PHYTOCHEMICAL INVESTIGATION OF FOUR KENYAN PLANTS FROM FAMILIES VERBENACEAE AND ASTERACEAE FOR THEIR ANTIPLASMODIAL, ANTILEISHMANIAL AND ANTIOXIDANT ACTIVITIES

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Sponsored by

DAAD-NAPRECA

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY AT THE UNIVERSITY
OF NAIROBI

2010
DECLARATION

This is the original work of the author, except where reference is made, carried out at University of Nairobi between November 2007 and June 2010. It has never been submitted anywhere for award of any degree or diploma.

Francis Machumi, 180/80008/2007

Approved by Supervisors

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Prof. Abiy Yenesew
ACKNOWLEDGEMENTS

I would like to sincerely thank my supervisors, Prof. Jacob O. Midiwo and Prof. Abiy Yenesew who have instructed, guided, supported and encouraged me throughout my studies. I also express my appreciation to the academic staff, technical staff and support staff of the Department of Chemistry University of Nairobi for their assistance in various aspects of my study.

Dr Matthias Heydenreich and Prof Erich Kleinpeter of Universit"at Potsdam hosted me in their laboratory where I did most of the spectroscopic work. I would like to sincerely thank them and their whole research group for all the hospitality and help they accorded to me. I should also sincerely thank Dr Ilia Muhammad of University of Mississippi, and his group, for helping with the antiplasmodial, antimicrobial and antileishmanial tests as well as some of the spectroscopic measurements.

I am also thanking Mr. Patrick Mutiso of Department of Botany for his assistance in collection and identification of the plants. I am also grateful to my lab mates, graduate students in the Department and all research students I met in the course of my study for the friendship they accorded to me, their help, advice and collaboration.

Finally, I sincerely thank DAAD-NAPRECA for supporting me and sponsoring my study.
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malaria complications such as cerebral and pulmonary oedema, anemia and poor eyesight [Taoufiq et al., 2006].

The emergence of resistance has made many of the first line drugs such as chloroquine not effective. The need for new drugs, preferably with new mode of action is therefore strongly felt. In this regard, several plants are being screened for anti-plasmodial activity.

In collaboration programme between University of Nairobi and University of Mississippi, 140 plants were screened out of which four plants namely Clerodendrum eriophyllum (Verbenaceae), Sphaeranthus bullatus (Asteraceae), Microglossa pyrifolia (Asteraceae) and Vernonia galamensis (Asteraceae) showed activities shown in Table 1:1. These activities were interpreted as good to moderate activities, based on WHO guidance on activity, which stipulates that extracts with IC50 of less than 10 µg/ml have good activity, whereas those with IC50 of 11-50 µg/ml have moderate activity.

Table 1.1: Anti-plasmodial activities of the selected plant extracts towards D6 (Chloroquin sensitive) and W2 (Chloroquin resistant) strains of Plasmodium falciparum

<table>
<thead>
<tr>
<th>Plant</th>
<th>In vitro activity (IC50, µg/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>P. falciparum D6</td>
</tr>
<tr>
<td>Microglossa pyrifolia (whole plant)</td>
<td>8.0</td>
</tr>
<tr>
<td>Sphaeranthus bullatus (whole plant)</td>
<td>9.7</td>
</tr>
<tr>
<td>Vernonia galamensis (aerial parts)</td>
<td>9.0</td>
</tr>
<tr>
<td>Clerodendrum eriopyllum (aerial parts)</td>
<td>8.8</td>
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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACT</td>
<td>Artemisinin based Combination Therapies</td>
</tr>
<tr>
<td>CC</td>
<td>Column Chromatography</td>
</tr>
<tr>
<td>CD</td>
<td>Circular Dichroism</td>
</tr>
<tr>
<td>COSY</td>
<td>Correlation Spectroscopy</td>
</tr>
<tr>
<td>DBE</td>
<td>Double Bond Equivalence</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DDT</td>
<td>Dichlorodiphenyltrichloroethane</td>
</tr>
<tr>
<td>DEPT</td>
<td>Distortionless Enhancement by Polarization Transfer</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>DPPH</td>
<td>2,2-Diphenyl-1-picrylhydrazyl</td>
</tr>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Effective Dose-50: Amount of material required to produce a specified effect on 50% of test animal</td>
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<tr>
<td>EI MS</td>
<td>Electron Impact Mass Spectrometry</td>
</tr>
<tr>
<td>FAB MS</td>
<td>Fast Atom Bombardment Mass Spectrometry</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
</tr>
<tr>
<td>GDP</td>
<td>Gross Domestic Product</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HMBC</td>
<td>Heteronuclear Multiple Bond Correlation</td>
</tr>
<tr>
<td>HMQC</td>
<td>Heteronuclear Multiple Quantum Correlation</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HR-EIMS</td>
<td>High Resolution Electron Impact Mass Spectrometry</td>
</tr>
<tr>
<td>HSQC</td>
<td>Heteronuclear Single Quantum Correlation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>Inhibition Concentration-50: Concentration of substance that produce 50% inhibition of certain process</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>LC$_{50}$</td>
<td>Lethal Concentration-50: Concentration that kills 50% of test animal</td>
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<tr>
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<td>Minimum Inhibition Concentration</td>
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<td>Mass Spectrometry</td>
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<td>Nuclear Overhauser effect</td>
</tr>
<tr>
<td>NOESY</td>
<td>Nuclear Overhauser effect Spectroscopy</td>
</tr>
<tr>
<td>pLDH</td>
<td>Plasmodium lactate dehydrogenase</td>
</tr>
<tr>
<td>PTLC</td>
<td>Preparative Thin Layer Chromatography</td>
</tr>
<tr>
<td>RBM</td>
<td>Roll Back Malaria</td>
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<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
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<tr>
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<td>Structure Activity Relationship</td>
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<td>Thin Layer Chromatography</td>
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<td>UNICEF</td>
<td>United Nation Children Fund</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra Violet</td>
</tr>
<tr>
<td>UV-VIS</td>
<td>Ultra Violet-Visible</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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In an effort made to address the problem of malaria and its associated complications, four Kenyan plants used to treat malaria were investigated for compounds with anti-plasmodial and anti-oxidant activities. The plants are *Clerodendrum eriophyllum* (Verbenaceae), *Sphaeranthus bullatus* (Asteraceae), *Microglossa pyrifolia* (Asteraceae) and *Vernonia galamensis* (Asteraceae). A total of forty-two compounds were isolated from the plants, two being new, namely 12-hydroxy-8,12-abietadiene-3,11,14-trione (220) from *Clerodendrum eriophyllum* and 8-acetoxyisochiliolide lactone (248) from *Microglossa pyrifolia*. The antifungal, antibacterial and antileishmanial activity of compounds were also investigated.

Separation of compounds was carried out using chromatographic methods and their identification done by spectroscopic methods. The *in vitro* anti-plasmodial tests were done by a colorimetric assay that determined the parasitic lactate dehydrogenase (pLDH) activity in 96-well microplate. Antioxidants were analysed by UV-VIS spectrometry method employing the stable radical compound DPPH.
From the roots of *Clerodendrum eriophyllum*, fifteen compounds were isolated, being twelve abietane diterpenoids, two triterpenoids and a long chain ester of tyrosol. Three abietane diterpenoids had anti-plasmodial activities, these are taxodione (222), 6-hydroxysalvinolone (226) and 6,16-dihydroxysalvinolone (227), with IC$_{50}$ of 1.2 $\mu$g/ml, 1.8 $\mu$g/ml and 3 $\mu$g/ml respectively against chloroquine sensitive D6 strain. 1.2 $\mu$g/ml, 2.5 $\mu$g/ml and 4.8 $\mu$g/ml respectively against chloroquine sensitive W2 strains of *P. falciparum*. Anti-oxidant activities were observed on four abietane diterpenoids, taxodione (222), 6-hydroxysalvinolone (226), 6,16-dihydroxysalvinolone (227) and Nellionol (228) with IC$_{50}$ of 31.5±0.8, 17.7±0.4, 20.5±0.5 and 10.2±0.5 respectively. Antifungal activities were observed on taxodione (222), 6-hydroxysalvinolone (226) and 6,16-dihydroxysalvinolone (227), the best activity observed with taxodione (222) on *Cryptococcus neoformans* (IC$_{50}$ 0.58 $\mu$g/ml).

The aerial parts of *Sphaeranthus bullatus* gave seventeen compounds being five carvotacetone monoterpenes, three $\beta$-isopropyltoluene derivatives, five flavonoids, two phenyl propanoids and two triterpenes. Four carvotacetone derivatives had anti-plasmodial activities. These were 3,5,7-trihydroxycarvotacetone (85), 3-acetoxy-5,7-dihydroxycarvotacetone (106), 3,7-dihydroxy-5-tigloyloxyacarvotacetone (88) and 3-acetoxy-7-hydroxy-5-tigloyloxyacarvotacetone (99), with IC$_{50}$ of 3.4 $\mu$g/ml, 0.6 $\mu$g/ml, 0.8 $\mu$g/ml and 1.4 $\mu$g/ml respectively against chloroquine sensitive D6 strain as well as 2.8 $\mu$g/ml, 0.7 $\mu$g/ml, 0.9 $\mu$g/ml and 2.0 $\mu$g/ml respectively against chloroquine sensitive W2 strains of *P. falciparum*. This is a first encounter of antiplasmodial activity on carvotacetone derivatives. The two phenyl propanoids, caffeic acid (240) and
coniferaldehyde (241) showed anti-oxidant activities, with IC$_{50}$ of 2.6±0.3 and 14.1±0.6 respectively.

The aerial parts of Microglossa pyrifolia afforded six compounds. These were two rearranged clerodane diterpenoids, two steroids, one triterpenoid and one eudesman sesquiterpene. The compounds from this plant showed no activities.

The aerial parts of Vernonia galamensis gave seven compounds; five flavanols, one lignan and one benzenoid. The compounds showed no anti-plasmodial activity, but showed anti-oxidant activities, with IC$_{50}$ of 6.0±0.2 for quercetin (235), 6.6±0.3 for quercetin 3-methylether (244), 10.4±0.5 for crysoplenol D (238), 9.3±0.4 for quercetin 3-$O$-$\beta$-galactopyranoside (212), 12.9 ± 0.4 for quercetin 3-$O$-$\beta$-rhamnopyranoside (245), 8.3 ± 0.4 for Syringic acid (247) and 21.0 ± 0.5 µg/ml for syringaresinol (246).

Anti-microbial activities were observed with the abietane diterpenoids taxodione (222), ferruginol (224), 6-hydroxysalvinolone (226) and 6,16-dihydroxysalvinolone (227). Best antifungal activity was shown by taxodione (222) and 6-hydroxysalvinolone (226) (IC$_{50}$ 0.58 and 0.96 µg/ml respectively against C. neoformans) where as best antibacterial activity was shown by ferruginol (224) on methicillin-resistant Staphylococcus aureus (MRS) (IC$_{50}$ 0.96 µg/ml). The best antileishmanial activity was shown by taxodione (222) (IC$_{50}$ 0.08 µg/ml against L. donovani).
CHAPTER ONE
INTRODUCTION

1.1 General introduction

Plants are known to perform photosynthesis whereby they absorb carbon dioxide, water and light energy to produce monosaccharides, which in turn undergo further chemical metabolism to give an enormous variety of metabolites. The metabolites vary from primary metabolites which are essential for plant growth and development, to secondary metabolites, also referred to as Natural Products, which fulfill ecological functions, aiding interaction between plants and their biotic and abiotic environments. Secondary metabolites/natural products therefore serve as defense compounds against pathogens and herbivores, as flower pigments that attract pollinators, and as hormone or signal molecules [Osbourn and Lanzotti, 2009].

In addition to their function in plants, the natural products have great impact on human and animal life. Mankind has throughout the ages used natural products for flavors, fragrances, dyes and most important, for medicine. The use of natural products for treatment of parasitic diseases is well known and documented since ancient times, and stems from the fact that some of these natural products are biosynthesized as defense agents against plant pathogens [Kaur et al., 2009]. Initially these medicines took a form of crude extracts contained in herbal formulations like teas, tinctures, poultices and powders [Balunas and Kinghorn, 2005]. With development of separation chemistry and pharmacological testing, the medicines now are made of active compounds isolated from the plants, or their synthetic equivalents.
Currently, with progress in synthetic and computational chemistry, some medicinal drugs are designed through molecular modeling process [Meyer et al., 2000]. However, plants still constitute an important source of pharmaceuticals. Without them there would be a significant therapeutic deficit in several important clinical areas, such as, neurodegenerative disease, cardiovascular disease, most solid tumours and immune-inflammatory diseases such as rheumatoid arthritis [Nisbet and Mooret, 1997]. Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world, including the treatment of malaria [Gurib-Fakim, 2006].

Malaria chemotherapy is one of the medicinal fields that are known to use pharmaceuticals originating from natural products research, examples are quinine from Cinchona succiriba and artemisinin from Artemisia annua. Many more compounds with significant anti-plasmodial activities have been isolated from plants and efforts are being made to develop some of these into future drugs [Kaur et al, 2009]. In underdeveloped communities, crude natural products made in the form of herbal formulations are still used for malaria therapy. Several plants with traditional use in curing malaria by different communities have been documented [Muthaura et al., 2007a; Gessler et al., 1995; Kvist et al., 2006].

The use of natural product-derived drugs and drugs from other sources in combating malaria has however been faced with several challenges, including the emergence of drug resistance parasites to drugs world-wide as well as complications arising from oxidative stress during malaria infections. In fact, oxidative stress has been linked to several
malaria complications such as cerebral and pulmonary oedema, anemia and poor eyesight [Taoufiq et al., 2006].

The emergence of resistance has made many of the first line drugs such as chloroquine not effective. The need for new drugs, preferably with new mode of action is therefore strongly felt. In this regard, several plants are being screened for anti-plasmodial activity.

In collaboration programme between University of Nairobi and University of Mississippi, 140 plants were screened out of which four plants namely Clerodendrum eriophyllum (Verbenaceae), Sphaeranthus bullatus (Asteraceae), Microglossa pyrifolia (Asteraceae) and Vernonia galamensis (Asteraceae) showed activities shown in Table 1:1. These activities were interpreted as good to moderate activities, based on WHO guidance on activity, which stipulates that extracts with IC$_{50}$ of less than 10 μg/ml have good activity, where as those with IC$_{50}$ of 11-50 μg/ml have moderate activity.

**Table 1.1:** Anti-plasmodial activities of the selected plant extracts towards D6 (Chloroquin sensitive) and W2 (Chloroquin resistant) strains of *Plasmodium falciparum*

<table>
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<th>Plant</th>
<th>In vitro activity (IC$_{50}$, μg/ml)</th>
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<td></td>
<td><em>P. falciparum</em> D6</td>
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<tr>
<td>Microglossa pyrifolia (whole plant)</td>
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<tr>
<td>Sphaeranthus bullatus (whole plant)</td>
<td>9.7</td>
</tr>
<tr>
<td>Vernonia galamensis (aerial parts)</td>
<td>9.0</td>
</tr>
<tr>
<td>Clerodendrum eriopyllum (aerial parts)</td>
<td>8.8</td>
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</table>
The supremacy of medicinal plants is encountered in a wide spectrum of diseases. It is therefore becoming a common practice to test the extracts and compounds from medicinal plants against several pathogens. In this regard, this work has also investigated antileishmanial, antibacterial and antifungal properties.

1.2 Statement of the problem

Malaria is a major contributor to the global burden of disease and a significant impediment to socioeconomic development in poor countries. It is estimated that 300 to 660 million clinical attacks of malaria occur globally each year (Geissbühler et al., 2007) and result in over a million deaths yearly (Hetzel et al., 2007), over 80% of these deaths occurring in Africa (Geissbühler et al., 2007). Most of fatalities are linked to oxidative stress, caused by elevated production of radicals in host cells [Taoufiq et al., 2006]. Efforts to combat the disease are hampered by growing resistance of malaria parasites to the readily available drugs. There is, therefore, a need for continued efforts in the search of antioxidants and compounds that are active against malaria parasites, that can be developed to new and more effective drugs.

1.3 Justification of the research

Research on medicinal plants represents a major strategy for the discovery of compounds that can be developed to new anti-malarial drugs. The most important antimalarial compounds that have been isolated from traditional medicinal plants are quinine (1) from Cinchona succiriba (Rubiaceae), and artemisinin (4) from the Artemisia annua...
(Asteraceae). Both compounds have or are being used as molecular templates in the syntheses of more potent antimalarial drugs.

The plants investigated under this study [*Clerodendrum eriophyllum* (Verbenaceae), *Sphaeranthus bullatus* (Asteraceae), *Microglossa pyrifolia* (Asteraceae) and *Vernonia galamensis* (Asteraceae)] are used traditionally to treat malaria in Kenya [Beentje, 1994; Kokwaro, 1976] and their extracts have shown anti-plasmodial activities [Midiwo *et al.*, unpublished results]. This makes them good candidates for investigation for compounds with anti-malarial compounds.

The previous phytochemical work on these plants leave a room for further research since not all parts were studied for compounds present. For *Clerodendrum eriophyllum*, no phytochemical work has been done at all. Moreover, compounds isolated from these plants have never been tested for antimalarial and anti-oxidant activities. It is therefore important that the plants should be investigated further for metabolites and tested for anti-plasmodial and anti-oxidant activities.

1.4 **Objectives**

1.4.1 **General objective**

To identify anti-plasmodial and anti-oxidant lead structures from plants used in traditional medicine to cure malaria.
1.4.2 Specific objectives

1. To isolate metabolites from four Kenyan plants used traditionally to treat malaria: namely *Clerodendrum eriopyllum*, *Sphaeranthus bullatus*, *Microglossa pyrifolia* and *Vernonia galamensis* by use of chromatographic methods.

2. To elucidate the structures of the isolated metabolites using spectroscopic methods.

3. To establish the *in vitro* anti-plasmodial activities of pure compounds and extracts.

4. To establish the radical scavenging activities of pure compounds.

5. To establish antimicrobial and antileishmanial activities of the compounds.

6. To predict structure/activity relationships for anti-plasmodial and anti-oxidant activities of some compounds isolated.
CHAPTER TWO

LITERATURE REVIEW

2.1 Background on malaria

Malaria is a global disease that is predominant in the tropics and caused by blood parasites *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium vivax* [Odugbemi et al., 2007]. The parasites are transmitted to humans by female *Anopheles* mosquito. Clinical malaria is manifested by a range of symptoms such as fever, vomiting, joint pain and convulsions [Nkuo-Akenji and Menang, 2005]. Besides contributing to over a million deaths yearly, malaria is known to be a cause of anaemia and its various complications, miscarriages, brain damage, decreased cognition and irreversible disabilities [Rugemalila et al., 2006].

In terms of economy, malaria is known to be both a disease and a cause of poverty. It is estimated that malaria is responsible for a GDP economic growth penalty of up to 1.3% per year in some African countries [Sachs and Malaney, 2002]. When compounded over the years, this penalty leads to substantial differences, totaling US$ 12 billion annually in GDP between countries without malaria and countries with malaria, and severely restrains the economic growth of entire Africa region [Kamau, 2006].

Efforts to overcome the problem are hindered by two obstacles. One is the growing resistance of malaria parasite *Plasmodium falciparum* to chloroquine and other commonly used cheap synthetic drugs, as well as the growing resistance of the vector *Anopheles* to DDT and other insecticides [Bilia, 2006]. The second obstacle is limited
availability, coupled with higher cost and greater toxicity of alternative drugs [Saidu et al., 2000].

In recognition of the gravity of the malaria problem, WHO and UNICEF launched the Roll Back Malaria (RBM) partnership in 1998, with the aim of reducing malaria burden by at least 50% by the year 2010 through application of evidence based interventions and strengthening health delivery services [Kamau, 2006]. This was supported by the Abuja declaration in 2000 where African heads of state agreed on the concerted effort to reduce malaria burden on the continent and endorsed the goals of RBM partnership of halving the number of malaria deaths by the year 2010 [Hetzel et al., 2007]. Currently, concerted efforts promote three core malaria control interventions: use of insecticide treated nets, intermittent preventive treatment during pregnancy and infancy, and effective treatment of clinical cases with artemisinin based combination therapies (ACT) [Kamau, 2006].

In Kenya, like other tropical countries, malaria is endemic and resistance of malaria parasites to readily available and cheap drugs like chloroquine is growing fast. According to the Ministry of Health, twenty four million Kenyans are at risk of malaria infections and majority cannot afford the costs of the adopted artemisinin based combination therapy. The government therefore found it necessary to provide free ACT therapy in health facilities alongside expansion of healthcare provision [Muthaura et al., 2007b; World Report, 2007]. Despite this effort however, many regions remain underserved and subsequently the communities use herbal remedies and other cheaply available alternatives [Muthaura et al., 2007b; Kirira et al., 2006].
Plants and natural products have had a great contribution to the fight against malaria since time immemorial. In Kenya and other developing countries, the use of herbal remedies in treatment of malaria is well known as evidenced by overwhelming literature on plants used traditionally to cure malaria [Kokwaro, 1976; Muregi et al., 2003; Kirira et al., 2006; Koch et al., 2005; Muthaura et al., 2007a; Muthaura et al., 2007b]. Apart from their use in traditional medicine, plants and natural products have also contributed to modern treatment of malaria, being the source of some commonly used anti-malarial drugs like quinine (1) and artemisinin (2) from Cinchona and Artemisia respectively and their synthetic modifications and analogues (Figure 2.1).

![Figure 2.1: Some of the Natural Products and synthetic modifications used against malaria.](image-url)
Malaria and oxidative stress

Malaria is normally accompanied by oxidative stress caused by elevated production of reactive oxygen species (ROS). Such oxidative stress will lead to tissue damages and are associated with several pathological phenomenon such as cerebral and pulmonary oedema, poor eyesight, atherosclerosis, cardiac ischermies, rheumatic diseases and cancer [Bahorun et al., 1996]. The major cause of elevated ROS production is heme, a byproduct of hemoglobin digestion degradation by Plasmodium, whereby the iron II in heme is oxidized to iron III releasing an electron that is used to ionize oxygen molecule and subsequently form peroxide which is easily broken to radicals (Figure 2.2).

