INVESTIGATION OF CHEMICAL CONSTITUENTS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES; AND PHARMACOGNOSTIC CHARACTERS OF THE LEAVES OF *MOMORDICA FOETIDA* AND *BERKHEYA BERGIANA*

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A THESIS

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DECLARATION

The experimental work described in this thesis was conducted in the Department of Chemistry, Faculty of Science and Agriculture, University of Zululand and at the Natural Product Research Laboratory, School of Chemistry, University of KwaZulu-Natal, Westville campus between March 2007 - December 2008, under the supervision of Prof. A.R. Opoku and Dr. (Mrs.) A.O. Oyedeji.

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I declare the above statement to be true.

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ABSTRACT

This research work was aimed at the isolation, structural elucidation and pharmacological evaluation of the chemical constituents of *M. foetida* (Cucurbitaceae) and *B. bergiana* (Asteraceae) and to determine the pharmacognotic profile of the plants.

Phytochemical screening of the crude ethanol extract of *Momordica foetida* revealed the presence of alkaloids, flavonoids, terpenoids, steroids, saponins and tannins while that of *B. bergiana* had flavonoids, terpenoids, saponins and tannins as their major secondary metabolites.

Six compounds were isolated from the various solvent extracts of the two plants, various chromatographic techniques were employed for the fractionation and purification of the isolates. Spectroscopic methods NMR, IR, MS were employed for the structural elucidation of the compounds. The NMR spectral pattern of four of the isolates suggests a triterpenoid based compound with the characteristics C_{30} carbon content. With the aid of the 1D and 2D NMR, MS and IR, these four compounds were identified as 3*β*,7*β*,25-trihydroxycucurbita-5,(23E)-dien-19-al; 3*β*,7*β*-dihydroxy-cucurbita-5(23E),23,25-triene-19-al; 3*β*-hydroxylup-20(29)-ene and lup-20(29)-en-3*β*-ol-1α-yl-acetate. The remaining 2 isolates were also identified with the help of full house NMR and Mass spectroscopy to be Kaempferol-3-*O*-*β*-*D*-glucopyranoside and 4-hydroxy-3-methoxy-benzaldehyde

Although Kaempferol-3-*O*-*β*-*D*-glucopyranoside has been reported isolated from *Tephrosia calophylla* and *Clitoria ternatea,* this is the first time that it is being reported and isolated from any *Momordica* species. Furthermore, the isolation of 3*β*,7*β*,25-trihydroxycucurbita-5,(23E)-dien-19-al and 3*β*,7*β*-dihydroxy-cucurbita-5(23E),23,25-triene-19-al is noteworthy because of the unique position of hydroxyl group at position 25 and the chromophore conjugation at position 23-25. These two compounds have not been reported isolate from *M. foetida* before.

In-vitro antimicrobial activities of the crude extract and five solvent fractions were screened against 32 bacterial strains using disc diffusion and microplate dilution methods. Ethyl aceate fractions of *M. foetida* exhibited the highest broad spectrum antibacterial activity against the tested bacteria. The antibacterial activity followed the order: of ethyl acetate > butanol > chloroform > aqueous > hexane fraction at a 5.0 mg mL^{-1}. The minimum inhibitory concentration (MIC) of *M. foetida* exhibited by the five

fractions against the bacterial strains ranged between 0.156 and 5.0 mg mL^{-1}. Chloroform extract of *B. bergiana,* exhibited the highest broad spectrum antibacterial activity against the tested bacteria and decreased in the order chloroform > ethyl acetate > butanol > hexane > aqueous extract at a concentration of 5.0 mg mL^{-1} while the minimum inhibitory concentration (MIC) of *B. bergiana* exhibited by successive extracts range between 0.078 mg mL^{-1} and 5.0 mg mL^{-1}.

Antioxidant activity of the extracts and fractions were investigated using four antioxidant assays namely DPPH, ABTS radical scavenging effect, reducing power and metal chelating effect on iron(II) ion. Total phenol, flavonoid and proanthocyanidin contents were also estimated.

This study showed that *M. foetida* and *B. bergiana* possess substantial antibacterial and antioxidant potentials. They may be good potential drugs in the treatment of infection-related diseases because of their antibacterial activity. The antioxidant activity exhibited by the plants also provides a rationale for the ethnomedical use of the plants for the treatment of inflammation, neurodegenerative and cardiovascular disease conditions in traditional medicine.

DEDICATION

This project is dedicated to the glory of God Almighty, the Alpha and Omega the author and finisher of the work of my salvation.

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Whenever one faces a task, it is natural that other people get involved directly or indirectly in its execution. The ideas and responses emanating from these people make significant impact on the successful execution of the task. In view of this, it has become important to mention some of those that have made constructive contributions to ensure the success of this research.

I thank God Almighty for the marvelous things He has done for me. I can do nothing without Him. "It is of the LORD's mercies that we are not consumed, because HIS compassions fail not" Lamentations 3:22

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MAY GOD ALMIGHTY BLESS YOU ALL. AMEN

LIST OF ABBREVIATIONS USED

1D	1-dimensional
2D	2-dimensional
ABTS	2,2-azinobis-3-ethylbenzothiazoline-6-sulphonate
ATCC	American Type Culture Collection
B.B	*Berkheya bergiana*
BuOH	n-Butanol
CC	Column chromatography
CE	Catechin equivalent
$CHCl_3$	Chloroform
COSY	Correlation spectroscopy
CSIR	Council for Scientific and Industrial Research
DEPT	Distortionless enhancement by polarization transfer
DPPH	1,1-diphenyl-2-picryl hydrazyl
EC_{50}	Effective concentration at 50%
EMW	Ethyl acetate methanol water
EtOAc	Ethyl acetate
EtOH	Ethanol
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic acid equivalent
HAT	Hydrogen atom transfer
HEX	Hexane
HMBC	Heteronuclear multiple bond correlation
HMQC	Heterronuclear multiple quantum coherence
IC_{50}	Inhibition concentration at 50%
INT	p-Iodonitrotetrazolium violet
IR	Infra-red
IPUF	Indigenous Plant Use Forum
LIO	Locally Isolated Organism
M.F	*Momordica foetida*

MeOH	Methanol
MIC	Minimum Inhibitory Concentration
MS	Mass Spectroscopy
NCCLS	National Committee for Clinical Laboratory Standards
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Enhancement Spectroscopy
PTLC	Preparative Thin Layer Chromatograpy
QE	Quercetin Equivalent
R_f	Retention Factor
ROS	Reactive Oxygen Species
TLC	Thin Layer Chromatography
UV	Ultraviolet Light
VLC	Vacuum Liquid Chromatography

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2007

- ***O. M. Odeleye***, and A. O. Oyedeji. **"**Preliminary Phytochemical Studies on the Leaves of *Berkheya bergiana***. "**Indigenous Plant Use Forum, Johannesburg, South Africa, 2nd -5th July, 2007.

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- ***O. M. Odeleye*** and O. A. Oyedeji and A.R. Opoku "Phytochemical Screening, Antioxidant and Antimicrobial Activities of *Berkheya Bergiana* Leaves" Joint Symposium IOCD-ISDNP kasane, Botswana, 25th -29th February 2008.

- ***O. M. Odeleye*** and O. A. Oyedeji "*In Vitro* Antibacterial Activities of the Crude and Fractions of *Momordica foetida* Shoot Extract" Plant-Microbial Interaction-2008, Krakow, Poland. 2nd-6th July, 2008.

- ***O. M. Odeleye,*** O. A. Oyedeji, F.O Shode and O.O. Oyedeji **"**Phytochemical Studies on the Leaves of *Momordica foetida***. "**Indigenous Plant Use Forum, Graaf reinet, South Africa, 7nd -10th July, 2008.

- ***O. M. Odeleye*** and O. A. Oyedeji and A.R. Opoku "Phenolic Content, Antioxidant Activity of *Berkheya bergiana* Leaves" 4TH World Congress on Medicinal and Aromatic Plants (WOCMAP IV 2008), held at the Cape Town International Convention Centre (CTICC) Cape Town, South Africa, 9th -14th November 2008

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- ***O. M. Odeleye,*** O. A. Oyedeji and F. O. Shode "Constituents of *Momordica foetida* and Evaluation of their Antimicrobial Activity" on 8th Annual Oxford International Conference on the Science of Botanicals (ICSB) held at Oxford Center, Mississippi, USA. 6th – 9th April, 2009.

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- ***O.M. Odeleye***, and O.A. Oyedeji (2008) *In-vitro* Antimicrobial activities of the crude and fractions of *Momordica foetida* leave extract. (International Journal of Biomedical and Pharmaceutical Sciences 2(2): 75-78)

- *O. M. Odeleye* and O. A. Oyedeji and A.R. Opoku (2011) Antimicrobial Activity of *Berkheya bergiana* Leaves Extracts (African Journal of Biotechnology, 10(20) 4226-4233)

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CHAPTER ONE

1.0 INTRODUCTION

Plants have been used by man for various purposes from time immemorial. They have been a source of food, furniture, shelter, fuel and clothing as well as for the prevention, management and cure of diseases.

Medicinal plant is a plant in which one or more of its organs contains substances that can be used for therapeutic purposes or which is a precursor for the synthesis of more useful drugs (Hamburger and Hostettmann, 1991). Herbal medicine which is the use of medicinal plants in the treatment and cure of sicknesses and diseased conditions, has been with man since the beginning of time. Medicinal plants have been also used for treating domestic animals aliment (Osai, 1998).

Herbal medicine can be classified into two broad categories. The first group constitutes preparations with complex mixtures containing a wide variety of compounds (e.g. Infusions, essential oils, tinctures or extracts), while the second group are used as pure, chemically defined active principles (Hamburger and Hostettmann, 1991). Modern and traditional health care often exist side by side but seldomly cooperate, despite the important contribution herbal medicine makes to primary health care. According to WHO (2002), traditional medicine does not keep pace with scientific and technological advancement, and its methods, techniques and training are often kept secret. The explanation of the use of traditional medicine is also not well defined, and often relies on ritual, mysticism and intangible forces such as witchcraft, with some aspects based on spiritual and moral principles which are difficult to explain. While these may be valid psychologically, they cannot be explained scientifically (Addae-Mensah, 1992). Other objections expressed towards herbal practitioners are that the herbalist is not competent to diagnose chronic illnesses in time (Addae-Mensah, 1992). Until the late 1980s, little attention was shown by the developed world in indigenous traditional knowledge, such that minimal assistance was provided to under-developed countries for the preservation, collection and systemisation of this knowledge. Recently this attitude has undergone a substantial change, and the current interest in natural products has led to increasing respect for the indigenous peoples and their knowledge (Cotton, 1996).

1.1 TRADITIONAL MEDICINE IN SOUTH AFRICA

There is still lack of detailed documentation on the use of medicinal plants in South Africa. This is becoming an urgent issue because of the fragility of oral-traditional knowledge and the rapid pace of urbanisation and acculturation in the country (van-Wyk *et al.,* 1997). The informal (oral) traditional medical systems of the Khoi-San, the Nguni and the Sotho-speaking peoples of South Africa have not yet been systemised. Formal and informal systems of medicine exist side by side in South Africa, the former dating back to the influx of European settlers 300 years ago and the latter possibly to palaeolithic times (van-Wyk *et al.,* 1997). According to WHO (2002), it is estimated that between 60 and 90% of Africa's population rely on medicinal plants to totally or partially meet their health care needs (Addae-Mensah, 1992; Sindiga, 1995). In South Africa about 60% of the population consult one of the estimated 200,000 traditional healers (van Wyk *et al.,* 1997), especially in rural areas where traditional healers are more numerous and accessible than Western doctors (Cunningham, 1988). In KwaZulu-Natal Province, approximately 80% of the population seek medical advice from traditional healers, in preference to or in addition to Western medicine. A large part of the common daily utilised medicines in South Africa are still derived from plants and large volumes of plants or their extracts are sold in informal and commercial sectors of the economy. It is also reported that in South Africa, more than 20,000 tons of plant material, are harvested, processed and sold annually as traditional medicine (Mander, 1997). Medicinal plants in KwaZulu-Natal province alone support traditional health services with a value of more than $30 million annually (Crouch and Arnold, 1997). More than 500 plant species are traded as medicinal plants on the Witwatersrand herbal market alone. These are mostly bark and roots, although stems, leaves, whole plants and bulbs are also sold. The active compounds in leaves, roots or bark can often differ considerably in content and/or concentration, with one plant part active and another inactive. For this reason, whole plants are rarely used in medical preparations (van Wyk *et al.,* 1997). In South Africa, and Africa as a whole, the evaluation and recognition of traditional medicine aims to improve its efficacy, safety, availability and wider application at low cost. Traditional medicine is not a static system, but is dynamic and adaptive and although it reflects the value and perceptions of the people, it is under pressure by the introduction of Western

culture (Iwu, 1993 and van Wyk, *et al.*, 1997). The South African flora consists of over 30,000 species of higher plants, with a biodiversity in the Cape comparable to the tropical rainforests in terms of species richness. Nearly 3 000 of these species are used as medicines with approximately 350 species forming the most commonly traded and used medicinal plants. Thus, there is a growing interest in natural and traditional medicines as a source of new commercial products (van Wyk, *et al.* 1997).

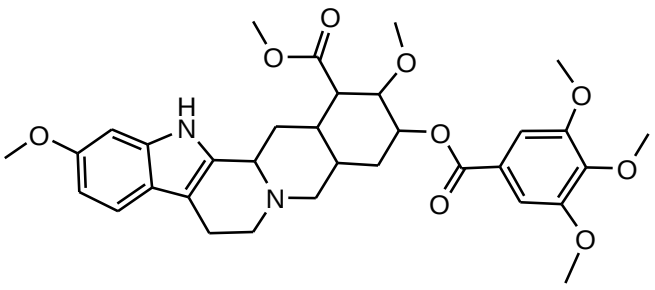
1.2 DRUG DISCOVERY FROM MEDICINAL PLANTS

Plants are valuable sources of new natural products. Despite the availability of different approaches for the discovery of therapeutics, natural products still remain one of the best reservoirs of new bioactive compounds. Pharmacognostic investigations of these plants are carried out to find novel drugs or templates for the development of new therapeutic agents and about 3300 million people use medicinal plants on a regular basis worldwide (Xie, *et al.*, 1998).

Medicinal components from plants also play an important role in conventional Western medicine. In 1984, at least 25% of the prescription drugs issued in the USA and Canada were derived from or modeled after plant natural products (Locker *et al.*, 1995). Farnsworth and Soejarto (1991) identified 119 secondary plant metabolites that are used globally as drugs. It has been estimated that 14 - 28% of higher plant species are used medicinally, out of which only 15% of all angiosperms have been investigated chemically. 74% of pharmacologically active plant derived components were discovered after following up on ethnomedical uses of plants (Locker *et al.*, 1995). Fourie *et al.* (1992) reported that about 300 plants were investigated by Noristan; 31% of these plants had good inhibitory activity, 48% were moderately active and 21% were in-active.

The quest for plants with medicinal properties continues to receive attention as scientists survey plants for a complete range of biological activities. Natural products from some plants, fungi, bacteria and other organisms continue to be used in pharmaceutical preparations either as pure compounds or as extracts. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacological studies leading to synthesis of more potent drugs with reduced toxicity (Khan and Omoloso, 1998; Omoregbe *et al.*, 1996). Some plants natural

products now used include reserpine **(1)** from *Rauwolfa* species as a sedative and aloin **(2)** from *Senna* and *Aloe* species used as a purgative and laxative (Elujoba *et al.*, 1998 and Odeleye *et al.*, 2008).



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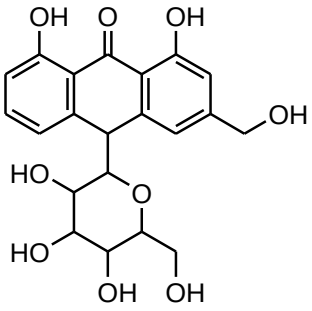
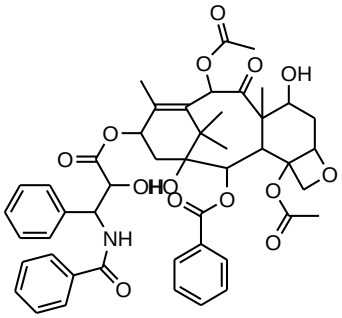


[2]

Recent successes in the field of plant-derived drugs include the anti-cancer agents, taxol **[3]** and camptothecin **[4]**, the Chinese anti-malarial drug, artemisinin **[5]**, and the East Indian Ayurvedic drug, forskolin **[6]** (Balandrin *et al.*, 1993).



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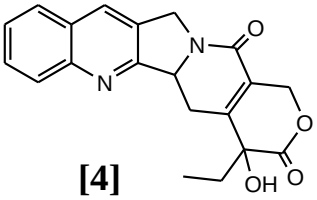

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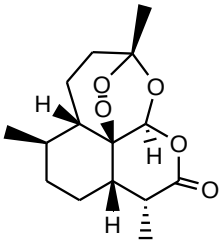

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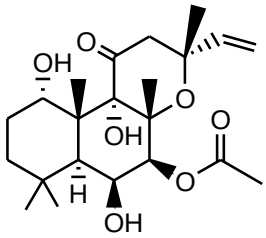


[6]

These illustrate the potential value and relevance of plant-derived secondary metabolites as viable compounds for modern drug development (Balandrin *et al.*, 1993).

Phytochemical studies are generally driven by the desire to discover beneficial compounds in order to add value to the plant resources that are investigated. Groups involved in drug discovery programs often use bioassay-guided fractionation to isolate and identify substances that have positive activity. Over the years several tests have been developed to serve such purposes. Simple bioassays like antibacterial, antioxidant, antifungal and brine shrimp lethality assay are employed (Yesilada, *et al.*, 1999).

With the increasing acceptance of traditional medicine as an alternative form of health care, the screening of medicinal plants for active compounds has intensified. The combination of biological and chemical screening provides important information about plant constituents but will not be a sufficient condition for the discovery of potent new drugs.

The role of plant-derived natural products in drug discovery has recently diminished by the advent of structure activity-guided organic synthesis, combinatorial chemistry and computational (in silico) drug design. Despite drug discovery technology diversification and reduced funding for natural product-based drug discovery, natural products from plants and other biological sources remain an undiminished source of new pharmaceuticals. The ethnobotanical approach to drug discovery has great potential for discovering potent new compounds; however, there are limitations on the type of drug likely to be discovered (Cox, 1994).

1.3 SECONDARY PLANT METABOLITES

Plant secondary metabolites are a generic term used for more than 30,000 different substances which are exclusively produced by plant. The compounds in living organism may be divided into two major groups: primary and secondary metabolites. Primary metabolites are plant compounds that are produced by and involved in primary metabolic processes such as respiration and photosynthesis; other metabolites clearly derived from primary metabolic pathways are considered “secondary”. In practice, there is overlap between the two terms. Secondary metabolites, produced by pathways derived from primary metabolic routes, are numerous and widespread, especially in higher plants. In practice, the difference between primary and secondary metabolites is fuzzy. Plant hormones such as gibberellic acid, indoleancetic acid (auxin), ethylene, kinetin and abscisic acid as well as compounds involved in plant cell wall structure such as cinnamic acid and its polymeric derivative, lignin, are intermediate between primary and secondary metabolism (Birch, 1973).

Secondary metabolites are organic compounds that are not directly involved in the normal growth development or reproduction of plant. Unlike primary metabolites, absence of secondary metabolites results not in immediate death, but in long-term

impairment of the plants survivability/fecundity or aesthetic or perhaps in no significant change at all (Raven, 1999). The amount of any plant secondary compound found in a plant is the result of equilibrium among synthesis, storage and degradation. Regulation of secondary metabolism is complex. The onset of secondary metabolism is linked to the developmental stage of the plant and often closely linked to morphological and cytological change (Haslam, 1986). In general, the formation of product in secondary metabolism appears to be enzymes limited but the level of substances present influences the production of other secondary metabolites, especially in artificial culture (Bu Luck, 1980). The degree to which any one prevails often depends on the developmental stage of the plant and a variety of other factors.

Secondary compounds are often involved in key interactions between plants and their abiotic and biotic environments that influence them. Formulation of many secondary compounds is influenced by environmental stress in both general and specific manners. Plant secondary metabolites respond to changes or differences in soil nutrients but not all plants respond in similar way. An increase in light intensity tends to increase production of phenol compounds and triterpenoids in plants while water stress has been reported to lead to increases in cyanogenic glycosides, glucosinolates, terpenoids, alkaloids and condensed tannins (Waterman and Mole, 1989).

Throughout history secondary metabolites of plants have been utilized by humanity. It is not surprising that many are used as pharmaceuticals, spices, fragrances, pesticides, poisons, hallucinogens, stimulants or colourants (Barz and Ellis, 1981; Luckner, 1990; Wink, 1988). Some still play exotic roles in other cultures as arrow poisons, piscicides (fish poisons) and hallucinogens. These plant metabolites also affect the human and animal health because of their significance in the diet, which has been ascribed to their antioxidant properties (Rice-Evans *et al.,* 1996), estrogenic action (Miksiciek, 1993) and a wide spectrum of antimicrobial and pharmacological activities (Wollenweber, 1988; Weidenborner and Jha, 1994; Dixon and Steele, 1999).

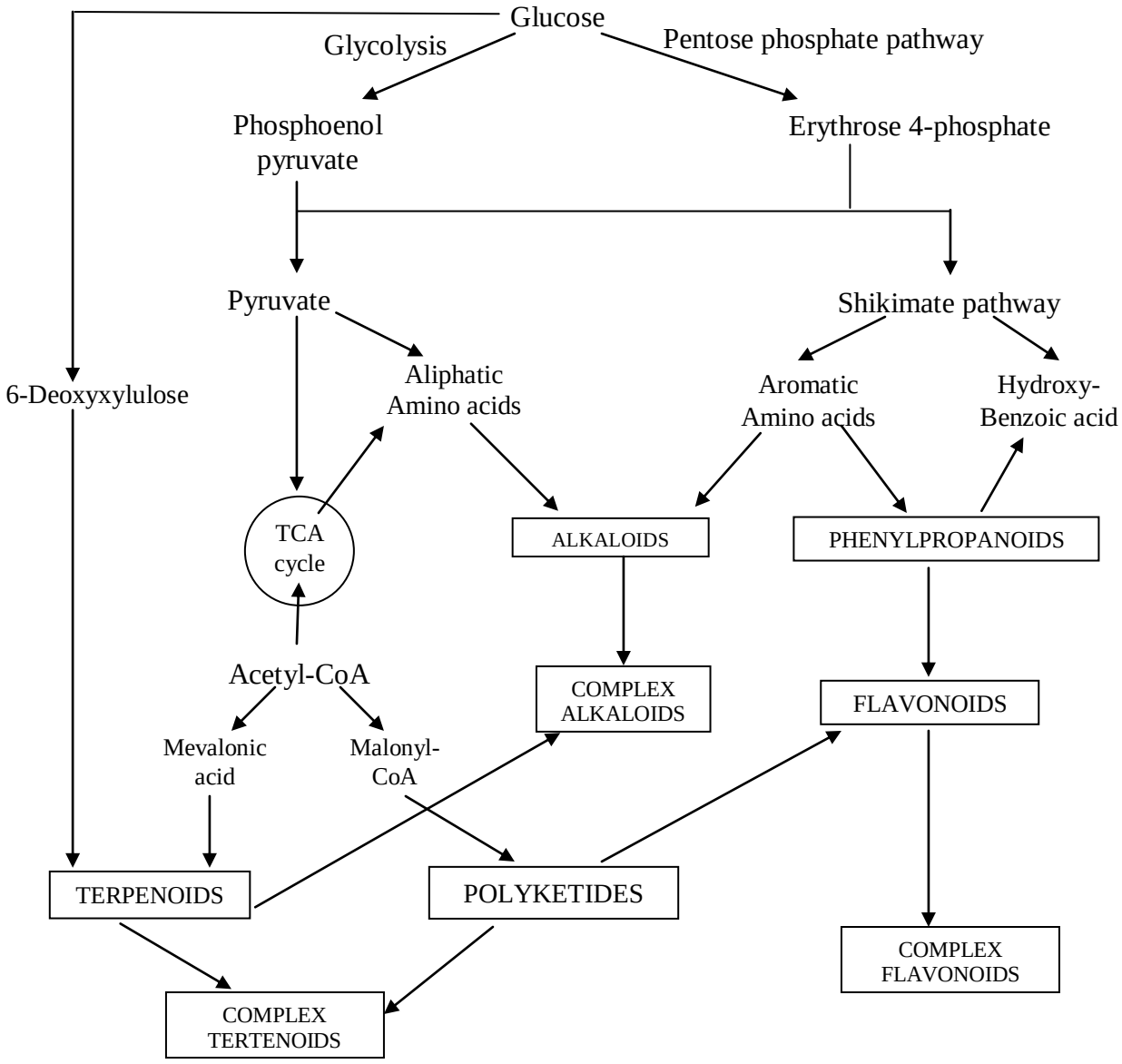
Secondary metabolites belong to one of a number of families, each of which has particular structural characteristics arising from the way in which they are built up in nature, i.e. from their biosynthesis. These classes of secondary metabolites are according to Koehn, *et al.,* 2005

- Polyketides and fatty acids;
- Terpenoids and steroids;
- Phenylpropanoids;
- Alkaloids;
- Specialised amino acids and peptides, and
- Specialized carbohydrates.

Polyketides are formed by the linear combination of acetate (ethanoate) units derived from the "building block", acetyl coenzyme A. Terpenoids and steroids are assembled in nature from isoprenoid (C_5) units derived from isopentenyl (3-methylbut-3-en1-yl) pyrophosphate. These C_5 units are linked together in a head-to-tail manner. They have a characteristic branched chain structure. A further group of natural products are those containing a phenylpropanoids (C_6–C_3) unit (Koehn, *et al.*, 2005).

The amino acids are building blocks for peptides and proteins. Although the amino acids are normally considered as primary metabolites, there are some unusual amino acids that are of restricted occurrence. Some antibiotics, such as the penicillins, are formed from small peptides. The alkaloids are a structurally diverse group of natural products containing nitrogen. The nitrogenous portions of the alkaloids are derived from amino acids such as arnithines, lysine, tyrosine or tryptophan.

Although sugars (carbohydrates), such as glucose, are typical primary metabolites. There are other sugars that are of a much more limited occurrence. Some of these common sugars are attached to natural products as part of a glycoside. The non-sugar portion is known as the aglycone and may be a terpenoid, alkaloid or polyketide (Koehn, *et al.*, 2005).



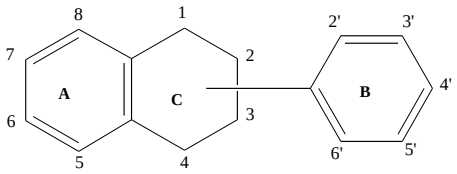
Adapted from Edwards and Gatehouse, 1999, p195

Figure 1.1: Secondary Metabolites pathways

The first stage in forming a secondary metabolite involves the formation of a branch-point enzyme, which determines the certain and the amount of the primary and secondary metabolisms and their pathways (Edwards and Gatehouse, 1999) (figure 1.1) Two main classes of secondary metabolites will be further discussed as these have close colouration with the finding of this study.

1.3.1 FLAVONOIDS

Flavonoids are ubiquitous secondary plant products that are best known by their characteristic red, blue and purple anthocyanin pigments of plant tissues (Winkel-Shirley, 2001). Flavonoids are a large family of compounds sharing the same basic structure i.e two benzene rings (A and B) linked through a heterocyclic pyran or pyrone ring (C) in the middle. They are subdivided primarily according to the varieties of ring C and the position of ring B i.e. they contain fifteen carbon atoms in their basic nucleus, arranged in a C_6-C_3-C_6 configuration consisting of two aromatic rings (A and B) linked by a three carbon unit which may or may not form a third ring (C) (Markham, 1982).



These compounds include six major subgroups that are found in higher plants: the flavones **[7]**, flavonols **[8]**, anthocyanins **[9]**, chalcones **[10]**, flavonodiols **[11,12]** and condensed tannins (or proanthocyanidins) **[13]**; a seventh group, the aurones **[14]**, is widespread, but not ubiquitous (Winkel-Shirley, 2001). Some plant species also synthesize specialized forms of flavonoids **[15-19]** such as the isoflavonoids **[18]** that are found in legumes and a small number of non-legume plants (figure 1.2).

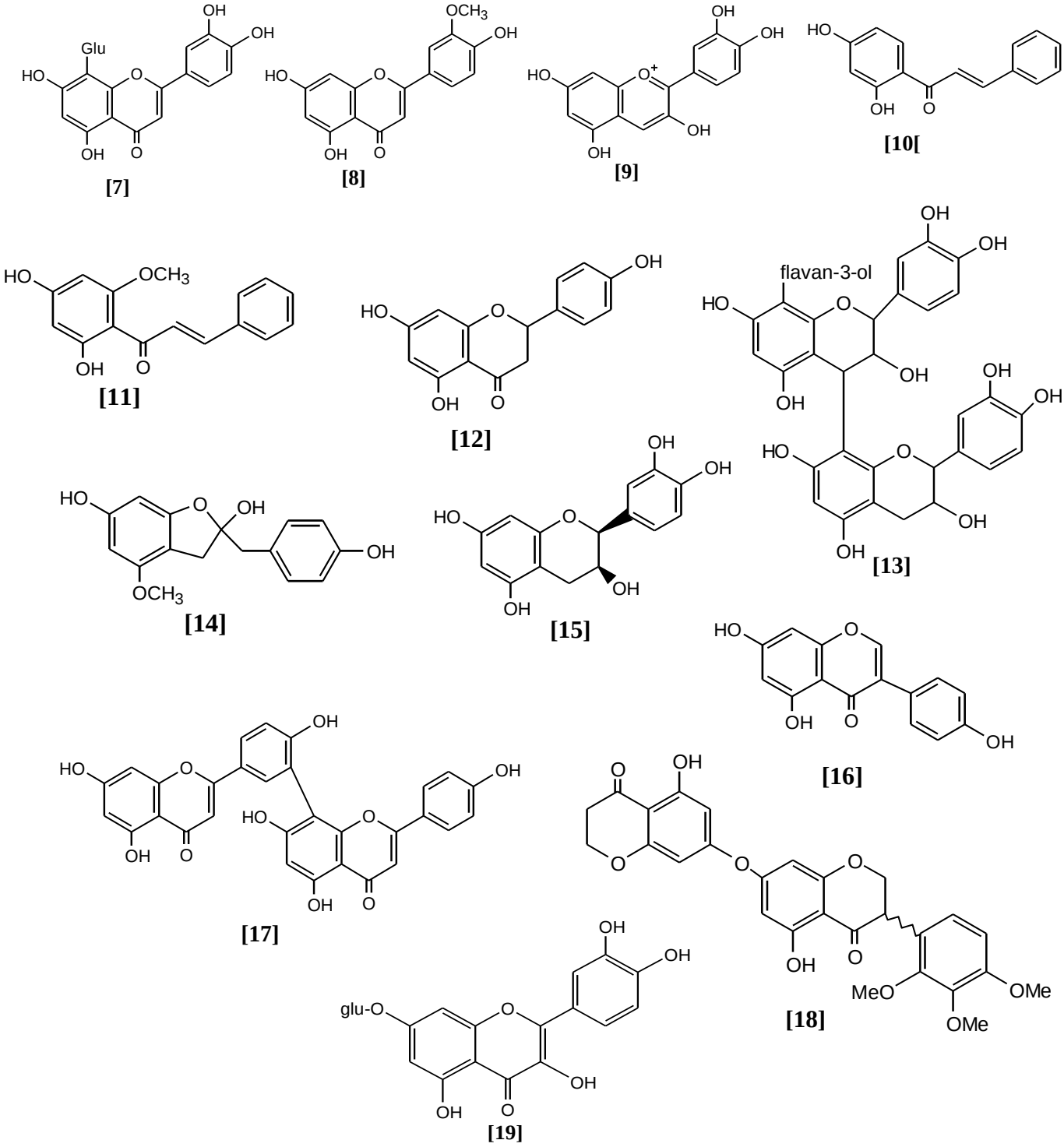


Figure 1.2: Examples of flavonoids structurebelonging to the different subgroups

Flavonoids generally occur in all higher plants where they occur both in the free state and as glycosides (Harborne and Baxter, 1999). Although, most flavonoids in plant cells are present as glycosides, sugar substitution on the flavonoid skeleton may occur through hydroxyl groups as in the case of *O*-glycosides or directly to carbon atoms in ring A of C-

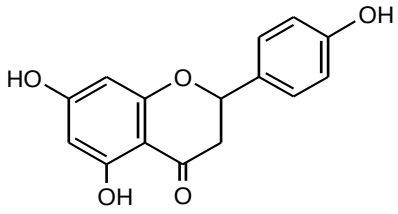
glycosides. The number of sugar rings substituted on the aglycone varies from one to four.

Flavonoids attracted growing global interest during the last decade and as a result of the upsurge in research, the number of known flavonoids has increased dramatically. About 800 different flavonoids as reported by Joachin Kuhnau in Harborne, 1998. At the start of the 1990s, the number of reported flavonoid structures had increased to 4000 (Harborne, 1998) and almost 6500 different flavonoids are known (Harborne and Baxter, 1999)

1.3.1.1 Biosynthesis of flavonoids

Flavonoids are biosynthesised via a combination of the shikimic acid and acylpolmalonate pathways. The mechanism of the biosynthesis involves

1. Deamination of phenyl-alanine by PAL (Phenylalanine ammonia-lyase) to yield cinnamic acid.
2. Conversion of cinnamic acid to p-courmaic acid by cinnamate-4-hydroxylase. Cinnamic acid is not released from PAL into the surrounding environment but is directly transferred to the active centre of the second enzyme-cinnamate-4-hydroxylase.
3. Addition of CoA, catalysed by p-coumarate/CoA ligase to yield *p*-coumaroyl-CoA.
4. p-Coumanyl-CoA new functions as the starting compound in a polyketides synthesis, catalysed by chalcone synthase in which three malonyl-CoA react to form naringenin chalcone **[13]**.
5. The final step is ring closure, catalysed by chalcone isomerase to yield the flavonone naringenin **[20]**.



[20]

Thus in flavonoids, the ring A is formed from acetate, whereas ring B originates from shikimic acid and three carbon atoms connecting rings A and B come from phosphoenol pyruvates. Subsequent hydroxylation of the B-ring occurs after formulation of the C_{15} skeleton and not as previously believed-by incorporation of properly substituted hydroxycinnamic acids. Specific oxygenases introducing the 3'- as well the 5'-hydroxyl group have been characterized. (Figure 1.3).

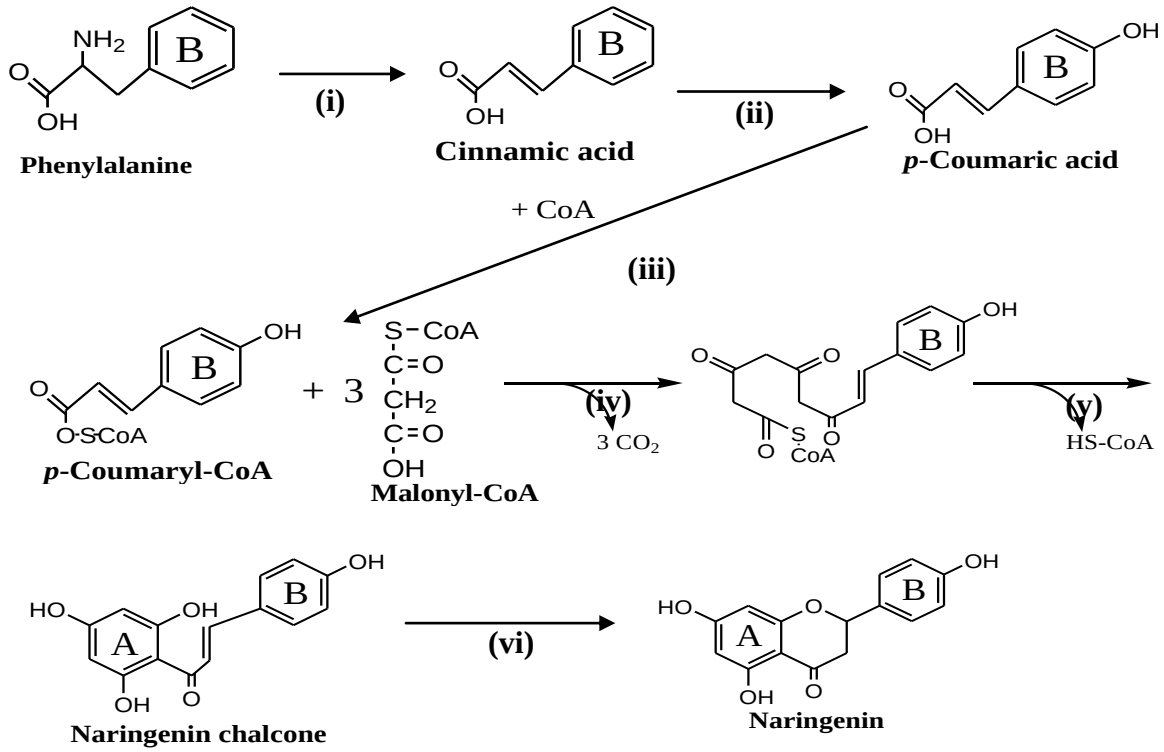


Figure 1.3: Biosynthesis of Flavonoids

1.3.1.2 Biological Activities of Flavonoids

The first suggestion of biological activity of flavonoids was presented by Szent-Gyorgyi in 1938 (Cook and Samman, 1996), who reported that citrus peel flavonoids were effective in preventing capillary bleeding and fragility associated with scurvy. Since then a great number of pharmacological effects have been ascribed to flavonoids and certain individual members of the group have been found to exert multiplicity of actions. Flavonoids exhibit biological effects to a wide range of both *in-vitro* and *in-vivo* activities. (Harborne, 1998; Harborne and William, 2000; Cook and Samman, 1996). These include anti-oxidation, anti-inflammation, anti-platelet, anti-thrombotic, anti-

allergic, anti-proliferation effects (Miean and Mohamed 2001); inhibition of carcinogenic cell invasion, effect on coronary heart disease, anti-microbial and anti-mutagenic (Benavente-Garcia *et al.,* 1997). In addition, they can also inhibit enzymes such as prostaglandin synthase, lipoxygenase, cycloxyganase and those closely related to tumourigenesis and induce detoxifiying enxyme systems such as glutathione S-transferase (Miean and Mohamed 2001).

The presence of flavonoids in vegetables and fruit is thought to be one of the reasons for the beneficial influence on human health of these components of the diet. Flavonoids have been shown to act as scavengers of various oxidizing species, i.e., superoxide anion (O_2^-), hydroxyl radical or peroxyl radicals. They may also act as quenchers of singlet oxygen. Flavonoids do not react specifically with a single species and so a number of different evaluation methods have been developed which makes comparison of the various studies very difficult. Often an overall antioxidant effect has been measured. Differences between the antioxidant potential of selected compounds can be measured using many different techniques. Because most phytochemicals are multifunctional, a reliable antioxidant protocol requires the measurement of more than one property relevant to either foods or biological systems (Frankel and Meyer, 2000).

Certain structure-antioxidant activity relationships of flavonoids have been reported in the literature. Das and Pereira (1990) studied the activity of carbonyl group and a double bond and found that they are important features for high antioxidant activity in flavonoids. Shahidi and Wanasundara (1992) also reported that the presence of 3-OH group attached to the double bond and its location adjacent to the 4-carbonyl in the C-ring, are required for the maximum effectiveness of radical scavenging. The weaker antioxidative potential of catechins can be enhanced to the stage of quercetin by incorporation of an additional 5'-OH group in the B ring (Salah *et al.,* 1995; Bouchet *et al.,* 1998; Guo *et al.,* 1999). This result in (-)-epigallocatechin-3-O-gallate being one of the most efficient scavengers of the superoxide radical (Jovanovic *et al.,* 1995). Another possible contributory mechanism to the antioxidant activity of flavonoids is their ability to stabilize membranes by decreasing membrane fluidity. Therefore, flavonoids have significant activities when ingested by animals and there is great interest in their potential health benefits particularly for compounds such as isoflavonoids, which have been linked

to the anticancer benefits of soya-based foods and the stilbenes in red wine that are believed to contribute to reduced heart disease.

1.3.2 Terpenoids

An enormous range of plant substances are covered by the word 'terpenoid', a term which is used to indicate that all such substances have a common biosynthetic origin. Terpenoids constitute the largest family of natural plant products with over 30,000 members (Sacchettini and Poulter, 1997; Dewick, 2002). Terpenoids have their carbon skeletons based on the isoprene molecule, $CH_2=C(CH_3)-CH=CH_2$, which could be from the union of two or more of these isoprene units. Terpenoids are classified by the homologous series of a number of five carbon isoprene units in their structure: hemiterpenes C_5 (1 isoprene unit), monoterpenes C_{10} (2 isoprene units), sesquiterpenes C_{15} (3 isoprene units), diterpenes C_{20} (4 isoprene units), triterpenes C_{30} (6 isoprene units), tetraterpenes C_{40} (8 isoprene units), polyterpenes $(C_5)n$ where 'n' may be 9 – 30,000 (McGarvey and Croteau, 1995).

1.3.2.1 Biosynthesis of Terpenoids

Biosynthetic origin of isoprene unit is known. Isopentenyl pyrophosphate exits in living cells in equilibrium with the isomeric dimethylallyl pyrophosphate $(CH_3)_2C=CHCH_2OPP$. Terpenoid biosynthesis involves mostly head to tail addition of isopentenyl diphosphate (IPP, the active C_5 isoprene unit), to its isomer dimethylallyl disophosphate (DMAPP) synthesizing geranyl diphosphate (GPP, C_{10}). Isopentenyl pyrophosphate (IPP) is formed itself from acetate via mevalonic acid, $CH_2OH-CH_2C(OH,CH_3)CH_2COOH$. Further, condensation of enzyme-bound geranyl diphosphate with additional IPP units forms successively larger prenyl diphosphates e.g. farnesly diphosphate (FPP, C_{15}), geranylgeranyl diphosphate (GGPP, C_{20}), that might undergo cyclisation, coupling and/or rearrangement to produce the parent carbon skeleton of sesquiterpenes and diterpene (Figure 1.4) (Singh *et al.,* 1989, McGarvey and Croteau, 1995; Luthra *et al.,* 1999). GPP and FPP yield monoterpene and sesquitepene skeletons, respectively. Furthermore, FPP and GGPP dimerize to product parental precursors are subjected to structural modification through oxidation, reduction, isomerization,

hydration, conjugation and/or other transformations to give rise to a variety of terpenoids (McGarvey and Croteau, 1995).

In summary, terpenoids biosynthesis can be divided into four stages. Firstly, there is the formation of the isoprene unit, isopentenyl pyrophosphate, followed by the association of these units to form the $(C_5)_n$ isoprenoid backbone of the terpenoid families, the cyclization of these to generate the carbon skeletons; and finally, there are the interrelationships, hydroxylations and oxidations that lead to the individual terpenoids (figure 1.4).

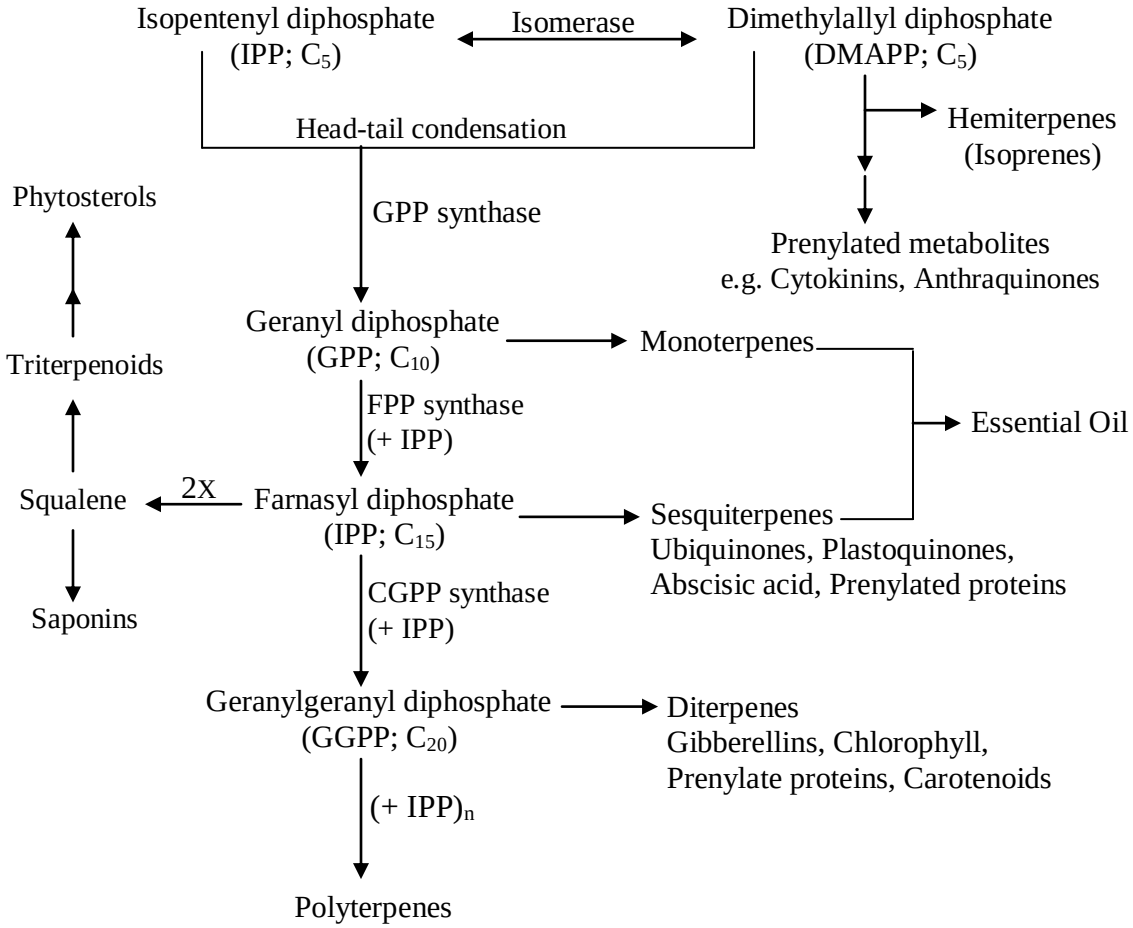


Figure 1.4: Biosynthesis of various classes of terpenoids

1.3.2.2 Biological Activities of Terpenoids

Terpenoids are known to have many important biological and physiological functions and they play multifunctional roles in plants, human health and commerce (Singh *et al.*, 1989; Dubey, 1999; Mahmoud and Croteau, 2002).

Those that are produced in nature because of their biological activity may well find commercial use as drugs or pest control agents. Canter *et al.,* (2005) discuss some areas within biotechnology to improve medicinal plant cultivation. Many commercial uses of terpenoids reflect their natural uses. Tian *et al.,* (2006) summarized the bioactivity of different kinds of terpenoids from all aspects including anti-tumour, anti-virus, antibacterial, anti-inflammation, immune-regulatory, cardiovascular system, hepatic protection, and so forth. For examples, α-santonin from *Artemisa maritime*, is used as an anthelmintic. The poisonous nature of foxglove is due to the presence of terpenoid glycosides which have strong stimulant action on heart muscles. Digitoxin is a glycoside of digitoxigenin used in treatment of certain heart condtions. The odourous terpenoids are used as fragmence ingredients in cosmetics, toiletries and household products. Cineole is used in perfumery as well as a nasal decongestant.

1.4 PHYTOCHEMICAL SCREENING

Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plant for particular secondary metabolites. Basic phytochemical screening consists of performing simple chemical tests to detect the presence of some of the secondary metabolites which include alkaloids, tannins, saponins, anthraquinones, cardenolide etc in a plant extract (Harborne, 1998).

Tests used in phytochemical screening should be simple and rapid. Most recent approaches to phytochemical screening have involved sophisticated instrumentation such as gas-liquid chromatography linked to mass spectrometry (GLC/MS) and high performance liquid chromatography (HPLC). The separation system in which HPLC is linked to mass spectrometry also allows for quick identification of natural products from small quantities of material (Wolfender *et al.,* 1993). The phytochemical screening of crude extracts therefore constitutes an efficient complemetary approach allowing localization and targeted isolation of new types of constituents with potential activities. This procedure also enables recognition of known metabolites at the earliest stage of separation, thus avoiding time consuming and expensive isolation of common constituents. One major drawback of bioassay-guided fractionation strategy is the frequent isolation of previously known secondary metabolites.

Phytochemical screenings are not only used to search for bioactive agents but also to predict the biological or pharmacological activities. However, it is more difficult to design an isolation protocol for a crude extract where the types of compounds present are totally unknown. The most important factor that has to be considered before designing an isolation protocol is the nature of the target compound present in the crude extracts or fractions. The large-scale isolation of a useful constituent discovered through phytochemical screening is usually achieved chemically (i.e. without biological monitoring) though trial and error.

1.5 CHEMICAL SCREENING

Isolation of pure, pharmacologically active constituents from plants remains a long and tedious process. For this reason, it is necessary to have methods available which eliminate unnecessary separation procedures. Chemical screening is thus performed to allow localization and targeted isolation of new or useful constituents with potential activities. This procedure enables recognition of known metabolites in extracts or at the earliest stages of separation. Chromatographic techniques play an important role as an analytical support in the work of phytochemists for the efficient localization and rapid characterization of natural products.

1.5.1 Extraction Methods

The extraction and characterization of active compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic values (Huic, 2002). Extraction is a process of bringing out or removal of the chemical constituents from the plant or animal tissues with or without the use of a menstruum (solvent), i.e. extraction is the withdrawing of an active agent or a waste substance from a solid or liquid mixture with a liquid solvent. The solvent is not or is only partial miscible with the solid or the liquid being extracted. By intensive contact the active agent transfers from the solid or liquid mixture (raffinate) into the solvent (extract). After mixing, the two phases are separated which is achieved either by gravity or centrifugation. The following methods of extraction exist depending on the phases:

- Solid-liquid extraction (SLE)

- Liquid-liquid extraction (LLE)

The precise mode of extraction naturally depends on the texture and water content of the plant material being extracted and on the type of substance that is being isolated. Plant constituents can be extracted from both dried and fresh materials. Dried materials must be powdered before extraction while fresh materials may be used directly or homogenized before extraction.

Sample preparation is the crucial first step in the analysis of herbs, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. A broad spectrum of solid-liquid extraction (SLE) techniques is widely used for the early purification of natural products from plant material and micro-organism. Classically, solid-liquid extraction can be divided into traditional and recent/modern methods. Traditional methods include soxhlet extraction, maceration, percolation, turbo-extraction (high speed mixing) and sonication. These techniques have been used for many decades; however they are very often time-consuming and require relatively large quantities of polluting solvents. Supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and pressurized solvent extraction (PSE) are fast and efficient unconventional extraction methods developed for extracting analytes from solid mixtures in solid-liquid extraction.

1.5.2 Choice of suitable extraction methods

There are various extraction methods for screening plants for activity. Selection of the solvent extraction approach is very important for a plant under investigation from an ethnobotanical perspective, than the extraction should mimic the traditional use. Several organic solvents with varying elution effects find common usage in phytochemical research for extracting plant metabolites. This is in addition to a variety of techniques that can be used to prepare extracts that provide sufficient scope for improvement in extraction methods (Cordell, 1993). The choice of the solvent is dependent on the end purpose of the extract. For example, if the plant is known to contain sugars, it would be pointless to extract with hexane. Ethyl acetate or ethanol, which are more polar are preferred. If all the constituents are to be examined, however, a series of solvents with increasing dielectric constant should be used sequential e.g hexane-chloroform-ether-

ethyl acetate in turn. If the extraction is with a view solely to isolating chemical components (without any bioassay), toxicity of the solvent is not critical, since the extract can be made solvent-free, before subsequent isolation procedure. If the extraction aims to screen for anti-microbial activity, it is essential that the solvent by itself should not inhibit the bioassay procedure (Eloff, 1998a). The biggest problem in drug development from plant extracts is the nature of the extract that requires testing. The extractants petroleum ether, chloroform, ethanol, methanol and water (Salie, *et al.*, 1996), 80% ethanol (Vlietinck *et al.*, 1995), methanol (Taylor, *et al.*, 1995), acetone and methanol-chloroform-water mixtures (Eloff, 1998a) have been used with varying success. The widely employed Soxhlet extraction of wet and dried plant material using solvents with increasing polarity has limitations for thermo-labile compounds. Although the problem can be overcome by extracting under reduced pressure, it is a tedious process. Some solvents, like water, take longer and, if not properly evaporated, could result in microbial contamination. Other advanced methods like microextraction employing, microwaves, help in separating several plant constituents in a short time, often in a few minutes. However, there are several disadvantages associated with these techniques. The advent of environmentally friendly 'green' technologies based on superheated water (that extracts under pressure above 100 ^{o}C, but below it critical temperature of 374 ^{o}C), carbon dioxide based supercritical fluid extraction (SCFE) at 31^{o}C and 73.8 bar pressure and SCFE-polar solvent mixtures is being increasingly adapted for greater efficiency in extraction yields and for obtaining value-added compounds of foods, cosmetic and agrochemical importance.

Once the extract is obtained, it is subjected to bioassay-guided fractionation. Pure constituents can also be obtained by subjecting the crude extract to separation techniques like open-column chromatography, HPLC, MPLC, GC and structural elucidation of various constituents carried out by analytical methods including mass spectroscopy (MS) and nuclear magnetic resonance (NMR), circular dichroism (CD) and X-ray crystallography as shown in Figure 1.5

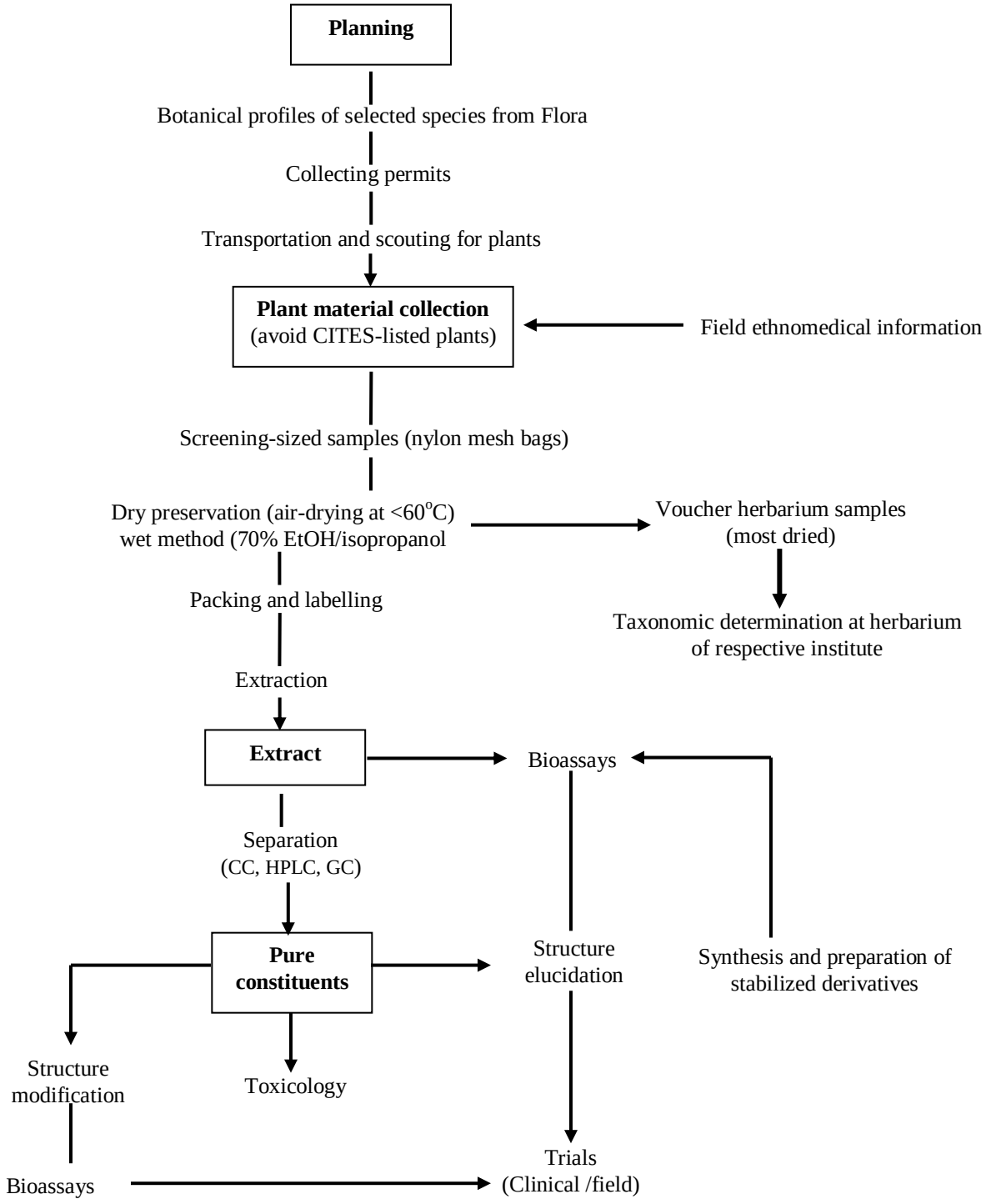


Figure 1.5: From plant to bioactive phytochemicals (modified from Soejarto and Hostettman *et al.,2000*)

1.5.3 Techniques for Isolation and Purification of Natural Products

The ability to separate and purify steady decreasing amounts of natural products from complex matrices has been a major factor in the burgeoning of known natural products since 1980 (Cordell, 1995). A crude natural product extract is literally a cocktail of compounds, i.e., natural product extracts in most cases are highly complex mixtures of neutral, acidic, basic, lipophilic, hydrophilic or amphiphilic compounds. Isolation and purification of natural products is the most vital, yet laborious and difficult, step in natural product research. It begins with the process of extraction followed by various separation techniques. It is difficult to apply a single separation technique to isolate individual compounds from this crude mixture. The most important factor that has to be considered before designing an isolation protocol is the nature of the target compound present in the crude extracts or fractions.

The separation, identification and structure determination of biologically active compounds has been facilitated by continual development of chromatographic and spectroscopic methods of analysis. It is important to keep in mind that the isolation of natural products differs from that of the more prevalent biological macromolecules. This is because natural products are typically secondary metabolites and as such are smaller in size, chemically more diverse in structure and present in smaller concentrations than the more homogeneous proteins, carbohydrates, lipids, nucleic acids and the like. A variety of different techniques can be used for the isolation and purification of natural product compounds. These techniques include, but are not limited to, solid-phase extraction, high-performance liquid chromatography, gradient high-performance liquid chromatography, bioautography; thin-layer chromatography (TLC), countercurrent chromatography (CCC), droplet countercurrent chromatography, vacuum column chromatography (VLC), desalting, liquid-liquid chromatography, paper chromatography, ion exchange chromatography, gel chromatography, gas liquid chromatography, flash column chromatography, etc.

In this study five techniques were used:

i. Solvent-Solvent fractionation
ii. Thin Layer Chromatography (TLC)
iii. Vacuum Liquid Chromatography (VLC)

iv. Preparative Thin layer chromatography (PTLC)

v. Column chromatography (CC)

1.5.3.1 Solvent-Solvent Fractionation

This technique is also known as partitioning. Partition is possibly the simplest separation method which is widely used as an initial extraction purification step. The purpose of solvent-solvent partitioning is to simplify extraction by fractionating the chemical compounds into broad groups based on their solubility (Suffness and Dous, 1982). All separation processes involve the division of a mixture into a number of discrete fractions. The type of fractionation depends on the individual sample and the aims of the separation.

A part from the available chromatographic separation techniques, solvent partitioning method can be successfully applied in the separation of different classes of natural products. In fact, before the introduction of chromatographic techniques, solvent partitioning method and recrytallization techniques were extensively used for the separation and purification of natural products. The separation technique using solvent partitioning involves primarily the use of two immiscible solvents in a separating funnel and the compounds are distributed in the two solvents according to their different partition coefficients. This method is relatively easy to perform and highly effective as the first step of the fairly large-scale separation of compounds from crude natural product extracts.

The fundamental strategy for separating these compounds is based on their physical and chemical properties that can be cleverly exploited to initially separate them into various chemical gropus. Alcoholic (MeOH or EtOH) extracts of plant materials contain a wide variety of moderately polar to polar compounds. By virtue of co-solubility many compounds which are insoluble individually in pure state in MeOH or EtOH can be extracted quite easily with these solvents. A dried alcoholic extract can also be extracted directly with a suitable solvent.

A typical partitioning scheme is presented in Figure 1.6. A MeOH extract is re-dissolved in small amount of water and the volume can be reduced to an appropriate level that can be handled easily with a separation funnel. Partitioning uses two immiscible

solvents to which the extract is added; this can be sequential by using immiscible organic solvents of increasing polarity. Typically, this may take place in two steps: (1) water/hexane to generate a non-polar fraction in the organic layer; (2) water/chloroform; water/ethyl acetate and water/butanol to give a medium-polar fraction in the organic layer. The remaining aqueous layer will contain polar water-soluble natural products. This is a soft separation method and relies on solubility of natural products and not a physical interaction with another medium

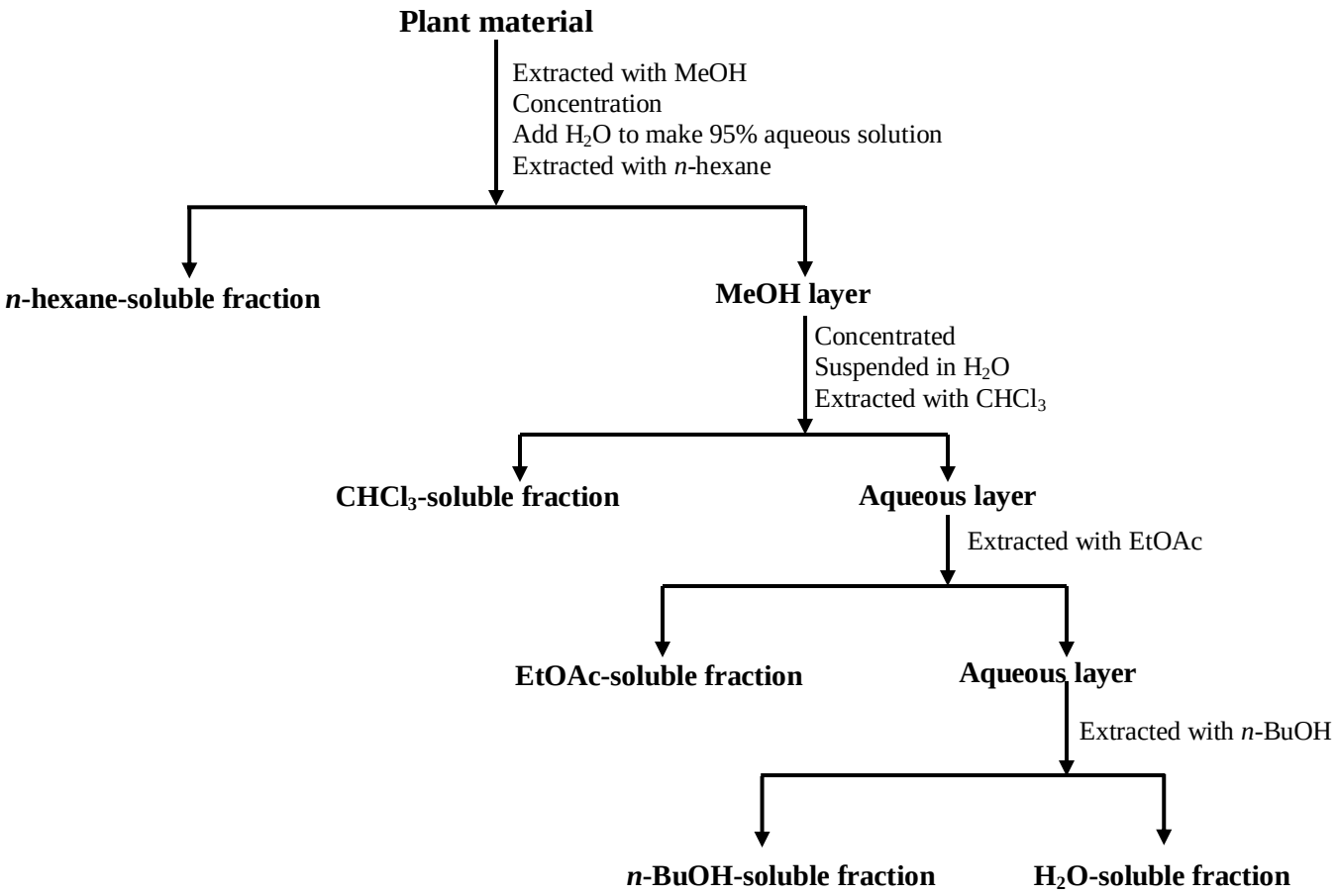


Figure 1.6: A typical partitioning scheme using immiscible solvents

1.5.3.2 Chromatographic techniques

Chromatography is one of the most useful means of separating mixtures of compounds, as a technique to both purify the components and identify them. In chromatography, the mixture is separated by differential distribution of the components between a stationary

phase and a mobile phase. Primary methods of chromatography employed in isolation and analysis of natural products include the following: thin-layer chromatography (TLC), liquid-column chromatography (CL), gas chromatography (GC), high-performance liquid chromatography (HPLC), fast protein liquid chromatography (FPLC), immobilized metal-ion affinity chromatography and antibody affinity chromatography (Heftmann, 1992a, 1992b; Poole, 1991; Porath, 1988). There are probably five major separation mechanisms of chromatography. They include adsorption chromatography, gas chromatography, liquid-liquid partition chromatography, ion-exchange chromatography and size-exclusion chromatography. Adsorption chromatography can be further subdivided into normal-phase chromatography and reversed-phase chromatography.

Chromatography as a technique is now probably the most widely used separation analytical method available to chemists. This is the lynchpin of phytochemistry and is the key to obtaining pure compounds for structure elucidation, for pharmacological test or for development into therapeuticals. It also plays a fundamental role as an analytical technique for isolation using other forms of chromatography. The advance made through the introduction of thermospray liquid chromatography mass spectrometry were reviewed by Hostettmam and colleagues (Wolfender *et al.,* 1994) and a summary given of the numerous diverse application in the analysis of almost every class of natural product.

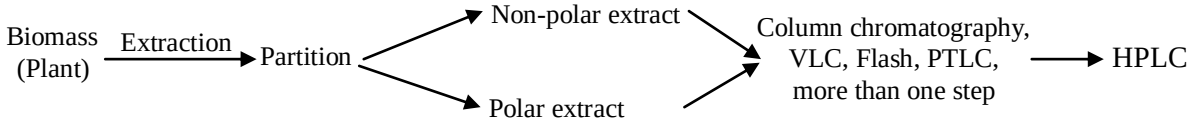
Thin Layer Chromatography (TLC)

Thin-layer chromatography (TLC) is the only chromatographic method offering the option of presenting the result in an image form, that is, TLC is the sole technique in which all the components of the sample are included in the chromatogram. TLC is the simplest and cheapest method of detecting plant constituents because the method is easy to run and requires little equipment (Marston, *et al.,* 1997). However, for efficient separation of metabolites, good selectivity and sensitivity of detection, together with the capability of providing on-line structural information, hyphenated high performance liquid chromatographic (HPLC) techniques are preferred (Hostettman *et al.,* 1997). The real breakthrough in TLC came through the work of Stahl, who introduced the use of calcium sulphate as binder and standardized layer thickness and chromatographic development (Stahl, 1958).

Not only does the TLC technique give visual result but it excels in its simplicity and its low cost. Parallel analysis of samples is possible, sample capacity is high and results are obtained rapidly. TLC is flexible and effective in multiple detection of sample components. It is an ideal screening method in biological and chemical analysis, providing identification and qualitative results, determination of adulteration and together with quantitative and semi-quantitative determination that this is the type of chromatographic technique that gives a clue as to how many components are in a mixture or an extract. Different solvent systems can be used. In conjunction with microorganisms and other biological agents, TLC bioautography can be used to screen for bioactivities (Hostettmann *et al.,* 1997). The disadvantages of TLC include lack of automation, the problems of reproducibility which sometimes occur and the lack of accuracy in quantitation. Nevertheless, TLC will remain a fast and simple micro technique of chromatography.

Preparative Thin-layer Chromatography (PTLC)

PTLC has long been a popular method of isolation, primarily because of its universal accessibility to researchers working in natural product chemistry. This popularity has diminished in recent years owing to the success of HPLC and counter current chromatography (CCC). Unlike these two techniques, PTLC does not, however require expensive equipment. Separations can be effected rapidly and the amount of material isolated generally falls into the 1 mg to 1 g range, which is certainly sufficient for structure elucidation purposes. Although separations depend on the level of complexity of an extract, HPLC is nearly always used as a final purification step of an isolation procedure. A broad procedure is given here



Vacuum Liquid Chromatography (VLC)

Vacuum liquid Chromatography (VLC), a name coined by Targett *et al.*, (1900). was developed because of the impatience of Australian chemist with classical column

chromatography. The technique is essentially a preparative layer chromatographic (PTLC) separation run as a column; the flow is activated by vacuum. It differs from flash chromatography in that VLC involves step-by-step gradient elution and the column is allowed to run dry after each fraction is collected. The similarity with PTLC then becomes obvious because PTLC separation may be enhanced by repetitive running of PTLC, plates dried between runs. Although Pelletier *et al.*, (1986) describe a very simple VLC apparatus for small-scale laboratory applications and a variant for larger scale separations, the most practical set-up is that shown in Figure 1.7 (Pelletier *et al.*, 1986).

