DECLARATION

I Zandile Lorraine Mthembu hereby declare that this work is my original dissertation and to my knowledge, it has not been submitted anywhere else for the award of a degree at any other University.

Date ……26 November 2018…….. Signed ……  ……..

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DEDICATION

I would like to thank God first and foremost, Thixo woSondisa, waMabongwe, woXaba woMthembu. God was and still with me. I am not shaken ngoba Thixo undingqingile wasibeka isandla sakhe kuba Ndikholose Ngaye.

Exceptional thanks to my Dear Husband Siyabonga, Gabadela, Ndaba and my children Unam and Amahle who has been an Inspiration to my struggle over the years. Thanks to my Mum Enkosi Maxaba ngemithandazo yakho and to my late Father Mbongwe, Sondisa, Aka -Cobral for your inspiration and to my loving sisters, my brother and his wife you were there for me.

The journey has just begun Mabongwe sakunqandwa liZulu

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LIST OF ABBREVIATIONS

COSY - Correlation Spectroscopy

$CDCl_3$ – Deuteriated Chloroform

CD_3OD - Deuteriated Methanol

DCM - Dichloromethane

DEPT - Distortion Enhanced by Polarization Transfer

DMSO - Dimethylsulfoxide

ES-MS - Electrospray Ionization Mass Spectrometry

EtOAc - Ethyl acetate

FDA - Food and Drug Administration

GC - Gas Chromatography

GC –MS - Gas chromatography coupled mass spectrometry

HMBC - Heteronuclear Multiple Bond Correlation

HMQC - Heteronuclear Multiple Quantum Coherence

HSQC - Hetero-nuclear Single Quantum Coherence

LC -MS - Liquid chromatography coupled mass spectrometry

LC_{50} - Lethal concentration 50

MeOH - Methanol

MIC - Minimum Inhibitory Concentration

MS - Mass Spectrometry

NMR - Nuclear Magnetic Resonance

TLC - Thin Layer Chromatography

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1 Introduction and Literature review

Phytochemistry or plant chemistry is an interdisciplinary field between chemistry of natural products, botany and pharmacy, which deals with the biochemistry, isolation, characterization, purification and process of determining chemical structure of plant constituents.

1.1 Chemical Constituents

The level of active ingredients, chemical constituents or natural products has been used as a standard or marker for quality of raw materials and value - added products. These active ingredients in medicinal plants are chemical compounds that act directly to prevent or treat disease. The active compound may be extracted and isolated from the plant in pure form. These phytomedicines are sold as extracts or powders in which the concentration of the active ingredient is standardised to ensure safety and efficacy. Medicinal plants are valuable and of great significance to the health of people in different community backgrounds. According to Edeoga *et al.*, (2005) the medicinal value of these plants depend on some chemical substances that produce a definite physiological action on the human body. The study further explain that the most important bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. According to Njoku *et al.* (2009), Penicillin from *Penicillium* species of fungi have great value to man as antibiotic, but appears to serve no useful purpose in the microorganisms producing it. They can be used in different applications in other fields such as cosmetics, food industries for example as herbs and spices, for animals in agriculture (Njoku *et al.*, 2009). In South Africa, a large part of medicine that is used every day is still derived from plants. Active ingredients from natural products and their derivatives represent more than

50% of all drugs in the world's clinical use. Quinine, morphine, aspirin, codeine, atropine, reserpine and cocaine are among the first well known drugs derived from plants. Recently new anti-cancer drugs such as taxol and vincristine have been developed (Van Wyk *et al.*, 2009).

The improvements and use of advanced technologies associated with the isolation, purification and identification of compounds, such as High Performance Liquid Chromatography (HPLC) instrument, and the capacity of Mass Spectrometer (MS) technology resulted in better identification of known compounds. The use of Liquid Chromatography LC Columns to enable a better separation and use of Nuclear Magnetic Resonance spectroscopy (NMR) for chemical structure elucidation was introduced by the growing interest in natural products from scientists. These techniques were introduced in order to discover chemical constituents with unique properties, which may be associated with quality of natural products possibly employed by traditional healers and sangomas, such that they may use a consistent dosage on their patients. Traditional healers and sangomas have been and are still using indigenous plants to heal infectious diseases and many other conditions (communicable and non-communicable diseases).

According to Singh and Barrett, (2006) there are two sources for antibiotics; natural products and synthetic compounds. They further elaborated that natural products have been the significant source in providing novel chemical scaffolds for many drugs as well as leads that were chemically modified and developed as antibacterial agents. (Singh *et al.*, 2006). The timeline of the discovery of the major classes of antibiotics and their source is shown in fig.1-1

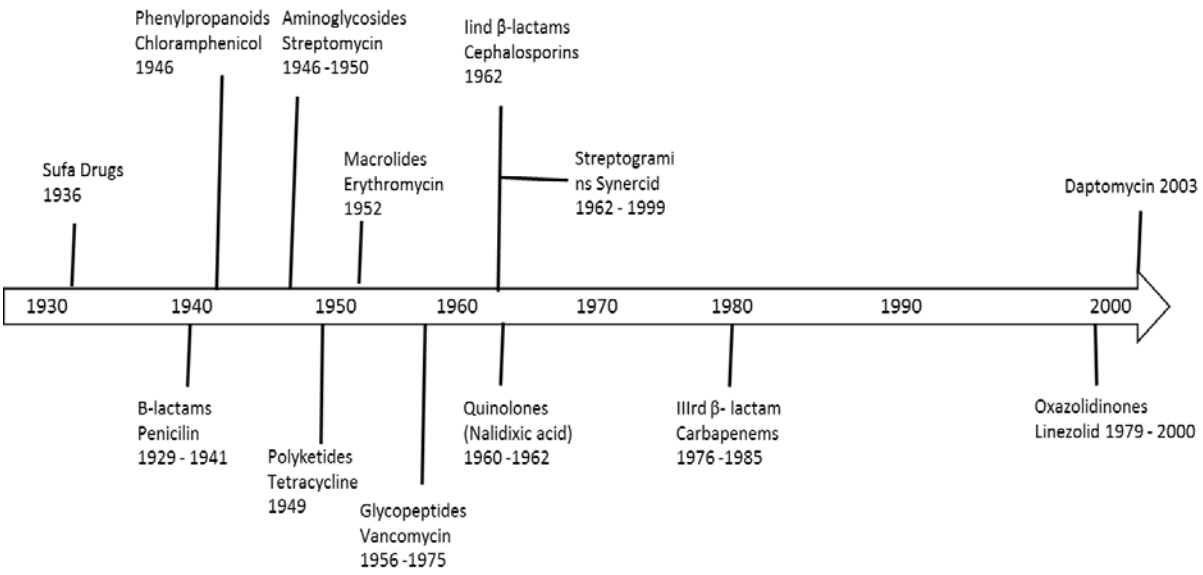
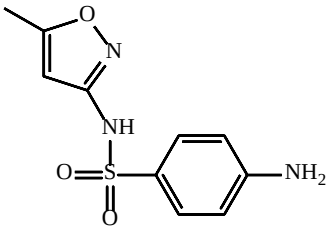


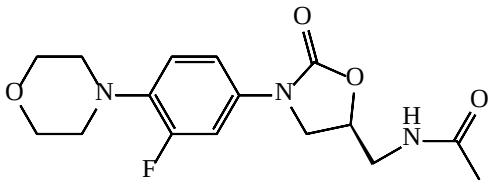
Figure 1-1: Timeline of discovery novel classes of antibiotics and introduction in clinic. Compounds listed in normal fonts are natural products and in italics from synthetic origin. (Singh *at el.*2006).

According to recent reviews by Newman and Cragg about drug development and processes they show that in the area of cancer small molecules, (73%) are other than synthetic with (47%) actually being either natural product or derived from them (Newman *at el.*, 2006).The examples of some antibiotics with a synthetic origin and natural product origin are shown Fig1-2 and Fig 1-3.

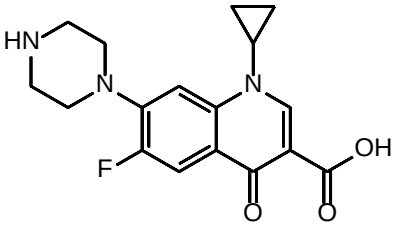
Sulfa drugs (e.g., Sulfamethoxazole)



Oxazolidinone (e.g., Linezolid)



Quinolones (e.g.,Ciprofloxacin)



Aurachin D (Quinolone Natural product)

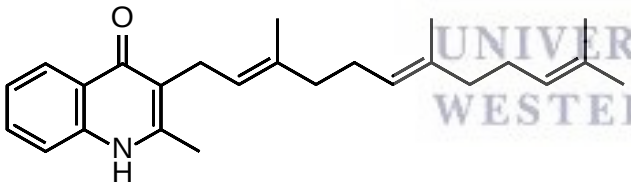
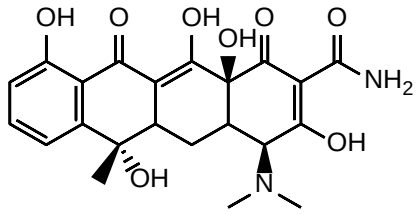
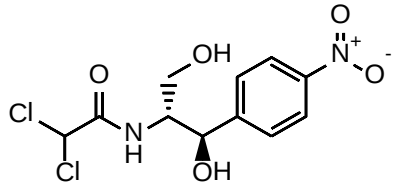


Figure 1-2: Some of antibiotic class with synthetic origin Singh *at el.*, (2006).

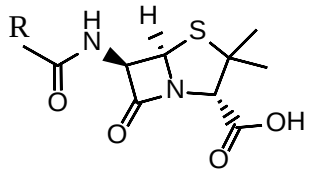
Tetracycline



Chloramphenicol



Penicillins



Cephalosporins

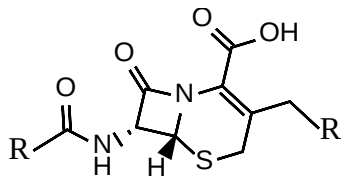


Figure 1-3: Some of antibiotics of Natural Product origin (Singh *at el.*, 2006).

1.2 Classification of chemical constituents

Through photosynthesis, plants produce carbohydrates and give off oxygen, and in this process they generate metabolic procedure that provide building blocks for the production of a vast array of compounds. In medicinal plants these include minerals, vitamins and trace elements, and a vast assortment of substances known to have specific therapeutic actions in the body. They have biological effects on other organisms. Some various classes of secondary metabolites that are known include: coumarins, tannins, flavonoids, terpenes, alkaloids, volatile oils saponins, etc.

1.3 List of some chemical constituents isolated from medicinal plants

1.3.1 Phenolics

Phenolics are defined as a class of polyphenols which are important secondary metabolites present in plants and are also responsible for their antioxidant action and various beneficial effects in multitude of diseases. Polyphenols are characterised as

- Phenolic compounds: are aromatic organic compounds with at least one hydroxyl group attached directly to the benzene ring. These are hydroxylated derivatives of benzoic acid, present in form of esters and glycoside.

- Phenolic acids: cinnamic acid derivatives. Often present in esterified form.

- Glycosidic phenylpropanoid esters.

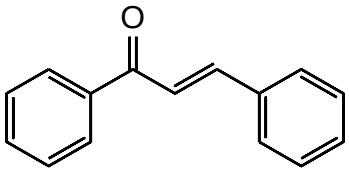
On the basis of C skeleton, polyphenols are classified as

● Flavonoids

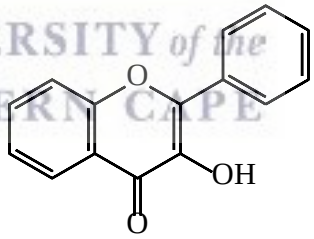
Flavonoids are low molecular weight bioactive polyphenols which play a vital role in photosynthesis cells (Kumar *at el.*, 2011) .The original flavonoid was discovered in 1936 by Albert Szent–Gyorgi (Kumar *at el.*, 2011). They are characterised by flavan nucleus and C6-C3-

C6 carbon skeleton. Flavonoids are distributed among the plant kingndom. Flavonoids are found in vegetables, fruits, nuts, tea, wine and other plant types and parts. These are an integral part of our daily diet. Flavonoids have been reported to exert wide range of biological activities, these include anti–inflammatory, antibacterial, antiviral, antiallergic, cytotoxic, antitumor, treatment of neurodegenerative diseases, and vasodilatory action (Kumar at el., 2011). According to Salucci *et al.*, (2002), the dietery flavonoids like epicatechin, epigallocatechin, galate gallic acid, and quercetin-3-O-glucoside possess strong antioxidant activity. According to the study review by Kumar, *et al.* (2011), that the average intake of flavanols and flavones was 23 mg per day among which quercetin contributed 16mg/day. Flavanoids protects against these and other diseases because they add to the overall antioxidant part of immune system. There are over 4000 flavanoids identified.

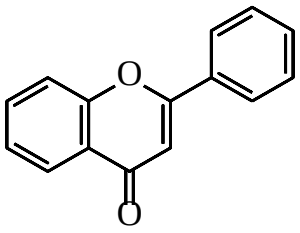
Chalcone



Flavonol



Flavone



Isoflavone

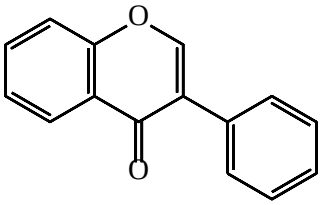


Figure1-4: Some classes of flavonoids found in medicinal plants (Kumar *et al.*, 2011).

1.3.2 Essential oils

Essential oils are aromatic and they are insoluble in water, they can be identified easily by colour some are yellow or green with a strong smell and this differs from each plant. According to (Ghannadi *et al.*, 2012), Essential oils are volatile, natural, complex compounds that are produced by plants as secondary metabolites for protection against bacteria, viruses, fungi and pets. They also have an important role in the dispersion of pollens and seeds by attracting some insects. In the middle ages essential oils were used for the preservation of foods and as a flavouring, antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and anesthetic remedies (Ghannadi *et al.*, 2012). At present, about 3000 essential oils are known and 300 of them are used commercially in different industries such as pharmaceutical, agronomic, food, sanitary, cosmetic and perfume. Current studies show interest in the antiviral and antitumor, antioxidant, antifungal and antibacterial activity of essential oils and their constituents. In South Africa, there are four provinces that are involved in the essential oil production KwaZulu Natal, Mpumalanga, Western Cape and Eastern Cape. The Amatole Pty Ltd is the company based in the Eastern Cape Province that is producing essential oils. (Mayekiso *et al.*, 2012).

1.3.3 Alkaloids

The chemicals in this diverse group contain nitrogen - bearing molecule and are pharmacologically very potent. Many of the more toxic plants contain alkaloids, such as atropine in belladonna and morphine in the opium poppy, the first alkaloid to be isolated in 1806. Caffeine, ephedrine, quinine, strychnine, piperine, nicotine and codeine are all alkaloids with diverse actions raging from stimulants, bronchodilators, antimicrobials and anti-inflammatories to narcotics and painkillers. (Anne McIntyre, 2010)

1.3.4 Tannins

Tannins occur extensively in nature, often as glycosides and they represent the largest group of polyphenols. Herbs rich in tannins are used to make mouthwashes for infected and bleeding gums, gargles for sore throats, eyewashes, remedies for catarrh, inflammation of gastrointestinal tract, diarrhoea and heavy menstrual bleeding. They can be used as compresses to heal burns, abrasions and cuts and lotions to bathe haemorrhoids and soothe inflamed skin. Tannins are found in green and black tea, red wine. Studies conducted shown that grape seed have a strong antioxidant activity that protects against free radical damage, cardiovascular disease and degeneration of connective tissue. (Anne McIntyre, 2010)

1.4 Medicinal Plants

According to Njoku and Obi, (2009) medicinal plants contain some organic compounds which produce fixed physiological action to human body and these bioactive substances include tannins, alkaloids, carbohydrates, tepernoids steroids and flavonoid. Sangoma's, herbalist and researchers use medicinal plants to develop medicinal drugs to treat diseases. The use of medicinal plants has become popular regardless of the advanced technological medicine that are produced by pharmaceutical companies. South Africa is home to over 30000 species of higher plants and 3000 of these species have been found to be used in traditional medicine across the country (Weitz *et al.*, 2006). According to Van staden *et al.* (2008), about 27 million South

			antitussive.
Passiflora incarnata L.	Passion flower	Passiflorine , flavonoids, maltol cyanogenic glycoside, indole Alkaloids	Sedative , relieve pain
P.capitatum (L)L'Her	Wild rose geranium	Geraniol esters, essential oils	Internally for minor digestive ailments, kidney and bladder disorders.
P.graveolens L'Her.ex *P.cripsum*(L)L Her	Lemon geranium		Externally for rashes and calloused and cracked skin
	Rose geranium		
Peltigera canina L.	Ground liverwort, Dog lichen	d-Usnic acid, thamnolic , nostoclide 1 and 11	Laxative effect , a liver tonic , treat throat congestion

1.5 The Family geraniaceae

Geraniaceae is a cosmopolitan family of mostly temperate and subtropical annual or perennial herbs and a few small shrubs. It was firstly described by Burmmann in 1738. A further sub-division into several genera was undertaken by the French botanist Charles- Louis L' Heritier de Brutelle in his work "Geraniologia" in 1788 (Kolodziej and Kayser, 1998, Aldasoro et al., 2002). The family comprises about 750 species belonging to five genera: Erodium L'Her., Geranium L., 'Monsonia L., Srcocaulon (DC.) Sweet and Pelargonium L'He'r. It is subdived by Hutchison (1969) into two tribes: Geranieae with primarily actinomorphic flowers (Erodium, Gerenium, Monsonia, and Sarcocaulon) and Pelargonieae (*Pelargonium*) with zygomorphic flowers (Shehata, 2008). Characters from the leaves and fruits are variable within the Graniaceae and usually not used to recognised different genera, but` they serve to distinguish infrageneric taxa (Aldasoro, 2008). Lwandiso *et al.*, 2010, further explain that the Geraniaceae family is regarded as the largest family in the plant kingndom whose species are well known for medicinal and fragrant properties. (Miller, 2002).

1.5.1 The genus: *Pelargonium*

All plants are divided into 'families' by means of taxonomic classification. *Pelargonium* species belong to the Geraniaceae family which encompasses 5 genera.L'Heritier (1789) described the genus *Pelargonium* but did not subdivide it, Sweet (1822) was the first to assemble a subdivision, he uplifted L'Heritier's genus to tribal level known as *Pelargonieae* included genera such as *Campylia* sweet. *Jenkinsonia* sweet and *Pelargonium* which are regarded presently as sections of the genus *Pelargonium* (Van der Walt, 1985).

The common name *geranium* has been incorrectly used instead of *Pelargonium* (Van der Walt, 1977). Of the over 250 *Pelargonium* (Geraniacea) species of herbaceous perennials shrubs and subshrubs, both evergreen and deciduous plants, but most scented in their habitats. Approximately 80% occur in South Africa with the highest concentration of species growing in the winter rainfall region of the south western Cape (Van der Walt and Vorster, 1988), Lis - Balchin, (1996) *Pelargonium* species are divided into 16 acknowledge parts (Bakker *et al.*, 2004) that are phylogenetically arranged. According to the investigation conducted by Van de Walt, (1985) taxonomically revised the section *Pelargonium*, which contains a total of twenty-four species. Many of these are aromatic and in particular, *P. capitatum*, *P. graveolens* and *P. radens* are used in cultivation programmes for the ennoblement of geranium oil.

Pelargonium species are a diverse group of plants with a wide variety of growth habits and habitats. Members of the family Geraniaceae, estimates of the total number of species and subspecies in the genus range from 230-300 most are native to Southern Africa, although few species occur naturally in Australia, eastern Africa, New Zealand, the Middle East, the islands of Madagascar, St Helena, and Tristan de Cuhna (Van der Walt and Vorster, 1985) *Pelargonium* height typically ranges from 1 to 6 feet depending on the species, hybrid or cultivar, but some grow even taller in their native habitats.

Pelargonium species grow in areas with low rainfall and low humidity (in a variety of habitats, from rocky slopes to grasslands, forests and long streams. *Pelargonium* species originating primarily in South Africa, and were introduced to the western world when naturalists, plant collectors and ship surgeons sailing trade route around the Cape of Good Hope brought plants back from their trips in the early seventeenth century. Many *Pelargonium* species were used as folk medicines: herbal tea and extracts of leaves, tubers and roots were used (Watt and Breyer, 1962) now are commercial produced for use in respiratory ailments (Kolodziej and Kaiser, 1997, Kolodziej *et al.*,1995) and one of these Umckaloabo, from *P. sidoides* or *P. reniforme* have been captivating gardeners for centuries. Today China produces most of the world's supply of rose geranium oil, followed by Egypt and the U.S. imports about 60-65 tons annually.

Pelargonium species are famous for their scents and have a history of medicinal use in their native Africa. These properties result from the chemical composition of the volatile/ essential oil found in glandular hairs in the leaves. Over 120 different chemical constituents have been isolated in *pelargonium* oil. The composition varies by species and growing conditions. The presence of phenols, coumarins, proanthocyanidis, phenolic compounds and tannins may account for some of the medicinal uses of *pelargonium species* in their native lands, and the reported antibacterial activity of some pelargonium oil may be due to the presence of monoterpenes. (http:www.herbsociety.org).

