Studies towards the development of African phytomedicines from *Combretum apiculatum* and *Galenia africana*

K.V. Phungula

Studies towards the development of African phytomedicines from *Combretum apiculatum* and *Galenia africana*

by

Khanya Valentine Phungula

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

Master of Science

In the Faculty of Natural and Agricultural Sciences

Department of Chemistry

at the

University of the Free State

Supervisors: Late Prof. Andrew Marston
Dr. Susan L. Bonnet

Co-Supervisor: Prof. Jan .H. van der Westhiuzen

January 2015

DECLARATION

I declare that the dissertation hereby submitted by me for the M.Sc degree at the University of the Free State is my own independent work and has not previously been submitted by me at another University/Faculty. I further more cede copyright of the dissertation in favour of the University of the Free State.

_____ _____

Khanya V. Phungula Date

Acknowledgements

At the end of this road, I would like to express my sincere gratitude and appreciation to the following people for their contributions towards this study:

- Above all I would like to thank my Heavenly Father for the grace and strength to finish this study. He truly is an amazing God
- My late supervisor Prof. Andrew Marston for support, encouragement, persistent, and guidance. You were a true inspiration. You may be gone but you will never be forgotten. I will keep you in my heart forever.
- My supervisor Dr S.L Bonnet for her assistance, guidance, and patience.
- My Co-supervisor Prof. J.H van de Westhuizen for his guidance and valuable advice
- The NRF, Framework 7 project (MUTHI), Framework 7 project(hERG Sreen), The University of the Free State for financial support.
- To Malefu for organising the Traditional Healers, Dr. P Zietsman for all his aid with collection and identification of plant material, and Prof. S van Vuuren for her considerable help in the running of antimicrobial and antimycobacterial test.
- My group colleagues for the help and the good times we shared and most of all the friendly environment conducive for this study;
- My parents Steven and Nolitha for their lifetime love, support and encouragement throughout my life and for giving me the opportunity to further my education. Thank you for believing in me when I sometimes ceased to believe in myself.
- My siblings, Bantu, Phumela, Lerato and Lithemba for their love and moral support.
- My friends, Pholani, Dika, Sibongile and Thembani for all the crazy time we shared.

Khanya V. Phungula

Table of contents

Abbreviations and symbols

1, 2, 3…	symbols used for the compounds cited in the literature
A, B, C…	symbols used for the isolates in this work
□	Chemical Shift
AChE	Acetylcholinesterase
bd	Twice a day
$CaCl_2$	Calcium Chloride
CC	Column Chromatography
cDNA	Complementary DNA
CHCL□	Chloroform
COSY	^1H, ^1H Homonuclear Correlation Spectroscopy
cRNA	Complementary Ribonucleic Acid
d	Doublet
dd	Doublet of Doublets
DCM	Dichloromethane
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic Acid
DPPH	1,1-Diphenyl-2-picrylhydrazyl
EAD	Early after depolarisation
ECG	Eletrocadiogram
EtOAc	Ethyl Acetate
GHMBC	^1H,^{13}C, Gradient Heteronuclear Multiple Bond Correlation
GHSQC	^1H,^{13}C, Gradient Heteronuclear Single Quantum Coherence
HEPEs	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
hERG	Human Ether –á-go-go Related Gene
HIV	Human Immunodeficiency Virus
Hz	Hertz
HSCCC	High-speed countercurrent chromatography
J	Coupling Constant
K^+	Pottasium
KCl	Potassium Chloride
lk	delayed rectifier Potassium current

LQTS	Long QT Syndrome
m	Multiples
MeOH	Methanol
MDR	Multidrug resistant
MDR-TB	Multidrug resistant tuberculosis
MIC	Minimal Inhibitory Concentration
mRNA	Messenger Ribonucleic Acid
MS	Mass Spectrometry
MW	Molecular Weight
m/z	Mass per Electronic Charge
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NMR	Nuclear Magnetic Resonance
Ppm	Parts Per Million
RNA	Ribonucleic Acid
RTO	Retrospective Treatment Outcome
s	Singlet
SPE	Solid Phase Extraction
Subsp	subspecies
t	Triplet
TB	Tuberculosis
tds	Three time a day
Tdp	Torsade de points
THPs	Traditional health Practitioners
TLC	Thin-Layer Chromatography
UV	Ultraviolet
VF	Ventricular Fibrillation
WHO	World Health Organization
XDR-TB	Extensively drug resistant tuberculosis

Abstract

Nearly 80% of the world population use traditional medicine, mainly medicinal plants, to treat diseases and ailments. In developing countries and rural communities, the use of traditional medicine is a valuable resource and a necessity and provides a real alternative for primary health care systems. These have commonly not been investigated for safety and efficacy and are not used in standardized doses. The active ingredients are often not known. The need thus exist to develop standardized phytomedicines.

In this dissertation we investigated the scientific experimental techniques required during the process to develop acceptable, standardized, efficacious and safe phytomedicines from South African Indigenous knowledge and African Traditional Medicine. Due to time and other constraints during this MSc dissertation we could not work on a single plant and disease but used different plants to investigate the different aspects. The following are important::

- Identification of plants and extracts extracts that can be used to develop phytomedicines.
- Establishment of safety and toxicity of a plant extract or isolated pure bioactive compounds
- Determination of efficacy
- Quality control

In **Chapter 1** we gave a brief introduction into the development of phytomedicines

In **Chapter 2** we performed an ethnobotanical investigation to identify plants used by traditional health practitioners (THPs) to treat tuberculosis in the districts of Mangaung Metro, Thabo Mofutsanyana and Lejweleputswa in the Free State province, South Africa. A total of 37 THPs were interviewed using guided questionnaires. The THPs reported 19 plant species used to treat tuberculosis, of which *Artemisia afra* was the most frequently administered. The practitioners formulate and dispense their own recipes, most frequently using the tubers, roots and leaves of plants, but there was low consensus among the THPs as to which plants (or mixture of plants) are most efficacious. We concluded that the three plants most frequently administered, *A. afra* and *H. caespititium* (both Asteraceae), and *L.*

lanceolata (Fabaceae), are candidates for further phytochemical investigation into their antimycobacterial and toxic properties, and to determine the efficacy of extracts and isolated compounds.

In **Chapter 3** we investigated toxicity issues by assessment of potentially cardiotoxic properties of extracts from traditionally used medicinal plants. hERG (human Ether-à-go-go Related Gene) is a gene that encodes the pore-forming α-subunit of a voltage-gated potassium K^+ channel expressed in the heart and nervous tissue. Mutations in hERG can lead to partial or complete loss of function, and may cause cardiac arrhythmia that can degenerate into ventricular fibrillation, leading to sudden death. We investigated the effect of plant extracts on ion channels expressed in heterologous *Xenopus laevis* oocytes by means of the two microelectrode voltage-clamp technique. The DCM and MeOH extracts from 129 plant species were screened on *Xenopus laevis* oocytes for their potential cardiotoxic risk. Plant extracts that reduced the peak tail current by ≥30% hERG were considered positive hERG channel blockers. Plant extracts showing an inhibition between 30-60% at a concentration of 100 μg/mL were identified.

In **Chapter 4**, a retrospective treatment outcomes based study was performed to investigate the efficacy of the plant preparations used by the population in the Thaba ‘Nchu district in the Free State, South Africa, for the treatment of diarrhoea. Thirty two patients were interviewed using guided questionnaires. Thirteen plant species were reported, of which *Xysmalobium undulatum* and *Punica granatum* were the most frequently used plant species. The majority of the patients (94.7%) had a successful treatment outcome even though the efficacy of the remedies varied.

In **Chapter 5**, phytochemical investigation of two medicinal plants, *Galenia africana* L. and *Combretum apiculatum* Subsp. *apiculatum*, led to the isolation of five known compounds, four of which were isolated from the plants for the first time. The isolated compounds were identified as 7,8-dimethoxy-2-phenyl-4*H*-chromen-4-one (**A**), 6,7-dimethoxy-2-phenyl-4*H*-chromen-4-one (**B**), 8-phenyl-6*H*-[1,3]dioxolo[4,5-*h*]chromen-6-one (**C**), 5,7-dihydroxy-2-phenyl-3-((3*S*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-methyltetra-hydro-2*H*-pyran-2-yloxy)-4*H*-chromen-4-one (**D**) and 5-hydroxy-7-methoxy-2-phenylchroman-4-one (**E**) via NMR spectroscopy and ESI mass spectrometry. It is important for quality control to

identify the active molecules responsible for the medicinal properties of a plant to ensure that these molecules occur in the same concentration in all phytomedicine batches.

In **Chapter 6**, the extracts and pure isolates were subjected to in-house TLC bioassays (antioxidant and inhibition of acetylcholinesterase), and antimicrobial and antimycobacterium testing (performed at the University of the Witwatersrand). *G. africana* and *C. apiculatum* have been used traditionally to treat infectious diseases, and our phytopharmacological study on the two plants has confirmed these practices. We found that,

- *G. africana* exhibits relatively low radical scavenging activity for the DCM extract and no activity for the MeOH extract, while both the seed and leave MeOH extracts from *C. apiculatum* displayed significant antioxidant activity
- The DCM extract of *G. africana* and isolated compounds **A** and **C** showed acetylcholine esterase (AChE) inhibition
- The DCM extract of *G. africana* showed significant activity against all four pathogens with the best activity observed against *C. neoformans*.
- The highest activity against *E. faecalis* and *K. pneumonia* was from the MeOH crude extract of the seeds of *C. apiculatum*, and against *C. neoformans* the BuOH partition fraction from the seed extract
- The seed and leave extracts of *C. apiculatum* and the partition fractions thereof all exhibited significant activity against *M. smegmatis*

We have thus researched protocols to develop standardized phytomedicines from traditionally used plants.

1. Literature review

1.1. Introduction

In recent years the evaluation of the efficacy of medicinal plants from Africa, which play an important role in the maintenance of health and in the introduction of new treatments, has led to an increasing number of reports in the literature. Even though medicinal plants still play an important role in the healthcare system in African countries, promising medicinal plants from the African continent have not yet been compiled in a reliable reference system. In this work we aimed to do a comprehensive investigation into the development of phytomedicines in South Africa.

1.2. History of phytomedicine and drug discovery

Phytomedicine, sometimes referred to as herbal medicine or botanical medicine, is the use of plants for their therapeutic or medicinal properties.[1,2] For centuries, plants have been used as a source of medicine to treat human diseases[3] and continue to play a significant role as therapeutic remedies in primary health care.[4] The first records, written on clay tablets in cuneiform script, are from Mesopotamia and date from about 2600 BC. Among the substances used were ales of the *Cedrus* species (cedar) and *Cupressus sempevirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh), and *Papaver somniferum* (poppy juice), which are all still used today to treat ailments ranging from cough and colds to parasitic infection and inflammation.[5]

Medicinal plants produce and contain a variety of chemical substances with varied physiological effects.[6] About 80% of the worlds population still rely on traditional medicine to treat diseases.[7] Estimates show that 25% of prescribed drugs contain at least one active compound derived from plant material; some are produced from plant extracts and other are synthesized to mimic a naturally occurring plant compound.[8] In recent years the research in medicinal plants has received much interest due to, among others, unmet therapeutic needs, the remarkable diversity of both chemical and biological activity of naturally occurring secondary metabolites and the utility of novel bioactive natural products as lead compounds in drug discovery. Conventional medicine is efficient, but a large percentage of the world's population does not have access to conventional treatment. Patient non-compliance and side effects also have an influence on the efficacy of modern medicine.[9] It is widely believed that phytomedicine offers fewer side effects since a large percentage of the world's population has been using phytomedicine for thousands of years.[10]

1.3. Approach for the development of phytomedicine

In phytomedicine, crude plant extracts in the form of infusions, decoctions and oils are traditionally used by the population for the treatment of diseases, including infectious diseases. Although their efficacy and mechanisms of action have not been scientifically investigated in most cases, the active chemical constituents of these herbal treatments often mediate positive responses.[11] Of the estimated 250 000 to 500 000 plant species, only a fraction (about 5000 species) has been scientifically investigated.[2]

Development of phytomedicine involves many fields and various methods of analysis. The process typically begins with a botanist, ethnobotanists, ethnopharmacologist or plant ecologist who collects and identifies the plant specimens of interest. The collection of plants may include species with known biological activity for which an active constituent(s) have not been isolated, e.g traditionally used herbal medicine, or may involve a taxa collected randomly for large screening programs.[12] Ethnobotany and ethnopharmacology play an important role towards this approach when aiming at popular information retrieval, i.e. the knowledge which has been transferred from generation to generation by all cultures. The ethno-oriented survey puts the popular information as an important reference for the experiments both with regards to the exploitation and use of herbal drugs and phytomedicines, and for the development of new remedies. **Figure 1** schematically represents the key steps for the development of herbal medicine, the starting point being the selection of medicinal plants according to the ethno-oriented method. [13]

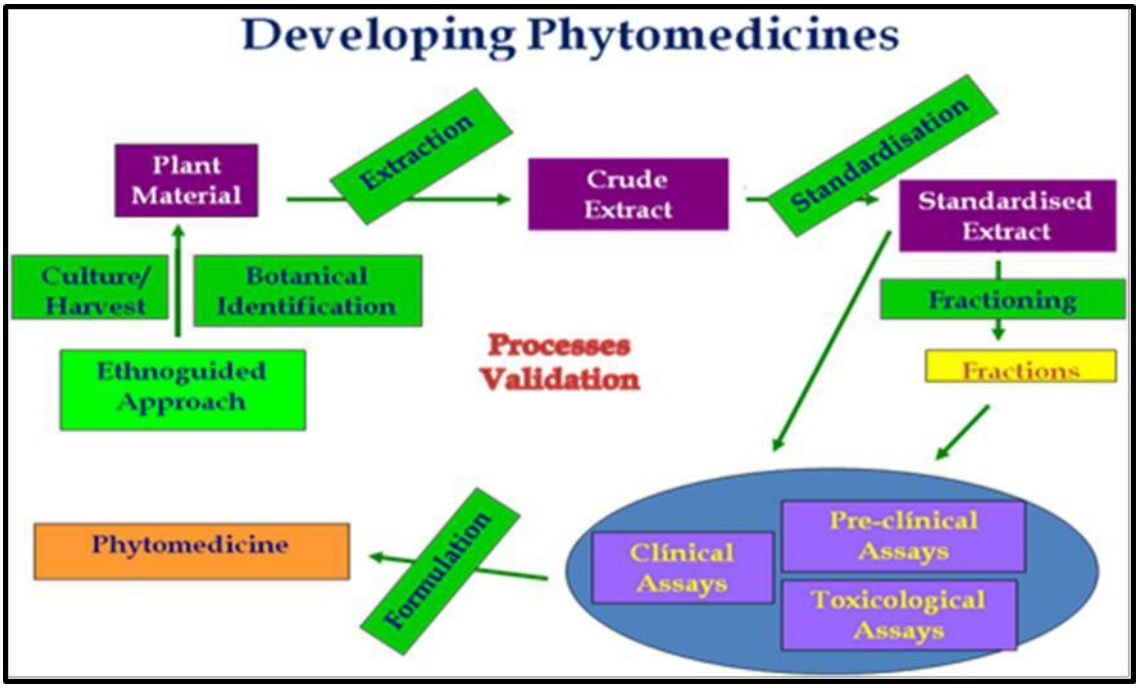


Figure 1.1. Schematic sequence of the steps in developing phytomedicine.[13]

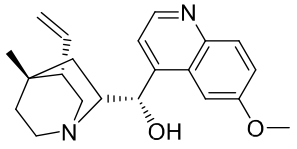
The plant extracts are prepared by phytochemists and then subjected to biological activity screening, followed by the process of isolation and characterization of active compounds.[12]

Extraction is the first step in drug discovery from plants. Either the whole plant or a specific part of the plant (root, leaf, stem, fruit, etc.) is extracted with a suitable solvent.[14] Several general extraction procedures have been proposed for obtaining extracts representing different ranges of polarity and enriched with the most common secondary metabolites such as alkaloids.[15]

1.4. Historical important natural products derived from higher plants

It has been estimated that only 5 to 15% of the ca. 250 000 plant species have been scientifically evaluated for the presence of biologically active compounds.[16] Plant-derived bioactive compounds have been developed directly as drugs, or serve as prototype drug molecules known as "lead compounds", and as pharmacological probes.[17]

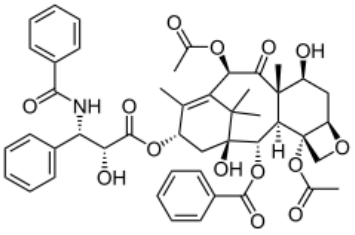
Well known examples of plant-derived drugs includes the antimalarial quinine, isolated from the bark of the *Cinchona* spp. Quinine was the first effective drug for the treatment of *Plasmodium falciparum* malaria, and the fatal parasitic disease caused by other species of plasmodium.[17]



Quinine

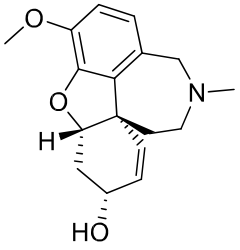
Vincristine, which is being used to treat certain types of cancer, was isolated from the "Madagascar periwinkle" (*Catharanthis roseus*). In 1819, the isolation of analgesic morphine, codeine and paregoric laid down the foundation for the isolation of pharmacologically active compounds for the treatment of diarrhoea from *Papaver somniferum*.[18]

The diterpenoid paclitaxel was isolated for the first time from the bark of *Taxus brevifolia* (Pacific yew) in the late 1960s, and approved for marketing as a cancer chemotherapeutic agent in 1992.[19]



Paclitaxel

Galanthamine is the most recently approved drug for the treatment of Alzheimer's disease. Galanthamine is an Amaryllidaceae-type alkaloid first isolated from the snowdrop (*Galanthus woronowii*) and later found in other plants of the Amaryllidaceae family. It acts as a cholinergic agent to improve the cerebral function and also inhibits acetylcholinesterase and modulates nicotinic acetylcholine receptors.



Galanthamine

1.5. Challenges in the development of phytomedicine

Ahmad[20] identified at least five major limitations in the development of herbal medicine: (i) reproducibility of biological activity of herbal extracts; (ii) toxicity and adverse effects; (iii) adulteration and contamination; (iv) conventional medicine interactions issues; (v) standardisation issues. We shall now briefly examine each of these limitations.

i) Reproducibility

Ahmad[20] reported that up to 40% of plant extracts lack reproducibility of activity. Herbal plants are often harvested at different locations and at different developmental stages during the growth seasons, influencing their biochemical profiles and thus their activity. Further influences include different extraction methods and different bioassays used. These factors

necessitate the evaluation of the quantitative and qualitative variations in the phytochemicals found in plant extracts. Pharmacodynamic synergism (activity due to a combination of phytocompounds) further complicates the standardization of extracts and thus variations in chemical composition of herbal medicine require careful chemical analysis to ensure consistency.

ii) Toxicity and adverse effects

Phytomedicine are often believed to be safer than modern medicine because it is "natural". Arlt[21], Wojcikowski[22] and Akinboro[23] reported herbal medicine preparations that are potentially toxic and carcinogenic. The toxic effect of herbal medicines may be due to the existence of phytotoxins in unadulterated herbal medicines, erroneous botanical identification and unsuitable combinations of plants. Some plant extracts interfere with conventional medicines, e.g. plants high in coumarin derivatives, tyramine and estrogenic compounds, and may lead to adverse events. Phytomedicines thus have a specific dosage threshold to be efficacious and/or toxic, just like conventional medicines.

iii) Adulteration and contamination

Adulteration and contamination of herbal medicine are unpredictable when strict regulations for production are not in place, and can cause serious medical problems. Herbal medicines may be contaminated with heavy metals from the use of inorganic compounds during preparation. This is combined with environmental heavy metal contamination, especially in developed countries. Contamination with conventional drug constituents has been reported by the FDA.

iv) Phytomedicine–conventional drug interaction

Fugh-Berman[24] reported that the pharmacokinetic profile of conventional pharmaceuticals can be changed by the usage of herbal medicine. The tempo of absorption and metabolism of drugs can be influenced and it can cause allergic reactions. The influence of the interactions of phytomedicines with drugs should be evaluated to determine the potential toxicity and efficacy reactions. The influence of the interactions of phytomedicines with drugs should be evaluated to determine the potential toxicity and efficacy.

v) Standardization of phytomedicine

Standardization of phytomedicines is not a facile task. The percentages of constituents of extracts are influenced, for example, by the time of year when plants are harvested, the

location and the climate during harvesting. Producers can improve the batch-to-batch consistency of their extracts by controlling the environment where the plants are cultivated, and using refined chromatographic techniques and marker compounds. However, the manufacturing processes among different producers are not standardized and leads to variability of constituent concentrations in the different brands. Owing to this, the efficacy of the preparations may differ from brand to brand, and the bioactivity of some brands even disappears during processing.[20]

1.6. Traditional medicine in South Africa

South Africa has an incredibly rich floral diversity, with over 30 000 species of higher plants of which ca. 3000 species are used therapeutically.[25,26] The flora is not only rich in diversity, but also largely endemic.[27] The Cape floristic region, with an estimated 6000 endemic species, is one of the world's richest floral regions, and many of the indigenous plants have been phytochemically investigated.[28] It is estimated that at least 70% of all South Africans consult one of the more than 200 000 traditional healers in the country. In many rural communities traditional medicine is still recognized as the primary health care, because of its accessibility, affordability and lack of modern medical alternatives. It has always been part of the cultural and religious life of the African people.[29]

1.7. Aim of this dissertation

During the development of commercially viable phytomedicines the following factors are important:

- Identification of plant species that can be developed commercially
- Establishment of safety and toxicity of a plant extract or isolated compounds
- Determination of efficacy
- Quality control

In this study, selected scientific methodologies were investigated to achieve the above aims. Due to time and capacity constrains we worked on different plants as examples.

Firstly, an ethnopharmacological investigation was performed to identify the plants used by traditional health practitioners to treat tuberculosis in the districts of Mangaung Metro, Thabo Mofutsanyana and Lejweleputswa in the Free State province of South Africa **(Chapter 2)**. This project was done in collaboration with C.I.E.A van't Klooster from the University of Amsterdam as part of an European Union funded Framework 7 project (MUTHI).

Secondly, we investigated toxicity issues by assessment of potential cardiotoxic natural botanicals via inhibition of the hERG channel by plant extracts. The presence of hERG modulators in the plant extracts increases the risk of ventricular arrhythmias that can lead to death **(Chapter 3)**. This investigation was performed during a four month stay in Vienna in collaboration with Prof. S. Hering (University of Vienna) as part of an European Union funded Framework 7 project (hERGscreen).

Thirdly, a retrospective treatment outcome based study (RTO) was performed to investigate the efficacy of the preparations used by the population in the Thaba 'Nchu area in the Free State, South Africa, for the treatment of diarrhoea **(Chapter 4)**. This project was done in collaboration with Dr M. Willcox from the University of Oxford as part of an European Union funded Framework 7 project (MUTHI)

Fourthly, a phytochemical investigation was performed on two medicinal plants, *Galenia africana* L. and *Combretum apiculatum* subsp. *apiculatum*, leading to the isolation of five known compounds, four of which were isolated from the plants for the first time **(Chapter 5)**.

Lastly, bioassays were performed on the extracts and isolated compounds **(Chapter 6)**. This process is an essential step towards the development of commercial phytomedicines. This project was performed in collaboration with Prof. D. Diallo from the National Institute of Research in Public Health in Bamako, Mali, as part of an European Union funded Framework 7 project (MUTHI).

Although the above investigation may somehow seem incoherent as it did not concentrate on a single plant and direction, the emphasis was on the investigation of the steps and technologies required to develop commercially acceptable, standardized, efficacious and safe phytomedicines from African indigenous knowledge.

1.8. References

1. Duke J.A. **2002**. *Handbook of medicinal herbs*. Maryland, USA.
2. Mohamed I., Shuid A., Borhanuddin B. and Fozi N. **2012**. The Application of phytomedicine in Modern drug development. *IJHPM* 1
3. Wendakoom C., Calderon P. and Gagnon D. **2012**. Evaluation of selected medicinal plants extracted in ethanol concentrations for antibacterial activity against human pathogens. *JMAP* 2: 60-68.
4. Sokmen A., Jones B.N. and Erturk M. **1999**. The in Vitro antibacterial activity of Turkish medicinal plants. *J. Ethnopharmacol.* 67: 79-86.
5. Newman D.J., Cragg G.M. and Snader K.M. **2000**. The influence of natural products upon discovery. *Nat. Prod. Rep*. 17: 215-234.
6. Saidu Y., Mwachukwu F.C., Bilbis L.S, Faruk U.Z. and Abbas A.Y. **2010**. Toxicity studies of the crude aqueous roots extract of *Albizzia Chevalieri* Herms in albino rats. *Nig. J. Basic Appl. Sci.* 18: 308-314.
7. Balick M.J. **1990**. Ethnobotany and the identification of therapeutic agents from the rainforest. *IEB* 154: 22-39.
8. Cragg G.M. and Newman D.J. **2002**. Drugs from nature: past achievements, future prospects. *In Advances in phytomedicine*; *Ethnomedicine and drug discovery*. By Iwu M.M. and Wootton J.C. 23-37.
9. Rates S.M.K. **2001**. Plants as source of drugs. *Toxicon.* 39: 603-613.
10. Okigbo R.N and Mmeka E.C. **2006**. An Appraisal of phytomedicine in Africa. *KMITL Sci. Tech. J*. 6: 53-94
11. Barnes J., Anderson L.A and Philipson D. **2007**. *Herbal Medicine*. Pharmaceutical press.
12. Balunas M.J. and Kinghorn A.D. **2005**. Drug discovery from medicinal plants. *Life Sci.* 78: 431-441.
13. Wagner L.R.B., do Nascimento M.S., do Nascimento L., Costa Maria L., Sousa A.J.A. and Monteiro M.M. **2011**. Selection of medicinal plants for development of phytomedicine and use in primary health care. *In Bioactive compounds in phytomedicine*. By Rosooli I.
14. Solanki R. and Nagori B.P. **2012**. New method for extracting phytoconstituents from plants. *Int J. of Biomed. & Adv. Res*. 3:770-774.
15. Brusotti G., Cesari I., Dentamaro A., Caccialanza G. and Massolini G. **2014**. Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach. *J. Pharm. Biomed. Anal.* 87: 218-228.

16. Manuel F.B., Kinghorn D.A. and Norman R.F. **1993**. *In human medicinal agents from plants*. By Kinghorn. Washington: American chemical society. 1-11.
17. Pan L., Carcache de Blanco E. and Kinghorn D.A. **2009**. Plant-derived natural products as leads for drug discovery. *In Plant-derived Natural products*. By Osbourn A.E and Lanzotti V. New York: Springer.
18. Jabeena K., Ahmad M.B., Ahmad S. and Cowan D.J. **2013**. Natural products as lead compounds in drug discovery. *J. Asian Nat. Prod. Res*. 15: 764-788.
19. McChesney J.D., Venkataraman S.K. and Henri J.T. **2007**. Plant natural products: back to the future or into extinction? *Phytochem*. 68: 2015-2022.
20. Ahmad I., Aqil F., Ahmad F. and Owais M. **2006**. Herbal medicine: Prospect and constraints in modern phytomedicine.
21. Arlt V.M., Stiborova M. and Schmeiser H.H. **2002**. Aristochic acid as a probable human cancer hazard in herbal remedies: a review. *Mutagenesis*. 17: 265-277.
22. Wojcikowski K., Johnson D.W. and Gobe G. **2004**. Medicinal herbal extracts- renal friend or foe? Part one: the toxicities of medicinal herbs. *Nephrology*. 9: 313-318.
23. Akinboro A. and Bakare A.A. **2007**. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on Allium cepa Linn. *J. Ethnopharmcol*. 112: 470-475.
24. Fugh-Berman A. **2000**. Herb-drug interactions. *Lancet*. 355: 134-138.
25. Van Wyk B-E.,Van Oudtshoorn B. and Gericke N. **1997**. *Medicinal plants of South Africa*, Briza, South Africa.
26. Light M.E., Sprarg S.G. Stafford G.I and van Staden J. **2005**. Riding the wave: South Africa's contribution to ethnopharmacology research over the last 25 years. *J. Ethnopharmacol*. 100: 127-130.
27. Van Vuuren S.F. **2008**. Antibacterial activity of South African medicinal plants. *J. Ethnopharmacol*. 119: 462-472.
28. Mulholland D.A. and Drewes S.E. **2004**. Global phytochemistry: indigenous medicinal chemistry on track in Southern Africa. *Phytochemistry*. 65: 769-782.
29. Steenkamp V. **2003**. Traditional herbal remedies used by South African women for gynecological complaints. *J. Ethnopharmacol*. 86: 97-108.

2. Ethnobotanical survey of plants used by traditional health practitioners to treat Tuberculosis in the Free State Province

2.1. Introduction

Tuberculosis (TB) is an infectious diseases caused by the *Mycobacterium tuberculosis* bacillus. It remains a major global health problem and ranks as the second leading cause of death due to an infectious disease. About 8.6 million new cases were reported in 2012 and approximately 1.3 million TB deaths.[1] South Africa ranks third in the number of TB incidents (0.4-0.6 million cases per year), following India (2.0 -2.4 million) and China (0.9-1.1 million).[1] The Free State province has the fourth highest TB incidence rate in the country (857 per 100 000), with the Lejweleputswa health district reporting the highest TB incidence rate and case load.[2]

The recent increase in the number of TB cases is associated with the increasing rate of infection by the human immunodeficiency virus (HIV) and the rapid spread of multidrug-resistant tuberculosis (MDR TB). People who are HIV positive are more at risk of getting infected with TB and TB treatment outcomes are worse among HIV-positive TB patients compared to HIV-negative TB patients. TB is the main cause of death among this population.[3,4]

As stated above, TB treatment has become more complicated because of the emergence of multi-drug resistant (MDR) *M. tuberculosis* strains. Anti-TB therapy is achieved with two groups of drugs: first-line drugs, which are usually employed for the treatment of TB patients who contract *M. tuberculosis* for the first time, and second-line anti-TB drugs used for the treatment of MDR-TB.[3] The second-line drugs are more expensive and have more serious adverse effects than the first-line drugs. The treatment can last up to 8 months. Misuse or mismanagement of these second-line drugs can lead to the development of extensively drug resistant tuberculosis (XDR-TB), and their effectiveness decrease. Due to the rise in the MDR- and XDR-TB strains, there is a growing need to discover alternative drugs that are effective against all forms of TB infections.[5,6]

The aim of this study was to collect and document medicinal plants used for the management of tuberculosis by traditional health practitioners (THPs) in the Free State Province of South Africa.

2.2. Materials and Methods

2.2.1. TB burden in the Free State Province

The Free State is located in the centre of South Africa and borders on Lesotho in the east (**Figure 2.1**). Even though it is the country's third-largest province, the population comprises only 5.3% of the population of South Africa. However, the province has the fourth highest TB incidence rate as mentioned above. Mining is the major industrial activity and employer in the Lejweleputswa district (around the city of Welkom), and this may explain why Lejweleputswa district reports the highest incidence of TB cases in the Free State. Sesotho is the main language spoken, followed by Afrikaans and Xhosa.

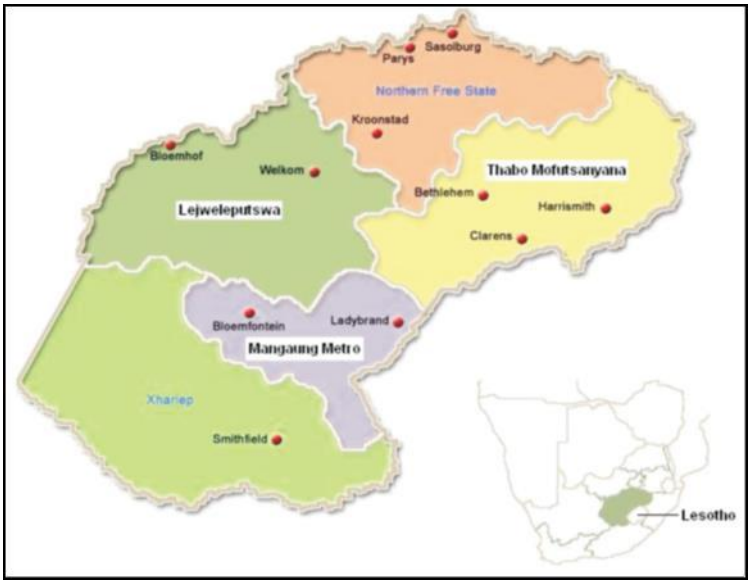


Figure 2.1. Map of the Free State Province, South Africa.

2.2.2. Data collection

We performed an ethnobotanical approach to explore the knowledge, diagnosis and treatment used to manage TB in the districts of Mangaung Metro, Thabo Mofutsanyana and Lejweleputswa in the Free State province of South Africa **(Figure 2.1)**. A selected group of THPs, identified with the assistance of the Traditional Practices Office, Department of Health, Free State, were interviewed using a guided questionnaire **(Appendix A)** to obtain the relevant ethnomedicinal data. The questionnaires were administered by trained interviewers.

There is two distinct types of THPs. Diviners (sangomas) are the most important intermediaries between humans and the supernatural, and they are mystically called to duty by the ancestors. They

regard themselves as servants of the ancestors. Their vocation is mainly that of divination, but they often also provide the medication for the specific cases they have diagnosed. Herbalists (inyanga) are ordinary people who have gained empirical knowledge to diagnose certain illnesses with certainty and thus prescribe medicines (healing herbs and plants) for everyday ailments and illnesses, to prevent and to alleviate misfortune or evil, to provide protection against witchcraft and misfortune, and to bring prosperity and happiness.[7]

The inclusion criteria of the respondents was based on their willingness to participate in the study, their reputation of treating patients with TB and respiratory problems, their number of years in practice (not less than 10 years) and a rich knowledge of traditional medicine. The data was collected in April and May 2012 from the three districts. Samples of all species mentioned during the interviews were collected and deposited at the National Museum, Bloemfontein, for identification.

The participation information sheets (in Sesotho, the home language of the THPs), which were handed out, clearly stated the purpose and objectives of the study, the methods followed and the expected outcome of the study **(Appendix A)**. The content of the information sheets and the accompanying consent forms **(Appendix A)** were explained in detail to the THPs in one-to-one interviews, after which they were asked to sign the consent form. They were informed that they would receive a report on completion of the survey.

From each participant, the following information was gathered and set on an identity card:

- Name, age, occupation (diviner or herbalist), village they originate from.
- Date and place where information was gathered.
- The local name of TB, the causes of TB, and diagnosic guidelines.
- The names of the plants used for TB (botanical name when possible and/or vernacular name).
- Ecological distribution: which district does the plant originate from?
- Parts used: leaves, bark, aerial parts, roots, tubers, seeds.
- Preparation and the form of the plant used (dried, powdered, decoction, infusion, fresh plant).
- Whether the plant is taken alone or as a mixture (contents of mixtures must be specified)
- Administration of the remedy, the frequency and quantity of doses and how long does the treatment last.

- Which symptoms are treated by the plant and precautions taken before administering remedies to TB patients.

2.3. Results and discussion

2.3.1. Respondent's biographic details

A total of 37 THPs were interviewed: 10 from Mangaung, 15 from Thabo Mofutsanyana and 12 from Lejweleputswa. Ages ranged between 35 and 60 years and there were 10 males and 27 females.

2.3.2. Knowledge of TB

Most THPs referred to TB by the local name "lefuba". THPs diagnose TB based on symptoms such as chronic cough, blood stained sputum, sweating, weight loss, loss of appetite, tiredness or loss of energy, and difficulty in breathing **(Figure 2.2)**. The THPs believe that TB is an air-borne disease that is primarily spread by interaction with infected people, smoking and drinking, polluted dust and smoking drugs **(Figure 2.3)**.