![Figure 2.2: Generation of ROS from heme](image)

ROS are also produced by host immune system for the purpose of suffocating the parasite, as the parasite is highly susceptible to oxidative burden [Kawazu et al, 2008]. Malaria parasites are therefore equipped with anti-oxidant defenses that are meant to establish redox equilibrium for their survival. Such defenses are the ones targeted in malaria control strategies, some of the anti-malarials like chloroquine and artemether are known to act by increasing the production of ROS [Taoufiq et al., 2006].
The effect of ROS to malaria patient therefore, though beneficial as it suffocates the parasite, it is also detrimental since it leads to the damage of some cells and hence bringing about more complications to the patient. It is therefore important that the cells are protected from oxidative burden through the use of effective antioxidants.

The antioxidants act by either suppressing the enzymes responsible for superoxide production, or chelate metal ions that induce free radical formation, or reduce the radicals by donation of hydrogen atoms (Figure 2.3) [Pieta, 2000].

![Mechanisms of antioxidant activity through chelation of metal ions and reduction by hydrogen donation.](image)

Antioxidants are usually taken in through diet of plants and plant products. Plants therefore make a good target to hunt for antioxidants.

### 2.4 Leishmaniasis

Leishmaniasis is a parasitic disease transmitted by a bite of some species of sand flies which infect the blood with parasites of the genus *Leishmania* [Tonui 2006]. Two common forms of leishmaniasis are known; cutaneous leishmaniasis (CL) which causes
sore (ulcers) at the bite site (Figure 2.4) and visceral leishmaniasis (VL) which affects vital organs.

Figure 2.4: Cutaneous leishmaniasis ulcer on the hand of an infected adult human being.

Leishmaniasis is found in 88 countries hosting 350 million people, being widespread in tropical and sub-tropical regions of the world where it is a serious health problem (Kigondu et al., 2009). More than 90% of the global cases of visceral leishmaniasis (VL) occur in India, Bangladesh, Nepal, Brazil and Africa (particularly East and North Africa including Sudan). Globally, the number of new cases of cutaneous leishmaniasis (CL) and VL is estimated at 1.5 million and 500,000 annually, respectively. People of all ages are at risk of infection with leishmaniasis if they live or travel where the disease is endemic. In Kenya, the disease has been reported in Baringo, Kitui, Machakos, Meru, West Pokot, Elgeyo Marakwet and Turkana districts, which are known to be endemic foci for kala-azar (VL) (Kigondu et al., 2009). Currently, the recommended drugs for both VL and CL are pentavalent antimonials: sodium stibogluconate (pentosam®) and meglumine antimoniate (glucantime®). Both drugs require long courses of parenteral administration and have toxic side effects (Kigondu et al., 2009).
2.5 Ethnobotanical, ethnopharmacological and phytochemical information on selected plants

2.5.1 *Clerodendrum eriopyllum* (Verbenaceae)

*Clerodendrum eriophyllum* Gürke [Verbenaceae (Lamiaceae)], a small tree 0.5 – 2 m high, is scattered in the dry bushlands of Eastern Kenya where it is used by local communities for the treatment of malaria [Kokwaro, 1976].

![Clerodendrum eriophyllum](image)

*Figure 2.4: Clerodendrum eriophyllum*

The plant has no record of previous phytochemical analysis. However, the methanol extract of its root bark is reported to have good *in vitro* activity against *Plasmodium falciparum* D6 and W2 clones (IC$_{50}$ 9.51- 10.56 μg/mL); its methanol and aqueous
extracts exhibited significant in vivo chemosuppression (i.e., 90.1% and 61.5%, respectively) against P. berghei infected mice treated intraperitoneally at a dose of 100 mg/kg body weight [Muthaura et al., 2007c].

The genus, Clerodendrum L. (Verbenaceae) is widely distributed in tropical and subtropical regions of the world. It is comprised of small trees, shrubs and herbs making more than five hundred species and varieties [Bashwira and Hootele, 1988]. The genus has a wide spectrum of folk and indigenous medicinal uses, with more than fifty species reported in the treatment of diseases like malaria, fevers, inflammatory diseases, venereal infections, skin diseases and internal body aches [Shrivastava and Patel, 2007].

Clerodendrum species used traditionally in treatment of malaria are C. colebrookianum whose leaves are used for fever and malaria in India [Rao and Jamir, 1982], C. cyrtophyllum which is used for malaria and common colds in China [Tian et al., 1993], C. eriophyllum, C. johnstonii and C. myricoides used for malaria in Kenya [Kokwaro, 1976], C. heterophyllum used for malaria and fever in Reunion Islands [Vera et al., 1990] as well as C. indicum and C. serratum used in China [Pei, 1985].

Other species of the genus used for malaria are C. multiflorum whose leaves are used for fever and malaria in India [Sikarwar and Kaushik, 1993], C. pleiosciadium whose leaves are used in Tanzania [Gessler et al., 1994], C. splendens used for cerebral malaria in Guinea [Akendengue, 1992] and C. viscosum whose leaves and roots are used for malaria and fever in India [Bhandary et al., 1995]. The extracts of C. inerme and C. serratum
have been found to possess anti-oxidant activities [Narayanan et al., 1999; Masuda et al., 1999].

Other than their therapeutic use, some of the species of this genus such as *C. inerme*, *C. thomsonae*, *C. indicum* and *C. speciosum* are also cultivated and used as ornamental plants [Shrivastava and Patel, 2007].

Efforts have been made by various researchers to isolate and identify biologically active principles and other major chemical constituents from various species of the genus. Compounds reported include abietane diterpenoids, *ent*-clerodane diterpenoids, iridoid glucosides, phenylpropanoid glycosides, macrocyclic alkaloids, flavones, steroids and triterpenes.

Previously reported abietane diterpenoids (Figure 2.5) are bungone A (7), bungone B (8) and sugiol (9) from *C. bungei* [Fan et al., 1999a]; cyrtophyllone A (12) and cyrtophyllone B (10) from *C. cyrtophyllum* [Tian et al., 1993]; mandarone A (11), mandarone B (13), mandarone D (18), mandarone E (14), mandarone F (15), mandarone G (16) and mandarone H (17) from *C. mandarinorum* [Fan et al., 1999b]; uncinatone (19) from *C. uncinatum* [Dorsaz et al., 1985]; clerodendrone (20) from *C. indicum* [Ravindranath et al., 2003] and royleanone (21) from *C. inerme* [Singh and Prakash 1983].
Figure 2.5: Abietane diterpenoids previously reported from the genus Clerodendrum

Reported *ent*-clerodane diterpenoids (Figure 2.6) from the genus Clerodendrum are clerodiol (22), clerodin (23), clerodinin A (25), clerodinin B (26), clerodinin C (27) and clerodinin D (28) from *C. brachyanthum* [Lin et al., 1989a, 1989b]; caryoptin (24) from *C. calamitosum* [Vigneron 1978]; clerodendrin A (29), clerodendrin B (33), clerodendrin C (34), clerodendrin D (35), clerodendrin E (30), clerodendrin F (31) and clerodendrin G (32) from *C. trichotomum* [Nishida et al., 1989].
The iridoid glucosides previously reported (Figure 2.7) are harpagide (36) isolated from *C. indicum* and *C. colebrookianum* [Jacke and Rimpler, 1983]; euphroside (37) and plantarenaloside (38) from *C. incisum* [Stenzel et al., 1986]; ajugoside (39), reptoside (40), aucubin (41) and melittoside (42) from *C. thomsonae* [Jacke and Rimpler, 1983] as well as serratoside B (43) from *C. serratum* [Yang et al., 2000a].

Phenylpropanoid glycosides isolated from the genus (Figure 2.8) are acteoside (44) and leucosceptoside A (45) from *C. inerme* [Kanchanapoom et al., 2001]; brachynoside (46)
from *C. brachyanthum* [Lin and Kuo, 1992]; martynoside (47) and clerodenoside (48) from *C. japonicum* [Tian and Sun, 1995] as well as calceolarioside A (49) from *C. buchananii* [Taoubi *et al.*, 1992].

![Iridoid glucosides previously reported from the genus Clerodendrum](image1)

**Figure 2.7:** Iridoid glucosides previously reported from the genus *Clerodendrum*

![Previous phenylpropanoid glycosides from the genus Clerodendrum](image2)

**Figure 2.8:** Previous phenylpropanoid glycosides from the genus *Clerodendrum*
Flavones isolated from *Clerodendrum* species (Figure 2.9) are apigenin (50), luteolin (51), cosmosin (52), cynacoside (53), salvigenin (54) and acacetin (55) from *C. inerme* [El-Shamy *et al.*, 1996; Raha *et al.*, 1989]; eupafolin (56), hispidulin (57) and seutellarein (58) from *C. indicum* [Tian and Sun, 1999]; cirsimaritin (59) from *C. mandarinorum* [Zhu *et al.*, 1996]; pectolinarigenin (60) from *C. neriifolium* [Ganapaty and Rao, 1989] and sorbifolin (61) from *C. fragrans* [Barua *et al.*, 1989].

![Figure 2.9: Flavones previously isolated from Clerodendrum species](image)

Previously reported steroids from this genus (Figure 2.10) are clerosterol (62) and stigmasta-5,22,25-trien-3β-ol (64) from *C. bungei, C. cyrtophyllum* and *C. brachyanthum* [Tian *et al.*, 1993; Lin *et al.*, 1989a]; colebrin A (63) and colebrin B (65) from *C. colebrookianum* [Yang *et al.*, 2000b]; stigmasterol (66), cholesterol (67), campesterol (68), cholestanol (69) and 24-methylcholestanol (70) from *C. fragrans*, *C. inerme*, *C. infortunatum*, *C. scandens* and *C. serratum* [Akihisa *et al.*, 1989].
Triterpenes reported from the genus *Clerodendrum* (Figure 2.11) are glutinol (71) from *C. brachyathum* [Lin et al., 1989a]; α-amyrin (72), 3-acetoxy-α-amyrin (73), β-amyrin (76) and betulin (81) from *C. inerme* [Singh et al., 1983]; Ursolic acid (74) from *C. japonicum* [Tian and Sun, 1995]; taraxerol (75), betulinic acid (80) and clerodone (82) from *C. bungei* [Yang et al., 2002; Dong et al., 1999; He et al., 1997]; 3-acetoxyoleanolic acid (78) and 3-acetoxyoleanolic aldehyde (79) from *C. indicim* [Ravindranath et al., 2003] and Clerodolone (83) *C. cyrtophyllum* [Tian et al., 1993] and *C. colebrookianum* [Joshi et al., 1979].
2.5.2 *Sphaeranthus bullatus* (*Asteraceae*)

*Sphaeranthus bullatus* is an annual or perennial herb, 0.2–1 m tall with aromatic leaves and purple flowers, native to tropical East Africa [Beentje, 1994]. The leaves of this plant are used against malaria [Kokwaro, 1976] and the 1:1 MeOH/CH₂Cl₂ extract of its aerial parts showed antiplasmodial activity [Midiwo *et al.*, unpublished results].
Previously reported phytochemicals from this plant are carvotacetone derivatives (84 - 90 and 94), derivatives of \( p \)-cymene (91 - 93), thiophene derivatives (95 - 96), a sesquiterpene \( \alpha \)-humulene (97) and a triterpene squalene (98) [Jakupovic et al., 1990] (Figure 2.13).

The genus comprises of about forty species distributed mainly in tropical areas of Africa, Southern Asia and Australia and [Jakupovic et al., 1990].
Several species of the genus are used traditionally as arbotrifacents or anti-conceptions and include *S. africanus* [Lal and Lata, 1980], *S. cyanthuloides* [Watt and Breyer-Brandwijk, 1962], *S. gomphrenoides* [Kokwaro, 1976], *S. indicus* [Sharma *et al*., 1992] and *S. suaveolens* [Amico, 1977]. Other ethnomedical uses of *Sphaeranthus* species includes treatment of stomach pains for *S. angolensis*, *S. indicus* and *S. senegalensis* [Samuelsson *et al*., 1992; Nagaraju and Rao, 1990; Manandhar, 1993], treatment of skin

Compounds reported from this genus are carvotacetone derivatives from S. bullatus, S. confertifolius, S. suaveolens and S. cyathuloides (84 – 90 and 99 – 110, Figures 2.13 and 2.14) [Jakupovic et al., 1990; Zdero et al., 1991; Ahmed and Mahmoud, 1997; Mwangi et al., 1995]; derivatives of thymol from S. bullatus and S. confertifolius (91 – 93 and 112, Figures 2.13 and 2.14) [Jakupovic et al., 1990; Zdero et al., 1991]; eudesmane sesquiterpene derivatives from S. indicus and S. suaveolens (114 – 129, Figure 2.15) [Rojatkar and Nagasampagi, 1992; Jakupovic et al., 1990; Pujar et al., 2000; Shekhani et al., 1991]; flavanols from S. confertifolius (130 – 132, Figure 2.16) [Zdero et al., 1991] and isoflavones from S. indicus (133 - 135, Figure 2.16) [Yadava and Kumar, 1999].

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**Figure 2.14:** Monoterpenes from *Sphaeranthus* species.
Figure 2.15: Eudesmane sesquiterpenoids from *S. indicus* and *S. suaveolens*.

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Figure 2.16: Flavanols and isoflavones from *S. conferifolius* and *S. indicus*.

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2.5.3 *Microglossa pyrifolia* (Asteraceae)

*Microglossa pyrifolia* is a climbing shrub indigenous in Africa and tropical Asia [Schmidt *et al*., 2003]. It is traditionally used against fever and malaria in Kenya and Ghana [Kokwaro, 1976; Kohler *et al*., 2002]. It is also used as an abortifacient, as an analgesic to relieve headaches and stomach pains as well as for treatment of colds, wounds and eyes [Akah and Ekekwe, 1995; Johns *et al*., 1990; Schmidt *et al*., 2003 Kuiate *et al*., 1999]. Its extract showed antiplasmodial activity [Kohler *et al*., 2002; Midiwo *et al*., unpublished results].

![Microglossa pyrifolia](image)

**Figure 2.17:** *Microglossa pyrifolia*
The plant is also called *Coryza pyrifolia* or *Microglossa volubilis* and it belongs to the genus *Microglossa*, with about ten species [Kuiate et al., 1999]. *M. pyrifolia* is the only species reported to have medicinal uses and biological activities. Phytochemical information however is available on *M. pyrifolia*, *M. angolensis*, *M. mespilifolia*, *M. pyrrhopappa* and *M. zeylanica*. Clerodane and seco-clerodane diterpenoids have been reported from *M. pyrifolia* (136 – 145, Figure 2.18) [Kohler et al., 2002; Zdero et al., 1990a], *M. pyrrhopappa* and *M. angolensis* (146 – 172, Figure 2.19) [Zdero et al., 1990b; Tene et al., 2005].

**Figure 2.18:** Clerodane and seco-clerodane diterpenoids from *Microglossa pyrifolia*
Figure 2.19: Clerodane and seco-clerodane diterpenoids from *M. pyrrhopappa* and *M. angolensis*
Another class of compounds isolated from the genus *Microglossa* is phytane diterpenoids. These have been isolated from *M. pyrifolia* (173–178, Figure 2.20) [Kohler et al., 2002; Zdero et al., 1990a], *M. pyrhopappa* (177–179, Figure 2.20) [Zdero et al., 1990b] and *M. zeylanica* (180–181, Figure 2.20) [Gunatilaka et al., 1987]. Out of these, phytol (173) and 6E-geranylgeraniol-19-oic acid (175) exhibited antiplasmodial activities of 8.5 and 12.9 µM respectively against chloroquine sensitive strains of *Plasmodium falciparum* (PoW), 11.5 and 15.6 µM respectively against chloroquine resistant strains of *Plasmodium falciparum* (Dd2) [Kohler et al., 2002].

![Figure 2.20: Phytane diterpenoids from *M. pyrifolia*, *M. Pyrhopappa* and *M. zeylanica*](image)

Other compounds reported from *Microglossa pyrifolia* are dihydrobenzofurans (182–187, Figure 2.21), triterpenoids (195–201, Figure 2.23) [Schmidt et al., 2003], aurone flavonoids (188–189, Figure 2.21) and sesquiterpenes (190–194, Figure 2.22) [Ruecker et al., 1992; Kohler et al., 2002; Zdero et al., 1990a] as well as acetylens and acetylenic
glucosides (202–209, Figure 2.24) [Ruecker et al., 1992]. Compound 203, namely 2-β-D-glucopyranosyl-1-hydroxytrideca-3,5,7,9,11-pentayne exhibited antibacterial activities against the bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Staphylococcus aureus* [Ruecker et al., 1992].

Figure 2.21: Dihydrobenzofurans and aurone flavonoids from *M. pyrifolia*

Figure 2.22: Sesquiterpenes from *Microglossa pyrifolia*
Figure 2.23: Triterpenoids from *Microglossa pyrifolia*

![Triterpenoids](image)

Figure 2.24: Acetylenes and acetylenic glucosides from *Microglossa pyrifolia*

![Acetylenes and acetylenic glucosides](image)
Vernonia galamensis ssp nairobensis (Asteraceae)

Vernonia galamensis ssp nairobensis is a shrub growing and native to Kenya and Tanzania [Miserez et al., 1996]. It is one of the six subspecies of Vernonia galamensis, the others being V. galamensis ssp afromontana, V. galamensis ssp galamensis, V. galamensis ssp gibbosa, V. galamensis ssp lushotoensis and V. galamensis ssp mutomonesis [Baye et al., 2001].

Figure 2.25: Vernonia galamensis ssp nairobensis

The plant belongs to the genus Vernonia with more than 500 species distributed in tropical and subtropical areas of the world [Miserez et al., 1996]. The species are widely used as folklore remedies for variety of human ailments. The most documented medicinal use includes treatment of malaria (more than 20 species including V. galamensis), fever,
venereal diseases, abdominal pains, wounds, diarrhea and hepatitis. Biological activities commonly encountered on the species are anti-bacterial, anti-yeast, antifungal, anti-inflammatory, anti-oxidant and anti-malarial activities [NAPRALERT, 2009].

Members of the genus are rich in epoxyfats and epoxyploy fatty acids that constitute vernonia oil used industrially for manufacturing paints and coatings [Baye et al., 2001]. They are also known to produce secondary metabolites like sesquiterpenoidss, triterpenoids, steroids, phenylpropanoids, flavones and flavanols [Erasto et al., 2006; Sanogo et al., 1998; Perdue Jr. et al., 1993].

Compounds isolated from Vernonia galamensis ssp nairobensis (Figure 2.26) are glaucolide-type sesquiterpene lactones glaucogalamensolide isobutyrate (210) and glaucogalamensolide isovalerate (211) [Zdero et al., 1990c] as well as flavanols glycosides quercetin 3-O-β-D-galactoside (212), quercetin 3-O-β-D-rhamnosyl(1→6)-β-galactoside (213) quercetin 3-O-β-D-apiosyl(1→2)-β-D-galactoside, (214) and isorhamnetin 3-O-β-D-apio-D-furanosyl(1→2)-β-D-galactopyranoside (215) [Miserez et al., 1996].

The other subspecies of Vernonia galamensis have been reported to produce vernolic acid, the steroids stigmasterol and β-sitosterol, triterpenoids lupeol, lupeol acetate, a-amyrin, β-amyrin, taraxasterol and taraxasterol acetate [Perdue Jr. et al., 1993]. They also contain prevernocietifolide sesquiterpenoids namely 8-isobutyrateprevernocietifolide (216) (V. galamensis ssp galamensis, V. galamensis ssp gibbosa), 8-senecioateprevernocietifolide (217) (V. galamensis ssp afromontana, V. galamensis ssp galamensis, V. galamensis ssp gibbosa) as well as 14-acetyl-8-
isobutyrateprevernocistifolide (218) and 14-acetyl-8-senecioateprevernocistifolide (219) from *V. galamensis* ssp *galamensis* var *petiti* (Figure 2.27) [Perdue Jr. *et al.*, 1993].

**Figure 2.26:** Glaucolide-type sesquiterpene lactones and flavanols from *Vernonia galamensis* ssp *nairobiensis*

**Figure 2.27:** Prevernocistifolide sesquiterpenoids from *Vernonia galamensis* subspecies
CHAPTER THREE

EXPERIMENTAL METHODS

3.1 General

Column chromatography (CC) used Merck Silica gel 60 (0.063-0.200 mm) and Fluka Sephadex LH-20 as stationary phases; PTLC on Merck Silica gel 60 PF254+366, coated on glass plates (20 x 20 cm) to make 1.0 mm layers; Analytical TLC was carried out using factory prepared aluminum plates (0.25 mm) coated with Silica gel (60 F254, Merck) and spots visualized by observing under UV light at 254 or 365 nm, followed by spraying with 1% vanillin-H$_2$SO$_4$ spray reagent.

Optical rotations were measured in CHCl$_3$ or MeOH using an AUTOPOL IV® instrument at ambient temperature. IR spectra were taken as films on a Bruker Tensor 27 FTIR instrument. UV spectra were obtained in MeCN using a Hawlett-Packard 8453 spectrophotometer; 1D and 2D NMR data were acquired on Bruker Avance 600 MHz or Bruker BioSpin instrument 500 MHz or Bruker Avance 300 MHz or Varian-Mercury 200 MHz. NMR measurements were done in deuterated solvents using the residual undeuterated chemical shifts as internal standard. EI-MS spectra were recorded on GC-MS TRACE DSQII single quadrupole mass spectrometer.