According to Watt and Breyer (1962), In South Africa many *Pelargonium* species with a pleasant smell or non-pleasant smell are used as traditional remedies by the Sotho, Xhosa Khoi-San and Zulus. They are used for wounds, abscesses, fever, colic, nephritis and suppression of urine, colds

and sore throats, haemorrhoids and gonorrhoea. They are also used for stimulating milk in breastfeeding mothers, for anti- helminthic infections and as an insecticide (Lis-Balchin, 1996). The overwhelming antimicrobial activity of Perlagoniums may partly explain their wound healing properties (Lis Balchin, 1996).

Pelargoniums or *Martha Washington* in the United States of America, have been produced for ornamental purposes (Van der Walt, 1985). The genus has a wide distribution worldwide distributed refer Fig. 1-6 below.*P. cucullatum* is also regarded as the ancestor of these hybrids as with *P. greveolens* and *P. radens* are known as the ancestors of the other group of hybrids known as Scentered-leaved *Pelargoniums.* Hybrids of *P. greveolens* are grown substantially for the commercial use of in the production of geranium oil. (Van der Walt, 1985).

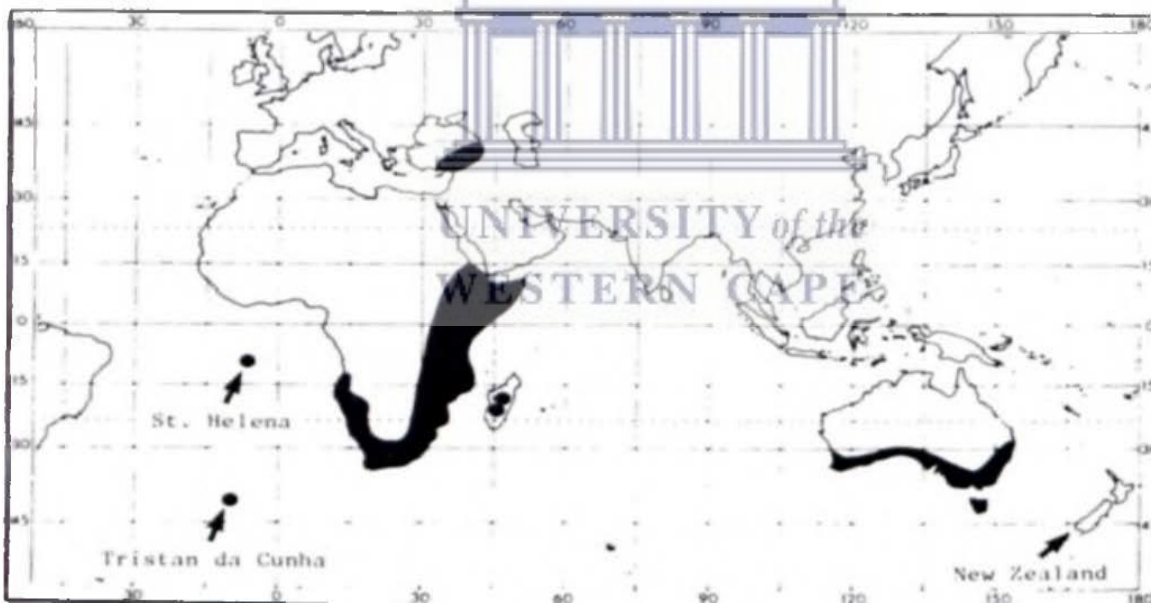


Fig 1-6. World distribution of the genus *Pelargonium* (Van der Walt *et al.*,1983).

1.5.2 The essential oil of genus *Pelargonium* ('geranium oil')

A large number of plant species are of commercial interest because they have the ability to produce essential oils. Hay and Waterman (1993) defined essential oil as the volatile oil stored in extracellular spaces in the epidermis or mesophyll cells of plants, has a low boiling point and can be recovered from the plant tissues by steam distillation. Geranium (*Pelargonium* species) is an essential oil plant that belongs to the geraniaceae family, that out of 25 species only four species are significant in the manufacturing of essential oil. These species *are P. graveolens, P. odoratissium, P capitatum and P. radens.* (Mayekiso *et al*., 2012).

Many of the natural *Pelargonium* species and the hybrids and cultivars derived from them have scented leaves producing essential oils (Williams and Harborne, 2002). The species particularly rich in essential oil belong to the sections *Pelargonium Polyactium* and *Cortusina* (Webb, 1984). High quality oil is contained in the leaves, stems and stalks of scented P*elargoniums*. Just before the plants bloom, they are harvested and processed to obtain the essential oil. *Pelargonium* essential oil is incorrectly declared as geranium oil, the species involved in the production of the oil are all *Pelargonium* and not *Geranium*. The geranium oil obtained from *Garanium macrorrhizum* in Bulgaria is both chemically and medicinally different to the commercial 'geranium oil' from *Pelargonium* species (Lis-Balchin, 2002b).'Geranium oil', as described by the International Standards Organization (ISO 4731), is the essential oil obtained by steam distillation from herbaceous parts of *Pelargonium graveolens* L'Heritier ex Aiton, *P. roseum* Willdenow and their cultivars and hybrids' *(Lis* Balchin, 1990).Since the early 1800's, the rose- scented 'geraniums' belonging to the genus *Pelargonium* have been cultivated commercially for their essential oil (Widmer and Collins, 1991). Commercial 'geranium oil' is derived from various *Pelargonium* cultivars growing mainly in Reunion, China, Egypt and

Morocco (Hart and Lis- Balchin, 2002). *Pelargonium capitatum*, *P. graveolens* and *P. radens* are considered to have been used in cultivation programs to create and ennoble the rose 'geranium' cultivars. A very important commercial essential oil producer is the cultivar known as *cv. Rose*, often used referred to as *P. graveolens*, obtained from the hybridization of *P. capitatum x P. radens* (Demarne *et al.*, 1989).

Other uses of geranium oil in treatment of dysentery, haemorrhoids, inflammation, heavy menstrual flows, and even cancer. The French medical community is currently treating diabetes, diarrhoea, gallbladder problems, gastric ulcers, jaundice, liver problems, sterility and urinary stones with this oil. The Chinese homeopathies, on the other hand, believe that essential oil opens up the liver charka and promote the expulsion of toxins that prohibit the achievement of balance within the body (Higley and Higley, 2001). In a recent study based on the essential oil of geranium and tropical capsaicin, a commonly prescribed conventional remedy for shingles pain was discovered that geranium essential oil was extremely useful in reducing pain due to post – herpetic neuralgia followed by shingles (Greenman *et al.*, 2003).

Studies conducted by Sayed and Fayed, (2009) on anticancer and antioxidant activities revealed that *P. greveolens* a plant species belonging to *Pelargonium* family can be used as a good source of anticancer and antioxidant properties because they are rich in citronellol and trans geraniol, compounds found in the essential oil.

1.5.3 Pelargonium sidoides

Synonym: None

Common name: Black geranium, rabassam

1.5.3.1 Description

*Pelargonium sidoides (*family Geraniaceae) is an herbaceous, perennial plant with a tuberous rootstock primarily found in the Eastern Cape province of South Africa but it is also scattered in areas of the Free State, southern and southern-western Gauteng and Lesotho (Colling *et al.*, 2010). Also found in coastal mountain rages up to 2300 m (Bladt and Wagner, 2007, Brendler and Van Wyk, 2008). *P. sidoides* is distinguished by thickened underground tubers or rhizomes which are red on the inside. Its flowers are arranged in a rosette of dark maroon red to black petals (Lewu *et al.*, 2007a, 2007b, Brendler and Van Wyk, 2008).

Figure 1-7: roots, leaves and flowers of the *P. sidoides* (Brown,2009)

Pelargonium sidoides occurs in the same domain and can be often confused with its sister plant *Pelargonium reniforme,* it can be distinguished by its flowers that are black red in colour while the *P. reniforme* has pink flowers. (Van Wyk and Wink 2004, Colling *et al.*, 2010). The plant is

very popular amongst different cultural group, hence it is recognised by variety of vernacular names such as rabas (Khoi -khoi), iCwayiba, Uvendle, Ikhubalo (isiXhosa), Khoarra eNyenyane (Sesotho) and Kalwerbossie (Afrikaans). The Sotho name "Khoaara e Nyenyane", exactly means "growing attached to stones and rocks".

1.5.3.2 Traditional and medicinal use

The roots of *P. sidoides* have been used for centuries as a traditional remedy across different cultural groups to treat diarrhoea and gastritis (Brendler and Van Wyk, 2008) They are also used in the treatment of ear, nose, throat disorders, respiratory tract infections, fatigue and weakness of the body, hepatic disorders and menstrual complaints (Kolodziej, 2002, 2007, Van Wyk and Wink, 2004). *Pelargonium sidoides* root extracts are also used as disinfect for cleaning wounds of livestock (Lewu *et a*l., 2006, Colling *et al*., 2010). This was confirmed by Smith, 1966 that the name Kalwerbossie used to refer a remedy for treating calves in the Free State Province. Matsiliza and Berker (2001) recorded the isiXhosa name iCwayiba that was used by Xhosa people to refer to their form of treatment for colic in infants.

Commercially, *P. sidoides* is available as Umcalaobo, an ethanolic extract used as a herbal tincture. A large part of the South African population is still reliant on traditional herbal remedies for healthcare and these tubers are used for multiple purposes as a traditional herbal medicine by several South African ethnic groups (Colling *et al*., 2010). In recent years, the commercial demand for *P. sidoides* tubers for local and international trade has caused an enormous increase in uncontrollable, illegal and indiscriminate wild harvesting of the species. This causes a reduction in the number of natural populations, thus posing a biodiversity threat. (Colling *et a*l., 2010). The roots of *P. sidoides* contain coumarins, flavonoids, phenolic and hydroxycinnamic acid derivatives, phytosterols and tannins. The root extracts display anti - bacterial, anti -fungal activity

and immunomodulating properties. (Collin *et al*., 2010).

Umckaloabo

According to the studies reported by Brendler and van Wyk (2008), the name was derived from the Zulu /Xhosa name Umkhuhlane and Uhlabo. When Stevens (Charles Henry Stevens, from Birmingham,UK) was cured from tuberculosis he invented Mckalaobo.According to Donald Brown ,(2009) reported that in the 19th century, a product prepared from the root extract , "Stevens 'Consumption Cure," gained some popularity in England as a cure for tuberculosis. He firther mentioned that in the 1920s, Dr. A.Sechehaye (Sechehaye, 1933, 1937) reportedly treated approximately 800 tuberculosis patients with the preparation of the root extract Eps 7630.It is a popular profitable and fully licensed herbal medicine by the Federal Institute for drugs and Medical device (BfArM) in Germany, it is the largest in the market, and it is listed in the European Pharmacopoeia. *P. sidoides* is available in South Africa and the product is commercially available as Licntagon, Phyto Nova cough syrup and natura Pentagon (Brendler and Van Wyk., 2008).

Eps 7630 is manufactured by Dr. Willmar Sechwabe Pharmaceuticals, Karlsruhe, Germany, and registered by ISO Pharmaceuticals, Ettlingen, Germany. The Eps 7630 extract contains primarily polyphenols (mainly catechin and gallocatechin), proteins and minerals and in lower concentrations, 7-hydroxycoumarin derivatives fig 1-8.These 7-hydroxycoumarins (including Umckalin) derivatives differ in chemical structure from the known anticoagulant coumarins and are not associated with anticoagulant activity or interaction with warfarin and its pharmacokinetics. Eps 7630 is used in the treatment of acute, uncomplicated upper respiratory tract infections.

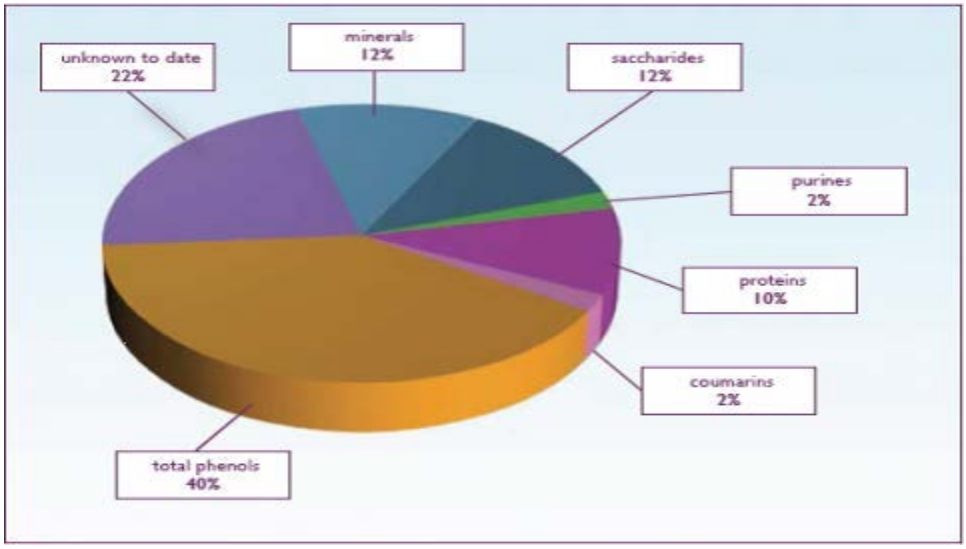
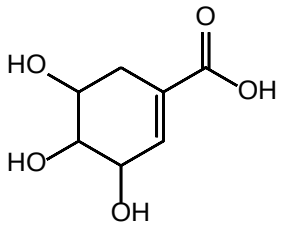


Figure 1-8: Chemical composition of Eps 7630 (Brown, 2009).

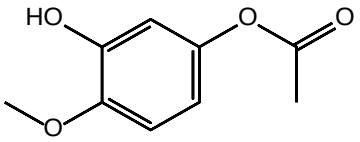
1.5.3.3 Chemical constituents of *Pelargonium sidoides*

According to Kayser *et al.* (1999), essential oil from *P. sidoides* leaves revealed that 102 components were identified with 60% to being sesquiterpenes with caryophellen and caryophellen epoxide were the most abundant. It also contained monoterpenes with 16% propanoids, 9 % methyleugenol and elemicin 3.6 %. Some of the constituents of *P. sidoides* root flavanoids such as quercetin, isovitexin, orientin, catechin were isolated by Kolodziej *et al*, (.2007). Below are typical structures of the main constituents of *P. sidoides* root, heb and of Eps 7630.

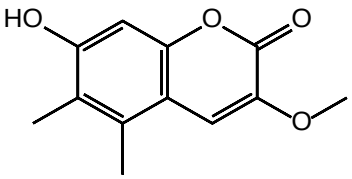
Shikimic acid



Scopoletin



7-Hydroxy-5,6-dimethylmethoxycoumarin



7-Hydroxy-6-methoxycoumarin

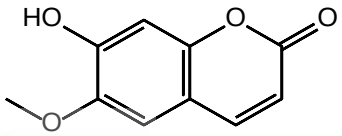


Figure 1-9: some selected constituents of *P. sidoides* and Eps 7630 (Kolodziej, 2007)

The presence of Umckalin and structurally related coumarins may be partly responsible for the activity of the medicine prepared from *P. reniforme* (Van Wyk et al., 2002). In December 2005, the Federal institute for Drugs and Medical Devices (BfArM, Bonn) approved a new license for the use of Umckaloabo (Eps 7630) as a drug. The drug is a fully licenced liquid herbal on the German market and it is listed in the European. Pharmacopoeia. A comprehensive study of all underground and areal parts of *P. sidoides* and *P. reniforme* was performed by Kolodziej *et al.*, (2007). Refer to the tables below for other examples of chemical constituents found in the species. Table 1- 2. Below some selected root constituent.

Table 1-2: Main root, constituents of *P. sidoides*, P. *reniforme* and Eps 7630 (Kolodziej *et al.*, 2007).

Compound	*P. reniforme*	*P.sidoides*	Eps 7630
Phenolic acids ,phenylpropanoids			
Gallic acid	+	+	+
Gallic acid methyl ester	+	+	+
p-Hydroxybenzoic acid	+		
Protocatechuic acid	+		
Vanillic acid	+		
Ferulic acid	+		
Caffeic acid	+		
p-Coumaraldehyde	+		
p-Coumaric acid	+		
Shikimic acid			+
Shikimic acid 3-O-gallate	+	+	+

Coumarin glycoside

Compound	*P. reniforme*	*P.sidoides*	Eps 7630
Magnolioside		+	
Isofraxoside		+	
Umckalin-7β-D-glucoside		+	+

Coumarin sulfates

Compound	*P. reniforme*	*P.sidoides*	Eps 7630
5,6-Dimethoxycoumarin-7-sulfate		+	+
6,7Dihydroxycoumarin-8-sulfate		+	
6-Hydroxy-5,7-dimethoxycoumarin-8-sulfate		+	
8-Hydroxy-5,7-dimethoxycoumarin-6-sulfate		+	

Coumarins

Compound	*P. reniforme*	*P.sidoides*	Eps 7630
Scopoletin	+	+	+
Fraxinol	+		
Isofraxetin	+		
Fraxidin	+		
Fraxetin		+	+
Artelin		+	+
Umckalin		+	+
6,7,8-Trihydroxycoumarin	+	+	+

Flavanoids

Compound	*P. reniforme*	*P.sidoides*	Eps 7630
Kaempferol-3-*O*-*β*-D-glucoside	+		+
Kaempferol-3-*O*-*β*-D-galactoside	+		
Quercetin-3-*O*-*β*-D-glucoside	+		
Myricetin-3-*O*-*β*-D-glucoside	+		

Flavan-3-ols and Proanthocyanidis

Compound	*P. reniforme*	*P.sidoides*	Eps 7630
Afzelechin	+		
Catechin	+	+	
Gallocatechin	+	+	
Proanthocynidins	+	+	+

Miscellaneous

Compound	*P. reniforme*	*P.sidoides*	Eps 7630
Reniformin	+		
β-Sitosterol	+	+	
β-Sitosterol-3-*O*-D-glucoside	+		+

NB: + stands for present, blank absent

1.5.3.4 Biological properties *P. sidoides*

The popular remedy umckalaobo based on the root extract of *P. sidoides* discovered by Charles Henry Stevens was used and still to date is used to treat respiratory conditions. The study conducted by Colling *et al.*, (2010) detected coumarins, flavonoids, phenolic and hydoxycinammic acid derevatives, phytosterols and tannins from the roots of *P. sidoides*. Mainly highly oxygenated coumarins that were identified such as Umckalin, 7 hydroxy-5.7- dimethoxycoumarin and 6, 8 dihydroxy -5, 7-dimethoxycoumarin. These coumarins are important in the pthytotherapy because they possess anti-bacteria and antifungal activity. (Kayser and Kolodziej, 1997, Lewu *et al.*, 2006, Brendler and Van Wyk, 2008).

Kolodzjie and Kiderlen (2007) reported antimicrobial activity of *P. sidoides* and *P. reniforme* against *Mycobacterium tuberculosis* in a primary radiorespirometric bioassay (BACTEC 460 system) using 12.5µg/ml methanol crude extract, a phenol and coumarin isolates. The crude extract of *P. sidoides* showed 96% inhibitory activity while *P. reniforme* together with the isolates were inactive. The extract and the isolates were further tested with the minimum

inhibitory concentrations. MIC of 100 µg/ml determined in a broth Alamar Blue assay the *P. sidoides* extract was found to be active and the isolated compounds and *P. reniforme* extract were inactive, when compared to 0.06 µg/ml of a rifampicin drug as a reference compound. Kolodjie and Kiderlen, (2007) further show the evaluated results of the extracts (methanol ethyl acetate water and butanol) and isolates against Gram-positive (*Staphylococcus aureus, Steptococcus pneumonia and* β-hemolytic Streptococcus 1451 and five Gram-negative bacteria (*Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa* and *Haemophilus influenza*) using the agar dilution method, the methanol extract was found to be moderately active against the MIC of 5 to 7.5µg/ml , while the water, ethyl acetate and butanol extract were found to be fairly high antibacterial effects (Kolodiej and Kiderlene, 2007).

The *Pelargonium endlicherianum* is the species that belongs to the *pelargonium* family and was used and discussed in Turkish folk medicine. The decoction prepared from the roots of *P. endlicherianum* plant known as '*solucanotu*' by local people in Turkey, has been used for the treatment of gastroinstestinal parasitism in small ruminants. This was revealed by in vitro studies conducted by Kozan *et al.*, 2016 on the methanol extract. The methanol extract was subjected to the female eggs of the parasite (*Haemonchus cortus*) and tested against the levamisole as a reference standard. The eggs were killed as the extract showed anthelmatic effect against *Haemonchus cortus* by causing paralysis. This confirmed that the tannin content of the plant species could be responsible from the thelmatic activity on the eggs and adult worms used and folkloric use of the plant.

1.6 Pelargonium reniforme

Synonym:None

Common name: rabassam, "rooirabas" or "rabas

1.6.1 Description

Pelargonium reniforme is a pink flowered erect shrublet that develop from tuberous rootstock belongs to the geraniaceaea family. The zygomorphous flower heads are borne on tall slender stalks, each flower has five lanceolate petals with two distinctive stripes on the upper two petals. The leaves of the species are the characteristic feature that is considered in its botanical name reniforme (Lis Balchin, 2002). The plant is amongst the other species such as *(P. antidysentericum, P. rapaceum, P. triste, and P. sidoides*) in the *pelargonium* family known with the Afrikaans names "rabassam (Van Wyk *et al.*, 1997). The species grows naturally in South Africa confined in Port Elizabeth extending in coastal regions further north, south and inland. (Kolodziej, 2007).