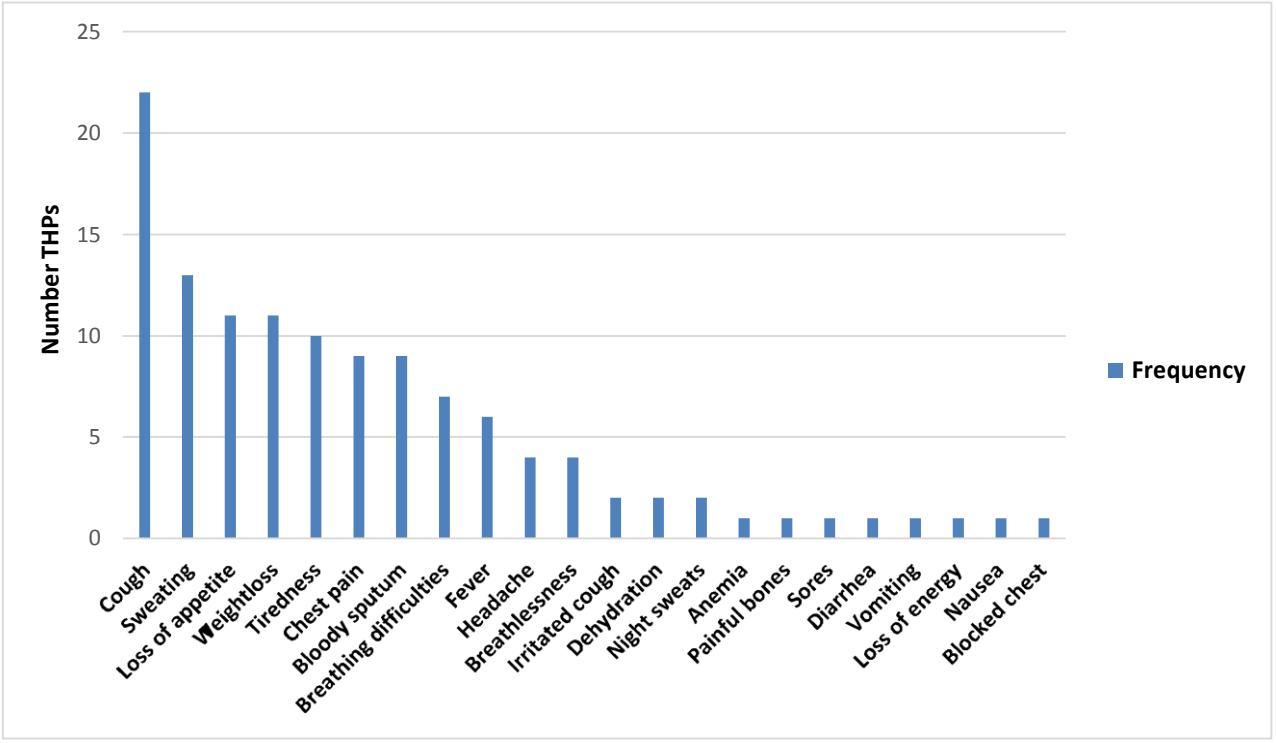


Figure 2.2. Signs and symptoms by which THPs recognize TB according to frequency mentioned by THP's

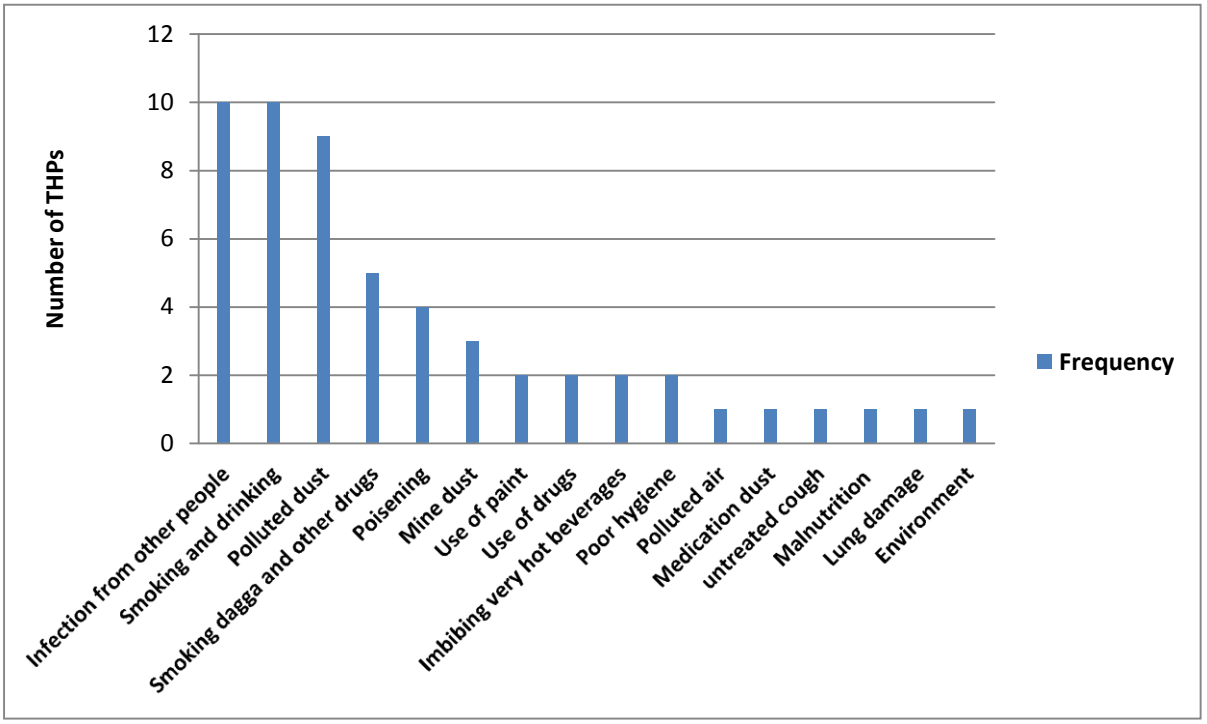


Figure 2.3. Causes of TB reported by THPs according to frequency (number of THPs)

2.3.3. Treatment practices and plant species used to treat TB

All THPs who participated in this study use plants to manage TB. We recorded 19 plants mentioned by THPs, but nine of those plant species were identified by correlating the Sesotho name, as stated by the THPs, with the scientific name from the plant list in "*Sesotho plant and animal names and plants used by the Basotho*" R. Moffett[8], **(Table 2.1)**. The plant family that is used most frequently is the Asteraceae (5 species), which is not surprising in view of the number and accessibility of these plants, followed by the Fabaceae (3 species) and Apocynaceae (2 species). These three families constitute 64% of plants used, while the seven remaining families are represented by one plant each **(Table 2.1)**. The popularity of Asteraceae is thought to be due to the large diversity of bioactive compounds in members of this family. Six of the plant species recorded were mentioned by several THPs and originated from more than one district **(Table 2.2)** Overall the most frequently used plant parts are tubers, roots, and leaves **(Figure 2.3)**.

Table 2.1. Plant species reported by THPs for the management of TB and allied diseases in the Free State Province.

Local name	Scientific name	Family	Collection Nr.	Prep[2]	Indication	Nr THPs	Nr. Of Districts
Bokgwe	*Sansevieria hyacinthoides*[*]	Agavaceae	§	D	cough	1	1
Hlokwanalatsela	*Thesium scirpoides*	Santalanaceae	5447	I	reverse severe illness, cough	2	1
Hlwenya[†]	*Dicoma anomala*[*]	Asteraceae	–	C	cough	1	1
Kgamane	*Rumex lanceolatis*[*]	Polygonaceae	–	I	sores, flu	1	1
Kgomayabadisa	*Bulbine narcissifolia*[*]	Asphodelaceae	–	D	cough	1	1
Kgonathi	*Leonotis lanceolata*	Fabaceae	5449	D	cough	8	2
Kgwara	*Pelargonium sidioides*	Geraniaceae	5451	D	cough, chest wounds, immunity	6	3
Leharaswana	*Sonchus dregeanus*[*]	Asteraceae	–	D	rash, cough	1	1
Lengana	*Artemisia afra*[*]	Asteraceae	§	I, V	Cough, blocked nose	10	3
Lesoko	*Alepidea amatymbika*[*]	Apiaceae	–	I, C	Cough	2	2
Moretele	*Drimia depressa*[*]	Hyacinthaceae	–	I	pain , appetite	1	1
Mositsane	*Elephantorhiza elephantine*	Fabaceae	5452	I	Anaemia	3	3
Phatheyangaka	*Helichrysum caespititium*	Asteraceae	5453	I	Pain	7	2
Poha/phowa	*Aster boekerianus*	Asteraceae	5445	D, P	sweat, pain	2	2
Pohotsehla	*Xysmalobium parviforum*	Apocynaceae	5448	P	cough	2	1
Seakga[†]	*Melolobium obcordatum*[*]	Fabaceae	–	M	cough, sores, tiredness	5	2
Sehamelapodi	*Parapodium costatum*[*]	Apocynaceae	–	D	breathlessness	1	1
Setimamollo	*Pentanisia pruneloides*	Rubiaceae	5446	D	breathlessness	1	1
Thola[†]	*Solanum aculeatissimum*[*]	Solanaceae	–	D	Cough	1	1

[*] Identified from the correlation of the Sesotho name with the scientific name from the plant list in Moffett.[8]

† Seakga also corresponds to Ipomoea bolusiana (Convolvulaceae), thola to Solanum lichtensteinii and Solanum supinum (Solanaceae) and hlwenya to Teedia lucida (Scrophulariaceae) Moffett [8]

§Collected plant material decomposed

1 A = aerial parts, B = bark, L = leaves, R = roots, T = tuber

2 C = chew, D = decoction, I = infusion, M = macerated, P = powder, V = vaporization

Table 2.2. Most frequently used plant species

Plant species	No. of THPs in %	No districts
Artemisia afra	10	3
Leonotis lanceolata	8	2
Helichysum caepititium	7	2
Pelargonium sidiodes	6	3
Melobium obcordatum	5	2

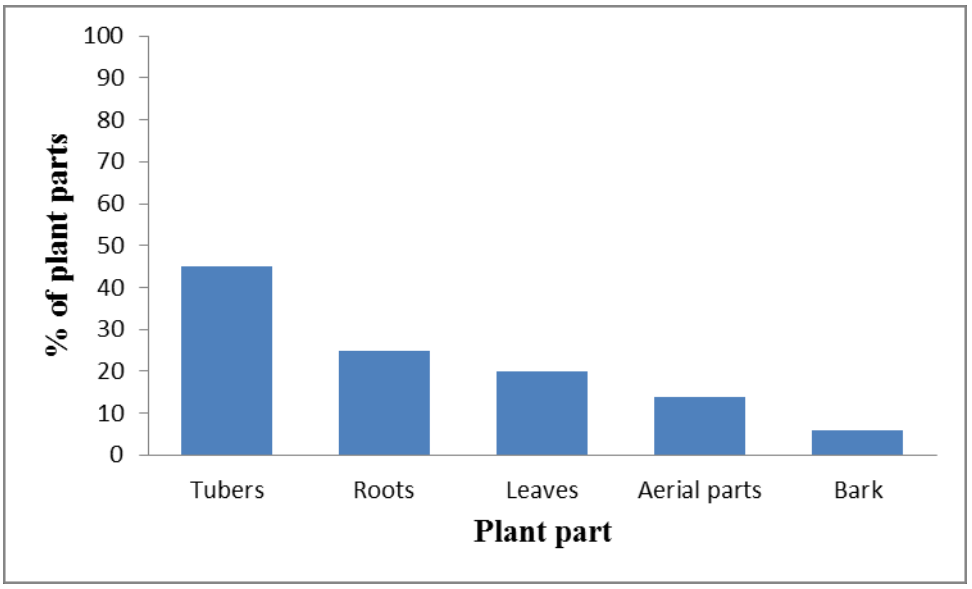


Figure 2.4. Most frequently used plant parts

3.3.4. Medicinal plant preparation and administration

Most THPs that were interviewed prepare the plant medicine as mixtures of two or more plant species. The plant material is mostly air dried and stored in glass and plastic containers in their "dumba", a workhouse and pharmacy. Decoctions and infusions are the most common preparation method for the medicines, followed by powders, macerated plant material, and vaporisation. Most medicines are administered orally, as a concoction, or solid plant parts can be chewed. In the single case of vaporization, the medicine is taken via inhalation.

The medicine is prepared by the THP and administered either by the healer in his hut, or the patient self-administers it at home. The dosage can differ for each remedy, e.g. 1 cup of decoction daily, or twice daily (bd) or three times daily (tds), ½ a cup bd, or tds, 3 tablespoons bd/tds. The periods of treatment vary from one week to six months. The treatment outcomes are assessed by client improvement, disappearance of TB signs and by the client reporting feeling better. If there is no improvement, the client is reassessed and put on new treatment; others refer the patient to a primary health care facility.

The three plants most frequently used for TB treatment were *A. afra* and *H. caespititium* (both Asteraceae), and *L. lanceolata* (Fabaceae). Although there is no history of the use of these three species for treatment of TB, *H. caespititium* has been shown to have antimycobacterial and antibacterial activity.[9] **Table 2.3** lists species in this study that had previously been found to be active against *M. tuberculosis* and coughs.

Table 2.3. Species reported in this study which have previously been found to be active against *Mycobacterium tuberculosis* (a) and related symptoms (b).

Plant species	**Citation**
(a) Activity dectected against *Mycobacterium tuberculosis*	
Pentanisia prunelloides (Klotzsch) Walp.	Madikizela et al.[10]
Helichrysum caespititium	Meyer et al.[11]
Pelargonium sidoides	Mativandlela et al. [12]
Artemisia afra	(*M. aurum*) Buwa and Afelayan [13]
(b) some species with unvaried traditional medicine claims that they treat coughs	
Alepidea amatymbica	(cough) Wintola and Aflolayan[14]
Artemisia afra	cough[15]

2.4. Conclusion

From this study, the following conclusions were made: that (i) the THPs have knowledge of the symptoms and management of TB by using plant species, that (ii) a large number of different plants are used for the treatment of TB in the three Free State districts investigated and that (iii) most THPs rely on mixtures as remedies. Most practitioners formulate and dispense their own recipes, most frequently using the tubers, roots and leaves of plants, but there was low consensus among the THPs as to which plants (or mixture of plants) are most efficacious for the treatment of TB. In conclusion, the three plants most frequently administered, *A. afra* and *H. caespititium* (both Asteraceae), and *L. lanceolata* (Fabaceae), are thus candidates for phytochemical investigation into their antimycobacterial and toxic properties, and to determine the efficacy of extracts and isolated compounds.

2.5. References

1. World Health Organization. **2013**. Global Tuberculosis Report.
2. Department of Health Free State Province. **2010**. HIV/AIDS, TB and STI Strategic Plan 2012 – 2016.
3. Arya V. 2011. A review on anti-tubercular plants. *Int. J. Pharm. Tech. Res*. 3:872-8800.
4. Mukadi Y.D., Maher D. and Harries A. **2001**. *AIDS*. 15: 143-152.
5. Keshavjee S. and Farmer P.E. **2012**. Tuberculosis, drug resistance and the history of Morden medicine. *N. Engl. J. Med.* 367: 931-936.
6. Singh H.B., Singh P., Gupta R., Thakur B., Sharma V.D., Katoch V.M. and Chauhan S.V.S. **2010**. Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant Mycobacterium tuberculosis isolates. *Indian J. Med.Res*. 131: 809-813.
7. Pretorius E. **1999**. Traditional healers. South African Health Review. Durban: Health system Trust. 249-256.
8. Moffett R. **2010**. *Sesotho plant and animal names and plants used by Basotho*. 1st ed. SUN MEDIA, Bloemfontein, South Africa. 7-119.
9. Mathekga A.D.M. **2001**. Antibacterial activity of Helichrysum species and the isolation of a new phloroglucinol from Helichrysum caespititium. Thesis (Ph.D) in Botany. University of Pretoria.
10. Madikizela B., Ndhlala A.R., Finnie J.F. and van Staden J. **2014**. Antimycobacetrial, anti-inflammatory and genotoxicity evaluation of plants used for the treatment of tuberculosis and related symptoms in South Africa. *J. Ethnopharmacol*. 153: 386-391.
11. Meyer J.J.M., Lall N. and Mathekga A.D.M. **2002**. In vitro inhibition of drug-resistant and drug- sensitive strains of Mycobacterium tuberculosis by *Helichrysum caespititium*. *S. Afr. J. Bot*. 63: 90-93.
12. Mativandlela S.P.N., Meyer J.J.M., Hussein A.A. and Lall N. **2007**. Antitubercular activity of compounds isolated from Pelargonium sidoides. *Pharm. Bio.* 45: 945-950.
13. Buwa L.V. and Afolayan A.J. **2009**. Antibacterial activity of some medicinal plants used for the treatment of tuberculosis in the Eastern Cape Province, South Africa. *Afri. J. biotechnol.* 8:6683-687.
14. Wintola O.A. and Afolayan A.J. **2014**. *Alepidea amatymbica* Eckl. & Zeyh.: A review of its traditional uses, phytochemistry, pharmacology and toxicology. *Evid. Based Complement. Alt. Med*. Hindawi Publishing Corporation. 1-12.
15. Van Wyk B-E. and Gericke N. *People's plants: a guide to useful plants of Southern Africa*. Briza puplishers, South Africa.

3. hERGscreen of the plant species consumed for their potential of blocking hERG channels.

3.1. Introduction

hERG (human Ether-à-go-go Related Gene) is a gene that encodes the pore-forming α-subunit of a voltage-gated potassium K^+ channel expressed in the heart and nervous tissue.[1] It is responsible for channels mediating the 'rapid' delayed rectifier K^+ current (IKI) which plays a critical role in the ventricular repolarisation phase of the cardiac action potential.[2] hERG was initially isolated by Warmke and Ganetzky in 1994 from the human hippocampal cDNA library with a mouse homologue of ether-a-go-go, a drosophila K^+ channel gene.[3,4] Mutations in hERG can lead to partial or complete loss of function, prolong the ventricular action potential and may cause an inherited cardiac arrhythmia, long QT syndrome (LQTS). LQTS is associated with torsade de pointes (TdP), a ventricular arrhythmia that can degenerate into ventricular fibrillation (VF), leading to sudden death **(Figure 3.1)**.[3,5,6,7]

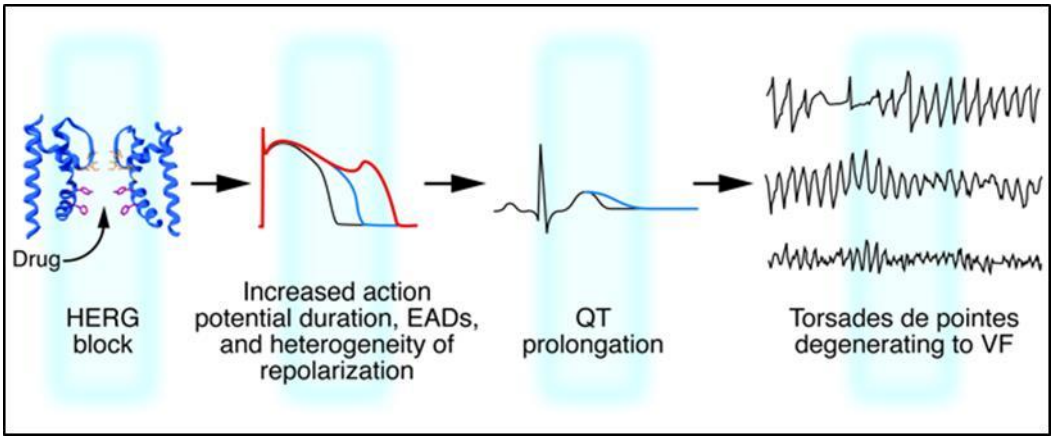


Figure 3.1. Mechanism of sudden cardiac death with drug blockade of the hERG channel. Drug blockade of a single hERG K^+ (left) produces prolonged action potential duration (blue) and soon after depolarisation EAD (red). These changes generate QT interval prolongation and torsade de points (right, upper panel). In this figure, the arrhythmia degenerates to ventricular fibrillation.[7]

3.1.1. Blockade of hERG potassium channel

The blockade of the hERG-encoded potassium channel is a major factor in pro-arrhythmic liability of a wide range of chemically diverse drugs. Several drugs from different chemical classes and therapeutic areas that show blockade of the hERG channels have been withdrawn from the

market or have had their use restricted because of a potential to trigger torsades.[8] For example, the potent hERG channel blocker Terfenadine, is an H1 receptor antogonist that was launched in 1992 and later removed from the market because it can cause a potentially life-threatening ventricular tachyarrhythmia, torsades de pointes.[8]

The link between hERG K^+ channel block and ventricular arrhythmias has prompted extensive screening studies during drug development. The risk that a drug will induce LQT has been investigated by recording action potentials in single myocardial cells and multi-cellular myocardial preparations, and electrocardiogram (ECG) measurements of the QT prolongation in animals. An important indicator for possible arrhythmogenic liability of a given compound or plant extract is the inhibition of hERG currents in heterologous expression systems.[9]

Voltage-gated ion channels are targets to a number of therapeutic drugs and are a focus for drug discovery. The effect of new compounds on ion channels expressed in heterologous *Xenopus laevis* oocytes is usually studied by means of the two microelectrode voltage-clamp technique.[10]

3.1.2. Micro-electrode using Xenopus oocytes in drug screening

X. laevis oocytes **(Figure 3.2)** are immature egg cells of the South African clawed frog *X. laevis* and have a striking appearance with a light vegetal pole and a dark animal pole, where the nucleus is situated. They are easy to handle, robust with a large diameter (1-1.2 mm) and can be obtained in large numbers.[11] *X. laevis* oocytes have become a popular expression system for ion channels, receptors and transporters. Ion channels expressed in oocytes can be electrophysiologically investigated by the voltage clamp technique.[12] They serve as a standard heterologous expression system for the study of cloned ion channels and have been successfully used to translate messenger RNA (mRNA) into respective membrane proteins, including post-translation modifications.[13]

Figure 3.1. *X. laevis* oocytes.[14]

Oocytes used in two-microelectrode experiments are injected with cRNA or cDNA of the molecular target and incubated to allow expression of the molecular target **(Figure 3.3)**. The oocytes are surgically removed from the female *X. laevis* and defollicated via collagenase treatment before injection.[11]

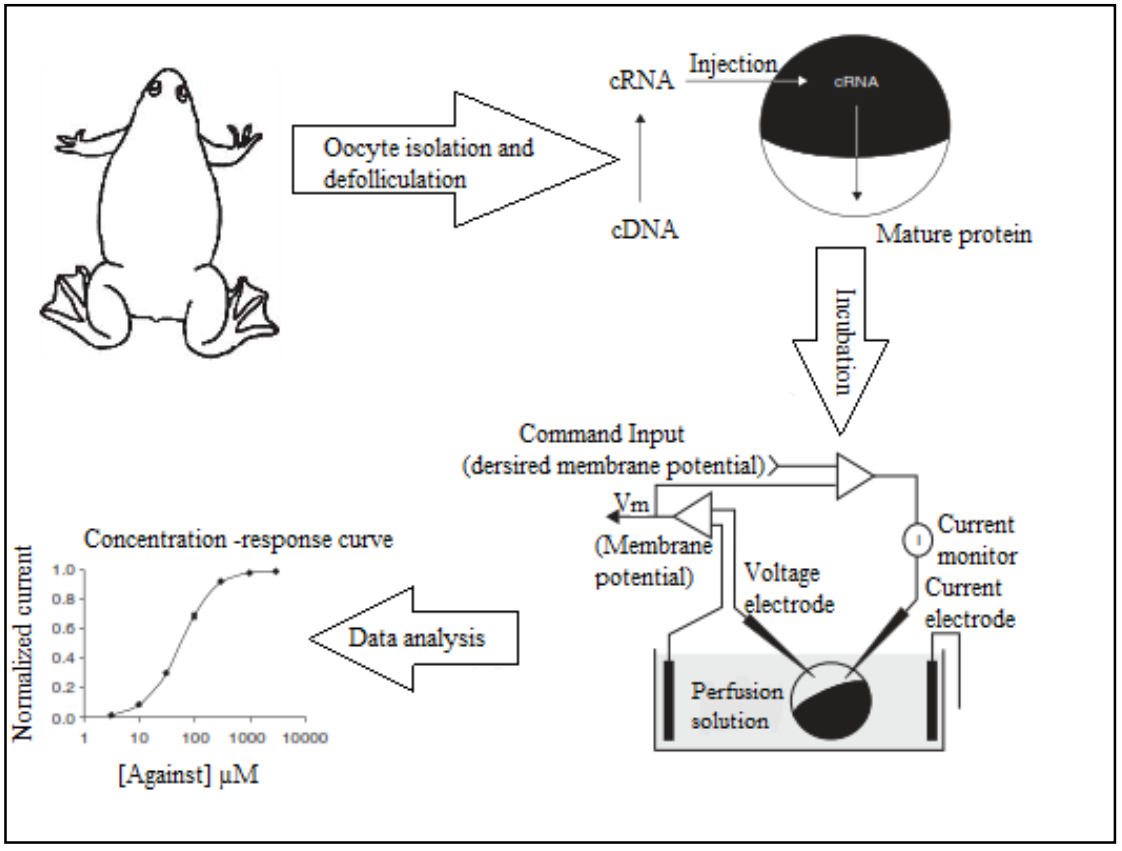


Figure 3.2. From *X. laevis* to data acquisition and analysis. Schematic representation of the work flow from a female *X. laevis* to data analysis. The oocytes are isolated from the female *X. laevis* by surgical excision. Following isolation, the oocytes are defolliculated by collagenase-treatment and sorted for injection. cRNA (transcripted from cDNA) is injected into the oocytes and the molecular target of interest is expressed at high levels. The expressing oocytes are used for two-microelectrode voltage clamp experiments.[11]

3.1.3. Two-microelectrode voltage clamp

In two-microelectrode voltage clamp experiments, the oocytes are placed in a circular or elongated bath with one or more tube inlets and a drain on the opposite side of the chamber. The test sample is applied via the individual tubes that are connected to the reservoirs. A schematic representation of the perfusion chamber is shown in **Figure 3.4**. The voltage clamp experiments on *X. laevis* oocytes were performed in a small (15 µL) bath that was covered with a glass plate. Two sloping inlet channels in the glass cover enable access of the two microelectrodes to the oocytes. A funnel reservoir for drug application surrounds the two microelectrodes.

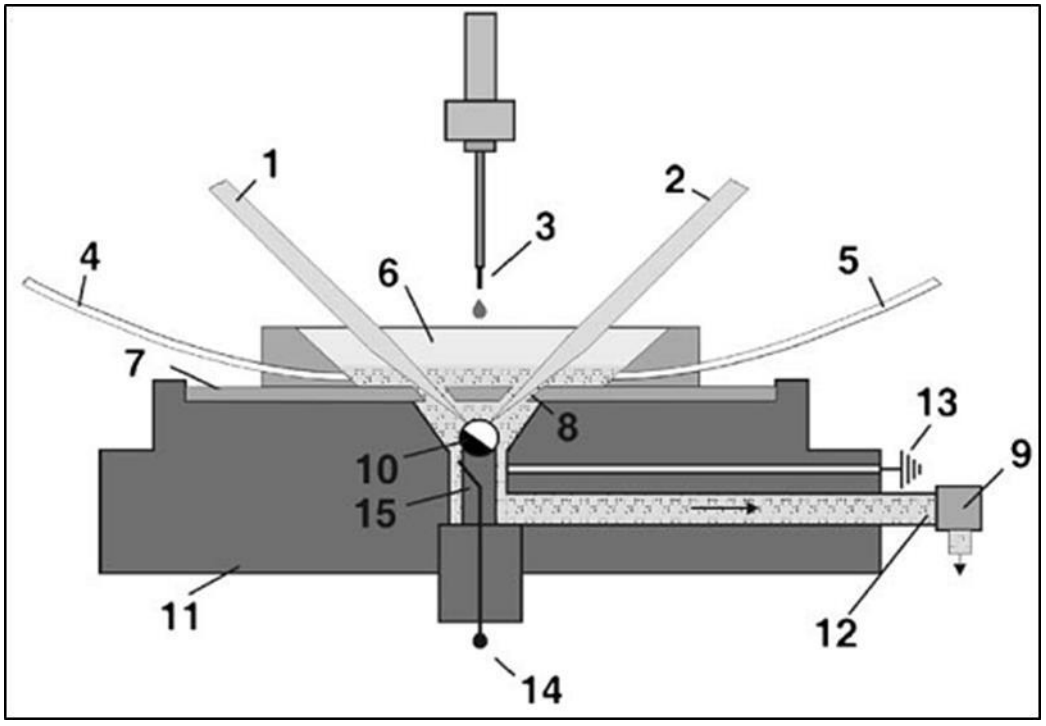


Figure 3.3. Schematic representation of the perfusion chamber. Two microelectrodes (*1* and *2*) are inserted via the sloping access inlets *(8)* through a glass cover plate *(7)* into the small (15 µL) oocytes chamber. The test sample is applied by the tip of the liquid handling arm *(3)* of a TECAN Minipren 60 to a funnel reservoir made of quartz *(6)* surrounding the microelectrode access holes. Perfusion of the oocyte (10) that is placed on the cylindrical holding device *(15)* is enabled by means of the syringe pump *(9)* of the TECAN connected to the chamber body *(11)* via outlet (12). Residual solution is removed from the funnel before drug application via the funnel outlets *(4* and *5*). In addition to the ground reference electrode *(13)*, the cylindrical holder for the oocyte contains a reference electrode *(14)* that serves as an extracellular reference for the potential electrode. Salt bridges can be inserted into the side outlet for the ground electrode *(13)*.[10]

3.2. Materials and Methods

3.2.1. Plant extraction

Ground plant material (500 mg) was defatted with *n*-hexane (5 mL) and centrifuged for 10 min. The extract was filtered and discarded. The same plant material was sequentially extracted with DCM (7 mL) followed by MeOH (13 mL) and stirred at room temperature for 2h. The extracts were filtered and evaporated.

3.2.2. Removal of tannins

Both extracts DCM and MeOH were loaded onto a Solid-Phase Extraction (SPE) cartridge (3 mL) packed with polyamide gel (CC-6; 900 mg) and washed with MeOH (8 mL). Both eluates were evaporated and dried.

3.2.3. Sample preparation

For each plant extract a stock solution of 20 mg/mL in dimethylsulfoxide (DMSO) was prepared. A concentration of 100 µg/mL was used for the screening experiments.

3.2.4. Voltage Clamp procedure

The plant extracts (DCM and MeOH) of the plants commonly consumed as medicine were tested for their potential of inhibiting hERG channels using *X. laevis* oocytes. Female *X. laevis* were anaesthetized for 15 min by placing them in an anaesthetic solution before surgically removing their ovaries. Thereafter the oocytes follicle membrane was enzymatically digested with 2 mg/mL collagenase and placed in an incubator for 90 min at 18˚C.

Oocytes were injected with wild type hERG RNA one day before use in the two-electrode voltage clamp technique using a Turbo Tec-03X npi amplifier and digitised with a Digidata 1440A. The currents were recorded at room temperature. The oocytes were placed in a small bath chamber, in a diluted Cl^- solution containing 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES and 1.8 mM $CaCl_2$. The pH was adjusted to 7.5 with 1 M NaOH. The chamber was covered by a glass plate with two channels for the microelectrodes that are surrounded by a quartz funnel serving as a reservoir for the test solutions. The two microelectrodes were filled with KCl (3 M) with a resistance between 0.1 and 4 MΩ. Only oocytes with an expression between 0.6 and 3 µA were used to screen the plant extracts.

The hERG channels were activated by a depolarisation to 20 mV and a repolarisation to -50 mV that induced a large tail current. Measurements were performed after stable peak amplitudes were reached over periods of 10 min with 0.3 Hz pulse trains. Plant extracts were applied with two slow perfusions of 800 µL. Oocytes were kept at a holding potential of -100 mV. After the two slow perfusions 1Hz pulse trains were applied until the steady stage was reached. The percentage block was determent in the reduction of the peak tail current. The data was analysed using pClamp 8.0 and Origin 7.0.

3.3. Results and discussion

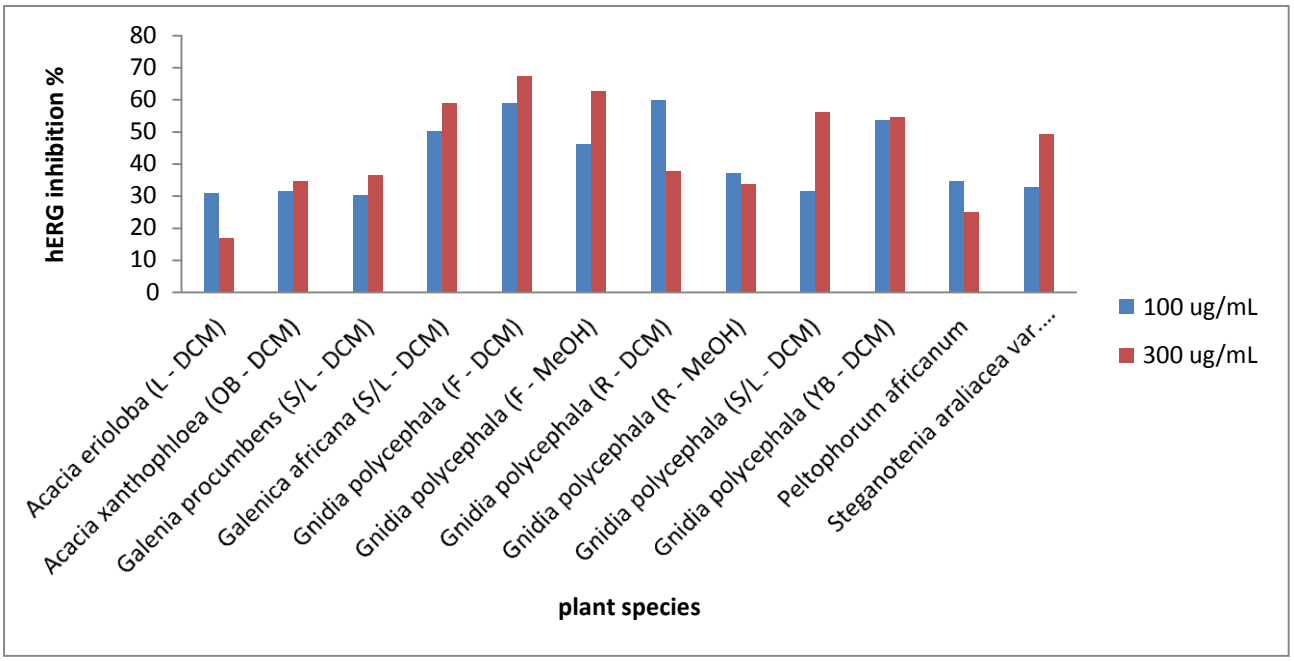
We investigated 129 plant species distributed in 48 families for their potential to block hERG K^+ channels. The plants were selected according to their use as traditional medicines and as ingredients in food and beverages. The DCM and MeOH extracts, respectively of different plant parts (350 extracts of both) were screened. Results of the extracts investigated are listed in **Appendix B**. Plant extracts that reduced the peak tail hERG current by ≥30% were considered positive hERG channel blockers. Ten plant extracts were identified showing an inhibition between 30-60% **(Table 3.1)**. The plant extract that showed the highest percentage of inhibition was *G. polycephala* even though the standard deviation was high. The genus Gnidia is rich in diterpene esters, coumarins, flavonoids,chromones, lignans, and neolignans. Phytochemical studies on some *Gnidia* species indicated the presence of toxic diterpene esters of daphnane type, which are the main types of plant orthoesters and have remarkable biological activities, such as antineoplastic and cytotoxic.[15]

Table 3.1. Plant extracts identified as positive hERG channel blockers at 100 and 300 µg/mL

Plants species	**Parts**	**Collection number**	**hERG inhibition (% Oocytes mean)**
Acacia xanthophloea	Old bark (DCM)	5625	31.665 ± 12.528
Galenia africana	Stems & leaves (DCM)	5238	50.396 ± 5.474
Galenia procumbens	Stems & leaves (DCM)	5102	30.446 ± 9.443
Gnidia polycephala	Flowers (DCM)	5031	58.889 ± 13.441
Gnidia polycephala	Flowers (MeOH)	5031	46.147 ± 10.707
Gnidia polycephala	Stems/twigs (DCM)	5031	31.383 ± 21.127
Gnidia polycephala	Young bark (DCM)	5031	53.765 ± 27.633
Sterganotenia araliacea var. araliacea	Stems/twigs (DCM)	5063	32.606 ± 9.504

Plant extracts which tested positive when screened at 100 µg/mL were further screened at 300 µg/mL to validate the results obtained. The activity of three plant extracts (*Peltophorum africanum*,

Acacia erioloba and *Gnidia polycephala* (roots) could not be confirmed because the inhibition percentage decreased when screened with an increased concentration **(Figure 3.5)**.



Abbreviations: L = leaves, S/L = stems/leaves, OB = old bark, F = flowers, R = roots, S/T = stems/twigs, YB = young bark

Figure 3.4. Comparison of percentage inhibition at concentrations of 100 µg/mL and 300 µg/mL, respectively

Comparing the peak tail reduction of DCM and MeOH extracts **(Table 3.2)** the DCM extracts showed a higher percentage of inhibition of the hERG channel compared to the MeOH extracts. However, 14.04% of the DCM extracts could not be screened because of their insolublility in DMSO. Some of the MeOH extracts destroyed the hERG channel, killing the oocytes.

Table 3.2. Comparison of the tail reduction results of the DCM and MeOH extracts

	DCM extracts (%)	**MeOH extract (%)**
No Inhibition	45.85	54.44
Not worthy inhibition <30 %	37.54	41.26
Insoluble in DMSO	14.04	2.29
Potential inhibition >30 %	2.58	0.86
Dead Oocytes	-	0.86
Unstable Oocytes	-	0.29

From the results obtained 50% of the plant extracts that were investigated on their hERG blocking potential showed no inhibition of the peak tail current and are therefore safe to be

consumed and free of cardiotoxic risk, 39% showed a non-worthy inhibition < 30% and only 1.7 % of the extracts had potential to inhibit hERG channels.

