Phytochemical and elemental analysis of *Cyrtanthus obliquus* and *Lippia javanica*

By

Nomfundo Thobeka Mahlangeni

Dissertation submitted in fulfillment of the requirements for the degree of Master of Science in Chemistry in the School of Chemistry and Physics, University of KwaZulu-Natal, Durban

2012

DECLARATION

I hereby declare that this dissertation is my own work, besides the assistance of project supervisors and has not been previously submitted by me to another institution to obtain any research qualification.

Author: _____

Nomfundo Thobeka Mahlangeni

Supervisor: _____

Prof. S.B. Jonnalagadda

Co-supervisor: _____

Roshila Moodley

DEDICATION

I dedicate this work to the loving memory of my dear sister Luyanda Thandeka Mahlangeni

Psalm 23: 1-6

ACKNOWLEDGEMENTS

Firstly, would like to thank God for giving me the strength and confidence to take such a big step in my life and getting me through the completion of the research successfully.

I would also like to convey my sincere gratitude to my supervisor Prof. S.B. Jonnalagadda for the opportunity to study under him and for the knowledge and wisdom I gained from his expertise. And also my co-supervisor, Mrs. R. Moodley for continuous support throughout my research and her being a great mentor and her sacrificial giving of her time to the research.

I would also like to acknowledge the following individuals for the success of my research

- Mr Phungula, traditional herbalist (Ndwedwe) for sharing his knowledge and experitises.
- All my friends: Letticia, Nerena, Zuzile and Ntombenhle, you all are amazing, thank you for your love, constant encouragement throughout the years.
- My colleagues in the natural products research group for moral support
- Vashti Reddy, Anita Naidoo, Greg Moodley and Dilip Jagjivan, School of chemistry (UKZN-Westville) technical team.
- National Research Foundation (NRF) for financial support.

Lastly, I would like to thank my family; my mother Mrs T.F. Mahlangeni for her love, support and encouragement throughout the research, my sampling partner and sister Phakamile, for her support and patience, my brother Andile and cousin Happiness for their support and my little nieces and nephews (Senamile, Aphiwe, Asanda, Sandiselwe, Siqalo, Nosipho and Minenhle) for their love, support and constant phone calls.

ABSTRACT

A growing number of South Africans are relying more and more on alternative medicine for their healthcare needs due to the high cost of commercially available medicines and lack of medical aid. To these people, traditional medicine has provided an alternative form of treatment with medicinal benefits that are claimed to be the same as conventional medicine but at a lower cost. Many herbal tonics and concoctions are used in traditional medicine, one of which is Imbiza, a herbal tonic comprising plant parts of different medicinal plants, which is deemed to be more effective than the use of a single medicinal plant. The safety and efficacy of these herbal preparations sold in the street markets as well as in rural areas have not yet been proven.

The study investigates two of the plants that are used to make Imbiza, namely *Cyrtanthus obliquus* bulbs and *Lippia javanica* leaves. Phytochemical studies of the extracts of *C. obliquus* bulbs yielded two new chalcones, two new dihydrochalcones and a lanostane triterpenoid. Antioxidant activity of the chalcones and dihydrochalcones was moderate and lower than ascorbic acid. GC-MS profiling of the various extracts of *L. javanica* leaves showed the presence of monoterpenes, sesquiterpenes and amino compounds.

Total and water extractable concentrations of selected elements were determined in *C. obliquus* bulbs collected from eight market sites around the KwaZulu-Natal province. The levels of the elements were found to be in decreasing order of Ca > Mg > Fe > Zn > Mn > Cu ≈ Se > Pb > Cr for total concentrations and Ca > Mg > Fe > Zn > Mn for water extractable forms. A high percentage of Zn (77.5-91.5 %) was shown to extract into water.

Total and water extractable concentrations of selected elements were determined in *L. javanica* leaves and corresponding soil samples collected from ten different locations around the KwaZulu-Natal province. The levels of the elements were found to be in decreasing order of Ca > Mg > Fe > Zn > Mn > Cu > Se > Cr > Pb > Co > Cd for total concentrations and Ca > Mg > Fe > Zn > Cu > Cr > Pb for water extractable forms. A high percentage of Cr (71.8 - 93.9 %) was shown to extract into water.

Imbiza has been recognized by traditional healers and herbalist for the treatment of minor and chronic illnesses, which range from chest infections to cancer. Previous studies have shown that the compounds identified in this research (chalcones, dihydrochalcones, monoterpenes and sesquiterpenes) have indeed anticancer activities. This study therefore adds to the growing body of research on indigenous medicinal plants.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ANOVA	analysis of variance
BAF	bioaccumulation factor
bs	broad singlet
cc	column chromatography
CEC	cation exchange capacity
^{13}C NMR	C-13 nuclear magnetic resonance spectroscopy
COSY	correlated spectroscopy
CRM	certified reference material
d	doublet
dd	double doublet
DEPT	distortionless enhancement by polarization transfer
DPPH	2,2-diphenyl-1-picrylhydrazyl
DRI	dietary reference intake
EDTA	ethylenediammine tetraacetic acid
LC-ESI-MS	liquid chromatography-electrospray ionization-mass spectrometry
Ex	exchangeable
FRAP	ferric reducing antioxidant potential
Hz	hertz
HMBC	heteronuclear multiple bond coherence

^{1}H NMR	proton nuclear magnetic resonance spectroscopy
HSQC	heteronuclear single quantum coherence
I_{geo}	geoaccumulation index
ICP-OES	inductively coupled plasma-optical emission spectroscopy
IR	infrared
m	multiplet
nd	no date
ND	not determinable
NOESY	nuclear overhauser effect spectroscopy
ppm	part per million
r	correlation coefficient
RDA	recommended dietary allowance
s	singlet
SOM	soil organic matter
t	triplet
tlc	thin layer chromatography
UL	tolerable upper intake level

CHAPTER 1

INTRODUCTION

Currently, a large majority of the world's population utilizes traditional or herbal medicines in some way or another; a large proportion of this population is in developing countries. The treatment of diseases began a long time ago with the use of herbs. Herbs are plants with leaves, stems, roots and flowers that can be used medicinally or for cooking purposes (IARC, 2002). These herbs are either used domestically or commercially.

Commercially, there has been a steady increase in the global trade of medicinal plants, which has increased by approximately 11.5% between 1997 and 2000. This emphasises the gain in popularity of traditional or herbal medicine, worldwide. Medicinal plants are increasingly being recognized as important resources; they are accessible and considered to be effective.

Table 1: Trends in the global nutrition products industry, 1997-2000 (in millions of US $).

	1997	**1998**	**1999**	**2000**
Vitamins/minerals	18 000	18 870	19 620	20 440
Herbs/botanicals	15 990	16 980	17 490	18 070
Sports, meal replacements, homeopathy, specialty	8 760	9 310	9 960	10 710
Natural foods[a]	16 690	19 910	22 700	25 420
Natural personal care	9 620	10 280	11 020	11 850
Functional foods[b]	40 320	43 940	47 670	51 480
Total	109 380	119 290	128 420	137 980

Source: (Nutrition Business Journal, 2000)
[a] Natural foods: foods derived from natural sources
[b] Functional foods: foods fortified with added or concentrated ingredients to improve health and/or performance

The use of medicinal plants plays a major role in traditional medicine. The medicinal value of these plants is attributed to their natural products. The natural products are the organic compounds found in the plants; they are divided into two categories namely primary metabolites and secondary metabolites. The primary metabolites consist of nucleic acids, some common amino acids and sugars, while the more sort after secondary metabolites consist of terpenoids, alkaloids, flavonoids and other polyphenols. They are said to work in synergy, that is, they work together and the sum of their effect is stronger than each individually (Nyam News, 2005).

As seen from the growing market interest in herbal remedies, herbal teas and infusions have also become more popular due to their detoxifying ability. Herbal teas include Chamomile tea, Green tea, Lavender tea, Lemon bush tea and Nettle; some of these teas are available commercially. Herbal infusions are said to be sources of polyphenolic compounds such as flavoniods and phenolic acids (Kohlmünzer, 2003). In South Africa herbal infusions are preferred over single medicinal plants; these infusions are commonly known as Imbiza. These infusions are prepared by boiling specific amounts of different plant parts from different plants in water for about 10 to 20 minutes at medium heat. A quarter of a cup of this solution is ingested.

Medicinal plants that are seen to have significant curative properties are extracted with solvents of differing polarity thereby extracting a wide range of compounds. These extracts are separated and single phytocompounds are eventually isolated as seen in Figure 1 (Hamburger & Hostettman, 1991). The plants extracts and/or the pure compounds undergo a series of biological tests, such as, antibacterial, antifungal, antioxidant and antibiofilm to

name a few, to determine the biological activity. Toxicology profiles are also determined in order to evaluate if these compounds have any adverse effects to human health.

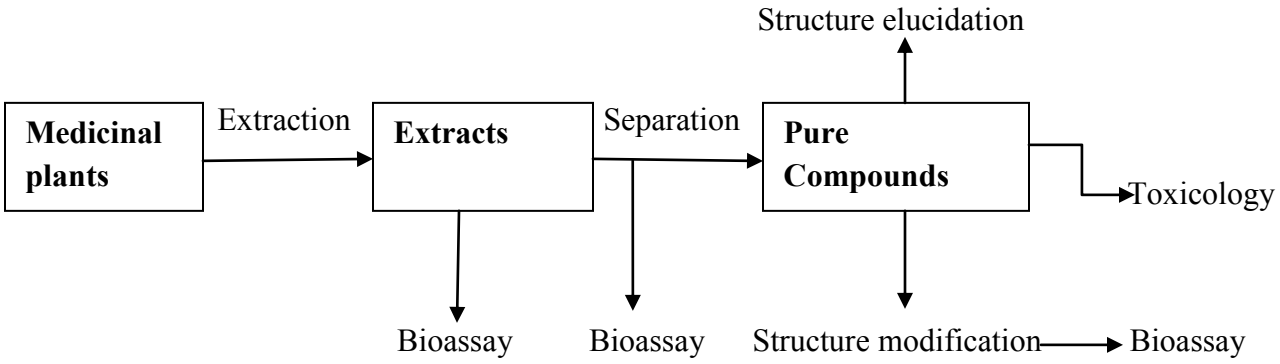


Figure 1: Route followed to obtain active constituents from plants (Hamburger & Hostettman, 1991)

There are approximately 500 000 plant species occurring worldwide but a mere 1% has been phytochemically analysed (Palombo, 2006). Numerous studies have been undertaken to assess whether isolated compounds possess antioxidant activity. Antioxidants are compounds that are able to trap free radicals and prevent the oxidative mechanism that lead to degenerative diseases from occurring. Dietary antioxidants include polyphenolic compounds, Vitamin C, Vitamin E and carotenoids, and are believed to be effective against chronic diseases such as heart disease, age-related illnesses and some cancers (Huang et al., 2005). There has been a great interest in antioxidants, particularly in dermatology and food science research which lead to the development of antioxidant studies on compounds obtained from medicinal plants.

Medicinal plants are often collected from the wild and ingested, therefore plant nutrition has to be taken into account and an assessment of the growth soil needs to be done to evaluate for

possible metal contamination. The metal content of the plants needs to be established to evaluate how much of these are ingested when these plants are consumed for medicinal purposes.

A plant essentially needs nutrients for growth and development, there are two types of inorganic nutrients namely macronutrients and micronutrients. Macronutrients are elements needed in relatively large amounts in living organisms which include N, P, K, S, Mg and Ca Micronutrients, on the other hand, define trace elements which are required in minimal amounts in living organisms which include As, Cr, Co, Cu, Fe, Mn, Mo, Ni, Se and Zn. Non-essential metals are Pb and Cd.

The mobility of elements in the soil is influenced by the physical and chemical properties of the soil. The chemical properties of the soil that need to be taken into account are water content, soil organic matter (SOM), pH and cation exchange capacity (CEC). The change in soil conditions influences availability of elements or nutrients for plant uptake. All trace elements have the potential to be toxic if concentrations are very high, therefore monitoring the soil properties is vital.

1.1 Problem statement

The number of South Africans using traditional medicine is growing each year as is evidenced by the increase in street trade of medicinal plants in the urban and rural markets of KwaZulu-Natal (KZN) (Mander & Le Breton, 2006). The more popular type of traditional medicine is the herbal tonic, Imbiza, which is reputed to cure a variety of ailments. Previously, studies have been conducted on the bioactivity and mutagenic effects of the plants used to prepare this tonic. However, information on the active compounds in the tonic is lacking which warrants the isolation and identification of the phytocompounds in the plants. Consumption of medicinal plants for the organic components does not preclude intake of the inorganic constituents. Heavy metals from the environment can be absorbed and stored by medicinal plants and consumption of these plants if contaminated can result in adverse health effects and metal toxicities. So, when consuming a medicinal plant it is important to consider both the organic and inorganic constituents to evaluate the plants therapeutic effectiveness. Several attempts have been made to determine the metal content of medicinal plants from other parts of the world but this is not true for South African medicinal plants.

1.2 Aim and objectives

The aim of the study was to phytochemically and analytically investigate two medicinal plant species used to make Imbiza, that is, *Cyrtanthus obliquus* (Umathunga) and *Lippia javanica* (uMsuzwane). The phytochemical investigation was done on plant parts that are used by traditional healers to determine if they contained any secondary metabolites. The analytical investigation was done to determine the elemental content of *C. obliquus* bulbs and *L. javanica* leaves and to evaluate the impact of soil quality parameters on the chemical composition of *L. javanica* leaves whose infusion is also taken as a substitute for tea by many South Africans in rural areas.

The research objectives were:

- To extract and isolate the phytocompounds from various morphological parts of the plants.
- To identify and characterise the isolated compounds using spectroscopic techniques (NMR, IR, UV, and MS).
- To identify suitable bioassays, based on classification of the compounds isolated and to test the isolated compounds for biological activity thereby promoting further use of the plants or validating their ethnomedicinal use.
- To determine and compare the total and water extractable concentrations of selected elements in *C. obliquus* bulbs purchased from eight different market sites and to assess for potential toxicities.
- To determine and compare the total and water extractable concentrations of selected elements in *L. javanica* leaves collected from ten different sites in KwaZulu-Natal and to assess for potential toxicities.

- To assess the elemental concentrations in *L. javanica* leaves as a function of geographic location and soil quality parameters to determine their impact on elemental uptake and to assess them for metal contamination.

CHAPTER 2

LITERATURE REVIEW

2.1 Worldview on traditional medicine

Traditional medicine is defined as a body of knowledge, skills and practices indigenous to different cultures based on theories, beliefs and experiences utilized to maintain good health (WHO, 2002). The World Health Organisation (WHO) has for decades encouraged the incorporation of traditional medicine into the primary state healthcare system, especially in the developing countries (Akerele, 1987). According to WHO, one in twenty one women has a likelihood of contracting and dying from pregnancy-related illnesses in Africa compared to one in fifty four in Asia and one in two thousand and eighty nine in Europe (Graham, 1991) due to underdeveloped healthcare systems. In some developing countries the healthcare system suffers many drawbacks such as poor training of staff, accessibility, affordability and cultural awareness (Graham, 1991). Because of these and many more reasons, the interest in traditional medicine is growing both in developing and developed countries. For example, Chinese traditional medicines reached US $14 billion in the year 2005, increasing by 24 % compared to the previous year (Zhang et al., 2009).

The Drug Discovery (DD) has focused research on medicinal plant-based drug development through traditional knowledge from traditional medicine systems following unsuccessful attempts in developing new drugs. The study of traditional medicine has resulted in the entry of a number of drugs in the international pharmacopoeia. Previous studies (Figure 2) show that between 1981 and 2002, of the 877 small molecules introduced, about half (49%) were

natural products (NPs), semi-synthetic NP analogues or synthetic compounds based on NPs (Newman et al., 2003).

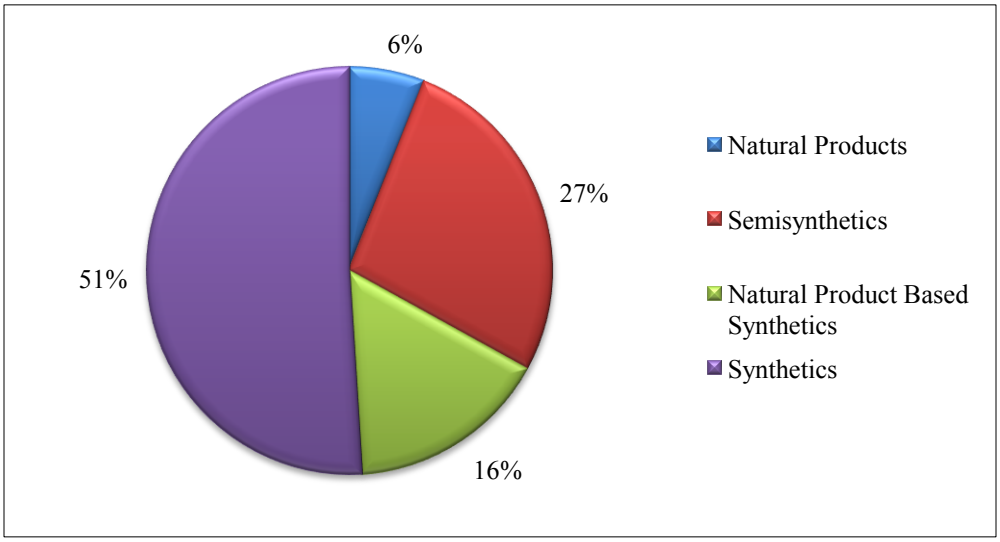


Figure 2: Small molecules introduced between the years 1981-2002 (Newman et al., 2003)

In many of the developing countries, a dual system exists, where both traditional and westernized healthcare systems are recognized. In this system, traditional health practitioners and doctors practice in clinics and hospitals and individuals can choose which type of treatment they prefer.

The African population is very familiar with indigenous knowledge and practices which includes traditional medicine. It has been within the African culture for decades. There is also large biological diversity within the African continent which provides a rich source of medicinal plants. Table 2 outlines the traditional uses of various medicinal plant species found in some parts of Africa, some of which are now commercially sold as herbal remedies (Vasisht & Kumar, 2004).

Table 2: List of some medicinal plant species popular in African countries (Vasisht & Kumar, 2004).

Botanical name	Plant part(s)	Traditional uses	Country
Aloe ferox (L.) Burm. Baill	Leaf	Conjunctivitis; venereal sores	South Africa
Brucea antidysenterica	Leaf, root, bark, fruit	Skin diseases; leprosy; dysentery; fever	Ethiopia
Citrullus colocynthis L. Schrad	Fruit pulp, seed	Purgative; gastro-intestinal stimulant	Egypt
Vernonia amaygdaline	Leaf	hypertension	Nigeria
Vernonia brachycalyx O. Hoff.M	Leaves	Anti-malaria	Kenya

WHO has been working in collaboration with 19 countries around the world to further validate the ethnomedicinal uses of medicinal plants. The quantity of research going into traditional medicine has also increased over the past years and medical science is also observing the importance of the old folk medicine (WHO, 2008).

2.2 Traditional medicine in South Africa

Studies indicate that 80% of South Africans utilize and rely on traditional medicine for their healthcare needs (Gqaleni et al., 2007; Goggin et al., 2009). Many South Africans regard traditional medicine as a desirable alternative to treating a range of health problems (Mander et al., 2007). A survey derived by Mander reports that 84% of clinic patients in Durban (KwaZulu-Natal) utilised traditional medicine and chose this form of medicine because of its holistic nature.

The number of traditional health practitioners (THPs) was estimated at 190 000 in 2007 (Mander et al., 2007); these include herbalists (Izinyanga), diviners (Izangoma), traditional surgeons (Ingcibi) and traditional birth attendants (Ababelethisi) (Traditional Health Practitioner Act, 2008). THPs are recognized as individuals who are competent to provide health care by using traditional medicine based on social, cultural and religious backgrounds. The source of medicine for traditional healers is indigenous medicinal plants. The bar graph below (Figure 3) shows the percentage of the different plant parts that are used in medicinal plant trade in South Africa (Mander, 1998).

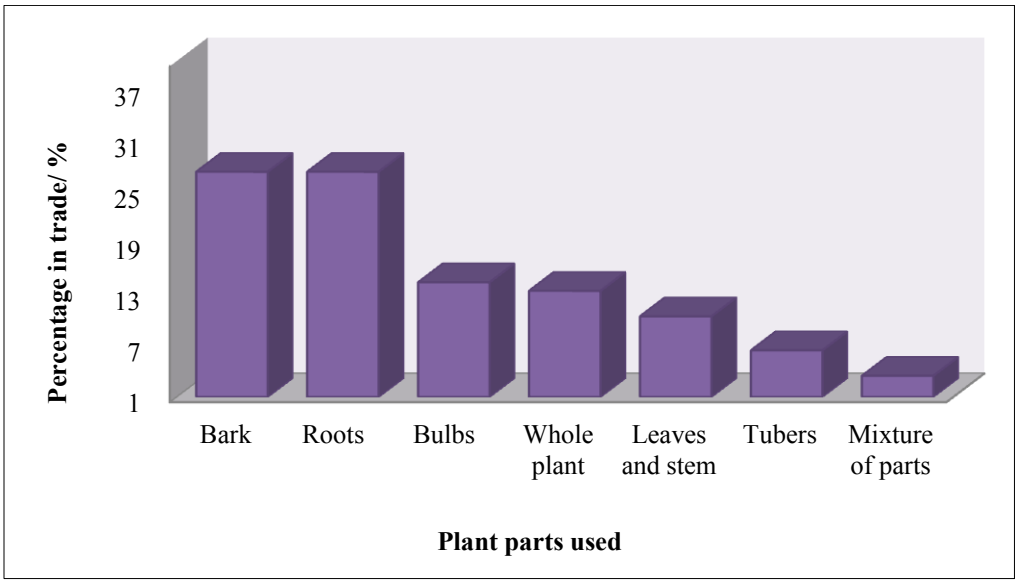


Figure 3: Percentage of the different plant parts used in medicinal plant trade in South Africa (Mander, 1998)

The plants are obtained from wild populations. Traditional healers or traders go out into the wild to collect the medicinal plants; different parts of the plants are either sold in the markets or used to make a decoction. Scarcity in knowledge on farming methods for the cultivation of these plants has been one of the reasons for collecting medicinal plants from the wild.

Figure 4: A- Medicinal plants sold on the informal market. B- Imbiza (herbal preparations) sold in street markets in Durban (Institute of Natural Resources, 2003)

The informal trade in medicinal plants and products in Southern Africa is dominated by between 400 000 to 500 000 traditional healers that dispense crude traditional medicine (Figure 4A) and herbal preparations (Figure 4B and Figure 5 (Institute of Natural Resources, 2003)) to between a staggering 50 to 100 million customers. For the past decade there has been a steady growth of formal and informal markets. Approximately 1000 medicinal plants are sold in informal markets in Southern Africa (Chen et al., 2004).

Figure 5: Imbiza packed in bottles in a traditional healers' shop (Institute of Natural Resources, 2003)

WHO has devised requirements for the labelling of traditional medicine for the African region. The label should cover the active ingredients, identify the plant name, dosage form, therapeutic indications, manufacturing and expiry date (WHO, 2005). According to Ngcobo et al. (2012) the packaging should be in accordance with good manufacturing practice requirements for manufacturers. Sahoo and Machikanti (2010) stated that the screening of heavy metals such as Pb, Hg, Cu, and As should be included among the standard protocols to test medicinal plants or finished products due to its abundance in traditional medicines as contaminants. Work by Govender et al. (2006) assessed the microbial quality of herbal medicines from shops in Port Elizabeth; the herbal medicines were found to be significantly contaminated with bacteria and fungi which suggested poor hygienic practices when preparaing these medicines.

2.3 Medicinal plants

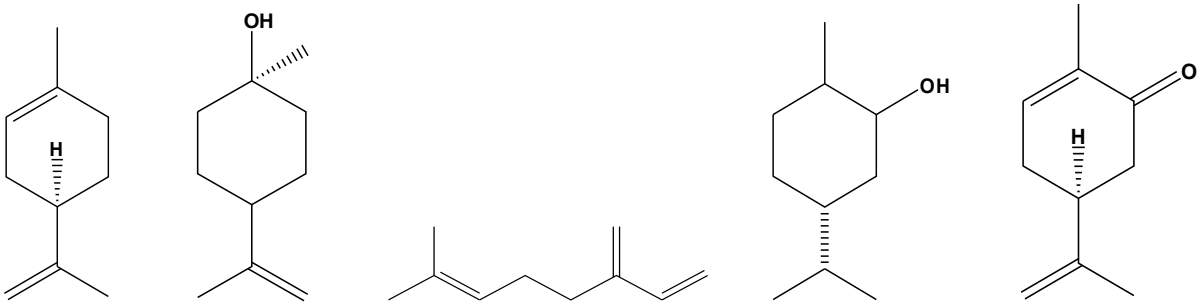
Medicinal plants are known to be the main source of drug therapy in traditional medicine (Tyagi, 2005). These plants are still today collected from wild populations. The selling of these indigenous plants has become highly commercial. More than 1000 medicinal plant and 150 animal species are used for traditional medicine in KwaZulu-Natal, of which approximately 450 plant species are sold intensively in informal and formal markets. Enforcement of conservation legislation prohibits plant gatherers from collecting species that are regarded as endangered. Thus, the legislation makes certain that endangered species do not become extinct. The government has also introduced programs to educate plant gatherers on the cultivation of indigenous medicinal plants with the hope of conserving these plants (KwaZulu-Natal Wildlife, 2012).

2.4 Phytochemicals in medicinal plants

Phytochemicals are naturally occurring and are known as secondary metabolites which are essential nutrients for plants. Phytochemicals are associated with the treatment and prevention of some of the deadliest diseases like cancer, diabetes, cardiovascular disease, and hypertension. They are recognized for various activities including antioxidant, antimicrobial and anti-inflammatory activities (Nyam news, 2005). Phytochemicals include inter alia alkaloids, flavonoids, chalcones, polyphenolic compounds, terpenoids and sterols.

2.4.1 Terpenoids

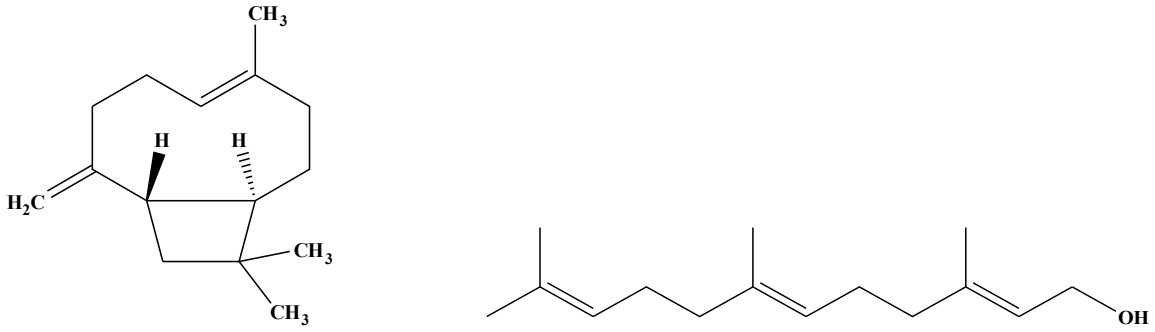
Terpernoids are found in abundance in higher plants. They contain a carbon backbone made up of isoprene units which contain five carbons (5 C). The different terpenoid groupings are monoterpenes (10 C), sesquiterpenes (15 C), triterpenes (30 C) and polyterpenoids (> 40 C). Monoterpenes and sesquiterpenoids are chief constituents of essential oils; these are volatile oils obtained from the tissue of certain plants and trees (Singh, 2007). Terpenoids are produced via the mevalonic acid pathway, but others are biosynthesized by a newly discovered mevalonate independent route (Harrewijn et al., 2001).



limonene **β-terpineol** **myrcene** **menthol** **carvone**

Figure 6: Some of the monoterpenes found in essential oils

Figure 6 shows some of the monoterpenes found in essential oils. The intermediate geranyl disphophate (GPP), which is formed from the compounds isopentenyl disphosphate (5 C) and dimethyallyl disphosphate by the GPP synthase (GPPS), is the precursor for all monoterpenes (Poulter & Rilling, 1981; Ogura & koyama, 1988).



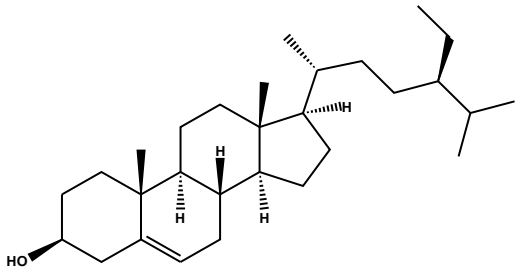
β-caryophyllene **farnesol**

Figure 7: Some of the sesqiuterpenes found in essential oils

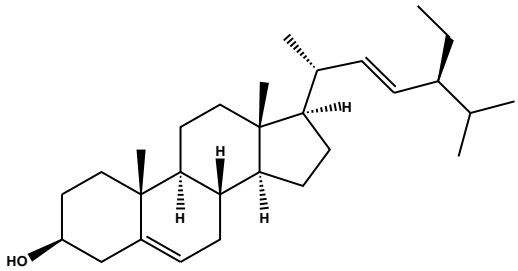
Figure 7 shows some of the sesqiuterpenes found in essential oils. In nature, sesquiterpenes occur as hydrocarbons or in oxygenated forms such as alcohols, ketones, aldehydes, acids and lactones. They have many applications, not only in medicine, but also in the soap and perfumery industry. The compounds are known for their biological and therapeutic activity, thus plants containing these compounds are commonly used in traditional medicine (Merfort, 2002).

2.4.2 Sterols

Sterols can be found in the fat soluble fractions of seeds, roots, stems, bulbs and leaves of plants. They are constituents of both edible and ornamental plants (Clifton, 2002). Sterols are essential components of cell membranes and both plants and animals produce them (Law, 2000). Amongst the most common sterols are β-sitosterol, stigmasterol, campesterol and lanosterol (Figure 8).



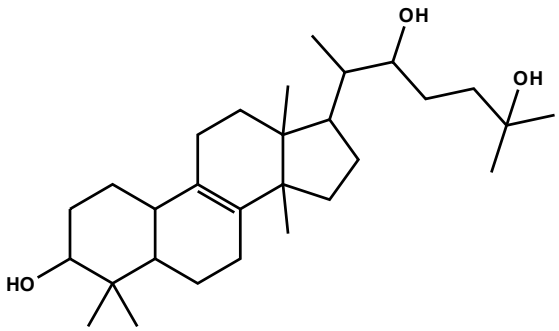
β-sitosterol



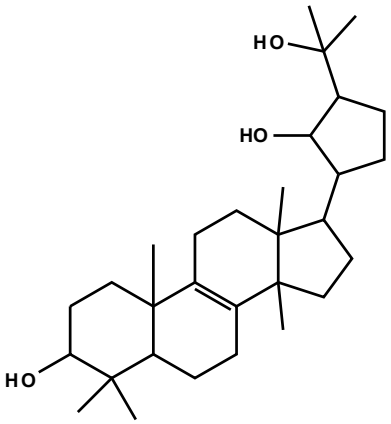
stigmasterol

Figure 8: Some of the more common sterols in plants

Lanosterol (C-30) is a key intermediate in the biosynthesis of cholesterol and bile acids. Related phytosterols are ingredients in traditional Chinese medicine commonly used to treat a variety of diseases. The C-30 sterols are known for their functions in numerous biological processes (Dias & Gao, 2009). Figure 9 shows some of the lanosterol derivatives isolated from *Inonotus obliquus* (Yusoo et al., 2001).



3-β-22,25-trihydroxy-lanosta-8-ene



21,24-cyclopenta-lanosta-3β,21,25-triol-8-ene

Figure 9: Lanosterol derivatives isolated from *Inonotus obliquus*

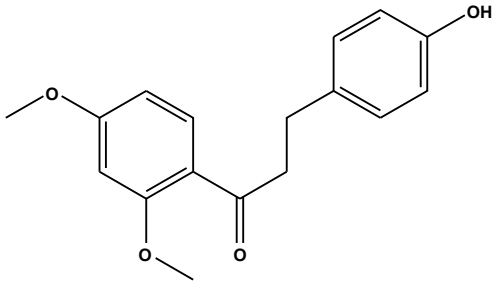
2.4.3 Chalcones

Chalcones and dihydrochalcones are distinguished from flavonoids by the open three-carbon structure linking the A and B-rings in place of a heterocylic C-ring. Chalcones and dihydrochalcones (Figure 10) are abundantly present in higher plants and some are present as polyhydroxylated chalcones. In plants chalcones are converted to corresponding (S)-flavanones in a sterospecific reaction catalysed by the enzyme *chalcone isomerase* (Veitch & Grayer, 2006).

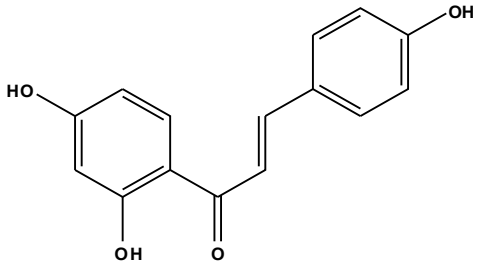
Chalcone **Dihydrochalcone**

Figure 10: Structure of chalcones and dihydrochalcone with the A and B-rings

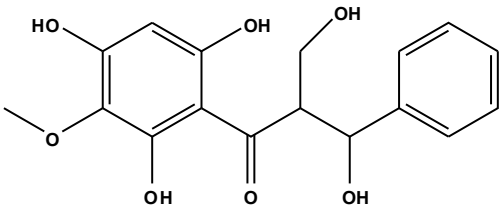
Chalcones are formed by the sequential condensation of three molecules of malonyl-CoA (acetate pathway) and ρ-coumaroyl-CoA (Shikimate pathway). The reaction is catalysed by the enzyme *chalcone synthase.* There is limited information on the biosynthesis of dihydrochalcones from chalcones (Veitch & Grayer, 2006). Figure 11 shows the compounds isolated from *Crinum bulbisperm* bulbs (A and B) (Ramadan et al., 2000) and *Polygonum ferrugineum* leaves (C) (López et al., 2006).



4-hydroxy-4',6'-dimethoxy-dihydrochalcone (A)



Isoliquiritigenin (B)



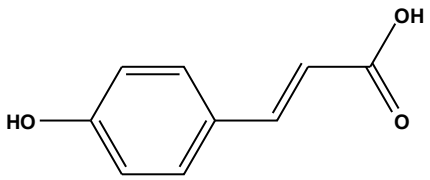
(-)-2',4',6'-trihydroxy-5'-methoxy-α-hydromethyl-β-hydroxy-dihydrochalcone (C)

Figure 11: Dihydrochalcones A, C and chalcone B isolated from *Crinum bulbisperm* bulbs and *Polygonum ferrugineum* leaves

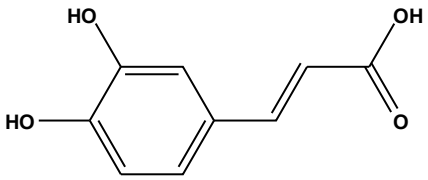
2.4.4 Polyphenolic compounds

These compounds vary from simple, single aromatic ringed compounds to large, complex polyphenols (Figure 12). They are found in higher plants and have numerous biological activities. The compounds are derived from many pathways including the phenylpropanoid, acetate, and Shikimate pathways (Handique et al., 2002). Biosynthesis produces a large variety of phenols as cinnamic acids and benzoic acids. Phenolic hydroxyl groups are known to be good H-donating antioxidants which scavenge reactive oxygen species. Phenolics act as antioxidants by inhibiting enzymes involved in radical generation (Castellano et al., 2012).

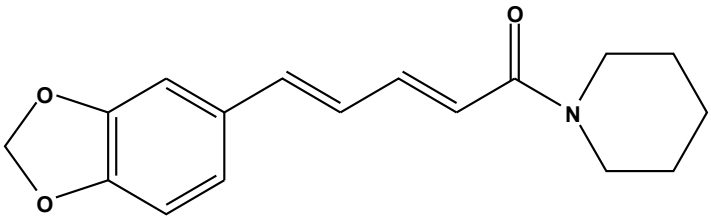
Stilbenes are also polyphenols; they have a C6-C2-C6 structure and have antifungal, antibacterial and antiviral activity (Handique et al., 2002).



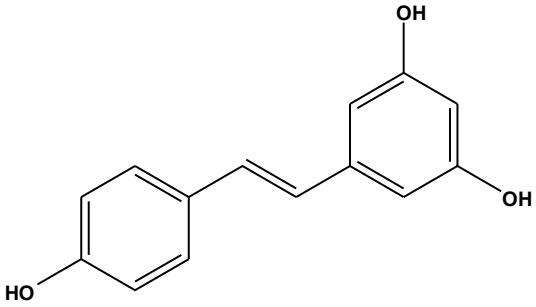
ρ-coumaric acid



caffeic acid



piperine



resveratrol

Figure 12: Polyphenolic compounds that are mostly found in essential oils

2.5 Botanical overview of the Amaryllidaceae family

Cyrtanthus is a genus of the Amaryllidaceae family. Amaryllidaceae are known to be herbaceous perennials that produce bulbs; they are widely distributed and represented by 59 genera and over 850 species all over the world. The regions with major diversity include South America (28 genera) and South Africa (18 genera). Some of the larger genera along with the number of their world-over reported species are *Crinum* (110), *Hippeasmum*(75), *Hymenocallis* (50), *Cyrtanthus* (47) and *Pancratium* (15) (Cedrón et al, 2010).

2.5.1 Compounds found in the Amaryllidaceae

Plants of the Amaryllidaceae have attracted considerable attention due to their content of alkaloids with interesting pharmacological activities. These compounds are known to be formed biogenetically by intramolecular oxidative coupling of norbelladines derived from the amino acids, L-phenylalanine and L-tyrosine, in this respect, are considered to be members of the large group of isoquinoline alkaloids (Hoshino, 1998).

Until recently, the Amaryllidaceae alkaloids have been classified structurally mainly into seven subgroups, namely, lycorenine, crinine, narciclastine, galanthamine, tazattine, lycorenine and montanine (Zhong, 2005). Renowned amongst the compounds is galanthamine, which is used in the treatment of Alzheimers disease (Pearson, 2001; Shechter et al., 2005). About 500 alkaloids with a wide range of physiological effects have been isolated up to date (Zhong, 2005).

Studies show a range of other compounds that have been isolated from the family (Table 3). Koorbanally et al. (2000), isolated cylcoartane compounds from *Ammocharis coranica* bulbs. Work done by Griffiths (2004) on *Crinum bulbisperm* bulbs probed the isolation of dihydrochalcones as well as flavonoids. Much work has been done on the isolation and characterisation of alkaloids from this family, at the expense of other phytochemicals that may have been present.

Table 3: Traditional uses of Amaryllidaceae plant species with the active constituents of each plant.

Plant species	Traditional uses	References	Active constituents	References
Boophone disticha (bulbs)	antibacterial, analgesia, anticholinesterase	Cheesman et al., 2012; Sandager et al., 2005	buphanidrine, buphanamine	Sandager et al., 2005
Crinum bulbisperm (bulbs)	antimicrobial, antimalarial, anti-inflammantory	Roberts, 1990; Griffiths, 2004	isoliquiritigenin, liquiritigenin, hippacine, isolarrien, lycorine, dihydrochalcones, flavonoids	Ramadan et al., 2000; Griffiths 2004
Ammocharis coranica (bulbs)	anticholinesterase	Koorbanally et al., 2000; Elisha, 2011	lycorine, hippadine, hamayne, demethylpluviine, 6α-hydroxypowelline, cycloartane compounds	Koorbanally et al., 2000

2.5.2 Cyrtanthus species

Cyrtanthus of the Cyrtantheae is the largest Amaryllidacea genus in Southern Africa, with 56 species (Arnold & De Weet, 1993; Snijman & Archer, 2003). The center of distribution is the South-Eastern Cape with smaller centers in the Western and Eastern Cape, Gauteng, Mpumalanga and KwaZulu-Natal (Du Plessis & Duncan, 1989; Meerow & Snijman, 1998; Snijman & Acher, 2003). The genus may be evergreen, winter-growing or summer-growing. The foliage varies among the species, from tubular and pendulous to widely bell-shaped, spreading or erect. Most bulbs of *Cyrtanthus* species are known to be rich in alkaloids (Brine

et al., 2002; Herrera et al., 2001; Nair et al., 2002) and extracts from these bulbs were shown to possess anticholinesterase activity (Table 4).

Table 4: Traditional uses of *Cyrtanthus* plant species with the active constituents of each plant.

Plant species	Traditional use	References	Active constituents	References
C. elatus (bulbs)	anticholinesterase	Herrera et al., 2001	zephyranthine, 1,2-O-diacetylzephyranthine, galanthamine, haemanthamine, haemanthidine	Herrera et al., 2001
C. contractus (bulbs)	anticholinesterase	Nair et al., 2011	narciprimine	Nair et al., 2011
C. obliquus (bulbs)	coughs, analgesic	Watt & Breyer-Brandwijk, 1962	obliquine, isoquinolinone, 11α-hydroxygalanthamine, 3-epimacronine, tazettine, narcissidine, trisphaeridine	Brine et al., 2002

2.5.3 Cyrtanthus obliquus

This study focuses on the plant species, *Cyrtanthus obliquus*, commonly known as Knysna lily (English), Knysnalelie (Afrikaans) and Umathunga (IsiZulu and IsiXhosa), which is an evergreen species with large pendulous flowers and grey-green leaves.

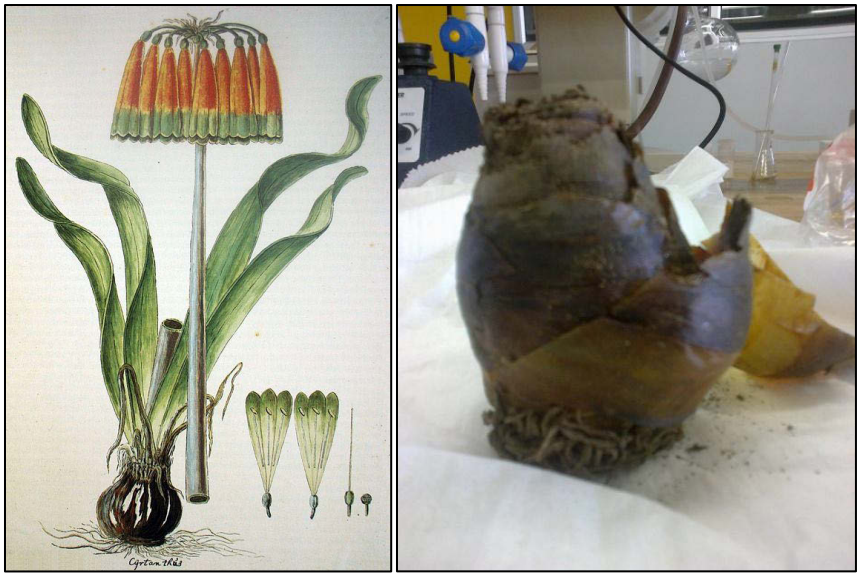


Figure 13: Bulbs of *Cyrtanthus obliquus*

The bulbs of *C. obliquus* are large and resemble onions. The flowers are pendulous bells or horizontal to upright flaring and funnel shaped, the colour of the flowers range from red to orange (Figure 13). The plant grows on dry, rocky, sloping ground or sandstone-derived soils. It is found in the coastal grasslands from KwaZulu-Natal to the Eastern Cape and through to the Western Cape. The plant is known to flower late in April and May (Du Plessis & Duncan, 1989; Hutchings et al., 1993; Leistner, 2000).

2.5.4 Medicinal uses of C. obliquus

The bulbs of *C. obliquus* are used to relieve chronic coughs and dry bulb layers are used as a snuff and to relieve headaches resulting from head wounds. The bulbs are also used medicinally to treat broken bones, cuts and abrasions (Watt & Breyer-Brandwijk, 1962).

2.6 Botanical overview of the Verbenaceae family

Lippia belongs to the Verbenaceae family. The Verbenaceae family is large with about 75 to 100 genera and more than 3000 species. It includes herbs, shrubs and trees with opposite, rarely whorled or alternate leaves, mostly found in the warmer regions of the world. The Verbenaceae are often hairy and characteristically the hairs are incrusted with calcium carbonate and/or silicic acid. Grandular hairs secreting essential oils are also common (Paulsen & Andersen, 1999).

The family is known for an abundance of polyphenolic compounds and terpenoids. These include iridoid glycosides, triterpenes, polyphenolics, isoverbascoside, and verbascoside (Table 5). Much research has been conducted on the isolation of the phytocompounds from this family and testing for biological activity. The plants are well known for their antioxidant, antimicrobial, antifungal and antihypertensive activities (Guerrera et al., 1995; Valentão et al., 2002; Deena et al., 2000; Hernadez et al., 2003; Khalifa et al., 2002)

Table 5: Traditional uses of three well known plant species found in Verbenaceae family with the active constituents.

Plant species	Traditional uses	References	Active constituents	References
Citharexylum spinosum L. (aerial parts)	antiulcer, antihypertensive, hepatoprotective	Khalifa et al. 2002	iridiod glycosides, lignan glucoside	Balázs et al. 2006
Lantana camara (leaves, roots)	antitumour, anticancer, antibacterial, antifungal, antiviral	Rwangabo et al. 1988; Deena et al. 2000; Hernadez et al. 2003; Inada et al. 1995	triterpenes, iridiod glycosides, oligosaccharides, oleanolic acid, isoverbascoside, verbascoside	Hart et al. 1976; Misra & Laatsch 2000; Ghisberti 2000
Aloysia triphylla (aerial parts)	antipyretic, antispasmodic, diuretic agent, antioxidant	Guerrera et al. 1995; Ragone et al. 2007; Valentão et al. 2002	artemitin, hesperidin, camphor, limonene, caryophyllene, luteolin 7-diglucuronide, polyphenolics	Qnais et al. 2009; Kim & Lee 2004; Carnat et al. 1995; 1999

2.6.1 Lippia species

Studies on the pharmacological activities of *Lippia* species show that some of these plants serve to treat stomach aches, influenza and other respiratory diseases (Table 6). The major compounds common in *Lippia* species are terpenoids, polyphenols, phenolic glycosides and flavonoids. Oil profiling of *Lippia* species by gas chromatography techniques afforded compounds limonene, carvone, myrcene, β-carvophyllene and α-pinene, amongst others (Dlamini, 2006; Mujovo et al., 2008; Rampier & Saubier, 1986). The essential oil has been

extensively shown to exhibit antimicrobial activity (Pascual et al., 2001). Phenylethanoid glycosides have also been isolated from *Lippia* species for example, verbacoside.

In vitro studies on extracts from *Lippia multiflora* showed the plant to possess antifungal activity whilst studies on a decoction and infusion obtained from the plant showed the plant to possess antimalarial activity (Valentín et al., 1995). Studies by Mwangi et al. (1992) indicated that *Lippia javanica* was active against *Aedes aegypti* larvae and *Sitophilus zeamais* Motan (maize weevil). Antimicrobial studies on *L. javanica* essential oils showed activity against *Klebsiella pneumonia*, *Crytococcus neoformans* and *Bacillus cereus* (Viljoen et al., 2005). The tea extracts of *L. javanica* showed the highest antibacterial activity when compared to other *Lippia* species, *Lippia wilmsii* and *Lippia scaberrima* (Shikanga, 2008).

Table 6: Traditional uses and active constituents from three plants from *Lippia* species abundant in Southern Africa

Plant species	Traditional uses	References	Active constituents	References
L. scaberrima (leaves)	respiratory diseases, antispasmodic	Combrinck et al. 2006	theviridoside, limonene, carvone	Combrinck et al. 2006
L. multiflora (aerial parts)	hepatic diseases, choleretic, vesicle ache remedy, antimalarial, antihypertensive, respiratory diseases	Pham Huu Chanh et al. 1988a, 1988b, Pousset 1989, Valentín et al. 1995, Tauobi et al. 1997, Abena et al. 1998, Mukherjee 1991, Forestieri et al. 1996	verbascoside, nerolidol, isoverbascoside, linalool, derhamnosylverbascoside, sterols, caretenoids, 1,8-cineole, β-farnesene, β-caryophyllene, germacrene-D	Valentín et al. 1995, Pham Huu Chanh et al. 1988a, 1988b, Tauobi et al. 1997
L. javanica (leaves)	analgesic, anti-inflammatory, antipyretic, antispasmodic, respiratory diseases	Mwangi et al. 1992, Hutchings & van Staden 1994	theveside, theviridoside, verbascoside, luteolin isoverbascoside, limonene, ocimene, piperitenone, linalool, *β*-caryophyllene, cirsimantin, apigenin, myrcenone, (E)-2(3)-tagetenone epoxide, 4-ethyl-nonacosane,	Rampier & Sauerbier 1986, Dlamini 2006, Mujovo et al. 2008

2.6.2 Lippia javanica

This plant belongs to the Verbenaceae family and is commonly known as the lemon bush (English), uMsuzwane (IsiZulu and IsiXhosa) or mosukudu (Tswana). It can grow up to 2m high as a woody shrub (Figure 14). The leaves of the plant have a strong lemon-like odour when crushed; it is said to be one of South Africa's aromatic indigenous shrubs (van Wyk & Gericke, 2000).

Figure 14: uMsuzwane (*L. javanica*) is known to grow in the tropics of Southern Africa

Figure 15: *L. javanica* leaves used to make tea (Dlamini, 2006)

In Botswana, *L. javanica* leaves are utilized as tea substitutes (Mosukudu tea bags (Figure 15)); this is also practised in some parts of KZN and the Eastern Cape (Dlamini, 2006). *L. javanica* is widespread throughout Southern Africa; it can be found growing from the Eastern Cape northwards towards Swaziland, Mozambique, Tanzania and Botswana. It grows well in most soil types and it grows faster in sunny areas (Palgrave et al., 2003).

2.6.3 Medicinal uses of L. javanica

The plant is known to possess analgesic, anti-inflammatory, antipyretic, and antispasmodic activities (Table 6). The Xhosa and Zulu people use aerial parts of the plant to make tea infusions to treat coughs, colds and bronchial problems. A study done by Palgrave et al. (2003) reports that the tea infusions of the leaves are used by patients in KwaZulu-Natal to treat common symptoms in HIV and Aids including the treatment of lung infections and diarrhea. *L. javanica* tea is commercially sold as caffeine-free health tea under the name 'Mosukudu tea' in Botswana (Shikanga, 2008). In some cases the tea infusion is cooled and applied as a lotion to irritated skin to treat rashes, sting and insect bites (van Wyk & Wink, 2004).

2.7. Oxidative stress

Oxidative stress is a chemical stress induced by the presence of large amounts of reactive oxygen species (ROS) in our bodies (Blomhoff, 2010). This could be caused by the increased production of ROS in our bodies or deficiency in the effectiveness of the natural antioxidant found in the human body (McCord, 2000; Sies, 1997). A consequence of this is oxidative damage, where ROS oxidize nucleic acids, proteins, lipids or DNA. This could then result in age-related illnesses and even cancer (Beckman & Ames, 1998). Antioxidants are therefore needed to counteract these processes. Antioxidants are essentially substances that are capable of reacting with the ROS, producing less harmful products. A large majority of phytochemicals found in plants are antioxidants.

Some methods of assessing a compounds' antioxidant activity are the DPPH (2,2-diphenyl-β-picrylhydrazyl) (Figure 16) assay and ferric reducing antioxidant potential (FRAP) assay. The DPPH method of analysis is known to be rapid, simple and inexpensive. It involves the use of a free radical, DPPH (purple in colour); the odd electron on the free radical gives a strong absorption maximum at 517 nm. The activity of the test compound is seen when it decolorizes the purple DPPH colour to yellow (Huang et al., 2005). The reaction is monitored by a spectrophotometer. The proposed reaction between ascorbic acid and the DPPH radical is given in Figure 17 (Wanasundara & Shahidi, 2005).

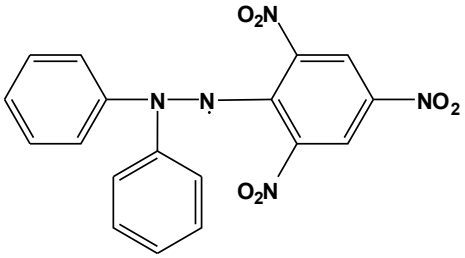


Figure 16: Structure of 2,2-diphenyl-β-picrylhydrazyl (DPPH)

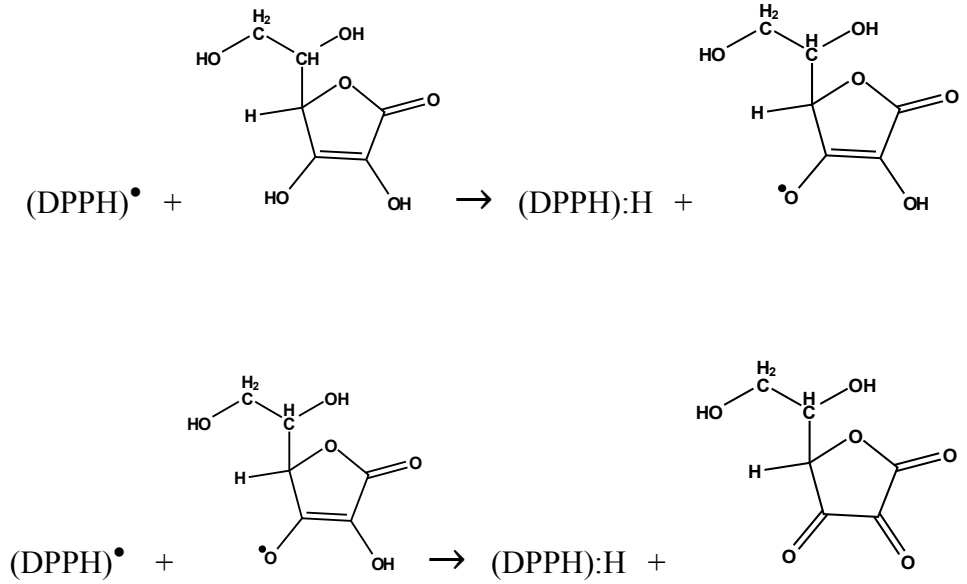


Figure 17: Proposed reaction between ascorbic acid and DPPH radical

The ferric reducing antioxidant potential measures the ferric to ferrous reduction in the presence of an antioxidant. The method is known to be simple and relatively inexpensive (Gupta et al., 2009).

$$Fe^{3+} + \text{Antioxidant} \rightarrow Fe^{2+} + \text{Oxidised antioxidant}$$

2.8 Soil

Soil is defined as weathered material on the earth's surface which may or may not contain organic matter and often also contains air and water. The primary constituents of soil are inorganic material, which is mostly produced by weathered parent rock, and different forms of organic matter, gas and water required by plants and soil organisms, and soluble nutrients used by plants (Gerrard, 2000). Figure 18 shows the characteristic soil profile which is subdivided into four horizons where plants and living organism in the soil obtain their nutrients and the composition by volume of topsoil of which 45% constitutes minerals. Soil is characterised according to its texture, colour, structure and thickness due to the merged contributions of the organic and inorganic constituents in the soil.

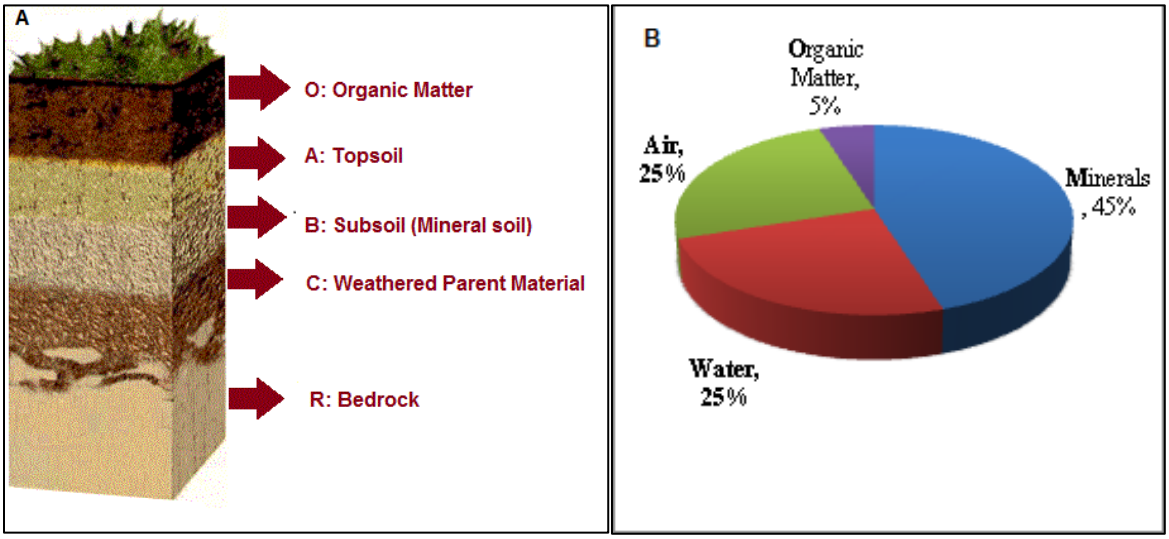


Figure 18: Soil profile showing the basic soil horizons (A) and typical composition, by volume, of an ideal topsoil (B). (AG Unlimited, nd)

The major organic constituent in the soil is humus. Humus is simply defined as decayed organic matter which breaks down into organic compounds. It gives the soil a dark brown/black colour. The organic matter helps in the ability of the soil to retain water. Soils with high humus content usually have high exchange capacities.

In order for the nutrients to be absorbed by the plant, these have to be in contact with the plant roots. The movement of nutrients from the soil to the plant root occurs in three basic ways. Firstly, through mass flow, where nutrient ions are transported to the root surface via flow with water; the transpiration process of the plant allows the nutrients in the water to be absorbed into the plant. Most common nutrient ions include Ca^{2+}, Mg^{2+}, NO_3^- and Cl^- ions.

Secondly, through diffusion which, in its basic description, are ions in solution moving from a region of high concentration to a region of low concentration. Basically, nutrients migrate from the soil (region of high concentration) towards plant roots (region of low concentration). Thirdly, through root interception which describes the process by which the roots of a plant are extended to unfamiliar parts of the soil which allows direct contact and therefore absorption of nutrients (Oxford, 2010).

Soil therefore plays an important part in the plant life cycle since it provides nutrition, protection and facilitates growth.

2.9 Soil analysis

Soil evaluations are done using two types of methods, that is, physical and chemical methods. Physical methods include the determination of soil moisture content, density, pore spaces and

mechanical analysis, just to name a few. However, chemical methods are divided into two basic kinds; the general methods involve the determination of the elemental distribution as well as composition, the specialized chemical methods include determination of organic matter, pH, salinity, cation exchange capacity (CEC) and primary nutrients (N, P, K). Chemical methods that may be investigated to establish soil quality are elemental composition, organic matter, pH and CEC (Wright, 1994).

2.10 Soil quality

The concept of soil quality looks into how well the soil performs the following functions

- A medium for plant growth
- A regulator of water flow in the environment
- An environmental filter
- Maintenance of animal and human health
- As a part of the global storage and cycling of nutrients

High quality soil usually has high organic matter and biological activity, are easily penetrated by plant roots and easily infiltrated by water. Some common indicators of soil quality are soil organic matter (SOM), pH and CEC (Lewandowski & Zumwinkle, 1999)

2.10.1 Soil organic matter (SOM)

SOM is the part of the soil that consists of plant and animal residue in various stages of decay (Poole, 2001). The functioning of the soil is influenced by the organic matter content in the soil (Carter, 2001). Organic matter increases the soil water holding capacity and water infiltration ability. It is also a good source of plant nutrients and helps to hold plant nutrients already in the soil from excessive leaching.

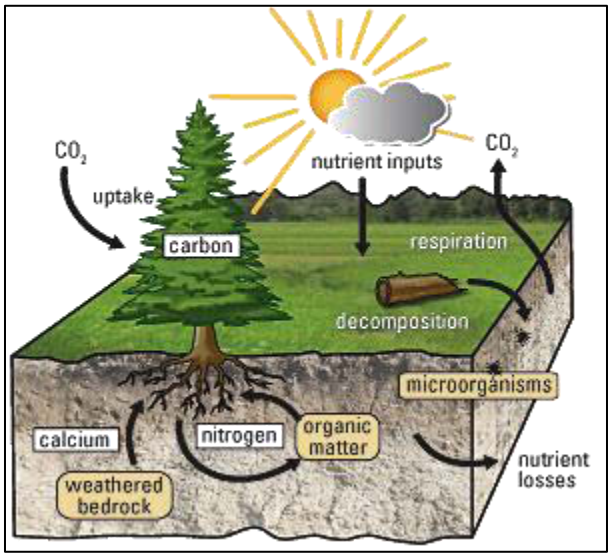


Figure 19: Basic nutrient cycle in a forest ecosystem showing the role of SOM (Beldin & Perkis, 2009)

Figure 19 shows the nutrient cycle where SOM is broken down into simpler organic compounds, which allows for the release of plant nutrients in the available form to be absorbed by plant roots (Beldin & Perkis, 2009). Once the plant withers or animal waste enters the soil, this becomes food for microorganisms in the soil where organic matter is broken; this triggers the cycle to occur all over again.

Extremes in soil pH (acid or alkaline) affect decomposition of humus thereby reducing additions of organic matter into the soil. Humus forms through biotic and abiotic processes. SOM tends to increase with an increase in the clay content (Kabata-Pendias, 2001).

2.10.2 Soil pH

Soil pH is an indication of the acidity or alkalinity of soil. The pH scale ranges from 0 to 14 and with pH 7 as the neutral point (McKenzie, 2003). Soil pH is influenced by both acid and base-forming ions in the soil. Acid conditions occur with soils having parent material high in silica content, high levels of sand with low buffering capacities and in places with high amounts of precipitation (which causes the increased leaching of base cations thus lowering the soil pH). Acid-forming cations include H^+, Al^{3+} and Fe^{2+}/Fe^{3+}.

Basic conditions arise due to the presence of base cations associated with carbonates and bicarbonates found naturally in soils. Common base-forming cations include Ca^{2+}, Mg^{2+}, K^+ and Na^+ (McCauley et al., 2009). The soil pH is normally reported with such interpretation as shown in Table 7, showing that the best suited range of the soil pH for most crops is between 6.5 and 7.0, close to the neutral point (McKenzie, 2003).

Table 7: Interpretation of soil pH (McKenzie, 2003)

5.0	**5.5**	**6.0**	**6.5**	**7.0**	**7.5**	**8.0**
Strongly acid	Medium acid	Slightly acid	Neutral	Neutral	Mildly alkaline	Moderately alkaline
			Best range for most crops			

Soil pH can be determined by using the calcium chloride or water method. Soil pH of 6.0 to 7.5 provides optimum conditions for agricultural plants. Soil pH is seen to affect the availability and the interaction of certain nutrients in the soil, for instance, low pH decreases the availability of Mo, P, Mg and Ca. Other elements such as Al, Fe and Mn may become more available and Al and Mn may reach levels that are toxic to plants. At pH greater than 7.5, Ca shows antagonistic effects on P and this gives rise to deficiencies of nutrients like Zn and Co (Lake, 2000).

2.10.3 Cation exchange capacity (CEC)

The CEC of soil is the measure of the number of sites on the soil surface that can retain positively charged ions (cations) by electrostatic forces. Cations retained electrostatically are easily exchangeable with other cations in the soil and are therefore readily available for plant uptake (Ross, 1995). The five most abundant exchangeable cations are Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and Al^{3+}. The clay minerals and organic matter of soil supply the negatively charged sites that cations are attracted to and retained. One cation on the surface of clay minerals and organic matter can be exchanged for another cation (Shaw & Andrew, 2001). As soil pH increases, the number of negative charges on clay mineral and organic matter increases, which increases the CEC. The CEC level varies according to soil type (Table 8) (Primary Industries Agricultural, 2002), soil pH and the quantity of organic matter in the soil.

Table 8: CEC levels with regards to soil type (Primary Industries Agricultural, 2002)

Soil type	CEC level	Outcome
Humus	Highest	Organic matter have large quantities of negative charges
Clay	High to low	Can attract and hold cations because of its chemical structure- but varies according to type of clay eg. Montmorillonite clay (high), Kaolinite clay (low)
Sand	Very low	Has no capacity to exchange cations because it has no electrical charge.

2.11 Total and bioavailable or exchangeable metals in soil

Heavy metals are elements that have densities greater than five and atomic weight of 23 and above. These include about 38 metals (Passow et al., 1961) amongst which are As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se and Zn. Heavy metals can enter the ecosystem through natural causes or by anthropogenic (human) activities. These metals can accumulate into the different compartments of the soil and therefore mobilization is possible due to changes in the environmental conditions inducing disorientation of the ecosystem and may cause adverse health effects to biota (Fedotov & Miró, 2008).

The total concentrations of trace elements present in soils depend on the type and intensity of weathering, the climate and other factors that predominated during soil formation. Parent rocks which are more prone to weathering form fine textured soils which are the main source of trace elements. Rocks resistant to weathering form coarse textured soils which generally have low micronutrient content (Sillanpää, 1982). Total metal concentrations in soils do not

generally correspond to bioavailable concentrations thus it is important that the mobility and availability of metals in soil is assessed.

Bioavailability is the portion of total metal concentration that is available for incorporation into biota (John & Leventhal, 1995). Only a small portion of the heavy metals are bioavailable. The mobility and availability of these metals is mostly governed by biochemical and chemical processes. These processes themselves are influenced by the pH, organic matter content, ionic exchange and other biological processes (Violante et al., 2010).

Table 9: Chemical forms of metals in soil (Gunn et al., 1988; Salomons, 1995)

	FRACTION	**MOBILITY**
T	In pore water (dissolved)	High
O	Weakly adsorbed (exchangeable)	High
T	Associated with carbonates	High
A	Associated with Fe, Mn oxides	Moderate
L	Complexed by organics	Moderate
	Associated with sulfide	Low
	In the mineral lattice	Low

Table 9 shows the chemical forms of metals in different solid phases in soil (Gunn et al., 1988; Salomons, 1995). Metals are associated with a number of sites in the soil; changes in the composition of cations may result in ion exchange which then releases weakly adsorbed cations into the soil. Changes in redox and environmental conditions may cause the release of

metal ions from soil sites thus increasing mobility and availability. Metals contained within the mineral lattice are generally not available to biota (John & Leventhal, 1995).

2.12 Soil extraction methods

Exchangeable ions in soil are determined by submerging the soil into an extractant which is usually an ionic solution. The ions weakly held on the soil surface are easily displaced by ions in the extractant solution. The extractant solution will now contain the exchangeable soil ions, in addition to its own. The choice of extractant solution is dependent on the target ions.

The most preferred extractant solution is 1M ammonium acetate (NH_4OAc) because of its relatively high concentration and the metal complexing power of acetate which prevents readsorption and precipitation of released metal ions (Ure, 1996). Acetic acid is known to dissolve exchangeable species, in addition it can release more tightly bound exchangeable forms (Rapin & Fösterner, 1983). Ethylenediamine tetraacetic acid (EDTA) is a powerful chelating agent and known to forms strong complexes with many metals (Rashid, 1974; Stover et al., 1976). Studies showed that the combination of EDTA and acetic acid attacked the carbonate phase and both extract metals in non-silicate bound phases. Metals extracted with an extractant solution that combines these three extractants can therefore better represent available forms.

2.13 Studies on heavy metal contamination in soil

Heavy metals can exist in a number of chemical forms; they can exist as free ions, as hydroxo complexes, adsorbed onto particles or complexes chelated with organic ligands. The oxidation state of a metal can change due to changes in the redox condition in the environment thus redox reactions are important as they can influence the chemical speciation of a number of metals. The free ion species is regarded as the most toxic thus monitoring its mobility within the soil and uptake by the plant is vital (Valentão et al. 2002).

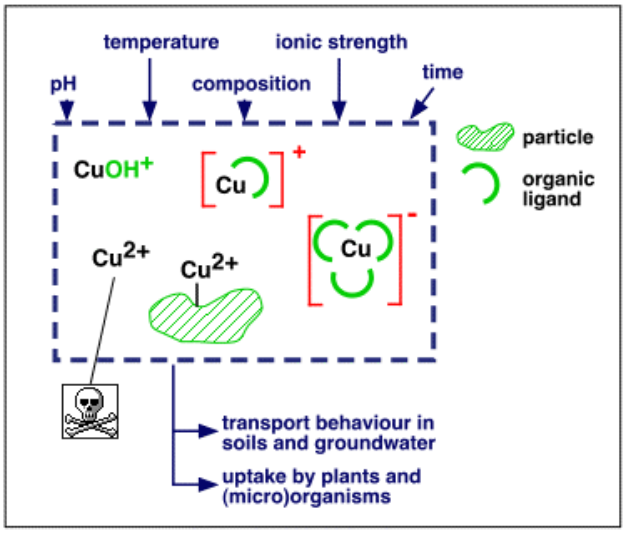


Figure 20: Various forms of copper in soil (http://www.polyql.ethz.ch)

Copper can exist in both the oxidised cupric (Cu^{2+}) and reduced cuprous (Cu^{+}) forms (Figure 20). In soil, the form of Cu present in the system is influenced by pH, temperature, redox conditions, ionic strength, time and composition. Copper can be adsorbed onto particles, complexed with organic ligands, or can be present as a hydroxide. However free Cu ions are considered to be potential toxicants due to their mobility, these ions can be absorbed by plants and microorganisms. Some other heavy metals that are considered to possess potential toxic

effects are As (As^{3+}, As^{5+}), Cd (Cd^{2+}), Cr (Cr^{6+}), Ni (Ni^{2+}), Pb (Pb^{2+}), Se (Se^{2-}, Se^{4+},Se^{6+}) and Zn (Zn^{2+}) (Blais et al. 2008).

South Africa has numerous mines and chemical industries where illegal dumping and unregulated disposal of industrial and mine waste into the soil and water system are practised. These industrial and mine wastes may contain heavy metals at high concentrations that can leach into the soil and water system (Naicker et al., 2003; Roychoudhury & Starke, 2006). Animals and humans are generally exposed to elevated levels of heavy metals by consuming or using plants that have grown on contaminated soils which then pose a serious health risk (Hussain et al, 2011). It is because of this reason that the World Health Organisation has recommended that medicinal plants be evaluated for heavy metals and other contaminants before processing to finished products (WHO, 1998).

2.14 Geoaccumulation index

Geoaccumulation index (I_{geo}) was initially introduced by Müller (1986) for the determination of the extent of metal accumulation in sediments. The I_{geo} of a metal in sediment can be calculated using the formula

$$I_{geo} \quad \log_2 \frac{C_n}{B_n \quad 1.}$$

Where C_n is the concentration of the heavy metal in the sample and B_n is the background/baseline concentration of the metal. The factor 1.5 is introduced to minimise the effect of possible variations in the background or control values which may be due to lithogenic variations in the sediment.

Table 10: Geoaccumulation index, classification and degree of metal contamination (Müller, 1986).

Geoaccumulation Index (I_{geo})	I_{geo} Classification	Degree of Metal Contamination
≤ 0	0	Uncontaminated
0-1	1	Uncontaminated to moderately contaminated
1-2	2	Moderately contaminated
2-3	3	Moderately to strongly contaminated
3-4	4	Strongly contaminated
4-5	5	Strongly to very strongly contaminated
≥	6	Very strongly contaminated

Table 9 shows the geoaccumulation indices with I_{geo} classification and associated degree of metal contamination (Müller, 1986). There are seven classes of metal contamination ranging from uncontaminated ($I_{geo} = 0$) to very strongly contaminated ($I_{geo} = 6$).

2.15 Soil-plant relationship

Nutrients are defined as major elements and trace elements that are essential for the growth of organisms (EPA, 2009). The nutrients are released into the soil through the weathering of parent rock or from the decaying of plants and other fractions. In order for nutrients to be absorbed by the plant, plant roots have to be in contact with the soil. Elemental uptake by the plant is dependent on the movement of elements from the soil to the plant root, the crossing of the elements through the root membrane and the transportation of elements by the plant cells to aerial parts of the plant.

2.16 Essential elements in plants

Essential minerals in plants include trace elements which are needed for metabolic functions within the plant. Table 11 shows some trace elements with their plant available forms, role in plants and deficiency symptoms (Jain, 2008). Although trace element deficiencies can lead to growth defects, excessive concentrations can also cause toxic effects. According to a study done by Foy et al. (1978), trace elements Mn, Zn, Cd and Se are readily translocated to the plant followed by Ni, Co and Cu and finally Cr and Pb which are the least readily translocated to the plant.

Competition arises between elements for sites in the plants. An example is the competition between As and P for uptake by plants, which is a result of their chemical similarities (Community Gardening, nd). The permeability of the cell membranes of plants may also change. The most toxic elements to higher plants are Hg, Cu, Ni, Pb, Co and Cd. Excessive concentrations of Co, Cu or Ni in most cases inhibit translocation of Fe from the plant roots to the shoots resulting in Fe deficiency (Foy et al., 1978).

Botanists have identified some plants that have a high tolerance for certain trace elements. These plants have special metal tolerance mechanisms that allow for the selective uptake of some ions and immobilization of other ions at the roots.

Table 11:Physiological function of trace elements in plants and deficiency symptoms (Jain, 2008).