Fig1-10: Leaves and flowers *P. reniforme* Curtis (@pza.sanbi.org/*pelargonium-reniforme*)

1.7 Traditional and medicinal use

This plant has been confused with its sister *Pelargonium sidoides* as it contains some of the chemical constituent's characteristics that the *P. sidoides* has and they are both used for the treatment of upper respiratory conditions such as tubercolousisis. This was confirmed by Kolodzjie and Kayser, (1998) that the successful origin of the drug umckaloabo from the roots of both species was a mixture prepared from them. The African native population use the roots of *Pelargonium reniforme* in the treatment of gastrointestinal diseases due to the high concentration of proanthocyanindis, hepatic diseases and the aerial parts are used to heal wounds. (Lis Balchin, 2002). Further explanation on the use of the aerial parts for the wound healing is the presence of tannins, while the therapeutic effects related to hepatic diseases may tentatively be explicable on the basis of the radical scavenging activities of the extensive domain of phenolic compounds. (Lis Balchin, 2002, Latte and Kolodziej, 2004).

1.8 Chemical constituents of *Pelargonium reniforme*

Studies conducted by Kolodziej, 2007 on the roots of *Pelargonium reniforme*, the aqueous acetone and methanol extracts afforded a total 24 abundant metabolites including ten simple phenolic acids, six courmarins, four flavonoids, two flavan-3-ols with associated proanthocyanidins, one phythosterol and an unrivalled dieterpin, reniformin.The roots of *Pelargonium reniforme* consists of highly oxygenated coumarins , this has been confirmed by Kolodziej ,2007 on the fascinating metabolic pools studies that the roots revealed a remarkable series of highly oxygenated simple courmarins as characteristic constituents of *P. reniforme*. Aside from the widely distributed scopoletin and all the courmarins have tri and disubstituted oxygenation patterns on the aromatic nucleus that are rarely found in the plant kingdom. The 6, 7,8-trihydroxycoumarin and 8-hydroxyl 5, 6, 7, trimethoxycoumarin represent a secondary novel class of coumarins from the roots of *P. reniforme*. The quality of the root metabolites in *P. reniforme* was further demonstrated by characterization of an

unrivalled diterpen ester, connected to the hydroxyl group of *p*-hydroxyphenethansulfonic acid which in turn represent an atypical natural acyl moiety. This special metabolite, reniformin was accompanied by analogous compounds (Kolodziej *et al.*, 2007).

Kolodziej, 2007 further explained that chemical studies on the aerial parts of the species in contrast to the roots have not thoroughly done which may be accredited atleast in part, to less relevant therapeutic uses. Benzoic and cinnamic acid derivatives, flavonoids and tannins are the main phenolic substances found in the aerial parts of the plant species. These aerial parts also showed a remarkable wide range O-galloylated compounds, some new and rarely found secondary products such as glycerol-lgallate which was evident only in *Mallotus japonicas* (Euphorbiaceae) and *Rheun* species (*Polygonaceae)*. Kolodziej 2007 further explain in the metabolic pool studies, with regards to flavonoid, the extracts of the species provided a complex mixture of flavonol, flavanones, dihydroflavonols and flavones. Furthermore, it was noted the presence of distinctive sequence of 2-O-galloyi derivatives of orientin, isoorientin, vitenxin and isovetixin respectively, constitute the first reported O-galloyl derivatives of C-glucosylflavones. Proanthocyanindis were correlated with ellagitannis such as strictinin and isostrictinin, which were clearly absent from the root material. According to Kolodziej *et al.*, (2007) the chemical study also led to the isolation of two structurally closely equivalent lignin glucosides, (+)-isolariciresinol-2a-β- glucopyranoside. The isomer of unfixed stereochemistry due to small amount of a sample and the following secondary products were the reported for the first time in the geraniaceae (4,6- dihydroxyacetophenone 2-O-β-D-glucopyranoside. Refer to the tables 1, 2 for other examples of chemical constituents found in the species. According to the studies conducted by Latte *et al.*, (2008) they discovered three new phenolic metabolites the corilagnin-based ellagitannin pelargonini E, *n*-butyl gallate and (-)-4,4'9-trihydroxy3'5'-dimethoxy and 2,7'-cyclolignan 9- O-β-glucopyranoside from the aerial parts of *P. reniforme* and the reniformin form the roots.

According to the investigation by Kayser *et al*., (1998) the essential oil of *P. reniforme* contain a relectively high sesquiterpene hydrocarbons 19.4 %such as α-muurolene (1.2%), cyclosativene (0.9%), calamenene (1.2%), β calamenene (1.2 %) and δ-selinene (4.2 %) and the following compound were not discovered in *P. sidoides* and were fairly high such as γ-cadinene, δ-cadenene. Monoterpenes found consists of 16.3% and 4.7 %of the essential oils, with linalol, geranylacetone and terpinen-4-ol were found in high concentrations and monoterpene ster being observed in the essential oil.

1.9 Biological properties *P. reniforme*

Pelargonium reniforme is used as herbal medicine to treat respiratory conditions (Kolodzjie, 2007). The studies conducted by Appidi *et al*., (2008) of 17 plant species, *P. reniforme* was amongst the species used for the treatment of diarrhoea. The concoction prepared from the fresh roots of *P. reniforme* used by Sangomas especially in the Eastern Cape Province where the species grows in abudnace. The Sangoma's prepare the concoction by boiling the roots in water and administered orally to the patient until full recovery and the dosage depends to the age of the patient.Studies conducted by Kolodziej and Kiderlen, (2007) revealed that there were no remarkable differences found between *P. sidoides* and *P. reniforme* with respect to the Gram positive and Gram bacteria activities. The investigation by (Kayser, 1998), the presence of anacardic acid, salicylic and phenolic compounds with antimicrobial effects confirms the insect detergency. Also the furan constituents were identified such as (furfural, 2-pentylfuran, and perillene) and naphthalene derivatives such as (naphthalene, 1, 2-dihydro-1, 1, 6-trimethyl-naphthalene) were recommended to present remarkably to plant protection against insects.

The vital class of the metabolic pool of the aerial parts of *P. reniforme* is constituted by a wealth of tannins, polymeric proanthocyanandis associated with ellagitannins, their occurrence explains the traditional use of aerial parts as wound healing agent. Ellagitannins exhibit a remarkable array of biochemical and pharmacological actions including the antiviral, antimicrobial and

antitumoral properties (Kolodziej, 2007). The safety use of the plant species was confirmed by study conducted by Adewusi and Afolayan, (2009) based on the evaluation in male wistar rats. 24 rats were selected for the study and the extract was administered at different concentration of (100, 200 and 400 mg/kg) respectively daily for three weeks. The study revealed that the amount of extract at different concentration given to the animals did not result in mortality, neither was there any behavioral change in the animals. All the observations suggested that the plant is safe for medicinal uses (Adewusi *et al*., 2009).

1.10 Pelargonium capitatum

Synonym:

Pelargonium drummondii (van der Walt, 1985)

Common name*:*

Rose - scented *pelargonium* (van der Walt, 1977)

1.10.1 Description

Pelargonium capitatum is indigenous to South Africa and is one of several species (including *Pelargonium graveolens*) known as rose-scented pelargonium in English. Some of the species are known as kusmalva (meaning, roughly, "coastal geranium") in Afrikaans. It is found in fynbos along the coast of South Africa, from Lamberts Bay east to Kwazulu-Natal. *Pelargonium capitatum* is considered as one of the most important species of *Pelargonium* in the cultivation of hybrids that are used commercially for production of essential oil (Viljoen *et al*., 1995). It is a parent species of the Bourbon cultivar grown in the Reunion Island for the production of high quality rose scented geranium oil (Hori, 2003).

Pelargonium capitatum plants was one of the earlier species imported to Europe and the literature indicate that it was brought into England from Holand in 1690. The species is rare

in gardens but represented by the cultivar 'Alta of Roses 'with a more upright habit, rougher but strongly aromatic foliage and pink flowers. (Maria Lis Balchin). It is a popular and convenient ornamental plant and it also is one of the species of *Pelargonium* cultivated as a source of essential oils (Van der Walt, 1977). *Pelargonium capitatum* is an aromatic low shrub up to about 100 cm (39 in) in height and 1.5 m across with a rose- like aroma. The stems are soft and coated in green, glandular hairs. The flowers range from white through various shades of pink to purple. Its preferred habitat is on sand dunes, but it grows fast on any reasonable base, including hard clayey soil, and it grows well in disturbed habitat as shown in Fig below captured at Belhar site in Cape Town.

Figure 1-11: *Pelargonium capitatum* in habitat on a disturbed Belhar area

Although its preferred habitat is sand dunes, it will also do well in a sunny position on any neutral to alkaline soil. *Pelargonium capitatum* will tolerate temperatures down to about 0 °c and need to be kept fairly dry in winter. Traditionally the plant is taken internally as a tea for minor digestive ailments, plus kidney and bladder disorders. The leaves have been used as functional ingredient of cosmetics and toiletries, for example is applied to rashes and cracked skin in aromatherapy and skin care.

1.10.2 Previously isolated compounds from *Pelargonium capitatum*

The studies conducted on the chemistry of *Pelargonium capitatum* have intensively focused on the chemical composition of essential oils. There is limited information on the chemical constituents of *Pelargonium capitatum* , hence , the study provided more information on the essential oil of the species. *Pelargonium* species are popular for essential oils and *P. capitatum* is one of the plant that is rich in essential oil and is used in the manufacturing because it contains major compounds such a citronellol and geraniol that makes it unique. (Alessandra et al., 2011), reported that thirty-seven compounds we isolated and citronellol and gereniol being the two major compounds found, and the study reveals that these two compounds are responsible for providing a good performance as antimicrobial and antibacterial hence they are used in perfumes and insect repellent. (Yannovitis-Agriadis et al., 1991) also reported on the study of the variation of essential oil in dwarf plants of *P. capitatum* and found the two major compounds having high components of terpene alcohols as citronellol, geraniol and their esters 2-phenyl alcohol that is responsible for the rose scent and uses of essential oil. Typical structure of geraniol and citronellol are shown Fig 1-12 below.

Figure 1-12: Typical structure of geraniol and citronellol

Some of the common and similar essential oil from *P. sidoides* that were unambiguously identified in literature compared to *P. capitatum* revealed that about 102 components were obtained with sesquiterpenes found in abundance such: caryophellen cadinene, α- copeane that were detected. Other components were geranial, citronellol, α - terpineol, geraniol, geranyl

acetate, myrcene, α -pinene etc. (Kayser *et al.*, 1998).

1.10.3 Biological properties of *Pelargonium capitatum* extracts.

Volatile oils of some plants are known to have antimicrobial activity (Deans *et al.*, 1992, Piccaglia et al., 1993). In general, the cytotoxicity of essential oils is commonly attributed to the presence of phenols, aldehydes and alcohols (Bruni et al., 2003; Sacchetti *et al.*, 2005). These activities act as a chemical defence against plant pathogens.

Studies were conducted (Djeddi *et al.*, 2009), of antimicrobiological activity of *P. capitatum* aerial parts against gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) and gram positive bacteria (*Micrococcus luteus* and *Enterococcus faecalis*) and the fungi *Candida albicans* and *Saccharomyces cerevisiae*. The essential oils showed strong activity against *S. cerevisiae (32.7* ± 0.02 mm*), C. albicans* (*25.1* ± 0.03 mm) and *P. aeruginosa (24.2* ± *0.01mm),* while the oil showed the highly successful against M. luteus *(17.2±0.01mm),* E. coli *(17.1* ± *0.01 mm)* and *E. faecalis (*15.0±0.01mm*).* Djeddi concluded that *P. capitatum* oil is of high quality in aroma and composition. This was confirmed by the results showed by Alessandra *et al.*, 2011, that the best bioactivity of the essential oil was detected against Enterococcus faecalis with MIC of (0.42 ± 0.11mg/ml). The oil showed remarkable effects against *Candida albicans* strains these shows the relevance of the oil being used in cosmetic industry towards natural substances with antiseptic properties for treating candidiasis in animals and humans with MIC values of 0.25 ± 0.07 to 0.60 ± 0.17 which confirms the study by Djeddi. The essential oil of *P. capitatum* was further tested for antioxidant using the DPPH (1, 1-diphenyl - 2-picrylhydrazyl), β-carotene bleaching test and PSL assay against the reference thyme oil. The results show poor to insignificant in all assays conducted showing applicable health perspectives. The safety and efficacy of the oil, mutagenic and antimutagenic properties were also assayed using the plate incorporation assay. No mutagenic activity of *P. capitatum* was

detected on *S. typhimurium* tester strains. The oil was tested further for mutagen-protective efficacy in the *Ames Salmonella*/microsome assay and it showed no significant statistical effects. The oil shows a statistical decrease when against mutagen 2-aminoanthracene which acts as a genotoxic compound. (Alessandra *et al.*, 2011).

1.11 Problem statement

Herbal medicine in South Africa is an important part of the culture and tradition of African people and is generally more accessible and affordable. However, only a few of these herbal medicinal plant species have been commercialized as branded medicinal products for example *P. sidoides*. According to Van Wyk and Wink (2004), Africa has only 83 medicinal plants that have been totally or partially commercialized. This can be accredited to a lack of substantial research studies and evolution activity regulated on most of the plants. As such, safety concerns on how to handle or administer the crude extracts from these plants are not fully understood, and this problem extends to their pharmacological efficacy and activity.

Traditionally, *P. capitatum* has been used as tea for the treatment of minor digestive ailments such as kidney and bladder disorders, stomach cramps, diarrhea and nausea. The plant is popular for its essential oil, the leaves used as functional ingredient of cosmetics and toiletries such as to soften horny, cracked skin, soothes rashes and in aromatherapy skin care etc. Thus, chemical studies on *P. capitatum* may steer to the isolation of some bioactive chemical constituents, which may be utilized as precursors in the synthesis of new drugs. *P. sidoides* has been fully developed into a highly successful evidence based phytomedicine, a part of this study is to obtain a comparative investigation of the chemical constituents present on both species and alternatively use the aerial parts of *P. capitatum* instead of the *P. sidoides* root metabolites as this hinder the growth of the species.

1.12 Hypothesis

The World's largest population depends on traditional medicine because of scarcity and cost effectiveness of pharmaceutical drugs. The terrifying amount at which drug-resistant pathogens are emerging, medicinal plants may be used as a possible source to conventional drugs, therefore, taking advantage of the chemical constituents present in such plants. Hence, isolation and characterization of such compounds may subscribe to a better understanding of their role either relevant pharmacological activities.

1.13 Aim of the study

Aim of the study is to isolate and characterize chemical constituents from the aerial parts of *Pelargonium capitatum* and to use *P. sidoides* as a prototype plant to evaluate the chemical constituents obtained.

1.14 Objectives of the study

1. To extract phytochemical constituents from the aerial parts of *P. capitatum*.
2. To fractionate extracts towards isolation of pure compounds using different chromatographic techniques.
3. To characterize isolated compounds using spectroscopic techniques.
4. To determine chemical composition of essential oils and provide a sound knowledge on the response of *P. capitatum* essential oil to seasonal changes.
5. To determine the biological activities of extracts and where possible that of compounds.

CHAPTER 2

MATERIALS AND METHODS

2 Reagents, equipment and materials

2.1 Reagents

Sulphuric acid, vanillin, glacial acetic acid, formic acid (Analytical grade), HPLC grade methanol and acetonitrile were purchased from Merck, South Africa. Quercetin std, Brine shrimp eggs sea salt, deuterated methanol and deuterated chloroform were purchased from Sigma-Aldrich, South Africa. Solvents: n-hexane, dichloromethane, ethyl acetate, butanol, methanol, Dimethyl sulphoxide were purchased from Kimix, South Africa.

2.2 Equipment

2.2.1 Solvent Evaporation

Buchi Rotavapor RE 111, with the water bath temperature maintained at 45 ^{0}C was used for solvent evaporation.

2.2.2 Chromatography

Column chromatography (CC)

Silica gel 60 (0.063 – 0.200mm) 70- 230 mesh particle size (Merck) was packed in glass columns (20-25mm diameter) for column chromatography.

Sephadex LH20 (Sigma Aldrich) column chromatography was used eluting with 80% methanol packed in glass column (20 – 25mm).

Thin layer chromatography (TLC)

Thin layer chromatography was carried out on pre-coated silica gel 60 F_{254} aluminum sheet plate 20x20 cm (Merck). The TLC spots were visualized under UV light at 254 nm and/or 366 nm, and further detection of compounds was achieved by spraying with vanillin (prepared by dissolving 15 g of vanillin in 250 ml ethanol and then added 2.5 ml concentrated sulphuric acid). After spraying, the TLC plates were heated on a hot plate until spots became visible.

Preparative Thin layer chromatography was carried out on pre-coated silica gel 60 F_{254} aluminium sheet plate 20x20 cm (Merck). The TLC bends were visualized under UV light at 254 nm and/or 366 nm, and piece of the plate was cut, for further detection of compounds was achieved by spraying with vanillin. After spraying, the piece of TLC plates was heated on a hot plate until spots became visible. Each band was scraped out dissolved in methanol and applied as spot on TLC plate.

Ascending Preparative Paper Chromatography

Ascending Preparative paper chromatography was carried out on 3MM CHR Whitman paper 46x57cm (Merck). The TLC bends were visualized under UV light at 254 nm and/or 366 nm. After detected under UV/VIS the paper was cut into small pieces and extracted in methanol and spotted on the TLC plate.

2.2.3 Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy

NMR spectra were recorded on Bruker-400 MHz NMR at the University of the Western Cape, deuterated methanol (CD_3OD) and deuterated chloroform ($CDCl_3$) was used as solvents. The chemical shifts were expressed in δ (ppm), and coupling constants (*J*) in Hz.

2.2.4 Mass Spectrometry (MS)

Gas Chromatography-mass spectrometry (GC-MS) and Liquid chromatography-mass spectrometry (LC-MS) were conducted at the University of Stellenbosch Central Analytical Facility.

LC –MS method

LC –MS analysis was conducted on a Waters Synapt G2 quadrupole time-of-flight mass spectrometer. The instrument was fitted with a Waters Ultra pressure liquid chromatograph and photo diode array detection. Separation was achieved on a Waters BEH C18, 2.1x100 mm column with 1.7 um particles. A gradient was applied using 0.1% (v/v) formic acid (solvent A) and acetonitrile containing 0.1% formic acid in water (solvent B). The gradient started at 100% solvent A for 1 minute and changed to 28 % B over 22 minutes in a linear way. It then went to 40% B over 50 seconds and a wash step of 1.5 minutes at 100% B, followed by re-equilibration to initial conditions for 4 minutes. The flow rate was 0.3 ml/min and the column was kept at 55 ºC. The injection volume was 2 µL.

Data was acquired in MS^E mode which consisted of a low collision energy scan (6V) from *m/z* 150 to 1500 and a high collision energy scan from *m/z* 40 to 1500. The high collision energy scan was done using a collision energy ramp of 30-60V. The photo diode array (PDA) detector was set to scan from 220-600 nm. The mass spectrometer was optimized for best sensitivity, a cone voltage of 15 V, desolvation gas was nitrogen at 650 l/hr and desolvation temperature 275 ºC. The instrument was operated with an electrospray ionization probe in the negative mode. Sodium formate was used for calibration and leucine encephalin was infused in the background as lock mass for accurate mass determinations.

GC –MS method

Separation of essential oils was attained using an Agilent 6890N GC instrument with CTC CombiPAL Autosampler and Agilent 5975B MS with a Rtx®-5MS (30 m, 0.25 mm ID, 0.5 µm film thickness) Restek 12723-127 column. The instrument settings are presented in

Table 1. The samples were previously prepared by diluting in hexane before injection into a GC- MS. Exactly 100 µl of the samples were diluted in 900 µl of hexane.

Table 2- 1: GC-MS instrument settings

Instrument settings	
Injector temperature	280 °C
Injection volume	1 µl
Injection mode	Split
Split ratio	5:1
Split flow	5 mL/min
Constant flow	1 ml/min
Total flow	8.7 mL/min
Nominal init pressure	60.7 kPa
Average velocity	37 cm/sec
Carrier gas	Helium
MS transfer	280 °C
Mode	EI+; Electron energy 70 eV
Acquistion Mode	Scan
Scanning mass range	40 to 550 m/z
Solvent delay	3 min

Oven Ramp	°C/min	Temp (°C)	Hold (min)
Initial		70	1
Ramp 1	3	142	0
Ramp 2	5	225	3
Ramp 3	5	300	3

Oven temperature program

2.3 Plant material

2.3.1 Collection and preliminary treatments

The plant material of *Pelargonium capitatum* was harvested in the wild by hand at Belhar, a suburb of Cape Town on the 19th of April 2012. The collected material was thoroughly sorted to remove sand, epiphytes and salt on the surface of the sample. Then it was spread on a plastic to remove excess water (allow to dry) at room temperature. The dried material was blended using the Blender machine prior to extraction.