3.4. Conclusions

The two-microelectrode voltage clamp experiment on *X. laevis* oocytes described in the study is a convenient and flexible microperfusion technique for the application of neurotransmitters, drugs and plant extracts to *X. laevis* oocytes expressing voltage gated ion channels. The main advantage of the perfusion chamber described in **(Figure. 5.4)** is that only a small test sample solution 50 µL is required for perfusion, compared to 2 mL test solution used in conventional gravity-flow perfusion.

In conclusion, we have demonstrated that our virtual screening approach (two-microelectrode voltage clamp) was successful in identifying novel hERG blockers. This experimentally validated model represents a valuable predictive tool in the assessment of potentially cardiotoxic natural botanicals.

3.5. References

1. Gutman G.A., Chandy K.G., Grissmer S., Lazdunski M., McKinnon D., Pardo L.A., Robertson G.A., Rudy B., Sangunetti M.C., Stuhmer W. and Wang X. **2005**. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol. Rev*. 57: 473-508.
2. Hancox J.C., McPate M.J., Harch A.E.L. and Zong Y-H. **2008**. The hERG potassium channel and hERG screening of drugs-induced torsades de pointes. *Pharmacol. Ther*. 119: 118-132.
3. Snguinetti M.C., Lang C., Curran M.E. and Keeting M.T. **1995**. A mechanistic link between an inherited and aquired cardiac arrhythmia: HERG encoded Ikr potassium channel. *Cell*. 81: 299-307.
4. Trudaeu M.C., Warmke W.J., Ganetzky B. and Robertson G.A.**1995**. hERG: A human Inward rectifier in the Voltage-gated potassium channel family. *Sci*. 269: 92-95.
5. Fernandez D., Ghanta A., Kauffman G.W. and Sanguinetti M.C. **2004**. Physiochemical features of the hERG channel drug binding site. *J. of Biol. Chem*. 270: 10120-10127.
6. Aronov M.A. and Goldman. **2004**. A model for identifying hERG K+ channel blockers. *Bioorg. Med. Chem*. 12: 2307-2315.
7. Roden D.M. and Viswanathan P.C. **2005**. Genetics of acquired long QT syndrome. *J. Clin. Invest*. 115: 2025-2032.
8. Danke T. and Moller C. **2014**. Early identification of hERG liability in drug discovery programs by automated patch clamp. *Front. Pharmacol*. 5:1-11.
9. Stork D., Timin E.N., Nerjukaw S., Huner A., Auer M. and Hering S. **2007**. State dependent dissociation of HERG channel inhibitors. *Br. J. Pharmacol*. 151: 1368-1376.
10. Barburlun I., Beyl S. and Hering S. **2006**. Automated fast perfusion of Xenopus oocytes for drug screening. *Pflugers Arch-Eur. J. Physiol*. 453: 117-123.
11. Kvist T., Hansen K.B., Bräuner-Osborne H. **2011**. The use of Xenopus oocytes in drug screening. Expert opn. *Drug discovery*. 6: 141-153.
12. Baumagartner W., Islas L.and Sigworth E.J. **1999**. Two-microelectrode voltage clamp of Xenopus Oocytes: Voltage errors and compensation for local current flow. *Biophys. J*. 77: 1980-1991.
13. Stűhmer W. **1988**. Electrophysiologic recordings from Xenopus oocytes. *Methods Enzymol*. 293: 280-300.
14. http://pixgood.com/xenopus-laevis-oocytes.html.
15. Bhandurge P., Rajaraje S, Ganapaty S. and Pattanshetti S. **2013**. The *Gnidia* genus :A Review. *Asian J. Biomed. Pharm. Sci*. 3: 1-13.

4. Retrospective treatment outcome (RTO) analysis on the use of medicinal plants to alleviate diarrhoea

4.1. Introduction

Diarrhoea continues to be one of the leading causes of mortality and morbidity in developing countries, including South Africa, especially in children under 5 years.[1] Worldwide, 3-4 million diarrhoeal related deaths are reported each year (WHO, 1996). It is estimated that 88% of diarrhoeal-related deaths are caused by inadequate sanitation and poor hygiene.[2] The major cause of intestinal infection is due to foodborne infections caused by *Salmonella* and *Campylobacter jejuini*, waterborne infections resulting from the contamination of domestic water supply with the cysts of *Giardia lamblia* and *Cryptosporidium parvum,* and *Escherichia coli,* which may be waterborne and/or foodborne.[3] The main cause of death from diarrhoea is dehydration, which results from the loss of electrolytes in diarrhoeal stools.[4]

There are three major diarrhoea syndromes; acute watery diarrhoea, resulting in varying degrees of dehydration; persistent diarrhoea, which lasts 14 days or more, manifested by malabsorption and nutrient losses, and dysentery, which results in intestinal damage caused by an infectious agent.[2] Acute watery diarrhoea accounts for approximately 80% of deaths, persistent diarrhoea for 10% and dysentery for up to 10%.[3] The emergence of multiple drug resistant strains of diarrhoeagenic pathogens has made the treatment of dysentery more difficult. In developing countries the majority of people living in rural areas almost exclusively use traditional medicine for treating all sorts of diseases including diarrhoea.[5]

A wide range of medicinal plants used to treat diarrhoea has been used by traditional healers of different tribes in South Africa. Only a few of those plant species have been phytochemically and pharmacologically investigated. *Bridelia micrantha* Hochst. Baill. (Phyllanthaceae) is also traditionally used for the treatment of gastro-intestinal diseases.[5] De Wet et al.[4] mention plant species which were recorded for the first time worldwide as medicinal plant for anti-diarrhoeal treatments (*Acacia burkei Benth.* (Leguminosae)*; Brachylaena transvaalensis* Hutch. ex E.Phillips & Schweick., (Compositae)*; Cissampelos hirta* Klotzsch, (Menispermaceae) *and Cynanchum viminale* (L.) L. (syn. Sarcostemma viminale), (Apocynaceae) *Psidium guajava L.* (Myrtaceae),

well studied species with respect to diarrhoea, is widely used in traditional medicine for the treatment of diarrhoea, dysentery and gastroenteritis.[6]

The aim of this study was to identify one or more medicinal plant(s) commonly used by participants in Sediba village, located in the Thaba ‘Nchu area in the Free State, South Africa, to reduce/alleviate diarrhoea, and how effective these remedies are.

4.2. Methods and materials

4.2.1. Study area

The study was conducted in Sediba village in Thaba ‘Nchu area in the Free State province. Thaba ‘Nchu is a small town located 60 km east of Bloemfontein. The population is largely made up of Tswana and Sotho people. Sediba is a small community situated in Thaba ‘Nchu its geographical coordinates -29.048649, 26.947983 located about 20 km from Thaba ‘Nchu town (**Figure. 6.1**)

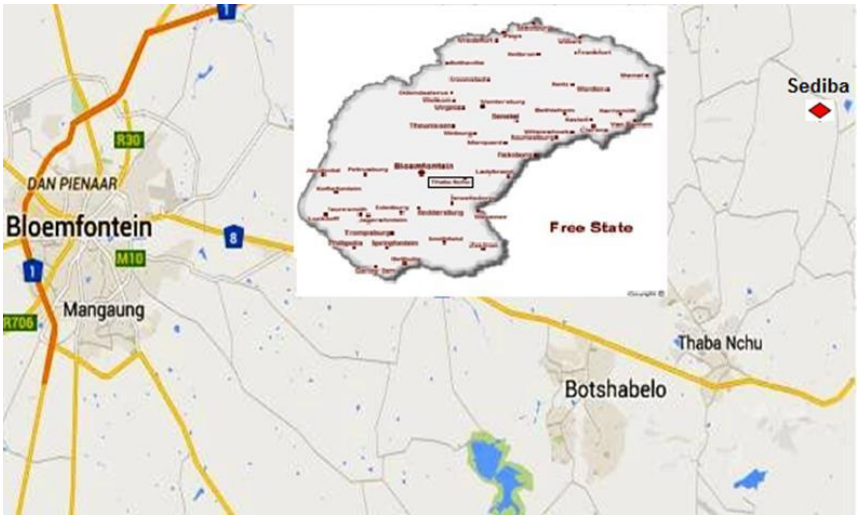


Figure 4.1. Position of Sediba village in Free State Province

4.2.2. Study design and data collection

The retrospective study was among patients who has experienced diarrhoea from the past 3 months. The chief of the Sediba village in the Thaba ‘Nchu district, was contacted and he gave us his assistance to recruit participants among the villagers. The data was collected using guided questionnaires **(Appendix C)**. Some of the interviews were conducted in the community hall, but the rest was house to house interviews. The participation information sheets **(Appendix C)** (in

Sesotho, the home language of the participants) clearly stated the purpose and objectives of the study, the methods followed and the expected outcome of the study. The content of the information sheets and the accompanying consent forms were explained, after which each participant signed the consent form **(Appendix C)**. The questionnaires were completed during the interviews by the research assistants. Ethical clearance was obtained from the University of the Free State.

4.3. Results and discussion

A total of 32 participants were interviewed. The participant group consisted of 12 males and 20 females. The age ranges of the males were from 30 – 40 years (5), 50 – 60 (2), 61 – 70 (2) and 71 – 80 (3) years. The age ranges of the females were from 20 – 30 (6), 31 – 40 (5), 50 – 60 (4) and 61 – 70 (4) years, with one participant not stating her age. Of the 32 participants 13 were excluded because they did not only use traditional medicine, but conventional medicine or a combination to treat diarrhoea; results are summarized in **(Table 4.1)**.

Table 4.1. Plants commonly used for the treatment of diarrhoea

Scientific name[†]	**Family**	**Local name**	**Part used**	**Preparation and use**	**Nr use**[*]	**%**[#]
Xysmalobium undulatum	Apocynaceae	Pohotsehla	Roots	Powdered with water. Infusion	10	50
Punica granatum L.	Punicaceae	Kgarinati (pomegranate)	Peel	Infusion	7	35
Dicoma anomala	Asteraceae	Hlwenya	Roots	Decoction or chew	4	20
Aloe ferox	Asphodelaceae	Mohalakane	Leaves	Infusion	3	15
Hermannia depressa	Sterculiaceae	Phateyangaka	Whole plant	Decoction usually used with other plants	2	10
Pelargoniuim sidoides	Geraniaceae	Kgwara	Tuber	Infusion	2	10
Dianthus basuticus	Caryophyllaceae	Hlokwanalatsela	Root	Decoction	1	5
Schkuhria pinnata	Asteraceae	Letswamadi		N/A	1	5
Alpidea amatymbica	Apiaceae	Lesoko	Roots	Chewed or decoction (painful joints, fever)	1	5
Scabiosa columbaria	Dipsacaceae	Selomi	Roots	Chewed	1	5
Artemisia afra	Asteraceae	Lengana	Leaves	Infusion or dried and powdered	1	5
Searsiae rosa	Anacardiaceae	Tshilabela	Leaves	Dried and powdered	1	5
Monsonia brevirostrata	Geraniaceae	Monkgane	Roots	Infusion	1	5

[†]Plants were identified from the plant list in Moffett[7] and the review by van Wyk and Moteetee[8] via correlation of the Sesotho name with the scientific name.

[*]Number of respondents using the preparation

[#]Percentage of respondents using the preparation

Table 4.1 indicates that the most commonly used plants are *Xysmalobium undulatum* (50%), *Punica granatum* L. (35%) and *Dicoma anomala*, (20%). These plants are often used together, with *P. granatum* (pomegranate peel) being used for abdominal pain. The treatments are mostly prepared at home by themselves or relatives. The roots if the plant can be chewed, taken as powder followed by water, or as an infusion. Most of the other plants are used in conjunction with *X. undulatum* and treats among other symptoms abdominal pain, flatulence and joint pain. Symptoms reported included loose and/or bloody stools (sometimes accompanied by nausea and vomiting), abdominal pain, dizziness, and headache. The reported causes of diarrhoea ranged from food poisoning (slaughtered meat, chicken livers, beans, pears and spinach) to stress, bile "nyongo", salty water and heat. The duration of the illness ranged from 1 – 7 days, but two participants reported that their illness continued for two weeks. Relief was usually experienced within 1 to 2 days after starting treatment and only one participant experienced side-effects **(Table 4.2)**.

The majority of participants (18/19) 94.7 % has a successful treatment outcome (cured) even though the effectiveness of the medication varied. 31.5% were cured one day after taking medication, 21.5% after 2 and 3 days and 10 % after 5 days of taking medication. Only one participant that was uncured and the diarrhoea got worse with severe abdominal pains.

Table 4.2. Treatment outcome for the selected participants

Participant Nr	Age	M/F	Days sick	Days medication	Plants used	Outcome	Side-effects
001	52	F	2-3	1	*Searsiae rosa* *Artemisia afra*	Cured	None
002	53	F	4	2	*Xysmalobium undulatum* *Punica granatum* L.	Cured	None
004	74	M	14	10	*Dicoma anomala* *Hermannia depressa* *Scabiosa columbaria* *Xysmalobium undulatum*	Cured	None
006	52	F	3	2	*Xysmalobium undulatum* *Punica granatum* L.	Cured	None
009	26	F	1	1	*Dicoma anomala*	Cured	None
011	80	M	7	3	*Xysmalobium undulatum*	Cured	None
012	32	M	3	3	*Xysmalobium undulatum*	Cured	None
013	36	F	7	5	*Punica granatum* L.	Cured	None
017	32	F	14	5	*Punica granatum* L. *Hermannia depressa* *Dicoma anomala*	Cured	None
018	30	M	3	1	*Aloe ferox*	Cured	None

020	36	M	1-2	1	*Aloe ferox*	Cured	None
021	68	M	2-3	3	*Xysmalobium undulatum*	Worsened	Abdominal pains
024	58	M	1	1	*Alpidea amatymbica* *Pelargoniuim sidoides* *Xysmalobium undulatum*	Cured	None
025	67	F	2	2	*Xysmalobium undulatum*	Cured	None
026	65	F	14	3	*Punica granatum* L	Cured	None
027	72	M	4-5	2	*Punica granatum* L	Cured	None
028	80	F	Unsure	Unsure	*Monsonia brevirostrate* *Pelargoniuim sidoides*	Cured	None
029	26	F	Unsure	Unsure	*Punica granatum* L. *Xysmalobium undulatum*	Cured	None
032	58	F	1	1	*Xysmalobium undulatum* *Dicoma anomala* *Aloe ferox*	Cured	None

Some of the species reported in this investigation have been studied before and found to have some antidiarrhoeal activities (*Xysmalobium undulatum*[9] and *Punica granatum* L[10]).

4.4. Conclusion

Although this was only a pilot study on a small sample size, preliminary indications are that the traditional remedies are effective for treating diarrhoea and, since no adverse effects were reported, the remedies have low toxicity. *X. undulatum* is indicated most frequently to treat diarrhoea in the Thaba ‘Nchu area and thus merits phytochemical investigation to determine the activity, toxicity and efficacy of the extract and isolated compounds.

4.5. References

1. Abdulkarim A. Sadiq Y. and Gabriel O. A. Abdulkadir U. Z. and Ezzeldin M. A. **2005**. Evaluation of five medicinal plants used in diarrhoea treatment in Nigeria. *J. Ethnopharmacol.* 101: 27-30.
2. Semenya S. S. and Maroyi A. **2012**. Medicinal plants used by the Bapedi traditional healers to treat diarrhoea in the Limpopo province, South Africa. *J. Ethnopharmacol*. 144: 395-401.
3. Mathebe, M. C., Nikolova, R. V., Lall, N.and Nyazema, N. Z. **2006**. Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo province, South Africa. *J. Ethnopharmacol*. 105: 286-293.
4. De Wet H., Nkwanyana M. N. and Van Vurren S. F. **2010**. Medicinal plants used for the treatment of diarrhoea in Northern Maputoland KwaZulu-Natal Province, South Africa. *J. Ethnopharmacol*. 130: 284-289.
5. Lin J. Puckree T. and Mvelase T.P. **2002**. Anti-diarrhoeal evaluation of some medicinal plants used by Zulu traditional healers. *J. Ethnopharmacol*. 79: 53-56.
6. Almeida C. E., Karnikowski M. G., Foleto R. and Baldisserotto B. **1995**. Analysis of antidiarrhoeic effect of plants used in popular medicine. *RSP* 29: 428-433.
7. Moffett R. **2010**. *Sesotho plant and animal names and plants used by Basotho*. 1st ed. SUN MEDIA, Bloemfontein, South Africa.7-119.
8. Van Wyk B-E., Moteetee A. **2011**. The medical ethnobotany of Lesotho: A review. *Bothalia* 41: 209-228.
9. Vermaak I., Enslin M.G., Idowu O.T., Viligoen A.M. **2014**. Review of antidiarrhoeal traditional medicine. *J. of Ethnopharmacol*. 156: 135-146.
10. Qnais E.Y., Elokda A.S., Ghalyun Y.Y. and Abdulla F.A. **2007**. Antidiarrheal activity of aqueous extract of Punica granatum (pomegranate) peels. *Pharm. Biol*. 45: 715-720.

5. Phytochemical investigation of bioactive compounds from *Galenia africana* L. and *Combretum apiculatum* subsp. *apiculatum*

5.1. Introduction

The use of medicinal plants in the form of crude extracts may present some difficulties. The bioactive compounds may vary with both locality and the season in which they are collected. Medicinal properties of some plants are rapidly lost on storage, e.g. foxglove leaf must be dried soon after collection, otherwise the bioactive compounds decompose. It is therefore important to isolate the bioactive compound(s) from the crude extract. Using pure isolates instead of crude extracts can be an advantage in terms of accurately prescribed dosage. Structural modification of isolated and identified bioactive compounds from crude extracts may allow improvement in the efficacy and moderation of side effects. Pure bioactive compounds can be synthesized, thus preventing dependence on the plant as source.[1]

G. africana and *C. apiculatum* are two medicinal plants that have not been investigated extensively. Taking into account the wide ethnomedicinal use of Combretaceae species in Africa and Asia, the pharmacological activity in related taxa and considering the significant inhibitory activity shown by the *G. africana* and *C. apiculatum* extracts against *M. smegmatis*, their antibacterial properties, AChE inhibition properties and radical scavenging activity (see **Chapter 6**), these plant species were selected for the identification of bioactive components.

5.2. Family Combretaceae

The plant family Combretaceae consists of approximately 20 genera with 600 species of which genus Combretum is the largest, with about 370 species followed by Terminalia with about 200 species.[2,3] The other genera are Anogissus, Buchemavia., Bucida, calopyxis, Calycopteris, Conocarpus, Dansiea, Gansiea, Laguncularia, Lummitzera, Macropteranthes, Melostemon, Pteleopsis, Quisqualis, Strephonema, Terminaliopsis and Thiloa.[4] The genus Combretum is widely spread in Africa and mainly tropical, about 133 *Combretum* species occur in tropical Africa and 31 in subtropical Africa.[4] Species of the Combretaceae are used medicinally in several continents in the world. They are characterised by the wing-shaped appendages of fruit and may be trees, shrubs

or climbers.[5] Traditional healers throughout southern Africa use *Combretum* species for many medicinal purposes such as fever, headache, abdominal pains, diarrhoea, chest cough, colds, conjunctivitis, dysmenorrhea earache, hookworm, pneumonia, infertility in women, syphilis, toothache, leprosy, scorpion and snake bites, fattening babies, swelling caused by mumps, backaches, gastric problems, constipation and general weakness.[6,7] The leaves and the bark of the Combretum species are the most commonly used part. Fruits and seeds do not feature in medicinal use due to their toxicity to humans.[8] Several antibacterial constituents have been confirmed in different Combretum species.[9,10] *Combretum erythrophyllum* leaf extract contains at least 14 antibacterial constituents.[11] Antioxidant activities from Combretum species have been confirmed.[12]

Antibacterial triterpenoids have been isolated from the leaves of *Combretum imberbe.* Stilbenes, flavonones and flavones with antibacterial activities have been isolated and appear to be major constituents in the leaf extracts of *Combretum* species. Interestingly, pinocembrim, isolated from *Combretum apiculatum*, is known to be responsible for the antibacterial activity of propolis (bee glue).[4] Further compounds include stilbenes

5.2.1. *Combretum apiculatum* subsp. *apiculatum*

Figure 5.1. *Combretum apiculatum* subsp. *apiculatum*[13]

5.2.1.1. Description and distribution

C. apiculatum is a small to medium sized tree, 3-10 meters high with a short bent trunk, a stem-diameter of 40 cm and fairly sparse spreading. The branches hang low, giving the tree a willow-like appearance.[14] The bark on the main stem is grey to dark grey or brownish grey, and smooth becoming scaly and rough with age. The leaves have sharp twisted tips. The fruits are borne in clusters. They are 4-winged, an average of 2.5 cm in length and 2 cm in breadth from the edge of one wing to the next, tapering towards both extremities and roughly oval in shape. The wings are

faintly ribbed crosswise, thin, and change colour from green to red-brown, and when matured from brown to dark-brown. In the centre a single seed is accommodated, about 5 mm in diameter and initially green but later pale brown.[15] *C. apiculatum* is a widespread in Africa, growing from KwaZulu-Natal, Mpumalanga and Limpopo (South Africa) to Botswana, Mozambique, Namibia, Zimbabwe and other parts of tropical Africa. It occurs in various ecosystems and is the dominant tree in Lowveld and Mopani savannah, South Africa.[15]

5.2.1.2. Medicinal uses

This plant has a wide range of traditional uses. The leaf extract is commonly used to treat an extensive range of ailments including abdominal disorder, conjunctivitis, infertility and venereal diseases. A decoction of the leaves has been used as a steam bath for stomach disorders. Whole leaves are placed in the vagina for body fatigue and are also used to clean the navel after birth.[16]

5.2.1.3. The chemical compounds

The species of the Combretaceae family have been largely investigated for their phytochemical and properties. Four antioxidant compounds, cardamonin **1**, pinocembrin **2**, kaempferol **3** and quercetrin **4 (Figure 5.2)** have been isolated from the ethyl acetate and butanol partitioned extracts of *C. apiculatum.*[12]

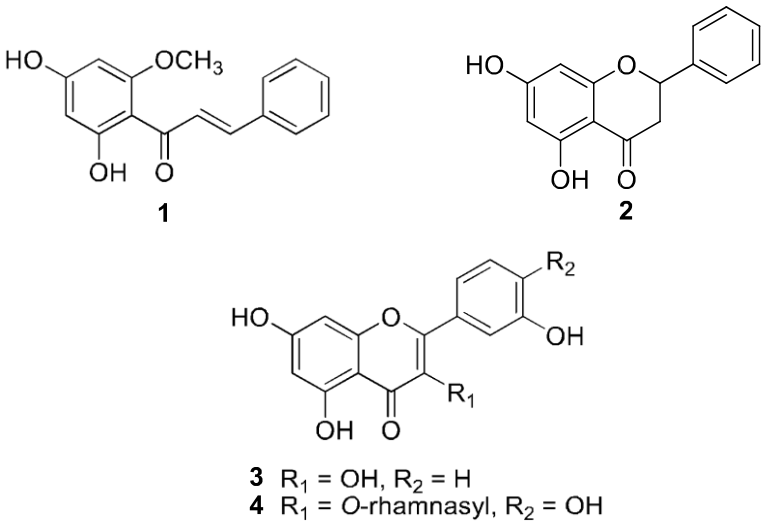


Figure 5.2. Chemical structures of compounds previously isolated from *C. apiculatum.*

5.3. Family Aizoaceae

The family Aizoaceae, formerly known as Ficaideae, is a family of dicotyledonous flowering plants. Aizoaceae is largely endemic to southern Africa, containing at least 120 genera and over 1800 species. A few species are found in Australia and the Central Pacific Area.[17] Most of the species are considered succulent and belong to the subfamilies Mesembryanthemoideae and Ruschioideae and are loosely termed mesems or mesembs. The highest number of genera and species occurs in a semi-arid climate. Aizoaceae are important elements in farming management in extensive winter rainfall areas and adjacent regions.[18]

5.3.1. *Galenia africana* L.

Figure 5.3. *Galenia africana* L

5.3.1.1. Description and distribution

G. africana, commonly known as yellow bush, belongs to the family Azoaceae and is distributed throughout Namaqualand, South Africa. *G. africana* is an aromatic, erect, woody, perennial sub-shrub growing to 0.5 to 1 m. The leaves are oppositely arranged, about 1.5-5.0 cm long and up to 0.5 cm wide. The leaves are green, but yellows with age, while the stems are light brown and smooth.[20]

5.3.1.2. Medicinal uses

The aerial parts of *Galenia africana* L. are used in South Africa to treat venereal sores, asthma, coughs, wounds, eye infection and TB, and is prepared as a decoction for skin diseases such as ringworm and to relieve inflammation of the eyes. Indigenous tribes chew the plant to relieve toothache.[11,21] *G. africana* has been associated with liver damage and sever ascites, a condition commonly known as “waterpens” in sheep and goats.[22]

5.3.1.3. Chemical compounds

Preliminary tests suggested the presence of alkaloids but not of saponins, tannins and reducing sugars. Flavonoids were isolated from the leaf extract of *G. africana*, namely; (2*S*)-5,7,2′-trihydroxyflavanone **5**, (*E*)-3,2′,4′-trihydroxychalcone **6**, (*E*)-2′,4′-dihydroxychalcone **7**, and (*E*)-3,2′,4′-trihydroxy-3′-methoxychalcone **8 (Figure 5.4)**. (2S)-5,7,2′-trihydroxyflavanone **5** and (E)-2′,4′-dihydroxychalcone **7** exhibited moderate antituberculosis activity. Crude extract from the aerial parts of *G. africana* also displayed moderate antifungal properties.[23]

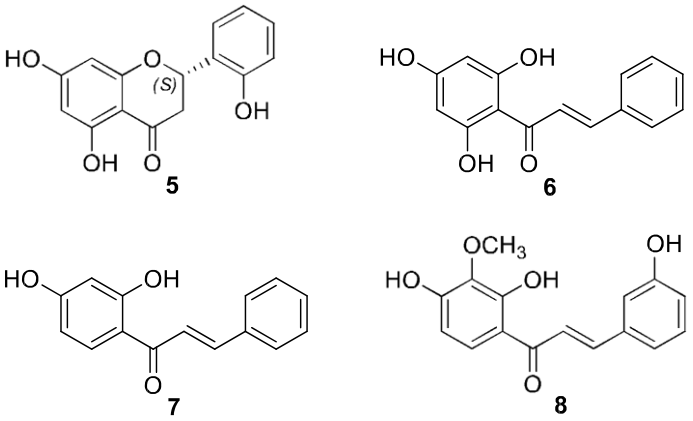


Figure 5.4. Previously isolated compounds from *G. africana*

5.4. Materials and methods

General

A Spectrum model (Dynamic Extractions, Slough, UK) multilayer coil-planet J-type centrifuge was used for hydrodynamic HSCCC chromatography according to the instrumental setup described in a previous report (Shikanga).[24] NMR spectra were recorded at 600 and 150 MHz for ^{1}H and ^{13}C, respectively, with a Bruker 600 NMR Avance II spectrometer (Bruker, Germany), using standard pulse sequence with TMS as internal standard. Chemical shifts were given in values of ppm and coupling constants in Hertz. ESI mass spectra were obtained with a QTRAP 3200 triple-quadrupole mass spectrometer (Applied Biosystems/MDS Sciex), coupled with an electrospray ionisation source. Sephadex LH-20 (25-100 µm, Sigma-Aldrich, St. Louis, MO, USA) was used for column chromatography (CC). Fractions obtained from CC were monitored by TLC with pre-coated silica gel GF_{254} plates (Merck) and detected by spraying with vanillin-sulfuric acid. All solvents used were of analytical grade.

Abbreviations:		
	Butanol	BuOH
	Dichloromethane	DCM
	Ethyl acetate	EtOAc
	Heptane	hept
	Methanol	MeOH
	Methanol-d_4	MeOD
	Chloroform-d_1	$CDCl_3$

5.4.1. Plant collection

The plant material were collected and identified by Dr Pieter Zietsman, National Museum, Bloemfontein, South Africa. Voucher specimens were deposited in the National Museum herbarium **(Table 5.1)**. The collected plant material was air dried at room temperature.

Table 5.1. Table of plants collected

Plant species	**Family**	**Collection Nr.**
Combretum apiculatum subsp. *apiculatum.* (leaves)	Combretaceae	5240
Combretum apiculatum. subsp. *apiculatum* (seeds)	Combretaceae	4718
Galenia africana L. (Aerial parts)	Aizoaceae	5238

5.4.2. Fractionation of *G. africana* L.

Powdered aerial plant material of *G. africana* (400 g) was extracted consecutively with DCM (500 mL, overnight, x3) and MeOH (500 mL, overnight, x3), with stirring. The respective extracts was filtered and concentrated under vacuum. This afforded the DCM extract (18.637 g) and the MeOH extract (30.939 g), respectively. Considering the preliminary bioactivities tested against *Enterococus faecalis* (ATCC 29212), *Klebsiella pneumoniae* (ATCC 13883), *Cryptococcus neoformans* (ATCC 90112) and *Mycobacterium tuberculosis* (see **Chapter 6**) the DCM extract was selected for further studies.

The TLC plate of the DCM crude extract was developed (Hept:EtOAc, 1:1) and sprayed with vanillin to determine the nature of the compounds present in the extract **(Figure 5.5)**.

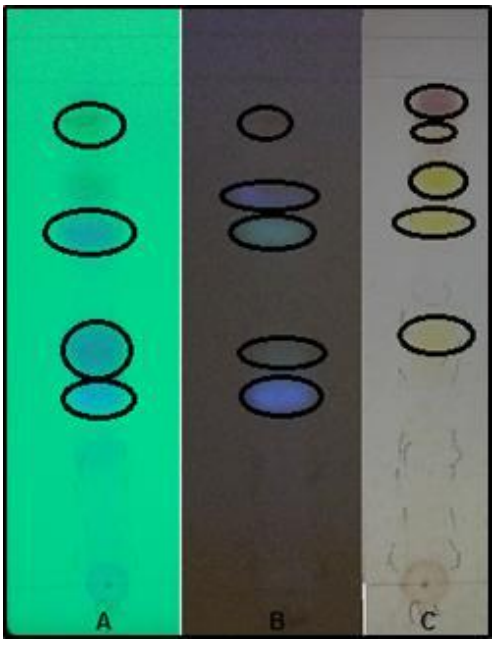


Figure 5.5. TLC plate (Heptane: EtOAc 1:1) of the DCM extract of *G. africana*, (**A**) UV at 366 nm, (**B**) UV at 254 nm and (**C**) sprayed with vanillin (90% H_2SO_4 in H_2O).

*7,8-dimethoxy-2-phenyl-4H-chromen-4-one (**A**)*:

The DCM extract of the aerial parts of *G. africana* (200 mg) was fractionated via high-speed countercurrent chromatography (HSCCC). This technique is an all-liquid method, without solid phases, which relies on the partition of the sample between two immiscible solvents to achieve separation. The relative proportion of the solute passing into each of the two phases is determined by the respective partition coefficients.[25] HSCCC chromatogram of the DCM extract of *G. africana* aerial parts. Solvent system: n-Heptane:EtOAc:MeOH:H_2O; 1:9:1:9 (mobile phase: upper phase), 1500 rpm, flow rate 3mL/min, sample weight: 200 mg. 23 fractions were collected. Fraction two was further for purified using Saphadex LH-20 using MeOH as an eluent.19 fractions were collected and detected using TLC plate. Compound **A** was isolated as a white amorphous powder (6 mg). ESIMS: *m/z* 282.9 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$) δ_H (ppm): 7.97 (1H, *d*, *overlapped*, *J* = 8.8 Hz, H-5), 7.96 (2H, *m*, *overlapped*, H-2′/6′), 7.55-7.53 (3H, *m*, H-3′/4′/5′), 7.06 (1H, *d*, *J* = 9.0 Hz, H-6), 6.78 (1H, *s*, H-3), 4.05 (3H, *s*, -OMe), 4.01 (3H, *s*, -OMe). ^{13}C NMR (150 MHz, $CDCl_3$) δ_C (ppm): 178.0 (C-4), 163.0 (C-2), 156.6 (C-7), 150.6 (C-9), 136.9 (C-8), 131.8 (C-1′), 131.5 (C-4′), 129.0 (C-3′/5′), 126.2 (C-2′/6′), 121.0 (C-5), 118.7 (C-10), 109.9 (C-6), 106.9 (C-3), 61.6 (C-OMe), 56.4 (C-OMe).

The schematic representation of the isolation of Compound **A** is depicted in **Figure 5.6.**

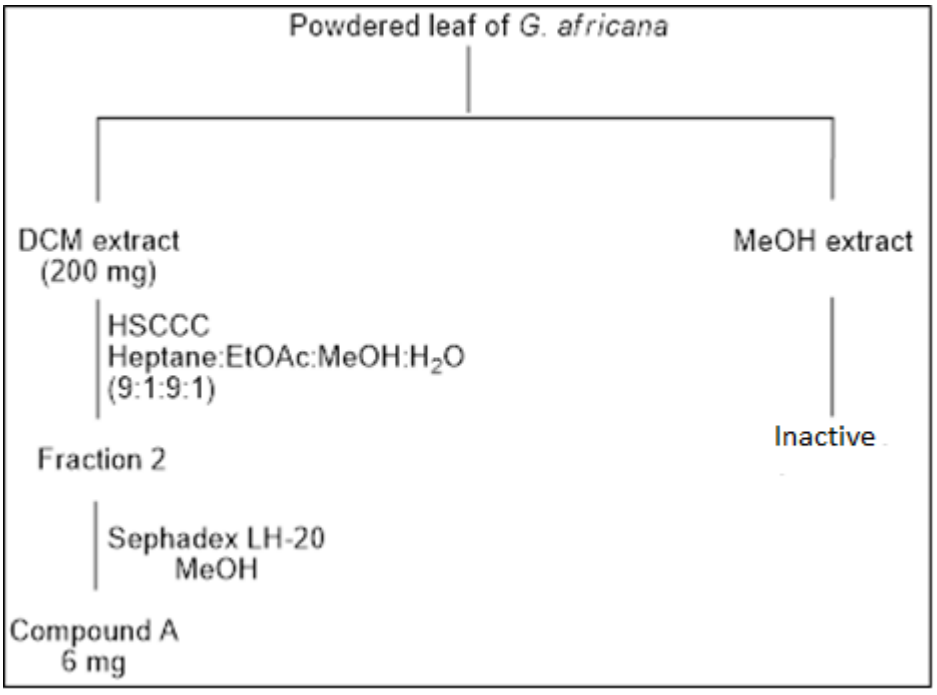


Figure 5.6. Schematic representation of the isolation of **A** from the DCM extract of *G. africana*.

The HSCCC chromatogram of the DCM extract of *G. africana* aerial parts is depicted in **Figure 5.7**.

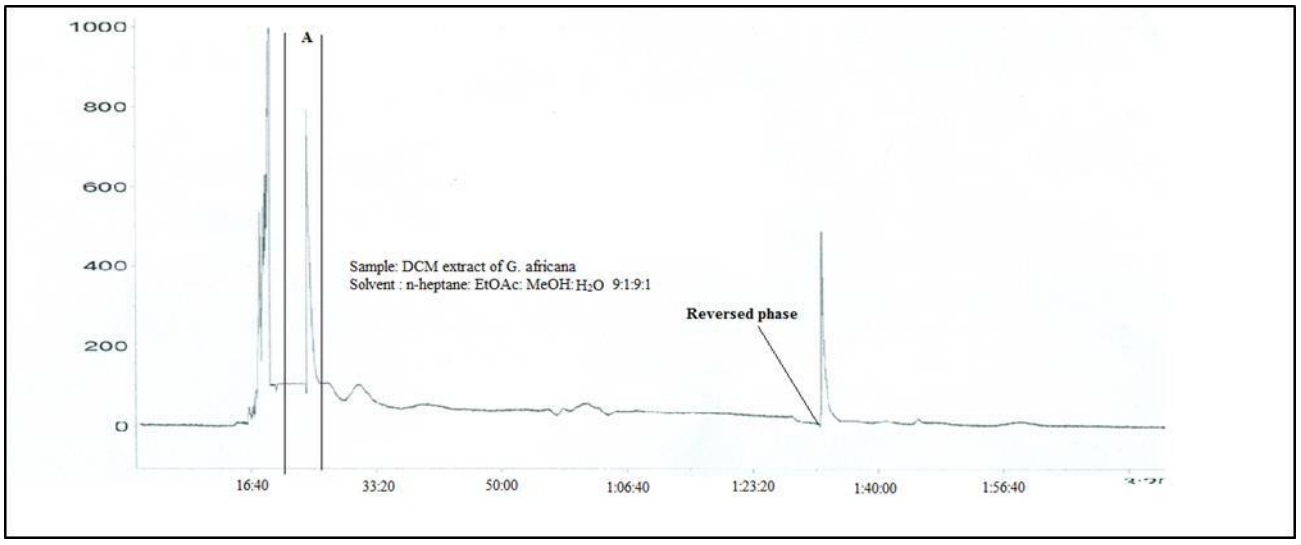


Figure 5.7. HSCCC chromatogram of the DCM extract of *G. africana* aerial parts. Solvent system: n-Heptane:EtOAc:MeOH:H_2O; 1:9:1:9 (mobile phase: upper phase), 1500 rpm, flow rate 3 mL/min, sample weight: 200 mg.