A short column or a Buchner filter funnel fitted with glass frit (10-20 um, porosity D or pororsity 2) is dry-packed with sorbent (19-20 µm of TLC grade, e.g. Merck 60H or 60G silica gel). The adsorbent is allowed to settle by gentle tapping under gravity. Then vacuum is applied via the three-way stopcock and the adsorbent compressed to a hard layer by pressing with a rubber stopper and tapping. The vacuum is released, solvent of low polarity is poured quickly onto the surface of the adsorbent and vacuum is re-applied. When the eluent is through, the column is sucked dry and is ready for loading. The sample in a suitable solvent is applied directly to the top of the column and is drawn gently into the packing by applying the vacuum. Alternatively, the sample is preadsorbed on silica gel, aluminium oxide or celite. The column is developed with appropriate solvent mixtures, starting with solvent of low polarity and gradually increasing the polarity, pulling the column dry between each fraction collected (this helps to avoid channeling).

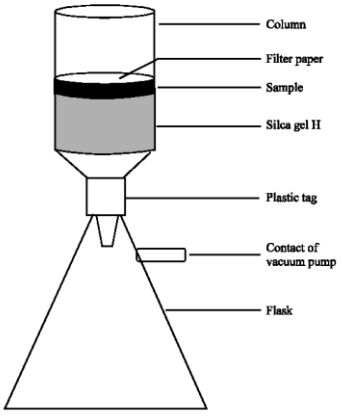


Figure 1.7: Vacuum Liquid Chromatographic Diagram

Fractions are collected in a round-bottomed flask or in a suitable separatory funnel. The use of a separatory funnel avoids the problem of changing the flask for each fraction. In contrast to methods which use pressure applied at the top of the column to increase flow rates, manipulations on the VLC column (e.g. solvent changes) are easy because the head of the column is at atmospheric pressure. Targett introduced a modification to avoid channeling (Targett *et al.*, 1979). This system was designed to operate under conditions of continuous vacuum and used a longer column to increase resolution. However, the simplicity of the original method is lost in this particular version. Finally, vacuum liquid chromatography method works extremely well and is very quick. The apparatus is simple and universally available and the saparations are efficient in terms of time, amount of adsorbent and volume of solvent (Pelletier *et al.*, 1986).

Column chromatography (CC)

This is the preparative application of chromatography. It is used to obtain pure chemical compounds from a mixture of compounds on a scale from micrograms up to kilograms using different sizes of columns. The classical preparative chromatography column is a glass tube with a diameter from 5 to 50 mm and a height of 50 cm to 1 m with a tap at the bottom. A slurry is prepared of the eluent with the stationary phase powder and then carefully poured into the column. Care must be taken to avoid air bubbles. A solution of the organic material is pipetted on top of the stationary phase. This layer is usually topped with a small layer of sand or with cotton or glass wool to protect the shape of the organic layer from the velocity of newly added eluent. Eluent is slowly passed through the column to advance the organic material. Often a spherical eluent reservoir or an eluent-filled and a closed separating funnel is put on top of the column. The individual components are retained by the stationary phase differently and separate from each other while they are running at different velocity through the column with the eluent. At the end of the column they elute one at a time. During the entire chromatography process the eluent is collected in a series of fractions. The composition of the eluent flow can be monitored and each fraction is analyzed for dissolved compounds.

1.6. STRUCTURAL ELUCIDATION

The chemical structures of natural product compounds are tremendously diverse and can be very simple, beautiful and well-made in their nature (Yu *et al.,* 2002). Such diversity can present a challenge to the analytical or medicinal chemists attempting to unravel the mystery of the chemical structure of an unknown material presented to them. The investigation of natural products chemistry has been and continues to be an integral part of the development of organic chemistry.

In most cases of extraction and isolation of natural products, the end point is the structure elucidation of the isolated compounds. Ideally, bioassay-guided methods should afford a pure natural product of known activity. However, structure elucidation of compounds isolated from plants or other organisms is generally time consuming and sometimes can be the "bottleneck" in natural product research. There are many useful spectroscopic methods of getting information about chemical structures but the interpretation of their spectra normally requires specialist with detailed spectroscopic knowledge and wide experience in natural products chemistry. With the remarkable advances made in the area of artificial intelligence and computing, there are a number of excellent automated structure elucidation programs available that could be extremely useful (Steinbeck, 2004; Blinov *et al.,* 2003).

Today, natural products chemists have an array of highly sophisticated instrumentation available to perform strucuture elucidation. The basic techniques for the structure eludication of natural products have changed very little in the past ten or fifteen years, they are still Infra-red (IR), Ultra-violet (UV), Proton and carbon-13 NMR spectroscopy, Mass spectroscopy (MS), optical rotation and circular dichroism. Mass spectrometry and nuclear magnetic resonance spectroscopy are the most widely used. A combination of the many forms of ^{1}H and ^{13}C NMR spectroscopy can provide extraordinary amounts of structural information and arguable, in most cases it is sufficient for characterization. It is worth mentioning that some particularly exciting developments in structure determination pertinent to the area of natural products have come from the field of Computer-Assisted Structure Elucidation (CASE).

If the target compound is known, it is often easy to compare preliminary spectroscopic data with literature data or to make direct comparison with the standard sample.

However, if the target compound is an unknown and complex natural product, a comprehensive and systematic approach involving a variety of physical, chemical and spectroscopic techniques is required. Information on the chemistry of the genus or the family of plant under investigation could sometimes provide additional hints regarding the possible chemical class of the unknown compound. The following spectroscopic techniques are generally used as mentioned above for the structure determication of natural products:

- **Ultraviolet-visible spectroscopy (UV-vis):** Provides information on chromophores present in the molecule. Some natural products e.g flavonoids, isoquinoline alkaloids and coumarins, to name a few, can be primarily characterized (chemical class) from characteristic absorption peaks.
- **Infrared spectroscopy (IR):** Determines different functional groups e.g –C=O, -OH, $-NH_2$, aromaticity and so on, present in a molecule.
- **Mass spectrometry (MS):** Gives information about the molecular mass, molecular formula and fragmentation pattern. Combining chromatography with mass spectrometry provides the advantage of both chromatography as a separation and mass spectrometry as an identification method.
- **Nuclear Magnetic Resonance (NMR):** NMR is the most powerful spectroscopic tool for obtaining structural details of complex organic compounds. It is a valuable strucuture elucidation tool for organic and biological molecules. This technique reaveals information on the number and types of protons and carbons present in the molecule and the relationships among these atoms (van de Ven, 1995). Besides qualitative information, NMR can provide valuable quantitative information about a sample. The NMR experiments used today can be classified into two major categories:

a) One-dimensional techniques: ^{1}H-NMR, ^{13}C-NMR, ^{13}C-DEPT. These ID ^{1}H-NMR spectrum are commonly recognized as reliable methods for quantification.
b) Two-dimensional techniques: ^{1}H-^{1}H COSY, ^{1}H-^{1}H DQF-COSY, ^{1}H-^{1}H COSY-IR, ^{1}H-^{1}H NOSY, ^{1}H-^{1}H ROESY, ^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HMBC, ^{1}H-^{13}C HMQC, ^{1}H-^{13}C HSQC, HSQC-TOCSY.

In addition to the above-mentioned spectroscopic techniques, X-ray crystallographic techniques provide information on the crystal structure of the molecule and polarimetry offers information on the optical activity of chiral compounds.

1.7 BIOLOGICAL SCREENING/ACTIVITIES OF MEDICINAL PLANTS

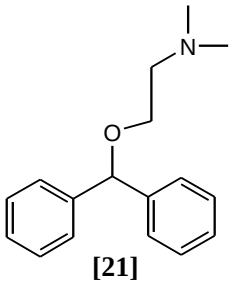
Detection of compounds with the desired activity in complex plant extracts depends on the reliability and sensitivity of the test systems used. Bioassays are also essential for monitoring the required effects throughout activity-guided fractionation, all fractions are tested and those exhibiting good activity are further isolated and purified until the active substances are obtained. The bioassays to be employed must be simple, inexpensive and rapid in order to cope with large number of samples – including extracts from the screening phase and all fractions obtained during the isolation procedure. They must also be sensitive enough to detect active principles which are generally present only in small concentrations in crude extracts. Their selectivity should be such that the number of false positives is reasonably small (Hostettmann *et al.,* 1995).

1.7.1 Anti-inflammatory activity

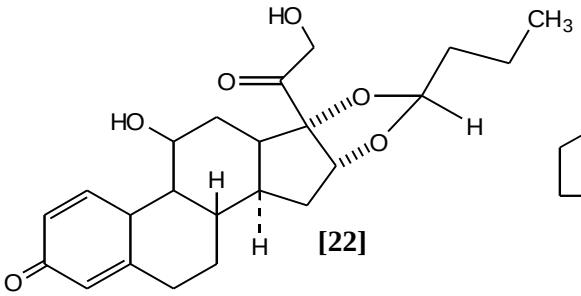
Inflammation is a localised protective reaction of cell/tissues of the body to allergic or chemical irritation, injury and/or infection. Inflammation is the body's response to acute or chronic injury. Acute inflammation is characterized by rapid onset and is of short duration. It is characterized by the exudation of fluids and plasma proteins, and the migration of leukocytes, most notably neutrophils into the unjured area. This acute inflammatory response is believed to be a defense mechanism aimed at killing of bacteria, virus and parasites while still facilitating repairs. Chronic inflammation is of a more prolonged duration and manifests historically by the presence of lymphocytes and macrophages, resulting in fibrosis and tissue necrosis. The persistent chronic inflammation increases the development of the degenerative diseases such as rheumatoid arthritis, atherosclerosis, heart diseases, asthma, cancer etc, all of which are associated with immunopathology that appears to play a key role in the onset of the condition (O'Byrne and Dalgleish, 2001; Dalgleish and O'Byrne, 2002)

The symptoms of inflammation are characterized by pain, heat, redness, swelling and loss of function that result from dilation of the blood vessels leading to an increased blood supply and increased intercellular spaces resulting in the movement of leukocytes, proteins and fluids into the inflamed regions (Parham, 2000). Similarly, cell from the tissues remove and consume the dead cell, sometimes with the production of pus and the process of healing commences. In certain circumstances healing does not occur and chronic inflammation ensures. Diseases and disorders are manifested through inflammatory responses as the body recognizes the injury and prepare to repair the damage.

Various groups of anti-inflammatory drugs act against one or more of the mediators that initiate or maintain inflammation (Gibbon and Swanepoel, 1995). Some groups suppress only certain aspects of the inflammatory response. The main groups of anti-inflammatory drugs are the antihistamines **[21]**, the glucocorticoids (Corticosteroids **[22]**) and the nonsteroidal **[23]** anti-inflammatory drugs (NSAID). Most of the action and problems of the NSAID's have been attributed to their ability to inhibit cyclooxygenase, the enzyme responsible for prostaglandin synthesis.



[21]



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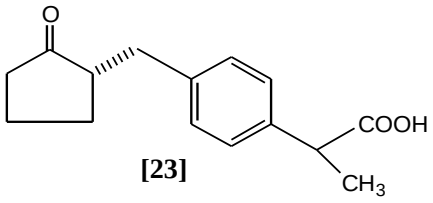


[23]

More than 115 plant species of 60 families are used in the treatment of inflammatory-pain disorders in South Africa (Iwalewa, 2007). The evidence pointing to the fact that these plants are used for inflammatory disorders arose from the various indications that emanated from ethnomedical surveys and the diverse arrays of chemical constituents found in these plants.

Plant secondary metabolites that are capable of modifying the activities of inflammatory cell include flavonoids, triterpenoids, sterols (phytosterols), tannins, alkaoloids, chalcones, carotenes, vitamins A, E and C, limonoids and essential/volatile oils.

1.7.2 Antimicrobial Activity

An antimicrobial compound is a substance that kills or inhibits the growth of microbes such as bacteria (antibacterial activity), fungi (antifungal activity), viruses (antiviral activity), or parasites (anti-parasitic activity). During the last ten years the pace of development of new antimicrobial drugs has slowed down while the prevalence of resistance has increased astronomically (Cunningham, 1988). The increase in number of antibiotic resistant bacteria is no longer matched by expansion in the arsenal of agents available to treat infections. Literature reports and ethnobotanical records suggest that plants are the sleeping giants of pharmaceutical industry. They may provide natural sources of antimicrobial drugs that will provide novel or lead compounds that may be employed in controlling some infections globally (Locker, *et al.*, 1995).

An antimicrobial review of the South African literature reveals a broad-spectrum of research activity where traditional medicinal practices are used to treat a variety of infections including skin disorders, tuberculosis, urinary tract infections and gastrointestinal disorders (van Vuuren, 2008). Majority of the studies dedicated to the antimicrobial activity of South African plants focus on extracts. Screening activities are initially the first choice of investigation with the intention of identifying plants with potential antimicrobial activity for further study. In addition to random screening studies, there have been reports dedicated to the investigation of various genus such as the antimicrobial activities from *Carpobrotus* species (Springfield *et al.*, 2006) and intensively studied *Helichrysum* genus (Afolayan *et al.*, 1997). Other microbial related botanical studies have involved the isolation of antimicrobial compounds from South African plant species. Collaboration between phytochemists, botanists and microbiologists has yielded some valuable contributions. Although a number of reports have focused on the isolation and identification of bio-active compounds, several studies on the isolation of bioactive compounds from South African plants have been published: (Afolayan and Meyer, 1997; Bremner and Meyer, 1998, 2000; Dilika *et al.*, 2000; Mathekga *et al.*, 2000; Drewes *et al.*,; 2005, 2006; van der Kooy *et al.*, 2006; and Kamatou *et al.*, 2007).

Assays for plants with antimicrobial activity are particularly important in developing countries due to the prevalence of infectious diseases of bacterial or fungal

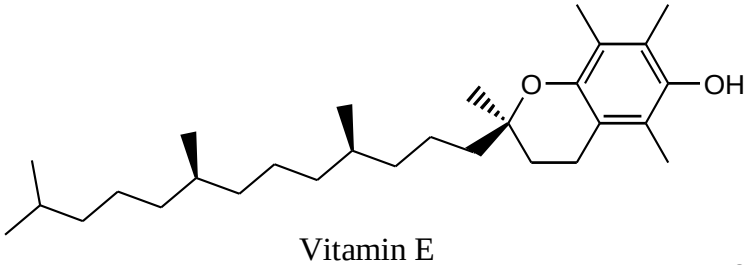
origin. For instance, there is a great need for cheaper, more effective antimicrobial and antifungal agents for topical. Antimicrobial assays can be classified into three groups, namely, agar diffusion, agar dilution and bioautographic methods (Onawunmi, 2002). These methods do not require any sophisticated infrastructure. The agar diffusion method has the advantage of the small size of sample used in the screening and is suitable for testing large numbers of samples for activity against a single organism. For screening plant extracts against a battery of microorganisms, the agar dilution method in a Petri dish with multipoint inoculation or using a 96-well microtitre plate is the most suitable technique. The two most common methods used to investigate the antimicrobial activity of South African medicinal plants are disc diffusion and minimum inhibition concentration (MIC) assay. Initially, disc diffusion studies were the first method of choice, possibly due to their simplicity and capacity to analyse a large number of test samples. The MIC measurement used is for quantitative determination of antimicrobial activity based on the principle of contact of a test organism to a series of dilutions of test extracts or fractions or isolated compounds. Assays involving MIC methodology are widely used as an accepted criterion for measuring the susceptibility of organism to inhibitors (Lambert and Pearson, 2002). The use of the more quantifiable MIC assay techniques are presently the preferred method of antimicrobial assessment (Kalemba and Kunicka, 2003).

Leaf extracts (water, ethanol, and methanol) of *Momoedica charantia* have clinically as well as experimentally demonstrated broad-spectrum antimicrobial activity (Khan *et al.*, 1998). *In vitro* antimicrobial activity of extracts of leaves of *Momordica charantia* showed good antimicrobial activity against *Escherichia coli, Salmonella paratyphi*, *Shigella dysenterae* and against *Streptomyces griseu* (Hugo and Russel, 1984) and an extract of the entire plant was also shown to have antiprotozoal activity against *Entamoeba histolytica*l (Khan *et al.,* 1998). Extract of fruit of *Momordica charantia* has also being reported to have inhibitory effect against *Helicobacter pylori-organism* with MICs range between 1.95 and 250 µg/mL (Hostettmann and Hamburger, 1991). In a phase II study, *M. Charantia* leaves extract showed inhibition of *Mycobacterium tuberculosis* growth using susceptibility test method (Khan *et al.,* 1998).

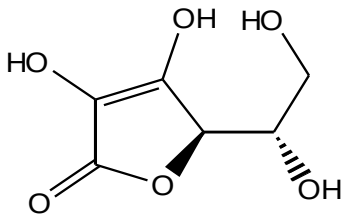
1.7.3 Antioxidant activity

As the name implies, antioxidants are substances that are capable of counteracting the damaging (but normal) effects of the physiological process of oxidation in animal tissue. Antioxidants are substances or nutrients in foods which can prevent or slow down the oxidative damage to our body. When our body cells use oxygen, they naturally produce free radicals (by-products) which can cause damage. Antioxidants are also nutrients (vitamins and minerals) as well as enzymes (proteins) in our body that assist in chemical reactions. Antioxidants act as "free radical scavengers" and hence prevent and repair damage done by these free radicals. Health problems such as heart disease, muscular degeneration, diabetes, cancer etc are all contributed to by oxidative damage.

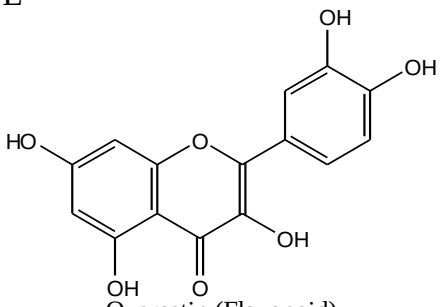
Free radical reactions, especially with participation of oxidative radicals have been shown to be involved in many biological processes that cause damage to lipids, proteins, membranes and nucleic acids, thus giving rise to a variety of diseases (Atoui, *et al.*, 2005). Reactive oxygen species (ROS) have been recognized as playing an important role in the initiation and/or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular disease. Thus, recent studies have investigated the potential of plant products to serve as antioxidants against various diseases induced by free radicals. Plants rich in antioxidants provide protection against cancer and cardio- and cerebrovascular diseases through their capacity to scavenge free radicals. In recent years, the prevention of cancer and cardiovascular diseases has been associated with the ingestion of fresh fruits, vegetables or tea rich in natural antioxidants. Plant constituents that often contain substaintial amounts of antioxidants include tocopherols (Vitamin E) **[24]**, carotenoids, ascorbic acid **[25]**, flavoinoids **[26]** and tannins (Geetha, *et al.*, 2007; Newmark, 1996; Kahkonen, *et al.*, 1999; Sugihara, *et al.*, 1999; Di Carlo, *et al.*, 1999; Brace, *et al.*, 2003; Oktay, *et al.*, 2003).



Vitamin E
[24]



Ascorbic acid
[25]



Quercetin (Flavonoid)
[26]

McCume and Johns in 2002 suggested that antioxidant action may be an important property of plant medicines associated with diabetes.

Phenolic compounds are among the largest and most ubiquitous groups of plant metabolites that possess an aromatic ring bearing one or more hydroxyl constituents. Current interest in this group of compounds stems from their antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic activities (McCune and Johns, 2002; Chung, *et al.,* 1998). Phenolic compounds exert their protective effects through diverse mechanisms like preventing the formation of carcinogens from precursor substances by acting as blocking agents or suppressory agents (Pietta, 2000; Pereira, *et al.,* 1996; Thompson, 2002 ; Atoui, *et al.,* 2005). There have been numerous studies on the biological activities of phenolics which are potent antioxidants and free radical scavenger (Newmark, 1996). Among naturally occurring phenolic compounds, flavonoids have gained a particular interest because of their broad pharmacological activity (Claudine, *et al.,* 2004). Reports reveal the antioxidant activity of flavonoids to be due to their ability to scavenge for oxygen radicals and inhibiting peroxidation (Kahkonen, *et al.,* 1999). The potential value of such antioxidants prompted investigators to study new flavonoids to improve the treatment of various diseases. Moreover, the list of newly discovered flavonoids is constantly growing due to the enormous structural diversity associated with these compounds (Sugihara, *et al.,* 1999). A variety of antioxidants are collectively

required for the removal of free radicals to protect the body from adverse effect of ROS. Certain enzymes as well as non-enzymatic cellular molecules are involved in the detoxification of ROS. Based on the nature of antioxidants, the human antioxidants system can be categorized into two broader classes: enzymatic and non-enzymatic (Sugihara, *et al.*, 1999).

The use of TLC for biological test is a means for discovering new antioxidants in higher plants (Cuenet, *et al.*, 1997). Antioxidant can be detected on a TLC plate by spraying with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Antioxidants reduce the radical, producing white spots on a purple background. A number of experimental models have been developed for the determination of antioxidant activities in different samples. These methods can be divided into two major categories (Vaya and Aviram, 2001) including measuring the potential of a sample to donate an electron or a hydrogen atom to a specific reactive oxygen species or to any electron acceptor and measuring the ability to remove any source of oxidative initiation such as inhibition of enzymes, chelation of transition metal ions and absorption of UV radiations. These are the most common used methods of antioxidant assay for measurement of antioxidant potential of South African medicinal plants

- The DPPH method
- ABTS radical cation scavenging activity
- Reducing power
- Metal chelating capacity
- *β*-Carotene–linoleic acid assay
- Scavenging of hydrogen peroxide

1.8 LITERATURE REVIEW

1.8.1 *Momordica foetida* Schumach and Thonn.

Genus: *Momordica*

Family: Cucurbitaceae

Common Name: Gifappel

Zulu names: intshungu, itshungu.

1.8.1.1 Origin and geographic distribution

Momordica foetida occurs in forest clearings and at outskirts of villages. *M. foetida* is widespread in tropical Africa and in South Africa. *M. foetida* occurs also in margins of swamps and on disturbed ground as a weed and colonizer, up to 2400 m altitude (Dokosi, 1998).

1.8.1.2 Botanical Description of *M. foetida*

M. foetida are dioecious, perennial herb rootstock, trailing or climbing plants with simple or bifid tendrils. The stem is up to 4.5 m long, with dark green flecks when young, woody when old, rooting at the nodes. The leaves are alternate, simple while stipules are absent The petiole is 1.5–17 cm long and the blade is broadly ovate-cordate to triangular-cordate of 1.5–16 cm × 1.5–17 cm and the base deeply cordate with rank of odour. Flowers are unisexual, regular, 5-merous and with obconic tube calyx and lobes up to 11 mm long and the petals free and obovate-lingulate of up to 3.5 cm long. The flowers are white, pale yellow to orange-yellow and about 3 with scales inside at base. Male flowers 1-9 (only 1 opening at a time) fasciculate at the apex of a 20-230 mm long peduncle and immediately subtended by an elliptic, obovate-spathulate or broadly ovate-cordiate 3-22 mm long, 1-26 mm broad bract enclosing the buds when large; pedicels glabrous to densely tomentose, 4-55 mm long; receptacle-tube broadly obconic, 2-5 mm long; lobes ovate or triangular, obtuse or rounded at the apex, convex and often with a few short soft spines in the lower part outside, shortly ciliate near the apex, brown to purplish-black with green margins, 5-9 mm long; petals caduceus, white, creem, yellow or orange, marked with dark green or brown or black at the base, obovate, 17-35 mm long, 9-27 mm broad, 3 with orange incurved scales inside at the base; stamens 3; thecae triplicate; anthers coherent in centre of flower. Female flowers on 14-130 mm long stalks are

usually bracteate in the lower half; bract 4-16 mm long; ovary ovoid and beaked, densely and softly papillose-spinose, 10-26 mm long, 4-11 mm broad; receptacle-tube broad and shallow, 2-2.5 mm long; lobes triangular to strap-shaped, acute, obtuse, rounded or somewhat dilated and green at the apex, black at the base, 6-11 mm long; petals similar to those of male flower, 20-35 mm long18-24 mm broad, 3 with scales inside at the base; staminodes 3. The fruit is a long-stalked, freshy, ellipsoid berry up to 7 cm × 5 cm and orange when ripe, densely and softly spiny, dehiscing with 3 valves, 35-68 mm long, 30-50 mm broad, densely and softy spiny; spines fleshy, pointed, somewhat recurved, 7-13 mm long, without an apical bristle; pulp scarlet, surrounding the exposed seeds at maturity with a sticky bright-red, sheath. Seeds are black in colour when busted, oblong, flattened, about 1 cm long, brown in colour, testa sculptured, margins 2-grooved, cotyledons hypogaeus (Jackson, 1990; Burkill, 1985; Dokosi, 1998).

Figure 2.1: *Momordica foetida* (flower and fruit)

1.8.1.3 General Uses of *M. foetida*

The plant is used as an ornamental. It is considered to be an indicator of a good coca soil in Ghana (Dokosi, 1998). In Gabon, Sudan, Uganda, Tanzania and Malawi the leaves of *M. foetida* are collected from the wild and eaten after boiling as a vegetable. The pulp of ripe fruits is eaten in Ghana, Gabon, Sudan, Kenya, Uganda and Tanzania (Jackson, 1990). The plants are grazed by cattle in Sudan. Leaves are used as fodder (Kenya,

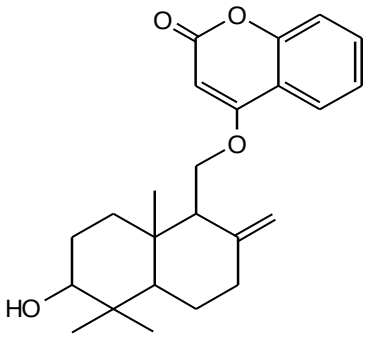
Tanzania) and are said to be suitable for fattening rabbits. However, there are reports from Kenya that cattle avoid it and that it is poisonous (Dokosi, 1998).

1.8.1.4 Ethnomedicinal uses of *M. foetida*

Traditional medicinal uses are numerous and many are shared with other *Momordica* species. The juice of crushed leaves is used to relieve cough, stomach-ache, abortifacient and toothache in Uganda, In Nigeria and South Africa the plant is used for intestinal disorders, headache and boils while in Burundi, Malawi and Tanzania it is used for earache, and as antidote for snakebites,. The leaves are used for skin problems caused by smallpox, as emmenagogue, ecbolic and aphrodisiac in Côte d'Ivoire (Jackson, 1990). It is also used against spitting cobra poison and malaria is treated with crushed leaves (in Côte d'Ivoire, Gabon, Uganda, Tanzania) (Jackson, 1990). In Ghana, the mashed leaves of the plant are mixed with water, native black soap and heated in the sun for two or three hours. This preparation is used as a bath for fever and parched leaves are administered to pregnant women in Southern.Nigeria (Dokosi, 1998). For joint pains in the joints, a decoction of the leaves is prepared and used as a steam bath. A person under treatment for fever or pains in the joints drinks an infusion of the plants (Jackson, 1990). The roots are said to be poisonous. Crushed seeds are used in East Africa to cure constipation. The fruit pulp is said to be poisonous to weevils, moths and ants, and is used as an insect repellent in Tanzania. The Karamajong (Uganda) use the whole plant on their cattle as an oxpecker repellent. In Gabon the leaves are soaked, dried in the sun and used to stuff cushions. Roots are used with *Strophanthus* species in arrow posions by the Benne (Burkill, 1985; Watt and Breye-Brabdwyk, 1962).

1.8.1.5 Chemical Constituents of *M. foetida*

Marquis *et al.*, (1977) reported that foetidin which were isolated from *M. foetida* are capable of reducing blood-glucose level in fasting albino rats.



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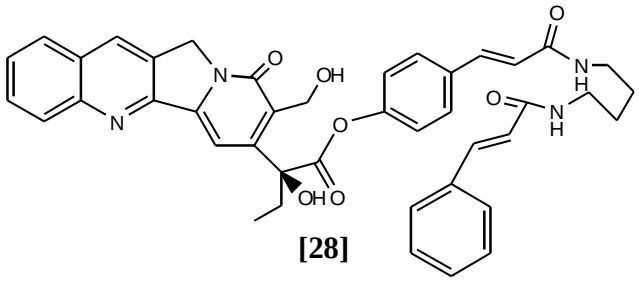

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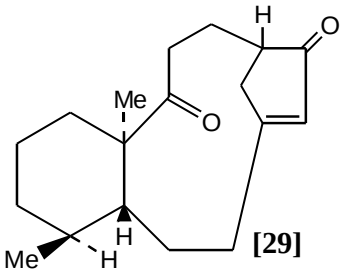
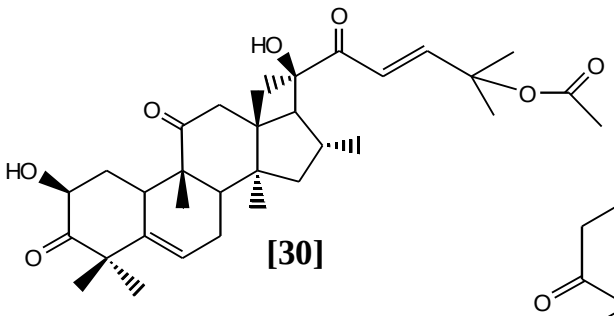


[29]

There are different structures of foetidin **[27, 28 and 29]** reported by different authors (El-Razek *et a.l*, 2004; Buddrus *et al.,* 1985; Pinar *et al.,* 1983; Pirillo *et al.,* 1995, Marquis *et al.,* 1977). Momordicines and foetidin (identical to charantin) were isolated from the fruits and leaves of *Momordica foetida*. Triterpenes of the cucurbitacin type, cucurbitacin **[30]** isolated from the fruits and seed of both *Momordica charantia* and *Momordica foetida*, are potentially cytotoxic. Momordocin **[31]** have been found to be insecticidal (Pinar *et al.*, 1983; Pirillo *et al.,* 1995, Marquis *et al.,* 1977, Olaniyi, 1975).



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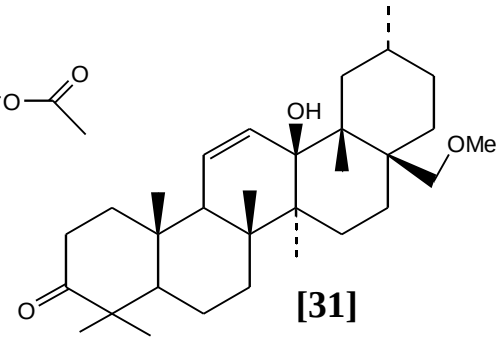
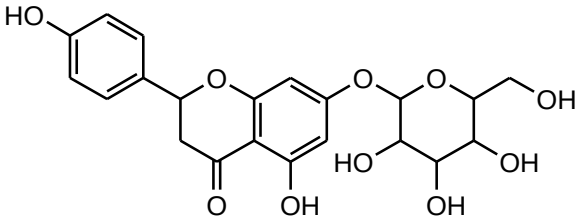


[31]

Foetidin **[28]** has been reported to have slight antispasmodic and anticholinergic effects (Marquis *et al.,* 1977). Recent phytochemical analysis of *M. foetida* by Froelich, *et. al.*

(2007) led to the isolation of a number of flavonoid and other glycosides namely prunin **[32]**, eriodictol-*7-O-β-D*-glucopyranoside **[33]**, 5,7-dihydroxychromone-*7-O-β-D*-glucopyranoside **[33]** and populnin **[35]**.



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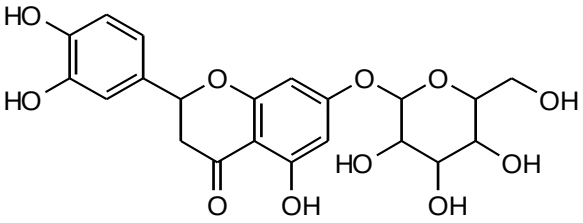

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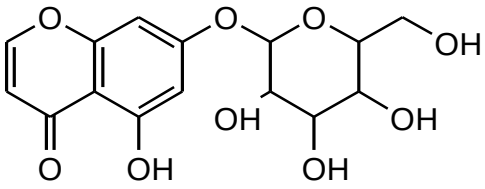

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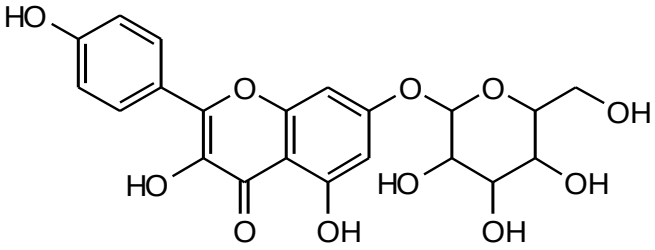


[35]

1.8.1.6 Pharmacological Uses of *M. foetida*

M. foetida is a plant with potential strong antiantinicotinic and antimuscarinic action. Foetidin **[28]** which was isolated from the plant, was reported to lower the blood glucose level in fasting rat up to 18 hours (Marquis, *et al.,* 1977). This effect was comparable to that produced by insulin. However, unlike insulin, foetidin had no antidiabetic action in alloxan-treated rats (Marquis, *et al.,* 1977). Foetidin also has a hypotensive activity (Nuralieva and Alimbaeva, 1970). Leaf extracts of *M. foetida* showed antitrichomonas activity against *Trichomonas vaginalis* (Burkill, 1985). Momordocin **[33]** have been found to be insecticidal; while foetidin has slight antispasmodic and anticholinergic effects (Marquis, *et al.,* 1977). *In vivo* studies with water extracts showed that *M. foetida*, given orally in different doses, prolonged the survival of *Plasmodium berghei* infected mice (Waako *et al.,* 2005). Froelich *et al.,* (2007) reported that the ethyl acetate extract of *M. foetida* showed about 88% inhibition of heme degradation, which is very similar to chloroquine (84%) patent drug. Eriodictyol-7-*O-B-D*-glucopyranoside isolated from this fraction also inhibited heme degradation by 86%.

1.8.2 *Berkheya bergiana* Berg.

Genus: *Berkheya*

Family: Asteraceae

Common Name: Nil

Zulu names: Nil

1.8.2.1 Origin and geographic distribution

The genus was named after Jan le Francq van *Berkheya* 1729-1812, a Dutch botanist (Pooley, 1993a). The genus *Berkheya* was formally under the family Compositae but now under the family Asteraceae. There are about 75 species of *Berkheya* while 71 species are widespread in Southern Africa and 30 species in Natal (Leistner, 2000). The genus *Berkheya* belongs to the group of very unusual plants termed hyperaccumulators (Leistner, 2000). Hyperaccumulator plants accumulate heavy metals from the soil into the aerial parts to concentrations in excess of threshold of 100 µg/g (pmm). (*B. coddii* has been recognized as one of the five hyperaccumulators of nickel metal from South Africa in Mpumalanga province). Its nickel content can exceed 3.7 wt % of dry mass (Augustyniak, *et al.*, 2002). *B. bergiana* is widely spread in Natal, from Kranskop, Eshowe and Mtunzini districts southwards to Port St Johns in the Transkei; also recorded from Mkupe Hill (Newcastle districts) and Estcourt Pasture Research Station as weed (Pooley, 1993b)

1.8.2.3 Botanical Description of *B. bergiana*

Berkheya species are perennial herbs or subshrubs, sometimes cobwebby or woolly. Leaves are alternate or radical, rarely opposite, sometimes decurrent, subentire, toothed, pinnatified or pinnatisect, teeth spinescent. Capitula is radiate or discoid, several to may-flowered, terminal on single stems or on branchlets, rarely axillary, rarely corymbose or umbellate. Involvcre is campanulate, bracts in few to many rows, connate at base, tips and often margins spiny. Receptacle is flat, honeycombed fimbrilliferous. Disflorets are bisexual, fertile or sometimes inner ones male, tubular, dilated above, deeply 5-lobed. Anthers are linear, sagittate at base, with lanceolate, apical appengage. Style is terete,

undivided or with linear, obtuse branches. Pappus of short or long scales or bristle (Leistner, 2000).

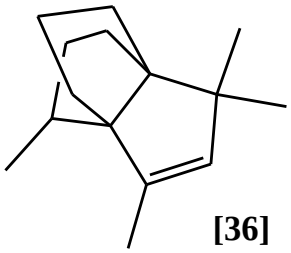
It is a short perennial herb, up to 2.5 m tall. Stem branching above into the compound inflorescence, pubescent, winged, leafy leaves up to + 150 x 50 mm, oblong in outline, slightly narrow to the base, subpetiolate or sessile, half-clasping and decurrent on the stem in broad spiny wings, pinnatilobate, lobes up to 6 each side, coarsely and deeply toothed, teeth triangular, spine-tipped, margins spinose-ciliate, upper surface harshly glandular-pubscent, lower grey- or white-cobwebby. Heads 4-6cm across the expanded rays, several corymbose-paniculately arranged. Involucral bracts lanceolate, up to 3mm broad, tip and margins spiny, very light cobwebby-glandular on the back. Flower heads; In branched inflorescences; 40-60mm diameter, yellow, bracts + 3mm wide, tips and margins spiny, lighty cobwebby beneath, flower mainly March – June (Pooley, 1993b).

Figure 2.2: *Berkheya bergiana*

1.8.2.4 Chemical constituents of *Berkheya* species

Bohlmann *et al.,* (1979) reported the chemical constituents of 18 *Berkheya* species. In addition to the known terpenes and thiophene derivatives, unusual sesquiterpene hydrocarbons, modhephene **[36]** and isocomenes **[37]** were isolated from *B. bergiana, B.*

rhapontica, B. setifera and B. sp. *novum*. β-Isocomene **[38]** was also isolated from the three latter species.



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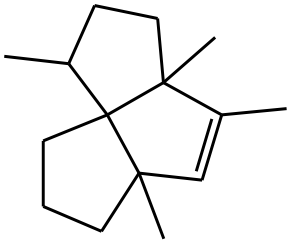

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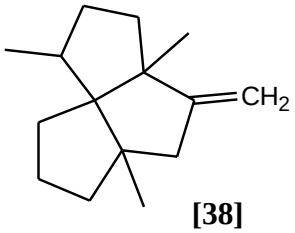
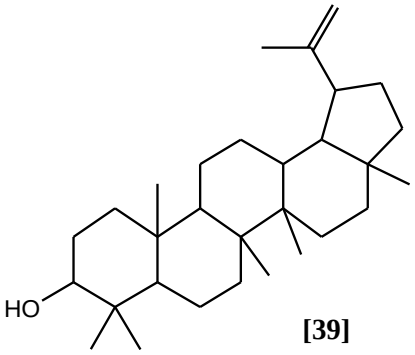


[38]

All species investageted contain the typical thiophene derivatives (Bohlmann *et al.*, 1979). Investigation of *B. zeyheri* root afforded lupeol **[39]** and its Δ^{12} isomer, taraxasterol **[40]**, 2-[thienyl(2)ethinyl]-5-[prop-1-in-1-yl]-thiophen, the bithienyl derivatives **[41]** and its dihydro derivatives **[42]** (Bohlmann and Zdero, 1972; Bohlmann and Klein, 1963) and 2-acetoxymethyl-5-[but-1-in-1-yl]-bithienyl-(2',5).



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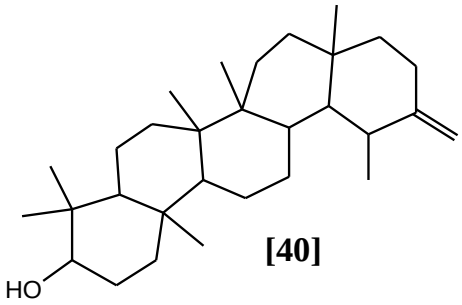

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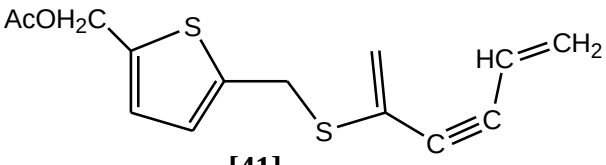

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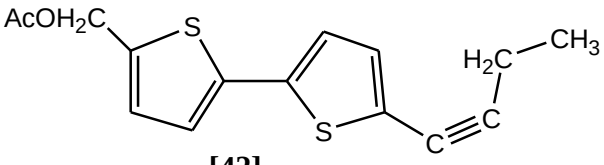
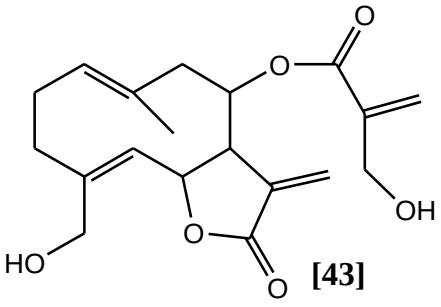


[42]

Also germacranolide onopordopicrin **[43]** was isolated from aerial parts of the same plant, indicating a relationship of the tribe Arctotheae to Cynareas. The roots of *B. radula* contain, beside known compounds, a new sesquiterpene hydrocarbon, berheyaradulene

[37], with an anomaly C skeleton. The structure was elucidated by spectroscopic methods and by transformation in alcohol **[44]** (Bohlmann *et al.,* 1979).



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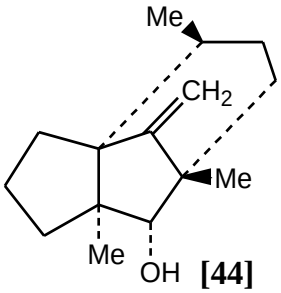
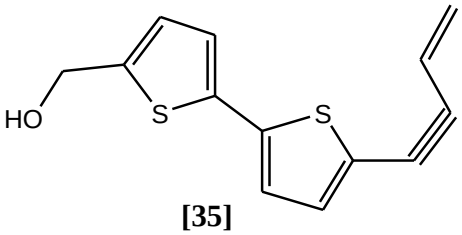


[44]

α-Terthienyl, a known nematocide **[45]**, was isolated from *B. adlamii* and *B. macrocephala* and also a second nematocide, 5-(3-buten-1-ynyl)-2,2'-bithienyl **[46]**, was also detected in the two species of *Berkheya*. A C-2-Substituted 5-[2-(2-thienyl) ethynyl]thiophene **[47]** and dihydro derivatives were isolated from various species of the South African *Berkheya* genus (Bohlmann and Zdero, 1977). Five typical thiophene derivatives were also reported from *Berkheya* species and *Platycarpha glomerta* (Bohlmann and Zdero, 1977).



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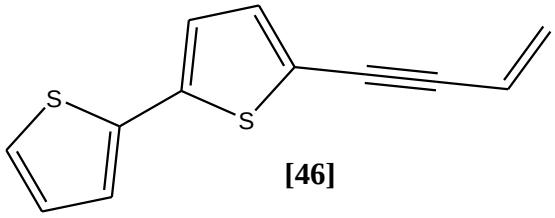

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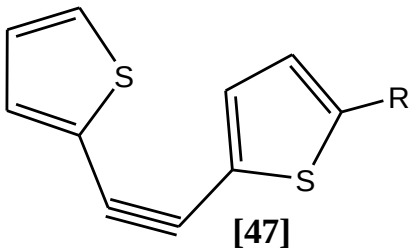
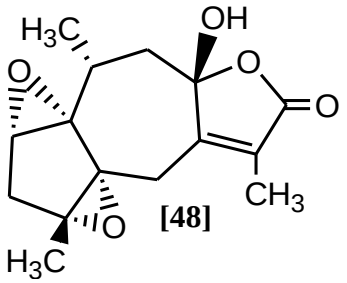


[47]

Also, the aerial parts of *B. carlinopsis* magalismontana afforded two new guaianolides **[48 and 49]** closely related to subluteolide while *B. pauciflora* gave desacetyl laurenobiolide **[50]** and a costic acid derivative **[51]** (Bohlmann *et al.*, 1983, 1984).



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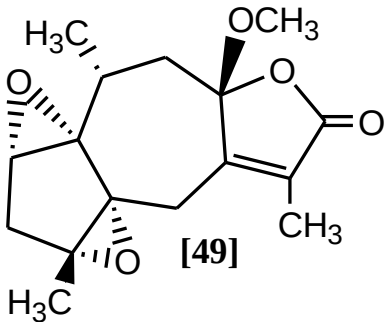

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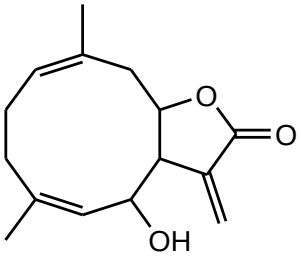

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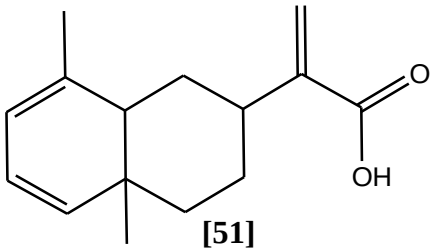


[51]

1.8.2.5 Ethnobatonical Uses of *B. bergiana*

The decoctions of *Berkheya* species roots mixed with parts of Arthrixian phylicoides are taken for dry hacking coughs (Watt and Breyer-Brandwijk, 1962) and anti-emetics. Also these decoctions are used by the Manyka for gonorrhoea and as a good luck charm and treatment of urinary complaints (Bryant, 1966). The roots infusions of *Berkheya* species are used for treatment of abdominal disorders, (Watt and Breyer-Brandwijk, 1962) schistosomiasis, eyes sore, enemas for children with stomach and intestinal complaints (Hulme, 1954), scented body lotions by young girls (Hulme, 1954). Infusions of the roots are also taken as blood puriier for skin problems while steam extract is used for eye and respiratory ailments (Hutchings, 1996). Crush roots of the plant are mixed with pounded leaves in cold water and applied as foments for rheumatism or cold astringents for sores. A steam leaf decoction is used for pustular ophthalma (Watt and Breyer-

Brandwijk, 1962). Powdered roots is applied directly to abscesses and burns and also used as a poultice to relieve aches and pains (Pujol, 1990).

1.9 RATIONALE OF THE STUDY/RESEARCH PROBLEM

Recently, various extracts of plants have provoked interest as sources of natural products. Many plant constituents are effective as remedy for some diseases and accounts for large number of pharmaceutically important compounds in Western Pharmacopoeia and a number of important drugs. For example, taxol and artermisinin were isolated from plants which are known for their anti-cancer and antimalarial activities respectively (Gulcin *et al.,* 2003a). In light of the recent emergence of bacteria which are resistant to multiple antimicrobial drugs, posing challenge to the treatment of infections (van de Watt and Pretorius, 2001), the need to discover new antimicrobial agents becomes pertinent. It is aimed that this study will contribute to the search of natural source of antimicrobial drugs that will provide novel or lead compounds that may be employed in controlling some infections globally. Furthermore, due to increasing number of resistant strains of microbial pathogens, coupled with the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections pose serious medical problems (Tshibangu *et al.,* 2002 and Afolayan and Meyer, 1995) this research will therefore investigate more medicinal plants for their antibacterial activities.

Antioxidants may have an important role in the prevention of diseases. Free radical reactions especially with participation of oxidative radicals have been shown to be involved in many biological processes that cause damage to lipids, proteins, membranes and nucleic acids, thus giving rise to a variety of diseases such as cancer, neurodegenerative disease, malaria and arteriosclerosis and pathological events in living organism (Atoui, *et al.,* 2005; Geetha *et al.,* 2007). In quest of finding novel antioxidant agents from plants, this study intends to isolate antioxidant compounds through bioactive guided-assay on the two plants extracts growing in KwaZulu-Natal province.

Although there is some information on the biological activity of *Momordica foetida* in literature, no information on the biological activity of *Berkheya bergiana* has been reported. Furthermore, some aspects of chemical constituents of South African *Momordica foetida* are available. However, the chemical constituents that have been

isolated and characteristised from *M. foetida* in South Africa were not linked to any of the biological activities. Thus, the rationale for this study based on the ethnomedicinal uses.

It is believed that these two plants (*Momordica foetida and Momordica charantia*) are being used interchangeably and substituted with each other in local communities. Likewise, *Berkheya* species also need to be differentiated from the other species. In the light of this finding, there is a need for detailed pharmacognostic investigation on the efficacy of *Momordica foetida* and *Berkheya bergiana* for their antimicrobial activities.

2.0 THE AIM AND OBJECTIVES OF THE STUDY

This research is aimed at studying the chemical constituents in relation to biological activities of *Momordica foetida* and *Berkheya bergiana* plants growing in KwaZulu-Natal region with the following objectives

- To investigate the phytochemical constituents *Momordica foetida and Berkheya bergiana* plants.
- To isolate possible chemical constituents from both *Momordica foetida and Berkheya bergiana* plants
- To evaluate and compare the antimicrobial and antioxidant activities of extracts, fractions and constituents (compounds) of both *Momordica foetida and Berkheya bergiana* plants
- To investigate and compare some other pharmacognostic characters of *Momordica foetida and Berkheya bergiana* plants investigated.

CHAPTER TWO

2.0 EXPERIMENTAL

2.1 MATERIAL

2.2 COLLECTION, AUTHENTICATION AND PREPARATION OF PLANT MATERIALS

2.2.1 *Momordica foetida*

M. foetida leaves were collected from Zululand within KwaZulu-Natal district in South Africa in May, 2007. Identification and authentication of the plant was carried out by Mrs Anne Hutchings a taxonomist and Research fellow at University of Zululand, KwaDlangezwa, South Africa and voucher specimen was deposited in the Univeristy herbarium. Leaves were carefully examined and old, insect damaged, fungus-infected leaves and twig were removed. Healthy leaves were used for the study by firstly oven drying for 18-24 h at 40 °C temperature. The leaves were then grounded to a fine powder in a Junkel and Kunkel Model A10 mill.

2.2.1.1 Preparation of extracts

The powdered plant material (700 g) was cold-extracted using 90% ethanol for 2 days with occasional shaking. This was repeated 3 times to ensure complete extraction of the chemical constituents of the plant. The extracts were combined after TLC analysis. The mixture was then filtered through Whatman filter paper No. 1 and the filtrate was evaporated to dryness using a rotary evaporator at 40 ^{o}C. This gave a yield of 123.44 g (17.63%).

2.2.1.2 Fractionation of the ethanol crude extract

The dried 90% ethanolic extract (118.44 g) above was dissolved in a mixture of methanol:water (2:3 v/v) and partitioned successively with hexane, chloroform, ethyl acetate and butanol with the aid of separating funnel. The hexane, chloroform, ethyl acetate and butanol fractions were taken to dryness in a rotary evaporator under reduced pressure. During partitioning, equal volumes of the solvents were used and the extraction or partition process repeated with a small volume approximately two or more times (Suffness and Douros, 1992). After the extractions were completed, the aqueous layer was evaporated using rotary evaporator under reduced pressure to a minimal volume and

then freeze dried. The following yields were obtained: hexane (17.58 g), chloroform (7.60 g), ethyl acetate (6.29 g) and butanol (11.66 g) and aqueous (75.76 g). The remaining 5 g ethanolic extract was reserved for antibacterial and antioxidant assays.

2.2.2 *Berkheya bergiana*

Berkheya bergiana Soderberg leaves were collected from Zululand within KwaZulu-Natal district in South Africa in June, 2007. Identification and authentication of the plants was carried out by Mrs Anne Hutchings, a taxonomist and Research fellow at University of Zululand, KwaDlangezwa, South Africa and voucher specimens deposited in the University herbarium. Leaves were carefully examined and old, insect damaged, fungus-infected leaves and twig were removed. Healthy leaves were used and allowed to dry for 2-3 weeks in the shade at room temperature. The leaves were grounded to a fine powder in a Junkel and Kunkel Model A10 mill.

2.2.2.1 Preparation of Extract of *B. bergiana*

The powdered plant material (100 g) was cold-extracted using 90 % methanol for 2 days with occasional shaking and which was then filtered and concentrated to obtain the crude extract, the procedure was repeated 3 times. The extracts obtained were combined after TLC analysis ensured complete extraction. The mixture was then filtered through Whatman filter paper No 1 and the filtrate was concentrated to dryness using a rotary evaporator at 40 ^{o}C. This gave a yield of 19.39 g (19.39 %).

2.2.2.2 Preparation of various extracts of *B. bergiana*

The air-dried and powered leaves of *Berkheya bergiana* (370 g) were extracted sequentially with hexane, chloroform, ethyl acetate, butanol and water at room temperature. The fractions were filtered through Whatman filter paper No 1 and the filtrates were evaporated to dryness using a rotary evaporator at 40 ^{o}C to give 4.99 g, 5.53 g, 1.66 g, 4.71 g and 2.78 g of crude extracts respectively.

2.3 Phytochemical screening of the extracts

Phytochemical screening was carried out on the crude extract of both *M. foetida* and *B. bergiana* and on their powdered specimens using standard procedures to identify the

constituents as described by Sofowora (1993), Trease and Evans (1989) and Harborne (1998) as follows:

Test for alkaloids

About 25 mg of extract was dissolved in water and filtered. The filterate was acidified with 1 M HCl. 1 mL of the filterate was treated with two drops of Mayer's reagent. A precipitate was taken as preliminary evidence of the presence of alkaloids. Another 1 mL of the filterate was treated with Dragendrorff's reagent. Precipitate was also taken as preliminary evidence for the presence of alkaloids.

Confirmatory test for alkaloids

About 0.5 g in 5 mL of the ethanolic extract in distilled water stirred with 1% HCl on steam bath and extracted with $CHCl_3$ to give $CHCl_3$ (I) fraction. The aqueous layer of each species was basified (pH 9-10) with ammonia solution and extracted with $CHCl_3$ to give $CHCl_3$ (II) fraction. Both $CHCl_3$ fractions separately concentrated and chromatographed on Silica gel- G using $CHCl_3$: MeOH (97:3), spraying with freshly prepared Dradendorff spray reagent. An orange spot in $CHCl_3$ I or II on pale yellow background is taken as confirmatory evidence for the presence of alkaloids.

Test for tannins: About 0.5g of ethanolic extract was boiled with 10 mL of water for 15 min, filtered and made up to 10 mL. To 2 mL of the filterate was added 10 mL of water and one drop of $FeCl_3$. Bluish-black green or blue-green precipitate was taken as preliminary evidence of the presence of tannins.

Test for phlobatannins: Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1 % aqueous hydrochloric acid was taken as evidence for the presence of phlobatinins.

Test for saponin:

(i) About 2.5 g of the plant material was extracted with boiling water. After cooling, the extract was shaken vigorously to froth and was then allowed to stand for 15-20 min and classified for saponin content as follows:

No froth = negative;

Froth less than 1 cm = weakly positive;

Froth 1.2 cm high = positive; and

Froth greater than 2 cm high = strongly positive.

(ii) **Blood heamolysis test:-** About 2 mL of methanolic extract of samples added to 5 mL of 10% freshly collected blood sample and 0.2 g in 2 mL of water of the extract was prepared as a control. Formation of a precipitate in the test-tube is taken for haemolysis saponins

Test for flavonoids: Three methods were used to determine the presence of flavonoids in the plant samples.

a) 5 mL of dilute ammonia solution were added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.
b) To the methanol extract of samples, some magnesium ribbons were added with about 1 mL of concentrated HCl. A red colouration is taken as an indication of the presence of flavonoid and flavones.
c) A portion of the powdered plant sample was in each case heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for steroids: 2 mL of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 mL H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids (Salkowski test): 5 mL of methanolic extract was mixed carefully with 2 mL of chloroform, and concentrated H_2SO_4 (3 mL) carefully added to form a layer. A reddish brown colouration at the interface was formed to show positive results for the presence of terpenoids.

Test for cardiac glycoside

(i) **Legal test:-** Aliquot of methanoic extract was dissolved in pyridine and a few drops of 2% sodium nitroprusside were added along side with few drops of 20% NaOH. A deep red colour, which faded to a brownish yellow, indicated the presence of cardenolides.

(ii) **Salkowski test:-** 0.5 g methanoic extract of the samples dissolved in 2 mL of $CHCl_3$ and H_2SO_4 was carefully added to form a layer. A reddish-brown colour at the interphase indicated the presence of steroidal ring.

(iii)**Keller-Killani test:** 5 mL of each extracts was treated with 2 mL of glacial acetic acid containing one drop of iron(III)chloride solution. This was underlayed with 1 mL of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form gradually throughout thin layer.

Test for Anthraquininones:-

(i) **Free Anthraquinones:-** 0.5 g of methanolic extract of *M. foetida* and *B. bergiana* was dissolved in a little distilled water and shaken with 5 mL of benzene, filtered and 5 mL of 10% ammonia solution was added to the filtrate and shaken. A pink colour in the ammonia layer was taken as evidence for the presence of free anthraquinones (Sofowora., 1982)

(ii) **Combined Anthraquinones:-** About 0.5 g extract methanolic extract of *M. foetida* and *B. bergiana* was dissolved in a little distilled water and boiled with iron(III) chloride solution (2.5mL, 25%) and 5 mL hydrochloric acid for 10 min. It was filtered hot, cooled and then filtered and treated with 5 mL of 10% ammonia solution was added to the filtrate and shaken. A pink colour in the ammonia layer was taken as evidence for the presence of combined anthraquinones.

2.4 BIOLOGICAL ACTIVITIY

2.4.1 Antibacterial Activity Test

2.4.1.1 Test Micro-Organisms

The test organisms used in this study consisted of reference strains obtained from the University of Fort Hare, namely *Escherichia coil* (ATCC 8739), *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 19582), *Staphylococcus aureus* (ATCC 6538), *S. faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 10702), *B. pumilus* (ATCC 14884), *Pseudomonas aeruginosa* (ATCC 7700), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumonia* (ATCC 10031), *K. pneumonia* (ATCC 4352), *Proteus vulgaris* (ATCC 6830), *P. vulgaris* (CSIR 0030), *Serratia marscens* (ATCC 9986), *Acinetobacter calcaoceuticus* (Aci1), *A. calcaoceuticus* (Aci2).

Environmental strains of *K. pneumonia*, *Bacillus subtilis*, *Shigella flexineri*, *Salmonella* sp., *Staphylococcus epidermidis*, *P. aeruginosa*, *P. vulgaris*, *Enterococcus faecalis*, *E. coli*, *S. aureus*, *Micrococcus kristinae* and *M. luteus* were also used which were also obtained from University of Fort Hare.

2.4.1.2 Preparation of Müeller-Hinton Medium

Müeller-Hinton Agar purchased from Sigma was prepared according to manufacturer's instructions: 38g of Müeller-Hinton agar powder was poured into a 1000 mL Duran bottle. The Duran bottle was filled with distilled water to give 1000 mL and to ensure proper mixing, occasional and vigorous shaking was done. This was immediately autoclaved for 30 mins and allowed to cool to 45 to 50 ^{o}C before dispensing into 90 mm plastic petri dishes. The agar medium in the various plates was allowed to cool to room temperature.

2.4.1.3 Preparation of Nutrient broth

This was prepared also according to manufacturer's instruction in which 16 g of nutrient broth powder was weighed and poured into a 1000 mL Duran bottle. The Duran bottle content was bought to 1000 mL by addition of distilled water and about 5 ml was dispensed into various test-tubes. The test-tubes containing nutrient broth were autoclaved and allowed to cool to room temperature. The bacteria were sub-cultured in test-tubes strains for disc diffusion assay. This procedure was repeated for the minimum

inhibitory concentration (MIC) in which 500 ml of Duran bottle was used to prepared nutrient broth according to manufacturarer's specification and autoclaved for 15 min and allowed to cool at room temperature and was ready for MIC assay.

2.4.1.4 Antibacterial bioassay: Disc Diffusion Assay

Antibacterial activity of the extract and fractions were evaluated using the agar disc diffusion method described by Bauer *et al.,* (1966). 5 mg/ml of each extract and fraction were prepared by dissolving 0.05g of the plant extracts and fractions in 10ml of methanol used for extraction. 5 mg/ml of Ampicillin, Neomycin and Tetracycline were used as the positive control and the extracting solvents were used as negative controls.

The cooled agar plates were inoculated with 100 µl (0.5 M McFarland i.e 1.5 x 10^6 CFU/mL) of the microorganism spreading with the aid of stirring rod. Sterile Whatman No. 1 (6 mm) discs impregnated with each sample were placed at the center of the plate. The plates were incubated for 24 hr at 37°C and zone of inhibition measured thereafter. The zone of inhibition of the negative controls was subtracted from the zone of the plant extracts so as to find the true zone of inhibition of the extract. This experiment was done in triplicates.

2.4.1.5 Microdilution assay: Minimum Inhibitory Concentration (MIC)

The MIC for each plant extract or fraction against a range of bacteria was determined by modified Eloff's method (Eloff 1998b). The assay was performed in 98-wells microlitre plates by adding 50 uL of sterile water to all wells. In row A, 50 µL of sample extract was added with a micropipette. Serial dilution from row A to H was done by taking 50 mL after proper mixing at each row to the next and 50 µL from row H was discarded. Two wells were used as control: one containing only water, and the other a growth control containing both water and test organism i.e water, broth with bacteria and solvent. Tetracycline, Neomycin and Ampicillin were used as positive control antibiotics. After adding 50 µL of the bacteria suspension to each row (except for the sterility control), the microplate was covered and incubated at 37 ^{o}C with 100% relative humidity for 24 h. After 24 h incubation, 40 µL of a 2 mg/ml solution of INT (Iodonitotetrazolium chloride) was added to each row and the plate was further incubated for 30 mins to ensure adequate colour development. INT is a dehydrogenase activity detecting reagent, which is

converted into an intensely colour red-purple formazan by metabolically active micro-organisms. Inhibition of growth was indicated by a clear solution or a definite decrease in colour reaction. The inhibition of growth indicated by a clear solution was taken as value of the minimum inhibitory concentration (MIC) of the extracts or fractions.

2.5 ANTIOXIDANT ASSAY

2.5.1 Antioxidant Qualitative Assay

Qualitative screening entails spraying the TLC chromatograms of the fractions and crude extracts with 0.2% DPPH in methanol. About 1 mg of each extract and fraction was weighed into a small test tube and 10ml of methanol added. The mixture was shaken together and by use of capillary was spotted carefully on the aluminum-coated plate. 5ml of each of the mixture was spotted on the coated aluminum plate about 10mm away from the bottom of the plate. The point of the spot was labeled and the plate allowed to dry in air and later put into a developing tank containing the mobile phase with various suitable solvents like (n-hexane:$CHCl_3$ 50:50, 30:70; $CHCl_3$ 100%; $CHCl_3$-EtOAc 60:40; $CHCl_3$-MeOH 80:20, 60:40; EtOAc-MeOH 90:10; EtOAc-MeOH-H_2O 100:17:13 and BuOH-AcOH-H2O 100:10:10, Ethyl acetate: formic Acid: water 85:15:10 depending on the extract or fractions). The above was allowed to dry and viewed under the UV light at 365 and 254 nm. The fluorescent points was marked at each wavelength after which the plates were sprayed with 2,2-Diphenyl-1-Picrylhydraxyl (DPPH) reagent in methanol (10 mg in 10 ml). After this, the plate was left to dry and the colouration produced on the plate was noted. The DPPH reagent in this case was used to detect the presence of antioxidants. This reagent form complexes with the free hydroxyl group present in the crude extract and fractions. Thus DPPH on forming these complexes show the observed colouration (yellow coloration) on the TLC plate. This was also repeated for the isolated compounds.

2.5.2 Antioxidant Quantitative Assay

Quantitative antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH), ABTS, Metal chelating and FRAP methods.

2.5.2.1 DPPH radical scavenging activity assay

Free radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was measured using the modified method Mensor *et al.,* (2001). 1 mL of various

concentrations of the extract and fraction in methanol was added to 4ml of 0.004% MeOH solution of DPPH. After incubation period at room temperature the absorbance was yellow against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I %) was calculated using the formula below:

$$\text{DPPH scavenging effect } (\%) = 100 - \left[\frac{A_0 + A_1}{A_0} \times 100 \right]$$

Where A_o is the absorbance of the control reaction (containing all reagents except the test compound)

A_1 is the absorbance of the test extracts, fractions or compound.

The IC_{50} value, defined as the amount of the sample sufficient to elicit 50% reduction of the initial DPPH concentration, was calculated from the linear regression of plots of concentration of test extracts and fractions (µg/mL) against the mean percentage of antioxidant activity obtained from the three replicate test of each extracts and fractions. The free radical scavenging activity of Ascorbic acid (Vitamins C), BHT and BHA were also measured under the same condition to serve as antioxidant agents (positive control).

2.5.2.2 Ferric reducing antioxidant power (FRAP) assay

Adopting the method of Oyaizu (1986), diluted extract and fractions (1 mL) were mixed with 2.5 mL of potassium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50^oC for 20 min. A total of 2.5 mL of 10% Trichloroacetic acid (TCA) was added to the mixture to stop the reaction which was then centrifuged at 1000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. The procedure was carried out in triplicate and allowed to stand for 30 min before measuring the absorbance at 700 nm. The higher the absorbance, the higher the FRAP

2.5.2.3 Metal chelating Activity assay

The chelation of ferrous ions by the extract was estimated by the method of Dinis *et al.*, (1994) with some modifications. 1 mL of sample at different concentrations was mixed with 3.7 mL of methanol and 0.1 mL of $FeCl_3$. The reaction was initiated by the addition of 0.2 mL of ferrozine, followed by shaking vigorously and left to react at room

temperature for 10mins. The absorbance was measured spectrophotometrically at 562 nm. Extracts and fractions concentration at 50% inhibition was calculated from the plot of inhibition (%) against extract concentration i.e. IC_{50}. Tests was carried out in triplicate.

2.5.2.4 ABTS Radical cation Scavenging activity assay

The free radical scavenging activity was determined by ABTS radical cation decolourisation assay using a method by Re *et al.,* (1999). It involved the generation of $ABTS^{+}$ chromophore by the oxidation of ABTS with potassium persulfate. $ABTS^{+}$ radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature in the dark for 12-16 h. The solution was then diluted with methanol to an absorbance of 0.7074 ± 0.02 at 734 nm using spectrophotometer. The reaction mixture (1 mL of extract/fractions and 1 mL of $ABTS^{+}$) was allowed to stand at room temperature for 6 min and the absorbance was immediately recorded at 734 nm. BHA standard solution was prepared and assayed at the same conditions as reference. The percentage inhibition of absorbance at 734 nm was calculated and plotted as a function of concentration of antioxidants.

Extracts and fractions concentration providing 50% inhibition was calculated from the plot of inhibition (%) against extract concentration i.e. IC_{50}. The test was carried out in triplicate.

2.5.2.5 Calibration graph for Phenolic content (Gallic acid equivalent) assay

A stock solution of gallic acid was prepared by dissolving Gallic acid in diethyl ether (0.01% w/v). Various concentrations of gallic acid in triplicate (0.01 mg to 0.15 mg in diethyl ether) were prepared from the stock solution and thereafter, the diethyl ether evaporated. The residue was dissolved in 1.5 mL of Folin-Ciocalteau reagent (diluted 10 times) and 1.2 mL of sodium carbonate (7.5 g/100mL). The contents of the tubes were mixed thoroughly and stored in the dark for 30 mins before the absorbance was measured at 765nm using visible spectrophotometer. A linear relationship was obtained by plotting the concentrations of the Gallic acid against the respective mean absorbance.

2.5.2.6 Determination of Total Phenolic Content:

The total phenolic content (TPC) of the plant extracts and fractions were determined spectrophotometrically using Folin-Ciocalteau's reagent according to the method described by Kahkonen *et al.,* (1999) with slight modifications. Three hundred microlitres of the samples (triplicate) were added into test-tube followed by 1.5 mL of Folin-Ciocalteau reagent (diluted 10 times) and 1.2 mL of sodium carbonate (7.5g/100mL). The contents of the tubes were mixed thoroughly and stored in the dark for 30 mins before the absorbance was measured at 765 nm using visible spectrophotometer. TPC was expressed as mg Gallic acid equivalents (GAE) of the samples based on the above caliberation curve.

2.5.2.7 Calibration graph for flavonoid (Quercetin equivalent) assay

A stock solution of quercetin was prepared by dissolving 5 mg of quercetin in 50 mL (0.01%w/v) diethyl ether and various concentrations of quercetin in triplicate (0.01 mg to 0.15 mg in diethyl ether) were placed into individual test tubes from the stock solution and the diethyl ether evaporated. The residue was dissolved in 0.5 mL of 2% $AlCl_3$ ethanol solution was added. After 1 h at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of quercetin flavonoid. A linear relationship was obtained by plotting the concentrations of the quercetin against the respective mean absorbance.

2.5.2.8 Determination of Total flavonoid Content

The flavonoid contents in the extracts were determined spectrophotometrically using the method of Ordon-Ez *et al.,* (2006) based on the formation of a complex flavonoid-aluminum. An amount of 0.5 mL of 2% $AlCl_3$ ethanol solution was added to 0.5 mL of sample. After 1 h at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/mL. Total flavonoid content was calculated as quercetin (mg/g) using the following equation based on the calibration curve above: $y = 0.0255x$, $R^2 = 0.9812$, where x was the absorbance and was the quercetin equivalent (mg/g).

2.5.2.9 Calibration graph for Proanthocyanidin (Catechin equivalent) assay

A stock solution of catechin concentration was prepared by dissolving 5mg of catechin in 50ml (0.01%w/v) diethyl ether and various concentrations of catechin in triplicate (0.01 mg to 0.15 mg in diethyl ether) were placed into individual test tubes from the stock solution and the diethyl ether evaporated. The residue was dissolved in 3 mL of 4% vanillin-methanol solution together with 1.5 mL hydrochloric acid and the mixture was allowed to stand for 15 min. Absorbance was measured at 500 nm. A linear relationship was obtained by plotting the concentrations of the catechin against the respective mean absorbance.

