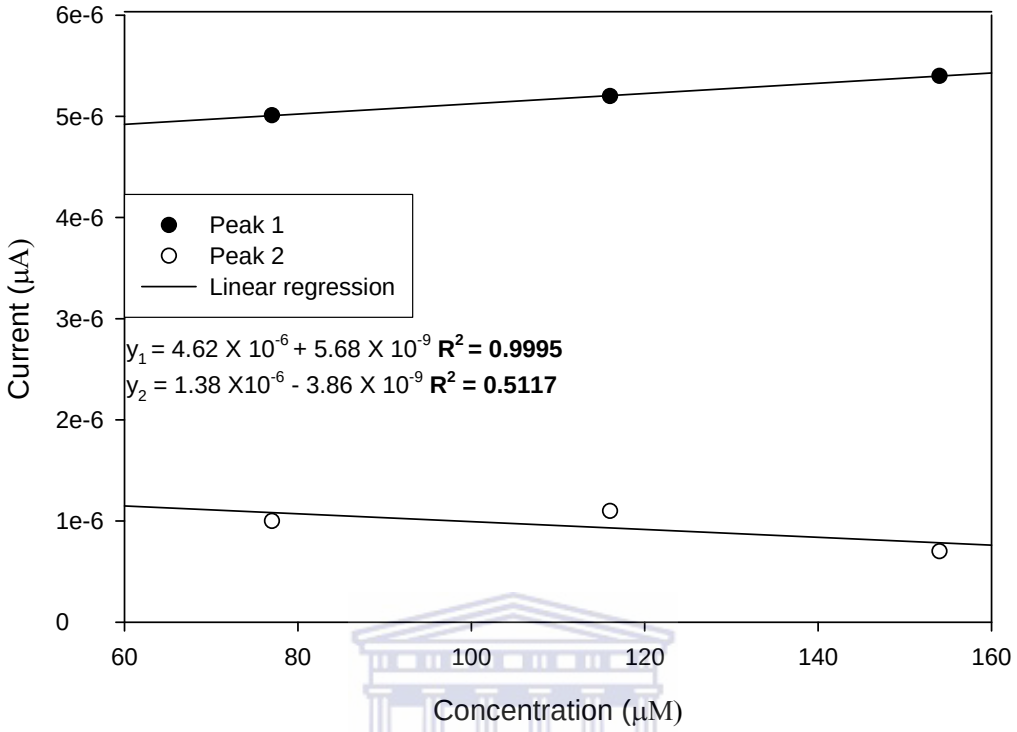


Figure 83*: Linear regression graph of compound 5.*

Figure 81 shows the cyclic voltammogram of compound **5** shows one anodic peak at 916 mV. When dealing with compound that behave as antioxidants anodic peaks are assigned to individual compounds based on the oxidation potentials [105]. The absence of a corresponding reduction peak for this oxidation peak at 916 mV shows the irreversibility of the oxidation reaction product produced in this reaction. The oxidation of mono phenol group occurs at high positive potentials forming a phenoxy radical or phenoxium ion and can successively undergo different secondary reactions [166]. The square wave voltammogram of compound **5** shows two sets of peaks one at 43 mV and the other at 916 mV. The first and second oxidation peaks were used as additional variables to analyze the effect of concentration by extracting the linear region between 77 and 154 μM and a multiple regression analysis was performed. The regression equation for peak 1 I_{p1} = 5.68 X 10^{-9} C^0 + 6.83 X 10^{-7} with a

correlation coefficient of 0.9995 and peak 2 has a regression equation of I_{p2} = 1.38 X 10^{-6} C^0 – 3.86 X 10^{-9} with a very low correlation coefficient of 0.5117.

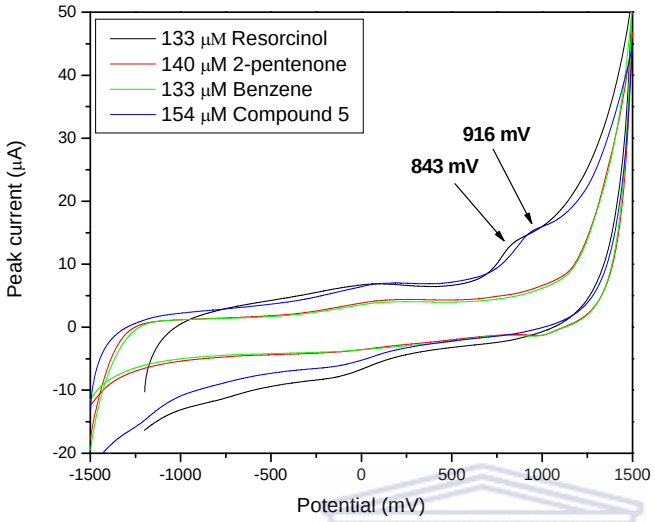


Figure 84*: Cyclic voltammograms of resorcinol, 2-pentenone, benzene and compound 5.*

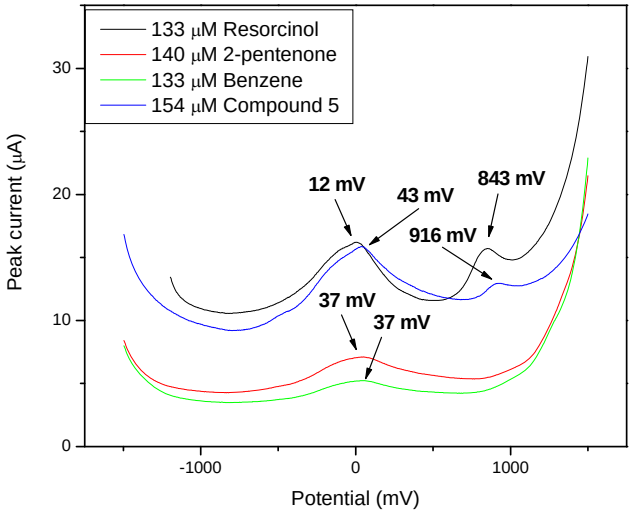


Figure 85*: Square wave voltammograms of resorcinol, 2-pentenone, benzene and compound 5.*

Compound **5** has the same number of oxidation peaks as resorcinol which can lead us to assume that the electrochemically active part of compound **5** is the resorcinol moiety due to the presence of hydroxyl groups which can act as electron donors [165]. The comparison of peak 1 and 2 of resorcinol, at 12 mV and 843 mV respectively, with those of compound **5**, at 43 mV and 916 mV shows us that resorcinol has a better antioxidant activity than compound **5** [17, 164].

5.1.6. Compound **6**: **5-acetoxy-2,3-dihydro-7-hydroxy-2-(2'-methoxyphenyl)-4H-1-Benzopyran-4-one (6)**

Formula: $C_{18}H_{16}O_6$

Molecular mass: 328 g/mol

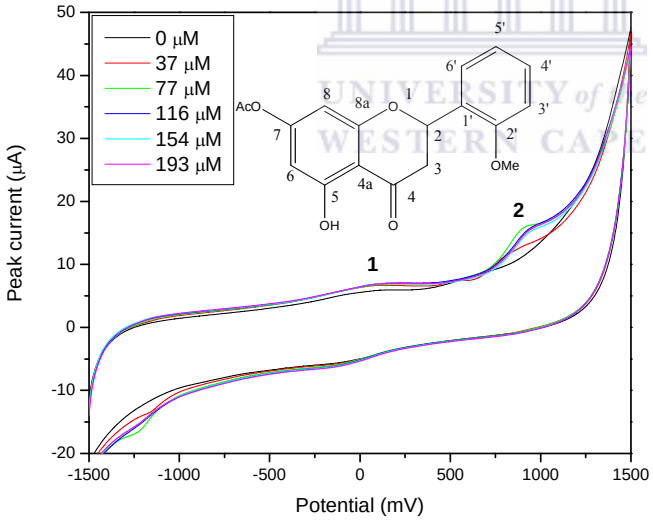


Figure 86: Cyclic voltammogram of compound 6, scan rate100 mV/s.

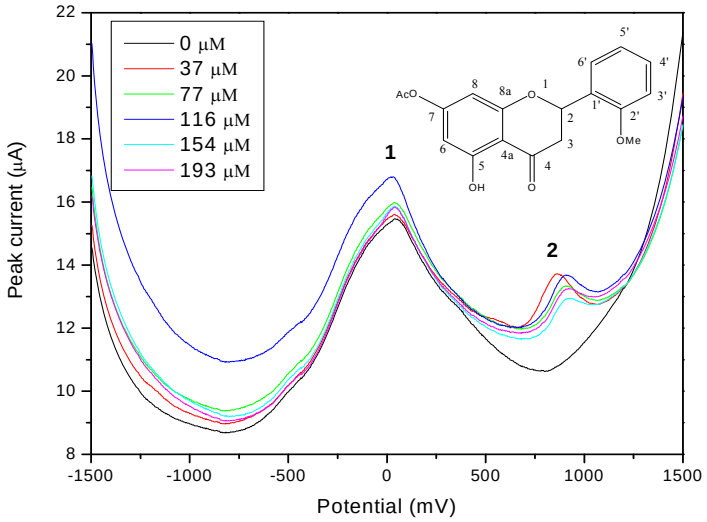


Figure 87: Square wave voltammogram of compound 6, scan rate 20 mV/s.

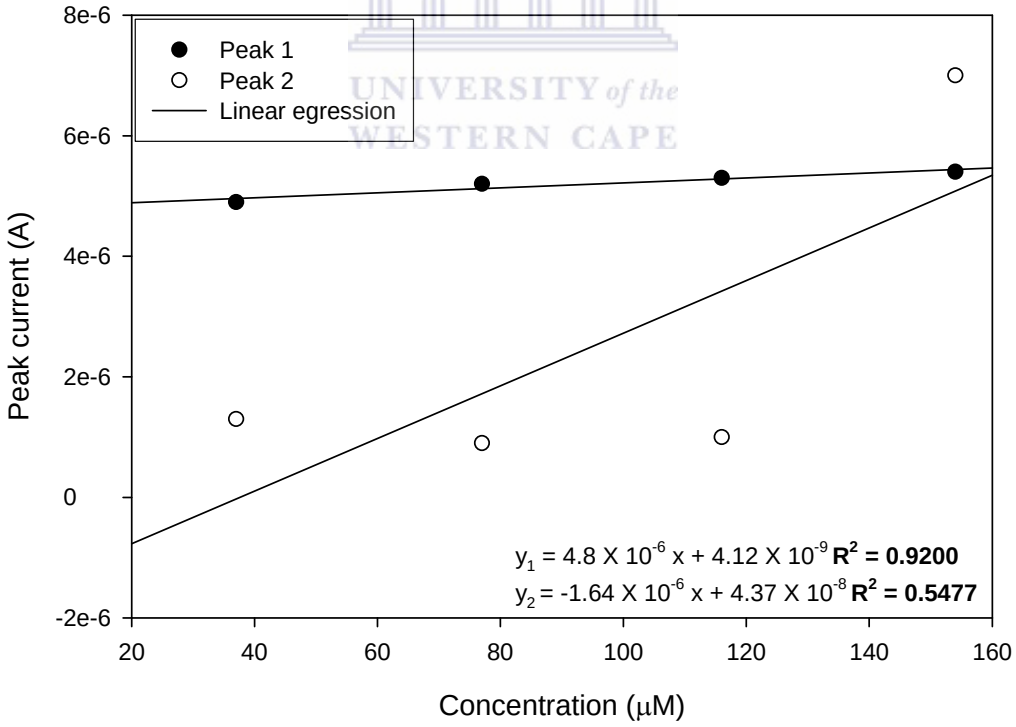


Figure 88: Linear regression graph of compound 6.

Figure 86 shows the cyclic voltammogram of compound **6** shows two anodic peaks. The absence of a corresponding reduction peak for this oxidation peak at 944 mV shows the irreversibility of the oxidation reaction product produced in this reaction. The square wave voltammogram of compound **6** shows two sets of peaks one at 122 mV and 916 mV respectively (**Figure 87**). The first and second oxidation peaks were used as additional variables to analyze the effect of concentration by extracting the linear region between 37 and 154 µM and a multiple regression analysis was performed. The regression equation for peak 1 $I_{p1} = 4.8 \text{ X } 10^{-6} C^0 + 4.12 \text{ X } 10^{-9}$ with a correlation coefficient of 0.9200 and peak 2 has a regression equation of $I_{p2} = -1.74 \text{ X } 10^{-6} C^0 + 4.37 \text{ X } 10^{-8}$ with a low correlation coefficient of 0.5477.

1,3 acetoxy phenol

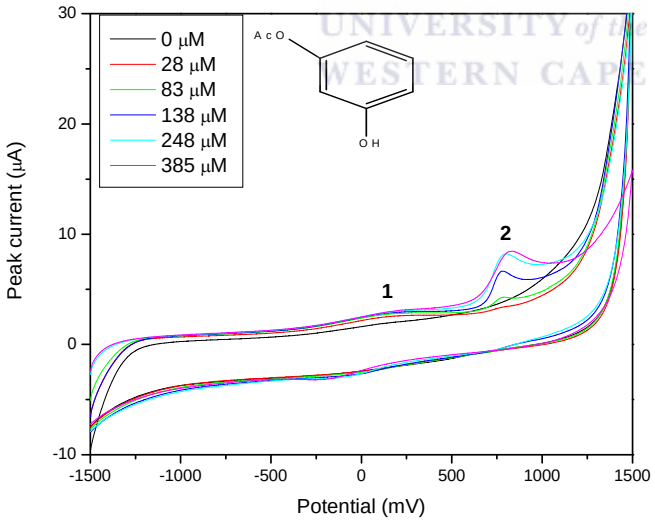


Figure 89: Cyclic voltammogram of 1,3-acetoxy phenol, scan rate100 mV/s.

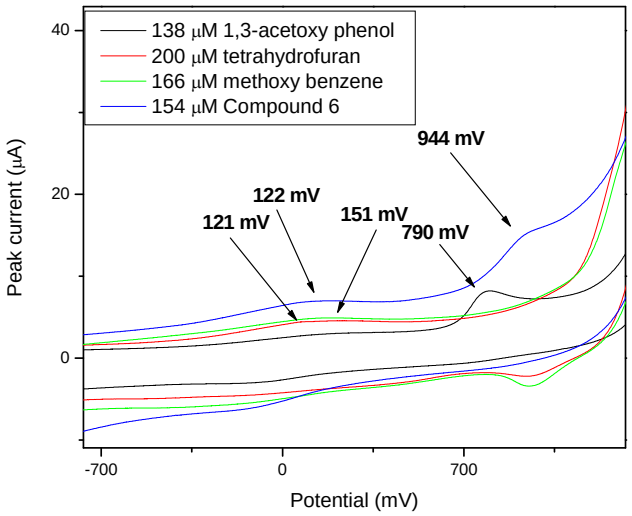


Figure 90: Cyclic voltammograms of 1,3-acetoxy phenol ,tetrahydrofuran, methoxy benzene and compound 6.