Element	Available form	Role	Deficiency symptoms
Copper	Cu^{2+}	Essential component of ascorbic acid oxidase and polyphenol oxidase; component of plastocynanin	Stunted growth; distortion of young leaves
Iron	Fe^{2+} or Fe^{3+}	Constituent of cytochrome and enzymes like catalase and peroxidase; constituent of non-haeme Fe proteins involved in photosynthesis, nitrogen fixation and respiration	Chlorosis of young leaves; degeneration of chloroplast structure
Manganese	Mn^{2+}	Required for activities of some enzymes (oxidases and peroxidases) and for photosynthetic oxygen evolution	Grey-speck leaves; reduction in photosynthetic oxygen evolution
Zinc	Zn^{2+}	Essential constituent of alcohol dehydrogenase, carbonic anhydrase and other enzymes	Chlorosis, stunted leaves and internodes

2.17 Accumulators and excluders

Accumulators are plants that accumulate heavy metals, extreme accumulators are known as hyperaccumulators. These plants can be found on heavily contaminated soils and near ore deposits. Excluders are plants that are insensitive to heavy metals over a wide concentration range; these plants have developed avoidance or exclusion mechanisms (Bradl, 2005). These mechanisms somehow result in internal detoxification (Baker, 1981).

Studies on a hyperaccumulator plant, *Alysuum bertolonii*, done by two groups of researchers showed that the plant contained >10% Ni (Negri & Hinchman, 1996) and >1% Ni (Robinson et al., 1997). These studies outline the fact that accumulation of a certain element is depended on the concentration of the contaminated area. The higher the contamination of a heavy metal in an area, the greater the concentration of the heavy metal in the accumulator plant.

2.18 Bioaccumulation factor

Bioaccumulation is described by the International Union of Pure and Applied Chemistry (IUPAC, 1993) as a progressive increase in the amount of a substance in an organism which occurs because the rate of intake exceeds the organism's ability to remove the substance from the body. It is an essential process since it allows organisms to take up and store certain nutrients that are important for growth and development, such as various vitamins, trace elements, essential fats and amino acids (Focus on Chlorine Science, 2011). Bioaccumulation is the net result of the interaction of uptake, storage and elimination of a chemical.

Bioaccumulation is dependent on solubility, mobility and interactions of metals with specific sites within the body of an organism. The predicament with bioaccumulation is that trace element such as Pb, Cu and Cd, can accumulate into an organism at high concentrations thus causing toxicity (Ginawi, 2007).

The bioaccumulation factor (BAF) for the relative accumulation of a metal taken up by the plant is described as the ratio of the concentration of metal in the plant to the concentration of metal in the soil (Timperley et al., 1973)

$$BAF \quad \frac{Metal_{plant}}{Metal_{soil}}$$

The BAF can be obtained for both the total and bioavailable metal concentrations in soil.

2.19 Essential elements in humans

These are minerals which include trace elements which are needed for physiological functions in the human body. At least 21 elements have been found to be essential in animal and human life (Abdulla et al., 1996), These include C, N, O, P, K, S, Ca, Mg, Fe, Cu, Co, Mn, Mo, B, Na, Cr, F, I, Ni, Se and Zn (Agrifax, 1998). These essential elements are commonly referred to as nutrients. A nutrient is either a chemical element or compound used in an organism's metabolism or physiology. Nutrients are divided into two categories, these are macronutrients and micronutrients. Macronutrients are elements that are needed in the human body in large amounts whilst micronutrients describe elements needed in small or trace amounts in the human body.

Elements such as Ca and Mg are needed in larger amounts compared to other elements in the human body (Table 12). Ca is required for normal growth and development of the human skeleton. Mg plays an important role in the development and maintenance of bones and is essential for a wide range of enzymatic reactions (Gibson, 2005).

Table 12: Recommended Daily Allowances (RDA) of individuals[a,b].

Lifestage	Ca (mg/d)	Cr (µg/d)	Cu (µg/d)	Fe (mg/d)	Mg (mg/d)	Mn (mg/d)	Se (µg/d)	Zn (mg/d)
Males								
14-18 y	1 300	35	890	11	410	2.2	55	11
19-50 y	1 000	35	900	8	400	2.3	55	11
>51 y	1 200	30	900	8	420	2.3	55	11
Females								
14-18 y	1 300	24	890	15	360	1.6	55	9
19-50 y	1 000	25	900	18	320	1.8	55	8
>51 y	1 200	20	900	8	320	1.8	55	8

[a] Sourced from: Food and nutrition board, Institute of Medicine, National Academies, 2011

[b] RDA- Average daily intake level sufficient to meet the requirement of 97-98% healthy individual in a group

Iron is found in haemoglobin within erythrocytes and also stored in macrophages. Iron is used up in cells in the body and is essential in certain enzymatic processes. Chromium in trivalent form is an essential nutrient that functions in carbohydrate, lipid and nucleic acid metabolism. It also has a role in the regulation of insulin in diabetic patients (Strain & Cashman, 2003).

Lead facilitates the absorption and utilization of Fe. Selenium is an antioxidant nutrient and has important interactions with other antioxidant micronutrients. Selenocysteine is a component of at least 30 selenoproteins. Zinc is essential for the synthesis of lean tissue in humans; it also has an essential role in many fundamental cellular processes. Zinc has three major groups of functions in the human body, that is, catalytic, structural and regulatory (Strain & Cashman, 2003)

Table 13: Tolerable Upper Intake levels (UL)[a,b].

Lifestages (M/F)	As (µg/d)	Ca (mg/d)	Cr (µg/d)	Cu (µg/d)	Fe (mg/d)	Mg (mg/d)[c]	Mn (mg/d)	Ni (mg/d)	Se (µg/d)	Zn (mg/d)
14-18 y	ND	3 000	ND	8 000	45	350	9	1	400	34
19-50 y	ND	2 500	ND	10 000	45	350	11	1	400	40
>51 y	ND	2 500	ND	10 000	45	350	11	1	400	40

[a] Sourced from: Food and nutrition board, Institute of Medicine, National Academies, 2011

[b]UL- Highest level of daily nutrient intake that is likely to cause no adverse health effects

[c] Represents intake from a pharmacological agent only

ND- Not determinable

2.20 Synergistic and antagonistic behavior of metals

Studies have illustrated that an increase in the level of one element can lead to the increase in the availability of another, for example an increase in Cu levels can lead to an increase in the availability of Pb in soil. This describes a synergistic relationship between the metals. Similarly, an antagonistic relationship was also found where high levels of one metal in soil caused a decrease in the availability of another metal; high levels of Co, Cu or Ni are antagonistically related to Fe (Foy et al., 1978).

Interactions between metals in plants can be where the increase or decrease in the content of one of the metals in a plant can result in an increase or decrease in other metals present in the plant therefore the content of one metal can affect the content of many other metals. It can also be where the increase or decrease in the content of one metal in the plant leads to the increase or decrease in the content of only one of the other metals in the plant (Kalavrouziotis et al., 2008a).

2.21 Phytochemical and analytical techniques

The following phytochemical and analytical techniques have been used to achieve the objectives of the study.

2.22 Separation and structure elucidation techniques

The characterisation of natural products by elemental analysis, melting point and optical rotation values are today being increasingly supported by parameters of spectroscopic tools such as nuclear magnetic resonance (NMR) spectroscopy, absorption spectroscopy and infrared (IR) spectroscopy (Voelter, 1976). The first step usually taken in a phytochemical analysis is obtaining the crude extracts from plant material; this is usually achieved by solvent extraction. Only certain secondary metabolites will be extracted into the solvent, depending on the polarity of the extractant solvent. The crude extract is then rechromatographed until pure phytocompounds are obtained. This is followed by structure elucidation and characterisation. This study makes use of the methods and techniques outlined below for the phytochemical analysis.

2.22.1 Chromatographic techniques

Chromatographic techniques are widely used for the separation, identification and determination of components of complex mixtures found in natural products. Methods in chromatography make use of a stationary and mobile phase. The components of a mixture are passed through the stationary phase with the aid of a mobile phase; this process is known as

elution. Chromatographic separation depends on the differential distribution of various components of a mixture between the mobile and stationary phases. The different migration rates will lead to their separation over a period of time and distance. There are two basic chromatographic types that are extensively used in natural products research (Skoog et al., 2004).

2.22.1.1 Thin-layer chromatography (TLC)

The stationary phase is supported on a flat plate, the mobile phase then flows through the stationary phase by capillary action. This method is used for identification purposes and for determining the purity of components. The stationary phase is a powdered adsorbent fixed to an aluminum, glass or plastic plate. The results obtained using TLC can inform the type of stationary phase being used in column chromatography. It is a useful tool for determining the best solvent system for preparative separations of mixtures (Tesso, 2005).

2.22.1.2 Column chromatography (CC)

In column chromatography (CC) the stationary phase is held on a narrow tube and the mobile phase is forced through the tube by gravity or under pressure (Figure 21). The stationary phase is fixed in place in the column; the most common stationary phases are silica gel and alumina. The stationary phase is dissolved in a suitable solvent (wet packing) and applied to the column or silica gel is packed into the column (dry packing) and then the solvent is loaded onto the column (Tesso, 2005). A solvent system is then chosen with the polarity increasing

with time as fractions are collected from the column. The fractions eluted from the column are monitored by TLC.

Figure 21: Columns used in column chromatography to separate the crude extracts

2.22.2 Spectroscopic techniques

Spectroscopic analytical methods are based on the interaction of radiation and matter. The amount of radiation produced or absorbed by molecular or atomic species is measured. These measurements are based on electromagnetic radiations (Figure 22).

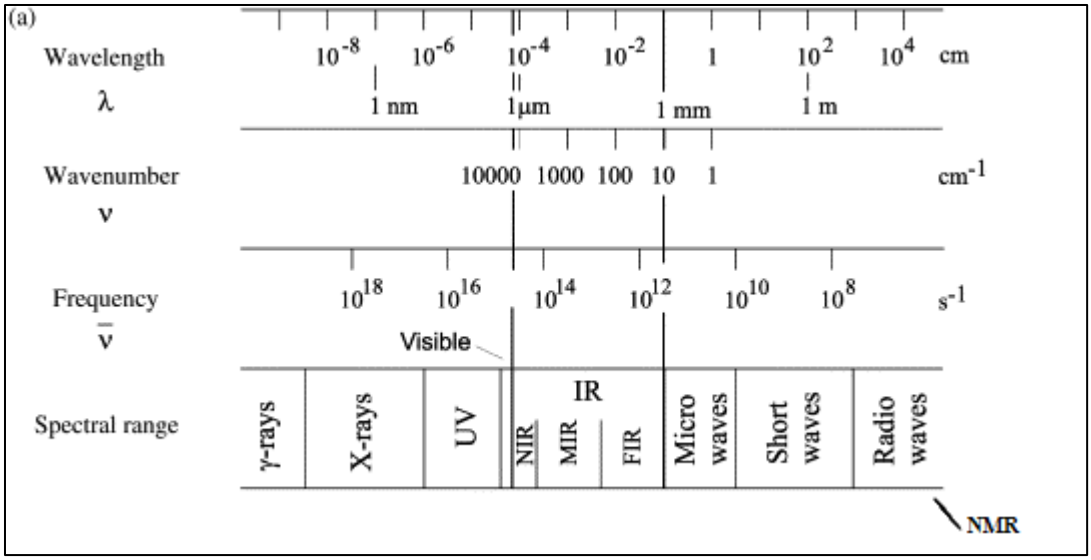


Figure 22: Regions of the electromagnetic spectrum

(http://www.ga.gov.au/minerals/disciplines/spectral-geology.html)

2.22.2.1 Nuclear magnetic resonance spectroscopy (NMR)

This technique is related on the ability of unpaired atomic nuclei to spin when interacting with radio frequency (RF) in an external magnetic field. When molecules are placed in a strong magnetic field the magnetic moment of nuclei aligns with the magnetic field. This equilibrium can be disturbed by applying RF, which brings the nuclei into an excited state. The nuclei returns back to equilibrium state by emitting RF radiations which are detected. The exact frequency of the radiation is depended upon the chemical environment.

The identity of chemical compounds can be determined by elucidating the detailed structural information obtained from one dimensional NMR (1D-NMR). In ^{13}C NMR a plot of signal arising from the different carbons as a function of chemical shift can be seen. The chemical shifts in ^{13}C NMR (0-230 ppm) are greater than those of ^{1}H NMR (0-13 ppm). The signals in

^{13}C NMR appear as singlets because of the decoupling of the attached proton. The other 1D-NMR techniques are Distortionless Enhancement by Polarisation Transfer (DEPT 90) where only signals for quaternary and tertiary carbons can be seen and are positive, whereas in DEPT 135 signals are for tertiary, secondary and primary carbons and the signals for secondary carbons appear as negative.

Two-dimensional $^{1}H,^{1}H$ Correlation Spectroscopy (COSY) gives correlation signals between crosspeaks with covalently bonded protons; these can be observed for distances up to three bonds away. The 2D-Nuclear Overhauser Enhancemnent Spectroscopy (NOESY) represents interactions between proton nuclei that are 5Å closer to each other in space (long-ranged correlations). There is also 2D-Heteronuclear Single Quantum Coherence (HSQC) spectroscopy where there are correlations between a carbon and its proton(s), ^{1}H-^{13}C one bond correlation. Correlations of protons with more distant carbons is referred to as 2D-Heteronuclear Multiple Bond Correlations (HMBC) spectroscopy.

2.22.2.2 Other spectroscopic techniques

IR spectroscopy is a tool used for the identification of pure organic and inorganic compounds; it commonly used for qualitative applications. IR energy can excite vibrational and rotational transitions but is insufficient to excite electronic transitions. The number of ways in which a molecule can vibrate is related to the number of atoms and therefore the number of bonds it contains. Investigation of absorption bands on the spectrum can provide information on the functional groups and the overall constitution of the molecule. Fourier-transform infrared (FTIR) is a spectrometer that has been proven to possess high sensitivity, resolution and great

speed. Attenuated total reflectance (ATR) sampling technique analyses both liquid and solid samples. The ATR accessory (eg. diamond) measures the changes that occur in a totally internally reflected infrared beam when the beam comes into contact with the sample when coupled with FTIR. Coupling of ATR with FTIR yields excellent quality of data, reproducibility and spectral acquisition (Perkin Elmer-FTIR-ATR, nd).

Ultraviolet/Visible (UV/Vis) Spectroscopy is useful in the determination of organic compounds containing one or more of the unsaturated heteroatoms or organic chromophores (unsaturated organic functional groups). When organic compounds absorb radiation between 180 nm to 780 nm there are interactions between photons and electrons that occur resulting in absorption bands (Skoog et al., 2004)

2.22.3 Gas chromatography-mass spectrometry (GC-MS)

The integration of gas chromatography (GC) and mass spectrometry (MS) into a single system has shown to have many advantages. The GC is based on the repeated partitioning or adsorption between a mobile phase and stationary phase, where components of a mixture are separated. The mobile phase is known as a carrier gas and the stationary phase can either be a solid or liquid.

The mass spectrometer measures the mass-to-charge ratio (m/z) of ions that have been produced from the sample. Most of the ions are singly charged ($z = 1$). Several ionization sources for MS are available; one of the most commonly used is electron impact source, where the molecules are bombarded with a high energy beam of electrons. This then results

in the fragmentation of the molecule producing positive ions, negative ions and neutral species. The positive ions are directed to the analyser by electrostatic repulsion and attractions. The fragments are very helpful in identifying molecular species entering the spectrometer (Hübschmann, 2009).

2.22.4 Liquid chromatography-electrospray ionisation-mass spectrometry (LC-ESI-MS)

LC-ESI-MS is a powerful tool for the analyses of small and large molecules of various polarities in a complex biological sample (Ho et al., 2003). The sample solution is brought into the LC system by an autosampler or otherwise the sample solution is injected into the system. The sample solution is then introduced to an ionization source (ESI), the ions that are produced in the source are directed to a mass analyser (ion trap) then to the ion detector (MS). The mass analyser seperates the ions according to their mass to charge (*m/z*) ratios.

Electrospray is a method used to dissipate liquid samples in a homogenous form (Wilm, 2011). A potential is applied to the liquid held at the nozzle of the spray chamber. The electric field produces charged sprays which desolvate, as they reduce in size the surface tension increases due to increased charge density. They then approach the Rayleigh limits which causes the eruption of the desolvated droplet, a large quantity of small droplets are produced which are most likely to be the major source of ions detected by a mass spectrometer (Wilm, 2011; Grimm & Beauchamp, 2010).

2.23 Instrumentation

Determination of the concentration of analytes in a sample requires the sample itself to be in aqueous form. The choice of reagents and techniques to carry out decomposition and dissolution of the sample is vital inorder to determine the content of the analytes in the sample. Specialised methods are then used to qualitatively and quantitatively determine the concentrations of analytes in the sample. Microwave digestion was used as a decomposition method in this study and inductively coupled plasma - optical emission spectrometry (ICP-OES) was the technique used to determine the elemental concentrations in the samples as it is a highly selective, rapid and convenient tool for elemental determination.

2.23.1 Microwave digestion

Microwave decompositions were first introduced in the mid-1970s, it has proved to be of extreme importance in the sample preparation step. The sample preparation step takes minutes rather than hours. Microwave digestion can be carried out in either open vessels or sealed vessels; however sealed vessels are much more popular due to the fact that the loss of volatile substances and contamination from external sources are minimal. Again, in sealed vessels higher pressures and higher temperatures can be achieved (Matusiewicz, 2003; Lamble & Hill, 1998; Levine et al., 1999).

The main advantage of microwave digestion is faster decomposition of the sample compared to the usage of a hotplate where it can take hours. Microwave energy is transferred directly to all the molecules of the solution at the same time without heating the vessels (Figure 23) whereas in hotplates (conventional heating) heat energy is transferred via conduction to the

vessels. These vessels are normally poor conductors thus more time is required for heating the vessels than the solutions in the vessels. Uneven heating of the solution is also a problem in conventional heating methods (Skoog et a.l, 2004).

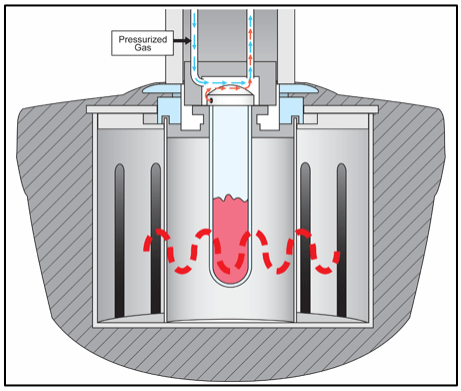


Figure 23: Diagram showing the path of microwave energy
(http://www.cem.com/discover-spd-features.html)

Microwave vessels consist of two-piece designs; liners and caps composed of high purity Teflon or PFA with outer jackets made of polyetheramide or other strong microwave transparent composite material. These materials are stable under high temperatures and pressures and are resistant to chemical attack by various acids used in digestion. Teflon is used in most cases since it has a melting point of 300˚C and is not attacked by many common acids. In cases where sulfuric or phosphoric acid is used then quartz or borosilicate glass vessels are used due to the acids high boiling points, above the melting point of Teflon (Matusiewicz, 2003).

Figure 24: CEM MARS 6 microwave
(http://www.uiw.edu/chemistry/chemfacilities.html)

Figure 24 shows a microwave designed to hold a maximum of 24 vessels, these vessels are held on a turnable that can rotate through 360 degrees so that the average energy received by each vessel is the same.

2.23.2 Inductively coupled plasma-optical emission spectrometry (ICP-OES)

The ICP-OES is a commonly used instrument for the determination of the concentration of various elements in a sample (Figure 25). The sample is introduced in liquid form; this means that solid samples have to be brought into solution by digesting in a suitable acid.

The sample is introduced into the capillary tube by a nebulising gas flow. The high velocity gas at the tip of the capillary breaks the sample solution into an aerosol. The droplets from the aerosol are then separated according to size, large droplets go to the drain and the fine droplets are transported to the plasma.

The plasma consists of three concentric quartz tubes through which streams of Ar gas flow. Surrounding the tube is an induction coil powered by a radio frequency generator capable of

producing 2 kW of energy (Figure 26). The plasma vapor contains atoms and ions which are highly excited to a state of radiated light (photon) emission. Spectral observations are generally made 15 to 20 mm above the induction coil, where temperatures of 5 000 to 6 000 K are reached.

Figure 25: Image of an ICP-OES Optima 5300 DV at the School of Chemistry and Physics (UKZN)

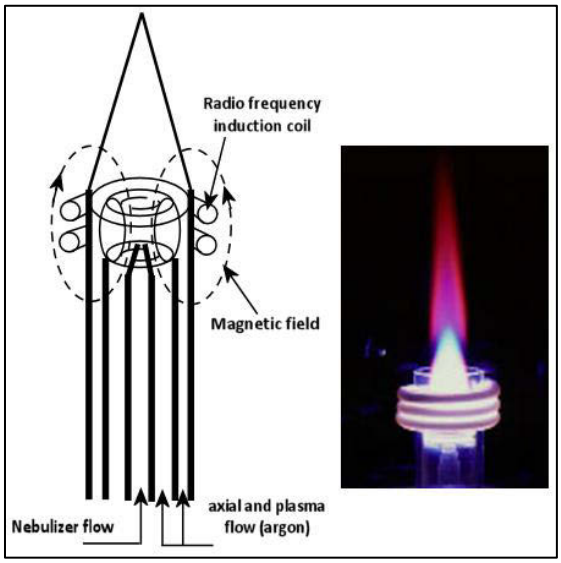


Figure 26: ICP source with a brilliant white opaque core topped by a flame-like tail (http://www.chemiasoft.com/chemd/node/52)

Atomic or ionic emission from the plasma is then separated into respective constituents' wavelengths by the wavelength isolation device. The separation can occur in a monochromator, a polychromator or a spectrograph. The simultaneous spectrometer uses polychromators or spectrographs where a range of wavelengths are scanned. The dispersive devices in theses spectrometers can be gratings or a combination of a grating and a prism (Figure 27). Multi-elements can then be determined instantaneously (Skoog et al, 2004).

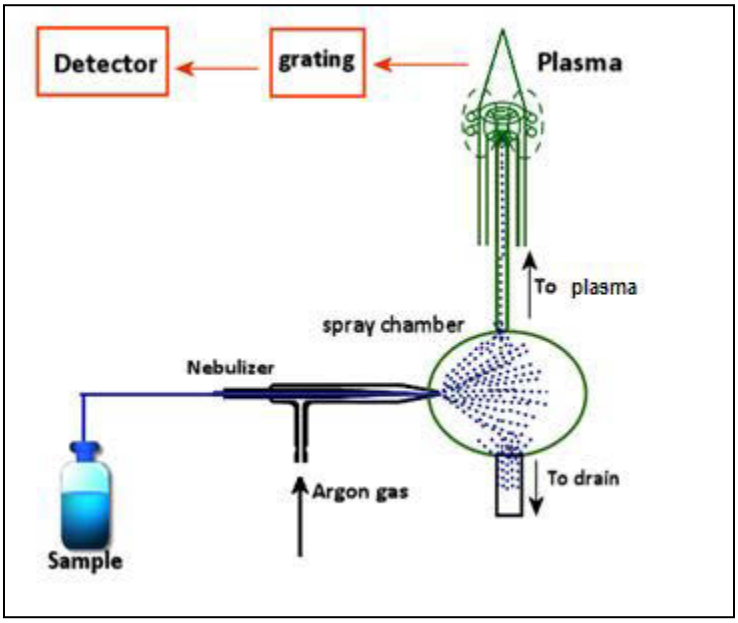


Figure 27: Diagram depicting the pathway of a sample solution through the ICP-OES (http://www.chemiasoft.com/chemd/node/52)

The charged coupled device (CCD) has become popular as an array detector for simultaneous and some sequential spectrometers. It uses a quantity of electrical charge to represent an analog quantity, such as light intensity, sampled at discrete times. The memory function comes from shifting these charges, simultaneously, down a row of cells, also in discrete time (Felber, 2002).

2.23.2.1 Detection limits

Detection limits (DL) is the smallest concentration that can be reported with a certain level of confidence. The detection limits for ICP-OES are in the sub ppb-ppm range.

Table 14: Typical detection limits reported in ICP-OES (www.perkinelmer.com.cn.46-74713PRD-Optima7000D.pdf).

Element	Wavelength (nm)	DL (ppb)
As	193.75	0.90
Cd	228.80	0.07
Co	228.61	0.25
Cr	267.71	0.25
Cu	324.75	0.90
Fe	259.94	0.20
Mn	257.61	0.03
Ni	221.64	0.37
Pb	220.35	1.40
Se	196.09	4.00
Zn	206.19	0.20

2.23.2.2 ICP-OES interferences

Spectral interferences in ICP-OES are caused by background emissions from an element other than the analyte, by causing a stray light to appear within the band-pass of the wavelength selection device, overlapping of the spectral line with that of the analyte, or unresolved overlap of molecular band spectra. Subtracting background emission is usually necessary for most analytical emission lines. Spectral overlap can be avoided by choosing alternative lines.

Physical interferences are caused by the alteration of the ionization process, where substances in the sample change the solutions viscosity. The flow rate and the efficiency of the nebulisation process of the sample solution will then be affected. Combustible constituents

such as organic solvents can change the atomizer temperature and thus affect the atomization efficiency indirectly. In other cases, matrices may not be similar; where the sample contains acid whilst standard solutions do not, this would result in differing flow rates of solutions.

Chemical interferences occur when one element supplies an excess of electrons to the plasma, boosting the neutral atom population of the less concentrated alkali metal, thus causing an enhancement in the emission intensity, relative to the standards (USA-EPA, 2004). In the few cases where this interference exists, it may be necessary to increase the RF power and/or reduce the inner argon flow to eliminate a chemical interference (Chemistry lab cookbook, nd).

2.24 Quality assurance

The best way to validate an analytical method is to analyse a standard whose analyte composition is reliably known. This standard should closely resemble the sample to be analysed with respect to the analyte concentration and overall composition (Skoog et al, 2004). Certified reference materials (CRMs) are therefore needed. The concentrations of analytes in CRMs are authenticated by a series of laboratories.

The international Organisation for Standardisation (ISO) defines CRMs as reference materials, accompanied by a certificate, one or more of whose property value are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by uncertainty at a stated level of confidence.

Method validation and accuracy of the trace element measurements in this study, were determined by the use of a standard reference material *lyophilized brown bread* (BCR 191), from the Community Bureau of Reference of the Commission of the European Communities (attached in the Appendix). This particular CRM was chosen due to matrix similarities. The CRM was provided as a fine dry powder. The CRM was digested and the obtained concentrations were compared to the certified concentrations. This also allowed the evaluation of the extent of the digestion method.

2.25 Walkley-Black method principles

This method is used for the determination of SOM. Potassium dichromate in acid medium is utilized as a digester. The chromate ions are in excess therefore a known amount of the reagent reacts with the organic matter of the soil. The amount left is then back-titrated with a known concentration of a ferrous solution, in order to estimate the quantity of organic matter in that particular soil (Schulte & Hoskins, 2009).

Chromate ions will react with carbon as follows:

$$2\,Cr_2^{\ 2}\quad 3C^0\quad 16\qquad Cr^3\quad 3C_{\ 2}\qquad {}_2$$

In order to convert from organic carbon to organic matter the following equation is used:

$$\text{organic matter}\quad \frac{\text{total C}\quad 1.2}{0.}$$

Ferrous ions react with chromate ions as follows:

$$Cr_2^{2} \quad 6Fe^{2} \quad 1 \qquad 2Cr^{3} \quad 6Fe^{3} \qquad _2$$

2.26 Chapman method principles

There are numerous methods for the determination of the CEC of soil; there is one by Chapman (1965) which utilises ammonium acetate at pH 7.0. The method has been widely used in the United States for decades and therefore a large database has been collected for soil CEC by this method (Ross, 1995). This method has three basic steps. Firstly, the soil is saturated with ammonium acetate solution, which then allows for the exchange of the metal cations adsorbed on the soil surface with the ammonium cations. Secondly, the excess ammonium acetate solution (saturating solution) is then removed by the addition of ethanol. Thirdly, the adsorbed ammonium cations are then replaced by potassium cations (KCl solution) and the amount of ammonium released is determined by Kjeldahl distillation. Kjeldahl distillation involves the conversion of organic nitrogen to ammonia by distillation.

Firstly ammonium ions are converted to gaseous ammonia

$$N \qquad N_{3} \quad _2$$

This is followed by the distillation and reaction of ammonia with a known concentration of hydrochloric acid (excess)

$$N_{3} \quad Cl_{xs} \quad N \quad Cl \quad Cl_{left}$$

The acid that is left-over is then back-titrated with a standard sodium hydroxide solution

$$Cl_{left} \quad Na \qquad Na \quad Cl \quad _2$$

CHAPTER 3

PHYTOCHEMICAL ANALYSIS OF CYRTANTHUS OBLIQUUS AND LIPPIA JAVANICA

3.1 Introduction

This section centers around the results and discussion obtained from the phytochemical analysis of *C. obliquus* bulbs and *L. javanica* leaves. These plants are commonly used to prepare the herbal tonic known as Imbiza in South Africa. The analysis of *C. obliquus* involved the isolation of the phytocompounds from the crude extracts obtained from the bulbs. These phytocompounds were then characterised by spectroscopic techniques. The DCM and EtOAc extracts from the leaves of *L. javanica* were profiled with the aid of GC-MS and identified using the NIST 05 Database library.

EXPERIMENTAL

This section of the chapter focuses on the methods utilized in the phytochemical analysis that is the extraction, isolation and characterisation of compounds found in *C. obliquus* bulbs *a*nd *L. javanica* leaves.

3.2 C. obliquus (L.f.) Aiton

Phytocompounds from *C. obliquus* bulbs where isolated and identified by the use of various analytical techniques such as 1D NMR, 2D NMR, IR spectroscopy, UV-vis spetrophotometry amd LC-ESI-MS. The antioxidant activity of selected isolated compounds was determined by the DPPH free radical and ferric reducing antioxidant power (FRAP) assays.

The melting points were recorded on an Ernst Leitz Wetzer micro-hot stage melting point apparatus. Specific rotations were measured at room temperature in methanol on a PerkinElmerTM, Model 341 Polarimeter with a 10 cm flow tube. IR spectra were recorded on a Perkin-Elmer Universal ATR Spectrometer. UV spectra were obtained in methanol on a UV-Vis-NIR Shimadzu UV-3600 Spectrophotometer. All 1D and 2D NMR spectra were recorded using a Bruker Avance^{III} 400 MHz NMR spectrometer. All LC-MS spectra were obtained from the Agilent LC/MSD Trap 1100 Series.

3.2.1 Collection and extraction

The bulbs of *C. obliquus* were purchased at Durban Berea market and identified by Mr Phungula (herbalist). The plant material was then cut into small pieces using a stainless steel knife, than air-dried for a week. Small pieces weighing 2041.78 g were than soaked in 500 mL of hexane and shaken on the orbital shaker for 48 hr. The mixture was then filtered; the residual plant material was kept aside and the filtrate was evaporated under reduced pressure until almost dryness, and then stored in the evaporation room for further analysis. The plant residue was soaked in turn in 500 mL DCM followed by 500 mL MeOH and treated in the same manner as above.

3.2.2 Sample fractionation and isolation of pure compounds

The hexane and DCM extracts were combined due to similar TLC profiles. The mass of the combined extracts was 16.39 g. The extract was loaded onto a column packed with silica gel slurry. The extract was then separated with hexane: ethyl acetate step gradient system starting from 100% hexane till 90% EtOAc in hexane was reached. The collected fractions were analysed using TLC to determine if separation had occurred. Fractions with similar TLC profiles were combined and concentrated using the rotary evaporator. The following compounds where obtained after separation with hexane: ethyl acetate (8:2) solvent system; compound **1** (214.5 mg) and compound **2** (45.7 mg).

The crude MeOH extract was mixed in 500 mL water then placed into a 2 L separating funnel and extracted with 500 mL DCM for 48 hr. The DCM fraction was run out and concentrated using a rotary evaporator and placed aside. Thereafter, the MeOH extract in the separating

funnel was extracted with 500 mL EtOAc for 48 hr, was run out and concentrated, similar to the DCM extract. The DCM and EtOAc fractions where loaded into separate columns. The column containing the DCM fraction (9.48 g) was separated with a hexane: EtOAc step gradient starting from 100% hexane till 100% EtOAc was reached. Again fractions with similar TLC profiles were combined and concentrated using the rotary evaporator. Compound **3 (**3.9 mg) was obtained when the solvent system was at 80:20.

The EtOAc fraction (3.40 g) was separated with a DCM: MeOH solvent system starting from 100% DCM which was gradually increased to 30% MeOH in DCM. Compound **4** (3.1 mg) was obtained as a white solid at 20% MeOH in DCM. Fractions 51-64 from this extract were combined and further purified using DCM: MeOH (98:2) yielding compound **5** (4.3 mg).

3.2.3 Physical data of Compound 1

2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone

Yellow crystals

Yield: 214.5 mg

Melting point: 90-92˚C

$[\alpha]^{20}$D: -0.11˚ (*c* 0.10, MeOH)

IR: 3391 (O-H), 2932 (-CH), 1638 (C=O), 1602 (-C=C-, aromatic), 1461, 1018 (C-O) cm^{-1}

UVλ_{max} (Me) nm (log ε): 216 (.20) , 292 (3.), 3 (3.)

LC-ESI-MS (negative mode): *m/z* 329.0 $[M^+ - (H_2O+1H^+)]^-$, (Calc. for $C_{18}H_{17}O_6$, 329.1)

3.2.4 Physical data of compound 2

2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-chalcone

Orange crystals

Yield: 45.7 mg

Melting point: 130-133˚C

$[\alpha]^{20}D$: 0.01˚ (*c* 0.10, MeOH)

IR: 3360 (O-H), 2931 (-CH), 1636 (C=O), 1600 (-C=C-, aromatic), 1513, 1460, 1019 (C-O) cm^{-1}

UVλ_{max} (Me) nm (log ε): 21 (.1) , 360 (3.30)

LC-ESI-MS (negative mode): *m/z*: 329.0 $[M-(OH^-)]^-$ (Calc. for $C_{18}H_{17}O_6$, 329.3)

3.2.5 Physical data of compound 3

2',4',6',4-tetrahydroxy-5'-methoxy-α- hydroxymethyl-β-methyl-dihydrochalcone

Pale yellow needles

Yield: 3.9 mg

Melting point: 194-196˚C

$[\alpha]^{20}D$: 0° (*c* 0.10, MeOH)

IR: 3288 (O-H), 2945 (-CH), 2831, 1693(C=O), 1586 (-C=C-, aromatic), 1450, 1019 (C-O) cm^{-1}

UVλ_{max} (Me) nm (log ε): 216 (.13) , 362 (3.)

LC-ESI-MS (negative mode): *m/z*: 282.9 $[M-(CH_2OH+(H_2O+1H^+))]^-$ (Calc. for $C_{16}H_{11}O_4$, 283.1)

3.2.6 Physical data of compound 4

3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene

White solid

Yield: 3.1 mg

Melting point: 245-2 ° C

$[\alpha]^{20}D$: 0.02° (*c* 0.10, MeOH)

IR: 3325 (O-H), 2943 (C-H), 2832, 1658, 1449($-CH_2$), 1410, 1108, 1019 (C-O)

UVλ_{max} (Me) nm (log ε): 213 (.36) , 262 (2. 3)

LC-ESI-MS (negative mode): *m/z*: 649.5 $[M-(C_3H_7O)^+]^-$ (Calc. for $C_{38}H_{65}O_8$, 649.5)

3.2.7 Physical data of compound 5

2',4',6',4-tetrahydroxy-α-hydroxymethyl-chalcone

Yellow crystals

Yield: 4.3 mg

Melting point: 205-20 ˚ C

$[\alpha]^{20}D$: 0.01˚ (*c* 0.10, MeOH)

IR: 3211 (O-H), 2942 (-CH), 2831, 1673 (C=O), 1587 (-C=C- aromatic), 1450, 1022 (C-O) cm^{-1}

UVλ_{max} (Me) nm (log ε): 213 (.09) , 292 (2.), 362 (3.20)

LC-ESI-MS (negative mode): *m/z*: 282.9 $[M-(H_2O+1H^+)]^-$ (Calc. for $C_{16}H_{11}O_4$, 283.1)

3.2.8 Antioxidant activity

The antioxidant activity of the compounds isolated from C. *obliquus* was determined by two methods, the FRAP and DPPH assays.

3.2.8.1 Measurement of free radical scavenging activity using the DPPH assay

The scavenging activity (antioxidant capacity) of the plant phytocompounds on the stable radical, DPPH, was evaluated according to a method by Murthy et al. (2012) with some modifications. A volume of 150 µl of methanolic solution of the compound at different

concentrations of the compounds (1000, 500, 200, 100, 50, 40, 30, 20 and 10 µg ml^{-1}) was mixed with 2850 µl of the methanolic solution of DPPH (0.1 mM). An equal amount of MeOH and DPPH without sample served as a control. After 30 min of reaction at room temperature in the dark, the absorbance was measured at 517 nm against methanol as a blank using a UV spectrophotometer as mentioned above. The percentage free radical scavenging activity was calculated according to the following equation:

$$\text{Scavenging activity} \quad \frac{Ac\ As}{Ac} \times 100$$

Where Ac = Absorbance of control and As = Absorbance of sample

3.2.8.2 Determination of the reducing potential using the FRAP assay

The total reducing power of the compounds from *C. obliquus* bulbs was determined according to the FRAP method as described by Murthy et al. (2012) with some modifications. A 2.5 mL volume of different concentrations of the compounds (500, 200, 100, 50, 40, 30, 20 and 10 µg ml^{-1}) was mixed with 2.5 mL phosphate buffer solution (0.2 M, pH = 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] in test tubes. The mixture was placed in a water bath of 50 ºC, for 20 min. A volume of 2.5 mL of 10% trichloroacetic acid (TCA) was added to the mixture and mixed thoroughly. A volume of 2.5 mL of this mixture was then mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ of 0.1% solution and allowed to stand for 10 min. The absorbance of the mixture was measured at 700 nm using a UV-VIS spectrophotometer (UV Spectrophotometer Biochrom Libra S11, Cambridge, England); the higher the absorbance of

the reaction mixture, the greater the reducing power. Ascorbic acid was used as a positive control for this assay. All procedures were performed in triplicate.

3.3 L. javanica (Brum.f.) Spreng

The extracts obtained from the L. javanica leaves were profiled by GC-MS. The mass spectra of compounds obtained from the extracts were compared to the instruments library National Institute of Standard and Technology data bank (NIST 05, 2005).

3.3.1 Collection and extraction

The leaves of *L. javanica* were collected from Eshowe in KwaZulu-Natal and identified by Mr Phungula (herbalist). The leaves where then air-dried for a week. Afterwards, the leaves where crushed into a fine powder by use of a food processor (*Russell Hobbs* range). The powdered leaves (128.27 g) were then soaked in 500 mL hexane and shaken on the orbital shaker for 48 hr. The mixture was then filtered; the plant residue was kept aside and the filtrate was then evaporated under reduced pressure until almost dryness, and then stored in the evaporation room for further analysisuse. The plant residue was then soaked in turn in 500 mL DCM followed by 500 mL MeOH and treated in the same manner as above.

The hexane and DCM extract where combined due to similar TLC profiles, the hexane/DCM extract weighed 3.5 g. The crude MeOH extract (10.1 g) combined with 500 mL water was placed into a 2 L separating funnel then extracted with 500 mL DCM for 48 hr. The DCM fraction was then run out and concentrated using a rotary evaporator and placed aside. This

fraction weighed 1.0 g. The MeOH extract was then extracted with 500 mL EtOAc for 48 hr, run out of the separating funnel and concentrated, similar to DCM fraction. This fraction weighed 0.3 g.

The hexane/DCM extract, the DCM fraction from MeOH extract and the EtOAc fraction from MeOH extract were all profiled by GC-MS.

3.3.2 GC-MS analysis

Samples were analysed on an Agilent GC–MSD apparatus equipped with a DB-5SIL MS (30 m x 0.25 mm i.d., 0.25 μm film thickness) fused-silica capillary column, operating in electron impact mode (EI) and the splitless method was utilised. The hexane/DCM extract and DCM fraction from MeOH extract were combined for this analysis. Then 10 mg each of the DCM and EtOAc fraction from MeOH extract were diluted into 10 mL volumetric flasks (10 ppm) with DCM and EtOAc, respectively, thereafter 1 μL of each sample solution was injected into the GC-MS. Table 14 shows the conditions for the analyses. The total GC-MS running time was 22 min. The mass spectra obtained were compared with the National Institute of Standard and Technology data bank (NIST 05, 2005).

Table 15: Conditions for GC-MS analysis.

Method conditions	
Column type	DB-5SIL MS (30 m x 0.25 mm i.d., 0.25 µm film thickness) fused-silica capillary column
Injection volume	1.0 µl
Injector temperature	2 ˚C
Carrier gas	Helium
Column flow	1.0 ml/min
Oven temperature	60˚C
Oven programme	60˚C ramped to 260˚C at ˚C/m in for 10 min, then held at 260˚C for 10 min

RESULTS AND DISCUSSION

3.4 Compounds isolated from C. obliquus bulbs

3.4.1 Isolation of compound 1

Compound **1** was isolated from the hexane/DCM extract by means of CC with silica gel as the stationary phase. The compound was eluted with a hexane: EtOAc solvent system (80: 20). The compound was isolated as yellow crystals with a mass of 214.5 mg. All spectra for compound **1** are found in the appendices. The NMR data is shown in Table 16.

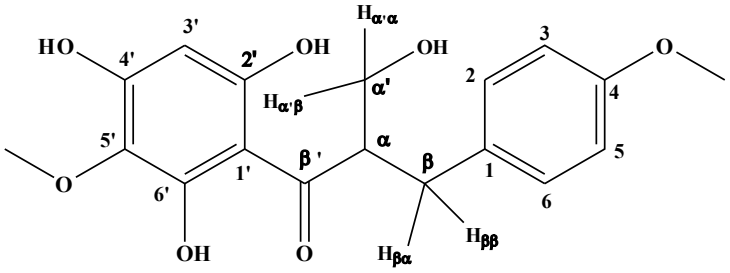


Figure 28: Compound **1** - 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone

The ^{1}H-NMR spectrum for compound **1** showed resonances in the aromatic region at δ_H 6.84 (d, H-3/5, *J* . 2 z) and δ_H 7.11 (d, H-2/6, J = 8.52 Hz), each integrating to two protons. The protons at δ_H 6. were coupled to those at δ_H 7.11 as confirmed by the COSY experiment. The ^{13}C-NMR spectrum for compound **1** showed strong signals at δ_C 113.9 and δ_C 129. which in the SQC experiment correlated to the protons at δ_H 6. and δ_H 7.11, respectively. The methoxy group singlet resonating at δ_H 3.77 was attributed to C-4 in the B

ring. This position was confirmed by MB C correlations where the carbon resonance at δ_C 158.2 (C-) correlated to protons at δ_H 3.77 (OCH_3) as well as δ_H 6.84 (H-3/) and δ_H 7.11 (H-2/6).

The ^{1}H NMR spectrum showed distinct resonances at δ_H 2.69 (dd, H-βα, *J* = 10.39 Hz, 13.79 z), δ_H 3.16 (dd, H-ββ, *J* .32 z , 13. z), δ_H 4.17 (dd, H-α'α, *J* = 7.10 Hz, 11.39 Hz) and δ_H 4.33 (dd, H-α'β, *J* = 4.10 Hz, 11.39 Hz), each integrating to one proton. The HSQC experiment confirmed the position of the protons at δ_H 2.69 (H-βα) and δ_H 3.16 (H-ββ) by their correlation to the carbon at δ_C 31.8 (C-β); this was further affirmed by the COSY experiment which showed coupling of the protons at δ_H 2.69 (H-βα) and δ_H 3.16 (H-ββ). Likewise, the SQC experiment confirmed the position of the protons at δ_H 4.17 (H-α'α) and δ_H 4.33 (H-α'β) by their correlation to the carbon at δ_C 69.1 (C-α'). The DEPT experiment showed methylene resonances at δ_C 31.8 (C-β) and δ_C 69.1 (C-α'), as expected, with the resonance at δ_C 69.1 shifting more downfield due to the attachment of the hydroxy group.

The ^{1}H-NMR spectrum showed a multiplet at δ_H 2.83 (H-α), integrating to one proton which correlated to the resonance at δ_C 46.6 (C-α) in the HSQC experiment. The NOESY experiment showed coupling of the protons at δ_H 2.83 (H-α) with protons at δ_H 4.17 (H-α'α) and δ_H 4.33 (H-α'β). This confirmed the presence of an –OC-CH(CH_2OH)-CH_2 – as was also observed by López et al. (2006). The carbon resonance at δ_C 197.3 was ascribed to the carbonyl at the C-β' position. The quartenary carbon resonance at δ_C 129.4 was ascribed to C-1 due to MB C correlations with the protons at δ_H 2.69 (H-βα), δ_H 3.16 (H-ββ) and δ_H 2.83 (H-α).

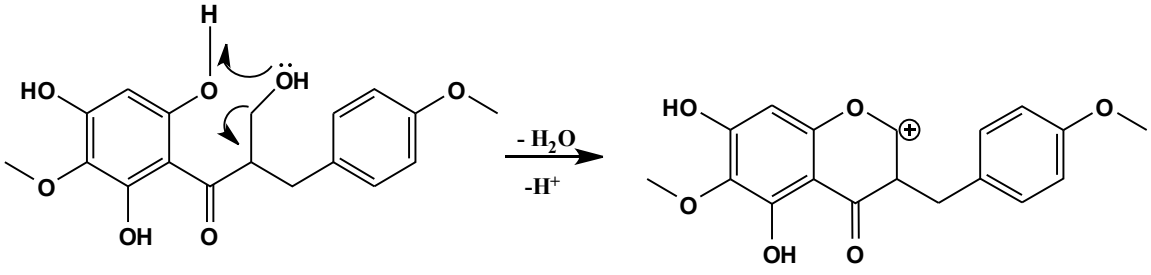
The ^{13}C NMR spectrum showed quaternary carbon resonances at δ_C 102.1, δ_C 127.2, δ_C 153.0, δ_C 1 . and δ_C 159.9 which were ascribed to the A-ring. The ^{1}H NMR spectrum showed a methoxy group resonance at δ_H 3.81 which correlated to the carbon resonance at δ_C 61.3 in the HSQC experiment and correlated to the carbon resonance at δ_C 127.2 in the HMBC experiment. The quartenary carbon resonance at δ_C 102.1 was assigned to position C-1', δ_C 127.2 was assigned to position C-5', δ_C 153.0 was assigned to position C-2', δ_C 157.8 was assigned to position C-4', and δ_C 159.9 was assigned to position C-6' due to HMBC, COSY and NOESY correlations. The singlet at δ_H 11.95 was due to the hydroxy group attached to C-6' as confirmed by the HMBC experiment. The ^{1}H NMR spectrum also showed a singlet at δ_H 6.10 which was due to H-3', as confirmed by HSQC and HMBC experiments.

Table 16: ^{1}H and ^{13}C NMR data of compound **1** in $CDCl_3$ (400 MHz).

Position	δ_C	DEPT	δ_H	HMBC Correlations
1	129.4	C	-	β, α
2, 6	129.8	CH	7.11 (2H, d, *J* = 8.52 Hz)	β, α
3, 5	113.9	CH	6.84 (2H, d, *J* = 8.52 Hz)	2, 6
4	158.2	C	-	4-OCH_3, 2, 6, 3, 5, 6'-OH
α	46.5	CH	2.83 (1H, m)	β, α'
α'	69.1	CH_2	4.17 (1H-α, dd, *J* = 7.10 Hz, 11.39 Hz)	β, α,
			4.33 (1H-β, dd, *J* = 4.10 Hz, 11.39 Hz)	
β	31.8	CH_2	2.69 (1H-α, dd, *J* = 10.39 Hz, 13.85 Hz)	α', 2, 6
			3.15 (1H-β, dd, *J* = 4.32 Hz, 13.85 Hz)	
β'	197.3	C	-	6'- , β, α'
1'	102.1	C	-	3', 6'-OH
2'	153.0	C	-	α’
3'	95.8	CH	6.10 (1H, s)	6’-OH
4'	157.8	C	-	3'
5'	127.2	C	-	5'-OCH_3
6'	159.9	C	-	3', 6'-OH, 5'-OCH_3
4-OCH_3	55.1	CH_3	3.77 (3H, s)	-
5'-OCH_3	61.3	CH_3	3.81 (3H, s)	-
6'-OH	-	-	11.96 (1H, s)	-

The IR spectrum showed absorption bands at 3391 cm^{-1} due to the hydroxy groups (OH stretching), 2932 cm^{-1} due to the aliphatic groups (–CH), 1638 cm^{-1} due to the carbonyl group (C=O stretching) which was lower than normal as is characteristic of chalcones (Tanaka et al, 1992), 1602 cm^{-1} due to the aromatic rings (C=C stretching) and 1018 cm^{-1} due to the presence of methoxy groups (C-O stretching). The UV spectrum showed maximum absorptions at 216 nm, 292 nm and 347 nm characteristic of dihydrochalcones (Srinath, 2011).

The melting point of the compound was 90-92˚C. The negative LC-ESI-MS showed a molecular ion peak at *m/z* 329.0 which is in agreement with the molecular formula $C_{18}H_{17}O_6$. The proposed pathway of molecular ion formation from LC-ESI- MS for compound **1** is given in Figure 30. This confirms the proposed structure of compound **1** which has molecular formula $C_{18}H_{20}O_7$ with a molecular mass of 348.1 g/mol. Compound **1** was therefore identified as 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone which has not previously been isolated.



348.1 g/mol *m/z* 329.0

Figure 29: Proposed pathway of molecular ion formation from LC-ESI-MS of compound **1**

3.4.2 Isolation of compound 2

Compound **2** was isolated from the hexane/DCM extract by means of CC. It was eluted after compound **1**, with a hexane: EtOAc solvent system (80:20) and the mass obtained was 45.7 mg. Spectra of compound **2** are found in appendices. The NMR data is shown in Table 17.

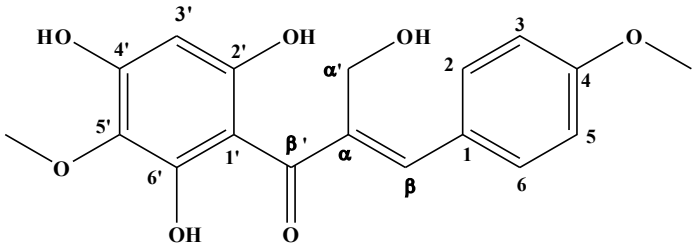


Figure 30: Compound **2 -** 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-chalcone

The ^{1}H NMR spectrum of compound **2** showed resonances at δ_H 6.87 (d, H-3/5, J = 9.70 Hz) and δ_H 7.80 (d, H-2/6, J = 8.80 Hz), each integrating to two protons, where the coupling of the protons to each other was confimed by the COSY spectrum. The coupling constant at δ_H 6.87 (H-3/5) was higher due to the overlapping of the peaks with δ_H 6.88 (s, H-β), therefore increasing its original value by 1.70 Hz. The ^{13}C-NMR spectrum for compound **2** showed strong resonances at δ_C 113. and δ_C 132.9 which, in the HSQC experiment, correlated to the protons at δ_H 6.87 (H-3/5) and δ_H 7.80 (H-2/6), respectively. The methoxy group singlet resonating at δ_H 3.82 was attributed to C-4 in the B ring. This position was confirmed by HMBC correlations, where the carbon resonance at δ_C 161.0 (C-4), correlated to protons at δ_H 3.82 (OCH_3), δ_H 6.87 (H-3/) and δ_H 7.80 (H-2/6). The carbon resonances at δ_C 161.0 (C-4) and δ_C 126.0 (C-1) were assigned due to HSQC, HMBC, and COSY correlations.

The ^{13}C NMR spectrum showed carbon resonances at δ_C 125.0 (C-α) and δ_C 141.0 (C-β) which are characteristic of chalcones (Sthothers et al., 1972). The ^{13}H NMR spectrum showed resonances at δ_H 6.88 (s, H-β) and δ_H 4.96 (d, H-α', J = 1.04 Hz) which integrated to two protons. The singlet at δ_H 6.88 (H-β) correlated to the carbon resonance at δ_C 141.0 (C-β) in the HSQC experiment and it correlated to the carbon resonance at δ_C 125.0 (C-α) in the HMBC experiment. The resonance at δ_H 4.96 (H-α') showed weak coupling to the resonance at δ_H 6.88 (H-β) in the NOESY spectrum. The HMBC experiment also confirmed the coupling of δ_C 125.0 (C-α) to the two protons at δ_H 4.96 (H-α'). The upfield shift in the carbonyl resonance at δ_C 187.0 (C-β') compared to compound **1** confirmed the α, β moiety to be unsaturated.

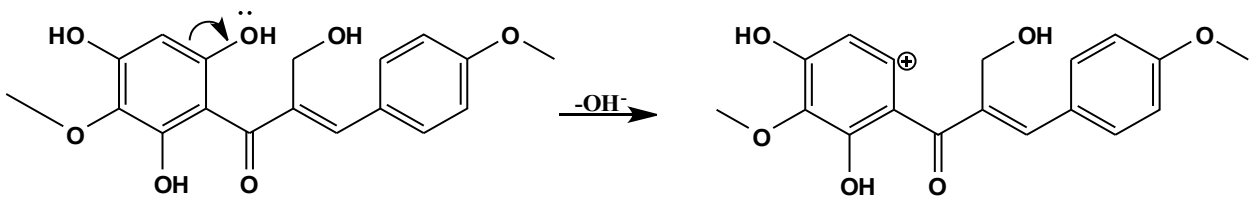
The ^{13}C NMR spectrum showed quaternary carbon resonances at δ_C 152.6 (C-2'), δ_C 157.3 (C-4') and δ_C 161.0 (C-6') that were seen to be hydroxylated. The ^{13}H NMR spectrum showed a singlet at δ_H 6.12 (H-3') which correlated to the carbons at δ_C 152.6 (C-2') and δ_C 157.3 (C-4') in the HMBC spectrum. The ^{1}H NMR spectrum showed a methoxy group resonance at δ_H 3.85 which correlated to the carbon resonance at δ_C 61.7 in the HSQC experiment and correlated to the carbon resonance at δ_C 161.0 in the HMBC spectrum. The quaternary carbon resonance at δ_C 104.5 was ascribed to position C-1', due to HMBC correlations with δ_H 6.12 (H-3') and δ_H 12.55 (6'-OH).

Table 17: ^{1}H and ^{13}C NMR data of compound **2** in $CDCl_3$ (400 MHz).

Position	**δ_C**	**DEPT**	**δ_H**	**HMBC Correlations**
1	126.3	C	-	β
2, 6	132.9	CH	7.80 (2H, d, *J* = 8.80 Hz)	3, 5
3, 5	113.4	CH	6.87 (2H, d, *J* = 9.70 Hz)	-
4	161.0	C	-	4-OCH_3
α	125.0	C	-	α',β
α'	75.8	CH_2	4.96 (2H, d, *J* = 1.04 Hz)	3, 5
β	141.0	CH	6.88 (1H, s)	2, 6, α'
β'	187.0	C	-	α', β
1'	104.5	C	-	3', 6'-OH
2'	152.8	C	-	α'
3'	96.1	CH	6.12 (1H, s)	2'/4'-OH
4'	157.3	C	-	3'
5'	127.2	C	-	3', 5'-OCH_3
6'	161.0	C	-	3', 6'-OH
4-OCH_3	55.4	CH_3	3.82 (3H, s)	-
5'-OCH_3	61.7	CH_3	3.85 (3H, s)	-
6'-OH	-	-	12.55 (1H, s)	-

The IR spectrum showed absorption bands at 3260 cm^{-1}due to the hydroxy groups (OH), 2931 cm^{-1} due to the aliphatic groups (–CH), 1636 cm^{-1} due to the carbonyl group (C=O) which was lower than normal as is characteristic of chalcones (Tanaka et al, 1992), 1600 cm^{-1} due to the aromatic rings (C=C) and 1019 cm^{-1} due to the methoxy group (C-O stretching). The UV spectrum showed maximum absorptions at 215 nm and 360 nm.

The melting point of the compound was 130-133˚C. The negative LC-ESI-MS showed a molecular ion peak at *m/z* 329.0 which was in agreement with the molecular formula $C_{18}H_{17}O_6$. The proposed pathway of molecular ion formation from LC-ESI-MS for compound **2** is given in Figure 32. This confirms the proposed structure of compound **2** which has molecular formula $C_{18}H_{18}O_7$ with a molecular mass of 346.3 g/mol. Compound **2** was therefore identified as 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-chalcone which has not previously been isolated.



346.3 g/mol *m/z* 329.0

Figure 31: Proposed pathway of molecular ion formation from LC-ESI-MS of compound **2**

3.4.3 Isolation of compound 3

Compound **3** was isolated from the DCM fraction of the MeOH extract by means of CC; it was eluted with a hexane: EtOAc (80:20) solvent system. The mass obtained was 3.9 mg. The spectra for compound **3** are attached at appendices. The NMR data is shown in Table 18.

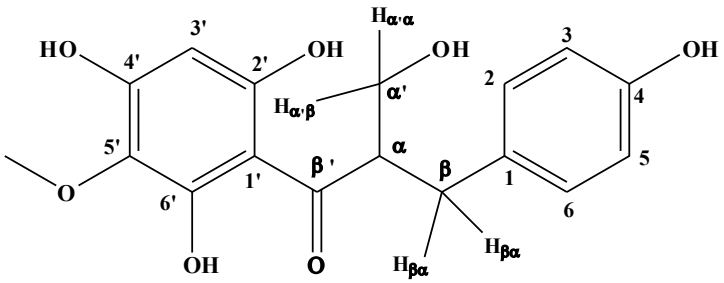


Figure 32: Compound **3** – 2',4',6',4-tetrahydroxy-5'-methoxy-α- hydroxymethyl-β-methyl-dihydrochalcone

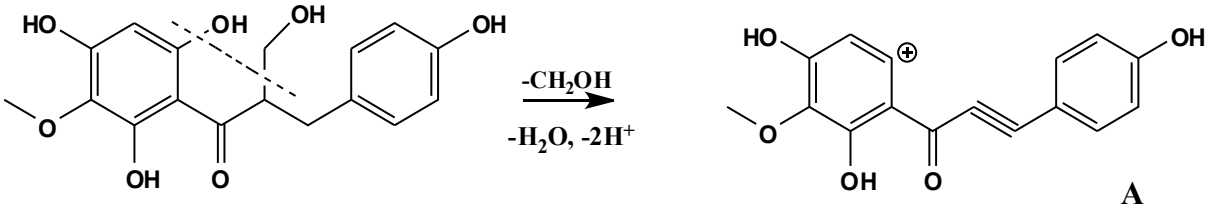
The ^{1}H NMR and ^{13}C NMR spectra of compound **3** showed a slight difference to that of compound **1**, in that there was only one methoxy resonance at δ_H 3.83 which was assigned to the carbon at position C-5' in the A ring due to HSQC and HMBC correlations. The methoxy group at C-4 in compound **1** was replaced by a hydroxy group at C-4 in compound **3** which was attached to the quartenary carbon resonance at δ_C 154.3.

Table 18: ^{1}H and ^{13}C NMR data of compound **3** in $CDCl_3$ (400 MHz).

Position	**δ_C**	**DEPT**	**δ_H**	**HMBC Correlations**
1	129.6	C	-	2, 6, β
2, 6	130.1	CH	7.08 (2H, d, *J* = 8.40 Hz)	3, 5, β
3, 5	115.4	CH	6.78 (2H, d, *J* = 8.40 Hz)	-
4	154.3	C	-	2, 6, 3, 5
α	46.6	CH	2.80 (1H, m)	α', H-βα
α'	69.3	CH_2	.1 (1 α , dd, *J* = 7.16 Hz, 11.41 Hz); .3 (1 β, dd, *J* = 4.20 Hz, 11.37 Hz)	β
β	31.8	CH_2	2.69 (1 α , dd, *J* = 10.38 Hz, 13.85 Hz); 3.17(1 β, dd, *J* = 4.34 Hz, 13.83 Hz)	α, -αβ, 2, 6
β'	197.6	C	-	H-ββ, α'
1'	102.3	C	-	3', 6'-OH
2'	152.8	C	-	α'
3'	95.7	CH	6.10 (1H, s)	6'-OH
4'	157.6	C	-	3'
5'	127.1	C	-	5'-OCH_3, 3'
6'	160.1	C	-	3', 6'-OH
5'-OCH_3	61.3	CH_3	3.83 (3H, s)	-
α'-OH	-	-	4.90 (1H, bs)	-
6'-OH	-	-	11.94 (1H, s)	-

The IR spectrum showed absorption bands at 3288 cm^{-1}due to the hydroxy groups (OH), 2945 and 2831cm^{-1} due to the aliphatic groups (CH), 1693 cm^{-1} due to the carbonyl group (C=O), 1586 cm^{-1} due to the aromatic rings (C=C) and 1019 cm^{-1} due to methoxy group (C-O stretching). The UV spectrum showed maximum absorptions at 216 nm and 362 nm.

The melting point of the compound was 194-196˚C. The negative LC-ESI-MS showed a molecular ion peak at *m/z* 282.9 and was in agreement with molecular formula $C_{17}H_{11}O_4$. The proposed pathway of molecular ion formation from LC-ESI-MS for compound **2** is given in Figure 34. This confirms the proposed structure of compound **3** which has molecular formula $C_{18}H_{18}O_6$ with a molecular mass of 334.1 g/mol. Compound **3** was therefore identified as 2',4',6',4-tetrahydroxy-5'-methoxy-α-hydroxymethyl-β-methyl-dihydrochalcone which has not previously been identified.



334.1 g/mol *m/z* 282.9

Figure 33: Proposed pathway of molecular ion formation from LC-ESI-MS of compound **3**

3.4.4 Isolation of compound 4

Compound **4** was isolated from the EtOAc fraction of the MeOH extract by means of CC with a DCM: MeOH (80:20) solvent system. The mass obtained was 3.1 mg. All spectra for compound **4** are found in appendices. The NMR data is shown in Table 19.

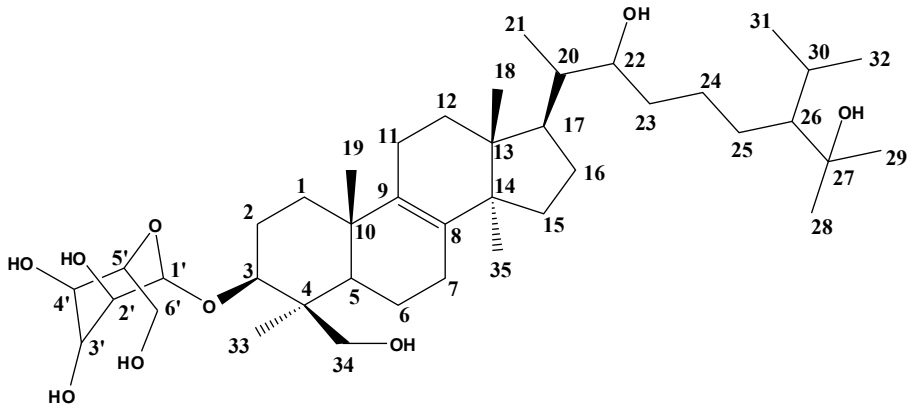


Figure 34: Compound **4 -** 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene

The ^{1}H NMR spectrum for compound **4** showed resonances at δ_H 0.69 (H-18), δ_H 0.92 (H-35), δ_H 0.98 (H-31/32), δ_H 0.99 (H-19), δ_H 1.21 (H-28/29/33), δ_H 1.91 (H-21) which were all singlets. The protons resonating at δ_H 0.69, δ_H 0.92, δ_H 1.21 and δ_H 0.99 were seen to be attached to unsaturated carbons. The resonances at δ_H 0.98 (H-31/32) and δ_H 1.21 (H-28/29/33) were integrating to six and nine protons, respectively therefore two carbons were resonanting at δ_C 19.5 (C-31/32) and three where resonating at δ_C 22.6 (C-28/29/33). The HMBC spectrum showed that the proton resonance at δ_H 0.69 (H-18) was correlating to the carbon resonances at δ_C 31.4 (C-2/15), δ_C 31.7 (C-1/16), δ_C 45.2 (C-13), δ_C 50.5 (C-14) and δ_C 51.8 (C-17), therefore was positioned at C-18. The HMBC spectrum also showed that the proton resonance at δ_H 1.21 (H-28/29/33) was correlating to the carbon resonances at δ_C 34.4

(C-20/30), δ_C 43.0 (C-4), δ_C 52.1 (C-5), δ_C 64.6 (C-34) and δ_C 80.6 (C-3), therefore was positioned at C-28, C-29 and C-33. The HSQC experiment showed that the proton resonances at δ_H 3.37 (H-34α, d, *J* = 11.25 Hz) and δ_h 3.41 (H-34β, d, *J* = 11.25 Hz) correlated to the carbon resonance at δ_C 64.6 (C-34). The characteristic resonance at δ_C 80.6 (C-3) correlated to these protons in the HMBC experiment. There was also coupling of the proton at δ_H 3.35 (H-3) with the carbon resonance at δ_C 100.0 (H-1'), which then prompted positioning at C-3. The heavily substituted 2-methylheptane side chain was found to be hydroxylated at C-22 and C-27, which led to the shift in the resonance peaks at δ_C 42.5 (C-23) and δ_C 52.1 (C-26). The NOESY experiments showed the coupling of the proton resonance at δ_H 3.35 (H-3) with the proton resonance at δ_H 1.30 (H-1, d, *J* = 14.49 Hz) as well as the coupling of the proton resonance at δ_H 1.30 (H-2, d, *J* = 14.49 Hz) with the proton resonance at δ_H 0.99 (H-19). The resonances at δ_C 135.2 (C-8) and δ_C 135.3 (C-9) confirmed that the skeleton structure is that of lanosterol with a substituted methylheptane side chain.

The ^{1}H NMR spectrum showed resonances at δ_H 3.16 (H-2'), δ_H 3.18 (H-3'), δ_H 3.28 (H-4'), δ_H 3.29 (H-5'), δ_H 3.69 (H-6'α), δ_H 3.88 (H-6'β) and δ_H 4.23 (H-1'). The HSQC spectrum showed that δ_H 3.69 (1 α, dd, *J* . 2, 11.9 z) and δ_H 3. (1 β, dd, *J* = 2.12, 11.89 Hz) were from the same carbon at δ_C 62.3 (C-6'). The HSQC spectrum showed that the proton resonances at δ_H 3.16, δ_H 3.29 and δ_H 4.23 correlated to carbon resonances at δ_C 74.6 (C-2'), δ_C 77.8 (C- ') and δ_C 100.0 (C-1'), respectively.

The C SY experiment showed the coupling of protons at δ_H 3.16 (H-2') with δ_H 4.23 (H-1') and protons at δ_H 3. (β-6') with δ_H 3.69 (α -6'). This led to the assumption of the presence of a glycosidic linkage at the C-3 position. There was weak coupling of the protons on the glucoside as seen from the weak HMBC correlations further upfield, therefore

correlations where minimal. The structural deduction of the entire molecule was found from the different experiments performed. The data for compound **4** was compared to NMR data for β-sitosterol glycoside (Akthar et al., 2010) and many similarities especially at the glycosidic linkage were found.

Table 19: ^{1}H and ^{13}C NMR data of compound **4** in MeOD (400 MHz)

Position	**δ_C**	**DEPT**	**δ_H**	**HMBC Correlations**	**β-sitosterol glycoside (δ_C)**
1	31.7	CH_2	1.30 (8H, d, *J* = 14.49 Hz)	-	36.9
2	31.4	CH_2	1.30 (8H, d, *J* = 14.49 Hz)	-	29.3
3	80.6	CH	3.35 (1H, s)	33, 1', 34	77.0
4	43.0	C	-	33	40.3
5	52.1	CH	1.18 (2H,d, *J* = 5.24 Hz)	33	100.5
6	19.1	CH_2	0.96 (2H, d, *J* =10.17 Hz)	-	121.2
7	27.4	CH_2	1.97 (6H, d, *J* = 5.52 Hz)	-	33.4
8	135.2	C	-	35, 19	31.4
9	135.3	C	-	35, 19	49.6
10	37.4	C	-	21	36.2
11	21.5	CH_2	2.06 (2H, s)	-	20.6
12	27.4	CH_2	1.97 (6H, d, *J* = 5.52 Hz)	35	38.3
13	45.2	C	-	18, 35	42.1
14	50.5	C	-	18, 35	56.2
15	31.4	CH_2	1.30 (2H, d, *J* = 14.49 Hz)	18	23.9
16	31.7	CH_2	1.30 (2H, d, *J* = 14.49 Hz)	-	28.7
17	51.8	CH	1.57 (1H, d, *J* = 9.21 Hz)	18	55.5
18	15.8	CH_3	0.69 (3H, s)	15, 16, 13, 14, 17	11.7

19	19.7	CH_3	0.99 (3H, s)	10, 5	19.1
20	34.4	CH	1.37 (2H, s)	21	36.2
21	13.7	CH_3	1.90 (3H, s)	-	18.6
22	72.1	CH	4.91 (1H, s)	-	35.5
23	42.5	CH_2	1.46 (2H, d, *J* = 4.56 Hz)	-	25.5
24	29.0	CH_2	2.04 (2H, s)	21	44.7
25	27.4	CH_2	1.97 (6H, d, *J* = 5.52 Hz)	33	27.8
26	52.1	CH	1.18 (2H,d, *J* = 5.24 Hz)	-	19.6
27	77.6	C	-	-	19.0
28	22.6	CH_3	1.21 (9H, s)	26	22.6
29	22.6	CH_3	1.21 (9H, s)	26	11.7
30	34.4	CH	1.37 (2H, s)	-	-
31	19.5	CH_3	0.98 (6H,s)	30, 26	-
32	19.5	CH_3	0.98 (6H, s)	30, 26	-
33	22.6	CH_3	1.21 (9H, s)	3, 4, 5, 34	-
34	64.6	CH_2	3.37 (1Hα, d, *J* = 11.25 Hz); 3.41 (1Hβ, d, *J* = 11.25 Hz,)	-	-
35	24.1	CH_3	0.92 (3H, s)	2, 13, 14, 8, 9	-
1'	100.0	CH	4.23 (1H, d, *J* = 7.84 Hz)	-	100.8
2'	74.6	CH	3.16 (1H, d, *J* = 4.72 Hz)	-	76.7
3'	77.6	CH	3.18 (1H, d, *J* = 4.68 Hz)	-	73.5
4'	71.2	CH	3.28 (1H, d, *J* = 4.68 Hz)	-	70.1
5'	77.8	CH	3.29 (1H, d, *J* = 4.68 Hz)	-	76.7
6'	62.3	CH_2	3.69 (1 α , dd, *J* = 5.72, 11.97 Hz)	-	61.1
			3. (1 β, dd, *J* = 2.12, 11.89 Hz)		

The IR spectrum showed absorption bands at 3325 cm^{-1} (OH stretching) due to the hydroxy groups and 2943 and 2832 cm^{-1} due to the –CH aliphatic stretch. The presence of the absorption band at 1658 cm^{-1} is indicative of unsaturation (C=C stretch). The UV spectrum showed maximum absorptions at 213 nm and 262 nm. Bagri et al. (2011) attributed the band at 262 nm (MeOH) to the glycosidic linkage.

The melting point of the compound was 245-248ºC which was close to that of β-sitosterol glycoside (252-253ºC) (Akthar et al., 2010). The proposed pathway of molecular ion formation from LC-ESI-MS for compound 4 is given in Figure 36 and the molecular ion peak at *m/z* 649.5, with others of *m/z* 573.1 and 451.0 are shown. This confirms the proposed structure of compound 4 which has molecular formula $C_{41}H_{72}O_9$ with a molecular mass of 708.5 g/mol. Compound **4** was therefore identified as 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene.

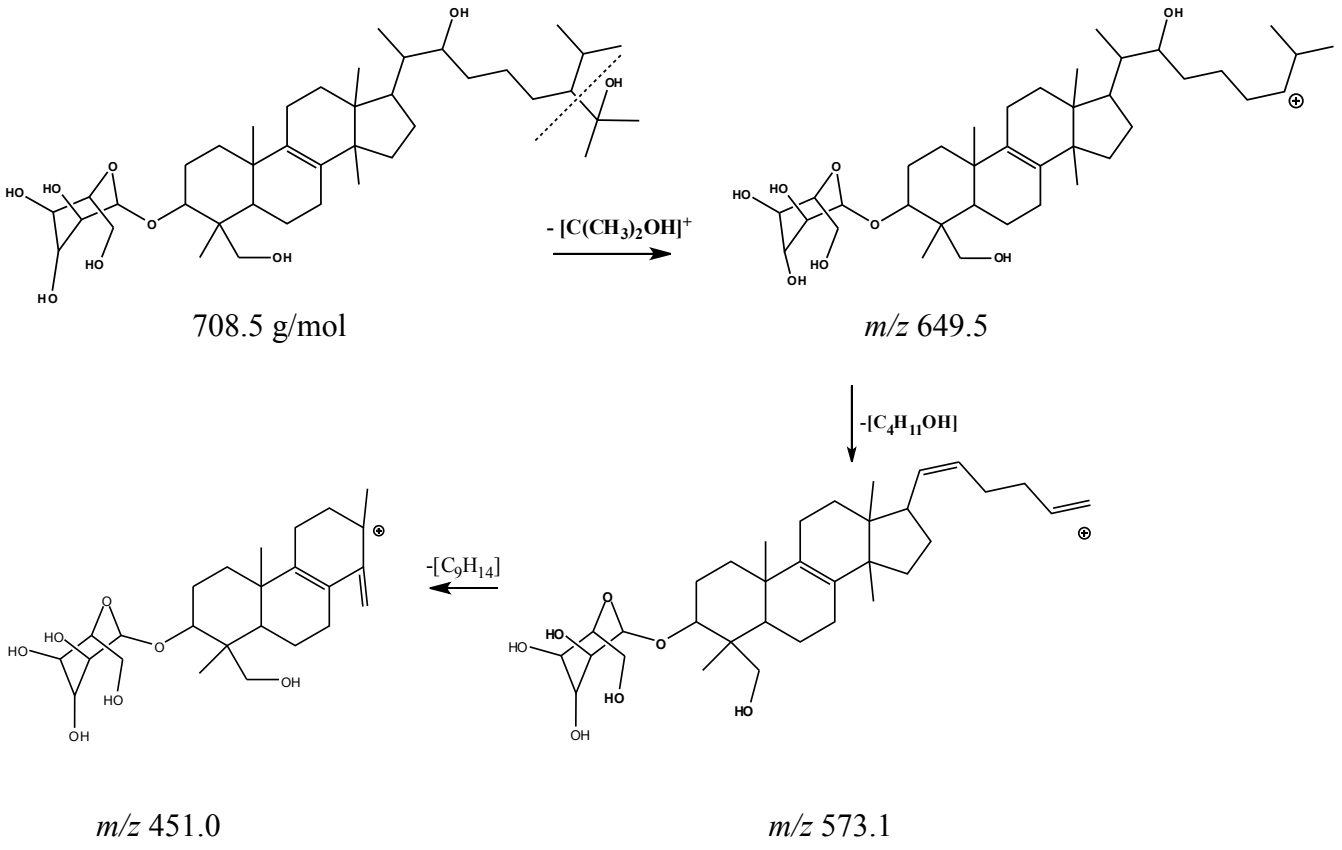


Figure 35: Proposed pathway of molecular ion formation from LC-ESI-MS of compound **4**

3.4.5 Isolation of compound 5

Compound **5** was purified from the EtOAc fraction of the MeOH extract; it was eluted with a DCM: MeOH (98:2) solvent system. The mass obtained was 4.3 mg. The spectra for the NMR, IR, UV and LC-ESI-MS are given in appendices. The NMR data of this compound are given in Table 20.