2.5.2.10 Determination of Total proanthocyanidin Content

Proanthocyanidin determination was based on the procedure reported by Sun *et al.,* (1998) in which a volume of 0.5 mL of 0.1 mg/mL of extract solution was mixed with 3 mL of 4% vanillin-methanol solution together with 1.5 mL hydrochloric acid and the mixture was allowed to stand for 15 min. Absorbance was measured at 500 nm. Extract and fractions were evaluated at a final concentration of 0.1 mg/mL. Total proanthocyanidin content were expressed as catechin equivalents (mg/g) using the following equation based on the above calibration curve: $y = 0.59153x$, $R^2 = 0.9371$, where x was the absorbance and y is the catechin equivalent (mg/g)

2.6 EXTRACTION AND ISOLATION OF COMPOUNDS

2.6.1 Isolation of Compounds from *M. foetida*

The plant material was extracted by adding 5L 90% Ethanol to 1700 g finely powdered leaf material to fully immerse and wet the plant powder in a 10 L glass container with closeable lid. The container was then vigorously shaken occassionally. The mixture was left for 2 days and the extract was decanted and filtered through a Buchner funnel into clean container. The process of extraction was repeated three times on the same plant material and the filterate combined. The extract was reduced to dryness using a rotary evaporator under reduced pressure at 40 ^{o}C. It was then successively partitioned between water/methanol and hexane (1 L x 3), chloroform (1 L x 3), ethyl acetate (1 L x 3) and n-butanol (1 L x 3). The fractions were evaporated to dryness using rotary evaporator at

40^{o}C to give hexane (17.58 g), chloroform (7.60 g), ethyl acetate (6.20 g), n-butanol (11.66 g) and water (75.76 g).

5.6 g of the chloroform fraction was mixed with small amount TLC grade silica gel (Merck) to form a slurry, which was then loaded onto a VLC, column (diameter 9.5 cm and length 20 cm) filled with silica gel as stationary phase. The column was eluted stepwise under vacuum with solvents of polarity ranging from hexane, mixtures of hexane and ethyl acetate to pure ethyl acetate. The solvents were eluted until it ran clear of the column. Fractions of 250 mL each were collected and numbered from 1 to 130. The mass of the eluted fractions was determined after the solvents were evaporated in a stream of air at the room temperature. The fractions obtained from the VLC were analysed by TLC and fractions with a similar profile were combined. Owing to the similarity of their composition as determined by TLC analysis, fractions 1 and 4, fractions 5 to 29, fractions 30 to 63, fractions 64 to 115 as well as fraction 116 to 130 were combined to give fractions C_A to C_E respectively. Fraction C_D (VLC fractions 64-115) due to its high crystalline nature and because it is well resolved components as seen in TLC, the fraction was subjected to column chromatography (CC) silica gel with hexane-ethyl acetate (4.5:5.5) and collected manually but the compound decomposed

Fraction C_E (VLC fractions 116-130) was also subjected to siligal gel CC eluted with hexane-ethyl acetate (4:6 to 5:5) and collected manually to yield 68 fractions. Similar fractions, based on TLC profile were combined in sub-fraction $C_{EA} – C_{EH}$. Sub-fraction C_{EE} (fraction 32-34) (176 mg) was further purified by silica gel Flash Column Chromatography (FCC) using hexane-ethyl acetate (5:5) to yield 3 major fractions and third fraction was undertaken. Further purification for third fraction afforded OM/11/40 (33 mg) as shown in figure 2.1. Sub-factions C_{EF} (fractions 35-40) (259 mg) was also further subjected to FCC using solvent system hexane-ethyl acetate (5:5) to give OM/12/12 which was further purified by prep-TLC to give 35 mg of OM/12/12.

5.0 g of ethyl acetate fraction was subjected to silica gel CC (30 x 2.0 cm) eluted with n-hexane/ethyl acetate (100/0 to 20/80) followed by EtOAc/MeOH (90/10 to 60/40) and fractions were collected manually. Collected fractions were examined by TLC. Due to their similar TLC profiles, fractions were pooled to give 5 major sub-fractions ($E_A – E_E$). The dried component of E_D was allowed to evaporate under a stream of cool air to

encourage crystallization. The crystals (0.351 g) was collected and subjected to silica gel CC (30 x 2 cm) and eluted with EtOAc/MeOH (9.5/0.5) to yield 598 mg of pure yellowish compound OM/E/T2.

A schematic representation of the procedure followed in the isolation of compounds from *M. foetida* is shown in figure 4.1.

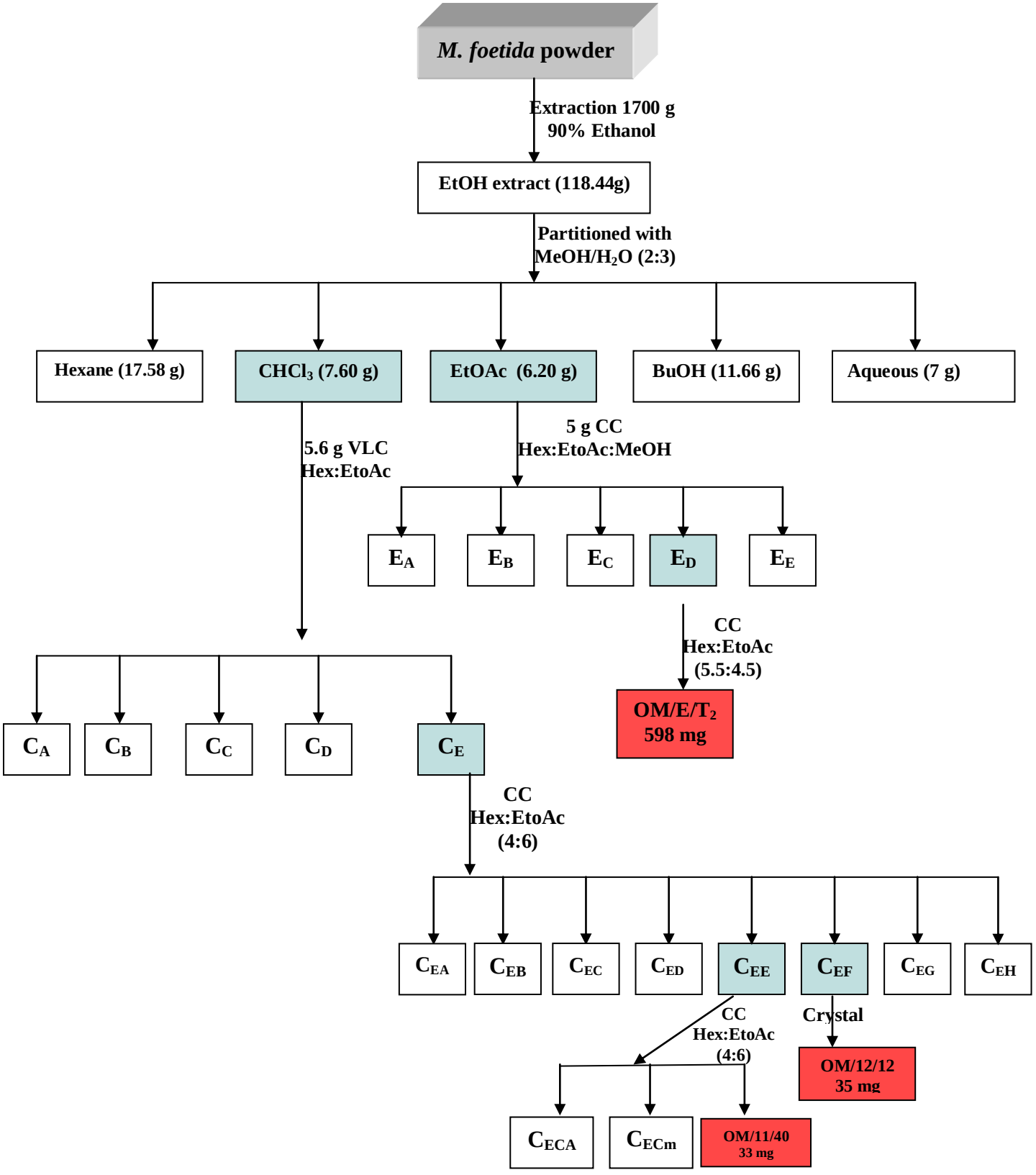


Figure 2.1: Diagrammatic summary of the isolation routes of *M. foetida* (Flow chart)

2.6.2 Isolation of Compounds from *B. bergiana*

The milled leaves (1370.0 g) of *B. bergiana* were extracted by maceration for 24 h x 2 seqentially with hexane, chlolorom, ethyl acetate, n-butanol and water. The solvent was removed under vacuum using a rotary evaporator under reduced pressure at 40 ^{o}C to yield the following extracts: hexane (5.70 g), chloroform (5.95 g), ethyl acetate (1.96 g), n-butanol (4.71 g) and water (2.78 g).

2.0 g of chloroform extract was subjected to silica gel CC (65 x 5 cm) eluted with hexane:ethyl acetate (9:1 to 8:2) to yield 98 fractions. TLC analysis of the fractions with sulphuric acid-anisaldeyde staining agentand heated at 150 ^{o}C, allowed the constitution of combined fractions $F_1 – F_6$. The fractions F_4 (fractions 40 – 49) was evaporated under vacuum, as a result of which rosette crystals (293 mg) were obtained. The crystals was subjected to silical gel flash column chromatography for further purification with solvent system hexane-ethyl acetate (8:2) to yield a pure compound in form of whitish crystals (OM/9/F3) (218 mg) as indicated in figure 4.2. 1031 mg of fraction F_5 (fractions 65-85) was subjected to silica gel flash column chromatography (FCC) and eluted with hexane:ethyl acetate (8.5:1.5) to yield 69 sub-fractions. Owning to their similar TLC profiles, the fractions were combined in sub-fractions $F_{5.1}$ - $F_{5.5}$. Sub-fraction $F_{5.2}$ (sub-fractions 4 - 8) (210 mg) was further subjected to FCC over silica gel (70-230 mesh) eluting with hexane-ethyl acetate (8.5:1.5) compound OM/10/F1 (158 mg). Sub-fraction $F_{5.5}$ (fractions 22 – 30) (453 mg) was subjected to FCC silica gel eluted with hexane-ethyl acetate (8.5:1.5) to obtained 60 sub-subfractions. And owing to the similarity of their composition, determined by TLC analysis, sub-subfractions were combined in fractions $F_{55.1} – F_{55.6}$. As a result of which rosette crystals was obtained from sub-subfraction $F_{55.5}$, the sub-subfractions were rechromatographed using silica gel to obtain OM/14/28 using solvent system hexane-ethyl acetate (8.5:1.5) and further purified by prep-TLC (8.5:1.5) hexane-ethyl acetate to give pure compound OM/14/28 (115 mg).

A schematic representation of the procedure followed in the isolation of compounds from *B. bergiana* is shown in figure 2.2 below.

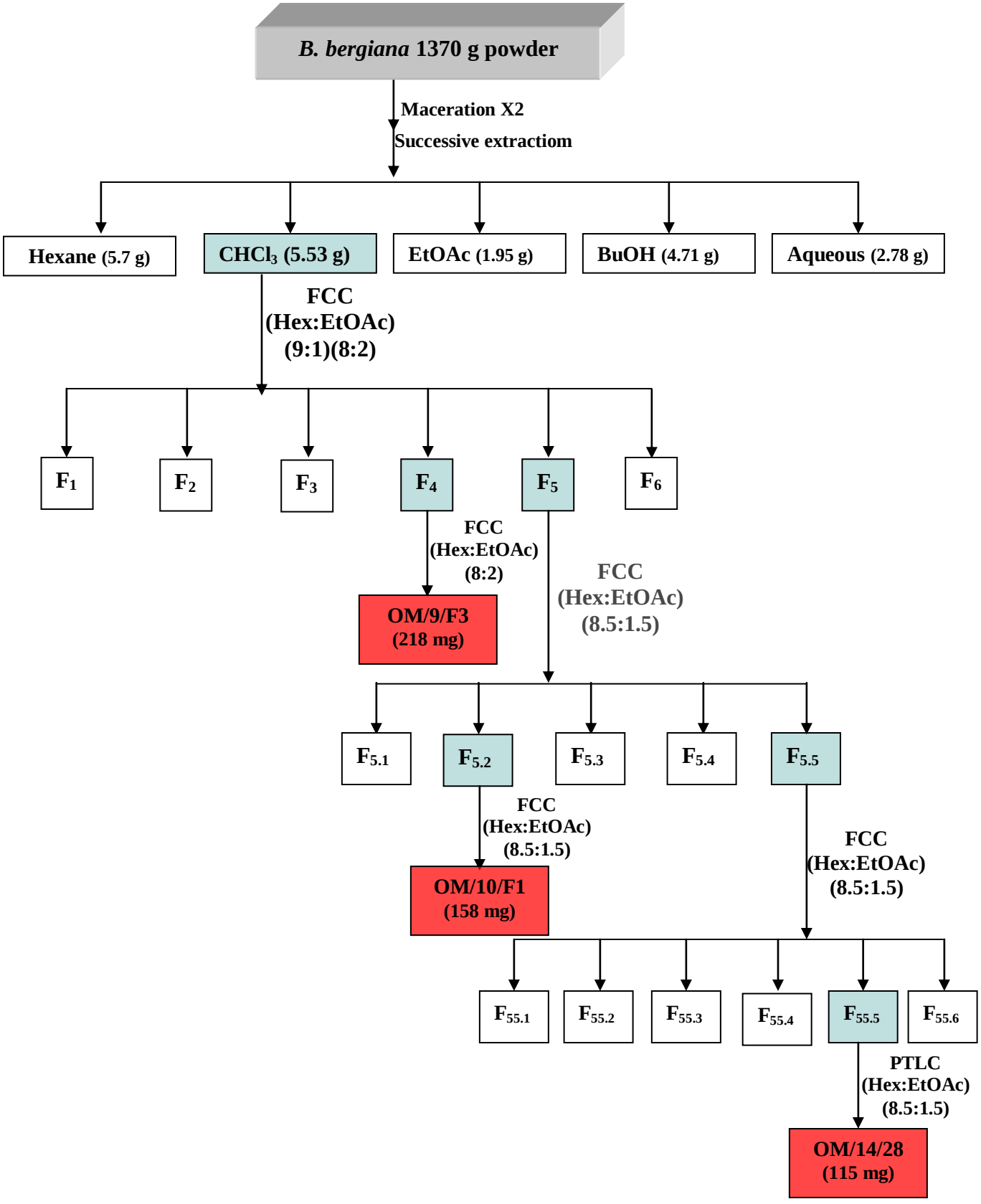


Figure 2.2: Diagrammatic summary of the isolation routes of *B. bergiana*

2.7 PHARMACOGNOSTIC ANALYSIS

2.7.1 Total Ash: 2.5 g of the ground air-dried plant material was weighed accurately in a clean tared white crucible. The sample was ignited by gradually increasing the heat from 500-600 ^{o}C in furnace overnight until the sample was carbon-free and white grey substance as ash obtained. The sample was transferred into a desiccator, cooled for 30 min and weighed immediately. The ash was calculated in mg per g of dried material and in percentage (African Pharmacopoeia, 1986).

2.7.2 Acid-Insoluble ash: The sample ash obtained above was boiled gently with 25 mL of 10% hydrochloric acid for 5 min. The sample was covered with watch-glass to prevent evaporation. After 5 min the sample was filtered to remove solution using ashless filter paper and was washed with hot water. The filter paper containing the insoluble matter was transferred to the original crucible and dried on hot-plate and ignited to constant weight. The sample was quickly transferred into a desiccator for 30 min and the weight was recorded without delay. The acid-insoluble ash was calculated in mg per g of air and percentage (African Pharmacopoeia, 1986).

2.7.3 Water-soluble Ash: The total ash obtained above was boiled gently with 25 mL of water in a conical flask for 5 min. The sample was filtered through ashless filter paper and washed with hot water. Then the process was followed as described above (African Pharmacopoeia, 1986).

2.7.4 Water-soluble extractive value: About 4.0 g of air-dried plant material was macerated with 100 mL of water for 6 h with vigorous shaking. The mixture was weighted to obtain the total weight. The mixture was allowed to stand for 18 h and filtered with No 1 whatman filter paper. 25 mL of filtrate was transferred to a tared flat-bottomed dish and allowed to evaporate on a water bath at 105^{o}C for 6 h until dry. The content of extractible matter was calculated in mg/g of air-dried material and in percentage (African Pharmacopoeia, 1986).

2.7.5 Alcohol-soluble extractive value: About 4.0 g of air-dried plant material was macerated with 100 ml of ethanol with 6 h shaking frequently. The mixture was weighted

to obtain the total weight. The mixture was allowed to stand for 18 h and filtered with No 1 Whatman filter paper. Then the process was followed as described above (African Pharmacopoeia, 1986).

2.7.6 Moisture Content: About 5 g of fresh leaves of plant was collected and cut into pieces and weighed. The sample was dried in the oven at 110 ^{o}C. The sample was weighed continuously at an interval of time until a constant weight was obtained. The sample was transferred immediately into a desiccator for 30 min and the weight was recorded. Percentage moisture content was calculated (African Pharmacopoeia, 1986).

CHAPTER THREE

3.0 RESULTS OF THE STUDY

Table 3.1: Phytochemical screening of *M. foetida* ethanolic and *B. bergiana* methanolic extract

PLANT METABOLITES	*M. foetida*	*B. bergiana*
Alkaloids: (a) Preliminary screening		
(i) Draggendorff's reagent	+	-
(ii) Mayer's reagent	+	-
(b) Confirmatory test (TLC)	+	-
Anthraquinones: (a) Free	-	-
(b) Combined	-	-
Carbohydrates: (a) Starch	+	++
(b) Cellulose	+	+
Cardiac glycosides (Keller-Kiliani test for deoxy sugars)	+++	+++
Flavonoids (i) Lead acetate test	++	++
(ii) Sodium hydroxide test	+	++
(iii) Ferric chloride test	++	++
(iv) HCl + Mg turning	+	++
(v) EtOAc + Heat +dil NH_3	++	+++
Flavonol (Shinoda reduction test)	-	++
Terpenoids (Liebermann-Buchard test)	+++	+++
Steroids and sterols (Salkowski test)	+++	+
Saponins Frothing test	+	++
Tannins: (a) True:		
(i) Phenazone test	++	++
(ii) Ferric chloride test	++	-
(b) Phlobatannins (Formaldehyde test)	+	+++

Key: +++: very strongly positive, ++: Strongly Positive, +: Positive, -: Negative,

Table 3.2: Zone of Inhibition of crude extract and fractions of *M. foetida* leaves

Extracts / Bacterial strains	ZONE OF INHIBITION (mm)								
	Crude	Hexane	$CHCl_3$	EtOAc	BuOH	Aqueous	Neomycin	Ampicilin	Tetracycline
E. coil (ATCC 8739)	8.0±0.25	0.0±0.00	12.0±0.00	14.0±2.00	12.0±0.00	9.5±0.75	17.5±1.50	17.5±2.00	22.0±2.50
E. coil (ATCC 25922)	10.0±0.75	9.0±0.00	0.0±0.00	10.0±1.00	12.5±0.75	10.0±1.00	0.0±0.00	0.0±0.00	0.0±0.00
P. aeruginosa(ATCC 19582)	10.0±1.00	12.0±1.50	15.0±1.00	13.0±3.00	12.0±1.00	9.0±0.00	18.0±0.75	10.0±0.25	17.0±0.25
S. aureus (ATCC 6538)	12.0±2.00	8.0±0.00	12.5±1.50	13.5±0.50	15.0±1.00	11.5±1.25	25.5±0.05	39.0±0.25	28.5±0.75
S. faecalis (ATCC 29212)	8.5±0.50	10.5±0.50	10.0±1.00	10.0±0.00	11.0±1.25	9.5±0.50	0.0±0.00	0.0±0.00	15.0±1.00
B. cereus (ATCC 10702)	11.5±0.75	12.0±0.00	12.5±0.50	10.0±0.50	9.5±0.75	11.5±0.50	21.0±1.00	17.0±0.25	29.0+1.25
B. pumilus (ATCC 14884)	10.0±1.00	0.0±0.00	0.0±0.00	9.5±0.50	7.5±0.50	13.0±0.50	24.5±0.25	36.0±0.75	20.5±0.50
P. aeruginosa (ATCC 7700)	11.5±1.50	12.0±0.00	7.0±0.50	13.5±1.00	11.0±1.00	13.5±2.70	ND	ND	ND
E. cloacae (ATCC 13047)	9.5±1.50	9.0±1.00	13.5±0.50	10.5±0.75	12.5±2.00	8.0±0.00	18.5±0.25	0.0±0.00	23.5±1.00
K. pneumonia(ATCC 10031)	12.5±0.50	15.0±1.00	16.5±0.50	17.0±1.00	14.0±0.50	12.0±0.25	23.5±0.50	15.0±0.25	29.5±2.00
K. pneumonia (ATCC 4352)	9.5±0.50	12.0±1.50	10.0±0.00	12.0±0.00	14.5±2.50	8.5±2.50	19.0±1.00	11.0±0.75	25.0±2.00
P. vulgaris (ATCC 6830)	9.5±0.50	12.0±0.00	12.5±0.50	14.0±2.00	9.0±1.00	8.0±0.00	21.5±1.55	24.0±0.50	20.0±0.25
P. vulgaris (CSIR 0030)	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	16.5±0.50	11.5±0.50	14.0±0.75
S. marscens (ATCC 9986)	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	16.5±0.00	0.0±0.00	19.5±0.25
A. calcaoceuticus Acil(CSIR)	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	9.0±0.25	0.0±0.00	0.0±0.00	0.0±0.00
A. calcaoceuticus Aci2(CSIR)	8.5±0.25	0.0±0.00	7.5±0.25	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
K. pneumonia§	11.0±0.00	11.0±0.50	0.0±0.00	9.0±0.25	8.5±0.25	9.5±0.50	0.0±0.00	0.0±0.00	0.0±0.00
B. subtilis§	0.0±0.00	0.0±0.00	9.5±0.50	0.0±0.00	0.0±0.00	0.0±0.00	25.5±1.00	43.0±2.00	40.0±2.50
S. flexineri§	16.5±4.50	22.0±4.50	11.0±1.00	11.0±0.50	9.5±2.50	11.5±1.25	12.5±0.50	25.5±0.75	30.0+0.25
Salmonella spp§	10.0±0.75	0.0±0.00	0.0±0.00	0.0±0.00	10.0±0.25	11.5±1.00	15.0±0.00	26.5±0.25	30.5±0.25
S. epididirmis§	9.0±0.25	9.5±0.50	13.0±2.00	12.5±0.75	11.5±0.50	13.0±0.75	30.0±1.50	29.5±0.75	17.5±0.50
P. aeruginosa§	8.0±0.00	0.0±0.00	8.0±0.00	10.5±0.75	0.0±0.00	13.0±0.00	26.5±1.00	30.0±1.50	32.5±2.00
P. vulgaris§	9.5±0.75	8.5±0.50	13.0±2.00	8.5±0.50	13.5±0.50	12.5±1.50	17.5±0.25	12.5±0.50	15.0±0.50
E. faecalis§	7.5±0.00	8.5±0.25	12.5±2.20	10.0±0.25	8.0±0.25	0.0±0.00	0.0±0.00	35.5±1.50	13.5±0.25
E. coil§	9.5±0.75	0.0±0.00	0.0±0.00	13.0±2.00	7.5±0.25	8.0±0.00	19.5±0.75	22.5±0.50	27.5±0.50
S. aureus§	8.0±0.00	11.0±1.00	8.5±0.25	8.5±0.00	10.5±1.75	10.5±0.75	21.0±1.75	41.5±3.00	34.5±2.50
*S. aureus OKOH2B*Ω	8.0±0.25	9.5±0.25	7.5±0.00	8.5±0.25	7.5±0.25	7.0±0.00	24.5±0.50	37.0±0.00	35.0±0.00
*S. aureus OKOH3*Ω	7.0±0.00	10.0±0.50	7.5±0.25	7.5±0.25	7.5±0.25	9.0±0.25	21.0±0.25	15.0±0.25	32.5±0.25
M. kristinae§	7.0±0.00	0.0±0.00	7.5±0.00	8.0±0.50	8.5±0.50	0.0±0.00	16.5±0.00	15.0±0.25	32.5±1.00
M. luteus§	8.5±0.00	0.0±0.00	14.5±2.50	8.0±0.00	7.0±0.00	0.0±0.00	26.0±0.00	16.0±0.00	24.5±0.50

Ω are clinical isolates; § are environmental strains. Dose: 5 mg mL^{-1}; Disc diameter: 6mm ; ND : not determined

Table 3.3: Minimum inhibitory concentration (MICs) of the crude extract and fractions of *M. foetida* leaves.

Extracts / Bacterial strains	MIC (mg mL^{-1})								
	Crude	Hexane	$CHCl_3$	EtOAc	BuOH	Aqueous	Neomycin	Ampicilin	Tetracycline
E. coil (ATCC 8739)	5.000	-	2.500	0.625	5.000	5.000	0.039	0.039	0.039
E. coil (ATCC 25922)	5.000	5.000	-	0.625	5.000	2.500	-	-	-
P. aeruginosa(ATCC 19582)	5.000	5.000	1.250	0.625	5.000	5.000	0.039	0.039	0.156
S. aureus (ATCC 6538)	2.500	2.500	1.250	0.078	0.625	1.250	0.039	2.500	0.039
S. faecalis (ATCC 29212)	0.313	0.313	0.078	0.078	2.500	2.500	-	-	0.078
B. cereus (ATCC 10702)	0.078	0.625	0.625	-	5.000	1.250	0.039	5.000	0.039
B. pumilus (ATCC 14884)	5.000	-	-	0.625	5.000	0.625	0.039	5.000	0.156
P. aeruginosa (ATCC 7700)	0.156	0.625	-	0.313	5.000	0.313	ND	ND	ND
E. cloacae (ATCC 13047)	5.000	5.000	0.625	1.250	5.000	5.000	0.039	-	0.078
K. pneumonia(ATCC 10031)	2.500	5.000	5.000	2.500	2.500	2.500	0.039	-	0.156
K. pneumonia (ATCC 4352)	0.039	0.313	0.313	0.625	1.250	2.500	0.039	1.250	0.039
P. vulgaris (ATCC 6830)	1.250	0.313	0.313	0.1563	0.313	0.625	0.039	-	0.039
P. vulgaris (CSIR 0030)	ND	ND	ND	ND	ND	ND	ND	ND	ND
S. marscens (ATCC 9986)	ND	ND	ND	ND	ND	ND	ND	ND	ND
A. calcaoceuticus Aci1 (LIO)	ND	ND	ND	ND	ND	ND	ND	ND	ND
A. calcaoceuticus Aci2 (LIO)	ND	ND	ND	ND	ND	ND	ND	ND	ND
K. pneumonia (LIO)	ND	ND	ND	ND	ND	ND	ND	ND	ND
B. subtilis (LIO)	ND	ND	ND	ND	ND	ND	ND	ND	ND
S. flexineri (LIO)	0.625	0.313	0.313	0.313	1.250	5.000	0.039	1.250	0.039
Salmonella spp (LIO)	1.250	-	-	-	1.250	2.500	0.039	0.156	0.039
S. epididirmis (LIO)	1.250	0.625	0.625	0.156	0.625	2.500	0.039	0.625	0.039
P. aeruginosa (LIO)	1.250	-	0.625	0.156	-	1.250	0.039	2.500	0.039
P. vulgaris (LIO)	0.625	0.625	0.625	0.313	2.500	1.250	0.039	0.313	0.039
E. faecalis (LIO)	1.250	0.625	0.625	0.156	2.500	-	-	0.039	0.039
E. coil (LIO)	0.625	-	-	0.078	1.250	5.000	0.039	0.625	0.039
S. aureus (LIO)	0.625	0.625	1.250	0.156	0.625	5.000	0.039	0.039	0.039
M. kristinae (LIO)	ND	ND	ND	ND	ND	ND	ND	ND	ND
M. luteus (LIO)	ND	ND	ND	ND	ND	ND	ND	ND	ND

ATCC=American Type Culture Collection; CSIR=Council for Scientific and Industrial Research; LIO=Locally Isolated Organism; $CHCl_3$=Chloroform; EtOAc=Ethyl acetate; BuOH=Butanol; ND=Not Determined. Dose: 5 mg mL^{-1}; -= no inhibition

Table 3.4: The antibacterial activities of crude and successive extract of *B. bergiana* leaves

Extracts / Bacterial	ZONE OF INHIBITION (mm)								
	Crude	Hexane	$CHCl_3$	EtOAc	BuOH	Aqueous	Neomycin	Ampicilin	Tetracycline
E. coil (ATCC 8739)	9.0±1.41	12.5±2.00	10.5±0.50	9.0±0.75	11.0±2.50	9.5±0.75	17.5±1.50	17.5±2.00	22.0±2.50
E. coil (ATCC 25922)	9.5±0.00	7.5±1.00	11.5±2.00	10.0±0.00	12.5±0.00	10.5±0.50	0.0±0.00	0.0±0.00	0.0±0.00
P. aeruginosa(ATCC 19582)	12.0±0.00	13.0±2.00	12.0±0.70	15.0±2.00	12.5±0.50	11.5±0.75	18.0±0.75	10.0±0.25	17.0±0.25
S. aureus (ATCC 6538)	13.0±0.00	13.5±2.50	10.5±1.00	15.5±2.50	9.0±0.25	12.0±0.50	25.5±0.50	39.0±0.25	28.5±0.75
S. faecalis (ATCC 29212)	9.0±1.10	16.0±2.00	0.0±0.00	10.5±1.00	0.0±0.00	8.0±0.75	0.0±0.00	0.0±0.00	15.0±1.00
B. cereus (ATCC 10702)	10.5±0.75	8.0±0.75	13.5±0.50	8.0±0.75	13.0±1.00	12.0±0.75	21.0±1.00	17.0±0.25	29.0+1.25
B. pumilus (ATCC 14884)	10.0±0.00	7.0±0.75	11.0±1.00	0.0±0.00	0.0±0.00	12.0±1.00	24.5±0.25	36.0±0.75	20.5±0.50
P. aeruginosa (ATCC 7700)	9.0±1.41	15.6±1.00	18.5±2.50	14.0±0.50	15.0±0.75	13.5±0.25	18.5±0.25	32.5±0.75	19.0±0.75
E. cloacae (ATCC 13047)	10.0±0.25	10.0±1.00	15.5±2.00	13.0±0.25	10.0±2.00	8.5±0.00	18.5±0.25	0.0±0.00	23.5±1.00
K. pneumonia(ATCC 10031)	11.0±0.75	16.0±2.00	16.0±0.50	13.0±0.50	14.5±0.00	13.0±2.50	23.5±0.50	15.0±0.25	29.5±2.00
K. pneumonia (ATCC 4352)	11.5±0.75	11.0±1.00	13.0±2.00	10.0±1.00	11.0±0.00	10.5±0.75	19.0±1.00	11.0±0.75	25.0±2.00
P. vulgaris (ATCC 6830)	10.0±3.53	10.0±0.50	10.0±1.00	11.0±0.75	10.0±0.50	9.0±0.00	21.5±1.55	24.0±0.50	20.0±0.25
P. vulgaris (CSIR 0030)	0.0±0.00	12.5±2.00	0.0±0.00	8.0±1.25	8.0±0.75	12.0±0.00	16.5±0.50	11.5±0.50	14.0±0.75
S. marscens (ATCC 9986)	0.0±0.00	9.5±1.00	0.0±0.00	9.0±0.50	9.0±0.25	11.0±1.00	16.5±0.00	0.0±0.00	19.5±0.25
A. calcaoceuticus Aci1	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
A. calcaoceuticus Aci2	0.0±0.00	9.5±2.00	0.0±0.00	0.0±0.00	8.0±0.50	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
K. pneumonia	8.0±1.00	11.0±0.50	0.0±0.00	0.0±0.00	7.5±0.50	11.5±2.00	0.0±0.00	0.0±0.00	0.0±0.00
B. subtilis	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	25.5±1.00	43.0±2.00	40.0±2.50
S. flexineri	11.5±0.25	0.0±0.00	8.0±0.75	15.5±2.50	12.0±2.00	0.0±0.00	12.5±0.50	25.5±0.75	30.0+0.25
Salmonella spp	9.0±0.75	10.5±1.00	10.0±0.50	10.0±1.00	9.5±0.75	0.0±0.00	15.0±0.00	26.5±0.25	30.5±0.25
S. epididirmis	9.0±0.75	9.0±0.75	8.5±1.50	9.0±0.50	10.0±1.00	8.5±0.25	30.0±1.50	29.5±0.75	17.5±0.50
P. aeruginosa	12.0±0.25	8.0±0.25	9.0±2.50	9.5±0.75	0.0±0.00	0.0±0.00	26.5±1.00	30.0±1.50	32.5±2.00
P. vulgaris	10.5±0.50	8.5±0.75	11.0±0.00	9.5±0.75	11.0±0.75	0.0±0.00	17.5±0.25	12.5±0.50	15.0±0.50
E. faecalis	0.0±0.00	0.0±0.00	13.0±1.00	10.0±0.75	0.0±0.00	0.0±0.00	0.0±0.00	35.5±1.50	13.5±0.25
E. coil	8.0±0.25	15.5±0.50	11.0±2.50	9.0±0.50	17.0±2.50	0.0±0.00	19.5±0.75	22.5±0.50	27.5±0.50
S. aureus	9.0±0.50	10.0±0.50	11.5±0.50	8.5±0.50	8.0±0.50	0.0±0.00	21.0±1.75	41.5±3.00	34.5±2.50
S. aureus OKOH2B$^{\Omega}$	8.0±0.25	0.0±0.00	9.0±0.25	7.5±0.25	10.5±0.25	10.5±0.50	24.5±0.50	37.0±0.00	35.0±0.00
S. aureus OKOH3$^{\Omega}$	7.0±0.00	7.5±0.25	10.5±0.50	9.5±0.50	9.0±0.50	8.5±0.75	21.0±0.25	15.0±0.25	32.5±0.25
M. kristinae	8.5±1.00	7.0±0.25	8.5±0.75	11.5±2.00	9.5±0.25	7.0±0.25	16.5±0.00	15.0±0.25	32.5±1.00
M. luteus	9.5±1.50	9.5±2.00	11.0±0.00	12.5±0.25	12.5±1.00	10.0±1.00	26.0±0.00	16.0±0.00	24.5±0.50

Ω are clinical isolates; *§* are environmental strains. Dose: 5 mg mL^{-1}; Disc diameter: 6mm

Table 3.5: Minimum inhibitory concentration (MICs) of the crude extract and fractions of *B. bergiana* leaves

Extracts / Bacterial	MIC (mg/ml)								
	Crude	Hexane	$CHCl_3$	EtOAc	BuOH	Aqueous	Neomycin	Ampicilin	Tetracycline
E. coil (ATCC 8739)	0.312	5.000	2.500	1.250	0.039	0.039	0.039	0.039	0.039
E. coil (ATCC 25922)	1.250	5.000	1.250	-	1.250	0.625	-	-	-
P. aeruginosa(ATCC 19582)	1.250	5.000	0.156	0.156	0.312	0.156	0.039	0.039	0.156
S. aureus (ATCC 6538)	0.625	5.000	0.625	0.312	0.312	-	0.039	2.500	0.039
S. faecalis (ATCC 29212)	2.500	5.000	-	0.625	-	1.250	-	-	0.078
B. cereus (ATCC 10702)	2.500	-	1.250	0.625	1.250	5.000	0.039	5.000	0.039
B. pumilus (ATCC 14884)	2.500	-	2.500	-	-	-	0.039	5.000	0.156
P. aeruginosa (ATCC 7700)	0.625	2.500	0.312	1.250	0.625	2.500	0.039	0.039	0.156
E. cloacae (ATCC 13047)	0.312	5.000	0.0780	0.078	0.078	0.312	0.039	-	0.078
K. pneumonia(ATCC 10031)	0.625	2.500	0.625	1.250	0.312	-	0.039	-	0.156
K. pneumonia (ATCC 4352)	2.500	5.000	1.250	0.625	0.625	-	0.039	1.250	0.039
P. vulgaris (ATCC 6830)	1.250	1.250	1.250	0.312	0.625	-	0.039	-	0.039
P. vulgaris (CSIR 0030)	ND	ND	ND	ND	ND	ND	ND	ND	ND
S. marscens (ATCC 9986)	ND	ND	ND	ND	ND	ND	ND	ND	ND
A. calcaoceuticus Aci1 (LIO)	ND	ND	ND	ND	ND	ND	ND	ND	ND
A. calcaoceuticus Aci2 (LIO)	ND	ND	ND	ND	ND	ND	ND	ND	ND
K. pneumonia (LIO)	ND	ND	ND	ND	ND	ND	ND	ND	ND
B. subtilis (LIO)	ND	ND	ND	ND	ND	ND	ND	ND	ND
S. flexineri (LIO)	0.625	-	0.625	0.625	0.625	-	0.039	1.25	0.039
Salmonella spp (LIO)	0.625	5.000	0.625	0.625	0.156	-	0.039	0.156	0.039
S. epididirmis (LIO)	0.312	5.000	0.156	0.156	1.250	0.312	0.039	0.625	0.039
P. aeruginosa (LIO)	0.312	5.000	0.312	0.312	-	-	0.039	2.500	0.039
P. vulgaris (LIO)	0.078	2.500	0.078	0.039	0.039	-	0.039	0.312	0.039
E. faecalis (LIO)	-	2.500	0.625	0.625	-	-	-	0.039	0.039
E. coil (LIO)	0.078	2.500	0.625	0.625	0.039	-	0.039	0.625	0.039
S. aureus (LIO)	0.078	-	2.500	2.500	2.500	-	0.039	0.039	0.039
M. kristinae (LIO)	ND	ND	ND	ND	ND	ND	ND	ND	ND
M. luteus (LIO)	ND	ND	ND	ND	ND	ND	ND	ND	ND

ATCC=American Type Culture Collection; CSIR=Council for Scientific and Industrial Research; LIO=Locally Isolated Organism; $CHCl_3$=Chloroform; EtOAc=Ethyl acetate; BuOH=Butanol; ND=Not Determined. Dose: 5 mg mL^{-1}; -= not active

Table 3.6: MIC units of Compounds Isolated from *M. foetida* and *B. bergiana*

	MIC (Mg/mL)					
Bacteria strains	**OM//E/T2**	**OM/14/37**	**OM/11/F9**	**OM/9/F3**	**0M/11/12**	**OM/10/F1**
E. coil (ATCC 8739)	0.0234	0.062	0.008	0.250	0.008	0.250
E. coil (ATCC 25922)	0.500	0.500	0.500	0.250	0.250	0.250
P. aeruginosa(ATCC 19582)	0.187	0.500	0.500	0.500	0.500	0.500
S. aureus (ATCC 6538)	0.187	0.250	0.500	0.500	0.500	0.375
S. faecalis (ATCC 29212)	0.125	0.500	0.500	0.500	0.500	0.250
B. cereus (ATCC 10702)	0.250	0.500	0.500	1.000	1.000	0.750
B. pumilus (ATCC 14884)	0.250	0.500	0.250	0.500	0.250	0.375
P. aeruginosa (ATCC 7700)	0.125	0.250	0.250	0.125	0.500	0.250
E. cloacae (ATCC 13047)	0.062	0.250	0.062	0.125	0.125	0.250
K. pneumonia(ATCC 10031)	0.062	0.125	0.062	0.250	0.125	0.125
K. pneumonia (ATCC 4352)	0.125	0.125	0.250	0.750	0.250	0.250
P. vulgaris (ATCC 6830)	0.125	0.125	0.125	0.125	0.250	0.375
S. marscens (ATCC 9986)	0.125	0.125	0.125	0.125	0.250	0.250
K. pneumonia	1.000	0.500	0.500	0.500	0.500	0.750
B. subtilis	0.500	0.500	0.500	0.500	0.500	0.750
Salmonella spp	0.125	0.250	0.250	0.375	0.125	0.125
S. epididirmis	0.125	0.250	0.125	0.125	0.125	0.250
P. aeruginosa	0.062	0.500	0.500	1.000	0.750	0.500
P. vulgaris	0.031	0.500	0.250	0.250	0.250	0.250
E. faecalis	0.008	0.008	0.008	0.008	0.008	0.008
E. coil	0.008	0.008	0.008	0.008	0.008	0.008
S. aureus	0.250	0.250	0.125	0.500	0.500	1.000
M. kristinae	0.0312	1.000	0.500	0.500	0.500	0.750
M. kristinae	0.0312	0.500	0.500	0.500	0.500	0.500

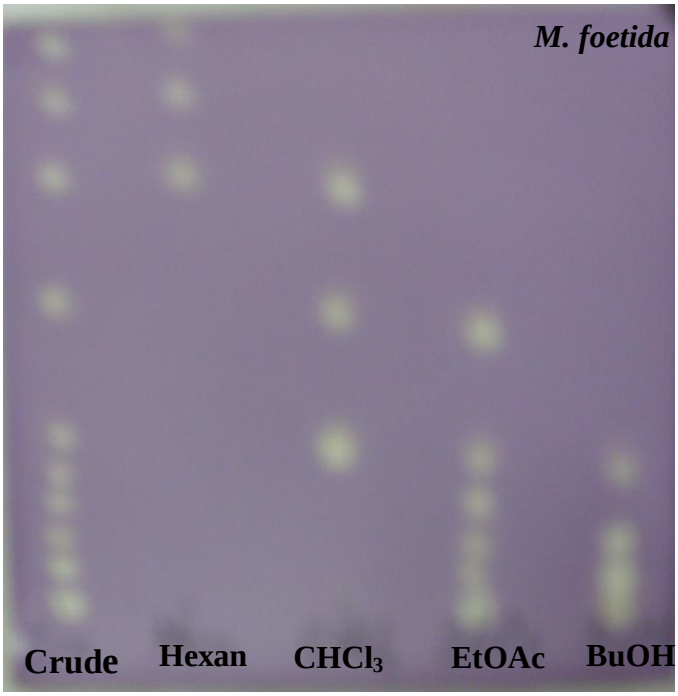


Figure 3.1: Chromatogram of Crude extract and fractions of *M. foetida* sprayed with DPPH.

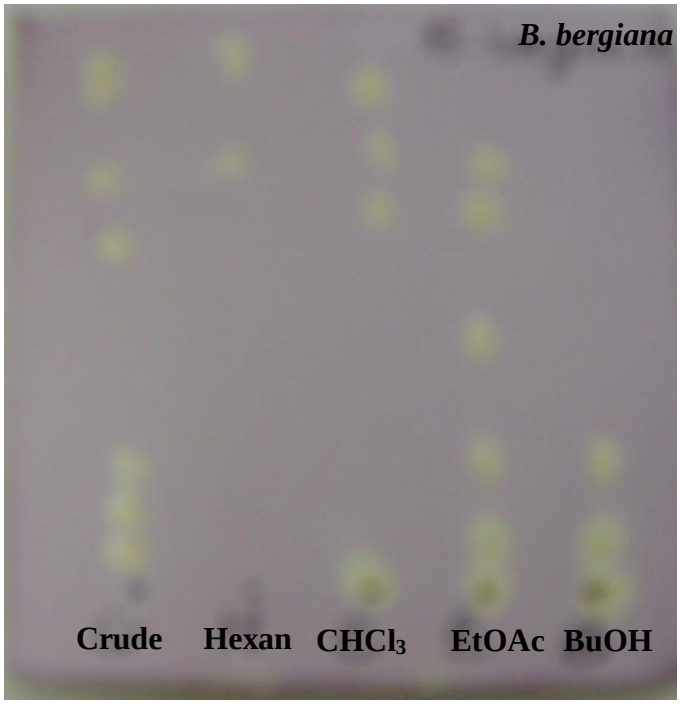


Figure 3.2: Chromatogram of crude and successive extracts of *B. bergiana* sprayed with DPPH

TABLE 3.7: Antioxidant: Qualitative Assay of crude extract of *M. foetida*

Major spots R_f value	Colour in daylight	Colour in UV254	Anisaldahyde Reagent	DPPH	
				Reaction uspeed	Intensity of spots
0.07	colourless	Fluorescence	light orange	Slow	++
0.10	colourless	fluorescence	orange	slow	+
0.15	colourrless	fluorescence	light purple	slow	++
0.34	v. light green	greenish	deep purple	-	-
0.19	colourless	fluorescence	purple	fast	++
0.23	colorless	fluorescence	violet	slow	++
0.48	colourless	fluorescence	violet	slow	+
0.65	light reddish	bright yellow	yellow	slow	+
0.78	colourless	blue	violet	-	-
0.80	colourless	fluorescence	purple	fast	++
0.85	v. light yellow	fluorescence	yellowish	fast	+++
0.91	yellowish green	greenish	green	-	-

TABLE 3.8: Antioxidant: Qualitative Assay of crude extract of *B. bergiana*

Major spots R_f value	Colour in daylight	Colour in UV254	Anisaldahyde Reagent	DPPH	
				Reaction uspeed	Intensity of spots
0.09	colourless	Fluorescence	light orange	Slow	++
0.13	colourless	fluorescence	orange	slow	+
0.15	colourrless	blue	purple	slow	++
0.23	light green	greenish	purple	-	-
0.29	colourless	fluorescence	purple	fast	++
0.34	colorless	fluorescence	violet	slow	++
0.44	colourless	fluorescence	violet	fast	+++
0.46	light yellow	bright yellow	yellow	fast	+
0.78	colourless	blue	violet	-	-
0.88	light yellow	fluorescence	yellowish	fast	+++
0.90	yellowish green	fluorescence	green	-	-

Keys -: no yellow colouration,
+: weak intensity of yellow colouration (15 – 30 mins before colour development)
++: intermediate intensity (1 – 15 mins before colour development)
+++: strong intensity (immediate reaction)
Keys same above

Table 3.9: Percentage DPPH scavenging potential of crude and fractions of *M. foetida*

	Percentage Inhibition of different Concentration						IC_{50}
	5 µg/mL	10 µg/mL	25 µg/Ml	50 µg/mL	125 µg/mL	250 µg/mL	DPPH
Crude Extract	40.58±0.04	40.83±0.02	43.64±0.01	45.56±0.01	49.09±0.01	59.15±0.02	114.87
Hexane fraction	14.93±0.02	16.57±0.03	16.65±0.01	16.88±0.00	22.25±0.13	24.72±0.02	>250
$CHCl_3$ fraction	12.43±0.04	12.92±0.04	13.90±0.04	15.97±0.04	18.11±0.03	19.98±0.01	>250
EtOAc fraction	11.87±0.01	16.37±0.05	17.02±0.01	22.82±0.02	40.36±0.01	59.76±0.06	110.73
BuOH fraction	13.94±0.03	14.87±0.02	15.40±0.02	21.17±0.04	31.74±0.03	42.67±0.06	103.27
Ascorbic acid	20.15± 0.05	23.17±0.02	37.94±0.07	51.76±0.03	71.56±0.02	91.43±0.04	47.13
BHT	23.03±0.06	45.38±0.04	91.19±0.02	97.68±0.02	100.00±0.00	100.00±0.00	12.47

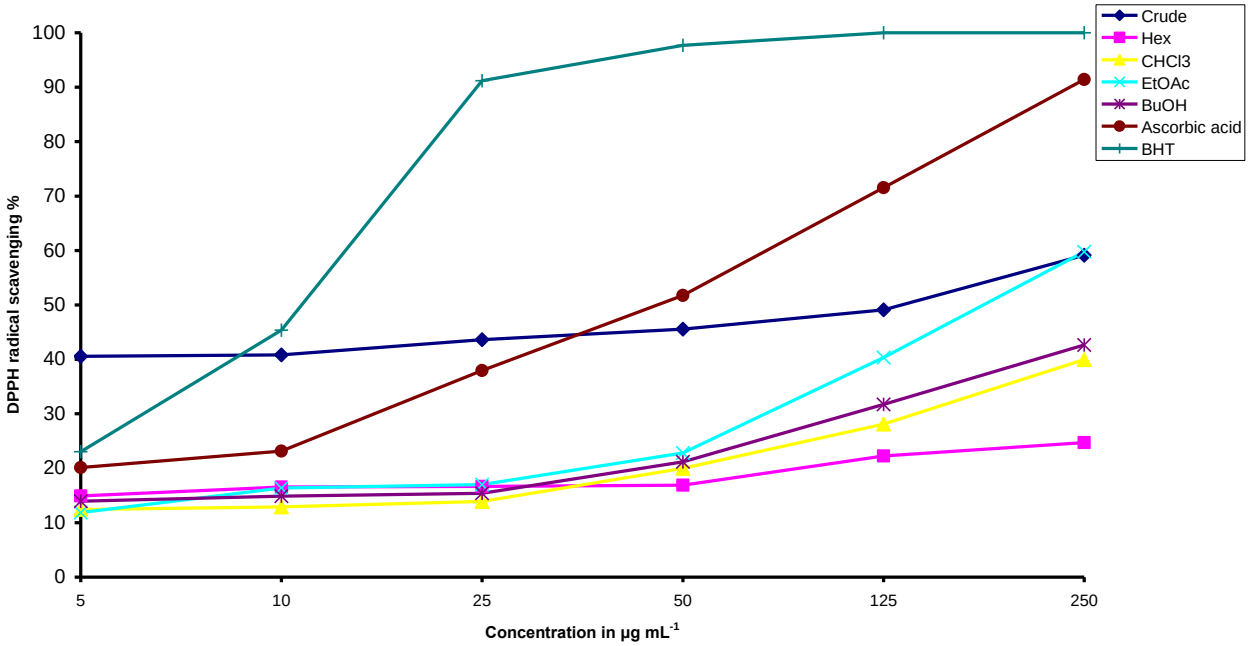


Figure 3.3: DPPH scavenging activities of the crude extract and fraction of *M. foetida* Leaves

Table 3.10: Percentage DPPH scavenging potential of sequential extracts of *B. bergiana*

	Percentage Inhibition of different Concentration						IC_{50}
	5 µg/mL	10 µg/mL	25 µg/Ml	50 µg/mL	125 µg/mL	250 µg/mL	DPPH
Crude Extract	15.66±0.01	16.71±0.03	21.81±0.04	26.97±0.05	53.41±0.00	86.66±0.03	114.87
Hexane fraction	0.40±0.02	0.47±0.02	1.13±0.02	2.24±0.01	5.81±0.01	7.88±0.04	>250
$CHCl_3$ fraction	2.33±0.04	5.00±0.01	9.75±0.02	10.54±0.01	22.38±0.03	42.57±0.00	>250
EtOAc fraction	7.16±0.01	9.27±0.02	11.96±0.02	17.08±0.01	57.86±0.02	94.48±0.00	110.73
BuOH fraction	5.79±0.04	9.91±0.02	20.56±0.03	24.74±0.07	60.19±0.09	93.67±0.02	103.27
Ascorbic acid	20.15±0.05	23.17±0.02	37.94±0.07	51.76±0.03	71.56±0.02	91.43±0.04	47.13
BHT	23.03±0.06	45.38±0.02	91.19±0.02	97.68±0.02	100.00±0.00	100.00±0.00	12.47

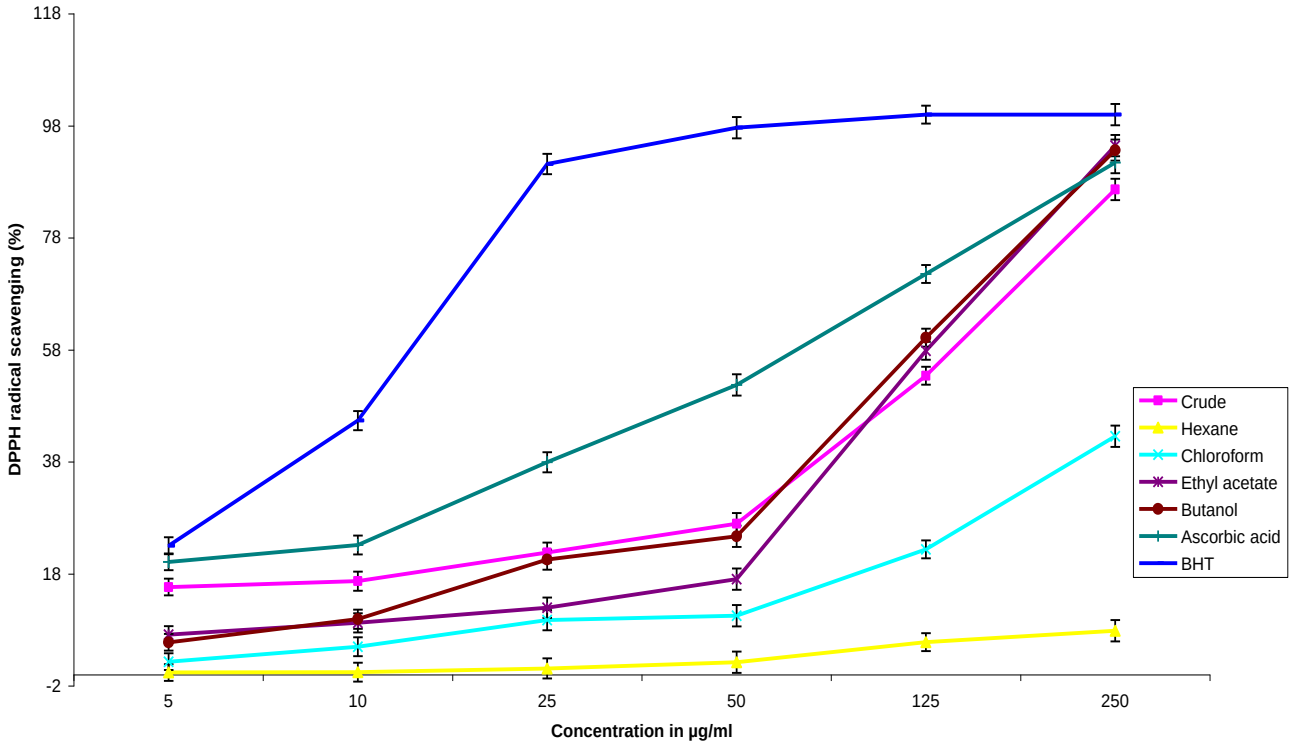


Figure 3.4: DPPH scavenging activities of the crude and successive extracts of *B. bergiana* Leaves

Table 3.11: Percentage ABTS scavenging potential of crude and fractions of *M. foetida*

	Percentage Inhibition of different Concentration						IC_{50}
	5 µg/mL	10 µg/mL	25 µg/Ml	50 µg/mL	125 µg/mL	250 µg/mL	ABTS
Crude Extract	7.95±0.00	11.48±0.01	23.06±0.00	56.04±0.04	80.67±0.01	99.80±0.00	45.53
Hexane fraction	4.49±0.01	4.20±0.01	11.82±0.01	25.19±0.00	53.81±0.01	86.43±0.00	114.07
$CHCl_3$ fraction	12.99±0.01	16.41±0.01	33.30±0.01	54.00±0.00	91.99±0.00	100.78±0.00	43.80
EtOAc fraction	12.79±0.01	33.10±0.02	48.54±0.01	78.71±0.00	100.00±0.00	100.78±0.01	24.87
BuOH fraction	14.35±0.02	19.24±0.01	38.48±0.01	60.35±0.00	98.34±0.01	100.78±0.00	38.07
BHA	95.49±0.01	98.71±0.00	99.03±0.00	99.14±0.00	100.00±0.00	100.00±0.00	<5
BHT	71.46±0.05	81.44±0.03	87.12±0.00	98.50±0.00	99.89±0.00	100.00±0.00	<5

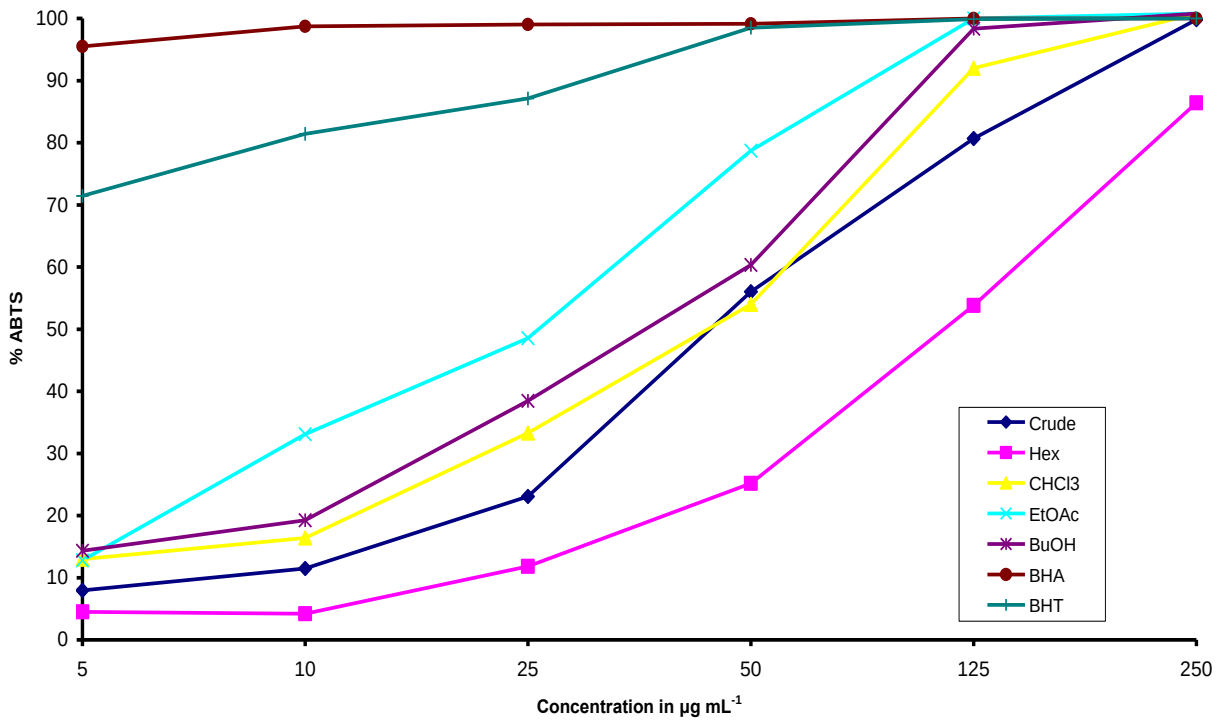


Figure 3.5: ABTS scavenging potential of the Crude extract and fractions of *M. foetida* leaves

Table 3.12: Percentage ABTS scavenging potential of sequential extracts of *B. bergiana*

	Percentage Inhibition of different Concentration						IC_{50}
	5 µg/mL	10 µg/mL	25 µg/Ml	50 µg/mL	125 µg/mL	250 µg/mL	ABTS
Crude Extract	11.58±0.00	27.58±0.02	49.66±0.01	87.93±0.01	100.29±0.00	100.00±0.00	217.27
Hexane fraction	1.47±0.01	0.39±0.01	7.16±0.01	11.78±0.00	24.24±0.01	59.27±0.00	26.33
$CHCl_3$ fraction	7.61±0.01	13.59±0.01	27.79±0.01	50.30±0.01	89.96±0.00	101.72±0.00	49.27
EtOAc fraction	23.12±0.00	39.45±0.01	95.54±0.00	100.30±0.00	100.20±0.00	100.10±0.00	13.27
BuOH fraction	9.432±0.01	12.58±0.01	43.61±0.01	75.96±0.03	101.12±0.00	100.91±0.00	29.67
BHA	95.49±0.01	98.71±0.00	99.03±0.00	99.14±0.00	100.00±0.00	100.00±0.00	<5
BHT	71.46±0.05	81.44±0.03	87.12±0.00	98.50±0.00	99.89±0.00	100.00±0.00	<5

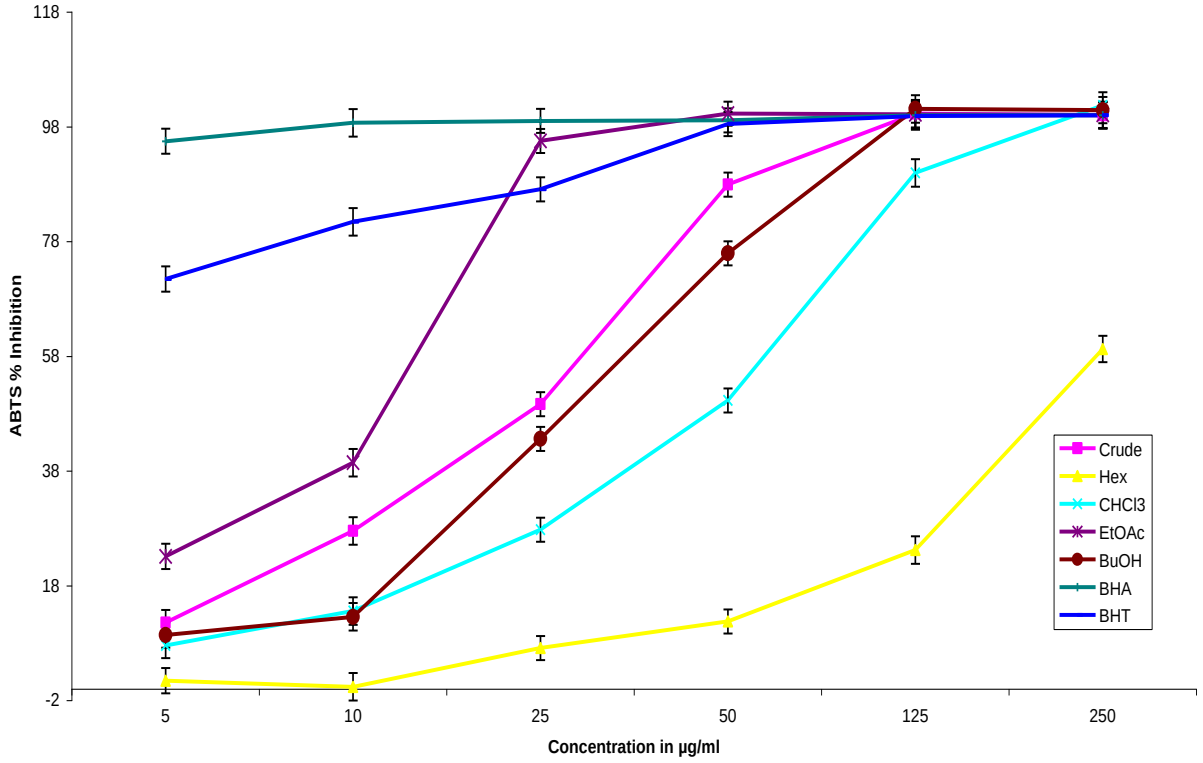


Figure 3.6: ABTS scavenging potential of the Crude and Successive extracts of *B. bergiana* leaves

Table 3.13: Reducing power ability of crude and different fractions of *M. foetida*

	Percentage Inhibition of different Concentration					
	5 µg/mL	**10 µg/mL**	**25 µg/mL**	**50 µg/mL**	**125 µg/mL**	**250 µg/mL**
Crude Extract	0.321±0.001	0.319±0.004	0.337±0.002	0.342±0.006	0.396±0.007	0.478±0.006
Hexane fraction	0.362±0.003	0.364±0.004	0.372±0.002	0.392±0.010	0.407±0.004	0.450±0.006
$CHCl_3$ fraction	0.761±0.046	0.803±0.014	0.820±0.005	0.833±0.016	0.858±0.027	0.928±0.025
EtOAc fraction	0.453±0.010	0.465±0.005	0.474±0.004	0.489±0.008	0.519±0.004	0.592±0.012
BuOH fraction	0.440±0.020	0.443±0.010	0.444±0.008	0.458±0.004	0.485±0.007	0.556±0.010
Ascorbic acid	0.472±0.002	0.537±0.003	0.612±0.004	0.725±0.007	0.781±0.003	0.932±0.004
BHT	0.419±0.004	0.434±0.004	0.528±0.006	0.723±0.006	1.128±0.003	1.683±0.012
BHA	0.140±0.007	0.279±0.004	0.703±0.004	0.854±0.034	1.074±0.041	1.509±0.058

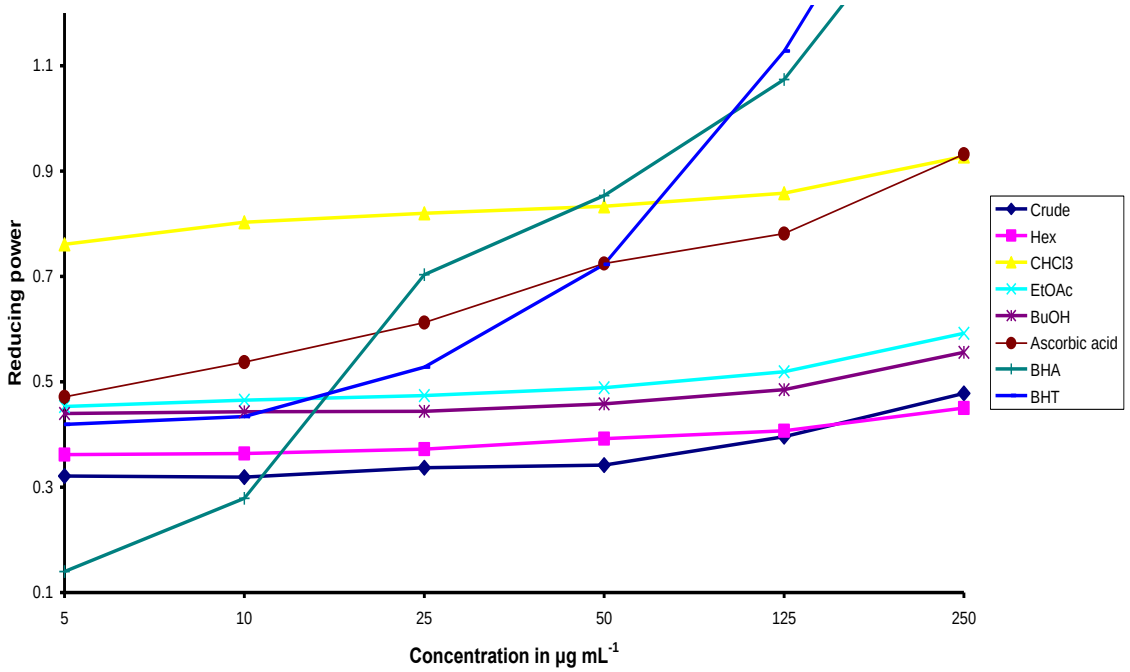


Figure 3.7: Reducing power of crude extract and fractions from *M. foetida* leaves

Table 3.14: Reducing power ability of crude and different fractions of *B.bergiana*

	Percentage Inhibition of different Concentration					
	5	**10**	**25**	**50**	**125**	**250**
Crude Extract	0.437±0.154	0.528±0.017	0.560±0.007	0.623±0.031	0.649±0.139	0.932±0.162
Hexane fraction	0.336±0.013	0.336±0.007	0.349±0.005	0.352±0.005	0.351±0.004	0.371±0.008
$CHCl_3$ fraction	0.142±0.002	0.162±0.006	0.169±0.006	0.178±0.002	0.188±0.004	0.209±0.004
EtOAc fraction	0.314±0.007	0.332±0.003	0.373±0.009	0.413±0.019	0.436±0.011	0.522±0.002
BuOH fraction	0.281±0.002	0.289±0.004	0.309±0.007	0.332±0.004	0.346±0.018	0.415±0.022
Ascorbic acid	0.472±0.002	0.537±0.003	0.612±0.004	0.725±0.007	0.781±0.003	0.932±0.004
BHT	0.419±0.004	0.434±0.004	0.528±0.006	0.723±0.006	1.128±0.003	1.683±0.012
BHA	0.140±0.007	0.279±0.004	0.703±0.004	0.854±0.034	1.074±0.041	1.509±0.058

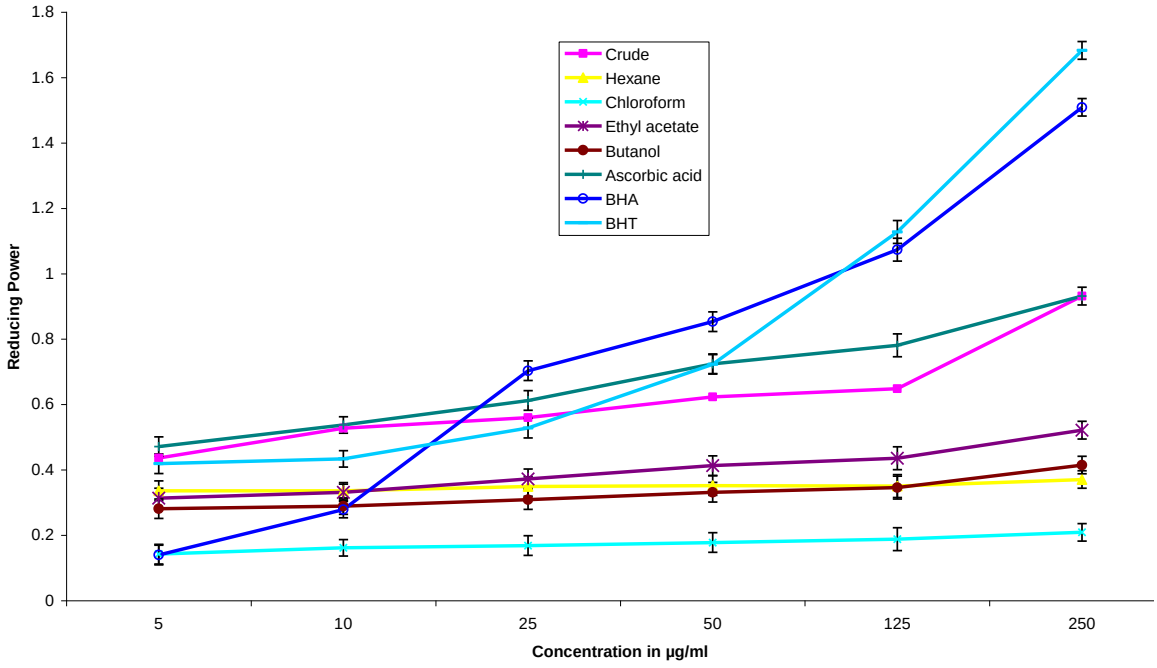


Figure 3.8: Reducing power of crude and successive extracts from *B. bergiana* leaves

Table 3.15: Percentage metal ion chelating potential of crude and fractions of *M. foetida*