The first and second oxidation peaks for compound **6** have higher oxidation potentials than all of the compounds in **figure 90**. This means that compound **6** has low antioxidant activity than all its standard solutions [17, 164]. The second oxidation peak of compound **6** is due to the irreversible oxidation of phenol in 1,3-acetoxyphenol at a potential of 790 mV [167].

5.1.7. Compound 7: **2,3-dihydro-7-hydroxy-2-phenyl-4H-1-Benzopyran-4-one (7)**

Formula: $C_{15}H_{12}O_3$

Molecular mass: 210 g/mol

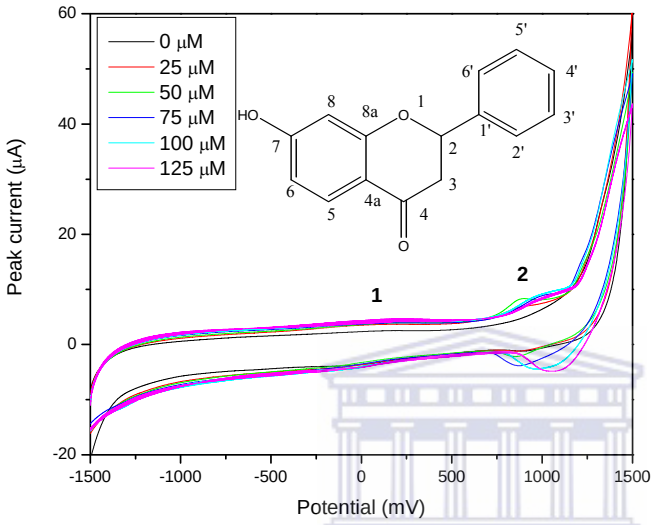


Figure 91: Cyclic voltammogram of compound 7, scan rate100 mV/s.

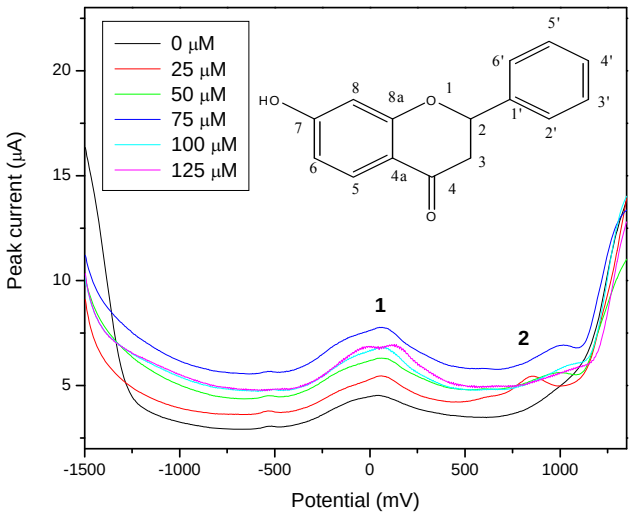


Figure 92: Square wave voltammogram of compound 7, scan rate 20 mV/s.

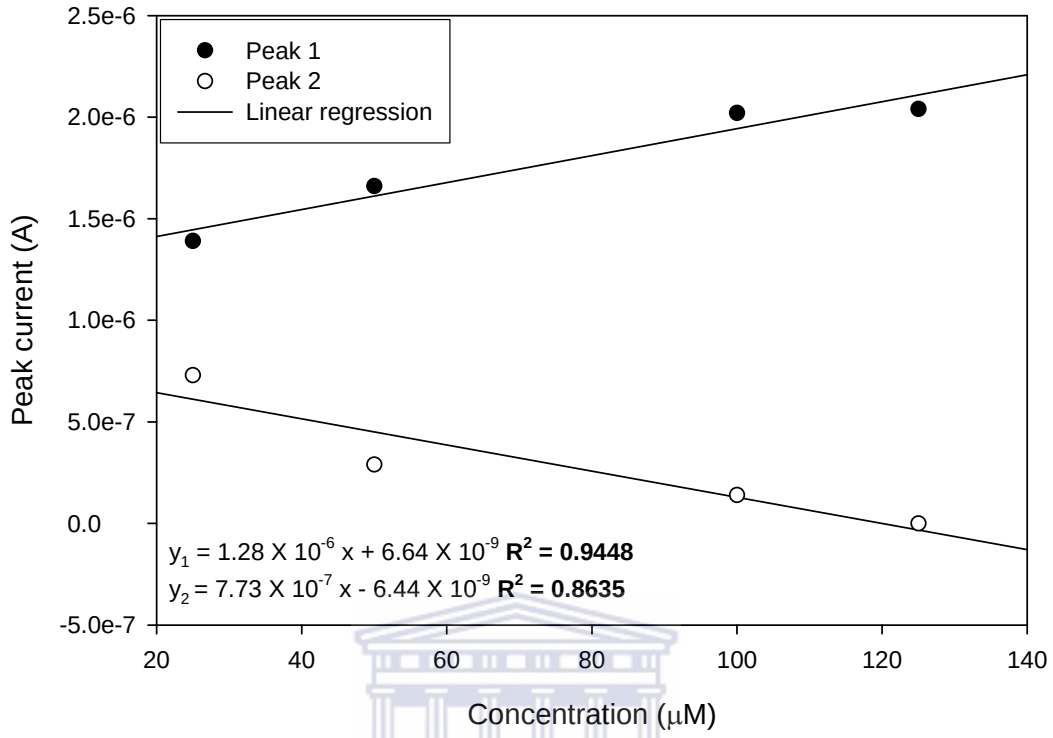


Figure 93: Linear regression graph of compound 7.

Figure 91 shows the cyclic voltammogram of compound **7** shows two anodic peaks. The presence reduction peak for this oxidation peak at 1012 mV shows the reversibility of the oxidation reaction product produced in this reaction. Filipiak (2001) carried out some studies on 7-hydroxyflavone and he reported peak 2 as dependant on pH, and for 7-hydroxyflavone at even higher potentials when reaction was ran at pH 7 [168]. The square wave voltammogram of compound **7** shows two sets of peaks one at 70 mV and 1012 mV respectively (**Figure 92**). The first and second oxidation peaks (I_{p1}, I_{p2}) were used as additional variables to analyze the effect of concentration by extracting the linear region between 25 and 125 μM and a multiple regression analysis was performed. The current of peak 1 (I_{p1}) increased with

increasing while the opposite occurs for the current of peak 2 (I_{p2}) which experiences a decrease with increasing concentration. The regression equation for peak 1 I_{p1} = 1.28 X 10^{-6} C^0 + 6.64 X 10^{-9} with a correlation coefficient of 0.9448 and peak 2 has a regression equation of I_{p2} = 7.73 X 10^{-7} C^0 + 6.44 X 10^{-9} with a low correlation coefficient of 0.8635.

Phenol

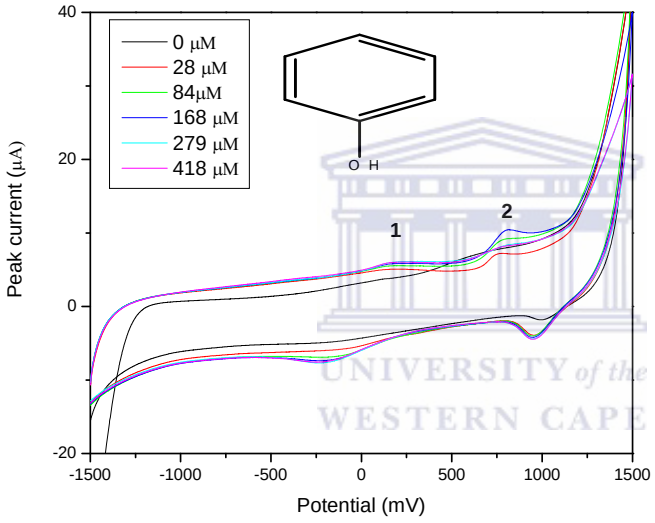


Figure 94: Cyclic voltammogram of phenol, scan rate100 mV/s.

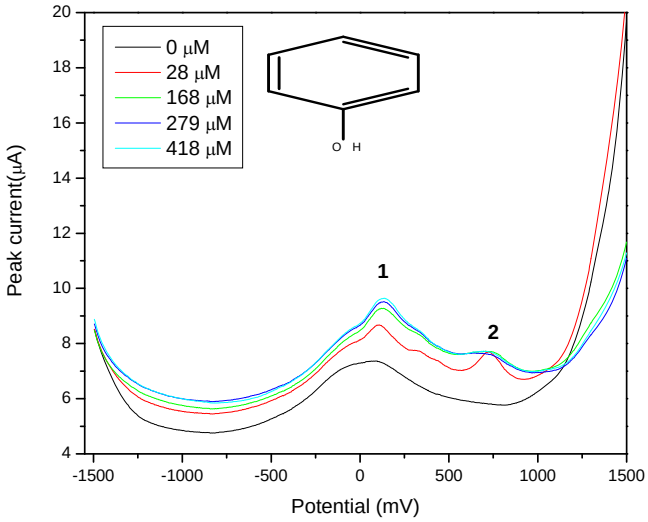


Figure 95: Square wave voltammogram of phenol, scan rate 20 mV/s.

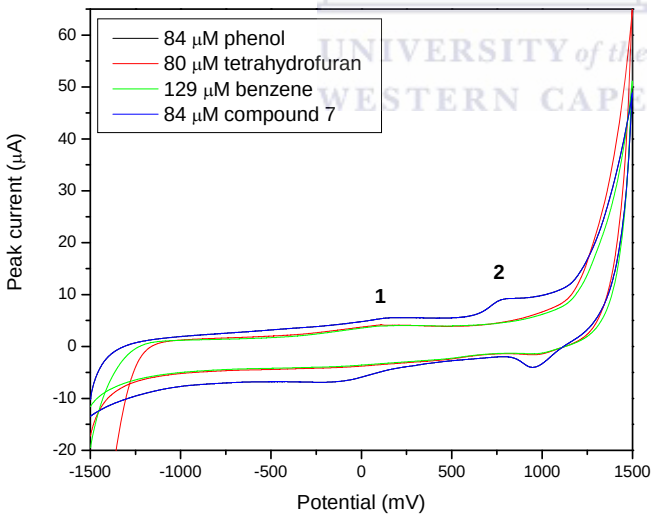


Figure 96: Cyclic voltammogram of phenol, tetrahydrofuran, benzene and compound 7.

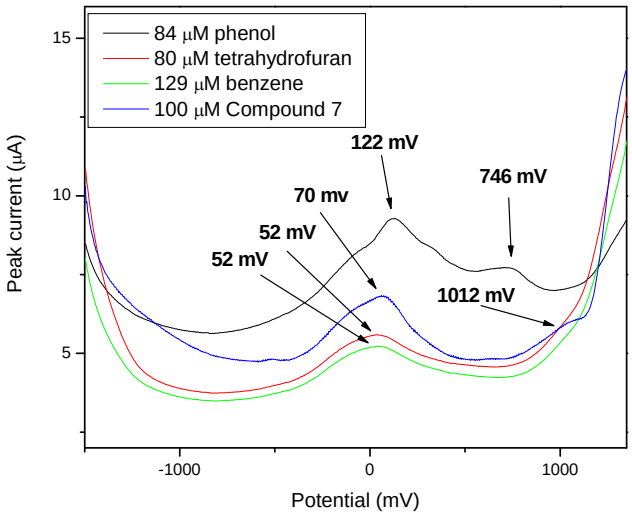


Figure 97: Square wave voltammograms of phenol, tetrahydrofuran, benzene and compound 7.

Although tetrahydrofuran and benzene have peak 1 they can not be considered as antioxidants because they do not contain hydroxyl groups which could behave as electron donors [165]. Compound **7** and phenol both contain hydroxyl groups so are able to behave as antioxidants, considering peak 1, compound **7** has a lower oxidation potential of 70 mV than phenol which has an oxidation potential of 122 mV proving that compound **7** is a better antioxidant than phenol [17, 164]. Peak 2 for compound **2** at 1012 mV is due to the oxidation of phenol and it shifts from a potential of 746 mV due to the presence of other substituents on the compound. In literature the oxidation potential of phenol is at 540 mV, the change in peak potential could be due to the solution used because when it comes to polyphenolic compounds a change in the pH of the solution leads to a shift of the peak potentials of the compounds [111].

5.1.8. Compound **8**: **5,7-(diacetoxy)-2,3-dihydro-2-phenyl-4H-1-benzopyran-4-one (8)**

Formula: $C_{19}H_{16}O_6$

Molecular mass: 340 g/mol

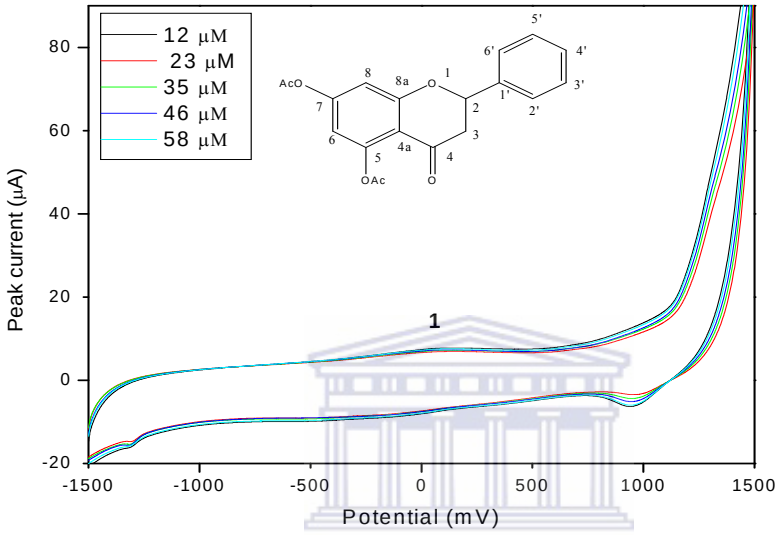


Figure 98: Cyclic voltammogram of compound 8, scan rate100 mV/s.