In order to have sufficient material for the isolation of pure compounds a portion of the DCM extract (8.0 g) was column chromatographed on silica gel (0.040-0.063 mm) using Hept: EtOAc; 1:1 as a solvent system. A total of 99 fractions were collected and analysed on TLC (Heptane:

EtOAc; 1:1) under UV light at 365 nm and 254 nm, respectively. Similar fractions were combined, which resulted in six fractions. A schematic representation of the purification steps is depicted in **Figure 5.8**.

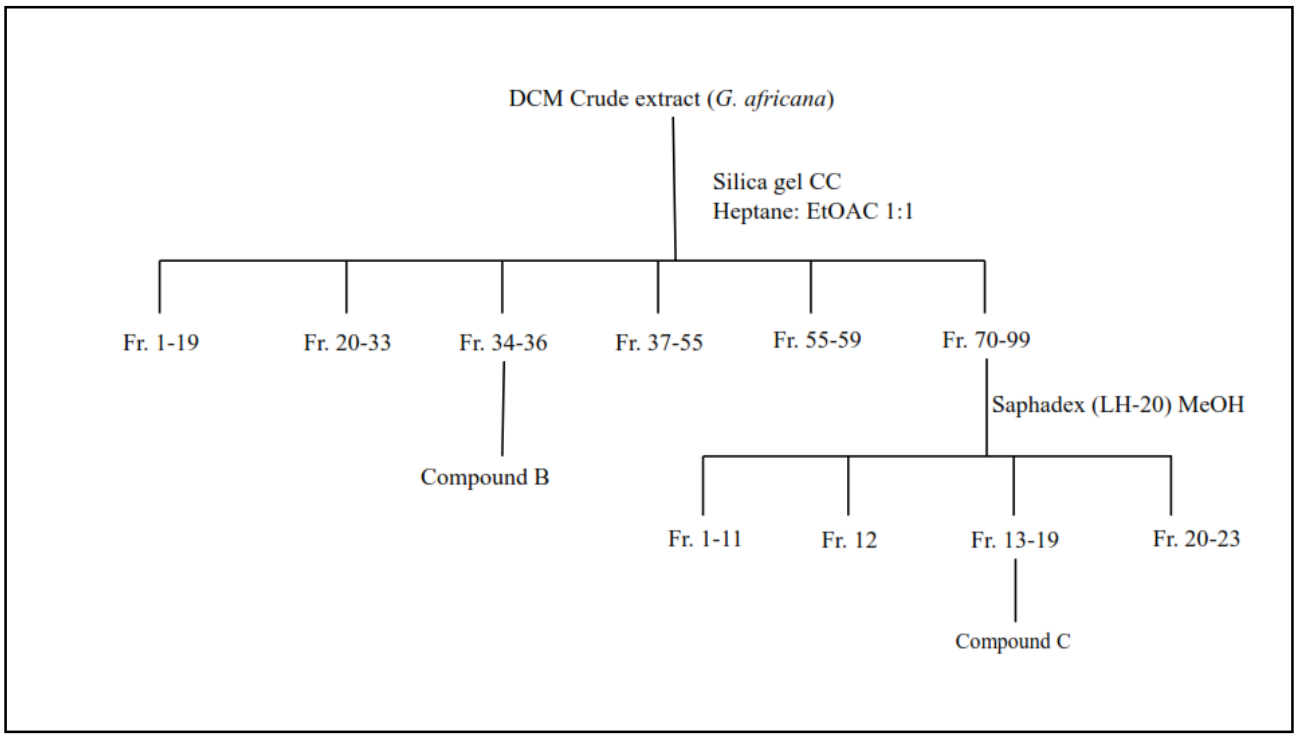


Figure 5.8. Schematic representation of the purification steps for the isolation of compounds **B** and **C** from the DCM extract of *G. africana*.

*6,7-dimethoxy-2-phenyl-4H-chromen-4-one (**B**)*

Combination of Fr. 34 – 36 yielded compound **B** as a pale green amorphous powder (6 mg). ESIMS: *m/z* 282.9 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$) δ_H (ppm): 7.91 (2H, *m*,H-2′/6′), 7.57 (1H, *s*, H-5), 7.55-7.53 (3H, *m*, H-3′/4′/5′), 7.01 (1H, *s*, H-8), 6.81 (1H, *s*, H-3), 4.03 (3H, *s*, -OMe), 4.00 (3H, *s*, -OMe). ^{13}C NMR (150 MHz,$CDCl_3$) δ_C (ppm): 177.7 (C-4), 162.8 (C-2), 154.5 (C-7), 152.3 (C-9), 147.6 (C-6), 131.9 (C-1′), 131.3 (C-4′), 129.0 (C-3′/5′), 126.0 (C-2′/6′), 117.3 (C-10), 107.1 (C-3), 104.3 (C-5) 99.7 (C-8), 56.4 (C-OMe), 56.3 (C-OMe).

*8-phenyl-6H-[1,3]dioxolo[4,5-h]chromen-6-one (**C**)*

Further purification of Fr. 70 – 99 on Sephadex LH-20 (MeOH) afforded **C** as white needle crystals (20 mg, mp. 208 – 110 ^{o}C).

ESIMS: *m/z* 266.9 [M+H]. ^{1}H NMR (600 MHz, $CDCl_3$) δ_H (ppm): 7.92 (2H, *m*, H-2′/6′), 7.82 (1H, *d*, *J* = 8.5 Hz, H-5), 7.56 -7.51 (3H, *m*, 3′/4′/5′), 6.97 (1H, *d*, *J* = 8.5 Hz, H-6), 6.75 (1H, *s*, H-3), 6.23 (2H, *s*, dioxo-CH_2). ^{13}C NMR (150 MHz,$CDCl_3$) δ_C (ppm): 177.4 (C-4), 162.6 (C-2), 152.4 (C-7), 141.1 (C-9), 134.8 (C-8), 131.6 (C-4′), 131.4 (C-1′), 129.0 (C-3′/5′), 126.6 (C-2’/6), 120.1

(C-5), 119.9 (C-10) 107.1 (C-6), 107.0 (C-3) 103.2 (CH_2diox). Resonances at δ 2.19 and 1.51 were attributed to artifacts.

5.4.3. Extraction of *C. apiculatum* subsp. *apiculatum* leaves and seeds

Ground leaves of *C. apiculatum* (80 g) were extracted with methanol (500 mL, overnight x3), with stirring. The extract was filtered and dried under vacuum. The concentrated crude extract was freeze-dried and subsequently suspended in distilled water and successively partitioned with heptane, EtOAc, BuOH and H_2O. This gave four partitioned fractions. The fractions were developed on a TLC plate and sprayed with vanillin in H_2SO_4 to determine the nature of the constituents **(Figure 5.9)**. The seeds of *C. apiculatum* were extracted following the same procedure and the developed TLC is depicted in **Figure 5.9.**

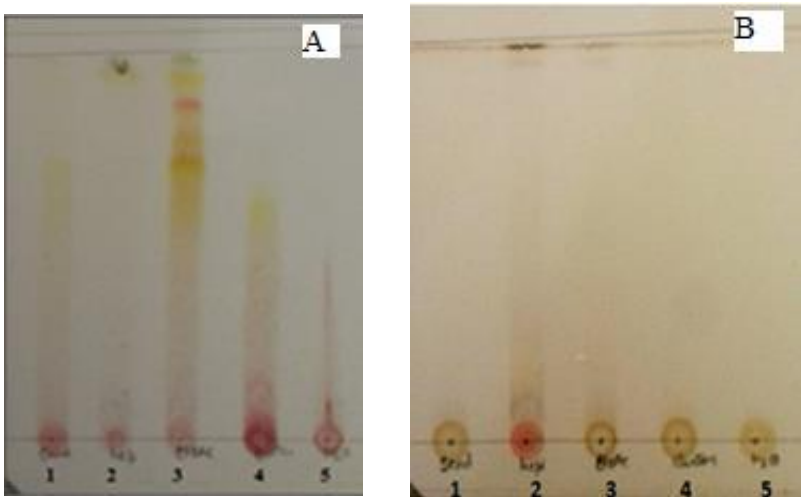


Figure 5.9. TLC **A** (EtOAc:MeOH:H_2O; 100:13.5:5) of *C. apiculatum* MeOH crude leaf extract and portioned fractions. MeOH crude extract **(1)**, heptane fraction **(2)**, EtOAc fraction **(3)**, BuOH fraction **(4)** and aqueous fraction **(5)**. TLC B is the seed extract and partitions. The TLC plates were sprayed with vanillin in H_2SO_4 to determine the nature of the chemical compounds.

After inspection of **Figure 5.9**, Fr.3 (EtOAc, leaf extract) was selected for the isolation of pure compounds. The EtOAc extract (3 g) was column chromatographed (EtOAc:MeOH:H_2O; 100:13.5:5) on silica gel (0.040-0.063 mm). A total of 40 test tubes were collected.

*5,7-dihydroxy-2-phenyl-3-((3S,4S,5S,6R)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yloxy)-4H-chromen-4-one (**D**)*

Fractions 31 – 33 were combined and separated on Sephadex LH-20 (MeOH) to yield compound **D** as a yellow powder (5 mg).

ESIMS: *m/z* 471 [M+Na], 447 [M-H]. ^{1}H NMR (600 MHz, MeOD) δ_H (ppm): 7.28 (1H, *d*, *J* = 2.1 Hz, H-2′), 7.24 (1H, *dd*, *J* = 8.3 and 2.1 Hz, H-6′), 6.86 (1H, *d*, *J* = 8.3, H-5′), 6.31 (1H, *d*, *J* = 2.0 Hz, H-8), 6.14 (1H, *d*, *J* = 2.0 Hz, H-6), 5.28 (1H, *d*, *J* = 1.5 Hz, H-1″), 4.16 (1H, *dd*, *J* = 1.6 and 3.4 Hz, H-2″), 3.70 (1H, *dd*, *J* = 9.4 and 3.4 Hz, H-3″), 3.35 (1H, *m*, H-4″), 3.28 (1H, *overlapped by solvent resonance*, H-5″), 0.88 (3H, *d*, *J* = 6.1 Hz, H-6″). ^{13}C NMR (150 MHz, MeOD) δ_C (ppm):179.9 (C-4), 167.0 (C-7), 163.4 (C-5), 158.9 (C-2), 158.8 (C-9), 150.1 (C-4′), 146.7 (C-3′), 136.4 (C-3), 123.2 (C-1′), 123.1 (C-6′), 117.2 (C-2′), 116.7 (C-5′), 105.9 (C-10), 103.8 (C-1″), 100.4 (C-6), 95.2 (C-8), 73.5 (C-4″), 72.4 (C-5″), 72.3 (C-3″), 72.2 (C-2″), 17.9 (C-6″)

5-hydroxy-7-methoxy-2-phenylchroman-4-one ***E*** (pinostrobin)

The same fraction mentioned above yielded **E** as a white powder (8 mg). ESIM: *m/*z 271. [M+H]. ^{1}H NMR (600 Hz, MeOD) 7.45 (2H, *d*, *J* = 7.1 Hz, H-2′/ 6′), 7.37 (2H, *td*, *J* = 7.1 and 1.5 Hz, H-3′/5′), 7.32 (1H, *m*, H-4’), 6.06 (1H, *d*, *J* = 2.2 Hz, H-6), 6.01 (1H, *d*, *J* = 2.2 Hz, H-8), 5.39 (1H, *dd*, *J* = 12.7 and 3.0 Hz, H-2), 4.59 (1H, *s*, OH), 3.79 (3H, *s*, OMe), 2.95 (1H, *dd*, *J*=16.6 and 12.7 Hz, H-3), 2.72 (1H, *dd*, *J* = 16.6 Hz and 3.0 Hz, H-3). **^{13}C** NMR (150 MHz, MeOD) δ_C (ppm): 191.8 (C-4), 167.4 (C-7), 166.6 (C-9), 164.3 (C-5), 140.6 (C-1′), 129.7 (C-3′/5′), 129.5 (C-4′), 127.3 (C-2′/6′), 105.7 (C-10), 97.2 (C-6), 94.4 (C-8), 80.2 (C-2), 56.2 (OCH_3), 46.5 (C-3).

The isolation of compound **D** and **E** is summarized in **Figure 5.10**.

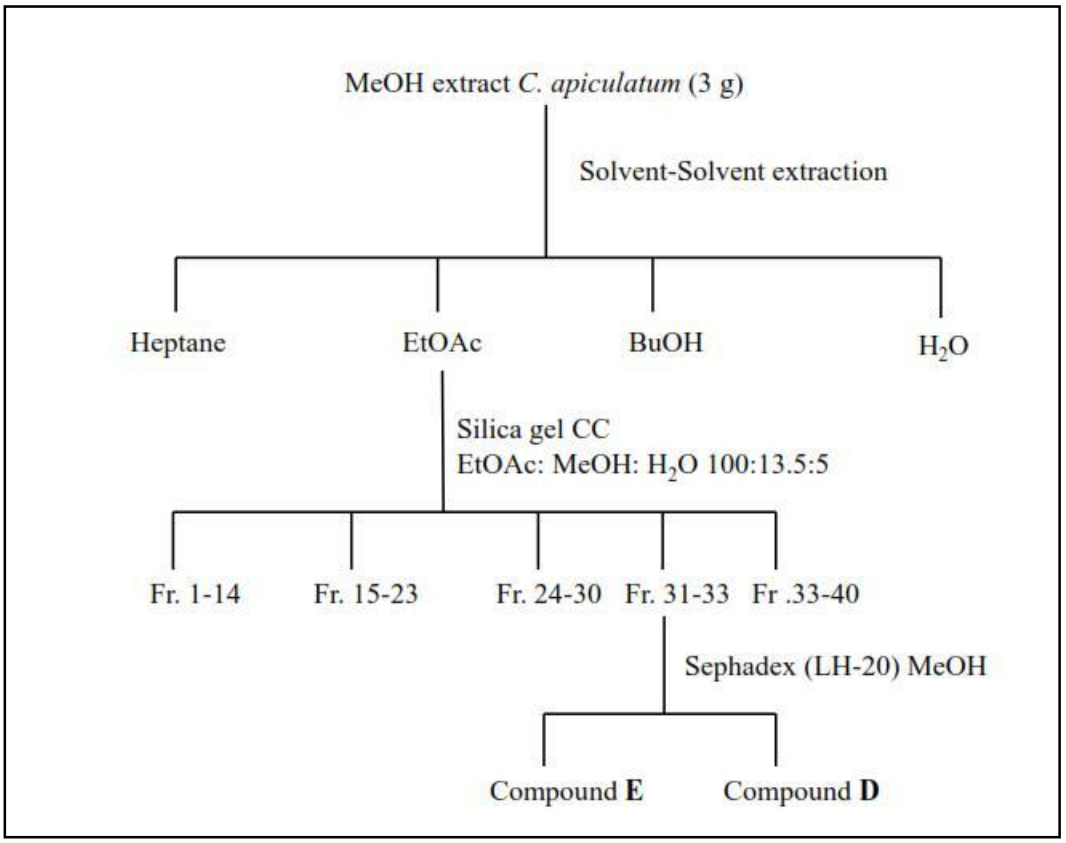


Figure 5.10: Schematic representation of the isolation and purification of **D** and **E** from the MeOH crude leaf extract of *C. apiculatum*

5.5. Discussion

The isolated compounds **A** – **E** were identified as 7,8-dimethoxy-2-phenyl-4*H*-chromen-4-one (**A**), 6,7-dimethoxy-2-phenyl-4*H*-chromen-4-one (**B**), 8-phenyl-6*H*-[1,3]dioxolo[4,5-*h*]chromen-6-one (**C**), 5,7-dihydroxy-2-phenyl-3-((3*S*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-methyltetrahydro-2*H*-pyran-2-yloxy)-4*H*-chromen-4-one (**D**) (quercetrin), and 5-hydroxy-7-methoxy-2-phenylchroman-4-one (**E**) via NMR spectroscopy and ESI mass spectrometry **(Figure 5.11)**. All compounds correlate to the C15 (C6-C3-C6) framework of flavonoids. A carbonyl resonance in the ^{13}C NMR spectra at $\delta_C \approx 180$ for flavones **A** - **D** and 191.8 for the flavanone **E** (see NMR spectra attached), indicate the presence of a carbonyl group on the heterocyclic C-ring. All five compounds were identified as known compounds, compounds **A** – **C** and compound **E** were isolated for the first time from *G. africana* and *C. apiculatum*, respectively.

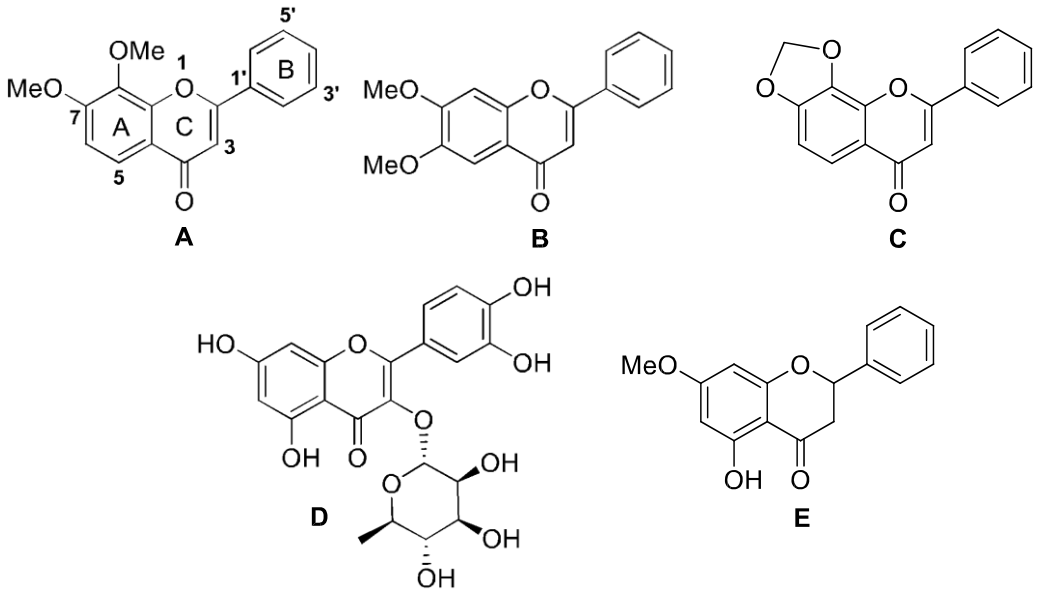


Figure 5.11. Structures of the isolated compounds **A** – **E**

For both **A** (MS **Plate 1**) and **B** (MS **Plate 2**) the [M+H] quasi-molecular ions at *m/z* 282.9 in the ESI-MS spectra are consistent with the molecular formula $C_{17}H_{14}O_4$, indicating structural isomers. The [M+H] quasi-molecular ion at *m/z* 266.9 in the ESI-MS spectrum of **C** (MS **Plate 3**) is consistent with the molecular formula $C_{16}H_{10}O_4$. Notable in the ^{1}H NMR spectra of **A** (NMR **Plate 1**) and **C** (NMR **Plate 5**) ($CDCl_3$) is an A-ring AB system corresponding to H-6 at δ_H 7.06 (*d*, *J* = 8.9 Hz) and 6.97 (d, *J* = 8.4 Hz), respectively, and H-5 at δ_H 7.97 (*d*, J = 8.9 Hz) and 7.82 (*d*, *J* = 8.4 Hz), respectively, while the A-ring of **B** (NMR **Plate 3**) shows two singlets at δ_H 7.57 and 7.01 corresponding to H-5 and H-8, respectively. The vinylic protons of **A**, **B** and **C** resonate as one proton singlets at δ_H 6.78, 6.81 and 6.75, respectively. All three compounds have an AA′BB′C system in their ^{1}H NMR spectra indicating an unsubstituted phenyl ring at C-2. The ^{1}H NMR spectra of both **A** and **B** have two singlets integrating for three protons each at δ_H 4.05 and 4.00, and δ_H 4.03 and 4.00, respectively, corresponding to two methoxy substituents on the A-ring for each. However, the ^{1}H NMR spectrum of **C** shows a downfield two proton singlet at δ_H 6.23 indicating a methylenedioxy group. 2D NMR experiments confirmed the position of the methoxy groups of **A** and the methylenedioxy groups of **C** to be at C-7 and C-8, but for B the methoxy groups are situated at C-6 and C-7. The 17 C-atoms for **A** and **B**, and 16 C-atoms of **C** were assigned via 2D NMR experiments (HSQC and HMBC), with C-3 resonating at δ_C 106.9, 107.1 and 107.0, respectively, and the carbonyl carbon at δ_C 178.1, 177.7 and 177.4, respectively (NMR

Plates 2, **4** and **6**, respectively). Compounds **A-C** are previously described as synthetic products.[26,27]

The [M+Na] quasi-molecular ion at *m/z* 471 and [M-H] quasi-molecular ion at *m/z* 447 in the ESIMS spectra of **D** (MS Plate 4 and Plate 5) is consistent with the molecular formula $C_{12}H_{20}O_{11}$. The ^{1}H NMR spectrum (NMR **Plate 7**) (MeOD) exhibited two doublets at δ_H 6.37 (*J* = 2.0 Hz) and 6.20 (*J* = 2.0 Hz), correlating with the carbons at δ_C 94.9 and 100.09, respectively, in the 2D HSQC spectrum, that were assigned to H-8 and H-6 of the A ring. The ^{1}H NMR spectrum exhibited an ABX system with resonances at δ_H 7.28 (*d*, *J* = 2.1 Hz, H-2′), 7.24 (*dd*, J = 8.2 and 2.1 Hz, H-6′) and 6.86 (*d*, *J* = 8.3 Hz, H-5′), Confirming the *orto* disubstituted B-ring. The 3-*O*-rhamnose moiety is confirmed by the presence of six aliphatic resonances, with the anomeric proton doublet resonating at δ_H 5.28 (*J* = 1.5 Hz), correlating to C-1″ at δ_C 103.8, and the methyl group doublet at δ_H 0.88 (*d*, *J* = 6.2 Hz). The small $J_{H1□□,H2□□}$ coupling of the anomeric proton indicates an α-sugar. The
^{13}C NMR spectrum (NMR **Plate 8**) showed 21 carbon resonances, with the carbonyl carbon C-4 resonating at δ_C 179.8 and the oxygenated carbons at δ_C 179.5 (C-4), 166.3 (C-7), 163.1 (C-5), 149.8 (C-4′), 146.4 (C-3′) and 136.1 (C-3). The spectral data obtained were identical to the quercetin 3-*O*-α-rhamnoside (quercetrin) previously reported.[28]

For compound **E** the [M+H] quasi-molecular ion at *m/z* 271.2 in the ESI-MS spectrum (MS **Plate 6**) is consistent with the molecular formula $C_{17}H_{14}O_4$. The ^{1}H NMR (NMR **Plate 9**) (MeOD) spectrum of **E** displays two meta-coupled doublets at δ_H 6.06 (*J* = 2.2 Hz, H-6) and 6.01 (*J* = 2.2 Hz, H-8) indicating the A-ring, while the unsubstituted B-ring is confirmed by a doublet at δ_H 7.45 (*J* = 7.1 Hz, H-2′/6′), a triplet of doublets at δ_H 7.37 (J = 7.1 and 1.5 Hz) and a multiplet at δ_H 7.32 (H-4′). The proton of the OH group resonates as a singlet at δ_H 4.64. Notable in the aliphatic region of the ^{1}H NMR spectrum is the presence of three doublet of doublets at δ_H 5.39 (*J* = 12.8 and 3.0 Hz), 2.95 (*J* = 16.6 and 12.8 Hz) and 2.68 (*J* = 16.6 and 3.0 Hz), corresponding to H-2 and the two geminal protons H-3_{eq} and H-3_{ax}. The three proton singlet at δ_H 3.79 was assigned to the 7-*O*-methoxy group via 2D experiments. The ^{13}C NMR spectrum (NMR **Plate 10**) exhibited 17 carbons with C-4 resonating at δ_C 191.6, the three oxygenated carbons at δ_C 167.4, 166.6 and 164.3, and the two aliphatic carbons C-2 and C-3 at δ_C 80.2 and 46.8, respectively. Compound E has been previous isolated.[29]

5.6. Conclusion

Natural products play an important role in the discovery of potential innovative lead compounds in drug discovery and in development of quality herbal medicine with proven efficacy. The phytochemical investigation of both *G. africana* and *C. apiculatum* yielded five known flavonoids namely; 7,8-dimethoxy-2-phenyl-4*H*-chromen-4-one (**A**), 6,7-dimethoxy-2-phenyl-4*H*-chromen-4-one (**B**), 8-phenyl-6*H*-[1,3]dioxolo[4,5-*h*]chromen-6-one (**C**) (from *G. Africana*), and 5,7-dihydroxy-2-phenyl-3-((3*S*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-methyltetrahydro-2*H*-pyran-2-yloxy)-4*H*-chromen-4-one (**D**), and 5-hydroxy-7-methoxy-2-phenylchroman-4-one (**E**) (from *C. apiculatum*). Biological activities of compounds **D** and **E** have been previously reported.[30,31] Various bioassays were conducted on the crude extracts and isolated compounds from *G. africana* and *C. apiculatum* and will be discussed in **Chapter 6**.

5.7. References

1. Kgatle D.T. **2007**. Isolation and characterization of antioxidant compounds from *Combretum apiculatum* subsp. *apiculatum*. M.sc thesis, University of Pretoria.
2. Pietrovski E.F., Rosa K.A., Facundo V.A., Rios K., Marques M.C.A and Santos A.R.S. **2006**. Antinociceptive properties of the ethanolic extract and tritepene 3β, 6β, 16β trihidroxilup-30(29)-ene obtained from flowers of *Combretum laprosum* in mice. *Pharmacol Biochem. Behav*. 83: 90-99.
3. McGaw I.J., Rabe T., Sparg S.G., Jager K.A., Eloff J.N. and Van Staden J. **2001**. An investigation of the biological activity of Combretum species. *J. Ethnopharmacol*. 75: 54-50.
4. Eloff J.N., Keterere D.R. and McGaw L.J. **2008**. The biological activity and chemistry of the Southern African Combretaceae. *J. Ethnopharmacol*. 119: 686-699.
5. De Morais Lima G.R., de Sales I.R., Calda F.M.R., de Jesus N.Z., de Sousa F.H., Barbosa-Fihlo J.M., Cabral A.G., Souto A.L., Taveares J.F. and Batista L.M. **2012**. Bioactivities of the genus Combretum (Combretaceae): a review. *Molecules*. 17: 9142-9206.
6. Hutchings A., Scott H., Lewis G. and Cunninghan A. **1996**. *Zulu medicinal plants*. An inventory. University press. Pietermaritzburg, South Africa.
7. Van Wyk B-E., Van Oudtshoorn B. and Gericke N. **1997**. *Medicinal plants of South Africa*. Briza, South Africa.
8. Roger C.B. and Verotta L. **1996**. Chemistry and biological properties of the African combretaceae. In Hostettman K., Chinyanganga F., Maillard A.M. and Wolfernder J. *Chemistry, biological and pharmacological properties of African medicinal plants*. University of Zimbabwe publications. Harare Zimbabwe.
9. Elgami A.A., El-Nima E.L., El-Tohami M.S. and Muddhir A.K. **2002**. Antibacterial activity of some species of the family Combretaceae. *Phytother. Res*. 16: 555-561.
10. Eloff J.N. **1999**. The antibacterial activity of 27 Southern African members of the Combretacea. SAJS. 95: 148-154.
11. Martin N. and Eloff J.N. **1998**. The preliminary isolation of several antibacterial compounds from *Combretum erythrophyllum* (Combretaceae). *J. Ethnopharmacol*. 62: 255-263.
12. Aderagba M.A., Kgatle D.T., McGaw L.J. and Eloff J.N. **2012**. Isolation of antioxiadant constituents from *Combretum apiculatum* subsp. *apiculatum*. *S. Afr. J. Bot*. 79: 125-131.
13. http://suntrees.co.za/wp-content/uploads/2014/10/Combretum-apiculatum-pods.jpg.
14. Palmer E. **1977**. *A field to the tress of Southern Africa*. Collins, Johannesburg.
15. Van Wyk P. **1974**. *Trees of the Kruger National Park*. Pumell, Cape Town.
16. Palgrave C.K. **1977**. *Trees of southern Africa*. Struik publishers, Cape Town.

17. http://succulent-plant.com/families/aizoaceae.html Accessed 19 August **2014**.
18. Hartman Heidrun E.K. **2001**. *Illustated handbook of succulent plants: Aizoaceae F-Z.* New York Springer.
19. http://www.plantzafrica.com/. Accessed 19 August **2014**
20. Watt J.M. and Breyer-Brandawijk M.G. **1962**. *The medicinal and poisonous plants of Southern and Eastern Africa*. Edited by 2nd edition. London, United Kingdom. Livingstone.
21. Mativandlela S.P.N., Muthivhi T., Kikuchi H., Oshima Y., Hamilton C. and Hussein A.A. **2009**. Antimycobacterial flavonoids from the leaf extract of *Galenia africana*. *J. Nat. Prod.* 72: 2169-2171.
22. Van der Lugt J.J., Schultz R.A., Fourie N., Hon L.J., Jordaan P. and Labuschagne L. **1992**. Galenia africana L. poisoning in sheep and goats: hepatic and cardiac changes. *Onderstepoort J. Vet. Res*. 59: 323-333.
23. Vries F.A., Bitar H.E., Green I.R., Klaasen J.A., Mabusela W.T., Bodo B. and Johnson Q. **2009**. An antifungal active extract from the aerial parts of *Galenia africana*. 11th NAPRECA Symposium book of proceedings, Antananarivo, Madagascar. 123-131.
24. Shikaga S. and Marston A. Isolation of Sceletum alkaloids by high-speed Counter Current Chromatography. *Phytochem. Lett.* 4: 190-193.
25. Marston A. and Hostettemann K. **2006**. Development in the application of countercurrent chromatography to plant analysis. *J. Chromatogr*. 1112: 181-194.
26. Visweswara R.K. and Viswanadham N. **1949**. A note on 7,8-dimethoxy flavones. *Proc. Indian acad. Scie*. 29: 218-220.
27. Yoon H., Eom S., Hyun J., Jo G., Hwang D., Lee S., Yong Y., Park J.C., Lee Y.H., and Lim Y. **2011**.^{1}H and ^{13}C NMR Data on Hydroxy/methoxy Flavonoids and the Effects of Substituents on Chemical Shifts. *Bull. Korean Chem. Soc*. 32: 2101-2104.
28. Bose S., Sushomasri M. and Chakroborty P. **2013**. Quercetrin from *Ixora cocinea* leaves and its anti-oxidant activity. *J. Pharmaceut. Sci.Tech.* 2: 72-74.
29. Ramirez J., Cartuche L., Morocho V., Aguilar S. and Malagon o. **2013**. Antifungal activity of raw extract and flavonons isolated from *Piper ecuadorense* from Ecuadar. *Rev. bras farmacogan.* 23: 370-373.
30. Dadi P.K., Ahmad M. and Ahmand Z. **2009** Inhibition of ATPase activity of *Eschorichia coli* ATP synthase by polyphenols. *Int .J. Biol. Macromol*. 45: 72-79.
31. Maridass M., RajuG., Thangavela K. and Ghanthikumar S. **2008**. Prediction of ant-HIV activity flavonoids constituents through PASS. *Ethnobot leaflets*. 12: 954-994.

6. Bioassays of crude extracts and isolated compounds of *G. africana* and *C. apiculatum*

6.1. Introduction

Bioassay, defined as the estimation of the concentration or potency of a substance by measurement of the biological response that it produces, has played a key role in the development of pharmacology and drug discovery. It is used to measure the pharmacological activity of new or chemically undefined substances, investigate the function of endogenous mediators and measure drug toxicity and unwanted effects.[1]

Extracts or pure isolated compounds from plant material serve as an important source of molecular diversity in drug discovery and several important drugs have been isolated from natural products. Such products can be heterogeneous due to the mixture of bioactive components present in the plant material.[2] Bioassay screening or pharmacological evaluation are used to guide the isolation process towards the pure bioactive component.[3] Pharmacological evaluation of extracts and pure isolates is an essential aspect of the drug development process in natural products. It offers an advantage in the standardization and quality control in botanical products.

Bioassays can be divided into two categories; primary bioassay screens and secondary bioassay screens. Primary bioassays are assays that are effectively applied to a large number of samples (crude extracts) to determine if any bioactivity of the desired type is present. They are low in cost and provide rapid results. Secondary bioassays are more detailed screening of the lead compounds in different model systems in order to select compounds for clinical trials. They are more expensive and time consuming.[3]

Four primary assays, (i) antioxidant, (ii) acetylcholine esterase inhibition test and (iii) antibacterial and antimycobacterial tests, were used for the purpose of this study. The assays were selected due to the facts that: (i) anti-oxidants can protect the human body from free radicals and retard the process of many chronic diseases and the general antioxidant activity of most flavonoids has been proven conclusively, (ii) both plants have been used traditionally as medicinal preparations against infections and chest complaints and (iii) Alzheimer's disease effect 37 million people worldwide and few synthetic drugs are available on the market.

6.2. TLC based bioassays

6.2.1. Radical scavenging (antioxidant activity)

6.2.1.1. Introduction

Free radicals are defined as molecules or molecular fragments containing one or more unpaired electrons in a molecular orbital. In the human body they are formed as by-products when the cell use oxygen to generate energy. Below is the formation reaction of reactive oxygen species (ROS) **(Figure 6.1)**

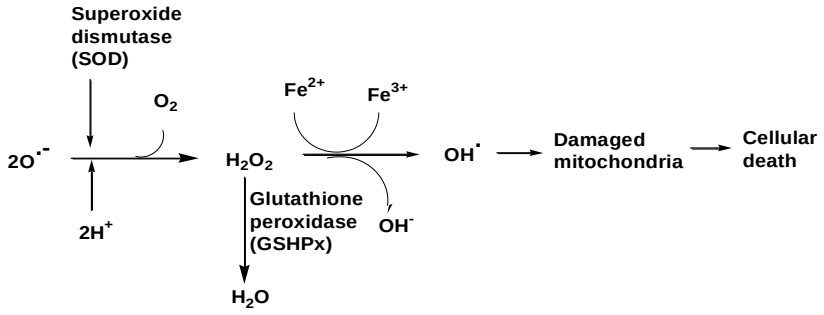


Figure 6.1. Formation reaction of reactive oxygen species

Reactive oxygen species, in particular free radicals, are believed to be a primary factor in various degenerative diseases as well as the normal process of aging. They are capable of damaging a wide range of essential biomolecules and if they are not effectively scavenged by cellular constituents, they can lead to disease conditions.[4,5] The harmful action of free radicals can be blocked by antioxidant substances which scavenge the free radicals and detoxify the organism. Antioxidants are substances that can delay or prevent the oxidation of cellular oxidizable substrates.[4]

Currently antioxidants and free radical scavenging from natural products are receiving increasing attention, since they can protect the human body from free radicals and retard the process of many chronic diseases. Prevention of cancer and cardiovascular diseases has been linked to the intake of food rich in natural antioxidants.[6] Natural antioxidants constitute a wide range of compounds including phenolic compounds, nitrogen compounds and carotenoids.[4] Many herbal medicines have antioxidative and pharmacological properties associated with the presence of phenolic compounds, especially flavonoids and therefore the search of newer natural antioxidants intensified.

6.2.1.2. Qualitative testing via TLC plates

In the radical scavenging assay, a solution of DPPH, a stable radical with a violet colour, is sprayed on the developed TLC plate. Any antiradical substances present, will capture and reduce the DPPH radicals and the violent colour will disappear **(Figure. 6.2)**. The active zone exhibited a pale yellow spots against the violet background.

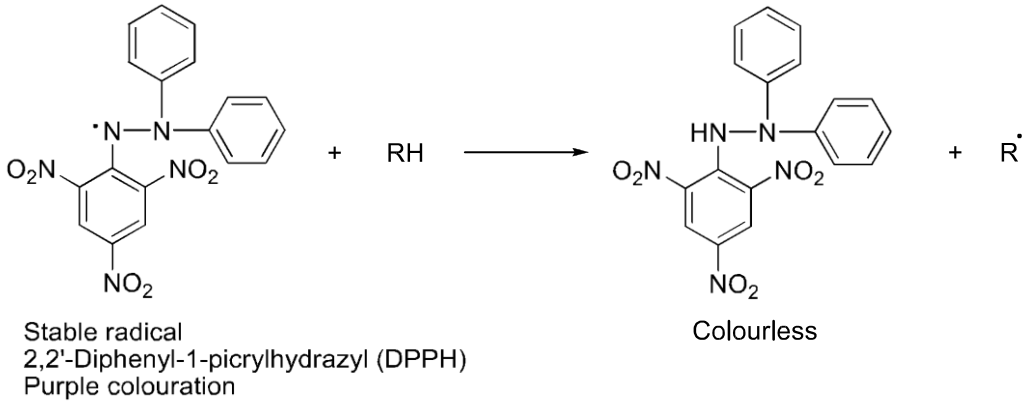


Figure 6.2. Reaction taking place during detection of radical scavengers

6.2.1.3. Quantitative antioxidant testing

The spectroscopic dilution test is used to quantitatively evaluate free radical scavenging activity. During quantitative testing for radical scavenging activities the absorbance of a standard methanolic DPPH solution is determined at 517 nm. A dilution series of the sample being tested is made up, the respective samples added to DMSO, which is subsequently added measured at 517 nm over period of 5 min. the respective percentage of radical scavenging is calculated from the decrease in absorbance at 517 nm using the following equation,

$$100 \times \left[\frac{(A0 \times 2.95/3.00) - At}{(A0 \times 2.95/3.00) - Ap}\right]$$

Where A_0 is the absorbance before addition of the test solution, A_t is the absorbance after 5 min reaction time and A_p is the absorbance after 5 min reaction time if all DPPH has been scavenged.