	Percentage Inhibition of different Concentration						IC_{50} Metal Chelating
	5 µg/mL	**10 µg/mL**	**25 µg/Ml**	**50 µg/Ml**	**125 µg/mL**	**250 µg/mL**	
Crude Extract	12.84±0.17	16.84±0.05	18.55±0.03	23.25±0.01	26.95±0.03	29.73±0.00	180.07
Hexane fraction	34.56±0.01	38.00±0.03	40.66±0.04	44.55±0.05	45.25±0.04	56.54±0.01	>250
$CHCl_3$ fraction	23.27±0.35	27.38±0.05	31.36±0.09	34.81±0.01	37.73±0.03	42.19±0.02	>250
EtOAc fraction	29.92±0.01	34.83±0.04	35.30±0.02	42.98±0.03	49.20±0.06	64.85±0.14	129.67
BuOH fraction	25.93±0.05	26.96±0.06	30.45±0.05	33.08±0.02	39.89±0.06	51.79±0.09	230
BHA	59.20±0.08	60.33±0.02	62.39±0.03	69.86±0.01	72.77±0.03	83.10±0.00	<5
BHT	19.16±0.04	39.47±0.06	44.64±0.02	55.03±0.06	83.81±0.04	98.94±0.06	30.07

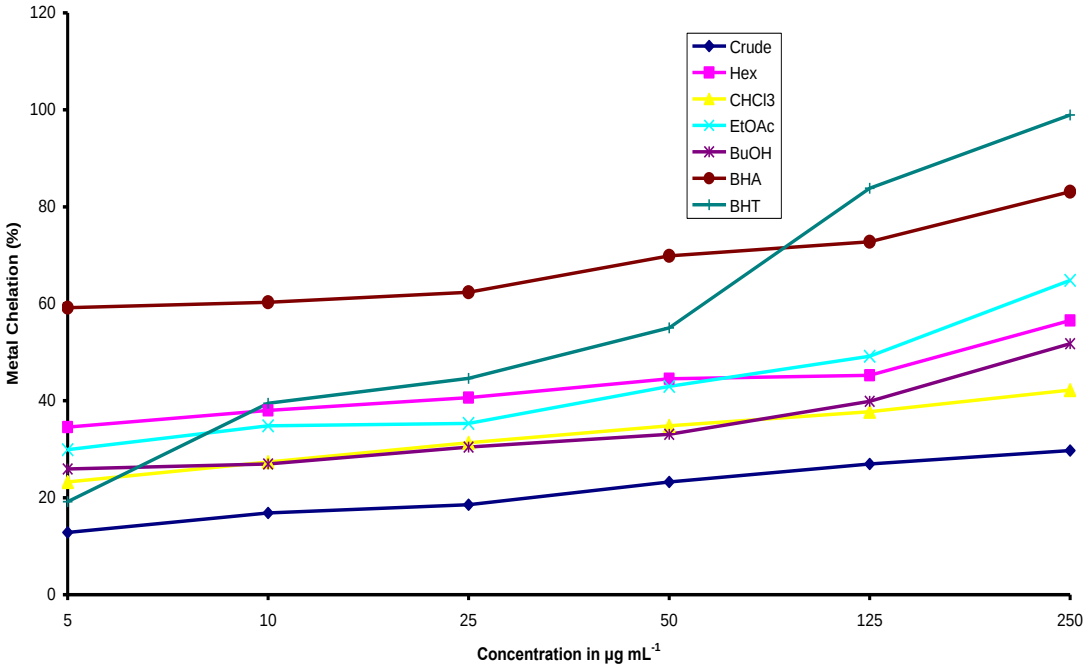


Figure 3.9: Metal Chelating ability of Crude extract and fractions from *M. foetida* leaves

Table 3.16: Percentage metal ion chelating potential of sequential extracts of *B. bergiana*

	Percentage Inhibition of different Concentration						IC_{50} Metal Chelating
	5 µg/mL	10 µg/mL	25 µg/mL	50 µg/mL	125 µg/mL	250 µg/mL	
Crude Extract	30.62±0.03	31.06±0.03	32.85±0.03	33.37±0.02	40.51±0.02	44.22±0.04	>250
Hexane fraction	33.10±0.07	35.49±0.03	37.55±0.02	38.19±0.01	41.20±0.02	45.69±0.07	>250
$CHCl_3$ fraction	42.32±0.07	44.50±0.06	45.70±0.00	49.68±0.19	54.07±0.06	62.83±0.05	52.6
EtOAc fraction	34.38±0.05	34.40±0.02	41.10±0.04	43.67±0.05	47.87±0.04	51.63±0.01	191.8
BuOH fraction	49.09±0.01	50.03±0.01	52.68±0.00	55.14±0.00	57.22±0.01	63.03±0.04	8.33
BHA	59.20±0.08	60.33±0.02	62.39±0.03	69.86±0.01	72.77±0.03	83.10±0.00	<5
BHT	19.16±0.04	39.47±0.06	44.64±0.02	55.03±0.06	83.81±0.04	98.94±0.06	38.6

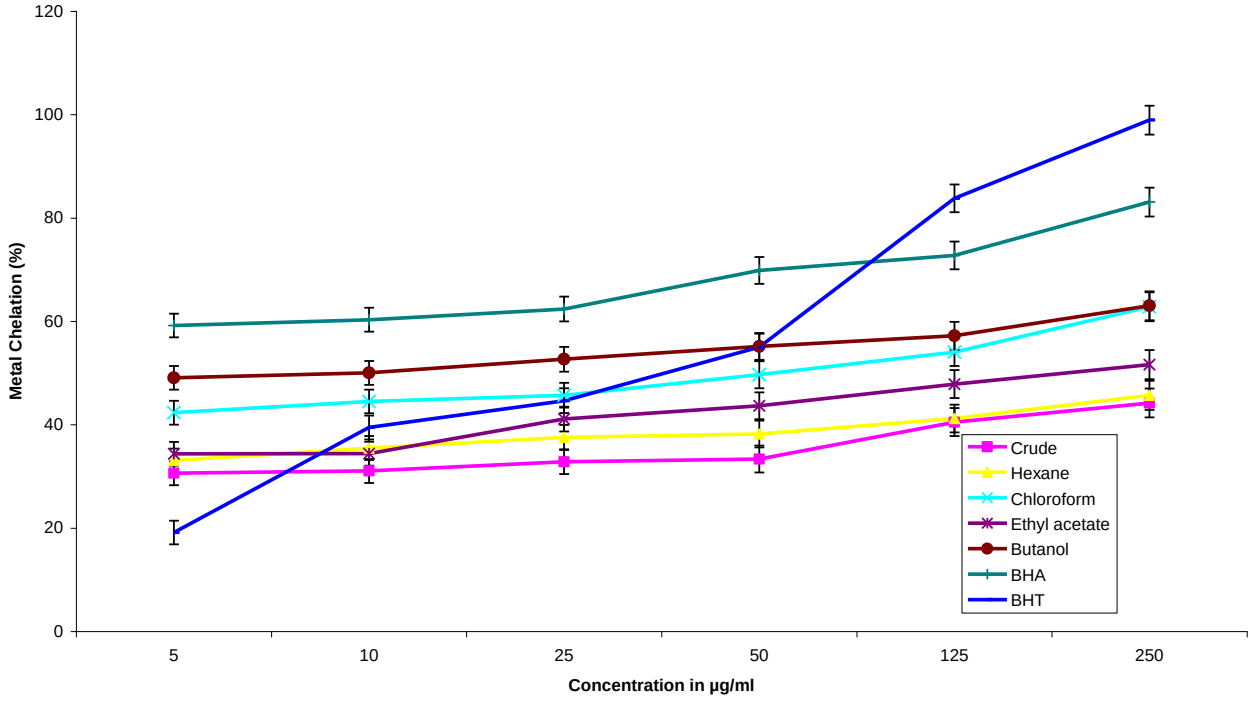


Figure 3.10: Metal Chelating ability of Crude and successive extracts from *B. bergiana* leaves

TABLE 3.17:- Absorbance Readings of graded concentrations of Gallic acid for caliberation graph

Volume (ml)	Gallic acid (mg)	1st Reading	2nd Reading	3rd Reading	Mean
0.10	0.01	0.212	0.209	0.213	0.211
1.50	0.15	1.699	1.691	1.700	1.697
2.00	0.20	2.217	2.219	2.210	2.215
2.50	0.25	2.956	2.932	2.945	2.944
3.00	0.30	3.276	3.270	3.271	3.272
4.00	0.40	4.428	4.429	4.427	4.428
4.50	0.45	4.859	4.853	4.855	4.856
5.00	0.50	5.305	5.307	5.303	5.305
6.00	0.60	6.427	6.432	6.434	6.431
6.50	0.65	6.969	6.969	6.970	6.969
7.00	0.70	7.498	7.501	7.492	7.497
8.00	0.80	8.453	8.457	8.451	8.454
8.50	0.85	9.080	9.084	9.082	9.082
9.00	0.90	9.412	9.412	9.406	9.410
10.00	1.00	10.464	10.466	10.471	10.467

$y = 10.564x + 0.103$

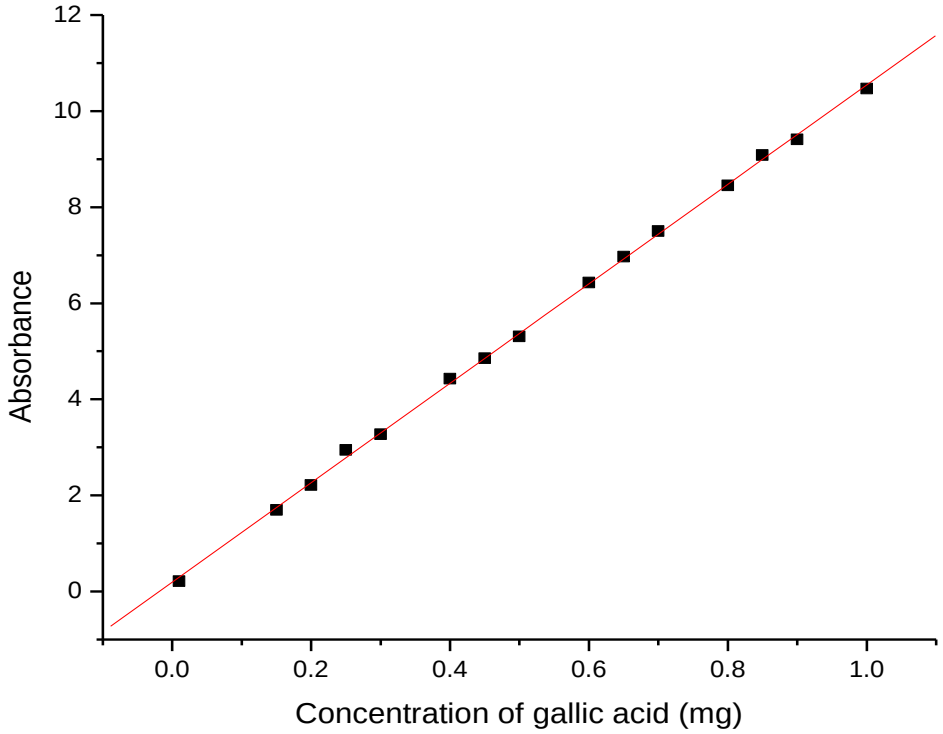


Figure 3.11: Graph of Gallic acid against concentration ($R^2 = 0.903$)

Table 3.18: Total phenolic contents of crude extract, successive extracts and their derived fraction of plant leaves

	Total Phenolic content (GAE mg g^{-1})	
Extracts/fractions	*M. foetida*	*B. bergiana*
Crude extract	12.295±0.054	10.863±0.004
Hexane fraction	9.498±0.045	7.634±0.044
$CHCl_3$ fraction	11.687±0.005	10.348±0.015
EtOAc fraction	12.295±0.045	12.295±0.045
BuOH fraction	11.194±0.065	8.566±0.025

TABLE 3.19:- Absorbance Readings of graded concentrations of Quercetin for caliberation graph

Volume (ml)	Quercetin (mg)	1st Reading	2nd Reading	3rd Reading	Mean
0.10	0.01	0.0225	0.0225	0.0225	0.0225
1.50	0.15	0.1155	0.1156	0.1157	0.1156
2.00	0.20	0.1488	0.1489	0.1488	0.1488
2.50	0.25	0.1821	0.1821	0.1821	0.1821
3.00	0.30	0.2153	0.2153	0.2153	0.2153
4.00	0.40	0.2818	0.2818	0.2818	0.2818
4.50	0.45	0.3150	0.3151	0.3151	0.3150
5.00	0.50	0.3483	0.3483	0.3483	0.3483
6.00	0.60	0.4148	0.4147	0.4148	0.4147
6.50	0.65	0.4480	0.4480	0.4480	0.4480
7.00	0.70	0.4812	0.4813	0.4812	0.4812
8.00	0.80	0.5477	0.5477	0.5476	0.5477
8.50	0.85	0.5810	0.5809	0.5809	0.5809
9.00	0.90	0.6142	0.6142	0.6142	0.6142
10.00	1.00	0.6807	0.6807	0.6806	0.6807

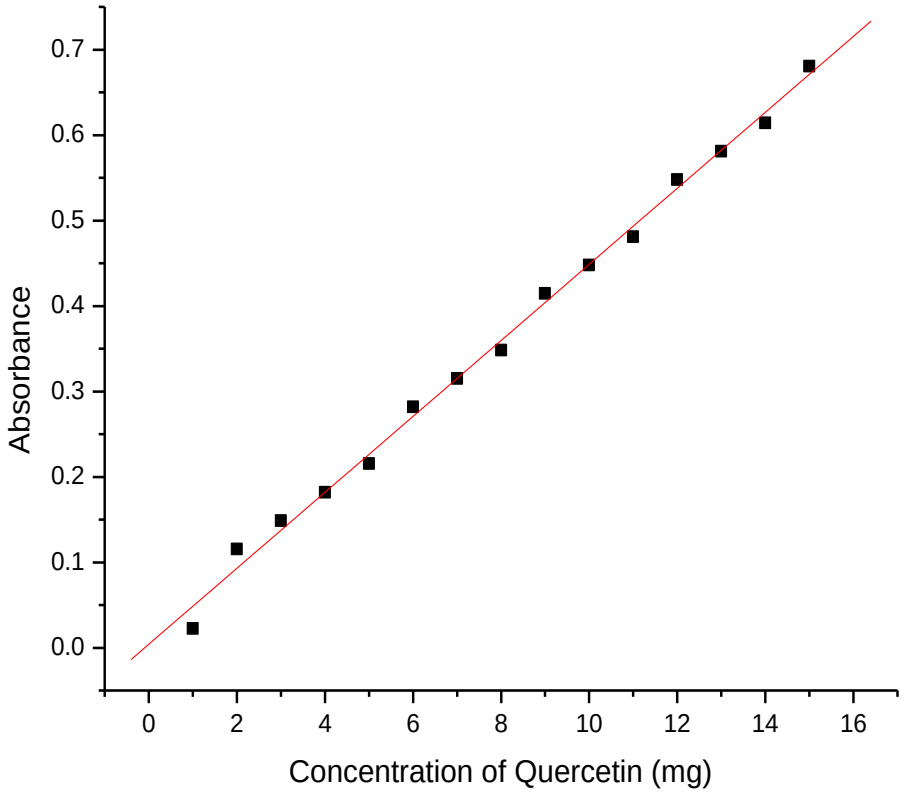


Figure 3.12: Graph of Quercetin at various concentrations against absorbance (R^2 = 0.9979)

Table 3.20: Total Flavonoids contents of crude extract, successive extracts and their derived fraction of plant leaves

	Total flavonoid (µg QE/mg extract)	
Extract/fractions	*M. foetida*	*B. bergiana*
Crude extract	23.75±0.05	26.54±0.25
Hexane fraction	24.66±0.04	14.86±0.24
$CHCl_3$ fraction	24.12±0.08	20.53±0.021
EtOAc fraction	30.95±0.06	25.10±0.061
BuOH fraction	5.27±0.05	18.10±0.25

TABLE 3.21:- Absorbance Readings of graded concentrations of Catechin for caliberation graph

Volume (ml)	Catechin (mg)	1st Reading	2nd Reading	3rd Reading	Mean
0.10	0.01	0.005	0.006	0.006	0.006
1.50	0.15	0.085	0.089	0.087	0.087
2.00	0.20	0.116	0.118	0.117	0.117
2.50	0.25	0.149	0.147	0.142	0.146
3.00	0.30	0.175	0.175	0.175	0.175
4.00	0.40	0.235	0.234	0.230	0.233
4.50	0.45	0.264	0.266	0.256	0.262
5.00	0.50	0.295	0.288	0.289	0.291
6.00	0.60	0.349	0.349	0.349	0.349
6.50	0.65	0.374	0.380	0.383	0.379
7.00	0.70	0.401	0.409	0.415	0.408
8.00	0.80	0.469	0.467	0.462	0.466
8.50	0.85	0.491	0.498	0.496	0.495
9.00	0.90	0.526	0.525	0.521	0.524
10.00	1.00	0.583	0.583	0.580	0.582

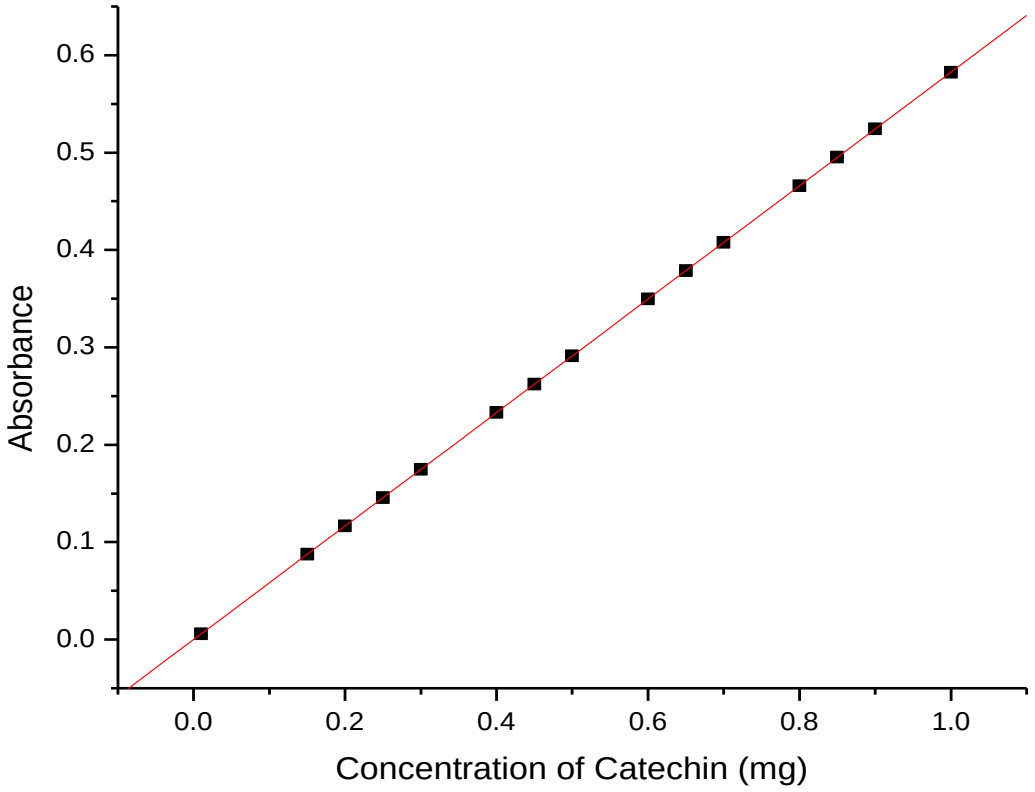


Figure 3.13: Graph of Absorbance against various concentration of Catechin (R^2 = 0.9277)

Table 3.22: Total polyphenolic contents of crude extract and their derived fraction of leaves

	Total proanthocyanidin (Catechin mg/g)	
Extract/fractions	***M. foetida***	***B. bergiana***
Crude extract	0.1430±0.0043	0.3479±0.0024
Hexane fraction	0.6249±0.0065	0.3445±0.0025
$CHCl_3$ fraction	0.0858±0.0043	0.4235±0.0034
EtOAc fraction	0.0641±0.0054	0.4658±0.0045
BuOH fraction	0.0664±0.0025	0.2586±0.0025

Table 3.23: Percentage inhibition of DPPH isolated compounds from *M. foetida* and *B. bergiana*

Concentration µg/Ml	**DPPH Radical scavenging activity (%)**							
	OM/1/E/T1	**OM/4**	**OM/11/F9**	**OM/12/12**	**OM/14/37**	**OM/9/F3**	**OM/10/F1**	**Ascorbic acid**
6.25	49.12±0.05	41.64±0.24	37.59±0.16	44.84±0.18	38.37±0.45	45.47±0.51	42.43±0.23	21.15±0.28
12.5	50.34±0.15	42.02±0.18	37.24±0.15	46.9±0.13	39.12±0.22	46.5±0.25	44.89±0.41	25.89±0.30
25	51.21±0.09	43.1±0.13	40.19±0.15	48.2±0.29	40.45±0.21	47.35±0.53	46.5±0.61	37.95±0.24
50	54.56±0.05	43.36±0.06	39.47±0.24	48.69±0.12	43.28±0.19	47.93±0.25	47.97±0.19	51.79±0.12
100	60.44±0.05	50.75±0.23	44.35±0.12	53.16±0.30	47.83±0.35	51.46±0.51	52.09±0.25	64.35±0.13
200	70.19±0.05	54.19±0.55	50.79±0.28	58.13±0.19	56.11±0.25	56.12±0.52	58.22±0.51	83.66±0.09
IC_{50}	10.60±0.03	94.70±0.05	187.84±0.02	64.05±0.05	125.98±0.02	79.38±0.03	74.64±0.05	49.33±0.05

Table 3.24: Results of Moisture content, Ash and Extractive values of the leaves of *M. foetida* and *B. bergiana*

Pharmacognostic Parameters	***M. foetida***	***B. bergiana***
	(%) W/W	***(%) W/W***
Moisture Content	12.65±0.17	8.91±0.45
Water-soluble extractive value	4.851±0.015	0.444±0.004
Alcohol-soluble extractive value	5.762±0.024	0.173±0.003
Total Ash value	8.26±0.52	13.67±0.26
Acid-Insoluble Ash	2.37±0.05	6.16±0.51
Water-Soluble Ash	0.66±0.05	2.67±0.07

CHAPTER FOUR

4.0 DISCUSSION

4.1 Phytochemical Screening

The result secondary metabolites present in the crude extracts of both plants are presented in Table 3.1. The presence of terpenoid, steroids, tannins, flavonoid's and cardiac glycosides in *M. foetida* is confimed while *B. bergiana* shows the presence of flavonoids, saponins, tannins, trace of alkaloids, phenolic compounds and glycosides. These classes of compounds have earlier been reported in some medicinal plants with their antimicrobial activity (Hostettman and Nakanishi, 1997). These compounds may be responsible for the antibacterial activity of the leaves extract and fractions of *M. foetida* and this confirm the scientific basis for the traditional use of *B. bergiana* in treatment of cough and gonorrhea infections. Tannins have been found to form irreversible complexes with proline-rich proteins (Hagerman and Butler, 1981) which may also justified the use of the plant for traditional purpose. Herbs that contain tannins as their main constituents are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003) which is one of the traditional uses of *M. foetida* plant. Another group of secondary metabolites identified in the screening is alkaloids. The presence of alkaloids supports the report of Olaniyi and Marquis (1974) in which alkaloids and glycosides were isolated from *M. foetida.* Quinlan *et al.,* (2002) and Neumann *et al.,* (2004) showed that steroids exhibit antibacterial and antiviral activities respectively. Thus, the presence of these compounds in *M. foetida* and *B. bergiana* corroborates their medicinal use by traditional healers.

4.2 DISCUSSION OF ANTIBACTERIAL ACTIVITY

Plants have been a source of medicinal compounds since pre-historic time. The biological screening of medicinal plant extracts and fractions has frequently been carried out to determine the antibacterial profile. These evaluations are usually done through different techniques to ascertain the inhibition effect on pathogenic and non-pathogenic bacteria.

4.2.1 Antibacterial activity of *M. foetida* extract and fractions

The crude extract and five fractions were screened against a panel of microorganisms for antibacterial activity at 5 mg/ml for the agar diffusion method MIC. The antibacterial activities of the extract and fractions of the plant *M. foetida* are presented in Table 3.2. The plant showed significant antibacterial activity against almost all the organisms. The hexane, chloroform, ethyl acetate, butanol and aqueous fractions of the leaves extract displayed good antibacterial activities against some of the tested bacterial strains at concentration of 5 mg/mL. The disc diffusion method result (Table 3.2) showed antibacterial activities with zone of inhibition ranging from 7.5 to 16.5 mm for the ethanolic extract at the concentration of 5 mg/mL. Fractions presented zone of inhibition ranging from 8.0 to 22.0 mm for hexane, 7.5 to 16.5 mm for chloroform, 8.0 to 17 mm for ethyl acetate, 7.5 to 14.5 mm for butanol and 8.0 to 13.5 mm for aqueous fraction at the same concentration. It is apparent that the crude extract and fractions of *M. foetida* leaves posses varying degree of different antibacterial activity against tested bacteria. The ethyl acetate fraction was the most active fraction across selected bacterial while the hexane fraction showed the least activity among the selected bacteria except *S. flexineri* pathogen (Table 3.3). *S. flexineri* was the most sensitive pathogen to ethanolic extract and hexane fraction however *P. aeruginosa, B. cereus, S. flexineri, S. faecalis, P. vulgaris* and *K. pneumonia* showed a broad spectrum of inhibition across the five fractions. All fractions and extract of this plant tested were inactive against *B. subtilis*. Such results were expected since these bacteria form resting spores and are more resistant to environmental conditions than any other tested bacteria (Nascimento, *et al.,* 2000). The antibacterial activities of the fractions compared favourably with those of the three standard antibiotics (Neomycin, Ampicillin and tetracyclines) and have appeared to be broad in nature as their activities were independent of Gram reaction.

M. foetida possesses promising antibacterial properties, especially against *S. aureus, B. subtilis* and *E. coli* which are major pathogens for human infections, varying from food poisoning or minor skin infections to severe life threatening infections. *Staphylococcus aureus* is one of the most common Gram-positve bacteria causing food poisoning (Vijloen *et al.,* 2004). Interestingly, *M. foetida* showed antibacterial activity against this bacterium. *Escherichia coli* is a Gram-negative bacterium, which causes

serious food poisoning and diarrhea. *M. bacillus cereus* and *Klebsiella pneumoniae* are respiratory pathogens commonly associated with colds and flu (Vijloen *et al.,* 2004), this implies that *M. foetida* plant can be used in treatment of various respiratory diseases since it showed high zones of inhibition. The result of the study reveals that *M. foetida* extract may be useful in management of opportunistic infections in which these bacteria are implicated.

The microplate method of Eloff (1998b) was used to determine the MIC values for plant extract and fraction. The n-hexane, chloroform, ethyl acetate, butanol and aqueous fractions and the active crude ethanolic extract of *M. foetida* showed MIC of < 5 mg/mL against some clinical and environmental isolates (Table 3.3). Extracts and fractions having activities where MIC values are below 8 mg/mL are considered to possess some antimicrobial activity (Fabry *et al.,* 1998) and natural products with MIC values below 1 mg/mL are considered noteworthy (Gibbons, 2004; Rios and Recios, 2005).

The antibacterial activity can be due to alkaloids, phenols, polyphenols, saponins, tannins, triterpenes and steroids found in crude extract and fractions according to the phytochemical results in chapter 3. These phytochemicals are known to posses' antibacterial activities (Watt and Breyer-Brandwijk, 1962; Bruneton, 1999; Cowan, 1999) thus, their presence in the plant extract and fractions could justify the observed antibacterial activity. Although very few investigations have been reported on this class of compounds flavonoids, glycosides and pentacyclic triterpenes have been shown to be present in many plants belonging to this genus *Momordica*. However, further research is required to establish the *in vivo* activities as well as the therapeutic index of *M. foetida* plant in respect to the management and possible cure of infectious diseases.

4.2.2 Antibacterial activity of *B. bergiana* crude and successive extracts

The crude and five successive extracts were screened against a panel of microorganisms for antibacterial activity at 5 mg/mL using the agar disc diffusion method. The antibacterial activities of the crude and successive extracts of the plant *B. bergiana* against 30 bacterial strains are presented in Table 3.4. The *B. bergiana* showed pronounced and significant antibacterial activity against almost all the organisms (Table

3.4). The hexane, chloroform, ethyl acetate, butanol and aqueous extracts of the leaves displaced good antibacterial activities against tested bacterial at 5 mg/mL and these successive extracts showed varying degrees of antibacterial activities. The chloroform and ethyl acetate extracts were the most active extracts. However, chloroform extract was more active with zones of inhibition ranging between 8.0 and 18.5 mm and ethyl acetate zone of inhibition ranged from 8.0 to 15.5 mm while the hexane and aqueous extracts showed low activity with the aqueous extract showing least activities with zones of inhibition ranging between 7.5 and 13.0 mm (Table 3.4). Water is the most commonly used solvent to extract biologically active compounds (due to its easy availability) by traditional healers and herbalists. Freiburghans (1996) has reported that different solvent system extracts of some plant may exhibit different pharmacological properties. The antibacterial property of *B. bergiana* leaves (inhibition zones of 8.0 - 18.5 mm diameter) was as effective as the commercial antibiotics Tetracycline, Neomycin and Ampicillin (inhibition zones of 11.0 - 43.0 mm) against some bacterial strains. The most sensitive bacterial strains to the six extracts were *P. aeruginosa, S. aureus, B. cereus, K. pneumonia, E. coli, P. vulgaris,* and *M. luteus.* All extracts of this plant tested were majorly not active against *B. subtilis*. Such results were expected since these bacteria form resting spores and are more resistant to environmental conditions than any other tested bacteria (Nascimento, *et al.,* 2000).

The MIC of the successive and crude extract of *B. bergiana* leaves range from 5.0 to 0.07812 mg/mL. The MIC of the most active fraction (chloroform) ranges from 2.5 to 0.07812 mg/mL. The ability of the extracts from *B. bergiana* to inhibit the growth of some of the tested bacterial strains like *E. coli, P. vulgaris* (ATCC 8830) and *P. aeruginosa* is an indication of the potential of the plant as a broad spectrum antibacterial agent. *B. bergiana* has been used traditionally for the treatment of various respiratory diseases by the people of KwaZulu-Natal for a long time. Common pathogenic bacteria, such as *S. aureus* and *P. aeruginosa,* which are predominant organisms in both leg ulcers and superficial wounds, showed increased resistance to commonly used antibiotics (Valencia, *et al.,* 2004). The potential of *B. bergiana* leaves crude and successive extracts against these multi-resistant and standard strains of *S. aureus* and *P. aeruginosa*

may be explored in order to develop a topical antimicrobial therapy to promote skin wounds healing.

The inhibitory property of extracts from *B. bergiana* against these pathogens, might have justified the use of this plant for the treatment of cough and influenza fever by people of KwaZulu-Natal. The results also indicated that the extracts exhibited antibacterial activity against *E. coli* and *S. aureus*, the causative microorganisms of abdominal disorder like diarrhoea. It is noteworthy that all the fractions of this plant tested in this study exhibited strong activity against *K. pneumoniae*, a virulent pathogen associated with infections of the respiratory tract.

4.2.3 Antibacterial potential of Isolated compounds

The compounds tested were OM/E/T2, OM/4, OM/11/F9 and 0M/11/12 from *M. foetida* while OM/9/F3, OM/10/F1 and OM/14/37 were from *B. bergiana*. There MIC values of the compounds tested against 24 bacterial strains ranged between 0.00718 to 1.000 µg/mL. All compounds exhibited varying antibacterial activity against the bacterial strains. Compounds OM/E/T2, OM/4, OM/9/F3 and OM/11/F9 showed the highest antibacterial activity than other compounds while compounds OM/E/T2 and OM/4 showed higher antibacterial activity against isolated strains than standard strains. The most active compounds were OM/E/T2 and OM/4. All the compounds active against *S. aureus* and *E. faecalis* and the results support the use of these plants in traditional medicine for relieving symptoms that are caused by these infective agents e.g bloody diarrhea, wounds and conjunctivitis (Gelfand *et al.*, 1985). OM/E/T3 is a flavonoid and flavonoids are known to show many biological activities useful both to human health and plant physiology. Yoichi *et al.*, (2000) suggested that apigenin and the related flavonoids are potentially useful for the development of therapeutic treatments of MRSA infections. They also found that apigenin related compounds tested against the following organisms *S. aureus, Bacillus cereus, E. faecalis, P. vulgaris* and *Salmonelk typhimurium* resulted in MIC less than 250 µg/mL, this result supported the finding of the study showing the OM/E/T2 showed higher activity less than 250 µg/mL (Table 3.6). Flavonoids are known to be synthesized by plants in response to microbial infection (Yoichi *et al.*, 2000). Hence, it is not be surprising that they have been found to be effective antimicrobial

agents against a wide array of microorganisms, when tested *in vitro*. Their activity is probably due to their ability to react with extracellular and insoluble proteins and to complex with bacterial cell walls (Cowan, 1999). OM/4, OM/11/F9 0M/11/12, OM/9/F3, and OM/10/F1 are terpenoids and terpenoids have been reported to have antimicrobial activity (Cowan, 1999). Pentacyclic and tetracyclic triterpenes are known for their action as molluscides, particularly in their monodesmosidic form (Marston, *et al.,* 1997). The triterpenoid betulinic acid is just one of several terpenoids which have been shown to inhibit HIV (Cowan, 1999).

The present results further confirm the activity of the constituents of *M. foetida* and *B. bergiana* against tested bacteria and justify the potential use of these two medicinal plants in folk medicine, as well as expand our knowledge on the bacterial activity of *M. foetida* and *B. bergiana*. Some of the compounds isolated are candidates for further work to evaluate their therapeutic potential.

4.3 ANTIOXIDANT ACTIVITY

4.3.1 Qualitative Antioxidant Discussion

Various assays have been used to test for antioxidant activity but the most widely used methods are those that involve generation of free radical species which are then neutralized by antioxidant compounds (Arnao *et al.,* 2001). In qualitative analysis of antioxidant activity, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay on TLC plates was used as a screening test for the radical scavenging ability of the compounds present in the different extracts and fractions.

All the extracts and fractions from qualitative screening contained compounds that exhibited considerable free radical scavenging activity, as shown by the yellow bands (of the antioxidant activity) on the DPPH chromatograms Figure 3.1 and 6.2. The polar solvent fractions and extracts had more antioxidant activity compared to the non-polar solvents.

Momordica foetida

The TLC-DPPH screening method indicated the presence of antioxidant compounds in some of the fractions tested, with ethyl actate and butanol showing the most prominent antioxidant

activity (Figure 3.1). This figure was used as the representative of all the fractions and extract tested. Visualization of the compound with antioxidant activity enables the localization and the subsequent identification of the potential active compounds. All the fractions displayed antioxidant activity after spraying the chromatograms with DPPH. Hexane and chloroform fractions of *M. foetida* showed little activity when compared with other fractions, although most of the active compounds were very polar. Most of the antioxidant compounds were observed in solvent system ethyl acetate/methanol/water (40:5.4:5). Ethyl actate, chloroform and butanol fractions of *M. foetida* (figure 3.1) are good candidates to isolate antioxidant active compounds from because they contained a number of compounds with a high degree of activity. The most prominent compounds among the ethyl actate, chloroform and butanol fractions were at R_f values of 0.25, 0.60 and 0.85 (EMW) as shown in Table 3.7. Hexane fraction of *M. foetida* showed active compounds with less activity.

The degree of activity of all the samples tested was determined qualitatively from observation of the yellow colour intensity (Table 3.7). Only ethyl acetate and butanol showed antioxidant activity. Hence, they are good candidate to isolate antioxidant compounds from and these active compounds were clearly visible is chloroform/ethyl acetate/formic acid and ethyl acetate/methanol/water solvent systems.

B. bergiana

All the crude and successive extracts of *B. bergiana* from qualitative screening, contained compounds that exhibited considerable free radical scavenging activity, as shown by the yellow bands (of antioxidant activity) on DPPH chromatograms Figure 3.2. The chloroform and ethyl acetate had more antioxidant activity compared to other successive extracts. Qualitative analysis revealed that the chloroform and ethyl acetate successive extracts reacted strongly and rapidly with the radical DPPH, almost in the same manner as the potent positive control. Hexane extract, however, gave a very weak reaction which progressed much slower when compared with the strong antioxidant successive extracts. The elution of the successive and crude extracts by TLC using solvent system ethyl acetate/methanol/water reavealed the presence of two compounds with strong antioxidant activity in both chloroform and ethyl acetate (R_f = 0.44 and 0.88) whilst in the hexane and butanol extracts, a single compound was shown in each (R_f = 0.88 and 0.44 respectively). The ethyl acetate extract was best

eluted with solvent system mentioned above, which revealed the presence of a more polar compound with antioxidant activity (R_f = 0.46).

The result demonstrated that various antioxidant compounds of different polarities can be isolated from *B. bergiana*, particularly from the chloroform and ethyl acetate extracts. The extracts were analysed using two spray reagents (DPPH and anisaldahyde) (as shown in Table 3.8) for the classes of compounds normally associated with antioxidant activity.

The leaves of the *M. foetida* and *B. bergiana* plants are known for their pharmacological activity and in this study, it showed that various solvent extracted antioxidant compounds from the leaves of the two medicinal plants that is chloroform, ethyl acetate and butanol extracts and fractions contain the highest number of antioxidant compounds based on DPPH-TLC analysis. Qualitative DPPH assay on TLC was successfully used in this study to systematically assess the total antioxidant activity of the two medicinal plants (*M. foetida* and *B. bergiana*) extracts and fractions. This qualitative method is effective and efficient and can be used for systematic screening of medicinal herbs and dietary plants for their relative antioxidant content. It is simple, fast, reliable, inexpensive and also very adaptable to identify antioxidant compounds in medicinal plants.

4.3.2 Quantitative Antioxidant Discussion

The phenolic compounds in plants are widely distributed, sometimes present in surprisingly high concentrations and have an antioxidant capacity (Lapornik *et al.*, 2005). They have the ability to scavenge free radicals such as the Reactive Oxygen Species (ROS) which are determined by their reactivity as hydrogen- or electron-donating agents (Fernandez-Pachon *et al.*, 2006). In the present study, the total antioxidant effect of extracts and fractions of two medicinal plants that are traditionally used in South Africa were evaluated and compared to the total phenolic contents.

The methods employed in this study are simple and provided reproductible results showing antioxidant properties of the extracts and fractions. It was important to examine the correlation between the content of the main antioxidant compounds (total phenolic) and the antioxidant capacity of the extracts and fractions.

4.3.2.1 DPPH Assays

DPPH assay is one of the most widely used method for screening of antioxidant activity of plant extracts and fractions (Nanjo *et al.*, 1996). DPPH is a stable, nitrogen-centered free radical which produces violet colour in ethanol or methanol solution. The violet colour was reduced to yellow coloured product, diphenylpicrylhydrazine, on the addition of the fractions in a concentration-dependent manner. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants. BHT and Ascorbic acid are the reagents used as standards. These percentages can be considered as a absorbance of full inhibition of DPPH as, after completing the reaction, the final solution always possessed some yellowish colour and thereafter, its absorbance inhibition compared to the colourless methanol solution could be not reach 100% inhibition which is accord with literature report (Miliauskas, et al., 2004).

M. foetida

The extract and fractions were able to reduce the stable radical DPPH to yellow coloured diphenylpicrylhydrazine. Table 3.9 showed the percentage inhibition of DPPH by various extracts for Hex, $CHCl_3$, EtOAc, BuOH, and crude extract of *M. foetida* at various concentrations as indicated in the table and that of standard drugs (BHT and Ascorbic acid). At 50 µg/mL concentration, among successive extracts, EtOAC and BuOH extract showed highest DPPH radical scavenging potential (22.82% and 21.17% respectively) followed by hexane extract with 16.88% DPPH radical scavenging activity whereas $CHCl_3$ extract had the lowest potential. Crude extract showed extremely high DPPH radical scavenging potential at this concentration. The scavenging effect of crude fractions and standard with the DPPH radical is in the following order BHT (97.68%) >Ascorbic acid (51.76%) > crude (45.56%) > EtOAc (22.82%) > BuOH (21.17%) > Hex (16.88%) > $CHCl_3$ (15.97%) at the dose of 50 µg/mL as shown in Table 3.9. The experimental data of these crude and fractions revealed that all these fractions and crude extract are likely to have the effect of scavenging free radical. From Figure 3.3, it was observed that a dose-response relationship is found in the DPPH radical scavenging

activity. The activity increased as the concentration increased for each fraction and crude. Based on the result obtained from data in Table 3.9 as well as those statistical analysed, it can be general inferred that crude extract of *M. foetida* showed lower IC_{50} values 137.13 µg/mL than other fractions. Furthermore, from Table 3.17, except for the ethyl acetate fraction that showed IC_{50} 188.33 µg/mL, all the test fractions showed IC_{50} greater than 250 µg/mL against the DPPH radical. The complete inhibition of DPPH radical by extract and fractions were observed at a range of 5-250 µg/mL. In addition, the IC_{50} values (the concentration required to inhibit radical formation by 50%) of the crude extract and fractions were 137.13, >250 (Hex, $CHCl_3$ and BuOH) and 188.33 µg/mL. The IC_{50} value of well-known antioxidant compounds used as references in this study, Ascorbic and BHT were 46.33 and 10.87 µg/mL respectively. Among the partitions, the more polar ones (ethyl acetate) had higher antioxidant activity while the smaller antioxidant activity was found for the non-polar partitions (hexane), the exception being the n-butanol partition of *M. foetida* which showed a very low antioxidant activity IC_{50} (>250 µg/mL) . In summary, it was apparent that the polarity of the extracts markedly influences the antioxidant activity of plant extracts.

B. bergiana

Table 3.10 show the percentage inhibition of DPPH by various extracts for Hex, $CHCl_3$, EtOAc, BuOH, and crude extract of the plant at various concentrations as indicated and that of standard drugs (BHT and Ascorbic acid). The complete inhibition of DPPH radical by crude extract and successive extracts were observed at a range of 5-250 µg/mL. All the successive extracts exhibited strong DPPH radical scavenging potential at the tested concentration.

The percentage inhibition of DPPH radical scavenging potential for various successive extracts and crude extract range from 0.40 to 94.48%. At 250 µg/mL concentration, EtOAC and BuOH successive extract showed highest DPPH radical scavenging potential (94.48 and 93.67% respectively) followed by crude extract with 86.66% DPPH radical scavenging activity. $CHCl_3$ also showed relatively high DPPH radical scavenging potential (42.57%) whereas, hexane extract had the lowest potential. The radical scavenging effects increased with increasing concentration. The activities

decreased in the following order: BHT > Ascorbic acid > EtOAc > BuOH > crude > $CHCl_3$ > Hexane. The radical scavenging activities of the successive extracts are significantly ($p > 0.05$) less than that of BHT and Ascorbic acid (controls). The significant differences in the percentage radical scavenging potential for different extracts at various concentration suggested that the solvent extractions might have significant influence on the antioxidant potential of the extract.

As shown in Table 3.10 and Figure 3.4, except for the ethyl acetate and butanol extracts that showed IC_{50} of 110.73 and 103.27 µg/mL respectively, all the test other extracts showed IC_{50} greater than 250 µg/mL against the DPPH radical. In addition, the IC_{50} values (the concentration required to inhibit radical formation by 50%) of the crude extracts and successive extracts were 137.13, >250 µg/mL (Hex and $CHCl_3$ respectively). The IC_{50} value of well-known antioxidant compounds used as references is this study, Ascorbic and BHT were 47.13 and 12.87 µg/mL respectively.

Based on the mechanism of reduction of the DPPH molecule described by Basnet *et al.,* (1997) which they correlated with the presence of hydroxyl groups on the antioxidant molecule, it can be inferred that very good activity of polar extracts and fractions is probably due to the presence of substances with an available hydroxyl group (phenolic or not). This could be linked to the presence of flavonols or condensed tannins which are known to occur in plant kingdom.

The screening of plant extracts and fractions using DPPH free radical method proved to be effective for the selection of those which could have an antioxidant activity. These extracts may be rich in radical scavengers, such as flavonoids, known as antioxidants. The involvement of free radicals especially their increased production, appears to be a feature of most, if not all human diseases including cardiovascular disease and cancer (Deighton *et al.,* 2000). It has been found that cysteine, glutathione, ascorbic acid, tocopherol, flavonoids, tannins and aromatic amines (p-phenylene diamine, p-aminophenol etc) reduce and declourise DPPH by their hydrogen donating ability (Yokozawa *et al.,* 1998). Therefore, these two medicinal plant can be used in the the treatment of the the above disesases since they posses strong antioxidant potential. Plant phenolics constitute one of the major groups of compounds acting as primary antioxidant free radical terminators (Agrawal, 1989). Phenolic compounds of the extracts and

fractions are probably involved in their antiradical activity. Flavonoids, as one of the most diverse and widespread groups of natural compounds, are probably the most natural phenolics (Shimoi, *et al.*, 1996). These compounds possess a wide spectrum of chemical and biological activities including radical-scavenging properties. A strong relationship between total phenolic content and antioxidant potential in fruits, vegetables and grains products has been reported (Dorman, *et al.*, 2003, Velioglu *et al.*, 1998). Therefore, the antioxidant potential of these plants may be due to the phenolic compounds presence in the plant according to phytochemical screening.

4.3.2.2 ABTS Radical Cation Scavenging Activity

ABTS radical cation is common organic radical that has been used to determine the antioxidant activity of single compounds and other complex mixture (Zhou *et al.*, 2004). Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm that decrease with the scavenging of the proton radicals (Matthew and Abraham, 2006). ABTS as another antioxidant activity screening method, applicable for both lipophilic and hydrophilic antioxidants. ABTS radical cation decolourizing assay show results similar to those obtained by the DPPH assay. However, the reaction with $ABTS^{\cdot+}$ is fast and in almost all cases is completed with 1 mins.

M. foetida

The activity of the extracts increased with increasing concentration (Figure 3.5). The extracts and fractions of *M. foetida* were most active as they nearly (100%) scavenged $ABTS^{\cdot+}$.(Table 3.11) The percentage scavenging potential after 6 min at 50 µg/mL concentration were 56.04%, 25.49%, 54.00%, 78.71%, 60.35%, 99.14% and 98.50% for crude extract, hexane, $CHCl_3$, EtOAc, BuOH, BHA and BHT respectively. High concentrations of the extract and fractions have been reported to be more effective in quenching free radical in the system (Ligangli, *et al.*, 2002). It is interesting to note that, the activity of *M. foetida* extract and fractions was the same with the standard compounds at higher concentration. The ABTS scavenging activity of extract and fractions expressed in the term of IC_{50} was in the range of < 5.0 to > 250 µg/mL (Table 6.11), with the strongest antioxidant potency for the ethyl acetate fraction. These results suggest that the

ethyl acetate fraction might contain the strongest free radical scavenger compounds. These values were considerably higher than those obtained from the positive control (BHA) at 125 µg/mL. This data show that ethyl acetate fraction might contain either the higher concentration or the most potent compounds having the most potent efficacy in radical scavenging activity. Thus, *M. foetida* extract and fractions showed high radical-scavenging activities.

B. bergiana

Activity of *B. bergiana* extracts were also measured and compared for their free radical scavenging potentials against ABTS radical cation as shown in Table 3.12. All successive extracts used in this study showed significant ABTS radical cation scavenging activity. The values of ABTS radical cation scavenging activity for 5 sequence extracts ranged from 0.39 to 100%. The results obtained by ABTS method has similar pattern in extract activity with those of the DPPH method. At 50 µg/mL concentration, EtOAc had the highest ABTS radical cation scavenging activity whereas the lowest was observed in hexane extract at the same concentration (50 µg/mL). BuOH and crude extracts also exhibited relatively higher ABTS radical cation scavenging activity than the other sequent extracts.

The IC_{50} values of *B. bergiana* crude and successive extracts are considered to be another good measure of the antioxidant efficiency of medicinal plants according to Figure 3.6. According to the IC_{50} values displayed in Table 3.12, the concentration of ABTS radical is very high for hexane and chloroform extracts (> 250 µg/mL) compared with the other successive extracts which gave IC_{50} values range of 103.27 - 114.87 µg/mL. The IC_{50} obtained for the hexane and chloroform extracts were significantly different ($p<0.05$) from the IC_{50} values obtained from crude, ethyl acetate and butanol extracts which were similar. Based on the IC_{50} values, the butanol extract contain reasonable quantities of antioxidant compounds since the observed properties different from those compounds present in the hexane and chloroform extracts.

The scavenging of the $ABTS^{\cdot+}$ radical by the extracts and fractions were found to be much higher than that of $DPPH^{\cdot}$ radical. Factors like stereoselectivity of the radicals or the solubility of the extracts in different tested systems have been reported to affect the

capacity of extracts to react and quench different radicals (Yu *et al.,* 2002). Wang *et al.*, (1998) found that some extracts/fractions/compounds which have $ABTS^{\cdot+}$ scavenging activity did not show DPPH scavenging activity. This is not the case in this study as the plant extracts were able to quench the two radicals. A correlation between these two was apparent in this study (R^2 = 0.9328). This further showed the capability of the extracts and fraction to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage.

Nevertheless, results obtained by ABTS method had some discrepancies with those of the DPPH method. The different results from two methods might be due to different reaction kinetics between phenol and DPPH as well as ABTS radical cation over a similar range of concentration (Campos and Lissi, 1996). This could also probably be due to the various phenolic compounds present in the extracts and fractions prepared from *M. foetida* and *B. bergiana* with different responses to various kinds of free radicals. All of these results suggested that the extracts and fractions might have significant influences on the antioxidant activity of *M.foetida* and *B. bergiana* when the antioxidant activity was estimated by ABTS assay

Both the $ABTS^{\cdot+}$ and DPPH assays measure the total antioxidant activity of the plant extracts and fractions. The results of both the assays are in agreement in that extracts and fractions of both plants displayed the antioxidant activities. The extracts and fractions of these plants were strong radical-scavengers, indicating that active compounds of different polarity are present in these plants. The high antioxidant activities of these plants might be due to their flavonoid and phenolic contents. According to Shimoi *et al.*, (1996) plant flavonoids show antioxidant activity *in vitro* also function as antioxidants *in vivo* and their radioprotective effect may be attributed to their radical-scavenging activity. ROS generated by ionizing radiation are scavenged by radioprotectors before they can intereat with biochemical molecules, thus reducing the harmful effects of radiation. The antioxidant mechanisms of radioprotection and free radical-scavenging have been attributed to flavonoids, orientin and vicenin (Uma Devi *et al.,* 2000). Radioprotective/antioxidative effects of various other natural product also been reported (Tripathi, *et al.,* 2007)

The results of the present investigation suggest that the antioxidant and free radical-scavenging activities of the plant extracts and fractions constitute a likely mechanism for their radioprotective effect. The different radioprotective and antioxidant activities of these plant extracts and fractions may be assigned to different chemical constituents.

4.3.2.3 Reducing Power

In the reducing power assay, the presence of reductants (antioxidants) in the test samples results in the reduction of Fe^{2+}/Ferricyanide complex to the iron II form (Fe^{2+}). The Fe^{2+} can therefore be monitored by measuring the formation of Perl's Prussian blue colour at 700 nm (Chang *et al.,* 2002). The antioxidant activity has been reported by some investigators to be concomitant with the development of reducing power (Duh, 1999).

Antioxidant activity of antioxidants has been attributed by various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997). Like the antioxidant activity, the reducing power of extract and fractions increases with amount of sample.

M. foetida

Table 3.13 and Figure 3.7 shows the reductive capacities of different fractions of *M. foetida* when compared to the standard compounds/drugs. The reducing power increased with increasing concentration of the extract and fractions. The reducing power of the extract, fractions and standard compounds followed the order of BHT> BHA >Ascorbic acid > $CHCl_3$ > EtOAc > BuOH > crude extract > Hex. Chloroform fraction of the leaves showed stronger reducing power than the other extracts. Moreover, the reducing power of EtOAc and BuOH were related and more pronounced than others and that of crude and hexane were lower than other fractions. The reducing properties are generally associated with the presence of reductones which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. This shows that the reducing ability of *M. foetida* extract and fractions was evident with the presence of

reductones which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom.

This suggests that the leaves extract is an electron donor and could neutralize free radical (Zhu et al., 2001). Reductones inhibit lipid peroxidation by donating a hydrogen atom and thereby terminating the free radical chain reaction (Yen and Chen, 1995).

B. bergiana

Previous studies have reported that the reducing capacity of a compound or extract may serve as a significant indicator of its potential antioxidant potentials (Jeong *et al.,* 2004). Thus, it appears necessary to determine the reducing power of the sequent extracts of *B. bergiana* to evaluate their antioxidant potentials. As shown in Table 3.14 and Figure 3.8, all the successive extracts and crude extract with different concentration showed significant reducing power compared to the standard. The values of reducing power for 5 extracts ranged from 0.142 to 0.932 nm. At 50 µg/mL concentration, crude extract showed the strongest reducing power, followed by EtOAc (0.413). The reducing power effects increased with increasing concentration. At 50 µg/mL concentration, the activities decease in the following order: Ascorbic acid > BHT > crude extract > EtOAc > Hex > BuOH > $CHCl_3$. The reducing power activities of the successive extracts are less than the control (i.e Ascorbic acid, BHA and BHT). These observations suggested that different extracts mighty have some influences on the reducing power of *B. bergiana*. It was notable that, all extracts showed lower reducing power than that of the comparison standards BHT and Ascorbic acid. These results suggested that the antioxidant activities of these extracts in *B. bergiana* were probably due to their reducing capacities. In other words, these compounds are electron donors and can act reduce the oxidized intermediates of lipid peroxidation processes, so they can as primary and secondary antioxidants and therefore inhibit lipid peroxidation (Ordoriez, *et al.,* 2006).

The reducing power of the two plants extracts and fractions was concentration dependent (Table 3.14 and Figure 3.8). The antioxidant principles present in the fractions/extracts of the two plants studied caused the reduction of Fe^{3+}/Ferricyanide complex to the ferrous form and thus proved the reducing power ability i.e. the transformation of Fe^{3+} into Fe^{2+} in the presence of various extracts was measured to

determine the reducing ability. The result of this study also support that reducing power is associated with antioxidant activity and served as a significant reflection of the antioxidant activity as reported by Oktay *et el.,* 2003. Extracts or fractions with reducing power indicated that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Yen and Chen, 1995). The reducing ability of a compound generally depends on the presence of reductones (antioxidants) which increase the antioxidant potential by breaking the free radical chain by donating a hydrogen atom (Meir *et al.*, 1995). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation.

4.3.2.4 Metal Chelating Activity

It has been well recognized that transition metal ions such as those of iron and copper are important catalysts for the generation of intermediates lipid peroxidation (Nawar, 1996). Measurement of the rate of reduction of the colour, therefore, allows estimation of the chelating activity of the co-existing chelator. In this assay, extracts, fractions and standard compounds interfered with the formation of ferrous complex with the reagent ferrozine, suggesting that it has chelating activity and capture the ferrous ion before ferrozine. The absorbance of Fe^{2+}-ferrozine complex is linearly decreased with the dose taken dose (from 5 to 250 µg/mL)

M. foetida

The data obtained from Table 3.15 revealed that extract and fraction demonstrated an effective capacity for iron binding. The percentages of metal scavenging capacity of 50 µg/mL doses of tested extract and fractions of *M. foetida* crude, Hex, $CHCl_3$, EtOAc, BuOH, BHA and BHT were found to be 23.25%, 44.55%, 35.81%, 42.93%, 30.08%, 69.86% and 55.03% respectively as shown in Table 3.15. The metal scavenging effect of the extract, fractions and standards follows the order BHA > BHT > Hex > EtOAc > $CHCl_3$ > BuOH > crude. As shown in figure 3.9, the hexane fraction showed a better inhibitory effect with 44.55% of inhibition at a concentration at 50 µg/mL, followed by EtOAc fraction (42.93%), $CHCl_3$ (35.81%), BuOH (30.08%) and crude extract (23.25%)

respectively. This result implies that *M. foetida* crude extract is a poor chelating agent. According to Duh *et al.*, (1999) metal chelating potential is pronounced as the concentration of catalysed transition metal in lipid peroxidation decreases. It was also reported by Gordon (1990) that chelating agents, which form σ-bonds with metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion.

One can infer from Figure 3.9 that the extract and fractions demonstrate an effective capacity for iron binding, suggesting that its action as antioxidant may be related to its iron-binding capacity. In addition, some phenolic compounds exhibit antioxidant activity through the chelation of metal ions. As shown in Table 3.15, the IC_{50} value of BHT, BHA (positive control), crude extract, hexane, $CHCl_3$, EtOAc and BuOH fractions were <5, 30.07, 180.07, >250, >250, 129.67 and 230 µg/gL respectively. Among the fractions tested, the highest metal chelating effect was observed for the EtOAc, fraction. These results indicate that *M. foetida* extract and EtOAc fraction have good performance in metal chelating activity implyinging that *M. foetida* extracts are a potential source of antioxidant.

B. bergiana

The chelating effects of successive extracts of *B. bergiana* are shown in Table 3.16 and Figure 3.10. The various extract demonstrated chelating effect in dose dependent manner with percentage activity ranging from 30.62 to 63.03 %. BHA and BHT used as positive control revealed a better chelating ability than the various extracts. In this assay, both crude extract, successive extracts and standard compounds interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and to capture ferrous ion before the formulation with ferrozine. The difference between the extracts and control was statistically significant ($p<0.05$). The percentage of metal scavenging capacity at 50 µg/mL dose of tested extracts of *B. bergiana* that is crude, Hexane, chloroform, ethyl acetate, butanol, BHA and BHT were found to be 33.37%, 38.19%, 49.68%, 43.67%, 55.14%, 69.86% and 55.03% respectively. The metal scavenging effect of crude, hexane, chloroform, ethyl acetate and butanol extracts of *B. bergiana* are lower than that of BHA and BHT except BuOH extract which showed higher metal chelating effect than BHT. The metal scavenging effect of the extracts and

standards therefore follows the order: BHA > BuOH > BHT > $CHCl_3$ > EtOAc > Hex > crude at 50 µg/mL concentration. Among the various successive extracts tested, BuOH extract showed the highest ferrous ion chelating ability followed by chloroform extract which were almost similar at higher concentration (250 µg/mL). The control showed highest ferrous ion chelating ability than various successive extracts (Table 3.16 and Figure 3.10). However, there was statistically a very significant difference between various extracts of *B. bergiana* and control (BHT and BHA) ($p<0.01$). As shown in table 3.18, the IC_{50} values of BHT, BHA, crude, Hex, $CHCl_3$, EtOAc and BuOH successive extracts were <5, 38.60, >250, 52.60, 191.8, 8.33 µg/mL respectively. Among the successive extracts tested, the lowest IC_{50} value of metal chelating effect was observed for the BuOH successive extract. These results reveals that the *B. bergiana* extract have good performance of metal chelating effect which implies that the plant has antioxidant potential.

The metal chelating ability of the various extracts and fractions of the two medicinal plants were measured by the formation of ferrous ion-ferrozine complex. The results obtained in this study demonstrate that the extracts and fractions have an effective capacity for iron binding, suggesting its antioxidant potential. In addition, the metal chelating ability of the various extracts and fractions demonstrated that they reduce the concentration of the catalyzing transition metal involved in the peroxidation of lipids. Most effective pro-oxidants present in food and drugs systems are ferrous ion (Yamagchi *et al.*, 1988). Metal ion chelating activity of an antioxidant molecule prevents oxyradical generation and the consequent oxidative damage.

4.3.3.1 Phenolic Content Discussion

As one of the most important antioxidant plants components, phenolic compounds are widely investigated in many medicinal plant and vegetables (Djeridane *et al.*, 2006). It is important to examine the correlation between the content of the total polyphenols and the total antioxidant potential because some authors have reported that there is no correlation between the content of these main antioxidant compounds and the radical scavenging capacity (Yu *et al.*, 2002). Phenolic compounds have been extensively investigated in the past 30 years. They have one or more aromatic rings bearing hydroxyl groups that are

potentially able to act as reducing agents, donating antioxidants and singlet oxygen quenchers (Mattei *et al.*, 1998 and Rice-Evan *et al.*, 1996)

Phenolic compounds were considered as a major group of compounds that contributed to the antioxidant activity of medicinal plants (Zielinski and Koztowska, 2000). The key role of phenolic compounds as scavenger of free radical is emphasized in several reports (Moller *et al.*, 1999; Madsen *et al.*, 1996). To better understand the relationship between the antioxidant activity and total phenolic content, the total phenolic content of the two medicinal plants extracts and fractions were determined using the Folin-Ciocalteu phenol reagent. The results are expressed in milligrams of Gallic Acid Equivalents (GAE) per gram of the two medicinal plants investigated. The results were presented in Table 3.17 and 3.18.

M. foetida

The content of total phenolics in each fraction was determined spectrometrically according to the Folin-Ciocateu method and calucated as gallic acid equivalent (GAE) using the caliberated curve above figure 3.11. The total phenolic content of the *M. foetida* extract and fractions was measured and the value extrapolated on the calibration graph (Figure 3.11) calculated as gallic acid equivalent in mg/g GAE and were found to be 12.295±0.054, 9.498±0.045, 11.687±0.005, 12.295±0.045 and 11.194±0.065 mg/g GAE for crude, hexane, chloroform, ethyl acetate and butanol respectively as shown in Table 3.17. Ethyl acetate fraction of the leaves of *M. foetida* (12.295 mg/g GAE) had a higher phenolic content that others while the least phenolic containing fraction was in hexane fraction (9.498 mg/g GAE). Total phenolic compound are the major natural antioxidant components found in the crude extract and fractions from *M. foetida* and their contents were in order of ethyl acetate = crude > chloroform > butanol > hexane. It was observed that the content of phenolic in the extract and fractions correlates with the antioxidant activity (R^2 = 0.903) with DPPH scavenging radical and highest were ethyl acetate and crude (12.295 mg/g GAE) and lowest in hexane fraction (9.498 mg/g GAE). These results indicated that free scavenging activity of ethanolic extracts of *M. foetida* leaves could be effectively enriched in the EtOAc fraction. Thus, the EtOAc fraction was further investigated for its phytochemical characteristies and *in vitro* antioxidant.

B. bergiana

The content of total phenolics in the crude and successive extracts of *B. bergiana* is determined using the Folin-Ciocalteu assay, calculated from regression equation of calibrated curve y = 10.564*x* + 0.103 and R^2 = 0.903 (Figure 3.11 above) and is expressed as Gallic Acid Equivalent (GAE). The total phenolic content of the *B. bergiana* successive extracts were measured and the value extrapolated on the calibration graph (Figure 3.11) calculated as gallic acid equivalent in mg/g GAE and were found to be 10.863±0.004, 7.634±0.044, 7.634±0.044, 12.295±0.045 and 8.566±0.025 mg/g GAE for crude, hexane, chloroform, ethyl acetate and butanol of *B. bergiana* respectively as shown in Table 3.17. Total phenolic components found in the *B. bergiana* successive extracts were in order of ethyl acetate > crude > chloroform > butanol > hexane as shown in Table 3.17 above. In this study, there seems to be little correlation between the phenolic content and antioxidant activity of the extracts because BuOH extract with lower phenolic content showed higher antioxidant activity (IC_{50} of DPPH 103.27 µg/mL) compared with other successive extracts. This may be due to the non-phenolic compounds; however, it is known that non-phenolic antioxidant could also contribute to the antioxidant activity of an extract according to Harish and Shivanandappa (2006); and Mariko *et al.,* (2005).

Phenolic compounds are known as powerful chain breaking antioxidants (Shahidi and Wanasundara, 1992). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in human, when up to 1.0 g daily ingested from a diet rich vegetables (Tanaka *et al.,* 1998).

These data were in accordance with previous research (Gorinstein *et al.,* 2003; Maisuthisakul *et al.,* 2007) which have shown that high total polyphenolics content increases with antioxidant activity and there was a linear correlation between phenolic content and antioxidant activity. The highest content of total polyphenolics in ethyl acetate, crude and butanol might be the key components accounting for the better results found in total antioxidant activity, reducing power and metal chelating as compared to other fractions. It has been shown that flavonoids and triterpenoids were efficient protector against lipid peroxidation (Adhikari *et al.,* 2006; Assimopoulou *et al.,* 2005).

In conclusion, phenolic compounds may appear to be responsible for the antioxidant activity of these two medicinal plants.

4.3.3.2 Flavonoids Content

A linear relationship was obtained by plotting the concentrations of the quercetin against the respective mean absorbance as shown in graph below (Figure 3.12). Like phenolic compounds, the contribution of flavonoids to antioxidant activity is well known. The amounts of total flavaonoid compounds in the extracts and fractions of the medicinal plant prepared from leaves are shown in Table 3.18 (*M. foetida*) and (*B. bergiana*). Total flavonoid content in the extracts and fractions were expressed in µg Quercetin (QE)/mg equivelant using the standard curve generated by quercetin ($R^2 = 0.9979$), $y = 0.6648x + 0.01586$ QE (Figure 3.12 below)

M. foetida

Total flavonoid content (µg quercetin/mg of *M. foetida*) using the standard curve generated, the total flavonoid content of *M. foetida* samples (µg of QE/mg) varied from 5.27 to 30.95 mg with ethyl acetate fraction with higher and BuOH fraction with lowest level of total flavonoid content. Flavonoid content in the ethanolic extract and fractions (Hex, $CHCl_3$, EtOAc and BuOH) of *M. foetida* were 23.75, 24.66, 24.12, 30.95 and 5.27 µg QE/mg respectively. The content of total flavonoids in the fractions decrease in the order of ethyl acetate > hexane > chloroform > crude > butanol (Table 3.20). In this study, a spectrophotometric quantification of flavonoids with aluminium chloride was used which has previously being described for the quantification of flavonoids in propolis extracts (Arvouet-Grand *et al.*, 1994, Chang *et al.*, 2002). Infact, plant containing flavonoids have been reported to possess strong antioxidant properties (Badami *et al.*, 2003). The results strongly suggest that flavonoids are important components of *M. foetida* and some of its pharmacological effects could be attributed to the presence of these valuable constituents.

B. bergiana

The concentration of flavonoids in the extracts was expressed in µg of quercentin equivalents per mg of the extract as shown in Table 3.20 above. Flavonoid content in the

methanolic and successive extracts (Hex, $CHCl_3$, EtOAc and BuOH) of *B. bergiana* were 26.54, 14.86, 20.53, 25.10 and 18.10 µg QE/mg respectively. The most flavonoid rich among the successive extract was found to be ethyl acetate extract of the plant (25.10 µg QE/mg) while hexane extract of the leaves (14.86 µg QE/mg) was the poorest. The content of total flavonoids in the fractions decrease in the order of crude > ethyl acetate > chloroform > butanol > hexane (Table 3.18 above). In this study, the antioxidant activity of the plant extracts was found to be fairly high which are rich in flavonoids content.

Flavonoids are group of polyphenolic compounds, which exhibit several biological effects such as anti-inflammatory, antihepatotxic, antiulcer, antiallergic, antiviral, anticancer activity. They also inhibit enzymes such as aldose reducatse and xanthine oxidase. They are capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups and potent antioxidants (Cao *et al.*, 1997). In view of their wide pharmacological and biological actions, they have a greater therapeutical potential, thus, presence of high flavonoid content in the fractions has contribution directly to the antioxidant activity by neutralizing in free radical

4.3.3.3 Total Proanthocyanidins discussion

Total proanthocyanidins contents were determined based on the procedure reported by sun *et al.,* (1998) and were expressed as catechin based equivalents (mg/g) using the following equation base on the calibration curve: $y = 0.5825x$, $R^2 = 0.9277$, where x is the absorbance and y is the catechin equivalent (mg/g) according to the figure below.

M. foetida

The total proanthocyanidin content of the crude and fractions of *M. foetida* were determined (Table 3.22). Total proanthocyanidin content in the ethanolic and fractions (Hex, $CHCl_3$, EtOAc and BuOH) of *M. foetida* were 0.1430, 0.6249, 0.0858, 0.0641 and 0.0664 mg/g Catechin equivalent respectively. The concentration of proanthocyanidins compounds was higher in hexane when compared with the other fractions. Results obtained in the current study revealed that level of this proanthocyanidin compounds were in the following order: crude > hexane > chloroform > butanol > ethyl acetate (Table 3.17). The results strong suggest proanthocyanidins are important components of

M. foetida and some of its pharmacological effects could be attributed to the presence of this valuable constituent.

B. bergiana

Linear regression analysis of calibration plot gave a correlation coefficient (R^2) of 0.9277 for catechin used as standards in the determination of proanthocyanidin. The total proanthocyanidin content was 0.4658 mg catechin/g of the ethyl acetate of successive extract. Total proanthocyanidin content in the crude and successive extracts (Hex, $CHCl_3$, EtOAc and BuOH) of *B. bergiana* were 0.3479, 0.445, 0.4235, 0.4658 and 0.2586 mg/g Catechin equivalent respectively. Results obtained in this current study revealed that level of proanthocyanidin compounds were in the following order ethyl acetate > chloroform > crude extract > hexane > butanol (Table 3.22). Total proanthocyanidin content of the extracts may contribute in a significant way to the radical scavenging activities and reducing powers of the extracts. Some of the pharmacological effects of *B. bergiana* extract could be attributed to the presence of this valuable constituent.

The correlation studies on the contribution of the different classes of these phenolic compounds to scavenging activity showed that the proanthocyanidins were involved. The pronounced antioxidant properties of proanthocyanidins have been reported (Luximon-Ramma *et al.*, 2003). Synergism of polyphenolic compounds in plant extracts may contribute to the overall antioxidant activity (Shahidi *et al.*, 1994)

4.3.4 Antioxidant Discussion of Isolated compounds

The free radical-scavenging activities of the isolated compounds (OM/E/T2, OM/4, OM/11/F9 and 0M/11/12 from *M. foetida* and OM/9/F3, OM/10/F1 and OM/14/37 from *B. bergiana*) were evaluated by assessing their ability to decolourise DPPH in methanol and percentages of decolourisation and concentration of the sample required to scavenge 50% DPPH (IC_{50}) represented in Table 3.23. All the compounds showed an appreciable DPPH radical scavenging effect at all concentration. The radical scavenging activity of all the compounds exhibited a concentration- and time- dependent reaction trend like the extracts and fractions reported in previous sections. Increasing the concentration of the assay sample increased the radical scavenging effect.