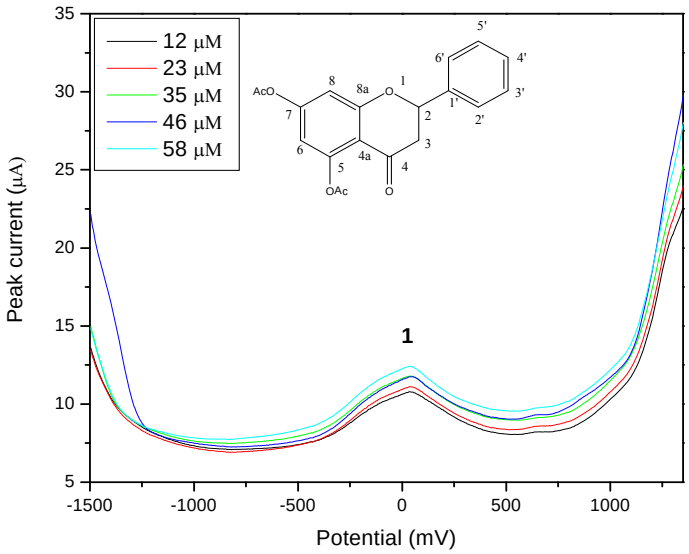


Figure 99: Square wave voltammogram of compound 8, scan rate 20 mV/s.

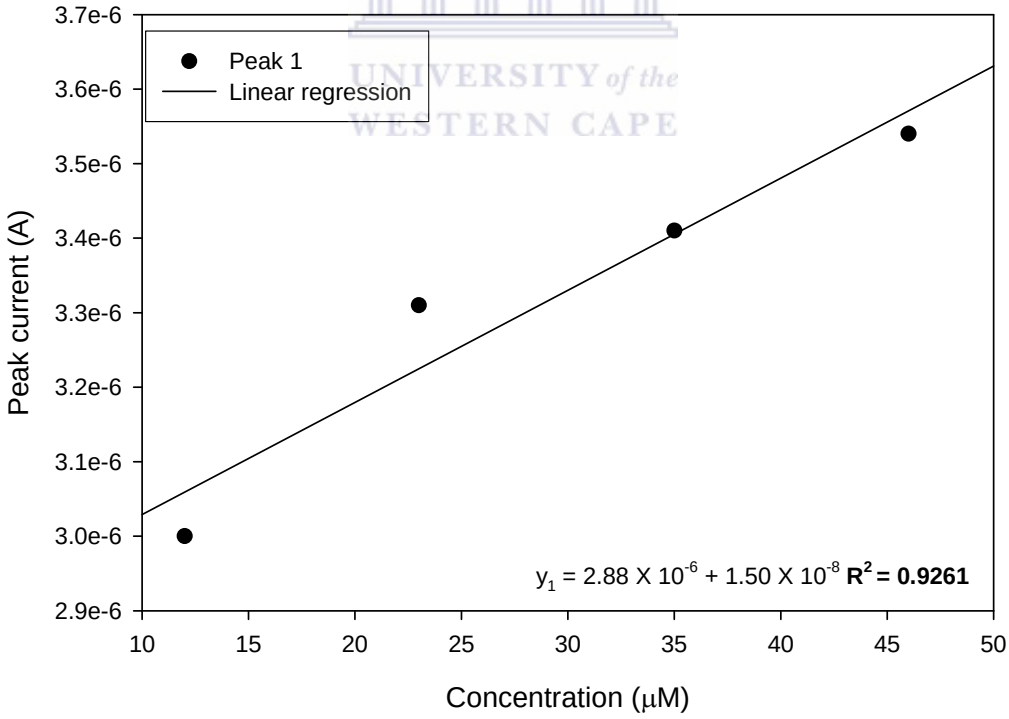


Figure 100: Linear regression graph of compound 8.

The square wave voltammogram of compound **8** (**Figure 99**) shows one sets of peaks at 50 mV. The first oxidation peaks (I_{p1}) was used to analyze the effect of concentration by extracting the linear region between 12 and 46 µM and a multiple regression analysis was performed. The regression equation for peak 1 was $I_{p1} = 2.88 \text{ X } 10^{-6} C^{0} + 1.50 \text{ X } 10^{-8}$ with a correlation coefficient of 0.9261.

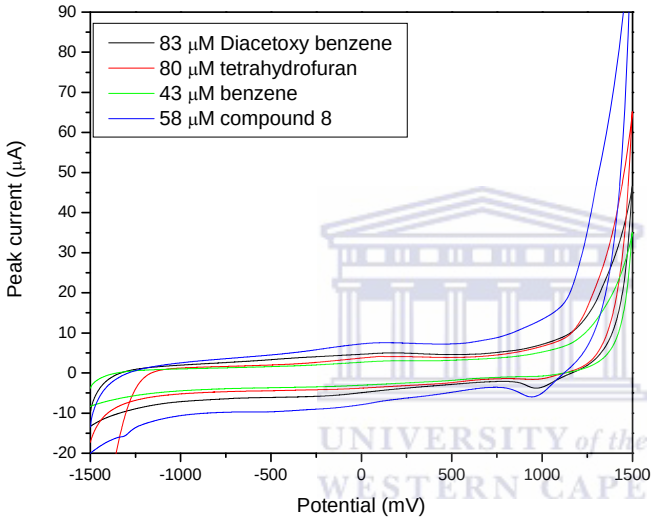


Figure 101: Cyclic voltammograms of diacetoxybenzene, tetrahydrofuran, benzene and compound 8.

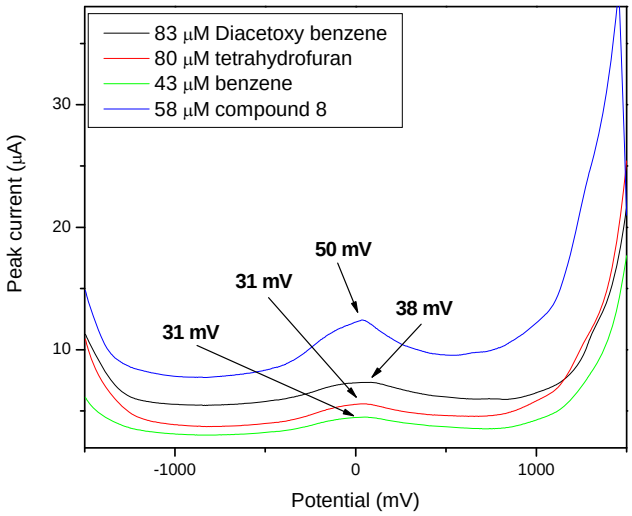


Figure 102: Square wave voltammograms of diacetoxybenzene, tetrahydrofuran, benzene and compound 8.

Cao (1997) proved that the presence of hydroxyl group is necessary for the antioxidant activity of a compound hence compound **8** is assumed not to have any antioxidant activity due to absence of a hydroxyl group that will behave as an electron donor. [165].

5.1.9. Compound **9**: **5,7-(diacetoxy)-2,3-dihydro-2-(2-methoxyphenyl)-4H-1-benzopyran-4-one (9)**

Formula: $C_{20}H_{19}O_7$

Molecular mass: 371 g/mol

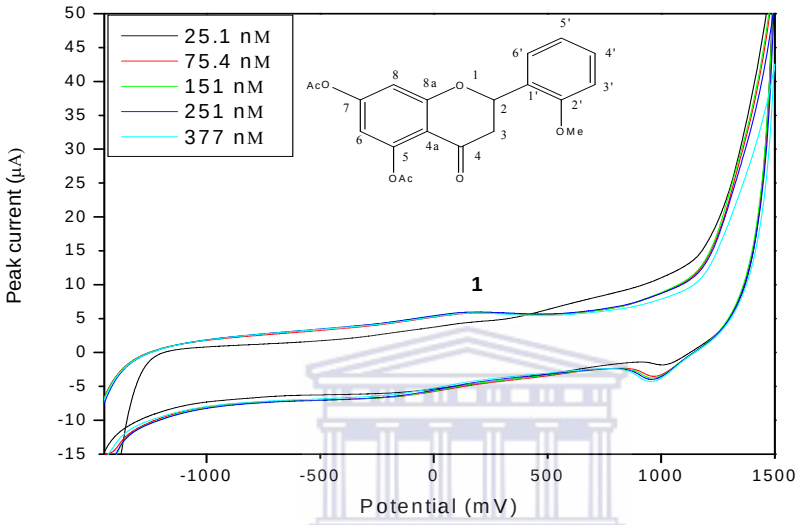


Figure 103: Cyclic voltammogram of compound 9, scan rate100 mV/s.

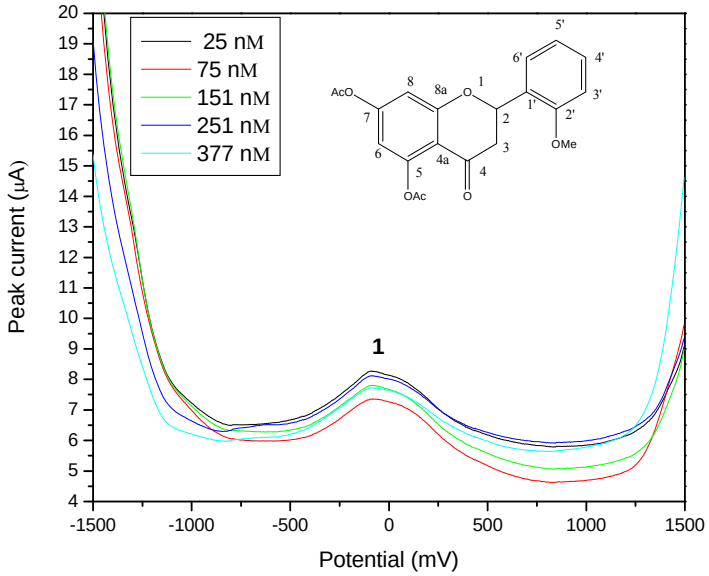


Figure 104: Square wave voltammogram of compound 9, scan rate 20 mV/s.

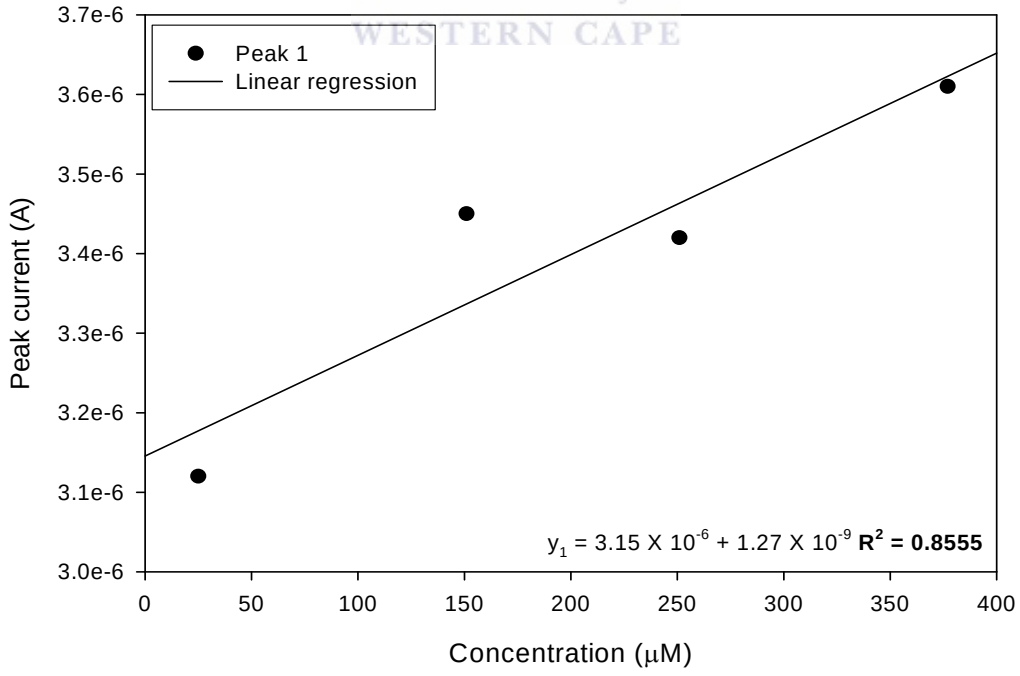


Figure 105: Linear regression graph compound 9.

The square wave voltammogram of compound **9** (**Figure 104**) has one set of peaks at -85 mV. The first oxidation peak (I_{p1}) was used to analyze the effect of concentration by extracting the linear region between 25 and 377 nM and a simple regression analysis was performed. The regression equation for peak 1 I_{p1} = 3.15 X 10^{-6} C^0 + 1.27 X 10^{-8} with a correlation coefficient of 0.8555.

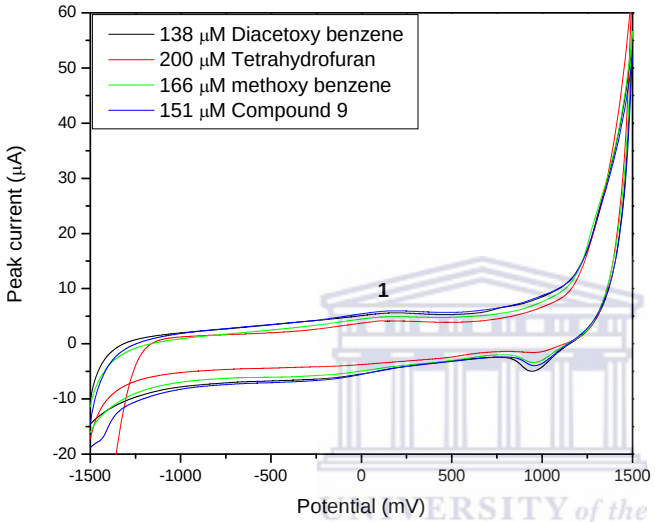


Figure 106: Cyclic voltammograms of diacetoxybenzene, tetrahydrofuran, anisole and compound 9.

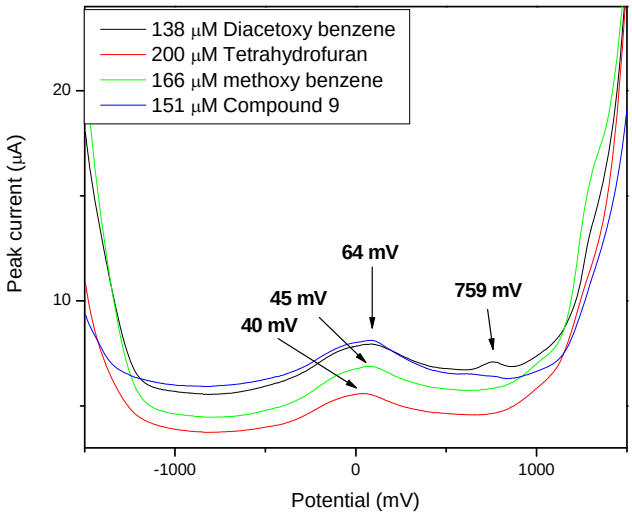


Figure 107: Square wave voltammograms of diacetoxybenzene, tetrahydrofuran, anisole and compound 9.