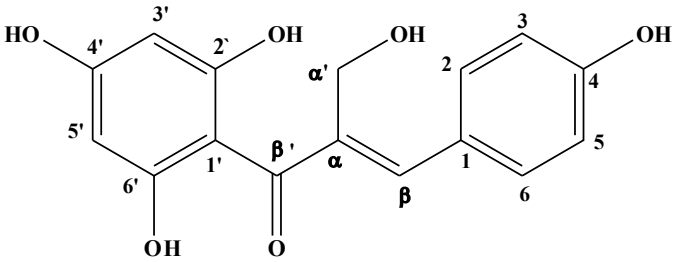


Figure 36: Compound **5 -** 2',4',6',4-tetrahydroxy-α-hydroxymethyl-chalcone

The ^{1}H NMR and ^{13}C NMR spectra of compound **5** showed slight differences to that of compound **2**, in that there were no methoxy resonances. The ^{1}H NMR spectrum showed a resonance at δ_H 5.84 (H-5', J = 1.76 Hz) that showed weak meta-coupling to the resonance at δ_H 5.91 (H-3', J = 2.20 Hz) in the COSY spectrum. The proton at C-5' in compound **5** replaced the methoxy group at C-5' in compound **2**. The methoxy group at C-4 in compound **2** was replaced by a hydroxy group at C-4 in compound **5** which was attached to the quartenary carbon resonance at δ_C 159.8 as confirmed by HMBC correlations.

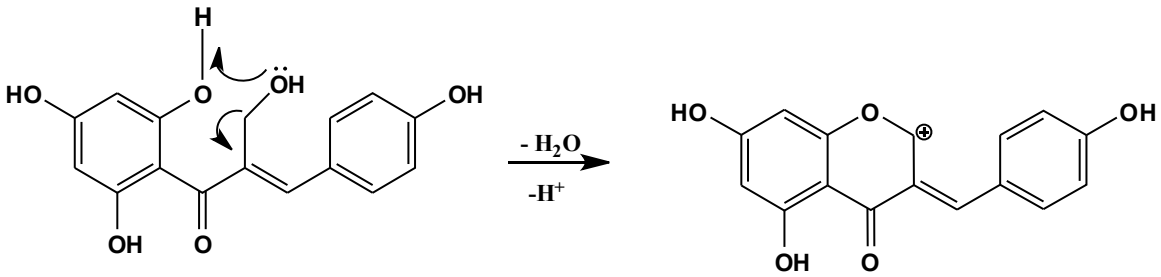
Table 20: ^{1}H and ^{13}C NMR data of compound **5** in MeOD (400 MHz).

Position	δ_C	DEPT	δ_H, *J* (Hz)	HMBC Correlations
1	126.1	C	-	3, 5
2, 6	132.7	CH	7.26 (2H, d, *J* = 8.60 Hz)	β
3, 5	115.9	CH	6.88 (2H, d, *J* = 8.60 Hz)	-
4	159.8	C	-	2, 6 ; 3, 5
α	127.4	C	-	α'
α'	67.7	CH_2	5.32 (2H, d, *J* = 1.72 Hz)	2, 6
β	137.2	CH	7.74 (1H, s)	2, 6
β'	185.5	C	-	β
1'	102.6	C	-	3, 5
2'	165.4	C	-	3'
3'	96.3	CH	5.91 (1H, d, *J* = 2.20 Hz)	5'
4'	167.4	C	-	3', 5'
5'	95.0	CH	5.84 (1H, d, *J* = 1.76 Hz)	3'
6'	163.0	C	-	α', 5'
α'-OH	-	-	4.65 (1H, bs)	-

The IR spectrum of compound **5** showed absorption bands at 3321 cm^{-1} due to hydroxy groups (O-H), 2942 and 2831 cm^{-1} due to –CH stretching, 1673 cm^{-1} due to the carbonyl group, 1022 cm^{-1} indicative of alcohols C-O stretching. The UV spectrum showed maximum absorption at 213, 292, 362 nm characteristic of chalcones (Srinath, 2011).

The negative LC-ESI-MS showed a molecular ion peak at *m/z* 282.9 and was in agreement with molecular formula $C_{16}H_{10}O_5$ which occurred as a result of the loss of H_2O and one

hydrogen which agreed with the calculated mass of 283.1 for $C_{16}H_{10}O_5$. The proposed pathway of molecular ion formation from LC-ESI-MS for compound **5** is given in Figure 38. This confirms the proposed structure of compound **5** which has molecular formula $C_{16}H_{13}O_6$ with a molecular mass of 302.8 g/mol. Compound **5** was therefore identified as 2',4',6',4-tetrahydroxy-α-hydroxymethyl-chalcone that has not previously been isolated.



302.8 g/mol *m/z* 282.9

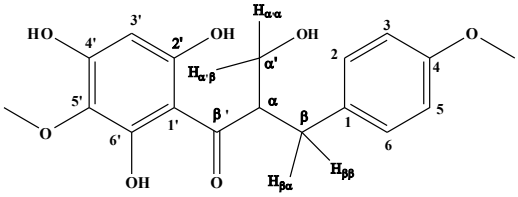
Figure 37: Proposed pathway of molecular ion formation from LC-ESI-MS of compound **5**

Studies on the hexane, DCM and MeOH extracts of the *C. obliquus* bulbs has led to the isolation and identification of two new chalcones and two new dihydrochalcones that are structurally-related. This has also led to the isolation of a lanosterol glycoside that has not previously been isolated from *C. obliquus* bulbs.

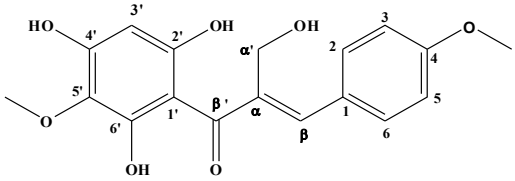
3.4.6 Antioxidant activity

The antioxidant activity of the four compounds isolated were determined by two methods namely, the DPPH radical scavenging assay and the FRAP assay. Compound **4** (3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene) was not tested due to insufficient amounts. The compounds antioxidant activities were compared to a standard, ascorbic acid.

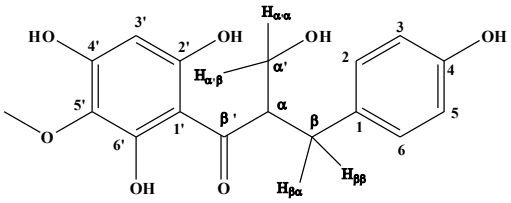
Compound 1



Compound 2



Compound 3



Compound 5

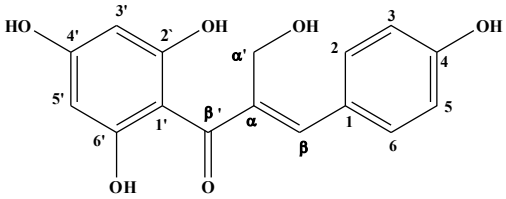


Figure 38: Compounds isolated from *C. obliquus* bulbs

The DPPH radical scavenging assay results are shown in Table 21 and represented graphically in Figure 39. The results reveal that ascorbic acid had the highest antioxidant activity compared to all the compounds. The antioxidant activities of the compounds were relatively low; studies conducted by Huang et al. (2012) indicated that compound davidigenin (2', 6', 4-

trihydroxychalcone) of various concentrations showed no significant antioxidant activity. Additionally Desire et al. (2012) indicated that IC_{50} of davidigenin was also insignificant (> 40 μg ml^{-1}) at compound concentrations between 0.4 to 40 μg ml^{-1}. Nonetheless, the antioxidant activity of the compounds increased steadily as concentrations increased (100-1000 μg ml^{-1}). At low concentration of 50 μg ml^{-1} the activity of compounds were in decreasing order of Compound **1** > **3** > **2** > **5**.

Table 21: Percent inhibition of compounds **1**, **2**, **3**, **5** and ascorbic acid with concentrations (μg ml^{-1}) from the DPPH assay[a].

Concentration/ μg ml^{-1}	% Inhibition				
	1	**2**	**3**	**5**	**Ascorbic acid**
10	2.20	0.45	1.42	0.74	5.88
20	4.56	2.59	2.36	1.07	7.22
30	5.86	2.65	2.85	1.33	7.36
40	7.20	3.01	2.96	2.01	8.13
50	7.99	3.17	3.33	1.81	12.76
100	9.93	4.08	3.97	3.11	51.77
200	11.29	6.12	7.94	4.53	96.12
500	13.02	7.15	18.05	5.89	96.46
1000	30.81	12.10	35.81	9.16	98.88

[a] n 3, standard deviations all ≤ 0.00

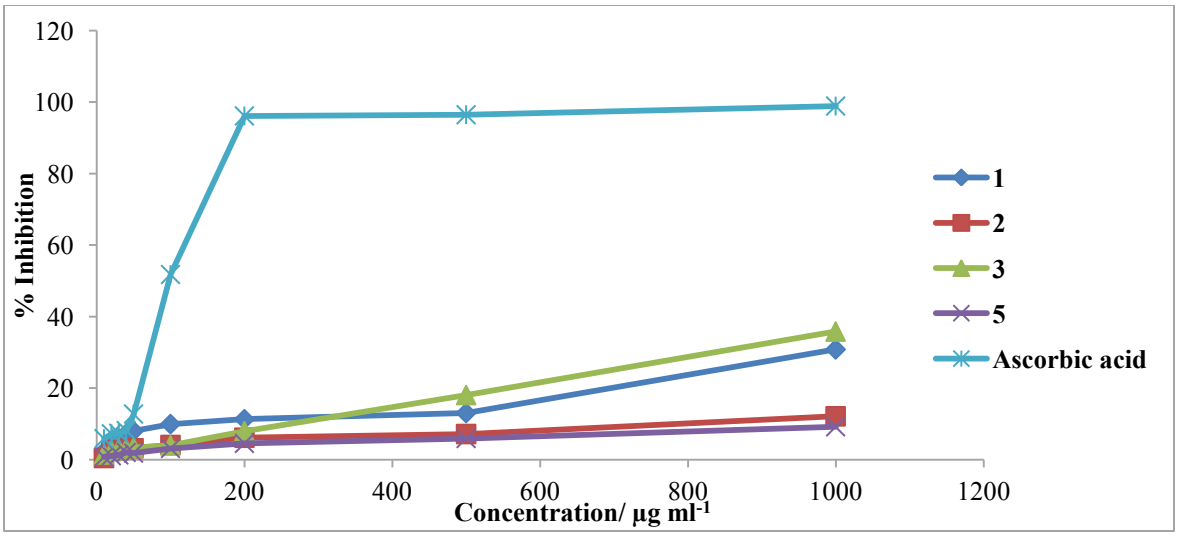


Figure 39: Antioxidant activity of compounds **1**, **2**, **3**, **5** and ascorbic acid standard, as measured by the DPPH method

At even lower concentration, 30 and 10 µg ml^{-1}, the compound with the highest percentage activity was compound **1** with percentage inhibition of 5.86 % (30 µg ml^{-1}), followed by compound **3** with percentage inhibition of 2.85 % (30 µg ml^{-1}). Both the compounds possess the -OC-CH(CH_2OH)-CH_2- moeity where the hydrogen may be easily donated to the DPPH radical to form DPPH-H.

Table 22: Absorbance of compounds, **1**, **2**, **3**, **5** and ascorbic acid with concentrations (µg ml^{-1}) from the ferric radical reducing potential assay[a].

Concentration/ µg ml^{-1}	Absorbance (700 nm)				
	1	**2**	**3**	**5**	**Ascorbic acid**
10	0.024	0.021	0.026	0.015	0.060
20	0.034	0.022	0.027	0.016	0.032
30	0.039	0.024	0.031	0.019	0.087
40	0.044	0.025	0.037	0.020	1.070
50	0.047	0.025	0.037	0.026	1.930
100	0.052	0.028	0.05	0.031	2.800
200	0.083	0.029	0.079	0.038	3.000
500	0.701	0.224	0.602	0.18	3.000

[a] n 3, standard deviations all ≤ 0.00

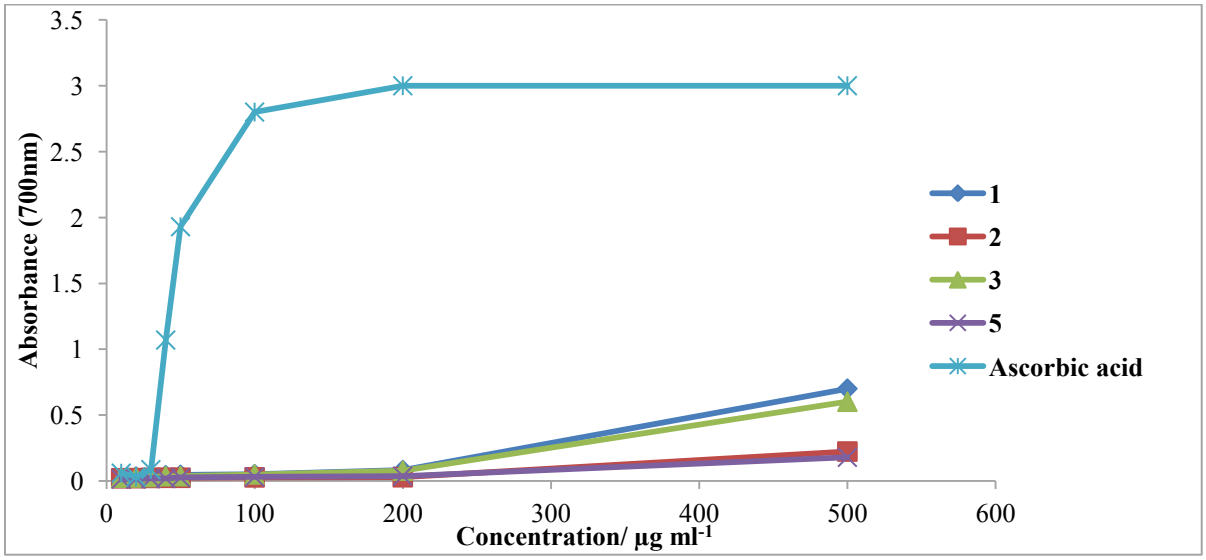


Figure 40: Ferric radical reducing potential of compounds **1**, **2**, **3**, **5** and ascorbic acid standard, as measured by the FRAP assay method

The FRAP assay is shown in Table 22 and is graphically represented in Figure 40. The results show that the ascorbic acid standard has the highest antioxidant activity as in the DPPH method. The antioxidant activity of the compounds increases steadily with an increase in their concentrations. At low concentrations of 50 μg ml^{-1} the activity of compounds were in decreasing order of compound **1** > **3** > **5** > **2**, which shows a similar trend to the DPPH assay. Though compounds **5** and **2** possessed a double bond at the α and β position, low antioxidant activity was observed.

3.5 Extract profiling by GC-MS: L. javanica

An amount of 10 mg each of the hexane/DCM extract and EtOAc fraction from MeOH extract of *L. javanica* leaves were diluted into 10 mL volumetric flasks with DCM and EtOAc, respectively. Exactly 1 µl of the extract solution were injected into the GC-MS. The mass range was from *m/z* 103 to 286, the base peaks were identified by comparison with the computerized mass spectra library. All MS spectra are found in appendices.

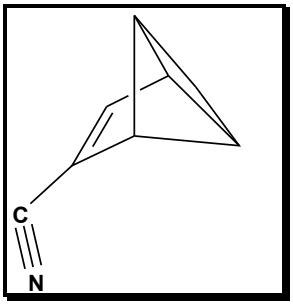
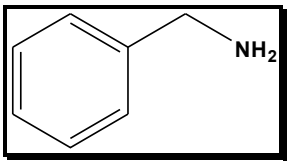
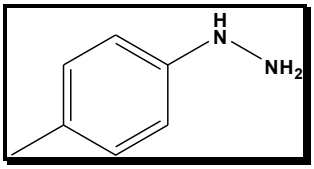
Studies have been done on the identity of volatile components in essential oils of *L. javanica* (Manenzhe et al., 2004; Mujovo et al., 2006); these were found to have low activity against gram-positive *Escherichia coli* and *Staphylococcus aureus* at 10 mg/mL concentrations. However, a study reported by Nkomo et al. (2011) showed that acetone, MeOH and ethanol extracts of *L. javanica* displayed antimicrobial activity against *Helicobacter pylori*.

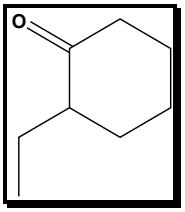
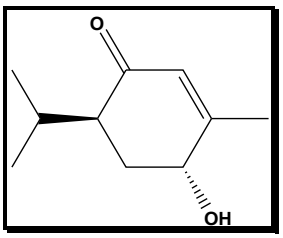
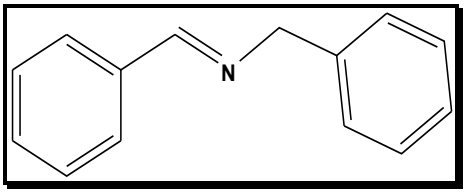
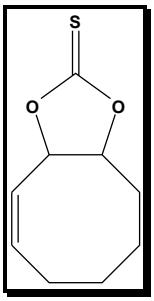
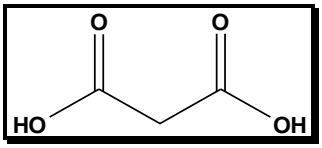
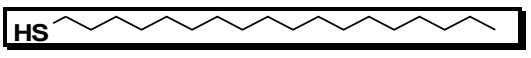
The chemical investigation of the extract by GC-MS indicated the presence of monoterpenoids, amino compounds, organic acids and some alcohols in both extracts of the plant; these have previously been reported (Niedler & Staehle, 1973; van Wyk et al., 1997). It is shown in Table 23 that the most common chemical compounds in the DCM extract are tricyclo[3.1.0.0(2,4)] hex-3-ene-carbonitrile, benzylamine, hydrazine (4-methylphenyl), cyclohexanone (2-ethyl), 2-cyclohexene-1-one-4-hydroxy-3-methyl-6-(1-methylethyl) trans, benzenemethanime-N-(phenylmethylene), 3a,4,5,6,7a-hexahydrocycloocta-1,3-dioxole-2-thione, propanedioic acid (malonic acid), 1-octadecanethiol and phytol.

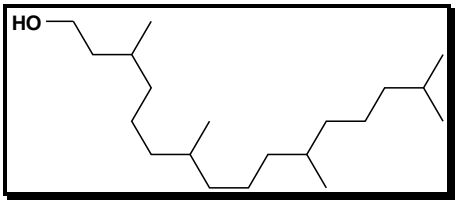
The compound 2-cyclohexene-1-one-4-hydroxy-3-methyl-6-(1-methylethyl), trans (4-hydroxypiperitone) was found to be a constituent of many essential oils of *Lippia* species (Mukoka, 2005), and had a similar structure to piperitone.

Phytol is a branched fatty alcohol which is a constituent of chlorophyll. It can be converted into phytanic acid in animals by the partial digestion of chlorophyll; the acid has a function in the regulation of lipid metabolism (Goto et al., 2009). Phytol is also reported for its antimicrobial, antiviral and antioxidant activities (McKay & Blumberg, 2006). It has also been found to have antibacterial activity against *S. aureus* (Inoue et al., 2005).

Table 23: The main compound identified by GC-MS in the dichloromethane extract of *L. javanica* leaves.

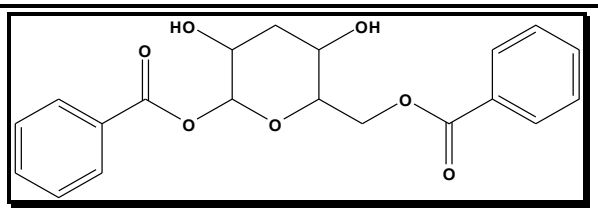
Compound	**Molecular ion/ Base ion peak**	**T_R/ min**	**Structure**
Tricyclo[3.1.0.0(2,4)] hex-3-ene-carbonitrile	103/ 76	4.44	
Benzylamine	106/ 30	5.26	
Hydrazine (4-methylphenyl)	122/ 77	8.23	

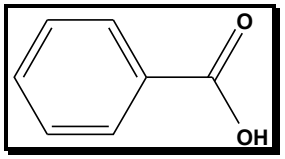
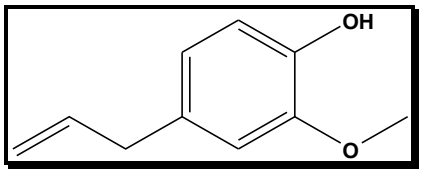
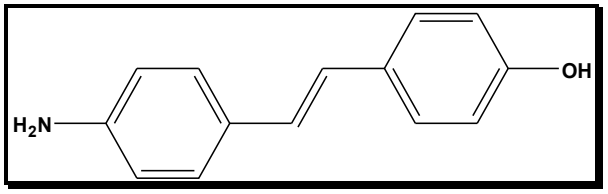
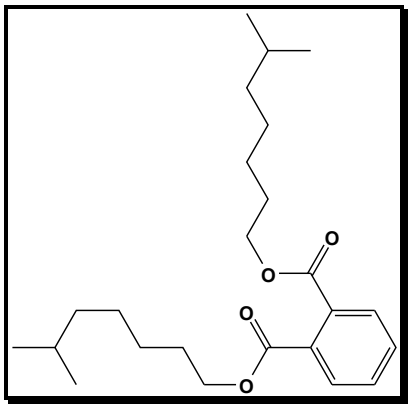
Cyclohexanone, 2-ethyl	126/ 98	10.17	
2-cyclohexene-1-one, 4-hydroxy-3-methyl-6-(1-methylethyl), trans	168/ 98	10.18	
Benzenemethanime, N-(phenylmethylene)-	195/ 91	14.12	
3a,4,5,6,7a-hexahydrocycloocta-1,3-dioxole-2-thione	184/ 95	14.87	
Propanedioic acid	207/ 149	15.17	
1-octadecanethiol	286/ 83	17.11	

Phytol	123/ 71	17.58	

The amino compounds benzylamine, hydrazine, benzenemethanime and 4-amino-4'-hydroxystilbene (Table 23 and 24) were found to be present in the extracts. Nitrogen is known to be a constituent in amino acids which are the building blocks of proteins. Studies have also shown that amino compounds form the part of the raw material from which essential oils are made, however the final product is said to contain trace amounts of these compounds (Stewart, 2005). These amino compounds therefore may form the part of the plant tissue, where they are recognized for their catalyses of enzymatic reactions in the plant.

Table 24: The main compound identified by GC-MS in the ethyl acetate extract of *L. javanica* leaves.

Compound	**Molecular ion/ Base ion peak**	**T_R/ min**	**Structure**
Beta-1,5-O-dibenzylribofuranose	149/ 105	6.99	

Benzenecarboxylic acid	121/ 105	7.04	
Eugenol	164/ 103	9.28	
4-amino-4'-hydroxystilbene	211/ 165	17.75	
1,2-benzenedicarboxylic acid, diisooctylester	279/ 149	21.19	

The compound 3a,4,5,6,7a-hexahydrocycloocta-1,3-dioxole-2-thione was found to be present in the EtOAc extract. Sulfur compounds are also recognized as constituents of essential oils in trace amounts. They are known to dominate the fragrance of the oil even in trace amounts (Stewart, 2005).

CHAPTER 4

ELEMENTAL COMPOSITION OF CYRTANTHUS OBLIQUUS AND LIPPIA JAVANICA

4.1 Introduction

The elemental concentrations in *C. obliquus* bulbs and *L. javanica* leaves and water extracts were investigated. Soil quality parameters were also evaluated to determine the impact of soil on elemental uptake in *L. javanica* leaves; the methods used to evaluate these parameters are known and well established. There are four common steps in an analytical method that is, sampling, sample storage, sample preparation and analysis. This part of the chapter explains the techniques and instrumentation that are used in such an analysis.

4.2 Sampling

The bulbs of *C. obliquus* were purchased from traders in eight different markets in KwaZulu-Natal, shown in Figure 42 with geographical coordinates shown in Table 25. The bulbs where purchased at the beginning of April.

Furthermore, *L. javanica* leaves and soil samples were collected from ten different sites in KwaZulu-Natal, shown in Figure 43 with geographical coordinates shown in Table 26. The leaves of *L. javanica* were collected at the beginning of April, the average temperature was 2 ˚C on the five day sampling period and there was no rain or wind but sunshine during this time. A soil sampling technique called coning and quartering was used to obtain a smaller sample size but one that correctly represented the soil. The soil sampling depth was 15 cm

which corresponded to the crop rooting depth. The soil was mixed using a plastic shovel into a uniform conical pile; the cone was then flattened from the center to form a disk. The disk was then divided into quarters using the shovel; two of the quarters were chosen randomly and then mixed into a conical pile again. This was done until the desired sample size was obtained. The soil samples where then placed in labelled polyethylene bags and placed in a fridge for analysis in the laboratory.

4.3 Sampling sites

Figure 41 shows the eight different market sites in KwaZulu-Natal where *C. obliquus* bulbs where purchased.

Figure 41: Map of sites where *C. obliquus* bulbs were purchased

Table 25: Geographical coordinates of the 8 chosen market sites where *C. obliquus* bulbs were purchased.

Sampling code	Site	Latitude	Longitude
A1	Durban	29° 1' 16" S	31° 0' 4" E
A2	Umzinto	30° 1 ' 27" S	30° 39' 58" E
A3	Eshowe	2 ° 3' 16" S	31° 2 ' 56" E
A4	Verulam	29° 3 ' 29" S	31° 2' 45" E
A5	Stanger	29° 21' 1" S	31° 1 ' 47" E
A6	Tongaat	29° 3 ' 16" S	31° 6' 58" E
A7	KwaNkalokazi	30° 26' 39" S	30° 10' 31" E
A8	Ixopo	30° 9' 25" S	30° 3' 49" E

Figure 42 shows the ten different sites in KwaZulu-Natal where *L. javanica* leaves and soil samples were collected.

Figure 42: Map of the 10 sampling sites in KwaZulu-Natal where *L. javanica* leaves and soil samples were collected

Table 26: Geographical coordinates for the 10 chosen sites where *L. javanica* leaves and soil samples were collected.

Sampling code	Site	Latitude	Longitude
B1	Amandawe	30° ' 58" S	30° 2' 51" E
B2	Eshowe	2 ° 3' 29" S	31° 29' 45" E
B3	Ntumeni	2 ° 1' 9" S	31° 19' 26" E
B4	Mangeti	29° 11' 11" S	31° 31' 32" E
B5	Maphumulo	29° 1 ' 47" S	31° ' 40" E
B6	Stanger	29° 20' 15" S	31° 1 ' 21" E
B7	Ndwedwe	29° 30' 35" S	30° 57' 20" E
B8	Ixopo	30° 13' 12" S	30° 2' 20" E
B9	KwaNkalokazi	30° 2 ' 17" S	30° 1 ' 30" E
B10	Highflats	30° 1 ' 56" S	30° 11' 52" E

4.4 Sample preparation and elemental analysis

Once the plant and soil samples were collected from the chosen sites, the samples were prepared for analysis then analysed using established techniques. Microwave digestion was utilized to digest solid samples. Elemental analysis was achieved by ICP-OES. Method validation was done to test the accuracy of the analytical methods.

4.5 Reagents and standards

All chemicals used were supplied by Merck and Sigma Chemical Companies and were of analytical-reagent grade**.** Double distilled water was used throughout the experiments. To minimize the risk of contamination all glassware and other equipment were cleaned with 6.0 M HNO_3 and rinsed off with double distilled water.

4.6 Sample preparation

Bulb and leaf samples were washed with doubly distilled water. Bulb samples were cut into smaller pieces with a stainless steel knife. All samples where then dried overnight in an oven at 0˚C. Thereafter, dried samples were crushed into a fine powder using a food processor (*Russell Hobbs* range) and stored in labelled polyethylene bags until required for analysis. Soil samples where frozen to prevent microbes from forming. Soil samples were dried in an oven at 0 ˚C, overnight. Thereafter, dried soil was sieved through a 2 mm mesh sieve to remove the gravel and debris. The 2 mm fraction was then placed into labelled polyethylene bags until required for analysis.

4.7 Digestion of samples

The bulb and leaf samples where digested prior to analysis using the microwave-assisted closed vessel technique. Digestions were performed using the CEM MARS (CEM Corporation, USA) microwave reaction system with patented Xpress technology. Five replicates of each sample where digested to improve accuracy and precision. The bulb and leaf samples were accurately weighed (0.5 g) into 50 mL liners (24 liners used) and 10 mL of 70% HNO_3 was added. For digestion of soil, 0.25 g was weighed accurately into the liners and 10 mL of 70% HNO_3 was added. The liners were capped, placed into sleeves, loaded into the 40-placed carousel accordingly to ensure a correct balance and then placed into the microwave.

4.7.1Programme for digestion of bulb and leaf samples

The power was set at 100% at 1600 W and the temperature was ramped to 1 0˚C (ramp time 15 min.) where it was held for 15 min. The digested samples where then removed from the vessels and transferred into 50 mL volumetric flasks, diluted to the mark with double distilled water and then stored into polyethylene bottles for elemental analysis.

4.7.2 Programme for digestion of soil samples

The power was set at 100% at 1600W and the temperature was ramped to 200˚C (ramp time 15 min.) where it was held for 15 min. The digested samples where then removed from the vessels, filtered through filter paper (to remove the undigested silicates) into transferred into

50 mL volumetric flasks, diluted to the mark with double distilled water and then stored into polyethylene bottles for elemental analysis.

4.8 Certified reference material (CRM)

The accuracy of the elemental determination was done by use of a CRM, *lyophilized brown* bread (BCR 191), from the Community Bureau of Reference of the Commission of the European Communities; the CRM was chosen to match the matrix and composition of the samples. The same analytical procedure was carried out for the CRM as the samples.

4.9 Extraction of exchangeable metals

A combination of chemical extractants, ammonium acetate, ethylenediaaminetetraacetic acid (EDTA) and acetic acid were used to release available metals from soil fractions. For good reproducibility, the soil/extractant ratio, temperature and duration of extraction were kept constant.

An extractant solution was prepared by dissolving 38.542 g of ammonium acetate ($NH_4CO_2CH_3$, 0.5 M), 25 mL of acetic acid (96% CH_3COOH) and 37.225 g of EDTA (0.1 M) in distilled water in a 1 L volumetric flask. Then 1.0 g of dry soil sample was mixed with 10 mL of extractant solution in a 50 mL polyethylene bottle and shaken in a laboratory shaker for 2 hours. The solution mixture was then filtered through a Millipore filter membrane (pore diameter 0.45 μm, membrane type HVLP) to permit the determination of the extracted elements. Thereafter, the filtrate was stored in labelled polyethylene bottles.

4.10 Imbiza (South African herbal tonic)

As per instructions by the herbalist, 0.375 g of *C. obliquus* bulbs was placed into a 200 mL beaker to which 50 mL of deionised water was added. The solution mixture was then placed onto a hot plate and brought to boil (at medium heat) for 10 min. The resulting solution was filtered by gravity (Whatman No. 4 filter paper) into a 50 mL volumetric flask and made up to the mark with deionised water. The solution was then transferred into labelled polyethylene bottles.

L. javanica leaves (0.200 g) were prepared in the same manner and the solution was stored in labelled polyethylene bottles.

4.11 Analytical methods used for elemental analysis

All the plant and soil samples where analysed for the following elements; As, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Se and Zn. Concentrations of As and Ni were below detection limits of the instrument therefore not determinable. Elemental analysis was by ICP-OES, Perkin Elmer Optima 5300 DV. The samples where determined in quintuplicates (n=5). Working standards were made up with doubly distilled water and 10 mL of 70 % HNO_3 to match the sample matrix. Emission lines were chosen based on minimal spectral interferences. Table 27 shows the selected wavelengths for the studied elements.

Table 27: Emission lines (Wavelengths) chosen for each element.

Element	Emission line /nm
Ca	317.93
Cd	228.80
Co	228.61
Cr	267.71
Cu	324.75
Fe	259.93
Mg	279.08
Mn	257.61
Pb	220.35
Se	203.98
Zn	206.20

4.12 Statistical analysis

The significance of plant-soil relationships was established by computing correlation coefficients (r) for the relationship between the concentration of the elements in *L. javanica* leaves and the total and exchangeable concentrations in the soil. Correlation coefficients were evaluated by Pearson's correlation analysis using the Statistical Package for the Social Science (SPSS) (PASW Statistics, Version 19, IBM Corporation, Cornell, New York).

4.13 Determination of soil quality

The following methods used for soil sample analysis are broadly explained in this section:

a) Determination of soil pH

b) Determination of SOM by Walkley-Black method

c) Determination of CEC by Chapman method

d) Kjeldahl Distillation

e) Extraction of bioavalable metals

4.13.1 Determination of soil pH

Soil pH was determined by the use of a 1:1 ratio of soil to diluted calcium chloride ($CaCl_2$). The pH meter fitted with a glass electrode was first calibrated with buffer solutions (pH 7 and pH 14), then 1.0 g of soil was weighed into a 100 ml plastic beaker. Thereafter 1 mL of 0.01 M $CaCl_2$ solution was added, the slurry was stirred vigorously and allowed to stand for 30 min. The pH electrode was then placed in the slurry, swirled carefully and the reading was taken immediately. The above experiment was done in quadruplicates.

4.13.2 Determination of SOM (Walkley-Black method)

Solutions

a) Potassium dichromate (0.167 M): 49.09 g of dried potassium dichromate ($K_2Cr_2O_7$) was dissolved in deionised water and diluted to 1 L.

b) Ferrous ammonium sulfate (0.5 M): 196.1 g of ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2.6H_2O$) was dissolved in 800 mL of deionised water containing 20 mL of concentrated sulfuric acid (H_2SO_4) and diluted to 1 L.

c) Diphenylamine indicator: 0.500 g of diphenylamine ($C_6H_5NHC_6H_5$) was dissolved in 20 mL deionised water, thereafter 100 mL of concentrated H_2SO_4 was added slowly. The solution was carefully mixed with a glass stirring rod.

Procedure

Depending on the type of soil used (light coloured soil or organic soil), 0.10 g to 2.0 g dried soil (passed through a 0.5 mm mesh sieve) was weighed and transferred into a 500 mL Erlenmeyer flask. Then 10 mL of 0.167 M $K_2Cr_2O_7$ was added into the flask by means of a pipette; thereafter 20 mL of concentrated H_2SO_4 was also added by means of a measuring cylinder. The contents in the flask were swirled gently to mix, avoiding excessive swirling which may result in the organic particles adhering to the sides of the flask out of the solution, and then it was allowed to stand for 30 min. The solution was then diluted to 200 mL with deionised water, thereafter 10 mL of 85% phosphoric acid (H_3PO_4) and 0.2 g of sodium fluoride (NaF) was added. Into the resulting solution 10 drops of diphenylamine indicator was added and the solution was rapidly titrated against 0.5 M $Fe(NH_4)_2(SO_4)_2.6H_2O$ until the dull green colour changed to turbid blue. Titration was then done dropwise until a brilliant green colour was reached, which signified the endpoint. The blank was prepared and titrated in the same manner.

Calculations

% Organic Carbon (C):

$$C \quad \frac{(B \; S) \quad M \text{ of } Fe^{2} \quad 12 \quad 100}{g \text{ of soil} \quad 000}$$

Where:

B = ml of Fe^{2+} solution used to titrate blank

S = ml of Fe^{2+} solution used to titrate sample

12/4000 = milliequivalent weight of C in g

Percent soil organic matter:

$$\text{Soil organic matter} \quad \frac{\text{total C} \quad 1.2}{0.}$$

4.13.3 Determination of CEC (Chapman method)

Solutions

a) Ammonium acetate (NH_4OAc, 1 M): In the fumehood 57 mL of glacial acetic acid (99.5%) was diluted with 800 mL of deionised water in a 1 L volumetric flask, thereafter 68 mL of concentrated ammonium hydroxide (NH_4OH) was added and the solution was mixed and cooled, then adjusted to pH 7.0 with NH_4OH if needed and diluted to 1 L.

b) Potassium Chloride (KCl, 1 M): 74.5 g of KCl was entirely dissolved in deionised water and diluted to a final volume of 1 L.

Procedure

Into a 500 mL Erlenymeyer flask, 25.0 g of soil was added along with 125 mL of NH_4OAc. The contents of the flask was shaken thoroughly then allowed to stand overnight. The solution was then filtered by suction filtration with the aid of a Buchner funnel. If the filtrate was not clear, the filtrate was refiltered through the soil. The soil was then washed with eight separate additions of 95% ethanol (EtOH) to remove excess saturating solution. The leachate was then discarded. The adsorbed ammonium ions (NH_4^+) were then extracted by leaching with 25 mL portions of 1 M KCl, leaching slowly. The soil was then discarded and the leachate was transferred into a 250 mL volumetric flask then diluted with KCl to the mark.

The concentration of NH_4-N in the KCl extract was determined by the Kjeldahl distillation method. The amount of NH_4-N in the original KCl extracting solution (blank) was also determined.

4.13.4 Kjeldahl distillation

The sample was transferred into a 500 mL Erlenmeyer flask and distilled water was added to give a total volume of 250 mL. Standardised 0.1 M hydrochloric acid (HCl) was added into the receiver flask by means of a pipette. The flask was clamped so that the tip of the adapter extended just below the surface of the acid. The water was circulated through the condenser. With the Kjeldahl flask tilted, 85 mL of concentrated NaOH, which was made by dissolving 45 g of NaOH pellets into 75 mL distilled water, was slowly poured down the walls of the flask to minimize mixing with the solution. About two pieces of granulated zinc were added along with red litmus paper. The flask was immediately reassembled with the spray trap and condenser. The solution was then mixed carefully by swirling, the litmus paper indicated that the solution was basic (red litmus paper turned blue). The solution was distilled at a steady rate, with controlled heating rates, until one-third of the original solution remained. After the distillation was judged complete, the heating was discontinued, the apparatus disconnected and the insides of the condenser and the adapter where washed with small amounts of distilled water. Then 2 drops of methyl orange indicator was added to the receiver flask and the residual HCl was titrated against standardised 0.1 M NaOH from pink to orange colour shift.

Calculations

$$C\,C\ \left(\frac{\text{meq}}{100}\text{g}\right)\quad \frac{(B\ S)\quad M\ \text{of Na}\qquad 100}{\text{g of sample}}$$

Where:

B = Titration of blank

S = Titration of sample

M = Molarity of standard alkali solution (NaOH)

CHAPTER 5

ELEMENTAL COMPOSITION OF CYRTANTHUS OBLIQUUS BULBS AND THEIR WATER EXTRACTS

5.1 Introduction

A large percentage of the communities living in rural areas utilize the herbal tonic, Imbiza for various ailments and diseases, from toothache, back pains, flu to kidney diseases, diabetes and even HIV/AIDS. This chapter focuses on the elemental composition of *C. obliquus* bulbs collected from different market sites in KwaZulu-Natal. Additionally, the concentration of the elements in *C. obliquus* bulbs will be compared to water extractable concentrations which are more closely related to the concentrations in the herbal tonic, Imbiza. The extraction percentages will be examined to evaluate how much of the total elemental load is transferred to the tonic/water which is the product consumed by humans.

Although samples were analysed for As and Ni, if present, they were below the instrument detection limits for all determinations. Therefore, these elements are omitted from further discussion. In this chapter, all the tables contain mean values with their standard deviations; standard deviations are omitted from the discussion for ease of reading.

5.2 Quality assurance

The accuracy of the trace element determinations were evaluated by comparison of the results of the CRM, *lyophilized brown bread* (BCR 191), obtained with the certified values (Table 28).

Table 28: Comparison of measured and certified values in the CRM (*lyophilized brown bread: BCR 191*).

Element	Wavelength/ nm	Concentration*	
		Certified**	**Measured****
Cu	324.76	2.6 ± 0.1 μg g^{-1}	2.8 ± 0.2 μg g^{-1}
Fe	259.93	40.7 ± 2.3 μg g^{-1}	40.6 ± 1.9 μg g^{-1}
Mn	257.61	20.3 ± 0.7 μg g^{-1}	20.1 ± 0.6 μg g^{-1}
Zn	260.20	19.5 ± 0.7 μg g^{-1}	19.0 ± 0.5 μg g^{-1}
Ca	317.93	0.41 mg g^{-1}	0.41 ± 0.02 mg g^{-1}
Mg	279.07	0.5 mg g^{-1}	0.5 ± 0.01 mg g^{-1}

*Based on dry mass ** Mean ± S.D, at 95% confidence interval, n=6

The values for Cu, Fe, Mn and Zn are certified concentration of the elements, however the values given for Ca and Mg are indicative therefore uncertainties were not provided for these elements. The measured values were found to be in good agreement with the certified values at 95% confidence interval.

5.3 Chemical composition of C. obliquus

Table 29: Elemental concentrations in μg g^{-1} (Mean (SD), n=5) of selected elements in the bulbs of *C. obliquus* and water extracts.

Element	*Site*[a]	*Bulb (B)*	*Water extract WE*	*[WE] /[B] %*
Ca	A 1	4617 (96)	458 (96)	9.9
	A 2	3043 (113)	955 (77)	31.4
	A 3	3059 (243)	484 (64)	15.8
	A 4	3949 (118)	362 (38)	9.2
	A 5	3067 (258)	408 (36)	13.3
	A 6	3765 (54)	706 (33)	18.8
	A 7	3736 (74)	636 (92)	17.0
	A 8	3022 (195)	406 (50)	13.4
Cr	A 1	0.202 (0.005)	ND	-
	A 2	0.340 (0.055)	ND	-
	A 3	0.640 (0.055)	ND	-
	A 4	0.140 (0.055)	ND	-
	A 5	ND	ND	-
	A 6	ND	ND	-
	A 7	0.360 (0.055)	ND	-
	A 8	0.110 (0.022)	ND	-
Cu	A 1	4.06 (0.09)	ND	-
	A 2	2.68 (0.29)	ND	-
	A 3	3.84 (0.11)	ND	-
	A 4	2.16 (0.21)	ND	-
	A 5	2.26 (0.15)	ND	-
	A 6	1.76 (0.27)	ND	-
	A 7	1.30 (0.07)	ND	-
	A 8	3.62 (0.15)	ND	-
Fe	A 1	61.0 (3.8)	27.8 (2.5)	45.6
	A 2	95.6 (7.5)	34.2 (1.4)	35.8
	A 3	119.4 (8.0)	53.2 (0.9)	44.6
	A 4	110.5 (22.4)	90.0 (2.2)	81.4
	A 5	37.7 (3.8)	27.5 (1.4)	72.9
	A 6	25.8 (3.1)	15.1 (0.4)	58.5
	A 7	97.7 (7.1)	60.1 (2.0)	61.5
	A 8	110.8 (2.7)	67.4 (2.0)	60.8
Mg	A 1	1007 (21)	180 (11)	17.8
	A 2	623 (14)	484 (18)	77.6
	A 3	613 (21)	232 (12)	37.8

	A 4	589 (14)	113 (3)	19.2
	A 5	815 (46)	270 (4)	33.1
	A 6	1316 (21)	510 (23)	38.8
	A 7	732 (6)	288 (20)	39.3
	A 8	506 (37)	182 (3)	36.0
Mn	A 1	16.12 (0.41)	1.77 (0.20)	11.0
	A 2	10.44 (0.48)	5.15 (0.47)	49.3
	A 3	5.24 (0.21)	1.72 (0.11)	32.8
	A 4	5.80 (0.22)	2.03 (0.22)	35.0
	A 5	2.46 (0.44)	0.46 (0.06)	18.7
	A 6	6.14 (0.11)	2.26 (0.22)	36.8
	A 7	7.96 (0.09)	2.24 (0.20)	28.1
	A 8	8.06 (0.45)	1.72 (0.15)	21.3
Pb	A 1	ND	ND	-
	A 2	0.54 (0.05)	ND	-
	A 3	0.66 (0.09)	ND	-
	A 4	0.42 (0.08)	ND	-
	A 5	0.42 (0.08)	ND	
	A 6	0.46 (0.05)	ND	-
	A 7	0.56 (0.05)	ND	-
	A 8	0.36 (0.05)	ND	-
Se	A 1	2.68 (0.24)	ND	-
	A 2	3.24 (0.24)	ND	-
	A 3	1.06 (0.21)	ND	-
	A 4	4.18 (0.18)	ND	-
	A 5	2.42 (0.26)	ND	-
	A 6	1.90 (0.27)	ND	-
	A 7	0.90 (0.07)	ND	-
	A 8	3.90 (0.34)	ND	-
Zn	A 1	13.5 (0.2)	6.9 (0.3)	51.1
	A 2	9.9 (0.9)	8.9 (0.2)	89.9
	A 3	42.2 (2.2)	23.6 (0.7)	55.9
	A 4	12.3 (1.0)	3.8 (0.2)	30.9
	A 5	12.9 (0.9)	10.0 (0.4)	77.5
	A 6	12.4 (1.3)	5.9 (0.3)	47.6
	A 7	8.2 (0.1)	7.5 (0.3)	91.5
	A 8	8.8 (0.3)	7.5 (0.2)	85.2

[a]Sites: A1-Durban, A2-Umzinto, A3-Eshowe, A4-Verulam, A5-Stanger, A6-Tongaat, A7-KwaNkalokazi and A8-Ixopo

ND: Not Determinable

In Table 29 the concentrations of the elements detected in the bulb samples of *C. obliquus* are shown. The bulbs were found to possess considerable amounts of Ca and Mg as compared to the other elements. Both these elements are said to be amongst the most abundant in plants (Sedaghathoor et al., 2009). Typical Ca concentrations in most plants are 5000 µg g^{-1} (Epstein, 1994); Ca concentrations in *C. obliquus* bulbs ranged from 3022 to 4617 µg g^{-1}, which was slightly lower than typical concentrations. Magnesium concentrations in the bulbs ranged from 506 to 1316 µg g^{-1}.

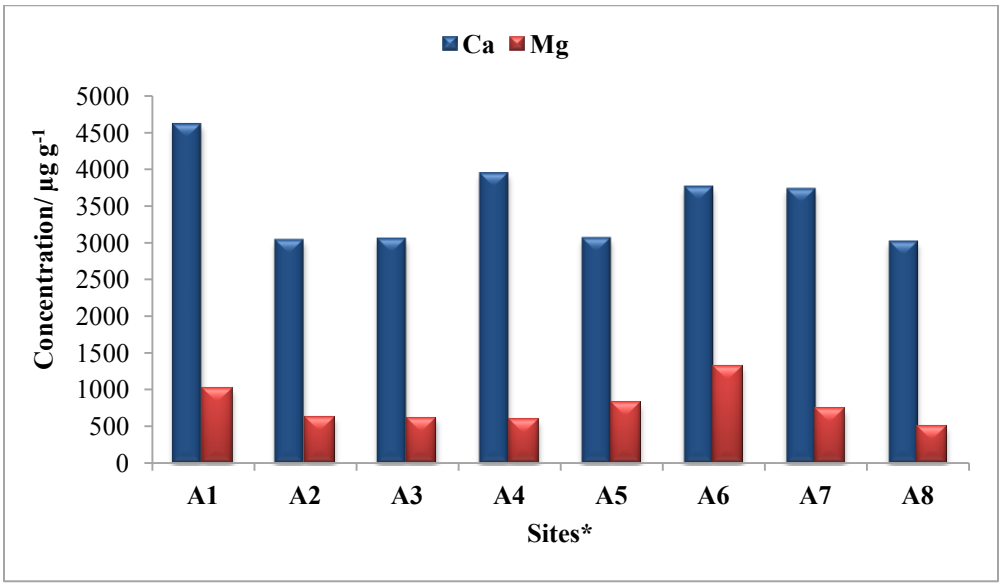


Figure 43: Distribution of the major elements in *C. obliquus* bulbs from the 8 different sites

Sites*: A1-Durban, A2-Umzinto, A3-Eshowe, A4-Verulam, A5-Stanger, A6-Tongaat, A7-KwaNkalokazi and A8-Ixopo

Figure 43 shows the distribution of the major elements, Ca and Mg, in *C. obliquus* bulbs at the eight different market sites in KZN. Calcium concentrations at all sites were much higher than Mg concentrations in the bulbs. Bulbs from site A1 (Durban) had the highest

concentration of Ca and those from site A6 (Tongaat) had the highest concentration of Mg. Though the exact location from which the bulbs were removed is not known, as these were purchased from traders who obtained them from suppliers, it is known that the bulbs were collected from the Eastern Cape in South Africa where the plant grows in abundance. The different concentrations in the bulbs from different market sites indicate that the bulbs were obtained from different sites with differing soil properties.

The minor elements that were assessed were Cr, Cu, Fe, Mn, Pb, Se and Zn (Figure 44). The bulbs contained relatively higher amounts of Fe than any other minor element. Sites A3 (Eshowe), A4 (Verulam) and A8 (Ixopo) had higher concentrations of Fe in the bulbs whilst A5 (Stanger) and A6 (Tongaat) contained the lowest concentrations. The distribution of Cr, Cu, Se and Pb in the bulbs was somewhat similar at the different sites except for sites A6 and A1 where no Cr and Pb were detected, respectively.

The concentrations of Mn and Zn varied somewhat at the different sites. Manganese concentrations were highest at site A1 and lowest at site A5. The variation in Mn concentration was not as significant as that of Zn were Zn concentration at the different sites ranged from 8.2 to 42.2 $\mu g\ g^{-1}$. Site A3 had the highest Zn concentration.

The concentration of the elements in *C. obliquus* bulbs was, typically, in decreasing order of Ca > Mg > Fe > Zn > Mn > Cu = Se > Pb > Cr.

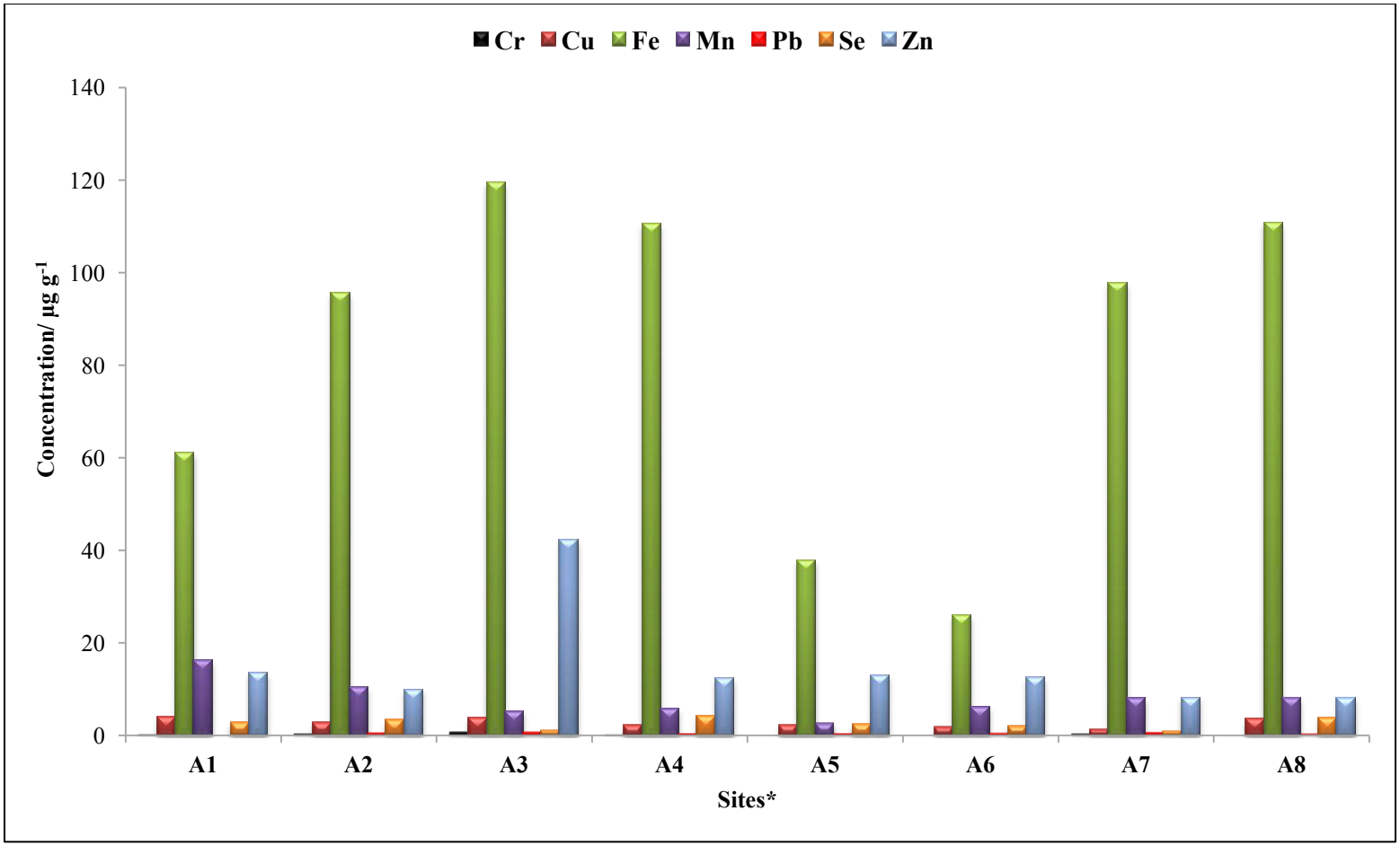


Figure 44: Distribution of the minor elements in *C. obliquus* bulbs at the 8 different sites

Sites*: A1-Durban, A2-Umzinto, A3-Eshowe, A4-Verulam, A5-Stanger, A6-Tongaat, A7-KwaNkalokazi and A8-Ixopo

Elemental concentrations of elements Ca, Fe, Mg, Mn and Zn in *C. obliquus* bulbs were compared to concentrations of these elements in the water extracts which more closely represents concentrations in the herbal tonic, Imbiza (Table 29). The extraction percentages of the elements were also determined to ascertain what fraction of the elements from the bulbs was extracted into solution. If present in the water extracts, Cr, Cu, Pb and Se were below the instrument detection limits.

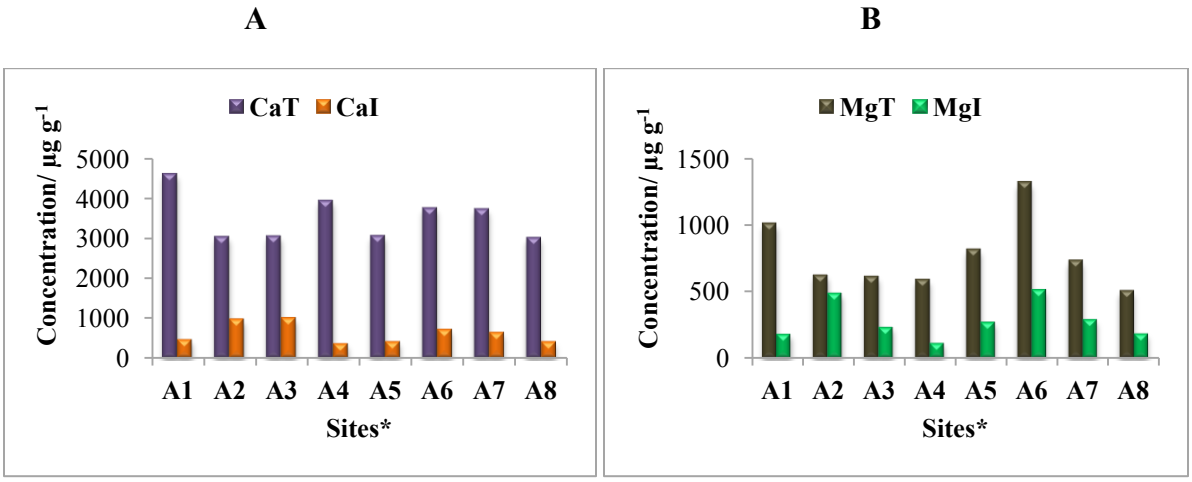


Figure 45: Total (T) concentrations of Ca and Mg in bulbs compared to concentrations in water extract/Imbiza (I)

Sites*: A1-Durban, A2-Umzinto, A3-Eshowe, A4-Verulam, A5-Stanger, A6-Tongaat, A7-KwaNkalokazi and A8-Ixopo

Calcium concentrations extracted from the bulbs at all sites were relatively low (Figure 45). The extraction percentages ranged from 9.2 to 31.4% (Table 29). The extraction percentage for Mg ranged from 17.7 to 77.6% with relatively high extraction percentages at most sites. Although the extraction percentages for Mg were high, concentrations of Mg in the solution were still lower than Ca.

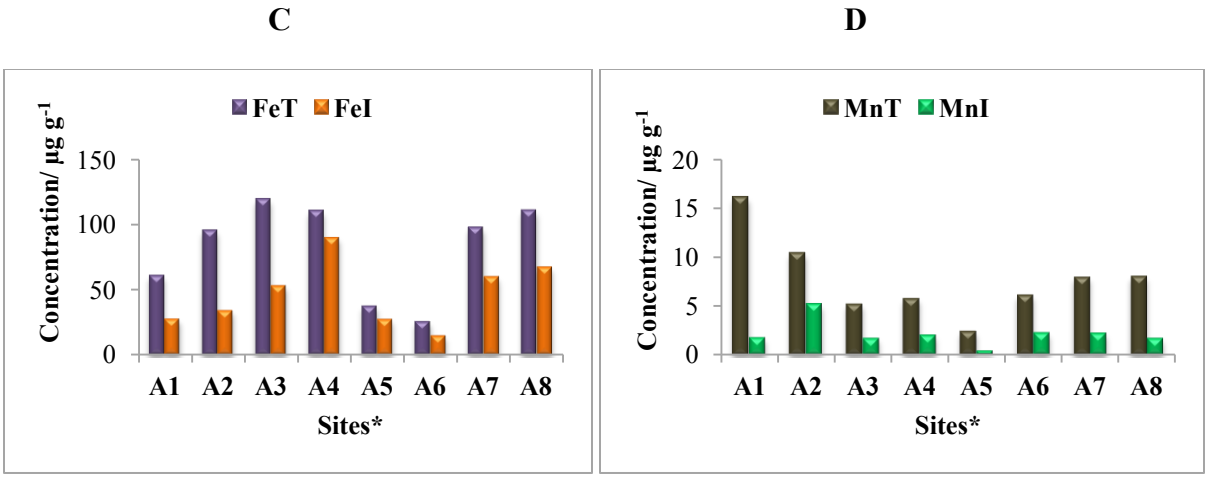


Figure 46: Total (T) concentrations of Fe and Mn in bulbs compared to concentrations in water extract/Imbiza (I)

Sites*: A1-Durban, A2-Umzinto, A3-Eshowe, A4-Verulam, A5-Stanger, A6-Tongaat, A7-KwaNkalokazi and A8-Ixopo

Extraction percentages of Fe at all sites ranged from 35.8 to 81.4 % (Figure 46) with more Fe being extracted from bulbs obtained at sites A4 and A8. The percentage of Mn extracted was between 11.0 to 49.3 %. This means that the extracted Fe concentrations more closely resembled bulb concentrations compared Mn.

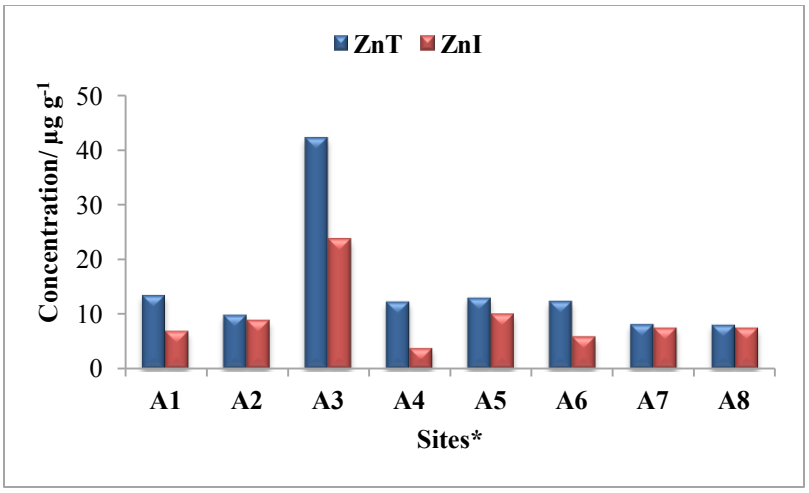


Figure 47: Total (T) concentration of Zn in bulbs compared to concentration in water extract/Imbiza (I)

Sites*: A1-Durban, A2-Umzinto, A3-Eshowe, A4-Verulam, A5-Stanger, A6-Tongaat, A7-KwaNkalokazi and A8-Ixopo

The percentage of the total Zn extracted was relatively high compared to the other elements with extraction percentages ranging from 30.9 to 91.5 %. For most sites, the concentration of Zn in the bulbs was more closely related to bulb concentrations.

The extraction percentages give an idea of what fraction of the elements from the bulbs are transferred to the solution used to make the herbal tonic, Imbiza. For the detected elements, the concentrations of elements in the water extracts were, generally, in decreasing order of Ca > Mg > Fe > Zn > Mn.

CHAPTER 6

ELEMENTAL COMPOSITION OF LIPPIA JAVANICA AND THEIR WATER EXTRACTS: IMPACT OF SOIL QUALITY

6.1 Introduction

An infusion of *L javanica* leaves, if ingested, are claimed to remedy colds, flu, fever and malaria. Due to the claimed medicinal value of the leaves, it is usually taken as a caffeine-free tea. This chapter focuses on the elemental composition of *L. javanica* leaves collected from various locations in KwaZulu-Natal. The concentration of the elements in the leaves will be compared to water extractable concentrations which are more closely related to the concentrations in the herbal tonic, Imbiza. The extraction percentages will be examined to evaluate how much of the total elemental load is transferred to the tonic/water. Since elemental concentrations in plants are dependent on growth soil, the impact of soil quality on elemental uptake and distribution will be assessed. The soil parameters that were assessed are total and exchangeable elemental concentrations, pH, SOM and CEC. Statistical analysis was necessary to evaluate the impact of soil quality parameters on elemental uptake in the plant. Furthermore, geoaccumulation indices were computed to evaluate for metal contamination in soil. In this chapter, all the tables contain mean values with their standard deviations; standard deviations are omitted from the discussion for ease of reading.

6.2 Chemical composition of L. javanica leaves and impact of soil quality

Table 30: Elemental concentrations in μg g^{-1} (Mean (SD), n=5) of selected elements in *L. javanica* leaves, soil (total (T) and exchangeable (Ex)) and water extracts.

Element	*Site*[a]	*Soil (T)* S_T	*Soil (Ex)* S_{Ex}	$[S]_{Ex}/[S]_T$ %	*Leaves L*	*Water extract WE*	*[WE]/[L]* %
Ca	B 1	3579 (267)	1115 (59)	31.2	5584 (313)	1309 (182)	23.4
	B 2	2333 (158)	2101 (233)	90.1	7974 (616)	1497 (59)	18.8
	B 3	3191 (85)	1313 (84)	41.1	2856 (93)	464 (54)	16.2
	B 4	2176 (125)	838 (61)	38.5	8642 (286)	1634 (58)	18.9
	B 5	2114 (76)	1603 (41)	75.8	4629 (58)	595 (33)	12.9
	B 6	1500 (73)	1333 (63)	88.9	6886 (110)	1479 (116)	21.5
	B 7	1395 (88)	1080 (97)	77.4	6538 (165)	1276 (117)	19.5
	B 8	1459 (29)	1210 (113)	82.9	9225 (549)	1378 (66)	14.9
	B 9	901 (85)	352 (21)	39.1	8370 (312)	1499 (121)	17.9
	B 10	966 (74)	425 (46)	44.0	8317 (71)	1361 (187)	16.4
Cd	B 1	1.64 (0.09)	ND	-	0.39 (0.03)	ND	-
	B 2	1.56 (0.09)	ND	-	0.64 (0.06)	ND	-
	B 3	1.44 (0.92)	ND	-	0.56 (0.06)	ND	-
	B 4	1.76 (0.09)	ND	-	ND	ND	-
	B 5	1.32 (0.18)	ND	-	ND	ND	-
	B 6	1.40 (0.14)	ND	-	ND	ND	-
	B 7	1.28 (0.11)	ND	-	ND	ND	-
	B 8	1.32 (0.18)	ND	-	ND	ND	-
	B 9	1.04 (0.17)	ND	-	ND	ND	-
	B 10	1.20 (0.14)	ND	-	ND	ND	-
Co	B 1	13.9 (0.5)	3.10 (0.20)	22.3	0.20 (0)	ND	-
	B 2	4.0 (0.1)	0.50 (0)	12.5	0.10 (0)	ND	-
	B 3	2.7 (0.4)	0.50 (0)	18.5	0.20 (0)	ND	-
	B 4	9.6 (0.7)	7.30 (0.67)	76.0	0.20 (0)	ND	-
	B 5	5.5 (0.3)	3.80 (0.45)	69.1	ND	ND	-
	B 6	4.7 (0.3)	1.90 (0.22)	40.4	0.26 (0.02)	ND	-
	B 7	3.1 (0.3)	0.48 (0.04)	15.5	0.19 (0.01)	ND	-
	B 8	3.8 (0.5)	0.45 (0.07)	11.8	ND	ND	-
	B 9	3.6 (0.4)	1.00 (0)	27.8	ND	ND	-
	B 10	3.1 (0.4)	1.40 (0.22)	45.2	ND	ND	-

Cr	B 1	52.7 (4.2)	ND	-	2.52 (0.22)	1.06 (0.08)	42.1
	B 2	39.8 (2.6)	ND	-	1.60 (0.12)	0.95 (0.07)	59.4
	B 3	20.6 (1.0)	ND	-	1.31 (0.02)	0.94 (0.05)	71.8
	B 4	72.0 (6.4)	ND	-	1.14 (0.09)	1.07 (0.05)	93.9
	B 5	12.3 (0.9)	ND	-	0.19 (0.02)	ND	-
	B 6	28.9 (2.9)	ND	-	0.66 (0.06)	ND	-
	B 7	45.9 (4.1)	ND	-	1.90 (0.19)	0.24 (0.02)	12.6
	B 8	6.2 (0.9)	ND	-	0.59 (0.02)	ND	-
	B 9	6.7 (0.6)	ND	-	ND	ND	-
	B 10	3.7 (0.9)	ND	-	ND	ND	-
Cu	B 1	34.7 (0.5)	3.20 (0.45)	9.2	4.06 (0.56)	2.33 (0.17)	57.4
	B 2	21.4 (0.5)	3.10 (0.42)	14.5	2.46 (0.28)	0.53 (0.06)	21.5
	B 3	20.6 (0.5)	2.20 (0.45)	10.7	4.78 (0.08)	0.74 (0.07)	15.5
	B 4	13.9 (1.1)	0.46 (0.11)	3.3	4.98 (0.19)	1.20 (0.07)	24.1
	B 5	8.3 (0.6)	4.06 (0.44)	48.9	8.48 (0.34)	0.26 (0.02)	3.1
	B 6	11.8 (0.8)	2.10 (0.42)	17.8	7.74 (0.40)	2.93 (0.11)	37.9
	B 7	8.7 (0.5)	0.50 (0)	5.7	3.40 (0.19)	ND	-
	B 8	5.4 (0.3)	ND	-	4.58 (0.48)	ND	-
	B 9	5.5 (0.6)	ND	-	5.74 (0.50)	2.80 (0.11)	48.8
	B 10	3.5 (0.3)	ND	-	1.92 (0.08)	ND	-
Fe	B 1	20284 (975)	87 (3)	0.4	146 (11)	40.7 (0.4)	27.9
	B 2	17389 (231)	275 (28)	1.6	166 (13)	47.7 (0.5)	28.7
	B 3	8793 (336)	216 (19)	2.5	186 (7)	42.8 (0.4)	23.0
	B 4	10133 (842)	109 (7)	1.1	266 (8)	78.7 (0.6)	29.6
	B 5	17090 (338)	397 (15)	2.3	178 (6)	41.2 (0.5)	23.1
	B 6	11336 (667)	259 (6)	2.3	167 (3)	38.6 (0.3)	23.1
	B 7	22085 (790)	141 (8)	0.6	742 (15)	30.5 (0.2)	4.1
	B 8	13427 (189)	168 (3)	1.3	59 (3)	16.3 (0.3)	27.5
	B 9	10677 (654)	32 (4)	0.3	195 (18)	22.9 (0.4)	11.7
	B 10	10000 (929)	51 (2)	0.5	64 (2)	20.3 (0.4)	31.7
Mg	B 1	6076 (167)	190 (8)	3.1	5619 (249)	2394 (66)	42.6
	B 2	1273 (26)	856 (33)	67.3	2619 (207)	1314 (20)	50.2
	B 3	1353 (62)	387 (13)	28.6	2242 (45)	435 (6)	19.4
	B 4	599 (61)	264 (16)	44.0	3599 (79)	1343 (31)	37.3
	B 5	1350 (78)	232 (4)	17.2	1598 (37)	501 (15)	31.4
	B 6	1511 (69)	567 (76)	37.5	4103 (56)	2016 (74)	49.1
	B 7	1186 (50)	511 (18)	43.1	2145 (90)	801 (32)	37.3
	B 8	1476 (38)	703 (19)	47.6	2446 (83)	834 (10)	34.1
	B 9	945 (73)	831 (22)	87.9	2968 (67)	1226 (14)	41.3
	B 10	944 (32)	621 (93)	65.8	3551 (74)	1073 (5)	30.2

Mn	B 1	368 (11)	116 (5)	31.6	18.9 (0.9)	ND	-
	B 2	119 (5)	35 (4)	29.6	15.1 (1.0)	ND	-
	B 3	118 (4)	75 (4)	63.8	45.1 (1.0)	ND	-
	B 4	197 (8)	196 (4)	99.2	58.4 (1.6)	ND	-
	B 5	197 (6)	193 (20)	98.3	13.6 (0.3)	ND	-
	B 6	92 (4)	67 (2)	72.3	60.4 (0.4)	ND	-
	B 7	117 (4)	54 (4)	46.4	34.1 (1.4)	ND	-
	B 8	86 (6)	37 (3)	42.3	57.4 (3.1)	ND	-
	B 9	89 (3)	40 (2)	44.7	64.2 (1.4)	ND	-
	B 10	87 (7)	39 (2)	45.4	33.7 (0.5)	ND	-
Pb	B 1	12.0 (0.9)	2.60 (0.50)	21.7	0.92 (0.08)	0.34 (0.04)	37.0
	B 2	11.7 (0.7)	4.20 (0.42)	35.9	0.38 (0.03)	0.06 (0.01)	15.8
	B 3	91.0 (5.7)	61.50 (3.49)	67.6	1.00 (0.07)	0.80 (0.01)	80.0
	B 4	5.3 (0.2)	1.40 (0.27)	26.4	0.54 (0.06)	0.30 (0.01)	55.6
	B 5	18.3 (0.9)	6.30 (0.57)	34.4	0.44 (0.04)	0.05 (0)	11.4
	B 6	10.8 (0.9)	5.30 (0.27)	49.1	0.45 (0.05)	ND	-
	B 7	11.1 (0.5)	4.40 (0.57)	39.6	1.18 (0.08)	0.06 (0)	5.1
	B 8	4.8 (0.5)	2.10 (0.35)	43.8	0.75 (0.07)	0.05 (0)	6.7
	B 9	5.1 (0.3)	1.70 (0.22)	33.3	0.80 (0.07)	0.55 (0)	68.8
	B 10	3.4 (0.3)	2.00 (0.55)	58.8	0.61 (0.02)	0.29 (0.03)	47.5
Se	B 1	7.0 (0.8)	ND	-	3.34 (0.37)	ND	-
	B 2	5.8 (0.9)	ND	-	4.96 (0.30)	ND	-
	B 3	ND	ND	-	1.68 (0.17)	ND	-
	B 4	24.8 (1.5)	10.50 (0.79)	42.3	1.58 (0.11)	ND	-
	B 5	ND	ND	-	3.40 (0.16)	ND	-
	B 6	5.6 (0.5)	1.05 (0.06)	18.8	2.20 (0.16)	ND	-
	B 7	12.6 (0.6)	7.50 (0.50)	59.5	0.86 (0.09)	ND	-
	B 8	ND	ND	-	ND	ND	-
	B 9	ND	ND	-	ND	ND	-
	B 10	ND	ND	-	ND	ND	-
Zn	B 1	39.6 (1.2)	5.8 (0.6)	14.6	18.2 (1.0)	6.3 (0.3)	34.6
	B 2	58.4 (1.6)	22.2 (1.4)	38.0	27.3 (2.2)	11.7 (0.5)	42.9
	B 3	115.5 (3.1)	50.9 (4.0)	44.1	20.6 (1.4)	6.7 (0.2)	32.5
	B 4	37.9 (1.8)	1.6 (0.1)	4.2	27.2 (1.6)	13.5 (0.3)	49.6
	B 5	75.0 (2.2)	27.4 (2.4)	36.5	26.2 (1.1)	9.1 (0.4)	34.7
	B 6	22.0 (1.2)	9.4 (1.0)	42.7	24.7 (1.4)	7.2 (0.3)	29.1
	B 7	30.6 (1.9)	4.2 (0.4)	13.7	16.5 (1.0)	11.2 (0.3)	67.9
	B 8	26.4 (1.9)	ND	-	15.6 (1.0)	13.9 (0.4)	89.1
	B 9	18.2 (1.9)	ND	-	25.1 (2.5)	11.0 (0.5)	43.8
	B 10	29.6 (1.0)	0.5 (0)	1.7	15.7 (0.4)	4.2 (0.2)	26.8

"Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats.
ND: Not Determinable

For the selected elements, concentrations in soil (both total and exchangeable), *L. javanica* leaves and water extracts from ten different sites in KZN are shown in Table 30. The exchangeable percentage gives the percentage of elements from soil that is available for plant uptake (Table 30). The extraction percentage gives the percentage of elements from leaves that have been transferred to solution (Table 30).