Of all the isolated compounds, OM/E/T2, OM/4, 0M/11/12 and OM/10/F1 had strong antioxidand activity with IC_{50} values of 10.60±0.03, 94.70±0.05, 64.05±0.05, 79.38 ±0.03 and 74.64±0.05 µg/mL respectiviely. The scavenging effect of the isolated compounds on the DPPH radical decreased in the order OM/E/T2 > Ascorbic acid > OM/11/12 > OM/10/F1 > OM/9/F3 > OM/4 > OM/14/37 > OM/11/F9 at concentration of 50 µg/mL. Based on the bioassay result, it can be concluded that compound OM/E/T2 posses higher antioxidant activity than the other isolated compounds. On a micromolar basis, OM/E/T2 was even active than L-ascobic acid, the positive control. Ascorbic acid showed lower IC_{50} more then other isolated compounds indicated that ascorbic acid posses antioxidant activity higher than the isolated compounds. It is noteworthy to mention that OM/E/T2 compound bleached the DPPH immediately suggesting that it could be classified as fast kinetic antioxidants (Brand-Williams *et al.,* 1995). Conditions for strong antioxidant found by structural studies in flavonoids: are the presence of catechol group (3',4'–OH) in ring B, the presence of 2,3 unsaturated along with 3-OH and keto group in position 4 (Op de Beck *et al.,* 2003 and Saskia *et al.,* 1996). OM/E/T2 fulfilled some of these requirements and had the highest activity comparable to L-ascorbic acid.

4.4 Pharmacognostic Analysis

The water-soluble and alcohol-soluble extractive values, moisture content and ash values (total acid-insoluble and total water soluble ash values) of the two medicinal plants (*M. foetida* and *B. bergiana*) are reported in table 3.24.

M. foetida

The mean value of moisture content of *M. foetida* leaves was 12.65±0.17 % w/w. Total ash of the leaves powder was 8.26±0.52 %w/w while the acid-insoluble ash and water-soluble ash were 2.37±0.05 and 0.66±0.06 %w/w respectively. The water and alcohol-soluble extractive of the leaves powder of *M. foetida* were 4.851±0.015 and 5.762±0.025 respectively. The aim of the total ash determination was to check the authenticity, purity and quality of the plant used for the study for reproducibility of the experimental result.

Momordica charntia and M. foetida are plant well known for their ethnopharmacological activities and it is believed that these two plants are being used interchangeably and substituted with each other in local communities. The results of these pharmacognostic investigations could, therefore, serve as a basis for proper identification, collection and investigation of the *Momordica foetida*.

B. bergiana

The mean of different extractive values are presented in Table 3.24. Extractives have higher value in the case of water (0.444±0.04 % on dry weight basis) that indicated the presence of polar compounds in the leaf parts. The extractive values are primarily useful for determination of exhausted or adulterated plants. Mean ash values (% w/w) were found to be 13.67±0.26 (total), 6.16±0.51 (acid-insoluble ash) and 2.67±0.07 (water-soluble ash) as indicated in Table 3.24. Total ash value was relatively higher due to the high content of carbonates, phosphates, silicates and silica. Loss on drying was found to be 0.91±0.45 % on the account of loss of water and volatile chemical.

B. bergiana is currently being used in the treatment of various disease conditions without standardization or concerns as to its level of quality. Standardization is an integral part of establishing the correct identification of the crude drug.

In conclusion, as there is no pharmacognostic work on record of these traditionally valued plants, this chapter was taken up for purposes of documentation, which could be useful in detecting the authenticity of these medicinal plants. The parameters are being reported for the first time and could be useful in the preparation of the herbal section of the South Africa Pharmacopoeia.

CHAPTER FIVE

CHEMICAL CHARACTERIZATION OF ISOLATED COMPOUNDS

5.1 Compound OM/1/E/T2

Compound OM/1/E/T2 was isolated as a yellow amorphous powder which gave colour reactions characteristic of flavonol glycoside with melting point of 178-180 ^{o}C. The IR spectrum showed absorption bands for the hydroxyl (3224 cm^{-1}) and a conjugate carbonyl (1644 cm^{-1}) groups (Appendix 1.1). The molecular ion peak was established as 448.1099 (calculated 448.3821) corresponding to the molecular formula $C_{21}H_{20}O_{11}$. Compound OM/1/E/T2 was identified by ^{1}H NMR, ^{13}C NMR, DEPT, HSQC and HMBC. The ^{13}C-NMR spectrum and distortionless enhancement by polarization transfer (DEPT 90 and 135) experiments showed the presence of 21 carbon resonances with one methylene, eleven methine and nine quaternary carbons.

The ^{1}H NMR spectrum of OM/1/E/T2 displayed signals for a kaempferol moiety, an AA 'XX' system for H-2' (H-6') δ_H 8.07 (1H, d, J=8.8Hz); δ_C 132.20 (d) and H-3' (H-5') δ_H 6.90 (1H, d, J=8.8Hz); δ_C 116.09 (d) and AB system for H-6 δ_H 6.22 (1H, d, J=1.6Hz); δ_C 99.89 (d) and H-8 δ_H 6.42 (1H, d, J=1.6Hz) δ_C 94.75 (d) and H-6 and H-8 are due to metacoupled protons of A-ring of flavonoid nucleus. The peak at δ_H 5.27 (1H, d, J=7.2Hz) suggests monoglycoside moiety with an β-linked sugar and a glucopyranosyl (glu) moiety H-1" δ_H 5.27 (1H, d, J=7.2Hz) δ_C 104 (d) and H-6" δ_H 0.86 2H, d, J=6.2Hz). A methylene signal (CH_2) was due to the C-6 of the glucose unit. The signals of glu- H-3" and glu- H-4", assigned by analyzing a COSY spectrum, were downfield shifted relative to those in the non-substituted glucose. Based on the chemical shifts obtained by HSQC spectrum Table 5.1 was drawn and the ^{1}H-NMR coupling pattern the sugar part of the compound was identified as a glucopyranosy moiety. The HMBC together with HSQC experiments as shown in the structure kaempferol-3-*O*-*β*-*D*-glucopyranoside were used to assign the carbon atoms of the aglycone. After irradiation of the H-2', 6' signal in the ^{1}H-NMR spectrum of compound OM/1/E/T2, a nuclear overhauser effect spectroscopy NOSEY was obtained at H-1" as shown in structure presented in Figure 5.1.

Table 5.1: ^{13}C (100Hz) and ^{1}H NMR (400Hz) spectral data of compound OM/1/E/T2

Position	C atom	δ ^{13}C (ppm)	δ ^{1}H (ppm)	J (Hz)
1	-	-		-
2	C	158.52		
3	C	135.46		
4	C	179.55		
5	C	163.10		
6	CH	99.89	6.22 (*d*)	1.6
7	C	166.00		
8	CH	94.75	6.42 (*d*)	1.6
9	C	159.09		
10	C	105.75		
1'	C	122.80		
2'	CH	132.30	8.07 (*d*)	8.8
3'	CH	116.09	6.90 (*d*)	8.8
4'	C	161.59		
5'	CH	116.09	6.90 (*d*)	8.8
6'	CH	132.32	8.07 (*d*)	8.8
1"	CH	104.06	5.27 (*d*)	7.2
2"	CH	75.75	3.46 (*dd*)	7.2, 9.0
3"	CH	78.05	3.43 (*t*)	9.0
4"	CH	71.36	3.34 (*t*)	9.0
5"	CH	78.44	3.21 (*ddd*)	2.3, 5.4, 9.0
6" a b	CH_2	62.63	3.70 (*dd*) 3.52 (*dd*)	2.3, 11.9 5.4, 11.9

.

Together with the shift correlation between H" and C-3 in the HMBC spectrum of OM/1/E/T2, the aglycone and the sugar moiety was assigned to the position. The complete assignment was aided by DEPT, HSQC and HMBC as discussed. Thus, compound OM/1/E/T2 was identified as Kaempferol-3-*O*-*β*-*D*-glucopyranoside (see appendixes for spectra). The molecular formula of this compound was identified by ESI-MS (positive mode) *m/z* (%) 449 $[M+H]^+$ (16); 287 $[M+H-162]^+$ (100); 241 (11), 231 (7), 213 (10), 203 (4), 165 (17), 153 (14), 137 (4), 127 (14), 121 (11) and 109 (7) and fragmentation pattern was shown in Figure 5.2. The synonym of this compound are 3-*O*-*D*-Glucopyranosyloxy-4',5,7-trihydroxyflavone and Kaempferol 3-glucoside. The spectra compare very well with those reported in literature (Kishore *et al*., 2003; Kazuma *et al*., 2003 and Awaad *et al*., 2006). This compound has never been detected in the genus *Momordica*. Kaempferol-3-*O*-*β*-*D*-glucopyranoside is an immunostimulant and shows strong antibacterial and anticandidal activity (Dictonary of Natural products, 2008).

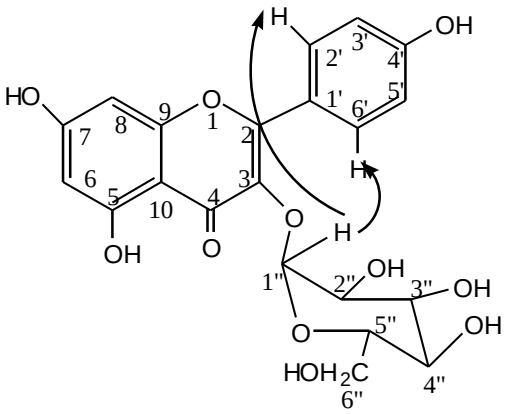
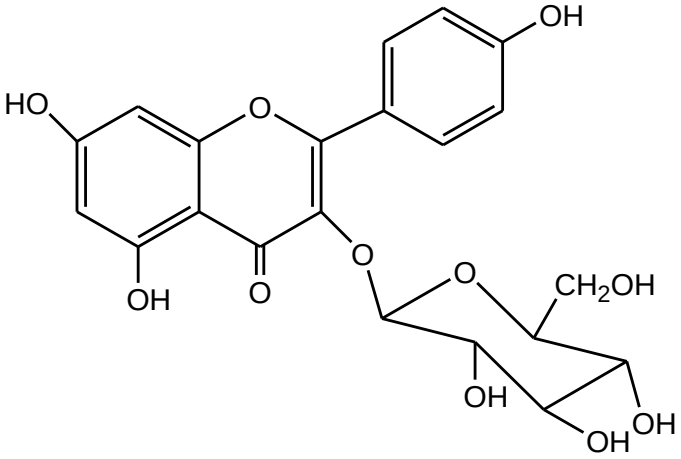


Figure 5.1: Important NOESY correlations in compound OM/1/ET2



Kaempferol-3-*O*-*β*-*D*-Glucospyranoside

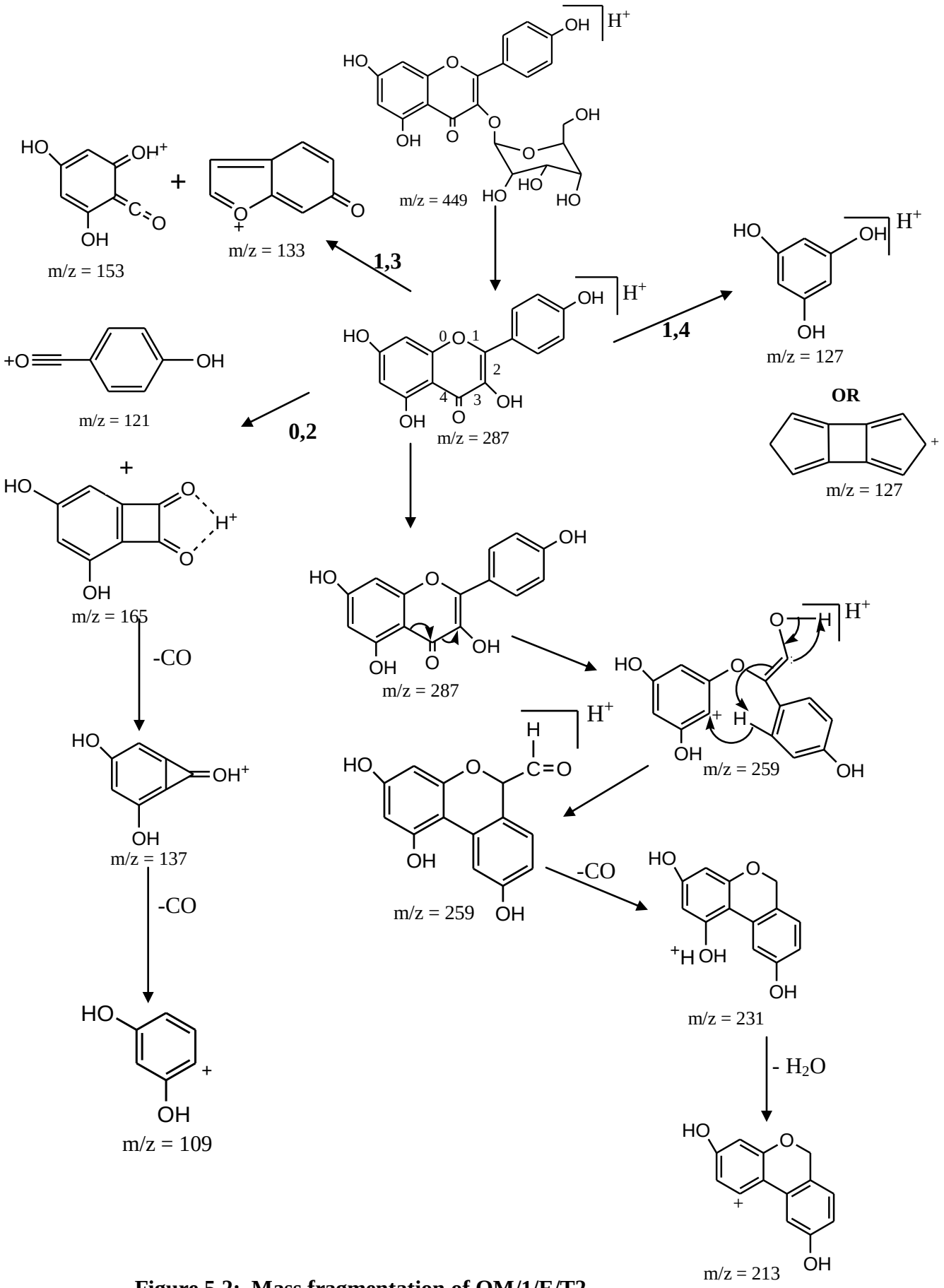


Figure 5.2: Mass fragmentation of OM/1/E/T2

5.2 Compound OM/11/40

Compound OM/11/40 was obtained as an amorphous white powder. The positive ESI-MS (positive mode) showed a pseudomolecular ion peak at *m/z* 495.3455 $[M+Na]^+$ and its molecular formula was established as $C_{30}H_{48}O_4$ (appendix 2.9a) with melting point 189-190 ^oC. Its IR spectrum showed absorptions of a hydroxyl group (3659 cm^{-1}), aldehyde group (2989 cm^{-1}), a non conjugated carbonyl group of aldehyde (1738 cm^{-1}) and a vinyl bond (1410 cm^{-1}) (Appendix 2.1).

The ^1H and ^{13}C NMR spectra (Appendix 2.2 and 2.3) of compound OM/11/40 (Table 5.2) showed the presence of six tertiary methyl group [δ_H 0.72, 0.86, 1.06, 1.25 (3H each, *s*) and 1.28 (3H x 2, *s*)] δ_C 17.93 (*q*), 14.88 (*q*), 27.09 (*q*), 25.39 (*q*), 28.96 (*q*) and 28.43 (*q*)], a secondary methyl group [δ_H 0.92 (3H, *d*, J=6.0 Hz); δ_C 18.71 (*q*)], three oxygenated carbons [δ_H 3.56 (1H, br, *s*), 3.98 (1H, *d*, J=5.6 Hz); δ_C 76.17 (*d*), 66.19 (*d*) and 70.77 (*s*)], an aldehyde group [δ_H 9.75 (1H, *s*); δ_C 207.99 (*d*)], a trans-oriented disubstituted double bond [δ_H 5.59 (2H, *m*); δ_C 125.13 (*d*) and 139.61 (*d*)] coupling to a neighbouring methylene [δ_H 1.75 (1H, *m*), 2.16 (1H, *m*); δ_C 39.04 (*t*)] and in addition, olefinic protons of a trisubstituted double bond [δ_H 5.90 (1H, *d*, J=6.4 Hz); δ_C 123.87 (*d*), 145.47 (*s*)].

The ^{13}C NMR spectrum of OM/11/40 revealed 30 carbon signals, which were assigned by DEPT analysis (appendix 2.4) as seven methyl, seven methylene, four methine, four quaternary, four olefinic and two tertiary and one quaternary oxygenated carbon. The downfield proton signals of two geminal methyl groups [δ_H 1.28 (3H x 2, s, H-26, 27)] suggested that the hydroxyl group was attached to C-25 [δ_C 70.77 (s)]. The structure of the side chain was confirmed by the HMBC correlations (appendix 2.8) between H-27 (δ_H 1.28)/C-24 (δ_C 139.61) and H-22 (δ_H 1.75, 2.16)/C-24. The HMBC spectrum of OM/11/40 also showed long-range correlations between H-3 (δ_H H-3 (δ_H 3.56)/C-1(δ_C 21.19), C-5(δ_C 145.47); H-7 (δ_H 3.98)/C-5, C-6 (δ_C 123.87), C-8 (δ_C 47.69), C-9 (δ_C 47.89); and H-6 (δ_H 5.90)/C-4 (δ_C 41.39), C-7 (δ_C 76.19), C-8 (δ_C 47.69), C-10 (δ_C 36.60), indicating that two hydroxyl groups were attached to C-3 and C-7. The relative configurations of stererogenic carbon atoms in the tetracyclic rings were determined by NOESY correlations between H-3 (δ_H 3.56)/H-2 (δ_H 1.70, 2.53), H-3/H-

28, (δ_H 1.25), H-3/H-29 (δ_H 1.06), H-7 (δ_H 3.98)/H-30 (δ_H 0.72), H-8 (δ_H 2.08)/H-18 (δ_H 0.86), H-10 (δ_H 2.53)/H-28 and H-10/H-30 in the NOSEY spectrum (Figure 5.3).

^1H and ^{13}C NMR chemical shifts were established by ^1H-^1H COSY, HMQC, HMBC and NOESY and compound 3 from Fatope *et al.,* (1990) and compound 2 from Mulholland *et al.,* (1997) were similar. Thus compound OM/11/40 was assigned as 3β, 7β, 25-trihydroxycucurbita-5,(23E)-dien-7-on-19-al (see appendices for spectra). The positive ESI-MS (positive mode) showed a pseudomolecular ion peak at m/z 495.345 $[M+Na]^+$ and its molecular formula was established as $C_{30}H_{48}O_4$ from the $[M]^+$ peak at m/z 472.36 (calculated 472.361) in the appendix 2.9a i.e. the ESI-MS (positive mode) m/z 495.345 $[M+Na]^+$ (100), 455.37 $[M+H-H_2O]^+$ (12), 437.34 $[M+H-2H_2O]^+$ (75), 419.35 $[M+H-3H_2O]^+$ (15) (Figure 5.4)

Table 5.2: Table of ^{13}C (100Hz) and ^{1}H NMR (400Hz) spectral data of compound OM/11/40

Position	C atom	δ ^{13}C (ppm)	δ ^{1}H (ppm)	J (Hz)
1.	CH_2	21.19	1.56 (*m*); 1.72 (*m*)	
2.	CH_2	29.96	1.70 (*m*); 2.53 (*m*)	
3.	CH	66.19	3.56 br, (*s*)	
4.	C	41.39	-	
5.	C	145.47	-	
6.	CH	123.87	5.90 (*d*)	6.4
7.	CH	76.19	3.98 (*d*)	5.6
8.	CH	47.69	2.08	
9.	C	47.89	-	
10.	CH	36.60	2.53 (*dd*)	10.0; 6.0
11.	CH_2	23.49	1.6 (*m*); 2.23 (*m*)	
12.	CH_2	29.87	1.31 (*m*); 1.39 (*m*)	
13.	C	45.30	-	
14.	C	47.53	-	
15.	CH_2	34.67	1.38 (*m*); 1.40(*m*)	
16.	CH_2	27.42	1.16 (*m*); 1.98 (*m*)	
17.	CH	49.85	1.49 (*m*)	
18.	CH_3	14.88	0.86 (*s*)	
19.	CH	207.99	9.75 (*s*)	
20.	CH	36.14	1.53 (*m*)	
21.	CH_3	18.71	0.92 (*d*)	6.0
22.	CH_2	39.04	1.75 (*m*); 2.16 (*m*)	
23.	CH	125.13	5.59 (*m*)	
24.	CH	139.61	5.59 (*m*)	
25.	C	70.77	-	
26.	CH_3	28.96	1.28 (*s*)	
27.	CH_3	28.43	1.28 (*s*)	
28.	CH_3	25.39	1.25 (*s*)	
29.	CH_3	27.09	1.06 (*s*)	
30.	CH_3	17.93	0.72 (*s*)	

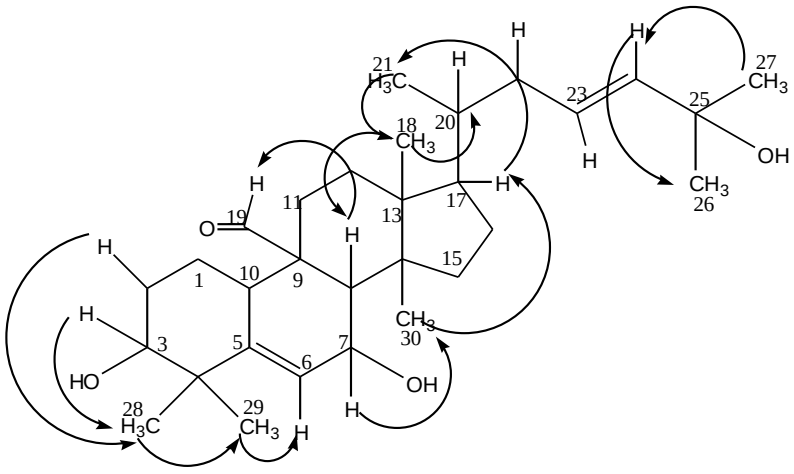
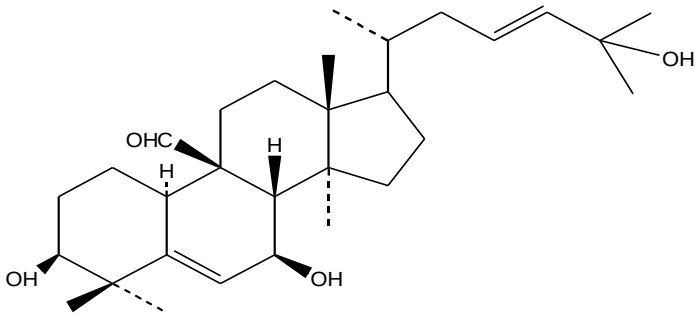


Figure 5.3: Important NOESY correlations in compound OM/11/40



3β, 7β, 25-trihydroxycucurbita-5,(23E)-dien-19-al

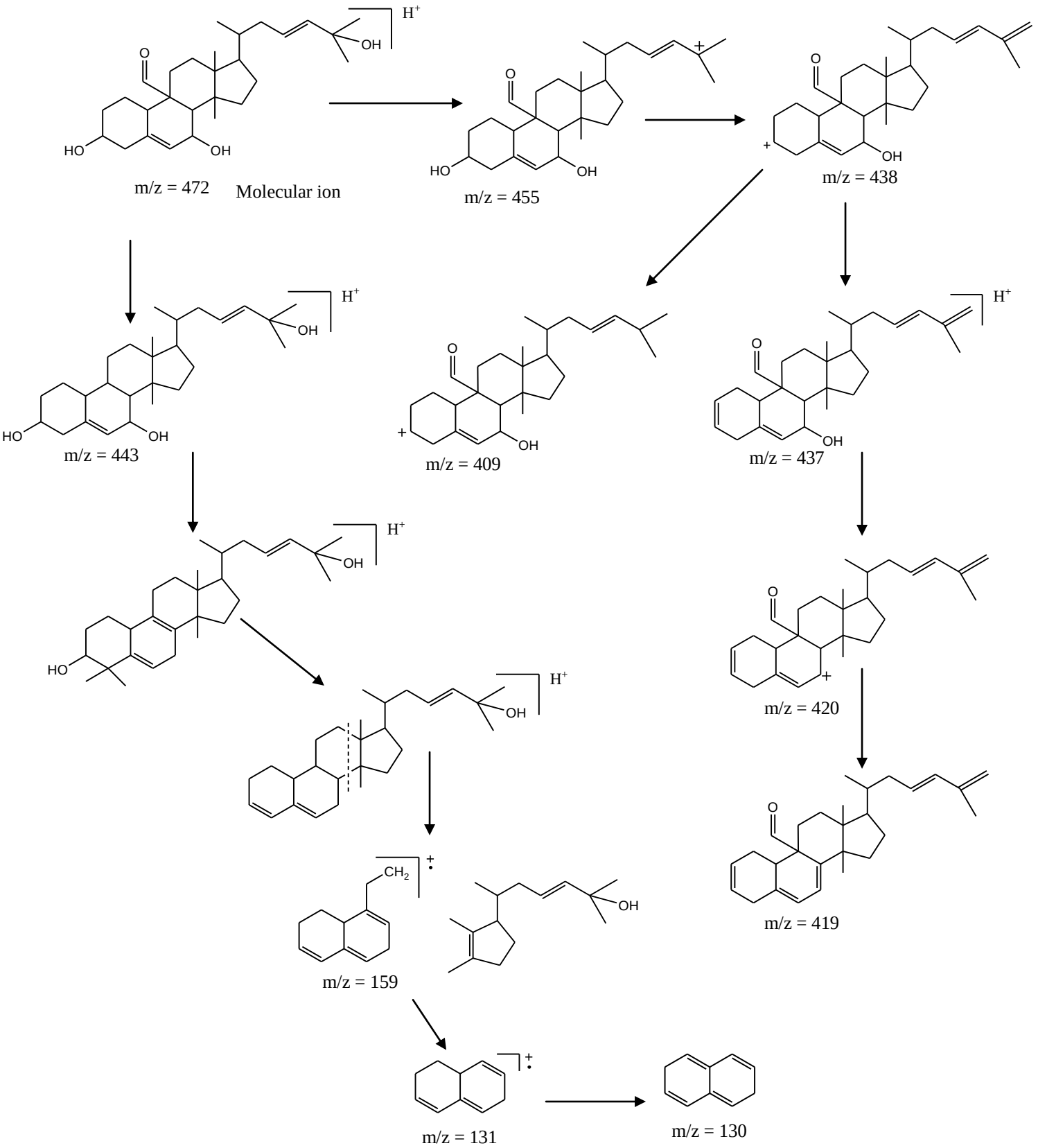


Figure 5.4: Mass fragmentation of OM/11/40

5.3 Compound OM/12/12

Compound OM/12/12 was obtained as an amorphous white powder with a melting point of 167-170^{o}C. The molecular formula of OM/12/12 gave a quasi-molecular ion at m/z 497.3335 $[M+Na]^{+}$ (Appendix 3.9) and fragmentation patterns similar to that of OM/11/40 corresponding to the molecular formula of $C_{30}H_{46}O_3$, which is 14 mass units less than the known compound (23E)-3β-hydroxyl-7-methyoxycucurbita-5,23,25-trien-19-al (Kimura *et al.*, 2005). Its IR spectrum showed absorptions of hydroxyl group (3659 cm^{-1}), aldehyde group (2989 cm^{-1}), non conjugated carbonyl group of aldehyde (1738 cm^{-1}) and vinyl bond (1410 cm^{-1}) (appendix 3.1).

The ^{1}H NMR spectrum (appendix 3.2) of compound OM/12/12 (Table 5.3) showed the presence of five methyl groups attached to a tertiary carbon atom [δ_H 0.68, 0.82, 0.98, 1.18 and 1.28 (3H each, *s*); δ_C 17.91 (*q*), 14.85 (*q*), 27.09 (*q*), 25.39 (*q*) and 18.84 (*q*)], a methyl group attached to a secondary carbon atom [δ_H 0.84 (3H, *d*, J=6.4 Hz); δ_C 18.74 (*q*)], two oxygenated carbons [δ_H 3.50 (1H, br, *s*), 3.91 (1H, *d*, J=4.4 Hz); δ_C 76.17 (*d*) and 66.14 (*d*)], an aldehyde group [δ_H 9.69 (1H, *s*); δ_C 208.05 (*d*)], two disubstituted double bond [δ_H 5.54 (1H, *m*), 6.04 (1H, *d*, J=16 Hz), 4.78 (2H, br *s*); δ_C 129.11 (*d*), 134.27 (*d*), 114.17 (*d*) and 142.15 (*s*)] coupling to a neighbouring methylene [δ_H 1.72 (1H, *m*), 2.18 (1H, *m*); δ_C 39.69 (*t*)] and in addition, olefinic protons of a trisubstituted double bond [δ_H 5.83 (1H, *d*, J = 4.8 Hz); δ_C 123.82 (*d*), 145.43 (*s*)].

The ^{13}C NMR spectrum of OM/11/40 (appendix 3.3) revealed 30 carbon signals, which were assigned by DEPT experiments (appendix 3.4) as six methyl, seven methylene, four methine, four quaternary, six olefinic and two tertiary oxygenated carbons. In a HMBC experiment, correlations between H-23 (δ_H 5.54) and C-20 (δ_C 36.53), C-22 (δ_C 39.69) and C-25 (δ_C 142.15) and between H-26 (δ_H 4.78) and C-23 (δ_C 129.11), C-25 (δ_C 142.15) and C-27 (δ_C 18.84) were observed. The relative configuration of methyl groups and other protons in the tetracyclic rings were determined by significant NOE correlations between H-3 (δ_H 3.50) and H-28 (δ_H 0.98), H-7 (δ_H 3.91) and H-30 (δ_H 0.68), and H-30 (δ_H 0.68) and H-17 (δ_H 1.40) in the NOESY spectrum (Appendix 3.5 and Figure 5.5).

Table 5.3: Table of ^{13}C (100Hz) and ^{1}H NMR (400Hz) data of compound OM/12/12

Position	C atom	δ ^{13}C (ppm)	δ ^{1}H (ppm)	J (Hz)
1.	CH_2	21.18	1.48 (*m*); 1.64 (*m*)	
2.	CH_2	28.96	1.70 (*m*); 2.53 (*m*)	
3.	CH	76.17	3.50 br, (*s*)	
4.	C	41.37	-	
5.	C	145.43	-	
6.	CH	123.82	5.83 (*d*)	4.8
7.	CH	66.14	3.91 (*d*)	4.4
8.	CH	47.53	2.03 br (*s*)	2.03
9.	C	50.08	-	
10.	CH	36.61	2.45 (*dd*)	12.8; 4.2
11.	CH_2	23.45	1.50 (*m*); 2.18 (*m*)	
12.	CH_2	28.98	1.61 (*m*)	
13.	C	45.35	-	
14.	C	47.81	-	
15.	CH_2	34.69	1.25 (*m*); 1.33 (*m*)	
16.	CH_2	28.44	1.70 (*m*); 1.84 (*m*)	
17.	CH	50.08	1.40 (*m*)	
18.	CH_3	14.85	0.82 (*s*)	
19.	CH	208.05	9.69 (*s*)	
20.	CH	36.53	1.48 (*m*)	
21.	CH_3	18.75	0.84 (*d*)	6.4
22.	CH_2	39.69	1.72 (*m*); 2.18 (*m*)	
23.	CH	129.11	5.54 (*m*)	
24.	CH	134.27	6.04 (*d*)	16.0
25.	C	142.15	-	
26.	CH_2	114.17	4.78 br (*s*)	
27.	CH_3	18.84	1.77 (*s*)	
28.	CH_3	27.04	0.98 (*s*)	
29.	CH_3	25.39	1.18 (*s*)	
30.	CH_3	17.91	0.68 (*s*)	

Thus, compound OM/12/12 was elucidated as (23E)-3β-7β-di hydroxylcucurbita-5,23,25-trien-19-al (see appendixes for spectral). The only difference between the ^{13}C NMR spectra of the OM/12/12 and that of Kimura *et al.*, (1990) compounds was that OM/12/12 lack a methoxy group at C-7 and the signal for C-7 was shifted upfield to δ_H 66.14 in OM/12/12. The HMBC correlation from δ_H 3.91 (br *d*, J= 4.4 Hz H-7) to C-5, C-6 and C-8 also substantiated the above structure. Also the NMR data obtained correlate well with

those of (23E)-cucurbita-5,23,25-triene-3β,7β-diol (Chang, *et al.*, 2006) and Kuguacin J (Chen, *et al.*, 2009). The only difference between the spectra of the and OM/11/40 and OM/12/12 compounds was lack a methoxyl group at C-7 and the signal for C-7 was shifted upfield to δ_c 66.14 in OM/12/12.

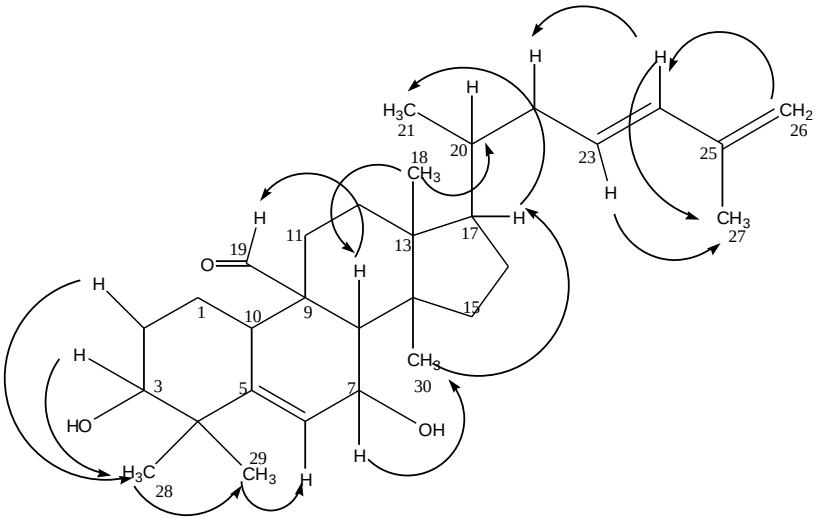
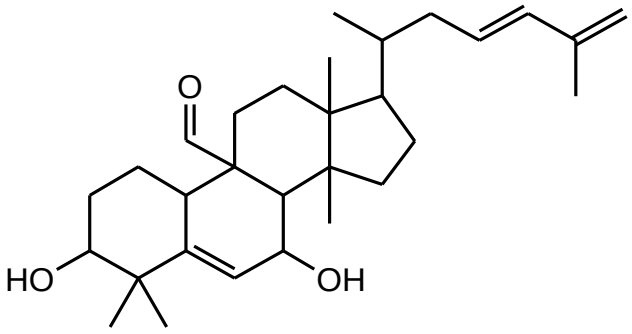


Figure 5.5: Important NOESY correlations in compound OM/12/12



(23E)-3β-7β-di hydroxylcucurbita-5,23,25-trien-19-al

5.4 Compound OM/9/F3

Compound OM/9/F3 (melting point of 215-217 ^{o}C) was obtained as a colourless amorphous white solid. Compound OM/9/F3 appeared to be the lupane-type triterpnoid, as suggested by the very intense fragment peak at *m/z* 189 in its ESI-MS (Ogunkoya, 1981). The molecular ion peak of OM/9/F3 at m/z 426 as shown in appendix 4.8 corresponds to the molecular formula of $C_{30}H_{50}O$ with important fragment ions shown in Figure 5.6. The IR spectrum indicated the presence of hydroxyl (3325 cm^{-1}), olefinic (1650, 1464 cm^{-1}) and C-O (1378 cm^{-1}) groups (appendix 4.1).

The ^{1}H-NMR spectrum (Appendix 4.2, Table 5.4) displayed six methyl group as singlet signals [δ_H 1.05, 0.95, 0.92, 0.83, 0.82 and 0.74 (3H each, *s*)], one isopropenyl group [δ_H 1.66 (3H, *s*), 4.54 and 4.67 (1H each, *d*, J= 2.4 Hz)] and one oxymethine proton (δ_H 3.17 (1H, *dd*, J=10.4 Hz) while the ^{13}C-NMR spectrum (Appendix 4.3) showed 30 carbon signals including seven methyls, ten methylenes, seven (one of which is oxygenated) methines, and six quaternary carbons (Table 5.4).

On the basis of the analysis of the ^{1}H-^{1}H COSY, HSQC, HMBC and DEPT spectra (Appendix 4.5 - 4.8), OM/3/F9 was proposed to be lupane-type triterpene bearing one hydroxyl group. The position of the hydroxyl group was established on the basis of the HSQC, HMBC and NOESY experiments. The HMBC spectrum of OM/9/F9 showed correlations between the oxymethine at 3.17 and the carbon C-2 (δ_C 38.78), C-4 (δ_C 38.87), C-5 (δ_C 55.29), C-23 (δ_C 27.99) and C-24 (15.37), confirming the location of only hydroxyl group at C-3. The 3β-OH equatorial orientation was established using H-3α coupling constant (J_{trans} = 10.6 Hz) and the interaction observed in the NOESY spectrum (Appendix 4.5, Figure 5.6), between the two axis protons H-3α (δ_H 3.17) and H-5 (δ_H 0.66). In addition, the ^{13}C-NMR spectrum of OM/9/F3 (Table 5.4) exhibited the important and characteristic signal for C-5 at δ_H 55.29 that was smaller (ca. 4-5 ppm) than reported data (*ca.* 60.4) in similar triterpenes with a 6β-OH group (Dongfack *et al.*, 2008) but characteristic signal for C-5 at δ_H 55.29 was similar to some reported data for C-5 (*ca.* 55.4 – 56.6 ppm) in similar triterpenes with and without a 6β-OH group (Zhi-Hong *et al.*, 1995, Ghulam *et al.*, 2005, Savona *et al.*, 1987). Therefore, the structure of compound OM/9/F3 was established as 3*β*-hydroxylup-20(29)-ene.

Table 5.4: Table of ^{13}C (100Hz) and ^{1}H NMR (400Hz) data of compound OM/9/F3

Position	C atom	δ ^{13}C (ppm)	δ ^{1}H (ppm)	J (Hz)
1.	CH_2	37.17	1.04 (*m*); 1.25 (*m*)	
2.	CH_2	38.78	0.99 (*m*); 1.65 (*m*)	
3.	CH	79.02	3.17 br, (*s*)	
4.	C	38.87	-	
5.	CH	55.29	0.66 (*d*)	10.3
6.	CH_2	18.32	1.33 (*m*); 1.55 (*m*)	
7.	CH_2	34.28	1.38 (*m*); 1.51 (*m*)	
8.	C	40.83	-	
9.	CH	50.44	1.24 (*m*)	
10.	C	38.71	-	
11.	CH_2	20.93	1.22 (*m*); 1.35 (*m*)	
12.	CH_2	25.14	1.05 (*m*); 1.65 (*m*)	
13.	CH	38.05	1.62 (*m*)	
14.	C	43.01	-	
15.	CH_2	27.45	1.00 (*m*)	
16.	CH_2	35.59	1.35 (*m*); 1.46 (*m*)	
17.	C	42.84	-	
18.	CH	48.31	1.35 (*m*)	
19.	CH	47.99	2.37 (*m*)	
20.	C	150.99	-	
21.	CH_2	29.85	1.25 (*m*); 1.89 (*m*)	
22.	CH_2	40.00	1.17 (*m*); 1.35 (*m*)	
23.	CH_3	27.99	0.94 (*s*)	
24.	CH_3	15.37	0.74 (*s*)	
25.	CH_3	15.99	0.82 (*s*)	
26.	CH_3	16.10	1.05 (*m*)	
27.	CH_3	14.55	0.92 (*s*)	
28.	CH_3	18.01	0.83 (*s*)	
29.	CH_2	109.32	4.54 (*d*); 4.67 (*d*)	2.4; 2.4
30.	CH_3	19.40	1.66 (*s*)	

This compound is an antineoplastic agent, and trypsin and chymotrypsin inhibitor (Dictionary of Natural products, 2008).

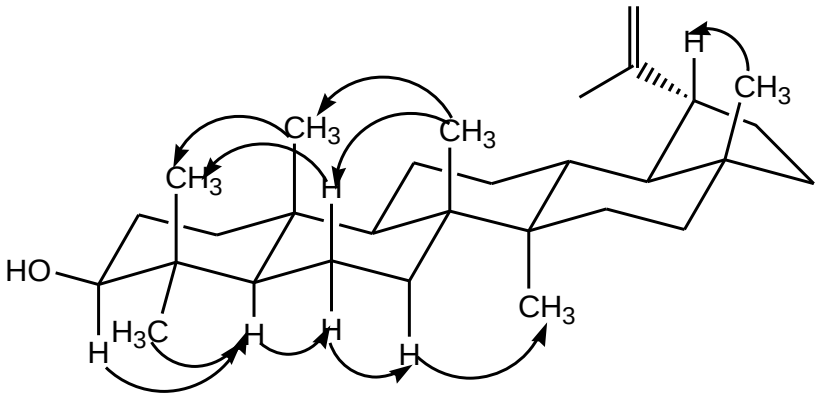
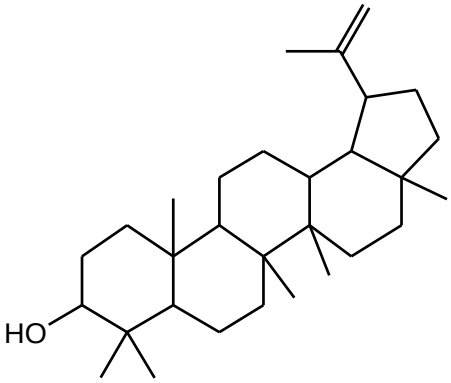


Figure 5.6: Important NOESY correlation of compound OM/9/F3



3*β*-hydroxylup-20(29)-ene

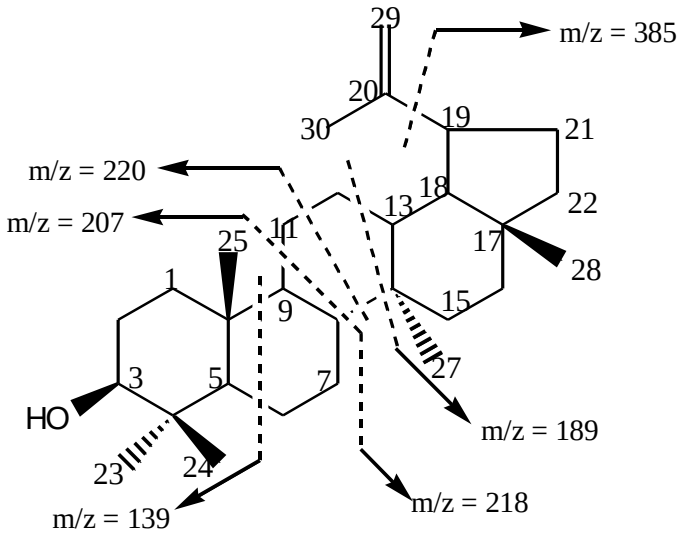


Figure 5.7: Mass fragmentation of OM/9/F3

5.5 Compound OM/10/F1

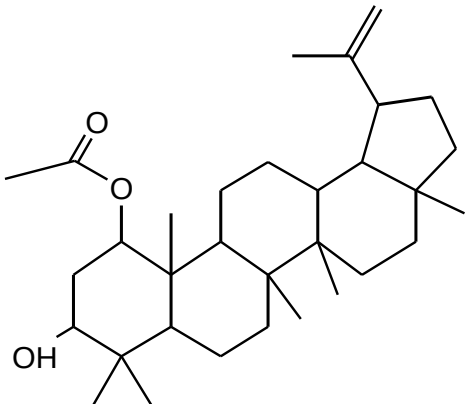
Compound OM/10/F1 (m.p. 215 - 217 ^{o}C) was obtained as a colourless amorphous solid with molecular mass 484.39 (Appendix 5.9). Its molecular formula is $C_{32}H_{52}O_3$. The IR spectrum (Appendix 5.1) indicated the presence of hydroxyl (3356 cm^{-1}), olefinic (2926; 1642 and 882 cm^{-1}) i.e. vinylidene group, keto group (1733 and 1188 cm^{-1} i.e. COOMe) and C-O (1378 cm^{-1}) groups.

The ^{1}H-NMR spectrum of 2 (Table 5.5) displayed six methyl groups attached to the tertiary carbon possed singlet signals [δ_H 1.05, 0.95, 0.92, 0.83, 0.82 and 0.74 (3H each, *s*)], one isopropenyl group [δ_H 1.66 (3H, *s*), 4.54 and 4.67 (1H each, *d*, J= 2.4 Hz)] and one oxymethine proton (δ_H 3.17 (1H, *dd*, J=10.4 Hz) while the ^{13}C-NMR spectrum (Appendix 5.3) showed 30 carbon signals including seven methyls, ten methylenes, seven (one of which is oxygenated) methines, and six quaternary carbons (Table 5.5).

On the basis of the analysis of the ^{1}H-^{1}H COSY, HSQC, HMBC and DEPT spectra (Appendix 4.4 – 4.8), OM/3/F9 was proposed to be lupane-type triterpene bearing one hydroxyl group. The position of the hydroxyl group was established on the basis of the HSQC, HMBC and NOESY experiments. The compound OM/10/F1 was identified by direct comparison of its spectral data to those published in literature (Hui and Li, 1978). Therefore, the structure of compound OM/10/F1 was established as lup-20(29)-en-3*β*-ol-1α-yl- acetate.

Table 5.5: Table of ^{13}C (100Hz) and ^{1}H NMR (400Hz) data of compound OM/10/F1

Position	C atom	δ ^{13}C (ppm)	δ ^{1}H (ppm)	J (Hz)
1.	CH_2	55.12	1.04 (*m*); 1.25 (*m*)	
2.	CH_2	38.79	0.99 (*m*); 1.65 (*m*)	
3.	CH	79.03	3.17 br, (*s*)	
4.	C	38.86	-	
5.	CH	55.30	0.66 (*d*)	10.3
6.	CH_2	18.32	1.33 (*m*); 1.55 (*m*)	
7.	CH_2	34.28	1.38 (*m*); 1.51 (*m*)	
8.	C	40.83	-	
9.	CH	50.44	1.24 (*m*)	
10.	C	38.70	-	
11.	CH_2	20.93	1.22 (*m*); 1.35 (*m*)	
12.	CH_2	25.14	1.05 (*m*); 1.65 (*m*)	
13.	CH	38.05	1.62 (*m*)	
14.	C	43.00	-	
15.	CH_2	27.45	1.00 (*m*)	
16.	CH_2	35.58	1.35 (*m*); 1.46 (*m*)	
17.	C	42.83	-	
18.	CH	48.70	1.35 (*m*)	
19.	CH	47.99	2.37 (*m*)	
20.	C	150.99	-	
21.	CH_2	29.85	1.25 (*m*); 1.89 (*m*)	
22.	CH_2	40.01	1.17 (*m*); 1.35 (*m*)	
23.	CH_3	27.99	0.94 (*s*)	
24.	CH_3	15.37	0.74 (*s*)	
25.	CH_3	15.98	0.82 (*s*)	
26.	CH_3	16.12	1.05 (*m*)	
27.	CH_3	14.55	0.92 (*s*)	
28.	CH_3	18.00	0.83 (*s*)	
29.	CH_2	109.32	4.54 (*d*); 4.67 (*d*)	2.4
30.	CH_3	19.33	1.66 (*s*)	
31.	C=O	121.72	-	
32.	-O-Ac	21.50	2.08 (*s*)	



Lup-20(29)-en-3*β*-ol-1*α*-yl- acetate

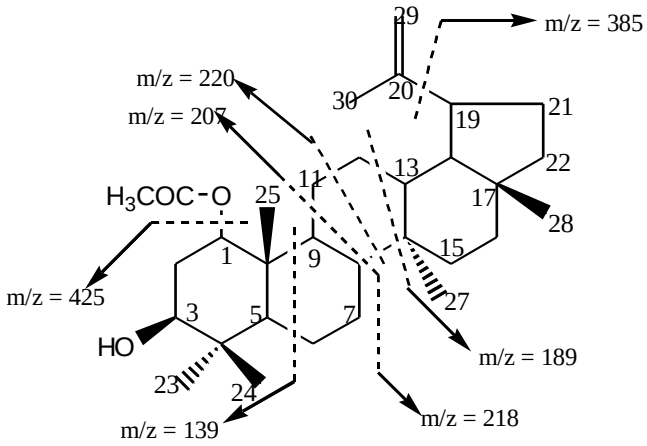


Figure 5.8: Mass fragmentation of compound OM/10/F1

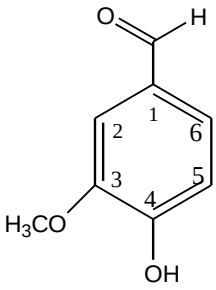
5.6 Compound OM/15/17

Compound OM/15/17 was obtained as white needle-like crystals with ESI-MS (positive mode) *m/z* (%) 141 (50), 137 (8), 123 (25), 109 (32) of molecular formula $C_8H_8O_3$ and molecular weight 152.15 based on the mass spectroscopic analysis (appendix 6.9a – 6.9b) with melting point 154-156 ^oC. The diagnostic fragment ion was shown in Figure 5.9.

The ^1H and ^{13}C NMR spectra of compound OM/15/17 (appendix 6.2 - 6.3) (Table 5.6) showed the presence of one methoxy group [δ_H 3.93 (3H, *s*); δ_C 58.12 (*q*)], three aromatic protons [δ_H 7.02 (1H, *d*, J=8.5Hz), 7.40 (1H, *dd*), 7.42 (1H, *dd*); δ_C 114 (*d*), 108.79 (*d*), 127.56 (*d*)], an aldehyde group [δ_H 9.80 (1H, *s*); δ_C 190.99 (*d*)] and three quaternary carbons [δ_C 129.85 (*s*), 147.18 (*s*), 151.74 (*s*)]. Additional signal included broad peak δ_H 6.33 (1H, *s*) indicating that it could be a hydroxyl group. The HMBC and HSQC (Appendix 6.7 - 6.8) were used to assign the carbon atoms and the NOESY (Figure 5.10) and COSY were used to assign the proton to the neighbouring protons. The complete assignment was aided by ^1H-NMR, ^{13}C-NMR, DEPT, HSQC and HMBC (appendix 6.2 - 6.8). Thus compound OM/15/17 was identified as 4-hydroxy-3-methoxyl-benzaldehyde, (see appendixes for spectra). The molecular formula of this compound was identified by ESI-MS (positive mode) *m/z* (%) 151 (100), 141 (50), 137 (8), 123 (25), 109 (32) This compound has never been detected in the genus *Bergiana*.

Table 5.6: Table of ^{13}C and ^1H NMR spectral data of compound OM/15/17

Position	C atom	δ ^{13}C (ppm)	δ ^1H (ppm)	J (Hz)
1.	C	129.85	-	
2.	CH	127.56	7.42 (*dd*)	
3.	C	14.18	-	
4.	C	151.74	-	
5.	CH	114.42	7.02 (*d*)	8.5
6.	CH	108.79	7.72 (*dd*)	
7.	CO	190.99	9.80 (*s*)	
8.	CH_3	58.12	3.94 (*s*)	



4-Hydroxy-3-methoxybenzaldehyde

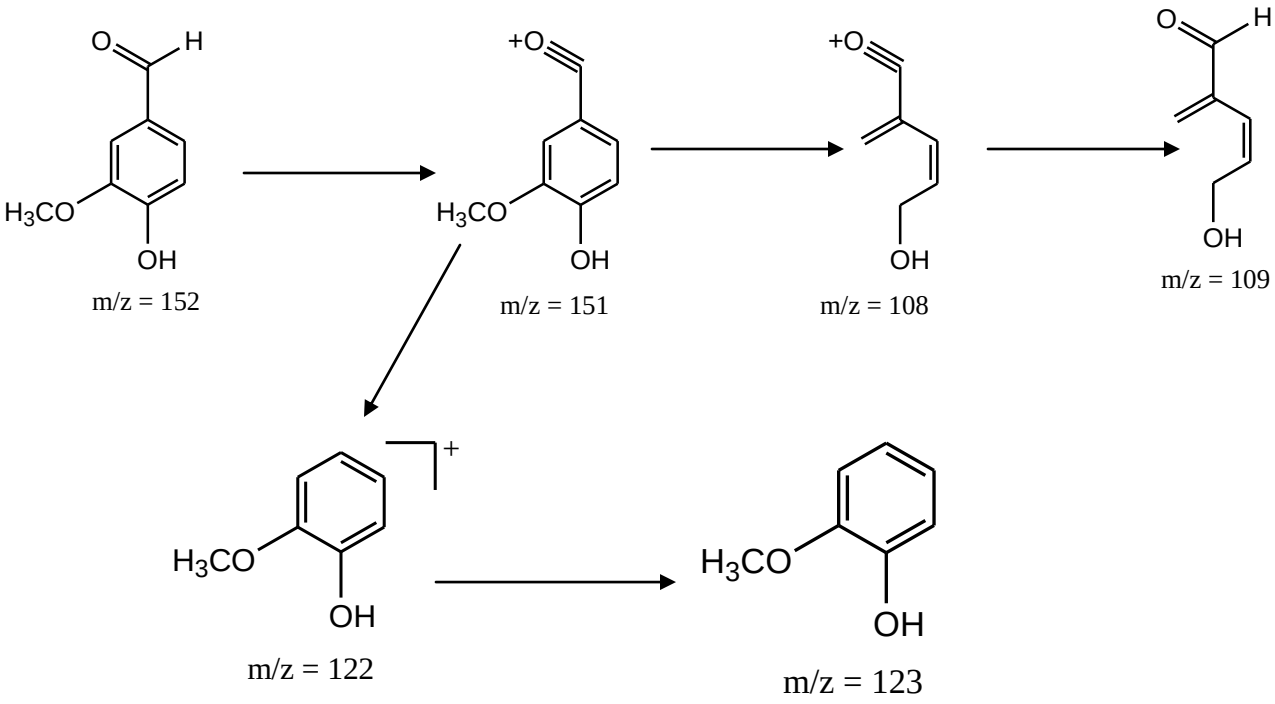


Figure 5.9: Mass fragmentation of OM/15/17

CHAPTER SIX

CONCLUSION, LIMITATION AND SUGGESTION FOR FURTHER WORK

6.1 CONCLUSION

Medicinal plants have been used for centuries as the primary mode of treatment of ailments caused by various organisms. This has provided the opportunity for researchers and scientists to evaluate the biological activities and safety of medicinal plants to promote their usage and for the development of new pharmaceuticals which may be less toxic and more potent than currently used.

Chromatographic Analysis/purification of extracts, successive extracts and fractions led to the isolation of six compounds, three from each plant. The structures of the isolated compounds were elucidated using the NMR, MS and IR techniques and the compounds were identified as Kaempferol-3-*O*-*β*-*D*-glucospyranoside (OM/1/E/T2); 3*β*,7*β*,25-trihydroxycucurbita-5,(23E)-dien-7-on-19-al (OM/11/40); 3*β*,7*β*-dihydroxy-cucurbita-5(23E),23,25-triene-on-19-al (OM/12/12); 3*β*-hydroxylup-20(29)-ene (OM/9/F3); lup-20(29)-en-3*β*-ol-1α-yl- acetate (OM/10/F1) and 4-hydroxy-3-methoxyl-benzaldehyde (OM/15/17). In the case of *M. foetida*, these compounds have been isolated previously from plants that belong to the same genus but different species except Kaempferol-3-*O*-*β*-*D*-Glucospyranoside which is now being reported for the first time in the genus *Momordica*. In the case of *B. bergiana*, these compounds had not been isolated from the plant. These compounds showed excellent antioxidant potential and this implies that they can serve as drugs in the treatment of antioxidant related diseases which include diabetes, inflammation, liver diseases and tumour. For example OM/9/F3 compound (3*β*-hydroxylup-20(29)-ene) is an antineoplastic agent, and trypsin and chymotrypsin inhibitor. These compounds can serve also as template in drug synthesis.

Research on natural products should be encouraged and funding should be made available for the development and production of new pharmaceuticals or herbal extracts of therapeutic value. The world is faced with devastating dieseases such as malaria, cancer, diabetes and tuberculosis and biological resources such as plants might provide solution to these diseases.

6.2 LIMITATIONS OF THE WORK

The four aims set out initially for this work, to establish the phytochemical, isolate, identify, and evaluate and compare the antioxidant and antimicrobial activities of extracts, fractions,

and constituents of *Momordica foetida and Berkheya bergiana* have been substantially achieved. However, because of the very low yields (at millgramme level) of pure components despite starting with kilogrammes of dry plant materials the extension of the work beyond the goals set have been impracticable. In the course of extractions, we isolated many unstable compounds some of which were also light sensitive that could not be analysed due to technical limitations. This also accounts for the limitation imposed on this work and the number of compounds reported. Further exploration of the chemistry of the pure isolates was therefore not contemplated.

It is well known that the chemical variability, the yield of chemical constituents, and the physiology of plants are affected by environmental conditions, geographic variations, genetic factors and evolution. Consequently the option to collect the plants from other locations to boost yield or replicate the work was anticipated would generate conflicting data that could compromise the conclusions drawn from the study. Subsequent visits to the area where the plants were collected revealed that the area was either burnt or devastated by extreme summer heat.

6.3 SUGGESTIONS FOR FURTHER WORK

This work forms the basis for further studies on the extracts and compounds to link the biological activity to the specific compounds identified using modern instrumentation like HPLC coupled with NMR (i.e. closed system to prevent decomposition of the compounds and identification of novel compounds) which should be made easily accessible. It would also be desirable to investigate chemical variability and the effect on biological activities in the plants due to seasonal changes. This could include the seasonal variation on the quantity and quality of the active compounds as well as the biological effect using modern instrumental analysis and working in collaboration with pharmacologists.

M. foetida and *B. bergiana* are widely distributed in Southern Africa and other tropical regions. Both plants are traditionally used to treat diarrhoea and this may be due to their antibacterial activities as revealed in this work. *In-vitro* activity of *M. foetida* and *B. bergiana* extracts, fractions and compounds indicated rich antioxidant content. The rich antioxidant content puts these plants in line for treatment of neurodegenerative and cardiovascular diseases. Future studies may therefore involve animal experiments to verify the results obtained here.

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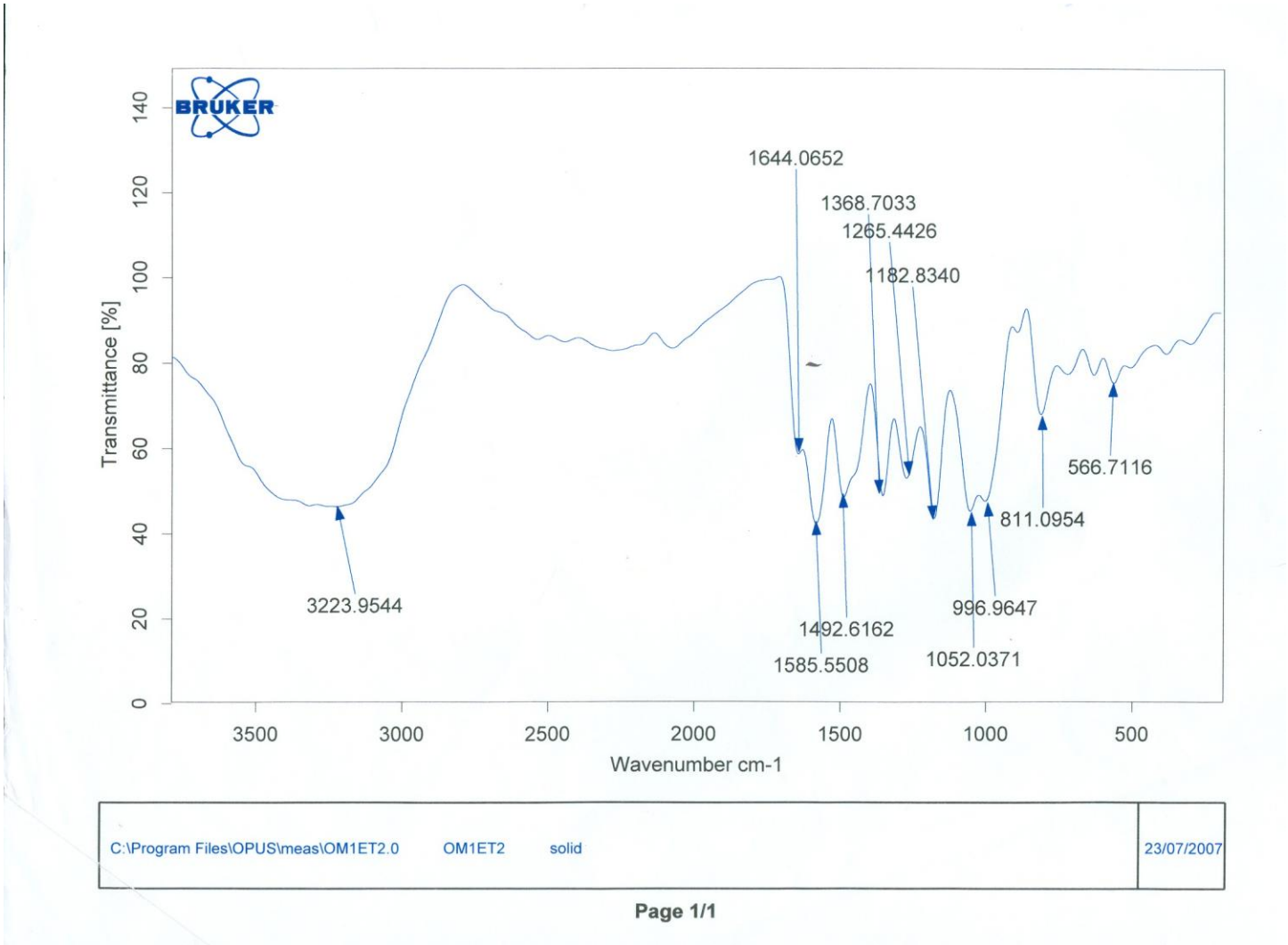
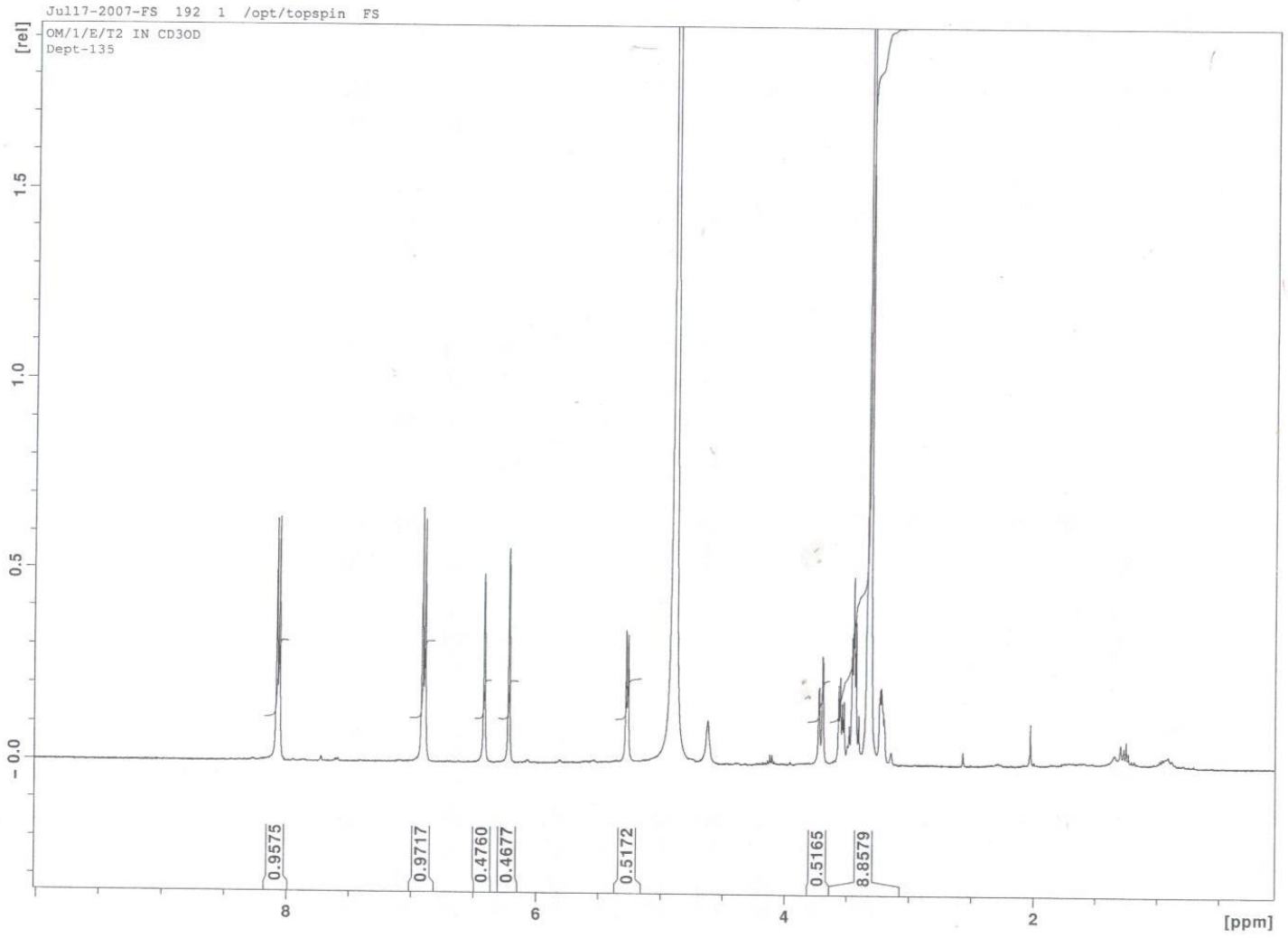
Appendix 1.0: Spectral for compound OM/1/E/T2

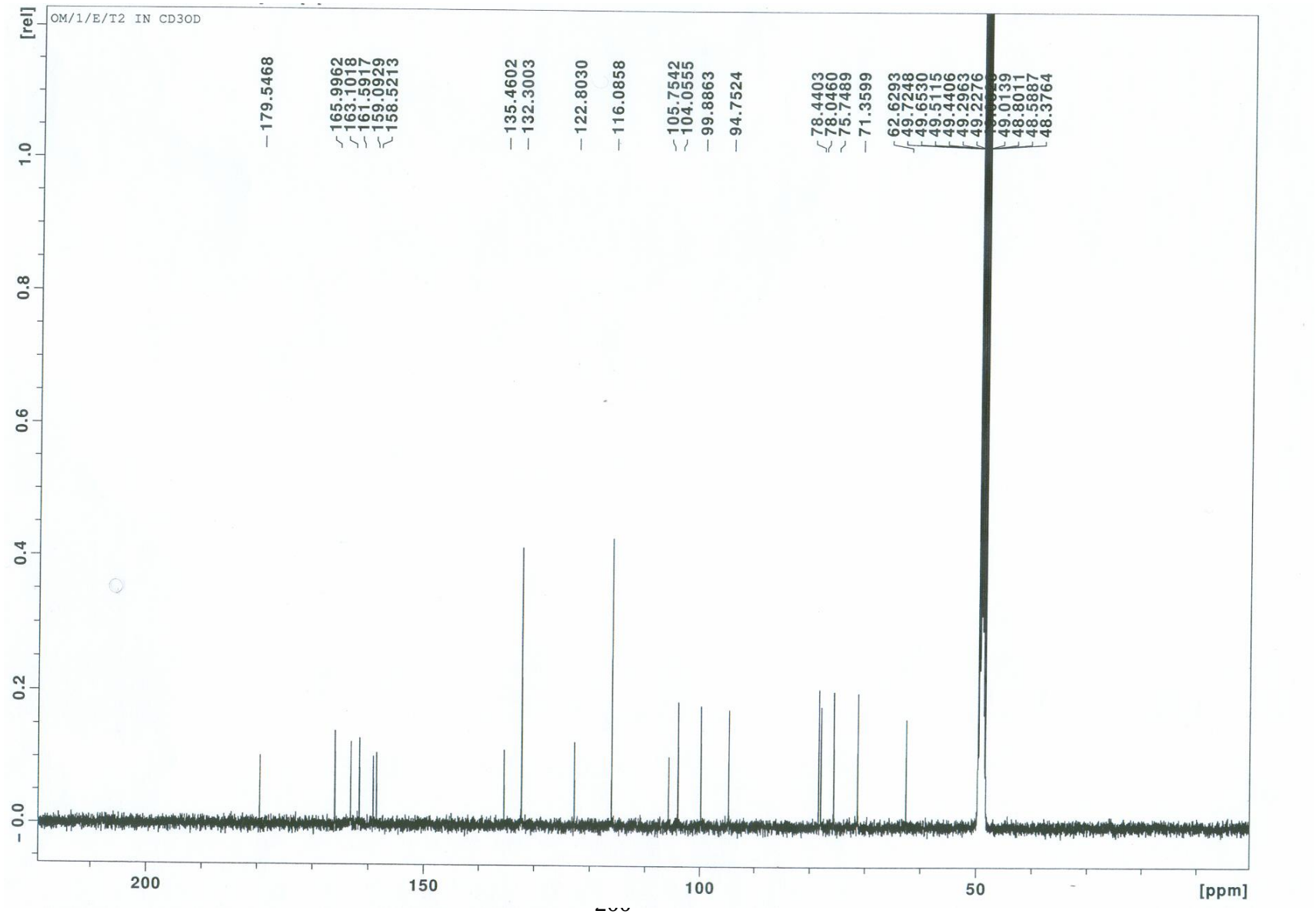
Appendix 1.1: IR Spectrum of compound OM/1/E/T2