The absence of peak two, between 600 mV and 750 mV, shows the absence of a hydroxyl group in the structure of compound **9**. Considering the antioxidant activity of a flavonoid that does not contain hydroxyl group as it is the main functional group that behaves as an electron donor [165]. If this compound contained a electron donating group it would the best antioxidant out of the group of flavanoids studied as it has an oxidation peak potential of – 85 mV for peak 1 [25].

5.2. Flavanoids from Elytropapus rhinocerotis

5.2.1. Compound **10**: 2,3-dihydro-5-hydroxy-2-(4'-hydroxy-3'-methoxyphenyl)-7-methoxy-4H-1-benzopyran-4-one (10)

Formula: $C_{17}H_{16}O_6$

Molecular mass: 316 g/mol

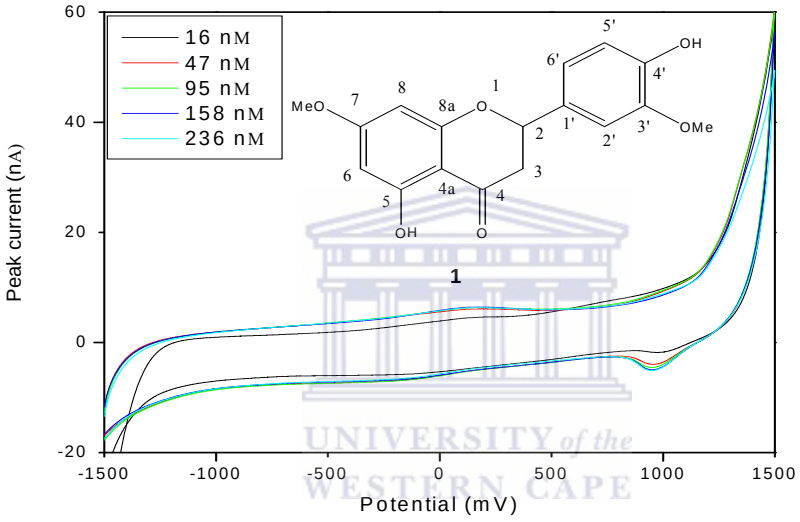


Figure 108: Cyclic voltammogram of compound 10, scan rate100 mV/s.

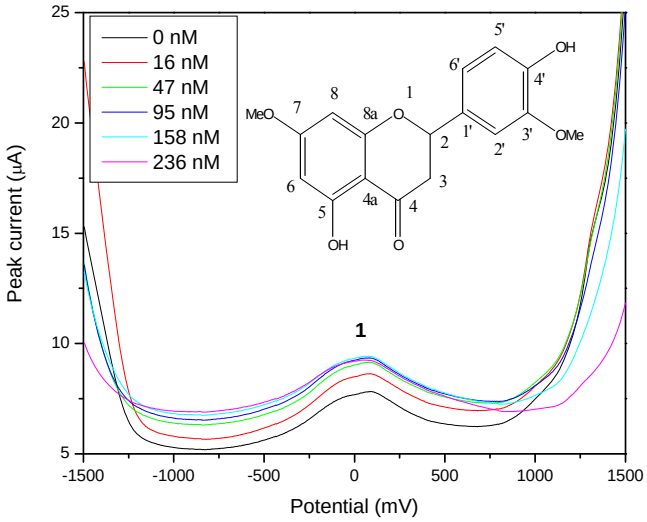


Figure 109 : Square wave voltammogram of compound 10, scan rate 20 mV/s.

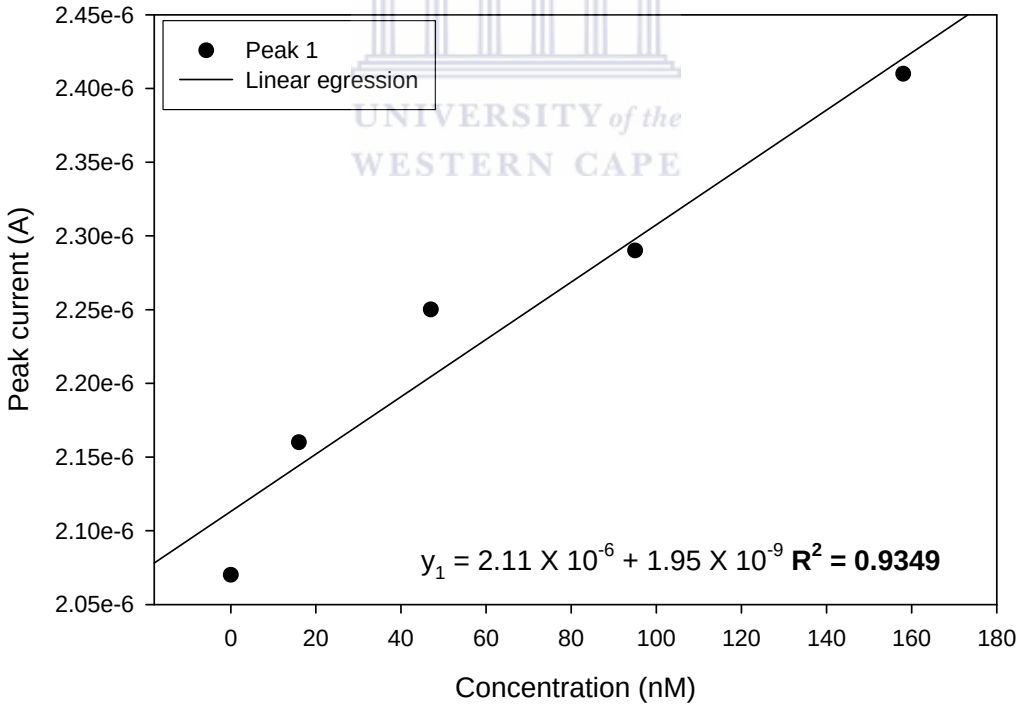


Figure 110: Linear regression graph of compound 10.

The square wave voltammogram of compound **10** (**Figure 109**) shows one set of peaks at 89 mV. The first oxidation peak (Ip1) was used to analyze the effect of concentration by extracting the linear region between 0 and 158 µM and a simple regression analysis was performed. The regression equation for peak 1 is I_{p1} = 2.11 X 10^{-6} C^0 + 1.95 X 10^{-9} with a correlation coefficient of 0.9349.

1,3-methoxy phenol

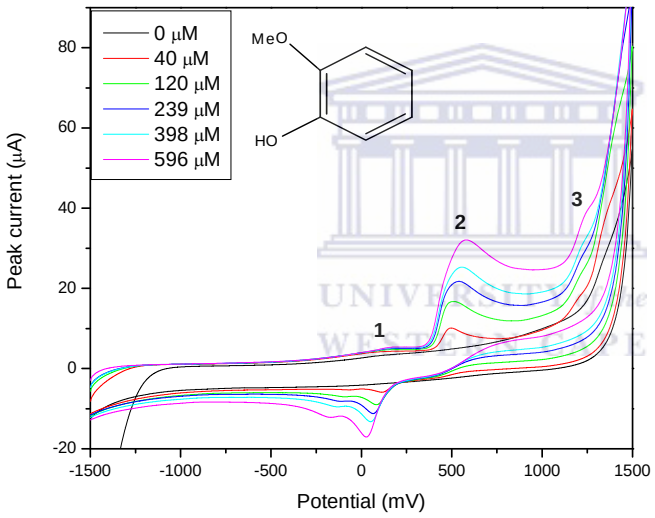


Figure 111: Cyclic voltammogram of 1,2 methoxy phenol, scan rate100 mV/s.

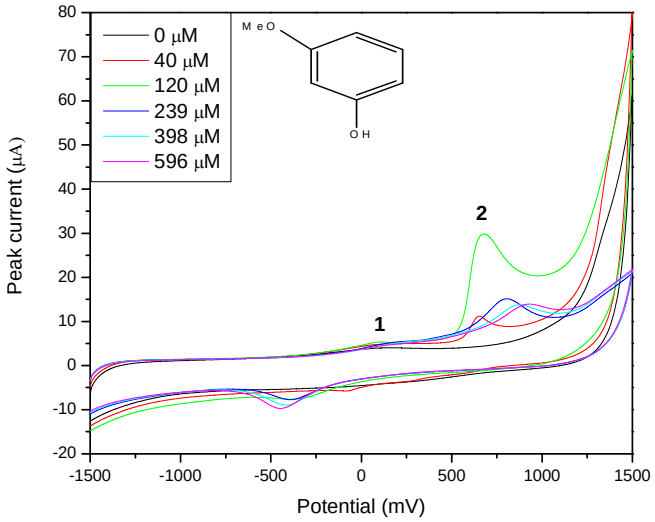


Figure 112: Cyclic voltammogram of 1,3 methoxy phenol, scan rate100 mV/s.

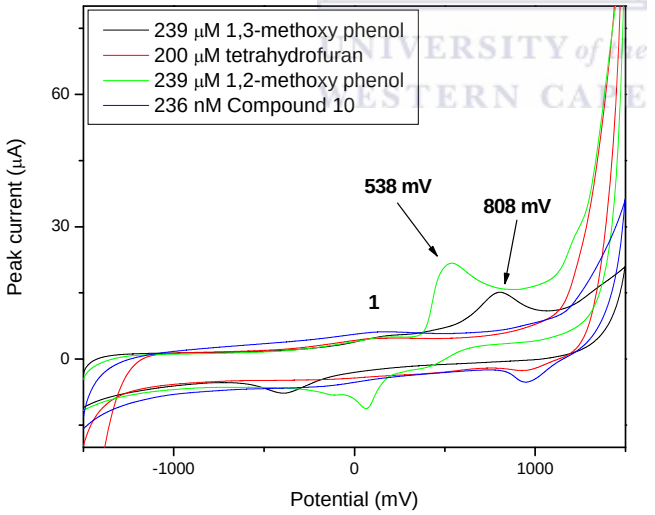


Figure 113: Cyclic voltammograms of 1,3 methoxy phenol, tetrahydrofuran, 1,2 methoxy phenol and compound 10.

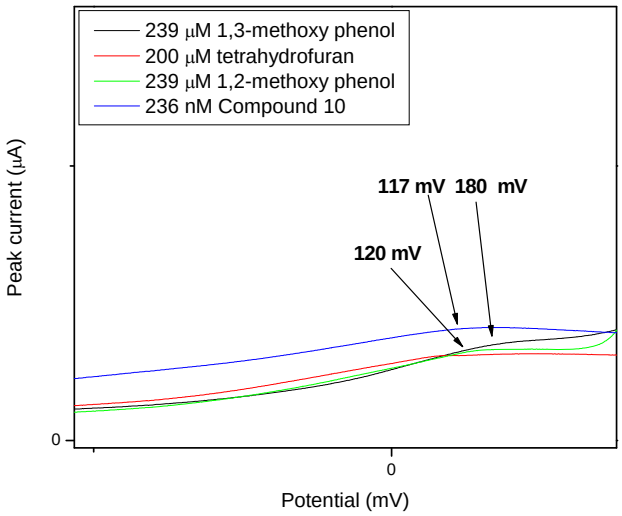


Figure 114: Magnified diagram of area labelled 1 on figure 123.

From **figure 114**, for peak 1, we can conclude that the antioxidant activity of compound **10** is greater than of all the standards and that the antioxidant activity of 1,2 methoxy phenol is greater than of 1,3-methoxy phenol due to the ortho arrangement of the methoxy and the hydroxyl on the benzene ring [17, 119, 163].

5.2.2. Compound **11**: **2,3-dihydro-5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-4H-1-benzopyran-4-one (11)**

Formula: $C_{16}H_{13}O_5$

Molecular mass: 285 g/mol

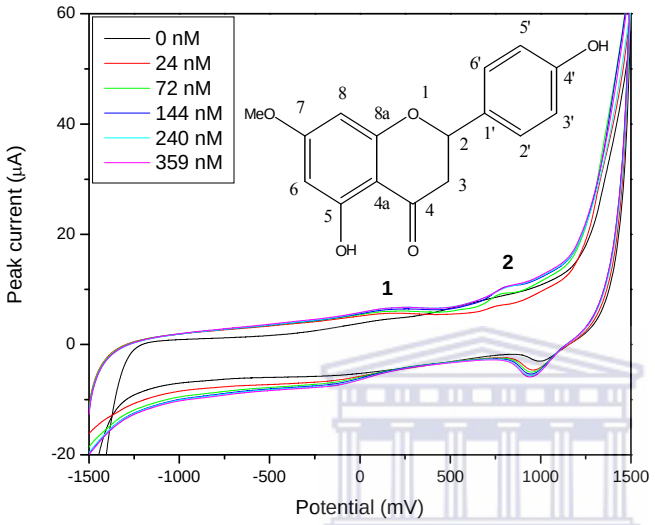


Figure 115: Cyclic voltammogram of compound 11, scan rate100 mV/s.

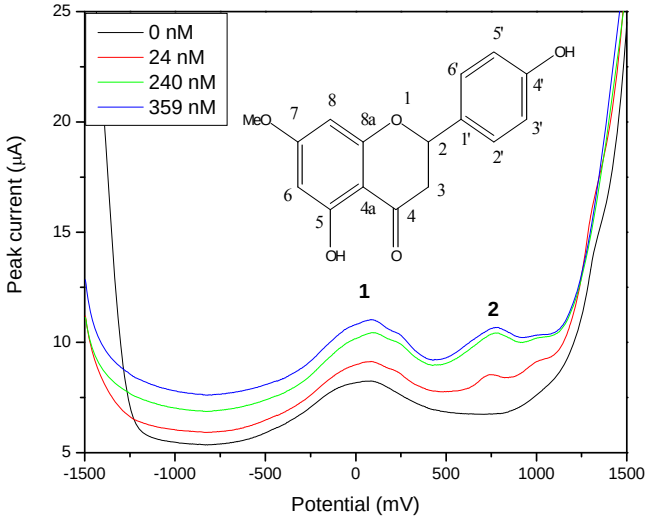


Figure 116: Square wave voltammogram of compound 11, scan rate 20 mV/s.