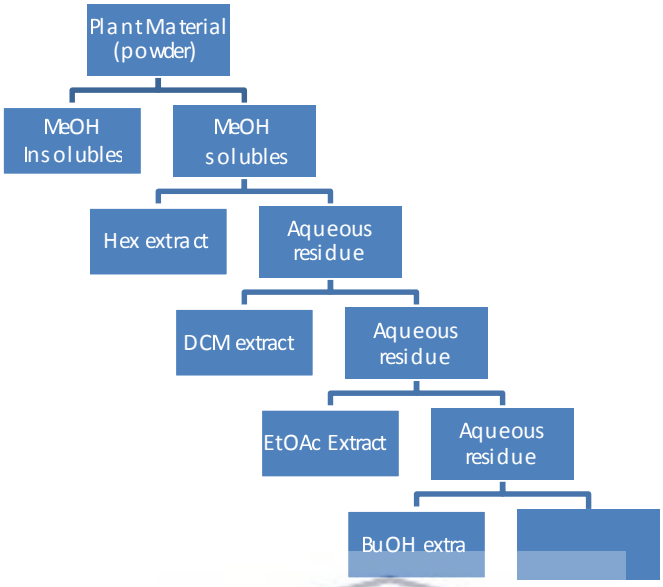
2.4 Preparation of extracts

The fresh blended material of ~1.1 kg were macerated with 80% MeOH for a period of about 48 hours. The extract was filtered using Whatman filter paper, the residue was washed with fresh MeOH (the process was repeated three times) and then centrifuged at 1500 rpm for 30 min. The total extract was concentrated under vacuum to remove the methanol and left to dry under the fumehood.

2.4.1 Preparation of crude extracts

The dried material weighed 151g as illustrated in Scheme 1 was suspended in water and partitioned successively with *n*-hexane (C_6H_{14}), dichloromethane (DCM), ethyl acetate (EtOAc) and butanol (BuOH). Each extract was concentrated to dryness under reduced pressure. Purification and isolation of natural products was achieved through one or a combination of chromatographic techniques.

Scheme 1: A typical flow diagram representing preparation of extracts.



2.5 Isolation of compounds

2.5.1 Fractionation of the Ethyl acetate extract

The Ethyl acetate extract (7.9 g) was adsorbed on silica gel before loading on a column. Fractionation was successfully reached through gravity column chromatography with the use of gradient elution. Eluents used were volumes of hexane and ethyl acetate elution mixtures at ratios of using the following solvent systems; 500ml of 100% hexane, followed by 250 ml volumes of mixtures with EtOAc at the following ratios (95:5), (90:10), (80:20), (70:30), (60:40), (50:50),(40:60), (30:70), (20:80), (10:90) and finally 100% EtOAc. The column fractionation yielded 45 fractions and the collected fractions were analyzed by TLC using chloroform: methanol: water (200:52:6 and hexane: ethyl acetate 7:3), Fractions with similar R_f values were pooled together. The combined fraction from vials 2-4 (50 mg) was loaded onto a sephadex column for further separation, using 10 % ethanol and then after preparative TLC was carried out for further isolation.

The bands were applied on the TLC plate eluted with chloroform: methanol: water (200:52:6). Fractionation resulted in the isolation of a pure compound **1** as a yellow powder (12mg).

Fractions 40-42 were also combined applied using Preparative paper chromatography (3MM CHR Whatman paper. The bands were visualized under UV light at 254 nm and/or 366 nm, and were cut into small pieces and extracted with hot methanol. All the fractions were pulled together subjected to sephadex column using saturated butanol to yield pure compound **2** as a yellow powder (9 mg).

2.5.2 Fractionation of the Hexane extract

The hexane extract (6.5g) was adsorbed on silica gel before loading on a column. Fractionation was successfully reached through gravity column chromatography with the use of gradient elution. Eluents used were volumes of Hexane and Ethyl acetate elution mixtures at ratios of using the following solvent systems; 500ml of 100% hexane, followed by 250ml volumes of mixtures with EtOAc: MeOH at ratios (95:5), (90:10), (80:20), (70:30), (60:40), (50:50), (40:60),(30:70), (20:80), (10:90) and finally 100% EtOAc. The column fractionation yielded 26 fractions and the collected fractions 9-10 and 14 -15 showed similar TLC profiles, and thus were pooled together for further purification on the Sephadex column, using Hexane –EtOAc (9:1). Fractions were collected and analyzed by TLC as previously described. Fractions with similar R_f values were pooled together. Pooling of the relevant fractions resulted in the isolation of a pure compound **3** as a white powder (10mg) and compound **4** (10.5 mg).

2.6 The Brine Shrimp Lethality test (BSLT)

2.6.1 Hatching of Brine-shrimp eggs

Cytotoxicity assessment of the crude extracts (HEX, EtOAc and pure compounds) was carried out as per the design used by Meyer *et al.*, (1982) when preparing the standards using DMSO. Artificial seawater was prepared using sea-salt (80 g) dissolved in di-ionized water (2 L). The chamber was made up of a shallow rectangular dish, which was separated with a plastic divider having a number of holes. One side of the chamber was covered with aluminium foil to prevent light and to obtain darkness. The Brine shrimp eggs (50.4mg) were weighed and subsequently added to the artificial seawater. Incubation of the eggs was kept for 48 hours at room temperature under continuous illumination (of the uncovered side) and an aquarium aerator pump for agitation and aeration of the eggs for oxygen (both sides). Following 48 hours incubation, the larvae (nauplii) were observed to be attracted to one side of the vessel that was illuminated with light.

2.6.2 Brine Shrimp exposure to extracts

Plant extracts and pure compounds were prepared at the following three different concentrations 1000,100, 10µg/ml from the stock solution containing 10 000µg/ml. Then 2.5µl was pipetted and made up to 5ml with dimethylsulphoxide (DMSO). Ten newly hatched Brine shrimp nauplii were collected with a Pasture pipette and placed in each of a series of the test tubes containing the extracts at varying concentrations (10, 100 and 1000 µg/mL). Each dosage level was tested in triplicates for all four extracts and a negative control, DMSO not exceeding 0.05%, was used for the (organic) extract. Furthermore, the suspensions of the nauplii were incubated for 24 hours at room temperature and the number thereof surviving nauplii was determined in order to generate lethality data LC_{50} at each dose (and control) level. The mortality values were used to calculate LC_{50} values i.e. the concentration at which 50% of the nauplii died, and data analysed using

Microsoft Excel 2010.The LC_{50} values greater than 1000ppm or in a range were considered

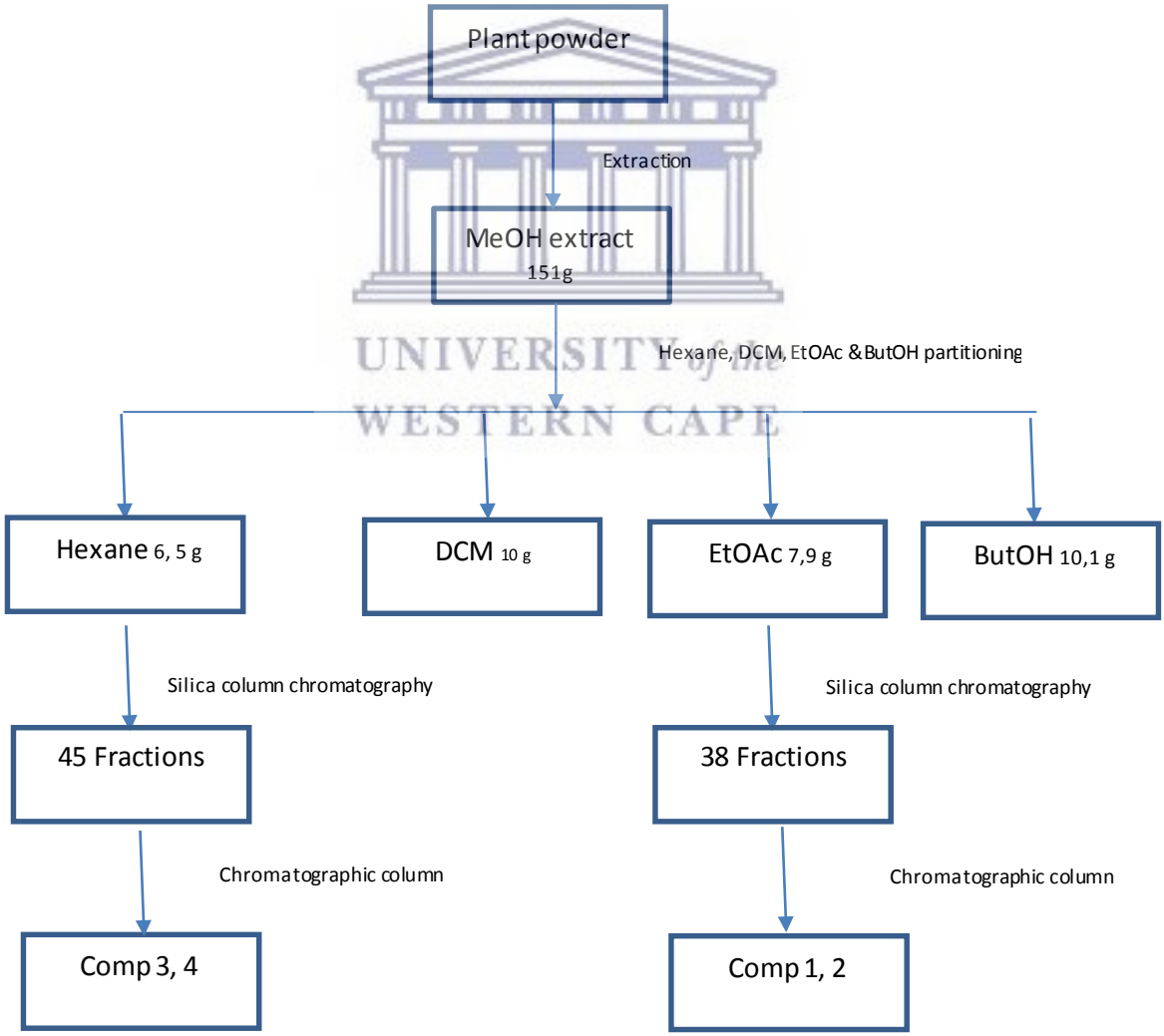
inactive.

CHAPTER 3

RESULTS AND DISCUSSION

3 RESULTS AND DISCUSSION

The plant material of *Pelargonium capitatum* was harvested in the wild by hand at Belhar and was extracted with eighty percent methanol. The processing of the methanolic extract according to the schematic diagram 2, figure 3.1 led to the isolation of four compounds and all compounds were elucidated using different spectroscopic techniques.



Scheme 3-1: A flow diagram representing a summarized fractionation process that led to isolation of chemical constituents

3.1 Structural elucidation of Compound 1

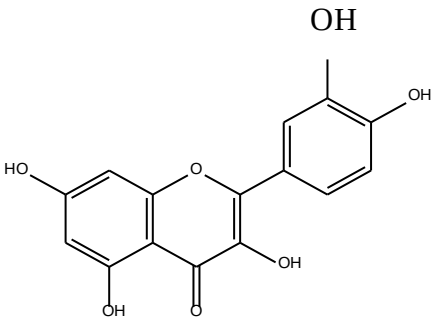


Fig 3-1 Chemical structure of compound P1

Compound 1 was identified based on the LC- MS and ^{1}H NMR analysis. The compound observed as a yellow powder, with a molecular ion base peak $(M)^{+}$ *m/z* 302.04 corresponding to $C_{15}H_{10}O_7$. The compound exhibits dark spot under UV (254nm) which indicated the phenolic nature of the compound, when sprayed with vanillin reagent it gave a yellow colour which is a very characteristic to a flavonoid. ^{1}H NMR exhibited only aromatic signals, indicating the absence of aliphatic substituents on the flavonoid nucleus. The spectrum showed the characteristic of 5-OH.

^{1}H NMR spectra showed two meta coupled aromatic protons at δ 6.29 and δ 6.08 ppm both doublets (J ~2.1Hz), assigned for H-6 that is deshielded because it is closer to the carbonyl and ether functional groups than H-8, confirming a 5, 7 dihydroxy substituted ring A. Other protons included in the typical 1, 3, 4 trisubstituted ring B.

^{1}H NMR (400MHz in CD_3OD): δ 7.64 (d, J= 2.1Hz, 1H), 7.54 (dd, J ~2.1, 8.5Hz, 1H), 6.78 (d, J~ 8.7 Hz, 1H), 6.29 (d, J~2.1Hz, 1H), 6.08 (d, J~ 2.1 Hz, 1H). The structure was further confirmed to be quercetin by comparing experimental data with literature (Mabry *et al.*, 1970).

Additionally, the identification of the compound was confirmed by coTLC with the commercial quercetin (Sigma). The TLC profile revealed same Rf values 0.40. Quercetin is a flavonoid that belongs to the flavonol class, which is a natural compound found in the plant kingdom occurring as aglycone or glycoside form. Quercetin had been isolated and reported previously, it is used in human dietary. It is found in tea, red wines, onion, olive oil, berries and grapefruit, which has been shown to prevent variety of human diseases due to their antihypertensive, anti-inflammatory and anti –tumor effects. (Kumar and Pandey, 2013). Quercetin has been reported from the sister species *P. sidoides*. (refer table 1.2)

3.2 Structural elucidation of Compound 2

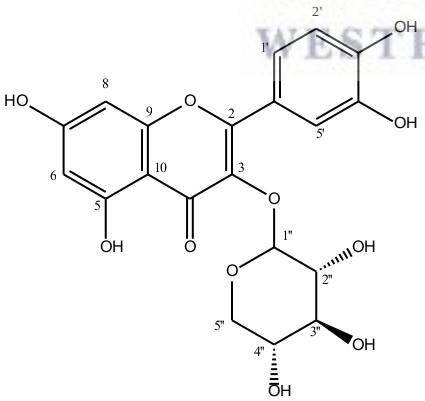


Fig 3-2 Chemical structure of compound 2

Compound 2 was identified based on the 1D (^{1}H, ^{13}C and DEPT135) and 2D (HSQC, COSY, and HMBC) NMR and MS data. The compound isolated as amorphous white yellowish powder with a negative ion mode *m/z* 435 corresponding $C_{20}H_{18}O_{11}$. The compound showed similar NMR data with the previous **compound 1**, the only difference is the presence of extra glycoside unit**.** The C-3 signal at 134.2 ppm indicating that the hydroxyl group at position 3 is attached to the glycoside unit. The anomeric proton observed at δ7.47 is corresponding to carbon atom 121.4 ppm from HSQC indicating that the pyranoside possessed β configuration. The glycoside unit demonstrated signals between (5H, m H-2` to H-5`) that appeared from δ3.33 – 3.82 ppm. According to HSQC and DEPT and ^{13}C there are five carbons that correlate with the corresponding protons as follows at H-2" to H-5" with δ (3.81/ 71.4 ppm) to δ (3.35,3.71 /65.65 p p m) and this confirms the sugar moiety. The spectra further showed the presence of methylene group CH_2 at5".

The above data is indicting the presence of quercetin 3-O-β-D-xylopyranoside and the structure was supported by the 2D analysis (HMBC, HMQC, and COSY). Further the structure was confirmed by correlating the obtained data with the corresponding compound in literature. Compound **2** is well known flavonoid and has been reported from many plant materials like *Psidium guajava* L. leaves (Zhu *et al.*, 2013). It is found in fruit such as berries, apples, pear, grapes and guava which are beneficial for health. It is used to protect kidney against diabetic progression by way of anti-oxidative, anti-inflammatory and anti –glycative effect and anti- cancer effects.

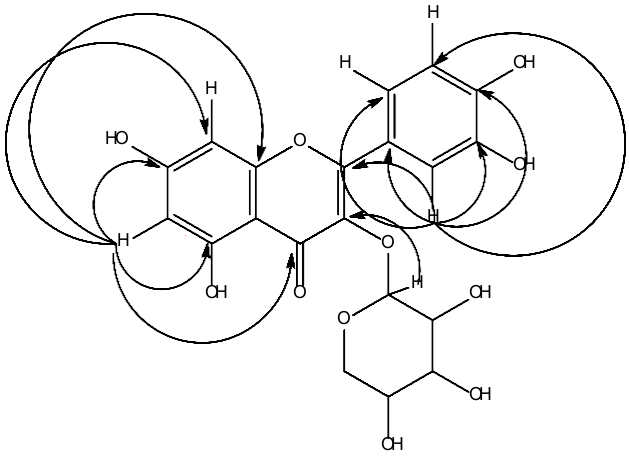


Figure 3-3: compound 2 showing key important HMBC correlations

Table 3-1.[I] 1D and 2D NMR data for **compound 2** CD_3OD

Position	δ ^{1}H	δ ^{13}C	δ ^{13}C DMSO d_6	COSY	HMBC
2	-	157.0	156.1	-	-
3	-	134.2	133.1	-	-
4	-	178.1	177.3	-	-
5	-	161.6	161.1	-	-
6	6.10	98.5	98.7	H-8	C-5, C-7, C-8, C9, C-10
7	-	164.7	164.1	-	
8	6.30	93.3	93.5	H-6	C-4, C-6, C-7, C-9, C-10
9	-	157.3	156.2	-	
10	-	104.2	103.8	-	
1’	7.47	121.4	120.9	H-2’, H-5’	C-2, C-2’, C-3’, C-4’, C-5’, C-6’
2’	7.65	116.1	116.1	H-1’	C-2, C-1’, C-3’, C-4’, C-5’, C-6’
3’	-	144.5	144.8	-	
4’	-	148.6	148.4	-	
5’	6.78	114.8	115.3	H-1’	C-2,C-1’, C-2’, C-3’, C-4’

6’	-	121.6	121.4	-	
1”	5.05	103.2	101.7	H-2”	C-3, C-3”, C-5”
2”	3.81	71.4	73.5	H-1”, H-3”	C-1”, C-3”, C-4”
3”	3.55	72.7	75.6	H-2”, H-4”	C-1”, C-2”
4”	3.74	67.7	69.3	H-3”, H-5”	C-2”, C-3”
5”	3.35, 3.71	65.6	66.0	H-4”	C-1”, C-3”, C-4”

[13]C (*Lit*) Zhu et al, 2013

3.3 Structural elucidation of Compound 3

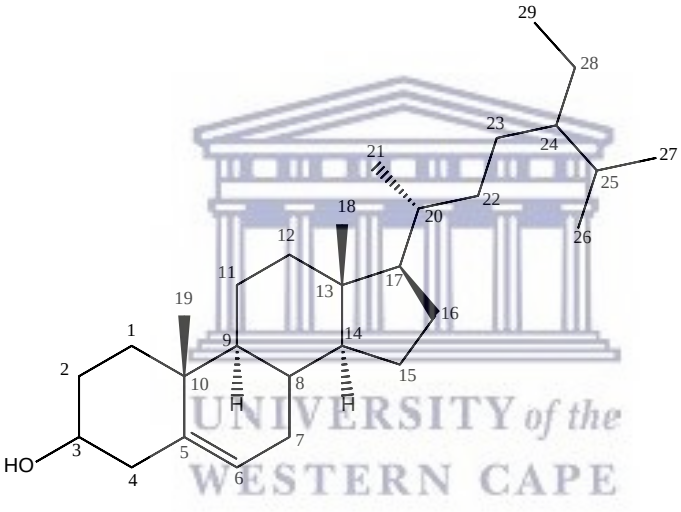


Fig 3-4 Chemical structure of compound P3

Compound 3 was identified based on the 1D (1H, ^{13}C and DEPT135) and 2D (HSQC, COSY HMBC) table 3.3. Compound 3 observed as a white powder. The data attainable from the spectroscopy gave effective confirmation of compound 3 with a molecular formula of $C_{29}H_{50}O$ (m/z 414, Fig 3) which was clearly identified as β –sitosterol. The NMR of compound 3 showed twenty-nine carbon signals, assignable to six methyls, nine methane, eleven methylene and three quaternary carbons, which was further confirmed by the DEPT 135 data (appendix 3). ^{1}H NMR (Appendix 3, Table 3-1.) review revealed the presence of two protons signals with shifts at 0.62ppm and 0.74ppm and,

respectively, which were due to singlets characteristic of methyls attached to the quaternary carbons at position 10 (δ C at 36.5ppm) and 13 (δ C at 42.3 ppm). The other three shielded doublets were observed at δ H 0.85(3H, d, J = 6.6Hz, Me -21), 0.74(3H, d, J = 1.8Hz, Me -26) and 0.77(3H, d, J = 1.8Hz, Me - 27), respectively. The spectrum further displayed a poor coupled triplet with δ H at 1.20 ppm (J= 2.2Hz Me - 28). Furthermore, the ^{1}H NMR also showed the presence of chemical shift δ H at 5.33 ppm (1H, d, J=2.36Hz) suggesting the presence of olefinic proton. This was confirmed by the presence of two signals with δ C at 140.8 ppm (C-5) highly de -shielded and 121.7 ppm (C -6) the olefinic region in the ^{13}C NMR spectrum. The de -shielded nature at C- 5 revealed the existence of a tri-substituted double bond at this position. The ^{1}H NMR also showed a proton at H-3 that appeared as a multiplet at δ 3.50 ppm, that revealed the existence of signals for olefinic proton at 71.8 ppm assignable to C-3.

COSY spectrum of compound **3** confirmed the presence of the four almost isolated ^{1}H spin systems, one spin system for each of the four rings A-D of the triterpene skeleton, ring There are cross peaks at H-1 (*δ* 1.79) and H-2 (*δ* 1.77), H -3 (*δ* 3.50), H-4 (*δ* 2.27) g that confirms that they are in one ring. H-6 (*δ* 5.33); H-7 (*δ* 1.37); H-7 (*δ* 1.96) are correlating in one ring (Maina, 2014). Further correlations are summarized in Table 3-2. Therefore, it was concluded that compound 3 was indeed β-setosterol. The results of compound 3 was achieved by comparing the experimental data with reference data (Chaturvedula *et a*l., 2012, Halilu *et al*., 2013 and Maina, M.H., 2014). Note: for COSY, HMBC and HSQC refer to appendix section, Table 3.2. Compound 3 is a phytosterol used to human health, is commonly known for its cholesterol lowering property. (Patra *et al*., 2010). β- sitosterol is also used as anti-inflammatory, anti -pyretic, antiarthritic, anti-cancer (reduce carcinogenic –induced cancer of the colon), anti - ulcer, insulin releasing and oestrgenic effects. (Patra *et al*., 2010). β -sitosterol has been reported from the sister species *P. sidoides*. (refer table 1.2).