3.2 Plant materials

The roots of Clerodendrum eriophyllum were collected from Machakos in November 2007, while the rest of the plants (Sphaeranthus bullatus, Microglossa pyrifolia and Vernonia galamensis) were collected from Ngong forest Nairobi, in November 2007.
Voucher specimens were deposited at the Department of Botany, University of Nairobi (No JMFM/2007/11 Clerodendrum eriophyllum, JMFM/2007/12 Microglossa pyrifolia, JMFM/2007/13 Sphaeranthus bullatus and JMFM/2007/14 Vernonia galamensis ssp nairobensis). The plants were air dried under shade and pulverized using a Willy mill.

3.3 Extraction and isolation

3.3.1 Clerodendrum eriophyllum

The shade air dried and pulverized roots of C. eriophyllum (1.8 Kg) were extracted by cold percolation at room temperature using 1:1 CH₂Cl₂/MeOH (3×4 L, 24 h each), followed by 100% methanol (1×4 L, 24 h). These were rotavaped and combined to give 65 g of brown gummy extract, of which 35 g was adsorbed on 40 g of Silica gel and subjected to CC on a silica gel column (300 g, 5×35 cm), eluted with n-hexane/CH₂Cl₂ (19:1, 3.5 L; 9:1, 1.25 L; 3:1, 2 L; 1:1, 3 L; 1:3, 1 L; 100% CH₂Cl₂, 1.5 L) followed by CH₂Cl₂/MeOH (99:1, 1.25 L; 49:1, 1 L; 19:1, 1 L).

Sixty-two fractions of eluents, collected in 250 mL aliquots, were collected and concentrated using a rotary evaporator with similar fractions combined on the basis of TLC analysis. Fraction 4 (76 mg) was rechromatographed over Silica gel (10 g, 1x12cm) eluted with n-hexane/CH₂Cl₂ (19:1) to give β-amyrinotacosoanoate (230) (21 mg). Combination of fraction 5-9 crystallized in n-hexane/CH₂Cl₂ (19:1) to give royleanone (21) (260 mg) which had 6,7-dehydroxyroyleanone (221) as a minor component. Fractions 11-19 formed crystals (9:1 n-hexane/CH₂Cl₂) which were filtered to give tyrosylotacosoanoate (231) (58 mg) and the filtrate was concentrated (640 mg) and rechromatographed over Silica gel (50 g, 2×30 cm) eluting with n-hexane/CH₂Cl₂ (19:1)
to give 11-hydroxy-7,9(11),13-abietatrien-12-one (223) (5 mg) after 0.6 L of elution, and ferruginol (224) (65 mg) after 1.4 L of elution. The latter was further purified by PTLC developed with n-hexane/CH₂Cl₂ (4:1).

Fraction 22-28 (160 mg) was purified on a Sephadex LH-20 column (20 g, 2.5 x 30 cm), eluted with CH₂Cl₂/MeOH 1:1 (0.3 L), followed by PTLC developed with n-hexane/CH₂Cl₂ 7:3 to give taxadione (222) (28 mg). Fraction 34-45 (600 mg) was subjected to Silica gel CC (50 g, 2.0 x 30 cm), eluted with n-hexane/CH₂Cl₂ (1:4) to give 12-hydroxy-8,12-abietadiene-3,11,14-trione (220) (4 mg) after 0.28 L of elution, uncinatone (19) (7 mg) after 0.35 L of elution, and sugiol (9) (10 g) after 0.8 L of elution.

Fractions 52-57 formed crystals which were filtered to give betulinic acid (229) (94 mg) and the mother-liquor was concentrated to 580 mg and rechromatographed over silica gel (50 g, 2 x 30 cm) eluted with CH₂Cl₂ to yield 6-hydroxysalvinolone (226) (7 mg), nellinol (228) (42 mg) and 6,16-dihydroxysalvinolone (227) (64 mg), both of which crystallized from CH₂Cl₂, after 0.5 L, 0.9 and 1.3 L of elution, respectively. 11-hydroxy-8,11,13-abietatriene-12-O-β-xylopyranoside (225) (15 mg) was obtained after purifying fractions 58-60 on a Sephadex LH-20 column (20 g, 2.5 x 30 cm) eluted with CH₂Cl₂/MeOH 1:1 (0.3 L).

3.3.2 Sphaeranthus bullatus

The shade dried and pulverized aerial parts of S. bullatus (2.3 Kg) were extracted by cold percolation at room temperature using 1:1 CH₂Cl₂/MeOH (3 x 5 L, 24 h each), followed by 100% methanol (1 x 4 L, 24 h). The filtrates were rotavaped and combined to give 168
g of black-brown gummy extract, of which 100 g were suspended in methanol/water (2:8) and partitioned successively with n-hexane, CH₂Cl₂ and EtOAc to give 31.5 g of n-hexane extract, 26 g of CH₂Cl₂ extract and 9.2 g of ethyl acetate extract.

The n-hexane soluble part (30 g) was adsorbed in 40 g of Silica gel and chromatographed over Silica gel (300 g, 5 x 35 cm) eluted with n-hexane/EtOAc mixtures (100:0, 2.0 L; 19:1, 2.5 L; 9:1, 2.0 L; 4:1, 1.5 L; 3:2, 2.5 L; 1:1, 2.0 L; 2:3, 1.5 L; 1:4, 1.5 L; 0:100, 1.0 L). A total of 65 fractions of 250 ml each were collected and combined on the basis of similarities observed on TLC analysis. Fraction 23-27 (164 mg) were rechromatographed over Silica gel (10 g, 1 x 12 cm) eluted with n-hexane/EtOAc 23:2 to give boehmery acetate (243) (37 mg) which was recrystallized from methanol. Fraction 29 and 30 gave crystals (n-hexane/EtOAc 4:1) which were filtered to make dammaradienyl acetate (242) (26 mg). Fractions 32-37 (420 mg) were passed on Sephadex LH-20 column (20 g, 2.5 x 30 cm; 1:1 CH₂Cl₂/MeOH) to remove chlorophylls and then chromatographed over Silica gel (20 g, 2.0 x 12 cm) eluted with n-hexane/CH₂Cl₂ (3:2, 1.2 L) to give 5-acetoxy-7-hydroxy-5-tigloyloxyacarvotacetone (99) (114 mg).

The dichloromethane soluble fraction of the original extract (25 g) was adsorbed on 30 g of Silica gel and chromatographed over Silica gel (300 g, 5 x 35 cm) eluted with n-hexane/CH₂Cl₂ mixtures (4:1, 2.5 L; 3:2, 2.5 L; 1:1, 2.25 L; 2:3, 2.0 L; 1:4, 1.50 L; 0:100, 2.0 L) followed by CH₂Cl₂/MeOH mixtures (99:1, 1.50 L; 49:1, 1.25; 19:1, 2.0 L; 9:1, 1.5 L; 4:1, 1.0 L). A total of 80 fractions of 250 ml each were collected and combined on the basis of TLC analysis.
Fraction 9-17 (1.7 g) were rechromatographed over Silica gel (50 g, 2 x 30 cm) eluted with \( n\text{-hexane/CH}_2\text{Cl}_2 \) (7:3, 2.0 L) to give 5-acetoxy-7-hydroxy-5-tigloyloxy carvotacetone (99) (326 mg). Fraction 20-22 (164 mg) was passed on Sephadex LH-20 column (20 g, 2.5 x 30 cm; 1:1 CH\(_2\text{Cl}_2\)/MeOH) to remove chlorophylls followed by purification with PTLC (three plates) developed with \( n\text{-hexane/CH}_2\text{Cl}_2 \) 1:1 to give 3,7-dihydroxy-5-tigloyloxy carvotacetone (106) (46 mg). Penduletin (237) (93 mg) crystallized from fractions 34-36 and was filtered off. The mother liquor was mixed with fractions 37-40 to give 432 g which was chromatographed over silicalgel (30 g, 2 x 20 cm) eluted with CH\(_2\text{Cl}_2\)/MeOH (19:1, 1.2 L) to give quercetin-3,7-dimethylether (236) (42 mg) and 4-hydroxylonchocarpin (239) (18 mg). The latter was purified by PTLC (two plates) developed with CH\(_2\text{Cl}_2\)/MeOH 19:1. Fraction 43-50 (535 mg) was passed on Sephadex LH-20 column (20 g, 2.5 x 30 cm) eluted with 1:1 CH\(_2\text{Cl}_2\)/MeOH. The major component was concentrated and re-crystallized in CH\(_2\text{Cl}_2\) to give chrysoplenol D (238) (177 mg). Fraction 54-55 (56 mg) was cleaned by PTLC to give 3,5,7-trihydroxy carvotetacene (85) (18 mg). Quercetin (235) (12 g) was collected as crystals from fraction 61-62 of the major column.

The ethyl acetate extract (9 g) was chromatographed over Silica gel (100 g, 2.5 x 30 cm) eluted with \( n\text{-hexane/CH}_2\text{Cl}_2 \) mixtures (1:1, 1.2 L; 1:3, 1.0 L, 100:0, 1.0 L) followed by CH\(_2\text{Cl}_2\)/MeOH mixtures (99:1, 0.80 L; 98:2, 0.60 L; 95:5, 0.80 L; 9:1, 0.60 L; 8:2, 0.50 L; 7:3, 0.40 L). Sixty-five fractions of 100 ml each were collected.

Fraction 14-17 (87 mg) was passed on Sephadex LH-20 column (20 g, 2.5 x 30 cm) eluted with 1:1 CH\(_2\text{Cl}_2\)/MeOH and the fractionated eluents concentrated and re-
crystallized in n-hexane/CH$_2$Cl$_2$ to give coniferaldehyde (241) (13 mg) and caffeic acid (240) (18 mg). Fraction 21-25 (107 mg) was rechromatographed over silicagel (50g, 2.0 x 30 cm) developed with CH$_2$Cl$_2$/MeOH 97:3 to give 5-O-β-glucopyranosylcarvotacetone (232) (8 mg) and thymol 3-O-β-glucopyranoside (233) (28 mg). Zetroside B (93) (283 mg) crystallized on fractions 29-36 of the main column and was filtered off. It contained zetaroside A (234) as a minor component.

3.3.3  

**Vernonia galamensis ssp nairobensis**

The pulverized and shade dried aerial parts (1.1 Kg) were extracted with 1:1 CH$_2$Cl$_2$/MeOH (3×3 L, 24 h each) followed by 100% methanol (1×3 L, 24 h). The filtrates were concentrated on a rotary evaporator and combined to give 101 g of black extract which was dissolved in methanol/water 1:4 and portioned successively with CH$_2$Cl$_2$ and ethyl acetate to give 23 g of CH$_2$Cl$_2$ extract and 8 g of ethyl acetate extract.

The CH$_2$Cl$_2$ soluble extract (20 g) was run on column chromatography using Silica gel (200 g, 4 x 30 cm) and eluted with n-hexane/CH$_2$Cl$_2$ mixtures (100:0, 1.6 L; 9:1, 1.4 L; 7:3, 1.2 L; 1:1, 1.6 L; 1:3, 1.2 L; 0:100, 1.8 L) and CH$_2$Cl$_2$/MeOH mixtures (99:1, 1.4 L; 49:1, 1.2 L; 19:1, 1.2 L; 9:1, 1.0 L). Seventy-two fractions of 200 ml each were collected and concentrated on a rotary evaporator. Similar fractions were combined on the basis of TLC analysis.

Fractions 24- 27 (126 mg) was passed through Sephadex LH-20 column (20 g, 2.5 x 30 cm) eluted with 1:1 CH$_2$Cl$_2$/MeOH to remove chlorophylls, followed by CC over silicagel (20 g, 2.0 x 12 cm) eluted with n-hexane/CH$_2$Cl$_2$ 3:2 to give syringaresinol
(246) (8 mg). Quercetin-3,7-dimethyl ether (17 mg) (236) crystallized in fractions 27-31 and was filtered off. Fractions 33-38 (242 mg) was chromatographed over Silica gel (20 g, 2.0 x 12 cm) eluted with 100% CH₂Cl₂ followed by 0.5% MeOH in CH₂Cl₂ to give a mixture of two compounds which were separated by PTLC to give quercetin (235) (9 mg) and quercetin-3-methyl ether (244) (34 mg).

The EtOAc extract (8 g) was chromatographed over Silica gel (100 g, 2.5 x 30 cm) eluted with n-hexane/CH₂Cl₂ mixtures (1:1, 1.0 L; 1:3, 1.0 L, 0:100, 0.8L) followed by CH₂Cl₂/MeOH mixtures (99:1, 0.80 L; 49:1, 0.60 L; 19:1, 0.60 L; 9:1, 0.60 L; 4:1, 0.50 L; 7:3, 0.40 L). Fifty-nine fractions of 100 ml each were collected. Syringic acid (247) (9 mg) was obtained after CC of fractions 27-31 over Sephadex LH-20 (20 g, 2.5 x 30 cm; 1:1 CH₂Cl₂/MeOH). Fractions 41-45 (n-hexane/CH₂Cl₂ 19:1) and 48-51 (n-hexane/CH₂Cl₂ 9:1) gave crystals which were filtered to give quercitrin (245) (124 mg) and quercetin-3-O-β-galactopyranoside (212) (92 mg) respectively.

3.3.4 Microglossa pyrifolia

The air dried and pulverized aerial parts of M. pyrifolia (2.6 Kg) was extracted by cold percolation at room temperature using 1:1 CH₂Cl₂/MeOH (3×5 L, 24 h each), followed by 100% methanol (1×4 L, 24 h). After filtering, the filtrates were concentrated on a rotary evaporator and combined to give 190 g of black gummy extract. Out of this, 120 g was taken and partitioned by suspending it in water/methanol 4:1 and extracted in turns with n-hexane and CH₂Cl₂ to give 53 g of n-hexane extract and 19 g of CH₂Cl₂ extract.
The n-hexane extract (50 g) was chromatographed over Silica gel (350 g, 5 x 40 cm) eluted with n-hexane/EtOAc mixtures (100: 0, 2.25 L; 19:1, 2.5 L; 9:1, 2.0 L; 4:1, 1.5 L; 3:2, 2.0 L; 1:1, 2.0 L; 2:3, 1.5 L; 1:4, 1.0 L) to give 55 fractions of 250 ml each. These gave friedelan-3-one (250) (25 mg), β-sitosterol (249) (14 mg) and stigmasterol (66) (66 mg) that crystallized in fractions 8-13, 19-22 and 24-36 respectively. The other fractions contained mainly chlorophylls, carotenoids and oils.

The CH$_2$Cl$_2$ extract (18 g) 35 g was adsorbed onto 20 g of Silica gel and subjected to CC on a silica gel column (200 g, 4 x 30 cm) eluted with n-hexane/CH$_2$Cl$_2$ mixtures (100:0, 1.8 L; 9:1, 1.4 L; 4:1, 1.4 L; 7:3, 1.2 L; 1:1, 1.6 L; 1:3, 1.2 L; 0:100, 1.4 L) and CH$_2$Cl$_2$/MeOH mixtures (99:1, 1.6 L; 49:1, 1.2 L; 19:1, 1.0 L; 9:1, 1.0 L) to give 70 fractions of 200 ml each. Repeated CC on fractions 33-41 (348 mg) (silicagel, 20 g, 2.0 x 12 cm) with n-hexane/CH$_2$Cl$_2$ 3:2 followed by n-hexane/CH$_2$Cl$_2$ 1:1 gave epoxyisochiliolide lactone (157) (57 mg) which crystallized in 1:1 n-hexane/CH$_2$Cl$_2$. The 8-acetoxyisochiliolide lactone (248) (97) crystallized in fractions 44-51 of the original column. Fraction 57-65 (183 mg) was chromatographed over sephadex LH-20 (20 g, 2.5 x 30 cm; 1:1 CH$_2$Cl$_2$/MeOH) followed by silicagel CC (20 g, 2.0 x 12 cm) to give 1,4-dihydroxy-7(11)-eudesmen-8-one (251) (9 mg).

3.4 In vitro anti-plasmodial activities
The in vitro anti-plasmodial activities were measured at the National Center for Natural Products Research of School of Pharmacy, University of Mississippi. The method used a colorimetric assay that determines the parasitic lactate dehydrogenase (pLDH) activity [Makler et al., 1993; Samoylenko et al., 2009]. The assay was performed in 96-well
microplate and included two *P. falciparum* strains [Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine-resistant)]. The IC₅₀ values were computed from the dose response curves generated by plotting percent growth against test concentrations. DMSO, artemisinin and chloroquine were included in each assay as vehicle and drug controls, respectively.

### 3.5 Cytotoxicity Assay

These were measured at the National Center for Natural Products Research of School of Pharmacy, University of Mississippi. The activity was determined against monkey kidney fibroblasts (VERO) obtained from the American Type Culture Collection (ATCC, Rockville, MD). The assay was performed in 96-well tissue culture-treated microplates. Cells were seeded at a density of 25000 cells/well and incubated for 24 hours. Samples at different concentrations were added and plates were again incubated for 48 hours. The number of viable cells was determined using Neutral Red according to a modification of the procedure of Borenfreund *et al.* [Borenfreund *et al.*, 1990]. IC₅₀ values were determined from dose response curves of percent growth inhibition against test concentrations. Doxorubicin was used as a positive control, while DMSO was used as the negative (vehicle) control.

### 3.6 Antioxidant test

Preliminary anti-oxidant test was done by spotting the compounds on a TLC followed by spraying the TLC plate with 0.2 mg/ml DPPH solution to view the active compounds which displayed white or yellowish sport on a purple background.
The active compounds were quantitatively analysed by UV-VIS spectrometry method adopted from Hou et al., (2002) with modifications made on the concentrations of the samples. For each compound, the concentration of the sample was varied by serial dilutions to give concentrations of 160, 80, 40, 20, 10, 5.0, 2.5, 1.25 and 0 μg/ml while the concentration of DPPH was kept constant at 100 μg/ml (Figure 3.1). These solutions were then measured for UV-VIS absorbance at DPPH absorbing wavelength (517 nm) half an hour after adding the DPPH. The absorbance measured at each of these intervals were converted into percentages of scavenged DPPH radicals using the following equation

\[
\% \text{ge of scavenged DPPH} = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100\%
\]

Where \(A_{\text{blank}}\) is the absorbance of DPPH solution without sample. The percentages of scavenged DPPH were then plotted against concentration of the compound to give graphs from which concentrations at half inhibition (IC\(_{50}\)) were determined. The tests were done in triplicates.

**Figure 3.1:** Samples with varied conc of compound but constant conc of DPPH
3.7 Antimicrobial Assay

The organisms are obtained from the American Type Culture Collection (Manassas, VA) and include the fungi *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113 and *Aspergillus fumigatus* ATCC 90906 as well as the bacteria *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* ATCC 43300 (MRS), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 and *Mycobacterium intracellulare* ATCC 23068. Susceptibility testing was performed at the National Center for Natural Products Research, School of Pharmacy, University of Mississippi, using a modified version of the CLSI methods as described by Samoylenko *et al* [Samoylenko *et al.*, 2009]. Drug controls ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi were included in each assay.

3.8 Antileishmanial Assay

Antileishmanial activity of the compounds was tested *in vitro* on a culture of *Leishmania donovani* promastigotes at the National Center for Natural Products Research of School of Pharmacy, University of Mississippi. In a 96 well microplate assay compounds with appropriate dilution were added to the Leishmania promastigotes culture (2×10⁶ cells/mL). The plates were incubated at 26°C for 72 hours and growth of *Leishmania* promastigotes was determined by Alamar blue assay [Mikus and Steverding, 2000]. Pentamidine and amphotericin B were used as standard antileishmanial agents. IC₅₀ values for each compound were computed from the growth inhibition curve.
CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Secondary metabolites isolated from Clerodendrum eriophyllum

4.1.1 Abietane diterpenoids

The roots of Clerodendrum eriophyllum afforded twelve abietane diterpenoids, which are 3-oxoroyleanone (220), royleanone (21), 6,7-dehydroroyleanone (221), taxodione (222), 11-hydroxy-7,9(11),13-abietatrien-12-one (223), ferruginol (224), 11-hydroxy-8,11,13-abietatriene 12-O-β-xylopyranoside (225), sugiol (9), 6-hydroxysalvinolone (226), 6,16-dihydroxysalvinolone (227), nellionol (228) and uncinatone (19).

Figure 4.1: Abietane diterpenoids isolated from the roots of C. eriophyllum
4.1.1.1 12-Hydroxy-8,12-abietadiene-3,11,14-trione (3-oxoroyleanone)

Compound 220 was isolated as pale yellow amorphous solid. Its molecular formula was established by HRESI to be C\textsubscript{20}H\textsubscript{26}O\textsubscript{4}, \textit{m/z} 331.1910 [M+H]\textsuperscript{+} requiring 331.1909. Its UV absorption maxima at 275 and 405 nm closely correlated that reported for a \(\pi\)-quinone chromophore of royleanone [Edwards \textit{et al.}, 1962].

The IR spectrum indicated presence of hydroxyl group (3080-3430 cm\(^{-1}\)), unconjugated carbonyl (1704 cm\(^{-1}\)) together with olefinic and conjugated carbonyl absorptions of a \(\pi\)-quinone moiety (1609, 1633 and 1650 cm\(^{-1}\)). The \(^{13}\)C NMR (Table 4.1, Appendix 1B) showed 20 signals with four sp\(^2\) quaternary carbons (\(\delta\text{C} 124.1, 144.1, 145.8, 150.6\)) and two conjugated carbonyls (\(\delta\text{C} 183.2, 186.9\)) assignable to the \(\pi\)-quinone moiety.

The \(^1\)H NMR (Table 4.1, Appendix 1A) did not show signals in the olefinic region, but it did show among other signals three methyl singlets at \(\delta\text{H} 1.09, 1.13\) and 1.24 assignable to C-18, C-19 and C-20 of abietane skeleton and two methyl doublets at \(\delta\text{H} 1.19\) and 1.20. The latter methyl doublets together with methine septet at \(\delta\text{H} 3.15\) are indicative of an isopropyl group attached to the \(\pi\)-quinone ring [Kabouche \textit{et al.}, 2007].