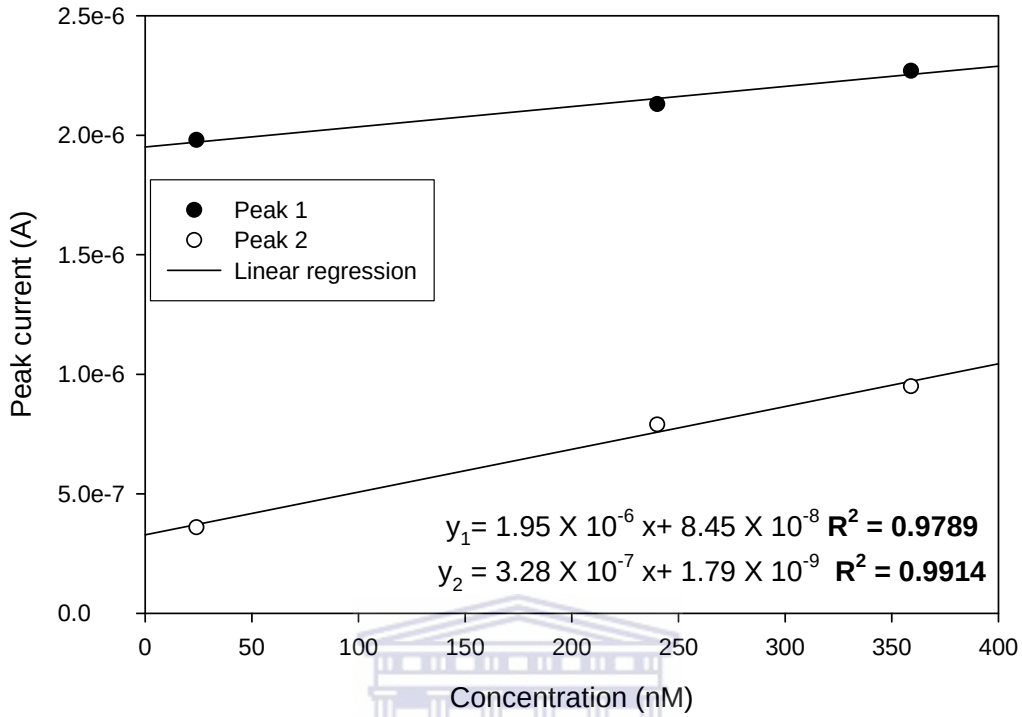


Figure 117: Linear regression of compound 11.

The square wave voltammogram of compound **11** (**Figure 116**) shows two sets of peaks at 98 mV and 782 mV respectively. The first and second oxidation peaks were used to analyze the effect of concentration by extracting the linear region between 24 and 359 μM and a multiple regression analysis was performed. The regression equation for peak 1 $I_{p1} = 1.95 \text{ X } 10^{-6} \text{ C}^0 + 8.45 \text{ X } 10^{-8}$ with a correlation coefficient of 0.9789 and peak 2 has a regression equation of $I_{p2} = 3.28 \text{ X } 10^{-7} \text{ C}^0 + 1.79 \text{ X } 10^{-9}$ with a correlation coefficient of 0.9914.

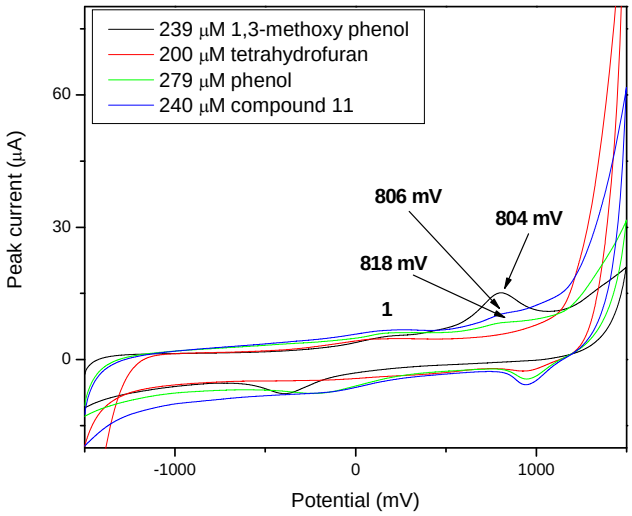


Figure 118: Cyclic voltammogram of 1,3 methoxy phenol, tetrahydrofuran, phenol and compound 11.

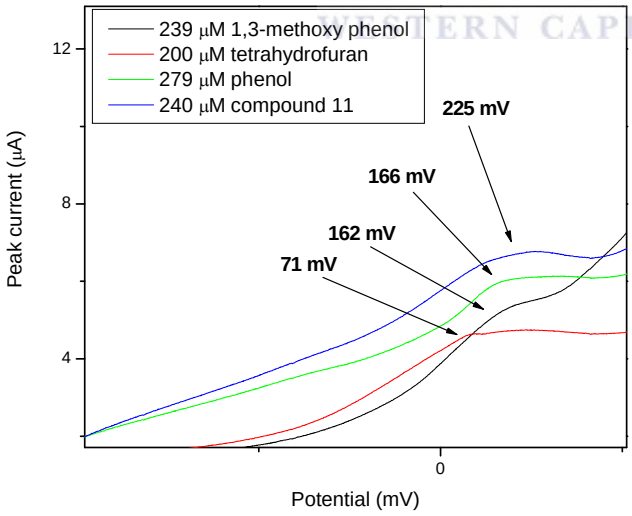


Figure 119: Magnified graph of area labelled 1 on figure129.

From **figure 118** we can observe that phenol, compound **11** and 1,3-methoxy phenol all have peak two at oxidation potentials of 818, 806 and 804 mV respectively. From **figure 130** we can see that compound **11** has a higher oxidation peak potential than its standards and hence would be not be a good antioxidant as compared to the standards [17, 163].

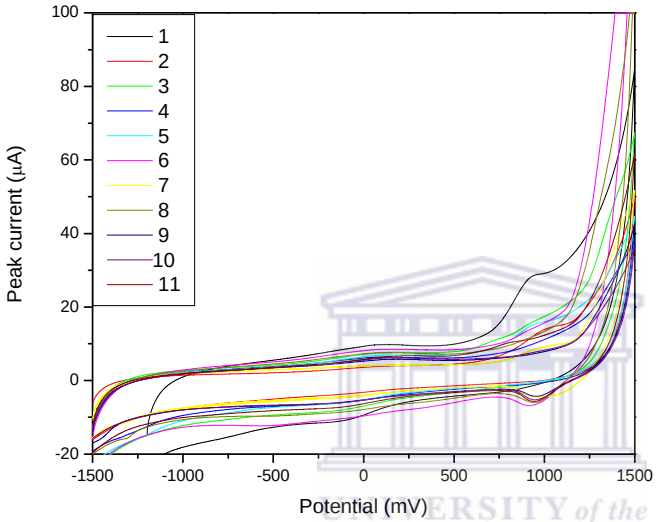


Figure 120: Cyclic voltammograms of all 11 compounds scan rate 100 mV/s.

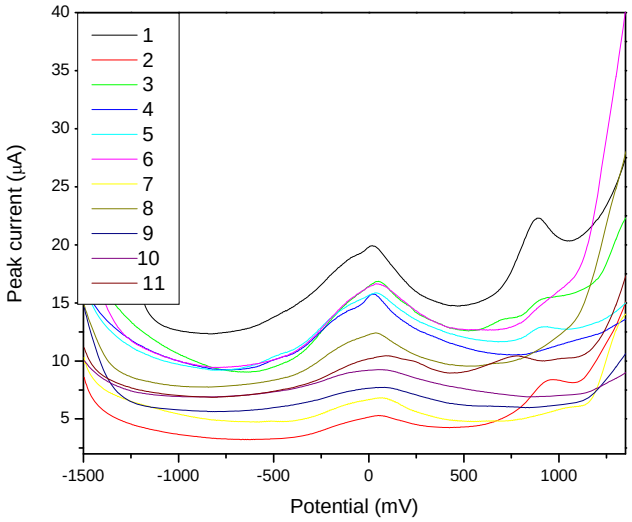


Figure 121: Square wave voltammograms of all 11 compounds, scan rate 20 mV/s.

Cyclic voltammetry is useful in determining the antioxidant activity of antioxidants due to their ability to donate electrons and produce an anodic peak. The current response due to phosphate buffer blank was measured before each experiment of sample solution and subtracted away from the response of the sample. Cyclic voltammetry is important as it helps us determine the antioxidant activity of low molecular weight antioxidant as is not the case with other techniques which determine antioxidant activity [169]. Square wave voltammetry was used to its high speed of analysis and low consumption of electrochemically active species. Square wave gave the best resolution of peaks as compared to C.V [107]. Vojtek et al (2007) proved that acetate buffer was not a suitable solution for determination of flavanoid compounds but instead that the best solution was phosphate buffer of pH 7 which gave a better signal , hence this solution was used for all my experiments [170].All flavonoids were prepared in 0.1 M phosphate buffer saline solution (PBS) at varying concentrations.

Linear regression curves were constructed to analyze current responses on increase of concentration for both standards and flavanoids. A scan rate of 100 mV/s was chosen as opposed to 50 mV/s and 20 mV/s due to the peak being more visible than with the other two scan rates. The faster the scan rate, the lower the amount of time that allows formation of a film on the electrode surface and the higher the current signal [148].

Flavanoids can electrochemically oxidised behave as antioxidants due to their ability to scavenge free radicals by an electron transfer process which involves the hydroxyl groups attached to either the A or B ring thereby forming an oxidation peak. In this reaction the phenol is converted into a phenoxy radical [1, 111]. In a scavenging reaction hydrogen atoms are donated to the radical and this involves the breaking of an O – H bond. It is generally believed that the presence of O – H is essential, for this reason compounds **4**, **8** and **9** which do not contain of any hydroxyl groups will be excluded from discussion involving antioxidant activity [5, 88]. Electrochemistry allows us to investigate the ability of flavanoids to donate electrons [119]. Cren-olive and colleagues (2002) showed that the resorcinol group is difficult to oxidise as it has high oxidation potentials as was evident in my results were compound **1**, **2**,**3** and **5** which contain the resorcinol group all had a second oxidation peak at potentials higher than 880 mV [111, 171]. In flavanoids that have an OH in ring B e.g. compound **10** and **11**, the rest of the flavonoid structure seems to be more important for the antioxidant activity. The presence of a methoxy in ring B as in compound **11** can activate the OH group hence the lower potential of compound **11** as compared to compound **10** [5]. Cren-olive et al (2002) and Slabbert (1977) proved that the ionisation of OH groups of ring system do not influence the ionisation of the other system if they are not conjugated as is the case with flavonoids involved in this

investigation and that the hydroxyl groups in Ring B are easily oxidizable as it is more basic than those in ring A. Since compound **10** and **11** are the only compounds which have hydroxyl groups in ring B. For compound **11**, we can assume that peak 1 at lower potential of 83 mV, is due to the hydroxyl on ring B and the second peak at a higher potential of 782 mV is due to the hydroxyl group on ring A [171-172].

Antioxidants can be oxidised on the surface of the electrode and the more powerful the reducing agent the lower its positive oxidation potential. Deprotonation increases the antioxidant action because during the reaction of the antioxidant the ionisation potential lowers instead of the bond dissociation energy and this leads us to the assumption that electron donation is important for antioxidant action. The oxidation potential determines the ability of a compound to act as a reducing agent and thus to function as an antioxidant and this depends on the chemical structure of the molecule [20, 25]. This is the principle I will use to arrange the remainder of my compounds in order from highest antioxidant activity to the lowest. The order would be as follows: **1** > **5** > **3** > **2** > **7** > **11** > **10** > **6**. Antioxidants may show more than one peak if further oxidations occur as in the case with square wave voltammograms of compounds **1**, **2**, **3**, **5**, **6**, **7**, **11** [25, 122, 169, 173]. Most flavanoids showed an obvious oxidation peak with square wave voltammetry between 15 mV to 122 mV for the first oxidation peak. The oxidation potential of flavonoids depended on the structure of flavonoids were flavanoids with two hydroxyl groups had the lowest oxidation potential than those with acetoxy groups. For most flavanoids there was a decrease in current of the second and third oxidation peaks and this maybe attributed to increase in concentration which results in adsorption of flavonoid to the electrode surface, blocking the surface after each scan [111]. Calibration of all **11** compounds (R^2 >

0.5117) was carried out. Of the **11** compounds, seven were successful calibrated at two oxidation potentials ($R^2 > 0.9200$). The calibration curve also gave information on the optimum oxidation potential suitable at certain concentrations.

Chapter 6

CONCLUSION

The intake of flavonoid-containing foods is beneficial to assist your body to combat the effect of free radicals in our bodies. In this study, the tested natural flavanoids are commonly found in plants, the flavanoids belonged to the sub classes of flavanones, flavones and chalcones. The data presented shows that the electrochemical characteristics of flavanoids may play an important role in their antioxidant activity and hence cyclic voltammetry and square wave voltammetry were used for the screening of the flavanoid antioxidant activity and for the estimation of the antioxidant activity of flavanoid containing plants. Column chromatography (CC), thin layer chromatography (TLC) and preparative layer chromatography (PLC) were successfully applied to isolate the 6 natural flavanoids namely, 1-(2,4-dihydroxyphenyl)-3-phenyl propan-1-one (**1**); 2,3-dihydro-5,7-dihydroxy-2-phenyl-4H-1-benzopyran-4-one (**2**); 2,3-dihydro-5,7-dihydroxy-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (**3**); 1-(2',4'-dihydroxyphenyl)-3-phenyl-2-propen-1-one (**5**); 2,3-dihydro-5-hydroxy-2-(4'-hydroxy-3'-methoxyphenyl)-7-methoxy-4H-1-benzopyran-4-one (**10**); 2,3-dihydro-5-hydroxy-2-(4'-hydroxyphenyl)-7-methoxy-4H-1-benzopyran-4-one (**11**) which are all known compounds. The structures of the above flavanoids were determined using proton and carbon nuclear magnetic resonance spectroscopy. The separation of compounds 2,3-dihydro-5,7-dihydroxy-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (**3**) and 1-(2',4'-dihydroxyphenyl)-3-phenyl-2-propen-1-one (**5**) gave a little difficulty during separation. A special mixture of EtOAc: hexane: CH_2Cl_2 in the ratio of 70: 30: 45 was discovered, it was this mixture of compounds that allowed successful separation of the two compounds after multiple re-runs using preparative layer chromatography (PLC). Of the five

synthesized compounds, 1-(2',4'-diacetoxyphenyl)-3-phenyl-2-propen-1-one (**4**); 2,3-dihydro-7-hydroxy-2-phenyl-4H-1-Benzopyran-4-one (**7**) and 5,7-(diacetoxy)-2,3-dihydro-2-phenyl-4H-1-benzopyran-4-one (**8**) were known but not extensively studied. Compounds, 5-acetoxy-2,3-dihydro-7-hydroxy-2-(2'-methoxyphenyl)-4H-1-Benzopyran-4-one (**6**) and 5,7-(diacetoxy)-2,3-dihydro-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (**9**) are novel compounds which have not been studied in literature. From all the eleven compounds studied only compounds **2**, **7** and **10** have been studied electrochemically.