Table 3-2.1D and 2D NMR data for compound 3 $CDCl_3$

Position	δ ^{13}C	δ ^{1}H	COSY	HSQC	HMBC	*β-sitosterol
1	37.3	1.79 (2H, *m*)	H-2	1.79	C-2, C-3, C- 5	37.5
2	31.7	1.77 (2H, *m*)	H-1, H- 3	1.77	C-3, C-4	319
3	71.8	3.50(1H, *m*)	H-2, H-4	3.50	C-2, C-4	72.0
4	42.3	2.2.7 (2H, m)	H-3	2.27	C-6, C-5, C- 3	42.5
5	140.8	-		-		140.9
6	121.7	5.33 (1H, *t, J*= 5.12Hz)	H-7	5.33	C-4, C-8, C- 10	121.9
7	31.9	1.37 , 1.96 (2H,m)	H-6	1.37		32.1
8	31.9 1	1.46(1H, *m*)		1.46		32.1
9	50.1	0.85 (1H,m)		0.85	C-12	50.3
10	36.5	-				36.7
11	21.1	1.42 (2H, m)		1.42	C-9	21.3
12	39.8	1.93 (2H, m)		1.93	C-13	39.9
13	42.3	-				42.6
14	56.8	0.94 (1H, m)		0.94		56.9
15	26.1	1.51 (2H, m)		1.51		26.3
16	28.3	1.75(2H, m)		1.75	C-13, C-14	28.5
17	56.1	1.04(1H, m)		1.04	C-20	56.3
18	11.9	0.62 s (3H, m)		0.62		12.0
19	18.8	0.93 (3H, *s*)		0.93	C-1, C-5	19.0
20	36.2	1.29(1H, m)		1.29	C-21	36.1
21	18.8	0.85 (3H, d , j = 6.4)		0.85		19.3
22	33.9	1.26(2H, m)		1.26		33.9
23	23.1	1.61(2H, m)		1.61		23.3
24	45.8	0.86 (1H, m)		0.86	C-28	45.8
25	29.1	1.60 (1H, m)		1.60		29.6
26	19.4	0.74 (3H, d ,j =6.4)	1.8)	0.74	C-27	21.0
27	19.8	0.77(3H, d,j= 1.8)		0.77	C-26	21.1
28	23.1	1.20, (2H, t)	H-29	1.20	C-29	23.0
29	12.0	0.78(3H, s)	H-28	0.78	C-28	12.2

3.4 Structural elucidation of Compound 4

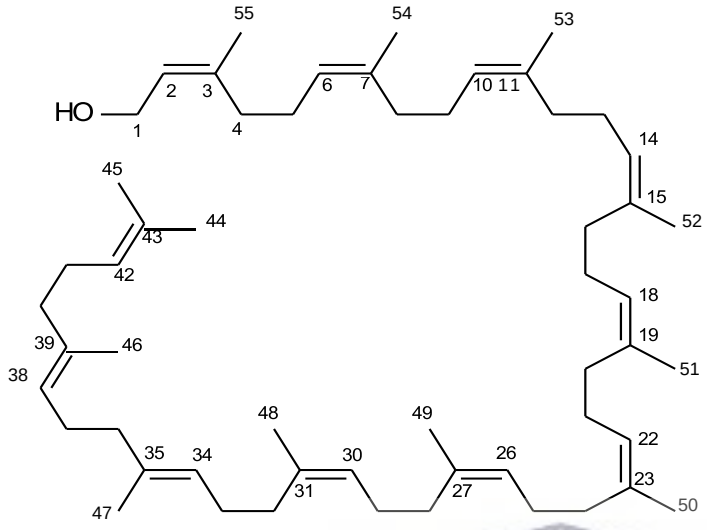


Fig 3-5 Chemical structure of compound P4

Compound 4 was isolated from the hexane fraction as clear oil (12 mg). Its chemical structure was elucidated by 1D (^{1}H, ^{13}C and DEPT 135) and 2D NMR experiment HSQC in addition to MS data the fragmentation at (43.1, 73.1, 207.1, 221. 0, 253.1, 281.1, 355.2, 405.2, 429.2, 503.3). Its chemical formula was determined to be $C_{55}H_{90}O$. The GC/MS molecular ion corresponding to the parent peak 766 ($C_{55}H_{90}O$) was not observed but other molecular ion fragments were observed.

The ^{1}H NMR spectrum of the compound **4** showed signals at δ 5.38 (1H, br. t, J=6.62, H-2) and 4.02 (2H, d, J=6.92, H-1) remained as the obvious pointers toward a terminal hydroxyl group. The signal resonating at δ 5.05 indicated the presence of olefinic protons (10H, br s, H-6, 10, 14, 18, 22, 26, 30, 34, 38, 42). The broad singlet signals at δ 1.61 was the characteristic peak of *cis*-methyls (br *s*, 12H, H-44, 45, 46, and 47) and δ 1.53 was assigned to all trans-methyls (*s*, 21H, H-48, 49, 50, 51, 52, 53, 54*)*. On the other hand, methyl at C-3 gave signal at δ 1.68 (*s*, 3H, H-3). The signal between δ 2.03-1.91 indicates the methylene hydrogens *(m*, 40H, H-4, 5, 8, 9,

12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, and 41). The integrals allowed the assignment of the number of *trans* vs *cis* double bonds but not their position.

The ^{13}C NMR and DEPT 135 spectral data indicated resonances for fifty - five carbons, suggesting a polyprenol. The ^{13}C NMR spectrum revealed the presence of olefinic carbons resonating at δ 124.5 and δ 139.9 which were attributed to C-2 and C-3 respectively.Thus other remaining carbon signals between δ 136.1 and δ 124.3 were attributed to the twenty olefinic carbons (refer appendix 4). The rest of the signals between δ 59.0 and δ 16.0 were assigned to the methylene and methyl carbons.

The correlations of the HSQC spectrum allowed interpretation of the ^{1}H- ^{13}C. The chemical structure of compound **4** was finally confirmed by comparison with the previously reported data by similar ^{13}C NMR spectral data (Sato et al., 1986) and (Rideout *et al.*, 2002). The compound was thus identified as undecaprenol. Compound **4** is a polyprenol, polyprenols are reported to be useful as pharmaceutical, particularly as an antiviral agent, immunomodulatory agent, cosmetics and for treating cancer. (Kwon, 2004). According to Rideout *et al.*;2002 undecaprenol was isolated successfully from the leaves of *Jatropha curcas L.*, family Eurphorbiaceae.The species leaves are used as a cough remedy, febrifuge and lactagogue (to enhance milk production). The juice from the stems is applied to the wounds, ulcers and shallow cuts and the bark is used as a poultice for sprains and dislocations. In addition, undecaprenol had been isolated and reported previously, it is a constituent of many leaf waxes. (Steyn., 1998). (Ibata *et al.*, 1983) from plants. To the best of our knowledge, this is the first report of undecaprenol from *Pelargonium capitatum*.

3.5 Isolation of essential oils of *Pelargonium capitatum*

The batch that was harvested in April 2012 of *Pelargonium capitatum* was subjected to hydro distillation using the Clevenger apparatus. The essential oil that was isolated was yellow in colour with a very strong scent and stored in hexane prior to analysis. The essential oil was analysed by GC- MS. Listed in the table 3-3 (leaves) and 3-4 (flowers) is the essential oil components of *Pelargonium capitatum* and their characteristics.

Table 3-3 GC –MS analysis of essential oil of *Pelargonium capitatum* (leaves)

Retention time	Compound	Quality match	Area percent (%)
16.43	Citronellol	98	6.11
21.34	αCubebene	99	0.15
21.45	2,6-Octadiene	98	0.21
21.66	Naphthalene	94	0.29
22.54	Copaene	99	0.43
23.84	1H-Cycloprop[e]azulene	99	0.22
24.43	Caryophyllene	99	3.82
24.99	Azulene	99	0.35
25.83	α Caryophyllene	97	0.54
27.21	(+)–Epi-bicyclosesquiphellandrene	94	0.21
27.41	Tridecanone	96	4.41
27.68	α -Farnesene	98	1.21
31.08	2- Naphthalnemethanol	93	0.18
36.76	2-Pentadecanone	99	0.52
38.54	Hexadecanoic acid	98	0.51
38.97	Isophytol	90	0.39
41.85	9,12-Octadecadienoic acid	99	0.34
41.97	9,12,15-Octadecatrienoic acid	98	0.43
46.05	Hexadecane	96	2.25
47.12	Tetracosane	98	0.25
47.92	Pentacosane	99	1.80
47.98	2,6-Octadiene	91	0.29
48.59	Hexacosane	98	0.24
49.31	Heptacosane	99	3.73
49.99	Octacosane	99	0.33
50.12	2,6,10,14,18,22-Tetracosahexaene	97	0.08

NB: Take note that all the compounds are tentively reported only on the Nist library match. The concentration of the compounds is reported in terms of area percentage

Table 3-4 GC –MS analysis of essential oil of *Pelargonium capitatum* (flowers)

Retention time	Compound	Quality match	Area percentage
30.65	Hexadecane	91	1.63
31.37	2 -Naphthaleinmethanol	93	11.34
31.92	Bicyclo[4.4.0]dec-1-ene	93	
35.79	Octadecane	95	1.43
38.53	Hexadecanoic acid	97	8.80
38.96	Isophytol	91	7.31
40.03	Eicosane	95	0.51
41.84	8,11-Octadecadienoic acid	99	2.65
41.95	9,12,15,-Octadecatrienal	96	9.57
47.90	Pentacosane	98	2.86
48.59	Heinicosane	91	0.86
49.28	Heptacosane	95	11.39

NB: Take note that all the compounds are tentively reported only on the Nist library match. The concentration of the compounds is reported in terms of area percentage

3.5.1 Discussion on Essential oils

About 107 components were detected 81 (flowers) and 16 (leaves) of which were dominated by sesquiterpenes, only the components with the quality match greater and equal to 90 are recorded in the tables (3-3) and (3-4), respectively.

Comparison of these results of essential oil obtained from the GC-MS to those obtained from the essential of the *Pelargonium* genus sister species for example *P. sidoides, reniforme, greveolens* etc. *Pelargonium capitatum* shows to have yielded common compounds namely octacosane, hexacosane, pentacosane 9, 12-otadecadienoic acid, 2-pentadecanone, azulene, copaene, α cubebene, displayed in abundance. Citronellol has been found as one of the abundant compound in the study that can be compared in literature amongst the following compounds with the same high oil content, Copaene, 2, 6-Octadiene, α - Farnesene, Hexadecanoic acid, Hexacosane, Tetracosane and 9, 12, 15-Octadecatrienoic acid.

Citronellol has been reported in literature as one of the most common compound found in essential oils, in *Pelargonium* species and it makes the species unique in manufacturing of essential oil (Alessandra et al., 2011) According to (Yannovitis -Agriadis et al., 1991) reported that citronellol as terpene alcohol is responsible for providing a good performance as the antimicrobial and antibacterial hence it is used in perfumes and insect repellent. That is responsible for the rose scent. Comparison of essential oil obtained from *P.sidoides* and *P. reniforme* it was found that the composition of sesquiterpene caryophellene was found to be similar but higher in *P.sidoides* than *reniforme* and to this study caryophyllene comparable that confirms the literature. Findings by Lalli et al, 2008 are in agreement with some of the components detected in the study citronellol, that it was the main compound in *Pelargonium glutinosum*

Studies conducted by Kayser *et al*, 1997 the essential oils of *Pelargonium sidoides and reniforme* revealed that 230 components have been detected on each, caryophyllene at 2.3% as the most abundant component amongst the following that were confirmed to this study namely, α-copaene, α-cubebene, β- farnesene, naphthalene, decanoic acid, and phytol. Kayser further concluded that the majority of the components were represented by sesquiterpenes (Kayser *et al*, 2007). According to Kayser *et al.*, (1997) the presence of naphthalene derivatives contributes remarkable to the protection of plant against insects.

Table 3-5 Some Essential oil composition of *Pelargonium capitatum* compared with *Pelargonium* and *Gerenium* species Aerial parts found in literature.

	Compound	***Species name***
1.	Decanoic acid	*Pelagonium hispidum, P capitatum,*
2.	Isophytol	*Geranium lucidum, G.culumbinum, G.sanguineum*
3.	Hexadecanioic acid	*Geranium lucidum, G.culumbinum, G.sanguineum, Geranium macrorrhizum*
4.	Eicosane ,Heneicosane , Pentacosane	*G.culumbinum, G.sylvaticum, Gerenium macrorrhizum aticum,P.capitatum*
5.	6-Octen-1-ol,3,7 dimethyl Formate	*P.groevelens*
6.	2,6-Octen-1-ol,3,7 dimethyl Formate	*P.groevelens*
7.	α-Copeane	*Psidoides, P.renirfome , Pelargonium sp capitatum, P.groevelens,rose scented Geranium.sylvaticum,G.sanguineum,Geranium macrorrhizum*
8.	Z 2.6-Dimethyl -2,6Octadiene	*P.groevelens*
9.	β-Caryophyllene	*P.rcseum, Pelargonium Capitatum,P.betulinum, P. hispidum,P.scabrum*
10.	2-Tridecanone	*G.sanguineum*
11.	α-Eudesmol	*P.groevelens*
12.	Octadecane , Hexadecane	*P.groevelens, G.sanguineum, Gerenium macrorrhizum*
13.	α-Farnasene	*Geranium .sylvaticum, Gerenium macrorrhizum, P. sidoides , P. reniforme , P. capitatum*
14.		
15.	1,2-dihydro-1,1,6-trimetylnaphthalene	*Pelargonium reniforme*
16.	Citronellol	*P. capitatum, P.glutinosum, P. vitiloliul, P. glutinosum , P.radens, P. graveolens*
17.	Caryophyllene	*P.Sidoides, P.renirfome, Geranium macrorrhizum, P. tomentosum*
18.	2,6-Octadiene-1-ol,3,7 dimethyl	*Rose scented Geranium*
19.	Tetracosane	*G.sanguineum, Geranium .Sylvaticum, G.robertianum*
20.	Pentacosane	*G.sanguineum, Geranium .Sylvaticum*

21. α-Cubebene	P. *quercetorum Agnew, G. macrorrhizum, G. sylvaticum, P. sidoides, P. reniforme, P. capitatum*
22. Octacosane	*G. macrorrhizum*
23. Naphthalene	*P. sidoides , P. reniforme , P. capitatum*

3.6 Biological activities

3.6.1 Brine shrimp lethality

Table 3-5 Quercetin Brine Shrimp Lethality Test Results

Quercitin	1000µg/ml	100µg/ml	10µg/ml	Control	%Mortality	LC_{50}	LC_{50}≥1000inactive LC_{50}≤1000 Active
	4	5	1	1	80	166	Active
	2	2	2	2	60		
	3	1	3	2	20		
Avarage	3	2.67	2	1.67			

Table 3-6Hexane Brine Shrimp Lethality Test Results

Hexane	1000µg/ml	100µg/ml	10µg/ml	Control	%Mortality	LC_{50}	LC_{50}≥1000inactive LC_{50}≤1000 Active
	3	1	1	1	99	2	Active
	5	3	2	2	40		
	2	3	3	2	20		
Avarage	3.33	2.33	2	1.67			

Table 3-7 Ethyl acetate Brine Shrimp Lethality Test Results

Ethyl Acetate	1000µg/ml	100µg/ml	10µg/ml	Control	%Mortality	LC_{50}	LC_{50}≥1000inactive LC_{50}≤1000 Active
	1	3	1	1	60	1.5	Active
	3	2	2	2	40		
	4	2	1	2	20		
Avarage	2.67	2.33	1.33	1.67			

Table 3-8 Methanol Brine Shrimp Lethality Test Results

Methanol	1000µg/ml	100µg/ml	10µg/ml	Control	%Mortality	LC_{50}	LC_{50}≥1000inactive LC_{50}≤1000 Active
	0	2	1	2	50	396	Active
	1	3	1	1	25		
	1	0	1	1	24.8		
Avarage	0.67	1.67	1	1.33			

High doses of bioactive compounds have been found occasionally to be poisonous or toxic and hence the pharmacology of these compounds can be preparatory measured or identified from their toxicology results. Therefore, the *in vivo* Brine shrimp larvae (nauplii), a zoological organism, have been used as suitable monitor for the screening of bioactive natural products (Meyer *et al.*, 1982). Predominantly, brine shrimp lethality test has been used for a number of bioassay system in which extracts, fractions or pure compounds of natural products are tested at three concentrations, 10, 100 and 1000µg/ml. These concentrations were prepared in test tubes containing 10 nauplii in triplicate for each concentration. Eventually, the number of mortalities counted after 24 hours in estimating the LC50.The technique was utilized as a bioassay for this study on four samples, namely, three extracts hexane, ethyl acetate and methanol and isolate, quercetin. The level of toxicity was interpreted as LC_{50} values >1000 µg/ml (non- toxic), ≥500≤1000 µg/ml (weak toxicity) and < 500 µg/ml (toxic) (Bastos *et al.*, 2009).

The results (Table 3-5 to 3-8) showed that all the extracts and the isolate were active (LC50≤.1000µg/ml). The ethyl acetate extract showed highest activity 1.5µg/ml (very active). From the results, it can be concluded that the crude ethyl acetate of the plant contains cytotoxic constituents, since the lethality of a test substance to brine shrimp nauplii has been linked to the possible ability of such substance to kill cancer cells (antitumor activity), as well as pesticidal and antibacterial activities (Meyer *et al.*, 1982) It may be concluded that all very active and active samples should be regarded as suitable for such activities.

CHAPTER 4
CONCLUSION

4 CONCLUSION

Pelargonium capitatum has been used since and is still to date used as an essential oil plant, however since there is a lack of knowledge on chemistry and biological activities a detailed investigation was conducted as alternative to its sister plant *Pelargonium sidoides* that is popular for its roots. Isolation of chemical constituents from *Pelargonium capitatum* was achieved successfully. In as far as the information collected during the study all four compounds are isolated for the first time in the plant species, in comparison to the sister plant *(P.sidoided*) that have previously found to have similar chemical constituents excluding undeprenol (Kolodziej,2007) . Four compounds quercetin, quercetin xylopyranoside (found in the leaves), β-sitosterol (roots) and undecaprenol were isolated and thoroughly characterized. With the flavonoids being abundant in the plant species it may be expected that *Pelargonium capitatum* to have antimicrobial effects. The isolated chemical constituents have been reported to show some important biological activities which include antibacterial, antifungal immunomodulatory antitumor and anti-flammatory antitumor, etc. This confirms that the isolated bioactives compounds from *Pelargonium capitatum* can be safe to use in nutritional diet. In conclusion the areal parts of the two species may be used as an alternative to the roots of P.*sidoides* in the medicinal formulations especially in the treatment of bacterial diseases like turbeculosis and bronchitis .The choice of the roots by the traditional healers over the leaves be haphazard or due to easy collection. (Lewu *et al.*, 2006).

The essential components were identified before from the leaves of *P. sidoides* (Kayser *et al.*, 1999), caryophellen was one of the major components detected. The seven essential oil obtained

in abudance such as Citronellol, α-copeane, α-farnesene etc, have been previously identified with citronellol as the main component. (Kayser *et al.*, 1997) (Guerrini *et al.*, 2011). The essential oil composition from *P.capitatum* differs from what has been reported in this study, this difference in essential oil composition might be due to geographical location, climate changes this finding is confirmed by Viljoene,(1995),(Viljoen *et al.*,1995).

Since most active plant compounds are toxic at elevated doses the Brine shrimp toxicity bioassay was conducted in order to detect a broad spectrum of pharmacologic activities in the plant species and, yet, can be employed by natural product chemists. The isolate and extracts were tested in the study (quercetin, ethyl acetate, hexane and methanol). The results revealed that all extracts and the isolate were active with ethyl acetate extract being very active with LC_{50} of 1.5µg/ml (LD50 ≤ 1000µ/ml). Brine shrimp lethality test has been associated to the feasible ability of the test substance to kill cancer cells (antitumor activity) as well as pesticidal and antibacterial activities. (Meyer *et al.*, 1982). It has been proposed that the total crude extract from *P.capitatum* with its content of so many bioactive compounds could be formulated for use in skin infections, microbial and viral activities.

As the recommendation both species can be used together (*P.capitatum* and *P.sidoides*) as they both contain similar chemical constituents used in phytotherapy. *P.capitatum* is rich in flavonoids and *P.sidoides* is unique as well with its highy oxygenated courmarins. Both species can be used in traditional medicine without using the roots as it threatens the species diversity. Furthemore, the isolated compounds from *P.capitatum* have not been investigated as yet for intracellular TB activities as *P. sidoides* It is therefore, recommended that these compounds should be analysed for intracellular activities against *M tuberculosis* in mice and or humans.