An exchangeable hydrogen singlet observed at \(\delta\text{H} 7.21\) showed NOE interaction with the two isopropyl methyl groups, indicating that the hydroxyl and isopropyl groups were on adjacent carbons, as confirmed by HMBC correlation between septet of H-15 and the oxygenated olefinic carbon at \(\delta\text{C} 150.6\) (C-12). The unconjugated carbonyl at \(\delta\text{C} 216.7\) was placed at C-3 from \(^3\)J HMBC correlation between CH\textsubscript{3}-18 and CH\textsubscript{3}-19 with this carbonyl carbon signal.
The relative configurations at C-5 and C-10 were established as follows; H-5 and CH$_3$-20 were in *axial-axial* orientation, as shown by NOESY experiments which displayed NOE correlations between H-5 and CH$_3$-18 as well as between CH$_3$-19 and CH$_3$-20 and no correlation between H-5 and CH$_3$-20 (Figure 4.2). This relative configuration at C-5 and C-10 is what is reported for natural abietane diterpenoids [Hirasawa *et al.*, 2007] and was the same for all of the isolated abietane diterpenoids.

Comparison of $^{13}$C NMR spectral data of 220 with those of known compound royleanone showed close correlation except for replacement of the methylene at C-3 with carbonyl and the corresponding deviations for neighbouring carbon signals, $\Delta$\text{C} -1.7, 16.4, 13.4 and -5.7 for C-1, C-2, C-4 and C-19 respectively. Thus, spectral evidence indicated compound 220 to be 12-hydroxy-8,12-abietadiene-3,11,14-trione (3-oxoroyleanone), which is a new compound.

![Figure 4.2: NOE correlations used to establish relative stereochemistry at C- 5 and C-10.](image)
Table 4.1: NMR data for compound 220 (CDCl3; 500 MHz for ^1H-NMR, 125 MHz for ^13C-NMR)

<table>
<thead>
<tr>
<th>H/C</th>
<th>δ_C</th>
<th>δ_H, m (J in Hz)</th>
<th>HMBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34.5</td>
<td>1.76, m</td>
<td>C-2, C-3, C-5, C-10, C-20</td>
</tr>
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<td></td>
<td></td>
<td>2.81, m</td>
<td>C-2, C-3, C-5, C-10, C-20</td>
</tr>
<tr>
<td>2</td>
<td>33.8</td>
<td>2.59, ddd (15.6, 9.2, 5.4); 2.51, dt (15.6, 7.2)</td>
<td>C-1, C-3, C-4, C-10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-1, C-3, C-4, C-10</td>
</tr>
<tr>
<td>3</td>
<td>216.7</td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td>46.9</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>50.8</td>
<td>1.76, m</td>
<td>C-1, C-3, C-7, C-19, C-20</td>
</tr>
<tr>
<td>6</td>
<td>18.6</td>
<td>1.46, dddd (21.8, 12.0, 4.5); 1.76, m</td>
<td>C-4, C-5, C-7, C-8, C-10</td>
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<td></td>
<td></td>
<td></td>
<td>C-4, C-5, C-7, C-8, C-10</td>
</tr>
<tr>
<td>7</td>
<td>26.0</td>
<td>2.31, dddd (19.6, 12.0, 6.0); 2.84, br dd (19.6, 5.4)</td>
<td>C-5, C-6, C-8, C-9, C-14</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>C-5, C-6, C-8, C-9, C-14</td>
</tr>
<tr>
<td>8</td>
<td>145.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>144.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>37.3</td>
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</tr>
<tr>
<td>11</td>
<td>183.2</td>
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<td></td>
</tr>
<tr>
<td>12</td>
<td>150.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>124.3</td>
<td></td>
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<tr>
<td>14</td>
<td>186.9</td>
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<td></td>
</tr>
<tr>
<td>15</td>
<td>24.1</td>
<td>3.15, sept (7.0)</td>
<td>C-12, C-13, C-14, C-16, C-17</td>
</tr>
<tr>
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<td>19.9</td>
<td>1.20, d (7.0)</td>
<td>C-13, C-15, C-17</td>
</tr>
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<td>17</td>
<td>19.8</td>
<td>1.19, d (7.0)</td>
<td>C-13, C-15, C-16</td>
</tr>
<tr>
<td>18</td>
<td>27.7</td>
<td>1.13, s</td>
<td>C-3, C-4, C-5, C-19</td>
</tr>
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<td>20.0</td>
<td>1.09, s</td>
<td>C-3, C-4, C-5, C-18</td>
</tr>
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<td>20.5</td>
<td>1.24, s</td>
<td>C-1, C-5, C-9, C-10</td>
</tr>
<tr>
<td>12-OH</td>
<td>7.21, s</td>
<td></td>
<td>C-11, C-12, C-13</td>
</tr>
</tbody>
</table>
4.1.1.2 12-Hydroxy-8,12-abietadiene-11,14-dione (royleanone)

Compound 21 was isolated as orange crystals. Its EI-MS spectrum (Appendix 2C) gave a molecular ion at $m/z$ 316 ($C_{20}H_{28}O_3$). The $^{13}$C-NMR (Table 4.4, Appendix 2B) showed twenty carbon signals, among them four quaternary olefinic and two conjugated carbonyl carbons representing a $p$-quinone moiety ($\delta_{C} 123.0, 145.5, 145.9, 149.9, 182.8, 187.0$). The presence of an isopropyl ($\delta_{H} 0.57$ and 0.58, both 3H doublets with $J = 7.0$ and $\delta_{H} 2.49$, 1H septet with $J = 7.0$) attached to $p$-quinone moiety was apparent and hydroxyl group (exchangeable $^1H$ singlet at $\delta_{H} 6.60$). The two groups were adjacent to each other from HMBC correlations.

The rest of the carbons constituted of three methyls, five methylenes and two methines which completed an abietane skeleton, as from HMBC, with ring C being the $p$-quinone moiety. The EI-MS retro Diels-Alder fragmentation at $m/z$ 231 and 84 (Figure 4.3) gave further proof for the structure. The compound was therefore characterized as 12-hydroxy-8,12-abietadiene-11,14-dione (royleanone, 21) previously reported from *Inula royleana* [Edwards et al., 1962].

![Figure 4.3: Fragmentation pattern for royleanone (21)](image-url)
Compound 221 was isolated as a minor component of a sample containing royleanone (21). The $^1$H-NMR (Appendix 2A) indicated that this compound made about 30% of the major compound. The difference between the two compounds was dehydrogenation at position 6 and 7 to form a double bond, which was observed by shift of carbon-13 chemicals shifts from 16.4 and 26.2 to 139.5 and 120.4 respectively for the two positions.

The olefinic protons were observed at $\delta_H$ 5.86 and 6.15, both being double doublets with coupling constants of 9.7 and 3.0 Hz. The mixture was consistently giving a single spot on a TLC over different solvent systems, a property that might have been attributed to hydrogen bonding (Figure 4.4), as it is observed with other similar organic molecules [Csehi and Palinko, 2004].

The minor compound was therefore 6,7-dehydroroyleanone, previously isolated from *Plectranthus sp.* [Hensch et al., 1975].

**Figure 4.4:** Hydrogen bonding between royleanone (21) and 6,7-Dehydroroyleanone (221) molecules
4.1.1.4 Taxodione

Compound 222 was isolated as yellow solid and gave a molecular ion peak of 314 on EI-MS analysis. The $^1$H-NMR (Table 4.2, Appendix 3A) showed among other signals two singlet olefinic protons (δ$_H$ 6.20 and 6.92, both 1H singlets), signals for isopropyl unit (two 3H doublets at δ$_H$ 1.15 & 1.16 and 1H septet at δ$_H$ 3.05, $J = 6.8$) as well as three methyl singlets at δ$_H$ 1.10, 1.249 and 1.253.

The $^{13}$C-NMR (Table 4.4, Appendix 3B) gave twenty signals for twenty carbons of an abietane diterpenoid, which included isopropyl signals (δ$_C$ 21.4$q$, 21.7$q$ and 27.6$d$) and three other methyls (δ$_C$ 22.0$q$, 22.2$q$ and 33.4$q$). Two conjugated carbonyls were observed at δ$_C$ 182.1 and 201.2. The carbonyl at 182.1 had HMBC correlation with the isopropyl 1H septet and an exchangeable proton of 11-OH suggesting that the carbonyl was at C-12 where as the carbonyl at 201.2 had HMBC correlation with a characteristic H-5 singlet at δ$_H$ 2.60 implying that the carbonyl was at C-6. The olefinic proton at δ$_H$ 6.92 (H-14) had HMBC correlation with C-12 (δ$_C$ 182.1) carbonyl where as the olefinic proton at δ$_H$ 6.20 (H-7) had HMBC correlation with C-6 (δ$_C$ 201.2) carbonyl. This suggested an extended conjugation from C-6 to C-12.

Consequently, based on the above spectroscopic evidence, the compound was identified as abieta-7,9(11),13-trien-6,12-dione (taxadione) previously reported from *Taxodium distichum* [Hirasawa et al., 2007]. The EI-MS fragmentation pattern (Figure 4.5, Appendix 3C) further confirmed the structure, with the molecular ion appearing at $m/z$ 314 (C$_{20}$H$_{26}$O$_3$), loss of isopropyl giving a fragment at $m/z$ 271 and retro Diels-Alder fragmentation resulting to the base peak at $m/z$ 149.
4.1.1.5 11-Hydroxy-7,9(11),13-abietatrien-12-one

Compound 223 was isolated from the roots of Clerodendrum eriophyllum as pale yellow crystals. Its NMR (Tables 4.2 and 4.4) features resembled those of taxadione (222), the difference being absence of a carbonyl at C-6 which resulted to major difference in $^{13}$C-NMR value at this carbon ($\delta_c$ 201.2 for 222 and 26.1 for 223) as well as differences for $^{13}$C-NMR values for C-5, C-7 and C-8 (Table 4.4). The compound was therefore identified as 11-hydroxy-7,9(11),13-abietatrien-12-one. These spectroscopic data are identical to those previously reported for the same compound isolated from Plectranthus elegans [Dellar et al., 1996].
Table 4.2: $^1$H-NMR data for abietane diterpenoids 21, 221, 222 and 223 (600 MHz)

<table>
<thead>
<tr>
<th></th>
<th>21</th>
<th>221</th>
<th>222</th>
<th>223</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>(C$_2$Cl$_4$D$_2$)</td>
<td>(C$_2$Cl$_4$D$_2$)</td>
<td>(CD$_2$Cl$_2$)</td>
<td>(CD$_2$Cl$_2$)</td>
</tr>
<tr>
<td>1</td>
<td>2.08, br d (13.6)</td>
<td>2.22, br d (13.3)</td>
<td>2.93, m</td>
<td>1.74, m</td>
</tr>
<tr>
<td></td>
<td>0.50, dd (13.6, 4.1)</td>
<td>0.83, m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.07, qt (13.6, 3.3)</td>
<td>0.98, m</td>
<td>1.74, m</td>
<td>1.58, m</td>
</tr>
<tr>
<td></td>
<td>0.89, m</td>
<td>0.89, m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.82, brd (13.4)</td>
<td>0.86, m</td>
<td>1.40, br d (11.4)</td>
<td>1.20, dd (11.0, 6.8)</td>
</tr>
<tr>
<td></td>
<td>0.53, dd (13.4, 4.1)</td>
<td>0.59, m</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>0.45, br d (12.2)</td>
<td>1.51, t (3.0)</td>
<td>2.60, s</td>
<td></td>
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<tr>
<td>6</td>
<td>1.22, dddd (25.0, 13.3, 7.2)</td>
<td>5.86, dd (9.7, 3.0)</td>
<td>2.59, dddd (20.8, 6.6, 4.2)</td>
<td>2.38, dddd (20.8, 12.0, 3.4)</td>
</tr>
<tr>
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<td>0.73, dddd (25.0, 12.2, 5.6)</td>
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<td>7</td>
<td>2.06, m</td>
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<td>6.20, s</td>
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<td></td>
<td>6.92, s</td>
<td>6.78, s</td>
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<tr>
<td>15</td>
<td>2.49, sept (7.0)</td>
<td>2.49, sept (7.0)</td>
<td>3.05, sept d (6.8, 1.0)</td>
<td>3.10, m</td>
</tr>
<tr>
<td>16</td>
<td>0.58, d (7.0)</td>
<td>0.59, d (7.0)</td>
<td>1.15, d (6.8)</td>
<td>1.13, d (6.8)</td>
</tr>
<tr>
<td>17</td>
<td>0.57, d (7.0)</td>
<td>0.58, d (7.0)</td>
<td>1.16, d (6.8)</td>
<td>1.15, d (6.8)</td>
</tr>
<tr>
<td>18</td>
<td>0.29, s</td>
<td>0.35, s</td>
<td>1.10, s</td>
<td>0.93, s</td>
</tr>
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<td>19</td>
<td>0.26, s</td>
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<td>20</td>
<td>0.61, s</td>
<td>0.40, s</td>
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<td>1.18, s</td>
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<tr>
<td>11-OH</td>
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<td>7.60, s</td>
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<tr>
<td>12-OH</td>
<td>6.60, s</td>
<td>6.71, s</td>
<td></td>
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</tr>
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</table>

4.1.1.6 Ferruginol

Compound 224 was isolated as pale yellow oil, having a molecular ion peak at m/z 286 (C$_{20}$H$_{30}$O, Appendix 5C). The $^{13}$C-NMR (Table 4.4, Appendix 5B) and DEPT spectra showed twenty signals of which five were methyls, five methylenes, four
methines and six quaternary carbons. Six of the twenty carbons appeared on the aromatic region (δC 111.5, 127.2, 127.9, 132.1, 149.4 and 151.4) assigned to an aromatic ring with two protonated carbons (111.5 and 127.2). The ¹H-NMR (Table 4.3, Appendix 5A) showed two aromatic singlets (δH 6.60 and 6.81) implying that the two were para protons. An isopropyl group attached to aromatic ring was evident from two ³H doublets at δH 1.19 and 1.21 as well as a ¹H septet at 3.10. Three singlet methyls were observed from ³H singlets at δH 0.92, 0.94 and 1.15.

It was evident that this was an abietane diterpenoid with ring C being aromatic and C-12 substituted with a hydroxyl group observed from an exchangeable proton signal (δH 4.67) showing HMBC correlation with C-12 and C-13. The compound was therefore identified as 12-hydroxyabieta-8,11,13-triene (ferruginal) previously reported from Cryptomeria japonica [Kondo et al., 1963] and Salvia syriaca [Ulubelen et al., 2000]. The structure was further confirmed by the EI-MS fragmentation pattern (Figure 4.6).

Figure 4.6: Fragmentation pattern for ferruginal (224)
Compound 225 was isolated as white crystals and gave a weak EI-MS molecular ion peak at \( m/z \) 435 [M+H] \((C_{25}H_{38}O_6)\). The \(^{13}\)C-NMR (Table 4.4, Appendix 6B) showed twenty-five carbon signals among them signals for a five-carbon sugar unit at \( \delta_c \) 68.2, 70.1, 73.0, 74.6 and 108.6. The sugar unit was deduced to be xylopyranoside through comparison of NMR data with literature values [Han et al., 2008].

The remaining twenty carbon signals included six in the aromatic region. The signals at \( \delta_c \) 142.2 and 149.0 implied adjacent oxygenated sp\(^2\) carbons of benzene ring. The \(^1\)H-NMR (Table 4.3, Appendix 6A) had one proton singlet in the aromatic region, implying that only one of the aromatic carbons was protonated. The \(^1\)H-NMR also had signals for isopropyl unit on the aromatic ring (two 3H doublets at \( \delta_H \) 1.10 and 1.11 as well as 1H septet at 3.61, \( J = 6.9 \) Hz) in addition to three methyl singlets (\( \delta_H \) 0.94, 0.96, 1.30) being indicative of abietane diterpenoid with ring C being aromatic.

The anomic proton of the xylopyranoside and the 1H septet of the isopropyl group showed HMBC correlation with C-12 (\( \delta_c \) 142.2) suggesting that the O-xylopyranoside is attached at (C-12) and the isopropyl group was attached to the adjacent carbon (C-13). The singlet at \( \delta_H \) 6.39 is assigned to H-14 from HMBC correlation with a methylene carbon at C-7 (\( \delta_c \) 34.0). The \( \beta \)-orientation of the xylopyranoside group was deduced from the large coupling constant of the anomic proton (\( J = 7.7 \) Hz) which meant that the proton was in axial position. The compound was therefore identified as 11-hydroxy-8,11,13-abietatriene 12-\( \beta\)-xylopyranoside previously reported from *Avicennia marina*. 

4.1.1.7 \( \text{11-Hydroxy-8,11,13-abietatriene-12-\( \beta\)-xylopyranoside (225)}\)
[Han et al., 2008]. The EI-MS fragment at m/z 302 represented the aglycone (Figure 4.7, Appendix 6C).

Figure 4.7: Fragmentation pattern for 11-hydroxy-8,11,13-abietatriene 12-O-β-xylpyranoside (225)

4.1.1.8 Sugiol

Compound 9 was isolated as white crystals; analysis by EI-MS showed a molecular ion peak at m/z 300 which also happened to be the base peak signifying the stability of the molecule. The 13C-NMR spectrum (Table 4.4, Appendix 7B) had twenty carbon signals for a diterpenoid, among them a carbonyl at δC 197.1 and six signals in the aromatic region. The chemical shift of the carbonyl (δC 197.1) is suggestive of conjugation and its HMBC correlation with an aromatic proton (δH 7.80, s) imply that it is conjugated to the aromatic ring.

That this compound had an abietane skeleton was deduced from the 1H-NMR isopropyl signals (two 3H doublets at δH 1.21 and 1.23 as well as 1H septet at δH 3.26, J = 6.9) accompanied by three methyl singlets of for H-18, H-19 and H-20 (δH 0.95, 1.10 and 1.22
respectively). The aromatic region of the $^1$H-NMR spectrum had two singlets ($\delta_H 6.87 \ s$ and $7.80 \ s$) signaling para protons of a tetrasubstituted benzene ring. The signal at $\delta_H 7.80$ proton had $^3J$ HMBC correlation with the isopropyl methine carbon suggesting that this proton was adjacent to the isopropyl group.

An oxygenated aromatic carbon atom ($\delta_C 160.6$) had HMBC correlation with isopropyl methine proton implying that a hydroxyl group was attached to a carbon (C-12) adjacent to the carbon bearing the isopropyl group. The position of the carbonyl was deduced to be C-7 to meet the requirement of conjugation with the aromatic ring. The compound was therefore identified as abieta-8,11,13-trien-7-one (trival name sugiol) with molecular formula $C_{20}H_{28}O_2$ consistent with EI-MS molecular ion. The compound was previously reported from *Caryopteris incana* [Gao and Han, 1997].

**Figure 4.8:** Fragmentation pattern for sugiol (9)
Compound 226 was isolated as pale yellow crystals from the roots of *Clerodendrum eriophyllum* with molecular ion peak at \( m/z \) 330 on EI-MS spectrum (Appendix 8C). The \(^{13}\)C-NMR spectrum of the compound (Table 4.4, Appendix 8B) had twenty signals for a diterpene constituting an aromatic ring (\( \delta_C \) 116.4, 121.7, 135.1, 139.2, 143.4, 148.1), two olefinic carbons (\( \delta_C \) 142.8, 143.8) conjugated to a carbonyl (\( \delta_C \) 180.5) and the rest being sp\(^3\) carbons including five methyls. From HSQC experiment, all the aromatic carbons were quartenary except for one carbon (\( \delta_C \) 116.4, \( \delta_H \) 7.66 s).

The \(^1\)H-NMR spectrum (Table 4.3, Appendix 8A) showed among other signals the aromatic proton (\( \delta_H \) 7.66 s), an isopropyl unit attached to aromatic ring (\( \delta_H \) 1.24 \text{ } d \text{ } J = 7.0, 1.26 \text{ } d \text{ } J = 7.0, 3.35 \text{ } \text{sept} \text{ } J = 7.0) and three more singlet methyls (\( \delta_H \) 1.45, 1.47, 1.70) all of which are indicative of abietane diterpenoid. The aromatic proton (H-14, \( \delta_H \) 7.66 s) showed HMBC correlation with the carbonyl and methine carbon of the isopropyl implying 7-oxo-13-isopropyl substitution as in compound 9. It was therefore established that the aromatic proton was at C-14, the carbonyl was C-7 and the isopropyl was attached at C-13.

An exchangeable hydrogen signal was observed at \( \delta_H \) 7.49 making HMBC correlation with the carbonyl and olefinic carbons implying that it was due to hydroxyl group attached at C-6 and that C-5 and C-6 were the olefinic carbons. Two more hydroxyl groups were on the aromatic ring at C-11 and C-12 of \( \delta_C \) 143.4 and 148.1 respectively. The compound was therefore identified as 6,11,12-trihydroxyabieta-5,8,11,13-tetraene-7-one (6-hydroxysalvinolone) previously reported from the roots of *Salvia montbretii*.
The structure of the compound was confirmed by EI-MS hypothetical fragmentation pattern shown in (Figure 4.9)

Figure 4.9: Fragmentation pattern for 6-hydroxysalvinolone (226)
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Table 4.4: $^{13}$C-NMR data for abietane diterpenoids from *C. eriophyllum*

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Key: $a = 150$ MHz, $b = 125$ MHz, $c = 75$ MHz, $d = 50$ MHz
4.1.1.10 6,16-Dihydroxysalvinolone

Compound 227 was isolated from the roots of Clerodendrum eriophyllum as pale yellow crystals with EI-MS molecular ion at $m/z$ 346 consistent with molecular formula C$_{20}$H$_{26}$O$_5$. NMR data (Tables 4.4, 4.5) were similar to those discussed for 6-hydroxysalvinolone (226), the only difference being on the isopropyl group where one of two methyl groups was oxidized to methylene ($\delta_c$ 69.0; $\delta_{H1}$ 3.99 $dd J = 10.2$ Hz & 3.0 Hz, 3.82 $dd J = 10.2$ Hz & 7.0 Hz). The methylene protons showed HMBC correlation with C-15 ($\delta_c$ 39.1), C-15 ($\delta_c$ 16.0) and C-13 ($\delta_c$ 131.1), implying that it was the methyl group of an isopropyl that was oxidized to hydroxymethylene.