6.1.Determination of antioxidant activity

Many methods have been developed to study the antioxidant activity of flavanoids. In general the antioxidant activity of flavanoids is related to its ability to donate electrons to free radicals and this is shown by its redox potentials. The mid point potentials ($E_{½}$) of the flavanoids were determined by cyclic and square wave voltammetry. When using cyclic voltammetry, some flavanoids were irreversibly oxidized, adsorption together with kinetic reaction lead to a change in cyclic voltammograms. In the case of square wave voltammetry due to the fast analysis time, a better current response was observed and therefore less interference was expected on the electrode process as compared to cyclic voltammetry. Hence the data from the square wave voltammograms was used to plot the linear regression graphs to relate current to concentration. CV and SWV have advantages because they require small amounts of sample, are easy to operate and have short analysis time as compared to other methods. These advantages made this the best method to screen flavanoids in plant extracts and to estimate the antioxidant activity of natural compounds. For flavonoids studied there was a range of oxidation potentials from the easiest to

oxidize at – 85 mV for compound **9** to the most difficult to oxidize at 1112 mV for compound **7**. It was evident that there are differences in oxidation potential for flavonoids having different structures. Flavonoids with oxidation potentials between 200 mV and 100 mV belong are considered moderate antioxidants and it is to this group that my compounds belong. An understanding of redox potentials of functional groups will allow us to improve the properties of new synthesized compounds.

6.2.Relationship between antioxidant activity and structure

Many researchers have tried to establish the relationship between flavonoid structure and antioxidant activity, i.e. the structure – antioxidant relationship of quercitin has been established and it showed that the ortho – dihydroxy groups of ring B were the most important for the antioxidant activity of this compound.

In this study, the important determinants of antioxidants flavanoids are the presence and number of hydroxyl groups as seen from the arrangement of compounds according to oxidation potential from the one with the lowest oxidation potential which would be the strongest antioxidant, i.e. compound **1** namely, 1-(2',4'-dihydroxyphenyl)-3-phenyl propan-1-one (**1**), which has two hydroxyl groups, a carbonyl and an open chain joining the A and B ring. While the compound with the highest oxidation potential which would be the weakest antioxidant , i.e. compound **6**, namely, 5-acetoxy-2,3-dihydro-7-hydroxy-2-(2'-methoxyphenyl)-4H-1-Benzopyran-4-one (**6**), has only one hydroxyl group, a 4 – oxo group and a closed C – ring. The open chain allows ease of delocalization of electrons across the molecule and this contributes to the stability of the products formed hence the irreversibility as seen for the second oxidation peak compound **1**. The opposite occurs with compound **7**, the 7 – hydroxy flavone which has the highest oxidation potential than compound **1** making

it a weaker antioxidant than compound **1**. Due to the closed C – ring in compound **7** electron delocalization does not occur easily due to instability of the products formed, hence the reversibility of the second oxidation peak of compound **7**. Flavanoids with a close ring that do not contain of ortho – dihydroxy groups in the B – ring or a 3 – hydroxyl group will have lower antioxidant activity than chalcones with an open chain joining the rings A and B and this will influence the ability of the compound to donate an electron. Compounds **1**, **5**, **3** and **2** have the strongest antioxidant activity because of the presence of two hydroxyl groups in those compounds as oppose to compound **7**, **6**, **10** and **11** which have a lower antioxidant activity than the first group as they only have one hydroxyl group. Compounds **4**, **8** and **9** are assumed to have little or no antioxidant activity due of absence of hydroxyl groups which can behave as electron donors although compound **9**, namely 5,7-(diacetoxy)-2,3-dihydro-2-(2'-methoxyphenyl)-4H-1-benzopyran-4-one (**9**), has the lowest oxidation potential of -85 mV, it is the low positive potentials that are important for the analysis of antioxidant activity of flavonoids.

REFERENCES

1. Yang, B., Kotani, A., Arai,K., and Kusu, F., *Estimation of antioxidant activities of flavonoids from their oxidation potentials.* Analytical sciences, 2001. **17**.(5): p. 599-604.

2. Pietta, P., Simonetti P., Gardana C., and Mauri P., *Trolox equivalent antioxidant capacity (TEAC) of Ginkgo biloba flavonol and Camellia sinensis catechin metabolites.* Journal of Pharmaceutical and Biomedical Analysis, 2000. **23**(1): p. 223-226.

3. Salah, N., Miller, N.J., Paganga, G., Tijburg, L., Bolwell, G.P. and Rice-Evans, C., *Polyphenolic Flavanols as Scavengers of Aqueous Phase Radicals and as Chain-Breaking Antioxidants.* Archives of Biochemistry and Biophysics, 1995. **322**(2): p. 339-346.

4. Ng, T.B., Liu, F. and Wang, Z.T., *Antioxidative activity of natural products from plants.* Life Sciences, 2000. **66**(8): p. 709-723.

5. Van Acker, S.A.B.E., van den Berg, D.J., Tromp, M.N., Griffioen, D.H., van Bennekom, W.P., van der Vijgh, W.J., and Bast, A., *Structural aspects of antioxidant activity of flavonoids.* Free Radical Biology and Medicine, 1996. **20**(3): p. 331-342.

6. http://www.friedli.com/herbs/phytochem/flavonoids.html.

7. Peng, Z.F., Strack, D., Baumert, A., Subramaniam, R., Goh, N.K., Chia, T.F., Tan, S.N., and Chia, L.S., Phytochemistry, 2003. **62**(2):219-28., *Antioxidant flavonoids from leaves of Polygonum hydropiper L*. Phytochemistry. 2003, **62**(2): p. 219-928.

8. Yamamura, S.; Ozawa, K.; Ohtani, K.; Kasai, R.; and Yamasaki, K., *Antihistaminic flavones and aliphatic glycosides from Mentha spicata.* Phytochemistry. 1998. **48**(1): p. 131-136.

9. Du, J., He, Z.D., Jiang, R.W., Ye, W.C., Xu, H.X., and But, P.P., *Antiviral flavonoids from the root bark of Morus alba L.* Phytochemistry, 2003. **62**(8): p. 1235-1238.

10. Karakaya, S. and Nehir El, S., *Quercetin, luteolin, apigenin and kaempferol contents of some foods.* Food Chemistry, 1999. **66**(3): p. 289-292.

11. Lim, C.F., Loidl, N.M., Kennedy, J.A., Topliss, D.J., and Stokigt, J.R., *Effect of loop diuretics and nonsteroidal antiinflammatory drugs on thyrotropin release by rat anterior pituitary cells in vitro.* Metabolism, 1995. **44**(8): p. 1008-1012.

12. http://ezinearticles.com/?Nutrition---Learn-Why-Flavonoid-Rich-Foods-Should-Be-Part-of-Your-Daily-Food-Menu&id=3728509.

13. http://en.wikipedia.org/wiki/Radical_(chemistry).

14. http://www.google.co.za/imgres?imgurl=http://shop.goji.co.za/UserFiles/Image/freeradicals.jpg&imgrefurl=http://shop.goji.co.za/custom.aspx%3Fid%3D2&usg.

15. Heim, K.E., Tagliaferro, A.R.and Bobilya, D.J., *Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships.* The Journal of Nutritional Biochemistry, 2002. **13**(10): p. 572-584.

16. Rice-Evans, C.A., N.J. Miller, and Paganga, G., *Structure-antioxidant activity relationships of flavonoids and phenolic acids.* Free Radical Biology and Medicine, 1996. **20**(7): p. 933-956.

17. Van Acker, S.A.B.E., van den Berg, D.J., Tromp, M.N., Griffioen, D.H., van Bennekom, W.P., van der Vijgh, W.J., and Bast, A., *Structural aspects of antioxidant activity of flavonoids.* Free Radical Biology and Medicine, 1996. **20**(3): p. 331-342.

18. http://www.google.co.za/imgres?imgurl=http://www.knowabouthealth.com/wp-content/uploads/2009/08/antioxidant_diag.gif&imgrefurl=http://www.knowab.

19. Harborne, J.B. and Williams, C.A., *Advances in flavonoid research since 1992.* Phytochemistry, 2000. **55**(6): p. 481-504.

20. Janeiro, P., and Brett, A.M.O., *Catechin electrochemical oxidation mechanisms.* Analytica Chimica Acta, 2004. **518**(2): p. 109-115.

21. Long, L.H., Evans, P.J., and Halliwell, B., *Hydrogen Peroxide in Human Urine: Implications for Antioxidant Defense and Redox Regulation.* Biochemical and Biophysical Research Communications, 1999. **262**(3): p. 605-609.

22. Pereira, T.A., and Das, N.P., *The effects of flavonoids on the thermal autoxidation of palm oil and other vegetable oils determined by differential scanning calorimetry.* Thermochimica Acta, 1990. **165**(1): p. 129-137.

23. Spencer, J.P.E., Schroeter, H., Crossthwaithe, A.J., Kuhnle, G., Williams, R.J., and Rice-Evans, C., *Contrasting influences of glucuronidation and O-methylation of epicatechin on hydrogen peroxide-induced cell death in neurons and fibroblasts.* Free Radical Biology and Medicine, 2001. **31**(9): p. 1139-1146.

24. Havsteen, B., *Flavonoids, a class of natural products of high pharmacological potency.* Biochemical Pharmacology, 1983. **32**(7): p. 1141-1148.

25. Kilmartin, P.A., and Wright, G.A., *Photoeffects to characterise polypyrrole electrodes and bilayers with polyaniline.* Electrochimica Acta, 2001. **46**(18): p. 2787-2794.

26. http://en.wikipedia.org/wiki/Herbalism.

27. Gurib-Fakim, A., *Medicinal plants: Traditions of yesterday and drugs of tomorrow.* Molecular Aspects of Medicine, 2006. **27**(1): p. 1-93.

28. Phillipson, J.D., *Phytochemistry and medicinal plants.* Phytochemistry, 2001. **56**(3): p. 237-243.

29. Brandão, M.G.L., *Antimalarial activity of extracts and fractions from Bidens pilosa and other Bidens species (Asteraceae) correlated with the presence of acetylene and flavonoid compounds.* Journal of Ethnopharmacology, 1997. **57**(2): p. 131-138.

30. Bracke, M.E., Castronovo, V., Van Cauwenberge, R.M., Coopman, P., Vakaet, L. Jr., Strojny, P., Foidart, J.M., and Mareel, M.M., *The anti-invasive flavonoid (+)-catechin binds to laminin and abrogates the effect of laminin on cell morphology and adhesion.* Experimental Cell Research, 1987. **173**(1): p. 193-205.

31. http://bank-ic.de/en/zellen-e.html.

32. McGaw, L.J., Lall, N., Meyer, J.J., and Eloff, J.N., *The potential of South African plants against Mycobacterium infections.* Journal of Ethnopharmacology, 2008. **119**(3): p. 482-500.

33. Anderson, P.M.L., Hoffmann M.T., and O'Farrell, P.J., *Above ground perennial plant biomass across an altitudinal and land-use gradient in Namaqualand, South Africa.* South African Journal of Botany, 2010. **76**(3): p. 471-481.

34. Rutherford, M.C., and Powrie, L.W., *Severely degraded rangeland: Implications for plant diversity from a case study in Succulent Karoo, South Africa.* Journal of Arid Environments, 2010. **74**(6): p. 692-701.

35. Desmet, P.G. and Cowling, R.M., *Patch creation by fossorial rodents: a key process in the revegetation of phytotoxic arid soils.* Journal of Arid Environments, 1999. **43**(1): p. 35-45.

36. Francis, M.L., Fey, M.V., Prinsloo, H.P., Ellis, F., Mills, A.J., and Medinski**,** T,V., *Soils of Namaqualand: Compensations for aridity.* Journal of Arid Environments, 2007. **70**(4): p. 588-603.

37. Botha, M.S., Carrick, P.J., and Allsopp, N., *Capturing lessons from land-users to aid the development of ecological restoration guidelines for lowland Namaqualand.* Biological Conservation, 2008. **141**(4): p. 885-895.

38. http://www.google.co.za/imgres?imgurl=http://www.whalewatching.co.za/images/namaqualandmap.gif.

39. http://plants.jstor.org/flora/floc004655.

40. http://www.google.co.za/imgres?imgurl=http://www.up.ac.za/dspace/bitstream/2263/8535/2/toxp_206.JPG

41. Vries, F.A, Green, I.R., Klaasen, J.A., Mabusela, W.T., Bodo, B., and Johnson, Q., *An antifungal active extract from the aerial parts of Galenia africana.* 11th NAPRECA Symposium Book of Proceedings, Antananarivo, Madagascar, 2001: p. 123-131.

42. http://www.plantzafrica.com/plantefg/elytrorhino.htm.

43. Levyns, M.R., *A revision of Elytropappus Cass.* ournal of South African Botany, 1935. **1**: p. 89-103.

44. http://www.google.co.za/imgres?imgurl=http://sophy.u-3mrs.fr/Afriqsud/PhotocpAFS/E/Elytropappus_rhinocerotis___03_09_2008_2.JPG

45. Rodriguez, R.J., Miranda, C.L., Stevens, J.F., Deinzer, M.L., Buhler, D.R., *Influence of prenylated and non-prenylated flavonoids on liver microsomal lipid peroxidation and oxidative injury in rat hepatocytes.* Food and Chemical Toxicology, 2001. **39**(5): p. 437-445.

46. Dziedzic, S.Z., and Hudson, B.J.F., *Hydroxy isoflavones as antioxidants for edible oils.* Food Chemistry, 1983. **11**(3): p. 161-166.

47. Barron, D., and Ibrahim, R.K, *Isophenylated flavonoids. A survey.* Phytochemistry, 1996. **43**(5): p. 921-982.

48. Edenharder, R., and Tang, X., *Inhibition of the mutagenicity of 2-nitrofluorene, 3-nitrofluoranthene and 1-nitropyrene by flavonoids, coumarins, quinones and other phenolic compounds.* Food and Chemical Toxicology. **35**(3-4): p. 357-372.

49. De la Rocha, N., María, A.O., Gianello, J.C., and Pelzer, L., *Cytoprotective effects of chalcones from Zuccagnia punctata and melatonin on the gastroduodenal tract in rats.* Pharmacological Research, 2003. **48**(1): p. 97-99.

50. Uchiumi, F., *Transcriptional suppression of the HIV promoter by natural compounds.* Antiviral Research, 2003. **58**(1): p. 89-98.

51. Wu, J.H., Wang, X.H., Yi, Y.H., and Lee, K.H., *Anti-AIDS agents 54. A potent anti-HIV chalcone and flavonoids from genus Desmos.* Bioorganic & Medicinal Chemistry Letters, 2003. **13**(10): p. 1813-1815.

52. Nerya, O., Musa, R., Khatib, S., Tamir, S., and Vaya, J., *Chalcones as potent tyrosinase inhibitors: the effect of hydroxyl positions and numbers.* Phytochemistry, 2004. **65**(10): p. 1389-1395.