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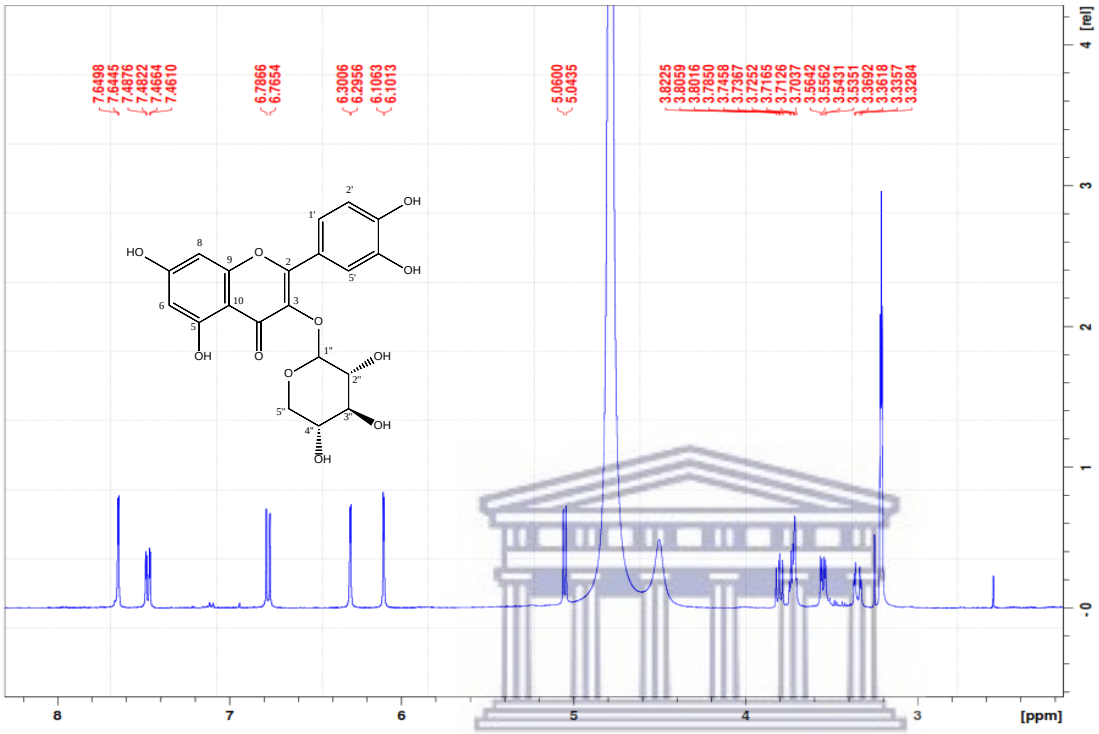
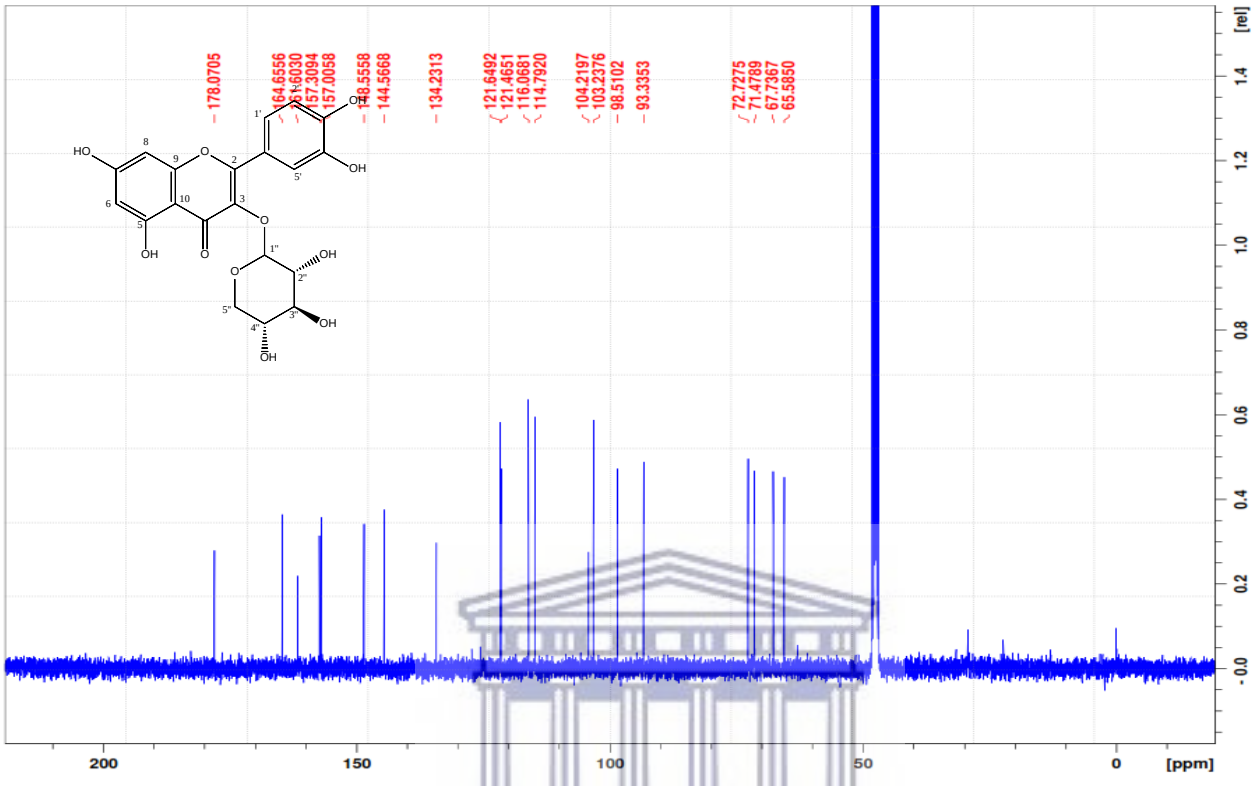
APPENDIX

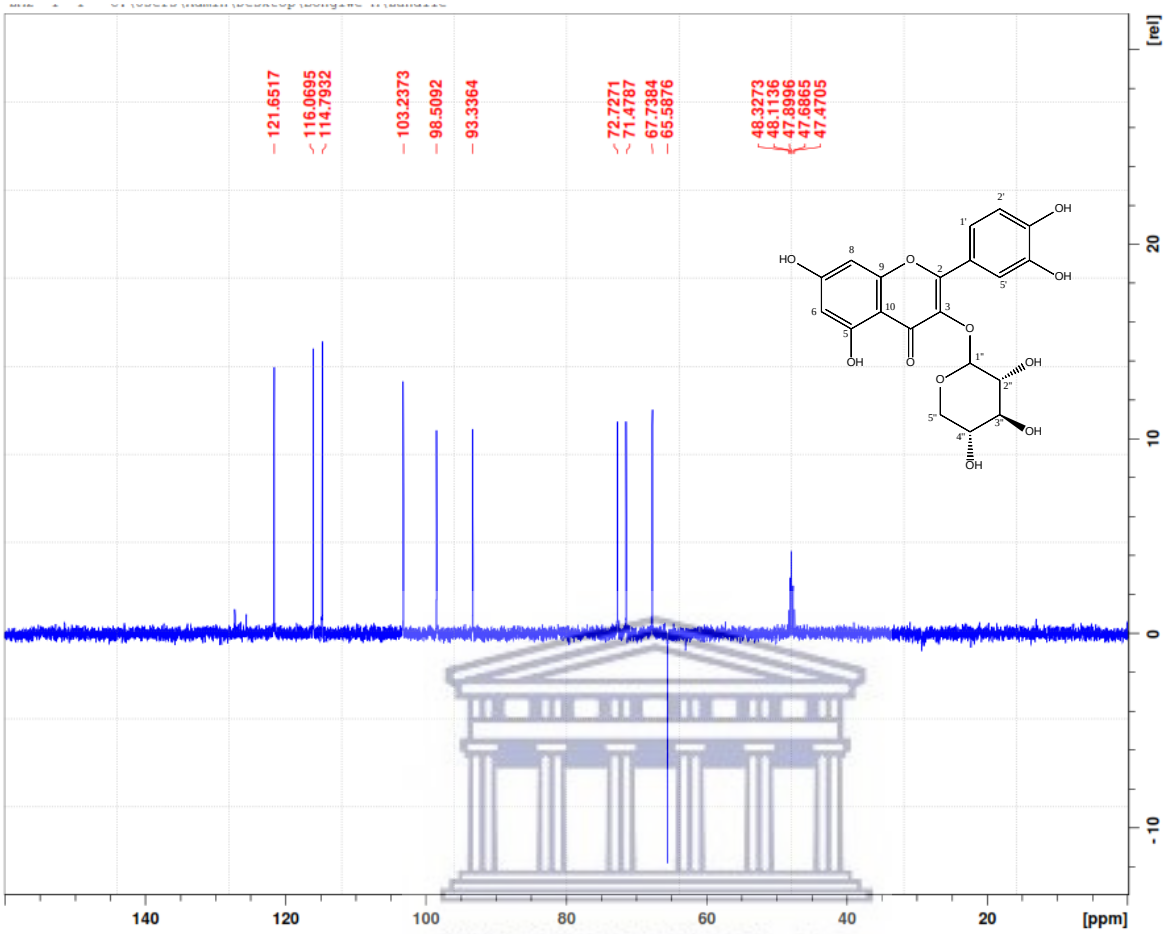
1
Appendix 2a. ^{1}HNMR of **compound 2**



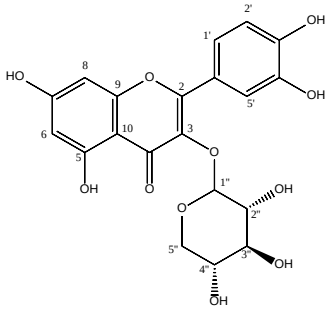
Appendix 2b. ^{13}C NMR of **compound 2**