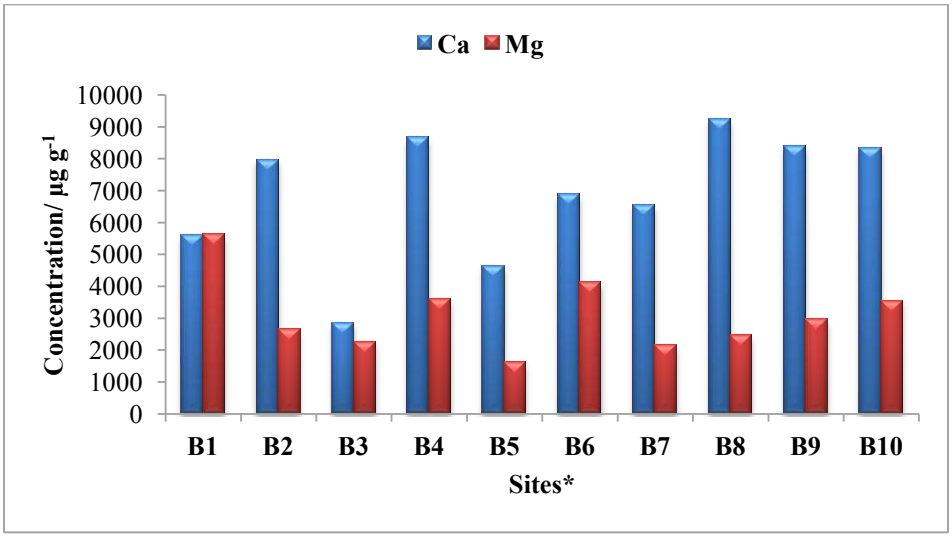


Figure 48: Distribution of the major elements in *L. javanica* leaves at the 10 different sites

*Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

The distribution of the major elements (Ca and Mg) in the leaves at the different locations is shown in Figure 49. The concentration of Ca in the leaves ranged from 2856 to 9225 µg g^{-1} and

Mg ranged from 1598 to 5619 μg g^{-1}. The ratio of Ca to Mg in the leaves at most sites was 1:3, except at B1 where the ratio is 1. According to Sedaghatoor et al. (2009), Ca and Mg are amongst the most abundant elements in tea plants.

Calcium concentrations in the soil were highest compared to the other elements with total soil Ca ranging from 901 to 3579 μg g^{-1}. Sites B9 and B10 were observed to have low levels of Ca. The percentage of total soil Ca that was in exchangeable form was between 31.2 and 90.1% at all sites. However, as seen from Table 30, the plant tended to accumulate Ca with concentrations in the leaves being much higher than the total amount in soil.

Total soil Mg ranged from 599 to 6076 μg g^{-1} with exchangeable concentrations ranging from 190 to 856 μg g^{-1}. The soils obtained from sites B9 and B10, similar to Ca, contained low levels of Mg. In spite of the estimated available concentrations of Mg, the concentrations in the leaves were quite high, even higher than total soil concentrations thereby indicating the plants tendency to accumulate this element.

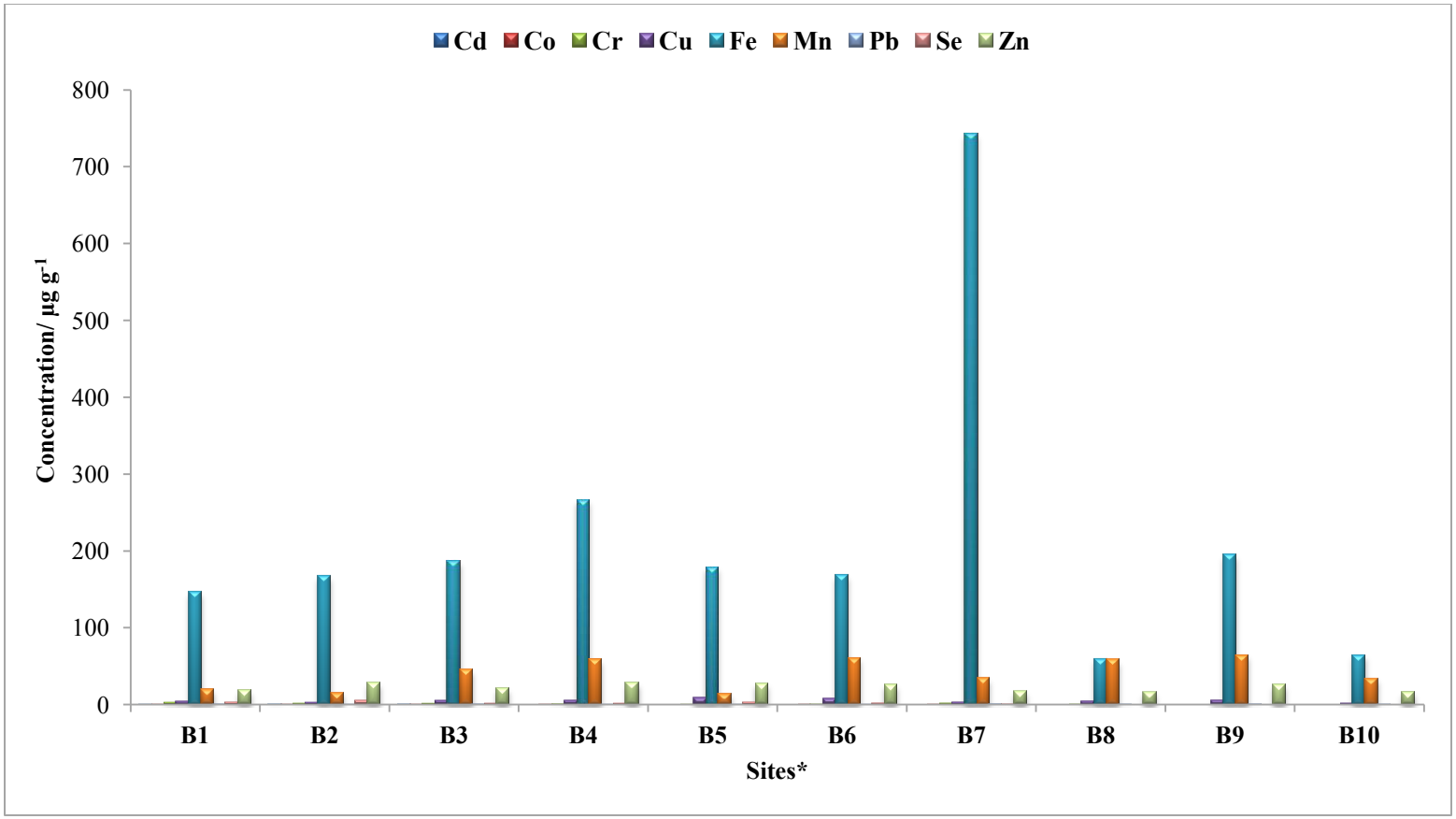


Figure 49: Distribution of the major elements in *L. javanica* leaves at the 10 different sites

*Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

The distribution of the minor elements in the leaves is presented in Figure 49. Amongst the minor elements, Fe was shown to have the highest concentration with concentrations ranging from 59 to 742 μg g^{-1}. Exchangeable Fe was observed to be extremely low compared to total soil Fe; Fe maybe bound in Al-Mn-hydroxide complexes or contained in the mineral lattice. Fe in the leaves was comparable to exchangeable Fe. Site B7 showed high levels of Fe in the leaves compared to the other sites.

There was a small range of variation between Mn in the leaves at all sites with Mn concentrations ranging from 13.7 to 64.2 μg g^{-1}. Total soil Mn ranged from 86 to 368 μg g^{-1} whilst exchangeable Mn ranged from 35 to 196 μg g^{-1}.

Total soil Cd ranged from 1.04 to 1.76 μg g^{-1}; this shows that soils from all the sites have similar Cd concentrations. Exchangeable Cd was not detected as it was below the instruments detection limit. Sites B1, B2 and B3 contained Cd in leaves with concentrations of 0.39, 0.64 and 0.56 μg g^{-1}, respectively whilst no Cd was detected in leaves obtained from the other sites.

Total soil Co ranged from 2.7 to 13.9 μg g^{-1}; the lowest concentration being at site B3. Exchangeable Co ranged from 0.45 to 7.30 μg g^{-1} and very little of the available form is taken up by the plant; Co concentrations in the leaves ranged from 0.10 to 0.26 μg g^{-1}. Elements like Cd and Co, amongst others, are required in minimal amounts in the plant as observed in this study.

Total soil Cr varied between 3.7 and 72.0 μg g^{-1} with the exchangeable form being below the instruments detection limit at all sites. Even though this was the case, the leaves at most sites seemed to be able to absorb the element from the soil, with Cr concentrations in leaves ranging from 0.59 to 2.52 μg g^{-1}.

Total soil Cu ranged from 3.5 to 34.7 μg g^{-1} and Cu in the leaves ranged from 1.92 to 8.48 μg g^{-1}. The typical concentration of Cu for most plants is 6 μg g^{-1} (Epstein, 1994), but leaves from sites B5 (8.48 μg g^{-1}) and B6 (7.74 μg g^{-1}) had higher than typical levels even though soil concentrations were relatively low.

Lead concentrations in the leaves from different sites were within a small range of variation between 0.38 to 1.18 μg g^{-1}. Total soil Pb was somewhat similar for the sites, except site B3 where total soil Pb was relatively high (91.0 μg g^{-1}). Although this was the case at site B3, Pb in the leaves was at 1.00 μg g^{-1}, which shows the plants tendency to exclude this non-essential element.

Selenium in soil was detected at five sites with exchangeable Se being detected in three of these sites. Selenium was detected in leaves at seven sites with concentrations ranging from 0.86 to 4.96 μg g^{-1}. At sites B3 and B5 no Se was detected in soil yet Se was detected in leaves (1.68 and 3.40 μg g^{-1}, respectively).

Total soil Zn varied from 18.2 to 115.5 μg g^{-1}, exchangeable Zn ranged from 0.50 to 50.9 μg g^{-1} and Zn in the leaves ranged from 15.6 to 27.3 μg g^{-1}. Zinc concentrations in the leaves reveal that the plant has a tendency to accumulate the element.

For the detected elements, the concentrations of elements in *L. javanica* leaves were typically in decreasing order of Ca > Mg > Fe > Zn > Mn > Cu > Se > Cr > Pb > Co > Cd.

From leaves, the elements extracted into water were Ca, Cr, Cu, Fe, Mg, Pb and Zn. If present in solution, other elements were below the instruments detection limits.

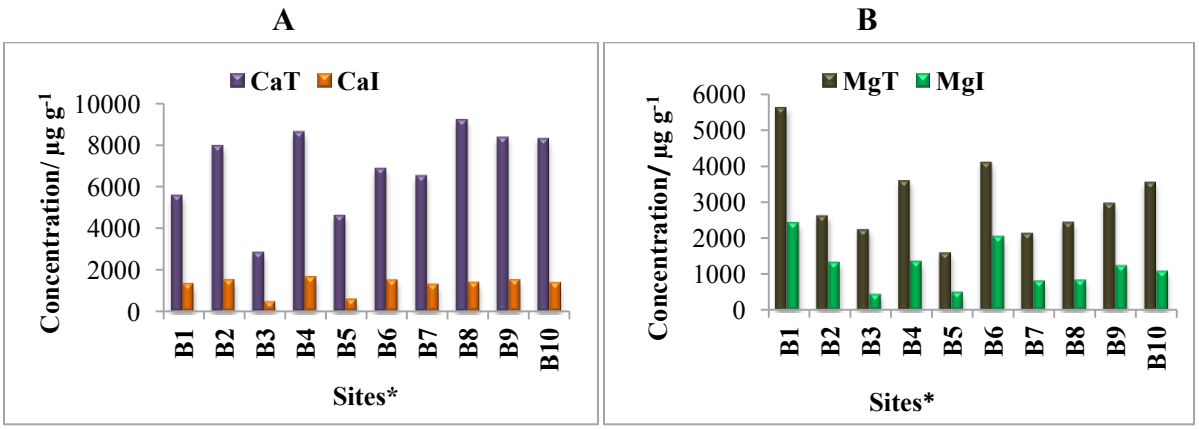


Figure 50: Total (T) concentrations of Ca and Mg in bulbs compared to concentrations in water extract/Imbiza (I)

*Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

Calcium concentrations in *L. javanica* leaves were high, but the amount extracted was considerably lower (Figure 50). The extraction percentage was between 16.2 to 23.4%, which was similar to that obtained for *C. obliquus* bulbs (Chapter 5). Even though the extraction percentages are similar, the concentrations of the element in the plants differ significantly with higher Ca concentrations in *L. javanica* leaves than *C. obliquus* bulbs. The extraction percentage for Mg was between 31.4 to 37.3% in the leaves which was also similar to that obtained for *C. obliquus* bulbs (Chapter 5). Again, the concentrations of the element in the plants differ significantly with higher Mg concentrations in *L. javanica* leaves than *C. obliquus* bulbs.

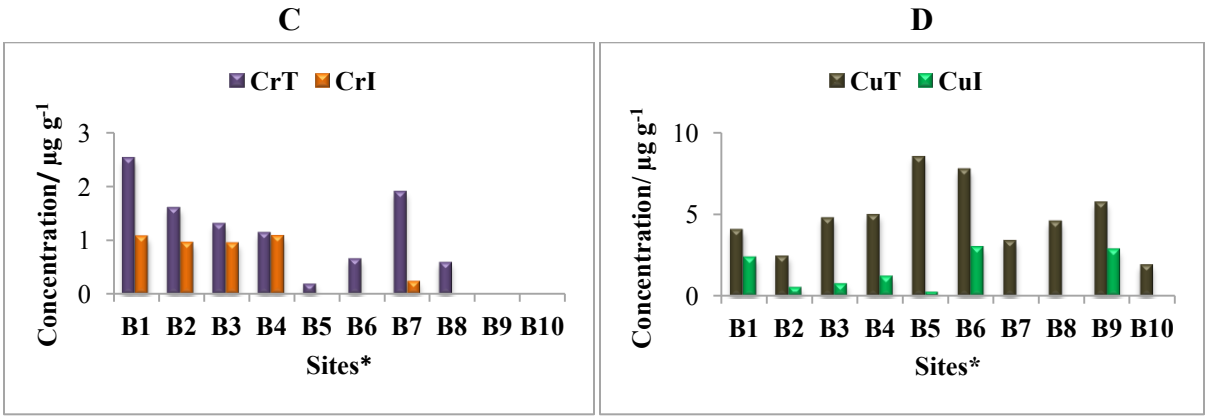


Figure 51: Total (T) concentrations of Cr and Cu in bulbs compared to concentrations in water extract/Imbiza (I)

*Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

The extraction percentage for Cr ranged from 71.8 to 93.9%, which is relatively high (Figure 51). This indicates that more than 70% of Cr from the leaves will be extracted into solution and consumed. Copper concentrations in the leaves were relatively low and for the very low concentrations Cu was not extracted by water. When Cu was extracted, it was observed that the estimated range was between 21.5 to 48.8%.

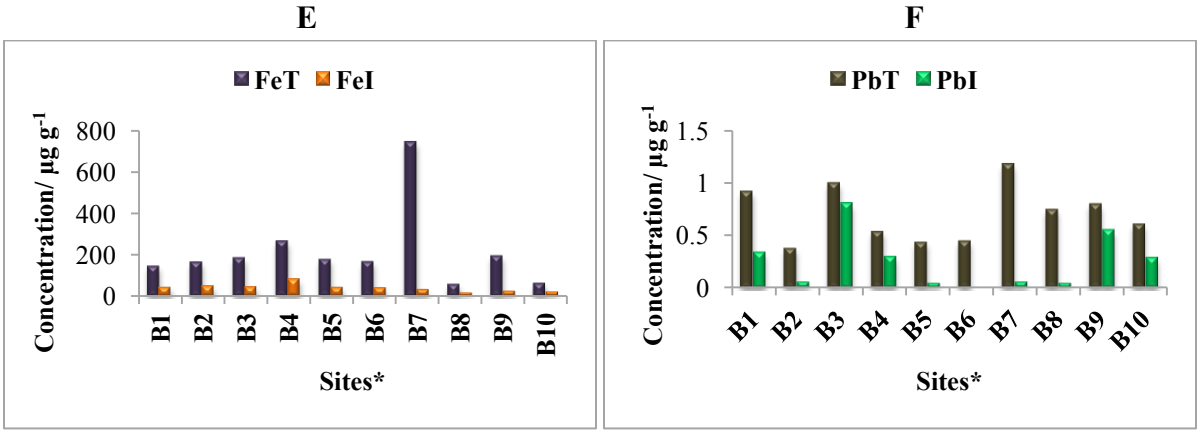


Figure 52: Total (T) concentrations of Fe and Pb in bulbs compared to concentrations in water extract/Imbiza (I)

The extraction percentage for Fe from *L. javanica* leaves was between 23.1 to 28.7% which was lower than that obtained for *C. obliquus* bulbs (44.6 to 61.5%, Chapter 5). The extraction percentage estimated for Pb was 37.0 to 68.8%; a very wide range was obtained probably due to the extremely low concentrations in the leaves.

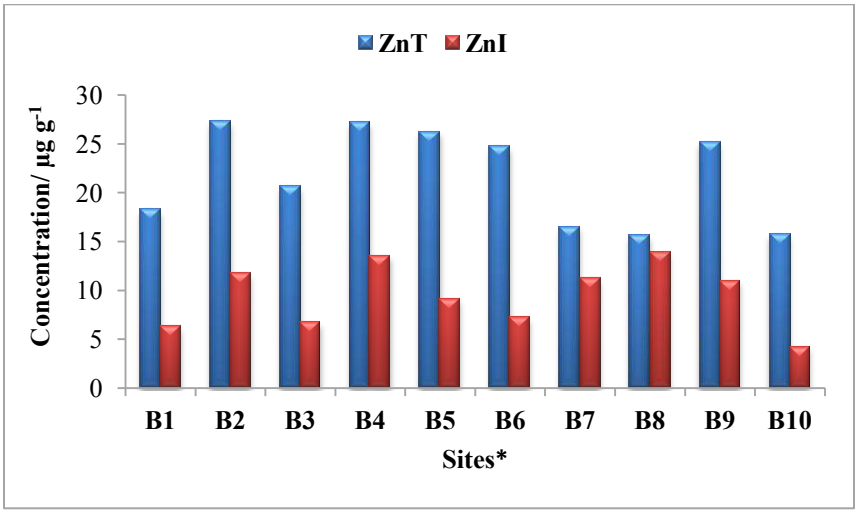


Figure 53: Total (T) concentration of Zn in bulbs compared to concentration in water extract/Imbiza (I)

*Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats.

For most sites, Zn in leaves was more than double the extracted amount. The extraction percentage for Zn ranged from 29.1 to 89.1%.

For the detected elements, the concentrations of elements in the water extracts were, generally, in decreasing order of Ca > Mg > Fe > Zn > Cu > Cr > Pb.

6.4 Bioaccumulation factors (BAFs)

The bioaccumulation factors (BAFs) of *L. javanica* leaves for selected elements are represented in tables 31 to 40 and the relative accumulation graphs are plotted in figures 54 to 63. Plants tend to accumulate an element until required levels are reached (Moodley et al., 2007), but once the level of the element exceeds required levels, adverse effects may occur. Accumulation of the element in the plant affects the absorption and transport of other essential elements therefore impacting the growth and reproduction of the plant (Xu & Shi, 2000). According to Timperley et al. (1973), a plot of relative accumulation as a function of total soil content indicates essentiality of the element if a rectangular hyperbola is produced and non-essentiality if a linear plot parallel to the x-axis is obtained.

In this section, BAFs are computed to determine whether the plant accumulates or excludes an element and BAFs are plotted against total and exchangeable soil concentrations to assess for essentiality and non-essentiality according to the trends of Timperley et al. (1973)

Table 31: Ca concentrations in µg g^{-1} (Mean (SD), n=5) in *L. javanica* leaves, soil (total (T) and exchangeable (Ex)) and water extracts, and bioaccumulation factors (BAFs).

Sites*				BAF	
	$Soil_T$	$Soil_{Ex}$	Leaves	$[Leaves]/[Soil]_{Ex}$	$[Leaves]/[Soil]_T$
B 1	3579 (267)	1115 (59)	5584 (313)	5.0	1.6
B 2	2333 (158)	2101 (233)	7974 (616)	3.8	3.4
B 3	3191 (84.8)	1313 (84)	2856 (93)	2.2	0.9
B 4	2176 (125)	838 (61)	8642 (286)	10.3	4.0
B 5	2114 (76)	1603 (41)	4629 (58)	2.9	2.2
B 6	1500 (73)	1333 (63)	6886 (110)	5.2	4.6
B 7	1395 (88)	1080 (97)	6538 (165)	6.1	4.7
B 8	1459 (29)	1210 (113)	9225 (549)	7.6	6.3
B 9	901 (85)	352 (21)	8370 (312)	23.8	9.3
B 10	966 (74)	425 (46)	8317 (71)	19.6	8.6

*Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

Table 30 shows the leaves to have higher concentrations of Ca than the soil (total and exchangeable); as a result the BAFs are relatively high. Plants with BAFs greater than one (>1) are deemed accumulators of that element (Ma et al., 2001; Cluis, 2004). Generally *L. javanica* would be considered to be an accumulator of Ca. *L. javanica* leaves from sites B9 and B10 showed higher accumulation of Ca compared to the other sites, for both total and exchangeable soil concentrations. The concentrations in the leaves were more comparable to total soil Ca than exchangeable soil Ca.

From the plot of BAF vs total and exchangeable concentration of Ca in soil, the shape of the curve points to essentiality of the element (Figure 54). Plants which are rich in Ca^{2+} are called calciotrophs eg. Crussulaceae (Ernest, 1982). The plants preference for Ca^{2+} is restricted to the leaves whilst the other parts of the plant have lesser amounts of the element (Popp, 1983); *L javanica* exhibits the characteristics of a calciotroph.

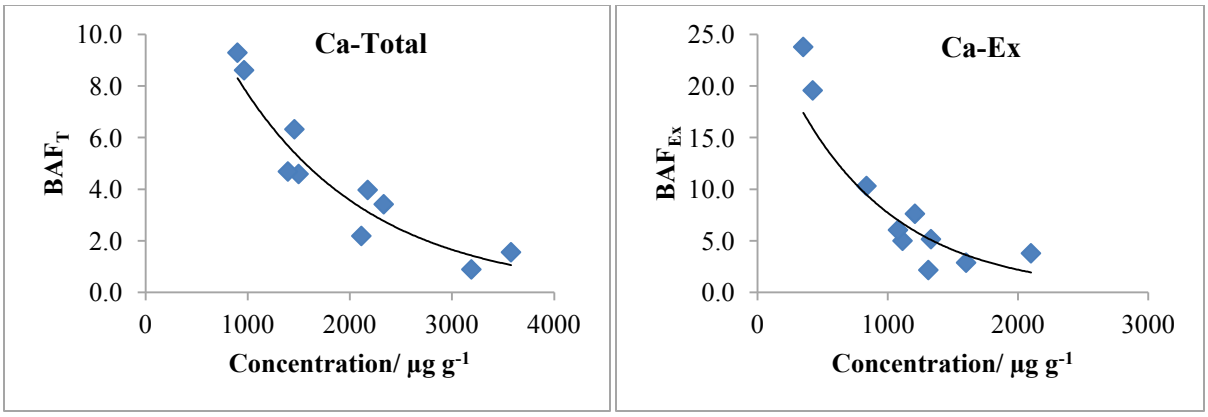


Figure 54: Bioaccumulation factors (BAF_T, BAF_{Ex}) versus Total and Exchangeable (Ex) concentration of Ca in soil

Table 32: Co concentrations in µg g^{-1} (Mean (SD), n=5) in *L. javanica* leaves, soil (total (T) and exchangeable (Ex)) and water extracts, and bioaccumulation factors (BAFs).

Sites*				BAF	
	$Soil_T$	$Soil_{Ex}$	Leaves	$[Leaves]/[Soil]_{Ex}$	$[Leaves]/[Soil]_T$
B 1	13.9 (0.5)	3.10 (0.20)	0.20 (0)	0.1	0.01
B 2	4.0 (0.1)	0.50 (0)	0.10 (0)	0.2	0.03
B 3	2.7 (0.4)	0.50 (0)	0.20 (0)	0.4	0.07
B 4	9.6 (0.7)	7.30 (0.67)	0.20 (0)	0.0	0.02
B 5	5.5 (0.3)	3.80 (0.45)	ND	-	-
B 6	4.7 (0.3)	1.90 (0.22)	0.26 (0.02)	0.1	0.06
B 7	3.1 (0.3)	0.48 (0.04)	0.19 (0.01)	0.4	0.06
B 8	3.8 (0.5)	0.45 (0.07)	ND	-	-
B 9	3.6 (0.4)	1.00 (0)	ND	-	-
B 10	3.1 (0.4)	1.40 (0.22)	ND	-	-

*Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

ND: Not Determinable

Co is an essential component of several enzymes and co-enzymes in plants (Palit et al., 1994). BAF for Co was less than one at all sites which means that the plant restricts uptake of this element to meet its metabolic needs, which is lower than soil concentrations. From the plot of

BAF vs total and exchangeable concentration of Co in soil, the shape of the curve points to essentiality of the element (Figure 55). Lower mobility of Co^{2+} in plants has been seen to restrict its transport to the leaves from the stems (Palit et al., 1994).

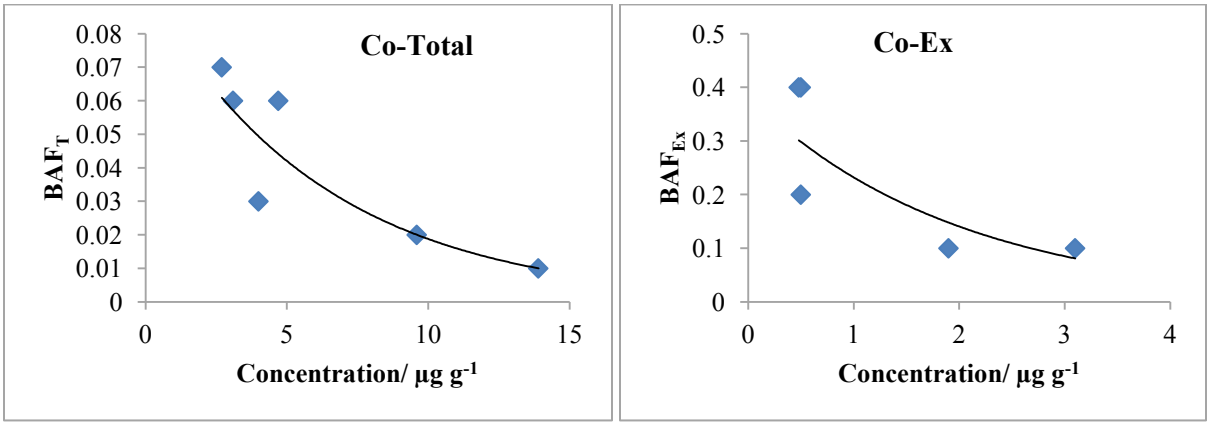


Figure 55: Bioaccumulation factors (BAF_T, BAF_{Ex}) versus Total and Exchangeable (Ex) concentration of Co in soil

Table 33: Cr concentrations in µg g^{-1} (Mean (SD), n=5) in *L. javanica* leaves, soil (total (T) and exchangeable (Ex)) and water extracts, and bioaccumulation factors (BAFs).

Sites*				BAF	
	$Soil_T$	$Soil_{Ex}$	Leaves	$[Leaves]/[Soil]_{Ex}$	$[Leaves]/[Soil]_T$
B 1	52.7 (4.2)	ND	2.52 (0.22)	-	0.05
B 2	39.8 (2.6)	ND	1.60 (0.12)	-	0.04
B 3	20.6 (1.0)	ND	1.31 (0.02)	-	0.06
B 4	72.0 (6.4)	ND	1.14 (0.09)	-	0.02
B 5	12.3 (0.9)	ND	0.19 (0.02)	-	0.02
B 6	28.9 (2.9)	ND	0.66 (0.06)	-	0.02
B 7	45.9 (4.1)	ND	1.90 (0.19)	-	0.04
B 8	6.2 (0.9)	ND	0.59 (0.02)	-	0.1
B 9	6.7 (0.6)	ND	ND	-	ND
B 10	3.7 (0.9)	ND	ND	-	ND

*Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

ND: Not Determinable

BAFs for Cr were below one. From the plot of BAF vs total and exchangeable concentrations of Cr in the soil, the shape of the curve points to non-essentiality of the element (Figure 56). There was no exchangeable form of Cr detected, however, the plant is observed to have the ability to extract its required amounts of Cr from the soil.

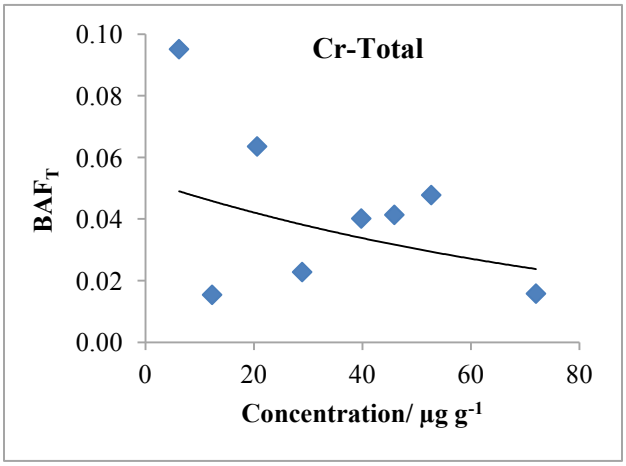


Figure 56: Bioaccumulation factors (BAF_T) versus Total concentration of Cr in soil

Table 34: Cu concentrations in µg g^{-1} (Mean (SD), n=5) in *L. javanica* leaves, soil (total (T) and exchangeable (Ex)) and water extracts, and bioaccumulation factors (BAFs).

Sites*				BAF	
	$Soil_T$	$Soil_{Ex}$	Leaves	$[plant]/[soil]_{Ex}$	$[plant]/[soil]_T$
B 1	34.7 (0.5)	3.20 (0.45)	4.06 (0.56)	1.3	0.1
B 2	21.4 (0.5)	3.10 (0.42)	2.46 (0.28)	0.8	0.1
B 3	20.6 (0.5)	2.20 (0.45)	4.78 (0.08)	2.2	0.2
B 4	13.9 (1.1)	0.46 (0.11)	4.98 (0.19)	10.8	0.4
B 5	8.3 (0.6)	4.06 (0.44)	8.48 (0.34)	2.1	1.0
B 6	11.8 (0.8)	2.10 (0.42)	7.74 (0.40)	3.7	0.7
B 7	8.7 (0.5)	0.50 (0)	3.40 (0.19)	6.8	0.4
B 8	5.4 (0.3)	ND	4.58 (0.48)	-	0.8
B 9	5.5 (0.6)	ND	5.74 (0.50)	-	1.0
B 10	3.5 (0.3)	ND	1.92 (0.08)	-	0.5

*Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

ND: Not Determinable

Where Cu was detected, for most sites, BAFs obtained with exchangeable concentrations were greater than one. For most sites BAFs obtained with total soil Cu were less than one. Probably the amount of Cu available for plant uptake was underestimated. The relative accumulation graphs (Figure 57) showed essentiality.

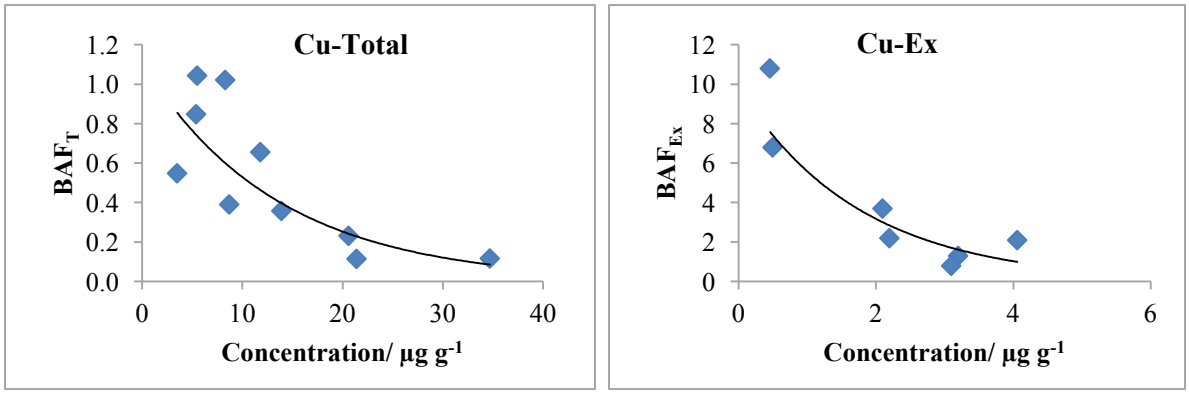


Figure 57: Bioaccumulation factors (BAF_T, BAF_{Ex}) versus Total and Exchangeable (Ex) concentration of Cu in soil

Table 35: Fe concentrations in µg g^{-1} (Mean (SD), n=5) in *L. javanica* leaves, soil (total (T) and exchangeable (Ex)) and water extracts, and bioaccumulation factors (BAFs)

Sites*				BAF	
	$Soil_T$	$Soil_{Ex}$	Leaves	$[plant]/[soil]_{Ex}$	$[plant]/[soil]_T$
B 1	20284 (975)	87 (3)	146 (11)	1.7	0.01
B 2	17389 (231)	275 (28)	166 (13)	0.6	0.01
B 3	8793 (336)	216 (19)	186 (7)	0.9	0.02
B 4	10133 (842)	109 (7)	266 (8)	2.4	0.03
B 5	17090 (338)	397 (15)	178 (6)	0.4	0.01
B 6	11336 (667)	259 (6)	167 (3)	0.6	0.01
B 7	22085 (790)	141 (8)	742 (15)	5.3	0.03
B 8	13427 (189)	168 (3)	59 (3)	0.4	0.00
B 9	10677 (654)	32 (4)	195 (18)	6.1	0.02
B 10	10000 (929)	51 (2)	64 (2)	1.3	0.01

*Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

ND: Not Determinable

From the BAFs obtained with total soil Fe, the plant appeared not to accumulate Fe. However, with exchangeable soil Fe, 50% of the sites showed accumulation of the element (BAFs > 1). This could be because at these sites (B1, B4, B7 and B9) the exchangeable Fe concentrations were well below that required by the plant. The relative accumulation graph for Fe (Figure 58) showed essentiality of the element.

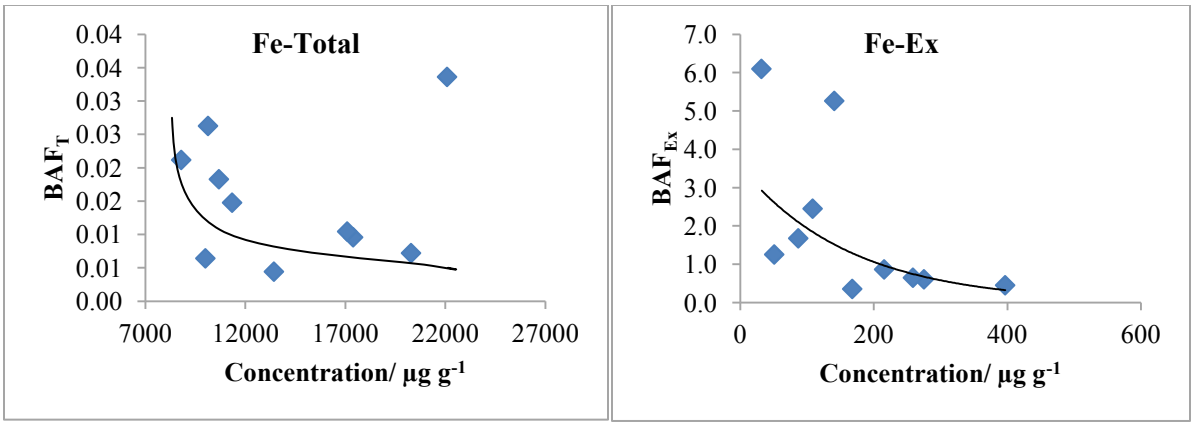


Figure 58: Bioaccumulation factors (BAF_T, BAF_{Ex}) versus Total and Exchangeable (Ex) concentration of Fe in soil

Table 36: Mg concentrations in µg g^{-1} (Mean (SD), n=5) in *L. javanica* leaves, soil (total (T) and exchangeable (Ex)) and water extracts, and bioaccumulation factors (BAFs).

Sites*				BAF	
	$Soil_T$	$Soil_{Ex}$	Leaves	$[plant]/[soil]_{Ex}$	$[plant]/[soil]_T$
B 1	6076 (167)	190 (8)	5619 (249)	29.6	0.9
B 2	1273 (26)	856 (33)	2619 (207)	3.1	2.1
B 3	1353 (62)	387 (13)	2242 (45)	5.8	1.7
B 4	599 (61)	263 (16)	3599 (79)	13.7	6.0
B 5	1350 (78)	232 (4)	1598 (37)	6.9	1.2
B 6	1511 (69)	567 (76)	4103 (56)	7.2	2.7
B 7	1186 (50)	511 (18)	2145 (90)	4.2	1.8
B 8	1476 (38)	703 (19)	2446 (83)	3.5	1.7
B 9	945 (73)	831 (22)	2968 (67)	3.6	3.1
B 10	944 (32)	621 (93)	3551 (74)	5.7	3.8

*Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

ND: Not Determinable

The BAFs for Mg were observed to be greater than one (BAF >1), for almost all sites and for both total and exchnangeable soil concentrations, following the same trend as Ca. The relative accumulation graph (Figure 59) for this element indicated essentiality. Site B4 showed higher accumulation of Mg than the other sites. At site B1, the amount of exchangeable Mg was somewhat underestimated which could explain the high BAF obtained. From the BAF it is clear that the leaves of *L. javanica* accumulate Ca and Mg in high amounts. Tea plants are known to contain substantial amounts of N, K, Ca and Mg (Sedaghathoor et al., 2009). Additionally, Mg is involved in almost all biological reactions in the plant which is why high concentrations are needed. The leaves of the plant can therefore be good sources of both Ca and Mg.

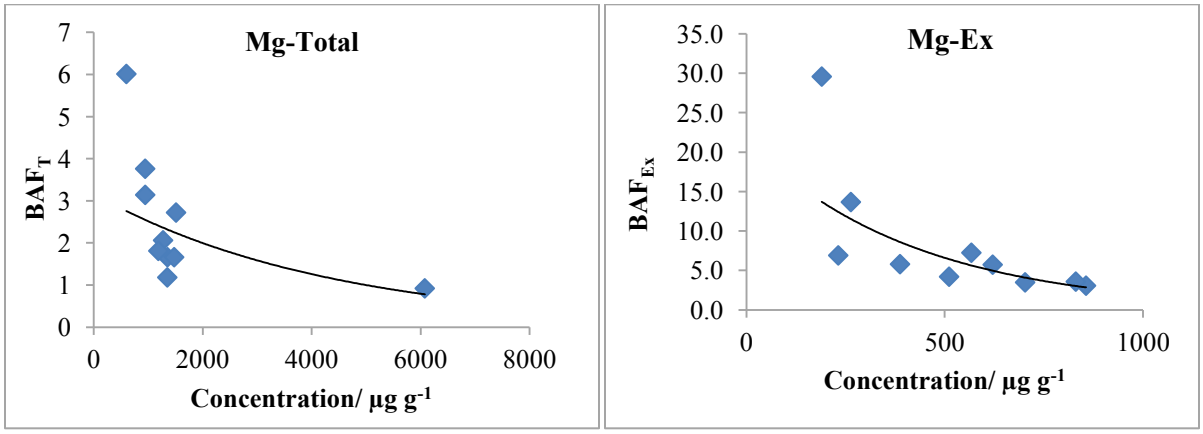


Figure 59: Bioaccumulation factors (BAF_T, BAF_{Ex}) versus Total and Exchangeable (Ex) concentration of Mg in soil.

Table 37: Mn concentrations in µg g^{-1} (Mean (SD), n=5) in *L. javanica* leaves, soil (total (T) and exchangeable (Ex)) and water extracts, and bioaccumulation factors (BAFs).

Sites*				BAF	
	$Soil_T$	$Soil_{Ex}$	Leaves	$[plant]/[soil]_{Ex}$	$[plant]/[soil]_T$
B 1	368 (11)	116 (5)	18.9 (0.9)	0.2	0.1
B 2	119 (5)	35 (4)	15.1 (1.0)	0.4	0.1
B 3	118 (4)	75 (4)	45.1 (1.0)	0.6	0.4
B 4	197 (8)	196 (4)	58.4 (1.6)	0.3	0.3
B 5	197 (6)	193 (20)	13.6 (0.3)	0.1	0.1
B 6	92 (4)	68 (2)	60.4 (0.4)	0.9	0.7
B 7	117 (4)	54 (4)	34.1 (1.4)	0.6	0.3
B 8	86 (6)	37 (3)	57.4 (3.1)	1.6	0.7
B 9	89 (3)	40 (2)	64.2 (1.4)	1.6	0.7
B 10	87 (7)	39 (2)	33.7 (0.5)	0.9	0.4

*Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

ND: Not Determinable

The BAFs for Mn were below one for most sites (BAF <1). The trend observed for Mn was somewhat similar to that observed for Co. The relative accumulation graphs for Mn (Figure 60) indicates essentiality.

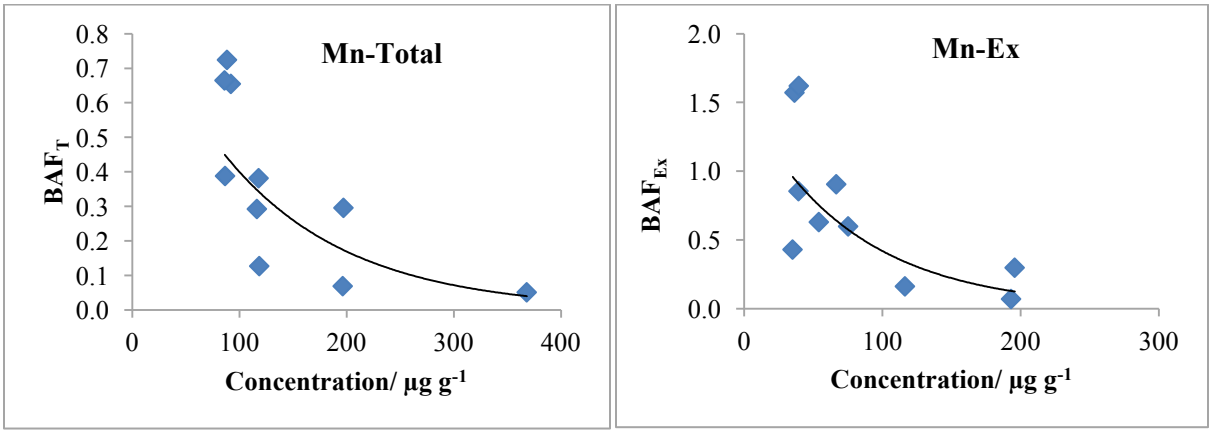


Figure 60: Bioaccumulation factors (BAF_T, BAF_{Ex}) versus Total and Exchangeable (Ex) concentration of Mn in soil

Table 38: Pb concentrations in µg g^{-1} (Mean (SD), n=5) in *L. javanica* leaves, soil (total (T) and exchangeable (Ex)) and water extracts, and bioaccumulation factors (BAFs).

Sites*				BAF	
	$Soil_T$	$Soil_{Ex}$	Leaves	$[plant]/[soil]_{Ex}$	$[plant]/[soil]_T$
B 1	12.0 (0.9)	2.60 (0.50)	0.92 (0.08)	0.35	0.08
B 2	11.7 (0.7)	4.20 (0.42)	0.38 (0.03)	0.09	0.03
B 3	91.0 (5.7)	61.50 (3.49)	1.00 (0.07)	0.02	0.01
B 4	5.3 (0.2)	1.40 (0.27)	0.54 (0.06)	0.39	0.10
B 5	18.3 (0.9)	6.30 (0.57)	0.44 (0.04)	0.07	0.02
B 6	10.8 (0.9)	5.30 (0.27)	0.45 (0.05)	0.08	0.04
B 7	11.1 (0.5)	4.40 (0.57)	1.18 (0.08)	0.27	0.11
B 8	4.8 (0.5)	2.10 (0.35)	0.75 (0.07)	0.36	0.16
B 9	5.1 (0.3)	1.70 (0.22)	0.80 (0.07)	0.47	0.16
B 10	3.4 (0.3)	2.00 (0.55)	0.61 (0.02)	0.31	0.18

*Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

ND: Not Determinable

Pb was also observed to follow the same trend as Fe and Mn, where BAFs are less than one. The relative accumulation graphs for Pb (Figure 61) indicated non-essentiality. Although total and exchangeable soil Pb was higher at site B3, the plant absorbed very little of this toxic metal and tended to exclude most of it.

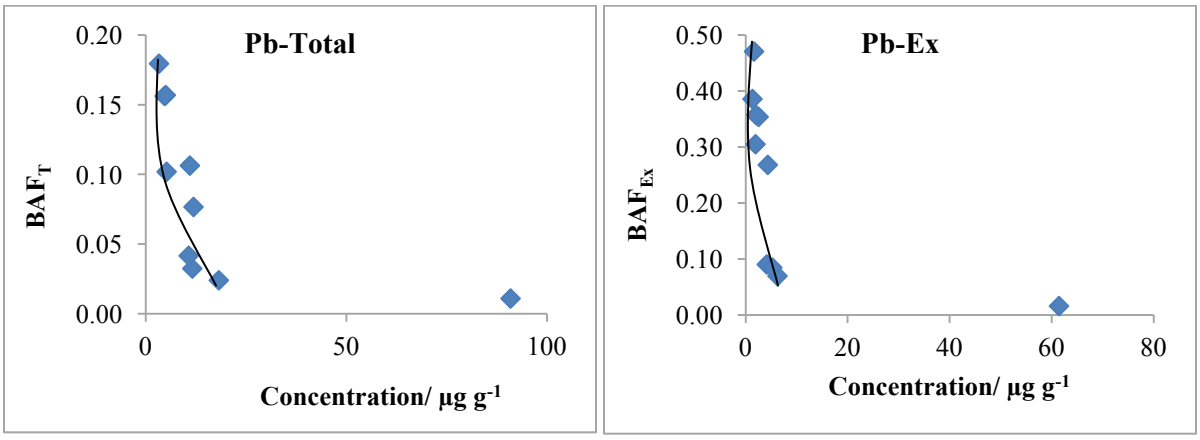


Figure 61: Bioaccumulation factors (BAF_T, BAF_{Ex}) versus Total and Exchangeable (Ex) concentration of Pb in soil

Se showed BAFs below one (BAF <1). It was observed that the concentrations of Se varied with site. At site B5, total soil Se was not determinable, but the plant had considerable amounts of Se, this implies that the plant has the ability to absorb and store Se in the leaves although soil concentrations are minimal. The relative accumulation graphs of Se (Figure 62) depict essentiality.

Table 39: Se concentrations in µg g^{-1} (Mean (SD), n=5) in *L. javanica* leaves, soil (total (T) and exchangeable (Ex)) and water extracts, and bioaccumulation factors (BAFs).

Sites*				BAF	
	$Soil_T$	$Soil_{Ex}$	Leaves	$[plant]/[soil]_{Ex}$	$[plant]/[soil]_T$
B 1	7.0 (0.8)	ND	3.34 (0.37)	-	0.48
B 2	5.8 (0.9)	ND	4.96 (0.30)	-	0.86
B 3	ND	ND	1.68 (0.17)	-	-
B 4	24.8 (1.5)	10.50 (0.79)	1.58 (0.11)	0.2	0.06
B 5	ND	ND	3.40 (0.16)	-	-
B 6	5.6 (0.5)	1.05 (0.06)	2.20 (0.16)	2.1	0.39
B 7	12.6 (0.6)	7.50 (0.50)	0.86 (0.09)	0.1	0.07
B 8	ND	ND	ND	-	-
B 9	ND	ND	ND	-	-
B 10	ND	ND	ND	-	-

*Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

ND: Not Determinable

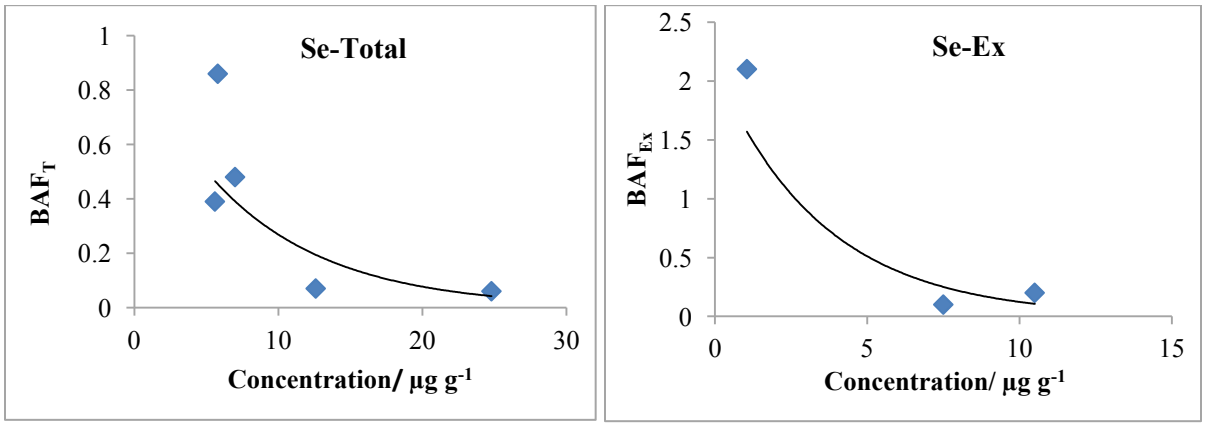


Figure 62: Bioaccumulation factors (BAF_T, BAF_{Ex}) versus Total and Exchangeable (Ex) concentration of Se in soil

The relative accumulation graphs for Zn (Figure 63) indicate the essentiality of Zn in the plant. Sites B7 and B9 were noted to have BAFs greater than 1 (BAF >1), otherwise the BAFs for [plant]/[soil]$_T$ were below one (BAF <1).

Table 40: Zn concentrations in µg g^{-1} (Mean (SD), n=5) in *L. javanica* leaves, soil (total (T) and exchangeable (Ex)) and water extracts, and bioaccumulation factors (BAFs).

Sites*				BAF	
	Soil$_T$	Soil$_{Ex}$	Leaves	[plant]/[soil]$_{Ex}$	[plant]/[soil]$_T$
B 1	39.6 (1.2)	5.8 (0.6)	18.2 (1.0)	3.1	0.46
B 2	58.4 (1.6)	22.2 (1.4)	27.3 (2.2)	1.2	0.47
B 3	115.5 (3.1)	50.9 (4.0)	20.6 (1.4)	0.4	0.18
B 4	37.9 (1.8)	1.6 (0.1)	27.2 (1.6)	17.0	0.72
B 5	75.0 (2.2)	27.4 (2.4)	26.2 (1.1)	1.0	0.35
B 6	22.0 (1.2)	9.4 (1.0)	24.7 (1.4)	2.6	1.12
B 7	30.6 (1.9)	4.2 (0.4)	16.5 (1.0)	3.9	0.54
B 8	26.4 (1.9)	ND	15.6 (1.0)	-	0.59
B 9	18.2 (1.9)	ND	25.1 (2.5)	-	1.38
B 10	29.6 (1.0)	0.5 (0)	15.7 (0.4)	31.4	0.53

*Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

ND: Not Determinable

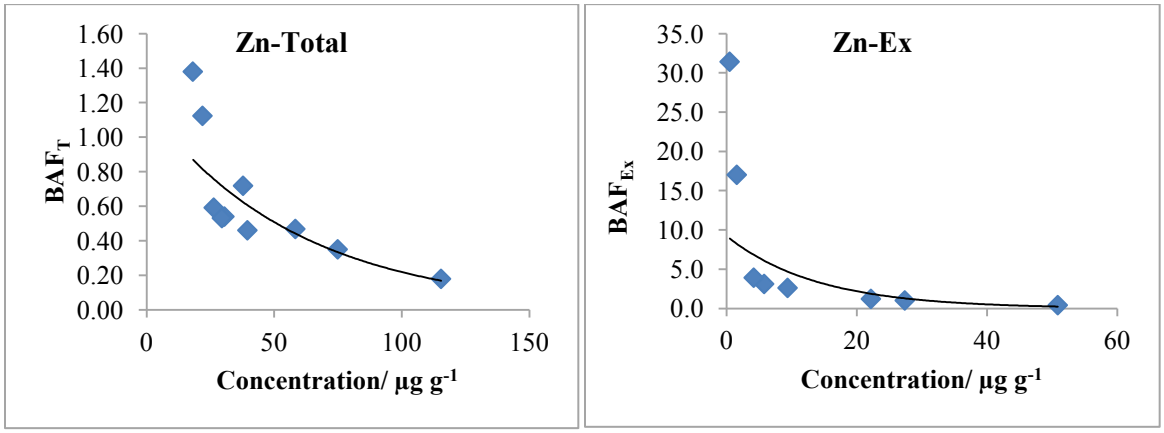


Figure 63: Bioaccumulation factors (BAF$_T$, BAF$_{Ex}$) versus Total and Exchangeable (Ex) concentration of Zn in soil

The relative accumulation plots (BAF_T, BAF_{Ex} vs. Total or Exchangeable soil concentrations) for *L. javanica* leaves showed essentiality for the following elements Ca, Co, Cu, Fe, Mg, Mn, Se and Zn. Accumulation of the elements was observed for Ca and Mg, making the leaves a rich source of both elements.

6.5 Soil quality assessment

6.5.1 Geoaccumulation index

Background concentrations of trace elements and heavy metals in soil are necessary in order to establish and manage polluted soils as well as deficiencies for plants and humans (Herselman, 2007). Studies by Herselman presented background/ baseline concentrations for South African soils, which then provide information on the natural range in soil concentrations that can be expected before contamination.

Table 41 shows the total concentration of each element in the soil and by calculation of the geoaccumulation indices (I_{geo}), the extent of contamination, if there is any, is then estimated by the I_{geo} value. According to Müller (1986), I_{geo} values below zero would mean that the soil is uncontaminated. An assessment of soil contamination by examining the I_{geo} values obtained from the ten different locations in KZN indicates that for the metals Cd, Co, Cr, Cu, Pb and Zn, the soils were uncontaminated.

Table 41: Total Baseline Concentrations of metals in South African soils (μg g^{-1}), total concentration of soils (μg g^{-1}), and geoaccumulation index (I_{geo}) for each sampling site.

Metal	TBC[a]	B1		B2		B3		B4		B5		B6		B7		B8		B9		B10	
		Soil (T)[b]	I_{geo}	Soil (T)	I_{geo}	Soil (T)	I_{geo}	Soil (T)	I_{geo}	Soil (T)	I_{geo}	Soil (T)	I_{geo}	Soil (T)	I_{geo}	Soil (T)	I_{geo}	Soil (T)	I_{geo}	Soil (T)	I_{geo}
Cd	2.7	1.6	-1.3	1.6	-1.4	1.4	-1.5	1.8	-1.2	1.3	-1.6	1.4	-1.5	1.3	-1.7	1.3	-1.6	1.0	-2.0	1.2	-1.8
Co	69	13.9	-2.9	4.0	-4.7	2.7	-5.3	9.6	-3.4	5.5	-4.2	4.7	-4.5	3.1	-5.1	3.8	-4.8	3.6	-4.8	3.1	-5.1
Cr	353	52.7	-3.3	39.8	-3.7	20.6	-4.7	72.0	-2.9	12.3	-5.4	28.9	-4.2	45.9	-3.5	6.2	-6.4	6.7	-6.3	3.7	-7.2
Cu	117	34.7	-2.3	21.4	-3.0	20.6	-3.1	13.9	-3.7	8.3	-4.4	11.8	-3.9	8.7	-4.3	5.4	-5.0	5.5	-5.0	3.5	-5.6
Pb	66	12.0	-3.0	11.7	-3.1	91.0	-0.1	5.3	-4.2	18.3	-2.4	10.8	-3.2	11.1	-3.2	4.8	-4.4	5.1	-4.3	3.4	-4.9
Zn	115	39.6	-2.1	58.4	-1.6	115.5	-0.6	37.9	-2.2	74.6	-1.2	22.0	-3.0	30.6	-2.5	26.4	-2.7	18.2	-3.2	29.6	-2.5

[a]TBC: Total Baseline Concentrations (Herselman, 2007)

[b]Soil T: Concentration/ μg g^{-1}

Sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

6.5.2 Soil pH, SOM and CEC

The soil properties pH, SOM and CEC were investigated and the results are shown in Table 42.

Table 42: pH, SOM and CEC of the soil samples obtained from 10 different sites in KwaZulu-Natal[a].

Sampling sites[b]	***pH($CaCl_2$)***	***SOM (%)***	***CEC (meq/100g)***
B1	6.52 (0.08)	4.80 (0.15)	5.71 (0.05)
B2	5.96 (0.02)	9.50 (0.30)	7.85 (0.01)
B3	6.09 (0.01)	10.72 (0.32)	6.69 (0.02)
B4	6.21 (0.03)	5.36 (0.03)	4.29 (0.04)
B5	6.34 (0.05)	5.09 (0.08)	6.00 (0.02)
B6	5.57 (0.01)	10.59 (0.22)	8.78 (0.03)
B7	6.11 (0.01)	6.82 (0.09)	6.22 (0.03)
B8	5.89 (0.03)	6.99 (0.31)	6.97 (0.04)
B9	5.84 (0.01)	2.71 (0.05)	4.57 (0.03)
B10	5.91 (0.02)	4.03 (0.07)	5.24 (0.04)

[a]Expressed as mean (SD), n= 4

[b]Sampling sites: B1-Amandawe, B2-Eshowe, B3-Ntumeni, B4-Mangeti, B5-Maphumulo, B6-Stanger, B7-Ndwedwe, B8-Ixopo, B9-KwaNkalokazi and B10-Highflats

The pH ranges from 5.57 to 6.52; this indicates that *L. javanica* plants grow in soils of medium acidity. Agricultural scientists report that for optimum growth and development of a plant the ideal pH range of the soil should be between 5.2 and 8.0; however some plants are sensitive to small variations in acidity and alkalinity in the soil (McKenzie, 2003). Low soil pH causes increased mobility and availability of certain heavy metals in the soil.

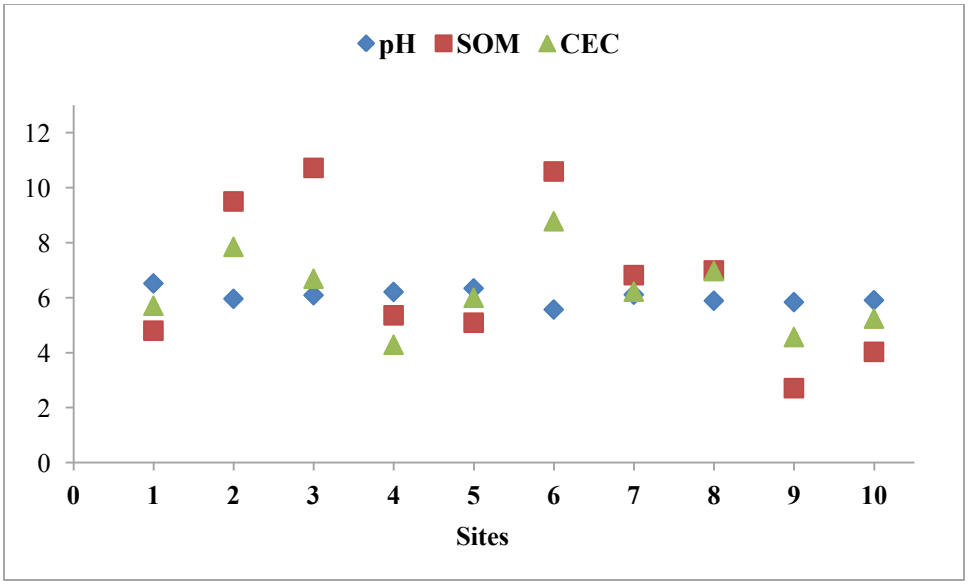


Figure 64: Comparing pH ($CaCl_2$), SOM (%) and CEC (meq/100g) in the soil for the 10 chosen sites

There was a wide variation in the SOM from the different sites which ranged from 2.71 to 10.72%. The amount of organic matter in the soil affects the pH and overall plant growth. The condition of the soil depends on the amount of organic matter contained in the soil, soil with a higher organic matter content tends to have higher water holding capacity and a higher content of essential nutrients as compared to soil with a low content of organic matter (Bot & Benites, 2005). There were no significant trends in the pH values of the soil and SOM from the different sites (Figure 64).

The CEC ranged from 4.29 to 8.78 meq/100g, the site with the lowest value of pH showed the highest CEC (B6). Studies indicate that the lower the CEC, the faster the soil pH decreases with time. Soils low in CEC are likely to possess reduced amounts of Mg, whilst high CEC soils are less susceptible to leaching losses of cations (Cornell University, 2007). The higher the CEC, the more organic matter present in the soil, therefore a positive relationship should be observed between the pH, SOM and CEC. It is noted that in Figure 64 the lower the pH

value, the higher the SOM and CEC. This trend is seen for sites B2, B3, B6 and B8. The sites B1, B5, B4 and B7 had slightly higher pH values ranging from 6.11 to 6.52. The SOM and CEC values however were lower than those of previous sites (B2, B3, B6 and B8). Sites B9 and B10 had lower pH values but the SOM and CEC were moderate. A positive relationship was only demonstrated between the SOM and CEC. This relationship is also supported by correlation analysis.

6.6 Statistical analysis of data

Table 43: Correlation matrix for the elemental concentrations in *L. javanica* leaves and soil (Total and Exchangeable).

	CaE	CaL	CdT	CoT	CoE	CrT	CrL	CuT	CuE	CuL	FeT	FeE	FeL	MgT	MgE	MgL	MnT	MnE	MnL	PbT	PbE	PbL	ZnT	ZnE	ZnL	pH	SOM	CEC
CaT	0.5	-0.6	0.7	0.6	0.2	0.5	0.7	0.9	0.7	0.0	0.2	0.2	-0.1	0.6	-0.6	0.3	0.7	0.4	-0.4	0.6	0.5	0.2	0.6	0.6	0.1	0.7	0.3	0.1
CaE		-0.3	0.4	0.0	-0.1	0.2	0.4	0.4	0.7	0.1	0.4	0.8	0.0	0.1	0.0	-0.3	0.1	0.1	-0.5	0.2	0.2	-0.3	0.5	0.6	0.3	0.1	0.7	0.7
CaL			-0.1	-0.1	0.1	0.0	-0.3	-0.4	-0.7	-0.3	-0.1	-0.5	-0.1	-0.3	0.6	0.1	-0.3	-0.3	0.4	-0.8	-0.7	-0.3	-0.8	-0.8	0.0	-0.4	-0.4	-0.2
CdT				0.7	0.6	0.8	0.7	0.7	0.4	-0.1	0.1	0.2	0.0	0.4	-0.5	0.4	0.6	0.5	-0.2	0.1	0.1	-0.2	0.3	0.2	0.3	0.5	0.3	0.1
CoT					0.7	0.6	0.5	0.7	0.3	0.1	0.3	-0.2	-0.1	0.8	-0.7	0.7	0.9	0.6	-0.2	-0.2	-0.3	0.0	-0.1	-0.2	0.1	0.7	-0.3	-0.3
CoE						0.6	0.0	0.1	0.1	0.3	-0.1	0.0	-0.1	0.1	-0.7	0.3	0.5	0.9	0.0	-0.2	-0.3	-0.4	0.0	-0.2	0.5	0.5	-0.3	-0.5
CrT							0.7	0.6	0.2	-0.1	0.3	-0.1	0.4	0.3	-0.5	0.4	0.6	0.5	-0.1	-0.1	-0.1	0.1	0.0	-0.1	0.3	0.4	0.1	-0.1
CrL								0.8	0.3	-0.4	0.6	0.0	0.4	0.6	-0.4	0.4	0.6	0.1	-0.4	0.2	0.1	0.4	0.2	0.1	-0.2	0.5	0.3	0.2
CuT									0.6	-0.2	0.3	0.1	-0.1	0.8	-0.4	0.5	0.8	0.2	-0.4	0.3	0.3	0.1	0.4	0.3	0.1	0.5	0.3	0.2
CuE										0.4	0.4	0.7	-0.2	0.4	-0.4	0.0	0.5	0.4	-0.7	0.3	0.2	-0.3	0.6	0.6	0.4	0.4	0.3	0.4
CuL											-0.1	0.5	-0.1	-0.1	-0.3	-0.1	0.1	0.5	0.2	0.1	0.0	-0.3	0.1	0.2	0.5	-0.1	0.1	0.2
FeT												0.2	0.5	0.5	-0.2	0.0	0.5	0.1	-0.7	-0.3	-0.4	0.3	-0.1	-0.1	-0.2	0.5	-0.1	0.1
FeE													-0.1	-0.1	-0.2	-0.5	0.0	0.3	-0.4	0.3	0.2	-0.5	0.5	0.6	0.4	0.0	0.5	0.6
FeL														-0.1	-0.1	-0.3	0.0	0.0	-0.1	0.0	0.0	0.6	-0.1	-0.1	-0.1	0.2	0.0	-0.1
MgT															-0.4	0.7	0.8	0.1	-0.4	0.0	-0.1	0.3	0.0	-0.1	-0.3	0.6	-0.1	0.0
MgE																-0.2	-0.7	-0.8	0.3	-0.3	-0.2	-0.2	-0.4	-0.2	0.0	-0.8	0.1	0.3
MgL																	0.6	0.0	0.1	-0.3	-0.3	0.0	-0.4	-0.4	-0.1	0.1	-0.2	-0.1
MnT																		0.6	-0.5	0.0	-0.1	0.1	0.1	0.0	0.0	0.8	-0.3	-0.3
MnE																			-0.2	0.0	0.0	-0.3	0.3	0.1	0.4	0.6	-0.2	-0.4
MnL																				0.0	0.0	0.1	-0.4	-0.3	0.0	-0.6	0.0	-0.1
PbT																					1.0	0.4	0.9	0.9	0.0	0.1	0.5	0.2
PbE																						0.4	0.8	0.9	-0.1	0.1	0.5	0.2
PbL																							0.1	0.0	-0.6	0.3	-0.1	-0.2
ZnT																								1.0	0.2	0.4	0.5	0.1
ZnE																									0.2	0.2	0.6	0.3
ZnL																										-0.1	0.1	0.0
pH																											-0.3	-0.5
SOM																												0.8

CaT: $[Soil\ Ca]_{Total}$
CaE: $[Soil\ Ca]_{Exchangeable}$
CaL: $[Ca]_{Leaf}$
SOM: Soil Organic Matter
CEC: Cation Exchange Capacity

A correlation matrix for the elemental concentration in *L. javanica* leaves and soil (total and exchangeable) are given in Table 43. Relationships with correlation coefficients ≥ 0. are positive and those ≤ -0.7 are negative. The correlation coefficients that are greater than 0.7 are strongly synergistic and less than -0.7 are strongly antagonistic.

Synergistic effects occur as a result of an increase in the levels of one or more interacting elements leading to the increase of the bioavailability of the other (Marschner, 2002). There is a statistically significant positive correlation between the concentration of elements Ca, Cd, Cr and Cu in the soil (Figure 65). This indicates that these elements have a common origin, that is, the parent material is likely to be the same (Lombnaes & Singh, 2003).

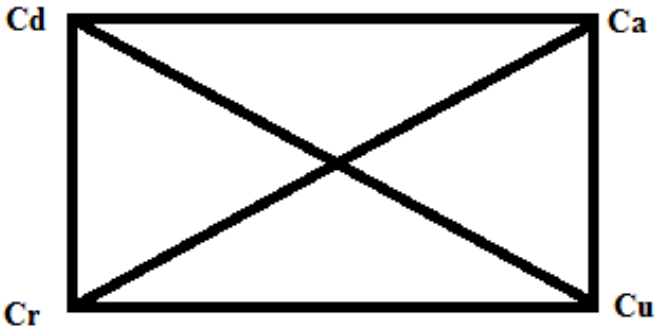


Figure 65: Diagram showing the intercorrelations between Ca, Cd, Cr and Cu in the soil.

A positive correlation was also observed between the levels of Co, Cu, Mg and Mn in the soil, eg. Mg in the soil is positively correlated to Co, Cu and Mn (Figure 66). Since the total soil concentrations of the three elements are closely related, although from different sites, according to Lombnaes & Singh (2003), this indicates that these elements are linked to a related geological parent material, therefore have a common origin.

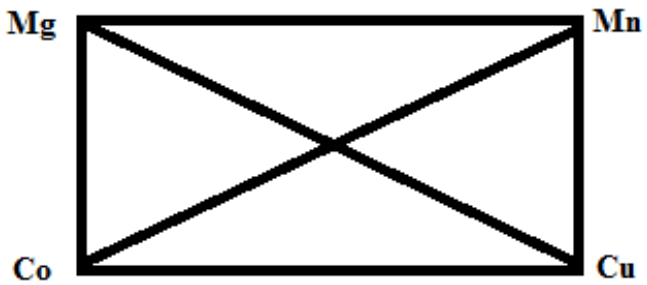


Figure 66: Diagram showing the intercorrelations between Co, Cu, Mg and Mn in the soil.

A synergistic relationship was observed between levels of Pb in the soil and the levels of exchangeable Zn in the soil. This is commonly known as a 'two way' synergy (Kalavrouziotis et al., 2008a). Negative correlations are related to antagonism amongst elements, that is, the increase in the levels of one or more of the interacting elements leads to the suppressed uptake of the other element (Kalavrouziotis et al., 2009). There was a significant negative relationship between total and exchangeable Mg in the soil. This significant inverse relationship shows that as the total Mg in soil increase the exchangeable Mg in the soil is reduced.

Strongly negative correlations were observed between Ca in the leaves with total soil Pb and Zn ($r = -0.8$). The increase in the levels of Ca in the leaves led to the decrease in the absorption of Pb and Zn from the soil to the plant leaves. The levels of Ca in the leaves were negatively related to exchangeable Cu, Pb and Zn in the soil. Therefore Ca accumulation in the leaves will be greater if exchangeable Cu, Pb and Zn are lower.

Statistically significant positive correlations existed between the levels of Cr in the leaves with the total levels of Ca, Cd and Cr in the soil. The Mg in the leaves was shown to positively correlate with the levels of total Co and Mg in the soil. Hence, high levels of Mg in

the leaves are a result of the high levels of Mg in the soil. The positive correlation between total Mg and Co levels in the soil has been previously outlined, which further validates the interaction between these elements.

A positive correlation between the soil parameters, CEC and SOM was observed. The levels of Ca exchangeable in the soil positively correlated with both SOM and CEC. No further significant correlations between SOM and CEC with the elements were obtained.

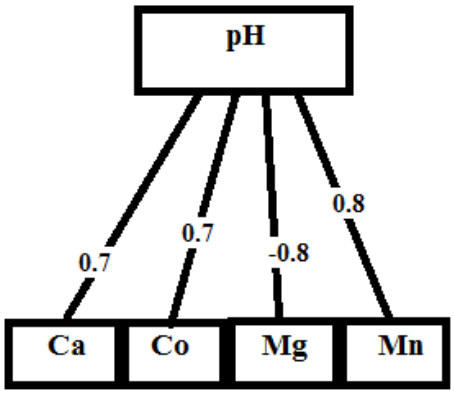


Figure 67: Diagram showing the correlations between pH and elements Ca, Co, Mg and Mn in the soil.

The pH of the soil was seen to positively correlate with total sol Ca, Co and Mn; however it negatively correlated with exchangeablethe Mg in the soil (Figure 67). It has been shown that the lower the pH of the soil, the less available the elements Ca and Mg become (Lake, 2000); the results show the opposite in the case of Mg. This could be attributed to the levels of Mg in the leaves. If the levels are high the microbes and exudates at the roots of the plant have specialized mechanisms to keep the element in bound form (Bais et al., 2006). Lower pH values have also been seen to increase the availability and mobility of Mn and Co in the soil (Lake, 2000), which is agreement with the results obtained.