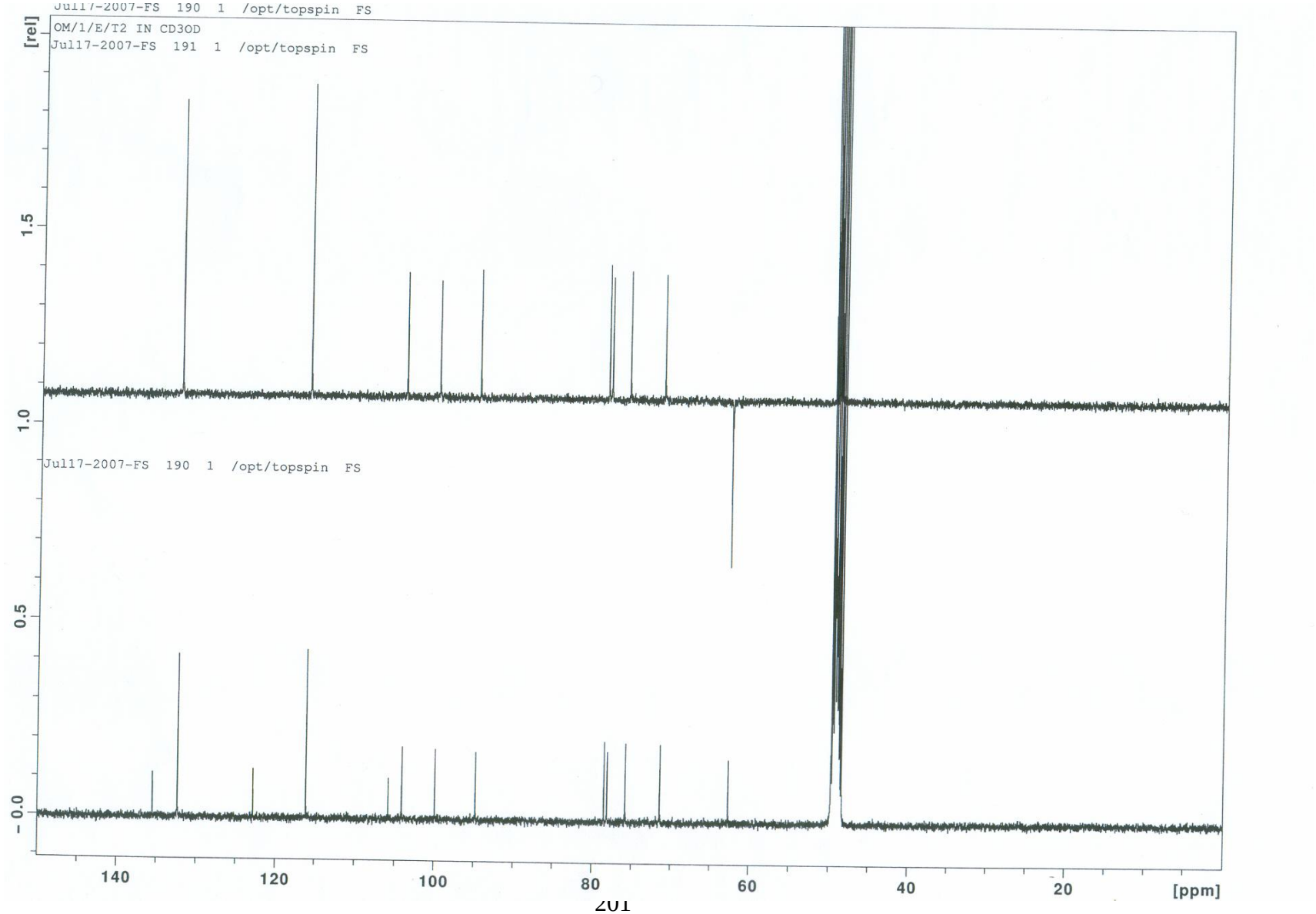
Appendix 1.2: ^{1}H NMR Spectrum of compound of OM/1/E/T2



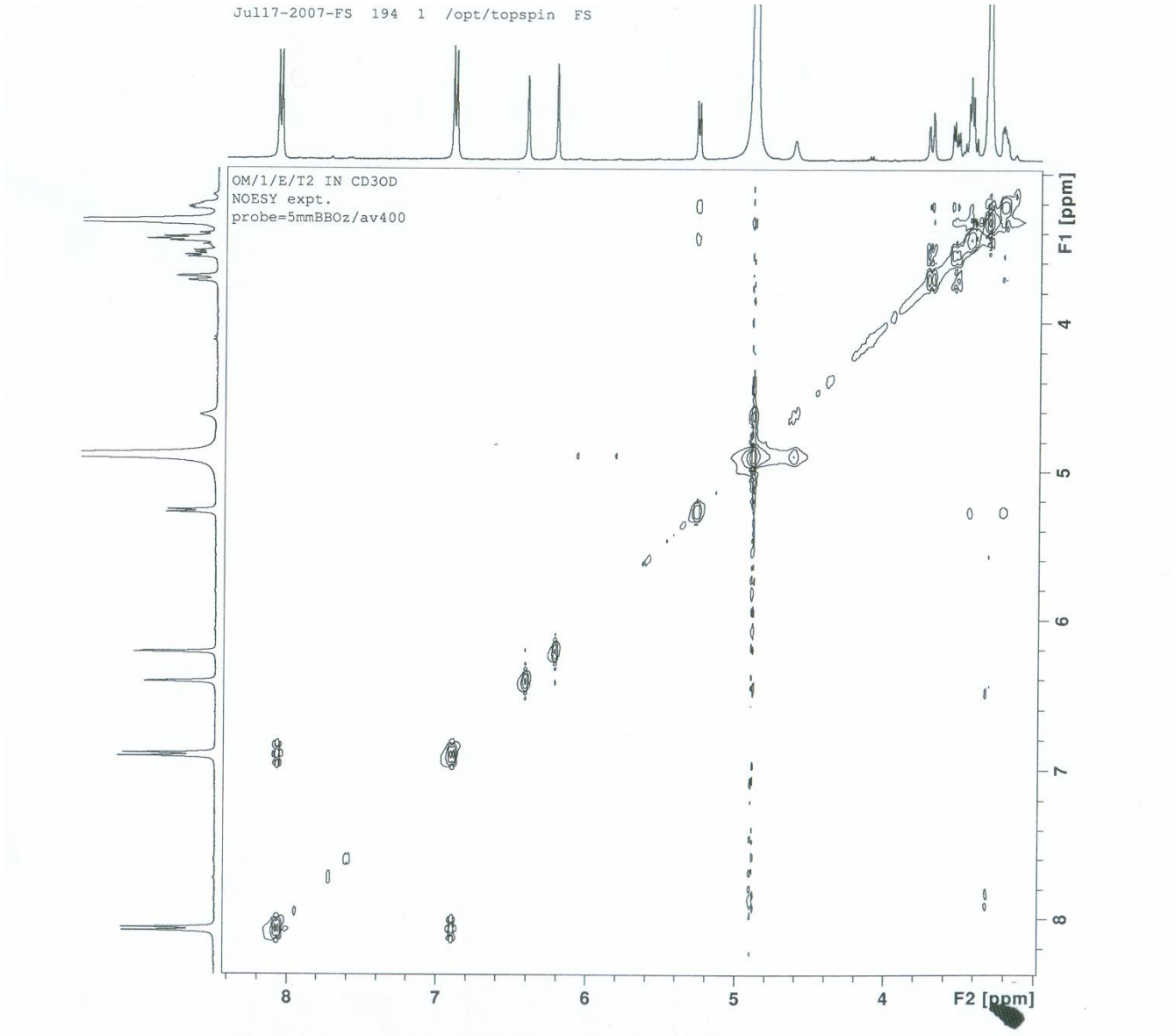
Appendix 1.3: ^{13}C NMR Spectrum of compound of OM/1/E/T2



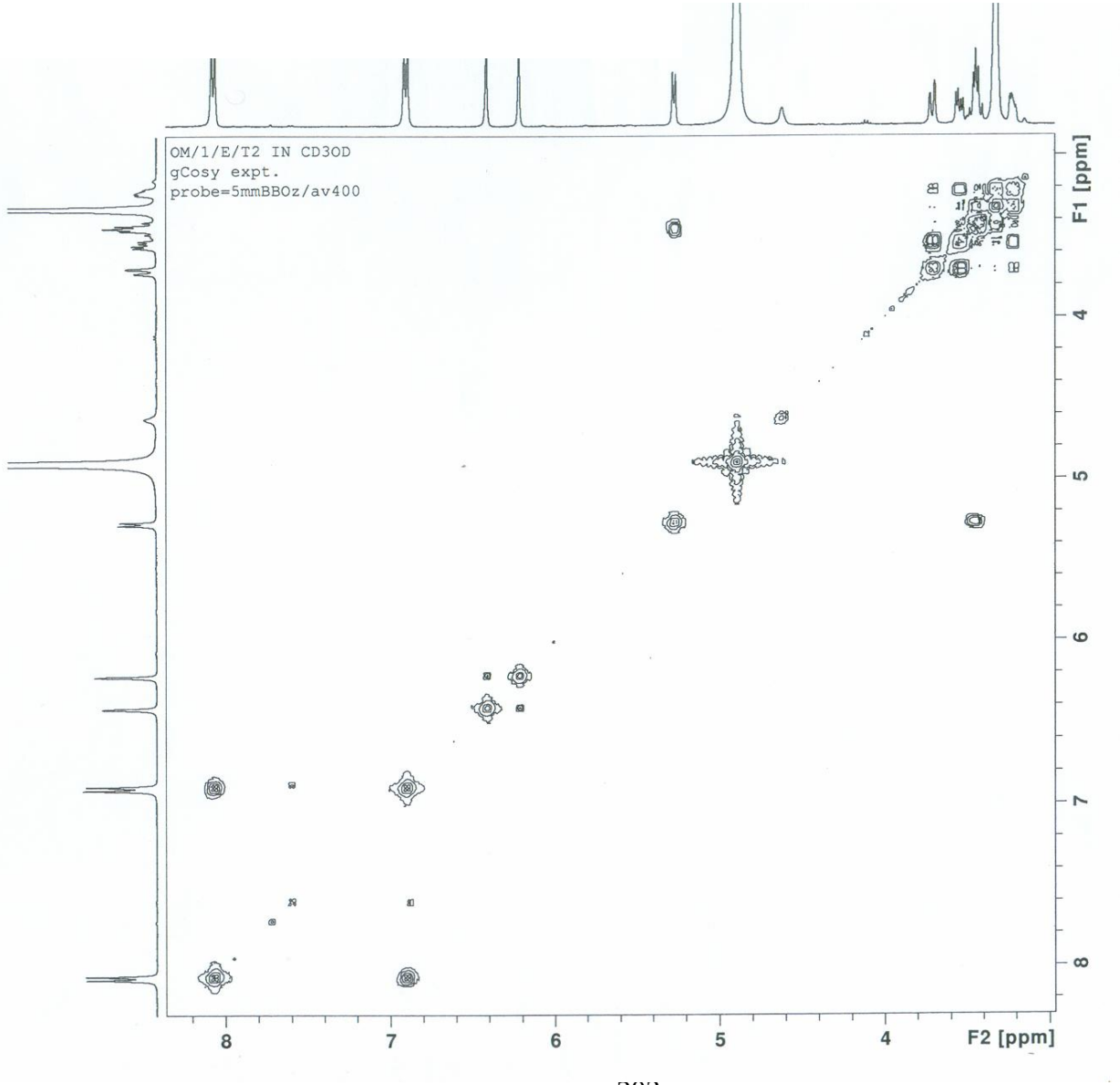
Appendix 1.4: DEPT NMR Spectrum of compound of OM/1/E/T2



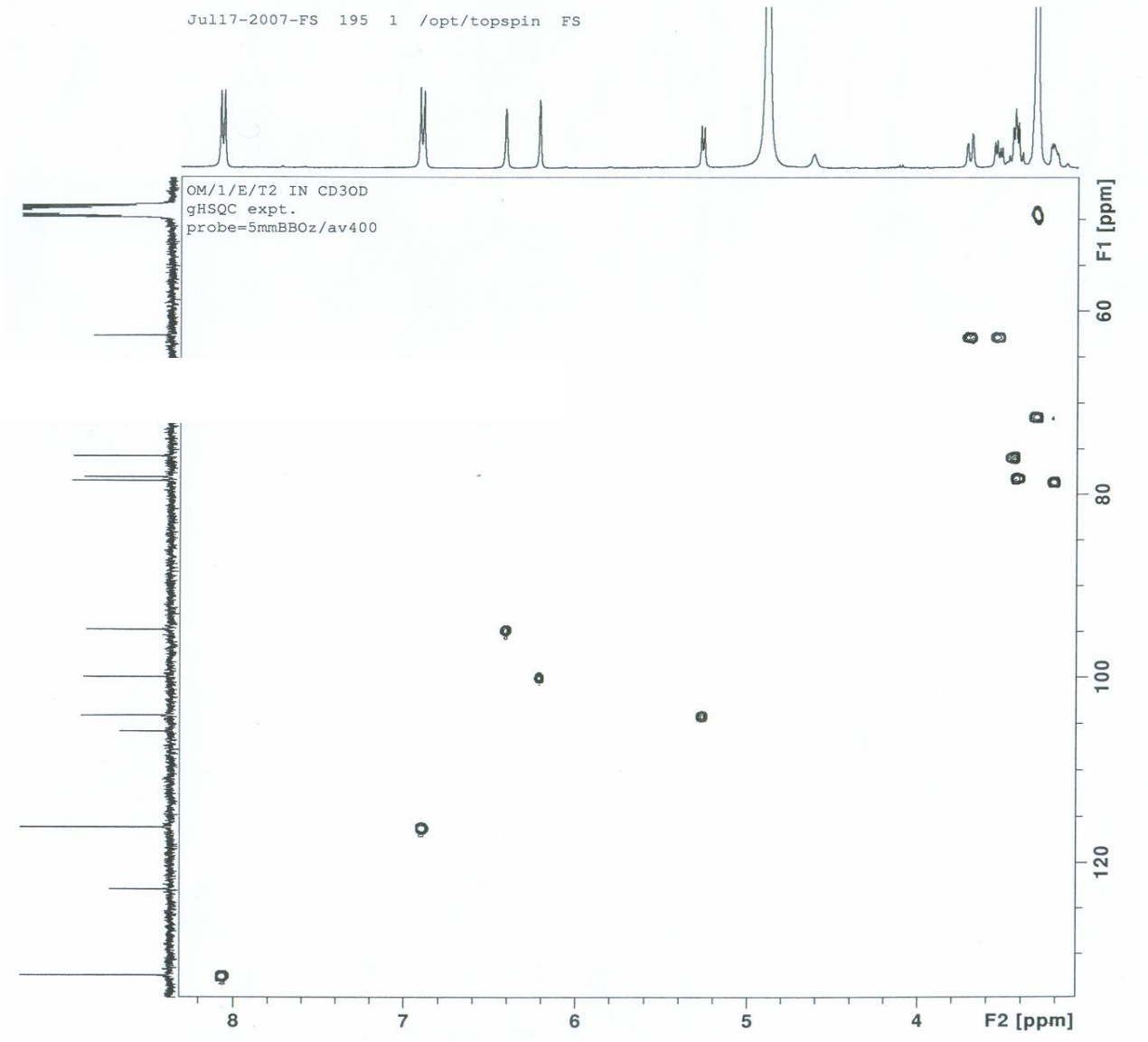
Appendix 1.5: NOSY NMR Spectrum of compound of OM/1/E/T2



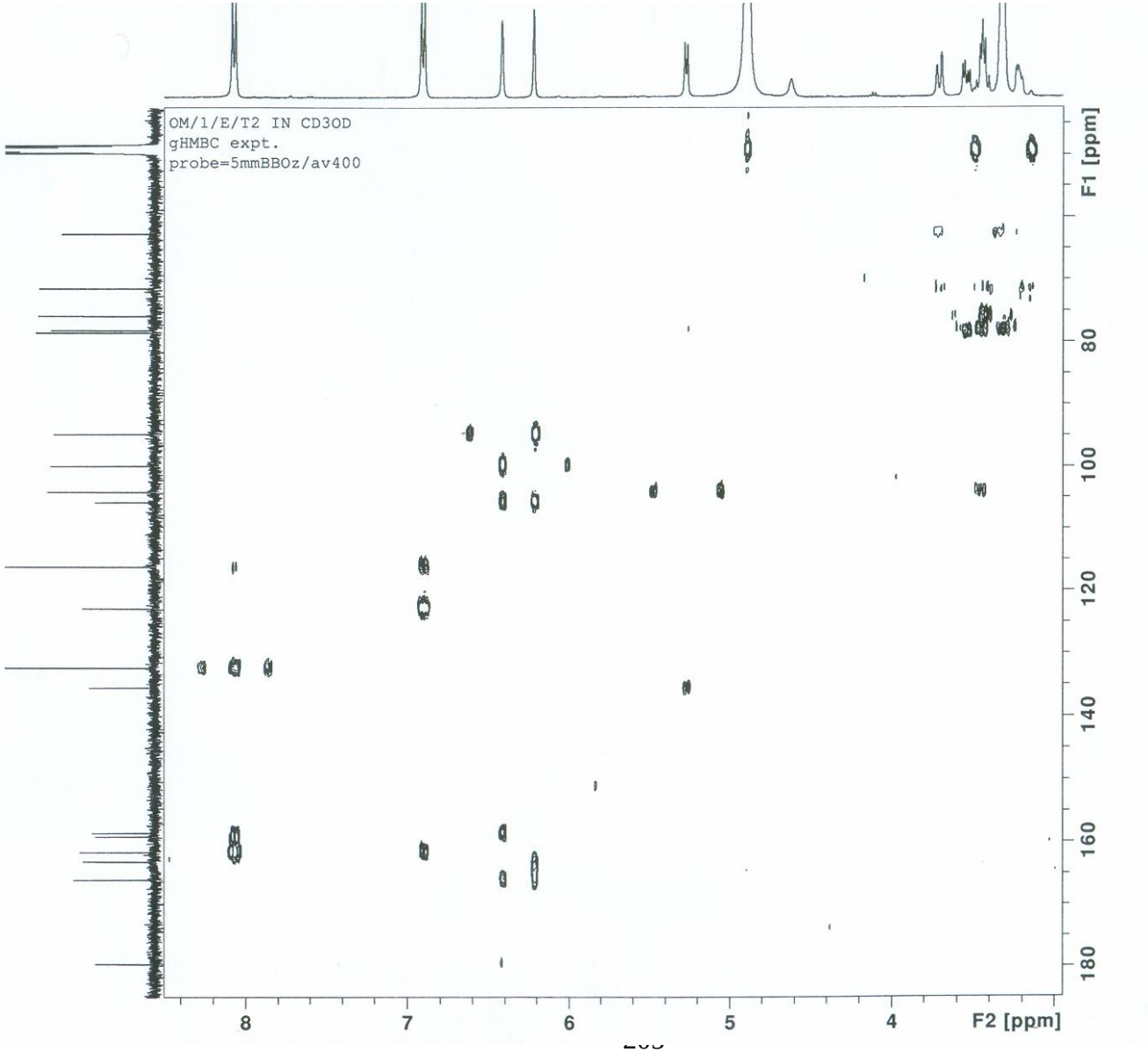
Appendix 1.6: gCOSY NMR Spectrum of compound of OM/1/E/T2



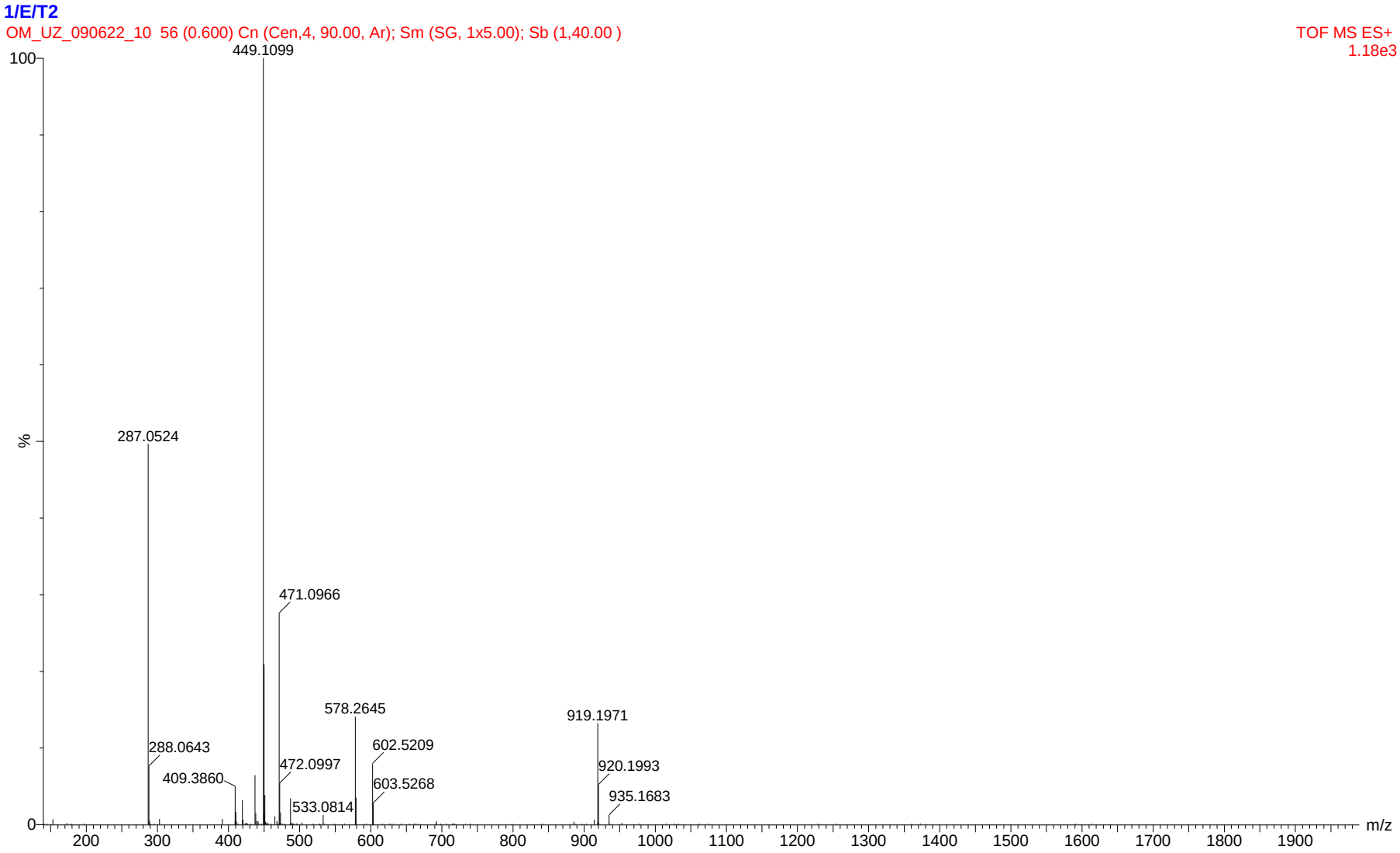
Appendix 1.7: HSQC NMR Spectrum of compound of OM/1/E/T2



Appendix 1.8: HMBC NMR Spectrum of compound of OM/1/E/T2



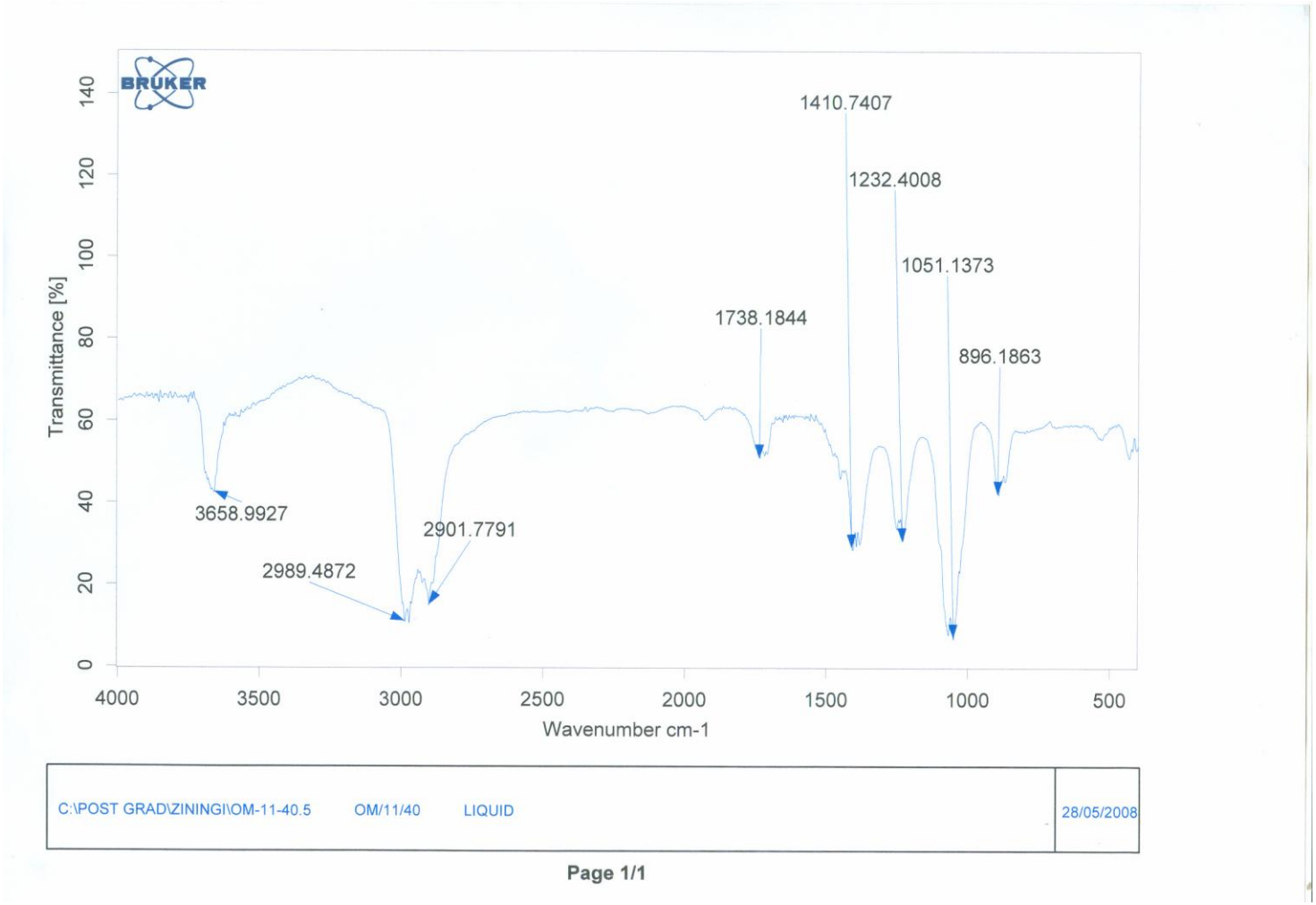
Appendix 1.9a: MS Spectrum of compound OM/1/E/T2



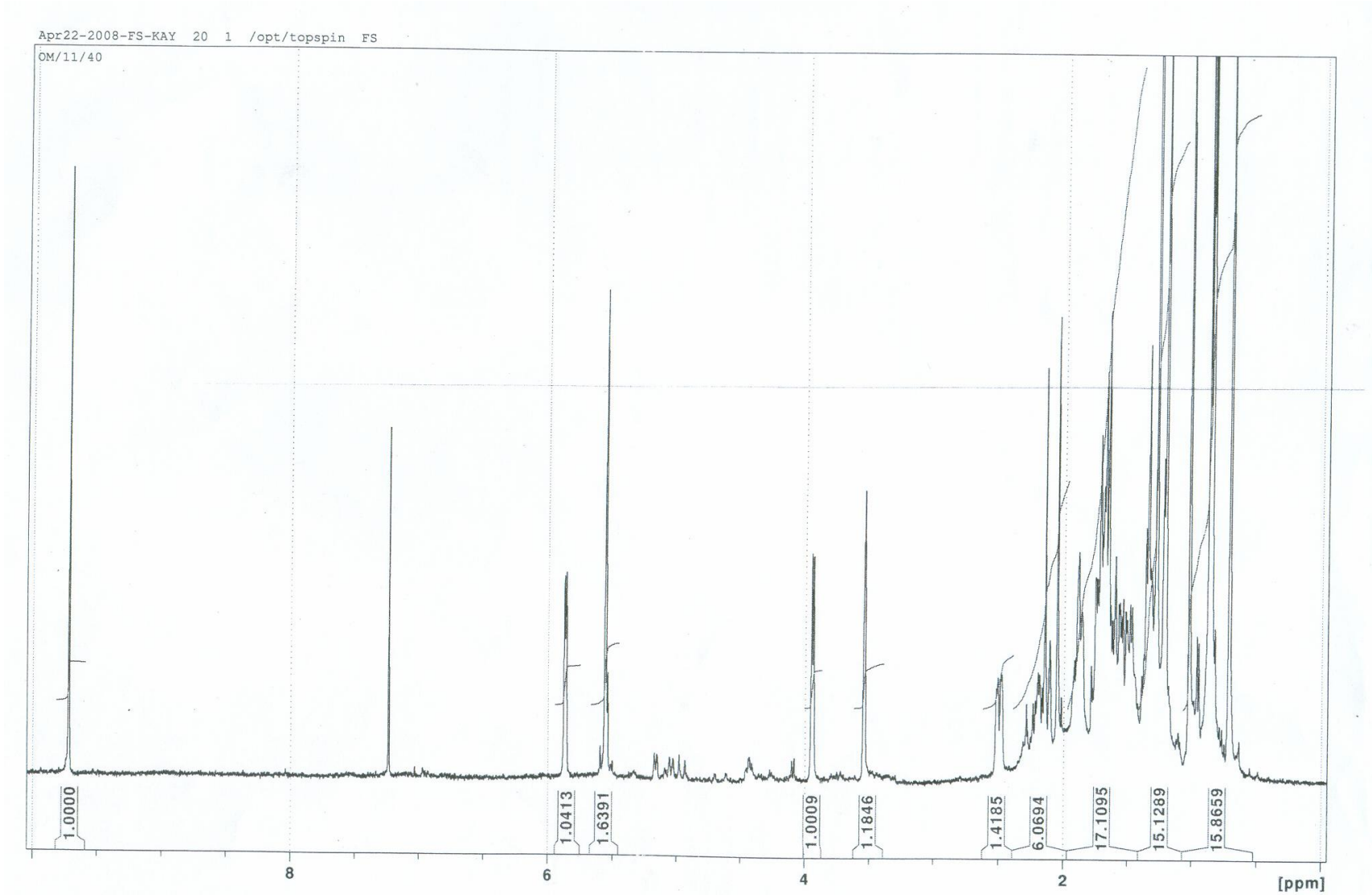
Appendix 1.9b: MS/MS Spectrum of compound OM/1/E/T2

Appendix 2.0: Spectral for compound OM/11/40

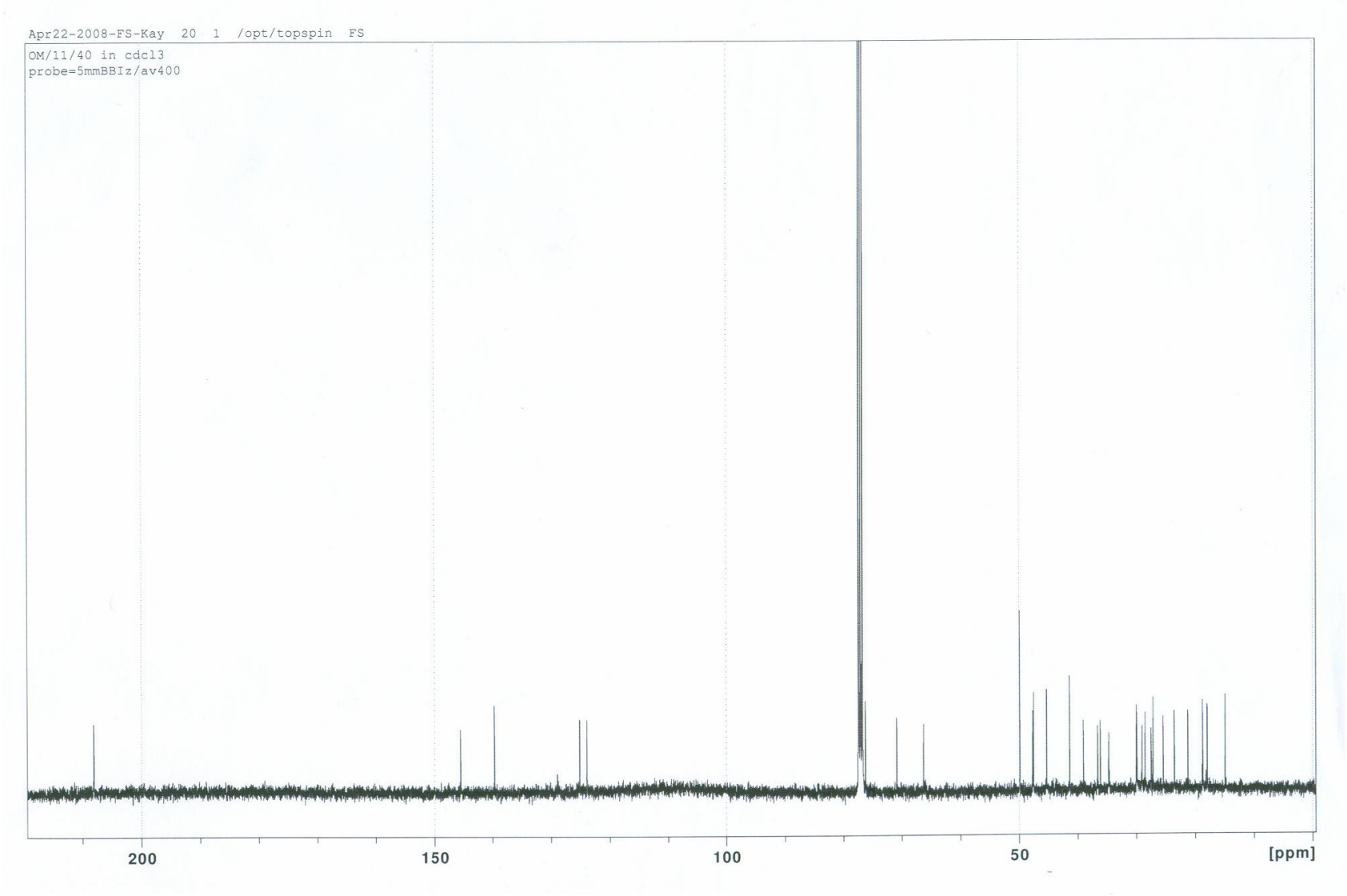
Appendix 2.1: IR Spectrum of compound OM/11/40



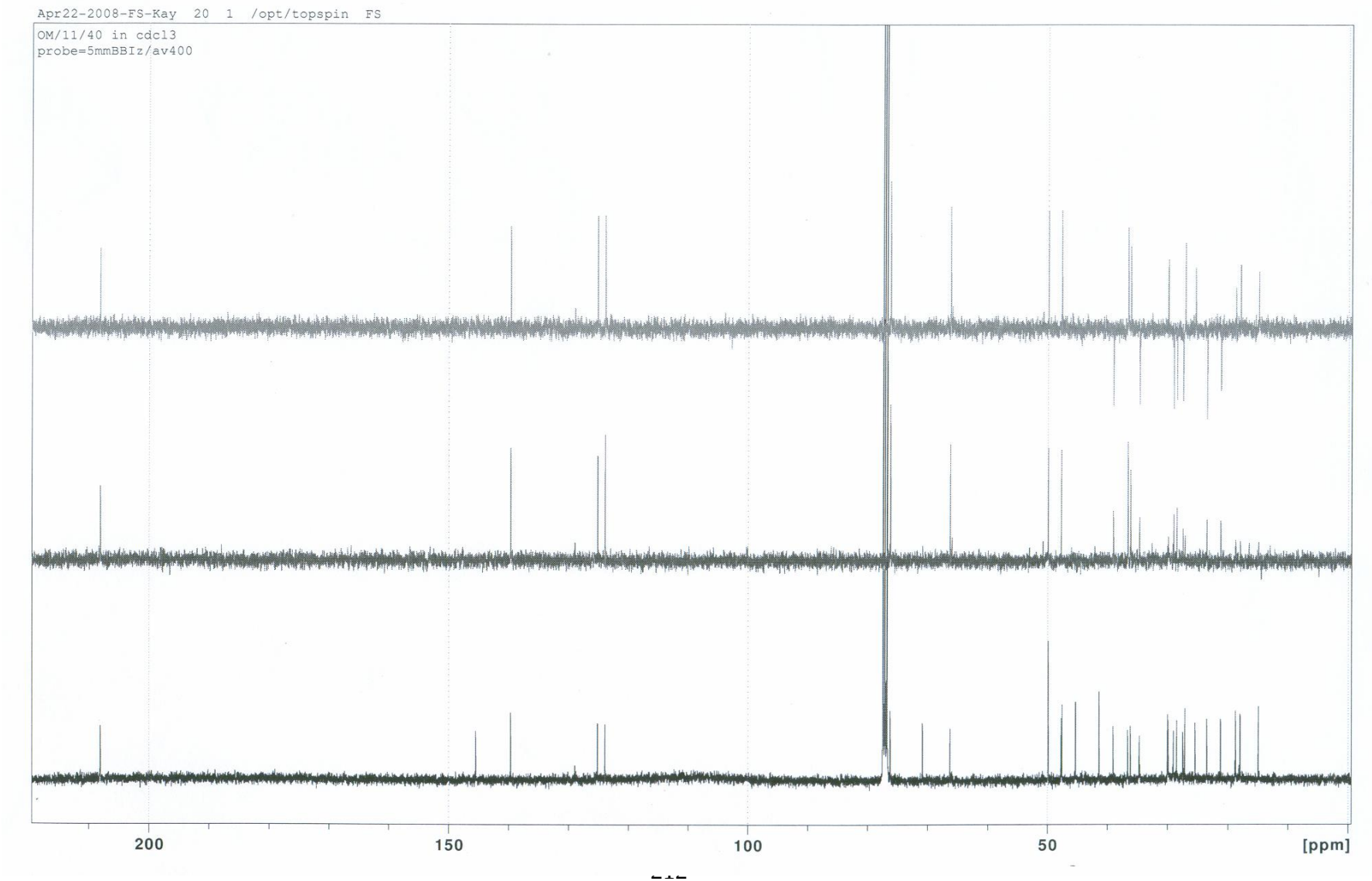
Appendix 2.2: ^{1}H NMR Spectrum of compound OM/11/40



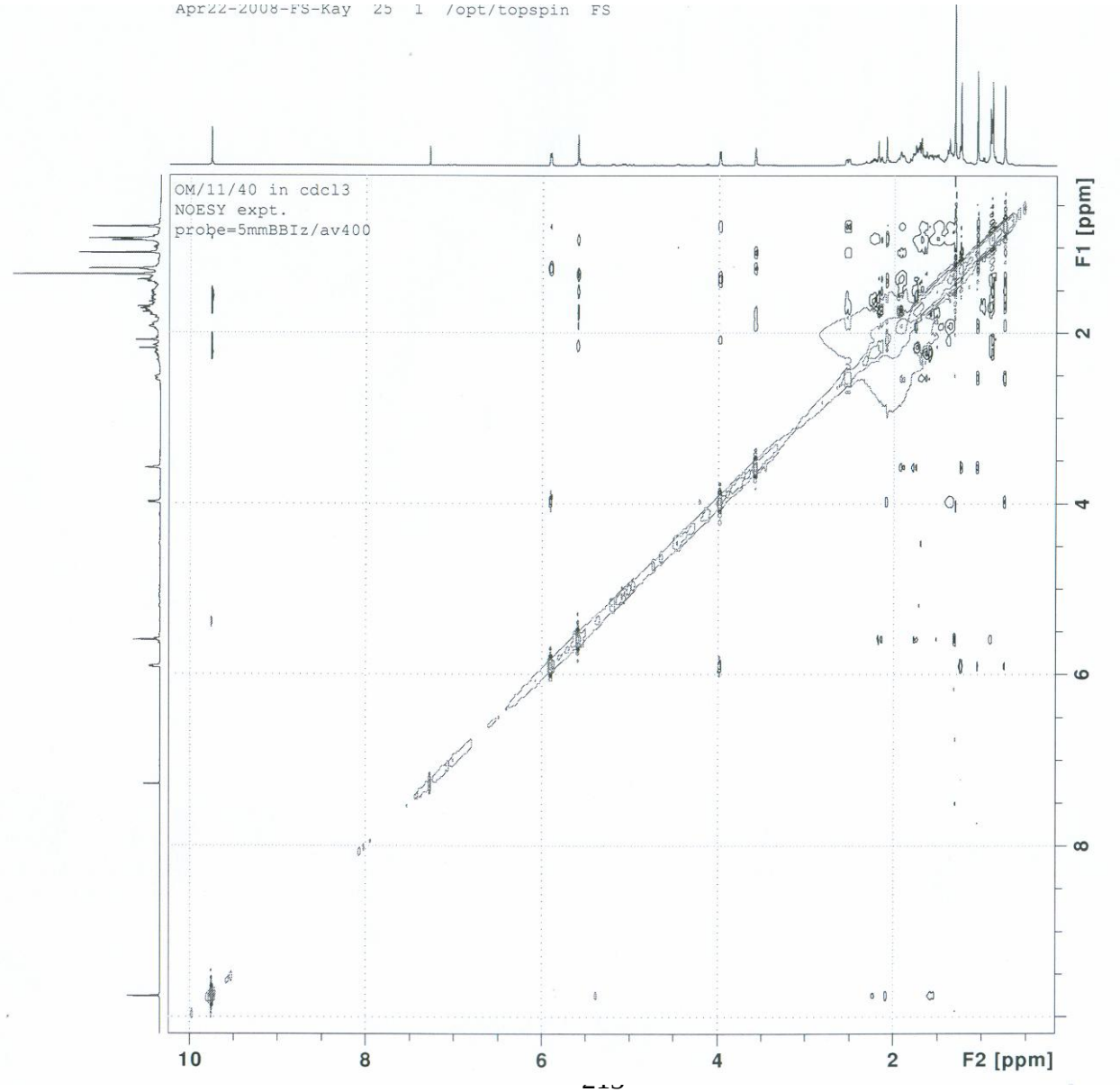
Appendix 2.3: ^{13}C NMR Spectrum of compound OM/11/40



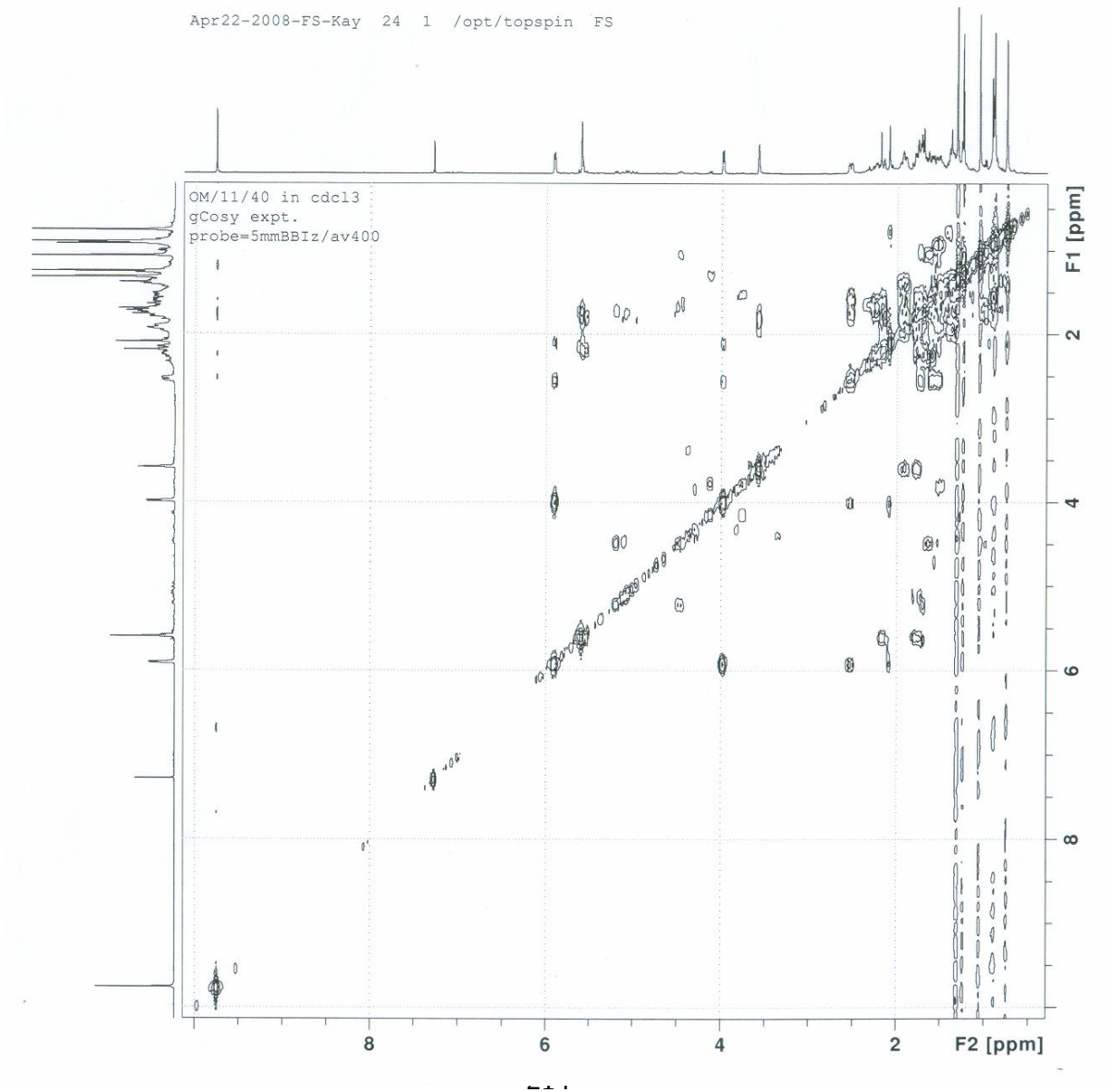
Appendix 2.4: DEPT NMR Spectrum of compound OM/11/40



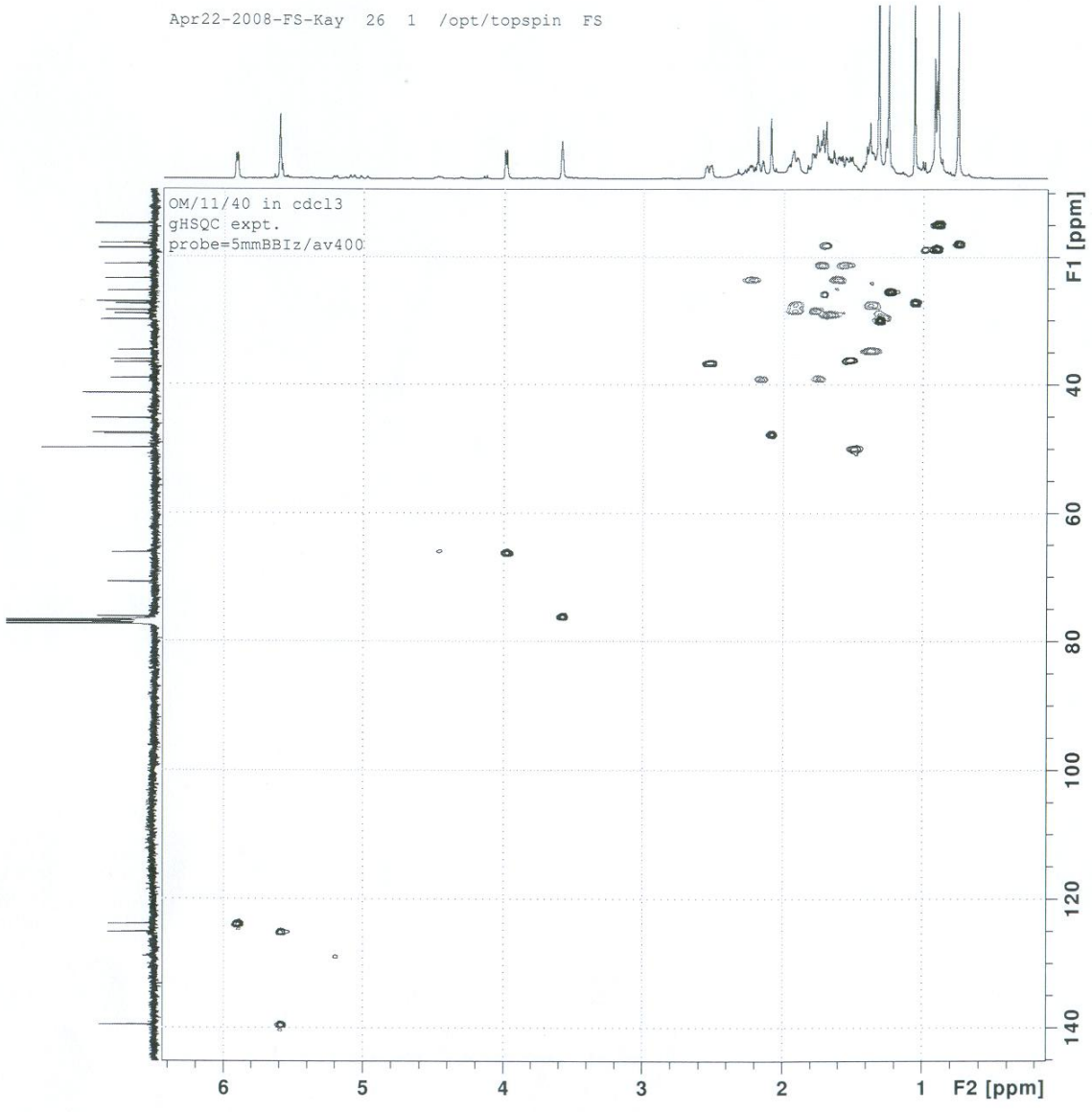
Appendix 2.5: NOSY NMR Spectrum of compound OM/11/40



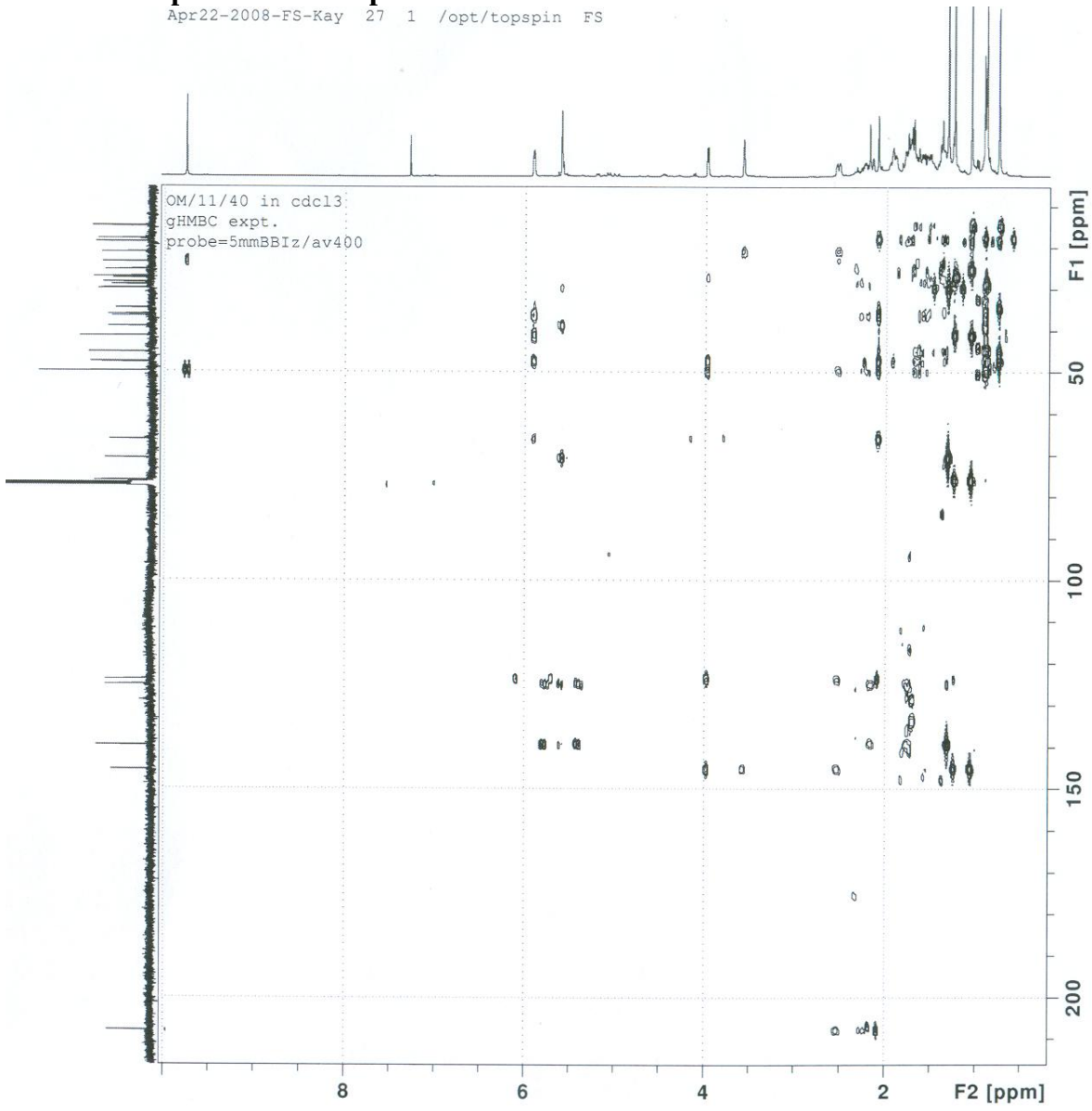
Appendix 2.6: gCOSY NMR Spectrum of compound OM/11/40



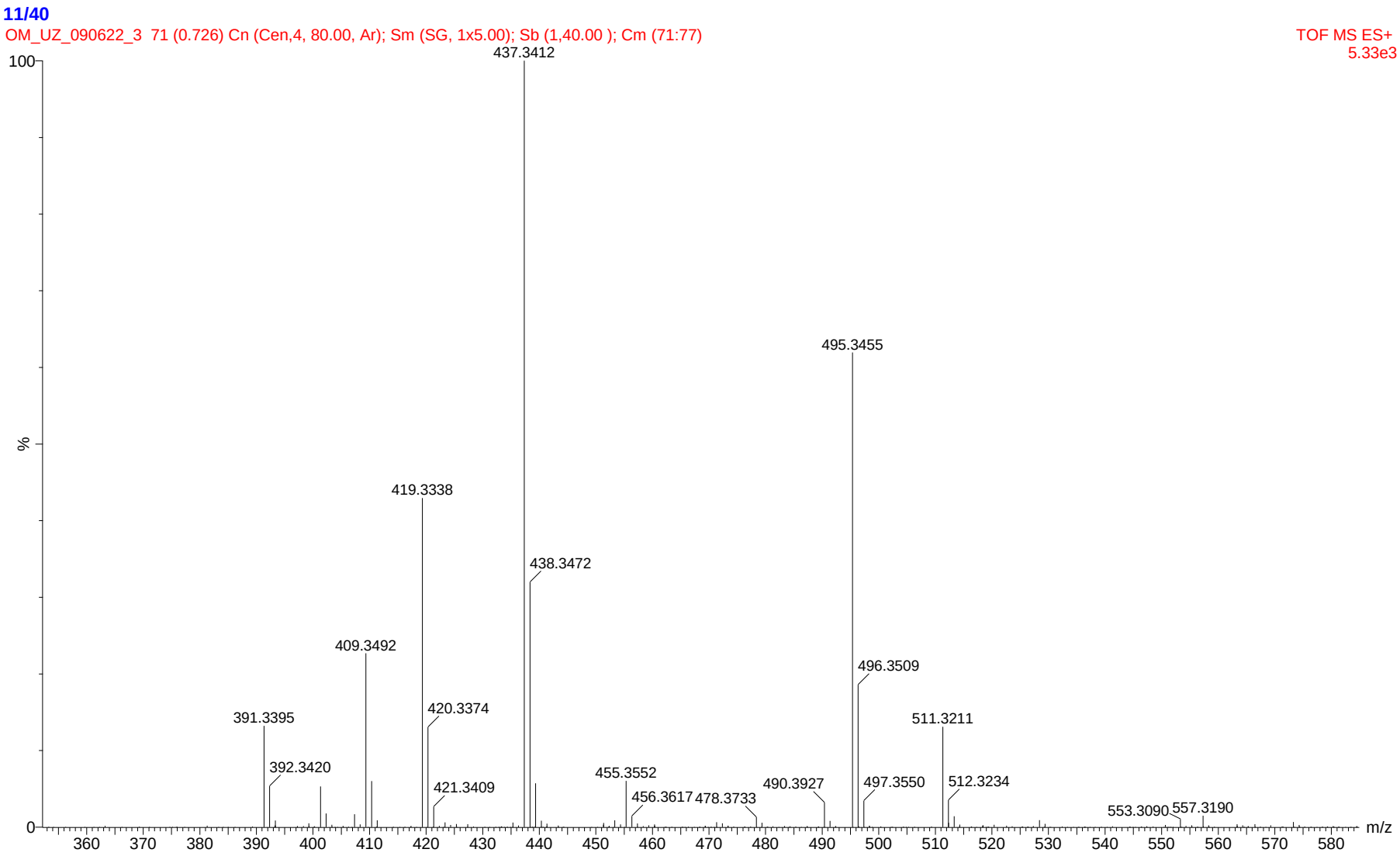
Appendix 2.7: HSQC NMR Spectrum of compound OM/11/40



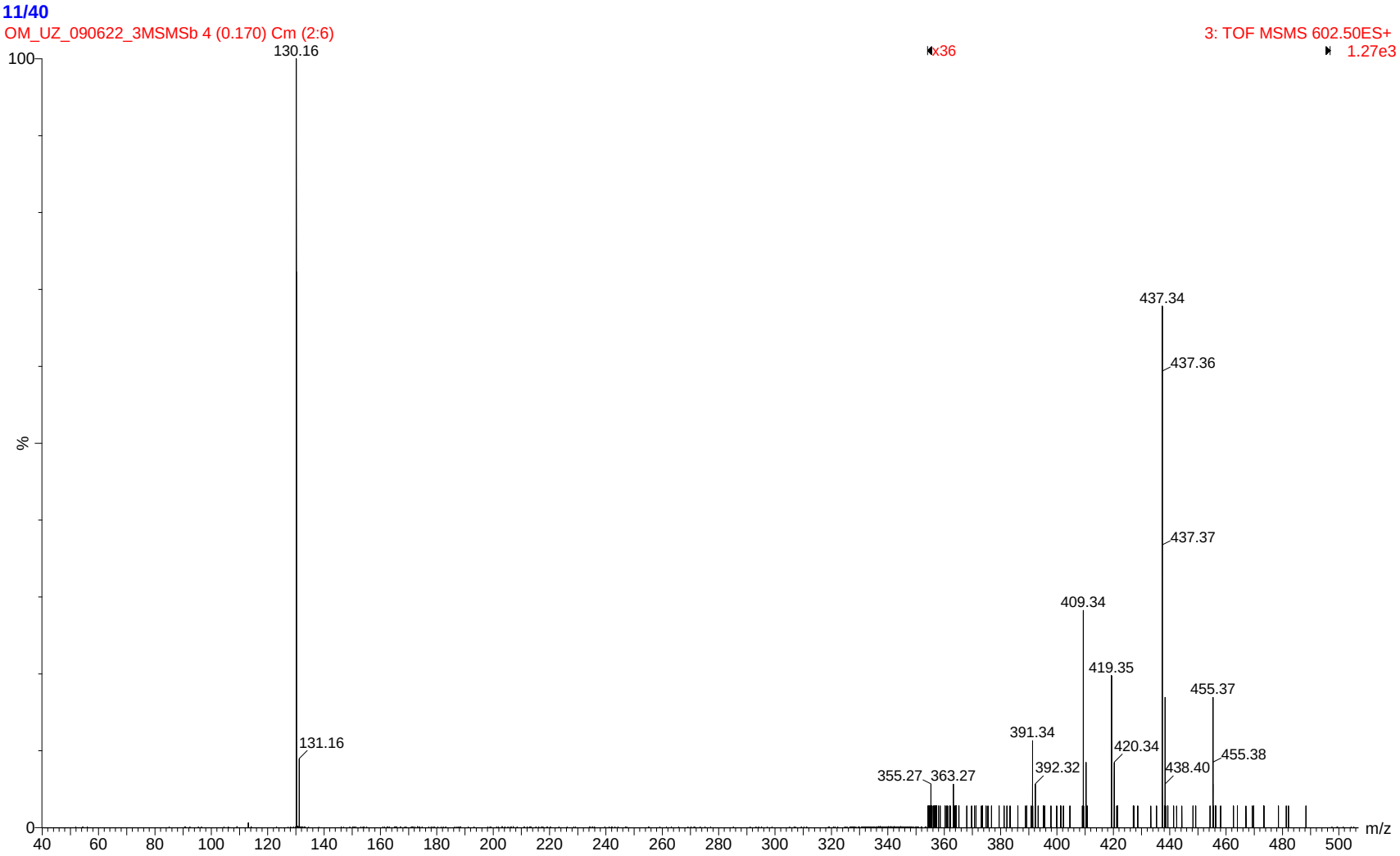
Appendix 2.8: HMBC NMR Spectrum of compound OM/11/40



Appendix 2.9a: MS Spectrum of compound OM/11/40

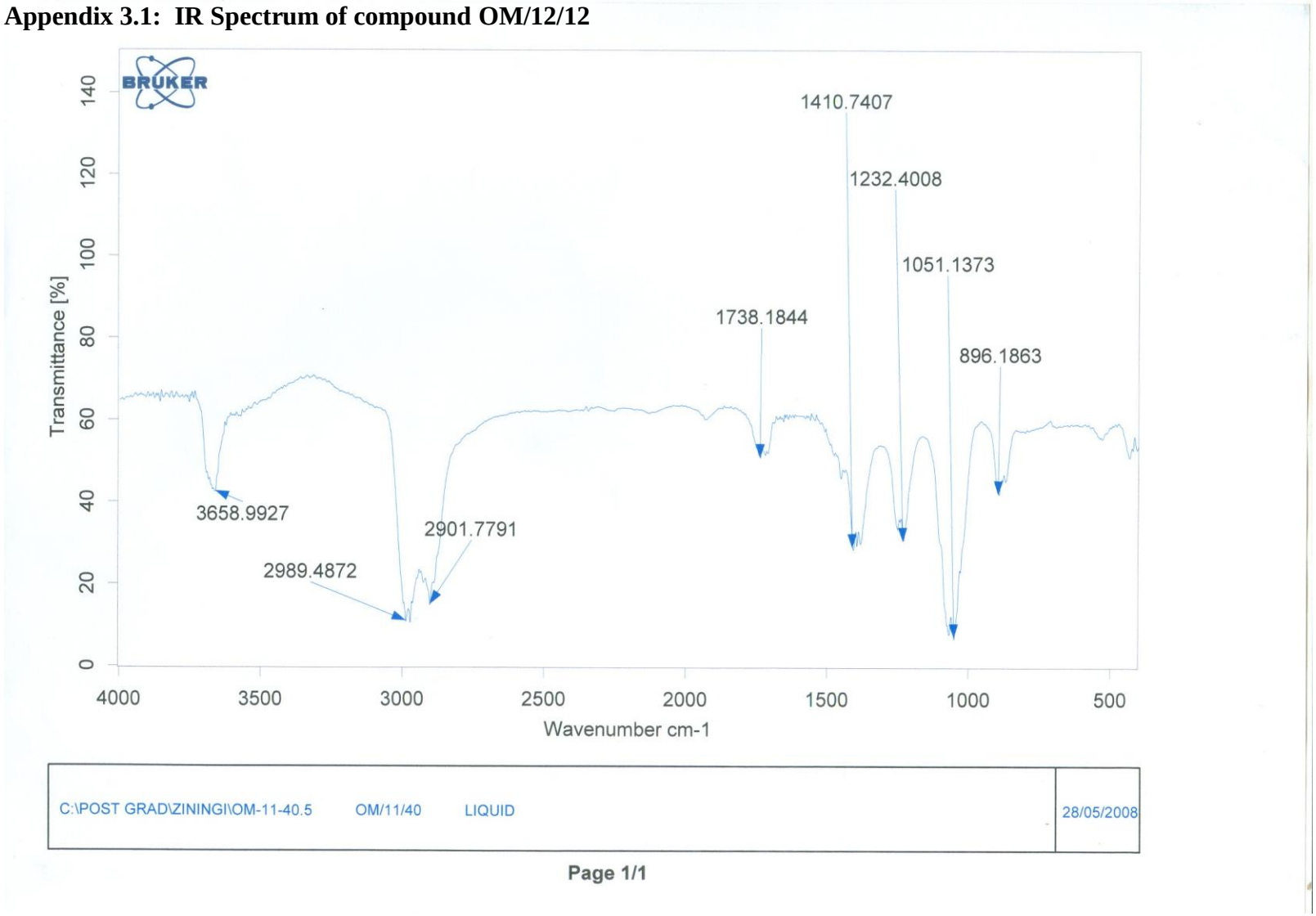


Appendix 2.9b: MS/MS Spectrum of compound OM/11/40

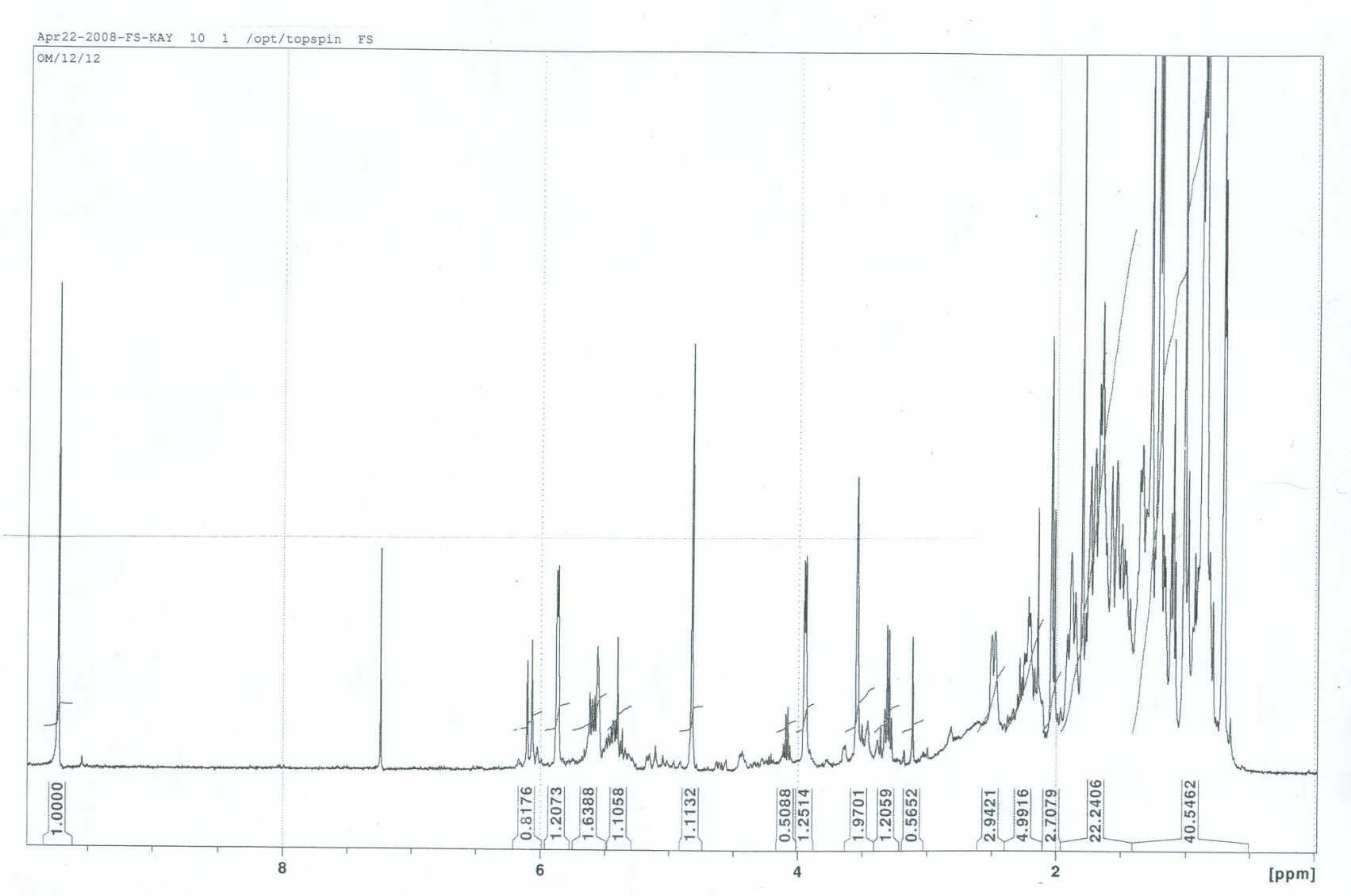


Appendix 3.0: Spectral for compound OM/12/12

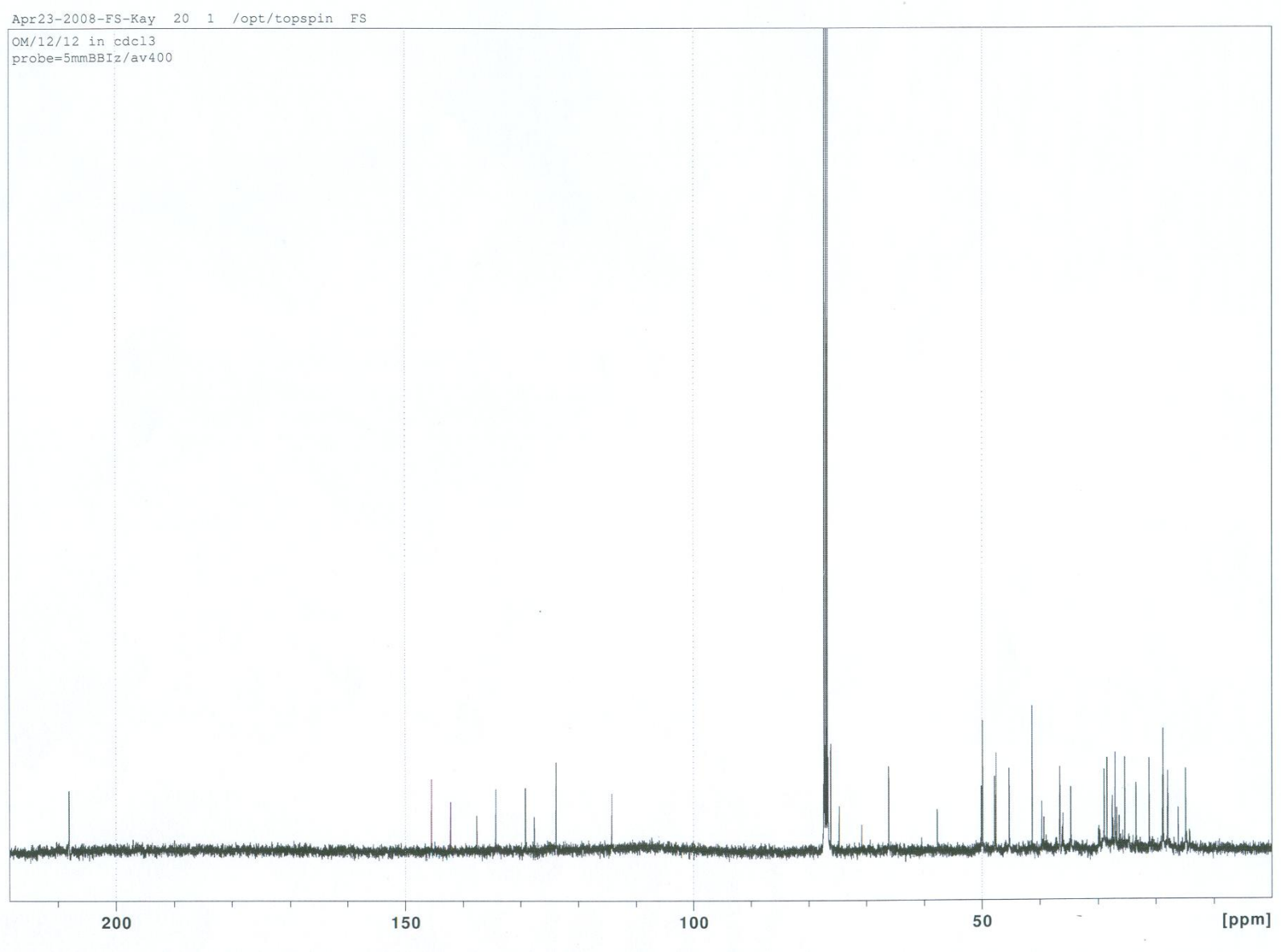
Appendix 3.1: IR Spectrum of compound OM/12/12