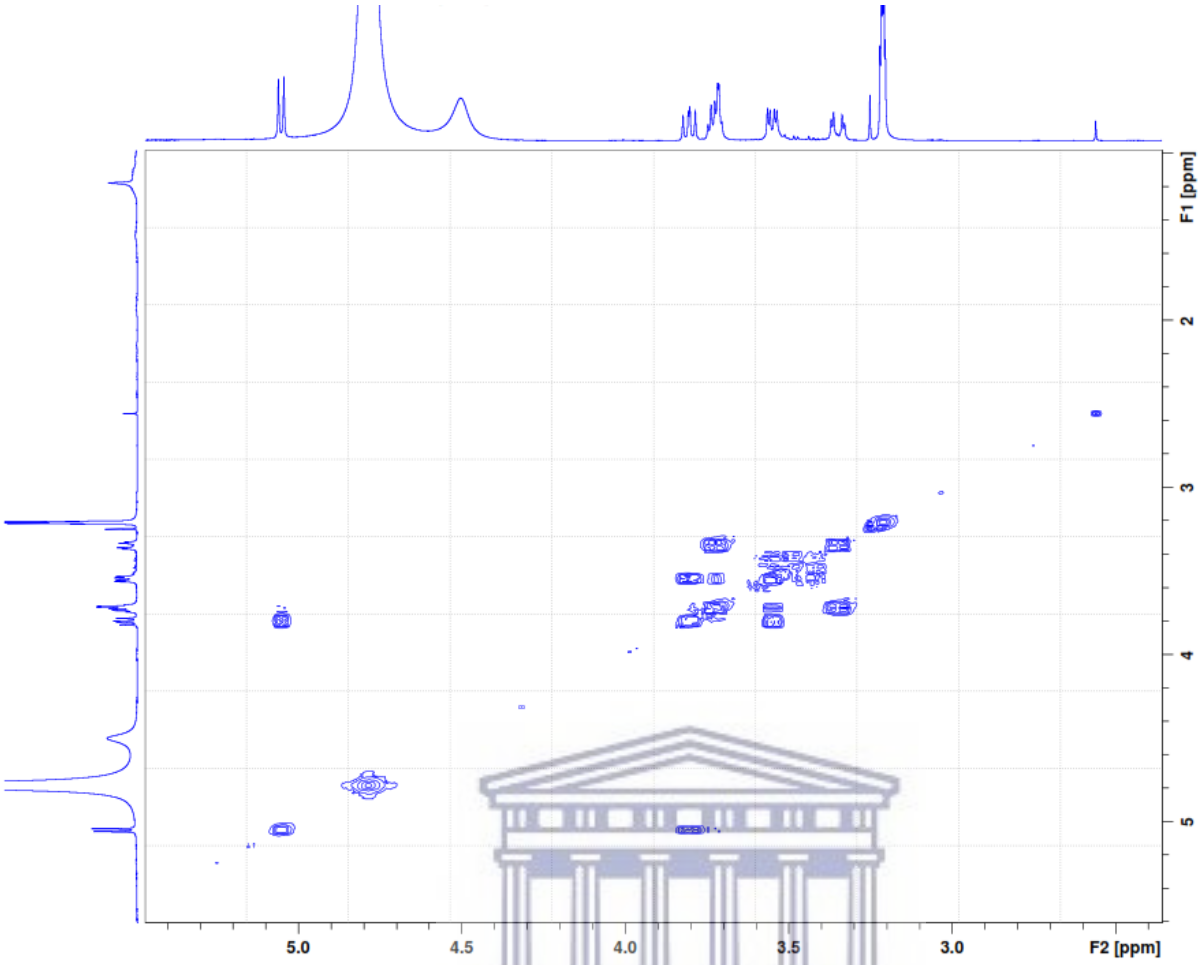


Appendix 2c. DEPT of **compound 2**

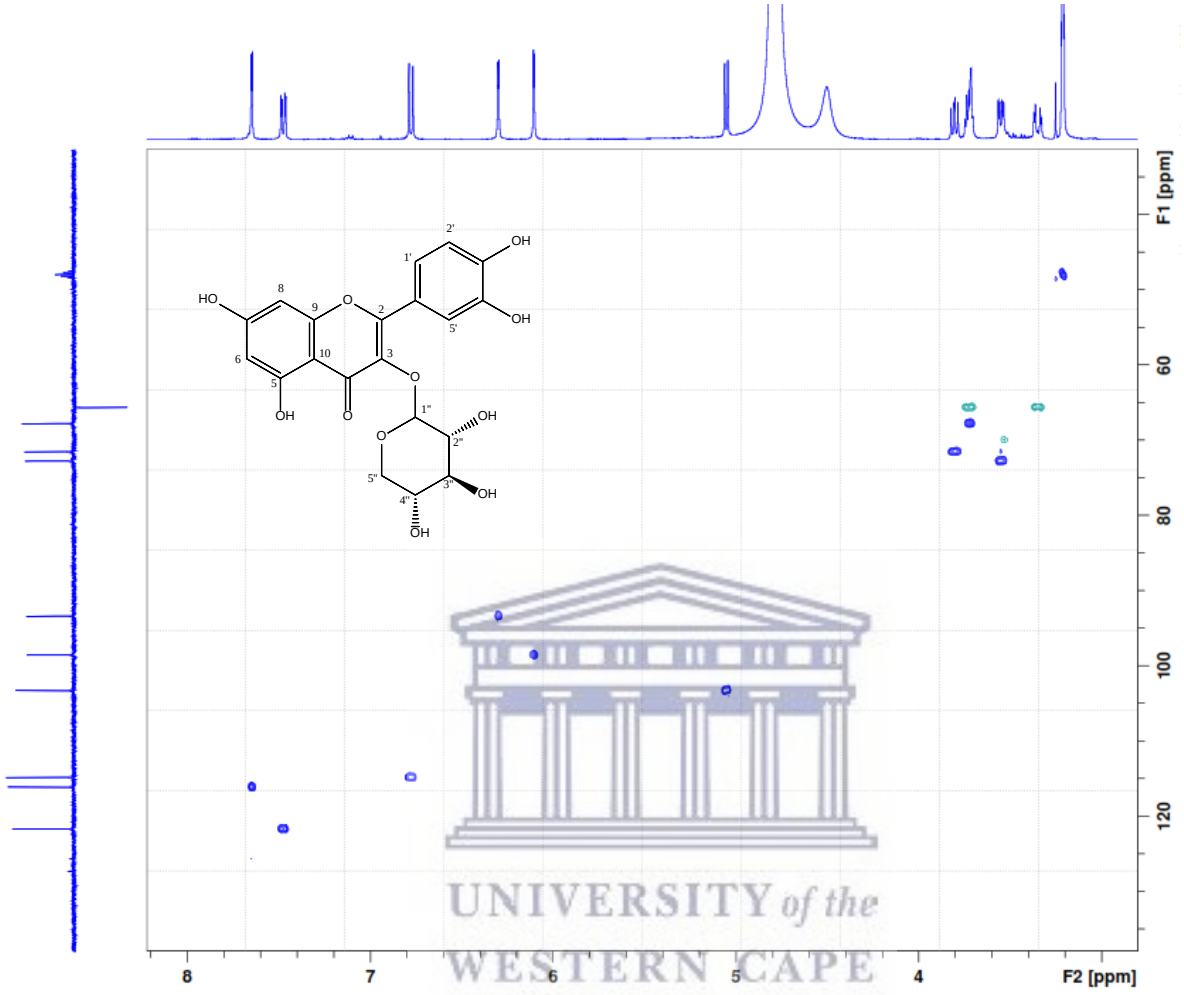


Appendix 2d. COSY of **compound 2**

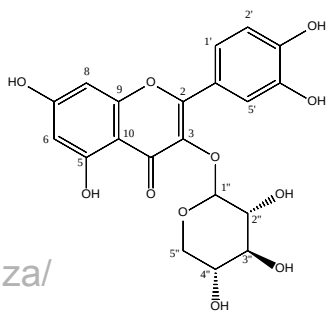


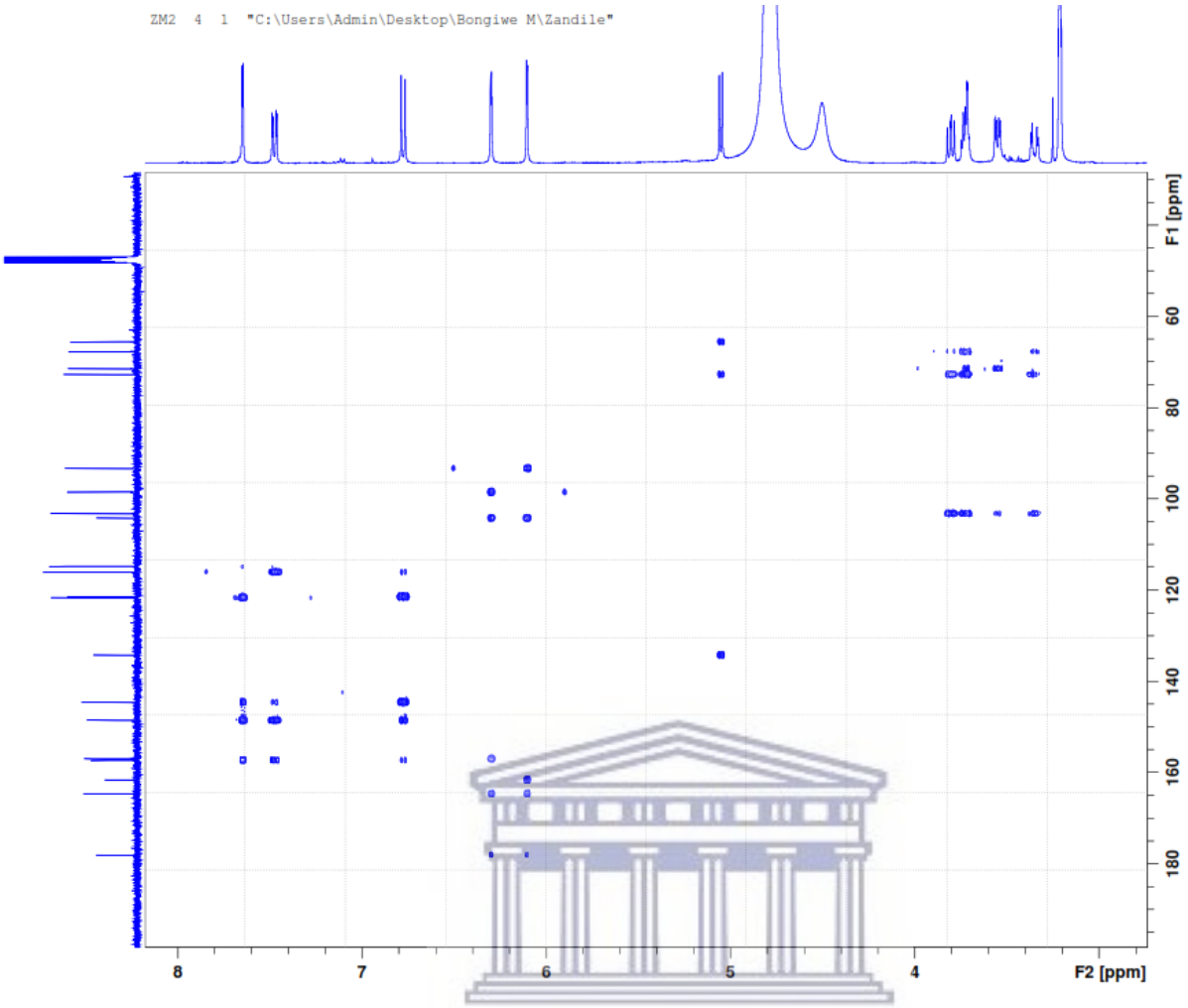


Appendix 2e. HSQC NMR of **compound 2**

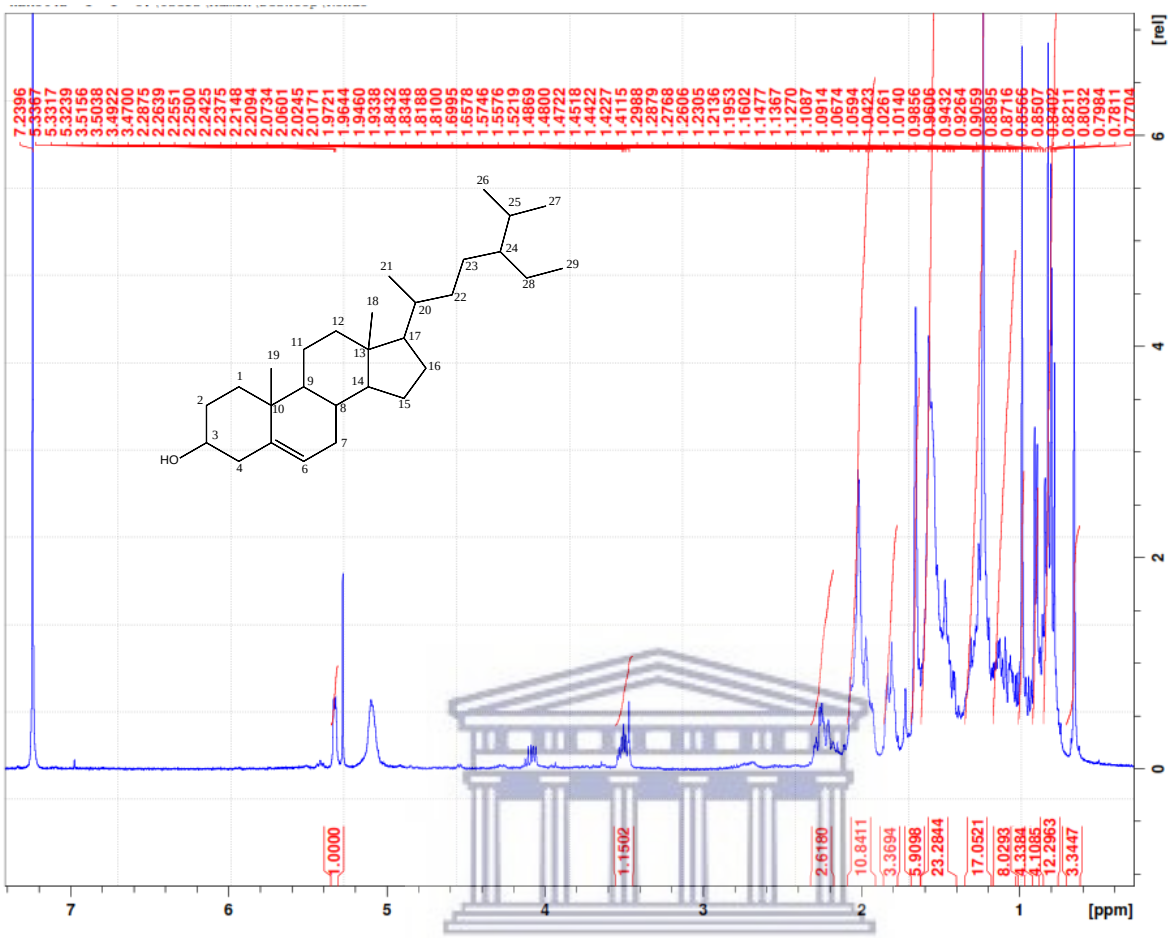


Appendix 2f. HMBC of **compound 2**

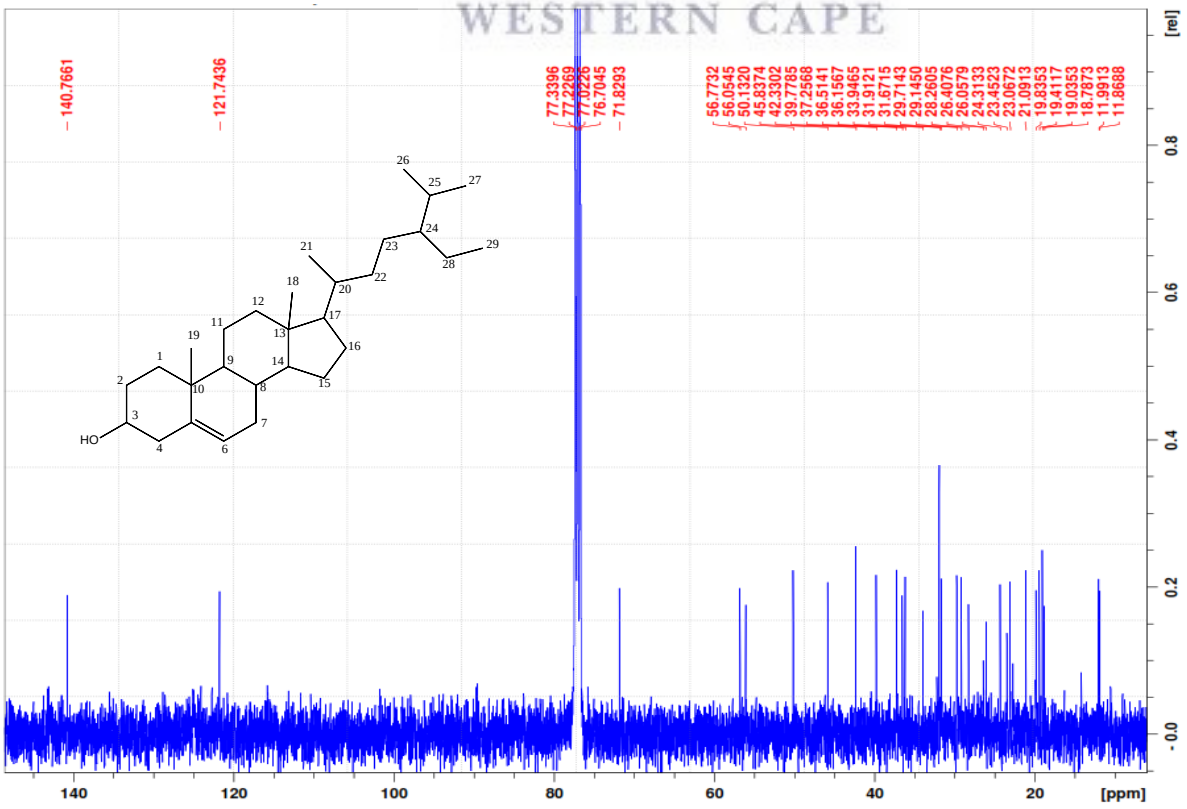




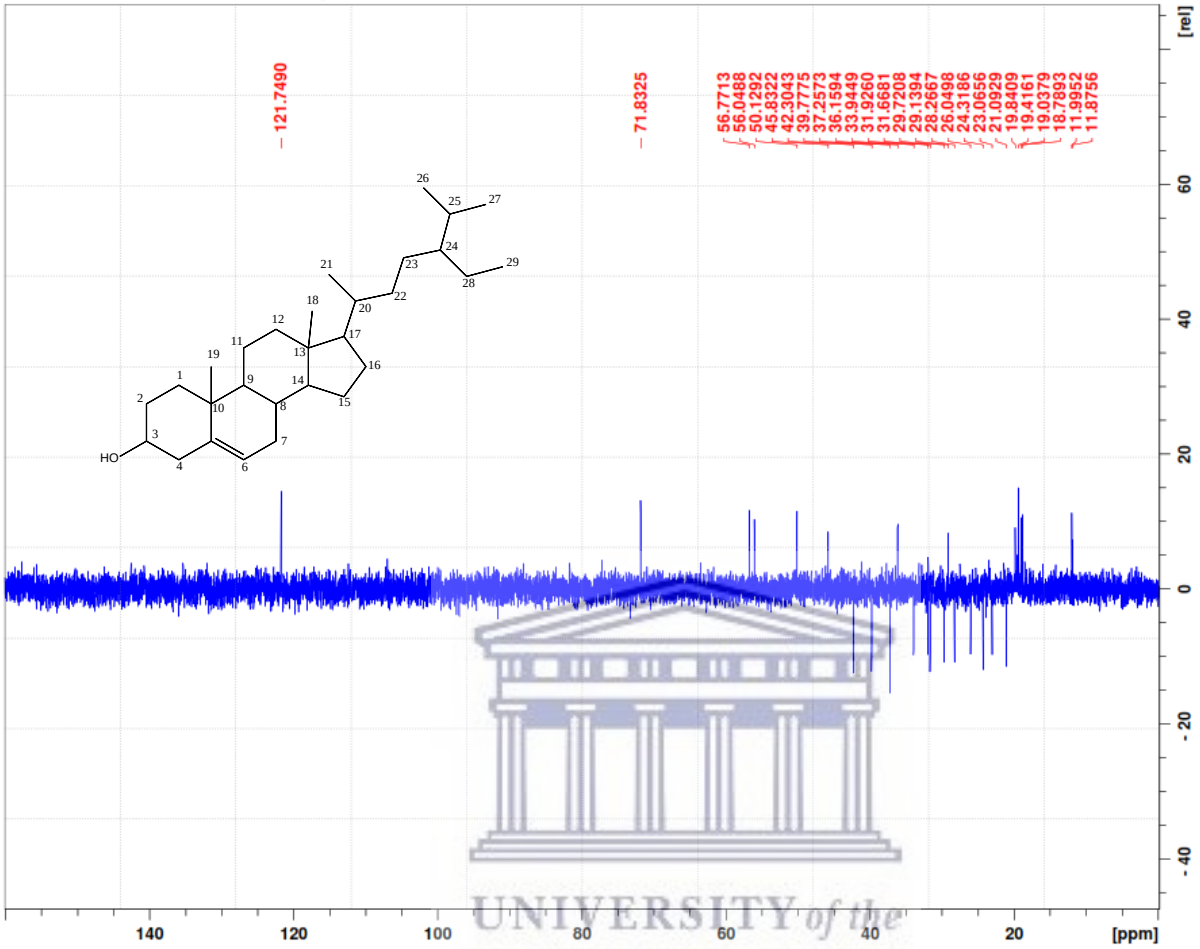
Appendix 3a. ^{1}HNMR of **compound 3**



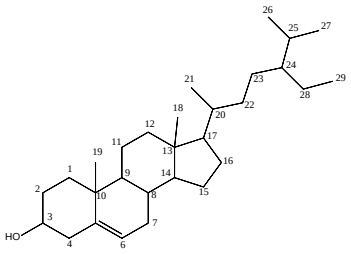
Appendix 3b. ^{13}C of **compound 3**

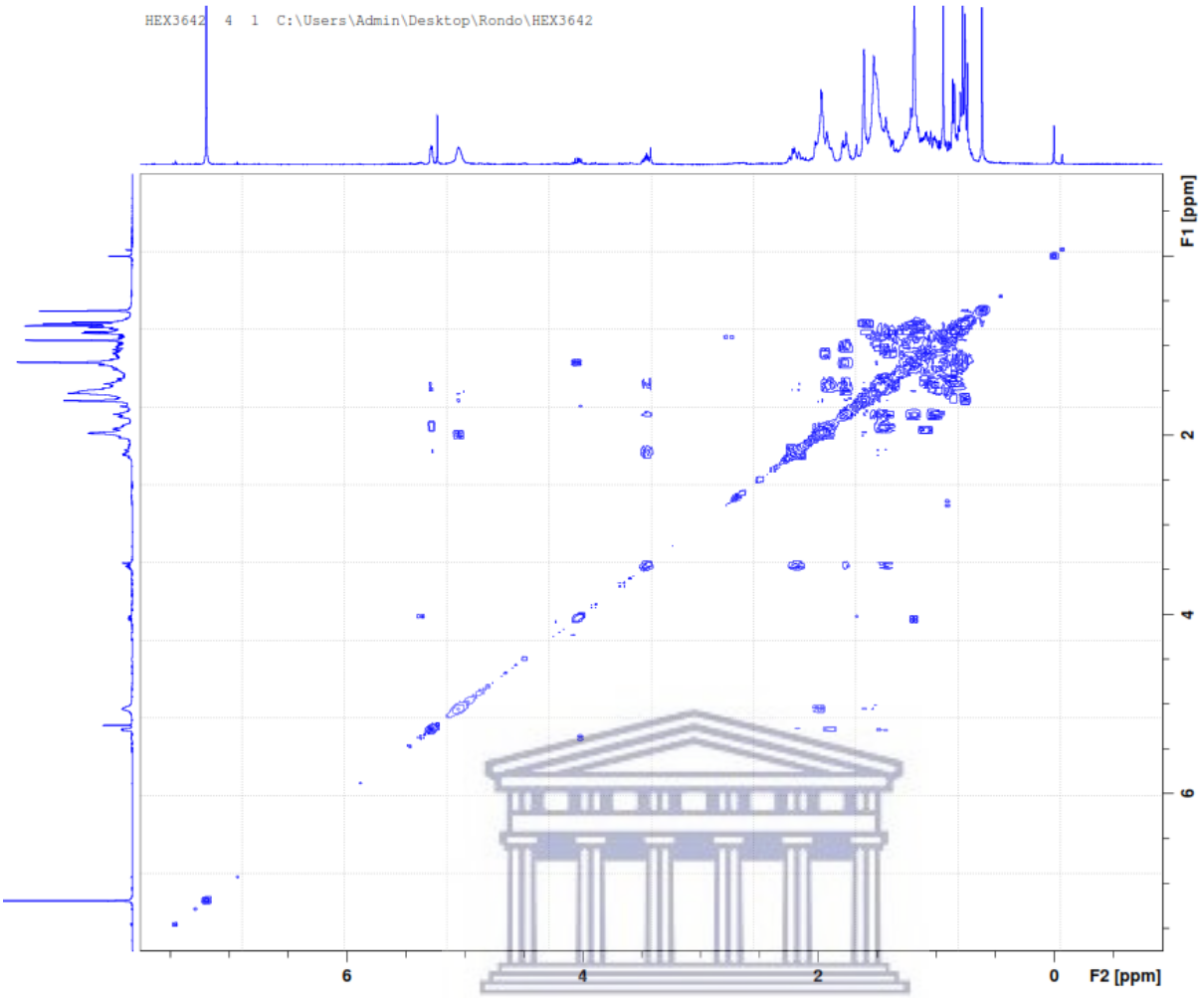


Appendix 3c. DEPT of **compound 3**

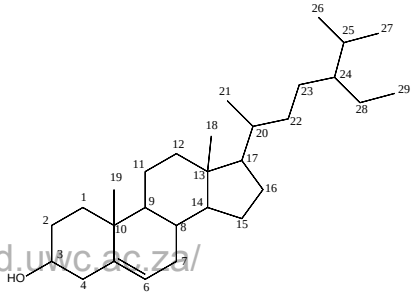


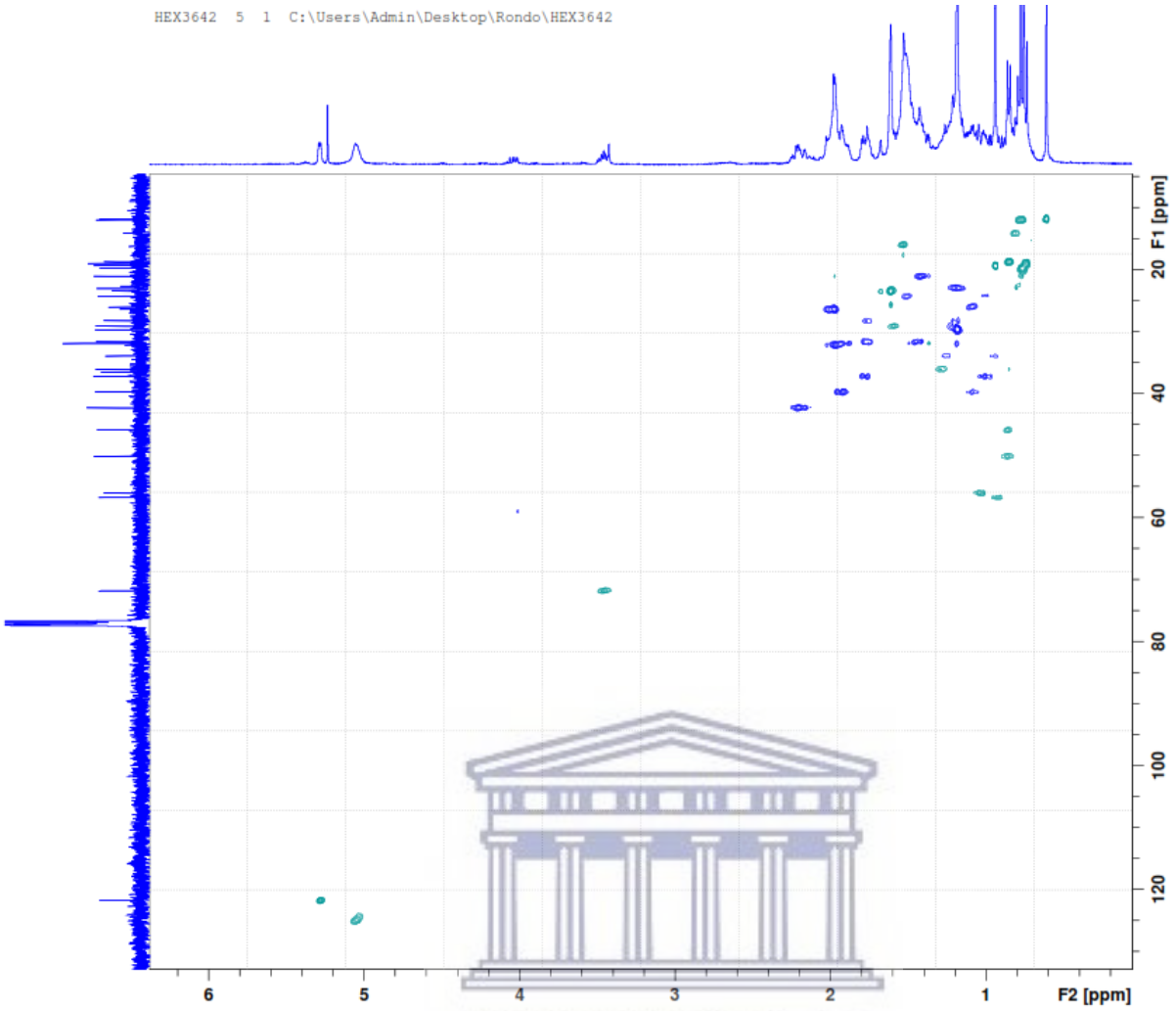
Appendix 3d. COSY of **compound 3**