53. Canesi, L., Canesi, L., Lorusso, L.C., Ciacci, C., Betti, M., Zampini, M., and Gallo, G., *Environmental estrogens can affect the function of mussel hemocytes through rapid modulation of kinase pathways.* General and Comparative Endocrinology, 2004. **138**(1): p. 58-69.

54. Zampini, I.C., Vattuone, M.A., and Isla, M.I., *Antibacterial activity of Zuccagnia punctata Cav. ethanolic extracts.* Journal of Ethnopharmacology, 2005. **102**(3): p. 450-456.

55. Nowakowska, Z., *A review of anti-infective and anti-inflammatory chalcones.* European Journal of Medicinal Chemistry, 2007. **42**(2): p. 125-137.

56. Dobashi, Y., Hirano, T., Hirano, M., and Ohkatsu**,** Y., *Antioxidant and photo-antioxidant abilities of catechins.* Journal of Photochemistry and Photobiology A: Chemistry, 2008. **197**(2-3): p. 141-148.

57. Zampini, I.C., Villarini, M., Moretti, M., Dominici, L., and Isla, M.I., *Evaluation of genotoxic and antigenotoxic effects of hydroalcoholic extracts of Zuccagnia punctata Cav.* Journal of Ethnopharmacology, 2008. **115**(2): p. 330-335.

58. Zhang, Y., and Huang, KK., *On the interactions of hydrated metal cations (Mg^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}) with guanine-cytosine Watson-Crick and guanine-guanine reverse-Hoogsteen DNA base pairs.* Journal of Molecular Structure: Theochem, 2007. **812**(1-3): p. 51-62.

59. Zhang, Y., Chen, J., Ma, X.M., and Shi, Y.P., *Simultaneous determination of flavonoids in Ixeridium gracile by micellar electrokinetic chromatography.* Journal of Pharmaceutical and Biomedical Analysis, 2007. **45**: p. 742-746.

60. Kumar Gupta, D., Krishnamurti, M., and Parthasarathi, L., *Purpurin, a new flavanone from Tephrosia purpurea seeds.* Phytochemistry, 1980. **19**(6): p. 1264-1264.

61. Rao, E.V., Murthy, M.S.R., and Ward, R.S., *Nine isoflavones from Tephrosia maxima.* Phytochemistry, 1984. **23**(7): p. 1493-1501.

62. Havsteen, B.H., *The biochemistry and medical significance of the flavonoids.* Pharmacology & Therapeutics, 2002. **96**: p. 67-202.

63. Cody, V., *Computer graphic modelling in rug design: conformational analysis of dihydrofolate reductase inhibitors.* Journal of Molecular Graphics, 1986. **4**(1): p. 69-73.

64. Saitô, N., Ôsawa,Y., and Hayashi, K., *Platyconin, a new acylated anthocyanin in Chinese bell-flower, Platycodon grandiflorum.* Phytochemistry, 1971. **10**(2): p. 445-447.

65. Shiono, M., Matsugaki, N., and Takeda, K., *Structure of commelinin, a blue complex pigmentfrom the blue flowers of Commelina communis.* Proc. Jpn. Acad Phys Biol Scie. 2008, **84**(10): p. 452-456.

66. Yoshida, K., Kondo, T., and Goto, T., *Intramolecular stacking conformation of gentiodelphin, a diacylated anthocyanin from Gentiana makinoi.* Tetrahedron, 1992. **48**(21): p. 4313-4326.

67. Mori, M., Kondo, T., and Yoshida, K., *Cyanosalvianin, a supramolecular blue metalloanthocyanin, from petals of Salvia uliginosa.* Phytochemistry, 2008. **69**(18): p. 3151-3158.

68. Takeda, K., Harborne, J.B., and Waterman, P.G., *Malonylated flavonoids and blue flower colour in lupin.* Phytochemistry, 1993. **34**(2): p. 421-423.

69. Basu-Modak, S., Gordon, M.J., Dobson, L.H., Spencer, J.P., Rice-Evans, C., and Tyrrell, R.M., *Epicatechin and its methylated metabolite attenuate UVA-induced oxidative damage to human skin fibroblasts.* Free Radical Biology and Medicine, 2003. **35**(8): p. 910-921.

70. http://en.wikipedia.org/wiki/Ultraviolet.

71. Hao, X., Hale, B.A., Ormrod, D.P., and Papadopoulos, A.P., *Effects of pre-exposure to ultraviolet-B radiation on responses of tomato (Lycopersicon esculentum cv. New Yorker) to ozone in ambient and elevated carbon dioxide.* Environmental Pollution, 2000. **110**(2): p. 217-224.

72. Cartwright, H.N., Baucom, C., Singh, P., Smith, K.L., and Stapleton, A.E., *Intraspecific comparisons reveal differences in the pattern of ultraviolet radiation responses in four maize (Zea mays L.) varieties.* Journal of Photochemistry and Photobiology B: Biology, 2001. **62**(1-2): p. 88-96.

73. Walenzyk, T., Carola, C., Buchholz, H., and König, B., *Chromone derivatives which bind to human hair.* Tetrahedron, 2005. **61**(31): p. 7366-7377.

74. Cuadra, P., Harborne, J.B., and Waterman, P.G., *Increases in surface flavonols and photosynthetic pigments in Gnaphalium luteo-album in response to UV-B radiation.* Phytochemistry, 1997. **45**(7): p. 1377-1383.

75. Gitz Iii, D.C., Liu, L., and McClure, J.W., *Phenolic metabolism, growth, and uv-b tolerance in phenylalanine ammonia-lyase-inhibited red cabbage seedlings.* Phytochemistry, 1998. **49**(2): p. 377-386.

76. Valko, M., Rhodes, C.J.,Moncol, J.,Izakovic, M., and Mazur, M., *Free radicals, metals and antioxidants in oxidative stress-induced cancer.* Chemico-Biological Interactions, 2006. **160**(1): p. 1-40.

77. Kuppusamy, U.R., Khoo, H.E., and Das, N.P., *Structure-activity studies of flavonoids as inhibitors of hyaluronidase.* Biochemical Pharmacology, 1990. **40**(2): p. 397-401.

78. Frankel, E.N., Waterhouse, A.L., and Kinsella, J.E., *Inhibition of human LDL oxidation by resveratrol.* The Lancet, 1993. **341**(8852): p. 1103-1104.

79. Sri Kantha, S., Matsumura, H., Kubo, E., Kawase, K., Takahata, R., Serhan, C.N., and Hayaishi, O., *Effects of prostaglandin D2, lipoxins and leukotrienes on sleep and brain temperature of rats.* Prostaglandins, Leukotrienes and Essential Fatty Acids, 1994. **51**(2): p. 87-93.

80. Samuelsson, B., Borgeat, P., Hammarstrom, S., and Murphy, R.C., *Introduction of a nomenclature: Leukotrienes.* Prostaglandins, 1979. **17**(6): p. 785-787.

81. Barnes, P.J., *Anti-leukotrienes: the promise for asthma?: Novel Inhibitors of Leukotrienes [Series: Progress in Inflammation Research (edited by Michael J. Parnham)] edited by G. Folco, B. Samuelsson and R.C. Murphy.* Trends in Pharmacological Sciences, 2000. **21**(5): p. 194-194.

82. German, J.B., Lokesh, B., and Kinsella, J.E., *The effect of dietary fish oils on eicosanoid biosynthesis in peritoneal macrophages is influenced by both dietary n-6 polyunsaturated fats and total dietary fat.* Prostaglandins, Leukotrienes and Essential Fatty Acids, 1988. **34**(1): p. 37-45.

83. Higgs, G.A. and Salmon, G.A., *Cyclo-oxygenase products in carrageenin-induced inflammation.* Prostaglandins, 1979. **17**(5): p. 737-746.

84. Higgs, E.A., Moncada, S., and Vane, J.R., *Prostaglandins and thromboxanes from fatty acids.* Progress in Lipid Research, 1986. **25**: p. 5-11.

85. Hamel, R., and Ford-Hutchinson, A.W., *Leukotriene A4-induced bronchoconstriction in the guinea pig.* Prostaglandins, 1985. **30**(6): p. 935-947.

86. http://themedicalbiochemistrypage.org/eicosanoids.html.

87. Harborne, J.B., *Journal of Chemical Ecology : ed. by R. M. Silverstein and J. B. Simeone, Vol. I, No. 1, January 1975.* Phytochemistry, 1976. **15**(1): p. 245-245.

88. Bors, W., Heller, W., Michel, C., and Saran, M., *Flavonoids as antioxidants: Determination of radical-scavenging efficiencies*, in *Methods in Enzymology*, A.N.G. Lester Packer, Editor. 1990, Academic Press. p. 343-355.

89. Alcaraz, L.E., Blanco, S.E., Puig, O.N., Tomas, F., and Ferretti, F.H., *Antibacterial Activity of Flavonoids Against Methicillin-resistant Staphylococcus aureus strains.* Journal of Theoretical Biology, 2000. **205**(2): p. 231-240.

90. Ono, K., Nakane, H., Fukushima, M., Chermann, J.C., and Barre-Sinoussi, F., *Differential inhibition of the activities of reverse transcriptase and various cellular DNA polymerases by a traditional Kampo drug, Sho-saiko-to.* Biomedecine & Pharmacotherapy, 1990. **44**(1): p. 13-16.

91. Hendrich, A.B., *Flavonoid-membrane interactions: possible consequences for biological effects of some polyphenolic compounds.* Acta Pharmacologica Sinica, 2006. **27**(1): p. 27-40.

92. Movileanu, L., Neagoe, I., and Flonta, M.L., *Interaction of the antioxidant flavonoid quercetin with planar lipid bilayers.* International Journal of Pharmaceutics, 2000. **205**(1-2): p. 135-146.

93. Kobiler, I., Prusky, D., Midland, S., Sims, J.J., and Keen, N.T., *Compartmentation of antifungal compounds in oil cells of avocado fruit mesocarp and its effect on susceptibility to Colletotrichum gloeosporioides.* Physiological and Molecular Plant Pathology, 1993. **43**(5): p. 319-328.

94. Laks, P.E., and Pruner, M.S., *Flavonoid biocides: Structure/activity relations of flavonoid phytoalexin analogues.* Phytochemistry, 1989. **28**(1): p. 87-91.

95. Ares, J.J., Outt, P.E., Randall, J.L., Johnston, J.N., Murray, P.D., O'Brien, L.M., Weisshaar, P.S., and Ems, B.L., *Synthesis and biological evaluation of flavonoids and related compounds as gastroprotective agents.* Bioorganic & Medicinal Chemistry Letters, 1996. **6**(8): p. 995-998.

96. Loa, J., Chow, P., and Zhang, K., *Studies of structure–activity relationship on plant polyphenol-induced suppression of human liver cancer cells.* Cancer Chemother Pharmacol, 2009. **63**.(6): p. 1007-1016.

97. Hodnick, W.F., Duval, D.L., and Pardini, R.S., *Inhibition of mitochondrial respiration and cyanide-stimulated generation of reactive oxygen species by selected flavonoids.* Biochemical Pharmacology, 1994. **47**(3): p. 573-580.

98. De Rijke, E., Out, P., Niessen, W.M.A., Ariese, F.,Goiijer, C., and Brinkman, U.A.T., *Analytical separation and detection methods for flavonoids.* Journal of Chromatography A, 2006. **1112**(1-2): p. 31-63.

99. Hendrickson, H.P., Sahafayen, M., Bell, M.A., Kaufman, A.D., Hadwiger, M.E., and Lunte, C.E., *Relationship of flavonoid oxidation potential and effect on rat hepatic microsomal metabolism of benzene and phenol.* Journal of Pharmaceutical and Biomedical Analysis, 1994. **12**(3): p. 335-341.

100. Pyrzynska, K., and Biesaga, M., *Analysis of phenolic acids and flavonoids in honey.* TrAC Trends in Analytical Chemistry, 2009. **28**(7): p. 893-902.

101. Sousa, F., Guebitz, G.M., and Kokol, V., *Antimicrobial and antioxidant properties of chitosan enzymatically functionalized with flavonoids.* Process Biochemistry, 2009. **44**(7): p. 749-756.

102. Makhotkina, O. and Kilmartin, P.A., *Uncovering the influence of antioxidants on polyphenol oxidation in wines using an electrochemical method: Cyclic voltammetry.* Journal of Electroanalytical Chemistry, 2009. **633**(1): p. 165-174.

103. Martinez, S., Valek, L., Petrovic, Z., Metikos-Hukovic, M., and Piljac, J., *Catechin antioxidant action at various pH studied by cyclic voltammetry and PM3 semi-empirical calculations.* Journal of Electroanalytical Chemistry, 2005. **584**(2): p. 92-99.

104. Piljac-Zegarac, J., Valek, L., Martinez, S., and Belscak, A., *Fluctuations in the phenolic content and antioxidant capacity of dark fruit juices in refrigerated storage.* Food Chemistry, 2009. **113**(2): p. 394-400.

105. Piljac-Zegarac, J., Valek, L., Stipcevic, T., and Martinez, S., *Electrochemical determination of antioxidant capacity of fruit tea infusions.* Food Chemistry, 2010. **121**(3): p. 820-825.

106. Oliveira-Brett, A.M., Diculescu, V., and Piedade, J.A.P., *Electrochemical oxidation mechanism of guanine and adenine using a glassy carbon microelectrode.* Bioelectrochemistry, 2002. **55**(1-2): p. 61-62.

107. Brett, C.M.A., Oliveira Brett, A.M., and Serrano, S.H.P., *On the adsorption and electrochemical oxidation of DNA at glassy carbon electrodes.* Journal of Electroanalytical Chemistry, 1994. **366**(1-2): p. 225-231.

108. Diculescu, V.C., Kumbhat, S., and Oliveira-Brett, A.M., *Electrochemical behaviour of isatin at a glassy carbon electrode.* Analytica Chimica Acta, 2006. **575**(2): p. 190-197.

109. Diculescu, V.C., Piedade, J.A.P., and Oliveira-Brett, A.M., *Electrochemical behaviour of 2,8-dihydroxyadenine at a glassy carbon electrode.* Bioelectrochemistry, 2007. **70**(1): p. 141-146.

110. Ghalkhani, M., Fernandes, I.P., Oliveira, S.C., Shahrokhian, S., and Oliveira-Brett, A.M., *Anodic behaviour of clioquinol at a glassy carbon electrode.* Bioelectrochemistry. In Press, Accepted Manuscript.2010.

111. Janeiro, P. and Oliveira Brett, A.M., *Catechin electrochemical oxidation mechanisms.* Analytica Chimica Acta, 2004. **518**(1-2): p. 109-115.