6.2.2. Acetylcholinesterase (AChE) inhibition

Alzheimer's disease (AD) is a neurodegenerative disorder which is characterized by loss of memory, cognitive decline, severe behavioural abnormalities and ultimately death.[7] AD is the most common form of dementia in our aging society, which affects more than 37 million people worldwide.[8] People suffering from AD often produce less acetylcholine, a neurotransmitter brain chemical that helps with memory and cognitive functions. Acetylcholinesterase inhibitor restore the level of acetylcholine by inhibiting acetylcholinesterase. Inhibition of acetylcholinesterase (AChE), the key enzyme in the breaking down of acetylcholine thus serves as a promising strategy for the treatment of neurological disorders such as AD, senile dementia, ataxia and myasthenia gravis.[9] The principal role of acetylcholinesterase is the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of acetylcholine (ACh).[10,11] Currently the newest drug available for the treatment of AD and dementia is based on the inhibition of acetylcholinesterase function by correcting a deficiency of the key enzyme in the breaking down of acetylcholine.

There are a few synthetic drugs available for treatment of cognitive dysfunction and memory loss associated with AD, such as tacrine, donepezil and the natural product-based rivastigmine.[12] These compounds have been reported to have adverse effects such as gastrointestinal disturbances and problems associated with bioavailability and therefore the development of better AChE inhibitors became essential.[9,10]

A TLC bioautographic assay was introduced for the screening of plant extracts for inhibition of acetylcholinesterase activity to the search for new potential drugs. The TLC bioautographic assay described by Marston[13] was used. The test relies on the cleavage by acetylcholinesterase of 1-naphthyl acetate to form 1-naphthol, which then reacts with Fast Blue salt B to give a purple coloured diazonium dye **(Figure. 6.3)**. Regions containing acetylcholinesterase inhibition appear as white spots under a purple background.

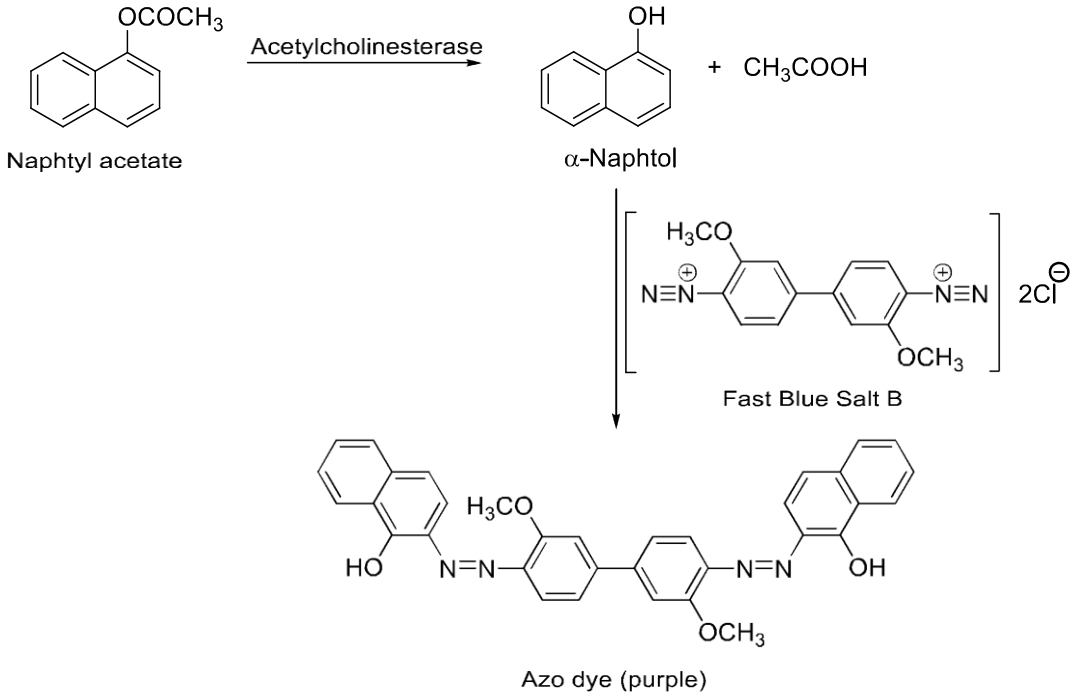


Figure 6.3. Reaction of AChE with naphthyl acetate and the substrate formation of the purple dye in the TLC assay.

6.3. Pathogen based bioassays

6.3.1. Antibacterial assays

The increasing incidence of drug-resistant pathogens has drawn the attention of the pharmaceutical and scientific communities towards studies on the potential antibacterial activity of plant-derived substances.[14] The Bacteria pathogen *Staphylococcus aureus* has become a major threat to public health. It is one of the most widespread and virulent pathogens in the world. The development of resistance to multiple antibiotics, impairs our ability to treat an increasing proportion of bacterial infections.[15] Although deaths from bacterial infections have decreased in the developed countries, it is still a major cause of death in developing countries. Infectious diseases account for approximately one-half of all deaths in tropical countries.[16] In the year 2009 the World Health Organization estimated that about 1.9 million children died worldwide of respiratory infections, with 70% of these deaths occurring in Africa and Asia. They also estimated that each year about 1.4 million children die from gut infection and diarrhoea resulting from these infections. Therefore the search of new strategies to control bacterial infections is highly desirable. Plant secondary metabolites have already demonstrated their potential as antibacterial agents.

Three representative bacterial strains were used for antibacterial testing: *Enterococus faecalis* (ATCC 29212), *Klebsiella pneumoniae* (ATCC 13883) and *Cryptococcus neoformans* (ATCC 90112).

6.3.2. Antimycobacterial tests

Tuberculosis (TB) once occurred as an epidemic disease among animals long before it affected humans. *Mycobacterium tuberculosis*, the cause of TB in humans, was first described in 1882 by Robert Koch.[17] It is a member of the tuberculosis complex, a group of closely related mycobacterium pathogens which includes *M. bovis* (which infects cattle and may also infect humans), *M. microti*, *M. africanum* (which causes TB in West Africa), *M. avium*, *M. intracellulare*, *M. leprae* (causes leprosy in man), *M. lepraemurium* (infection in rats and cats), and *M. scrofulaceum* (causing opportunistic infection diseases in patients with AIDS).[18]

Today, TB remains a serious health problem in many regions of the world, especially in developing countries. It is a contagious disease and is becoming epidemic in some parts of the world. The emergence of multidrug-resistant tuberculosis (MDR) and extensively drug resistant tuberculosis (XDR) strains, has complicated the treatment of TB, leading to increased interest in the possible discovery of more effective remedies from natural products.[19]

In this study plant extracts were tested against *M. smegmatis*, a gram positive bacteria that is generally considered as a fast growing non-pathogen microorganism that can be cultured in a laboratory. However, in some cases it can cause disease, mostly in animals.[20] *M. smegmatis* possesses a limited degree of similarities to *M. tuberculosis* with regard to drug susceptibility.[21]

6.4. Materials and Methods

General

The quantitative analysis for radical scavenging activity of extracts was done with a UV microplate reader (Molecular Devices, SpectraMax M2, USA).

Abbreviations:

Acetone	Acet
Butanol	BuOH
Dichloromethane	DCM
Ethyl acetate	EtOAc
Heptane	hept
Methanol	MeOH

Methanol-d_4	MeOD
Chloroform-d_1	$CDCl_3$

6.4.1. Radical scavenging assays

6.4.1.1. Qualitative TLC assay

A TLC plate was spotted with the respective test samples, developed (EtOAc:MeOH:H_2O; 10:1.4:10) and left to dry. After spraying with a methanolic DPPH solution (3 mg/mL), the active zone was observed as yellow spots on the violet background.

6.4.1.2. Quantitative assay

The quantitative radical scavenging abilities of the crude extracts from *C. apiculatum* were evaluated using a single cell (Becman Coulter Du 800, Fullerton, CA, USA) spectrophotometer. For each extract, respectively, the extract was dissolved in DMSO (10 mg/mL) and a dilution series were made up (5.0, 2.5, 1.25, 0.626 and 0.313 mg/mL). The absorbance of a 0.022% DPPH (Sigma-Aldrich) methanolic solution was measured. The respective test samples in the dilution series (50 µL) were added to the DPPH solution in the spectrophotometer. The mixture was stirred for some seconds and the decrease in absorbance at 517 nm measured over a period of 5 min. The percentage of radical scavenging was calculated. All measurements were carried out in triplicate. Quercetin (Sigma-Aldrich) was used as the positive control.

6.4.2. Acetylcholinesterase

A solution of each sample from each crude extract and isolated compounds (10 mg/mL) in MeOH was prepared and 10 µL of these test solutions was loaded on a TLC plate (10 cm x 10 cm) and migrated (*G. africana*, DCM and MeOH extracts; heptane:EtOAc; 1:1. *C. apiculatum*, MeOH extract; $CHCl_3$:MeOH:H_2O; 50:10:1). The TLC plates were dried and sprayed evenly with an AChE solution (5 mL) (from solution of 440 U ACHE in 70 mL, pH 7.8 Tris) (Sigma C3389). The plates were left for 5 min. A solution of α-naphthyl acetate (6 mg) in ethanol (2.5 mL) was added to a solution of Fast Blue Salts B (25 mg) (Sigma D9805) in H_2O (10 mL). The mixture was sprayed onto the TLC plates and after 5 min. the active extracts appeared as white spots on the purple background. Huperzine (1mg/100mL in MeOH) was used as reference.

6.4.3. Antibacterial screening

Minimum inhibitory concentrations were determined using the INT micro-well method (NCCLS, 2003).[22] The plant extracts were diluted in either acetone or DMSO so that starting concentrations of 5 mg/mL or 32 mg/mL, respectively, were introduced into the first well of a microtiter plate. The starting concentrations were diluted two fold in each successive serial dilution. Where necessary, further dilutions were performed so that valid endpoint MIC values could be determined. Positive antibacterial controls, ciprofloxacin and amphotericin B, at starting stock concentrations of 0.01 mg/mL and 0.10 mg/mL, respectively, were included in each assay to confirm antibacterial susceptibility. Negative controls (solvents: Acet or DMSO) were included to evaluate the effect of solvent on the growth of test micro-organisms. Broth controls (media incubated without test organism) were included to confirm sterility and cultures were streaked out on solid media to confirm purity. The bacterial culture was grown overnight at 37 °C, diluted 1:100 and inoculated into all wells at approximate inoculums concentration of 1 × 106 colony forming units/mL. Incubation followed at 37 °C for 24 hr. After incubation, a *p*-iodonitrotetrazolium violet solution (0.4 mg/mL) was transferred into all inoculated wells (40 µl) and examined to determine a colour change in relation to the concentration of microbial growth after examination and comparison with the culture controls. Tests were performed at least in duplicate and in triplicate where results varied by more than one dilution factor.

6.4.4. Antimycobacterial screening

Broth micro-dilution MIC determination

Middlebrook 7H11 agar (Becton Dickinson, Sparks, MD, USA) supplemented with 10% OADC (oleic acid, albumin, dextrose, catalase) (Remel, Lenexa, KS, USA) plates were used for the routine maintenance and growth of *Mycobacterium smegmatis* (Pasteur Institute, France) from glycerol stocks stored at -70 °C. For experimentation purposes, a single colony of growth was then used to inoculate supplemented Middlebrook 7H9 broth (Becton Dickinson, Sparks, MD, USA) and incubated at 37 °C for 72 hours. Sterile 96-well microtitre plates with flat-bottomed wells were used for these experiments. Supplemented 7H9 broth (100 µL) was added to each well. Ciprofloxacin (100 µL, 1 mg/mL) (Sigma, St Louis, MO, USA) was used as a positive control for *M. smegmatis*. The MIC values of the positive control required to be within acceptable limits for experimental success and subsequent interpretation of results. Broth (100 µL) was added to all the wells of the first row which served as a negative culture-free control. Each extract (100 µL, 64 mg/mL) was dissolved in the same solvent used for the initial extraction, and solvent controls were added to the first well of respective rows. A dilution series was then prepared by transferring 100 µL aliquots

from the top to the bottom of the plate using a multi-channel pipette. The organism (100 µL), diluted twenty-fold in supplemented broth to an optical density of 0.125 at 550 nm to yield approximately 1x105 cfu/mL, was added to each well of the 96-well plate. Extract concentrations therefore ranged from 16mg/mL to 125µg/mL, unless insufficient extract was available. The plates were sealed in plastic bags and incubated for 48 hours at 37ºC. Each extract was tested in duplicate against each organism. INT (40 µL, 0.4 mg/mL) (Sigma, St Louis, MO, USA) was added to each well and the plates were left at room temperature for eight hours. The results were determined by visual inspection of the wells with the MIC being reported as the lowest concentration of extract containing no red.

6.5. Results and discussion

6.5.1. Radical scavenging assay

6.5.1.1. Qualitative tests

The results of the DPPH TLC radical scavenging activity of *G. africana* (DCM and MeOH crude extracts), and *C. apiculatum* seeds and leaves (MeOH crude extracts) (TLC on the left), and of the isolated compounds **A - E** are depicted in **Figure 6.4.**

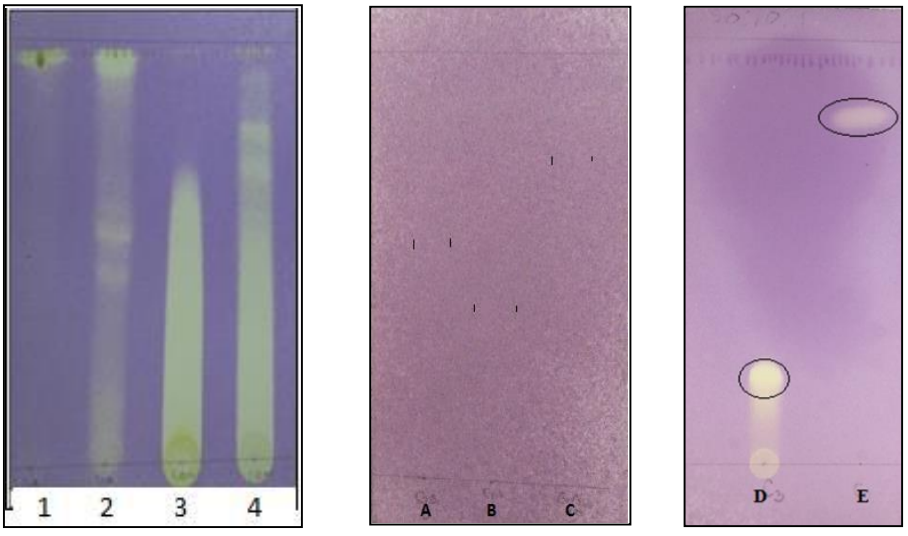


Figure 6.4. TLC of radical scavenging activity of *G. africana* aerial parts MeOH and DCM crude extracts (**1** and **2**, repectively), and *C. apiculatum* seeds and leaves MeOH crude extract (**3** and **4**, respectively), and radical scavenging activity of compounds **A**-**E**.

The above shown results indicate clearly that the MeOH crude extract (1) of *G. africana* has almost no antioxidant actitivy, and the DCM extract (2) shows relatively low activity. In contrast to this, the crude MeOH extracts of both the seeds (3) and leaves (4) from *C. apiculatum* show

significant activity. The compounds **A – C**, isolated from the DCM extract of *G. africana*, displayed no activity, as can be expected from the low activity of the crude extract because most antioxidants are polar secondary metabolites. However, compound **D**, isolated from the EtOAc partition fraction of the leave extract of *C. apiculatum*, showed significant activity, and **E** moderate activity.

The relationship between the structure and antioxidant activity of phenolic compounds especially flavonoids has been investigated for years. Flavonoids have been long regoconized to possess antioxidant properties. It has been indicated that the antioxidant activity of a compound is determined by the presence of free hydroxyl group and their mutual location.[23] It has been suggested that flavonols with a 3-OH substituent, are the strongest antioxidants among flavonoids. Regarding the reaction of quecetin with DPPH radical, it was reported that its' high radical scavenging activity is determined by the presence of a 1,2 dihydroxybenzene moiety in the ring B.[24]

6.5.1.2. Quantitative tests

The crude DCM extract from *G. africana* and the crude MeOH extracts from the seeds and leaves of *C. apiculatum* were subjected to the quantitative test. **Table 6.1** and **Figure 6.4** summarizes the results of the quantitative radical scavenging test. The quantative test confirmed the significant radical scavenging activity of the MeOH crude extract of both the seeds and leaves of *C. apiculatum*, and the weak activity of the DCM extract from *G. africana*. In **Figure 6.4** crude extract of seeds and leaves of *C. apiculatum* were tested against the DPPH radical in a spectrophotometric assay using quercetin as a reference compound.

Table 6.1. Radical scavenging activities with DPPH of the extracts from *G. africana* and *C. apiculatum*

Plant species	**Radical scavenging**
G. africana	+
C. apiculatum Seeds	+++
C. apiculatum Leaves	+++

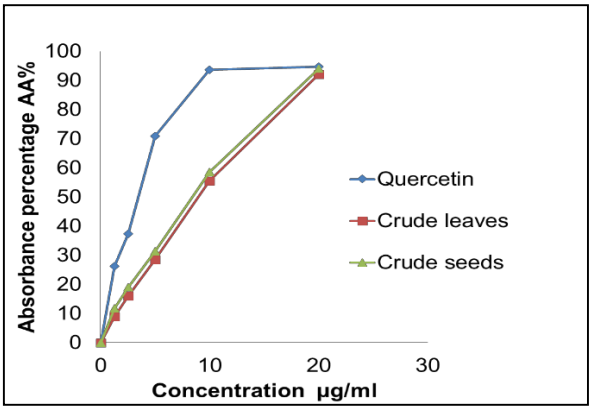


Figure 6.5. Radical scavenging from the seeds and leaves of *C. apiculatum* using quercetin as reference

It is clear from the graph that there is a linear correlation between %absorption and concentration for the MeOH extract of both the seeds and leaves of *C. apiculatum* with 90% absorbance occurring at a concentration of 20 µg/mL. Thus, the antioxidant activity of reference compound quercetin is better than those of our samples with maximum absorption at a concentration of 10 µg/mL.

6.5.2. TLC acetylcholinesterase inhibition bioassay

The crude DCM and MeOH extracts from *G. africana* and the MeOH extract from *C. apiculatum* were subjected to the TLC AChE inhibition bioassay. It was determined that only the DCM fraction of *G. africana* showed any positive result when compared to the reference, huperzine (**Figure 6.6**). Consequently only compounds **A** – **C** from *G. africana* were tested against acetylcholine inhibition and the TLC result showed that **C** strongly inhibits acetylcholinesterase, **A** has medium activity and **B** showed no inhibition **(Figure 6.7)**.

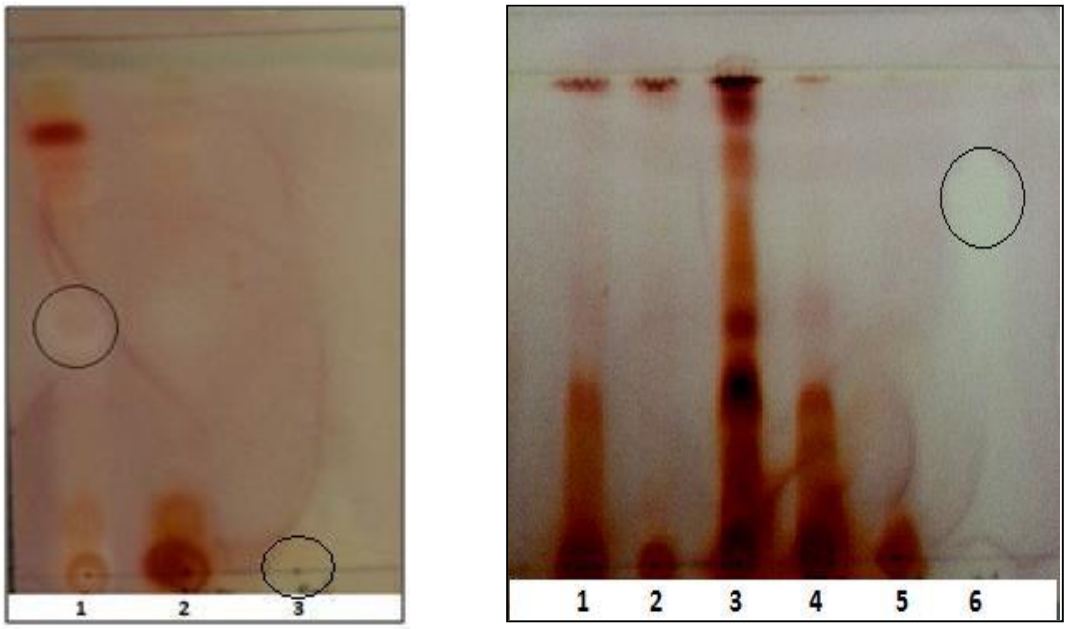


Figure 6.6. AChE inhibition of *G. africana* DCM and MeOH extracts (TLC on the left), and the MeOH leaf extract and partitioned fractions of *C. apiculatum* (TLC on the right), with MeOH crude **(1)**, heptane fraction **(2)**, EtOAc fraction **(3)**, BuOH fraction **(4)**, aqueous fraction **(5)** and reference hurpezine**(6)**.

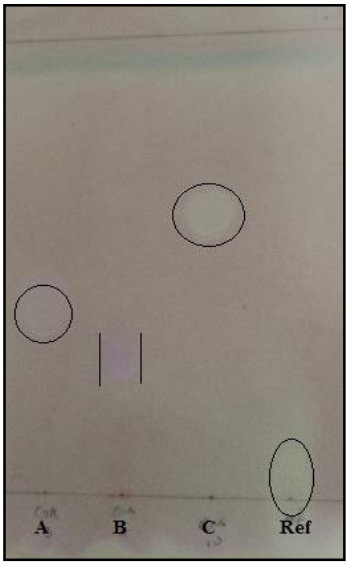


Figure 6.7. Results of the AChE inhibition test of the isolated compounds from the DCM fraction of *G. africana* when compared to the reference, huperzine

A review by Uriarte-Pueyo,[25] describing the acetylcholinesterase inhibitory activity of flavonoids, reported activity of 41 flavones, 21 flavanones, 35 flavonols, 25 isoflavones and 6 chalchones. Among these, flavones with the same general structure as **C**, included the following: **(Figure 6.8)**.

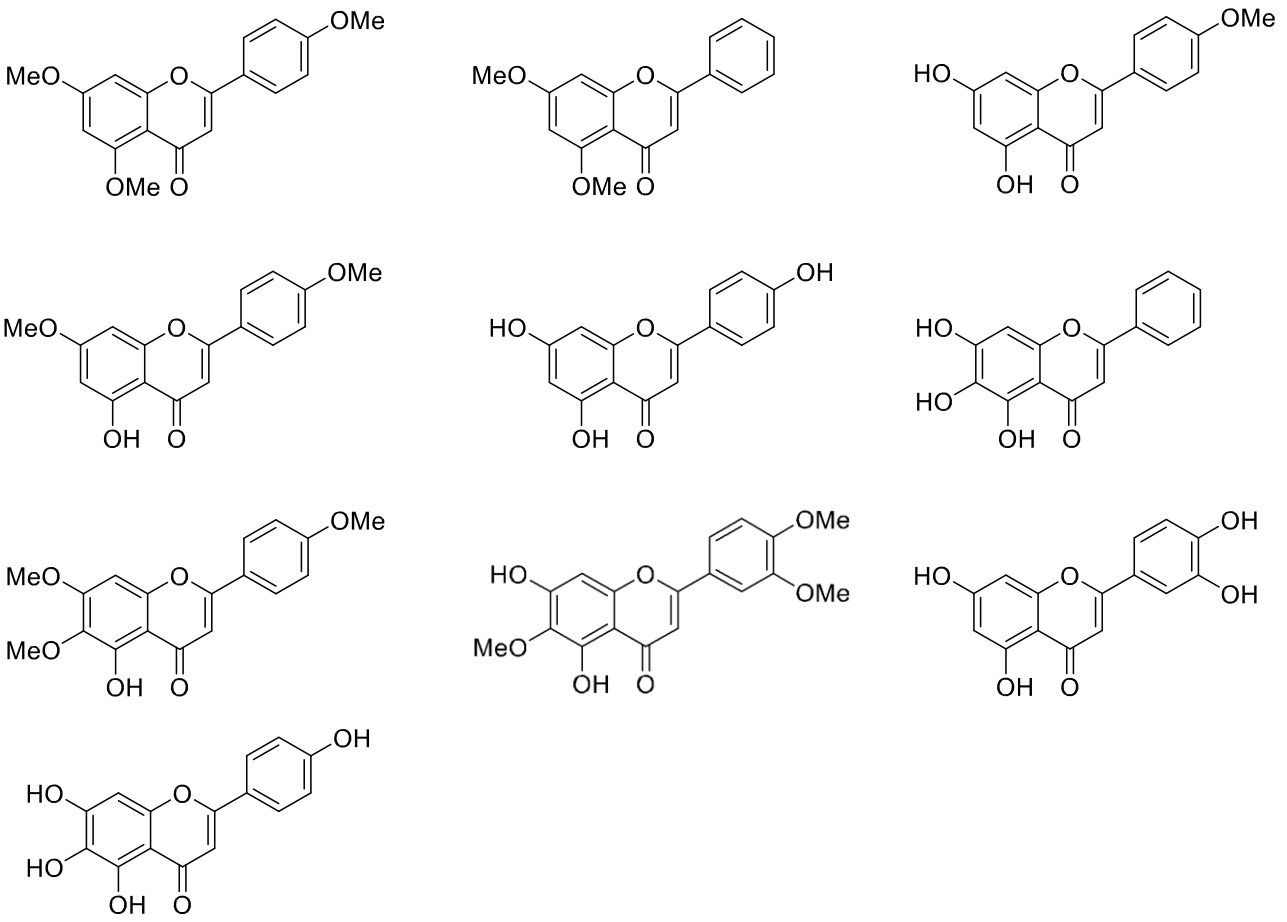


Figure 6.8. Flavones with the same general structure as **C** demonstrating AChE inhibition

No reference of the activity of compound **C** could be found. **Table 6.2** gives a summary of the results. Compounds **D** and **E** were not tested for acetylcholinesterase inhibition because the crude methanol extract showed no inhibition of acetylcholinesterase.

Table 6.2. AChE inhibition of crude DCM and MeOH extracts and isolated compounds from *G. africana*, and of the crude MeOH extracts of the seeds and leaves of *C. apiculatum*.

Plant species	Plant part	AChE inhibition
G. Africana (DCM extract)	Aerial	+++
G. Africana Compound **A**	Isolated	++
G. Africana Compound **B**	Isolated	-
G. Africana Compound **C**	Isolated	+++
C. apiculatum (MeOH extract)	Seeds	-
C. apiculatum (MeOH extract)	Leaves	-

6.5.3. Antibacterial and antimycobacterial activity

A summary of the antibacterial activity of the plant extracts against three reference pathogens, *E. faecalis* (ATCC 29212), *K. pneumonia* (ATCC 13883) and *C. neoformans* (ATCC 90112), and the antimycobacterial activity against reference pathogen *M. smegmatis*, are given in **Table 6.3.** The mean MIC values of the plant crude and partitioned extracts are indicated in mg/mL for the antibacterial pathogens, and in µg/mL in the case of the mycobacterium, *M. smegmatis*. After reviewing the literature, van Vuuren[26] proposed that if MIC values of plant extracts are below 8.0 mg/mL, they are to be considered as having some antibacterial activity, while MIC values below 1 mg/mL indicates noteworthy activity. Plant extracts with MIC values ranging from 250 µg/mL and below are considered active against *M. smegmatis*. The majority of the plant extracts demonstrated significant antibacterial activity.

The DCM extract of *G. africana* showed significant activity against all three antibacterial test pathogens of which the highest activity was observed against the *C. neoformans* strain with an MIC value of 0.09 mg/mL. The DCM extract of *G. africana* also showed good antimycobacterial activity against *M. smegmatis* with an MIC value of 250 µg/mL. The antimycobacterial properties of *G. africana* against *M. smegmatis* and *M. tuberculosis* have been previously reported by Mativandle[27], with MIC values of 0.781 µg/mL against *M. smegmatis*, and 1.2 mg/mL against *M. tuberculosis*, respectively. No antibacterial activity against *E. faecalis*, *K. pneumonia* and *C. neoformans* has previously been reported for *G. africana*. The MeOH extract from *G. africana* showed no noteworthy activity.

For *C. apiculatum* the crude MeOH seed extract displayed significant activity against all pathogens, except *C. neoformans*, which could not be tested due to lack of enough sample. Interestingly, the activity of the partitioned fractions from the MeOH extract of the seeds showed better activities than the crude extract against *K. pneumonia*. Furthermore, the partitioned fractions from the MeOH extract of the seeds showed very good activity against *C. neoformans* with MIC values between 0.02 to 0.48 mg/mL. In contrast to this finding, the crude MeOH leave extract displayed very little activity (the only noteworthy activity was against *C. neoformans* with an MIC value of 1 mg/mL), but almost all the partition fractions had noteworthy activity against all pathogens. The best activity against *E. faecalis* and *K. pneumonia* microbials was from the MeOH crude extract of the seeds of *C. apiculatum* (MIC = 0.13 mg/mL against *E. faecalis* and 0.25 mg/mL against *K. pneumonia*), and against C. *neoformans* the BuOH partition fraction from the seed extract (MIC = 0.03 mg/mL). The crude extracts and partitioned fractions of both the seeds and the leaves from *C. apiculatum* displayed notable antimycobacterial activity against *M. smegmatis*. The highest activities were from the H_2O fraction from the seeds (MIC = 39 µg/mL) and the Hept and EtOAc fractions from the leaves (MIC = 20 and 39 µg/mL, respectively). No studies on the mycobacterial properties of *C. apiculatum* have been reported. Antibacterial and antimycobacterial testing of the isolated products A - E will be conducted in future work due to time constraints.

Table 6.3.MIC values of the crude plant extracts, partitioned fractions and isolated compounds against *E. faecalis*, *K. pneumonia*, *C. neoformans* (expressed in mg/mL) and *M. smegmatis*(expressed in µg/mL).

Plant name	**Parts**	**Extract**	**Antibacterials (mg/mL)**			**Antimycobacterial (µg/mL)**
			E. faecalis ATCC 29212	*K. pneumonia* ATCC 13883	*C. neoformans* ATCC 90112	*M. smegmatis* ATCC 14468
C. apiculatum	Seeds	MeOH	0.13	0.25	*	100
C. apiculatum	Leaves	MeOH	2.00	4.00	1.00	250
G. africana	Aerial Parts	DCM	0.50	1.00	0.09	250
		MeOH	4.00	>8.00	4.00	500
C. apiculatum	Seeds	Part. fractions				
		Hept Fr.	1.25	0.63	0.48	59
		EtOAc Fr.	0.94	0.32	0.03	135
		BuOH Fr.	>1.25	0.48	0.02	67
		H_2O Fr.	>1.25	1.25	0.08	39
C. apiculatum	leaves	Part. fractions				
		Hept Fr.	>1.25	>1.25	0.08	20
		EtOAc Fr.	0.63	0.63	0.32	39
		BuOH Fr.	>1.25	0.32	0.04	94
		H_2O Fr.	0.63	0.63	0.32	*
Positive control (Ciprofloxacin/Amphotericine B)			4.00	4.00	4.00	0.31
Solvent control (Acetone)			>8.00	>8.00	>8.00	>8000
Solvent control (DMSO)			>8.00	>8.00	>8.00	>8000

*Not enough sample

6.6. Conclusion

G. africana and *C. apiculatum* have been used traditionally to treat infectious diseases, and our phytopharmacological study on the two plants has confirmed these practices. During our investigation we determined that:

- *G. africana* exhibits relatively low radical scavenging activity for the DCM extract and no activity for the MeOH extract, while both the seed and leave MeOH extracts from *C. apiculatum* displayed significant antioxidant activity.
- The DCM extract of *G. africana* and isolated compounds **A** and **C** showed the only AChE inhibition.
- The DCM extract of *G. africana* showed significant activity against all four pathogens with the best activity observed against *C. neoformans*.
- The highest activity against *E. faecalis* and *K. pneumonia* was from the MeOH crude extract of the seeds of *C. apiculatum*, and against *C. neoformans* the BuOH partition fraction from the seed extract.
- The seed and leave extracts of *G. africana* and the partition fractions thereof all exhibited significant activity against *M. smegmatis*.

These positive results is an essential step towards the development of efficacious commercial phytomedicines.

6.7. References

1. Rang H.P., Dale M.M., Flower R.J. and Henderson G. Rand and Dale's Pharmacology. 7th edition.
2. McLaughlin J.L. and Rogers L.L. **1998**. *Drug. Inf. J.* 32: 513-528.
3. Rahman A-U., Chouldhary M. I. and Thomson W. J. **2005**. *Bioassay techniques of drug development*. Harwood academic publishers.
4. Kumaran A. and Karunakaran R.J. **2006**. Antioxidants and free radical scavenging activity of an aqueous extract of Coleus aromaticus. Food Chem. 97: 109-114.
5. Nindita S. and Rajini P.S. **2004**. Free radical scavenging activity of an aqueous extract of potato peel. *Food Chem.*85: 611-616.
6. Johnson I.T. 2001. Antioxidants and antitumor properties. In Antioxidants in food. By Yanishliera N.N., Gordon M. and Pokorny J. 100-123. Cambridge: Woodhead publishing Ltd.
7. Mankil J. and Moonsoon P. **2007**. Acetylcholinesterase inhibition by flavonoids from Agrimonia pilosa. *Molecules*. 12: 2130-2139.
8. Kumar S., Seal J.C. and Okello E.J. **2011**. Kinetics of Acetylcholinesterase inhibition by an aqueous extract of Withania Somnifera roots. *IJPSR*. 2: 1188-1192.
9. Rajaseree P.H., Singh R. and Sankar C. **2012**. Screening for acetylcholinesterase inhibitory activity of methanolic extract of Cassia fistula roots. *Inter. of Pharm. life scie*.3:1976-1978.
10. Mukherjee P.K., Kumar V., Mal M. and Houghton P.J. **2007**. Acetylcholinesterase inhibitors from plants. *Phytomedicine*. 14: 289-300.
11. Rahman A.U. and Choudhary M.I. **2001**. Bioactive natural products as a potential source of new pharmacophores. A theory of memory. *Pure Appl. Chem*. 73: 555-560.
12. Oh M.H., Houghton P.J., Whang W.K. and Cho J.H. **2004**. Screening of Korean herbal medicines used to improve cognitive function for ant-chlolinesterase activity. *Phytomedicine*. 11: 544-548.
13. Marston A., Kissling J. and Hostettmann K. **2002**. A rapid TLC bioautographic methods for the detection of acetylcholinesterase and butyrylcholinesterase inhibitors in plants. *Phytochem. Anal*.13: 51-54.
14. Savoia D. **2012**. Plant-derived antibacterial compounds: alternative to antibiotics. *Future Microbiology*. 7: 979-990.
15. Osamana K., Evangelopoulos D., Basavannacharya C., Gupta A., McHugh T.D., Bhakta S. and Gibbons S. **2012**. An antibacterial from *Hypericum acmosepoilum* inhibits ATP-dependent *MurE lifase* from *Mycobacterium tuberculosis.* 39: 124-129.