CHAPTER 7

CONCLUSIONS

Firstly, phytochemical studies were done on *C. obliquus* bulbs; two new chalcones were obtained namely, 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-chalcone (**2**), and 2',4',6',4-tetrahydroxy-α-hydroxymethyl-chalcone (**5**) with two new dihydrochalcones; 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (**1**) and 2',4',6',4-tetrahydroxy-5'-methoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (**3**), including a 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene (**4**). Hence the study showed that the plant is not only rich in alkaloids but has considerable amounts of other phytochemicals. The antioxidant potentials of the four chalconoids were tested with the DPPH and FRAP assays but showed minimal activity. However, studies have shown that chalcones are good anticancer and antitubercular agents. Leaves from *L. javanica* were shown to possess an abundance of monoterpenes, sesquiterpenes and amino compounds, which are known to possess high therapeutic activity.

Secondly, the elemental composition of *C. obliquus* bulbs was determined. The study revealed that the concentration of elements, Ca, Cr, Cu, Fe, Mg, Mn, Pb, Se and Zn were controlled and variations according to site locations were moderate. The water extractable elements found in the herbal tonic, Imbiza, would contain high amounts of Ca and Mg. A high percentage of Zn can be extracted into the herbal tonic as seen from the study.

Thirdly, the elemental composition of *L. javanica* leaves and soil samples were determined. The leaf concentrations of the elements, Cd, Cr, Co, Cu, Fe, Mn, Pb and Zn were present in

moderate amounts. The water extractable elements found in the herbal tonic, Imbiza, would contain high amounts of Ca and Mg. A high percentage of Cr can be extracted into the herbal tonic as seen from the study.

There was no accumulation of the elements Cd, Cr, Co, Cu, Fe, Mn, Pb and Zn. However, Ca and Mg accumulation was evident in the plant. Statistical analysis revealed the antagonistic and synergistic effects the elements have in the soil-plant interface. Soil pH had an effect on the availability of specific metals present in the soil. This study therefore revealed that the herbal tonic, Imbiza, is rich in essential elements, especially Ca and Mg and contains low levels of toxic metals. It is also rich in secondary metabolites that are responsible for various therapeutic effects. This study therefore lends scientific credence and validity to the ethnomedicinal use of these plants and reveals the medicinal benefits of consuming the herbal tonic, Imbiza. It also adds to the growing body of research on indigenous medicinal plants.

RECOMMENDATIONS FOR FURTHER STUDY

- There is a need for the determination of the antibiofilm, antifungal and anticancer activity of the four new compounds isolated from *C. obliquus* bulbs.
- It is suggested that the elemental distribution in the other plants found in the herbal tonic, Imbiza, be assessed and therefore determine the total elemental composition of the preparation in the presence of all the plants in question.
- Since *L. javanica* leaves are a rich source of Ca and Mg which are used as tea leaves, the potential of commercializing such tea in South Africa can be examined.
- Speciation analysis of the elements studied in the research can also be undertaken.
- Studies on the safety, efficacy, dosage control and potential health benefits of the herbal tonic, Imbiza, sold in the streets needs thorough investigation.

REFERENCES

Abena, AA, Ngondzo-Kombeti, GR, Bioka, D. 1998. Psychopharmacologic properties of *Lippia multiflora*. Encephale, 24: 449–454.

Abdulla, M, Khan, AH, Reis, MF. 1996. Trace element nutrition in developing countries. Asia Pacific Journal of Clinical Nutrition. 3: 186-190.

AG Unlimited. Soil profile. Available online: http://www.agunlimited.com/consulting-soil-profile.html, accessed (04/09/12).

Agrifax. 1998. Minerals for plants, animals and man. Alberta Agriculture, Food and Rural Development. Agdex 531-3, pp 1-4.

Akerele, O. 1987. The best of both Worlds: bringing traditional medicine up to date. Social science and medicine, 24 (2): 177-181.

Akhtar, P, Ali, M, Sharma, MP, Farooqi, H, Khan, HN. 2010. Phytochemical investigation of fruits of *Corylus coriuna* Linn. Journal of Phytology Phytochemistry, 2(3): 89-100.

Arnord, TH, De Wet, BC. 1993. Plants of Southern Africa: names and distribution. Memoirs of the Botanical Survey of South Africa No. 62. NBI, Pretoria.

Australian Government: Geoscience Australia. 2012. Spectral geology. Available from: http://www.ga.gov.au/minerals/disciplines/spectral-geology.html, accessed (07/09/12).

Bagri, P, Ali, M, Sultana, S, Aeri, V. 2011. A new flavonoid glycoside from the seeds of *Cider arietinum* Linn. Acta Poloniae Pharmaceutica-Drug Research, 68(4): 605-608.

Bais, HP, Weir, TL, Perry, LG, Gilroy, S, Vivanco, JM. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology, 57: 233-266.

Baker, AJM. 1981. Accumulators and excluder: Strategies in the response of plants to heavy metals. Journal of Plant Nutrition, 3(1-4): 643-654.

Balázs, B, Toth, G, Duddeck, H, Soliman, HS. 2006. Iridoid and lignan glycosides from *Citharexylum spinosum* L. Natural Product Research, 20: 201-205.

Beckman, KB, Ames, BN. 1998. The free radical theory of aging matures. Physiological Reviews, 78(2): 547-581

Beldin, S, Perakis, S. 2009. Unearthing the secrets of the forest. United States Geological Survey. Factsheet 3078, pp 1-4.

Berker, KI, Güçlü, K, Demirata, B, Apak, R. 2010. A novel antioxidant assay of ferric reducing capacity measurement using ferrozine as a colour forming complexing agent. Analytical Methods, 2: 1770-1778.

Blais, JF, Djedidi, Z, Cheikh, RB, Tyagi, RD, Mercier, G. 2008. Metals precipitation from effluents: Review. Practice Periodical of Hazardous, Toxic and Radioactive Waste Management, 12(3): 135-149.

Blomhoff, R. 2010. Role of dietary phytochemicals in oxidative stress. In: Bioactive compounds in plants- Benefits and risks for man and animals. Bernhoff, A. (ed.). The Norwegian Academy of Science and Letters. Oslo. Norway, pp 52-68.

Bot, A, Benites, J. 2005. The importance of soil organic matter: key to drought resistant soil and sustained food production. Food and Agriculture Organisation of the United Nations. Bulletin 80. Rome, pp 1-78.

Bradl, H. 2005. Heavy metals in the environment: Origin, interaction and remediation. 1st edition. Academic Press. United Kingdom, p 90.

Brine, ND, Campbell, WE, Bastida, J, Herrera, MR, Viladomat, F, Codina, C, Smith, PJ. 2002. A dinitrogenous alkaloid from *Cyrtanthus obliquus*. Phytochemistry, 61: 443-447.

Carnat, A, Carnat, AP, Chavignon, O, Heitz, A, Wylde, R, Lamaison, JL. 1995. Luteolin 7-diglucuronide, the major flavonoid compound from *Aloysia triphylla* and *Verbena officinalis*. Planta Medica, 61(5): 490.

Carnat, A, Carnat, P, Fraiss, D, Lamaison, L. 1999. The aromatic and polyphenolic composition of lemon verbena tea. Fitoterapia, 70 (1): 44-49.

Carter, MR. 2001. Organic matter and sustainability. In: Sustainable management of soil organic matter. Rees, RM, Ball, BC, Campbell, CD, Watson, CA (eds). CABI publishing: New York, pp 1-34.

Castellano, G, Tena, J, Torrens, F. 2012. Classification of phenolic compounds by chemical structural indicators and its relation to antioxidant properties of *Posidonia oceania* (L.) Delile. Communications in Mathematical and in Computer Chemistry, 67: 231-250.

Cedrón, JC, Del Arco-Aguilar, M, Estévez-Braun, A, Ravelo, AG. 2010. Chemistry and biology of *Pancratium* alkaloids. Alkaloids: Chemistry and Biology, 63: 1-37.

CEM. Discover SP-D: closed vessel microwave. Available from: http://www.cem.com/discover-spd-features.html, accessed (04/09/12).

Chapman, HD. 1965. Cation exchange capacity. In: Methods of soil analysis: Chemical and microbiological properties. Black, CA, (ed). Agronomy 9: 891-901.

Cheesman, L, Nair, JJ, van Staden, J. 2012. Antibacterial activity of crinane alkaloids from *Boophone disticha* (Amaryllidaceae). Journal of Ethnopharmacology, 140(2): 405-408.

Chemiasoft. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Available from: http://www.chemiasoft.com/chemd/node/52, accessed (04/09/12).

Chemistry Lab Cookbook. Available online: http://docsfiles.com/pdf_icp.html , accessed (03/09/12).

Chen, MA, Vanek, J, Carr, M. 2004. Mainstreaming informal employment and gender in poverty reduction. Commonwealth secretariat. London:United Kingdom, Available online: www.idrc.ca/pub@idr.ca, accessed (04/09/12).

Clifton, P. 2002. Plant sterols and stanols: Comparison and contrasts. Sterol versus stanols in cholesterol lowering: Is there a difference?. Atherosclerosis, 3: 5-9.

Cluis, C. 2004. Junk-greedy greens: phytoremediation as a new option for soil decontamination. Journal of Biotechnology, 2: 60-67.

Combrinck, S, Bosman, AA, Botha, BM, Du Plooy, W, McCrindle, RI, Retief, E. 2006. Effects of post-harvest drying on the essential oil and glandular trichomes of *Lippia scaberrima* Sond. Journal of Essential Oil Research, 18: 80–84.

Community Gardening. Arsenic contaminated soils, Available online: www.comingalongside.org/Home_files/arsenic_lead_gardening.pdf, accessed (04/09/12)

Cornell University. 2007. Cation exchange capacity (CEC). Cooperative extension-Agronomy factsheet series. Factsheet 22, pp 1-2.

Deena, MJ, Thoppil, JE. 2000. Antimicrobial activity of the essential oil of *L. camara*. Fitoterapia, 71: 453-455.

Desire, O, Riviére, C, Razafindrazaka, R, Goossens, L, Moreau, S, Gillon, J, Uvery-Ratsimamanga, S, Andriamandro, P, Moore, N, Randriantsoa, A, Raharisololalao, A. 2010. Antispasmodic and antioxidant activities of fractions and bioactive constituent davidigenin isolated from *Mascarenhasia arborescens.* Journal of Ethnopharmacology, 130(1): 320-328.

Dias, JR, Gao, H. 2009. ^{13}C nuclear magnetic resonace data of lanosterol derivates profiling the steric topology of the steroid skeleton via substituent effects on its ^{13}C NMR. Spectrochimica Acta Part A, 74: 1064-1071.

Dlamini, TP. 2006. Isolation and characterization of bio-active compounds from *Lippia javanica*. M. Tech. (Chemistry) dissertation. University of Johannesburg. Johannesburg, South Africa.

Du Plessis, N, Duncan, G. 1989. Bulbous plants of Southern Africa. Tafelberg publishers Ltd. Cape Town, South Africa.

Ducki, S. 2007. The development of chalcones as promising anticancer agents. IDrugs: The Investigational Drugs Journal, 10(1): 42-46.

Elisha, IL. 2011. Characterization of an acetylcholinesterase inhibitor isolated from *Ammocharis coranica* (Amaryllidaceae). MSc. (Veterinary sciences) dissertation. University of Pretoria. Pretoria, South Africa.

Epstein, E. 1994. The anomally of silicon in plant biology. Proceedings of the National Academy of Science of the United State of America, 91: 11-17.

Ernest, W. 1982. Schwermetallpflanzen. In: Pflanzenökologie and mineralstoff weschsel. Kinzel, W. (ed.). Verlag Eugen Ulmer, Stuttgart.

Fedotov, PS, Mirò, M. 2008. Fractionation and mobility of trace elements in soils and sediments. In: Biophysico-chemical processes of heavy metals and metalloids in soil environments. Violante, A, Huang, PM, Gadd, GM (eds). Vol 1. John Wiley & Sons: New York, pp 467-520.

Focus on chlorine science. 2011. Bioaccumulation. Issue 8, pp 1-4, Available online: http://www.eurochlor.org/download-centre/focus-on-chlorine-science-(focs).aspx, accessed (05/09/12).

Food and Nutrition Board. 2011. Dietary reference intakes: Tolerable upper intake levels and recommended dietary allowance of elements. Institute of Medicine. National Academies.

Forestieri, AM, Monforte, MT, Ragusa, S, Trovato, A, Iauk, L. 1996. Anti-inflammatory, analgesic and antipyretic activity in rodents of plant extracts used in African medicine. Phytotherapy Research, 10: 100–106.

Foy, CD, Chaney, RL, White, MC. 1978. The physiology of metal toxicity in plants. Annual Review of Plant Biology, 103: 695-702.

Gerrard, J. 2000. Fundamentals of soils. Taylor & Francis Group. New York. USA, pp 1-22.

Ghisberti, EL . 2000. *Lantana camara* L.(Verbenaceae). Fitoterapia, 71: 467-486.

Gibson, RS. 2005. Principles of nutritional assessment. Chapter 23: Assessment of calcium, phosphorus and magnesium status. 2nd edition . Oxford University Press, pp 641-672.

Ginawi, O. 2007. PHL 473: Bioaccumulation and biomagnification, available online: http://faculty.ksu.edu.sa/58803/ginawis%20library/Forms/AllItems.aspx, accessed (07/09/12).

Goggin, K, Pinkston, M, Gqaleni, N, Puoane, T, Wilson, D, Berkley-Patton, J, Martinez, DA. 2009. The role of South African traditional health practitioners in HIV/AIDS prevention and treatment. In: HIV/AIDS global frontiers in prevention/ intervention. Taylor & Francis: New York.

Goto, T, Takahashi, N, Hirai, S, Kawada, T. 2009. Isoprenols. In: Nutrigenomics and proteomics in health and disease: Food factors and gene interactions. Mine, Y, Miyashila, K, Shahidi, F (eds). Wiley-Blackwell. USA, p 35.

Gould, MN. 1997. Cancer chemoprevention and therapy by monoterpenes. Environmental Health Perspective, 105(4): 977-979.

Govender, S, du Plessis-Stoman, D, Downing, TG, van de Venter, M. 2006. Traditional herbal medicines: microbial contamination, consumer safety and the need of standards. South African Journal of Science, 102: 253-255.

Gqaleni, N, Moodley, I, Kruger, H, Ntuli, A, McLeod, H. 2007. Traditional and complementary medicine. In: South African Health Review 2007. Harrison, S, Bhana, R, Ntuli, A (eds), pp 175-185.

Graham, WJ. 1991. Maternal mortality. Levels, trends and data deficiencies. In: Disease mortality in Sub-Sahara Africa. Feachem, RG, Jarrison, DT (eds). Oxford: New York, pp 101-116 .

Griffiths, S. 2004. Antimalarial compounds from *Crinum bulbisperm*. MSc. (Pharmaceutical Chemistry) dissertation. North West University. Potschefstroom, South Africa.

Grimm, RL, Beauchamp, JL. 2010. Evaporation and discharge dynamics of highly charged multicomponent droplets generated by electrospray ionization. Journal of Physical Chemistry A, 1411-1419.

Guerrera, PM, Leporatti, ML, Foddai, S, Moretto, D, Mercantini, R. 1995. Antimycotic activity of essential oil of *Lippia citriodora* Kunt (*Aloysia triphylla* Britton). Rivista Italiana EPPOS, 15: 23-25.

Gunn, AM, Winnard, DA, Hunt, DTE. 1988. Trace metal speciation in sediments and soils. In: Metal speciation: Theory, analysis and application. Kramer, JR, Allen, HE. (eds). Lewis Publications: Boca Raton, pp 261-294.

Gupta, R, Sharma, M, Lakshmay, R, Prabhakaran, D, Reddy, KS. 2009. Improved method of total antioxidant assay. Indian Journal of biochemistry and Biophysics, 46: 126-129.

Hamburger, M, Hostettmann, K.. 1991. Bioactivity in plants: The link between Phytochemistry and medicine. Phytochemistry . Harcourt Publisher. Edinburgh, 30: 3864-3874.

Handique, JG, Baruah, JB. 2002. Polyphenolic compounds: An overview. Reactive and Functional Polymers, 52: 163-188.

Harrewijn, P, van Oosten, AM, Piron, PGM. 2001. Natural terpenoids as messengers: A multidisciplinary study of their production, biological functions and practical applications. Kluwer Academic Publishers. Netherlands, pp 1-54.

Hart, NK, Lamberton, JA, Sioumis, AA, Suares, H, Seawright, AA. 1976b. Triterpenes of toxic and non toxic taxa of *L.camara*. Experientia, 32(4): 412-413.

Hernádez, T, Canales, M, Avila, JG, Duran, A, Caballero, J, Romo de Vivar, A, Lira, A. 2003. Ethnobotany and antibacterial activity of some plants used in traditional medicine of Zapotitlán de las Salinas, Puebla (México). Journal of Ethnopharmacology, 88(2-3): 181-188.

Herrera, MR, Machocho, AK, Nair, JJ, Campbell, WE, Brun, R, Viladomat, F, Codina, C, Bastida, J. 2001. Alkaloids from *Cyrtanthus elatus*. Fitoterapia, 72(4): 444-448.

Herselman, JE. 2007. The concentration of selected trace metals in South African soils. PhD (Soil Science) dissertation. University of Stellenbosch. Stellenbosch, South Africa.

Ho, CS, Lam, CWK, Chan, MHM, Cheung, RCK, Law, LK, Lit, LCW, Ng, KF, Suen, MWM, Tai, HL. 2003. Electrospray ionization mass spectrometry: principles and clinical applications. Clinical Biochemist Review, 24: 3-12.

Hoshino, O. 1998. The Amaryllidaceae alkaloids. In: The alkaloids, chemistry and biology. Cordell GA (ed.). Academic Press. San Diego, California.

Huang, D, Ou, B, Prior, RL. 2005. The chemistry behind antioxidant capacity assays: reviews. Journal Agricultural and Food Chemistry, 53: 1841-1856.

Huang, HY, Ko, HH, Jin, YY, Yang, SZ, Shin, YA, Chen, IS. 2012. Dihydrochalcone glucosides and antioxidant activity from the roots of *Annestea fragans* var. *lanceolata.* Phytochemistry, 78: 120-125.

Hübschmann, H. 2009. Handbook of GC/MS: Fundamentals and applications. 2nd edition. Wiley-VCH Verlag: Weinheim, pp 1-2.

Hussain, I, Khattak, M, Khan, K., Rehman, I, Khan, F, Khan, U. 2011. Analysis of heavy metals in selected medicinal plants from Dir, Swat and Peshawar Districts of Khyber Pakhtunkhwa. Journal of the Chemical Society of Pakistan, 33(4): 495-498.

Hutchings, A, Scott, AH, Lewis, G, Cunningham, A. 1993. Zulu medicinal plants. University of Natal Press. Scottsville, Pietermaritzburg.

Hutchings, A, van Staden, J. 1994. Plants used for stress-related ailments in traditional Zulu, Xhosa and Sotho medicine. Part 1: Plants used for headaches. Journal of Ethnopharmacology, 43: 89–124.

Hydrology Project. 2000. How to measure ammonia and organic nitrogen: Kjeldahl method. Training module WQ-38. New Delhi, 1-32.

Inada, A, Nakanishi, T, Tokuda, H, Nishino, H, Iwashima, A, Sharma, OP. 1995. Inhibitory effects of lantadenes andrelated triterpenoids on Epstein-Barr virus activation, Planta Medica, 61(6): 558-559.

Inoue, Y, Hada, T, Shiraishi, A, Hirose, K, Hamashima, H, Kobayashi, S. 2005. Biphasic effects of geranylgeraniol, terprenone, phytol on the growth of *S. aureus*. Journal of Antimicrobial Chemotheraphy, 49: 1770-1774.

Institute of Natural Resources. 2003. Indigenous medicinal plant trade: Sector analysis. Water and Forestry Support Programme. Investigation report No. 248. Scottsville. Pietermaritzburg, pp 1-16.

International Agency for Research on Cancer. 2002. IARC monographs on the evaluation of carcinogenic risks to humans. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Vol. 82. IARC Press: France, pp 43-68.

International Union of Pure and Applied Chemistry. 1993. Glossary for chemists of terms used in toxicology. Pure and Applied Chemistry, 65(9): 2003-2122.

Jain, M. 2008. Competition science vision magazine. Pratiyogita Darpan Printing: New Delhi. Issue 123, pp 379-381.

John, DA, Leventhal, JS. 1995. Bioavailability of metals. In: Preliminary compilation of descriptive geoenvironmental mineral deposit models. du Bray, E (ed). USGS: Denver, pp10-18.

Kabata-Pendias, A. 2001. Trace elements in soils and plants. 3rd edition. CRC Press: New York, USA.

Kalavrouziotis, IK, Koukoulakis, PH, Robolas, PK, Papadopoulos, AH, Pantazis, V. 2008a. Essential plant nutrients interactions in a soil cropped with *Brassica oleracea* var. Italica, irrigated with treated municipal wastewater, and their environmental implications. Fresenius Environmental Bulletin, 17(9a):1272-1280.

Kalavrouziotis, IK, Koukoulakis, PH, Papadopoulos, AH. 2009. Heavy metal interrelationship in soil in the presence of treated wastewater. Global NEST Journal, 11(4): 497-509.

Khalifa, TI, El-Gindi, OD, Ammar, HA, El-Naggar, DM. 2002. Iridiod glycosides from *Citharexylum quadrangular*. Asian Journal of Chemistry, 14(1): 197.

Kim, NS, Lee, DS. 2004. Headspace solid-phase microextraction for characterization of fragrances of lemon verbena (*Aloysia triphylla*) by gas chromatography-mass spectrometry. Journal of Separation Science, 27(1-2): 96-100.

Kohlmünzer, S. 2003. Pharmacognosy: Biological meaning and pharmacological activitiy of phenolic acids. 5th edition. PZWL Warsawa (in Polish).

Koorbanally, NA, Mulholland, DA, Crouch, NR. 2000. Alkaloids and triterpenoids from *Ammocharis coranica* (Amaryllidaceae). Phytochemistry, 54(1): 93-97.

KwaZulu-Natal Wildlife. 2012. The Medicinal plant trade in KwaZulu-Natal: Conservation, concerns and action. Available online: http://www.kznwildlife.com/old/index.php?/The-Medicinal-Plant-Trade-in-KwaZulu-Natal-Conservation-Concerns-and-Actions.html accessed (05/09/12).

Lake, B. 2000. Understanding soil pH. Acid soil action. NSW Agriculture. Leaflet No. 2, pp 1-4.

Lamble, KJ, Hill, SJ. 1998. Microwave digestion procedures from environmental matrices. Analyst 123: 103R-133R.

Law, M. 2000. Plant sterol margarines and health. British Medical Journal, 320: 861-864.

Leistner, OA. 2000. Seed plants of Southern Africa: families and genera. Strelizia 10. National Botanical Institute, Pretoria.

Levine, KE, Batchelor, JD, Rhoades, CB, Jones, BT. 1999. Evaluation of a high pressure, high temperature microwave digestion system. Journal of Analytical Atomic Spectrometry, 14: 49-59.

Lewandowski, A, Zumwinkle, M. 1999. Assessing the soil system: A review of soil quality. Fish, A (ed.). Minnesota Department of Agriculture, pp 1-65.

Lombnaes, P, Singh, BR. 2003. Predicting Zn and Cu status in cereal-potential for a multiple regression model using soil parameters. Journal of Agricultural Science, 141: 349-357

López, SN, Sierra, MG, Gattuso, SJ, Furlán, RL, Zacchino, SA. 2006. An unusual homoisoflavanone and structurally-related dihydrochalcones from *Polygonum ferrigeneum* (Polygonaceae). Phytochemistry, 67: 2152-2158.

Ma, LQ, Komar, KM, Tu, C, Zhang, W, Cai, Y, Kennelly, ED. 2001. A fern that hyperaccumulates arsenic. Nature. 409: 579-582.

Mander, M. 1998. Marketing of indigenous medicinal plants in South Africa. A case study in KwaZulu-Natal. Food and Agriculture Organization of the United Nations. Rome.

Mander, M, Le Breton, G. 2006. Overview of the medicinal plants industry in Southern Africa. In: Commercializing medicinalpPlants: A Southern African Guide. Diederichs, N (ed.). Sun Press. Stellenbosch, South Africa, pp 4-6.

Mander, M, Ntuli, L, Diederichs, N, Mavundla, K. 2007. Economics of the traditional medicine trade in South Africa. In: Traditional and complementary medicine. Harrison, S, Bhana, R, Ntuli, A. (eds). South African Health Review 2007. Durban: Health systems trusts, pp 189-200.

Manenzhe, NJ, Potgieter, N, van Ree, T. 2004. Composition and antimicrobial activities of volatile components of *Lippia javanica*. Phytochemistry, 65: 2333-2336.

Marschner, H. 2002. Mineral nutrition of higher plants. 2nd edition. Academic Press, Amsterdam.

Matusiewicz, H. 2003. Chapter 6: Wet digestion methods. In: Sample preparation for trace element analysis. Mester, Z, Sturgeon, R. (eds). Elsevier: Amsterdam, Vol 41: pp 193-233.

McCauley, A, Jones, C, Jacobsen, J. 2009. Soil pH and organic matter. Nutrient management module no. 8. Montana State University extension, pp 1-12.

McCord, JM. 2000. The evolution of free radicals and oxudatve stress. American Journal of Medicine, 108: 652-659.

McKay, DL, Blumberg, JB. 2006. A review of the bioactivity and health benefits of peppermint tea (*Mentha pipenta L.*). Phytotherapy Research, 20: 619-633.

McKenzie, RH. 2003. Soil pH and plant nutrients. Agri-Facts: Agdex 531-4, pp 1-2.

Meerow, AW, Snijman, DA. 1998. Amaryllidacea. In: The families and genera of vascular plants. Kubitzki, K (ed.). Springer-Verlang. Vol. III. Berlin, pp 83-110.

Merfort, I. 2002. Review of the analytical techniques of sesquiterpenes and sesquiterpene lactones. Journal of Chromatography A, 967: 115-130.

Misra, L, Laatsch, H. 2000. Triterpenoids, essential oil and photooxidative lactonization of oleanolic acid from *L. camara*. Phytochemistry, 54: 969-974.

Modzelewska, A, Sur, S, Kumar, SK, Khan, SR. 2005. Sesquiterpenes: natural products that decrease cancer growth. Current Medicinal Chemistry- Anti-cancer Agent, 5(5): 477-499.

Mokoka, NN. 2005. Indigenous knowledge of fever tea (*Lippia javanica*) and effect of shade netting on plant growth, oil yield and compound composition. M. Inst. (Agrarian Agronomy) dissertation. University of Pretoria, Pretoria.

Moodley, R, Kindness, A, Jonnalagadda, SB. 2007. Chemical composition of Macadamia nuts (*Macadamia integrifolia*) and impact of soil quality. Journal of Environmental Science and Health Part A, 42: 2097-2104.

Mujovo, SF, Hussein, AA, Meyer, JJM, Fourie, B, Muthivhi, T, Lall, N. 2008. Bioactive compounds from *Lippia javanica* and *Hoslundia opposite*. Natural product Research, 22(12): 1047-1054.

Mukherjee, T. 1991. Antimalarial herbal drugs: A review. Fitoterapia, 62: 197–204.

Müller, G. 1986. Schadstotte in sedimenten-Sedimente als schadstotte. Mitteilungen der Österreichische Geologische Gesellschaft, 79: 107-126.

Murthy, PS, Manjunatha, MR, Sulochannama, G, Naidu, MM. 2012. Extraction, characterisation and bioactivity of coffee anthocyanins. European journal of Biological Science, 4(1): 13-19.

Mwangi, JW, Addae-Mensah, I, Muriuki, G, Munavu, R, Lwande, W, Hassanali, A. 1992. Essential oils of *Lippia* species in Kenya. IV. Maize weevil (*Sitophilus zeamais*) repellancy and larvicidal activity. International Journal of Pharmacognosy, 30: 9–16.

Naicker, K, Cukrowska, E, McCarthy, TS. 2003. Acid mine drainage arising from gold mining activity in Johannesburg, South Africa and the environment. Environmental Pollution, 122: 29-40.

Nair, JJ, Aremu, AO, van Staden, J. 2011. Isolation of nacriprimine from *Cyrtanthus concractus* (Amaryllidaceae) and evaluation of its acetylcholinesterase inhibitory activity. Journal of Ethnopharmacology, 137(3): 1102-1106.

Negri, MC, Hichman, RR. 1996. Plants that remove contaminants from the environment. Laboratory Medicine, 27: 36-40.

Newman, DJ, Cragg, SM, Snader, KM. 2003. Natural Products as sources of new drugs over the period 1981-2002. Journal of Natural Products, 66 (7): 1022-1037.

Ngcobo, M, Nkala, B, moodley, I, Gqaleni, N. 2012. Recommendations for the development of regulatory guideline for registration of traditional medicine in South Africa. African Journal of Traditional Complementary and Alternative Medicine, 9(1): 59-66.

Niedlen, R, Staehle, R. 1973. Constituents of *Lippia javanica*. Deutschen Apotheker Zeitung, 113 (26): 993-997.

Nkomo, LP, Green, E, Ndip, RN. 2011. Preliminary phytochemical screening and *in vitro* anti-*Helicobacter pylori* activity of extracts of the leaves of *Lippia javanica*. African Journal of Pharmacy and Pharmacology, 5(20): 2184-2192.

Nutrition Business Journal. 2000. Global nutrition industry. San Diego. CA.

Nyam news. 2005. Phytochemicals. Caribbean Food and Nutrition Institute. No. 1-2: pp 1-4.

Ogura, K, Koyama, T. 1998. Enzymatic aspects of isoprenoid chain elongation. Chemical Reviews, 98: 1263-1276.

Orjala, J, Wright, AD, Behrends, H, Folkers, G, Sticher, O, Rüegger, H, Rali, T. 1994. Cytotoxic and antibacterial dihydrochalcones from *Piper adunum*. Journal of Natural Products, 57(1): 18-26.

Oxford, A, Oxford, E. 2010. Chemistry in soil-plant relationships. Available online: www.alaskafb.org/~akaitc/alaskaAITC/pdf/9_12/chemistry_soil.pdf, accessed (04/09/12).

Oxford, A, Oxford, E. Available online: www.agclassroom.org, accessed (05/09/12).

Padmini, E, Valarmathi, A, Usha Rani, M. 2010. Comparative analysis of chemical composition and antibacterial activities of *Mentha spicata* and *Camellia sinensis*. Asian Journal of Experimental Biological Sciences, 14: 722-781.

Palgrave, MC, Drummond, RB, Moll, EJ. 2003. Trees of Southern Africa. 3rd edition. Cape Town: Struik.

Palit, S, Sharma A, Talukder, G. 1994. Effects of cobalt on plants. Botanical Review. 60(2): 150-171.

Palombo, EA. 2006. Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intenstinal function. Phytotherapy Research, 20(9): 717-724.

Pascual, ME, Slowing, K, Carretero, E, Sánchez Mata, D, Villar, A. 2001. *Lippia*: traditional uses, chemistry and pharmacology: A review. Journal of Ethnopharmacology, 76:201-214.

Passow, HA, Rothstein, H, Clarkson, TW. 1961. The general pharmacology of the heavy metals. Pharmacological Reviews, 13:185-225.

Paulsen, E, Andersen, KE. 1999. Chapter 23: Verbenaceae. In: Dermatologic botany. Avalos, J, Maibach, HI (eds). CRC Press: Florida.

Pearson, VE. 2001. Galantamine: a new Alzheimer drug with a past life. Annuals of Pharmacotheraphy, 35: 1406-1413.

Pham Huu Chanh, A, Koffi, Y. 1988a. Comparative hypotensive effects of compounds extracted from *Lippia multiflora* leaves. Planta Medica, 54: 294–296.

Pham Huu Chanh, A, Koffi, Y. 1988b. Comparative effects on TXA_2 biosynthesis of products extracted from *Lippia multiflora* moldenke leaves. Prostaglandins, Leukotrienes and Essential Fatty Acids, 34(2): 83–88.

Perkin Elmer. FTIR-spectroscopy: Attenuated total reflectance. Available from: www.utsc.ca/~tracelabs/interpretation%20of%20FTIR%20spectra.pdf, accessed (06/09/12).

Perkin Elmer. ICP-OES: Optima 7000D. Available from: www.perkinelmer.com.cn.46-74713PRD-Optima7000D.pdf, accessed (06/09/12).

PolyQL. What is chemical speciation and what do we know about it. Available online: http://www.polyql.ethz.ch/pmwiki/pmwiki.php?n=Main.ConceptsAndPrimersChemicalSpeciation, accessed (04/09/12).

Poole, TE. 2001. Soil organic matter. Natural resources and water publications. Factsheet 783, pp 1-4 .

Popp, M. 1983. Genotypic differences in the mineral metabolism of plants adapted to extreme habitats. In: Genetic aspects of plant nutrition. Saric, MR (ed.). Kluwer Academic Publishers. Netherlands, 197-198.

Poulter, CD, Rilling, HC. 1981. Prenyl transferase and isomerase. In: Biosynthesis of isoprenoid compounds. Porter, JW, Spurgeon, SL (eds). John Wiley & sons. New York, pp 161-224.

Pousset, JL. 1989. Plantes Me´dicinales Africaines. Utilisation pratiques. Ellipses-ACCT: Paris, pp 102–103.

Primary Industries Agriculture. 2002. Cation exchange capacity. Available online: http://www.dpi.nsw.gov.au/agriculture/resources/soils/structure/cec, accessed (04/09/12).

Qnais, E, Abu-Safieh, K, Abu-Dieyeh, MH, Abdulla, FA. 2009. Jordan Journal of Biological Science, 2(4): 167-170.

Ragone, M, Sella, M, Conforti, P, Volonté, M, Consolini, A. 2007. The spasmolytic effect of *Aloysia citriodora*, Palau (South American cedrón) is partially due to its vitexin but not isovitexin on rat duodenums. Journal of Ethnopharmacology, 113: 258–266.

Ramadan, MA, Kamel, MS, Ohtani, K, Kasai, R, Yamasaki, K. 2000. Minor phenolics from *Crinum bulbisperm* bulbs. Phytochemistry, 54(8): 891-896.

Rapin, F, Förstner, U. 1983. Sequential leaching technique for particulate metal speciation: The selectivity of various extractants. In: Proceedings of the 4th International Conference on Heavy Metals in the Environment. Edinburgh, 2: 1074-1077.

Rashid, MA. 1974. Adsorption of metals on sedimentary and peat humic substances. Chemical Geology, 13: 115-123.

Rimpler, H, Sauerbier, H. 1986. Iridoid glucosides as taxonomic markers in the genera *Lantana*, *Lippia*, *Aloysia* and *Phyla*. Biochemical Systematics and Ecology, 14: 307–310.

Roberts, M. 1990. Indigenous healing plants. Halfway House, Southern publishers.

Robinson, BH, Chiarucci, A, Brooks, RR, Petit, C, Kirkman, JH, Gregg, PEH, Dominicis, V. 1997. The nickel hyperaccumulator plant *Alyssum bertolonii* as a potential agent for phytoremediation and phytomining of nickel. Journal of Geochemical Exploration, 59: 75-86.

Ross, DS. 1995. Chapter 9: Recommended methods for determining soil exchange capacity. In: Recommended soil testing procedures for the Northeastern United States. Horton, ML (ed). 2nd edition. Northeastern Regional Publication No. 493: pp 62-69.

Roychoudhury, AN, Starke, MF. 2006. Partitioning and mobility of trace metals in the Blesbokspruit: impact assessment of dewatering of mine waters in the East Rand, South Africa. Applied Geochemistry, 21: 1044-1063.

Rwangabo, PC. 1988. Umuhengerin, a new antimicrobially active flavonoid from *L.trifolia*. Journal of Natural Products, 51(5): 966-968.

Sahoo, N, Machikanti, P. 2010. Herbal drugs : Standards and regulations. Fitoterapia, 81: 462-471.

Salomons, W. 1995. Environmental impact of metals derived from mining activities: Processes, predictions and prevention. Journal of Geochemical Exploration, 52: 5-23.

Sandager, M, Nielsen, ND, Stafford, GI, van Staden, J, Jäger, AK. 2005. Alkaloids from *Boophone disticha* with affinity to the serotonin transporter in rat brain. Journal of Ethnopharmacology, 98(3): 367-370.

Schulte, EE, Hoskins, B. 2009. Recommended soil organic matter tests. Chapter 8. Cooperative Bulletin No. 493, pp 63-74.

Sedaghathoor, S, Torkashuand, AM, Hashemabadi, D, Kaviani, B. 2009. Yield and quality response of tea plant to fertilizers. African Journal of Agricultural Research, 4(6): 568-570.

Sharma, V, Kumar, V, Kumar, P. 2012. Heterocyclic chalcones analogues as potential anticancer agents. Anticancer Agents in Medicinal Chemistry, (Ahead of print).

Shaw, JW, Andrew, RD. 2001. Cation exchange capacity affects green's turf growth. Golf course Management, pp 73-77.

Shechter, SM, Bryce, CL, Alagoz, O, Kreke, JE, Stahl, JE, Schaefer, AJ, Angus, DC, Roberts, MS. 2005. A clinically based discrete event stimulation of end stage liver disease and organ allocation process. Medical Decision Making, 25(2): 199-209.

Shikanga, EA. 2008. Bioactive polar compounds from South African *Lippia* species. MTech. (Chemistry) dissertation. Tshwane University of Technology. Pretoria, South Africa.

Sies, H. 1997. Oxidative stress- oxidants and antioxidants. Exp. Physiol., 82: 291-295.

Sillanpää, M. 1982. Micronutrients and the nutrient status of soils: a global study. FAO soils. Bulletin 48: Rome.

Singh, G. 2007. Chemistry of terpenoids and carotenoids. Discovery Publishing House. New Delhi, pp 1-156.

Skoog, DA, West, DM, Holler, FJ, Crouch, SR. 2004. Fundamentals of analytical chemistry. Brooks/Cole. 8th edition. USA.

Snijman, DA, Archer, RH. 2003. Amaryllidaceae. In: Plants of Southern Africa: an annotated checklist. Germishuizen, G, Meyer, NL (eds). National Botanical Institute. Pretoria. South Africa. Strelitzia, pp 957-967.

Srinath, N. 2011. Synthesis and biological evaluation of new chalcones, pyrimidines and pyrazolines. PhD (Pharmaceutical Science) dissertation. Andhra University, India.

Stewart, D. 2005. The chemistry of essential oils made simple: God's love manifest in molecules. Care Publications, pp 127-141.

Sthother, JB. 1972. ^{13}C NMR Spectroscopy. Academic press.

Stover, RC, Sommers, LE, Silveira, J. 1976. Evaluation of metals in waste water sludge. Journal of Water Pollution Control Federation, 48: 2165-2175.

Strain, JJS, Cashman, KD. 2009. Chapter 9: Minerals and trace elements. In: Introduction to human nutrition. Gibney, MJ, Vorster, HH, Kok, FJ (eds). 2nd edition. John Wiley & sons. United Kingdom, p188-235.

Szliska, E, Czuba, ZP, Mazur, B, Paradysz, A, Krol, W. 2010. Chalcones and dihydrochalcones augment TRAIL-mediated apoptosis in prostate cancer cells. Molecules, 15: 5336-5353.

Tanaka, T, Iinuma, M, Yuki, K, Fujii, Y, Mizuno, M. 1992. Flavonoids in root bark of *Pongamia pinnata*. Phytochemistry, 31: 993-998.

Taoubi, K., Fauvel, MT, Gleye, J, Moulis, C, Fouraste´, I. 1997. Phenylpropanoid glycosides from *Lantana camara* and *Lippia multiflora*. Planta Medica, 63: 192–193.

Tesso, H. 2005. Isolation and structural elucidation of natural products from plants. PhD (Organic Chemistry) dissertation. University of Hamburg, Germany.

Timperley, MH, Brooks, RR, Peterson, PJ. 1973. The significance of essential and non-essential trace elements in plants in relation to biogeochemical prospecting. Journal of Applied Ecology, 7: 429-439.

Traditional Health Practitioners Act 2007: Republic of South Africa. 2008. Government Gazette. Available online: http://us-cdn.creamermedia.co.za/assets/articles/attachments/11034_tradhealpraca22.pdf, accessed (04/09/12).

Tyagi, DK. 2005. Field guide to medicinal plants. PharmaForestry. Atlantic Publishers and Distributors: New Delhi, p 20.

USA Environmental Protection Agency. 2004. Standard operating procedure for the analysis of metals in waters and wastewaters by ICP method 200.7 using Perkin Elmer Optima 3300DV and 4300DV. Chapter 2. LG 213, pp 1-25.

United States Environmental Protection Agency. 2009. Glossary list. Available online: http://www.epa.gov/oust/cat/TUMGLOSS.HTM, accessed (05/09/12).

University of the Incarnate World. Microwave acid digestion: CEM Mars Xpress 230/60. Available from: http://www.uiw.edu/chemistry/chemfacilities.html, accessed (05/09/12).

Ure, AM. 1996. Single extraction schemes for soil analysis and related applications. The Science of the Total Environment, 178: 3-10.

Valentão, P, Fernandes, E, Carvalho, F, Andrade, PB, Seabra, RM, de Lourdes Basto, M. 2002. Studies on the antioxidant activity of *Lippia citriodora* infusion: scavenging effect on superoxide radical, hydroxyl radical and hypochlorous acid. Biological and Pharmaceutical Bulletin, 25(10): 1324-1327.

Valentín, A, Pélissier, Y, Benoit, F, Marion, C, Kone, D, Mallie, M, Bastide, JM, Bessie`res, JM. 1995. Composition and antimalarial activity in vitro of volatile components of *Lippia multiflora*. Phytochemistry, 40: 1439–1442.

Van Wyk, BE, van Oudshoorn, B, Gericke, N. 1997. Medicinal plants of Southern Africa. Briza publications. Pretoria, South Africa.

Vasisht, K, Kumar, V. 2004. Compendium of medicinal and aromatic plants: Africa. Earth, Environmental and Marine Sciences and Technologies. Italy, pp 1-124.

Veitch, NC, Grayer, RJ. 2006. Chalcones, Dihydrochalcones and aurones. In: Flavonoids: Chemistry, Biochemistry and Applications. Anderson, ØM, Markham, KR (eds). Taylor & Francis Group, pp 1003-1100.

Viljoen, AM, Subramoney, S, van Vuuren, SF, Başer, KHL, Demirci, B. 2005. The composition, geographical variation and antimicrobial activity of *Lippia javanica* (Verbenaceae) leaf essential oils. Journal of Ethnopharmacology, 96: 271-277.

Voelter, W. 1976. Some recent aspects in the structure elucidation of natural products. Pure and Applied Chemistry, 48:105-126.

Walkley, A, Black, IA. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science, 37: 29-37.

Wanasundara, PKJPD, Shahidi, F. 2005. Antioxidants: science, technology and applications. In: Bailey's industrial oil and fat products. Shahidi, F. (ed.). 6th edition. John Wiley & son, pp 431-489.

Watt, JM, Breyer-Brandwijk, MG. 1962. The medicinal and poisonous plants of Southern and Eastern Africa. E. and S. Livingston Ltd: London.

Wilm, M. 2011. Principles of electrospray ionization. American Society for biochemistry and Molecular Biology, 1-25.

World Health Organisation. 1998. Quality control methods for medicinal plant material. Geneva: Switzerland, pp 62-63.

World Health Organisation. 2002. Traditional Medicine Strategy 2002-2005, Geneva 1: p 7.

World Health Organisation. 2005. National policy on traditional medicine and regulation of herbal medicine: Report of a WHO global survey, Geneva .

World Health Organisation. 2008. Traditional medicine: Factsheet N 134. Geneva.

Wright, H. 1994. A handbook of soil analysis. Concept Publishing company: New Delhi. 2nd edition, pp 1-2.

Xu, Q, Shi, G. 2000. The toxic effects of single Cd and interaction of Cd and Zn on some physiological index of *Oenanthe javanica* (Blume) DC. Journal of Nanjing Normal university (Natural Science), 23(4): 97-100.

Yusoo, S, Yutaka, T, Minoru, T. 2001. Chemical constituents of *Inonotus obliquus* IV: Terpenes and steroids from cultural mycelia. Eurasian Journal of Forest Research, 2: 27-30.

Zhang, X, Taubman, A, Conejo, M, Rao, R. 2009. Economics and Social Council Chamber. Panel discussion on the contribution of traditional medicine to the realization of the international development objectives related to global public health. Annual Ministerial Review (AMR): New York.

Zhong, J. 2005. Amaryllidaceae and *Sceletium* alkaloids. Natural Products Report, 22: 111-126.

APPENDIX

COMMISSION OF THE EUROPEAN COMMUNITIES

COMMUNITY BUREAU OF REFERENCE - BCR

CERTIFIED REFERENCE MATERIAL

CERTIFICATE OF ANALYSIS

3e4

BCR No 191

TRACE ELEMENTS IN LYOPHILISED BROWN BREAD

Element	Mass fraction (based on dry mass)		Number of accepted sets of results p
	Certified Value (1)	Uncertainty (2)	
Cd	28.4 $ng.g^{-1}$	± 1.4 $ng.g^{-1}$	12
Pb	187 $ng.g^{-1}$	± 14 $ng.g^{-1}$	12
Cu	2.6 $\mu g.g^{-1}$	± 0.1 $\mu g.g^{-1}$	8
Zn	19.5 $\mu g.g^{-1}$	± 0.5 $\mu g.g^{-1}$	13
Fe	40.7 $\mu g.g^{-1}$	± 2.3 $\mu g.g^{-1}$	12
Mn	20.3 $\mu g.g^{-1}$	± 0.7 $\mu g.g^{-1}$	11

(1) *This value is the unweighted mean of p values, each value being the mean of a set of results as obtained by different laboratories and methods.*

(2) *The uncertainty is taken as the 95% confidence interval of the mean value (1) and is applicable when the reference material is used for calibration purposes. When the reference material is used to assess the performance of a method, the user should refer to the recommendations laid down in the last chapter (Instructions for use) of the certification report.*

DESCRIPTION OF THE SAMPLE

The sample is a homogeneous powder consisting of particles that have passed through a 125 µm sieve. It is provided in screw-cap, dark glass bottles in units of approximately 40 g.

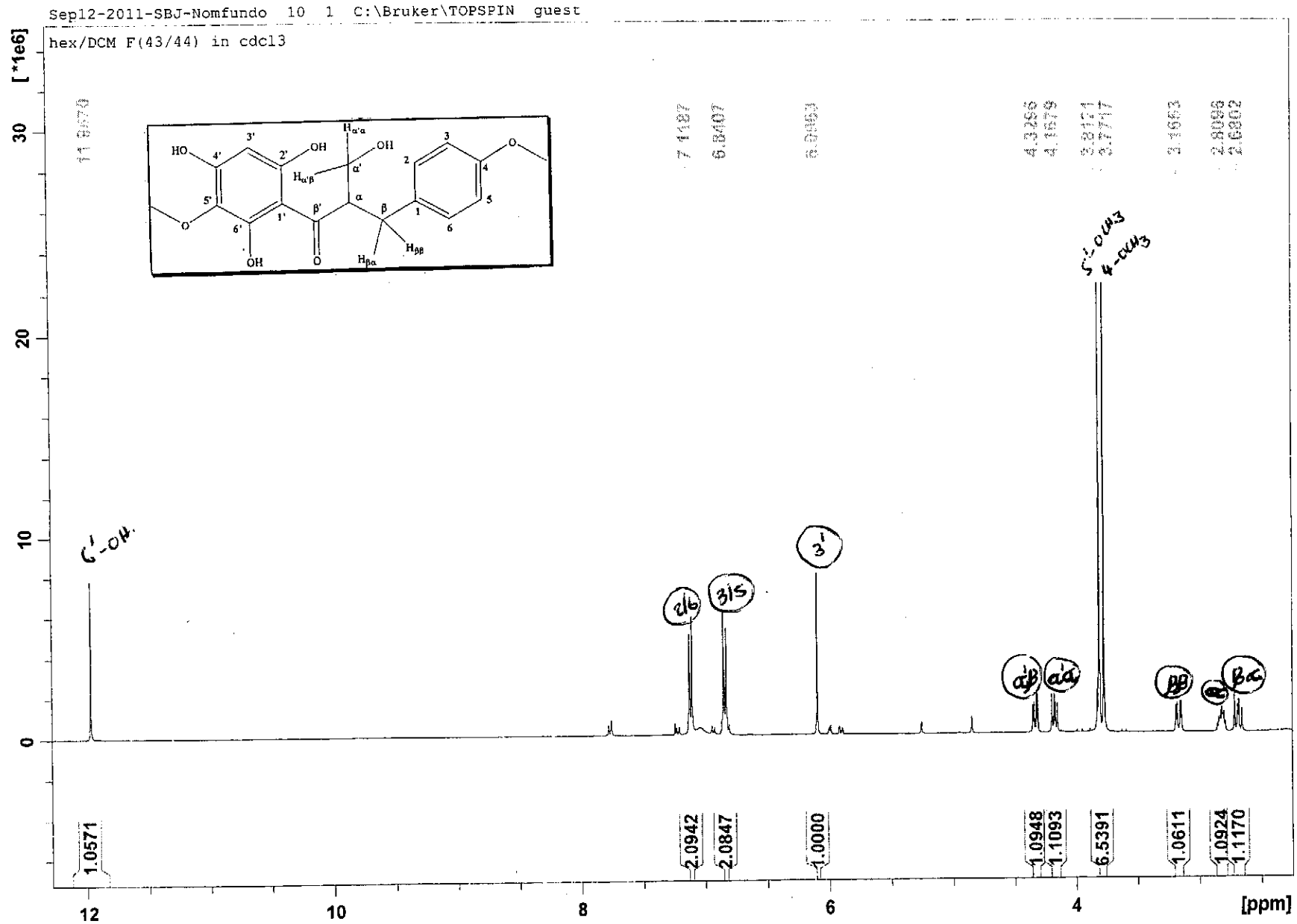
INSTRUCTIONS FOR USE

The portion for analysis should be taken after mixing the contents of the bottle. The moisture content is to be determined by drying another portion of the sample at 103 ± 2°C as described in the certification report (Chapter 11, Instructions for use). The recommended minimum sample intake is 200 mg.

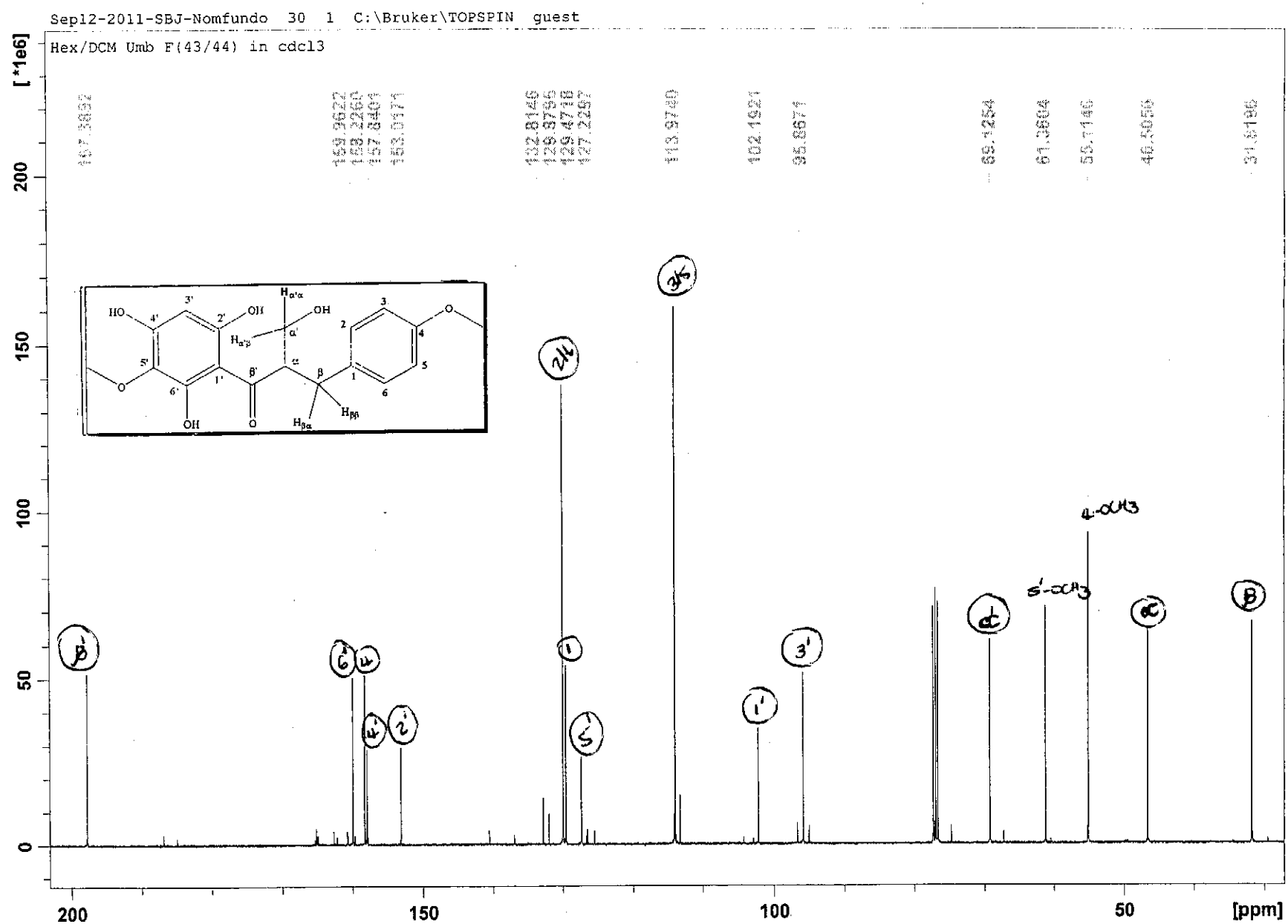
All care must be taken to avoid contamination during opening of the bottle and handling of the material. The bottle should be stored in a dark and cool place.

Brussels, December 1986

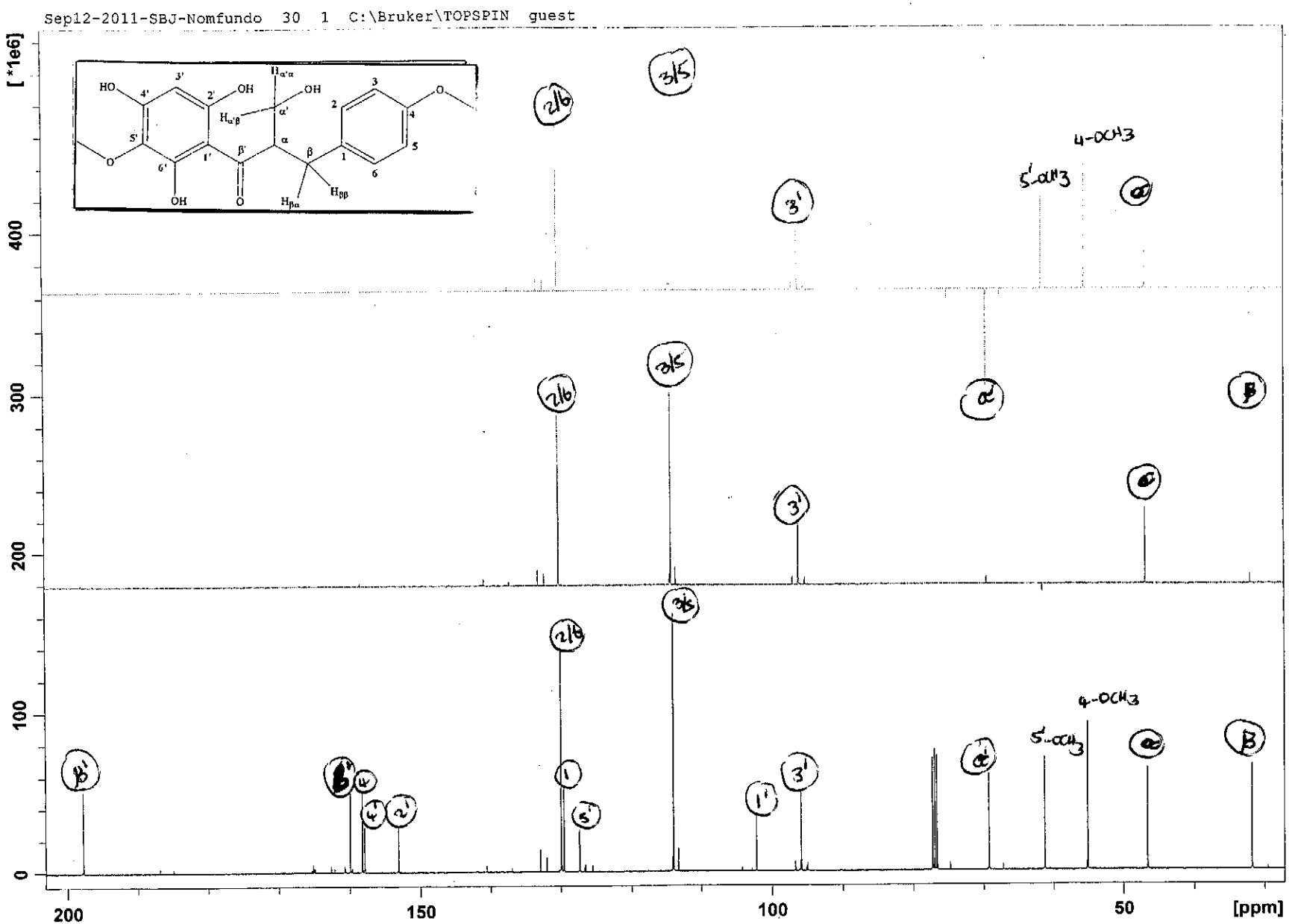
BCR
for certified true copy



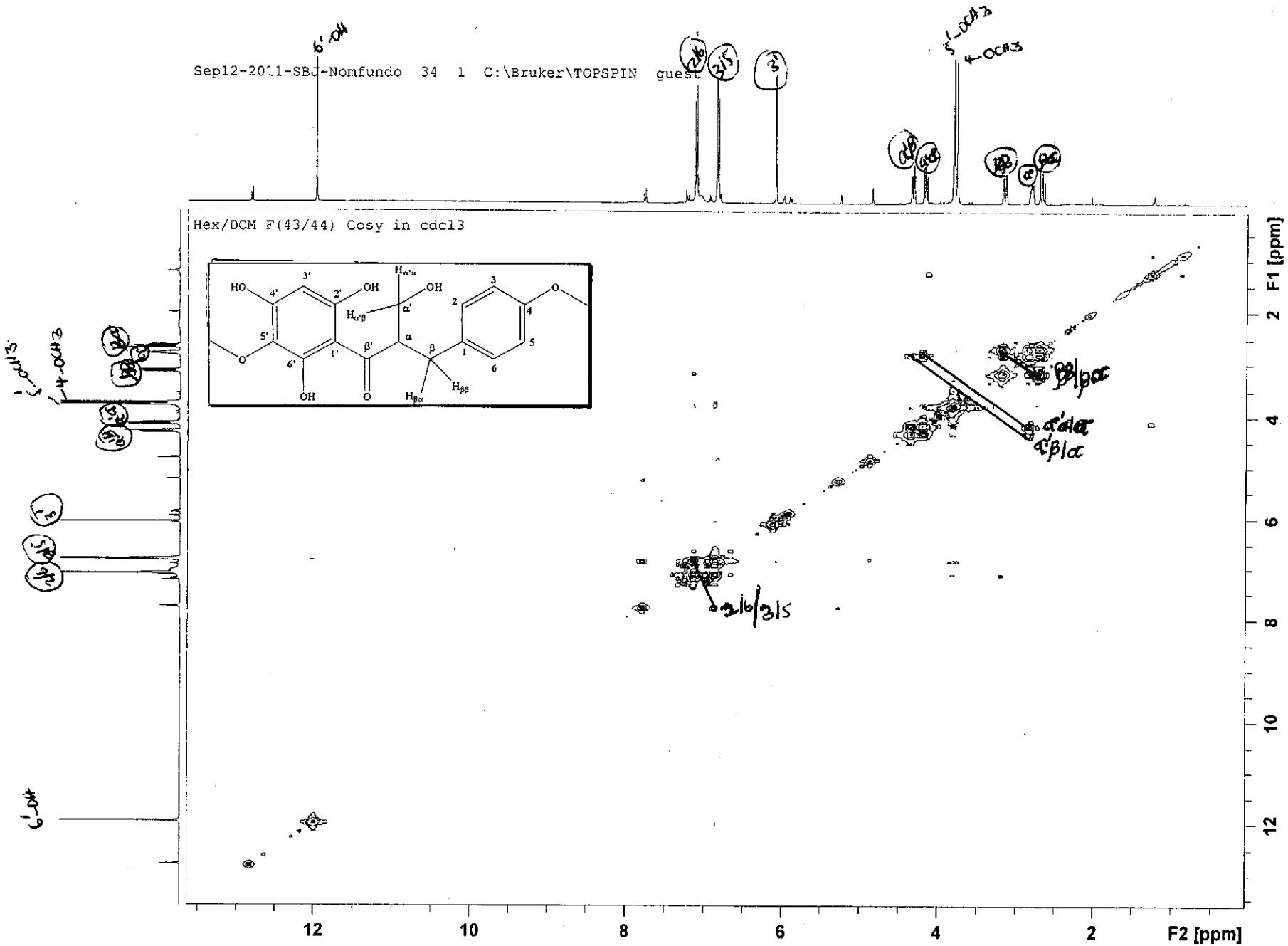
^{1}H NMR spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (1)



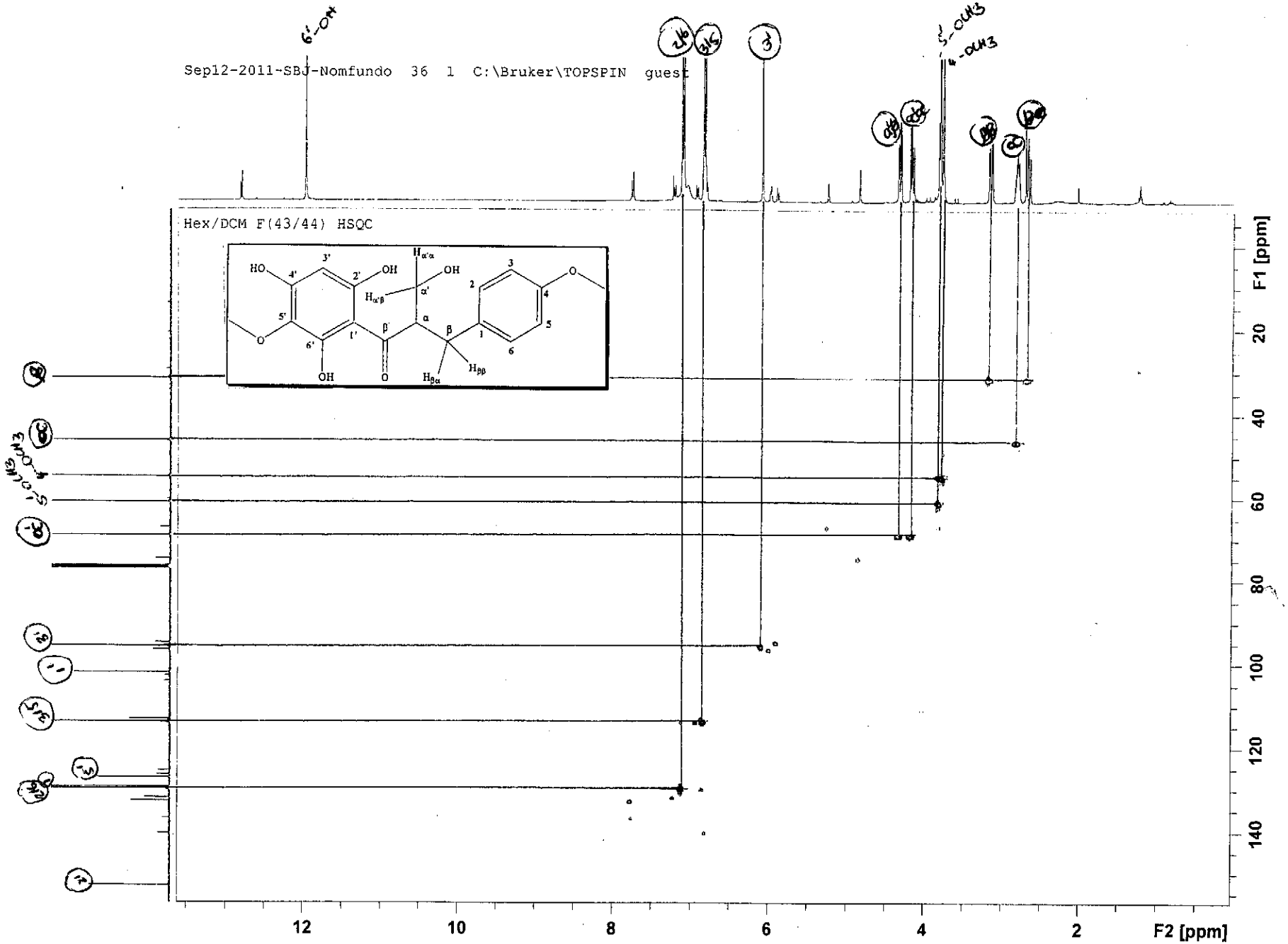
^{13}C NMR spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (1)



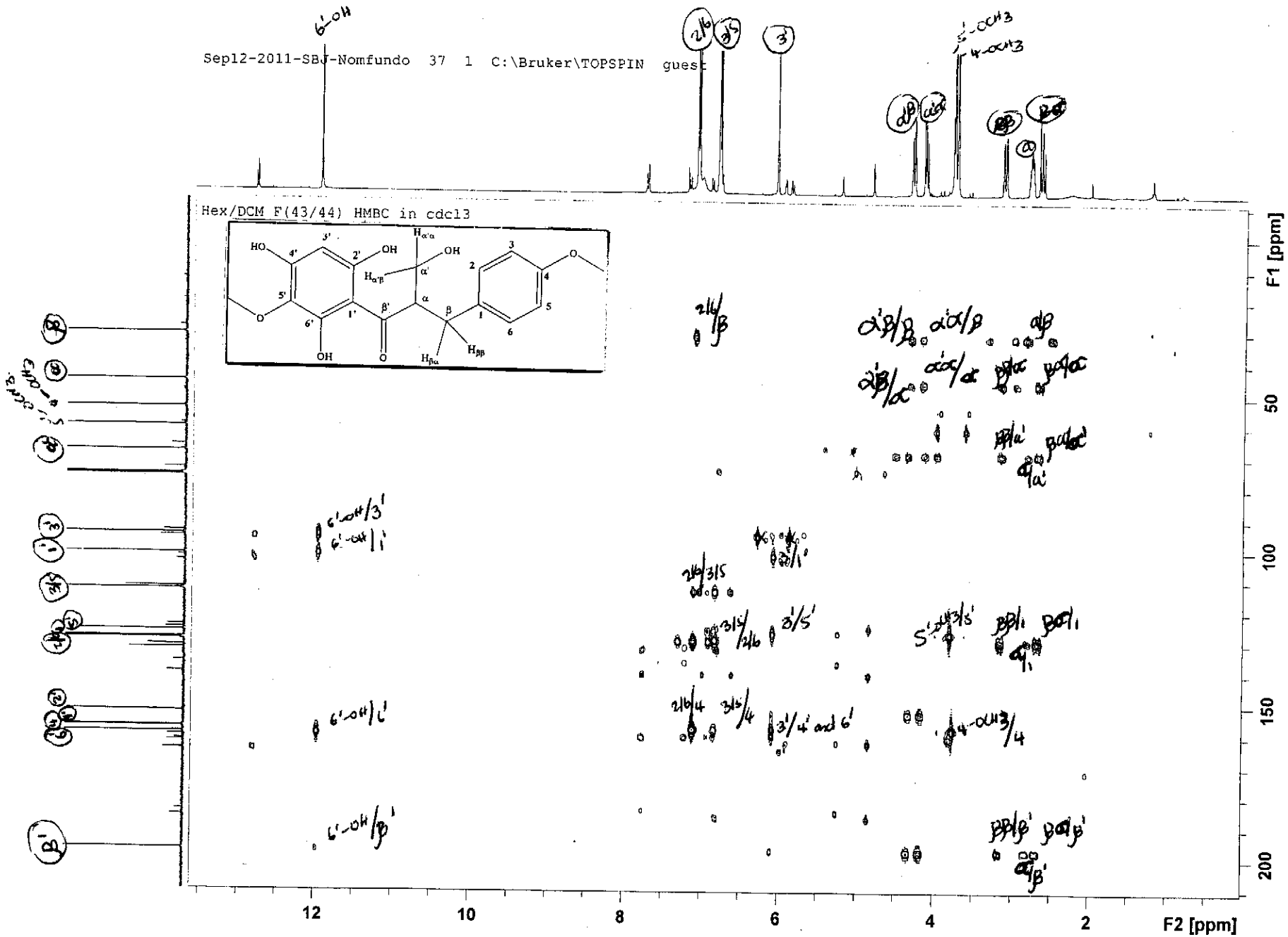
DEPT spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (1)



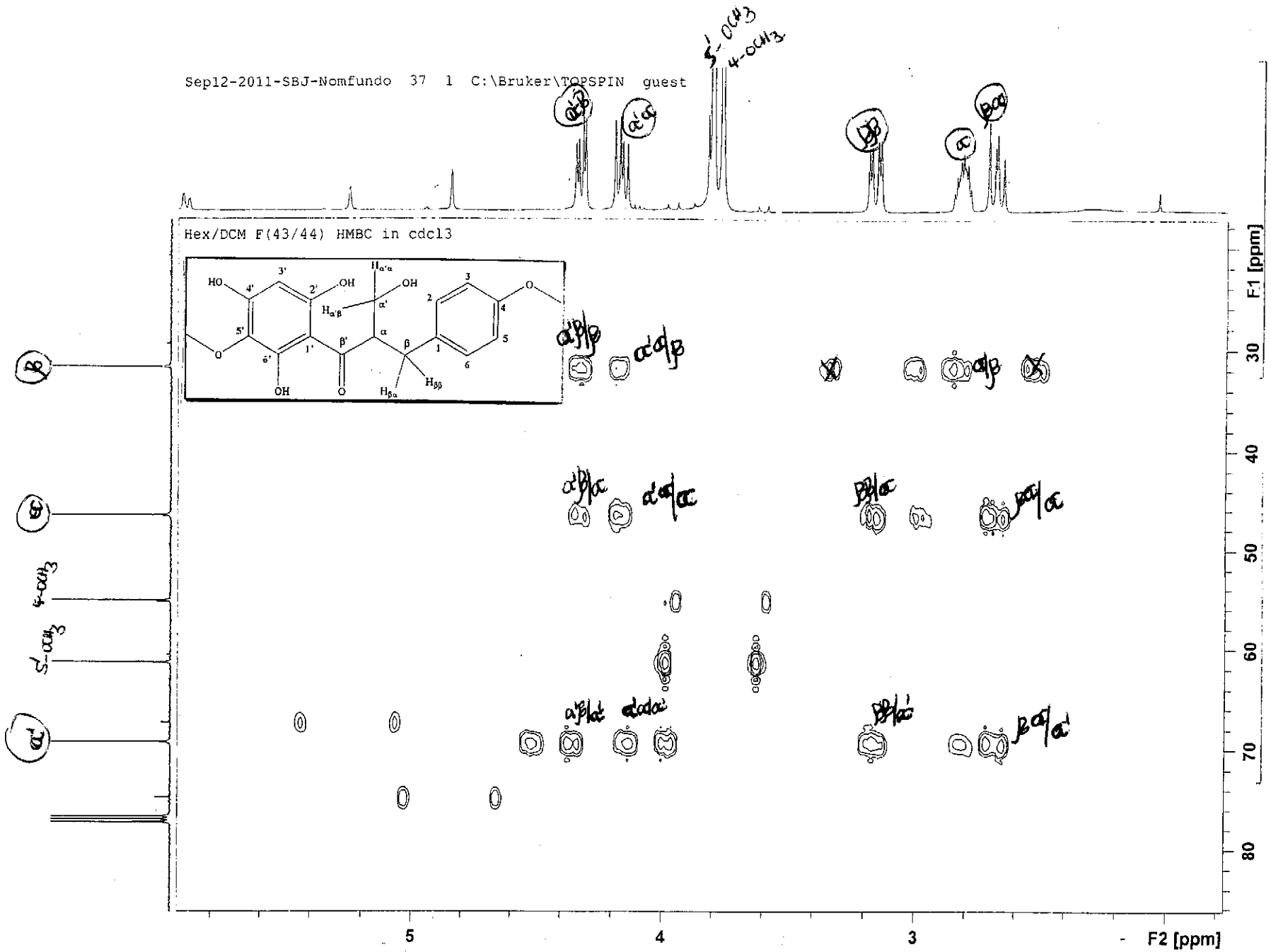
COSY spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (1)