112. Manuela Garrido, E.,Costa-Lima, J.L., Delerue-Matos, M.C., and Oliveira-Brett, A.M., *Electrochemical oxidation of bentazon at a glassy carbon electrode: Application to the determination of a commercial herbicide.* Talanta, 1998. **46**(5): p. 1131-1135.

113. Oliveira Brett, A.M., Macedo, T.R.A., Raimundo, D., Marques, M.H., and Serrano, S.H.P., *Electrochemical oxidation of mitoxantrone at a glassy carbon electrode.* Analytica Chimica Acta, 1999. **385**(1-3): p. 401-408.

114. Oliveira Brett, A.M. and Matysik, F.M., *Voltammetric and sonovoltammetric studies on the oxidation of thymine and cytosine at a glassy carbon electrode.* Journal of Electroanalytical Chemistry, 1997. **429**(1-2): p. 95-99.

115. Rebelo, I., Piedade, J.A.P., and Oliveira Brett, A.M., *Electrochemical determination of 8-oxoguanine in the presence of uric acid.* Bioelectrochemistry, 2004. **63**(1-2): p. 267-270.

116. Airado-Rodríguez, D., Galeano-Diaz, T., and Durán-Merás, I., *Determination of trans-resveratrol in red wine by adsorptive stripping square-wave voltammetry with medium exchange.* Food Chemistry, 2010. **122** (4): p. 1320-1326.

117. Hodek, P., Hanustiak, P., Krizkova, J., Mikelova, R., Krizkova, S., Stiborova, M., Trnkova, L., Horna, L., Beklova, M., and Kizek, R., *Toxicological aspects of flavonoid interaction with biomacromolecules.* Neuroendocrinol Lett, 2006. **27**: p. 14-17.

118. Korbut, O., Buckova, P., Tarapcik, P., Labuda, J., and Grundler, P., *Damage to DNA indicated by an electrically heated DNA-modified carbon paste electrode.* Journal of Electroanalytical Chemistry, 2001. **506**(2): p. 143-148.

119. Maoela, M.S.,Arotiba, O.A., Baker, P.G.L., Mabusela, W.T., Jahed, N., Iwuoha, E.I., and Songa, E.A., *Electroanalytical Determination of Catechin Flavonoid in EthylAcetate Extracts of Medicinal Plants.* Int. J. Electrochem. Sci., 2009. **4**: p. 1497-1510.

120. Vaya, J., Mahmood, S., Goldblum, A., Aviram, A., Volkova, N., Shaalan, A., Musa, R., and Tamir, S., *Inhibition of LDL oxidation by flavonoids in relation to their structure and calculated enthalpy.* Phytochemistry, 2003. **62**(1): p. 89-99.

121. Hotta, H.,Sakamoto, H., Nagano, S., Osakai, T., and Tsujino, Y., *Unusually large numbers of electrons for the oxidation of polyphenolic antioxidants.* Biochimica et Biophysica Acta (BBA) - General Subjects, 2001. **1526**(2): p. 159-167.

122. Chevion, S., Roberts, M.A., and Chevion, M.,*The use of cyclic voltammetry for the evaluation of antioxidant capacity.* Free Radical Biology and Medicine, 2000. **28**(6): p. 860-870.

123. Tanrisever, N., Fronczek, F.R., Fischer, N.H., and Williamson, G.B., *Ceratiolin and other flavonoids from Ceratiola ericoides.* Phytochemistry, 1986. **26**(1): p. 175-179.

124. Adityachaudhury, N., *Aurentiacin, a new chalcone from Didymocarpus aurentiaca.* Phytochemistry, 1976. **15**(1): p. 229-230.

125. Gregoris, E., and Stevanato, R., *Correlations between polyphenolic composition and antioxidant activity of Venetian propolis.* Food and Chemical Toxicology, 2010. **48**(1): p. 76-82.

126. Falcão S.I., Vilas-Boas, M., Barros, C., Domingues, R.M., and Cardoso, S.M., *Phenolic characterization of Northeast Portuguese propolis: usual and unusual compounds.* Anal Bioanal Chem, 2010. **396** (2): p. 887-897.

127. Narender, T., Papi Reddy, R., and Kumar, B., *BF3·OEt2 mediated regioselective deacetylation of polyacetoxyacetophenones and its application*

in the synthesis of natural products. Tetrahedron Letters, 2008. **49**(28): p. 4409-4415.

128. Rotelli, A.E., Guardia, T., Juarez, A.O., De la rocha, N.E., and Pelzer, L.E., *Comparative study of flavonoids in experimental models of inflammation.* Pharmacological Research, 2003. **48**(6): p. 601-606.

129. Barrero, A.F., Sánchez, J.F., and Rodríguez, I., *N-[Delta]13-Docosenoylanthranilic acid and alkylresorcinols from Ononis natrix subsp. Hispanica.* Phytochemistry, 1990. **29**(6): p. 1967-1969.

130. Bao-Ning Sua, E.J.P., Vigob, J.S., Grahama, J.G., Cabiesesb, F., Fonga, H.H.S., Pezzutoa, J.M., and Kinghorna, A.D., *Activity-guided isolation of the chemical constituents of Muntingia calabura using a quinone reductase induction assay.* Phytochemistry, 2003. **63**.(3): p. 335-341.

131. Roy, R. and Pandey, V.B., *A chalcone glycoside from Clerodendron phlomidis.* Phytochemistry, 1994. **37**(6): p. 1775-1776.

132. Lopes, N.P., Kato, M.J., and Yoshida, M., *Antifungal constituents from roots of Virolasurinamensis.* Phytochemistry, 1999. **51**(1): p. 29-33.

133. Braga de Oliveira, A., Fonseca e Silva, L.G., and Gottlieb, O.R., *Flavonoids and coumarins from Platymiscium praecox.* Phytochemistry, 1972. **11**(12): p. 3515-3519.

134. Yang, S. F., Yang, W.E., Kuo, W.H., Chang, H.R., Chu, S.C., and Hsieh, Y.S., *Antimetastatic potentials of flavones on oral cancer cell via an inhibition of matrix-degrading proteases.* Archives of Oral Biology, 2008. **53**(3): p. 287-294.

135. Si-Ahmed, K., Tazerouti, F., Badjah-Hadj-Ahmed, A.J., Aturki, Z., D'Orazio, G., Rocco, A., and Fanali, S., *Optical isomer separation of flavanones and flavanone glycosides by nano-liquid chromatography using a phenyl-carbamate-propyl-[beta]-cyclodextrin chiral stationary phase.* Journal of Chromatography A, 2010. **1217**(7): p. 1175-1182.

136. Del Rayo Camacho-Corona, M., De .Jesus Favela-Hernandez, J.M., González-Santiago, O., Garza-González, E., Said-Fernández, S., Delgado, G., and Luna-Herrerae, J., *Evaluation of Some Plant-derived Secondary Metabolites Against Sensitive and Multidrug-resistant Mycobacterium tuberculosis.* J. Mex. Chem. Soc., 2009. **53**(2): p. 71-75.

137. Chen, J., Liu, Y., and Shi, Y.P., *Determination of flavonoids in the flowers of Paulownia tomentosa by high-performance liquid chromatography.* Journal of Analytical Chemistry, 2009. **64**(3): p. 282-288.

138. Garo, E., Maillard, M., Antus, S., Mavi, S., and Hostettmann, K., *Five flavans from Mariscus psilostachys.* Phytochemistry, 1996. **43**(6): p. 1265-1269.

139. Hare, J.D., *Seasonal variation in the leaf resin components of Mimulus aurantiacus.* Biochemical Systematics and Ecology, 2002. **30**(8): p. 709-720.

140. Delaportea, D.H., Sarragiotto, M.H., Takemuraa, O.S., S´anchezc, G.M., and Filhod, B.P.,*Evaluation of the antioedematogenic, free radical scavenging and antimicrobial activities of aerial parts of Tillandsia streptocarpa Baker – Bromeliaceae.* Journal of Ethnopharmacology, 2004. **95**: p. 229-233.

141. Jinyoung, K., Chaewoon L., and Chong, Y., *Synthesis of a Complete Series of O-Methyl Analogues of Naringenin and Apigenin.* Bull. Korean Chem. Soc., 2007. **28**(12).

142. Oyama, K., and Kondo, T., *Total Synthesis of Flavocommelin, a Component of the Blue Supramolecular Pigment from Commelina communis, on the Basis of Direct 6-C-Glycosylation of Flavan.* The Journal of Organic Chemistry, 2004. **69**(16): p. 5240-5246.

143. Malakhova, N.A., Miroshnikova, E.G., Stojko, N,Y., and Brainina, K.Z., *Long-lived sensors with replaceable surface for stripping voltammetric analysis: part II.* Analytica Chimica Acta, 2004. **516**(1-2): p. 49-60.

144. De Andrade, D.F., Fernandes, D.R., and Miranda, J.L., *Methods for the determination of conjugated dienes in petroleum products: A review.* Fuel, 2010. **89**(8): p. 1796-1805.

145. http://en.wikipedia.org/wiki/Potentiostat.

146. Rezai, S. *http://www.chem.ucla.edu/~bacher/CHEM174/equipment/CV1.html*. 2003.

147. http://www.als-japan.com/1037.html.

148. http://en.wikipedia.org/wiki/Glassy_carbon.

149. Bard, A.J.F., Larry R, *Electrochemical Methods: Fundamentals and Applications. 2nd edition.* 2000, Wiley.

150.http://www.google.co.za/imgres?imgurl=http://chemwiki.ucdavis.edu/%40api/deki/files/6146/%3Drefelect.gif.

151. Lower, S.K., *Electrochemistry*. Chemical reactions at an electrode, galvanic and electrolytic cells.

152. http://en.wikipedia.org/wiki/Cyclic_voltammetry#cite_note-0.

153. http://www.abc.chemistry.bsu.by/vi//.

154. Lundquist, J.J.T. and Nicholson, R.S., *Theory of the potential step-linear scan electrolysis method with a comparison of rate constants determined*

electrochemically and by classical methods. Journal of Electroanalytical Chemistry, 1968. **16**(4): p. 445-456.

155. Heinze, J., Störzbach, M., and Mortensen, J., *Digital simulation of cyclic voltammetric curves by the implicit Crank-Nicolson technique.* Journal of Electroanalytical Chemistry, 1984. **165**(1-2): p. 61-70.

156. Jacobsen, N.E., *NMR spectroscopy explained: simplified theory, aplications and examples for organic and structural biology*. 2007: Wiley -interscience.

157. http://www.offresonance.com/?p=9.

158. Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T.,Hartzfeld, P.W., and Riechel, T.L., *High Molecular Weight Plant Polyphenolics (Tannins) as Biological Antioxidants.* Journal of Agricultural and Food Chemistry, 1998. **46**(5): p. 1887-1892.

159. Vries, F.A., Green, I.R., Klaasen, J.A., Mabusela , W.T., Bodo B., and Johnson, Q., *An antifungal active extract from the aerial parts of Galenia africana*, in *11th NAPRECA Symposium Book of Proceedings, Antananarivo, Madagascar*. p. 123-131.

160. Ichino, K., Tanaka, H., and Ito, K., *Two novel flavonoids from the leaves of var. and.* Tetrahedron, 1988. **44**(11): p. 3251-3260.

161. Areche, C., San-Martin, A., Rovirosa, J., Soto-Delgado, J., and Contreras, R., *An unusual halogenated meroditerpenoid from Stypopodium flabelliforme: Studies by NMR spectroscopic and computational methods.* Phytochemistry, 2009. **70**(10): p. 1315-1320.

162. Yaw-Lung, H., Jim-Min, F., and Yu-Shia, C., *Terpenoids and flavonoids from Pseudotsuga wilsoniana.* Phytochemistry, 1998. **47**(5): p. 845-850.

163. Zhang, P., Feng, Z., and Wang, Y., *Flavonoids, including an unusual flavonoid-Mg2+ salt, from roots of Cudrania cochinchinensis.* Phytochemistry, 2005. **66**(23): p. 2759-2765.

164. Vestergaard, M.d., Kerman, K., and Tamiya, E., *An electrochemical approach for detecting copper-chelating properties of flavonoids using disposable pencil graphite electrodes: Possible implications in copper-mediated illnesses.* Analytica Chimica Acta, 2005. **538**(1-2): p. 273-281.

165. Hotta, H., Nagano, S., Ueda, M., Tsujino, Y., Koyama, J., and Osakai, T., *Higher radical scavenging activities of polyphenolic antioxidants can be ascribed to chemical reactions following their oxidation.* Biochimica et Biophysica Acta (BBA) - General Subjects, 2002. **1572**(1): p. 123-132.

166. Cao, G., Sofic, E., and Prior, R.L., *Antioxidant and Prooxidant Behavior of Flavonoids: Structure-Activity Relationships.* Free Radical Biology and Medicine, 1997. **22**(5): p. 749-760.

167. Firuzi, O., Lacanna, A., Petrucci, R., and Saso, L., *Evaluation of the antioxidant activity of flavonoids by "ferric reducing antioxidant power" assay and cyclic voltammetry.* Biochimica et Biophysica Acta (BBA) - General Subjects, 2005. **1721**(1-3): p. 174-184.

168. Corduneanu, O., Chiorcea-Paquim, A.M., Garnett, M., and Oliveira-Brett, A.M., *Lipoic acid-palladium complex interaction with DNA, voltammetric and AFM characterization.* Talanta, 2009. **77**(5): p. 1843-1853.

169. Filipiak, M., *Electrochemical analysis of polyphenolic compounds.* Anal. Sci, 2001. **17**: p. 1667–1670.

170. Sun-Waterhouse, D., Smith, B.G., O'Connor, C.J., and Melton, L.D., *Effect of raw and cooked onion dietary fibre on the antioxidant activity of ascorbic acid and quercetin.* Food Chemistry, 2008. **111**(3): p. 580-585.

171. Adam, V., Mikelova, R., Hubalek, J., Hanustiak, P., Beklova, M., Hodek, P., Horna, A., and Trnkova, L., *Utilizing of Square Wave Voltammetry to Detect Flavonoids in the Presence of Human Urine.* Sensors, 2007. **7**(10): p. 2402-2418.

172. Cren-Olivé, C., Wieruszeski, J.M., Maes, E., and Rolando, C., *Catechin and epicatechin deprotonation followed by ^{13}C NMR.* Tetrahedron Letters, 2002. **43**(25): p. 4545-4549.

173. Slabbert, N.P., *Ionisation of some flavanols and dihydroflavonols.* Tetrahedron, 1977. **33**(7): p. 821-824.

174. Blasco, A.J., González, M.C., and Escarpa, A., *Electrochemical approach for discriminating and measuring predominant flavonoids and phenolic acids using differential pulse voltammetry: towards an electrochemical index of natural antioxidants.* Analytica Chimica Acta, 2004. **511**(1): p. 71-81.