16. Iwu M.M., Duncan A.R. and Okunji C.O. 1999. New antimycobactrials of plant origin. In Perspectives on new crops and uses. By Janick J., 457-462. Alexandria: ASHS press.
17. Talay G.M., Stewart G.R., Cooke M., Chaplin S., Ladva S., Kirkup J., Palmer S. and Young D.B. **2003**. Koch's bacillus- a look at the first isolate of mycobacterium tuberculosis from a morden perspective. *Microbiol.* 149: 3213-3220.
18. Horne N. **1996**. Tuberculosis and other Mycobacterial diseases. InManson's *Tropical diseases*. By Cook G.C.
19. Sigh R., Hussain S., Verman R. and Sharma P. **2013**. Anti-mycobacterial screening of five Indian medicinal plants and partial purification of active extracts of Cassia sophera and Urtica diocica. *Asian Pac J. Tro Med*. 6: 366-37.
20. Niederweis M., Ehrt S.S., Heinz C., Klöcker U., Swiderk K.M., Riley L.W. and Benz R. **1999**.Cloning of the mspA gene enconding the porin from Mycobacterium smegmatis. *Molecular Microbiol*. 33: 933-954.
21. Gautam R., Saklani A. and Jachak S.A. **2007**. Indian medicinal plants as source of antimycobacterialagents. *J. Ethnopharmacol*. 110: 200-234.
22. NCCLS.**2002**. Performance standards for antibacterial susceptibility testing twelf information supplement. NCCLS document M100-512.
23. Rice-Evens C.A., Miller J. and Paganga G. **1997**. Antioxidant properties of phenolic compounds. *Tends Plant Sci*. 2: 152-159.
24. Bruda S. and Oleszek W. **2001**. Antioxidant and anti-radical activities of flavonoids. *J. Agric .Food Chem.*49: 2774-2779.
25. Uriarte-Pueyo I and Calva M.I. **2011**. Flavonoids as Actylcholinesteras inhibition. *Curr. Med. Chem*. 18: 5289-5802.
26. Van Vuuren S.F. **2008**. Antibacterial activity of South African medicinal plants. *J Ethnopharmacol.* 119: 462-472.
27. Mativandlela S.P.N., Muthivhi T., Kikuchi H., Oshima Y., Hamilton C. and Hussein A.A. **2009**. Antimycobacterial flavonoids from the leaf extract of *Galenia africana*. *J. Nat. Prod.* 72: 2169-2171.

Appendix A

EXPLORATION OF MEDICINAL PLANTS USED FOR TB MANAGEMENT IN THE FREE STATE

Researchers: Khanya Phungula, Malefu Khamane and Professor Andrew Marston

University of the Free State, Bloemfontein, South Africa

Contact details: +27 071 156 5485/ +27 083 952 5020/ +27 076 046 0577

phungulakhanya@gmail.com,

malefu.khamane@gmail.com

marstona@ufs.ac.za

Participation information sheet

Purpose

You are kindly asked to participate in a survey on medicinal plants used in the Free Statefor the management of TB in order to find more effective cures for the disease. This is necessary because South Africa has the third highest incidence of TB in the world and the Free State has the third highest burden in South Africa. You were selected to participate in this survey because of your extensive knowledge of medicinal plants. The purpose of the survey is to collect samples of medicinal plants you use to treat clients and to document information on how you manage TB. The samples will be sent for identification and classification.

Risks, injuries and compensation

It is expected that you will do your collections in the veld as you usually do when collecting for your clients. We will not compensate for injuries sustained during the survey, but if necessary we can provide for transport to the nearest public health facility.

Benefits

When the documentation is done, all those who participated will be acknowledged and provided with the compiled report within 6 months. It is expected that from the information provided, a study of two of the medicinal plants will be madein the laboratory. We hope to have a long and continuous partnership in trying to improve your medicines scientifically for future benefits to your practices.

Confidentiality

All information provided will be kept confidential. However, the results will be published in ethnobotany journals.

Participation

Participation in this survey is **voluntary.** If you decide to participate, you can withdraw your consent at any time without penalty.

Future plans

More laboratory studies to be done on the medicinal plants used for TB management.

Consent Form

WHAT YOU SHOULD KNOW ABOUT THIS RESEARCH:

- We give you this consent form so that you read about the purpose, risks, and benefits of this survey.
- The main goal of this research is to document your knowledge on and collect samples of medicinal plants that you use on your patients.
- The collected samples will be sent for identification and classification.
- Herbarium samples will be made.
- We cannot promise that this research will benefit you as an individual.
- Your participation in this research is ***voluntary***.
- Whatever your decision, it will not affect your current or future relationships with us.
- You are sincerely required to read this consent form *carefully*, to ask any questions *before* making your decision to participate or not to participate in this survey.

Looking forward to working with you!

AUTHORIZATION

I, the undersigned, agree to participate in this survey. I have read and understood the information provided above.

(You are allowed to choose someone to sign this form for you, if necessary)

Full names of resource person (please print):

Date: Place:

--- ---

Signature of participant or authorized representative:

Signature of project leader obtaining consent:

Interview record sheet (Questionaire)

EXPLORATION OF MEDICINAL PLANTS IN THE FREE STATE FOR ANTI-TUBERCULOSIS ACTIVITY

District: .....

Date of Interview: .....

Personal Information of Participant

Full Name: ……

Village: ……

Category of participant:

(i)INYANGA ☐

(ii) ISANGOMA ☐

Number of years of practising: …… years

Instructions

- *Kindly answer all questions*
- *If you need more space, please use the reverse of the pages and appropriately number your answer*

TUBERCULOSIS (TB)

1. What is the local name of TB?

2. What causes TB?

3. How do you know that a patient is suffering from TB? Give as many symptoms as possible, and indicate the most significant.

4. What are the names of the plants you use for TB?

5. Which plant part is used?

6. Where is the plant obtained?

7. What is the form of the plant used (dried, powdered, syrup, fresh plant)?

8. Is the plant is taken alone or as a mixture (with what?)

9. How do you administer the remedy?

10. How long does the treatment last?

11. What symptoms are treated by the plant?

12. What precautions do you take before administering tuberculosis remedies to patients?

13. How do you know when the remedy has failed?

14. What do you do when the remedy fails?

15. Do you have plants that prevent people from TBinfection? How they are used?

16. Any further comments?

THANK YOU!

Appendix B: Results of investigated plant species for hERG channel blockers

Plant species	Family	Parts	Collection NO.	DCM extract		MeOH extracts	
				Yield (mg)	hERG inhibition (Mean %)	Yield(mg)	hERG inhibition (Mean %)
Acacia caffra	Fabaceae	D	No nr	8.8	no inhibition	12.5	18.79
Acacia caffra	Fabaceae	S	No nr	17.4	no inhibition	14.2	no inhibition
Acacia caffra	Fabaceae	L	No nr	10.5	insoluble in DMSO	7.4	insoluble in DMSO
Acacia caffra	Fabaceae	YB	No nr	10	no inhibition	6.6	no inhibition
Acacia caffra	Fabaceae	OB	5643	7.8	no inhibition	6.2	no inhibition
Acacia erioloba	Fabaceae	F	5644	10.9	31,007 ±5,442	9.9	no inhibition
Acacia erioloba	Fabaceae	L	5644	8.7	4.434	7.5	2.729
Acacia erioloba	Fabaceae	OB	No nr	37.1	8.962	11.2	19,466 ± 9,22
Acacia erioloba E. Mey	Fabaceae	SD	X1	17.10	no inhibition	7	no inhibition
Acacia erubescens	Fabaceae	OB	5647	15.7	5.471	4	2.879
Acacia erubescens	Fabaceae	B	5647	19.3	no inhibition	41.2	insoluble in DMSO
Acacia erubescens	Fabaceae	L	5647	6.5	no inhibition	21.8	4.86
Acacia erubescens	Fabaceae	YB	5647	16.2	no inhibition	4.2	no inhibition
Acacia erubescens	Fabaceae	D	5647	10.4	1.502	4.9	3.354

Acacia erubescens	Fabaceae	S	No nr	18.2	1.664	15.7	no inhibition
Acacia grandicornuta	Fabaceae	L	5642	6.3	no inhibition	6.3	no inhibition
Acacia grandicornuta	Fabaceae	D	5642	7.4	no inhibition	2.9	no inhibition
Acacia grandicornuta	Fabaceae	YB	5625	12.2	no inhibition	4.3	no inhibition
Acacia grandicornuta	Fabaceae	OB	5642	3.9	no inhibition	2.1	no inhibition
Acacia haematoxylon	Fabaceae	YB	5551	16.8	3.802	2	10.053
Acacia haematoxylon	Fabaceae	L	5551	3.3	6.262	4.6	5.705
Acacia haematoxylon	Fabaceae	F	No nr	24.4	insoluble in DMSO	35.7	5.396
Acacia haematoxylon Willd.	Fabaceae	S	5099	19.50	no inhibition	32.1	no inhibition
Acacia karroo	Fabaceae	G	5467A	13.20	no inhibition	4.5	no inhibition
Acacia karroo	Fabaceae	YB	No nr	7.1	9.104	2.7	no inhibition
Acacia mellifera ssp *detinens*	Fabaceae	L	5643	24.3	15.297	33.7	insoluble in DMSO
Acacia mellifera ssp *detinens*	Fabaceae	F	5643	9.7	6.13	50.5	insoluble in DMSO
Acacia mellifera ssp *detinens*	Fabaceae	B	5643	6	no inhibition	5.1	16.699
Acacia mellifera ssp *detinens*	Fabaceae	D	5031	10.5	2.743	28.2	no inhibition
Acacia mellifera ssp *detinens*	Fabaceae	OB	5643	13.3	2.68	3.6	no inhibition
Acacia mellifera ssp *detinens*	Fabaceae	YB	5643	9.3	no inhibition	31.8	11.188
Acacia nigrescens	Fabaceae	OB	5640	14	3.67	1	0.843

Acacia sieberana	Fabaceae	OB	5627	15.2	insoluble in DMSO	5.3	no inhibition
Acacia tortilis	Fabaceae	YB	5635	15.3	insoluble in DMSO	6.4	10.518
Acacia xanthophloea	Fabaceae	OB	5625	3.4	31,665 ±12,528	9.6	no inhibition
Acacia xanthophloea	Fabaceae	LD	5625	4.3	no inhibition	12.8	19,042 ±20,181
Acacia xanthophloea	Fabaceae	YB	5644	8.5	3.536	4.2	no inhibition
Acanthosicyosnaudinianus (Sond.) C. Jeffrey	Cucurtitaceae	O	4417	54.30	no inhibition	8.9	no inhibition
Afzeliaquanzensis Welw.	Fabaceae(Caesalpinioidaee	O	4742	29.40	no inhibition	4.4	no inhibition
Agapanthus africanus	Agapanthaceae	R	No nr	6.7	1.764	10	oocytes die
Agapanthus africanus	Agapanthaceae	F	4688D	19.5	no inhibition	15.9	no inhibition
Albizia harveyi E. Fourn.	Fabaceae	P	4717B	5.90	no inhibition	5.7	no inhibition
Albiziaharveyi E. Fourn.	Fabaceae	O	4717A	14.20	no inhibition	17	oocytes die
Ammocharis coranica(Ker Gawl.) Herb.	Amarylidaceae	R	4411	24.60	no inhibition	9	7,056 ±6,55
Bauhinia galpini N.E.Br	Fabaceae	P	5097	8.80	no inhibition	6.4	no inhibition
Berchemia discolour Klotzsch ex Mart	Rhamnaceae	O	4695	25.10	no inhibition	69.7	no inhibition
Boophane disticha	Amarylidaceae	R	4415	22.90	16,085 ±22,471	14.4	25,074 ± 15,333
Boscia albitrunca	Capparaceae	OB	5646	6.00	4.017	3.6	2.838
Boscia albitrunca	Capparaceae	F	5646	12.4	no inhibition	13.7	no inhibition
Boscia albitrunca	Capparaceae	L	5646	6.2	6.983	7.2	7.95

Boscia albitrunca	Capparaceae	B	5646	6.8	3.325	4.7	9
Boscia albitrunca	Capparaceae	YB	5032	6.5	7.474	7.4	no inhibition
Boscia cf.*foetida*	Capparaceae	YB	5628	50.9	2.108	6.6	no inhibition
Boscia cf.*foetida*	Capparaceae	L	5628	16.7	7.655	7.2	no inhibition
Boscia cf.*foetida*	Capparaceae	F	4501	14.6	insoluble	22.1	no inhibition
Boscia foetida Schinz subsp. *Foetida*	Capparaceae	O	5101	22.90	no inhibition	58.5	no inhibition
Brachylaena discolour	Asteraceae	F	No nr	5.9	insoluble in DMSO	4.1	20.877
Brachylaena discolour	Asteraceae	YB	No nr	11.3	2.774	25.3	3.434
Brachylaena discolour	Asteraceae	L	4693	16.7	4.897	21.7	4.301
Buddleja saligna	Buddlejaceae	F	No nr	44.7	insoluble in DMSO	33.8	no inhibition
Buddleja saligna	Buddlejaceae	OB	No nr	15.4	insoluble in DMSO	4.5	9.354
Buddleja saligna	Buddlejaceae	YB	No nr	13.5	no inhibition	24.8	no inhibition
Buddleja saligna	Buddlejaceae	L	5647	5.5	0.632	26.8	no inhibition
Burkea africana	Leguminosae(Mimosoidaceae)	YB	No nr	27.9	19.157	24.7	2.77
Burkea africana	Leguminosae(Mimosoidaceae)	L	5092	18.3	6.023	17.1	no inhibition
Carissa bispinosa	Apocynaceae	YB	5634	13.4	20.476	36.2	1.815
Carissa bispinosa	Apocynaceae	L	5634	18.6	no inhibition	20.9	no inhibition

Carissa bispinosa	Apocynaceae	F	No nr	41.6	no inhibition	41.4	1.97
Celtis africana	Celtidaceae	YB	No nr	5.2	17.006	3.2	1.182
Celtis africana	Celtidaceae	F	No nr	7.9	no inhibition	9.3	no inhibition
Celtis africana	Celtidaceae	L	No nr	7.1	no inhibition	4.5	no inhibition
Celtis africana	Celtidaceae	O	5631	3.6	4.149	6.6	4.566
cf. *Litogyne gariepina*	Asteraceae	YB	5626	11.1	3.232	7.7	7.827
cf. *Litogyne gariepina*	Asteraceae	L	5626	17.8	insoluble in DMSO	41.6	11.638
cf. *Litogyne gariepina*	Asteraceae	F	No nr	29.1	no inhibition	17	no inhibition
Chrysocoma ciliata	Asteraceae	B	5467B	66.90	no inhibition	80.7	no inhibition
Chrysocoma ciliata	Asteraceae	L	5467C	20.50	no inhibition	9.8	12,114 ± 2,294
Chrysocoma ciliata	Asteraceae	F	5470A	97.60	13,756 ± 0,552	50.9	no inhibition
Chrysocoma ciliata	Asteraceae	L	5464B	22.40	Insoluble	5.9	no inhibition
Citrillus lanatus (Thunb.) Matsum. & Nakai	Cucurbitaceae	O	X3	60.70	no inhibition	3.5	no inhibition
Colophospermum mopane	Fabaceae	OB	5241	12.4	no inhibition	3.5	no inhibition
Colophospermum mopane	Fabaceae	L	5241	6.7	14.318	20.1	8.579
Colophospermum mopane	Fabaceae	G	4713	12.1	insoluble	40.7	9.125
Colophospermum mopane	Fabaceae	F	No nr	24	18.752	59.4	2.75
Colophospermum mopane (J.Kirk ex Benth.) J.Kirk ex J. Léonard	Fabaceae	F	4688B	19.00	22.398 ±1.531	25.5	1.251±1.407

Colophospermum mopane (J.Kirk ex Benth.) J.Kirk ex J. Léonard	Fabaceae	O	4688A	41.30	6,342 ±0,222	20.2	5,643 ±0,004
Colophospermum mopane, (J.Kirk ex Benth.) J.Kirk ex J. Léonard,	Fabaceae	S	4688C	39.20	1,833 ± 1,81	26.2	no inhibition
Colophospermum mopane, (J.Kirk ex Benth.) J.Kirk ex J. Léonard,	Fabaceae	P	5469A	50.10	no inhibition	15.5	no inhibition
Combretum apiculatum	Combretaceae	S	5240B	21.80	20,225 ± 12,227	12.6	no inhibition
Combretum apiculatum	Combretaceae	G	5240C	12.60	no inhibition	3.4	no inhibition
Combretum apiculatum	Combretaceae	F	5240D	38.00	no inhibition	42	no inhibition
Combretum apiculatum Sond. subsp. *apiculatum*	Combretaceae	P	4718A	11.60	11,137 ±6,224	52.8	no inhibition
Combretum apiculatum Sond. subsp. *Apiculatum*	Combretaceae	O	4718B	40.70	no inhibition	18.3	no inhibition
Combretum hereroense Schinz	Combretaceae	SP	4745	41.60	no inhibition	13.6	6,829 ±6,163
Combretum hereroensis	Combretaceae	B	5649	17.5	no inhibition	33.5	7.725
Combretum hereroensis	Combretaceae	B	5649	18.1	7.145	25.2	4.452
Combretum hereroensis	Combretaceae	F	5649	25.9	insoluble in DMSO	8.4	20.309
Combretum hereroensis	Combretaceae	YB	5649	14.6	no inhibition	6.4	0.498
Combretum hereroensis	Combretaceae	OB	5649	13.4	insoluble in DMSO	17.8	no inhibition
Combretum hereroensis	Combretaceae	L	4832	11.1	7.97	8.4	2.249
Combretum imberbe	Combretaceae	F	5631	48.9	no inhibition	8.8	no inhibition

Combretum imberbe	Combretaceae	L	5631	17.7	insoluble in DMSO	6	no inhibition
Combretum imberbe	Combretaceae	YB	5631	8.3	3.191	3.9	no inhibition
Combretum imberbe	Combretaceae	B	5631	6	no inhibition	7.5	0.527
Combretum imberbe	Combretaceae	OB	5094	11	no inhibition	15.3	no inhibition
Combretum zeyheri Sond.	Combretaceae	S	4347A	19.60	no inhibition	11.1	no inhibition
Combretum zeyheri Sond.	Combretaceae	P	4347B	4.60	6,385 ±0,266	15.3	8,103 ±8,688
Combretumapiculatum (roots)	Combretaceae	R	5240A	9.90	no inhibition	10.7	no inhibition
Commiphora mollis (Oliv.) Engl.	Burseraceae	O	4705	9.50	no inhibition	5.6	no inhibition
Commiphora pyracanthoides Engl.	Burseraceae	O	4704	51.40	11,511 ±10,093	11.2	no inhibition
Cotyledon orbiculata	Crassulaceae	B	No nr	14.6	no inhibition	39.7	no inhibition
Cotyledon orbiculata	Crassulaceae	L	No nr	21.6	no inhibition	26	no inhibition
Cotyledon orbiculata	Crassulaceae	F	5510	20.2	no inhibition	25.9	no inhibition
Craibia zimmermannii (Harms) Dunn	Fabaceae	SP	4759	19.80	no inhibition	14.7	no inhibition
Crinum buphanoides Welw. Ex Baker	Amaryllidaceae	R	4530	17.50	no inhibition	13.3	no inhibition
Crinum minimum Milne-Redh.	Amaryllidaceae	R	5234	11.90	no inhibition	29.6	no inhibition
Cucumis africanus L.f	Cucurbitaceae	S	X2	113.30	no inhibition	1.4	no inhibition
Cucumis metuliferis E.Mey ex Naudin	Cucurbitaceae	S	4987	50.50	no inhibition	15.5	no inhibition
Cussonia spicata	Araliaceae	F	No nr	21.4	no inhibition	23.6	no inhibition

Deverra burchelli	Umbrelliferae	L	No nr	16.7	insoluble in DMSO	10.7	no inhibition
Dicerocarynum senecioides	Pedaliaceae	F	5510	33	14.419	23.5	no inhibition
Dicerocarynum senecioides	Pedaliaceae	L	5510	12.3	no inhibition	8.6	0.664
Dicerocarynum senecioides	Pedaliaceae	S	5510	42.9	insoluble in DMSO	7.2	no inhibition
Dicerocarynum senecioides	Pedaliaceae	P	5649	17.2	insoluble in DMSO	8.7	1.971
Dicerocaryum senecioides (Klotzsch) Abels	Pedaliaceae	C	4716B	11.80	no inhibition	7.7	no inhibition
Dicerocaryum senecioides (Klotzsch) Abels	Pedaliaceae	S	4716A	52.80	no inhibition	3.5	no inhibition
Dichrostachys cinerea (L.) Wight & Arn.subsp. africana	Fabaceae	O	4715	12.40	2,023 ±0,652	6.4	no inhibition
Diospyros lycioides	Ebenaceae	O	5469B	20.90	no inhibition	3.4	9,563 ± 6,572
Diospyros lycioides	Ebenaceae	G	5463C	17.60	no inhibition	3.2	no inhibition
Diospyros lycioides	Ebenaceae	F	5467D	20.00	insoluble	14.3	no inhibition
Diospyros lycioides (Desf,) subsp. *Lycioides*	Ebenaceae	O	4712	20.10	no inhibition	1.1	no inhibition
Diospyros mespiliformes	Ebenaceae	S	4520	16.50	no inhibition	9.1	no inhibition
Diospyrosaustro-africana De Winter var. *micorphylla*	Ebenaceae	S	4724	6.70	no inhibition	21.8	no inhibition
Dodonaea viscosa	Sapindaceae	O	No nr	44	7.859	100	3.452
Dodonaea viscosa	Sapindaceae	YB	No nr	22.9	6.86	2.6	9.449

Dodonaea viscosa	Sapindaceae	F	No nr	36.1	no inhibition	24.4	14.529
Dodonaea viscosa	Sapindaceae	L	5623	17.1	11.398	10.5	9.548
Dodonaea viscosa Jacq. var*angustifolia*	Sapindaceae	S	5074	19.30	16,773± 2.907	19	13 ± 6,479
Dombeyaburgessiae	Sterculiaceae	YB	No nr	8.6	no inhibition	5.3	no inhibition
Dombeyaburgessiae	Sterculiaceae	L	No nr	17.8	no inhibition	26.8	1.988
Dombeyaburgessiae	Sterculiaceae	F	No nr	24.9	4.793	39.2	no inhibition
Dombeyarotundifolius	Sterculiaceae	OB	No nr	10.3	insoluble in DMSO	2.4	4.405
Dombeyarotundifolius	Sterculiaceae	YB	5634	15	insoluble in DMSO	5.6	0.701
Drimia sp	Asparagaceae	R	5559	4.4	no inhibition	3.9	no inhibition
Englerophytummagalismontanum(Sond) T.D. Penn	Sapotaceae	O	4508	16.60	no inhibition	76.6	no inhibition
Eriocephalus ericoides	Asteraceae	L	4501	14.8	no inhibition	19.6	no inhibition
Eriocephalus ericoides	Asteraceae	F	No nr	18.5	11.36	5	3.553
Erythrinalysistemon	Fabaceae (lenguminosae)	F	5623	21.8	11,484 ±15,828	11.7	no inhibition
Erythrinalysistemon	Fabaceae (lenguminosae)	S	5623	33.5	no inhibition	9.2	no inhibition
Erythrinalysistemon	Fabaceae (lenguminosae)	B	5623	12.1	9.181	9.7	no inhibition
Erythrinalysistemon	Fabaceae (lenguminosae)	D	5623	8.1	6.871	3.2	no inhibition
Erythrinalysistemon	Fabaceae (lenguminosae)	G	5623	22.1	insoluble in DMSO	4.6	5.713
Erythrinalysistemon	Fabaceae (lenguminosae)	L	5646	9.4	6.254	3.3	no inhibition

Euclea divinorum Hiern	Ebenaceae	O	4525	13.90	no inhibition	33.6	no inhibition
Euclea sp	Ebenaceae	F	5636	37.1	no inhibition	63.1	2.6
Euclea sp	Ebenaceae	L	5636	16.6	12.906	42.4	insoluble in DMSO
Euclea sp	Ebenaceae	YB	5629	22.8	11.022	13.8	11.816
Euphorbia mauritanica	Euphorbiaceae	YB	No nr	24.4	21,354 ±16,899	17.9	19,559 ±10,554
Euphorbia mauritanica	Euphorbiaceae	L	5241	27.1	14.857	27.1	no inhibition
Faidherbiaalbida	Fabaceae	OB	5092	12	insoluble in DMSO	2.4	1.397
Faidherbiaalbida	Fabaceae	D	5092	9.7	no inhibition	15.9	16,971±9,336
Faidherbiaalbida	Fabaceae	S	5626	25.1	insoluble in DMSO	14.7	no inhibition
Freyliniatropica	Scrophulariacea	YB	No nr	13.1	10.369	29.7	3.863
Freyliniatropica	Scrophulariacea	L	No nr	13.7	22.625	27.7	no inhibition
Freyliniatropica	Scrophulariacea	F	No nr	56.1	insoluble in DMSO	69.1	4.916
Freyliniatropica	Scrophulariacea	B	No nr	23.3	4.014	56.3	no inhibition
Galenia procumbens L.f.	Aizoaceae	L	5102	31.30	15,274 ±10,253	26.8	30,446 ±9,443
Galenica africana	Aizoaceae	L	5238	51.00	50,393± 5,474	93.9	no inhibition
Gardenia cf. *volkensi*	Rubiaceae	OB	5637	13	insoluble in DMSO	2.7	4.667
Gardenia cf. *volkensi*	Rubiaceae	YB	5637	15	insoluble in DMSO	8.7	3.178
Gardenia cf. *volkensi*	Rubiaceae	L	5637	17	2.713	9.6	no inhibition

Gardenia cf. *volkensi*	Rubiaceae	F	No nr	15.4	3.888	6.7	no inhibition
Gnaphaliumfilagopsis	Asteraceae	B	5464A	18.80	6,553 ± 2,537	28.9	no inhibition
Gnidia polycephala	Thymelaeaceae	R	5031	11.6	58,889 ±13,441	19.1	46,147 ±10,707
Gnidia polycephala	Thymelaeaceae	B	5031	12.4	59,853±22,73	11.9	37,223 ±5,79
Gnidia polycephala	Thymelaeaceae	L	5031	14.3	31,383 ±21,127	30.1	insoluble in DMSO
Gnidia polycephala	Thymelaeaceae	YB	No nr	20.4	53,765 ± 27,633	21.2	27,384 ± 32,421
Gomphostigma virgatum	Buddlejaceae	YB	No nr	19.2	no inhibition	46.6	no inhibition
Gomphostigma virgatum	Buddlejaceae	L	No nr	11.6	no inhibition	29.8	no inhibition
Gomphostigma virgatum	Buddlejaceae	F	5636	8.2	no inhibition	7.5	no inhibition
Gossypiumherbaceum spp *africanum*	Malvaceae	YB	5630	7.9	18.779	11.2	3.546
Gossypiumherbaceum spp *africanum*	Malvaceae	L	5630	11.1	5.42	17	10.266
Gossypiumherbaceum spp *africanum*	Malvaceae	F	5630	21.6	insoluble in DMSO	53.7	0.956
Gossypiumherbaceum spp *africanum*	Malvaceae	D	5630	15.2	insoluble in DMSO	16.8	4.262
Gossypiumherbaceum spp *africanum*	Malvaceae	S	No nr	19.3	insoluble in DMSO	2.5	no inhibition
Grewia sp.	Malvaceae	YB	5632	16.5	4.91	8.3	5.523
Grewia sp.	Malvaceae	F	5632	21.7	insoluble in DMSO	19.6	4.223
Grewia sp.	Malvaceae	S	5632	12.7	9.427	14.4	10.716
Grewia sp.	Malvaceae	L	No nr	12	27,109±1,829	14.9	11.073

Haemanthus cf. *montanus*	Amaryllidaceae	R	4911	13.00	no inhibition	54.7	no inhibition
Harpagophytum procumbens (KijKij)	Pediliaceae	S	4414B	54.70	no inhibition	7.7	no inhibition
Harpagophytum procumbens (Loffiesdraai)	Pedaliaceae	S	4414A	38.90	no inhibition	4.2	no inhibition
Hertia pallens	Asteraceae	F	5468	56.30	no inhibition	10.8	no inhibition
Hertia pallens	Asteraceae	L	5460	12.20	no inhibition	42.7	8,311 ± 0,17
Hertia pallens	Asteraceae	L	5032	16	no inhibition	16.1	7.825
Hertia pallens	Asteraceae	F	5032	29.6	6.844	25.7	3.776
Hertia pallens	Asteraceae	G	5032	18.3	no inhibition	17.2	7.313
Hertia pallens	Asteraceae	B	5096	24.4	no inhibition	30.2	no inhibition
Heteromorpha arborescens	Apiaceae	F	No nr	26	2.137	33.4	no inhibition
Heteromorpha arborescens	Apiaceae	L	No nr	24.7	6.332	4.4	4.864
Heteromorpha arborescens	Apiaceae	YB	No nr	42.8	insoluble in DMSO	30.9	2.959
Holarrhena pubscens ZIM	Apocynaceae	G	5540	13.6	7.806	21.1	no inhibition
Hyphaene petersiana (Klotzsch) Hemsl.	Arecaceae	O	4701	17.70	no inhibition	1.5	3,088 ±3,495
Indigofera alternans	Fabaceae	F	5473	18.00	no inhibition	19.7	no inhibition
Indigofera sp.	Fabaceae	L	5463A	12.20	no inhibition	5	no inhibition
Kigelia africana	Bignoniaceae	U	5096B	7.70	2,181 ± 2,604	77.9	no inhibition
Kigelia africana	Bignoniaceae	P	5096C	9.70	no inhibition	48.8	no inhibition

Kigelia africana	Bignoniaceae	S	5096D	11.10	no inhibition	32.4	no inhibition
Kigelia africana	Bignoniaceae	B	5096	12.3	4.714	8.4	15,844±11,863
Kigelia africana	Bignoniaceae	YB	5096	9.6	insoluble in DMSO	27.5	no inhibition
Kigelia africana	Bignoniaceae	F	5096	10.7	8.389	9.4	5.861
Kigelia africana	Bignoniaceae	L	5641	5.2	no inhibition	10.1	13.534
*Kigelia africana (*Lam.) Benth.	Bignoniaceae	S	5096A	98.00	no inhibition	1	no inhibition
Kiggelaria africana	Achariaceae	B	No nr	15.9	no inhibition	8.8	no inhibition
Kiggelaria africana	Achariaceae	D	No nr	12.8	no inhibition	10.8	18.185
Kiggelaria africana	Achariaceae	YB	No nr	11.9	10.526	7.6	no inhibition
Kiggelaria africana	Achariaceae	F	5645	21.4	no inhibition	24.9	8.714
Lannea discolor Sond. Engl.	Anacardiaceae	O	5076	12.00	no inhibition	7.4	no inhibition
Lannea edulis (Sond.) Engl. var. edulis	Anacardiaceae	S	4505	8.50	no inhibition	5.4	no inhibition
Leonotis leonurus	Lamiaceae	S	No nr	84.2	no inhibition	22.1	19.475
Leonotis leonurus	Lamiaceae	L	No nr	7.8	no inhibition	10.1	no inhibition
Leonotis leonurus	Lamiaceae	F	No nr	33.7	1.786	8	8.749
Leonotis leonurus	Lamiaceae	YB	5608	9.8	no inhibition	34.4	no inhibition
Leonotisintermedia	Lamiaceae	N	5248	32.00	6,038 ± 3,282	78.6	no inhibition
Mackayabella	Acanthaceae	B	No nr	13.9	no inhibition	21.4	no inhibition

Mackayabella	Acanthaceae	L	No nr	3	no inhibition	25.1	no inhibition
Mackayabella	Acanthaceae	F	No nr	12.9	insoluble	65	3.559
Mackayabella	Acanthaceae	YB	No nr	6.8	15.822	23.4	no inhibition
Maeruaangolensis	Capparaceae	YB	5624	6.9	no inhibition	8.3	no inhibition
Maeruaangolensis	Capparaceae	L	5624	13.4	19,688± 27,098	12.8	1.623
Maeruaangolensis	Capparaceae	F	5624	13.5	no inhibition	2	8.14
Maeruaangolensis	Capparaceae	B	4722	10.1	no inhibition	46.1	no inhibition
Merwilla plumbea	Hyacinthaceae	R	5258	14.90	no inhibition	19.7	no inhibition
Mimusops zeyheri	Sapotaceae	F	4713	15.3	insoluble in DMSO	42.3	no inhibition
Mimusops zeyheri	Sapotaceae	YB	4713	20.6	15.195	12.4	6.649
Mimusops zeyheri	Sapotaceae	OB	4713	9.6	12.459	2.2	8.828
Mimusops zeyheri	Sapotaceae	L	No nr	15.6	22.458	11.1	5.084
Mimusops zeyhiri (Sond.)	Sapotaceae	P	5057	11.90	no inhibition	42.5	no inhibition
Myrsine africana	Myrsinaceae	L	No nr	11.3	3.564	30.6	5.583
Myrsine africana	Myrsinaceae	F	5624	22.6	no inhibition	41.7	6.608
Nerine laticoma (Ker Gawl.) T.Durand & Schinz	Amaryllidaceae	R	4535	10.10	no inhibition	14.4	11,259 ±1,963
Nidorella resedifolia ssp. *Resedifolia*	Asteraceae	B	5474A	17.80	no inhibition	38.2	no inhibition
Nuxia floribunda	Buddlejaceae	YB	No nr	11.1	27.072	8	14.274

Nuxia floribunda	Buddlejaceae	F	No nr	18.8	no inhibition	52.3	20.294
Nuxia floribunda	Buddlejaceae	L	5241	8.3	no inhibition	20	8.19
Ochnapulchra	Ochnaceae	F	5635	16.4	3.122	22.4	1.122
Ochnapulchra	Ochnaceae	YB	5635	8.2	4.633	9.8	no inhibition
Ochnapulchra	Ochnaceae	OB	5635	9.9	1.405	18.4	1.246
Ochnapulchra	Ochnaceae	L	No nr	7	18.279	21.2	0.429
Oleaeuropaea spp *africana*	Oleaceae	YB	No nr	11.8	15.879	20.4	20.136
Oleaeuropaea spp *africana*	Oleaceae	F	No nr	19.2	no inhibition	24.4	8.529
Oleaeuropaea spp *africana*	Oleaceae	L	No nr	12.8	20.862	9.5	3.311
Ormocarpumtrichocarpum	Leguminosae (papilionoideae)	P	4719A	12.80	25,441 ±11,52	7.6	18,023 ±1,432
Ormocarpumtrichocarpum	Leguminosae (papilionoideae)	S	4719B	32.20	no inhibition	14.3	18,423 ±24,511
Ozoroa dispar (C.Presl) R.Fern. & A.Fern	Anacardiaceae	O	4746	55.50	no inhibition	11.2	no inhibition
Pappea capensis	Sapindaceae	YB	5639	7.3	3.692	10.3	
Pappea capensis	Sapindaceae	L	5639	13.2	no inhibition	18	3.667
Pappea capensis	Sapindaceae	OB	5639	11.8	1.997	3.1	no inhibition
Pappea capensis	Sapindaceae	F	No nr	19.1	8.057	60.4	no inhibition
Pappea capensis Eckl. & Zeyh.	Sapindaceae	O	5090	123.10	no inhibition	12.3	no inhibition

Pechuel-Loeschealeubnitziae	Asteraceae	B	5627	22.4	6.883	43.8	4.527
Pechuel-Loeschealeubnitziae	Asteraceae	YB	5627	5.4	3.424	67.9	insoluble in DMSO
Pechuel-Loeschealeubnitziae	Asteraceae	F	5627	37.4	2.255	20	2.768
Pechuel-Loeschealeubnitziae	Asteraceae	L	5630	13	3.81	16.6	no inhibition
Pelargonium sidoides	Geraniaceae	R	5632	20.8	5.808	8.1	8.957
Peltophorum africanum	Fabaceae	OB	5648	13.9	insoluble in DMSO	7.6	11.666
Peltophorum africanum	Fabaceae	F	5648	11.3	insoluble in DMSO	19.8	no inhibition
Peltophorum africanum	Fabaceae	S	5648	23.8	insoluble in DMSO	10.3	no inhibition
Peltophorum africanum	Fabaceae	D	5648	11	17.662	6.6	15.473
Peltophorum africanum	Fabaceae	YB	5648	9.2	21.603	15.3	unstable
Peltophorum africanum	Fabaceae	L	No nr	5.3	34,738±15,859	9.7	17.934
*Peltophorumafricanum*Sond.	Fabaceae	S	4510	16.60	no inhibition	10.9	no inhibition
Persicaria hystricula	Polygonaceae	L	5462	9.30	no inhibition	4.5	no inhibition
*Phaeoptilumspinosum*Radlk.	Nyctaginaceae	S	4502	30.60	no inhibition	16.4	26,883 ±4,7
Pollichia campestris	Caryapylaceae	L	5642	23.50	no inhibition	8	no inhibition
Pseudolachnostylismaprouneifoila Pax. var. glabra (Pax) Brenan	Euphorbiaceae	E	5239A	5.60	no inhibition	6	no inhibition
Pseudolachnostylismaprouneifoila Pax.var. glabra (Pax) Brenan	Euphorbiaceae	C	5239C	11.80	no inhibition	89.8	no inhibition
Pseudolachnostylismaprouneifoila	Euphorbiaceae	S	5239B	44.90	no inhibition	12.1	no inhibition