Therefore the compound was 6,11,12,16-tetrahydroabieta-5,8,11,13-tetraen-7-one (6,16-dihydroxysalvinolone). The loss of oxidized isopropyl group and carbonyl gave a fragment at $m/z$ 259 (Figure 4.10, Appendix 9C). The compound was previously reported from Avicennia marina [Han et al., 2008].

![Figure 4.10: Fragmentation pattern for 6,16-dihydroxysalvinolone (227)](image)
4.1.1.11 Nellionol

Compound 228 was isolated as white crystals and had almost similar NMR features with 6,16-dihydroxyalsvinolone (227), except that the signals of olefinic group at 5 and 6 were replaced with signals for saturated carbons ($\delta_c$ 56.2 and 73.5) with their respective protons appearing at $\delta_h$ 1.76 ($d$, 13.4 Hz) and 4.58 ($dd$, 13.4 and 2.6 Hz). The coupling constant of 13.4 for H-5 and H-6 meant that two protons were in axial positions, a fact used to establish the relative stereochemistry at position 6. The EI-MS molecular ion peak at $m/z$ 348 (Appendix 1IC) was 2H units more than that of 227, giving further proof that this compound is a 5,6-dihydroderivative of 227. The compound was therefore identified as 6,11,12,16-tetrahydroxyabieta-8,11,13-trien-7-one (trival name nellionol) previously reported from Premna latifolia [Rao et al., 1979].

![Figure 4.11: Fragmentation pattern for nellionol (228)](image)

5.1.1.12 Uncinatone

Compound 19 was isolated as orange crystals. A total of twenty signals were present in the $^{13}$C-NMR spectrum (Table 4.4, Appendix 1IB) suggesting a diterpenoid, among them signals for an $\alpha$-methyldihydrobenzofuran, ie six quartenary carbons of benzene ring and signals at 83.0$d$, 34.5$t$ and 22.0$q$ for a -OCH(CH$_3$)CH$_2$- moiety, which was
suspected to be a result of rearrangement and cyclization of isopropyl group observed in abietane diterpenoids. A conjugated carbonyl was observed at 190.1 ppm, established to be at C-7 of abietane skeleton from chelation with 14-OH as well as HMBC correlation with the olefinic H-5 at 6.24 ppm.

The $^1$H-NMR spectrum (Table 4.5, Appendix 11A) showed a strongly H-bonded OH group at $\delta_H$ 13.74 ppm, another OH group at $\delta_H$ 4.97 ppm, an olefinic proton signal at $\delta_H$ 6.24 ppm, together with signals at $\delta_H$ 1.90 and 1.93 ppm, typical for non-equivalent vinylic CH$_3$ groups established by HMBC to be on adjacent carbons. Further two CH$_3$ groups were observed, one at $\delta_H$ 1.52 ppm and the other giving rise to a 6.5 Hz doublet at $\delta_H$ 1.54 ppm coupling to a 1H methine proton at 5.14 ppm, which was in turn coupling with two methylene protons at $\delta_H$ 2.91 and 3.42 ppm. The connection of the above methyl doublet, methine and methylene suggested a -OCH(CH$_3$)CH$_2$- partial structure as may occur in an $\alpha$-methylidihydrobenzofuran system, as evidenced by the HMBC correlation of the methine proton at 5.14 ppm with aromatic carbons of benzene ring at 111.0 and 154.1 ppm. Spectroscopic evidence suggested this compound was uncinatone. The data correlated perfectly with those reported for the same compound from Clerodendrum uncinatum [Dorsaz et al., 1985].
Table 4.5: $^1$H-NMR for abietane diterpenoids 227, 228 (600 MHz) and 19 (500 MHz)

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</tbody>
</table>

4.1.2 Triterpenoids

Two triterpenoids were isolated from the roots of *C. eriophyllum*. These are betulinic acid (229) and $\beta$-amyrin octacosanoate (230).

Figure 4.12: Triterpenoids from *Clerodendrum eriophyllum*
4.1.2.1  

Betulinic acid

Compound 229 was isolated as white crystals from the roots of Clerodendrum eriophyllum. Its EI-MS molecular ion peak at m/z 456 corresponded to molecular formula of C₃₀H₄₈O₃ (Appendix 12C). The ¹³C-NMR (Table 4.6, Appendix 12B) gave thirty signals for a triterpene including a carboxylic carbonyl (δC 177.2) and terminal alkene (δC 150.3 s, 109.6 t). The ¹H-NMR (Appendix 12A, Table 4.6) showed six methyl groups, all singlets, two protons in the olefinic region for the terminal alkene carbon (δH 4.68 d, J = 1.8, 4.55 brs) and a characteristic H-3 proton (δH 2.95 m) of triterpenes with OH at C-3.

Using combination of 1D ¹H-NMR, ¹³C-NMR and DEPT as well as 2D HMBC, HSQC and COSY, the compound was identified as betulinic acid, widely distributed in the Verbenaceae family. The spectroscopic data correlated with those reported for the same compound from leaves of Nerium oleander [Siddiqui et al., 1988].

4.1.2.1  

β-amyrin octacosanoate

Compound 230 was isolated as white solids from the roots of Clerodendrum eriophyllum. The ¹³C-NMR (Table 4.7, Appendix 13B) had more than forty signals; the presence of a long alkanoate chain being evident from the ester carbonyl at δC 173.7 ppm, intense peak for multiple methylenes at δC 30.1 and a terminal methyl carbon at δC 14.3 which had 3H proton signal resonating at δH 0.88 (t, J = 7.2Hz). The rest of the molecule was deduced to be β-amyrin through comparison of the NMR data with literature [Matsunaga et al., 1988]. The alkanoate was connected to β-amyrin through ester bond at the hydroxyl group of position 3 of the β-amyrin as verified by HMBC correlation between H-3 of the β-amyrin at δH 4.46 (dd, J = 10.0, 5.8 Hz) and the alkanoate carbonyl at δC 173.7.
The alkanoate was identified to be octacosanoate by EI-MS molecular ion at \( m/z \) 833 [M+H]+, \textit{retro} Diels-Alder fragmentation at \( m/z \) 614 and acyllium ion at \( m/z \) 408 (Figure 4.13 and Appendix 13C). The compound was therefore identified as \( \beta \)-amyrin octacosanoate previously found in bast fibres of industrial hemp \textit{Cannabis sativa} [Gutierrez \textit{et al.}, 2006].

Figure 4.13: Fragmentation pattern for \( \beta \)-amyrin octacosanoate (230)

4.1.3 Tyrosol derivative

One tyrosol derivative was isolated from \textit{C. eriophyllum}, namely tyrosyl octacosanoate (bongardol) (231)
4.1.3.1 Bongardol

Compound 231 was isolated as white crystals. The presence of 2-(4-hydroxyphenyl)ethanoate unit was observed from the $^1$H-NMR (Appendix 14A) showing AA'XX' spin system of a 1,4-disubstituted benzene at $\delta_H$ 6.77 and 7.08 as well as signals for two adjacent methylenes, one attached to aromatic ring at $\delta_H$ 2.88 ($\tau, J = 7.2$ Hz) and the other attached to oxygen at $\delta_H$ 4.23 ($\tau, J = 7.2$ Hz). There was also an alkanoate group evidenced by the ester carbonyl at $\delta_C$ 173.0 ppm, intense peak for accumulated methylenes at $\delta_C$ 28.7 and a terminal methyl carbon at $\delta_C$ 13.1 which had 3H proton signal resonating at $\delta_H$ 0.88 ppm.

That 2-(4-hydroxyphenyl)ethanoate was esterified to the alkanoate was established from HMBC correlation of methylene proton signal at $\delta_H$ 4.23 and the ester carbonyl. The alkanoate was identified as octacosanoate from EI-MS weak molecular ion peak at $m/z$ 545 [M+H]$^+$ as well as a peak for octacosanoic acid at $m/z$ 425 (+H) resulting from McLaffert rearrangement (Figure 4.15 and Appendix 14C). The compound was therefore identified as 2-(4-hydroxyphenyl)ethanoyloctacosanoate (bongardol), previously from Bongardia chrysogonum [Alfatafta et al., 1989].
Figure 4.15: Fragmentation pattern for Bongardol (231)

Table 4.6: $^1$H-NMR (600 MHz) and $^{13}$C-NMR (150 MHz) data for betulinic acid (229) in DMSO

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Table 4.7: $^1$H-NMR (600 MHz) and $^{13}$C-NMR (150 MHz) data for $\beta$-amyrrn octacosanoate (230) in CD$_2$Cl$_2$

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<td></td>
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</table>
5.1.4 **General observations on compounds of *C. eriophyllum***

The phytochemistry of the plant coincides with that of other species of the genus, giving abietane diterpenoids as the major class of metabolites (twelve abietane diterpenoids). These are biosynthesized through mevalonate pathway, involving cyclization of geranylgeranylipyrophosphate as in Figure 4.16. Out of those, one was a new compound (12-hydroxy-8,12-abietadiene-3,11,14-trione, 220). Eleven compounds were new to the genus, these are 6,7-dehydroroyleanone (221), taxodione (222), 11-hydroxy-7,9(11),13-abietatrien-12-one (223), ferruginol (224), 11-hydroxy-8,11,13-abietatriene 12-(O-β-xylopyranoside (225), 6-hydroxysalvinolone (226), 6,16-dihydroxysalvinolone (227), nellionol (228), betulinic acid (229), β-amyrin octacosanoate (230) and bongardol (231).

![Figure 4.16: Biosynthetic route for abietane diterpenoids](image)

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*Figure 4.16: Biosynthetic route for abietane diterpenoids*
4.2 Secondary metabolites isolated from *Sphaeranthus bullatus*

4.2.1 Carvotacetone derivatives

The aerial parts of *Sphaeranthus bullatus* afforded a total of seventeen compounds, among them five carvotacetone derivatives, which are 3,5,7-trihydroxycarvotacetone (85), 3-acetoxy-5,7-dihydroxycarvotacetone (106), 3,7-dihydroxy-5-tigloyloxyoxycarvotacetone (88), 3-acetoxy-7-hydroxy-5-tigloyloxyoxycarvot acetone (99) and 5-O-β-glucopyranosylcarvot acetone (232).

![Carvotacetones isolated from *Sphaeranthus bullatus*](image)

**Figure 4.17:** Carvotacetones isolated from *Sphaeranthus bullatus*

4.2.1.1 3,5,7-Trihydroxycarvot acetone

Compound 85 was isolated as pale yellow gum having a molecular ion peak at *m/z* 201 (C_{10}H_{16}O_{4}) [M+H]^+ on EI-MS spectrum (Appendix 15C). The ^1H-NMR (Table 4.8, Appendix 15A) showed among other signals an isopropyl unit (δ_H 1.18 d J = 6.0 Hz, 1.16 d J = 6.0 Hz, 2.18 m) and two protons of an oxymethylene (-OCH_{2}-, δ_H 4.27 and 4.20, both doublets with J = 15.0 Hz) as well as an olefinic proton at δ_H 6.96 (dd J = 5.4, 1.8 Hz).
The $13^\text{C}$-NMR (Table 4.8, Appendix 15B) had ten signals for ten carbon atoms, including three for the isopropyl unit ($\delta_C$ 20.3 $q$, 20.8 $q$, 29.2 $d$) and an oxy-methylene (-OCH$_2$-), $\delta_C$ 59.1). These two groups were connected to 1,4 positions of a six carbon ring containing a carbonyl conjugated to an a double bond ($\delta_C$ 202.1, 143.6, 137.1) forming a carvotacetone moiety hydroxylated at positions 3 and 5. The methine proton of the isopropyl group made HMBC correlation with the two hydroxylated carbons (H-8→C-3, H-8→C-5) implying that the isopropyl unit was positioned between hydroxyl groups.

The HMBC correlations between the oxymethylene protons and the olefinic carbons as well as with the carbonyl (H-7→C-1, H-7→C-2, H-7→C-6) implied that the oxymethylene was attached to an olefinic carbon and adjacent to a carbonyl. The relative stereochemistry at positions 3, 4 and 5 were established on the basis of the coupling constants and NOESY results. H-3 and H-4 showed NOE interactions (cis) and axial-equatorial coupling ($J = 3.8$ Hz) while H-4 and H-5 had axial-axial coupling ($J = 12.0$ Hz). The compound was therefore identified as 3,5,7-trihydroxycarvotacetone previously isolated from Sphaeranthus bullatus [Jakupovic et al., 1990]. The EI-MS retro Diels-Alder fragment ions at $m/z = 114$, 96 and 68 (Figure 4.18) confirmed the structure.

![Figure 4.18: Fragmentation pattern for 3,5,7-trihydroxycarvotacetone (85)](image)
4.2.1.2 3-Acetoxy-5,7-dihydroxycarvotacetone

Compound 106 was isolated as pale yellow gum. Spectroscopic features resemble those of 3,5,7-trihydroxycarvotacetone (85) except for additional acetyl signals at $\delta_c$ 170.5 and 21.3 ppm as well as $\delta_H$ 2.07 (3H, s) making an acetoxy derivative of 85. The acetoxy group was fixed at C-3 from HMBC correlation of the de-shielded H-3 ($\delta_H$ 5.57, $dd$ $J = 5.8, 3.6$ Hz) and the acetyl carbonyl. The presence of acetyl group was also evidenced by EI-MS fragment ion which is also a base peak at $m/z$ 43 corresponding to the acetyl group (Figure 4.19, Appendix 16C). The compound was therefore identified as 3-acetoxy-5,7-dihydroxycarvotacetone previously isolated from *Sphaeranthus suaveolens* [Ahmed and Mahmoud, 1997].

![Figure 4.19: Fragmentation pattern for 3-acetoxy-5,7-dihydroxycarvotacetone (106)](image)

4.2.1.3 3,7-Dihydroxy-5-tigloyloxyxarvotacetone

Compound 88 was isolated as pale yellow oil. Its EI-MS molecular ion at $m/z$ 283 (Appendix 17C) corresponded to the molecular formula of $C_{15}H_{22}O_5 [M+H]^+$. The $^{13}$C-NMR and $^1H$-NMR spectra (Table 4.8, 4.10 and Appendices 17A, 17B) are similar to those for 3,5,7-trihydroxycarvotacetone (85) discussed above. The principle difference was the presence of additional signals for five more carbon atoms at $\delta_c$ 167.7, 138.9,
128.0, 14.4 and 11.9 constituting two sp³ carbons and a carbonyl conjugated to two olefinic carbons.

The corresponding ¹H-NMR indicated that the additional signals constituted two methyl groups (1.82, 3H, dd, J = 7.2, 1.0 Hz; 1.86, 3H, d, J = 1.2 Hz) as well as an olefinic proton (6.93, dq, J = 7.2, 1.2 Hz). These data suggested either a tigloyl group with cis methyls or an angeloyl group with trans methyls [Zdero et al., 1991]. The NOESY interaction between the two methyls suggested that they were cis to each other and hence a tigloyl group. The HMBC correlation of the doublet at 5.76 (H-5) with the tigloyl carbonyl (C-1', δc 167.7) indicated that the tigloyl group was connected to C-5. The EI-MS retro Diels-Alder fragment ions at m/z 114 and 169 (Figure 4.20, Appendix 17C) is consistent with such substitution pattern. The compound was therefore identified as 3,7-dihydroxy-5-tigloyloxy-carvotacetone previously isolated from aerial parts of Sphaeranthus bullatus [Jakupovic et al., 1990].

Figure 4.20: Fragmentation pattern for 3,7-dihydroxy-5-tigloyloxy-carvotacetone (88)
4.2.1.4 3-Acetoxy-7-hydroxy-5-tigloyloxyacarvotacetone

Compound 99 was isolated as pale yellow oil with EI-MS $[M+H]^+$ at $m/z$ 325 ($C_{17}H_{24}O_6$).

The NMR spectroscopic data (Tables 4.8 and 5.10) resembled those for 3,7-dihydroxy-5-tigloyloxyacarvotacetone 88 discussed above. There was however additional signals for an acetyl group ($\delta_C$ 170.3, 20.9; $\delta_H$ 2.08, 3H, $s$). The acetyl carbonyl made HMBC correlation with H-3 (5.65, $dd$, $J = 6.0$, 3.8 Hz) implying that the acetoxy group was connected to C-3.

The compound was therefore identified as 3-acetoxy-7-hydroxy-5-tigloyloxyacarvotacetone previously isolated from *Sphaeranthus confertfolius* [Zdero *et al.*, 1991]. The presence of acetyl and tiglyl was confirmed by EI-MS fragmentations which gave acyllium ions of the acetyl and tiglyl at $m/z$ 43 and 83 respectively (Figure 4.21, Appendix 18C).

![Figure 4.21: Fragmentation pattern for 3-acetoxy-7-hydroxy-5-tigloyloxyacarvotacetone (99)](image-url)
4.2.1.5 5-O-β-D-glucopyranosylcarvotacetone

Compound 232 was isolated as colorless gum with EI-MS [M+H]+ at m/z 331 consistent to the molecular formula of C₁₆H₂₆O₇ (Appendix 19C). The ¹H-NMR (Table 4.9, Appendix 19,) showed among other signals an isopropyl unit (3H δₗ 0.90 d J = 7.0, 3H δₗ 0.95 d J = 7.0, 1H δₗ 2.24 m), a methyl attached to olefinic carbon (3H δₗ 1.77) and an olefinic proton at δₗ 6.87. The ¹³C-NMR had 16 signals including six in the sugar region (δC 105.3, 77.6, 76.1, 74.7, 70.5 and 62.8) assignable to a glucopyranosyl moiety [Yang et al., 2007].
There were also ten carbons forming a carvotacetone moiety defined by the isopropyl unit ($\delta_C$ 15.4, 20.2, 26.2) and the methyl group ($\delta_C$ 15.6) attached to the opposite ends of a six carbon ring containing a carbonyl conjugated to an olefinic group ($\delta_C$ 201.8, 147.2, 134.1) [Onayade et al., 1990]. The sp$^3$ carbon attached to an oxygen at $\delta_C$ 83.5 was established by HMBC (H-5$\rightarrow$C-6) to be adjacent the carbonyl group and was the point of attachment for the glucopyranosyl group (HMBC H-1$\rightarrow$C-5). The link of the glucopyranosyl was established to be $\beta$-linkage from the coupling constant of the anomeric proton which was 7.8 Hz. H-4 and H-5 had axial-axial coupling ($J = 13.0$), a fact used in establishing the relative stereochemistry at position 4 and 5.

The structure was confirmed by HMBC correlations to be 5-O-$\beta$-D-glucopyranosylcarvotanacetone and the NMR data closely correlated to those reported for the same compound from aerial parts of Laggera pterodonta [Yang et al., 2007].

### 4.2.2 $p$-Cymene derivatives

Three $p$-cymene derivatives were isolated from the aerial parts of Sphaerantus bullatus, these are thymol 3-O-$\beta$-glucopyranoside (233), zetaroside B (93) and zetaroside A (234).

![Figure 4.22: $p$-Cymene derivatives isolated from Sphaeranthus bullatus](image)


4.2.2.1 **Thymol-3-β-D-glucopyranoside**

Compound 233 was isolated as a white solid having a molecular ion peak at $m/z$ 312 (C$_{16}$H$_{24}$O$_{6}$). The $^{13}$C-NMR (Table 4.10, Appendix 20B) showed signals corresponding to sixteen carbons. The aromatic ring was evident from the $^{13}$C-NMR spectrum which showed six signals in the aromatic region ($\delta_c$ 116.9, 123.5, 126.3, 135.4, 136.9 and 155.8) and $^1$H-NMR signals for a 1,3,4 trisubstituted benzene ring ($\delta_h$ 7.08 1H $d J = 7.8$ Hz, 6.78 1H $brd J = 7.8$ Hz and 6.99 1H $d J = 0.6$ Hz). Attached to the benzene ring were β-0-D-glucopyranosyl ($\delta_c$ 102.4, 78.1, 77.6, 74.8, 71.4, 62.6); an isopropyl ($\delta_h$ 1.17 3H $d J = 7.2$ Hz, 1.16 3H $d J = 7.2$ Hz, 3.44 1H $sept J = 7.2$ Hz) and a methyl group ($\delta_c$ 21.3, $\delta_h$ 2.25 3H $s$).

The HMBC spectrum revealed that the methyl group at C-1 and the isopropyl group were para to each other constituting a p-cymene skeleton. Thus the O-glucopyranosyl at C-3 was ortho to the isopropyl group evidenced by the HMBC correlations of glucosidic anomeric proton and isopropyl septet with C-3 ($\delta_c$ 155.8). The identity of the sugar was confirmed by hydrolyzing 1 mg of the sample in 0.1 M HCl and comparing the products with authentic sample of D-glucose on the TLC, as well as presence of EI-MS fragment ion due loss of glucopyranosyl radical at $m/z$ 150 (Appendix 20C).

The compound was therefore identified as thymol-3-β-D-glucopyranoside. The spectroscopic data closely correlated to those reported for the same compound reported from aerial parts of *Jasonia montana* [Ahmed and Jakupovic, 1990] as well as from *Sphaeranthus bullatus* [Jakupovic et al., 1990].

80
4.2.2.2  

Zataroside B

Compound 93 was isolated from the aerial parts of *Sphaeranthus bullatus* as white solid, with a weak molecular ion peak observed in the EI-MS spectrum at \( m/z \) 329 (C_{16}H_{24}O_{7}) \([M+H]^+\) (Appendix 21C). The \(^{13}\)C-NMR (Table 4.10, Appendix 21B) had sixteen signals corresponding to sixteen carbons, six of those being for an aromatic ring. The aromatic carbons consisted of two protonated carbons (\( \delta_C 115.9 \) and 117.7) two C-substituted carbons (\( \delta_C 127.0 \) and 134.0) and two O-substituted carbons (\( \delta_C 150.4 \) and 150.5). The other six carbons were oxygen-substituted sp\(^3\) carbons of the glucose unit (\( \delta_C 62.5, 71.4, 75.0, 77.9, 78.1 \) and 104.3). The remaining four carbons were three methyl carbons and a methine carbon.