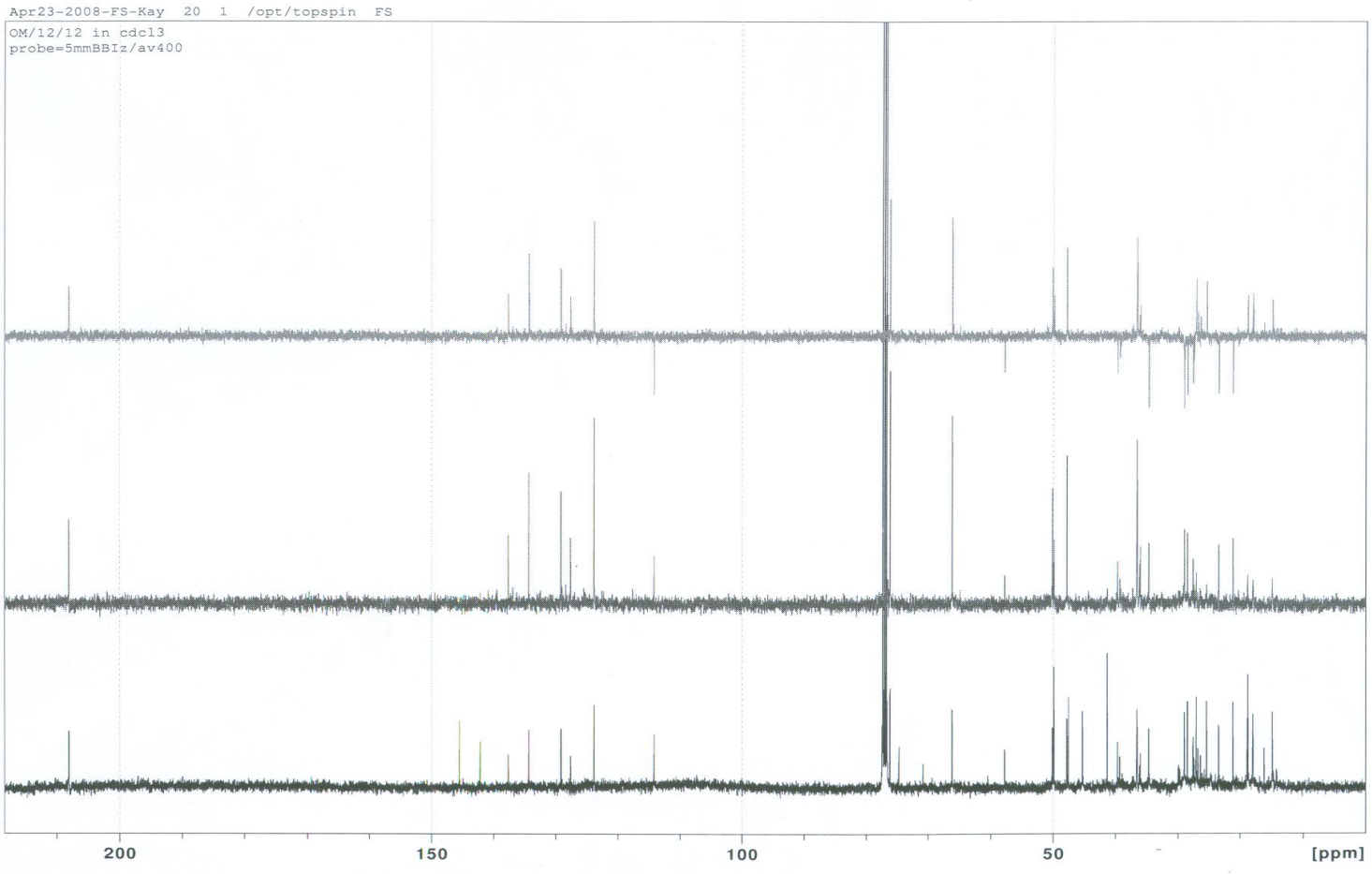
Appendix 3.2: ^{1}H NMR Spectrum of compound OM/12/12



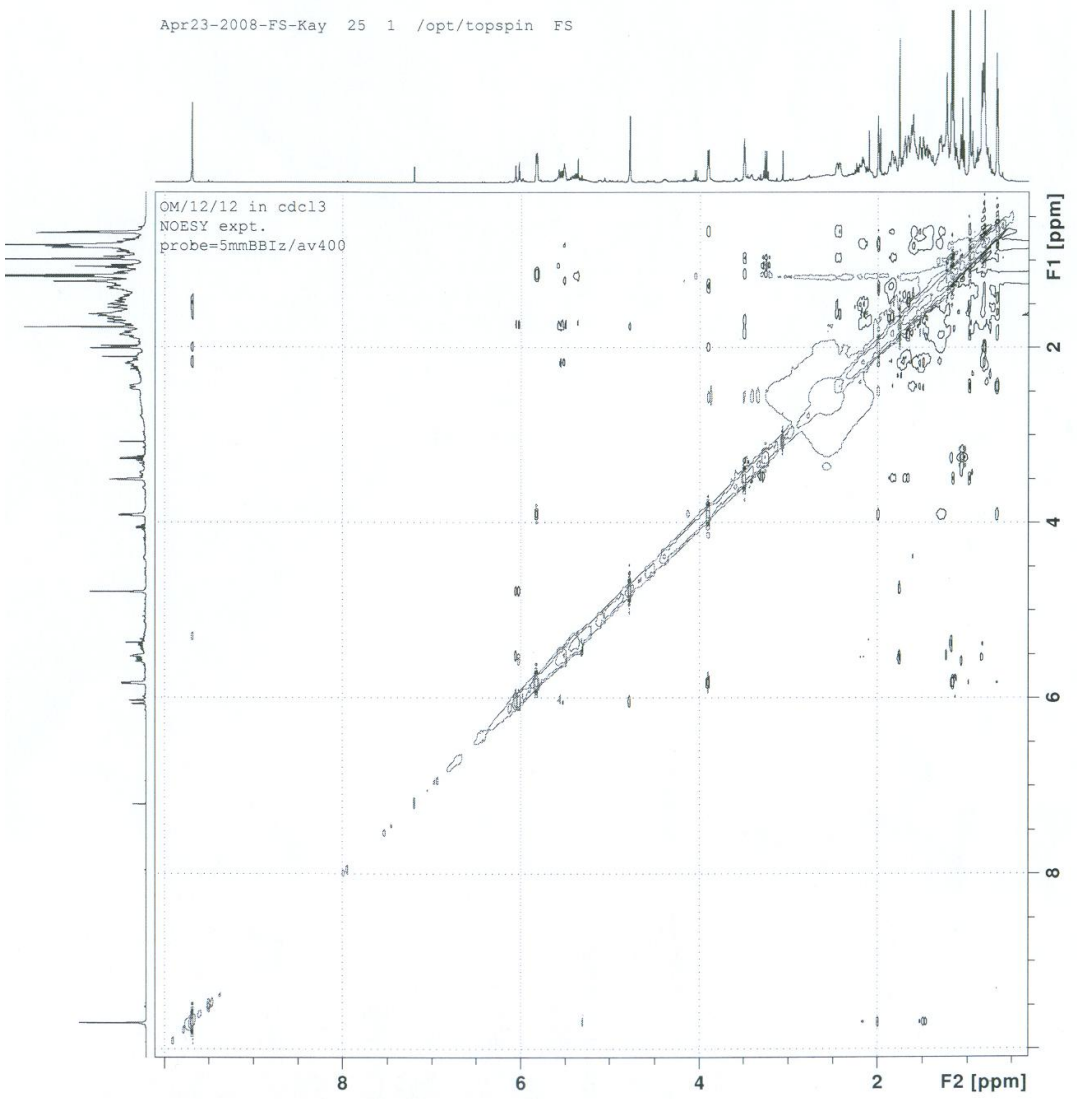
Appendix 3.3: ^{13}C NMR Spectrum of compound OM/12/12



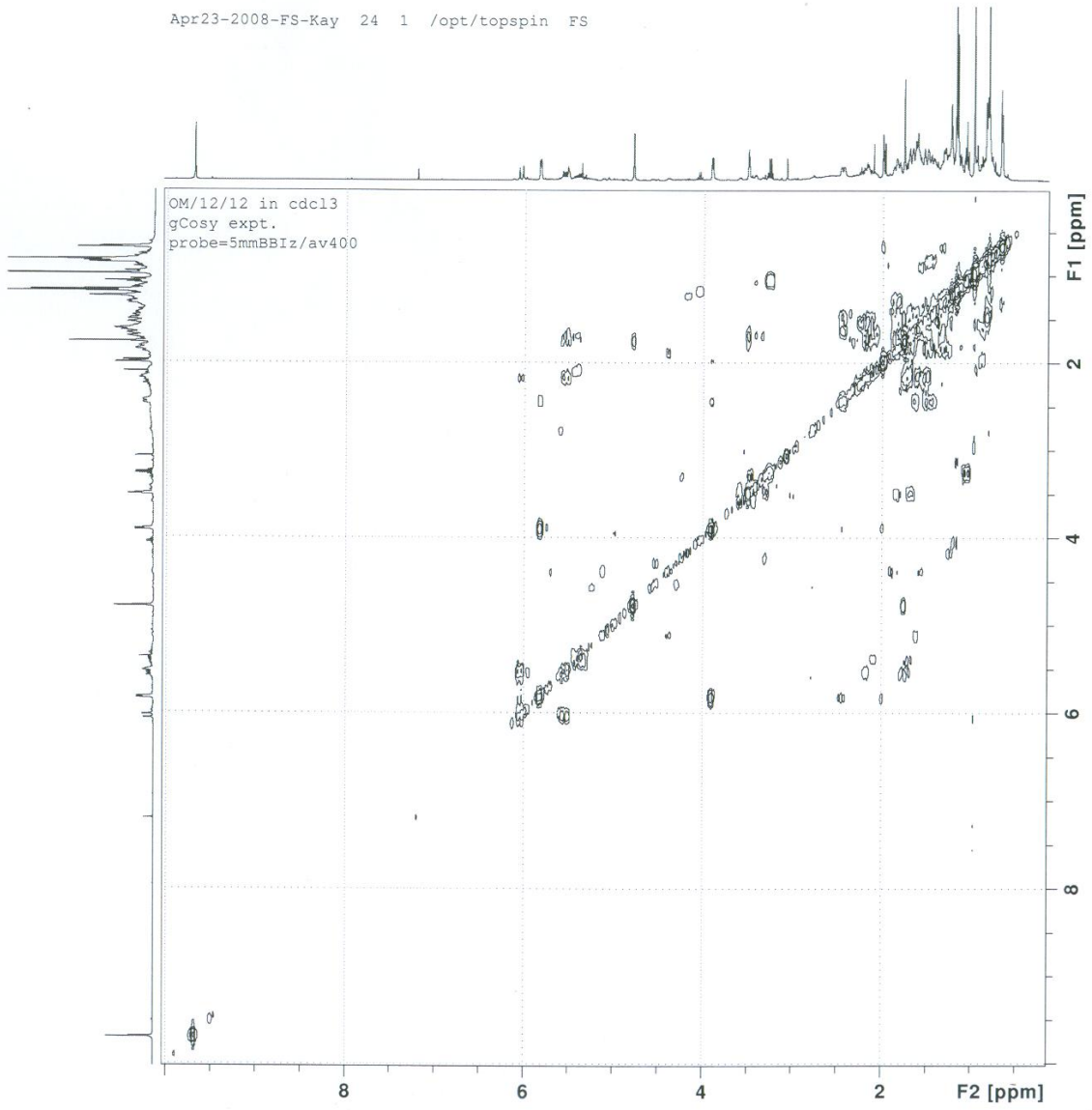
Appendix 3.4: DEPT NMR Spectrum of compound OM/12/12



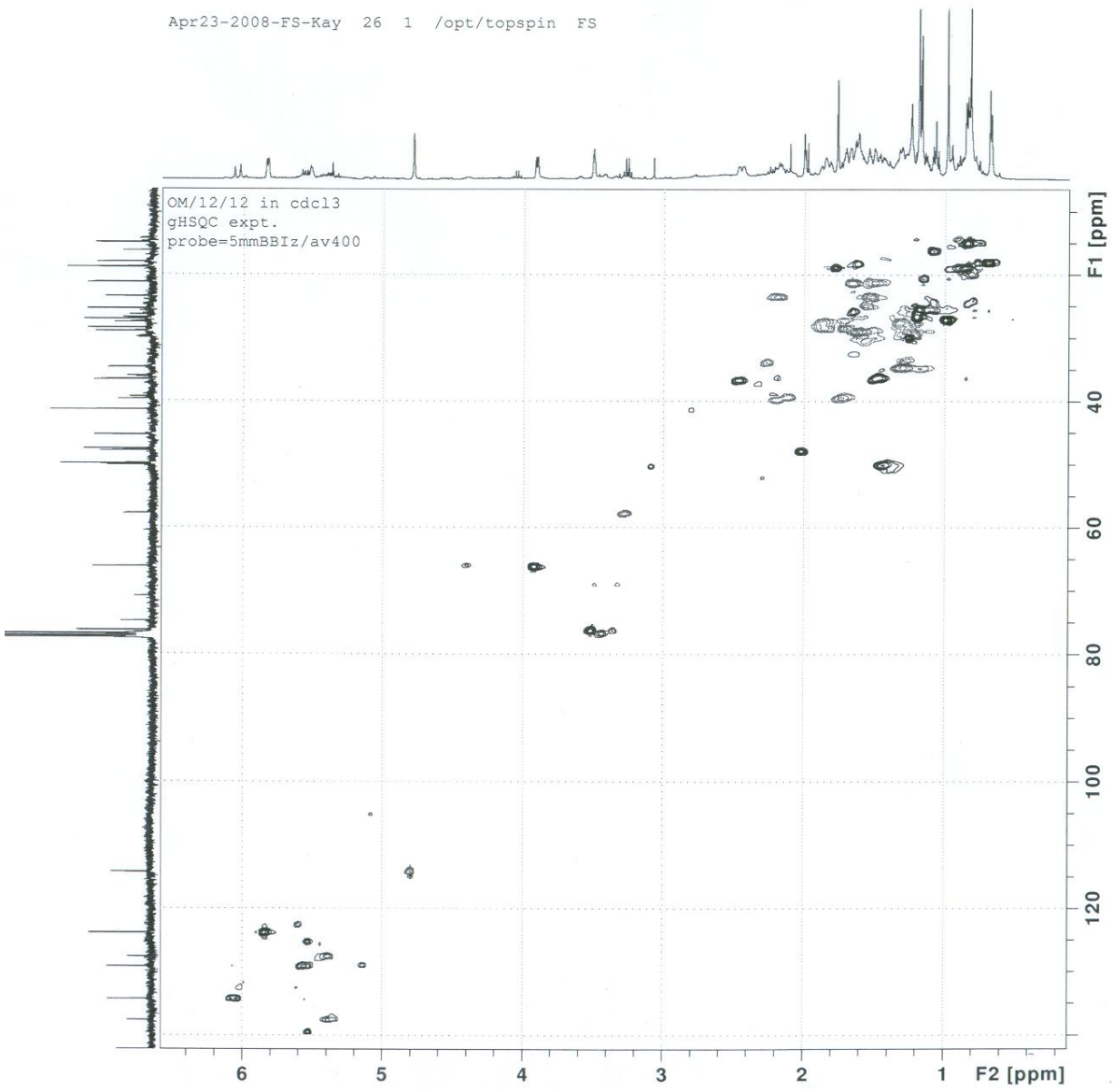
Appendix 3.5: NOSY NMR Spectrum of compound OM/12/12



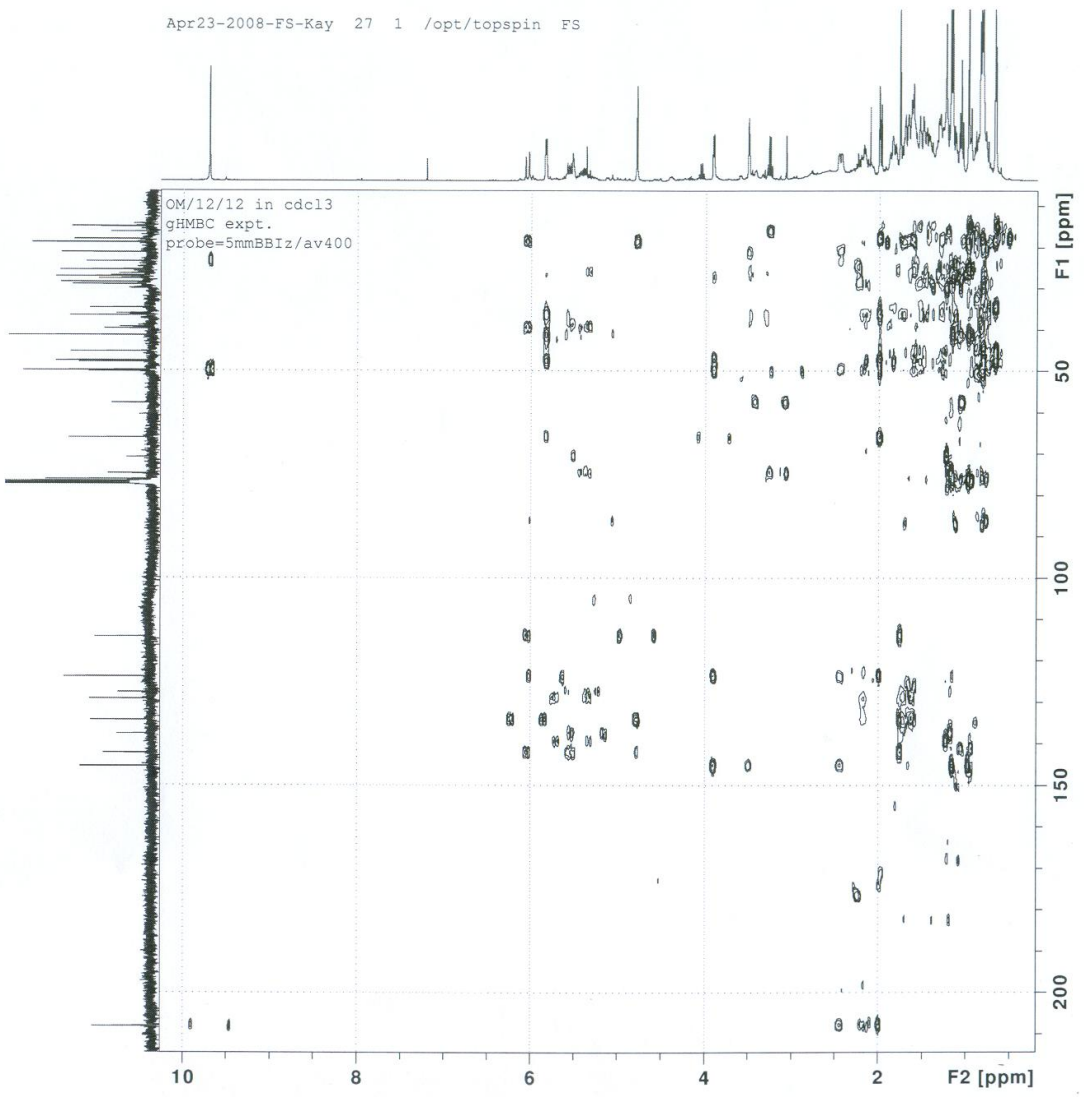
Appendix 3.6: gCOSY NMR Spectrum of compound OM/12/12



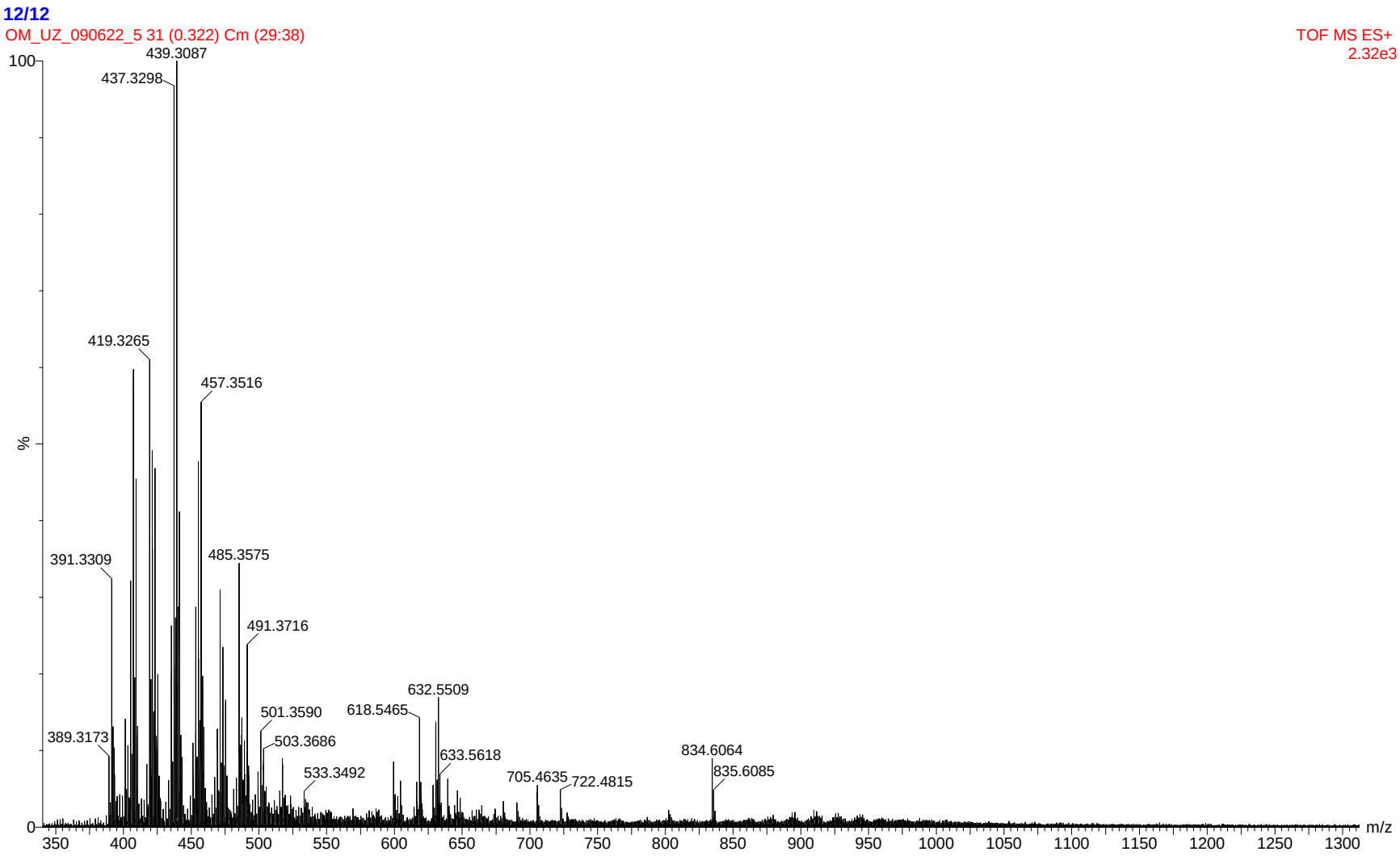
Appendix 3.7: HSQC NMR Spectrum of compound OM/12/12



Appendix 3.8: HMBC NMR Spectrum of compound OM/12/12

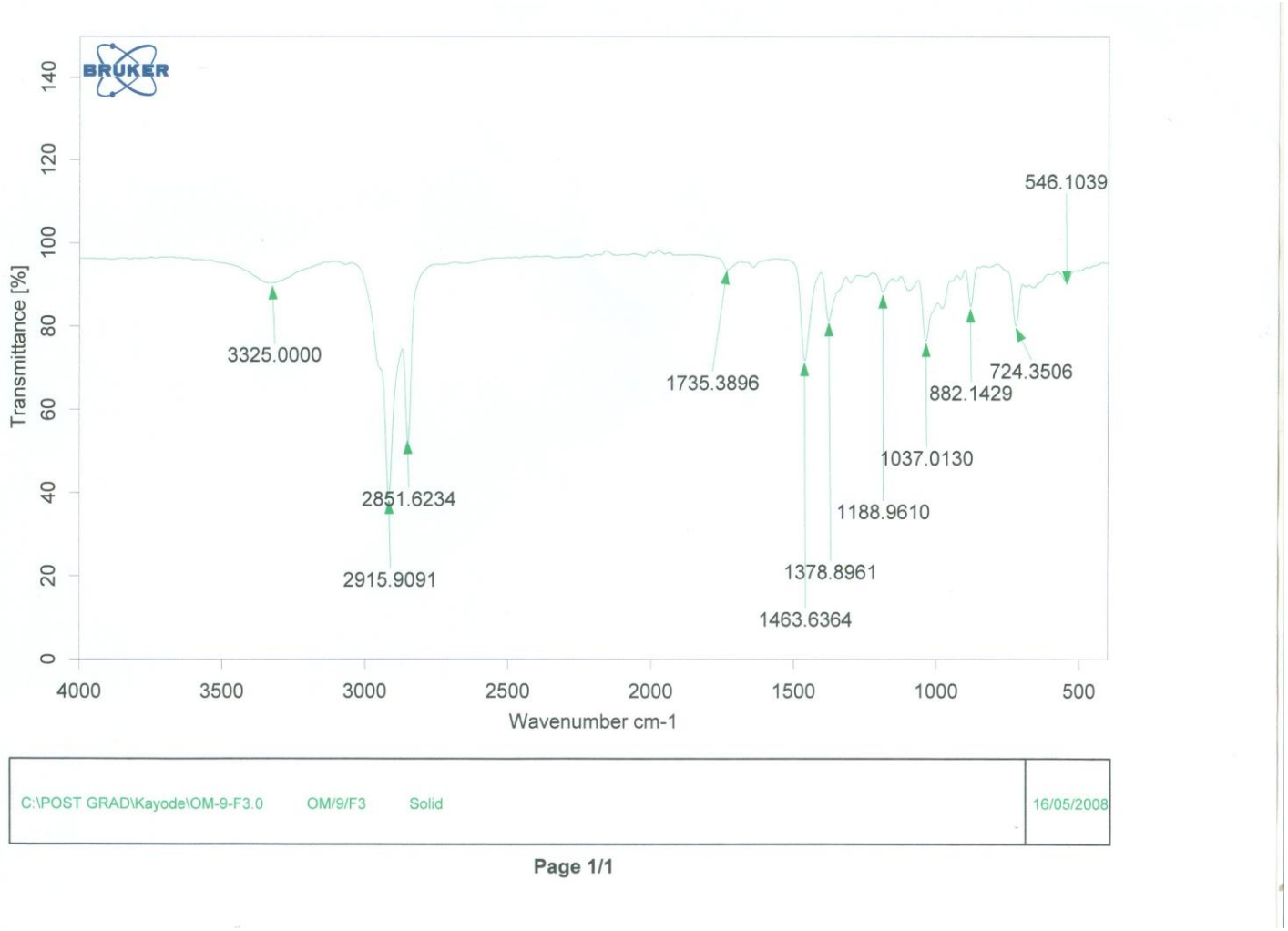


Appendix 3.9: MS Spectrum of compound OM/12/12

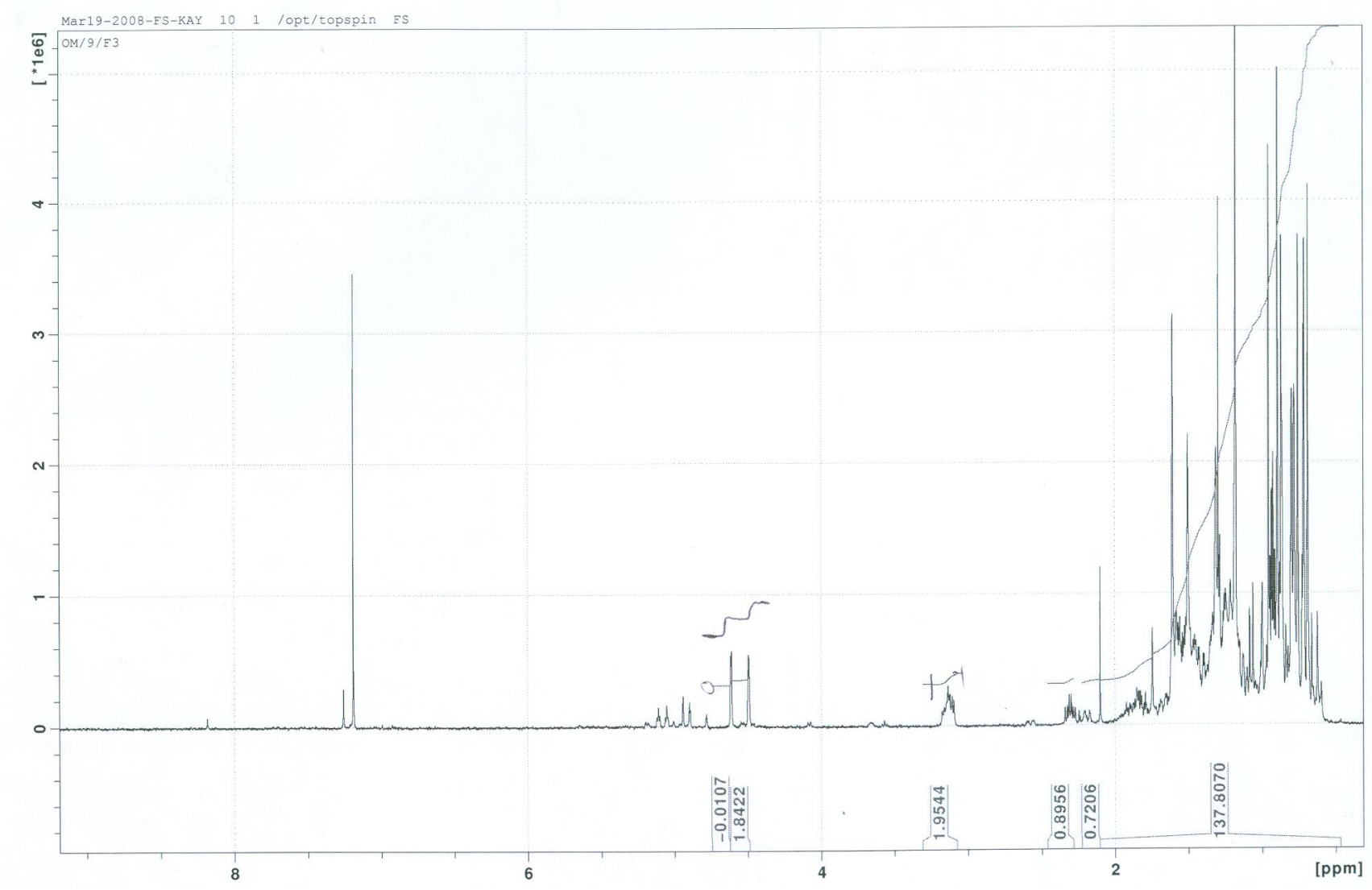


Appendix 4.0: Spectral for compound OM/9/F3

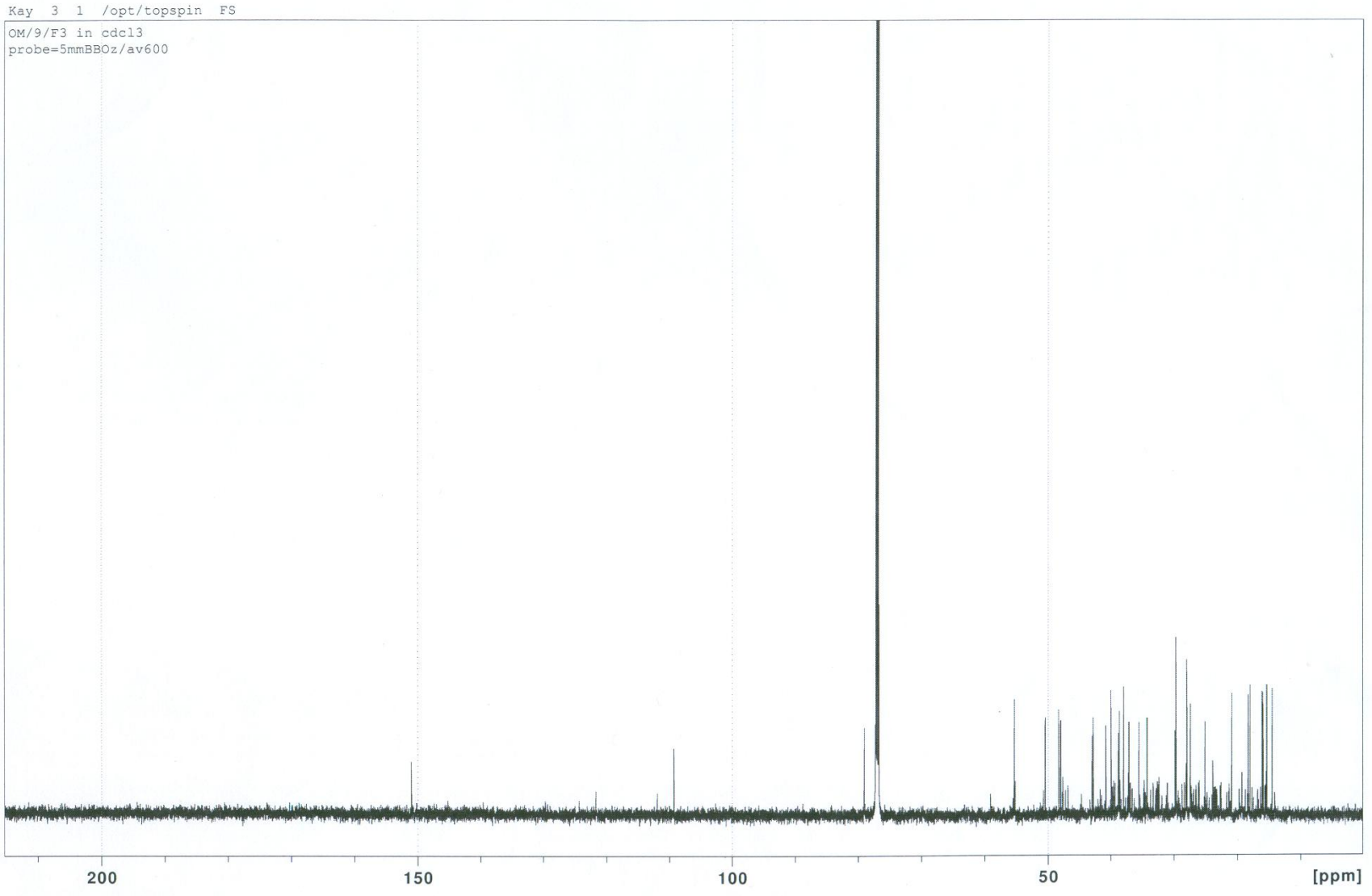
Appendix 4.1: IR Spectrum of compound of OM/9/F3



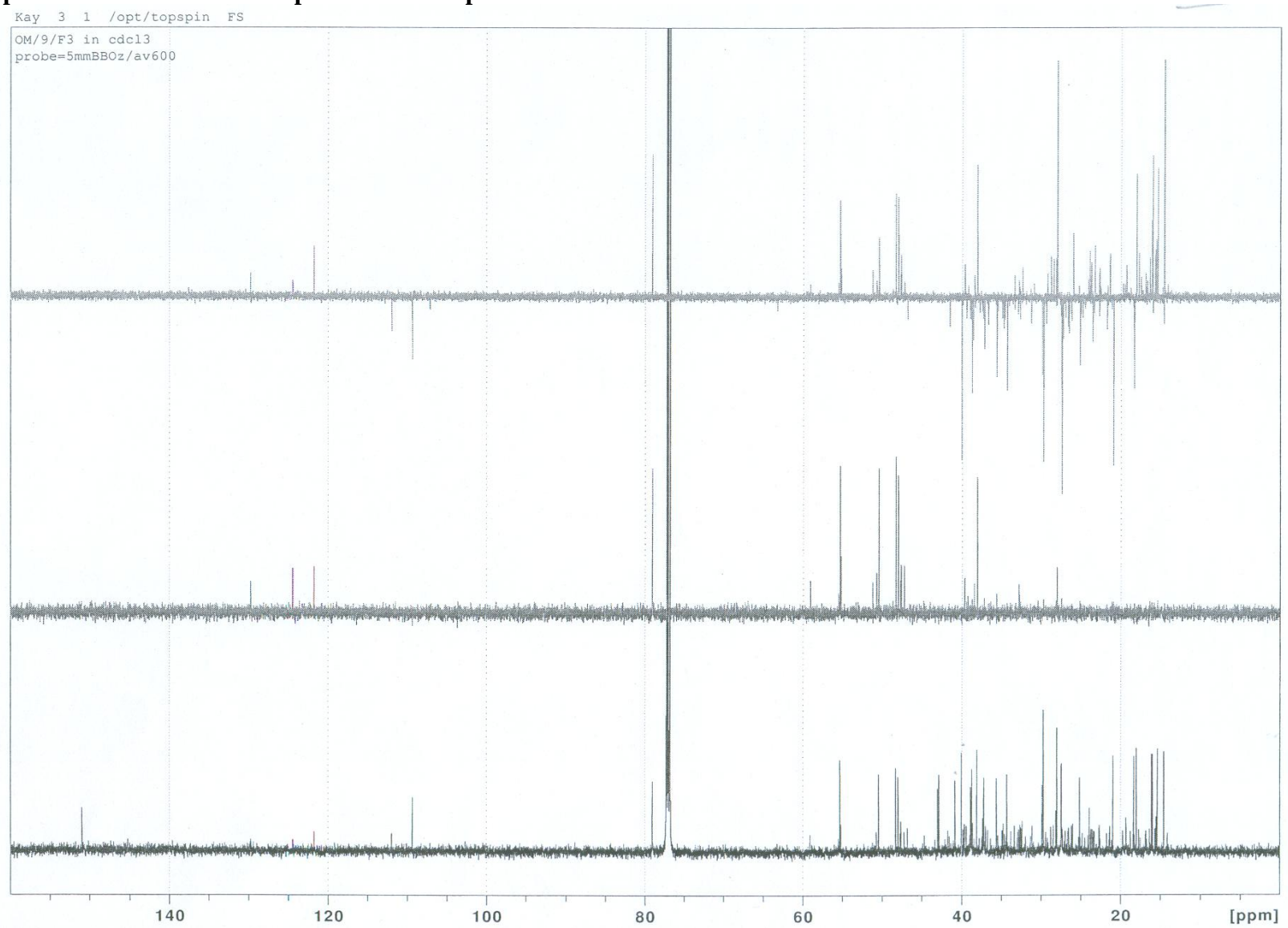
Appendix 4.2: ^{1}H NMR Spectrum of compound OM/9/F3



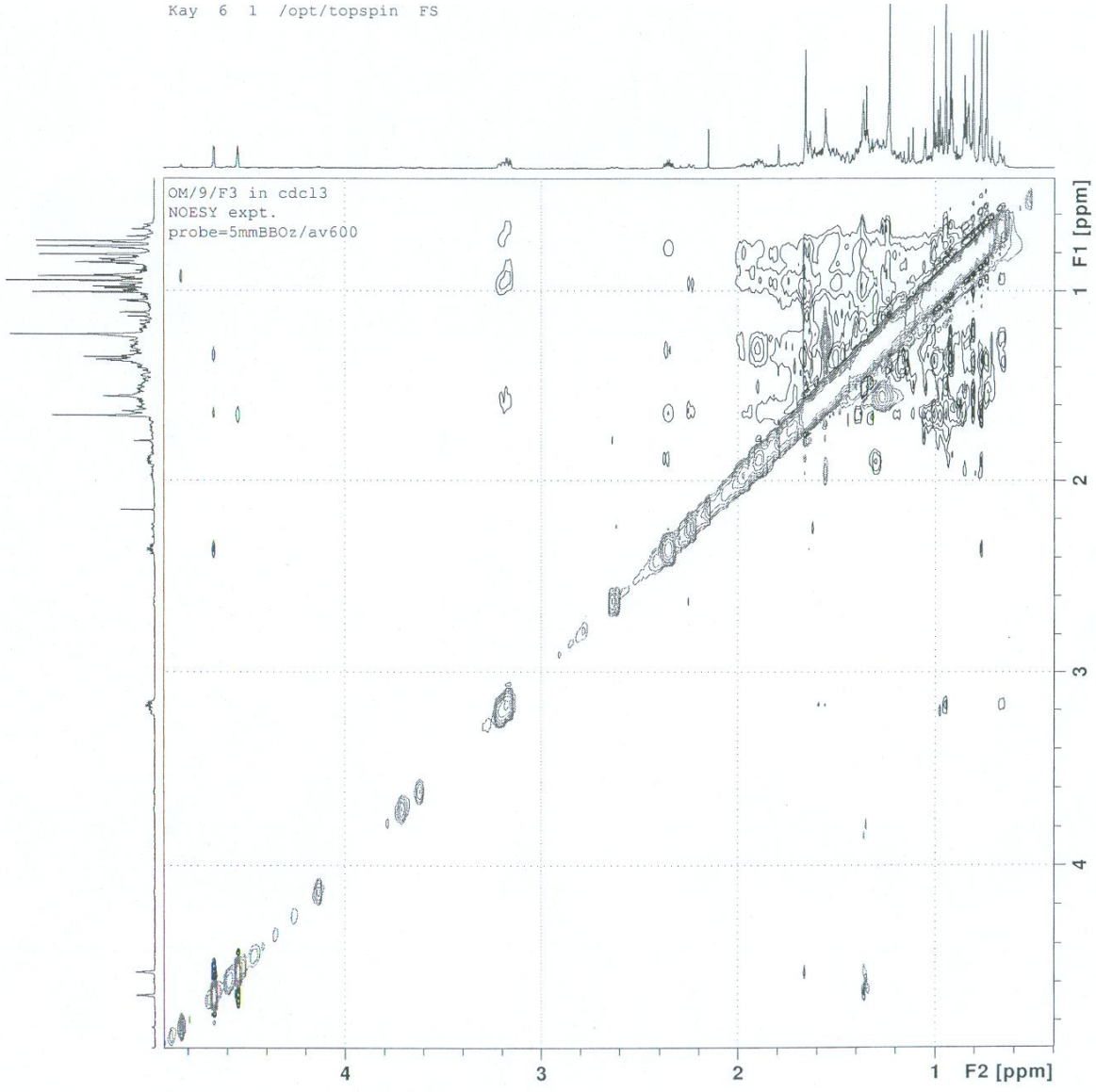
Appendix 4.3: ^{13}C NMR Spectrum of compound OM/9/F3



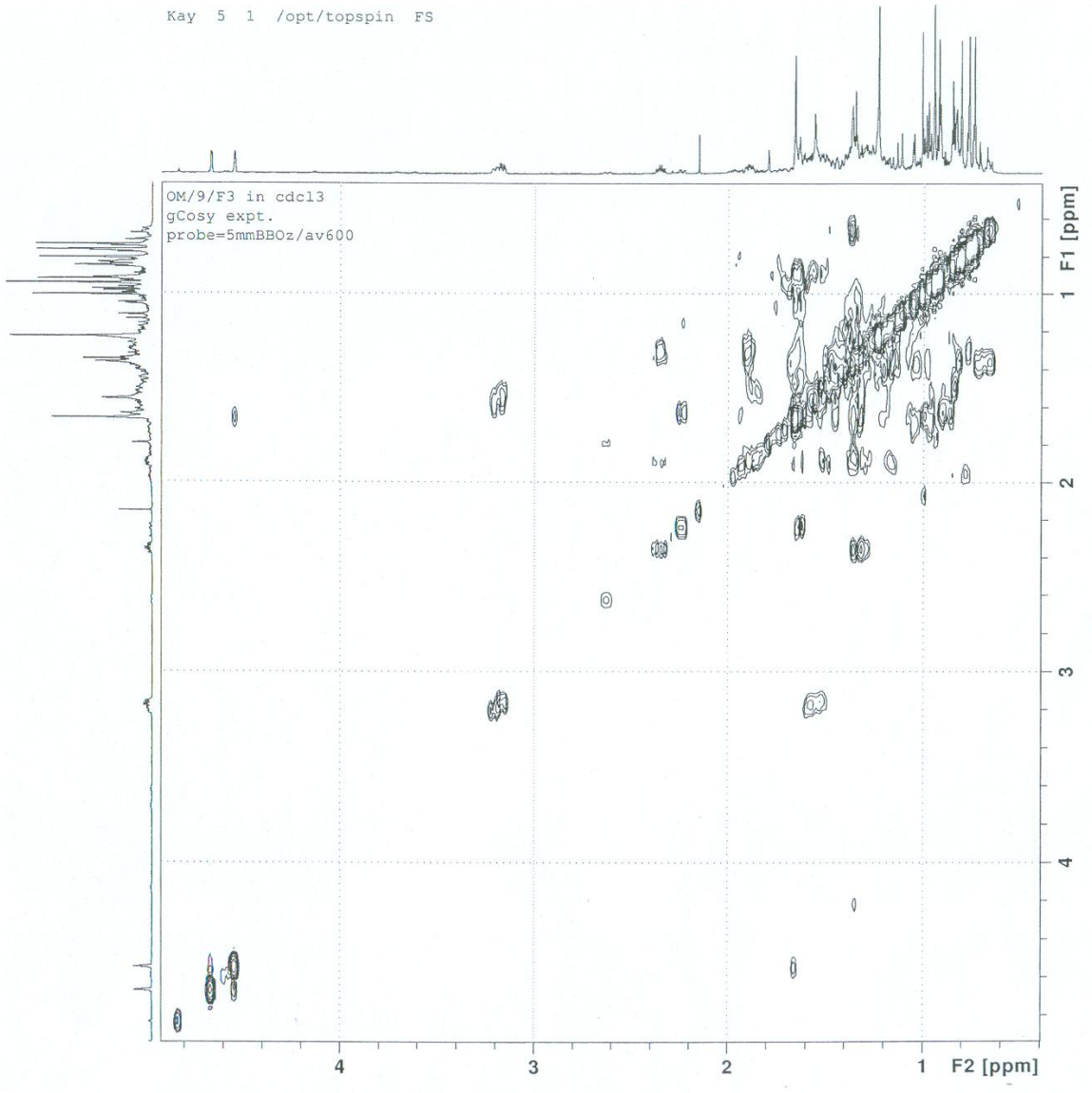
Appendix 4.4: DEPT NMR Spectrum of compound OM/9/F3



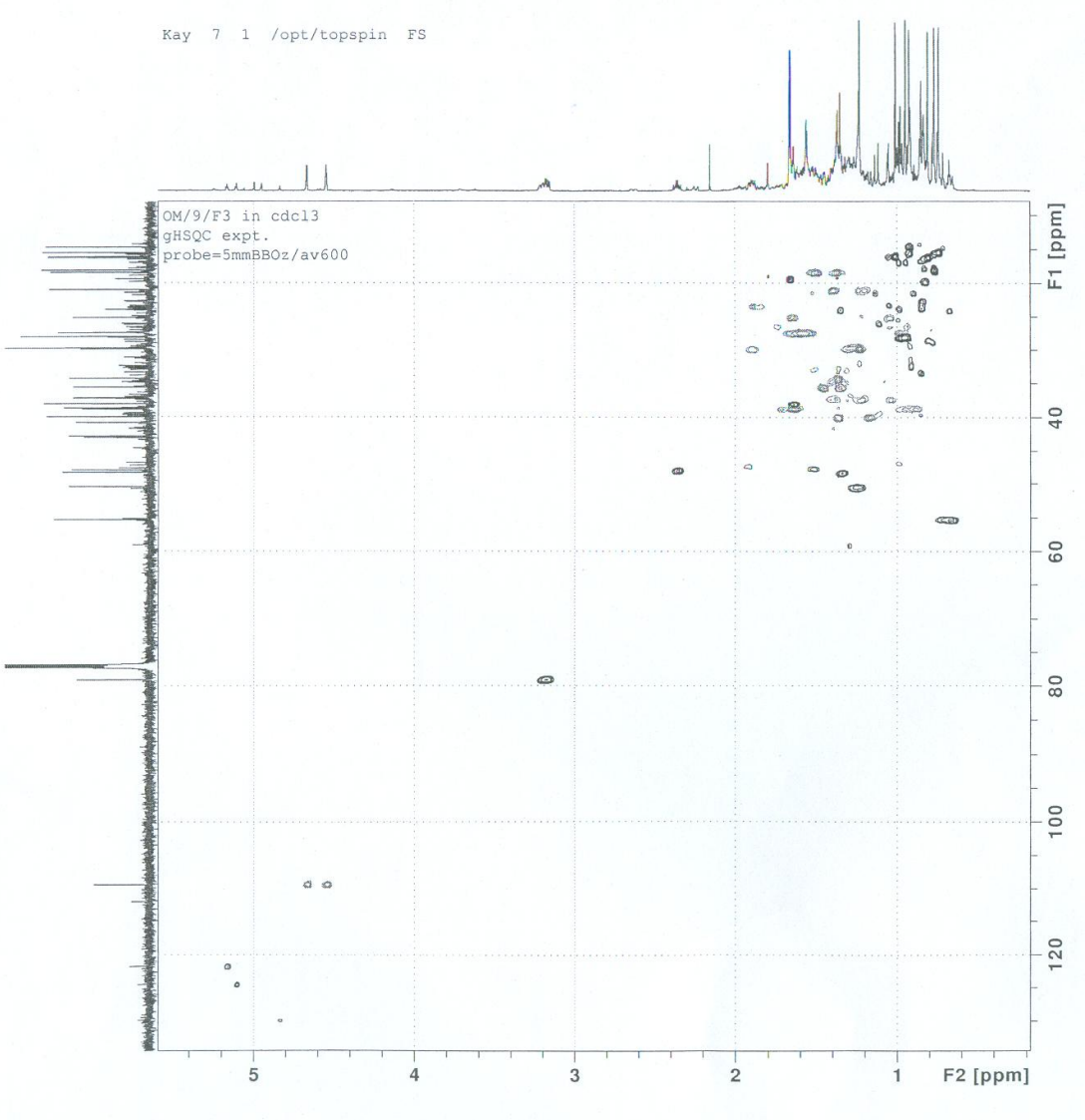
Appendix 4.5: NOSY NMR Spectrum of compound OM/9/F3



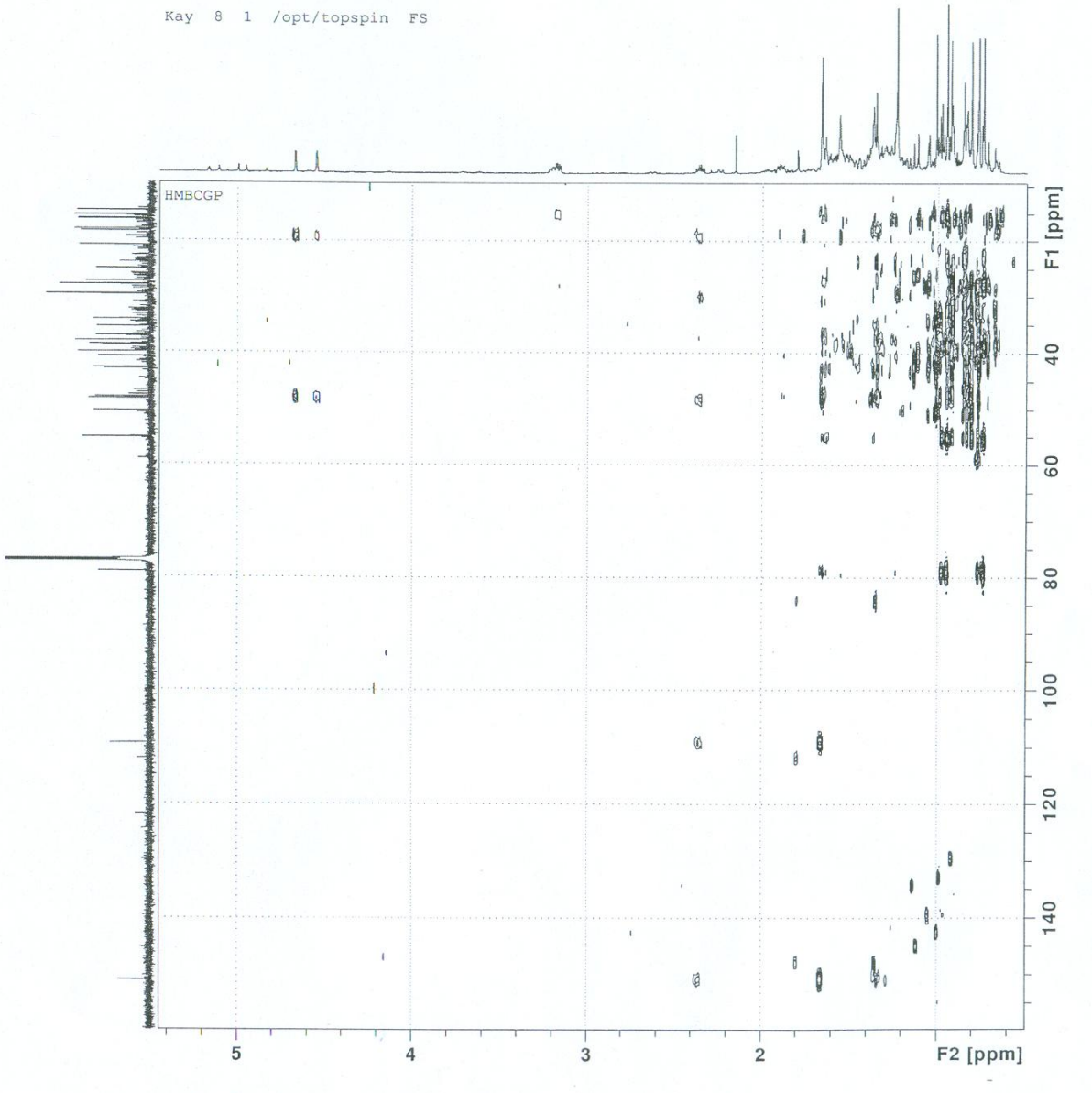
Appendix 4.6: gCOSY NMR Spectrum of compound OM/9/F3



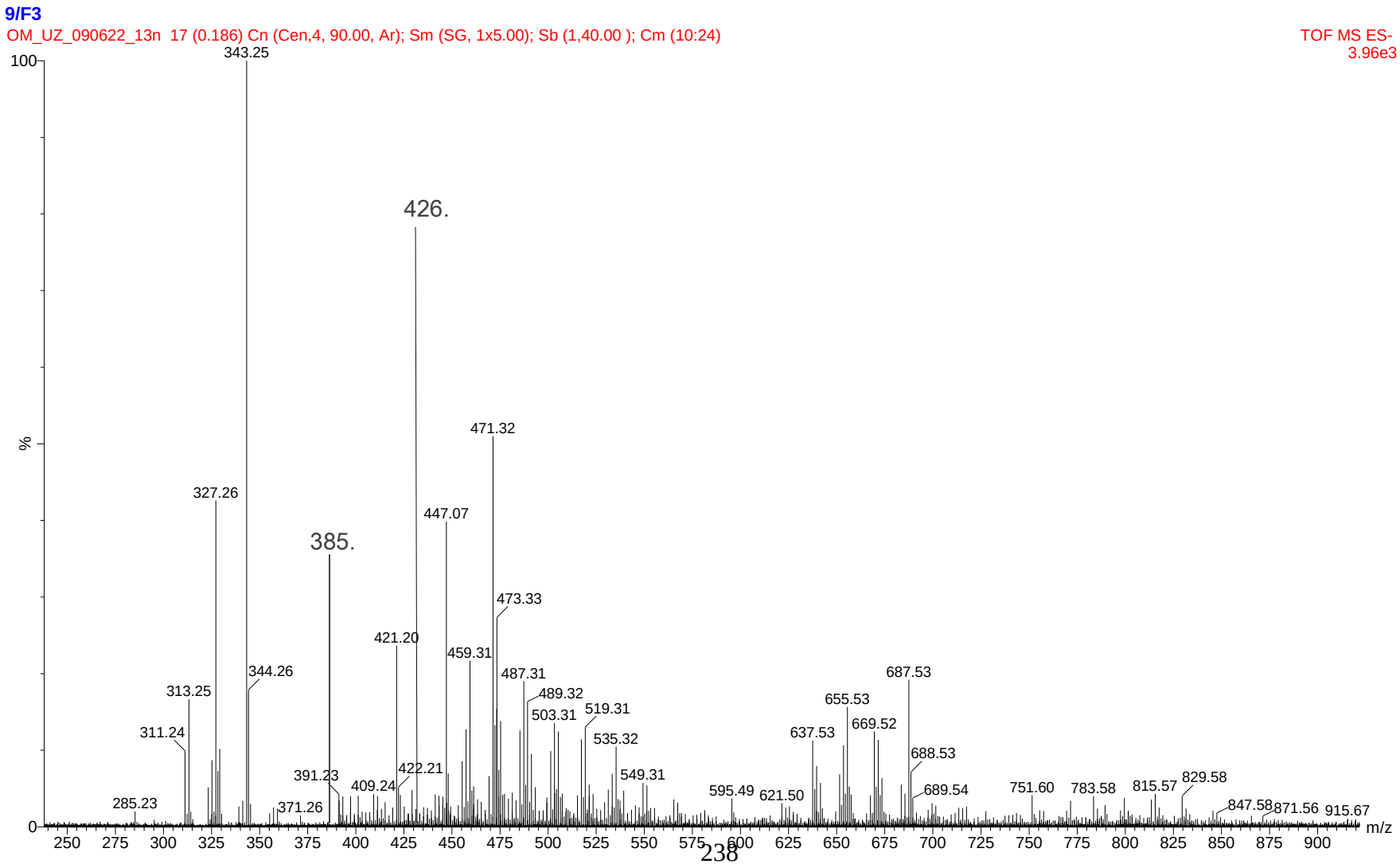
Appendix 4.7: HSQC NMR Spectrum of compound of OM/9/F3



Appendix 4.8: HMBC NMR Spectrum of compound of OM/9/F3

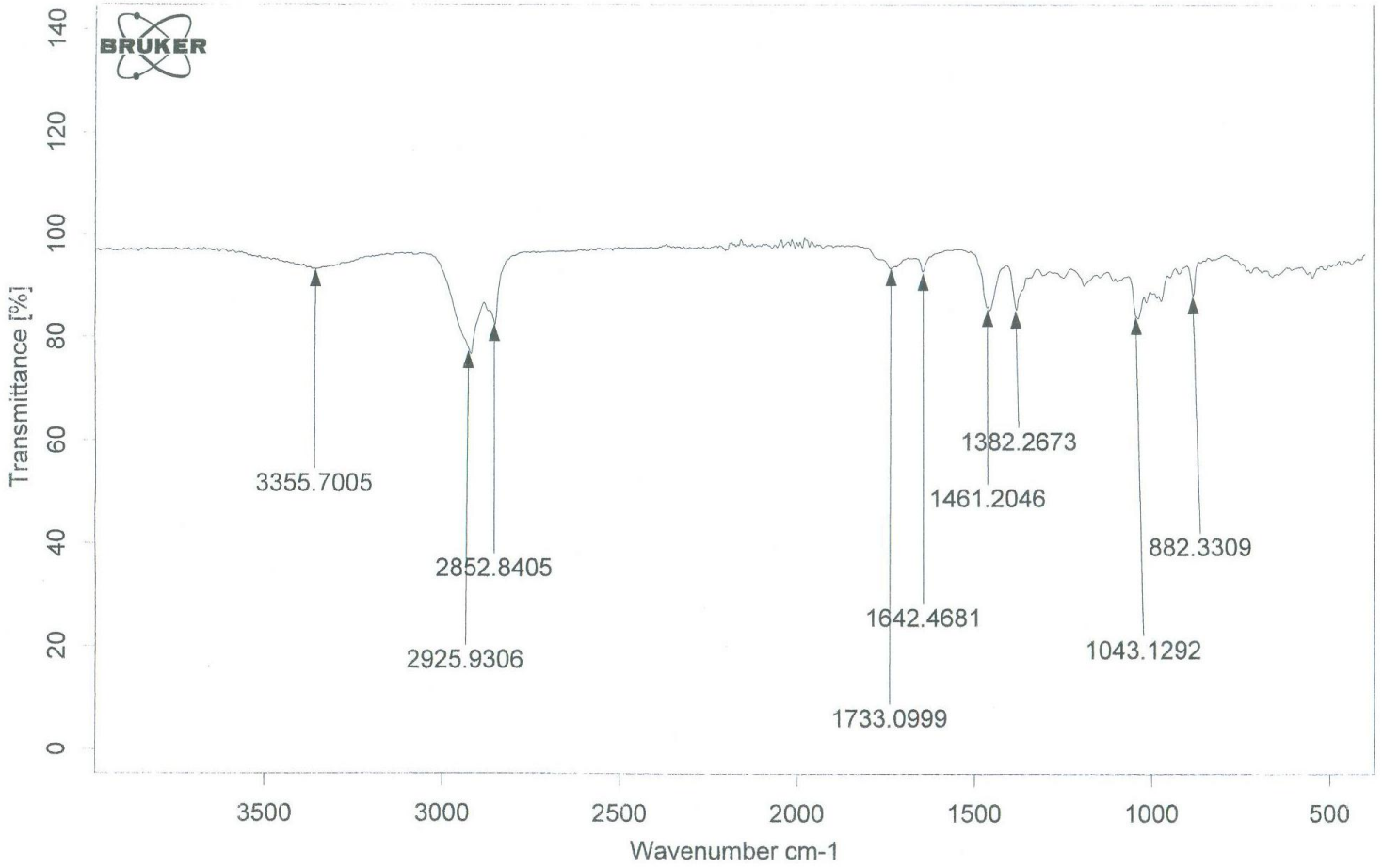


Appendix 4.9: MS Spectrum of compound OM/9/F9



Appendix 5.0: Spectral for compound OM/10/F1

Appendix 5.1: IR Spectrum of compound OM/10/F1



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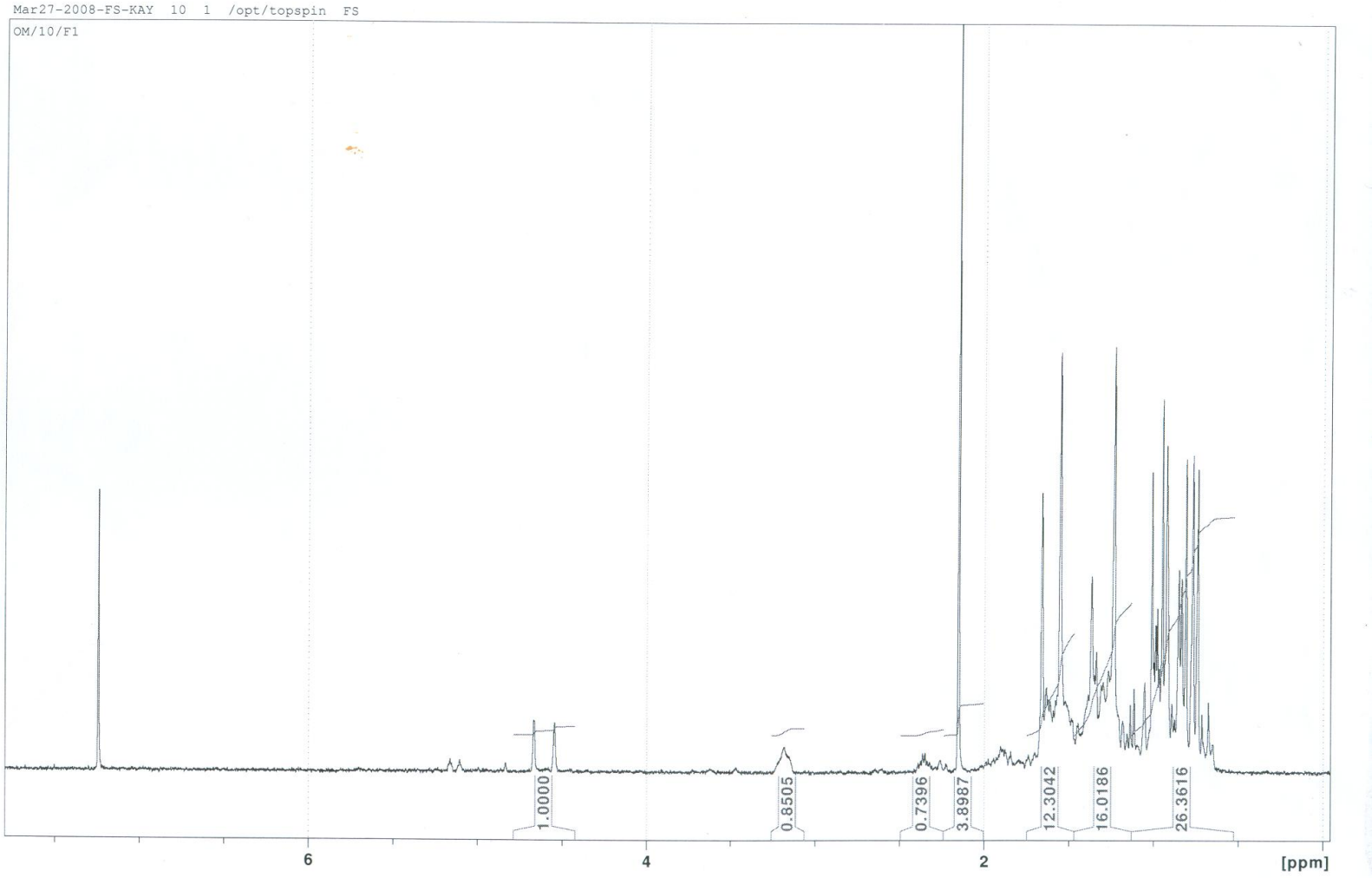
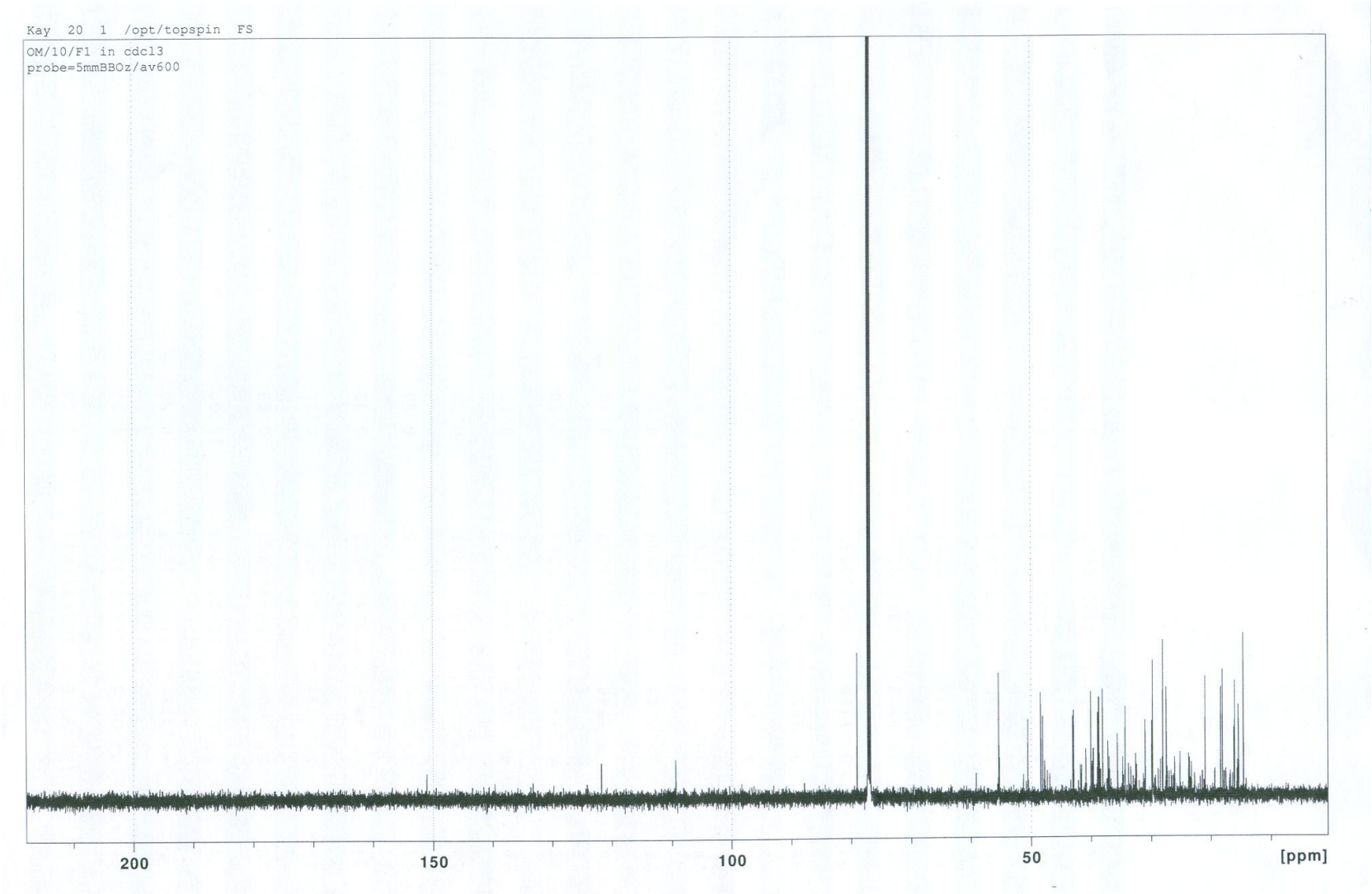
Appendix 5.2: ^{1}H NMR Spectrum of compound of OM/10/F1