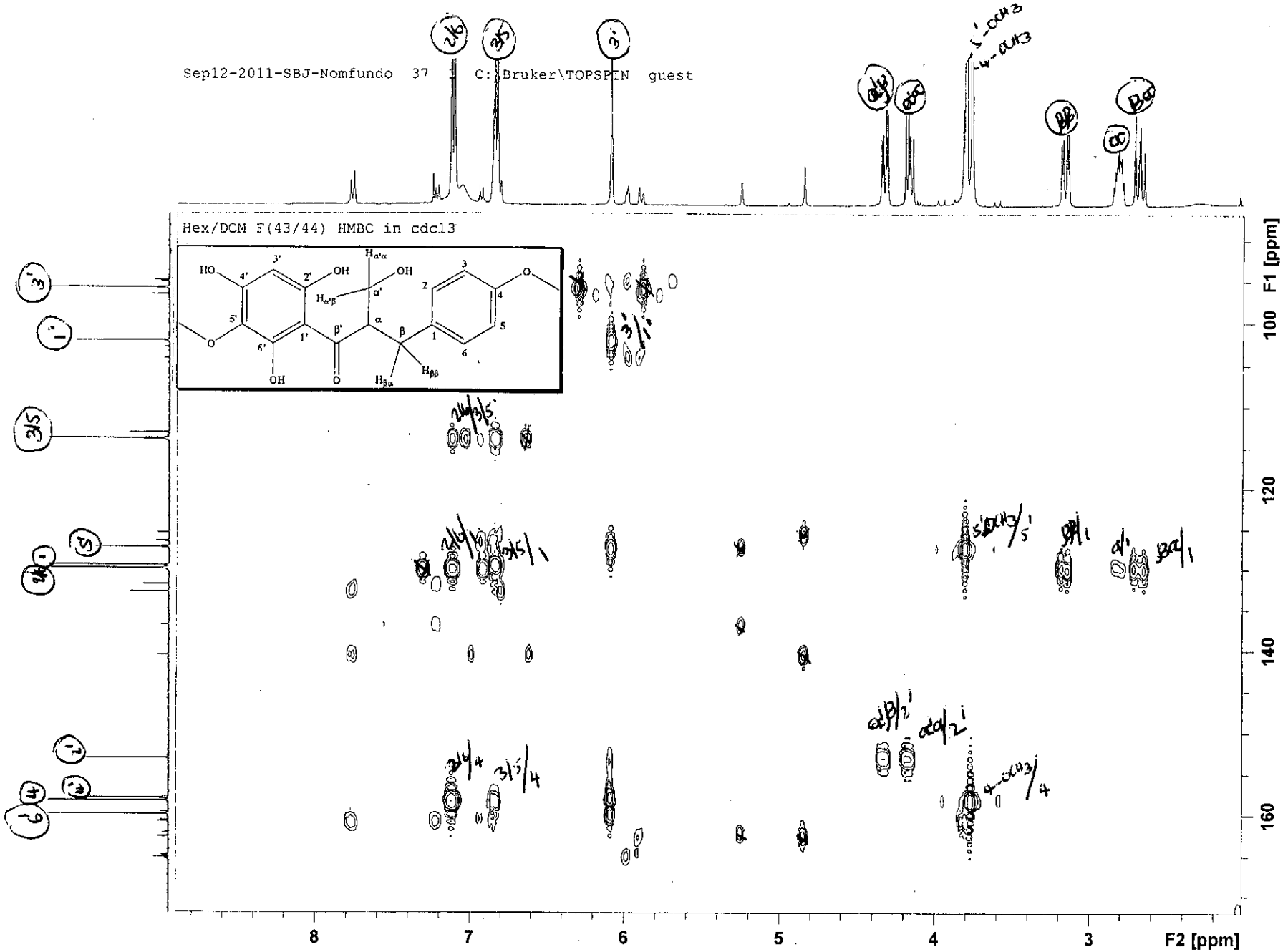
HSQC spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (1)



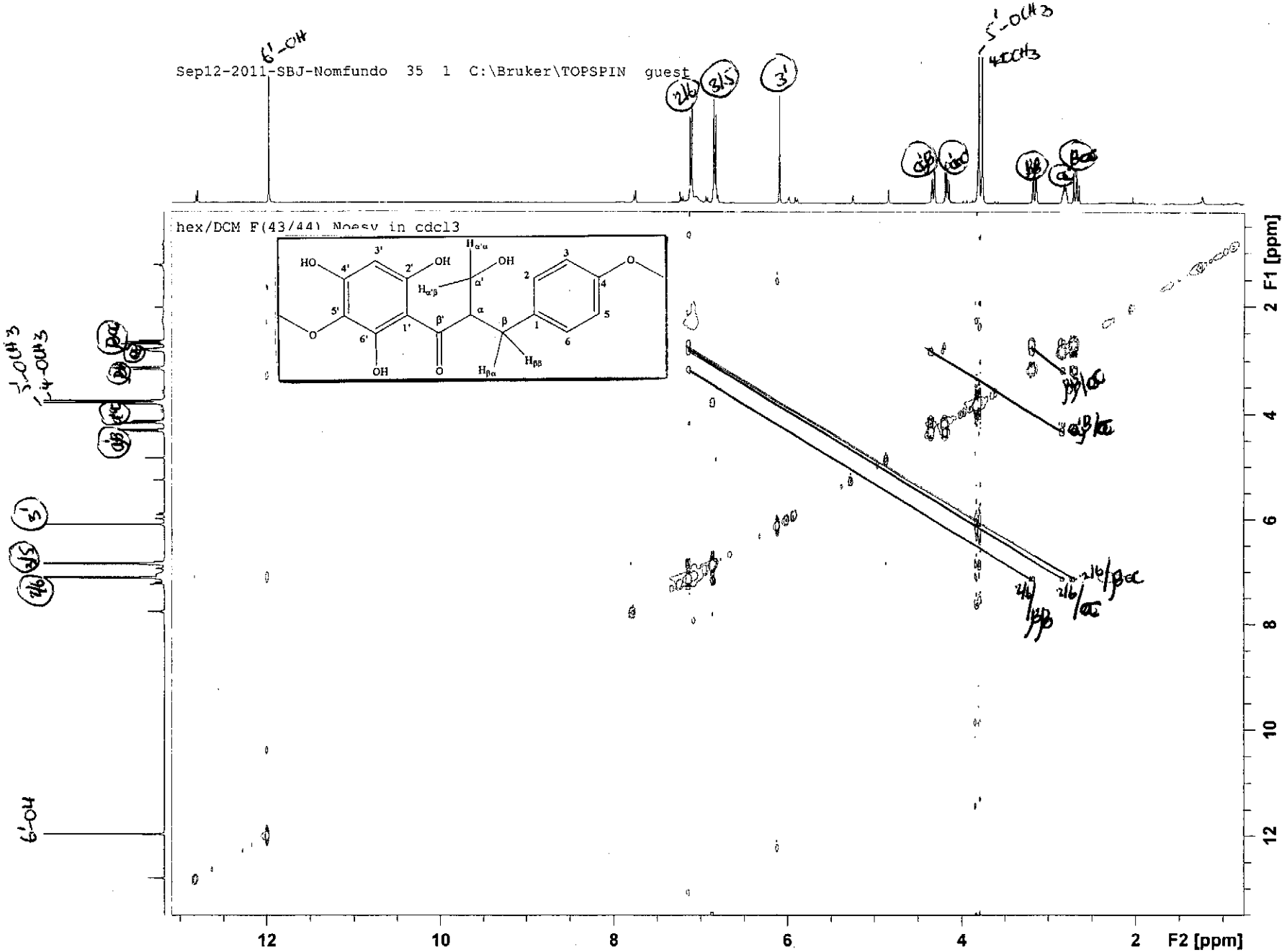
HMBC spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (1)



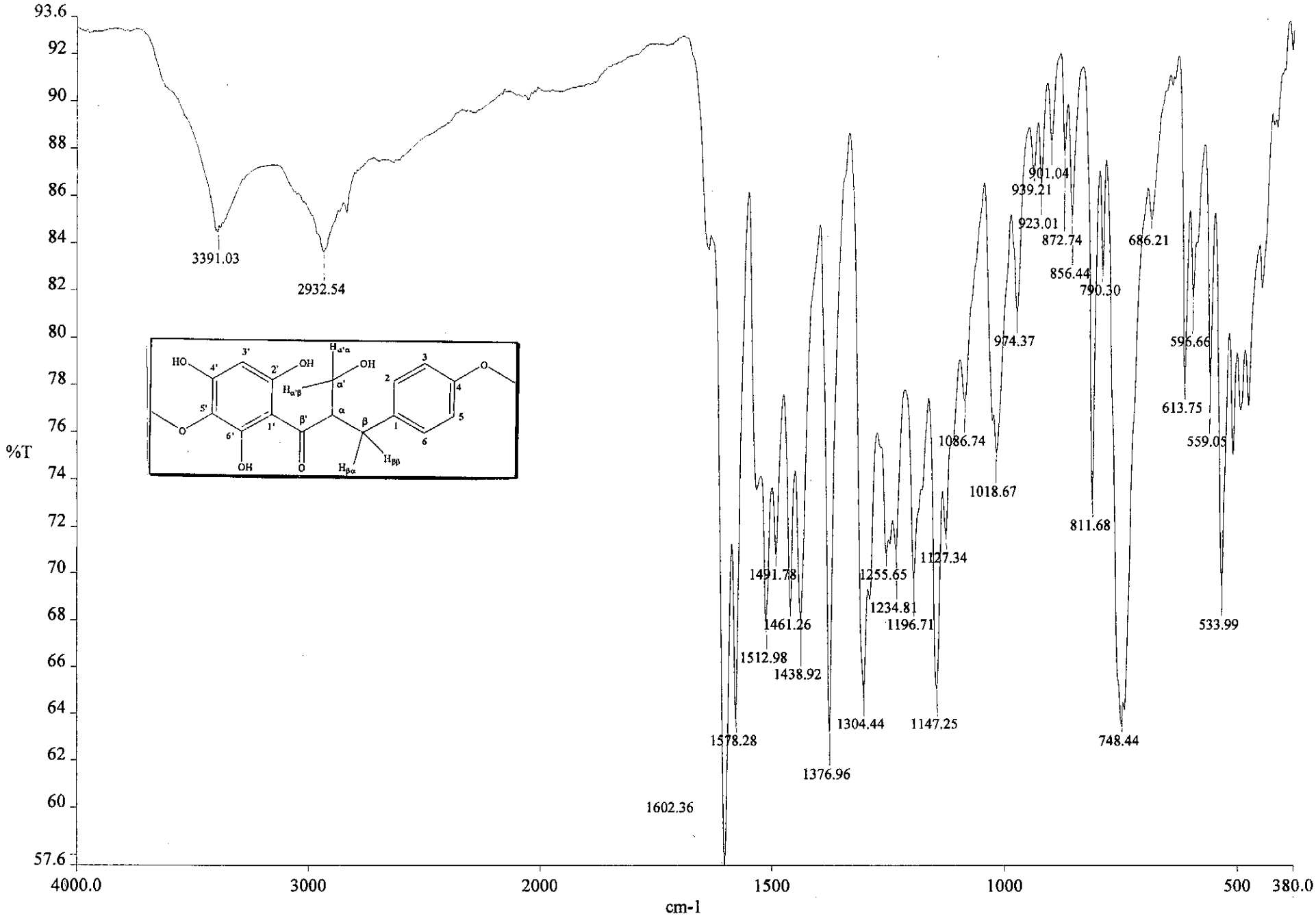
Expanded HMBC spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (1)



Expanded HMBC spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (1)

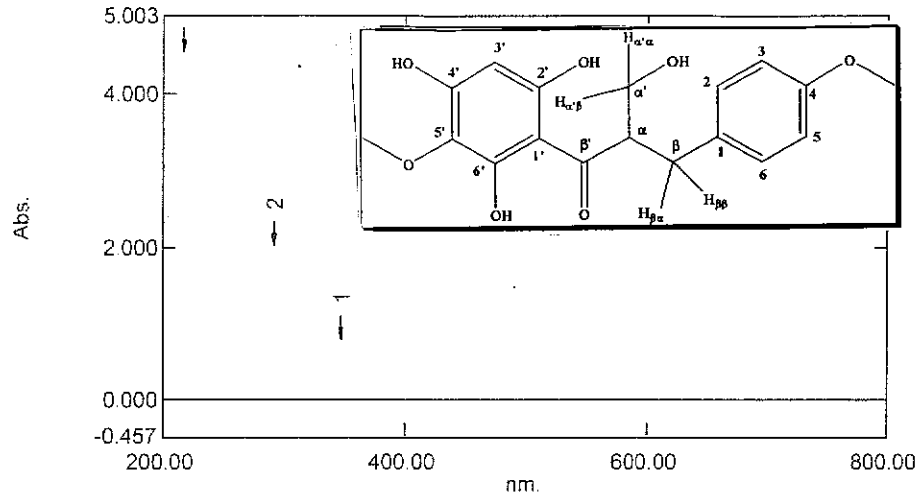


NOESY spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (1)



IR spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (1)

UNIVERSITY OF KWAZULU-NATAL
SCHOOL OF CHEMISTRY
ANALYSIS REPORT



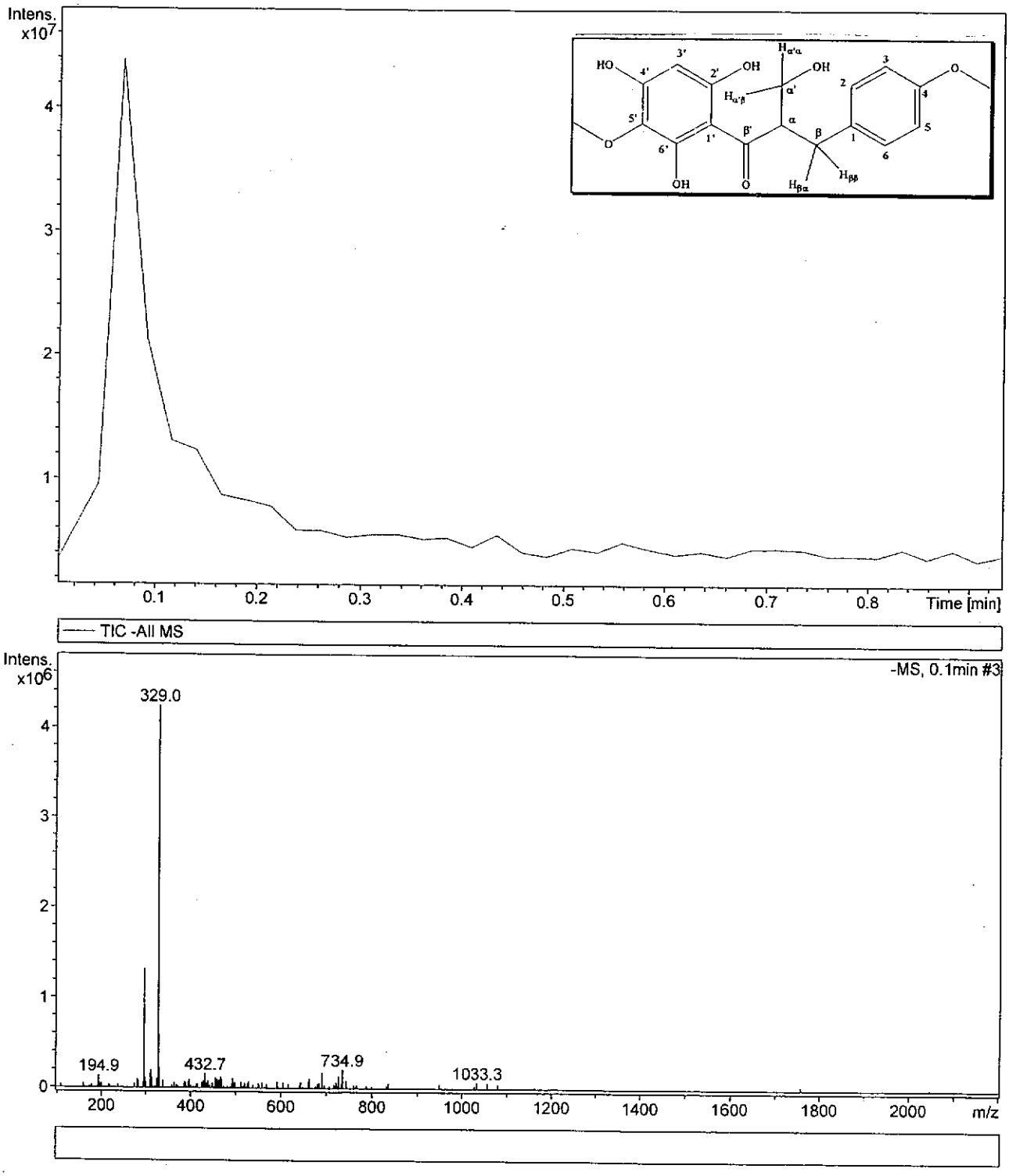
No.	P/V	Wavelength	Abs.	Description
1	⊕	347.00	0.790	
2	⊕	292.00	2.005	
3	⊕	216.00	4.548	
4	◯	638.00	-0.000	
5	◯	320.00	0.604	
6	◯	255.00	0.344	

File_121018_43NO2.spc - Storage 0

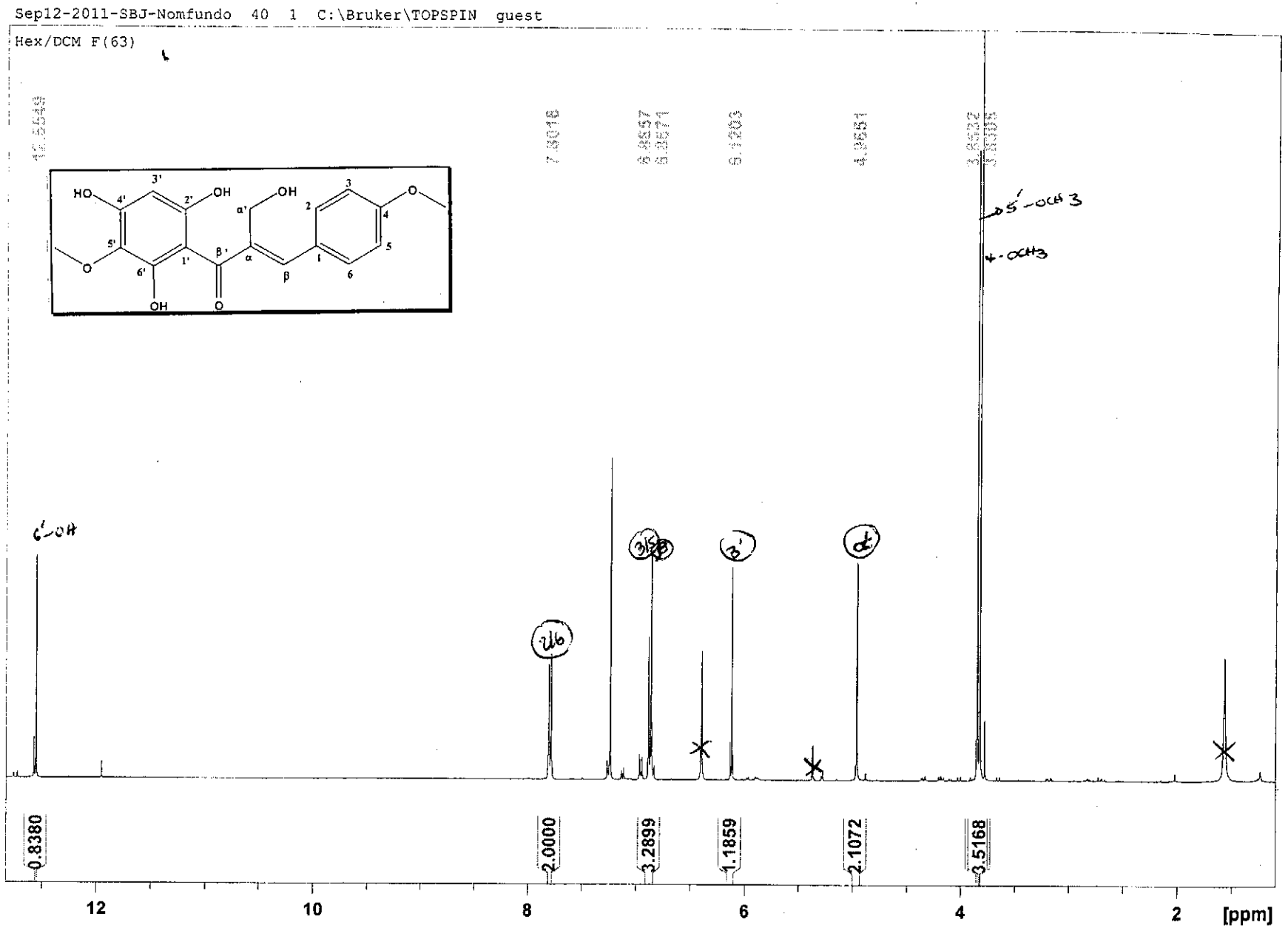
UV spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (1)

Display Report - All Windows Selected Analysis

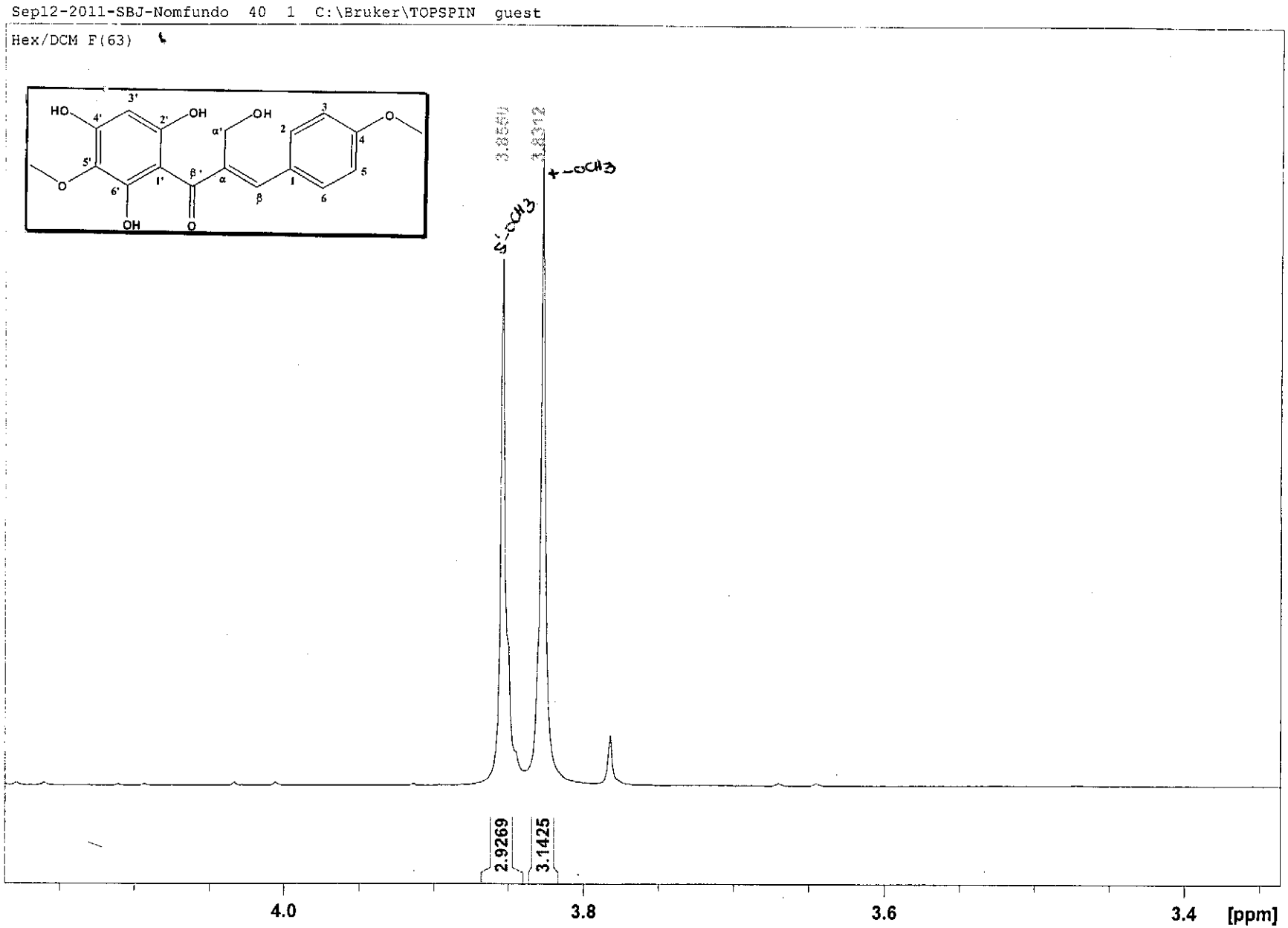
Analysis Name: F43000001.D **Instrument:** LC-MSD-Trap-VL **Print Date:** 11/12/2012 11:30:29 AM
Method: AN 2MIN.M **Operator:** Operator **Acq. Date:** 11/12/2012 10:32:26 AM
Sample Name: F43
Analysis Info: repeat 5ul



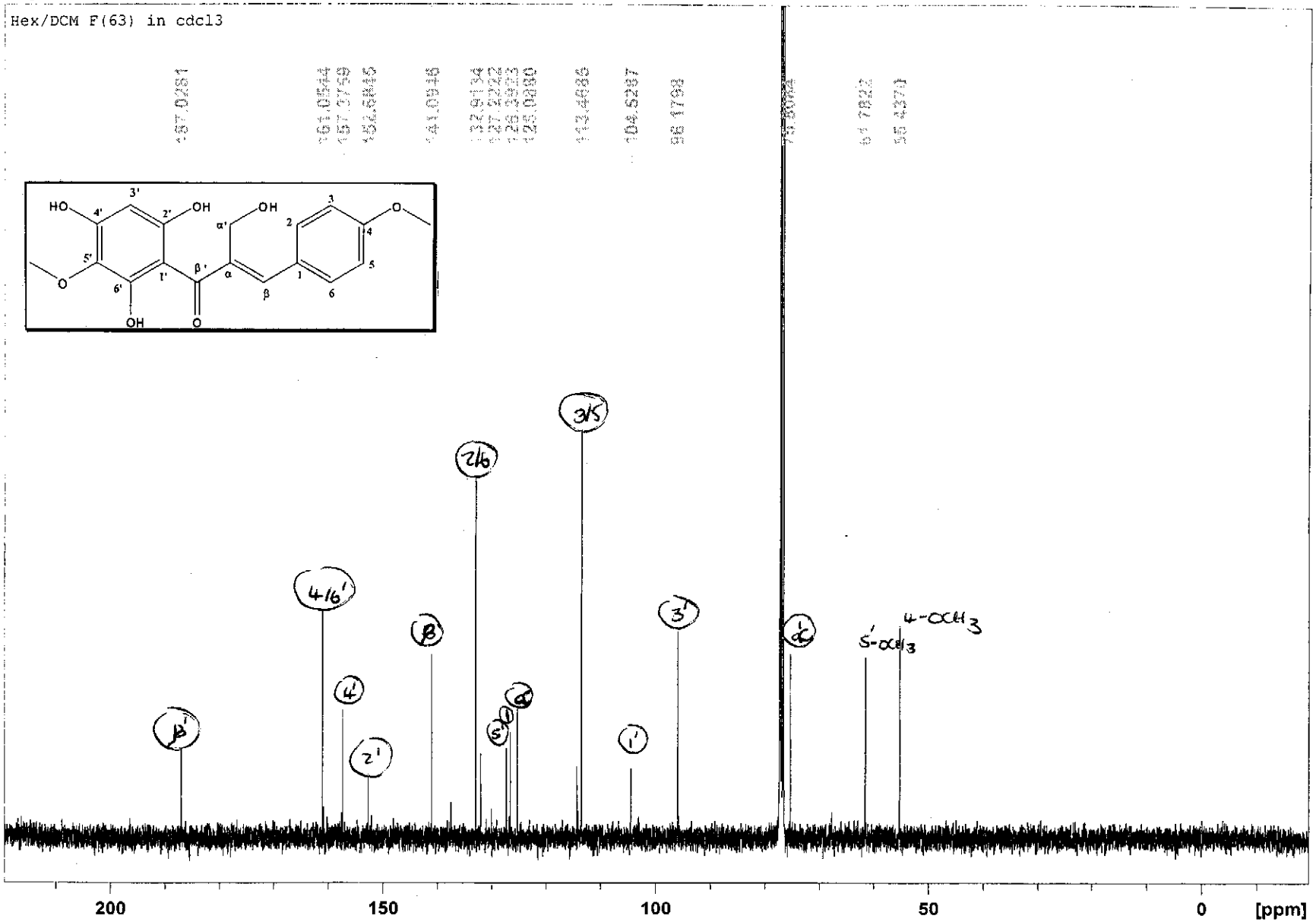
LC-MS spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (1)



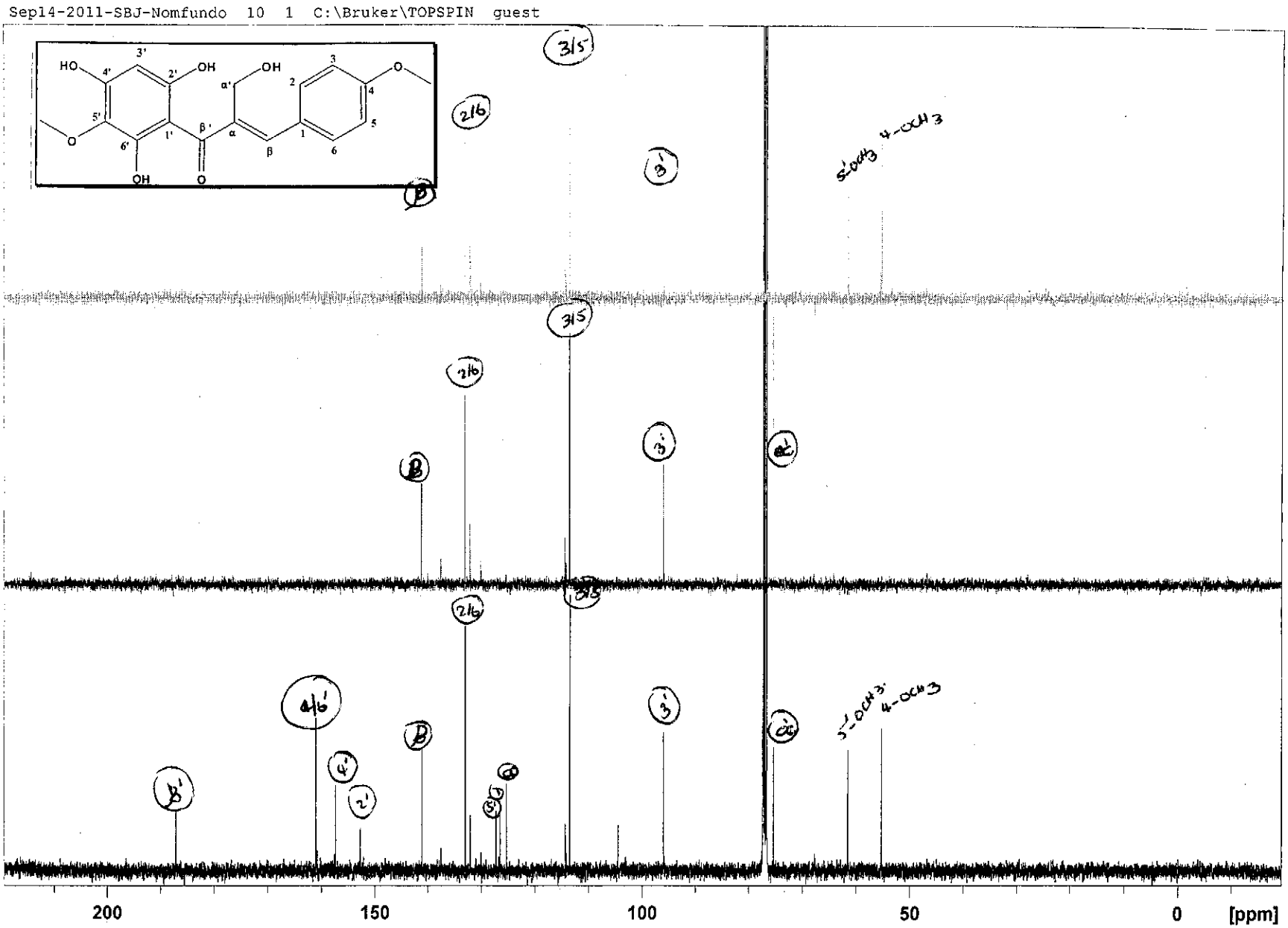
^{1}H NMR spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-chalcone (2)



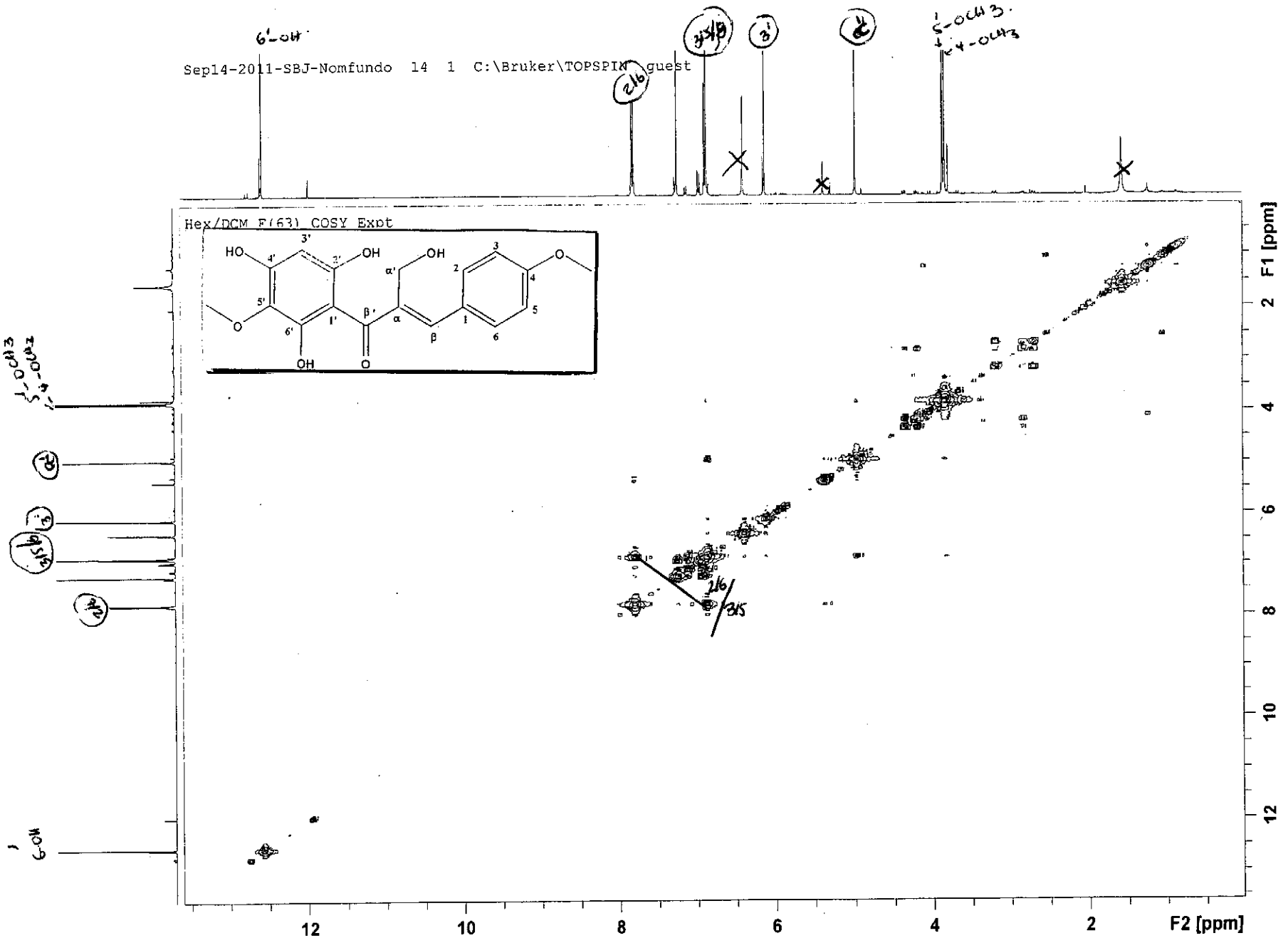
Expanded ^{1}H NMR spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-chalcone (2)



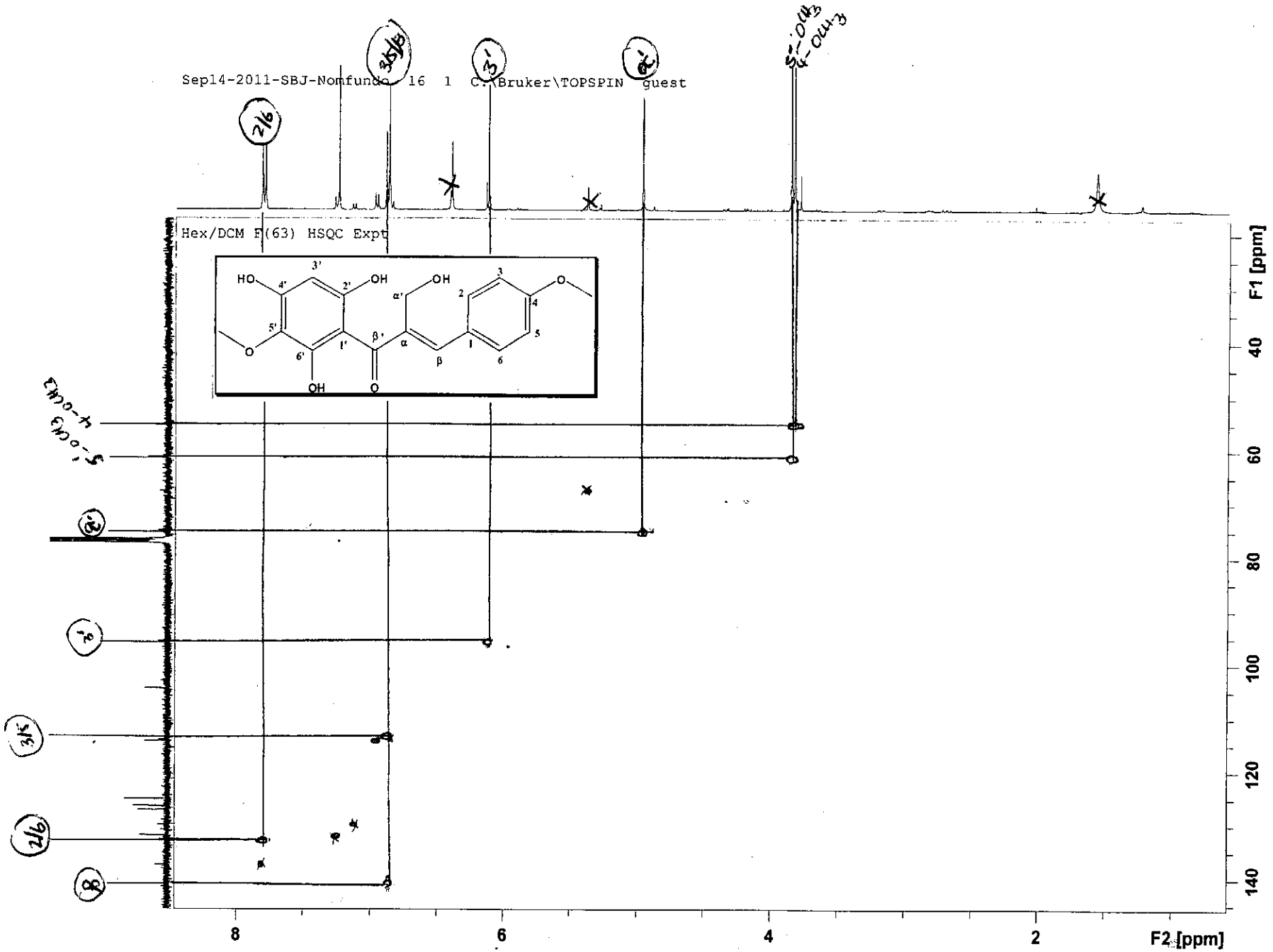
^{13}C NMR spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-chalcone (2)



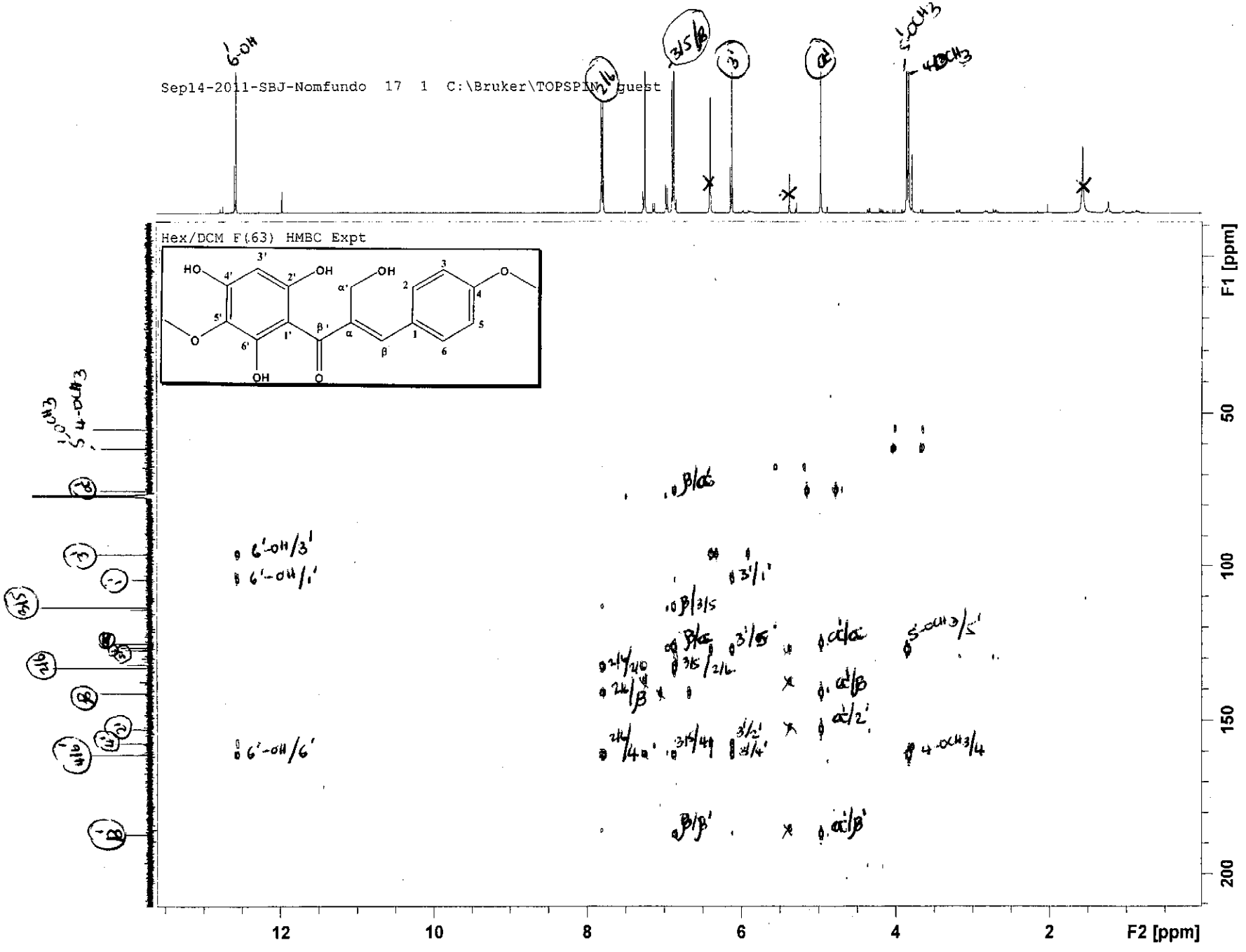
DEPT spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-chalcone (2)



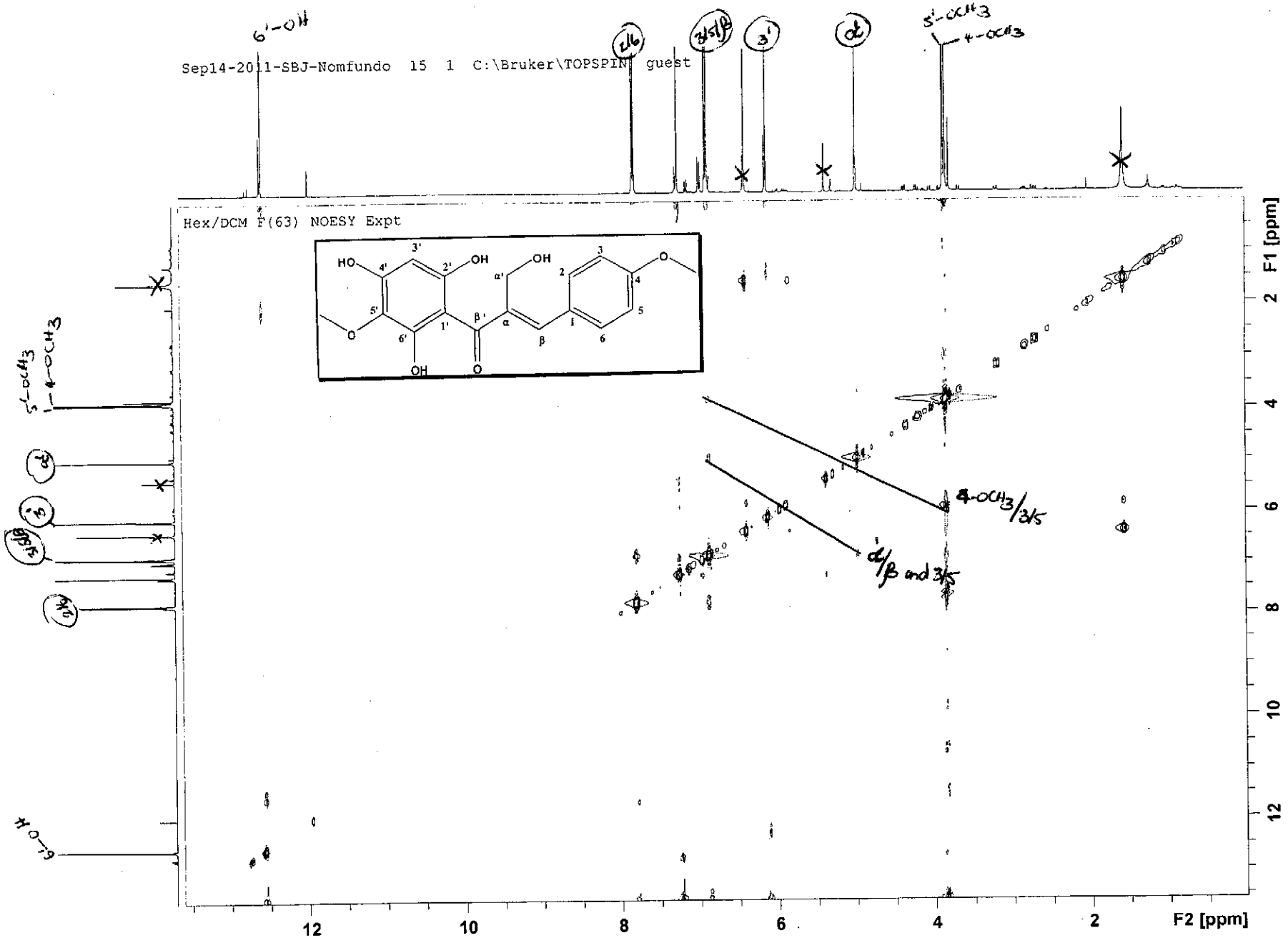
COSY spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-chalcone (2)



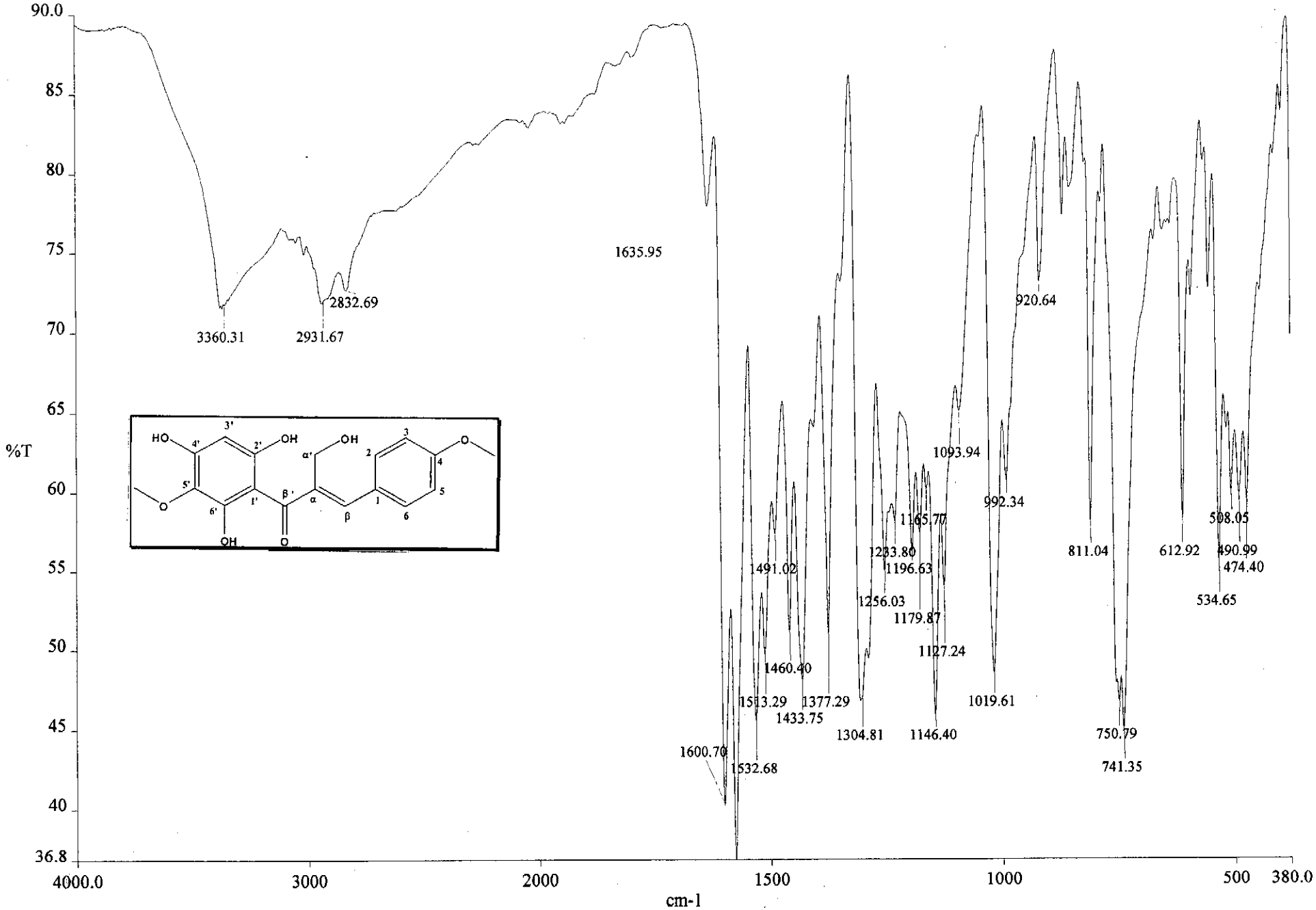
HSQC spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-chalcone(2)



HMBC spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-chalcone (2)

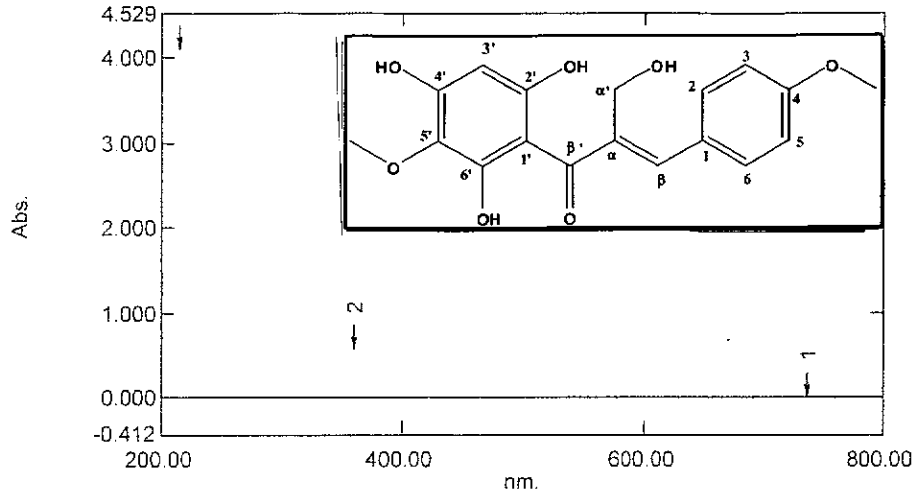


NOESY spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-chalcone (2)



IR spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-chalcone (2)

UNIVERSITY OF KWAZULU-NATAL
SCHOOL OF CHEMISTRY
ANALYSIS REPORT



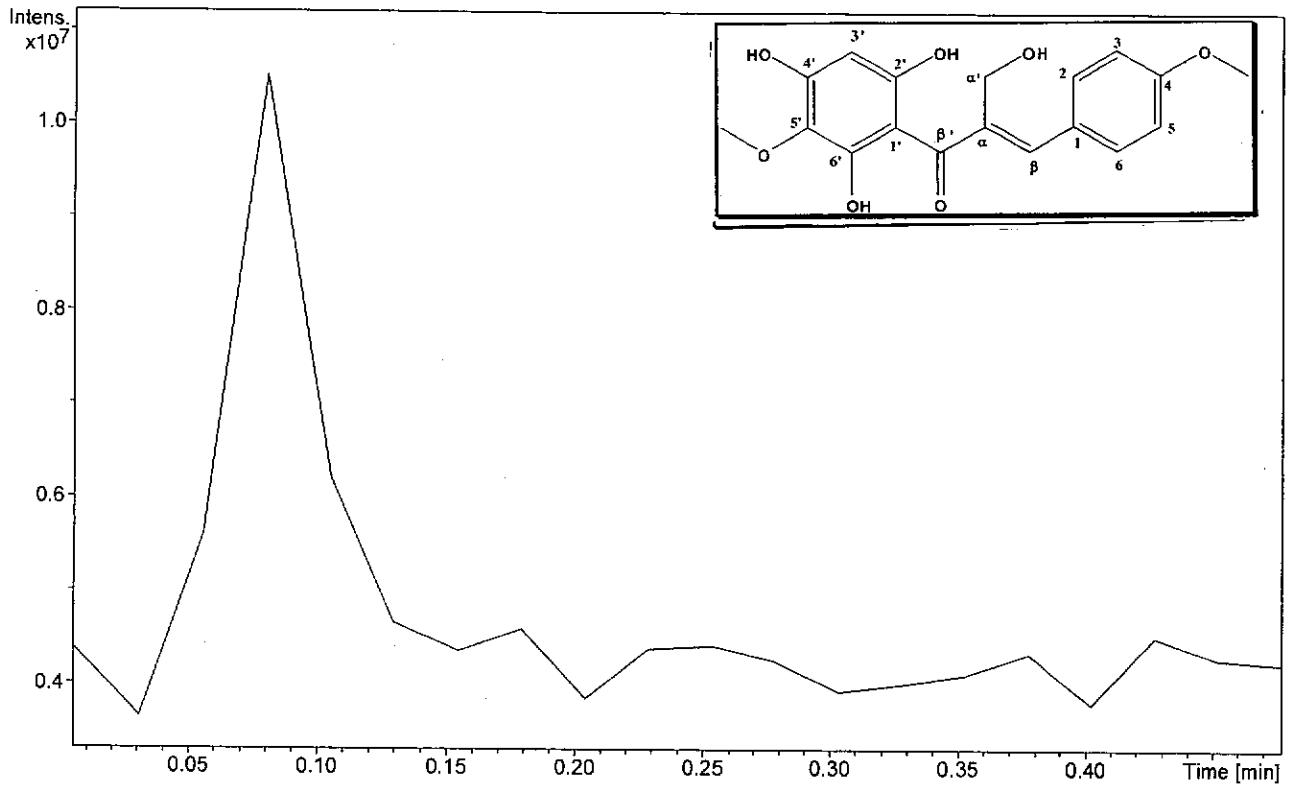
No.	P/V	Wavelength	Abs.	Description
1	⊕	737.00	0.002	
2	⊕	360.00	0.578	
3	⊕	215.00	4.118	
4	◯	277.00	0.161	

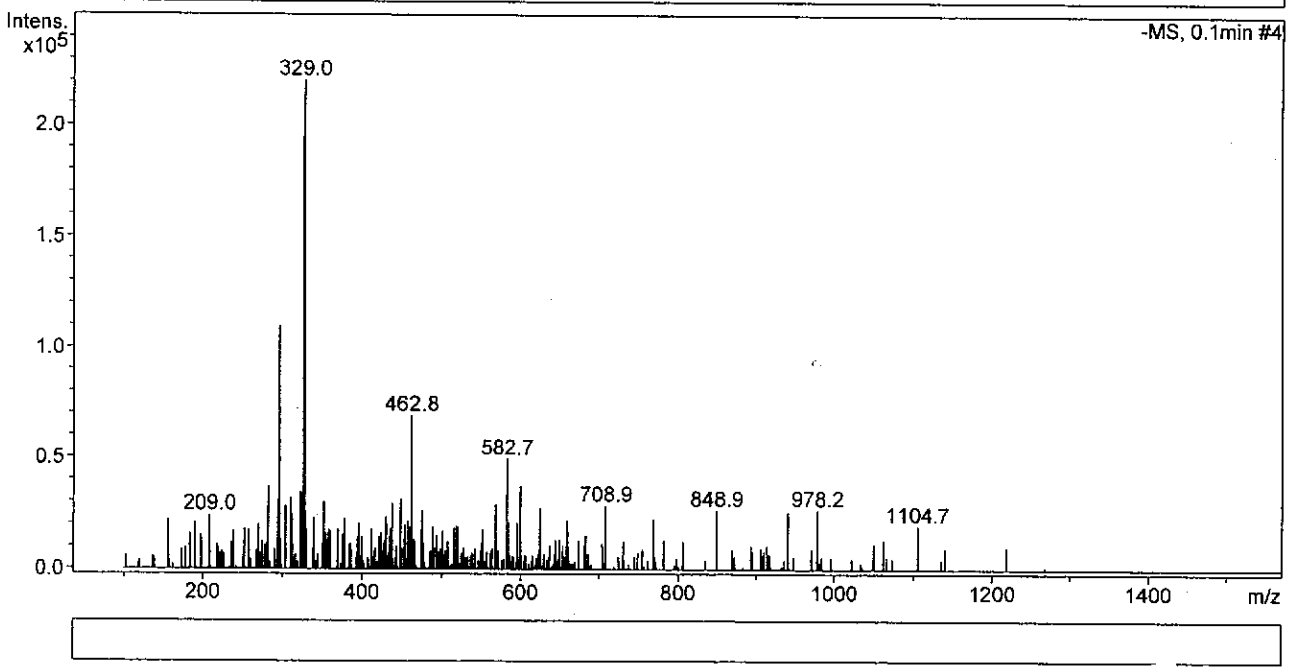
File_121018_C63.spc - Storage 0259

UV spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-chalcone (2)

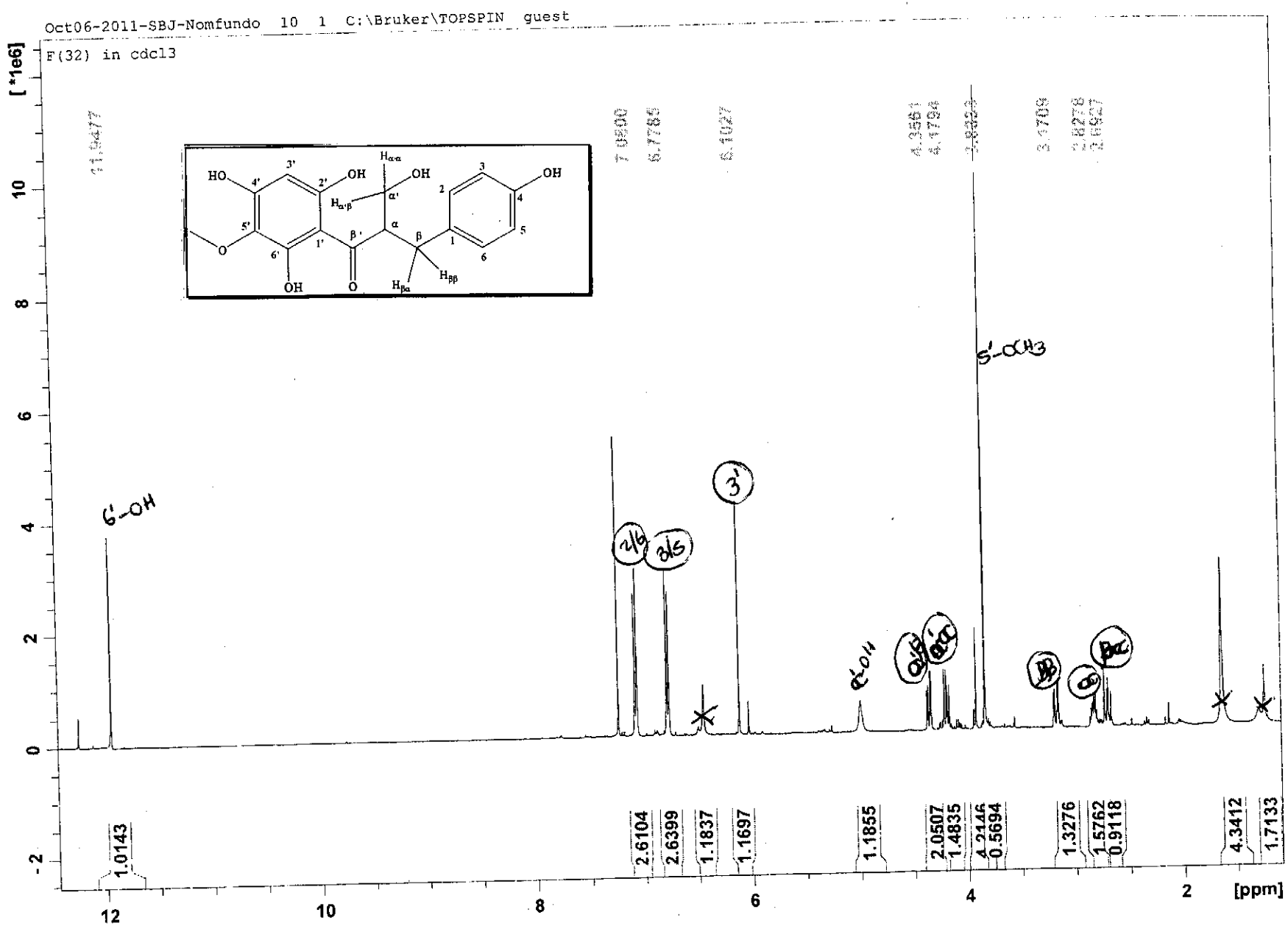
Display Report - All Windows Selected Analysis

Analysis Name: F63000005.D **Instrument:** LC-MSD-Trap-VL **Print Date:** 11/12/2012 10:28:22 AM
Method: AN 2MIN.M **Operator:** Operator **Acq. Date:** 11/12/2012 10:24:11 AM
Sample Name: F63
Analysis Info: repeat 5ul

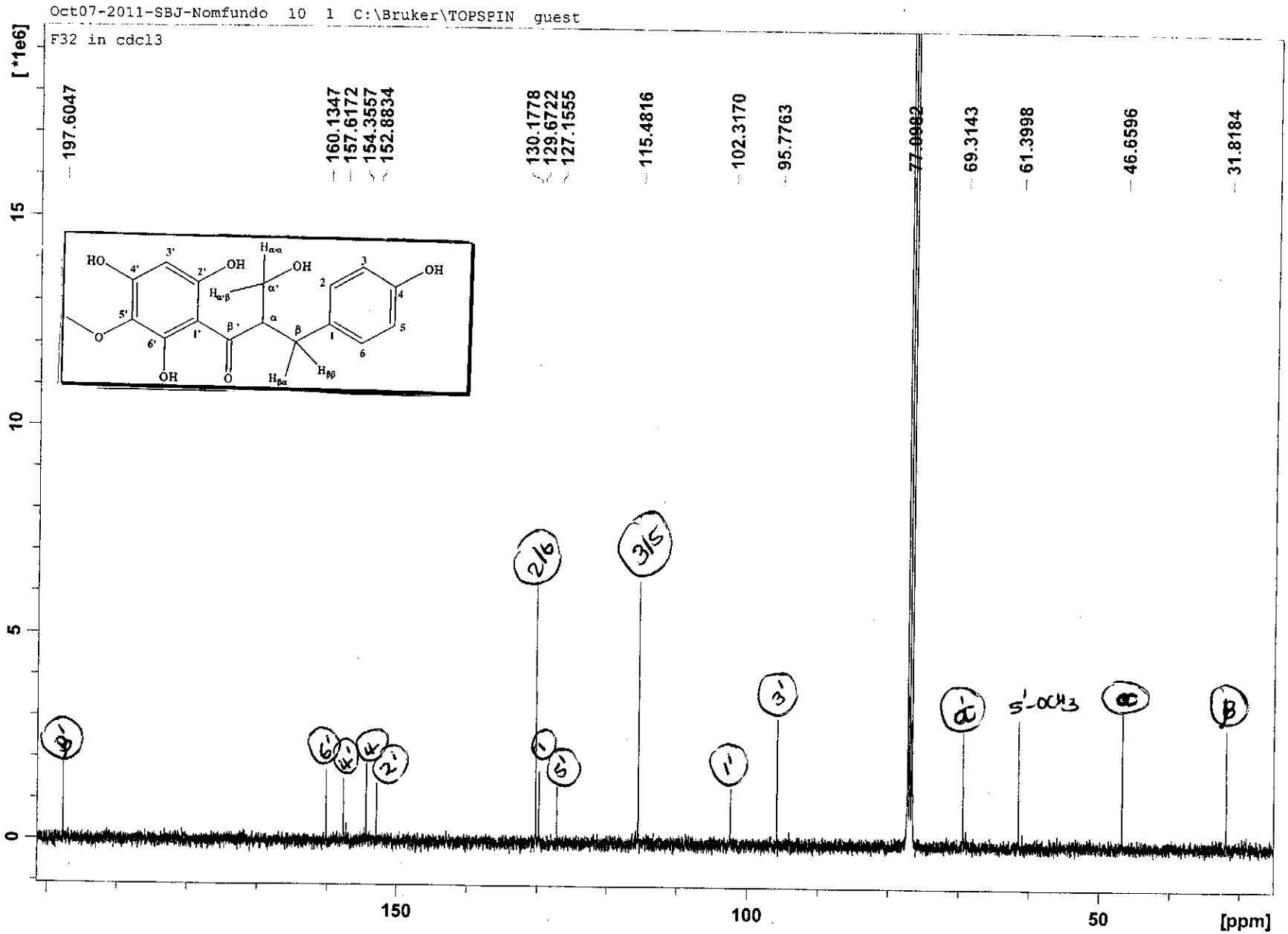




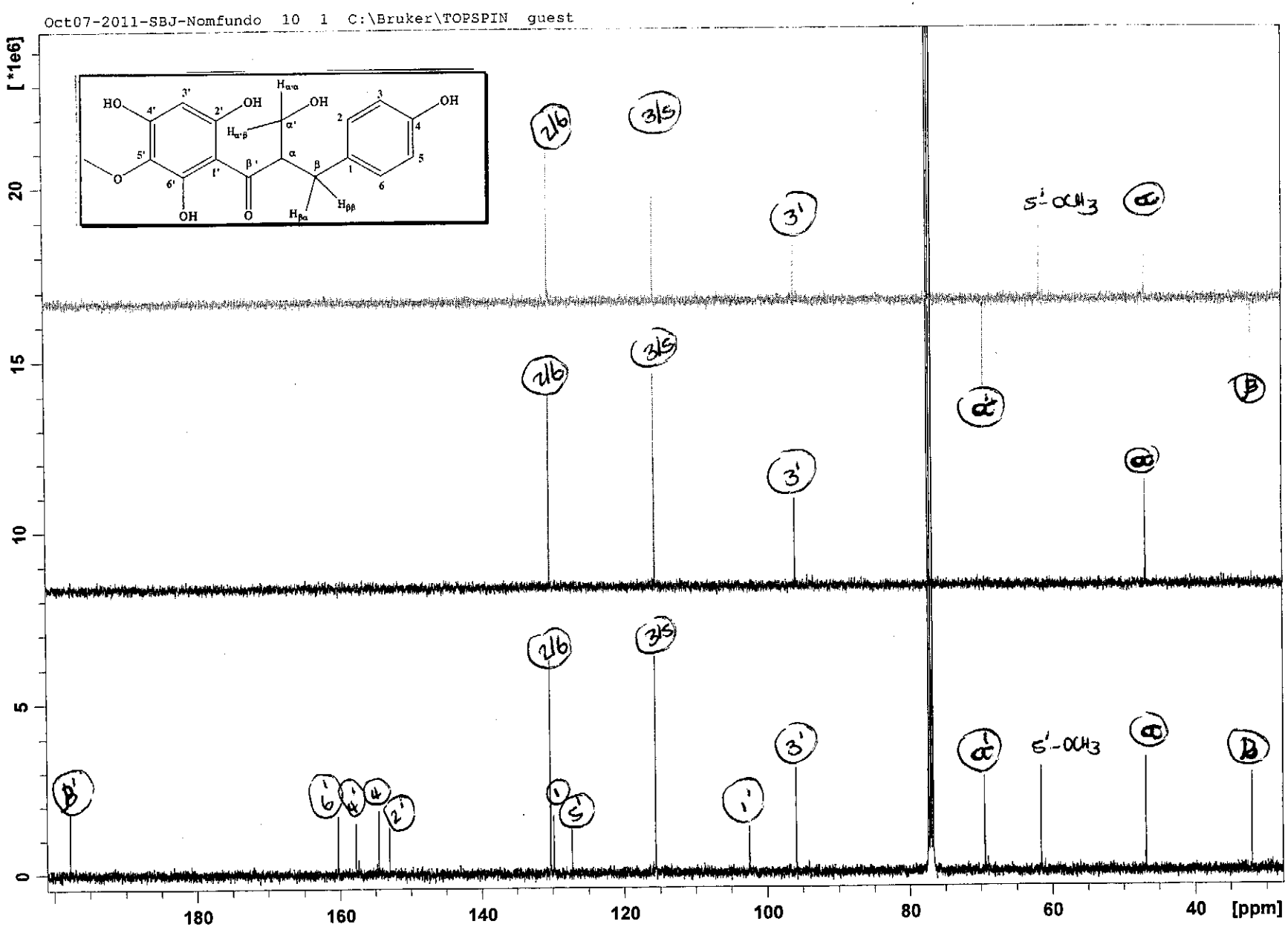
LC-MS spectrum of 2',4',6'-trihydroxy-5',4-dimethoxy-α-hydroxymethyl-chalcone (2)



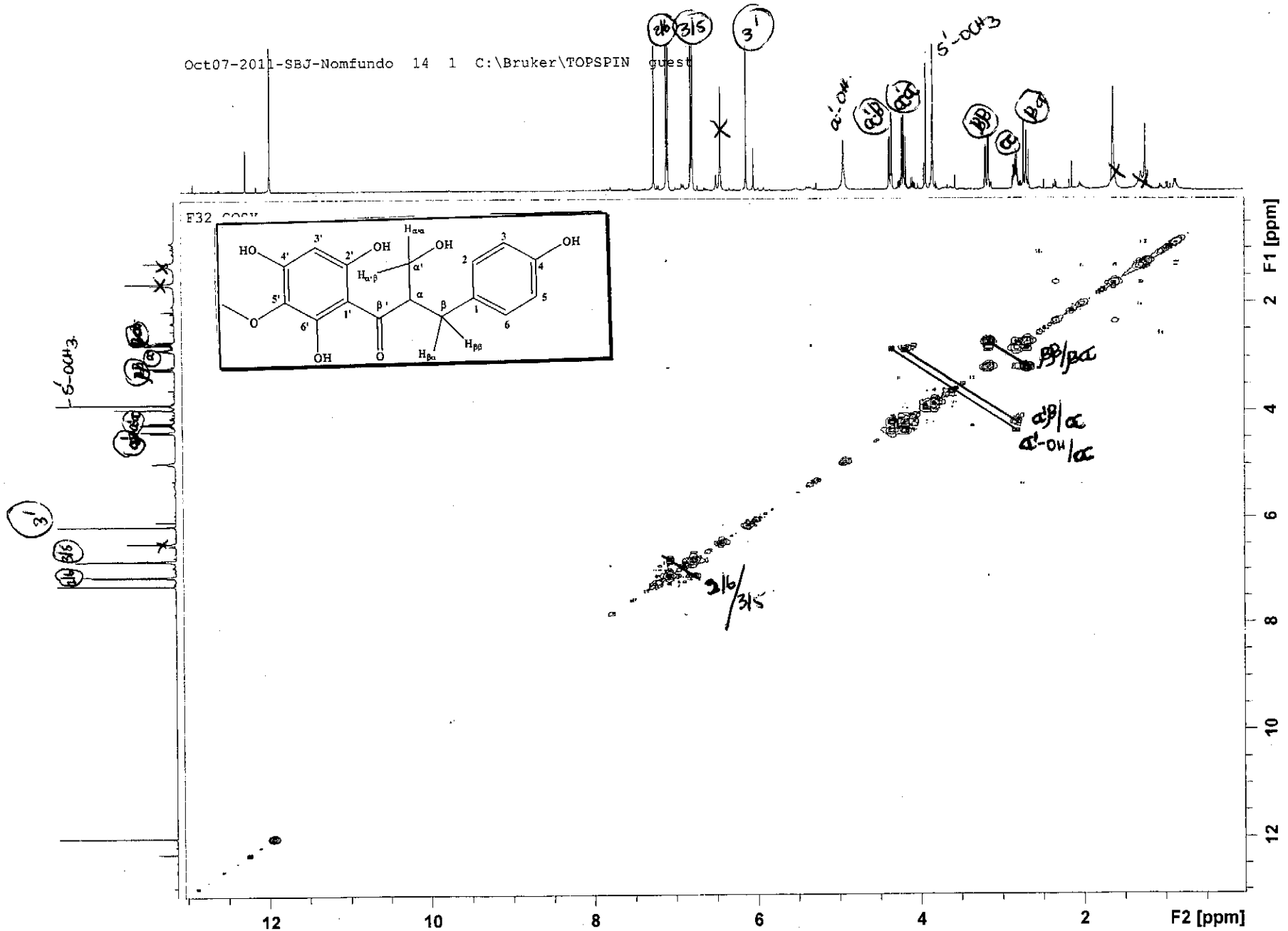
^{1}H NMR spectrum of 2',4',6',4-tetrahydroxy-5'-methoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (3)