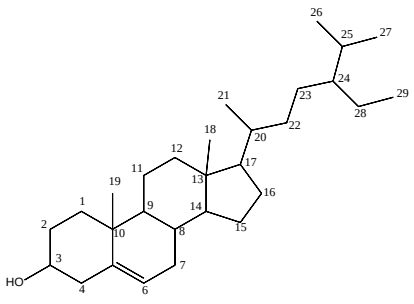


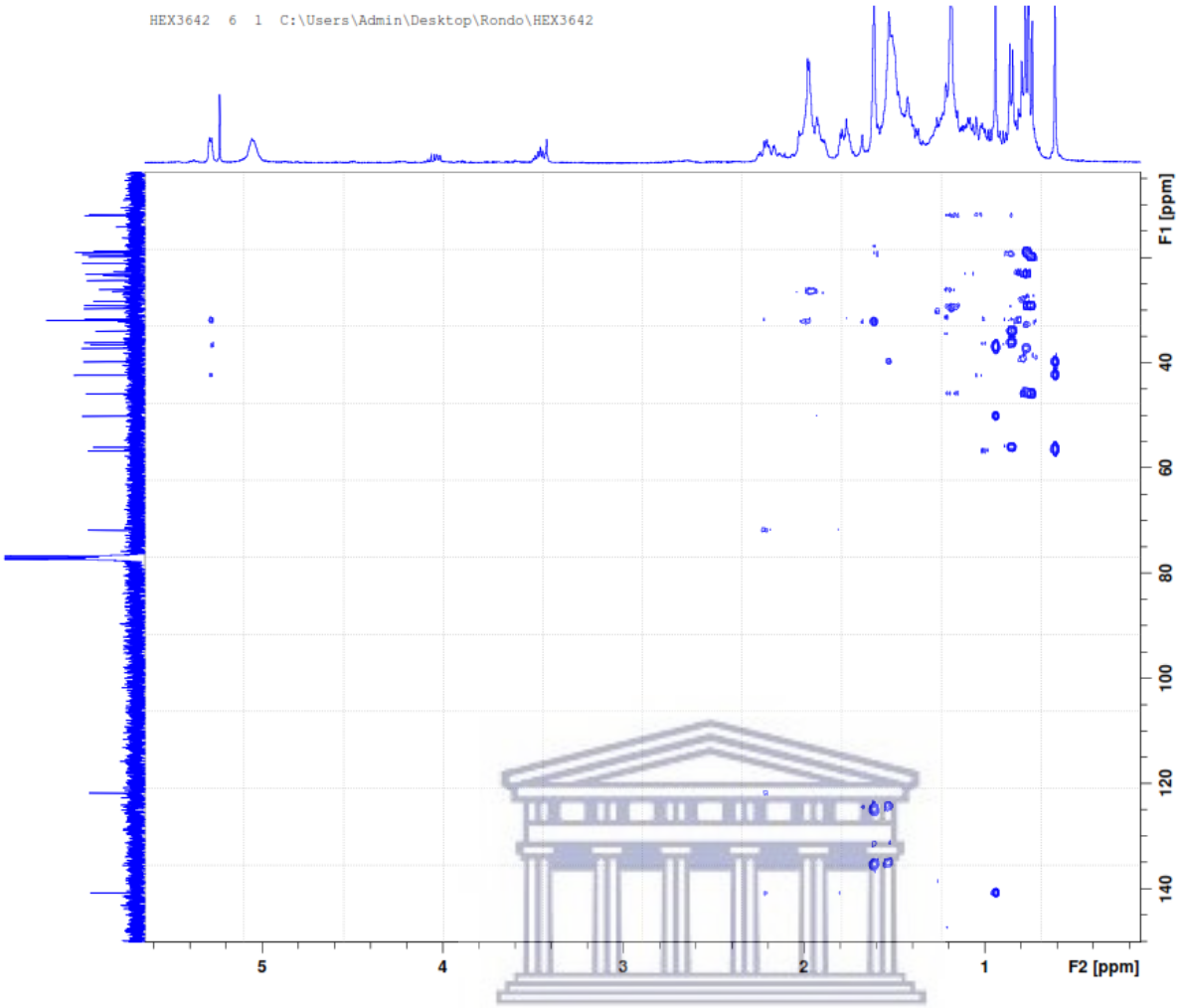
Appendix 3e. HSQC of **compound 3**



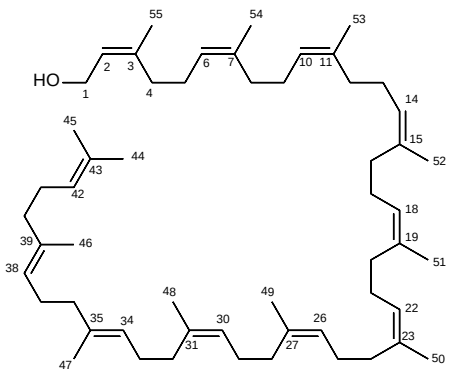


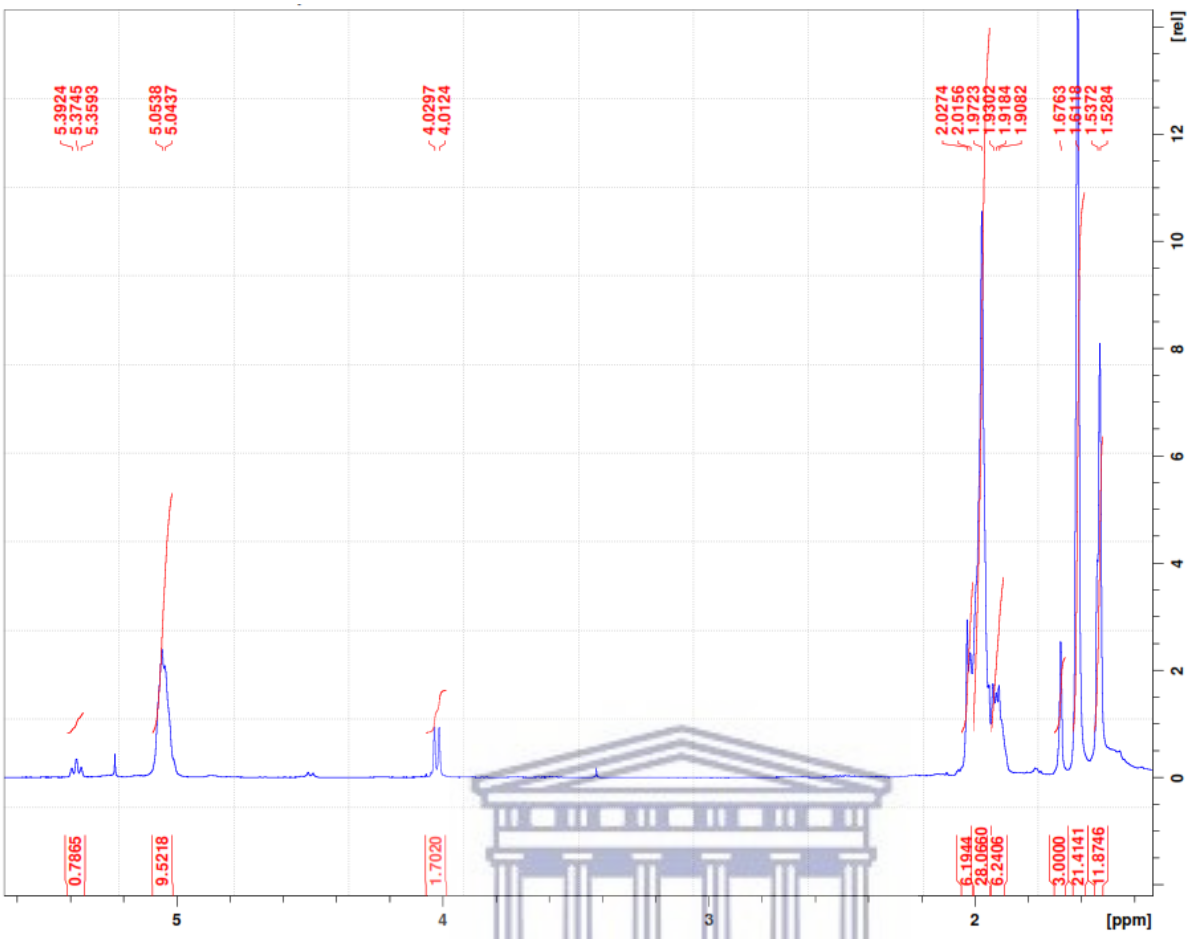
Appendix 3f. HMBC of **compound 3**



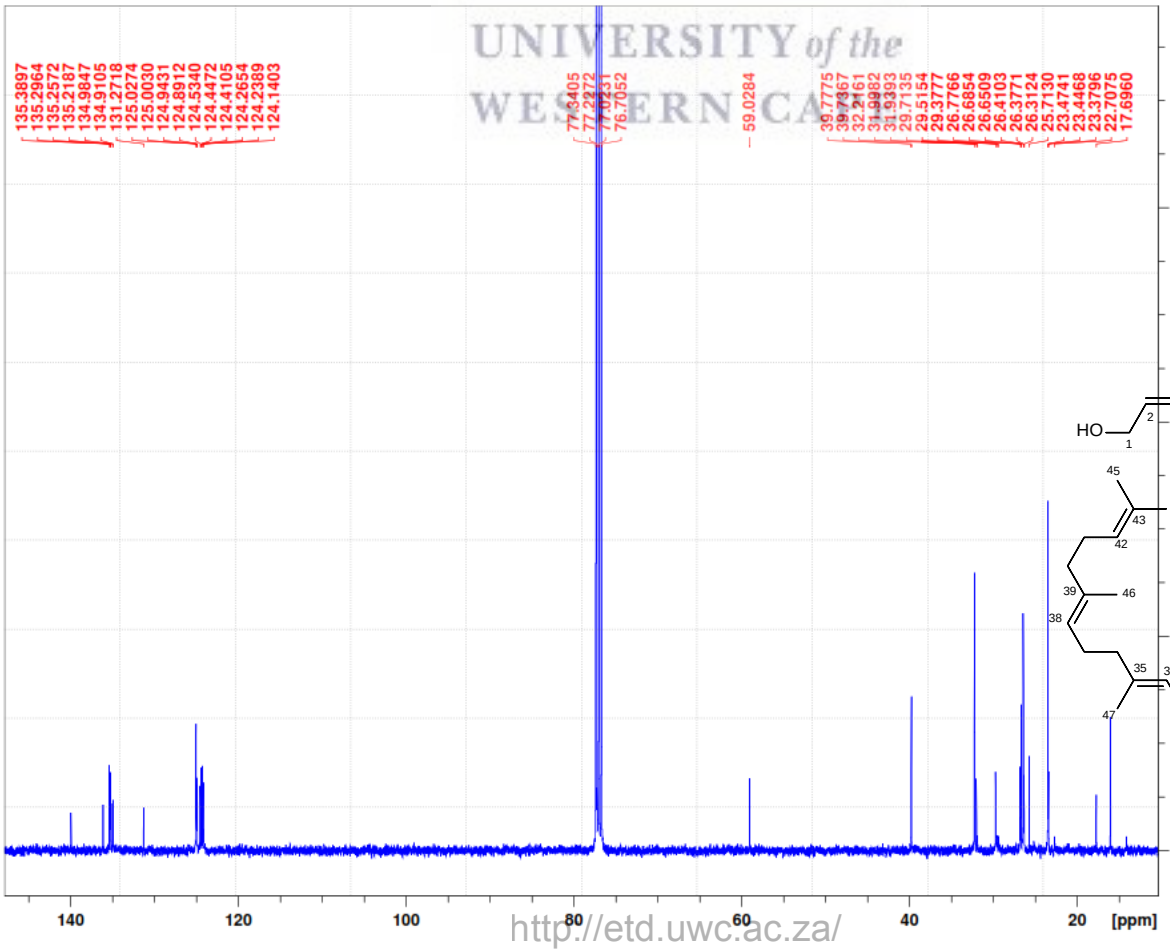


Appendix 4a. ^{1}H NMR of **compound 4**

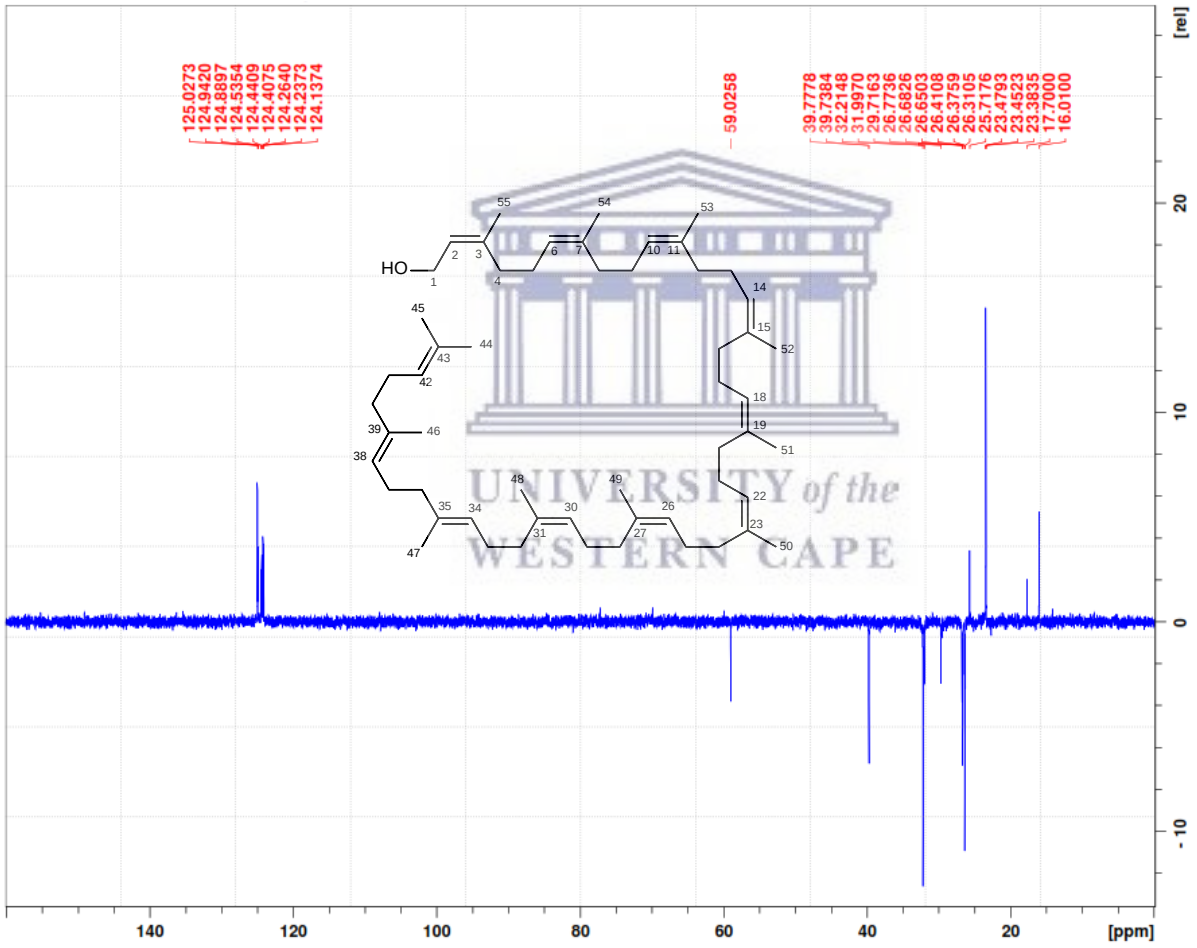




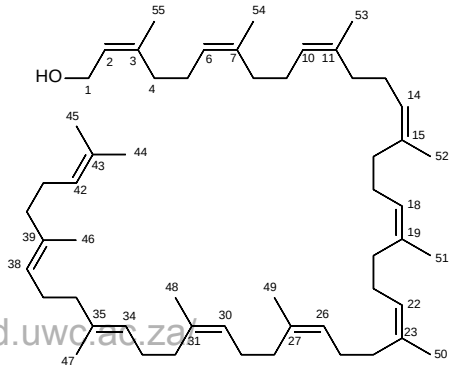
Appendix 4b. ^{13}C NMR of **compound 4**

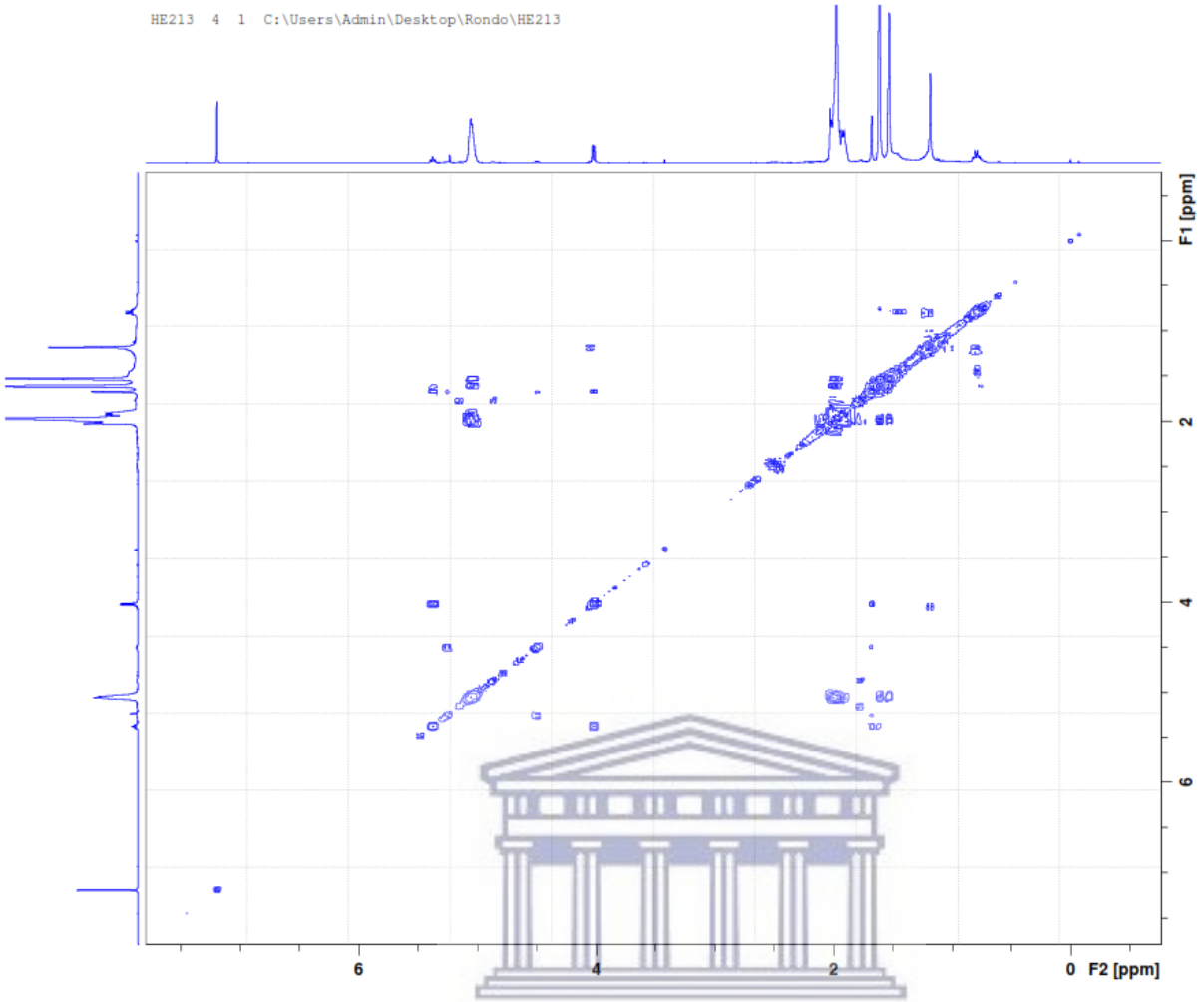


Appendix 4c. DEPT 135NMR of **compound 4**

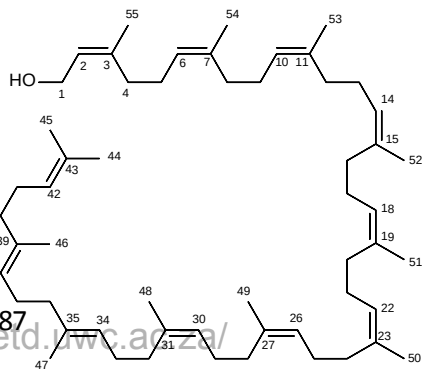


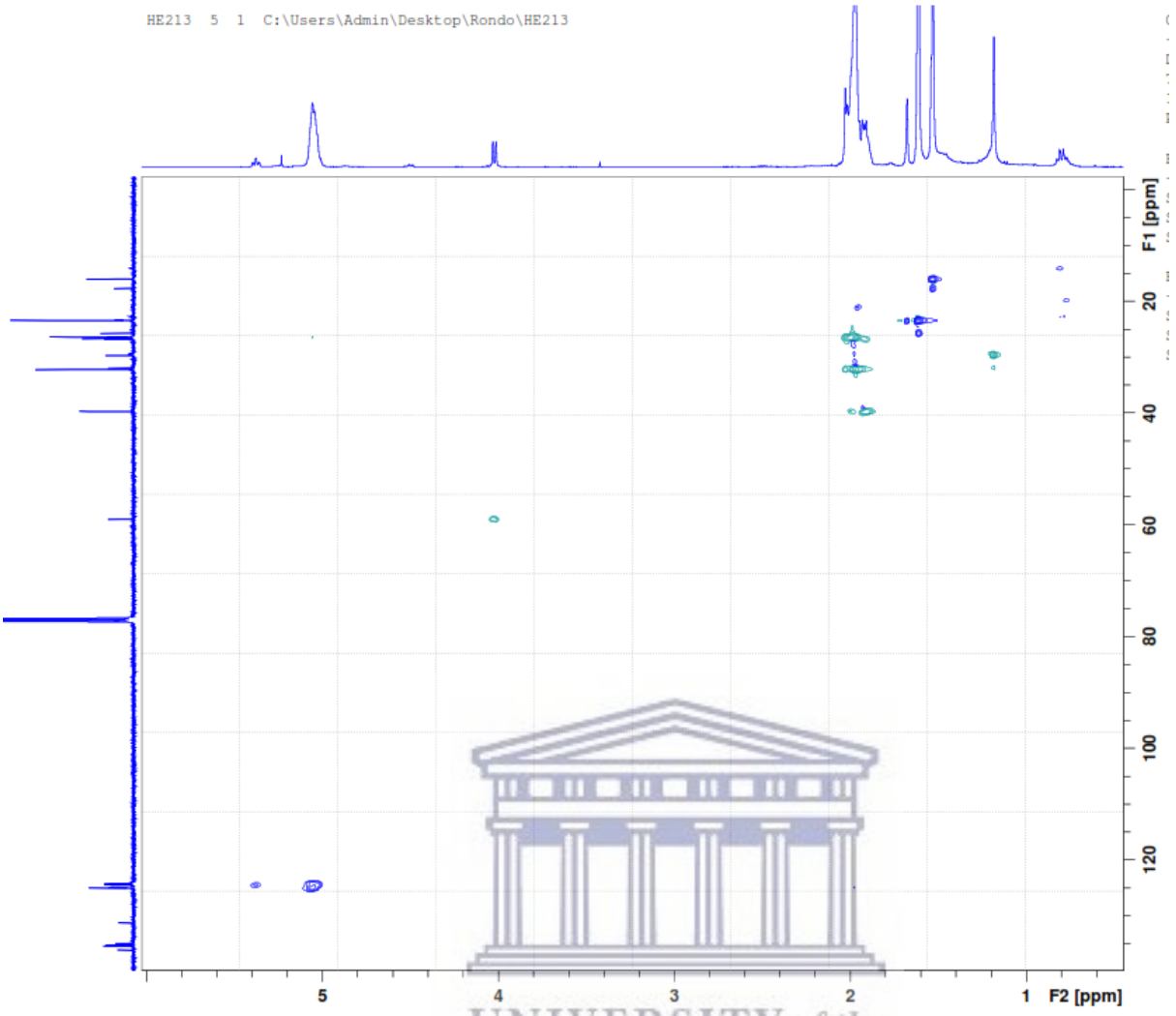
Appendix 4d. COSY NMR of **compound 4**





Appendix 4e. HSQC NMR of **compound 4**





Appendix 4f. HMBC NMR of **compound 4**