The \(^1\)H-NMR (Table 4.9, Appendix 21A) showed two aromatic singlets at \( \delta_H 6.51 \) and 6.98 implying a tetrasubstituted benzene ring with two *para* protons. The methyl doublets at \( \delta_H 1.17 \) and 1.18 coupling with a septet at 3.17 were indicative of an isopropyl unit attached to the aromatic ring. A methyl singlet also attached to the aromatic ring was observed at \( \delta_H 2.18 \). HMBC correlations established that the methyl and isopropyl unit were *para* to each other, constituting a monoterpenoid skeleton of *p*-cymene.

The O-glucopyranoside was attached to C-2 (\( \delta_C 150.5 \)), adjacent to the methyl group since both the methyl protons and anomeric proton were making HMBC correlation with C-2. The 7.8 Hz coupling constant of the anomeric proton implied that the glucosidic link was \( \beta \)-linkage.

Compound 93 was therefore identified as 2-O-glucopyranosyl-5-hydroxy-4-isopropyltoluene (Zataroside B) previously from *Zataria multiflora* [Ali et al., 1999].
Table 4.9: \(^1\)H-NMR data for compounds 232, 233, 93 and 234 (600 MHz)

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### Table 4.10: $^{13}$C-NMR data for carvotacetones and $p$-cymene derivatives (150 MHz)

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#### 4.2.2.3 Zataroside A

Compound 234 was isolated as a minor component of zatroside B (93), accounting for about 8% of the mixture. The NMR features (Tables 4.9 and 5.10) were similar to those of 93, the difference being the point of attachment of the $O$-glucopyranosyl unit, where in...
this case it was at position 5, as evidenced by HMBC correlation of anomeric proton ($\delta_H$ 4.66, $d J = 7.8$) with C-5 ($\delta_C$ 149.0) The compound was therefore identified as 5-O-glucopyranosyl-2-hydroxy-4-isopropyltoluene (Zataroside A). The NMR data were compared to those of the same compound reported from Zataria multiflora [Ali et al., 1999] and they matched.

### 4.2.3 Flavonoids

From the aerial parts of S. bullatus, four flavanols and one chalcone were isolated (Figure 4.23). The flavanols are quercetin (235), quercetin-3,7-dimethylether (236), penduletin (237), crysoplenol D (238) and the chalcone 4-hydroxylonchocarpin (239).

![Flavonoids isolated from Sphaeranthus bullatus](image)

**Figure 4.23:** Flavonoids isolated from *Sphaeranthus bullatus*
4.2.3.1 Quercetin

Compound 235 was isolated as yellow crystals from the aerial parts of *Sphaeranthus bullatus*. The $^{13}$C-NMR (Appendix 22B, Table 4.12) gave fifteen signals of a flavonoid, being twelve aromatic carbons for the two benzene rings and two quaternary olefinic carbons conjugated to a carbonyl. The chemical shifts for C-2, C-3 and C-4 ($\delta_c 147.0, 136.7$ and 176.6 respectively) suggested a flavanol, with C-3 bearing a hydroxyl group.

Ring A had hydroxyl group at C-5 (chelated proton of hydroxyl group at $\delta_H 12.17$, s) and another hydroxyl group at C-7, leaving two protonated carbon atoms at meta positions ($\delta_H 6.56$ $d J = 2.2$ Hz, $6.28$ $d J = 2.2$ Hz). Ring B had ortho hydroxyl groups as observed from adjacent oxygenated $sp^2$ carbons of benzene ring ($\delta_c 145.9$ and 148.5; C-3' and C-4' respectively) making a 1,3,4-trisubstituted benzene ring ($\delta_H 7.83$ $d J = 2.0$ Hz, $7.70$ $dd J = 8.4$ & 2.0 Hz, $7.01$ $d J = 8.4$ Hz).

The compound was therefore identified as quercetin, a dietary antioxidant widely found in fruity plants and vegetables [Ameho *et al.*, 2008]. The structure was confirmed by HMBC correlations and comparison with literature NMR data [Shen *et al.*, 1993].

4.2.3.2 Quercetin-3,7-dimethyl ether

Compound 236 was obtained as yellow crystals from the aerial parts of *Sphaeranthus bullatus*. The observed EI-MS molecular ion at m/z 330 corresponded to its molecular formula C_{17}H_{14}O_{7}. The $^1$H and $^{13}$C-NMR spectra (Tables 4.11, 4.12 and Appendices 23A, 23B) resembled those of quercetin (235), the difference being additional signals for two methoxyl groups ($\delta_H 3.82$ 3H s and 3.91 3H s; $\delta_c 60.4$ and 56.3). The methoxyl group at
δ_H 3.82 and δ_C 60.4 was placed at C-3 from HMBC correlation between this signal and C-3 (δ_C 139.6). The second methoxyl group (δ_H 3.91, δ_C 56.3) was at C-7 from HMBC correlation between the methoxy signal (δ_H 3.91) and C-7 (δ_C 167.2) as well as NOE interactions between the methoxy protons with H-6 and H-8.

The compound was therefore identified as quercetin-3,7-dimethyl ether. The NMR data matched those reported in literature for the synthesized compound [Bouktaib et al., 2002]. It is previously reported from Haplopappus integerrimus var. punctatus and other many other plants of the family Asteraceae [Ayanoglu et al., 1981].

4.2.3.3 Penduletin

Compound 237 was isolated from the aerial parts of Sphaeranthus bullatus as yellow crystals. The EI-MS molecular ion was observed at m/z 344 (C_{18}H_{16}O_{7}). The ¹³C-NMR (Table 4.12, Appendix 24B) showed signals for a flavanol as compounds 235 and 236, differences observed in substitution patterns and the number of methoxyl groups where in this case was three (δ_C 57.0, 60.5 and 61.1).

The ¹H-NMR (Table 4.11, Appendix 24A) showed two signals of a symmetrical 1,4-disubstituted benzene ring at δ_H 8.02 and 6.93 (both 2H doublets with J = 9.0 Hz) as well as an aromatic 1H singlet at δ_H 6.76 and three methoxy proton signals at δ_H 3.79, 3.82 and 3.96. The 1,4-disubstituted benzene was ring B from HMBC correlation of H-2' and H-6' (δ_H 8.02 d J = 9.0 Hz) with C-2 (δ_C 158.5). Ring A had one protonated carbon (δ_H 6.76 s, δ_C 92.1) established to be H-8 from HMBC correlations of this proton with C-7.
C-9 and C-10. Positions 5, 6 and 7 were therefore bearing oxygenated carbons, with δC
154.0, 133.4 and 160.6 respectively.

The methoxyl groups were at C-3, C-6 and C-7, from HMBC correlations of δH 3.79 with
C-3 (δC 139.5); δH 3.82 with C-6 (δC 133.4) and δH 3.96 with C-7 (δC 160.6). The
compound was therefore identified as 5,4’-dihydroxy-3,6,7-trimethoxyflavone
(penduletin). NMR data matched those reported for a synthesized compound [Horie et
al., 1998].

4.2.3.4 Cryosplenol D

Compound 238 (M+ 260, C18H16O8) was isolated from aerial parts of Sphaeranthis
bullatus as yellow crystals. NMR features (Tables 4.11, 4.12, Appendices 25A, 25B)
resembled those of penduletin (237), differences being on the substitution pattern of ring
B where in this case there were signals for a 1,3,4-trisubstituted benzene ring (δH 7.00 d J
= 8.4 Hz, 7.61 dd J = 8.4, 2.0 Hz and 7.73 d J = 2.0 Hz). It was therefore established that
ring B had hydroxyl groups at C-3’ and C-4’ (δC 145.9 and 149.1 respectively).
Substitution pattern in ring A and C as well as the position of three metoxyl groups were
as in 237.

The compound was therefore identified to be 5,3’,4’-trihydroxy-3,6,7-trimethoxyflavone
(cryosplenol D) previously isolated from species of the genus Cryosplenium [Jay et al.,
1976].
4.23.5 4-Hydroxylonchocarpin

Compound 239 was isolated as yellow solid from Sphaeranthus bullatus. The $^{13}$C-NMR showed two aromatic rings (twelve signals in the aromatic region) and carbonyl carbon ($\delta_c$ 192.4) conjugated to olefinic carbons ($\delta_c$ 118.3 and 144.3) with trans coupled protons ($\delta_H$ 7.47 $d J = 15.2$ Hz and $7.83 \ d J = 15.2$ Hz) characteristic of a chalcone skeleton.

The $^1$H-NMR (Appendix 26A, Table 4.11) had signals for a symmetrical 1,4-disubstituted benzene ring ($\delta_H$ 7.60 $d J = 8.4$ Hz and 6.90 $d J = 8.4$ Hz) assigned as ring B from HMBC correlation of H-2 and H-6 ($\delta_H$ 7.60 $d J = 8.4$ Hz) with $\beta$-carbon ($\delta_c$ 144.3). The chemical shift of C-4 ($\delta_c$ 158.6) suggested that it was oxygenated, bearing a hydroxyl group as there were no alkoxy signals.

Ring A was 1,2,3,4-tetrasubstituted as observed from two ortho coupled protons ($\delta_H$ 6.38 $d J = 8.8$ Hz and 7.75 $d J = 8.8$ Hz). A cyclized prenyl ($\delta_H$ 1.45 s, 5.62 $d J = 9.8$ Hz, 6.72 $d J = 9.8$ Hz) was attached to ring A as confirmed by the EI-MS acyllium ion fragment at $m/z$ 203 (Figure 4.24, Appendix 26C). The carbon at C-2' had hydroxyl group that gave rise to a chelated proton signal ($\delta_H$ 13.81 s). HMBC correlation of H-1'' ($\delta_H$ 6.72 $d J = 9.8$ Hz) with C-3' ($\delta_c$ 109.7) and C-2' ($\delta_c$ 161.3) implied that the prenyl was connected at C-3' and cyclizing at C-4'.

The compound was therefore identified as 4,2'-dihydroxy-4',3'-(2,2-dimethylpyrano)chalcone (4-hydroxylonchocarpin) with molecular formula of C$_{20}$H$_{18}$O$_4$ and molecular weight of 322 as indicated by EI-MS molecular ion. Physical and
spectroscopic data closely correlated to those reported for the same compound from *Mundulea sericea* [Luyengi et al., 1994].

![Fragmentation pattern for 4-hydroxylonchocarpin (239)](image)

**Figure 4.24:** Fragmentation pattern for 4-hydroxylonchocarpin (239)

**Table 4.11:** $^1$H-NMR data for flavonoids from *S. bullatus* (600 MHz)

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<th>237 MeOD</th>
<th>238 Acetone- $d_6$</th>
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4.2.4 Phenyl propanoids

Two phenyl propanoids were isolated, caffèic acid (240) and coniferaldehyde (241).

![Figure 4.25: Phenyl propanoids isolated from Sphaeranthus bullatus](image)

4.2.4.1 Caffeic acid

Compound 240 was isolated as pale brownish crystals. The $^{13}$C-NMR (Table 4.12, Appendix 27B) had a total of nine signals for a phenyl propanoid, being six signals for an aromatic ring and two for olefinic carbons conjugated carboxylic acid carbonyl at $\delta_c$ 168.3 ppm. The ortho hydroxyl groups were evident from the adjacent oxygenated aromatic carbons ($\delta_c$ 146.3 and 148.7).

The $^1$H-NMR (Appendix 27A) had signals for a 1,3,4-trisubstituted benzene ring ($\delta_H$ 6.88 $d J = 8.2$ Hz, 7.05 $dd J = 8.2$, 1.8 Hz, 7.17 $d J = 1.8$ Hz) and signals for trans olefinic protons ($\delta_H$ 6.07 $d J = 15.8$ Hz, 7.55 $d J = 15.8$ Hz). The compound was therefore identified as 3-(3,4-dihydroxyphenyl)-2-propenoic acid (caffeic acid), an antioxidant commonly consumed through coffee beverages [Andueza et al., 2009].
4.2.4.2 Coniferaldehyde

Compound 241 (M⁺ 178, C₁₀H₁₀O₃) was isolated as light yellow crystals. Its NMR spectral features (Table 4.12, Appendices 28A and 28B) resembled those of caffeic acid (240), the difference being replacement of carboxylic acid by aldehyde functionality (δC 192.7, δH 9.63, d = 7.8) and presence of methoxy signal (δC 55.3, δH 3.93, s). The methoxy group was attached to C-3 as shown by HMBC correlation (H-OMe at δH 3.93 → C-3 at 146.3). The compound was therefore identified as 3-(4-hydroxy-3-methoxyphenyl)-2-propenal (coniferaldehyde). The NMR data matched those reported for the same compound isolated from *Artemisia annua* [Sy and Brown, 1999].

Table 4.12: $^{13}$C-NMR data for 235, 236, 237, 238, 240 and 241 (150 MHz)

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<th>237 MeOD</th>
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4.2.5 Triterpenoids

Two triterpenoids were isolated from the aerial parts of *S. bullatus*, dammara-20,24-dienylacetate (242) and boehmery acetate (243).

![Diagram of triterpenoids]

**Figure 4.26:** Triterpenoids isolated from *Sphaeranthus bullatus*

4.2.5.1 Dammara-20,24-dienylacetate

Compound 242 was isolated as white crystals from the roots of *Sphaeranthus bullatus*. The thirty-two carbon signals in the 13C-NMR (Table 4.13, Appendix 29B) which included acetoxy carbons (δc 171.0, 21.4) suggested that it was a triterpene with an acetoxy group. The olefinic region had four carbon signals, two of them being quaternary (δc 150.2, 131.7), one methine (δc 124.9, δh 5.13 J = 7.2) and one methylene (δc 107.7, δh 4.70 and 4.74, both doublets with J = 1.0 Hz). The sp³ region of 1H-NMR showed, among other signals, eight methyl groups which were all singlets as well as a characteristic down field shifted H-3 signal (δh 4.44 dd J = 10.0, 6.6 Hz) of triterpenes having acetoxy group at C-3.

The molecular formula of the triterpene (C32H52O2) determined from MS (m/z 468) and DEPT spectra corresponds to DBE of seven, suggesting that it was a tetracyclic triterpene with two double bonds and a carbonyl which belonged to the acetoxy group. The carbon
connectivity was established using combination of HMBC, HSQC and COSY experiments to give a dammarane skeleton with doublebonds at positions 20(21) and 24(25) [Leong and Harrison, 1999], as well as an acetoxy group at C-3. The compound was therefore identified as 3-acetoxydammara-20,24-diene previously isolated from Olearia paniculata [Corbett et al., 1964], and Santolina corsica [Ferrari et al., 2005].

4.2.5.2  Boehrmyry acetate

Compound 243 was isolated as white crystals from the roots of Sphaeranthus bullatus. It had thirty-two carbons from the $^{13}$C-NMR (Table 4.13 Appendix 30B), being two acetoxy carbons ($\delta_\text{C}$ 171.1, 21.4), two olefinic quarternary carbons ($\delta_\text{C}$ 131.6, 142.1) and twenty-eight $sp^3$ carbons. The connectivity of carbons was followed using HSQC, HMBC and COSY spectra to give a neohopane triterpene [Mahato and Sen, 1997] having an acetoxy at C-3 and double bond at C-13(18) ($\delta_\text{C}$ 131.6, 142.1) [Chakravarty, 1994].

The $^1$H-NMR (Table 4.13, Appendix 30A) showed a total of nine methyl groups, seven being singlets and two doublets ($\delta_\text{H}$ 0.90 $d J = 6.6$ Hz, 0.94 $d J = 6.6$ Hz) assignable to an isopropyl unit attached at C-21. It also showed a characteristic deshielded signal of H-3 proton ($\delta_\text{H}$ 4.47 $dd J = 11.6, 5.3$ Hz) for a triterpenes oxygenated at C-3. This proton (H-3) was making HMBC correlation with acetoxy carbonyl ($\delta_\text{C}$ 171.1) suggesting that the acetoxy is at C-3. The triterpene was therefore identified as boehrmyry acetate, with molecular formula $C_{32}H_{52}O_{2}$ consistent with EI-MS molecular ion peak at $m/z$ 468. It was previously reported from Pluchea lanceolata [Chawla et al., 1990].

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Table 4.13: $^1$H-NMR (600 MHz) and $^{13}$C-NMR (150 MHz) data for triterpenoids from *S. bullatus* (CD$_2$Cl$_2$)

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4.2.6 General observations on compounds of *S. bullatus*

Out of the seventeen compounds isolated from *Sphaeranthus bullatus*, five were known to the genus, being the carvotacetones 85, 88, 99 and 106 as well as the *p*-cymene 93. Twelve compounds were thus new to the genus. These are 5-*O*-β-glucopyranosylcarvotacetone (232), thymol 3-*O*-β-glucopyranoside (233), zetaroside A (234), quercetin (235), quercetin-3,7-dimethylether (236), penduletin (237), crysoplenol D (238), 4-hydroxylonchocarpin (239), caffeic acid (240), coniferaldehyde (241), dammara-20,24-dienylacetate (242) and boehmery acetate (243).

For the carvotacetones, the relative stereochemistry at positions 3, 4 and 5 were the same, as established by NOESY experiments, with H-3 and H-4 showing NOE interactions indicating that the two protons are *cis* to each other, as supported by their axial-equatorial coupling constants in the range of 3.4 – 4.0 Hz. H-4 and H-5 had no NOE interactions, implying that they were *trans* to each other as indicated by their axial-axial coupling constants in the range of 12 – 13 Hz.

4.3 Secondary metabolites isolated from *Vernonia galamensis* ssp *nairobensis*

4.3.1 Flavanols

Chromatographic separations on the extract of aerial parts of *Vernonia galamensis* gave seven compounds, five of them being flavanols of quercetin (235) and derivatives of quercetin namely quercetin 3-methylether (244), quercetin 3,7-dimethylether (236), quercetin 3-*O*-β-*D*-galactoside (212) and quercetin 3-*O*-a-rhamnoside (245).
Figure 4.27: Flavanols isolated from *V. galamensis* ssp *nairobensis*

Two of the compounds, quercetin (235) and quercetin 3,7-dimethylether (236), were also isolated from *Sphaeranthus bullatus* and have been discussed above in sections 4.2.9 and 4.2.10 respectively.

### 4.3.1.1 Quercetin 3-methyl ether

Compound 244 was isolated as yellow crystals from the aerial parts of *vernonia galamensis*. Its EI-MS molecular ion at *m/z* 316 (Appendix 31C) corresponded to molecular formula C_{16}H_{12}O_{7}. The $^1$H and $^{13}$C-NMR data (Tables 4.14 and 4.15, Appendices 31A and 31B) closely correlated to those of quercetin (235) except for additional signals of a methoxyl group ($\delta_{C}$ 59.1, $\delta_{H}$ 3.77 s). The methoxy proton signal showed HMBC correlation with C-3 ($\delta_{C}$ 138.1) consistent to its placement at C-3.
The compound was therefore identified as quercetin-3-methyl ether, mostly found alongside other similar flavonoids in fruits, vegetables and plant derived products like wine and tea [Duenas et al., 2010]. Physical and spectroscopic data matched with those reported in literature [Bouktaib et al., 2002].

4.3.1.2 Quercetin-3-O-β-galactopyranoside

Compound 212 was isolated from the aerial parts of *Vernonia galamensis* as pale yellow crystals. The EI-MS spectrum showed a weak molecular ion peak at m/z 464 (C_{21}H_{20}O_{12}).

The aromatic and olefinic regions of both proton and carbon-13 NMR spectra (Tables 4.14, 4.15; Appendices 32A, 32B) had characteristic signal peaks of quercetin (235). The sp^3 region however had six signals in the ^{13}C-NMR including a methine acetal carbon at δ_c 101.8, four other oxy-methine carbons (δ_c 68.0, 71.2, 73.2, 75.9) and a hydroxylmethylene carbon at δ_c 60.2. It was evident that there was a cyclic hexose sugar unit. The anomeric proton was observed at δ_H 5.39, with a coupling constant of 7.6 Hz implying that the sugar unit had β-linkage to the quercetin aglycone.

The identity of the sugar was established through analysis of coupling constants, showing axial coupling for the anomeric proton (H-1”), axial-axial coupling for H-2”, axial-equitorial for H-3” and equatorial-axial for H-4”. The H-4” proton was therefore in equatorial position, making it a β-D-galactopyranosyl. The anomeric proton showed HMBC correlation with C-3 of quercetin aglycone (δ_C 133.5) implying that the β-D-galactopyranosyl was attached at C-3. The compound was therefore identified as quercetin-3-O-β-galactopyranoside. The NMR data were in agreement with those reported for the same compound from the flowers of *Erica sinerea* [Bennini et al., 1992].
The compound had also been isolated before from the aerial parts of *Vernonia galamensis* ssp *nairobensis* [Miserez *et al.*, 1996].

### 4.3.1.3 Quercetin 3-0-α-rhamnoside

Compound 245 was isolated from the aerial parts of *Vernonia galamensis* as pale yellow powder. The aromatic and olefinic regions of both proton and carbon-13 NMR were similar to those of quercetin (235). The sp³ region displayed signals for sugar moiety; the acetal methine signals (δc 101.8, δh 5.26 d J = 1.2 Hz), four other oxygen-attached methine carbons (δc 70.1, 70.2, 70.4, 70.6) and signals for a methyl (δc 17.5, δh 0.82 d J = 6.0 Hz). The connectivity of a sugar unit was followed by COSY, coupling constants and HMBC to establish a rhamnosyl moiety.