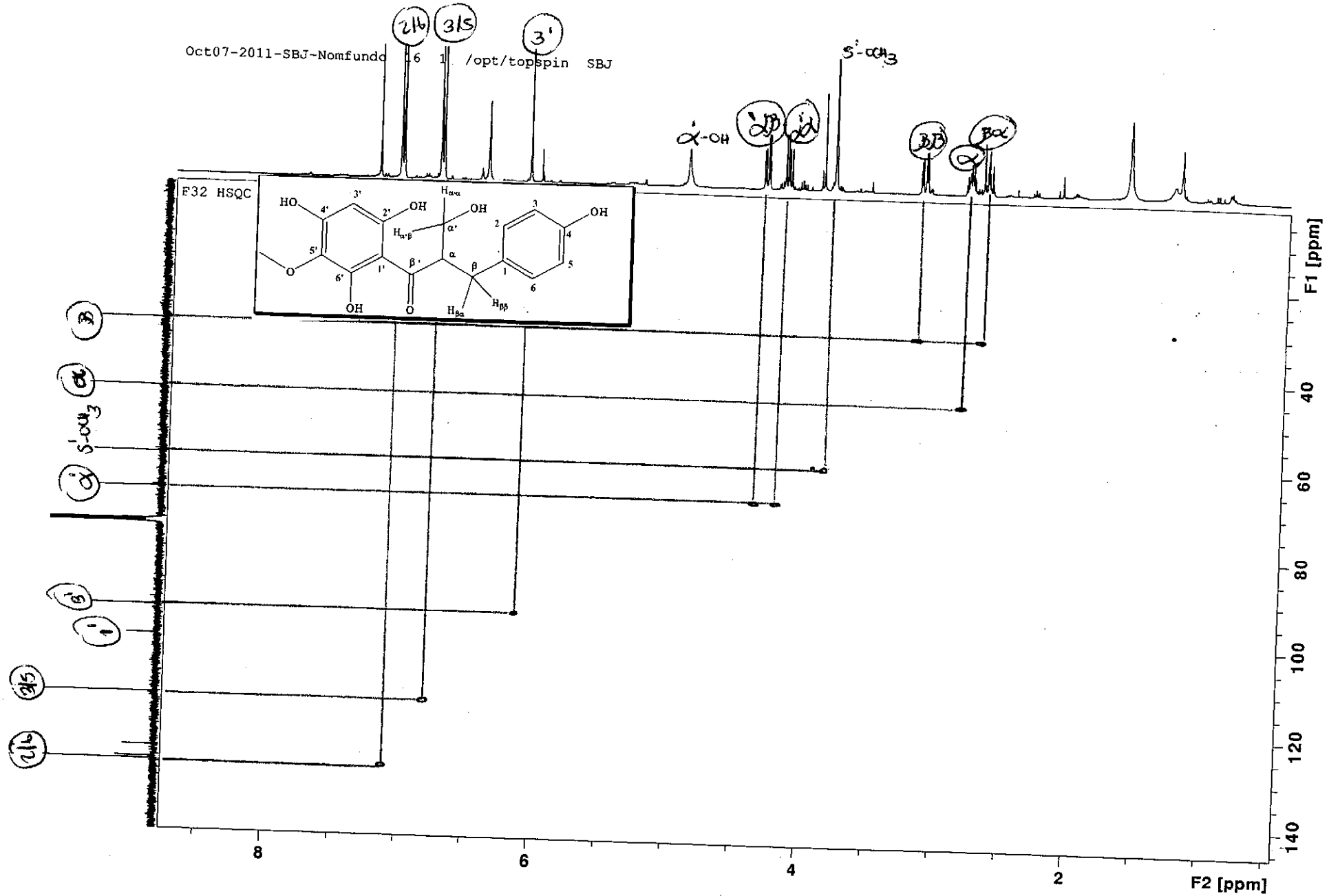
^{13}C NMR spectrum of 2',4',6',4-tetrahydroxy-5'-methoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (3)



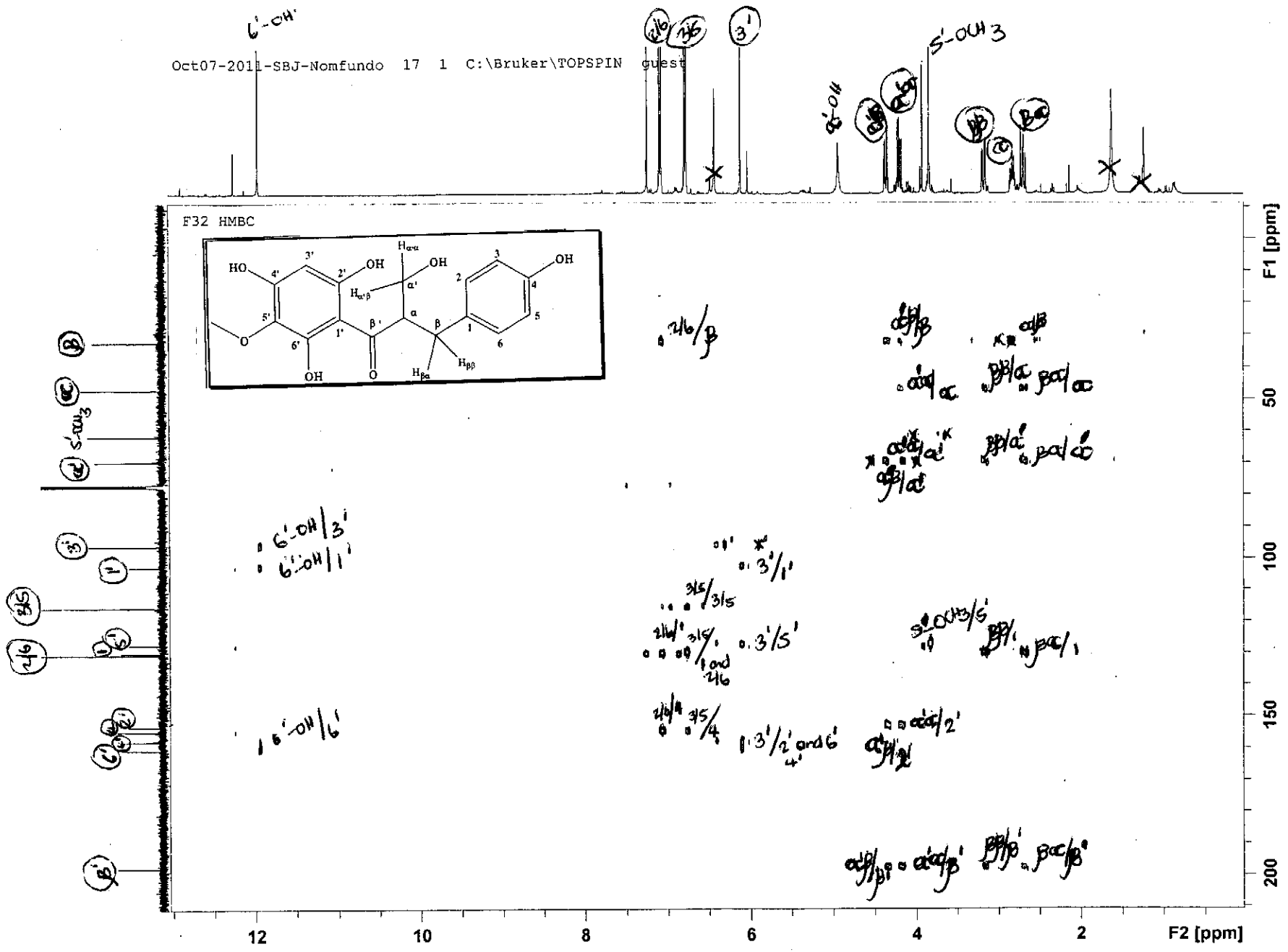
DEPT spectrum of 2',4',6',4-tetrahydroxy-5'-methoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (3)



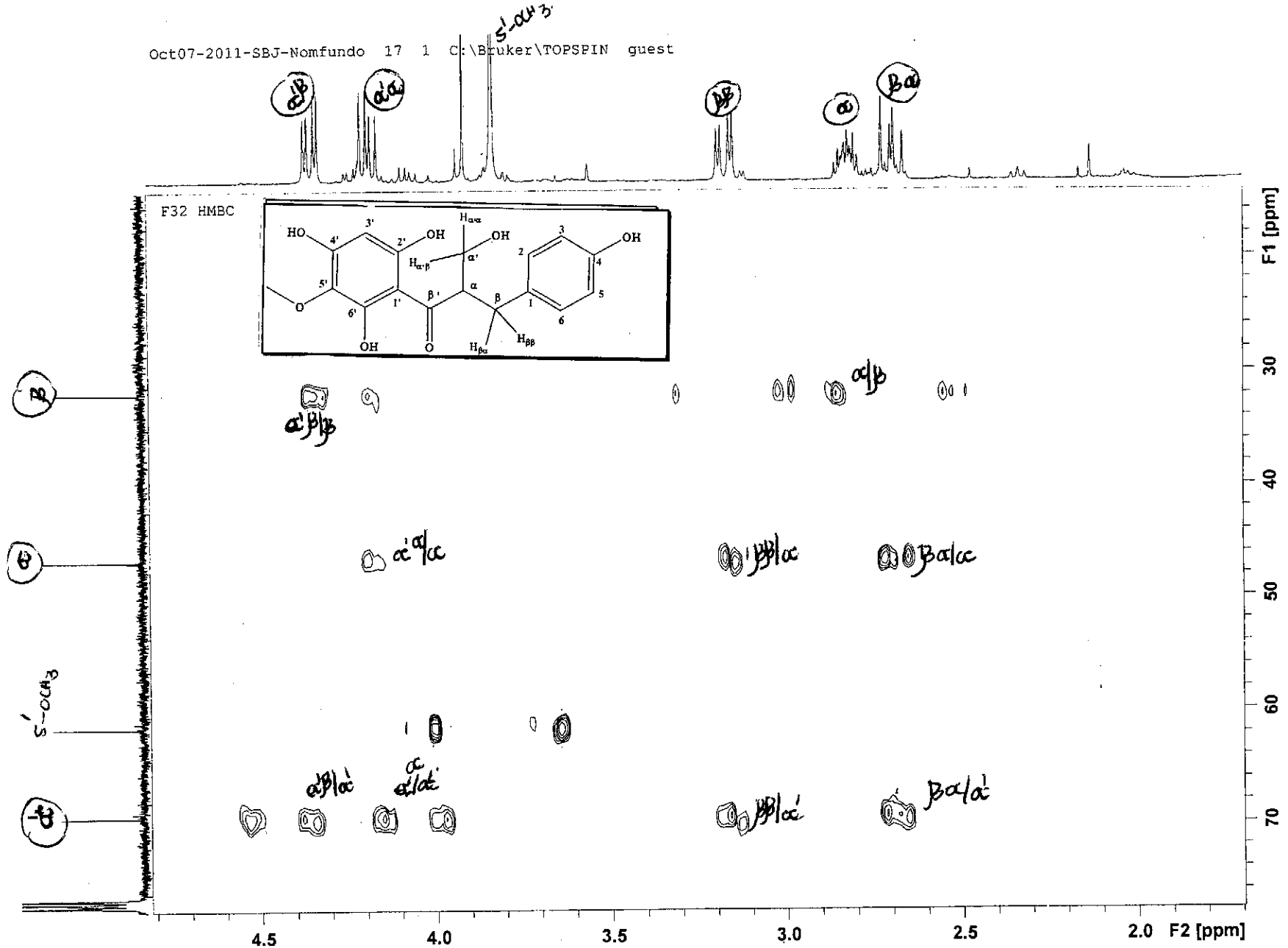
COSY spectrum of 2',4',6',4-tetrahydroxy-5'-methoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (3)



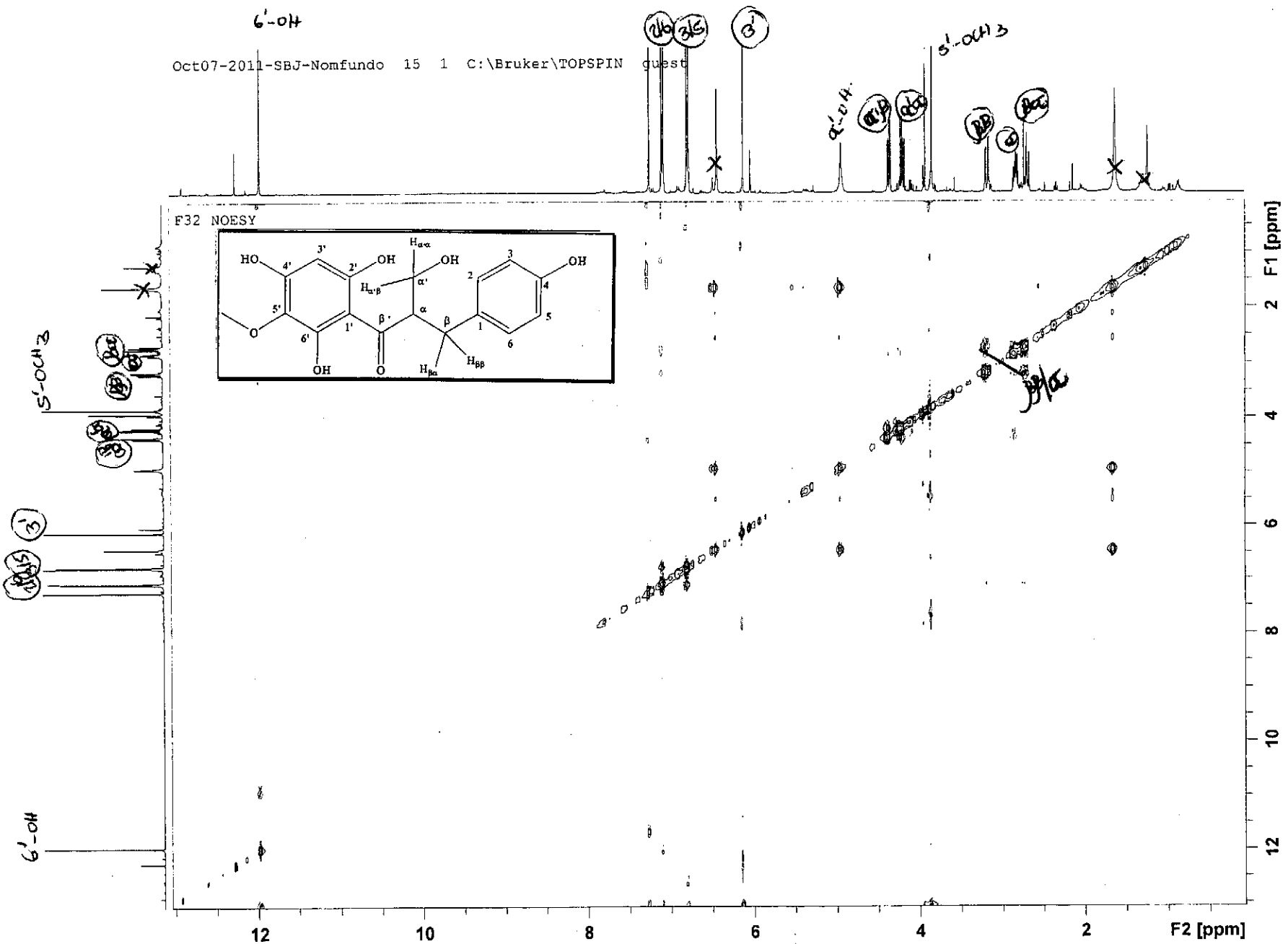
HSQC spectrum of 2',4',6',4-tetrahydroxy-5'-methoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (3)



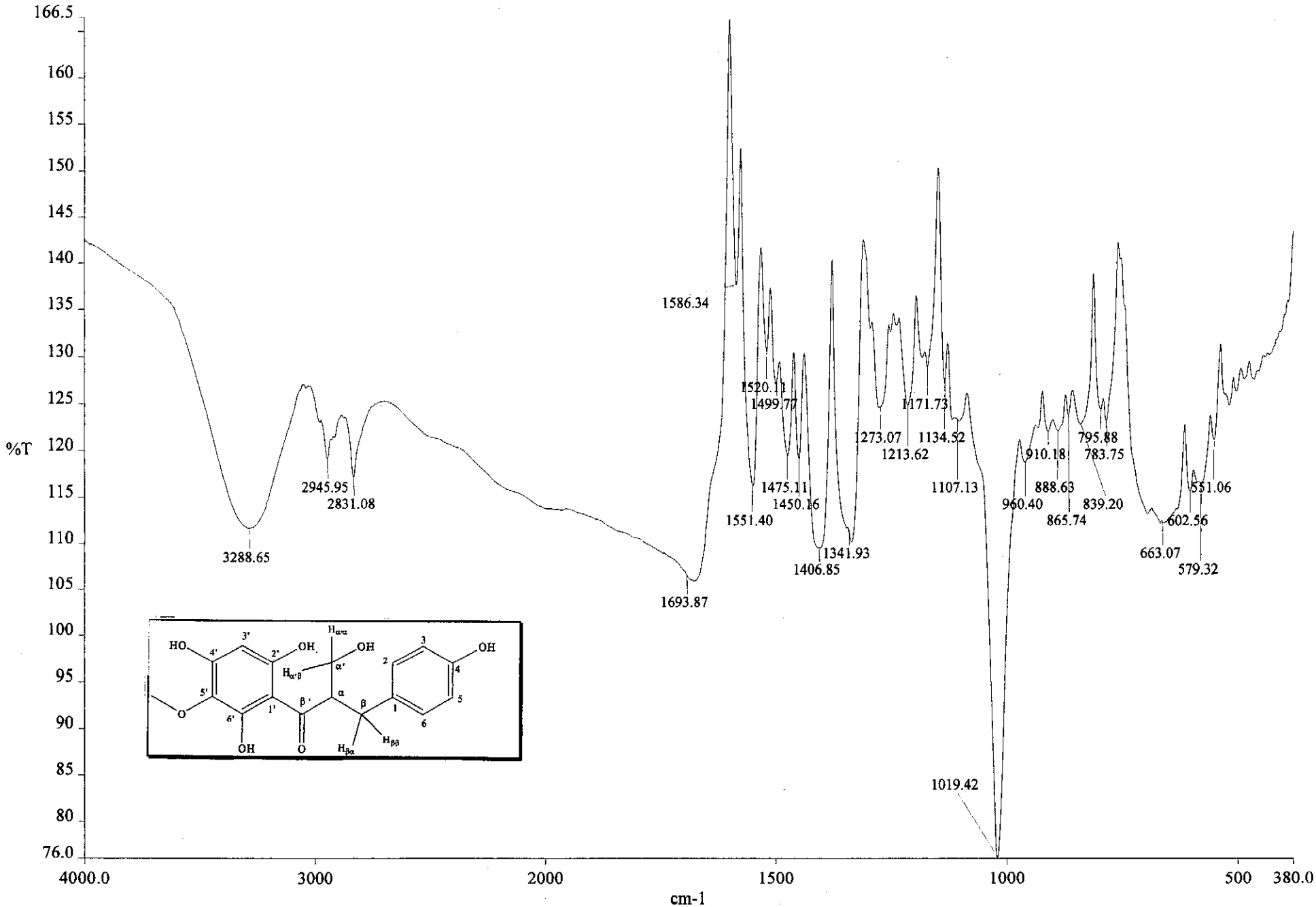
HMBC spectrum of 2',4',6',4-tetrahydroxy-5'-methoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (3)



Expanded HMBC spectrum of 2',4',6',4-tetrahydroxy-5'-methoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (3)

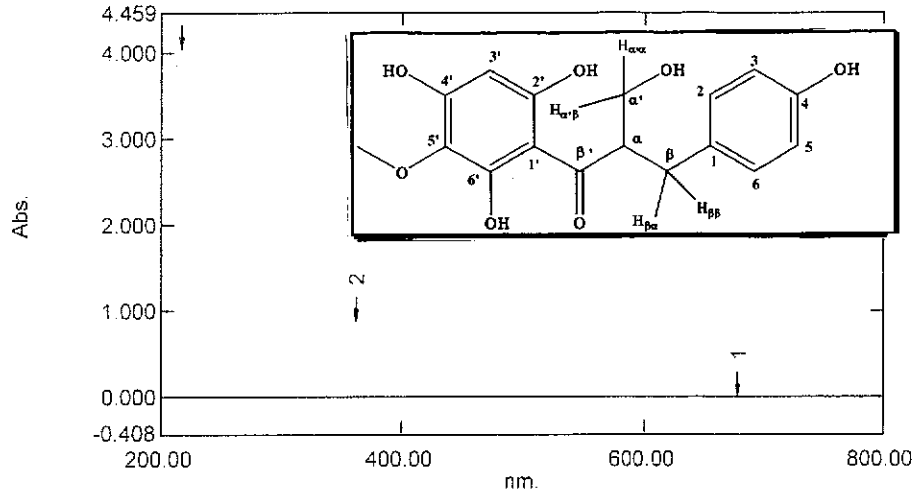


NOESY spectrum of 2',4',6',4-tetrahydroxy-5'-methoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (3)



IR spectrum of 2',4',6',4-tetrahydroxy-5'-methoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (3)

UNIVERSITY OF KWAZULU-NATAL
SCHOOL OF CHEMISTRY
ANALYSIS REPORT



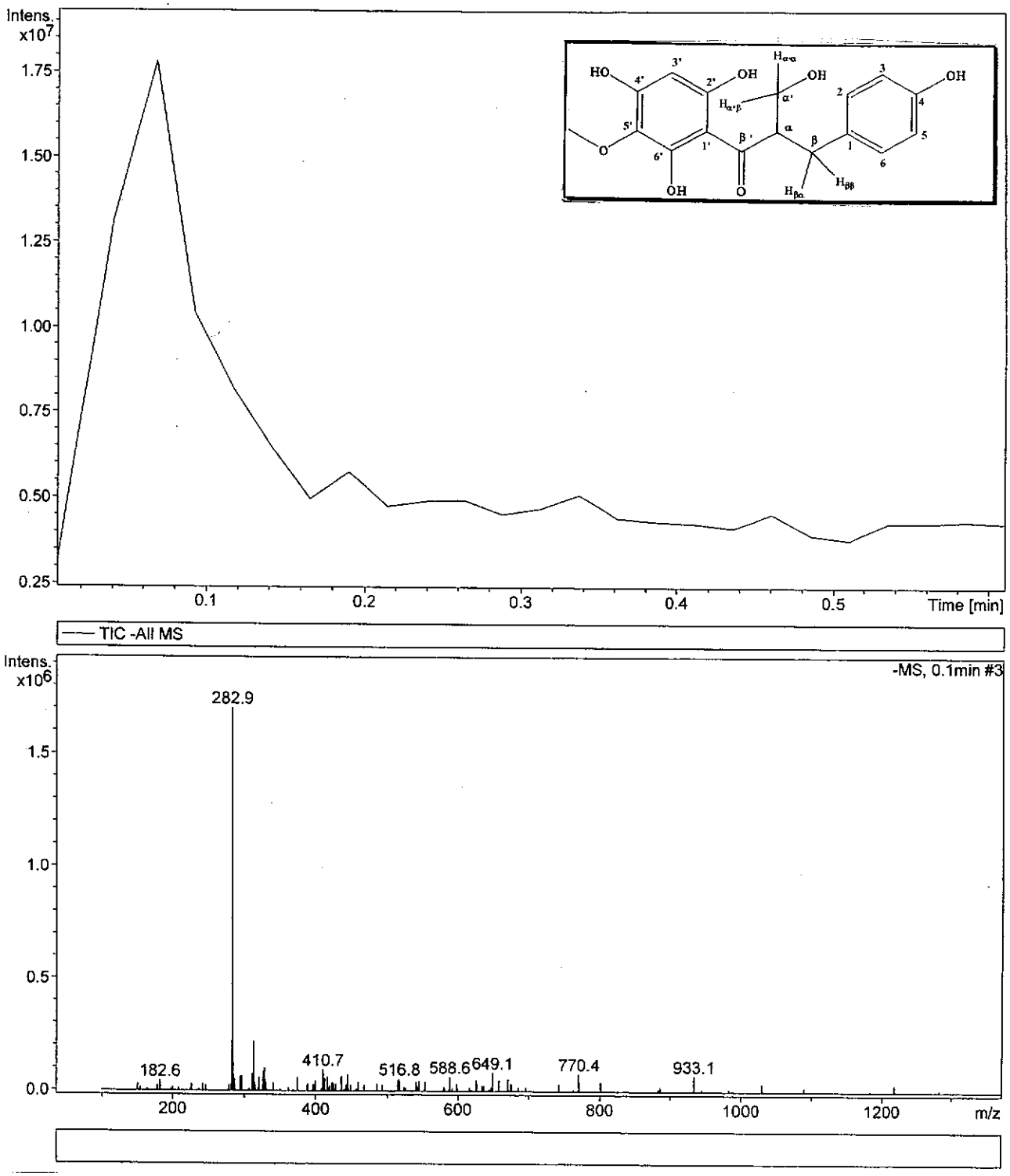
No.	P/V	Wavelength	Abs.	Description
1	⊕	678.00	0.000	
2	⊕	362.00	0.888	
3	⊕	216.00	4.054	
4	◯	638.00	-0.001	
5	◯	275.00	0.175	

File_121018_C32.spc - Storage 0256

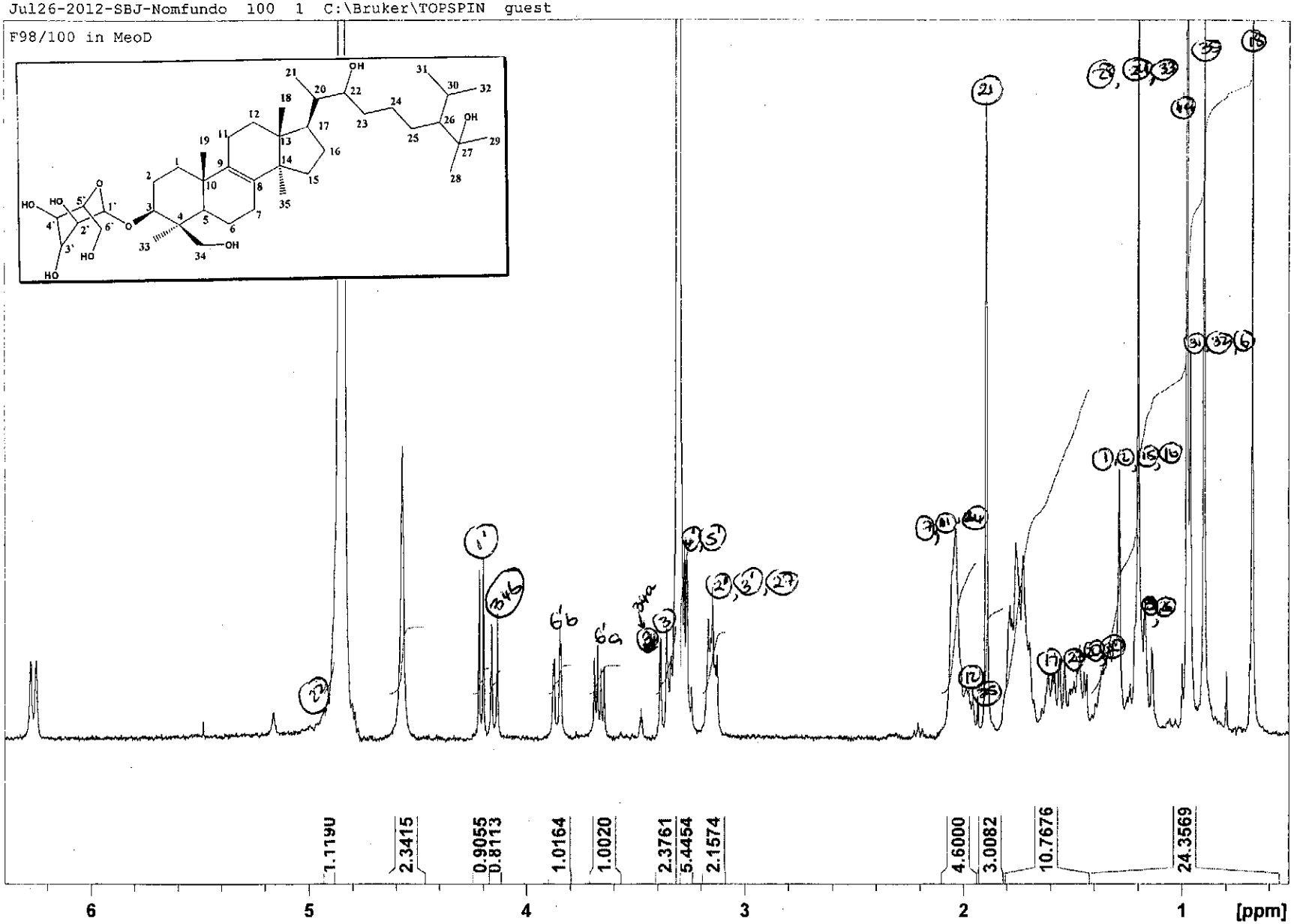
UV spectrum of 2',4',6',4-tetrahydroxy-5'-methoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (3)

Display Report - All Windows Selected Analysis

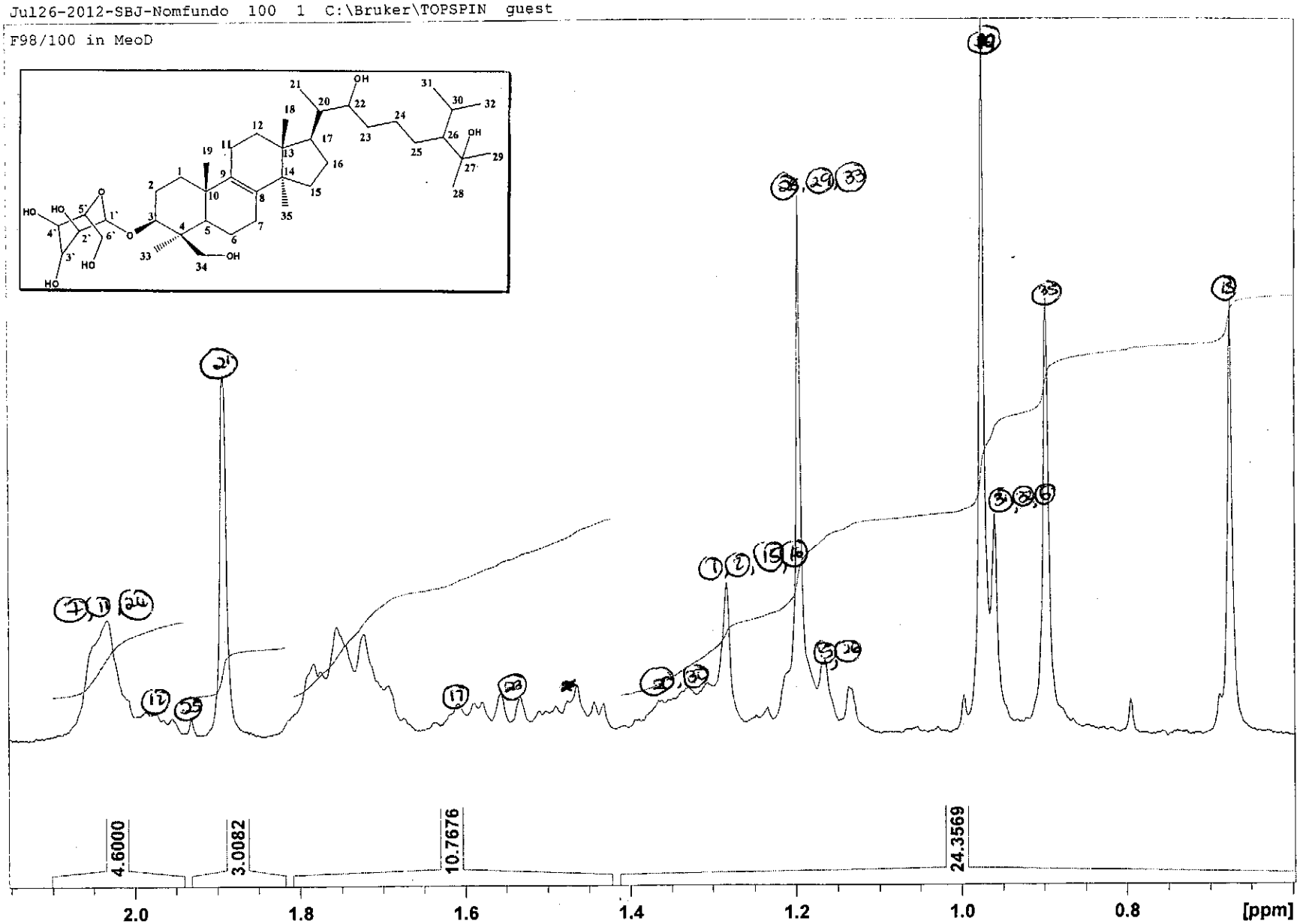
Analysis Name: F32000001.D **Instrument:** LC-MSD-Trap-VL **Print Date:** 11/12/2012 10:35:55 AM
Method: AN 2MIN.M **Operator:** Operator **Acq. Date:** 11/12/2012 10:35:03 AM
Sample Name: F32
Analysis Info: repeat 5ul



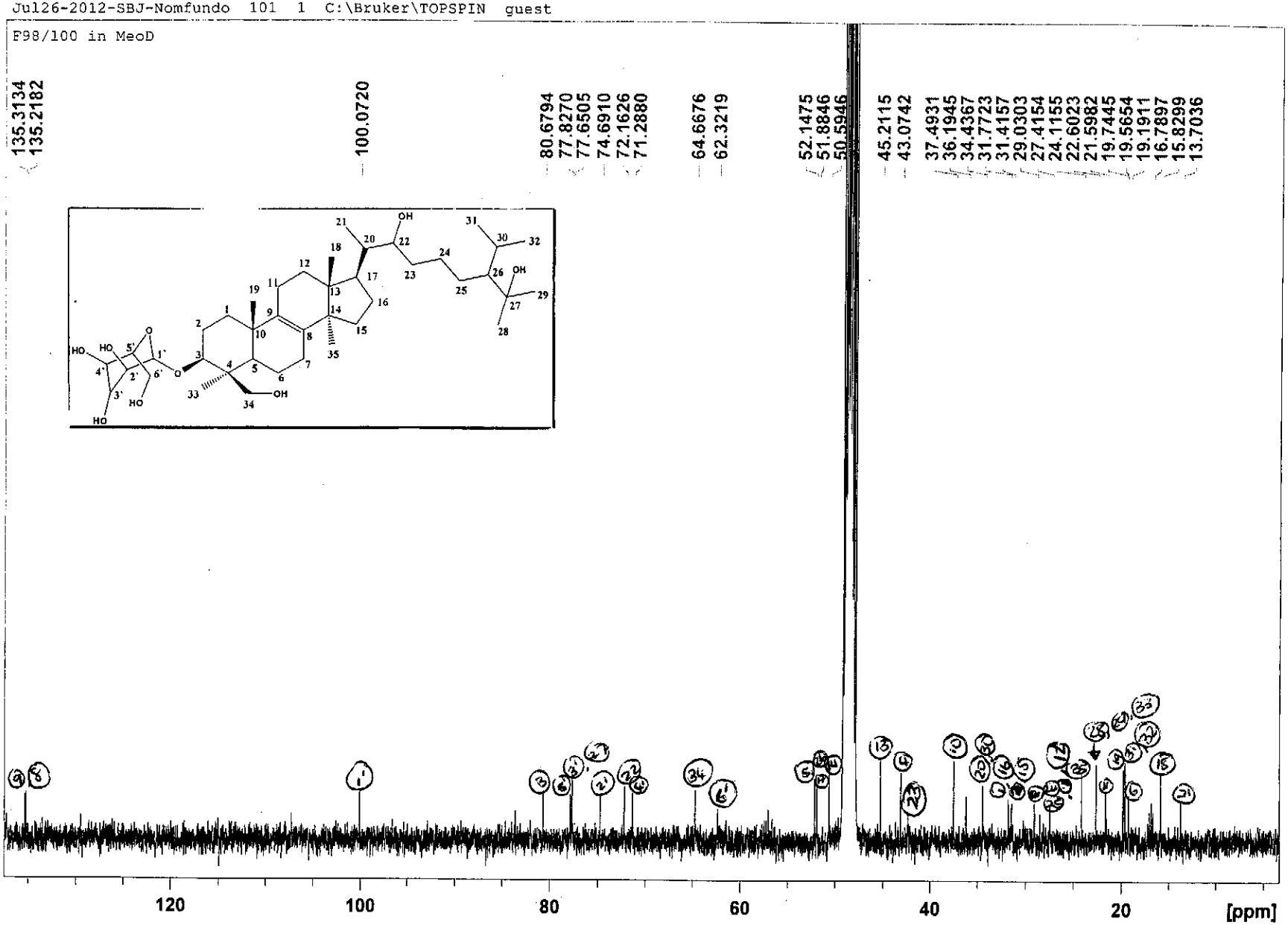
LC-MS spectrum 2',4',6',4-tetrahydroxy-5'-methoxy-α-hydroxymethyl-β-methyl-dihydrochalcone (3)



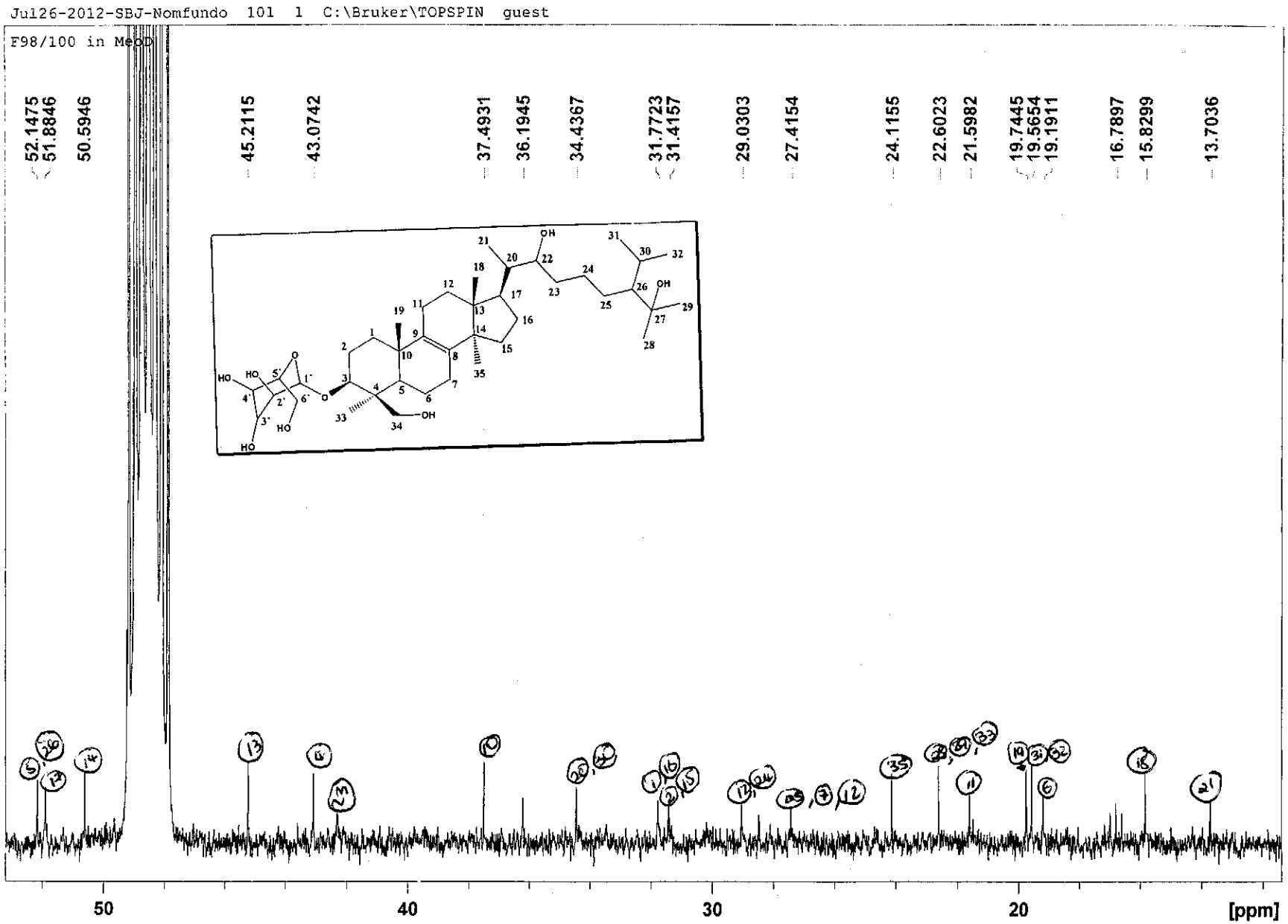
^{1}H NMR spectrum of 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene (4)



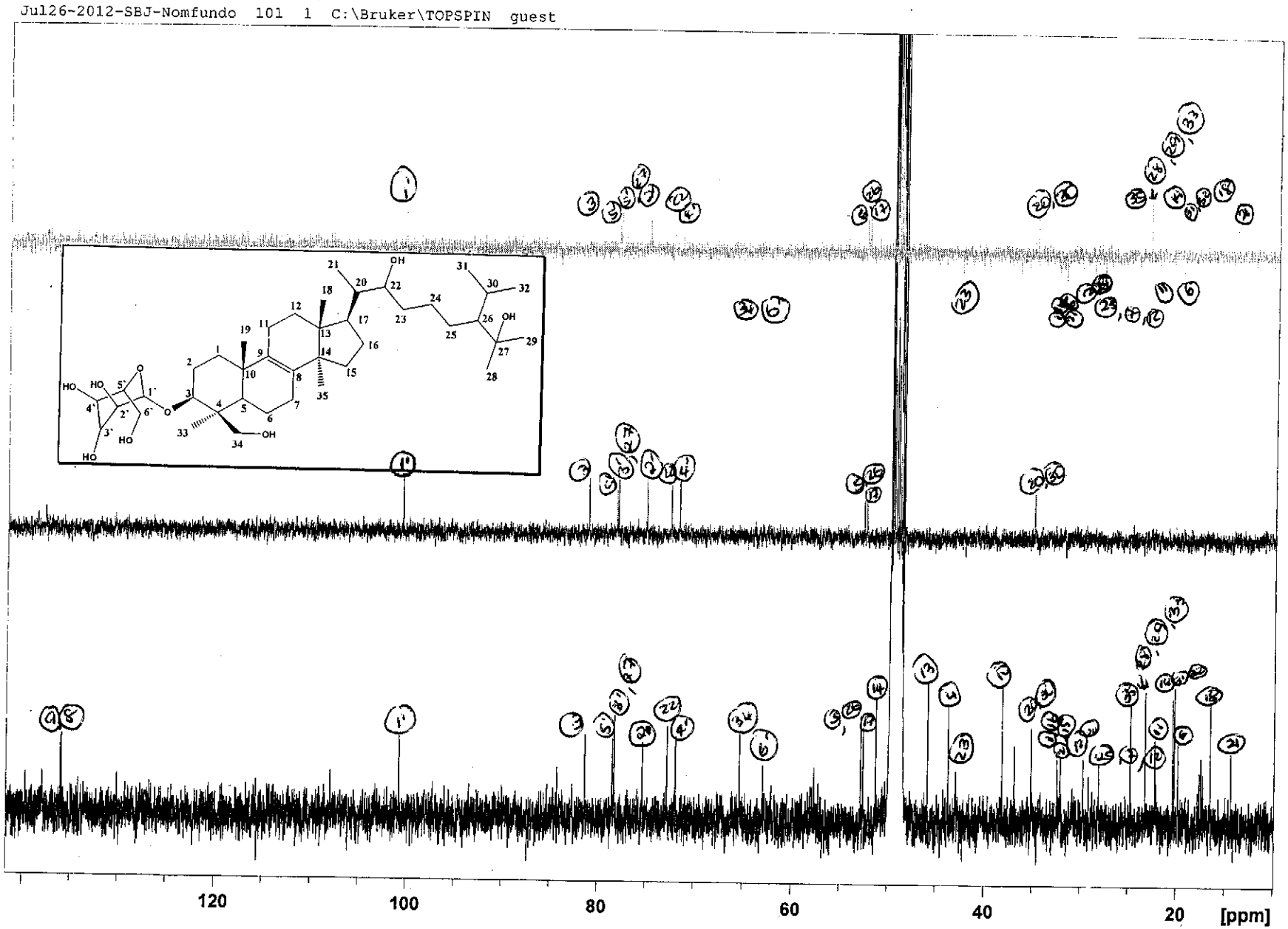
Expanded ^{1}H NMR spectrum of 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene (4)



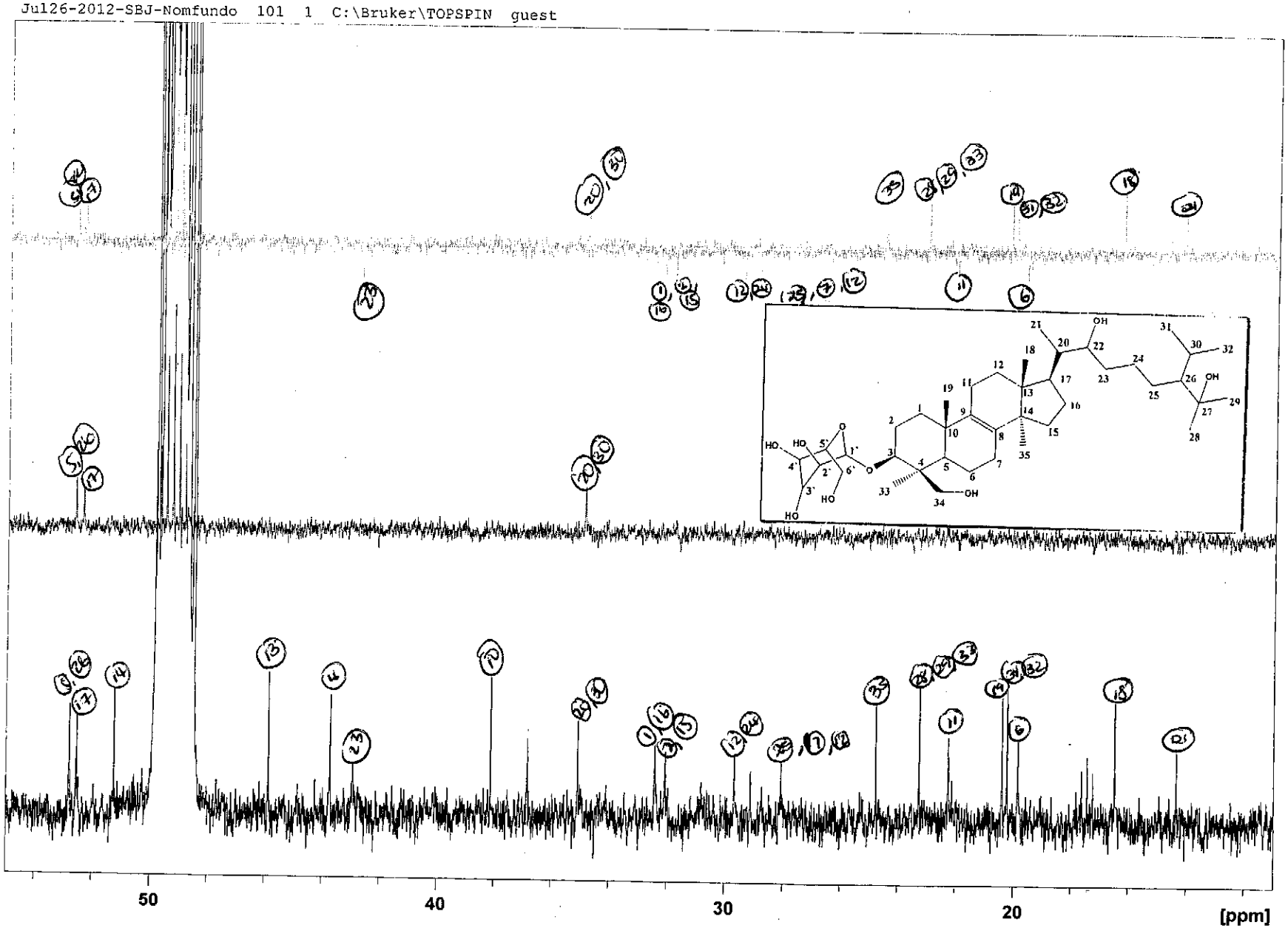
^{13}C NMR spectrum of 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene (4)



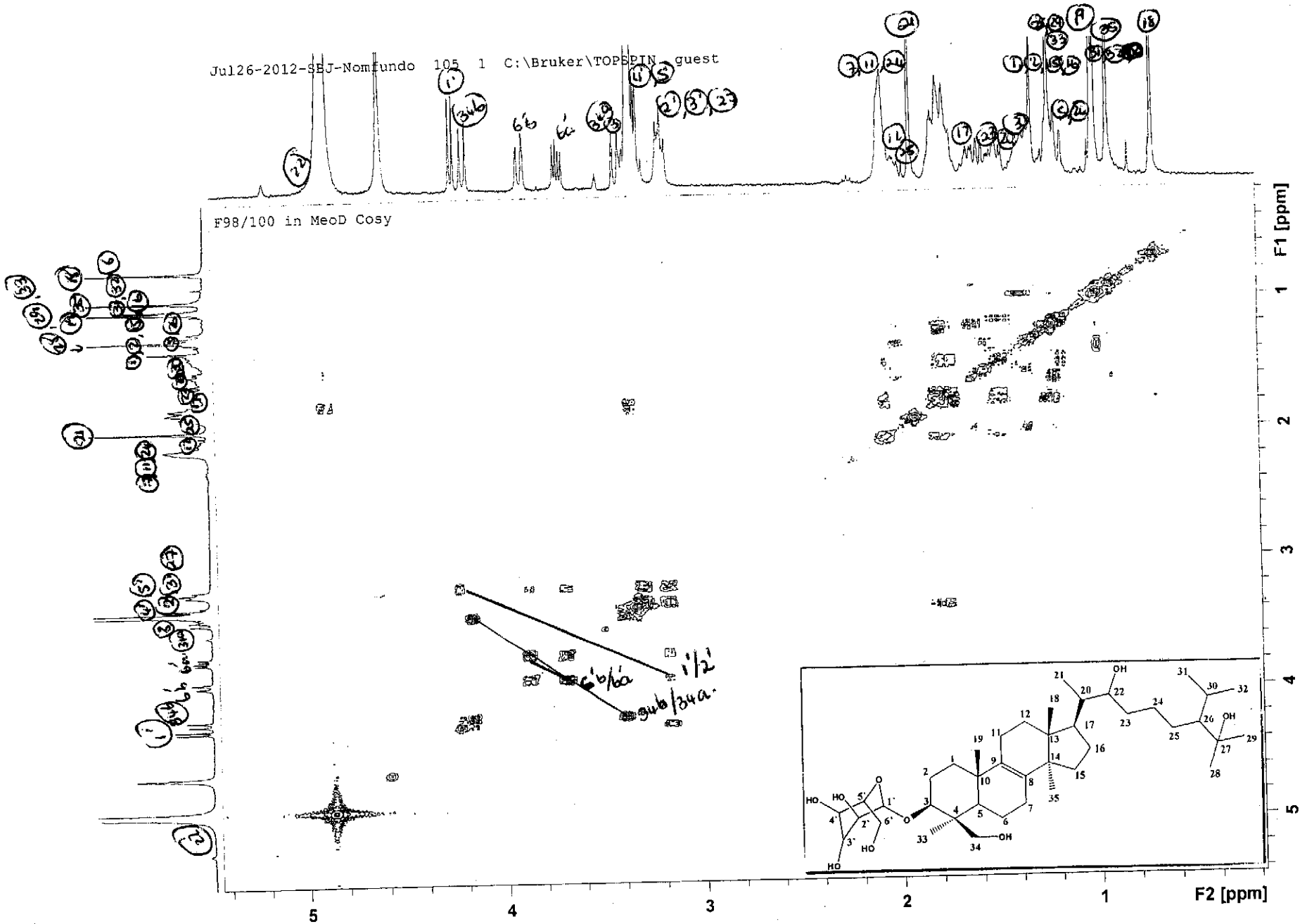
Expanded ^{13}C NMR spectrum of 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene (4)



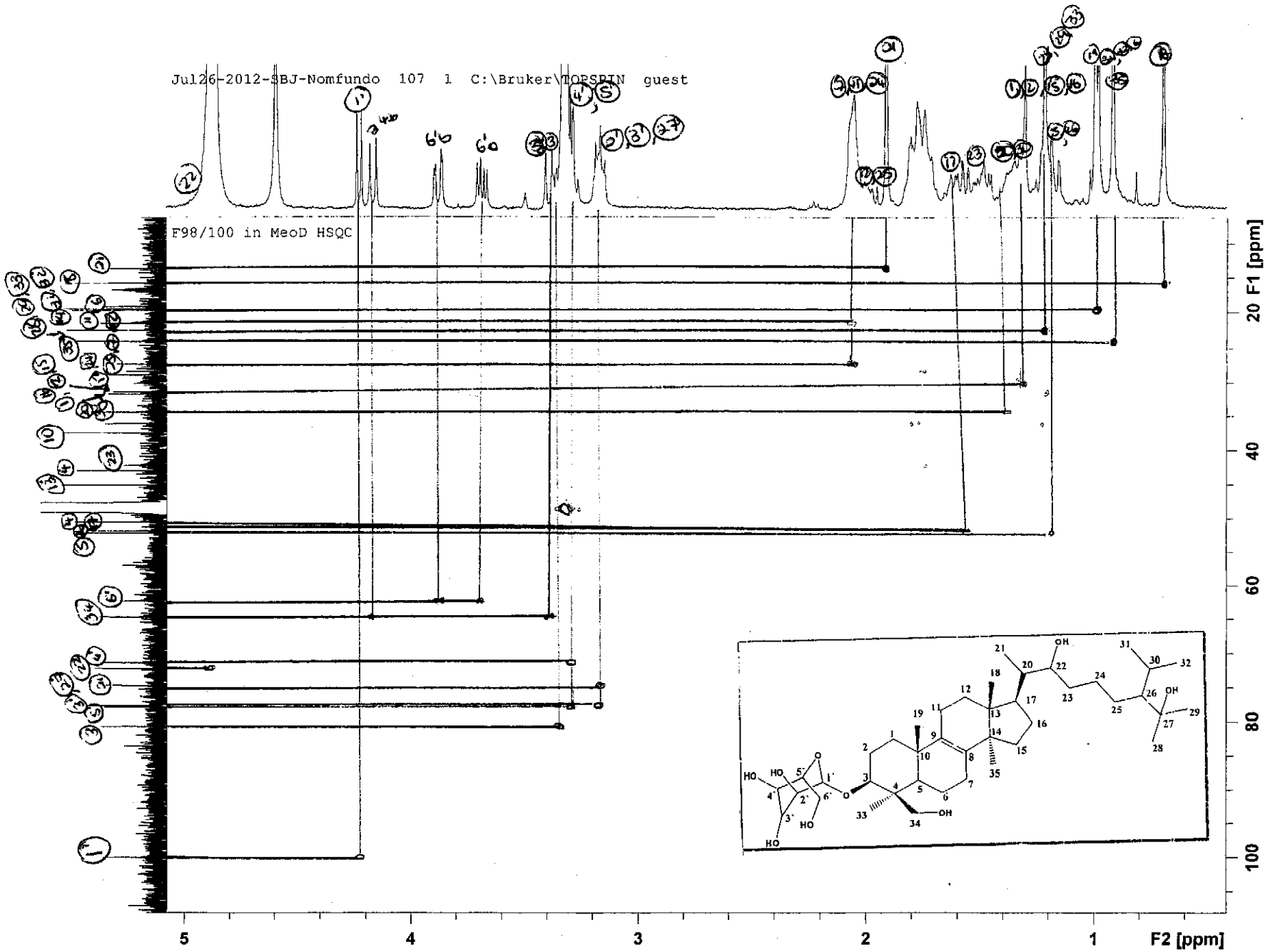
DEPT spectrum of 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene ((4)



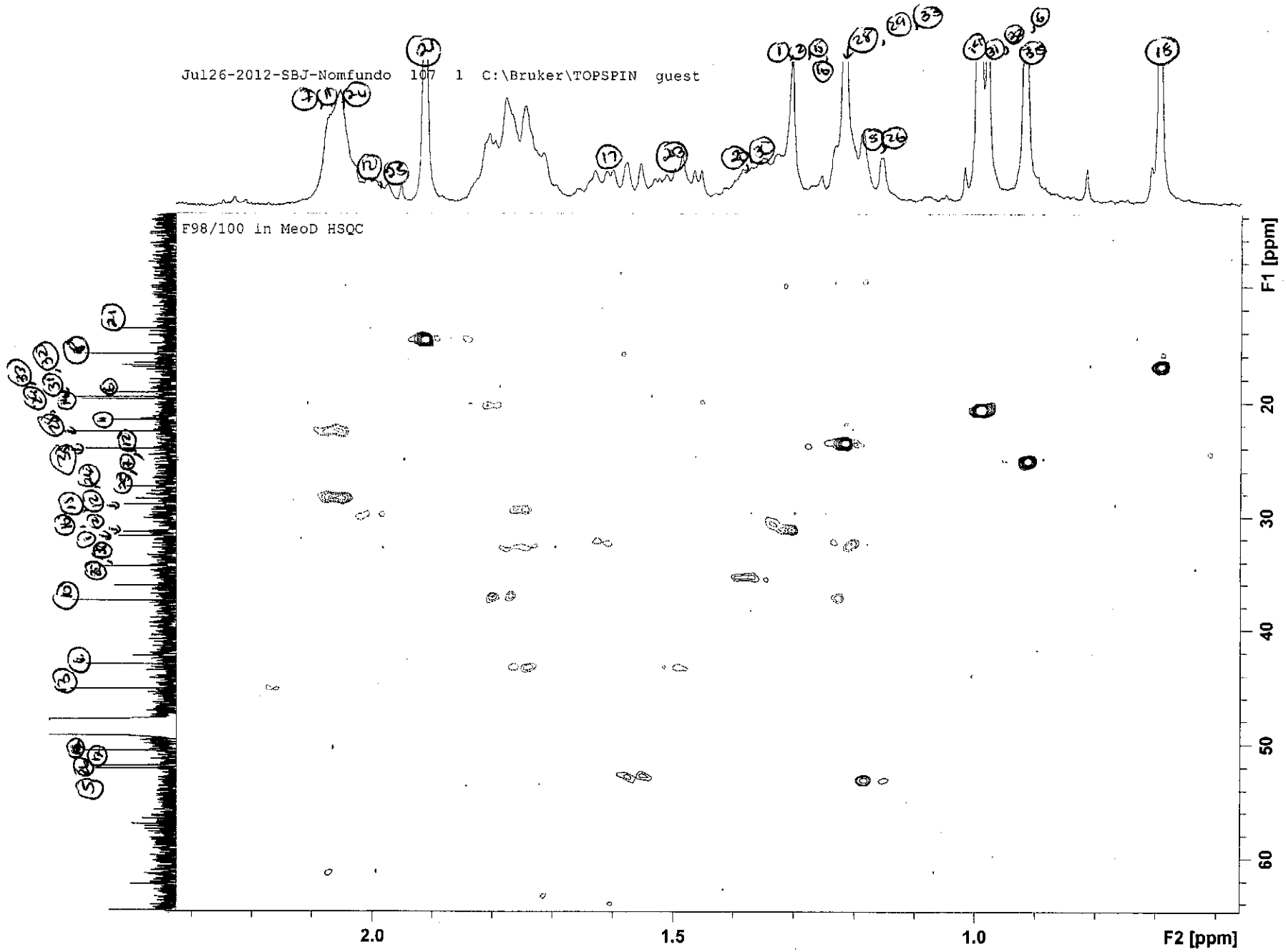
Expanded DEPT spectrum of 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene (4)



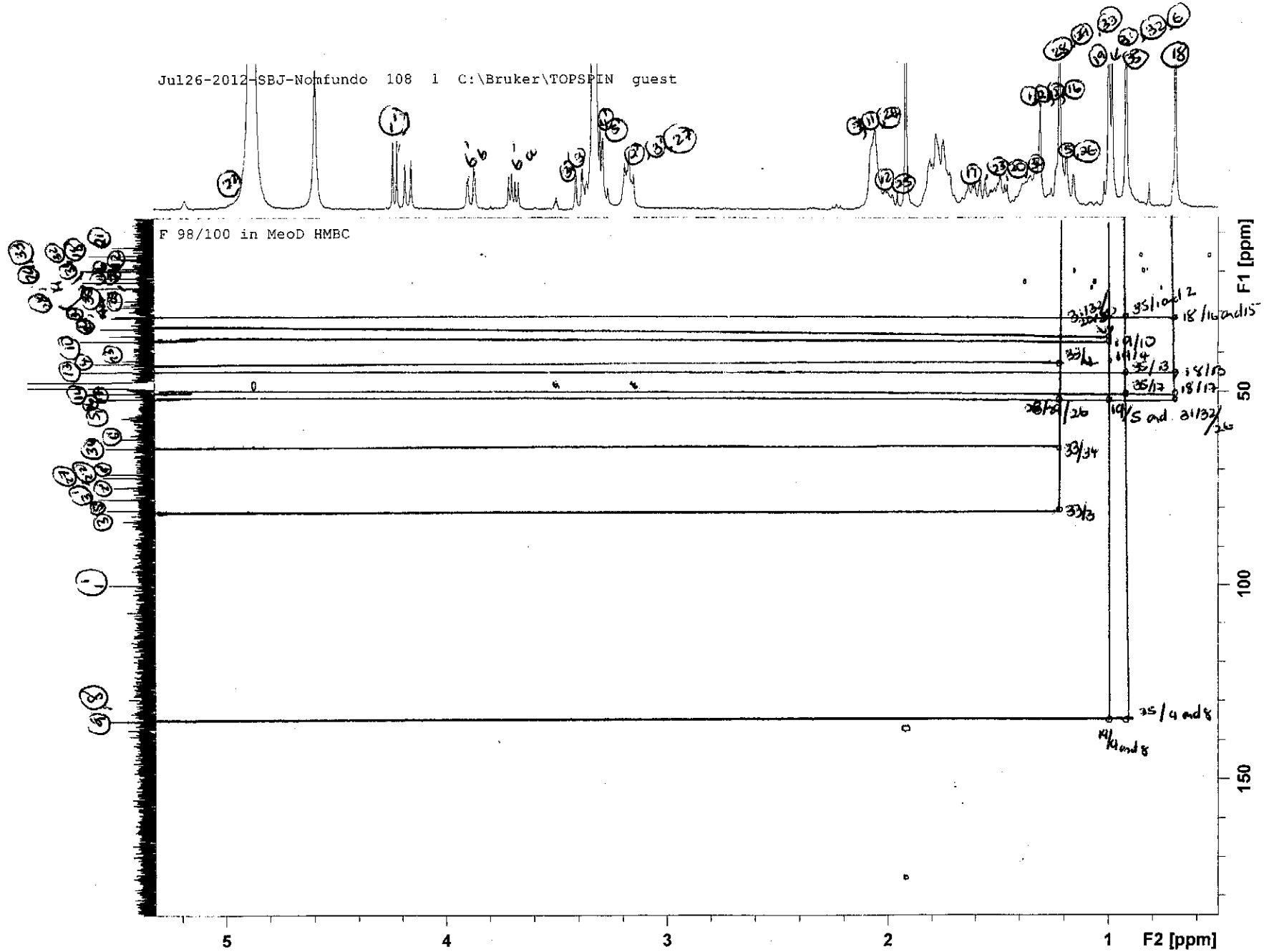
COSY spectrum of 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene (4)



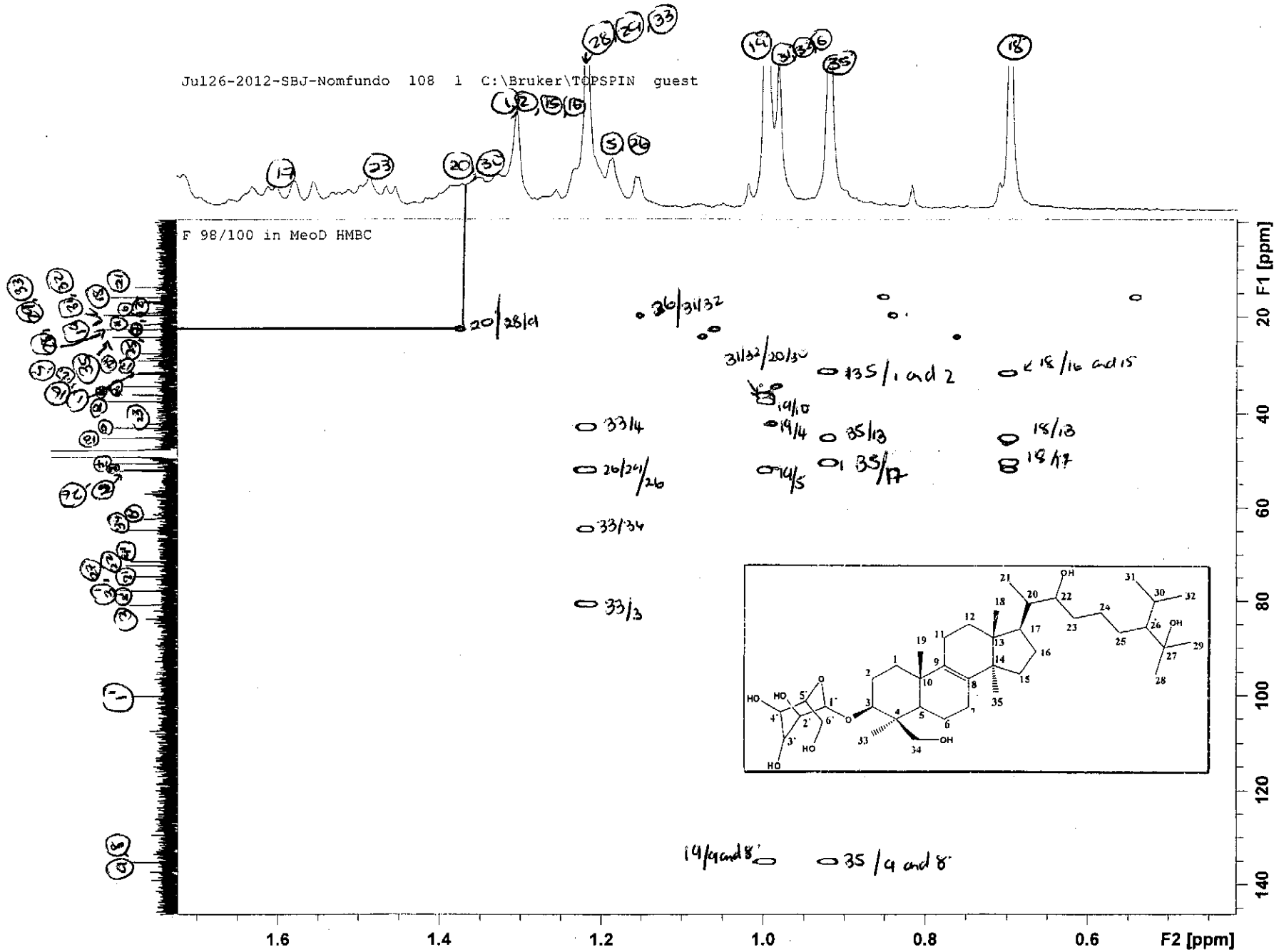
HSQC spectrum of 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene (4)



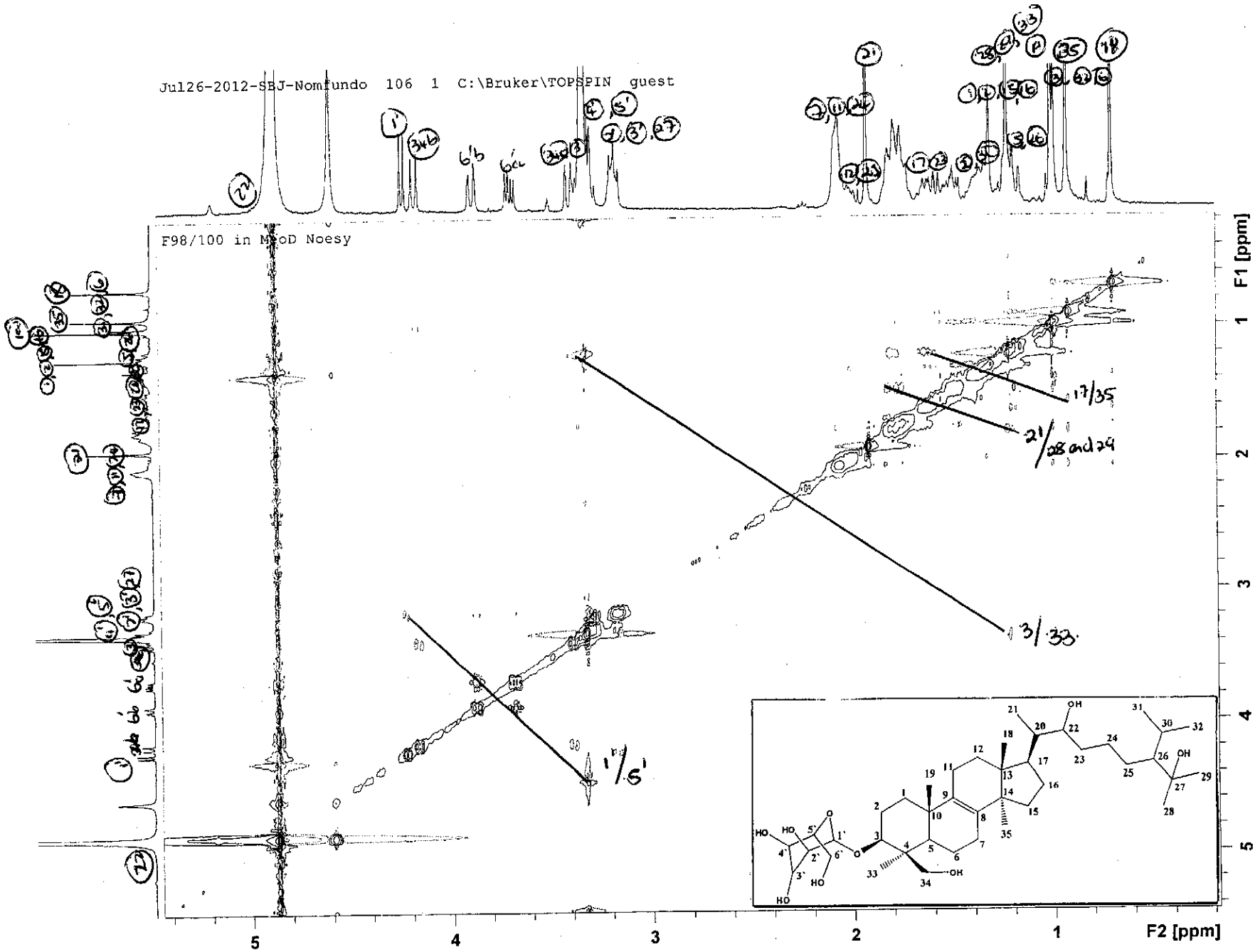
Expanded HSQC spectrum of 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene (4)



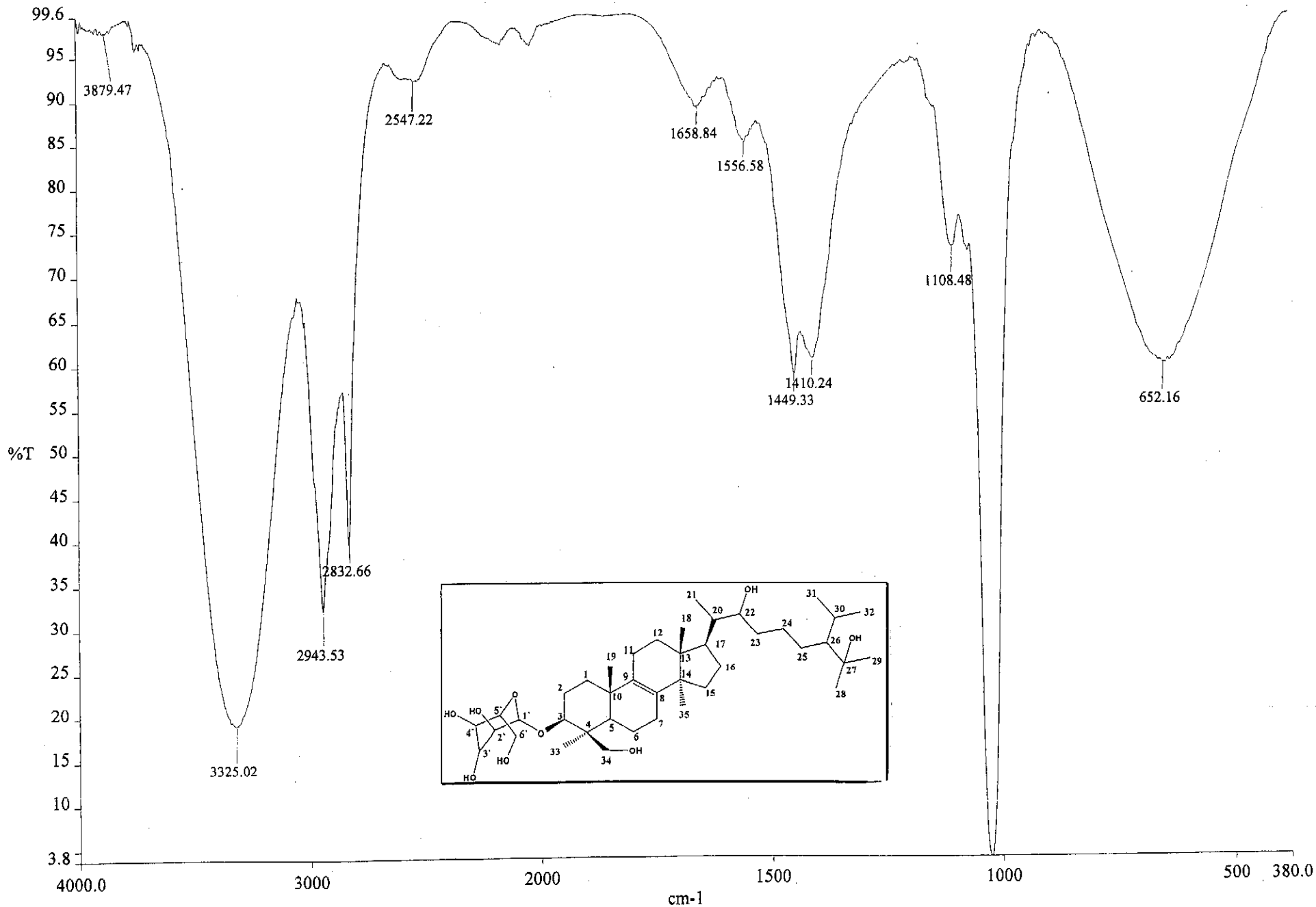
HMBC spectrum of 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene (4)