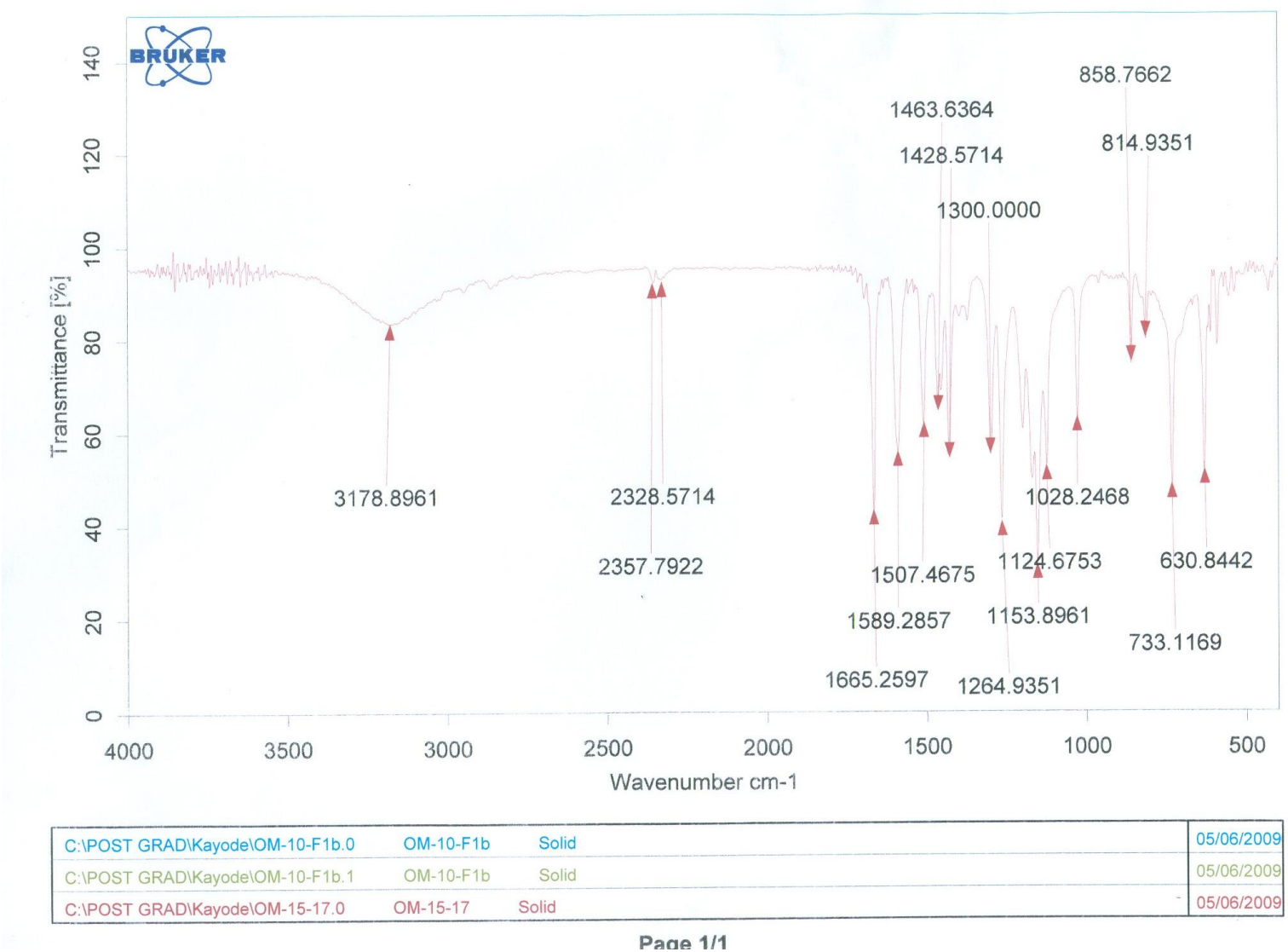


Appendix 5.2: ^{13}C NMR Spectrum of compound of OM/10/F1

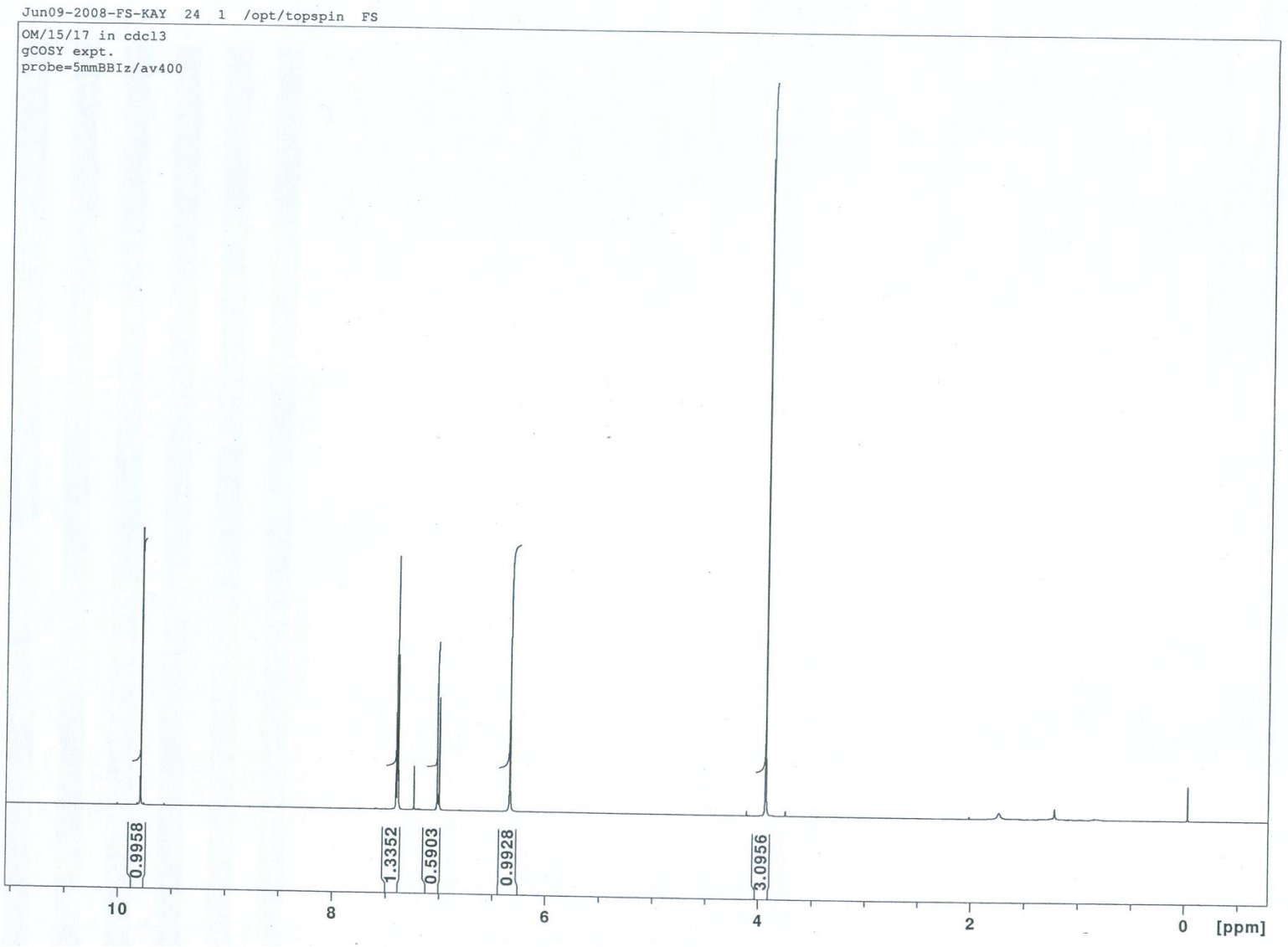


Appendix 6.0: Spectral for compound OM/15/17

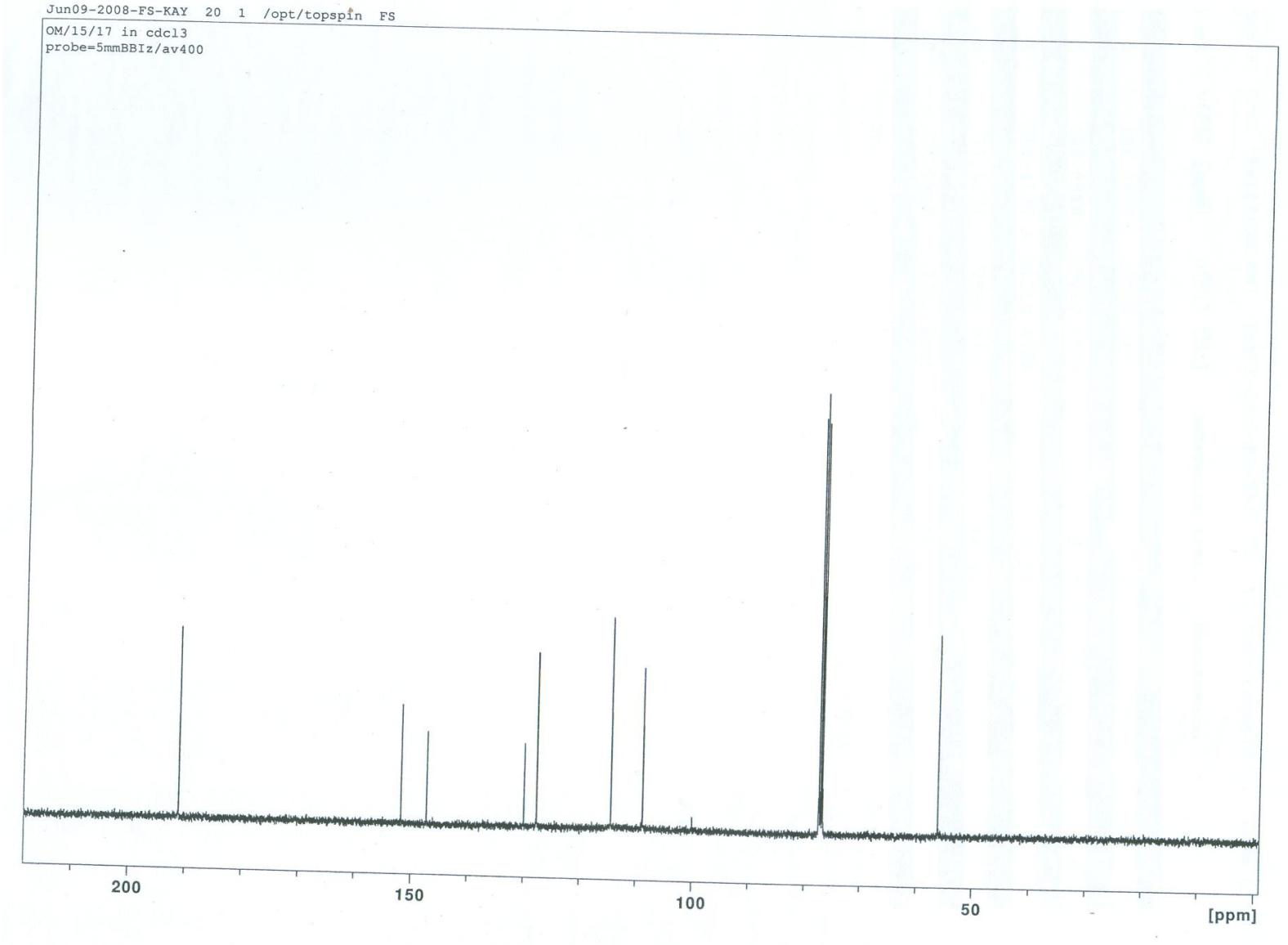
Appendix 6.1: IR Spectrum of compound OM/15/17



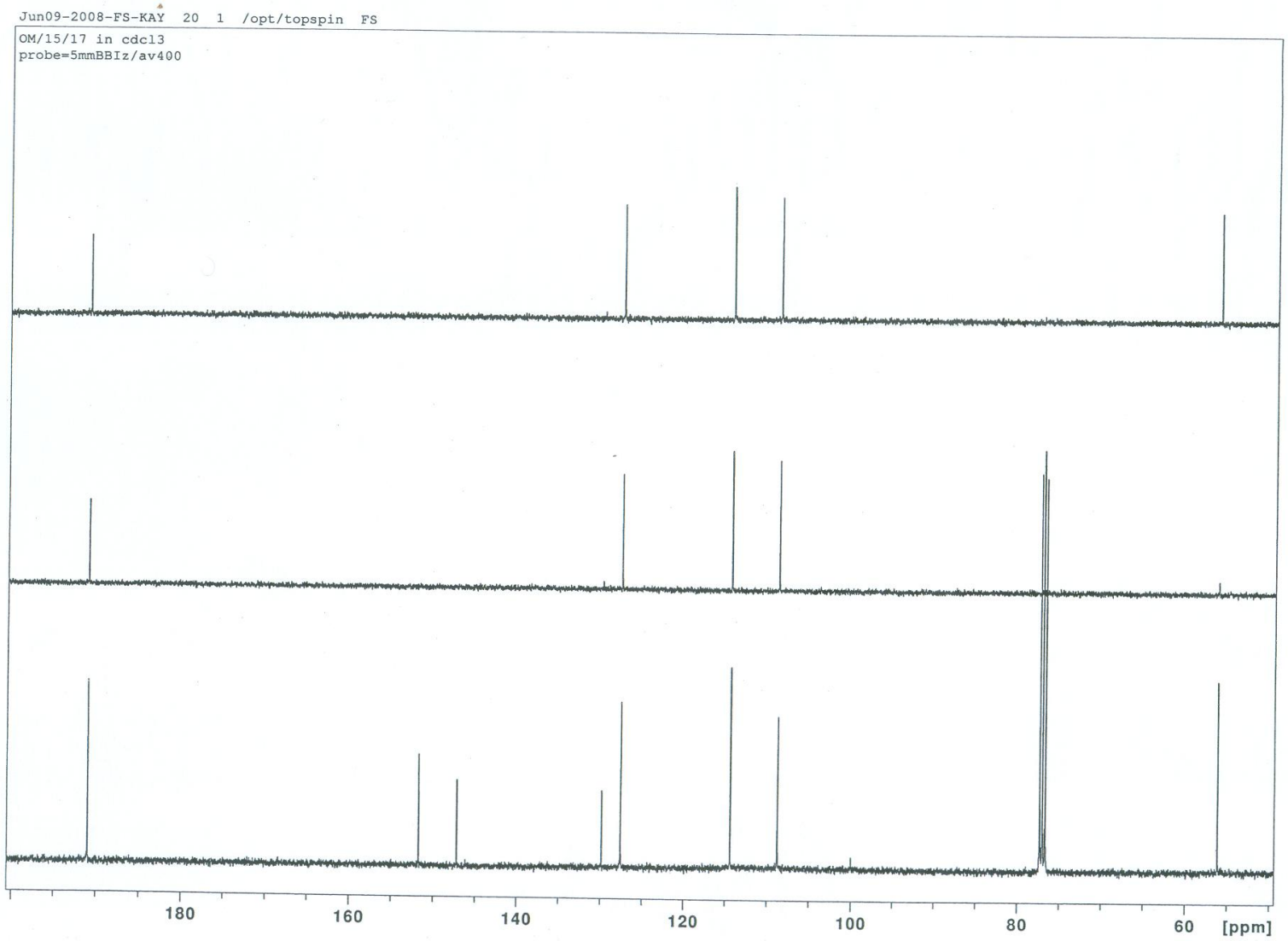
Appendix 6.2: ^{1}H NMR Spectrum of compound of OM/15/17



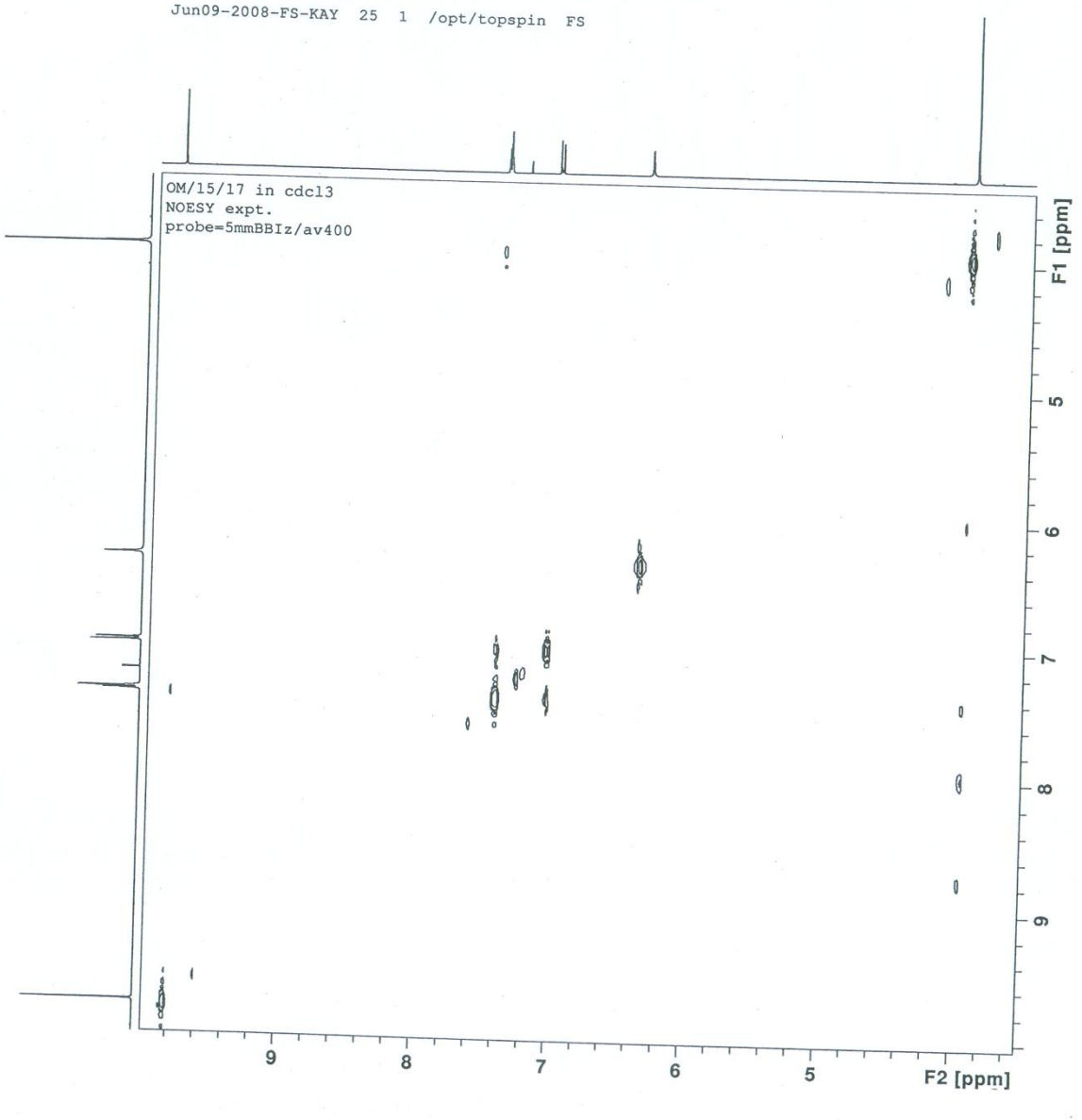
Appendix 6.3: ^{13}C NMR Spectrum of compound of OM/15/17



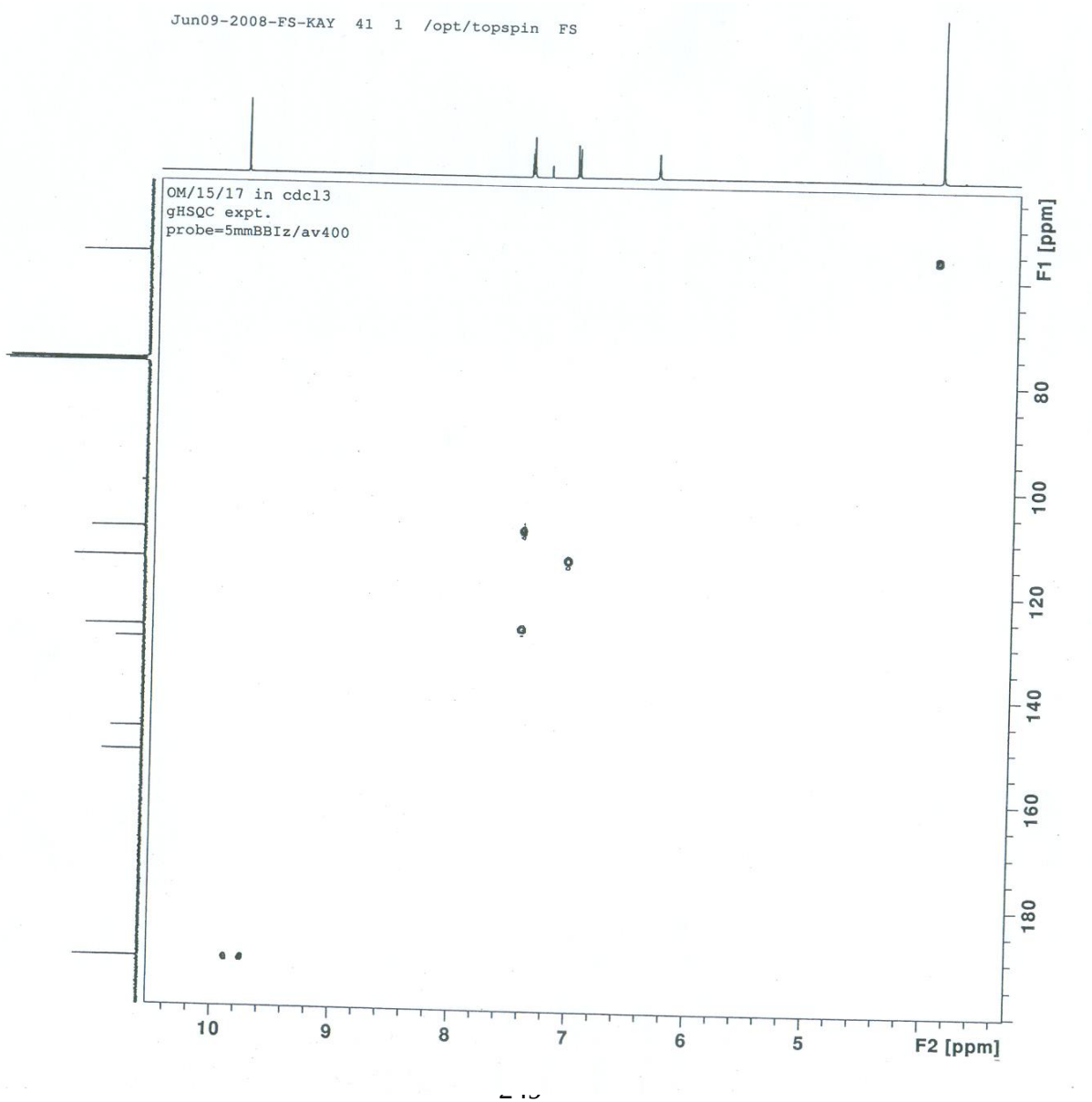
Appendix 6.4: DEPT NMR Spectrum of compound of OM/15/17



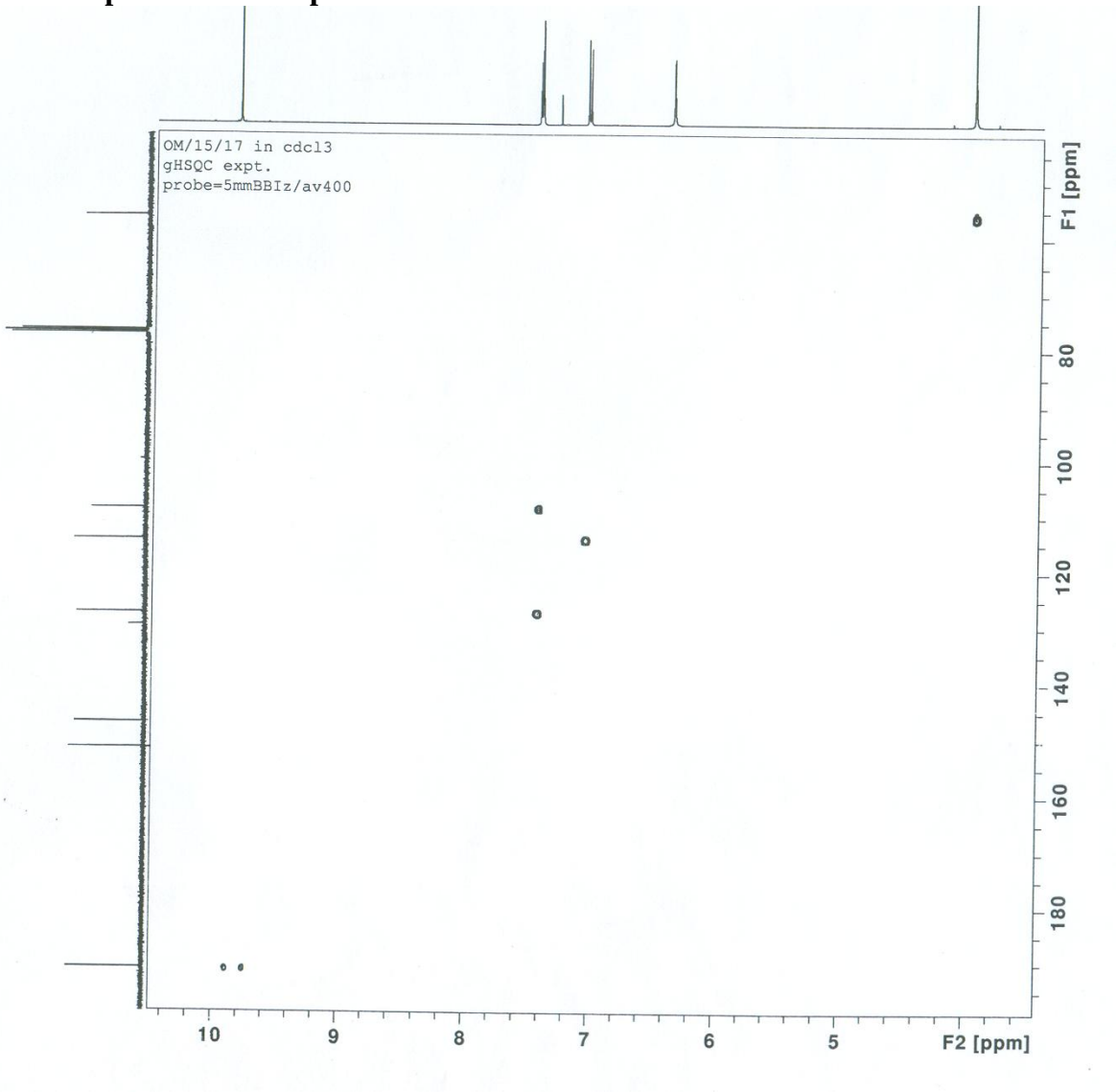
Appendix 6.5: NOSY NMR Spectrum of compound of OM/15/17



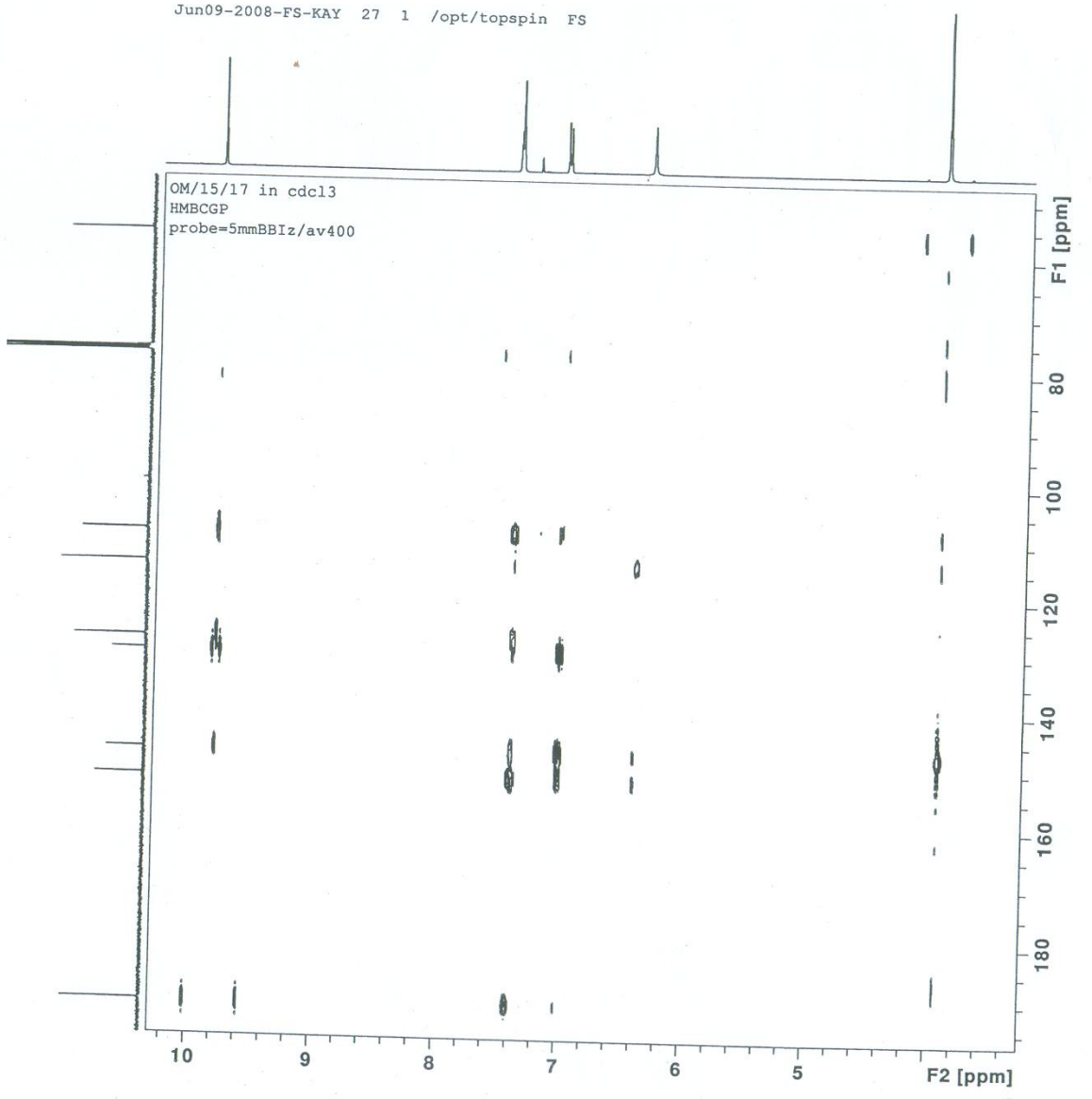
Appendix 6.6: gCosy NMR Spectrum of compound of OM/15/17



Appendix 6.7: HSQC NMR Spectrum of compound of OM/15/17

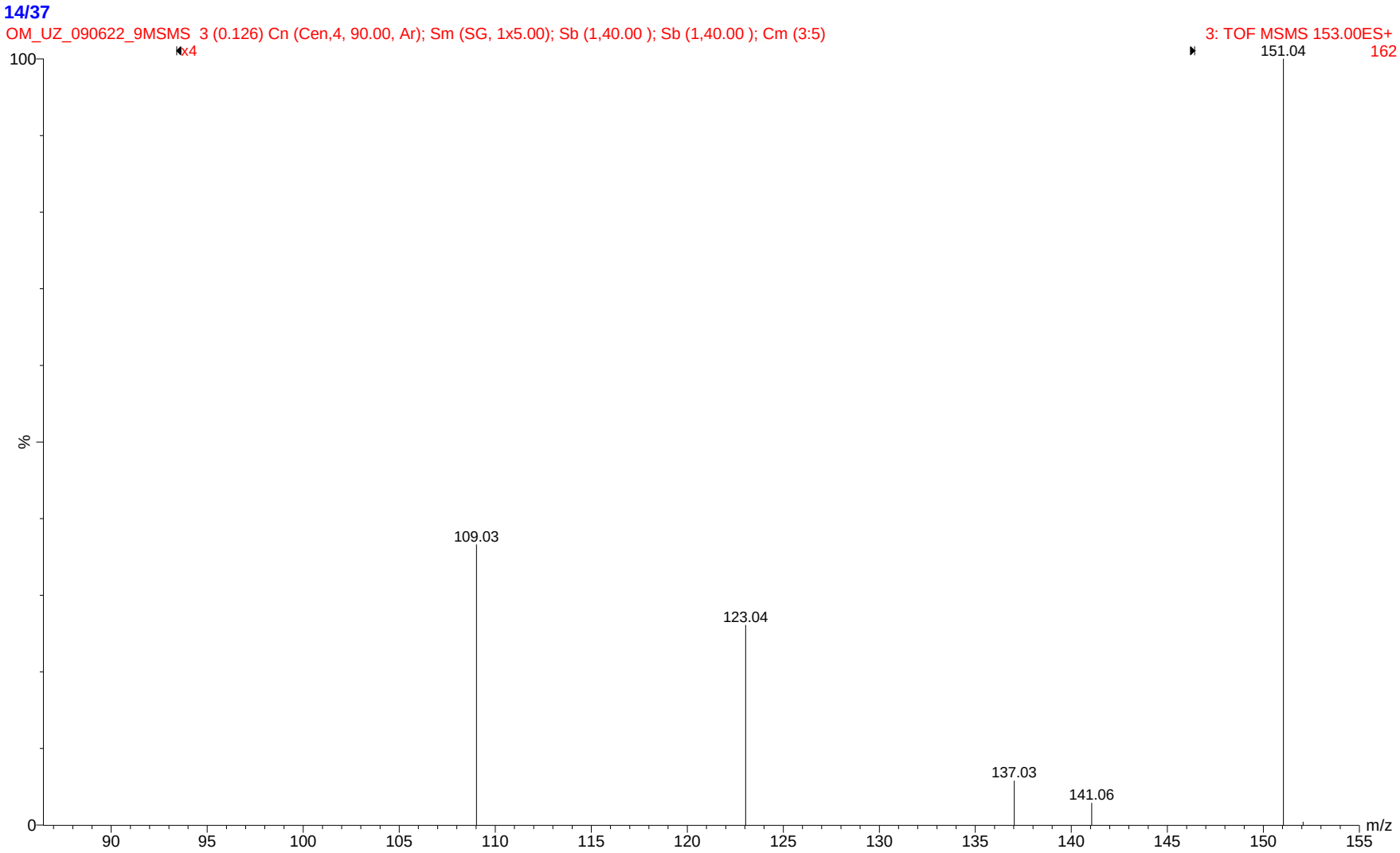


Appendix 6.8: HMBC NMR Spectrum of compound of OM/15/17



Appendix 6.9a: MS Spectrum of compound OM/15/17

Appendix 6.9b: MS/MS Spectrum of compound OM/15/17



Preliminary Phytochemical Studies on the Leaves of *Berkheya bergiana*

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ABSTRACT

The use of medicinal plants in the world and especially in South Africa, contributes significantly to Primary Health Care. This paper presents the finding of the phytochemical screening, and antioxidant and antioxidant activities of the *B. bergiana* use traditionally in KwaZulu Natal Province, South Africa. Antioxidant screening of the methanolic extract of *B. bergiana* was evaluated using various antioxidant assays, which includes free radical scavenging, hydrogen peroxide, reducing power. Phytochemical screening of the methanolic extract of the plant reveals the presence of tannins, flavonoids, carbohydrate, terpenoids, saponins and cardic-glycosides.

The various antioxidant activities were compared to standard antioxidant as ascorbic acid. The extract showed strong antioxidant activity in all the tested methods. This is the first report of the phytochemical, and antioxidant activity. Studies are in progress to evaluate the effective of the extract/fractions in antioxidant activities and identify the factors responsible for the activity.

INTRODUCTION

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases[1]. The genus *Berkheya* was formally under the family compositae but now under the family Asteraceae. There is about 75 species of *Berkheya* while 71 species are widespread in Southern Africa and 30 species in Natal[2]. *B. bergiana* is an asteraceous perennial herb, leaves rough above with white cobwebby beneath, margins sharply hairy, teeth spine tipped. Flower is yellow tips and margins spiny, lightly cobwebby beneath. Leaves and stem are used as traditional medicine. Decoction of roots for treatment coughs, as anti-emetics, for gonorrhoea and for abdominal disorders especially for pains after eating[3,4]. This study was to discuss the finding of the phytochemical screening and antioxidant activities of the *B. bergiana* use traditionally in KwaZulu Natal province, South Africa.

METHODOLOGY

Plant collection and authentication:
The plant material collected from Zululand within KwaZulu-Natal province in South Africa, identification and authenticated. The plant materials air-dried, powdered and extracted with methanol.

Phytochemical screening: was carried out on the methanolic extract using standard procedures described by Sofowara[5] for the determination of some of the secondary metabolites.

Antioxidant Assay: was carried out both qualitatively and quantitatively. Qualitative screening entails running TLC plates with various solvent systems and spray with 0.2% DPPH in Methanol while quantitative assay was determined by various methods which include DPPH method, Reducing power Method and Hydrogen peroxide Scavenging Method

RESULTS

Table 1: Phytochemical Screening of *B. bergiana* Methanol Extract

PLANT METABOLITES	*B. bergiana*
Alkaloids: (a) Preliminary screening	
(i) Draggendorff's reagent	-
(ii) Mayer's reagent	-
(b) Confirmatory test (TLC)	-
Anthraquinones: (a) Free	-
(b) Combined (i) O-glycosides)	-
(ii) C-glycosides	-
Carbohydrates: (a) Starch	++
(b) Cellulose	+
Cardiac glycosides (Keller-Kiliani test for deoxy sugars)	+++
Flavonoids (i) Lead acetate test	++
(ii) Sodium hydroxide test	++
(iii) Ferric chloride test	++
(iv) HCl + Mg turning	++
(v) EtoAc + Heat +dil NH_3	+++
Flavonol (Shinoda reduction test)	++
Terpenoids (Liebermann-Buchard test)	+++
Steroids and sterols (Salkowski test)	+
Saponins (i) Frothing test	++
(ii) Blood haemolysis test	++
Tannins: (a) True:	
(i) Phenazone test	-
(ii) Ferric chloride test	+++
(b) Phlobatannins (Formaldehyde test)	+

Keys: +++: very strongly positive, ++: Strongly Positive, +: Positive, ±: Trace, -: Negative

TABLE 2: ANTIOXIDANT:QUALITATIVE ASSAY

Major spots Rf value	Colour in daylight	Colour in UV_{254}	Anisaldahyde reagent	DPPH	
				Reaction speed	Intensity of spots
0.07	colourless	fluorescence	light orange	slow	++
0.10	colourless	fluorescence	orange	slow	+
0.15	colourless	fluorescence	light purple	slow	++
0.34	v. light green	greenish	deep purple	-	-
0.19	colourless	fluorescence	purple	fast	++
0.23	colorless	fluorescence	violet	slow	++
0.48	colourless	fluorescence	violet	slow	+
0.65	light reddish	bright yellow	yellow	slow	+
0.78	colourless	blue	violet	-	-
0.80	colourless	fluorescence	purple	fast	++
0.85	v. light yellow	fluorescence	yellowish	fast	+++
0.91	yellowish green	greenish	green	-	-

Solvent system: EtOAc:MeOH:H2O (8.0:1.1:1.0)

QUANTITATIVE ASSAY

Fig 1:Scavenging Activity of Methanol extract of *B. bergiana* against DPPH

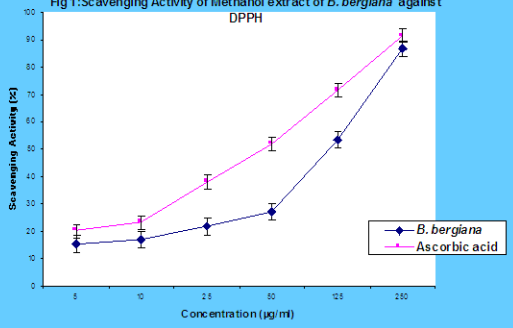


Fig 2:Reducing power of methanol extract of *B. bergiana*

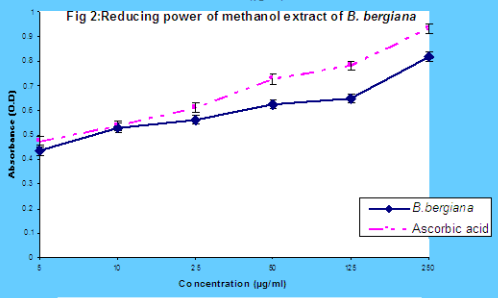
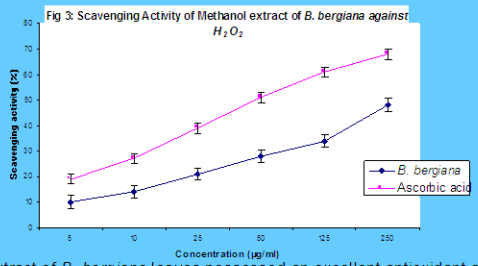


Fig 3: Scavenging Activity of Methanol extract of *B. bergiana* against H_2O_2



CONCLUSION

In this study, it was demonstrated for the first time that the methanolic extract of *B. bergiana* leaves possessed an excellent antioxidant activity. Results confirm and validate the traditional uses of the plant. Further studies on the various fractions and isolation of such bioactive components which could perhaps clarify the antioxidant properties of *B. bergiana* are in progress in our laboratory.

Acknowledgement: The authors are grateful to the NRF, South Africa and University of Zululand Research Committees for financial support.

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Antioxidant Properties of Natural Product from *Berkheya* species using Different Methods

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INTRODUCTION

- Oxygen free radical contribute to tissue damage in the events following skin injury
- Overproduction of reactive oxygen species(ROS) results in oxidative stress thereby causing cytotoxicity and delayed wound healing
- Antioxidants are known to counter the excess ROS and protease
- Many studies have focused on medicinal plants in searching for natural antioxidant principles
- The genus *Berkheya* was formally under the family Asteraceae.
- There is about 75 species of *Berkheya* while 71 species are widespread in Southern Africa and 30 species in Natal[2].
- *B. bergiana* an asteraceous perennial herb with leaves rough and white cobwebby beneath, margins sharply hairy, teeth spine tipped. Flower is yellow tips.
- Leaves and stem are used as traditional medicine. Root Decoction for treatment coughs, as anti-emetics, for gonorrhoea and for abdominal disorders [3,4].
- This study was to discuss the finding of the phytochemical screening and antioxidant activities of the *B. bergiana* use traditionally in KwaZulu Natal province, South Africa

METHODOLOGY

Plant collection and authentication:
The plant material collected from Zululand within KwaZulu-Natal province in South Africa, identification and authenticated. The plant materials air-dried, powdered and extracted with methanol. Sequential extraction with different solvent with increasing polarity

Phytochemical screening: was carried out on the methanolic extract using standard procedures described by Sofowara[5] for the determination of some of the secondary metabolites.

Antioxidant Assay: was carried out both qualitatively and quantitatively. Qualitative screening entails running TLC plates with various solvent systems and spray with 0.2% DPPH in Methanol while quantitative assay was determined by various methods which include DPPH method, Reducing power Method and Metal Chelating Method for methanol extract and various fractions

RESULTS

Table 1: Phytochemical Screening of *B. bergiana* Methanol Extract

PLANT METABOLITES	*B. bergiana*
Alkaloids: (a) Preliminary screening (i) Draggendorff's reagent (ii) Mayer's reagent (b) Confirmatory test (TLC)	- - - -
Anthraquinones: (a) Free (b) Combined (i) O-glycosides) (ii) C-glycosides	- - -
Carbohydrates: (a) Starch (b) Cellulose	++ +
Cardiac glycosides (Keller-Kiliani test for deoxy sugars)	+++
Flavonoids (i) Lead acetate test (ii) Sodium hydroxide test (iii) Ferric chloride test (iv) HCl + Mg turning (v) EtoAc + Heat +dil NH_3	++ ++ ++ ++ +++
Flavonol (Shinoda reduction test)	++
Terpenoids (Liebermann-Buchard test)	+++
Steroids and sterols (Salkowski test)	+
Saponins (i) Frothing test (ii) Blood haemolysis test	++ ++
Tannins: (a) True: (i) Phenazone test (ii) Ferric chloride test (b) Phlobatannins (Formaldehyde test)	 - +++ +

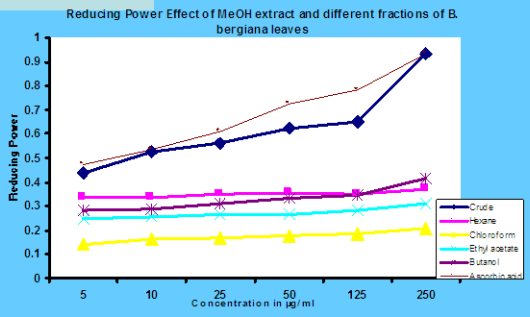
Keys: +++: very strongly positive, ++: Strongly Positive, +: Positive, ± Trace, -: Negative

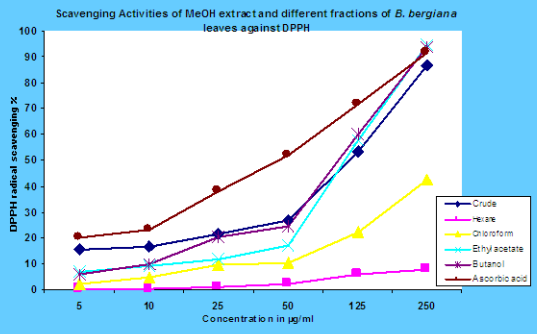
TABLE 2: ANTIOXIDANT:QUALITATIVE ASSAY

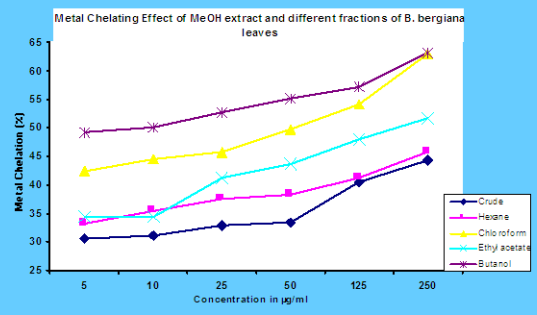
Major spots Rf value	Colour in daylight	Colour in UV_{254}	Anisaldahyde reagent	DPPH	
				Reaction speed	Intensity of spots
0.07	colourless	fluorescence	light orange	slow	++
0.10	colourless	fluorescence	orange	slow	+
0.15	colourrless	fluorescence	light purple	slow	++
0.34	v. light green	greenish	deep purple	-	-
0.19	colourless	fluorescence	purple	fast	++
0.23	colorless	fluorescence	violet	slow	++
0.48	colourless	fluorescence	violet	slow	+
0.65	light reddish	bright yellow	yellow	slow	+
0.78	colourless	blue	violet	-	-
0.80	colourless	fluorescence	purple	fast	++
0.85	v. light yellow	fluorescence	yellowish	fast	+++
0.91	yellowish green	greenish	green	-	-

Solvent system: EtOAc:MeOH:H2O (8.0:1.1:1.0)

QUANTITATIVE ASSAY







CONCLUSION *B. bergiana* has been used as a medicinal plant in South Africa. This study demonstrated for the first time that the methanolic extract and fractions of *B. bergiana* leaves possessed an excellent antioxidant activity. The methanol extract and various fractions are found to have different levels of antioxidant activity in all the system tested. The result showed that methanol extract of *B. bergiana* leaves might have good potential as a source of natural health products due to its antioxidant activities. Results confirm and validate the traditional uses of the plant. Further studies on the isolation of such bioactive components which could perhaps clarify the antioxidant properties of *B. bergiana* is in progress in our laboratory.

Acknowledgement: The authors are grateful to the NRF, South Africa and University of Zululand Research Committees for financial support.

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5. Sofowara A (1993) Medicinal Plants and Traditional medicine in Africa. Spectrum Book Ltd, Ibadan, Nigeria.

Phenolic Content, Antioxidant Activity of *Berkheya bergiana* Leaves

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INTRODUCTION

- Oxygen free radical contribute to tissue damage in the events following skin injury.
- Overproduction of reactive oxygen species (ROS) results in oxidative stress thereby causing cytotoxicity and delayed wound healing.
- Antioxidants are known to counter the excess ROS and protease.
- Many studies have focused on medicinal plants in searching for natural antioxidant principles
- The genus *Berkheya* belongs to the family Astereceae.
- *B. bergiana* an asteraceous perennial herb with leaves rough and white cobwebby beneath, margins sharply hairy, teeth spine tipped. Flower is yellow tips.
- Leaves and stem are used as traditional medicine. Root Decoction for treatment coughs, as anti-emetics, for gonorrhoea and for abdominal disorders.

AIM

This study was to discuss the finding of the phytochemical screening and antioxidant activities of the *B. bergiana* use traditionally in KwaZulu Natal province, South Africa.

METHODOLOGY

Plant collection and authentication: The plant material collected from Zululand within KwaZulu-Natal province in South Africa was identified and authenticated. The plant materials air-dried, powdered and extracted with methanol. Sequentially extracted with different solvents with increasing polarity.

Antioxidant Assay: was carried out both qualitatively and quantitatively. Qualitative screening entails running TLC plates with various solvent systems and spray with 0.2% DPPH in Methanol while quantitative assay was determined by various methods which include DPPH method, ABTS Method, FRAP Method and Metal Chelating Method for methanol extract and various fractions

FLOW CHART

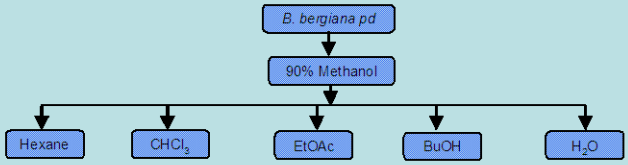

TABLE 1. ANTIOXIDANT:QUALITATIVE ASSAY

R_f value	Colour in daylight	Colour in UV 254	Anisaldahyde reagent	DPPH	
				speed	Intensity
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0.10	colourless	fluorescence	orange	slow	+
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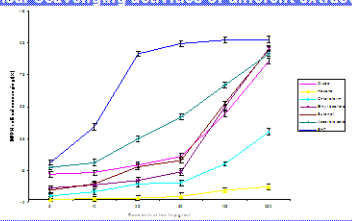
0.34	v. light green	greenish	deep purple	-	-
0.19	colourless	fluorescence	purple	fast	++
0.23	colorless	fluorescence	violet	slow	++
0.48	colourless	fluorescence	violet	slow	+
0.65	light reddish	bright yellow	yellow	slow	+
0.78	colourless	blue	violet	-	-
0.80	colourless	fluorescence	purple	fast	++
0.85	v. light yellow	fluorescence	yellowish	fast	+++
0.91	yellowish green	greenish	green	-	-

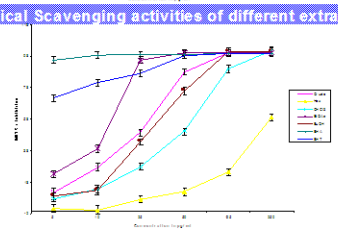
Table 2: Polyphenol contents and EC_{50} of different extracts of *B. bergiana*

Extract	Phenolic content (GAE mg g^{-1})	Flavonoid (µg QE/mg)	Proanthocyanidin (Catechin/mg/g)	EC_{50} (µg mL^{-1})		
				DPPH	ABTS	Metal Chelating
Crude	10.863±0.35	26.54±0.78	0.348±0.053	114.87±0.02	217.27±0.02	>250
Hexane	7.634 ±0.10	14.86±0.33	0.345±0.023	>250	26.33±0.02	>250
$CHCl_3$	10.348 ± 0.04	20.53±0.31	0.424±0.030	>250	49.27±0.03	52.60±0.01
EtOAc	12.295 ± 0.00	25.10±0.57	0.466±0.075	110.73±0.02	13.27±0.00	191.80±0.03
BuOH	8.566 ± 0.10	18.10±0.63	0.259±0.005	103.27±0.02	29.67±0.01	8.33±0.02
BHT	-	-	-	12.47±0.01	<5	<5
BHA	-	-	-	-	<5	38.6

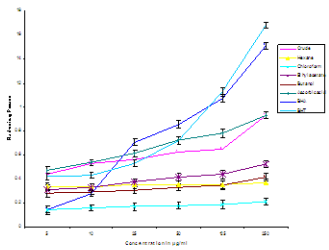
DPPH Radical Scavenging activities of different extracts of *B. bergiana*



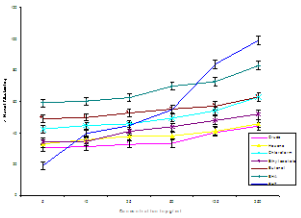
ABTS Radical Scavenging activities of different extracts of *B. bergiana*



Reducing power (FRAP) activities of different extracts of *B. bergiana*



Metal Chelating activities of different extracts of *B. bergiana*



CONCLUSION

B. bergiana has been used as a medicinal plant in South Africa. This study demonstrated for the first time that the methanolic extract and fractions of *B. bergiana* leaves possessed an excellent antioxidant activity. The methanol extract and various fractions are found to have different levels of antioxidant activity in all the system tested. The result showed that methanol extract of *B. bergiana* leaves might have good potential as a source of natural health products due to its antioxidant activities. Results confirm and validate the traditional uses of the plant.

FUTURE WORK

Further studies on the isolation of such bioactive components which could perhaps clarify the antioxidant properties of *B. bergiana* is in progress in our laboratory.

ACKNOWLEDGEMENT

The authors are grateful to the NRF, South Africa and University of Zululand Research Committees for financial support.

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Antimicrobial activity of pentacyclic triterpenes isolated from Berkheya bergiana

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The use of medicinal plants in the world and especially in South Africa, contributes significantly to Primary Health Care[1]. The genus *Berkheya* belongs to the family Astereceae[2]. *B. bergiana* leaves and stem are used as traditional medicine. Decoction of leaves and roots are used for the treatment of coughs, gonorrhoea, rheumatism and abdominal disorders especially for pains after eating. It is also used as anti-emetics[3]. Unusual sesquiterpenoids and thiophene derivatives have been isolated from *Berkheya* species[3]. The aim of the study was to provide scientific rationale for the use of the plant in traditional medicine through bioassay-guided fractionation of *B. bergiana* leaves. Bioactivity testing was done against selected microbes using disc diffusion technique as outlined in Clinical Laboratory Standard Institute (CLSI). Structure elucidation of the isolated compounds was based primarily on 1D and 2D NMR analyses, including HMQC, HMBC and NOESY correlations. Fractionation yielded some triterpenoids; 20(29)-Lupene-1,3-diol, 3-Methoxy-20(29)-lupene and 17-Epilupenyl acetate. The compounds were active against 25 bacterial strains both standard and isolates and were active against *P. aeruginosa* ATCC 7700, *P. vulgaris* ATCC 6830, *S. marscens* ATCC 9986, *E. coil* ATCC 8739 *S. epididirmis, Salmonella* spp, *E. faecalis* etc. These results explain the support the use of *B. Bergiana* leaves for the treatment of infectious diseases in traditional South Africa medicine. It also shows that the antimicrobial activity is concentrated in the triterpenoid fractions.

Acknowledgements: The authors are grateful to the NRF, South Africa and University of Zululand Research Committees for financial support

References: [1] Van der Watt, E., Pretorius, J.C. (2001)J. Ethnopharmacol. 76:87-91.

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Constituents of Momordica foetida and Evaluation of their Antimicrobial Activity

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Plants are a potential source of antimicrobial compounds. In this research, a plant from the family Cucurbitaceae was studied. *Momordica foetida* Schumch. Et Thonn is a climber commonly found in swampy areas in Central and Southern Africa. It has medicinal uses ranging from spiritual and psychiatric conditions to physical diseases. Drinking of aqueous leaf extracts of the plant for the treatment of malaria is reported in East and Central Africa [1,2]. The leaves were extracted using 70% ethanol and partitioned into hexane, chloroform, ethyl acetate, butanol and aqueous then screened for antimicrobial activity against 32 bacterial strains for both standard and isolates. Thus, ethyl acetate and chloroform fractions were chosen for further studies due to higher antimicrobial activity with minimum inhibitory concentration (MIC) values for 32 bacterial strains ranging from 0.156 and 2.5 mg mL^{-1}. Active fractions were further purified using chromatographic techniques. A detailed phytochemical investigation resulted into isolation of four curcubitane triterpenoids and flavonoids compounds from chloroform and ethyl acetate fractions respectively. The chemical structures of the isolated compounds were established through UV, IR, MS, ^{1}H, ^{13}C, COSY and 2D NMR spectroscopic data. Antimicrobial investigations were carried out on the isolated compounds against 25 bacterial strains of which 3β,7β-dihydroxyl-cucurbita-5,23,25-trien-19-al followed by Kaempferol-*3*-*O*-β-D-glucopyranoside displayed minimum inhibitory concentration (MIC) values for 25 bacterial strains ranging from 7.8 to 250 µg mL^{-1}. *Acknowledgement:* We are grateful to the National Research Foundation and University of Zululand, South Africa for financial support. *References:* [1] Hakizamungu E, et al. (1992) J Ethnopharmacology 36: 143-146. [2] Rwangabo PC, (1993) La medicine traditionnelle au Rwanda. Edition Karthala and ACCT, Paris, France.

PA21 Phytochemical screening, antioxidant and antimicrobial activities of *Berkheya bergiana* leaves

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The use of medicinal plants in the world and especially in South Africa, contributes significantly to Primary Health Care [1]. The genus *Berkheya* belongs to the family Astereceae [2]. The plant material collected from Zululand within KwaZulu-Natal province in South Africa, was air-dried, powdered and extracted with methanol. Fractions were obtained by successive extraction with n-Hexane (Hex), Chloroform ($CHCl_3$), Ethyl acetate (EtOAc), and n-Butanol (BuOH). Antioxidant activity was investigated by DPPH radical scavenging effect, reducing power and metal chelating effect on ferrous ion. Antimicrobial test was carried out by disc-diffusion method on some selected bacterials. Phytochemical screening [3] carried out on *B. bergiana* revealed the presence of carbohydrate, flavonoids, terpenoids, saponins and tannins but absence of anthraquinones and alkaloids. The result demonstrated that methanolic extract of *B. bergiana* have excellent antioxidant activities and also shows that crude and fractions found to have different levels of antioxidant activity in all the system tested. Results revealed that BuOH fraction exhibited the best performance in DPPH and Metal chelating assay, 93.7% and 63.0% respectively. Total phenolic and flavonoid contents in the crude and fractions were also determined in which EtOAc fraction (12.29 ± 0.11) has the highest total phenolic content. Strong correlation was recorded between DPPH/GAE (R^2= 0.85). Antimicrobial activity was highest on gram *E. coli, P. aeruginosa, E. cloacae, K. pneumonia, B. cereus* and *S. aureus*. The results of this study suggested that the antioxidant potential of *B. bergiana* leaf extract could be due to its strong proton donating ability and thus justified its use for the treatment of bacterial infections in ethnomedicine. References:1. Van der Watt, Pretorius, J.C. (2001) J. Ethnopharmacol. 76:87 – 91. 2. Van Wyk B.K., Gericke N. (2000) People's Plants. Briza Publications, Pretoria, South Africa. 3. Sofowara A. (1993) Medicinal Plants and Traditional medicine in Africa. Spectrum Book Ltd, Ibadan, Nigeria.

PA22 *In vivo* antiplasmodial activity of a crude ethanolic stem bark extract of *Nauclea pobeguinii*

Mesia K[1,2], Tona L[2], Cimanga K[1], Kuypers K[3], Pieters L[1], Vlietinck A[1], Maes L[3]
[1]Laboratory of Pharmacognosy and Pharmaceutical Analysis, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium; [2]Faculty of Pharmacy, University of Kinshasa, Kinshasa, DR Congo; [3]Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium

A crude ethanolic extract of the bark of the African tree *Nauclea pobeguinii* (Rubiaceae), used in traditional medicine in DR Congo against malaria, containing 5.6% (w/w) strictosamide as the active principle, was evaluated *in vivo* in the *Plasmodium berghei* mouse model in a suppressive treatment regimen. The test substance was formulated in PEG400 and orally dosed (PO) at 300 mg/kg for two times 5 daily doses. One group received the treatment by the intraperitoneal (IP) route using the same dosing regimen. The untreated infected control animals all developed typical malaria and died during the course of the experiment (mean survival time (MST): 8.4 – 11.2 days). The animals treated with chloroquine at 10 mg/kg did not develop malaria during dosing, but subsequently relapsed with 2 animals dying before termination of the experiment at day 21. Treatment with the crude extract, either after oral of intraperitoneal dosing, resulted in moderate depression of parasitaemia during dosing, however quickly followed by a full relapse (MST = about 13 days). At termination of the experiment at day 21, a single survivor in the PO group was apparently cured (no parasitaemia), the single survivor in the IP group showed high parasitaemia and was in a moribund state. It can be concluded that the crude extract of *N. pobeguinii* has slight antimalarial potential when administered orally in a suppressive dosing regimen of 2 x 5 days at 300 mg/kg. Its action is likely to be static since full relapse occurs quickly after ending the daily dosing.

PA23 Antimicrobial activity of *Jatropha curcas* L. and *Jatropha multifida* L. against bacteria and fungi s.t.d. organisms

Aiyelaagbe OO[1], Fatunsin OF[1], Oguntuase BJ[1], Adeniyi BA[2], Gibbons S[3]
[1]Department of Chemistry, University of Ibadan, Ibadan, 200284, Nigeria; [2]Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, 200284, Nigeria; [3]Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, U.K

Jatropha curcas and *Jatropha multifida* are ornamental, medicinal plants belonging to the family Euphorbiaceae. Many *Jatropha* plants have toxic and irritant properties and are used in folklore medicines to cure various infections in Africa, Asia and Latin America [1]. As part of a continuing investigation of the biological activity of *Jatropha* species [2], this study was carried out to investigate the antimicrobial activity of these plants against different microorganisms especially those responsible for sexually transmitted infections and isolate the bioactive constituents. Hexane, ethyl acetate and methanol extracts of the plants were analyzed phytochemically and screened against microorganisms comprising gram positive and gram negative bacteria and fungi. The extracts and compounds displayed potent antimicrobial activity against *Gardnerella vaginalis, Neisseria gonorrhoea* and *Candida albicans* giving Minimum Inhibitory Concentration (MIC) as low as 12.5 µg/mL. Phytochemical investigation resulted in the isolation of different compounds including a coumarin, 8-hydroxy-6,7-dimethoxy coumarin. The structures of the compounds were determined by MS, 1D and 2D NMR experiments. The results confirmed the potency of these plants in treating different infections including sexually transmitted diseases. References: 1. Burkill, H.M. (1994) The useful plants of West Tropical Africa. Vol.2, Royal Botanical Gardens, Kew. 2. Aiyelaagbe, O.O. et al. (2000) Phytother. Res. 14:60 – 62.

PA24 Bioassays in biodiscovery

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University of Queensland, Institute for Molecular Bioscience, Carmody Rd, St Lucia, 4072, Australia

Biodiscovery, the exploration of biodiversity, to detect natural products with biological properties, requires a multi-disciplinary commitment to guide biodiversity choices through bioassays and chemistry. We have focused on innovative biology, using high content read out methodologies, across the indications of cancer, infectious diseases (bacterial, fungal, parasitic and viral), diabetes and obesity, inflammatory conditions, and CNS-related diseases. More than eighteen assays have been identified and regrouped around these indications. Most are *in vitro* cell-based functional assays, with validated molecular targets of high to very high interest. The Institute for Molecular Bioscience (IMB) is also the repository of several chemical libraries containing thousands of yet undescribed chemical structures available for screening. These include marine and bacterial natural products, venom extracts and "combichem" libraries. Two examples of cell-based assays and one example of purified target-based assay, successfully used against these libraries, are presented: glycine-gated chloride channel inhibition assay (CNS-related indication), tumour necrosis factor alpha (TNFα) trafficking/delivery assay (inflammation) and pneumococcal surface antigen A (PsaA) competitive zinc binding assay (antibacterial). The first two assays are high-content-screenings. Their low-throughput (180 extracts tested per week in duplicate) is compensated by a high frequency of active extracts identified (~ 0.8%). Cell-based assays show surprisingly good robustness with natural extracts (cytotoxicity, reproducibility) and good signal-to-noise ratio. The last assay in a 384 well plate format uses recombinant PsaA, a fundamental determinant of *Streptococcus pneumoniae* virulence. Here again, a number of active extracts containing a new class of allosteric inhibitors were identified. References:1. Kruger, W. et al. (2005) Neurosci Lett. 380:340 – 5. 2. Murray, RZ. et al. (2005) J Biol Chem. 280:10478 – 83. 3. Tseng, HJ. et al. (2002) Infect Immun. 70:1635 – 9.

Title:

Antioxidant Activity of Triterpenoids and flavonoids from the leaves of *Momordica foetida*

Authors & affiliations:

Odeleye O.M and Oyedeji O.A
Department of Chemistry, Faculty of Science and Agriculture, University of Zululand, KwaDlangezwa, 3886, South Africa, E-mail:odeleyeom@yahoo.com.

Abstract: (Your abstract must use **Normal style** and must fit in this box. Your abstract should be no longer than 300 words. The box will 'expand' over 2 pages as you add text/diagrams into it.)

The use of natural products with therapeutic properties is an ancient as human civilization and for a longtime, mineral, plant and animal product were the main source of drug. Herbal medicines used to treat many diseases including several infections. In this research, a plant from the family Cucurbitaceae was studied. *Momordica foetida* Schumch. Et Thonn is a climber commonly found in swampy areas in Central and Southern Africa. It has medicinal uses ranging from spiritual and psychiatric conditions to physical diseases. Drinking of aqueous leaf extracts of the plant malaria treatment is reported in East and Central Africa[1,2]. The leaves was extracted using 70% Ethanol and partitioned into hexane, chloroform, ethyl acetate, butanol and aqueous and screened for antioxidant potential using four *in vitro* assays namely DPPH, ABTS, Metal chelating and FRAP assays . Thus, ethyl acetate and chloroform fractions were chosen for further studies due to higher antioxidant potential for DPPH, IC_{50} 188 and 250 mg mL^{-1} respectively. Active fractions were further purified using chromatographic techniques. A detailed phytochemical investigation resulted into isolation of four curcubitane triterpenoids and flavonoid compounds from chloroform and ethyl acetate respectively. The chemical structures of the isolated compounds were established through UV, IR, MS, ^{1}H, ^{13}C, COSY and 2D NMR spectroscopic data. Antioxidant investigations was carried out on the isolated compounds using DPPH method in which Kaempferol-*3-O*-β-D-glucopyranoside followed by 3β,7β-dihydroxyl-cucurbita-5,23,25-trien-19-al with IC_{50} values of 67 and 56 µg mL^{-1}.

Acknowledgement: We are grateful to the National Research Foundation and University of Zululand, South Africa for financial support.

References: [1] Hakizamungu E, Van Puyvelde L, Wery M (1992) Journal of Ethnopharmacology Vol. 36, p. 143–146. [2] Rwangabo PC (1993) La medicine traditionnelle au Rwanda. Edition Karthala and ACCT, Paris, France.