Pax.var. glabra (Pax) Brenan							
Pterocarpus rotundifolius	Fabaceae	F	5641	19.5	no inhibition	8.6	no inhibition
Pterocarpus rotundifolius	Fabaceae	OB	5641	6.1	insoluble	8.1	no inhibition
Pterocarpus rotundifolius	Fabaceae	YB	5641	4.8	13.488	8.2	9.217
Pterocarpus rotundifolius	Fabaceae	L	5648	6	insoluble in DMSO	6.3	insoluble in DMSO
Rhamnus prinoides	Rhamnaceae	F	No nr	30	no inhibition	53.8	no inhibition
Rhamnus prinoides	Rhamnaceae	YB	No nr	13.2	7.251	40.2	7.695
Rhamnus prinoides	Rhamnaceae	L	5637	10.9	4.851	32	no inhibition
Schotia brachypetala Sond.	Fabaceae	D	4700	9.40	no inhibition	10.1	no inhibition
Searsia (Rhus) lancea	Anacardiaceae	OB	5608	10.2	no inhibition	2.5	2.219
Searsia (Rhus) lancea	Anacardiaceae	YB	5638	5.6	4.979	26.5	no inhibition
Searsia (Rhus) lancea	Anacardiaceae	F	5638	22.9	insoluble	21.1	4.379
Searsia (Rhus) lancea	Anacardiaceae	O	5638	5.2	no inhibition	8.8	no inhibition
Searsia (Rhus) lancea	Anacardiaceae	L	No nr	5.6	33.425	21	14.119
Searsia dentate (Thunb.) F.A. Barkley	Anacardiaceae	S	4507	5.90	no inhibition	17.0	no inhibition
Searsia lancea	Anacardiaceae	F	5465	13.50	1,756± 2,484	55	no inhibition
Searsia lancea	Anacardiaceae	G	5472	19.00	no inhibition	2.8	no inhibition
Searsia pyroides(Burch.) Moffet var. *pyroides*	Anacardiaceae	S	4509	8.80	no inhibition	7.5	no inhibition

Seemannaralia gerrardii (Seem.) Harms	Araliaceae	L	5087A	11.60	16,793± 9,556	16.2	no inhibition
Seemannaralia gerrardii (Seem.) Harms	Araliaceae	S	5087B	24.60	no inhibition	19.4	no inhibition
Senna italica Mill. subsp. *arachoides* (Burch.) Lock	Fabaceae	N	5100	16.90	no inhibition	7.8	no inhibition
Sesamothamus lugardii	Pedaliaceae	OB	5094	12.9	no inhibition	7.5	no inhibition
Sesamothamus lugardii	Pedaliaceae	YB	5094	3.7	1.511	6.8	8.084
Sesamothamus lugardii	Pedaliaceae	D	5094	6.1	8.603	6	8.318
Sesamothamus lugardii	Pedaliaceae	L	No nr	3.2	9.255	4.4	7.474
Spirostachys africana	Euphorbiaceae	OB	No nr	22.1	insoluble	3.2	6.572
Spirostachys africana	Euphorbiaceae	YB	No nr	6.3	no inhibition	13.1	no inhibition
Spirostachys africana	Euphorbiaceae	L	No nr	5.5	9.842	2	11.248
Steganotenia araliacea Hochst. var. *araliacea*	Apiaceae	S	5068	21.80	32,606 ±9,504	16.2	no inhibition
Strychnos pungens Soler	Loganiaceae	U	4385A	16.30	no inhibition	51.1	no inhibition
Strychnos pungens Soler	Loganiaceae	S	4385B	9.80	no inhibition	27.6	no inhibition
Stumariatenella(L.f.) Snijman subsp. *Orientalis* Snijman	Amaryllidaceae	R	4409	12.50	4,494 ±4,461	40.2	no inhibition
Sutherlandia frutescens	Fabaceae	S	No nr	18.1	7.134	17.8	0.853
Sutherlandia frutescens	Fabaceae	YB	No nr	14.1	3.156	3.9	0.668
Sutherlandia frutescens	Fabaceae	D	No nr	12.8	2.703	16.4	no inhibition

Sutherlandia frutescens	Fabaceae	F	No nr	23.7	insoluble in DMSO	37.1	3.161
Sutherlandia frutescens	Fabaceae	L	No nr	11.8	no inhibition	20.2	no inhibition
Tapinanthusoleifolius	Loranthaceae	F	5559	11.8	no inhibition	5.1	no inhibition
Tapinanthusoleifolius	Loranthaceae	YB	5559	8.6	14.231	13	no inhibition
Tapinanthusoleifolius	Loranthaceae	L	No nr	6.6	23,117 ±16,864	18.1	no inhibition
Terminalia prunioides M.A. Lawson	Combretaceae	O	4693	8.40	no inhibition	7.6	no inhibition
Terminalia prunoides	Combretaceae	YB	4693	13.3	9.87	13.2	9.036
Terminalia prunoides	Combretaceae	L	4693	8.2	5.512	3.8	5.788
Terminalia prunoides	Combretaceae	F	4687	27.1	insoluble in DMSO	20.6	no inhibition
Terminalia sericea	Combretaceae	OB	4722	7.5	15.144	2.2	no inhibition
Terminalia sericea	Combretaceae	YB	5628	6.8	insoluble in DMSO	16.8	6.798
Terminalia sericea Burch. Ex DC.	Combretaceae	S	4722A	31.00	no inhibition	7.1	no inhibition
Terminalia sericea Burch. Ex DC.	Combretaceae	P	4722B	12.50	no inhibition	8.4	9,077±4,582
Trichiliaemetica	Meliaceae	O	4533	55.20	no inhibition	16.1	no inhibition
Trichiliaemetica	Meliaceae	YB	5629	16.1	insoluble in DMSO	8.9	9.055
Trichiliaemetica	Meliaceae	L	5629	17.9	no inhibition	6.5	no inhibition
Trichiliaemetica	Meliaceae	B	5629	25.3	no inhibition	24	1.929
Trichiliaemetica	Meliaceae	F	5551	36	insoluble in DMSO	26.8	12.374

Tulbaghia violacea	Alliaceae	F	No nr	19.4	8.611	23	no inhibition
Tulbaghia violacea	Alliaceae	R	5451	11.8	16.113	7.8	14.631
Tylosemafassoglense (Schweinf.) Torre &Hillc.	Fabaceae	P	4714A	14.20	no inhibition	6.5	no inhibition
Vahlia capensis ssp. *vulgaris* var. *linearis*	Vahliaceae	R	5470B	11.50	no inhibition	7.7	no inhibition
Vahlia capensis ssp. *vulgaris* var.*linearis*	Vahliaceae	F	5471	18.90	no inhibition	36.1	no inhibition
Vangueria infausta Burch. subsp. *infausta*	Rubiaceae	S	4511	7.80	no inhibition	36.5	no inhibition
Vernonia glabra ZIM	Asteraceae	R	No nr	10.6	insoluble in DMSO	5	15.517
Vernonia oligocephala	Asteraceae	R	5474B	13.30	8,604± 4,882	3.3	no inhibition
Vernonia oligocephala	Asteraceae	F	5463B	19.30	no inhibition	5.4	no inhibition
Viscum rotundifolium L.f.	Santalaceae	L	4733	8.70	no inhibition	7.8	3,378 ±0,856
Viscum sp	Santalaceae	YB	5645	23.3	no inhibition	21	no inhibition
Viscum sp	Santalaceae	L	No nr	18.5	no inhibition	20.7	no inhibition
Xanthocerciszambesiaca (Baker) Dumaz-le-Grand	Fabaceae	O	4687	62.60	no inhibition	56.4	oocytes die
Xantocerciszambesiaca	Fabaceae	F	4687	22.3	insoluble in DMSO	42.2	2.713
Xantocerciszambesiaca	Fabaceae	L	5639	13.1	1.036	24.2	no inhibition
Ziziphus mucronata	Rhamnaceae	OB	No nr	23.9	insoluble	2.8	16.932

Ziziphus mucronata	Rhamnaceae	F	No nr	16	no inhibition	46.8	no inhibition
Ziziphus mucronata	Rhamnaceae	L	No nr	6.6	no inhibition	5.1	no inhibition
Ziziphus mucronata	Rhamnaceae	YB	No nr	13.6	18.657	7.6	2.063

B – flos (flowers), F – folium (leaves), R – radix/rhizome/bulbus (roots/bulbs), S – semen (seeds), O – fructus (fruit), C – cortex, P– pericarp, D – pods, N – whole plant, C – meso&exocarp, L – stipites (stems & leaves), G –stem bark, SP – seeds & pericarp, U –pulp, S – seeds, OB – old bark, YB – young bark.

Appendix C

The use of medicinal plants by patients suffering from diarrhea

Principal Investigators: Dr SL Bonnet, Dr A Wilhelm-Mouton, Dr R Street. Ms M Khamane and Me KV Phungula

Organization: University of the Free State and the South African Medical Research Council

Sponsor: Multi-disciplinary University Traditional Health Initiative (MUTHI)

Questionaire

No.	Question			
1.	When last did you have diarrhea?	0-3 months ago	4-6 months ago (Exclude)	7-12 months ago (Exclude)
2.	Is the cause of the diarrhea known?	Wait for response (e.g. change of diet, medication, etc		
3.	How long did your diarrhea last?			
4.	How many times per day did you have diarrhea?			
5.	Did you notice blood when you had diarrhea?			
6.	Did you experience any other symptoms such as vomiting or abdominal pain?			
7.	To treat did you use:	Modern medicine (Exclude)	Traditional medicine	Both (*please explain*)
8.	Did you self-medicate or visit at THP?	Self-medicate	Visit THP	Both (*please explain*)
9.	Did you prepare the traditional medicine yourself?	Yes, self (Continue to 10 &12)	No, THP prepared (Continue to 11 & 12)	
10.	**If prepared by SELF:**			
10.1	How did you prepare it?			
11.	**If prepared by other**			
11.1	Who prepared it?			
11.2	Did you see it being			

	prepared, if yes, explain?			
12.	**If prepared by SELF or by other:**			
12.1	Can you list any ingredients or plants used?			
12.2	How many days did you take it for?			
12.3	How many days later did your diarrhoea stop?			
12.4	Route of administration?			
12.5	How many doses per day?			
12.6	What was the treatment outcome? (choose one)	Cured	No change in condition	Improvement of the condition but not cured
12.7	Were there any side effects?			

Prior Informed Consent Form

This Prior Informed Consent Form has two parts:

- Information Sheet (to share information about the research with you)
- Certificate of Consent (for signatures if you agree to take part)

You will be given a copy of the complete Prior Informed Consent Form

PART I: Information Sheet

Introduction

I am _____, working for the University of the Free State. We are doing a study on the use of medicinal plants by patients suffering from diarrhoea. I am going to give you information and invite you to be part of this study. You do not have to decide today whether or not you will take part in the research. Before you decide, you can talk to anyone you feel comfortable with about the study.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them to me, the study doctor or the staff.

The reason we are doing this study is to find out which of the medicinal plants used (from your traditional healer or market or the veld) help to relieve diarrhea. In this way we may identify the best remedies.

We are inviting all patients who have recently suffered from diarrhea to participate in the study on the effectiveness of the medicine plants used.

Your participation in this study is entirely voluntary. It is your choice whether to participate or not. You may change your mind later and stop participating even if you agreed earlier.

If you participate in the study you will need to meet with us only once (at the designated venue) and we will help you to fill in a form (questionnaire). We will ask you questions about your illness and treatment and explain them if you are not sure.

We will give you money to pay for your travel to the venue and we will give you a snack and a drink. You will not be given any other money or gifts to take part in this research.

Confidentiality

With this research, something out of the ordinary is being done in your community. It is possible that if others in the community are aware that you are participating, they may ask you questions. We will not be sharing the identity of those participating in the study.

The information that we collect from this research project will be kept confidential. Personal information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and the files will be placed in a locked cabinet. It will not be shared with or given to anyone.

However, it is important that you understand that the names of the plants you mention to us will not be kept a secret, and the names of these plants will be published. Therefore if there are any remedies whose ingredients you do not want to publish, let us know and we will exclude you from the interview. If any commercial product results from this research, we will agree an equitable scheme of benefit-sharing with the community.

Sharing the Results

The knowledge or information that we get from doing this study will be shared with you personally before it is made widely available to the public. Confidential information will not be shared. There will be small meetings in the community and these will be announced. After these meetings, we will publish the results in order that other interested people may learn from our research.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so and refusing to participate will not affect you in any way. You may stop participating in the research at any time you wish.

Certificate of Prior Informed Consent for RTO: The use of medicinal plants by patients suffering from diarrhea

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to take part as a participant in this research.

Print Name of Participant_____

Signature of Participant _____

Date _____

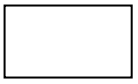
Day/month/year

If illiterate

A literate witness must sign. Participants who are illiterate should include their thumb-print.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ Thumb print of participant



Signature of witness _____

Date _____

Day/month/year

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. We will have one meeting at the designated venue in Thaba Nchu.
2. We will explain the research in depth.
3. We will use the information you give us to fill in a questionnaire.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

Appendix D

Plate 1 CDCl3 1H

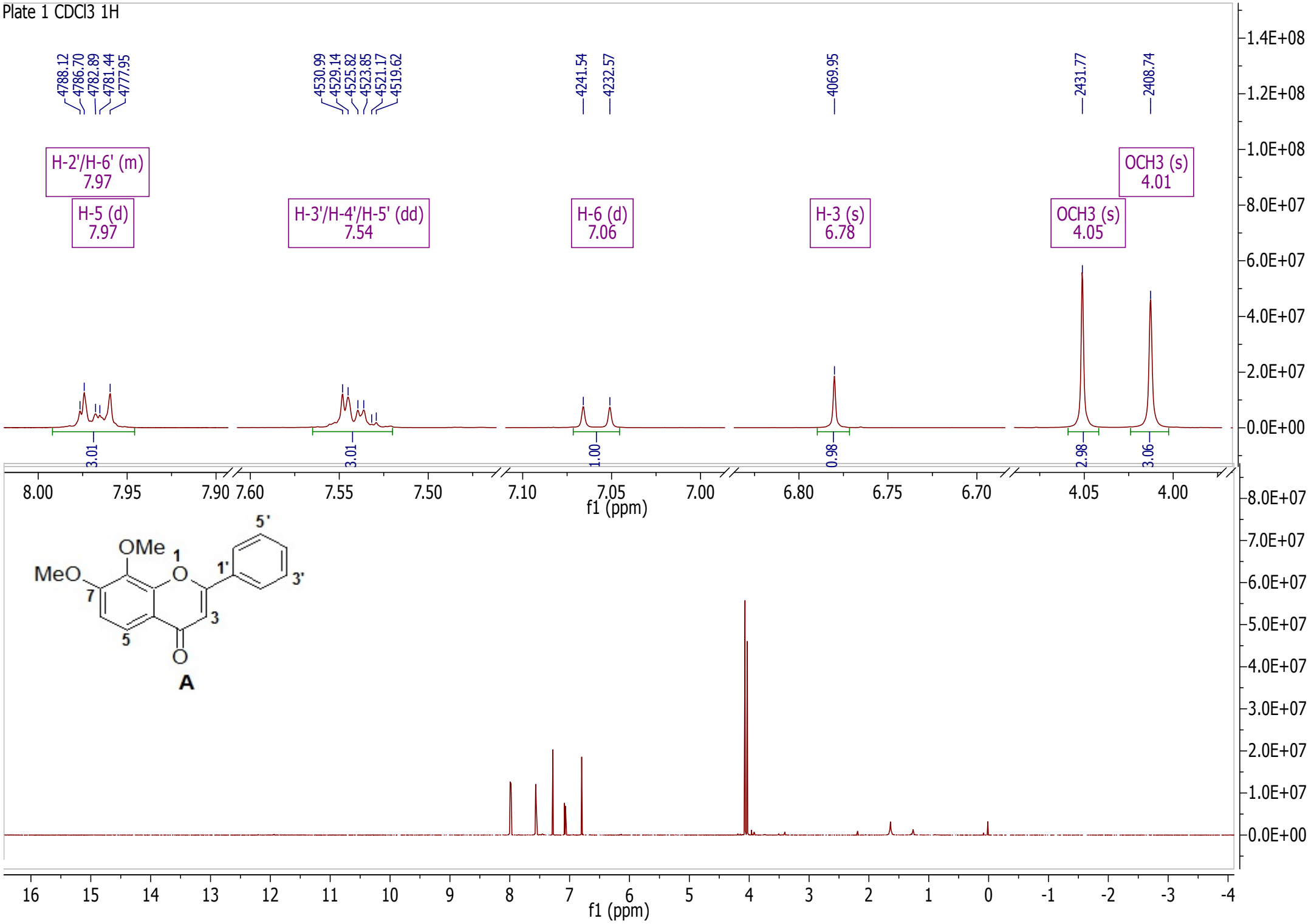


Plate 2 CDCl3 , 13C

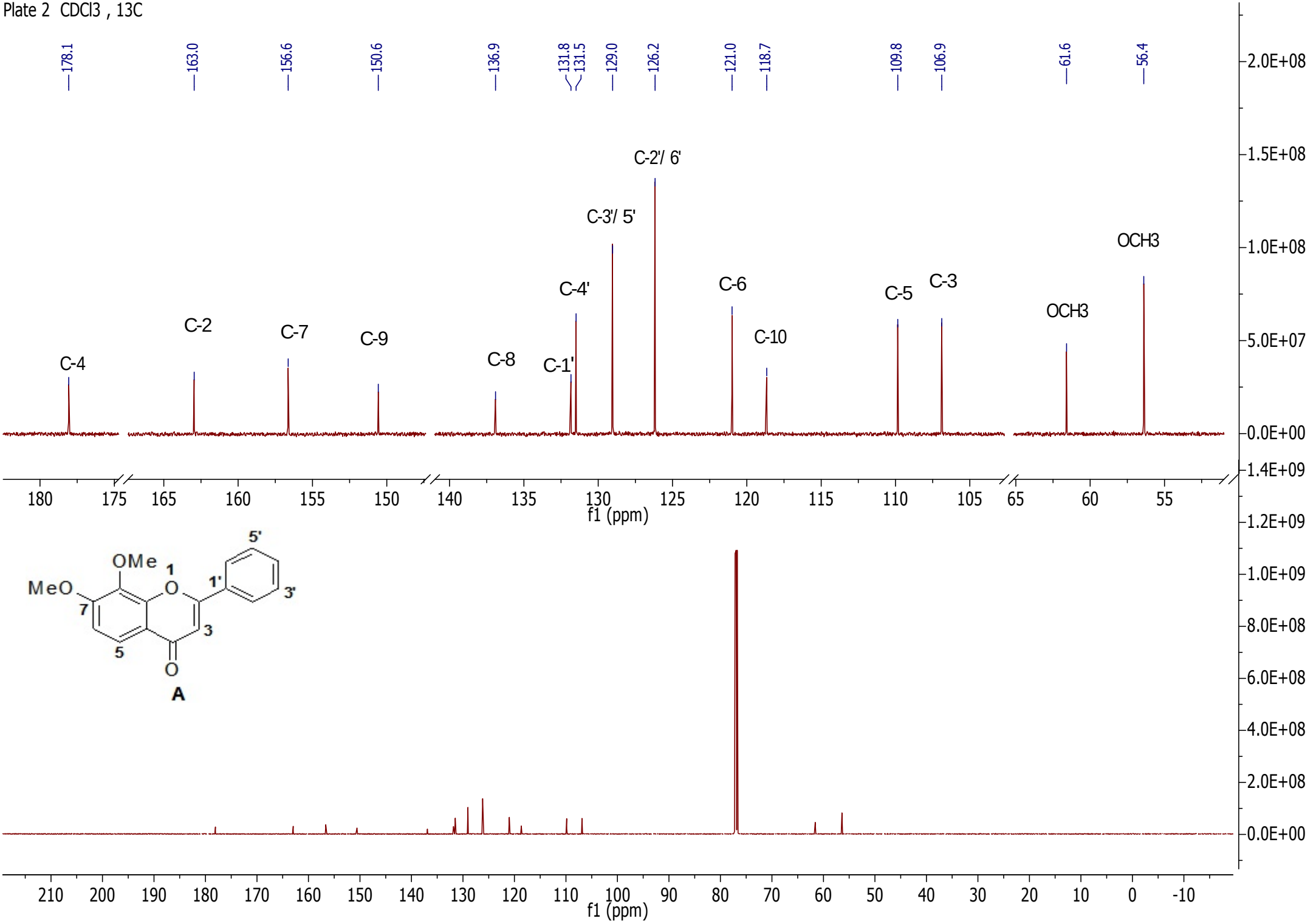


Plate 3 CDCl3 1H

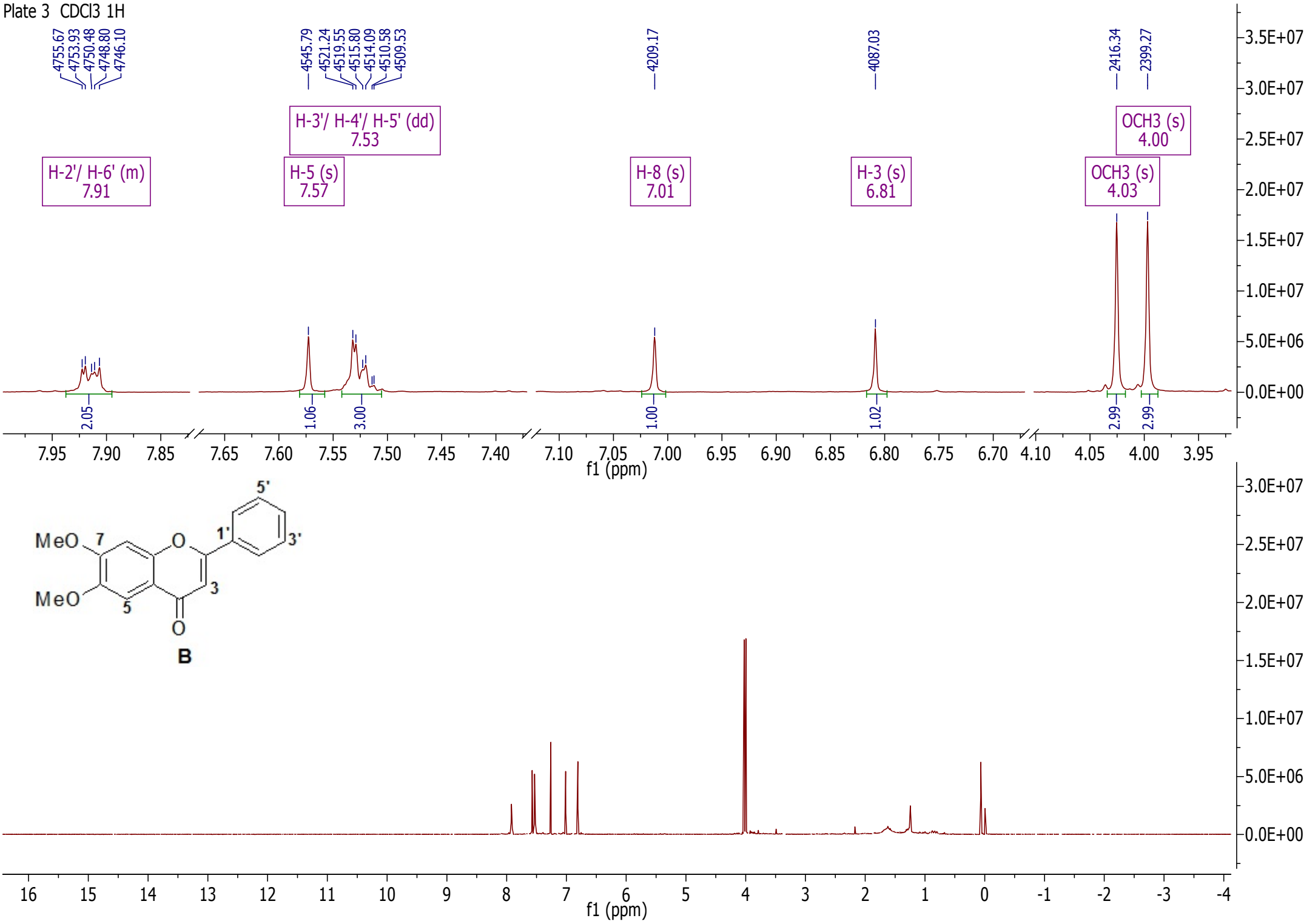


Plate 4 CDCl3 13C

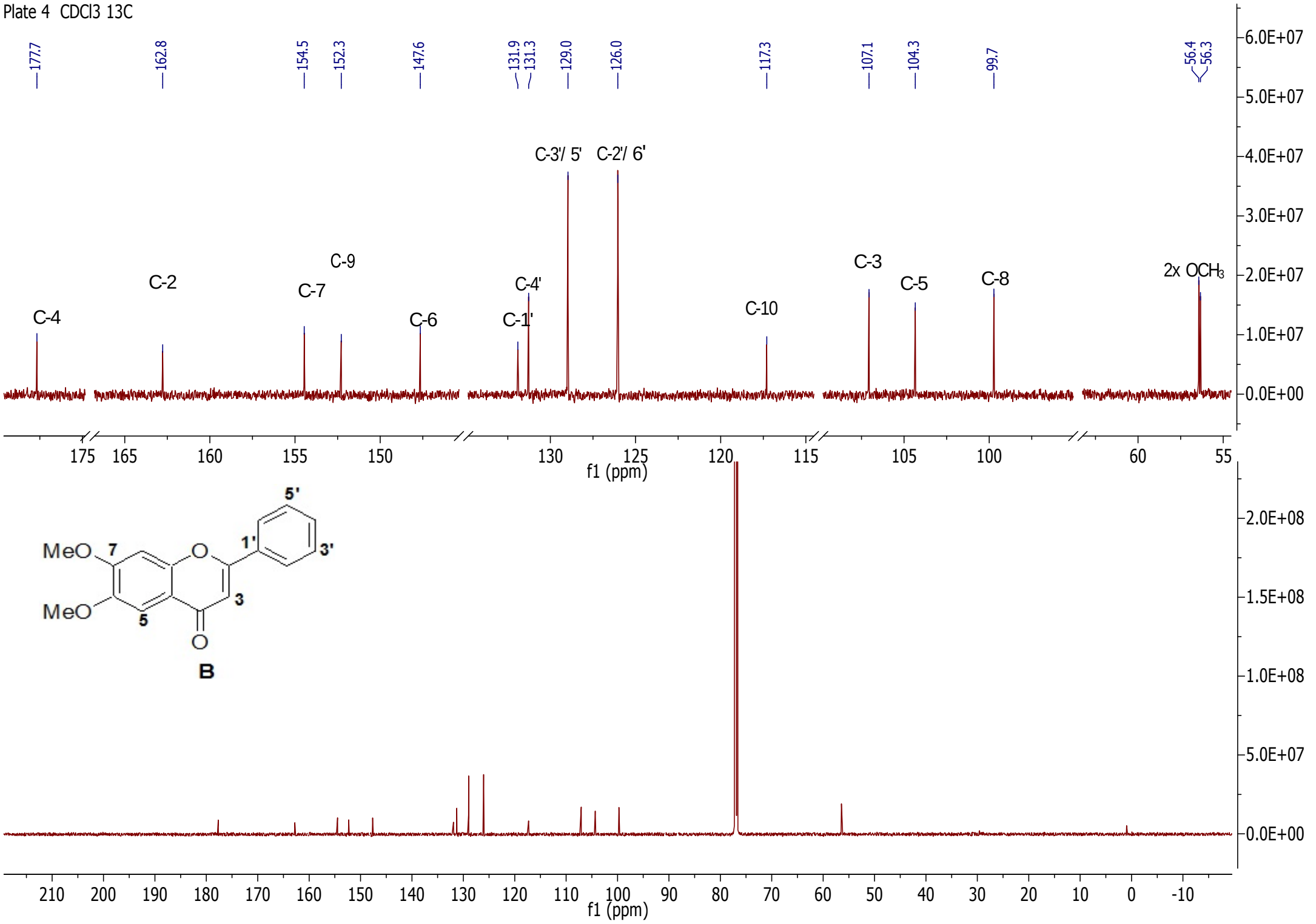
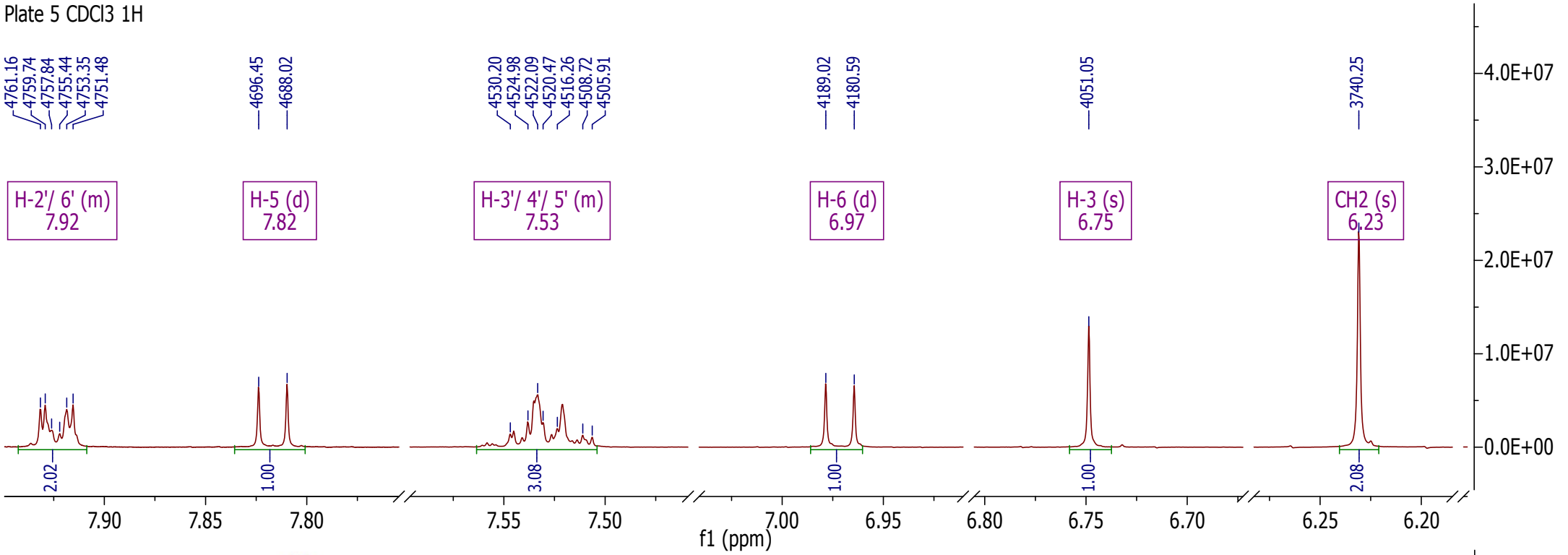
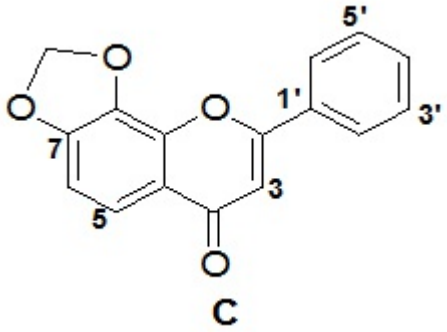


Plate 5 CDCl3 1H





C

Plate 6 CDCl3 13C

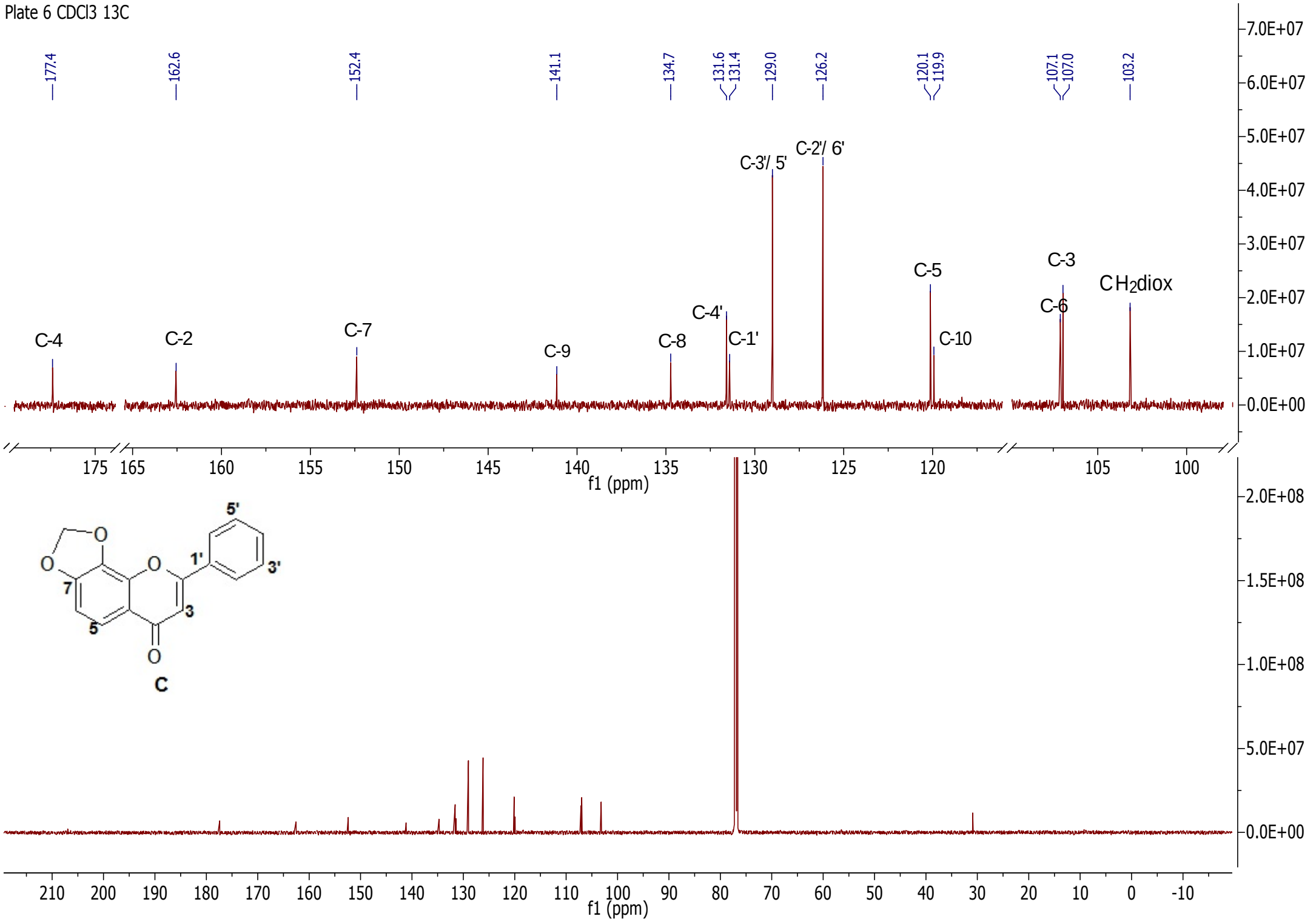
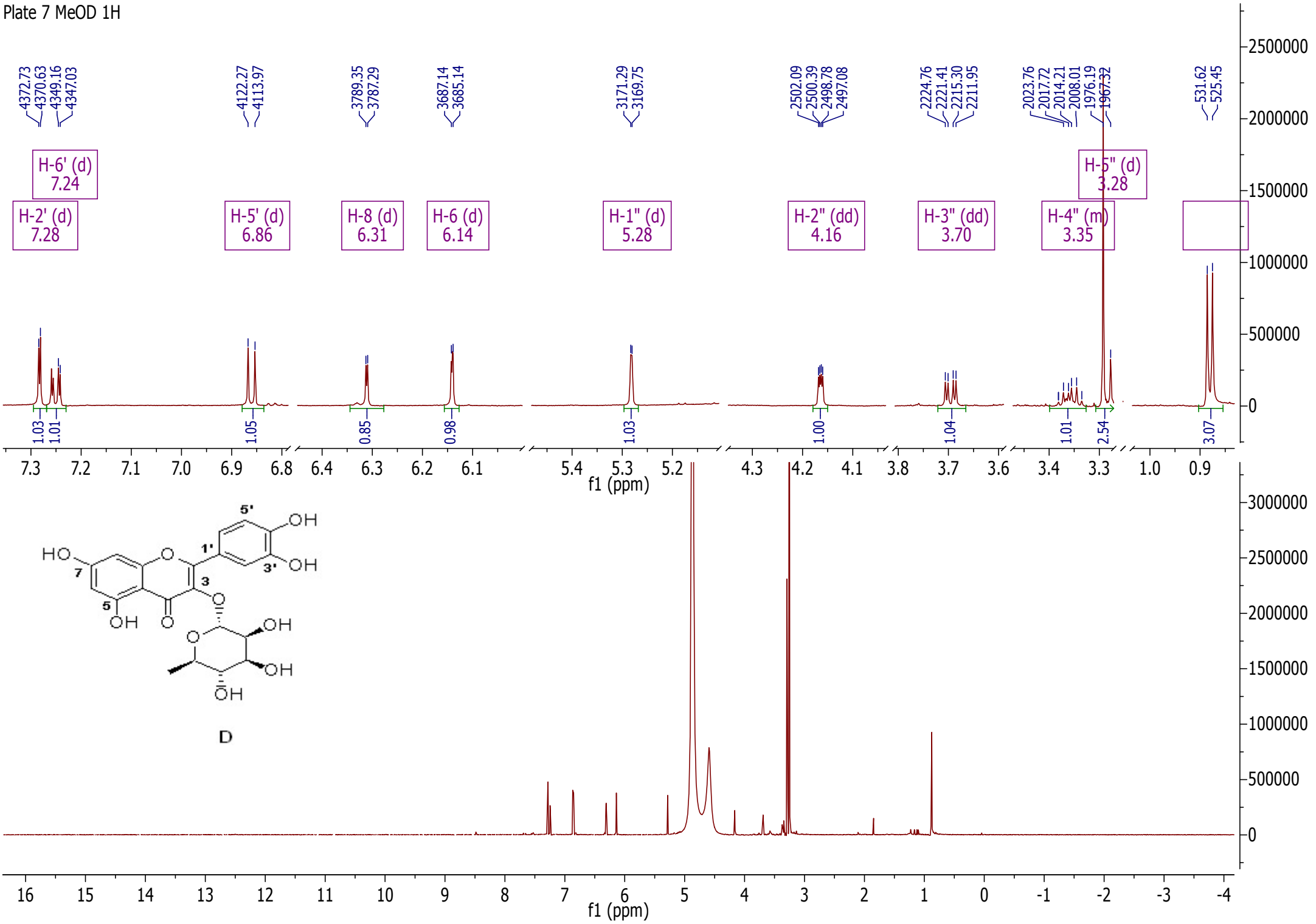
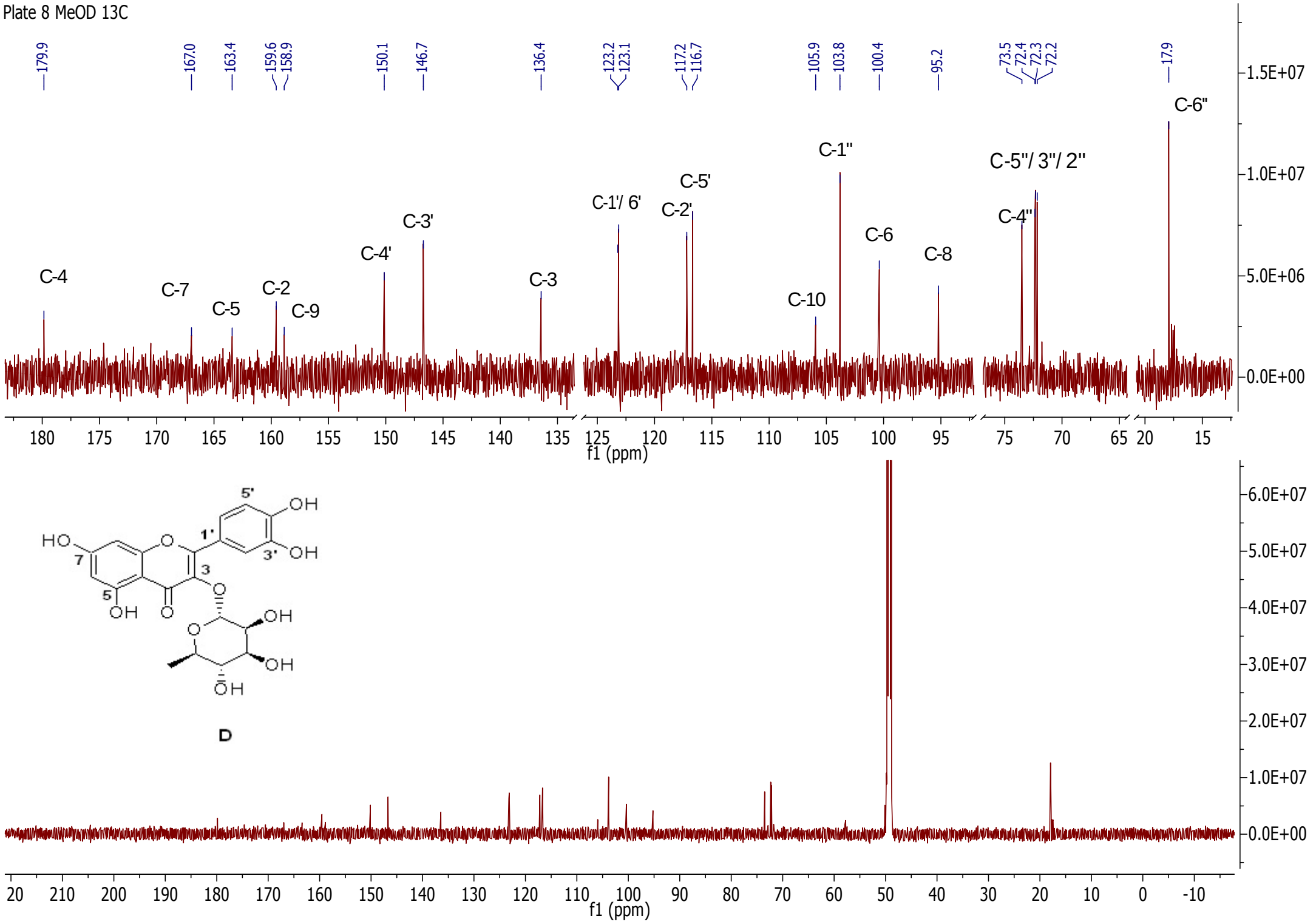