The linkage between the sugar moiety and the quercetin aglycone was established to be at C-3 of the quercetin from the HMBC correlation between the anomeric proton and C-3 (δc 134.2) of the quercetin aglycone. The linkage was established to be α-linkage from the coupling constant of the anomeric proton which was 1.2 Hz. NMR data correlated to those reported for quercetin 3-0-α-rhamnoside (quercitrin) from leaves of *Myrsine seguini* [Zhong *et al.*, 1997].

### 4.3.2 Lignan and benzenoid

One lignan and one benzenoid were isolated from the aerial parts of *V. galamensis* ssp *nairobensis*. The lignan was identified as syringaresinol (246) while the benzenoid was syringic acid (247).
4.3.2.1 Syringaresinal

Compound 246 was isolated as yellowish oil. The EI-MS molecular ion peak was observed at $m/z$ 418 ($C_{22}H_{26}O_{8}$) though the $^{13}$C-NMR (Appendix 34B) had only eight signals, this suggested presence of symmetry within the molecule as observed among symmetrically substituted lignans [Abe and Yamauchi, 1988]. NMR data (Tables 4.14, 1.15; Appendices 34A, 34B) showed a 3,5-dimethoxy-4-hydroxyphenyl from the aromatic signals ($\delta_C$ 103.1, 132.9, 134.6, 147.5; $\delta_H$ 6.5 brs) and methoxy signal ($\delta_C$ 56.7; $\delta_H$ 3.87 s). There was also an oxy-methylene (-OCH$_2$-, $\delta_C$ 72.2; $\delta_H$ 4.25 $dd$ $J = 9.0, 7.2$ Hz; 3.88 $ddd$ $J = 9.0, 3.8$ Hz) and two methines, one attached to oxygen ($\delta_C$ 86.4; $\delta_H$ 4.69 $d$ $J = 4.2$ Hz) and the other attached to carbons ($\delta_C$ 54.9; $\delta_H$ 3.07 $ddd$, $J = 7.2, 4.4, 3.8$ Hz) characteristic of furanoid rings of lignans [Das et al., 1998].

These NMR data resembled those obtained for furanoid lignans having 3,5-dimethoxyphenyls [Abe and Yamauchi, 1988]. HMBC correlations and coupling constants confirmed the compound to be the lignan furanoid called syringaresinal.
previously reported from *Allamanda neriifolia* [Abe and Yamauchi, 1988] and *Parthenium hysterophorus* [Das et al., 1998].

### Table 4.14: $^1$H-NMR data for flavanols and lignan from *V. galamensis* (600 MHz)

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<td>4.25, $dd$ (9.0, 7.2) 3.88, $dd$ (9.0, 3.8) 6.59, $s$</td>
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Table 4.15: $^{13}$C-NMR data for flavanols and lignan from *V. galamensis* ssp *nairobensis* (150 MHz)

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3-OMe | 59.1  | 56.7  |
5-OMe |      | 56.7  |
3'-OMe|      | 56.7  |
5'-OMe|      | 56.7  |
Compound **247** (M⁺ 198, C₉H₁₀O₅) was isolated from the aerial parts of *Vernonia galamensis* as white powder. Its ¹H-NMR (Appendix 35A) had only two signals, a singlet for aromatic proton at δₜ 7.33 and a singlet for methoxy protons at δₜ 3.89. The ¹³C-NMR (Appendix 35B) had six signals, a carboxylic acid carbonyl at δₗ 167.6, four aromatic carbon signals at δₗ 148.3, 141.5, 121.4 and 108.0 as well as a signal for methoxy carbon at 56.6.

The aromatic proton was showing HMBC correlation with its carbon of attachment implying existence of symmetrically identical carbon atom two or three bonds away from the proton. The presence of carboxylic acid carbonyl signal alongside four aromatic carbon signals suggested a symmetrical benzoic acid derivative. The position of the aromatic protons on the benzoic acid skeleton was established to be C-2 and C-6 following the HMBC correlation between the aromatic protons signal and the carbonyl C-1; δₗ 167.6) meeting the symmetrical requirements. The methoxyl groups, which were two to fit the symmetry, were at C-3 and C-5 following the HMBC correlation between the methoxyl protons signal and C-3, C-5 (δₗ 141.3) The carbon at C-4 (δₗ 148.3) was bearing a hydroxyl group. The compound was therefore identified to be 4-hydroxy-3,5-dimethoxybenzoic acid (syringic acid), an antioxidant constituent of soybean (*Glycine max*) [Porter et al., 1986].
Secondary metabolites isolated from Microglossa pyrifolia

4.4.1 Clerodane diterpenoids

From the aerial parts of Microglossa pyrifolia, six compounds were isolated. Two were rearranged clerodane diterpenoids, 8-acetoxyisochiliolide lactone (248) and epoxyisochiliolide lactone (157).

Figure 4.29: Rearranged clerodane diterpenoids from Microglossa pyrifolia

4.4.1.1 8-Acetoxyisochiliolide lactone

Compound 248 was isolated from the CH2Cl2 extract of Microglossa pyrifolia as white crystals. Its molecular formula was deduced to be C22H26O7 from the HR-EIMS molecular ion peak at 402.1700 (calc for 402.1679). Its DBE of 10 was accounted by two olefinic double bonds (four olefinic carbons at δC 108.5, 126.0, 140.0 and 144.5), three ester carbonyls (δC 169.6, 172.5 and 177.5) and the remaining five was presumed to be for five rings.

The four olefinic carbons made a furanyl ring, whose protons appered at δH 6.45 (d, 1.5 Hz), 7.45 (d, 1.5 Hz) and 7.49 (s). Two of the ester carbonyls (δC 172.5 and 177.5) were part of two lactone rings where as the one at δC 169.6 was an acetoxy carbonyl as it had
HMBC correlation with a methyl singlet at $\delta_H$ 1.79. Three more methyl singlets were observed, two of which being vicinal methyls at $\delta_H$ 1.08 and 1.13 and the third methyl at $\delta_H$ 1.93. These NMR data resembled those reported for the pentacyclic isochiliolide lactone diterpenoid, 8-hydroxyisochiliolide lactone (161) reported from *M. pyrrhopappa* [Zdero *et al.*, 1990].

The difference from 116 was the additional acetoxy signals in 248 ($\delta_C$ 22.2, 169.6; $\delta_H$ 1.79). It was evident that the hydroxyl in 161 had been replaced by acetoxy in 248. The compound was therefore identified as 8-acetoxyisochiliolide lactone, which is a new compound. The structure was confirmed by HMBC correlations presented in Table 4.16.

### 4.4.1.2 7,8-Epoxyisochiliolide lactone

Compound 157 was isolated as white crystals from the dichloromethane extract of the aerial parts of *Microglossa pyrifolia*. The $^{13}$C-NMR (Table 4.16, Appendix 37B) had twenty signals implying that it was a diterpenoid with twenty carbons. Among the carbons were four olefinic carbons ($\delta_C$ 144.8, 140.8, 124.8, 108.5) for a furanyl ring ($\delta_H$ 7.58 $d_J$ = 1.2 Hz, 7.51 br.s, 6.51 br.s), two lactone carbonyls at $\delta_C$ 172.2, 178.1 and three methyls at $\delta_C$ 9.7, 17.4 and 20.8, suggesting a clerodane diterpenoid of isochiliolide lactone skeleton [Zdero *et al.*, 1990]. The adjacent oxygen bearing carbons at position 7 and 8 ($\delta_C$ 62.8, 60.8) were deduced to be forming an epoxide from the EIMS molecular ion at $m/z$ 360 (Appendix 37C) and absence of OH stretch frequencies in the IR spectrum. The spectroscopic data correlated to the data reported for 7,8-epoxyisochiliolide lactone isolate from *M. pyrrhopappa* [Zdero *et al.*, 1990] confirming that it was the same compound.
Table 4.16: $^1$H-NMR (600 MHz) and $^{13}$C-NMR (150 MHz) data for isochiliolide lactones 248 and 157 (CD$_2$Cl$_2$)

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$^1$H and $^{13}$C NMR data for isochiliolide lactones 248 and 157 in CD$_2$Cl$_2$. The HMBC column indicates the correlations observed in the 2D NMR experiments.
4.4.2 Steroids

Two steroids were isolated from the aerial parts of *Microglossa pyrifolia*, stigmasterol (66) and β-sitosterol (249).

![Figure 4.30: Steroids from Microglossa pyrifolia](image)

4.4.2.1 Stigmasterol

Compound 66 was isolated as clear white crystals. The $^{13}$C-NMR (Table 4.17, Appendix 38B) had twenty nine signals for a steroid, among them four olefinic carbons ($\delta_C 141.3 \ s, 121.8 \ d, 138.8 \ d, 129.6 \ d$), the rest were $sp^3$ carbons, one ($\delta_C 72.0 \ d$) being attached to oxygen. The DEPT experiment showed that the compound has six methyl groups, nine methylenes, eleven methines and three quaternary carbons. All spectroscopic data matched well with those reported for stigmasterol [Blunt and Stothers, 1977], a steroid that is widespread in plants.

5.4.2.2 β-Sitosterol

Compound 249 was isolated as clear white crystals. The $^{13}$C-NMR (Table 4.17, Appendix 39B) had twenty nine signals for a steroid, among them two olefinic carbons ($\delta_C 141.4 \ s, 121.8 \ d$), the rest were $sp^3$ carbons, one ($\delta_C 72.1 \ d$) being attached to oxygen. The DEPT experiment showed that the compound has six methyl groups, eleven methylenes, nine
methines and three quaternary carbons. NMR data matched well with those reported for 
\(\beta\)-sitosterol [Blunt and Stothers, 1977], a steroid that is wide spread in plants.

### 4.4.3 Triterpenoid and sesquiterpenoid

One triterpenoid and one eudesman sesquiterpene were also isolated from the aerial parts of *Microglossa pyrifolia*. The triterpenoid was friedelan-3-one (250) and the sesquiterpenoid was 1,4-dihydroxy-7(11)-eudesmen-8-one (251).

**Figure 4.31: Triterpenoid and sesquiterpenoid from *Microglossa pyrifolia***

5.4.3.1 Friedelan-3-one

Compound 250 was isolated as white crystals from \(n\)-hexane extract of aerial parts of *Microglossa pyrifolia*. The \(^{13}\)C-NMR (Appendix 40B, Table 4.17) had thirty carbon signals of a triterpene, all of them being \(sp^3\) carbons except for a carbonyl carbon at \(\delta_C\) 212.9. DEPT experiment showed seven quaternary carbons, four methines, eleven methylenes and eight methyls. Of these, the only carbon attached to an electronegative atom was the carbonyl carbon, as deduced from chemical shift analysis. The molecular
formula was therefore deduced to be C_{30}H_{50}O (M^+ 426) with DBE of six, implying that it was a pentacyclic triterpene with one carbonyl.

The ^1H-NMR indicated that of the eight methyl groups, seven were singlets and one was a doublet with coupling constant of 6.6 Hz, showing HMBC correlation with the carbonyl. This implied that the triterpene was a 6-6-6-6-6 pentacyclic triterpene of friedelane skeleton with carbonyl at position three. Physical and spectroscopic data matched those reported for friedelan-3-one isolated from *Salacia beddomei* [Hisham et al., 1995].

**Table 4.17:** $^{13}$C-NMR data for Compounds 66, 249, 250 and 251 (150 MHz, CD$_2$Cl$_2$)

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Table 4.18: $^1$H-NMR data for Compounds 66, 249 and 250 (600 MHz, CD$_2$Cl$_2$)

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<td>1.50, m</td>
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<td>3.45, s</td>
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<td>1.00, s</td>
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<td>1.52, m</td>
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<td></td>
<td></td>
<td>1.03, m</td>
<td>0.93, m</td>
</tr>
<tr>
<td>23</td>
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<td>1.18, m</td>
<td>0.83, d (6.6)</td>
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<td>0.95, m</td>
<td>0.78, s</td>
</tr>
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<td>1.67, m</td>
<td>0.87, s</td>
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<td>0.82, d (6.9)</td>
<td>1.01, s</td>
</tr>
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<td>0.84, d (6.9)</td>
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<td>1.18, s</td>
</tr>
<tr>
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<td>0.85, t (7.4)</td>
<td>1.00, s</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>0.95, s</td>
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</table>
Compound 251 was isolated from the aerial parts of *Microglossa pyrifolia* as pale yellow oil. The $^{13}$C-NMR (Appendix 41B, Table 5.17) had fifteen signals of a sesquiterpene with a carbonyl at $\delta_C$ 207.4, two quaternary olefinic carbons at $\delta_C$ 134.6 and 138.2 as well as twelve sp$^3$ carbons. The HMBC spectrum showed that the olefinic carbon at $\delta_C$ 138.2 was attached to two methyl groups of $\delta_H$ 1.78 and 1.85 ($\delta_C$ 21.0 and 23.2 respectively). Two more methyl groups were observed at $\delta_H$ 1.10 and 1.40, both being singlets implying that they are attached to quaternary carbons. With the aid of coupling constants, COSY, HSQC and HMBC the compound was established to be an eudesman sesquiterpene derivative namely 1,4-dihydroxy-7(11)-eudesmen-8-one.
5.1 **Antiplasmodial activity of compounds**

As a follow up to the antiplasmodial activities shown by the extracts of the investigated plants (Table 1.1), *in-vitro* antiplasmodial tests were done for the compounds isolated from the plants. The tests aimed at identifying active components which contributed to the observed activities of crude extracts. The compounds tested are the abietane diterpenoids from *C. eriophyllum*, isochiliolide lactone diterpenoids from *M. pyrifolia*, carvotacetone and *p*-cymene derivatives from *S. bullatus* and flavanols from *S. bullatus* and *V. galamensis*. Two *P. falciparum* strains were used. Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine-resistant). Activities were observed for three abietane diterpenoids and two carvotacetone derivatives.

The abietane diterpenoids with antiplasmodial activities were taxodione (222), 6-hydroxysalvinolone (226) and 6,16-dihydroxysalvinolone (227) whereas the carvotacetone derivatives were 3,5,7-trihydroxycarvotacetone (85), 3-acetoxy-5,7-dihydroxycarvotacetone (106), 3,7-dihydroxy-5-tigloyloxycarvotacetone (88) and 3-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (99). Cytotoxicities of the compound were measured simultaneously, and the only compounds found with cytotoxicity were 6-hydroxysalvinolone (226) and 3-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (99). These results are summarized in Table 5.1 and Figure 5.1.
Table 5.1: *In-vitro* antiplasmodial activity and cytotoxicity

<p>| Compound                                                       | <em>In-vitro</em> antiplasmodial activity (IC₅₀) | Cytotoxicity (IC₅₀) |</p>
<table>
<thead>
<tr>
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<th>D6 (µg/ml)</th>
<th>W2 (µg/ml)</th>
<th>(µg/ml)</th>
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<tbody>
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<td>Royleanone (21)</td>
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<td>NA</td>
<td>NC</td>
</tr>
<tr>
<td>Taxodione (222)</td>
<td>1.2</td>
<td>1.2</td>
<td>NC</td>
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<tr>
<td>Ferruginol (224)</td>
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<td>NA</td>
<td>NC</td>
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<td>6,16-Dihydroxysalvinolone (227)</td>
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<td>Friedelan-3-one (250)</td>
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<td>NC</td>
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<tr>
<td>Chloroquine (standard)</td>
<td>&lt;0.026</td>
<td>0.14</td>
<td>NC</td>
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</table>

NA = Not Active; NC = Not Cytotoxic (up to the maximum dose tested 5.0 µg/ml).
Compounds found active are all highly oxygenated, with hydroxyl, carbonyl and ester groups. Literature shows that these groups enhance lipophilicity of compounds hence making transportation of these compounds from site of application to site of action easier [Lacks and Pruner, 1989], and that the hydroxyls enhance binding to the receptor site and act as inhibitors of parasitic enzymes, therefore contributing to bioactivities [Collins et al., 2005]. However, other structural properties have role in bioactivities, as there were no proper correlations between the number of these groups in the molecule and activity of the compound, and some compounds with these groups were inactive.
Compounds from *Microglossa pyrifolia* and *Vernonia galamensis* did not show antiplasmodial activities. This interprets that the activity of their extracts are either due to synergistic effect or contributed by trace compounds which were not isolated.

5.2 **Radical scavenging activity of compounds**

Thirty-eight of the isolated compounds were tested for anti-oxidant activity. The compounds were first subjected to preliminary qualitative testing on a TLC, and all triterpenoids, steroids, clerodane diterpenoids and carvotacetone derivatives were inactive. Activities were observed among the abietane diterpenoids, flavanols, phenyl propanoids, *p*-cymene derivatives and benzenoids in general. Compounds which showed activities on TLC assays were quantitatively analysed by UV-VIS absorption measurements on the compound solutions with DPPH. The results are presented in Table 5.2, 5.3, and 5.4 as well as Figure 5.2.

**Table 5.2**: Radical scavenging activity of flavonoids against DPPH

<table>
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</thead>
<tbody>
<tr>
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<td>µg/ml</td>
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<tr>
<td>Quercetin (standard)</td>
<td>6.0 ± 0.2</td>
</tr>
<tr>
<td>Quercetin 3,7-dimethylether (236)</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>Quercetin 3-methylether (244)</td>
<td>6.6 ± 0.3</td>
</tr>
<tr>
<td>Quercetin 3-α-D-galactopyranoside (212)</td>
<td>9.3 ± 0.4</td>
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<tr>
<td>Crysoplenol D (238)</td>
<td>10.4 ± 0.5</td>
</tr>
<tr>
<td>Quercetin 3-α-D-rhamnopyranoside (245)</td>
<td>12.9 ± 0.4</td>
</tr>
<tr>
<td>Penduletin (237)</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>4-Hydroxylonchocarpin (239)</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>
Table 5.3: Radical scavenging activity of abietane diterpenoids against DPPH

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µg/ml)</th>
<th>µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nellionol (228)</td>
<td>10.2 ± 0.5</td>
<td>29.3</td>
</tr>
<tr>
<td>6-Hydroxysalvinolone (226)</td>
<td>17.7 ± 0.4</td>
<td>53.6</td>
</tr>
<tr>
<td>6,16-Dihydroxysalvinolone (227)</td>
<td>20.5 ± 0.5</td>
<td>59.2</td>
</tr>
<tr>
<td>Taxodione (222)</td>
<td>31.5 ± 0.8</td>
<td>100.0</td>
</tr>
<tr>
<td>Ferruginol (224)</td>
<td>76.3 ± 3.6</td>
<td>267</td>
</tr>
<tr>
<td>Sugiol (9)</td>
<td>&gt; 100</td>
<td>-</td>
</tr>
<tr>
<td>Royleanone (21)</td>
<td>&gt; 100</td>
<td>-</td>
</tr>
<tr>
<td>11-Hydroxy-7,9,13-abietatrien-12-one (223)</td>
<td>&gt; 100</td>
<td>-</td>
</tr>
<tr>
<td>11-hydroxy-8,11,13-abietatriene 12-O-β-xylopyranoside (225)</td>
<td>&gt; 100</td>
<td>-</td>
</tr>
<tr>
<td>Uncinatone (19)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>3-Oxoroyleanone (220)</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

Table 5.4: Radical scavenging activity of other phenolics against DPPH

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µg/ml)</th>
<th>µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid (240)</td>
<td>2.6 ± 0.3</td>
<td>14.4</td>
</tr>
<tr>
<td>Syringic acid (247)</td>
<td>8.3 ± 0.4</td>
<td>41.9</td>
</tr>
<tr>
<td>Coniferaldehyde (241)</td>
<td>14.1 ± 0.6</td>
<td>79.2</td>
</tr>
<tr>
<td>Syringaresinol (246)</td>
<td>21.0 ± 0.5</td>
<td>50.2</td>
</tr>
<tr>
<td>Zetroside B (93)</td>
<td>28.9 ± 0.6</td>
<td>88.1</td>
</tr>
<tr>
<td>Tyrosylocosanoate (231)</td>
<td>&gt; 100</td>
<td>-</td>
</tr>
<tr>
<td>Thymol 3-O-β-glucopyranoside (233)</td>
<td>&gt; 100</td>
<td>-</td>
</tr>
</tbody>
</table>
The most active compound was caffeic acid (240), an established anti-oxidant found in coffee beverages [Andueza et al., 2009]. Its radical scavenging activity of 2.6 µg/ml (14.4 µM) was more than that of the standard used, quercetin (IC₅₀ 6.0 µg/ml; 19.9 µM).
All of the compounds found to have radical scavenging activities had phenolic moiety oxygenated at either ortho or para positions, explained by the ability of hydroxyl groups at these positions to reduce radicals and form quinones [Chen et al., 1996], as illustrated for compounds 226 and 93 in Figure 5.3.

![Figure 5.3: Reduction of radicals by compounds with phenolic ortho or para oxygenation.](image)

### 5.3 Antimicrobial activities

The isolated compounds were tested for antifungal and antibacterial activities, using fungi *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Cryptococcus neoformans*, *Aspergillus fumigatus* as well as the bacteria *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRS), *Escherichia coli*, *Pseudomonas aeruginosa* and *Mycobacterium intracellulare*. The results are summarized in Table 5.5 for antifungal activities and Table 5.6 for antibacterial activities. Taxodione (222), 6-
hydroxysalvinolone (226) and 6,16-dihydroxysalvinolone (227) showed antifungal activity, the best activity observed with taxodione (222) on *Cryptococcus neoformans* (IC₅₀ 0.58 µg/ml).