Plate 7 MeOD 1H



D

Plate 8 MeOD 13C



D

Plate 9 MeOD 1H

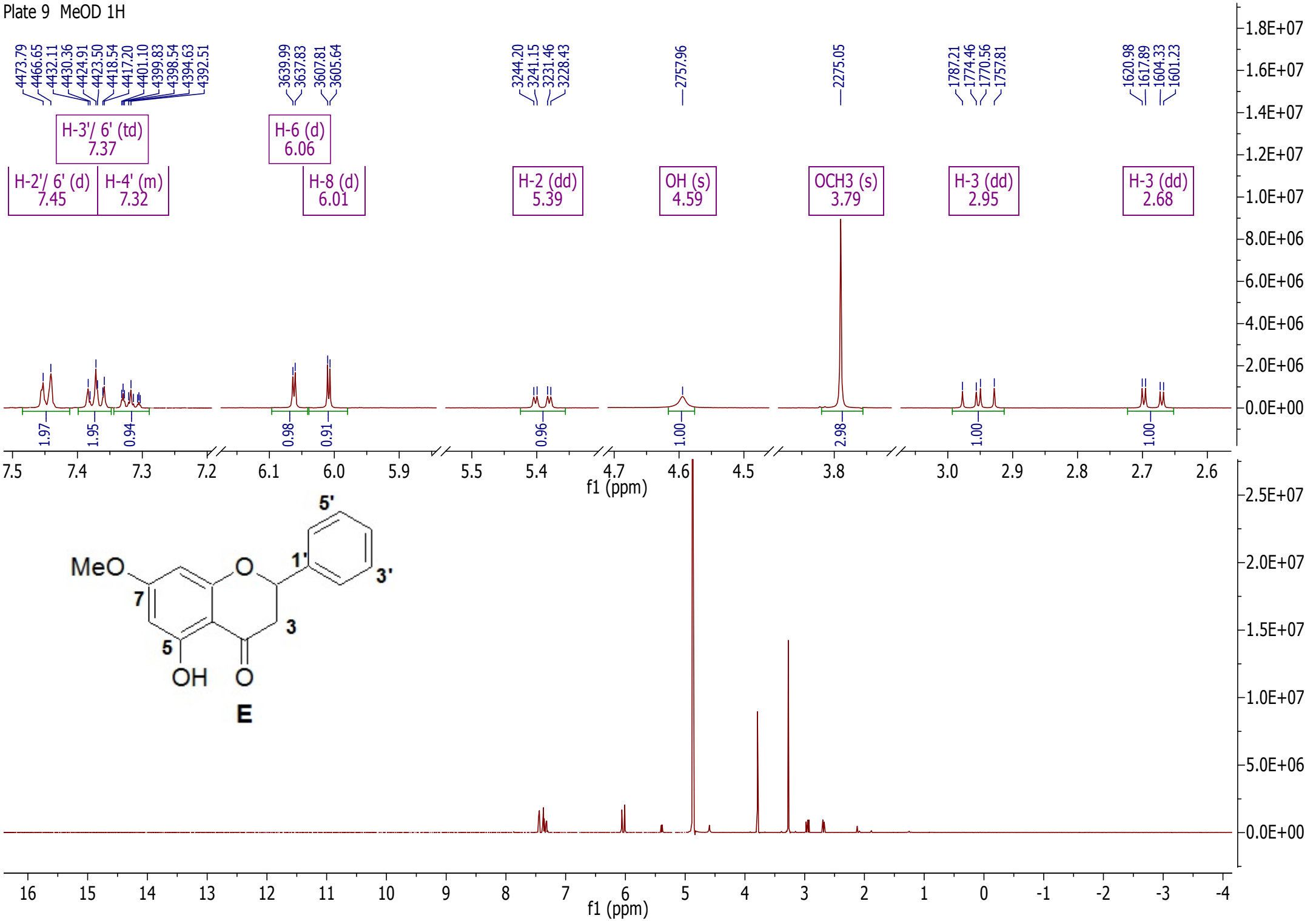
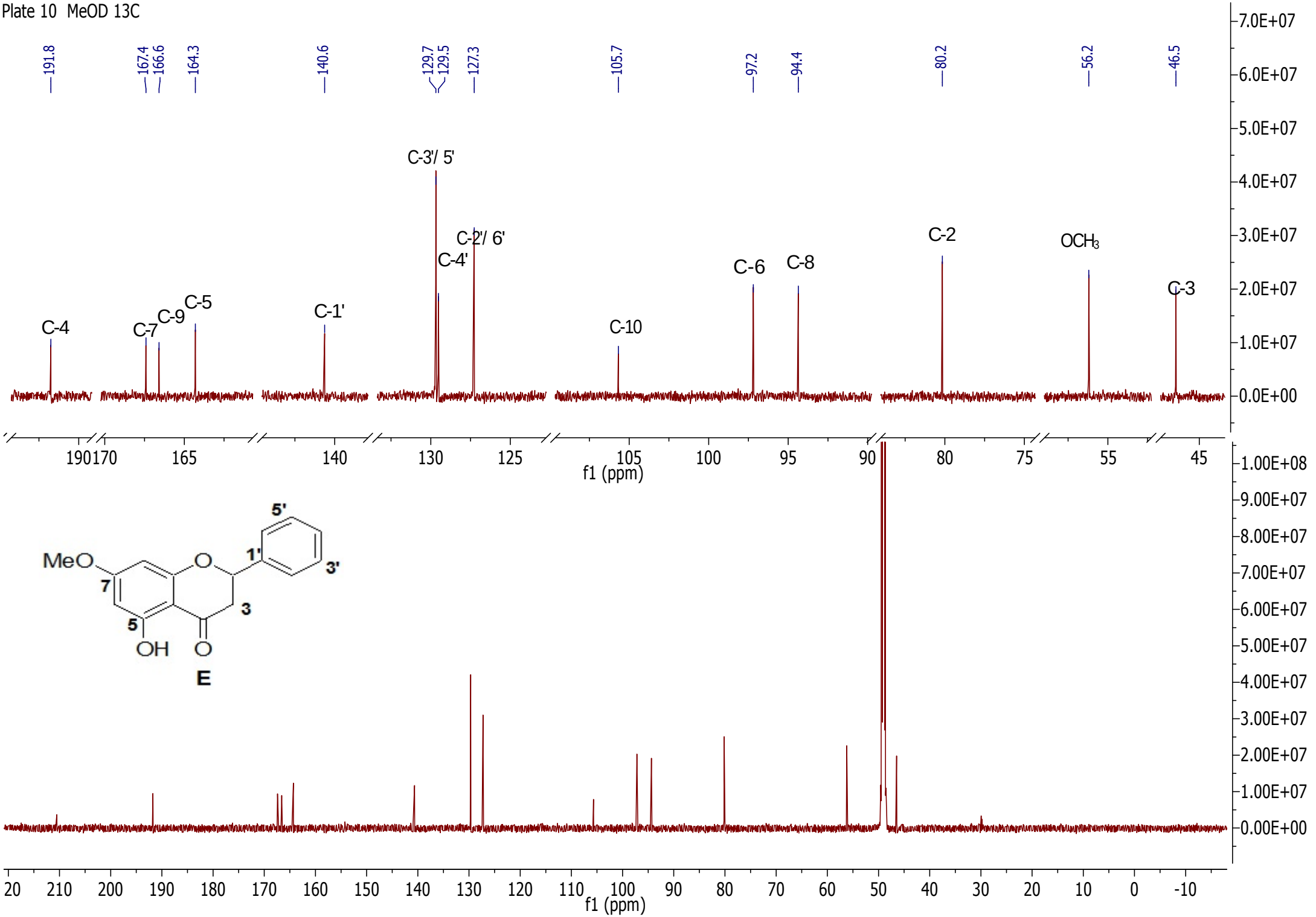
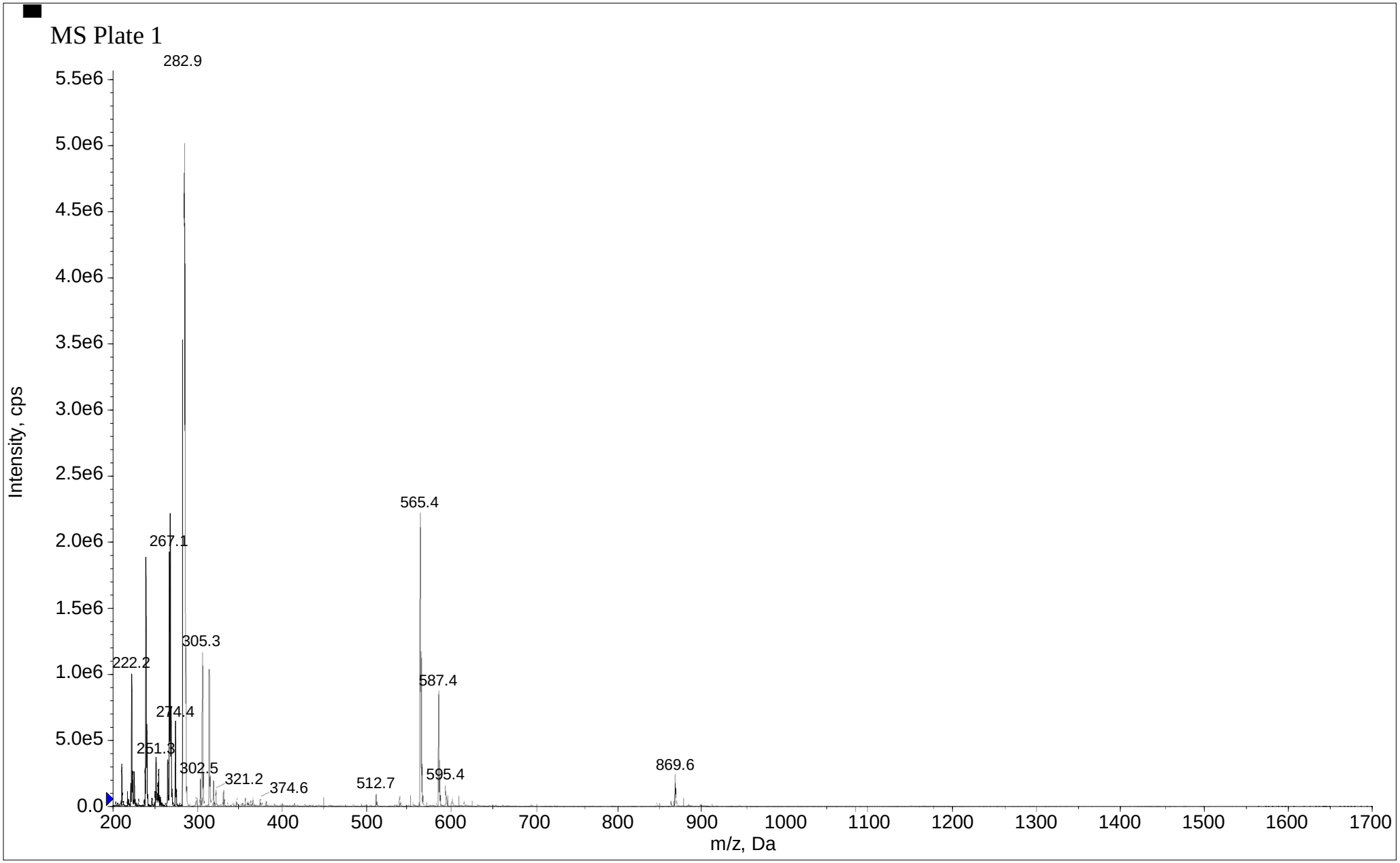
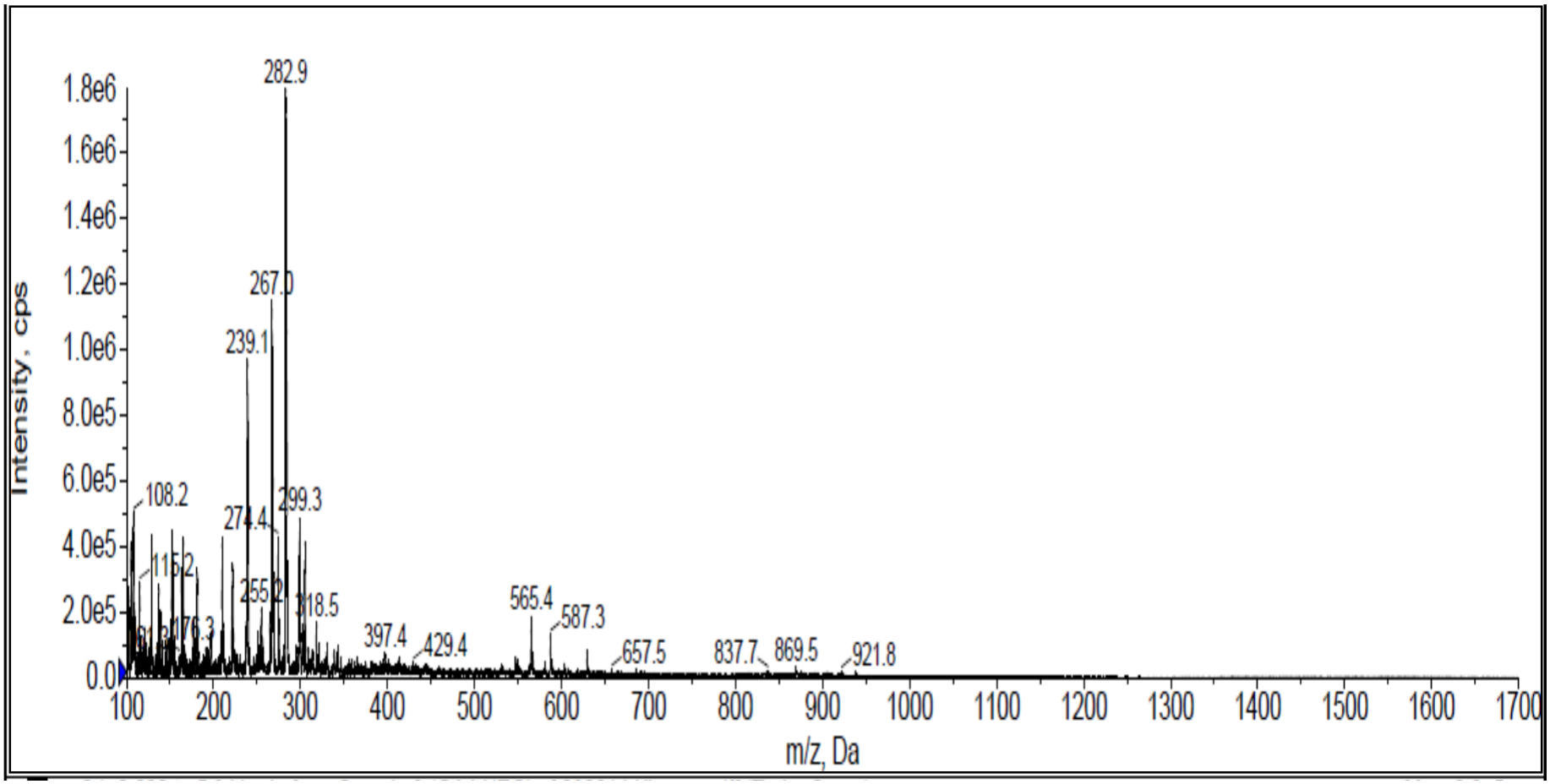


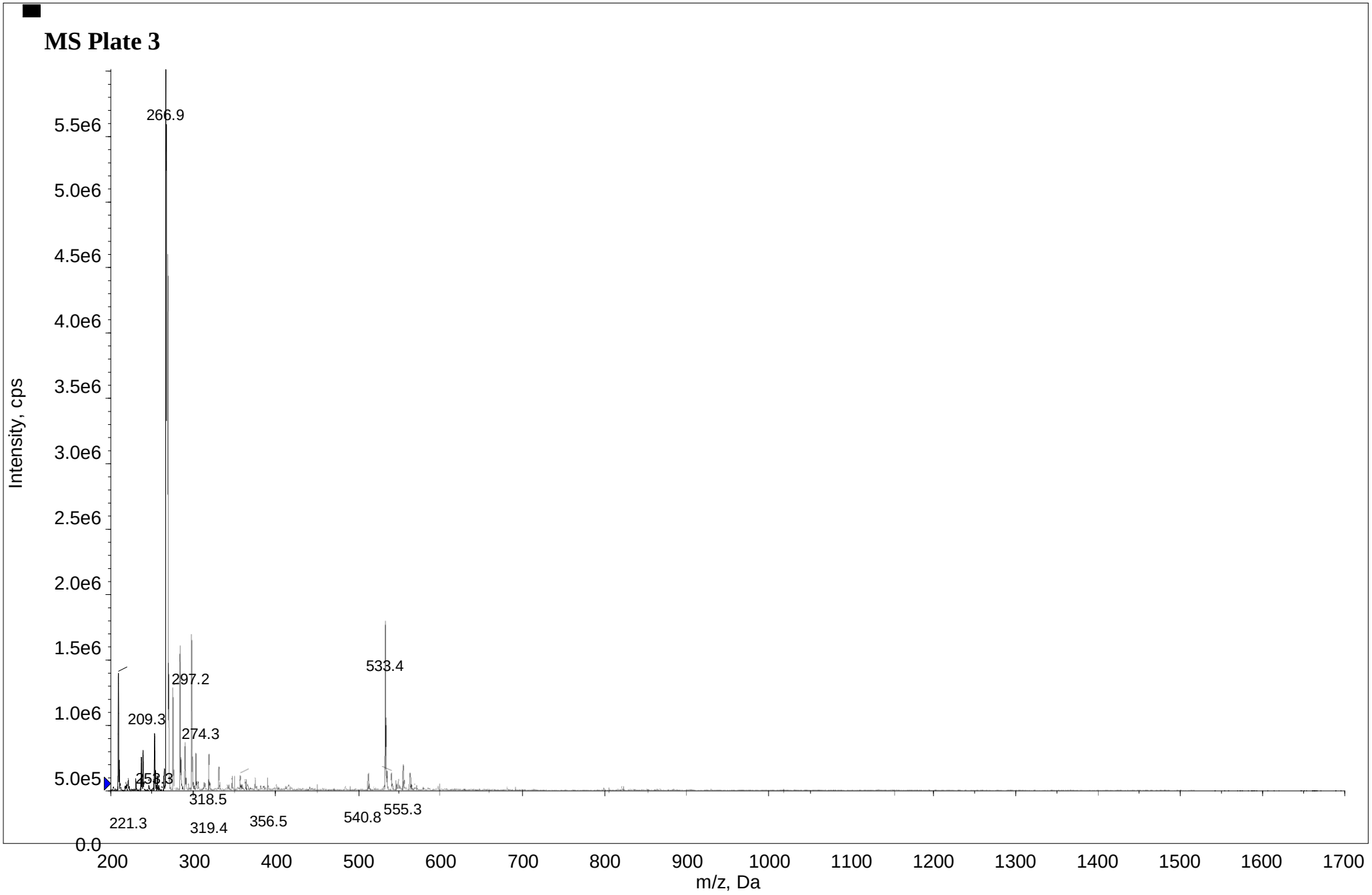
Plate 10 MeOD 13C



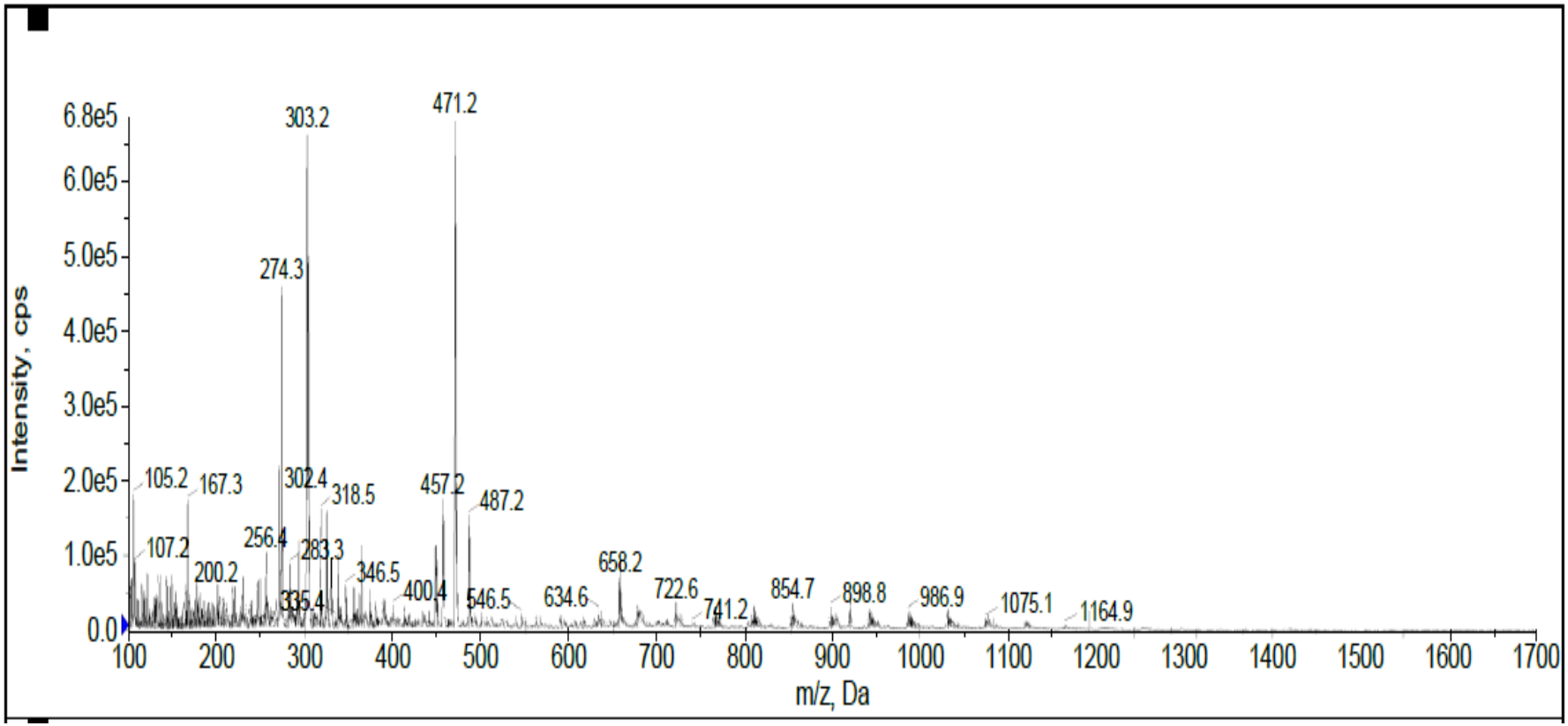

MS Plate 1
282.9
565.4
267.1
305.3
222.2
587.4
274.4
251.3
302.5
321.2
374.6
512.7
595.4
869.6
Intensity, cps
m/z, Da
5.5e6
5.0e6
4.5e6
4.0e6
3.5e6
3.0e6
2.5e6
2.0e6
1.5e6
1.0e6
5.0e5
0.0
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700

MS Plate 2

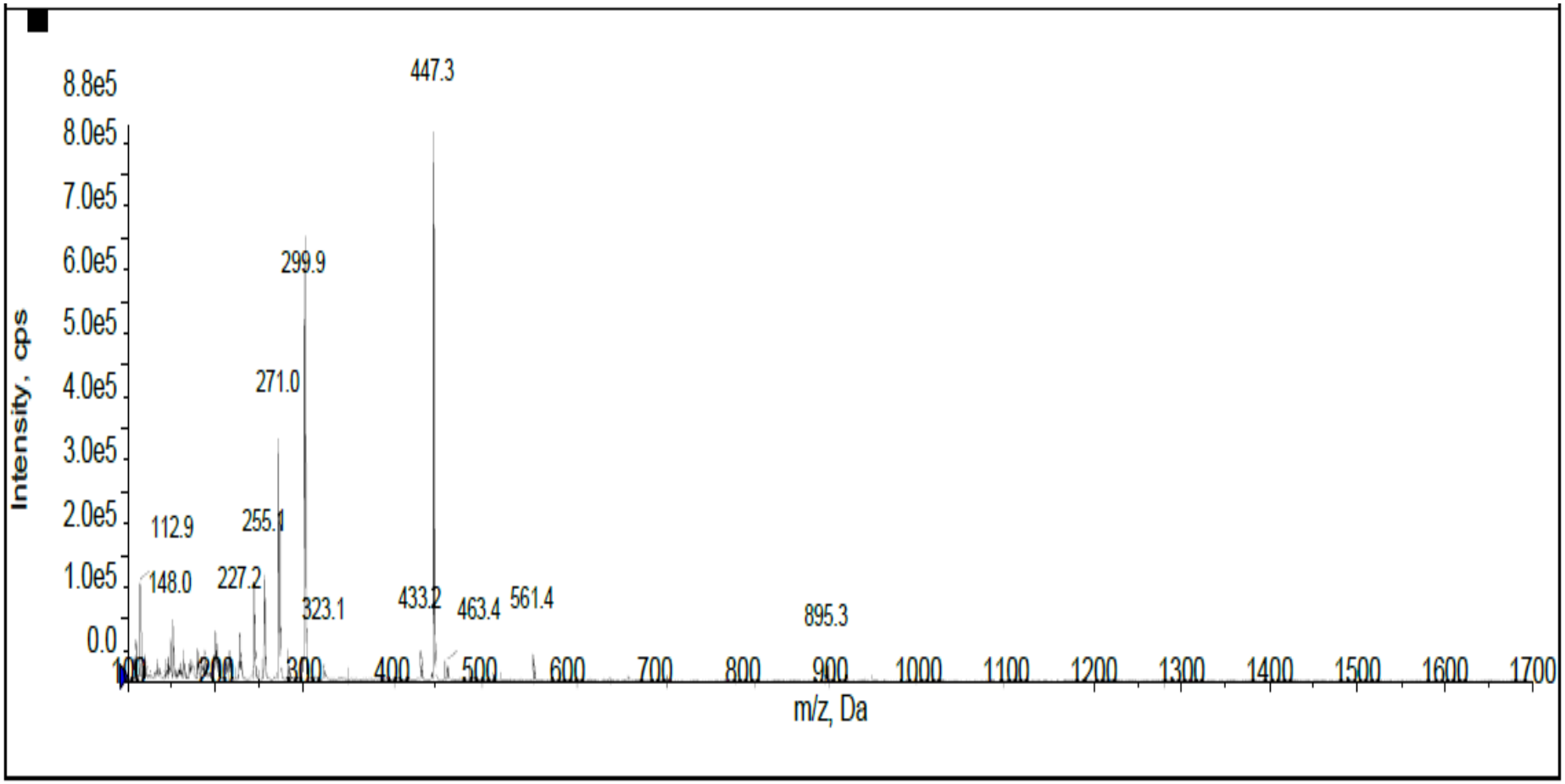

Intensity, cps
1.8e6
1.6e6
1.4e6
1.2e6
1.0e6
8.0e5
6.0e5
4.0e5
2.0e5
0.0
282.9
267.0
239.1
108.2
299.3
274.4
115.2
255.2
318.5
565.4
587.3
397.4
429.4
657.5
837.7
869.5
921.8
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
m/z, Da


MS Plate 3
Intensity, cps
5.5e6
5.0e6
4.5e6
4.0e6
3.5e6
3.0e6
2.5e6
2.0e6
1.5e6
1.0e6
5.0e5
0.0
266.9
533.4
297.2
209.3
274.3
253.3
318.5
221.3
319.4
356.5
540.8
555.3
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
m/z, Da

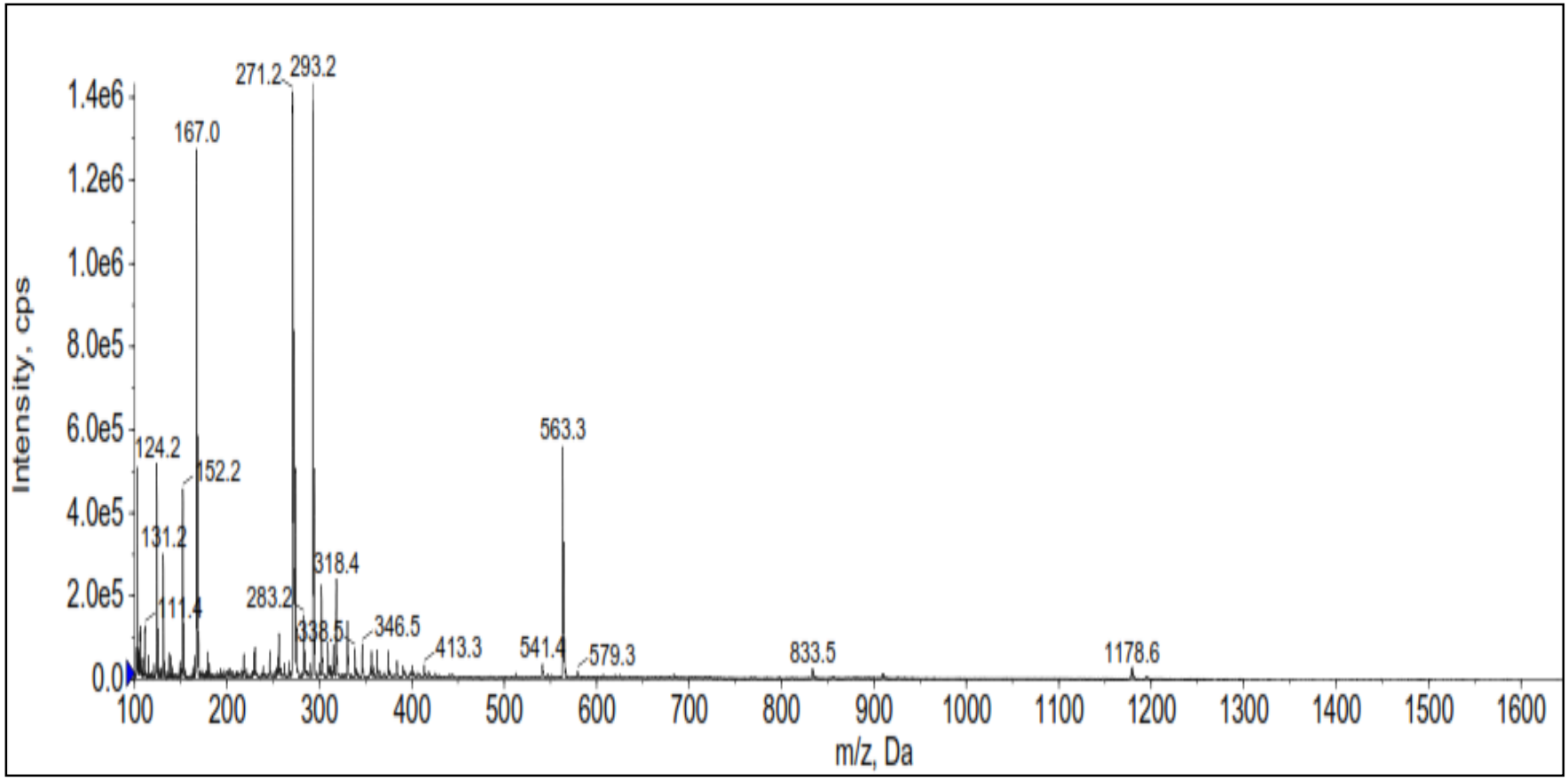
MS Plate 4


Intensity, cps
6.8e5
6.0e5
5.0e5
4.0e5
3.0e5
2.0e5
1.0e5
0.0
303.2
471.2
274.3
105.2
167.3
302.4
318.5
457.2
487.2
107.2
256.4
283.3
658.2
200.2
346.5
722.6
400.4
546.5
634.6
741.2
854.7
898.8
986.9
1075.1
1164.9
335.4
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
m/z, Da

MS Plate 5


Intensity, cps
8.8e5
8.0e5
7.0e5
6.0e5
5.0e5
4.0e5
3.0e5
2.0e5
1.0e5
0.0
447.3
299.9
271.0
112.9
255.1
148.0
227.2
323.1
433.2
463.4
561.4
895.3
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
m/z, Da

MS Plate 6


271.2
293.2
167.0
563.3
124.2
152.2
131.2
318.4
283.2
111.4
338.5
346.5
413.3
541.4
579.3
833.5
1178.6
1.4e6
1.2e6
1.0e6
8.0e5
6.0e5
4.0e5
2.0e5
0.0
Intensity, cps
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
m/z, Da