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀/MIC (µg/ml)</th>
<th>C. glabrata</th>
<th>C. krusei</th>
<th>C. neoformans</th>
<th>A. fumigatus</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Royleanone (21)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Taxodione (222)</td>
<td>5.2/10.0</td>
<td>12.0/-</td>
<td>0.58/1.25</td>
<td>8.9/-</td>
<td>12.5/-</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Ferruginol (224)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>11-hydroxy-8,11,13-abietatriene 12-O-β-xylopyranoside (225)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>6-Hydroxysalvinolone (226)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>0.96/2.5</td>
<td>11.2/-</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>6,16-Dihydroxysalvinolone (227)</td>
<td>14.9/20.0</td>
<td>14.5/20.0</td>
<td>5.9/20.0</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>uncinitone (19)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Bongardol (231)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>3,5,7-Trihydroxycarvotaceton (85)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>3-Acetoxy-7-hydroxy-5-tigloyloxyacarvotaceton (99)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>3-O-β-Glucopyranoside (233)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Zetaroside B (93)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Quercetin-3,7-dimethylether (236)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Penduletin (237),</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Cryosplenol D (238)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Quercetin 3-O-β-D-galactoside (212)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Quercetin 3-O-α-rhamnoside (245)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>8-Acetoxyisochiliolide lactone (248)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Epoxysalvinolone lactone (157)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Stigmasterol (66)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Friedelan-3-one (250)</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Amphotericin B (standard)</td>
<td>0.31/0.65</td>
<td>0.95/1.25</td>
<td>0.44/1.25</td>
<td>1.29/2.50</td>
<td>0.43/1.25</td>
<td>NA/NA</td>
</tr>
</tbody>
</table>

NA = Not Active
Antibacterial activities were exhibited by taxodione (222), Ferruginol (224), 6-hydroxysalvinolone (226) and 6,16-dihydroxysalvinolone (227), the best activity being of ferruginol (224) on methicillin-resistant *Staphylococcus aureus* (MRS) (IC$_{50}$ 0.96 µg/ml).

**Table 5.6: Antibacterial activities**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$/MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>Royleanone (21)</td>
<td>NA</td>
</tr>
<tr>
<td>Taxodione (222)</td>
<td>1.35/5</td>
</tr>
<tr>
<td>Ferruginol (224)</td>
<td>1.33/2.5</td>
</tr>
<tr>
<td>11-hydroxy-8,11,13-abietatriene</td>
<td>NA</td>
</tr>
<tr>
<td>12-O-β-xylopyranoside (225)</td>
<td>NA</td>
</tr>
<tr>
<td>6-Hydroxysalvinolone (226)</td>
<td>1.75/5</td>
</tr>
<tr>
<td>6,16-Dihydroxysalvinolone (227)</td>
<td>6.8/20</td>
</tr>
<tr>
<td>Uncinatone (19)</td>
<td>NA</td>
</tr>
<tr>
<td>Bongardol (231)</td>
<td>NA</td>
</tr>
<tr>
<td>3,5,7-Trihydroxycarvotacetone (85)</td>
<td>NA</td>
</tr>
<tr>
<td>3-Acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (99).</td>
<td>NA</td>
</tr>
<tr>
<td>3-O-β-Glucopyranoside (233)</td>
<td>NA</td>
</tr>
<tr>
<td>Zetaroside B (93)</td>
<td>NA</td>
</tr>
<tr>
<td>Quercetin-3,7-dimethylether (236)</td>
<td>NA</td>
</tr>
<tr>
<td>Penduletin (237)</td>
<td>NA</td>
</tr>
<tr>
<td>Cryosplenol D (238)</td>
<td>NA</td>
</tr>
<tr>
<td>Quercetin 3-O-β-D-galactoside (212)</td>
<td>NA</td>
</tr>
<tr>
<td>Quercetin 3-O-α-rhamnoside (245)</td>
<td>NA</td>
</tr>
<tr>
<td>8-Acetoxyisochiliolide lactone (248)</td>
<td>NA</td>
</tr>
<tr>
<td>Epoxyisochiliolide lactone (157)</td>
<td>NA</td>
</tr>
<tr>
<td>Stigmasterol (66)</td>
<td>NA</td>
</tr>
<tr>
<td>Friedelan-3-one (250)</td>
<td>NA</td>
</tr>
<tr>
<td>Ciprofloxacin (standard)</td>
<td>0.1/0.25</td>
</tr>
</tbody>
</table>

NA = Not Active
Antileishmanial activity of the compounds was tested in vitro on a culture of *Leishmania donovani* promastigotes. Taxodione (222) and uncinatone (19) exhibited strong antileishmanial activity (IC$_{50}$ 0.08 and 0.20 μg/ml respectively). Results for antileishmanial activity are summarized in Table 5.7.

**Table 5.7: Antileishmanial activities**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Activity against <em>L. donovani</em> (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC$_{50}$</td>
</tr>
<tr>
<td>3,5,7-trihydroxycarvotacetone (85)</td>
<td>17.0</td>
</tr>
<tr>
<td>3-acetoxy-5,7-dihydroxycarvotacetone (106)</td>
<td>0.7</td>
</tr>
<tr>
<td>3,7-dihydroxy-5-tigloyloxyacetone (88)</td>
<td>3.0</td>
</tr>
<tr>
<td>3-acetoxy-7-hydroxy-5-tigloyloxyacetone (99)</td>
<td>0.7</td>
</tr>
<tr>
<td>Royleanone (21)</td>
<td>NA</td>
</tr>
<tr>
<td>Taxodione (222)</td>
<td>0.08</td>
</tr>
<tr>
<td>Ferruginol (224)</td>
<td>4.0</td>
</tr>
<tr>
<td>11-hydroxy-8,11,13-abietatriene</td>
<td>NA</td>
</tr>
<tr>
<td>12-O-β-xylopyranoside (225)</td>
<td></td>
</tr>
<tr>
<td>6-Hydroxysalvinolone (226)</td>
<td>3.2</td>
</tr>
<tr>
<td>6,16-Dihydroxysalvinolone (227)</td>
<td>3.2</td>
</tr>
<tr>
<td>uncinatone (19)</td>
<td>0.2</td>
</tr>
<tr>
<td>Bongardol (231)</td>
<td>NA</td>
</tr>
<tr>
<td>8-Acetoxyisochiliolide lactone (248)</td>
<td>NA</td>
</tr>
<tr>
<td>Epoxyisochiliolide lactone (157)</td>
<td>38</td>
</tr>
<tr>
<td>Pentamidine (standard)</td>
<td>1.4</td>
</tr>
<tr>
<td>Amphotericin B (standard)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

NA = Not Active
CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In this research, four Kenyan plants used traditionally to cure malaria were investigated. The plants are *Clerodendrum eriophyllum* (Verbenaceae), *Sphaeranthus hullatus* (Asteraceae), *Microglossa pyrifolia* (Asteraceae) and *Vernonia galamensis* (Asteraceae). The investigation involved isolation and identification of secondary metabolites, *in-vitro* antiplasmodial testing, antimicrobial testing and antioxidant testing of the isolated compounds. A total of forty-four compounds were isolated, out of which two were new.

*Clerodendrum eriophyllum* gave fifteen compounds. One was a new compound (12-hydroxy-8,12-abietadiene-3,11,14-trione, 220) and eleven compounds were new to the genus, being 6,7-dehydroroyleanone (221), taxodione (222), 11-hydroxy-7,9(11),13-abietatrien-12-one (223), ferruginol (224), 11-hydroxy-8,11,13-abietatriene 12-O-β-xylopyranoside (225), 6-hydroxysalvinolone (226), 6,16-dihydroxysalvinolone (227), nellionol (228), betulinic acid (229), β-amyrin octacosanoate (230) and bongardol (231).

The plant had not been investigated before this study, the study therefore helped in understanding the chemistry of the plant, revealing that the plant contained abietane diterpenoids as its major class of compounds.

*Sphaeranthus hullatus* gave seventeen compounds. Twelve compounds were new to the species and genus, being 5-O-β-glucopyranosylcarvotacetone (232), thymol 3-O-β-glucopyranoside (233), zetaroside A (234), quercetin (235), quercetin-3,7-dimethylether
(236), penduletin (237), crysoplenol D (238), 4-hydroxylonchocarpin (239), caffeic acid (240), coniferaldehyde (241), dammara-20,24-dienylacetate (242) and boehmery acetate (243). Compound 106 (3-acetoxy-5,7-dihydroxycarvotacetone) was new to the species but known to the genus.

*Vernonia galamensis* ssp *nairobiensis* afforded seven known compounds, four of them being new to the species and genus, namely quercetin 3,7-dimethylether (236), quercetin 3-0-β-D-galactoside (212), syringaresinol (246) and syringic acid (247). Quercetin 3-methylether (244) is new to the species but known to the genus. *Microglossa pyrifolia* gave six compounds, 8-acetoxyisochiliolide lactone (248) being new compound and 1,4-dihydroxy-7(11)-eudesmen-8-one (251) new to the genus.

*In-vitro* anti-plasmodial testing showed that abietane diterpenoids from *C. eriophyllum* and carvotacetone derivatives from *S. bullatus* were potential anti-malarial agents, as they were the classes with active compounds. The abietane diterpenoids with antiplasmodial activities were taxodione (222) (IC$_{50}$ = 1.2 μg/ml against both D2 and W6 strains), 6-hydroxysalvinolone (226) (IC$_{50}$ = 1.8 μg/ml and 2.5 μg/ml against D2 and W6 strains respectively) and 6,16-dihydroxysalvinolone (227) (IC$_{50}$ = 3.0 μg/ml and 4.8 μg/ml against D2 and W6 strains respectively), where as the carvotacetone derivatives were 3,5,7-trihydroxycarvotacetone (85) (IC$_{50}$ = 3.4 μg/ml and 2.8 μg/ml against D2 and W6 strains respectively) and 3-acetoxy-7-hydroxy-5-tigloyloxy carvotacetone (99) (IC$_{50}$ = 1.4 μg/ml and 2.0 μg/ml against D2 and W6 strains respectively).
Anti-oxidant testing revealed that phenolic compounds with oxygenations in \textit{ortho} or \textit{para} positions were active. These were some of the flavonoids, some of the abietane diterpenoids, phenyl propanoids, \textit{p}-cymeme derivatives, the lignan syringaresinol (246) and the benzenoid syringic acid (247). The most active anti-oxidant was caffeic acid (240), with \textit{IC}_{50} of 2.6 ± 0.3 \text{μg/ml} (14.4 \text{μM}) as compared to 6.0 ± 0.2 \text{μg/ml} (19.9 \text{μM}) of quercetin, the standard used.

Taxodione (222) and 6-hydroxysalvinolone (226) exhibited potent antifungal activity (\textit{IC}_{50} 0.58 and 0.96 \text{μg/ml} respectively) against \textit{C. neoformans} whereas taxodione (222) and uncinatone (19) exhibited potent antileishmanial activity (\textit{IC}_{50} 0.08 and 0.20 \text{μg/ml} respectively) against \textit{L. donovani}. Taxodione was a more potent antileishmanial compound, being active than of amphotericin B, the standard used.

The observed antiplasmodial activities for extracts and compounds from the investigated plants justifies the traditional use of the plants as cure for malaria. However, compounds from \textit{Microglossa pyrifolia} and \textit{Vernonia galamensis} did not show antiplasmodial activities. This interprets that the activity of their extracts are either due to synergistic effect or contributed by trace compounds which were not isolated.

Three abietane diterpenoids displayed both antiplasmodial and antioxidant activities. These are taxodione (222) 6-hydroxysalvinolone (226) and 6,16-dihydroxysalvinolone (227). This gives a revelation for a possibility of having an anti-malarial which is also an
anti-oxidant, and hence giving a possibility of fighting malaria while minimizing the chances of developing complications from oxidative stress.

6.2 **Recommendations**

The compounds which displayed potent antiplasmodial and antioxidant activities, taxodione (222) 6-hydroxysalvinolone (226) and 6,16-dihydroxysalvinolone (227) should be tested for *in-vivo* activities and investigated further for possibilities of developing them to anti-malarial drugs.

Other parts of *Clerodendrum eriophyllum* as well as other species of the genus should be investigated for active compounds, since the roots of *Clerodendrum eriophyllum* gave more active compounds than the other plants investigated in this study.

Taxodione (222) should therefore be investigated for *in-vivo* activity and possibility of being developed to an antileishmanial drug, since it showed potent antileishmanial activity, being active than amphotericin B, a commercial drug.
REFERENCES


Appendix 1A: $^1$H-NMR spectrum of 12-hydroxy-8,12-abietadiene-3,11,14-trione (220), CDCl$_3$, 500 MHz
Appendix 1B: $^{13}$C-NMR spectrum of 12-hydroxy-8,12-abietadiene-3,11,14-trione (220), CDCl$_3$, 125 MHz
Appendix 1C: HMQC spectrum of 12-hydroxy-8,12-abietadiene-3,11,14-trione (220), CDCl$_3$, 500 MHz
Appendix 1D: HMBC spectrum of 12-hydroxy-8,12-abietadiene-3,11,14-trione (220), CDCl₃, 500 MHz
Appendix 2A: $^1$H-NMR spectrum of royleanone (21), C$_2$Cl$_4$D$_2$, 600 MHz
Appendix 2B: $^{13}$C-NMR spectrum of royleanone (21), C$_2$Cl$_4$D$_2$, 150 MHz
Appendix 2C: El-MS spectrum of royleanone (21)
Appendix 3A: $^1$H-NMR spectrum of taxodione (222), CD$_2$Cl$_2$, 600 MHz
Appendix 3B: $^{13}$C-NMR spectrum of taxodione (222), CD$_2$Cl$_2$, 150 MHz
Appendix 3C: EI-MS spectrum of taxodione (222)
Appendix 4A: $^{13}$C-NMR spectrum of 11-Hydroxy-7,9(11),13-abietatrien-12-one (223) CDCl$_3$, 50 MHz
Appendix 5A: $^1$H-NMR spectrum of ferruginol (224), CD$_2$Cl$_2$, 600 MHz
Appendix 5B. \(^{13}\text{C}\)-NMR spectrum of ferruginol (224), CD\(_2\)Cl\(_2\), 150 MHz
Appendix 5C: EI-MS spectrum of ferruginol (224)
Appendix 6A: $^1$H-NMR spectrum of 11-hydroxy-8,11,13-abietatriene 12-$O$-$\beta$-xylopyranoside (225) MeOD, 600 MHz
Appendix 6B: $^{13}$C-NMR spectrum of 11-hydroxy-8,11,13-abietatriene $12-O-\beta$-xylopyranoside (225) MeOD, 150 MHz
Appendix 6C: EI-MS spectrum of 11-hydroxy-8,11,13-abietatriene 12-O-β-xylopyranoside (225)
Appendix 7A: $^1$H-NMR spectrum of sugiol (9), Acetone-$d_6$, 600 MHz
Appendix 7B: $^{13}$C-NMR spectrum of sugiol (6), Acetone- $d_6$, $150$ MHz
Appendix 7C: EI-MS spectrum of sugiol (9)
Appendix 8A: $^1$H-NMR spectrum of 6-hydroxy salvinolone (226), Acetone-$d_6$, 300 MHz
Appendix 8B: $^{13}$C-NMR spectrum of 6-hydroxysalvinolone (226), Acetone-$d_6$, 75 MHz
Appendix 8C: EI-MS spectrum of 6-hydroxysalvinolone (226)
Appendix 9A: $^1$H-NMR spectrum of 6,16-dihydroxysalvinolone (227), Acetone-$d_6$, 600 MHz
Appendix 9B: $^{13}$C-NMR spectrum of 6,16-dihydroxysalvinolone (227), Acetone-$d_6$, 150 MHz
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Appendix 10A: $^1$H-NMR spectrum of nellionol (228), Acetone-$d_6$, 600 MHz
Appendix 10B: $^{13}$C-NMR spectrum of nellionol (228), Acetone-$d_6$, 150 MHz
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Appendix 11A: $^1$H-NMR spectrum of uncinatone (19), CDCl$_3$, 500 MHz
Appendix 11B. $^{13}$C-NMR spectrum of uncinatone (19), CDCl$_3$, 125 MHz
Appendix 12A: $^1$H-NMR spectrum of betulinic acid (229), DMSO, 600 MHz
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Appendix 13A: $^1$H-NMR spectrum of $\beta$-amyrin octacosanoate (230), CD$_2$Cl$_2$, 600 MHz
Appendix 13B: $^{13}$C-NMR spectrum of $\beta$-amyrin octacosanoate (230), CD$_2$Cl$_2$, 600 MHz
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Appendix 15A: $^1$H-NMR spectrum of 3,5,7-trihydroxycarvotacetone (85), Acetone d₆, 600 MHz
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Appendix 16A: $^1$H-NMR spectrum of 3-acetoxy-5,7-dihydroxycarvotacetone (106), CD$_2$Cl$_2$, 600 MHz
Appendix 16B: $^{13}$C-NMR spectrum of 3-acetoxy-5,7-dihydroxy-carvotacetone (106), CD$_2$Cl$_2$, 150 MHz
Appendix 16C: EI-MS spectrum of 3-acetoxy-5,7-dihydroxy-carvotearose (106)
Appendix 17A: $^1$H-NMR spectrum of 3,7-dihydroxy-5-tigloyloxycarvotacetone (88), CD$_2$Cl$_2$, 600 MHz
Appendix 17B: $^{13}$C-NMR spectrum of 3,7-dihydroxy-5-tigloyloxycarvotacetone (88), CD$_2$Cl$_2$, 150 MHz
Appendix 17C: EI-MS spectrum of 3,7-dihydroxy-5-tigloyloxyacarvotacetone (88)
Appendix 18A: $^1$H-NMR spectrum of 3-acetoxy-7-hydroxy-5-tigloyloxy-carvotacetone (99), CD$_2$Cl$_2$, 600 MHz
Appendix 18B: $^{13}$C-NMR spectrum of 3-acetoxy-7-hydroxy-5-tigloyloxyacetone (99), CD$_2$Cl$_2$, 150 MHz
Appendix 18C: EI-MS spectrum of 3-acetoxy-7-hydroxy-5-tigloyloxycarvotacetone (99)
Appendix 19A: $^1$H-NMR spectrum of 5-0-$\beta$-glucopyranosylcarvotacetone (232), CD$_2$Cl$_2$, 600 MHz
Appendix 19B: $^{13}$C-NMR spectrum of 5-O-\(\beta\)-glucopyranosylcarvotacetone (232), CD$_2$Cl$_2$, 150 MHz
Appendix 19C: EI-MS spectrum of 5-\(O\-\beta\-\)glucopyranosylcarvotacetone (232)
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Appendix 20B: $^{13}$C-NMR spectrum of thymol 3-0-β-glucopyranoside (233), Acetone d$_6$, 150 MHz
Appendix 20C: EI-MS spectrum of thymol 3-\(\alpha\)-\(\beta\)-glucopyranoside (233)
Appendix 21A: $^1$H-NMR spectrum of zetaroside B (93), MeOD, 600 MHz
Appendix 21B: $^{13}$C-NMR spectrum of zetaroside B (93), MeOD, 150 MHz
Appendix 21C: EI-MS spectrum of zetaroside B (93)
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Appendix 22B: $^{13}$C-NMR spectrum of quercetin (235), Acetone $d_6$, 150 MHz
Appendix 23A: $^1$H-NMR spectrum of quercetin-3,7-dimethylether (236). MeOD, 600 MHz
Appendix 23B: $^{13}$C-NMR spectrum of quercetin-3,7-dimethylether (236), MeOD, 150 MHz
Appendix 23C: EI-MS spectrum of quercetin-3,7-dimethylether (236)
Appendix 24A: $^1$H-NMR spectrum of penduletin (237), MeOD, 600 MHz
Appendix 24B: $^{13}$C-NMR spectrum of penduletin (237), MeOD, 150 MHz
Appendix 24C: EI-MS spectrum of penduletin (237)
Appendix 25A: $^1$H-NMR spectrum of crysoplenol D (238), Acetone $d_6$, 600 MHz
Appendix 25B: $^{13}$C-NMR spectrum of crysoplenol D (238), Acetone d$_6$, 150 MHz
Appendix 25C: Ei-MS spectrum of crysoplenol D (238)
Appendix 26A: $^1$H-NMR spectrum of 4-hydroxylonchocarpin (239), CD$_2$Cl$_2$, 600 MHz
Appendix 26B: $^{13}$C-NMR spectrum of 4-hydroxylonchocarpin (239), CD$_2$Cl$_2$, 150 MHz
Appendix 26C: EI-MS spectrum of 4-hydroxylonchocarpin (239)
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Appendix 30B: $^1$J$_{13}$C-$^1$H NMR spectrum of boehmeria acetate (243), CD$_2$Cl$_2$, 150 MHz
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Appendix 32A: $^1$H-NMR spectrum of quercetin 3-O-$\beta$-D-galactoside (212) DMSO $d_6$, 600 MHz
Appendix 32B: $^{13}$C-NMR spectrum of quercetin 3-$O$-$\beta$-$D$-galactoside (212) DMSO d$_6$, 150 MHz
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Appendix 33A: $^1$H-NMR spectrum of quercetin 3-O-α-rhamnoside (245), DMSO d$_6$, 600 MHz
Appendix 33B: $^{13}$C-NMR spectrum of quercetin 3-O-α-rhamnioside (245), DMSO d₆, 150 MHz
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Appendix 34A: $^1$H-NMR spectrum of syringaresinol (246), CD$_2$Cl$_2$, 600 MHz
Appendix 34B: $^{13}$C-NMR spectrum of syringaresinol (246), CD$_2$Cl$_2$, 150 MHz
Appendix XXXIVC: Ei-MS spectrum of syringaresinol (246)

![EI-MS spectrum of syringaresinol (246)](image-url)
Appendix 35A: $^1$H-NMR spectrum of syringic acid (247), Acetone $d_6$, 600 MHz
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Appendix 38A: $^1$H-NMR spectrum of stigmasterol (66), CD$_2$Cl$_2$, 600 MHz
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Appendix 40C: EI-MS spectrum of friedelan-3-one (250)
Appendix 41A: $^1$H-NMR spectrum of 1,4-dihydroxy-7(11)-eudesmen-8-one (251), CD$_2$Cl$_2$, 600 MHz
Appendix 41B: $^{13}$C-NMR spectrum of 1,4-dihydroxy-7(11)-eudesmen-8-one (251), CD$_2$Cl$_2$, 150 MHz