Expanded HMBC spectrum of 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene (4)

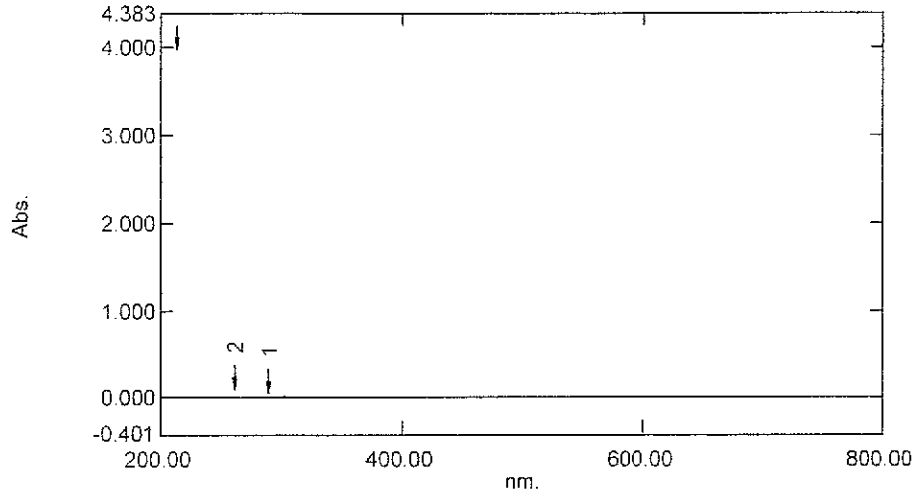


NOESY spectrum of 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene (4)



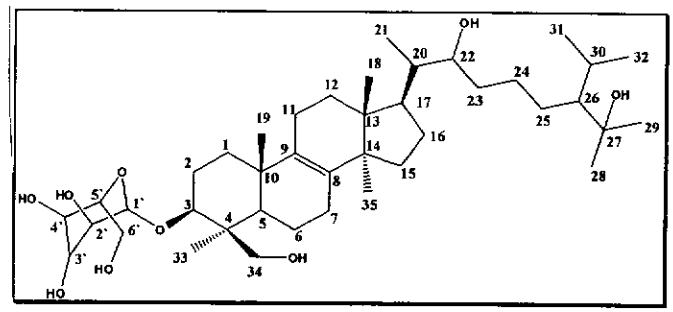
IR spectrum of 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene (4)

UNIVERSITY OF KWAZULU-NATAL
SCHOOL OF CHEMISTRY
ANALYSIS REPORT



No.	P/V	Wavelength	Abs.	Description
1	ⓣ	290.00	0.057	
2	ⓣ	262.00	0.093	
3	ⓣ	213.00	3.985	
4	◯	281.00	0.036	
5	◯	250.00	0.061	

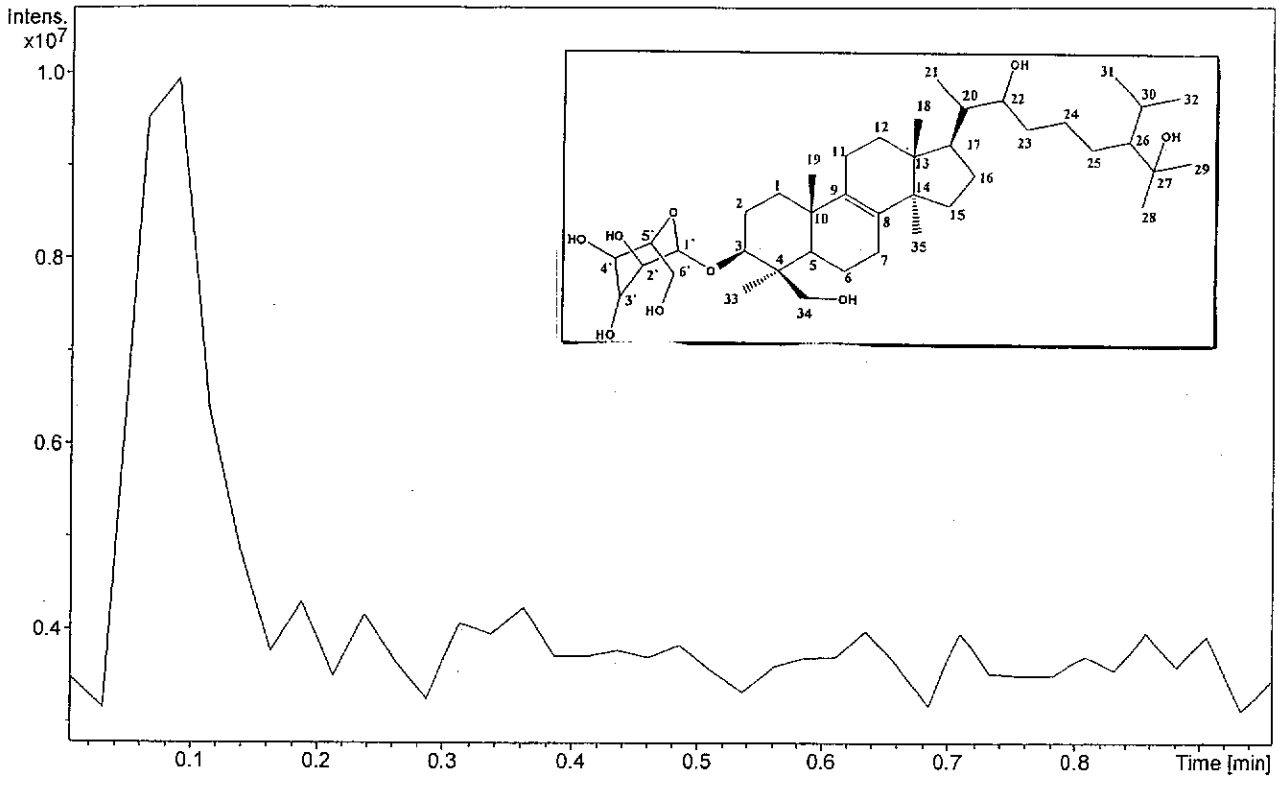
File_121018_C98100.spc - Storage 0

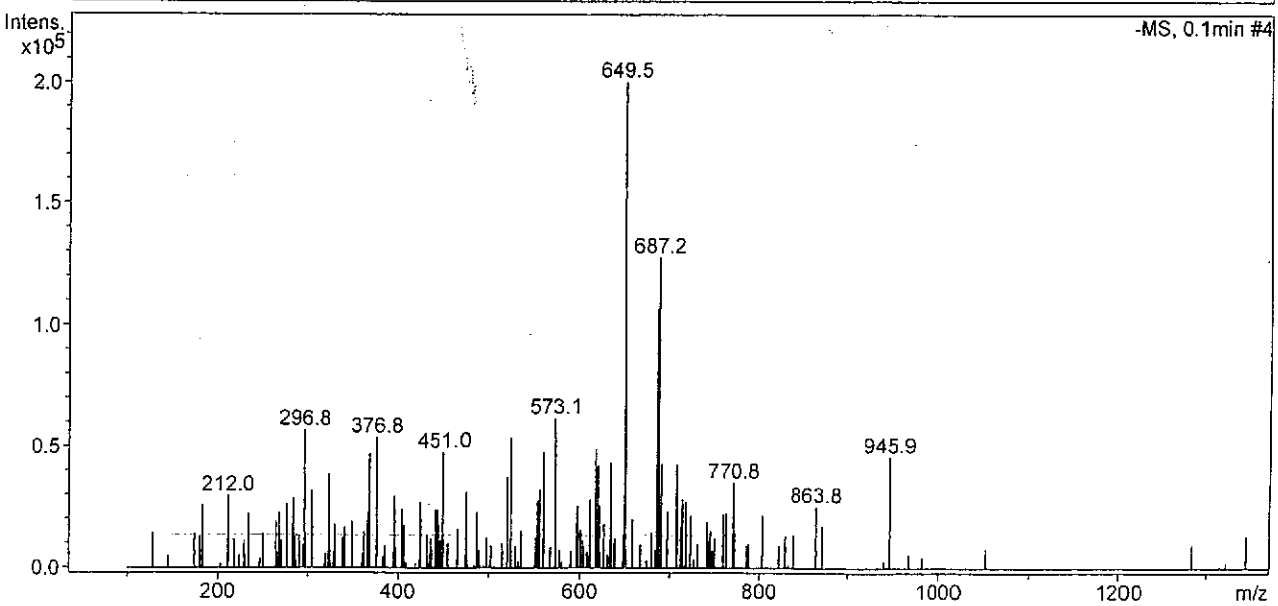


UV spectrum of 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene (4)

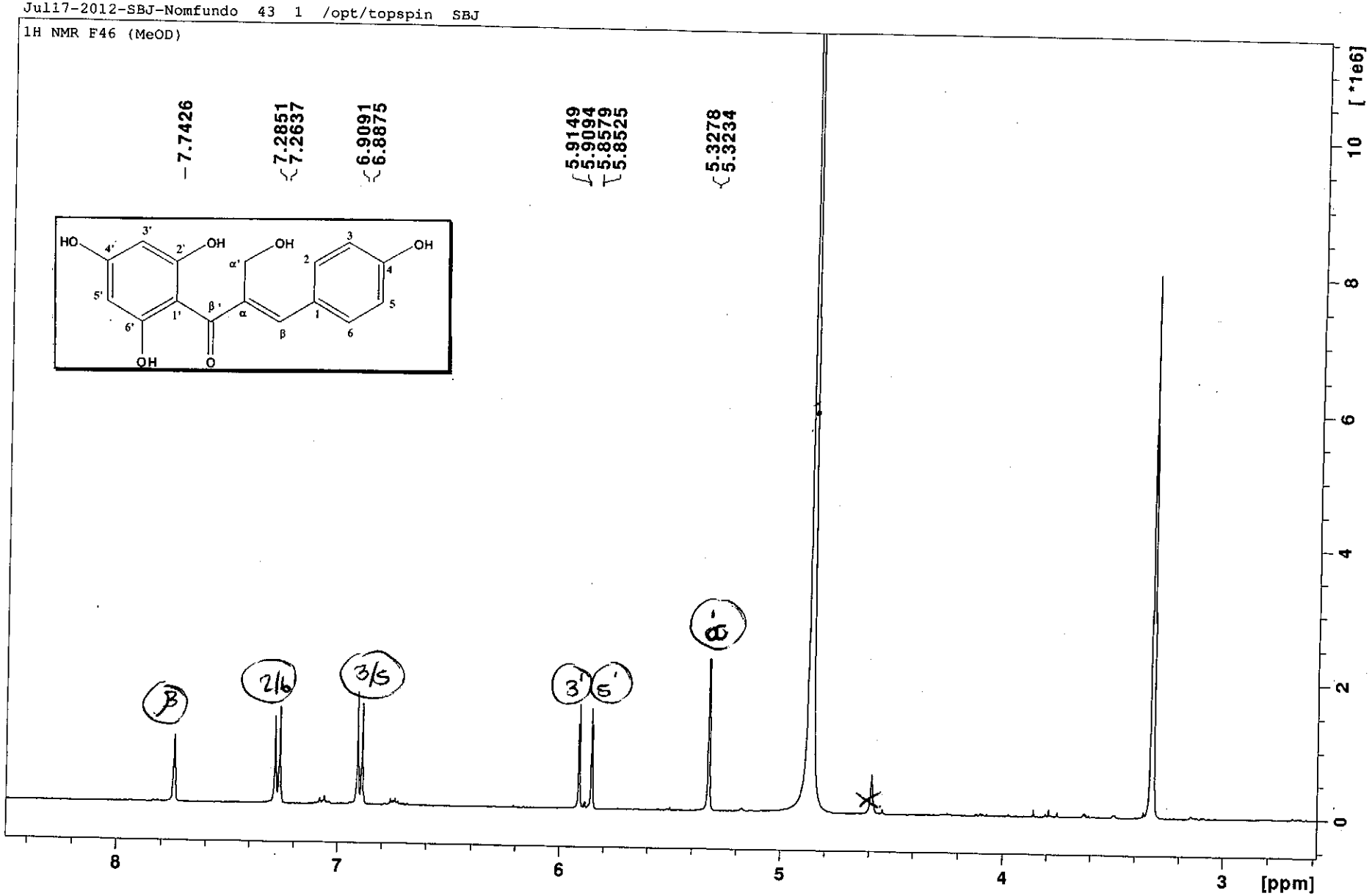
Display Report - All Windows Selected Analysis

Analysis Name: F98000001.D **Instrument:** LC-MSD-Trap-VL **Print Date:** 11/12/2012 10:42:46 AM
Method: AN 2MIN.M **Operator:** Operator **Acq. Date:** 11/12/2012 10:37:48 AM
Sample Name: F98
Analysis Info: repeat 5ul

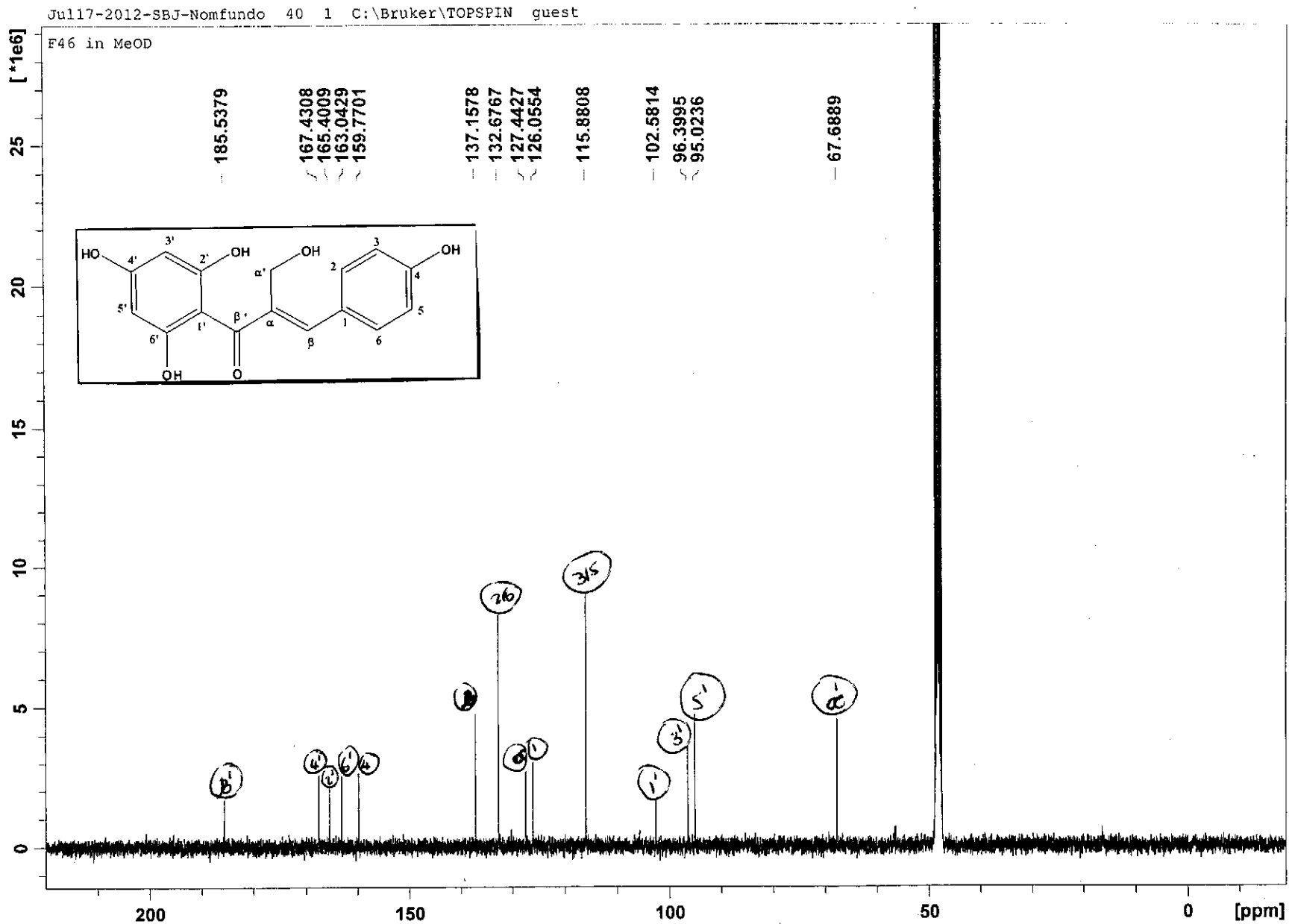




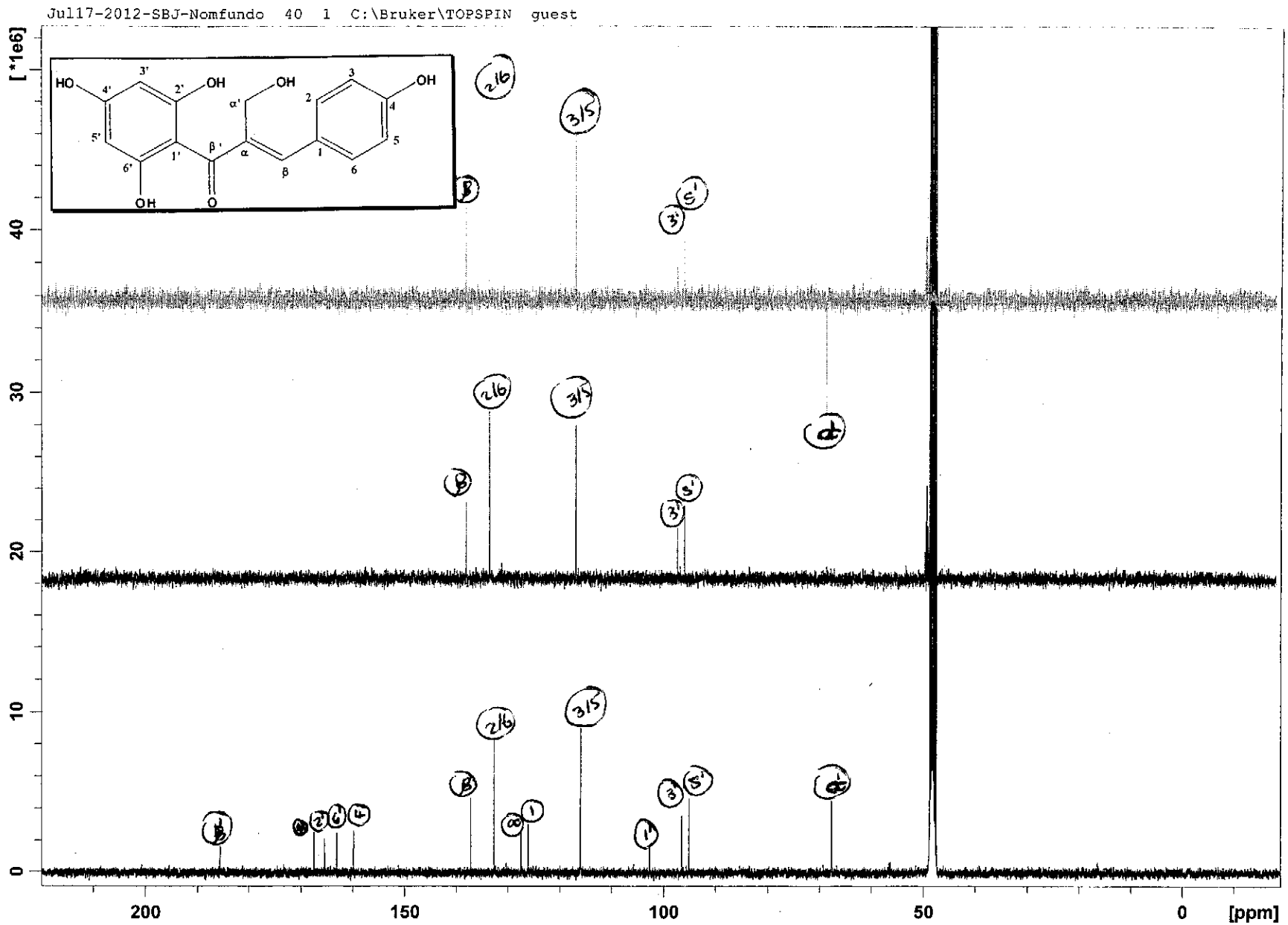
LC-MS spectrum of 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene (4)



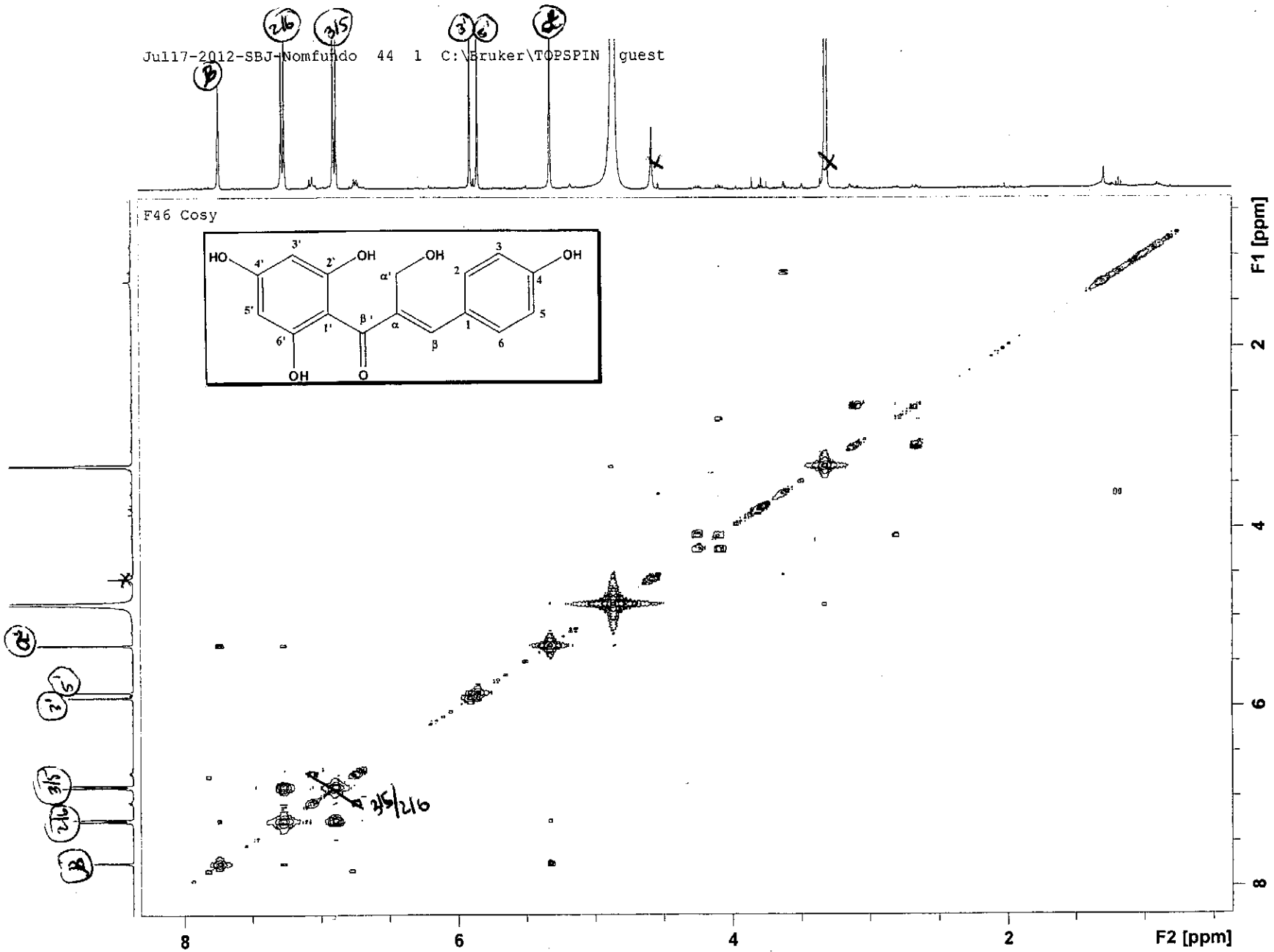
^{1}H NMR spectrum of 2',4',6',4-tetrahydroxy-α-hydroxymethyl-chalcone (5)



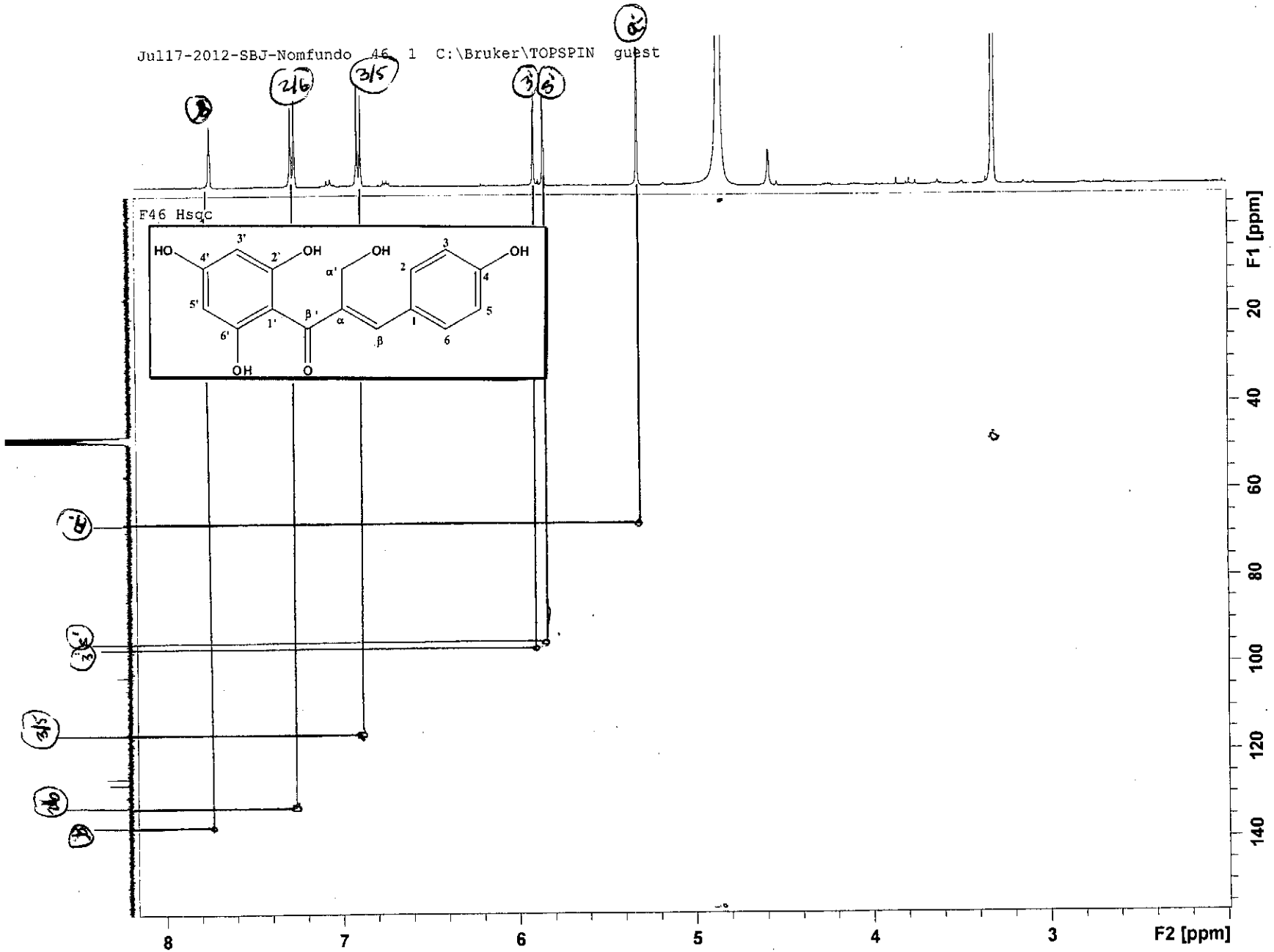
^{13}C NMR spectrum of 2',4',6',4-tetrahydroxy-α-hydroxymethyl-chalcone (5)



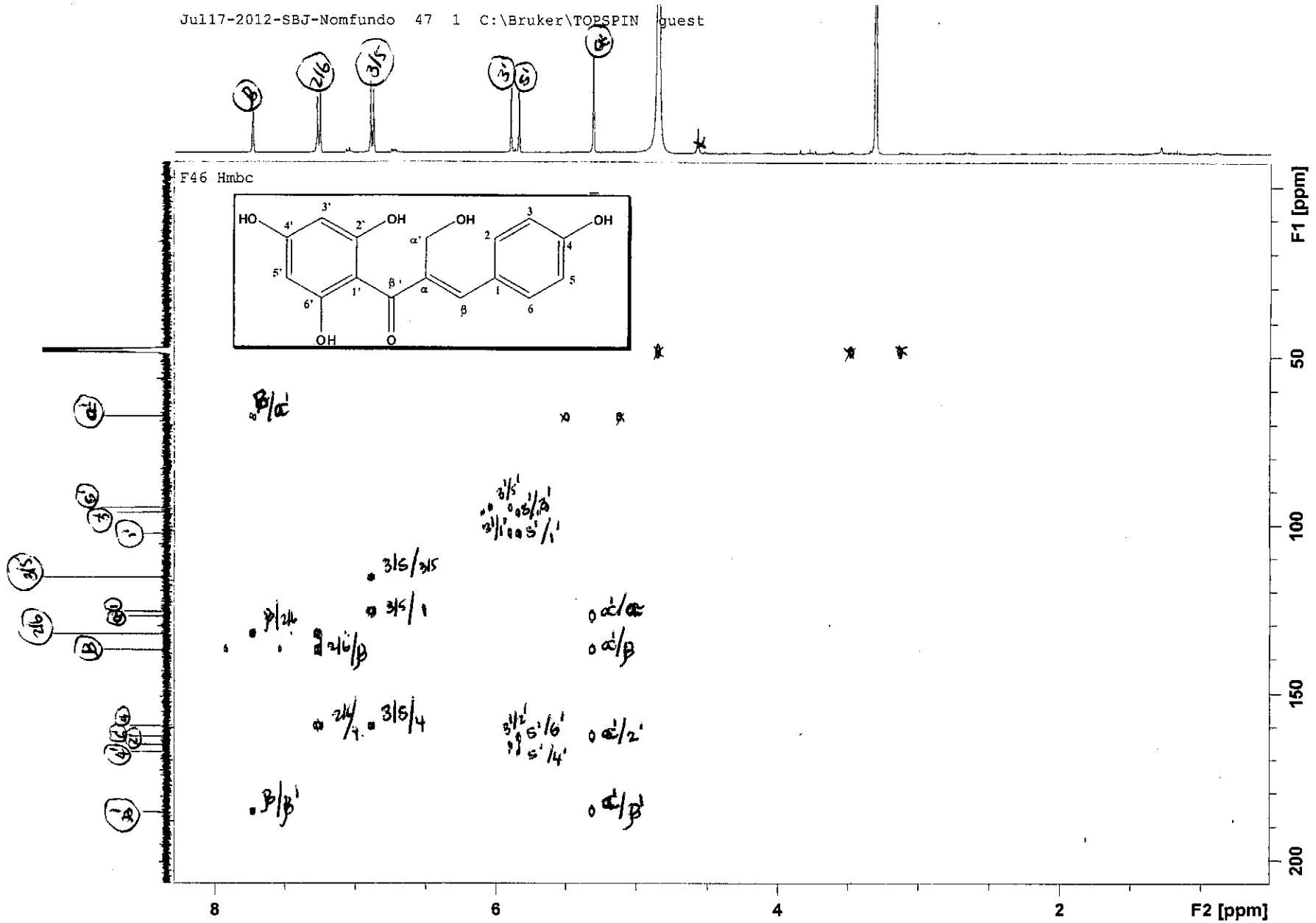
DEPT spectrum of 2',4',6',4-tetrahydroxy-α-hydroxymethyl-chalcone (5)



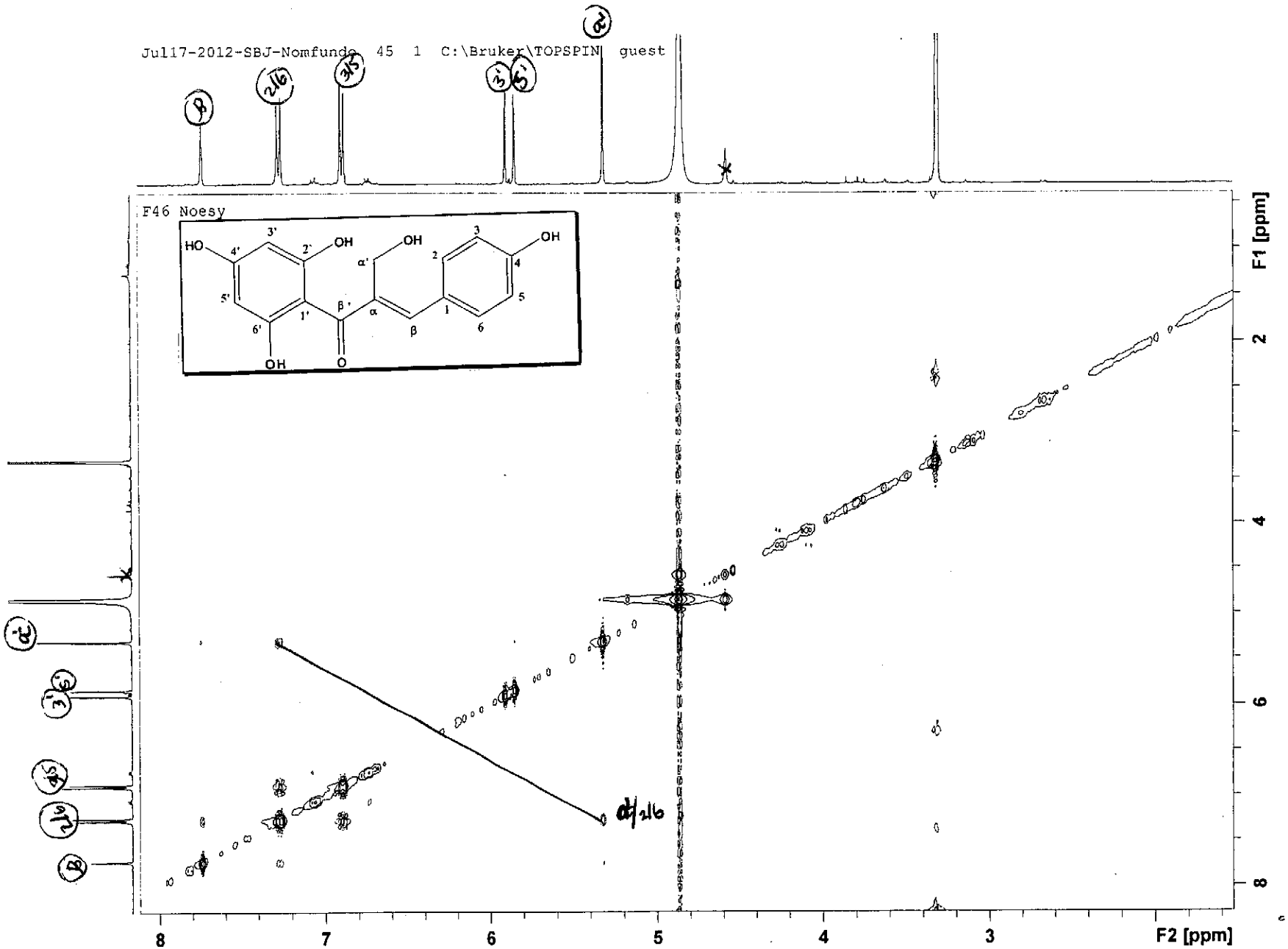
COSY spectrum of 2',4',6',4-tetrahydroxy-α-hydroxymethyl-chalcone (5)



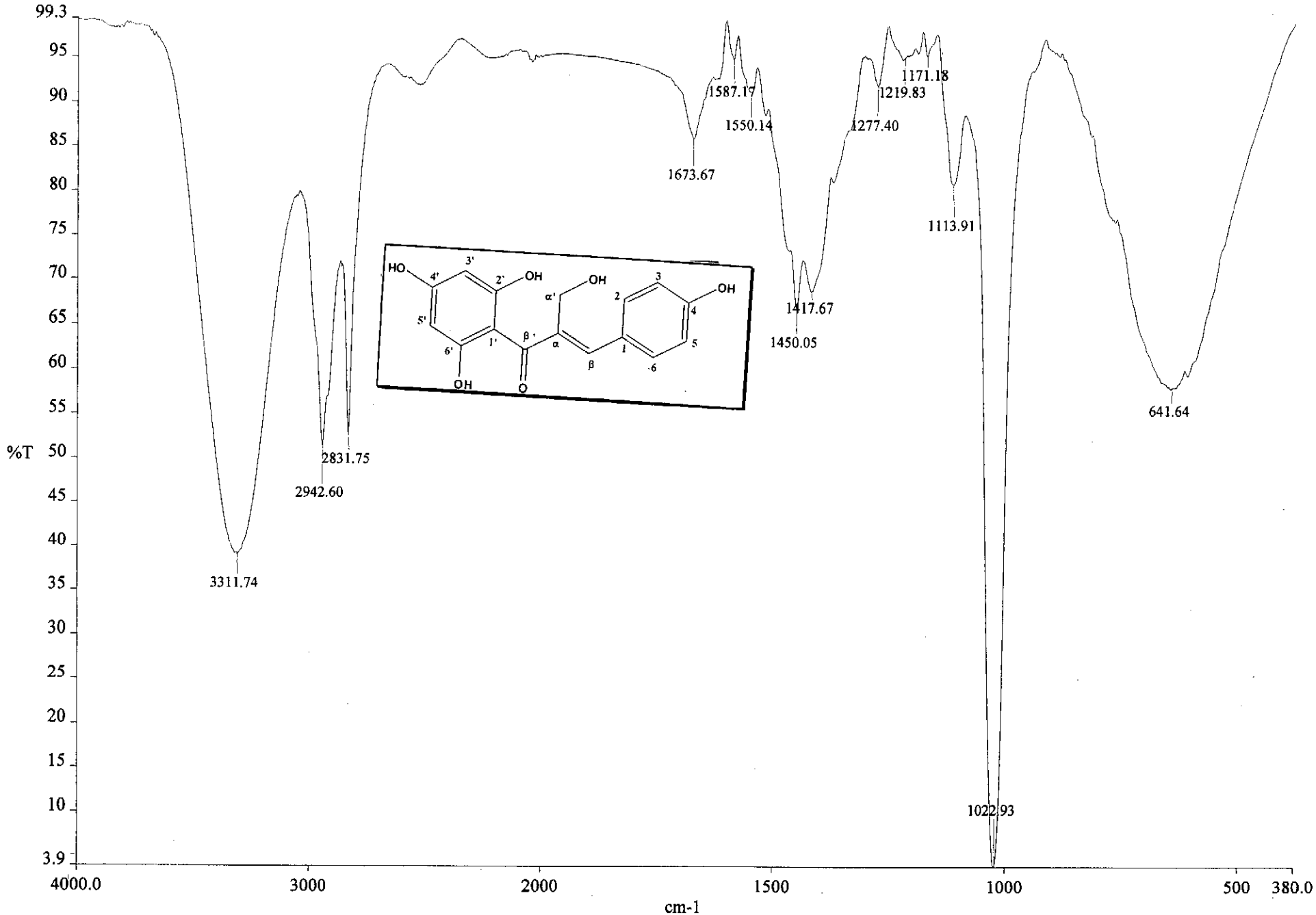
HSQC spectrum of 2',4',6',4-tetrahydroxy-α-hydroxymethyl-chalcone (5)



HMBC spectrum 2',4',6',4-tetrahydroxy-α-hydroxymethyl-chalcone (5)

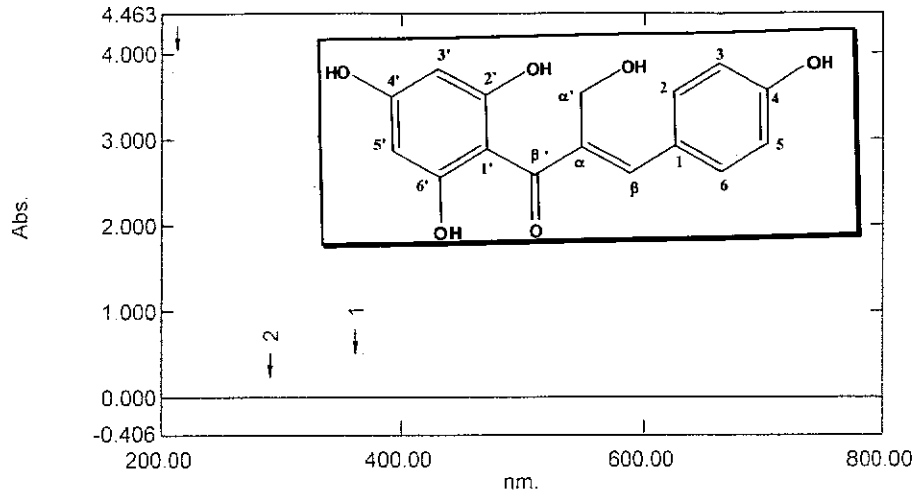


NOESY spectrum 2',4',6',4-tetrahydroxy-α-hydroxymethyl-chalcone (5)



IR spectrum of 2',4',6',4-tetrahydroxy-α-hydroxymethyl-chalcone (5)

UNIVERSITY OF KWAZULU-NATAL
SCHOOL OF CHEMISTRY
ANALYSIS REPORT



No.	P/V	Wavelength	Abs.	Description
1	⊕	362.00	0.526	
2	⊕	292.00	0.253	
3	⊕	213.00	4.058	
4	◯	306.00	0.238	
5	◯	270.00	0.176	

File_121018_C46.spc - Storage 0303

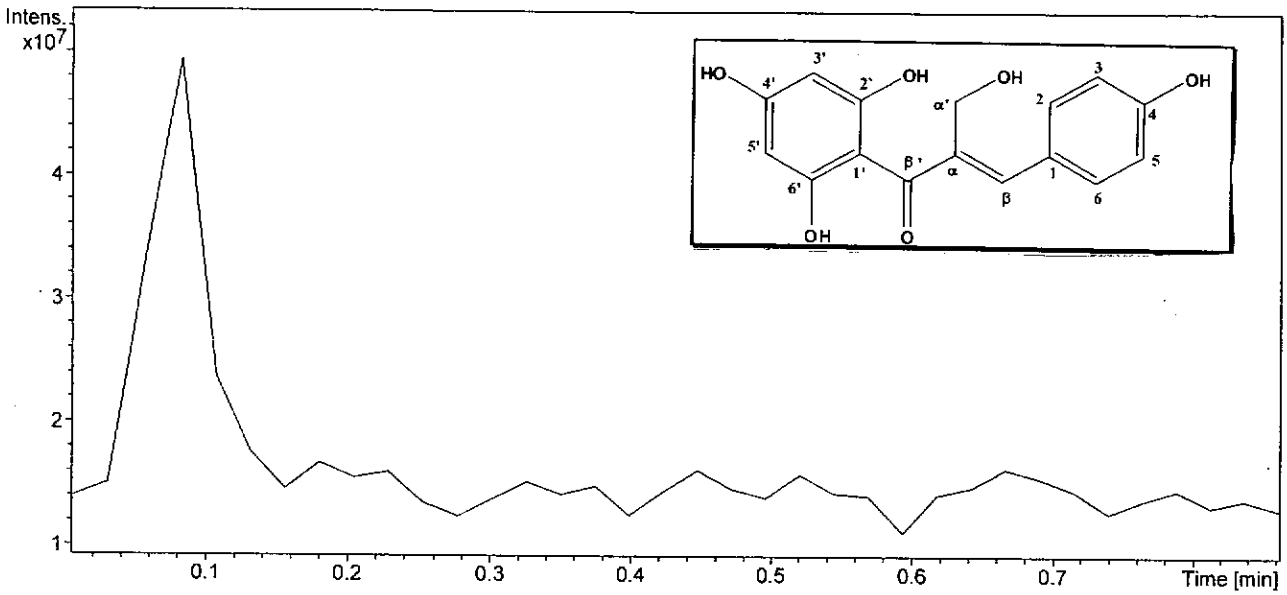
UV spectrum of 2',4',6',4-tetrahydroxy-α-hydroxymethyl-chalcone (5)

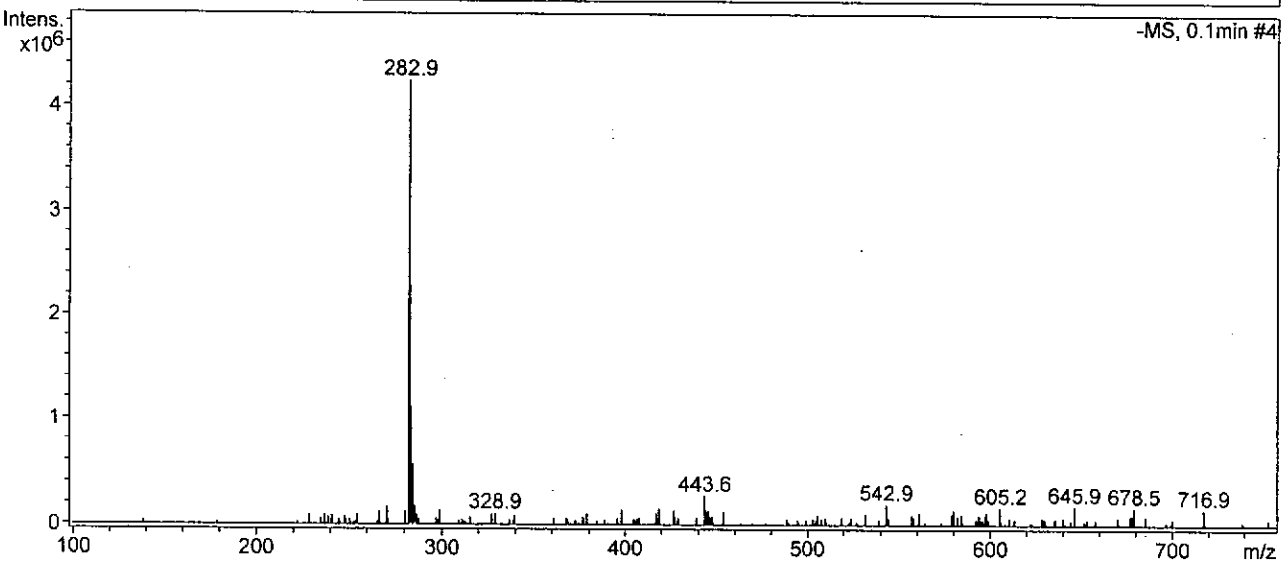
Display Report - All Windows Selected Analysis

Analysis Name: F46000002.D
Method: AN 2MIN.M
Sample Name: Default
Analysis Info: - mode

Instrument: LC-MSD-Trap-VL
Operator: Operator

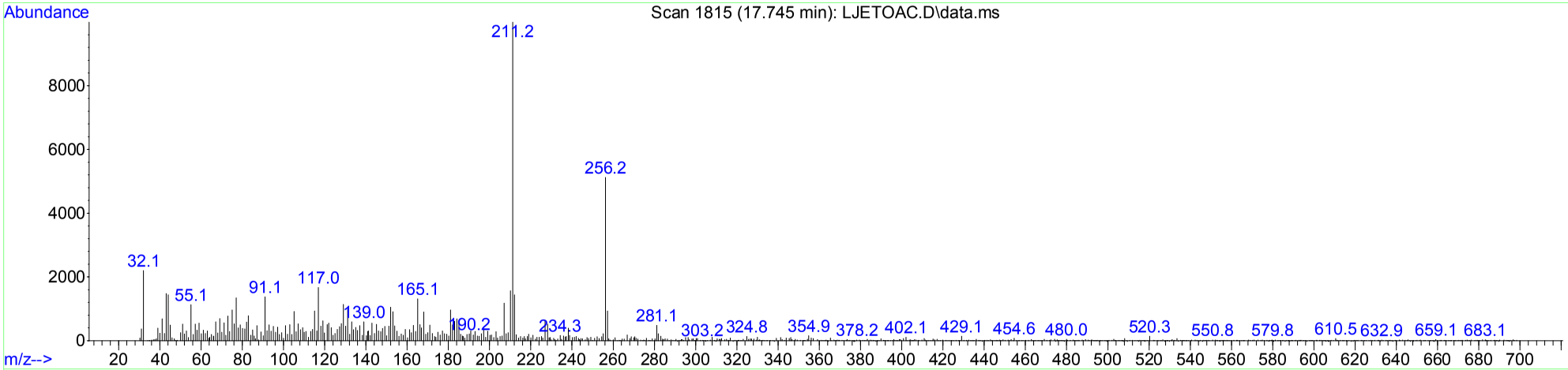
Print Date: 11/7/2012 8:12:53 AM
Acq. Date: 11/7/2012 8:11:47 AM

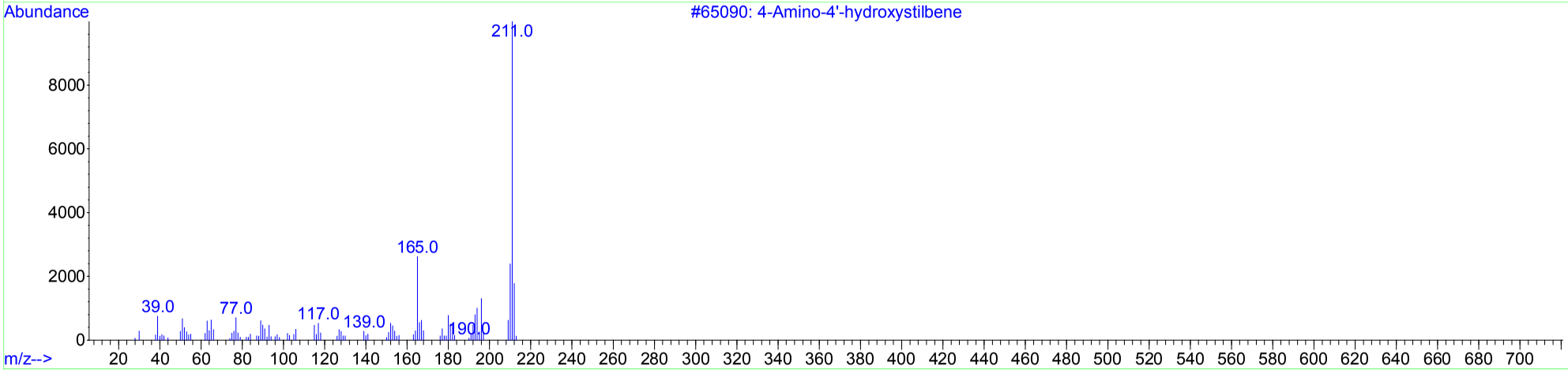


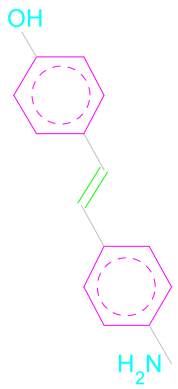


LC-MS spectrum of 2',4',6',4-tetrahydroxy-α-hydroxymethyl-chalcone (5)

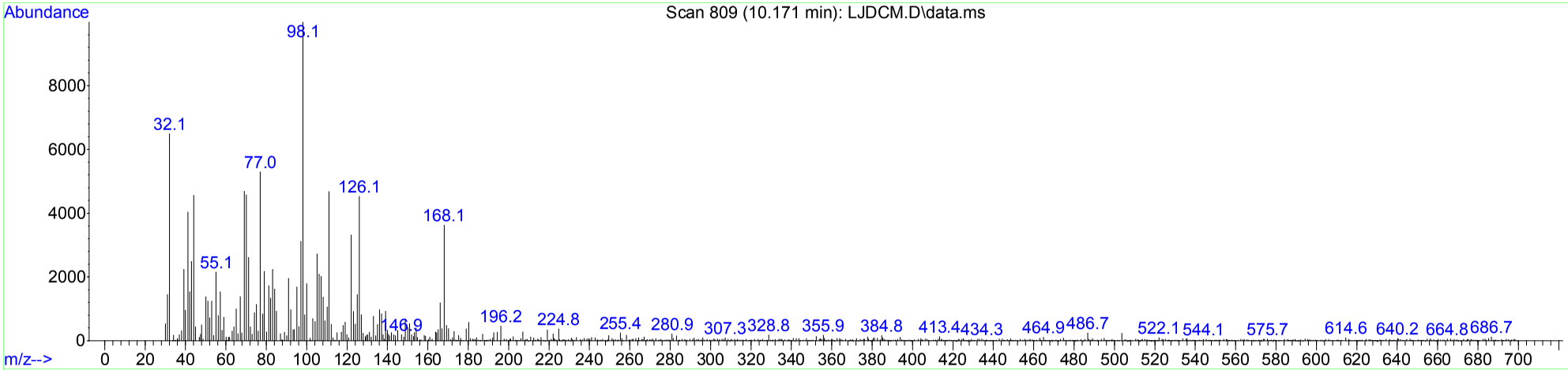
Library Searched : C:\Database\NIST05a.L
Quality : 53
ID : 4-Amino-4'-hydroxystilbene

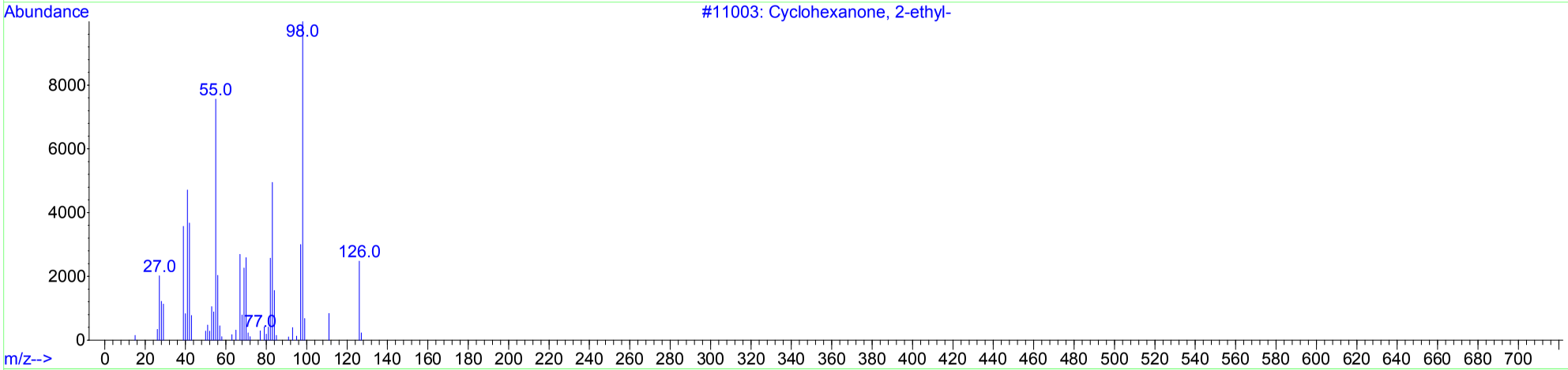


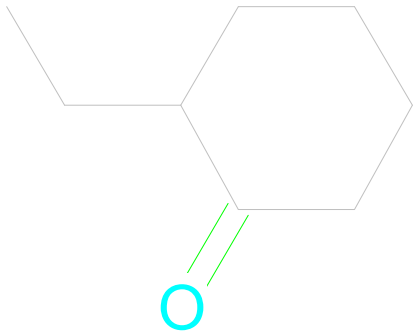




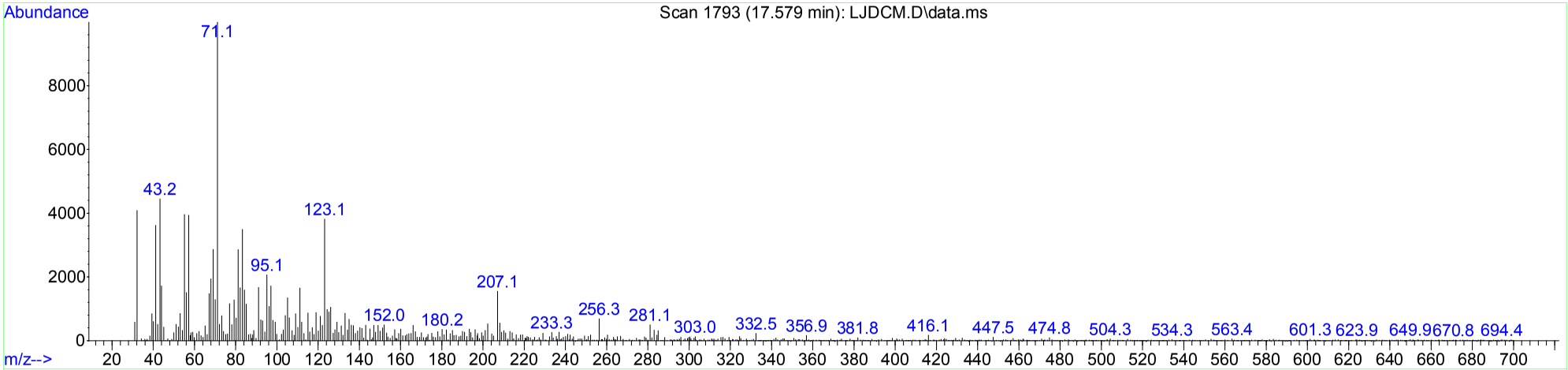
Library Searched : C:\Database\NIST05a.L
Quality : 53
ID : Cyclohexanone, 2-ethyl-

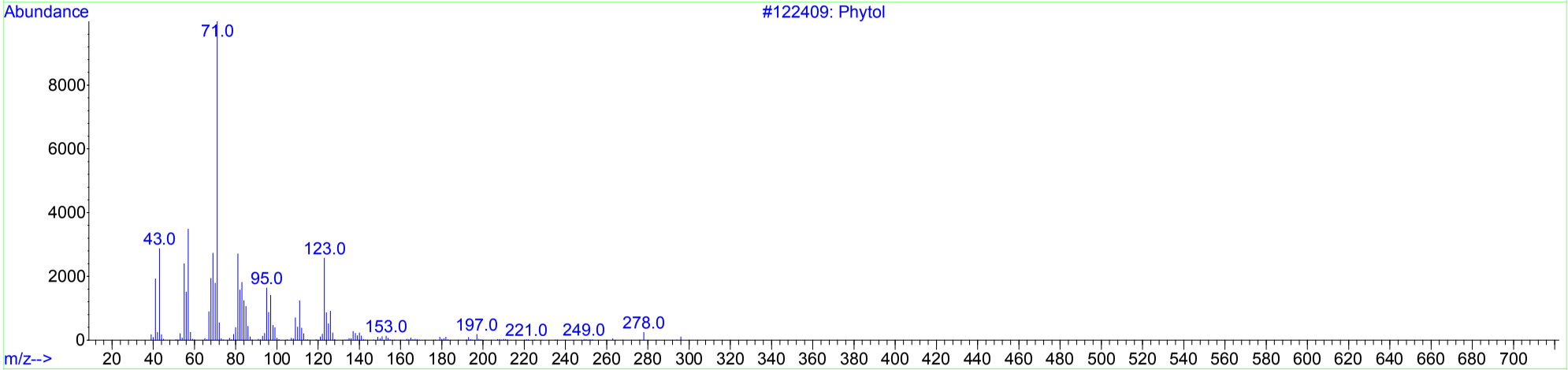




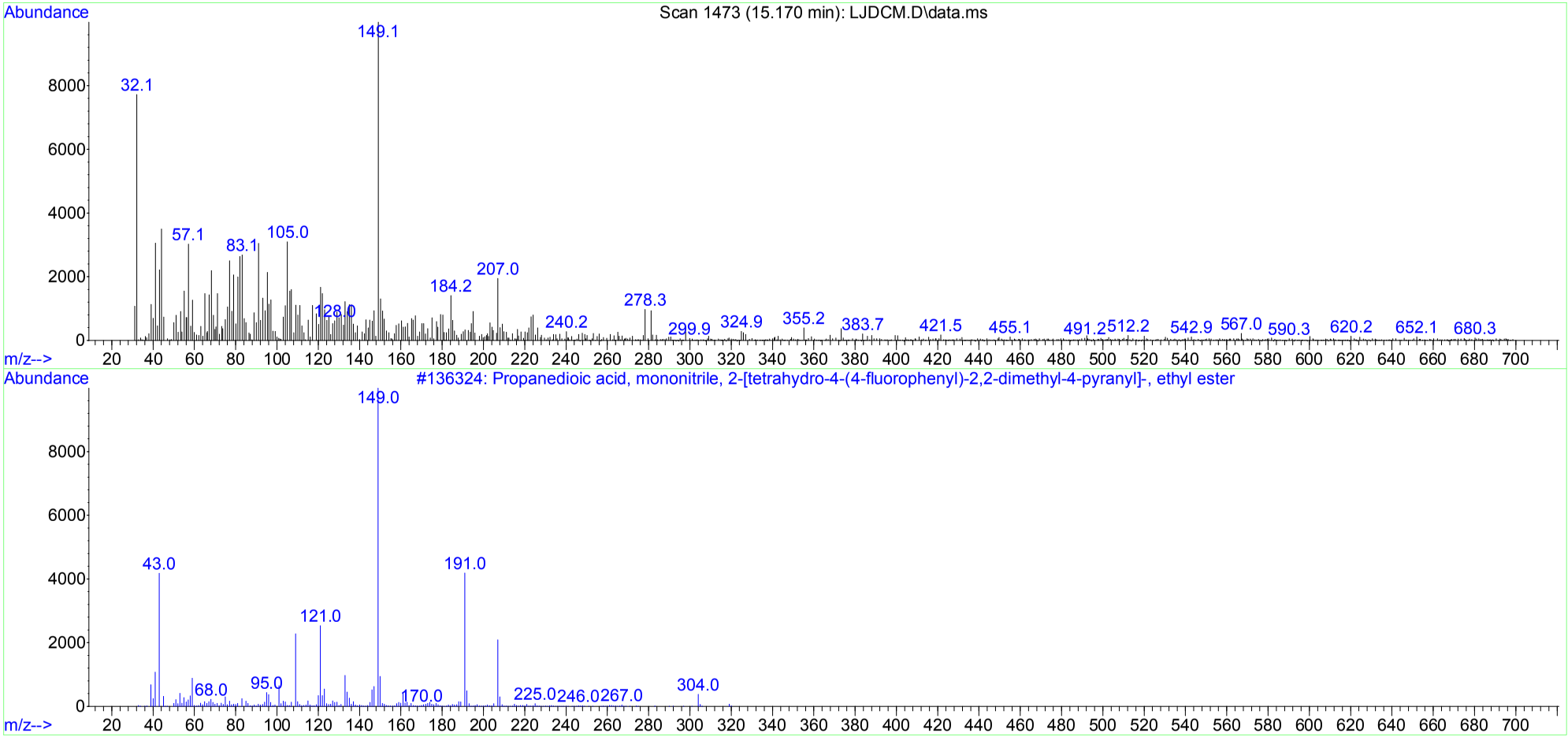


Library Searched : C:\Database\NIST05a.L
Quality : 95
ID : Phytol

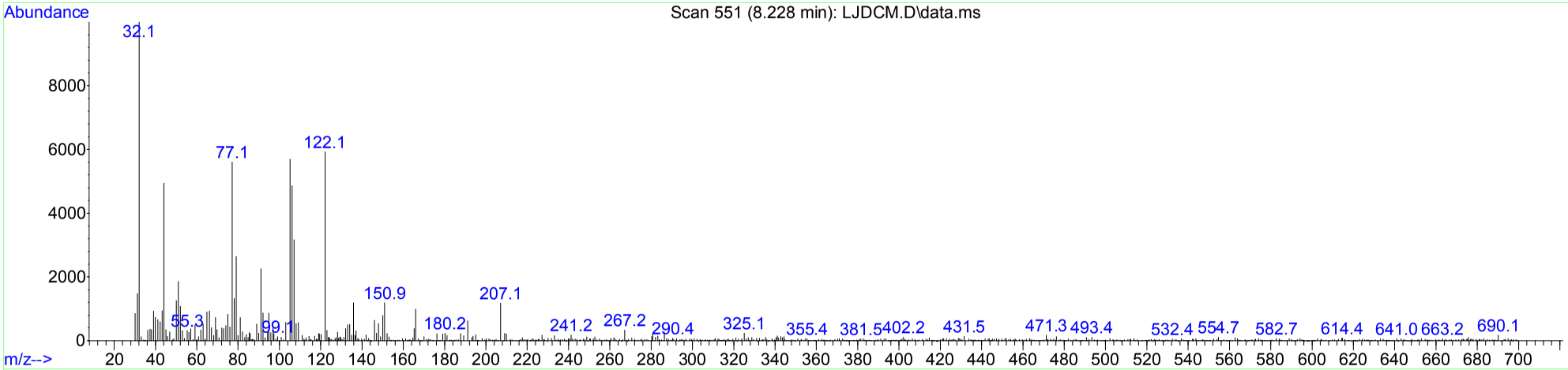


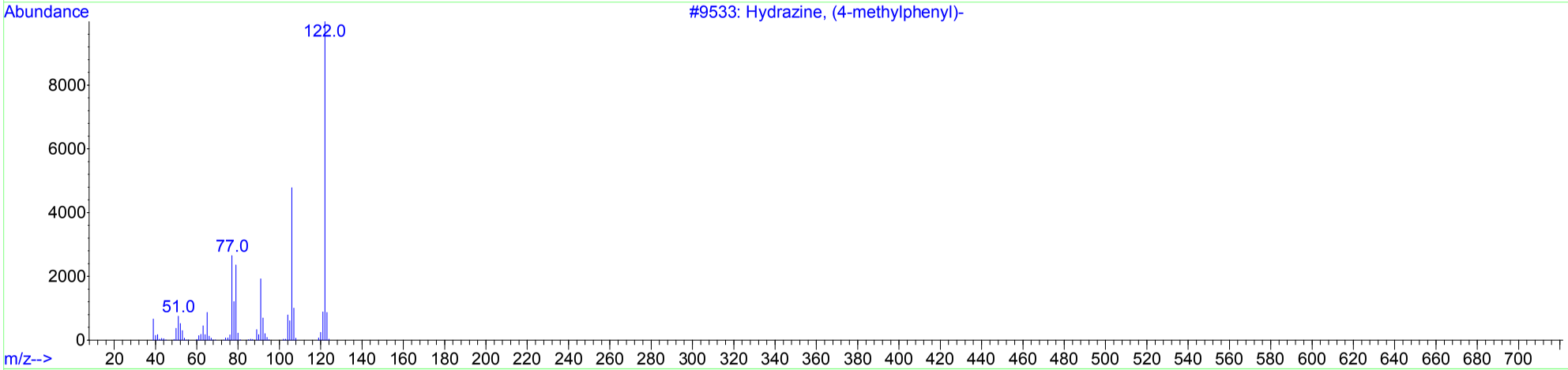


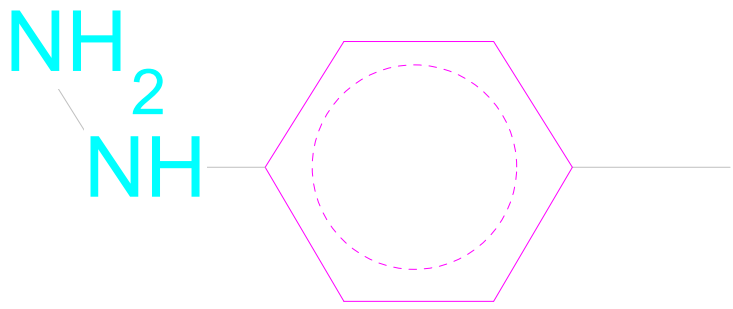
Library Searched : C:\Database\NIST05a.L
Quality : 89
ID : Propanedioic acid, mononitrile, 2-[tetrahydro-4-(4-fluorophenyl)-2,2-dimethyl-4-pyranyl]-, ethyl ester



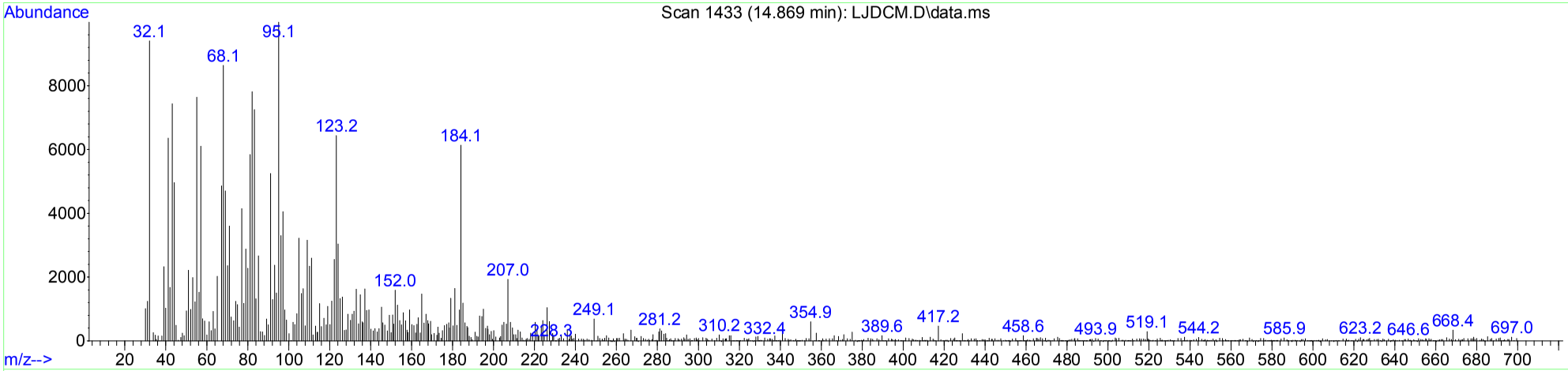
Library Searched : C:\Database\NIST05a.L
Quality : 87
ID : Hydrazine, (4-methylphenyl)-

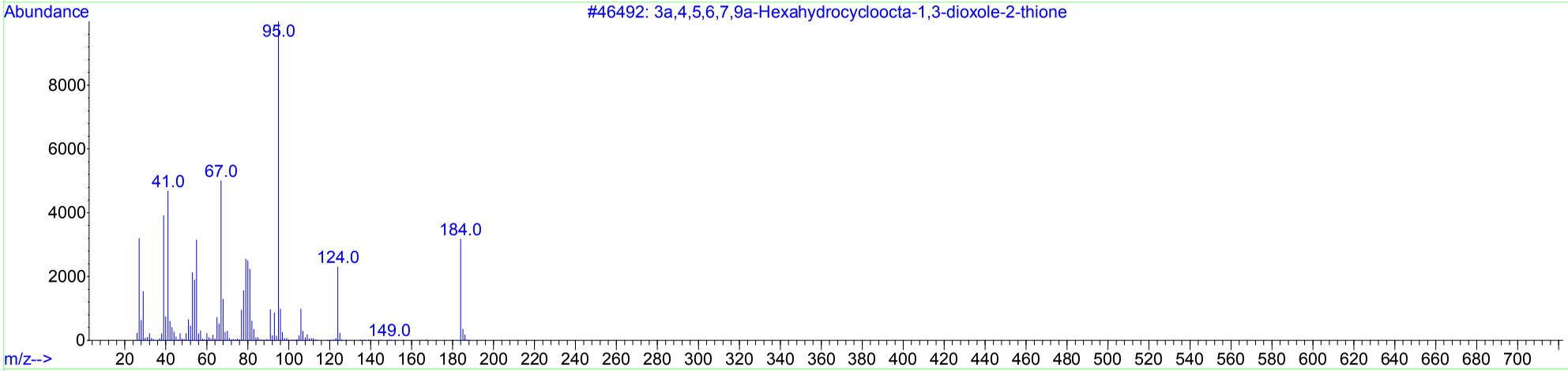


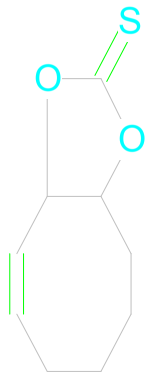




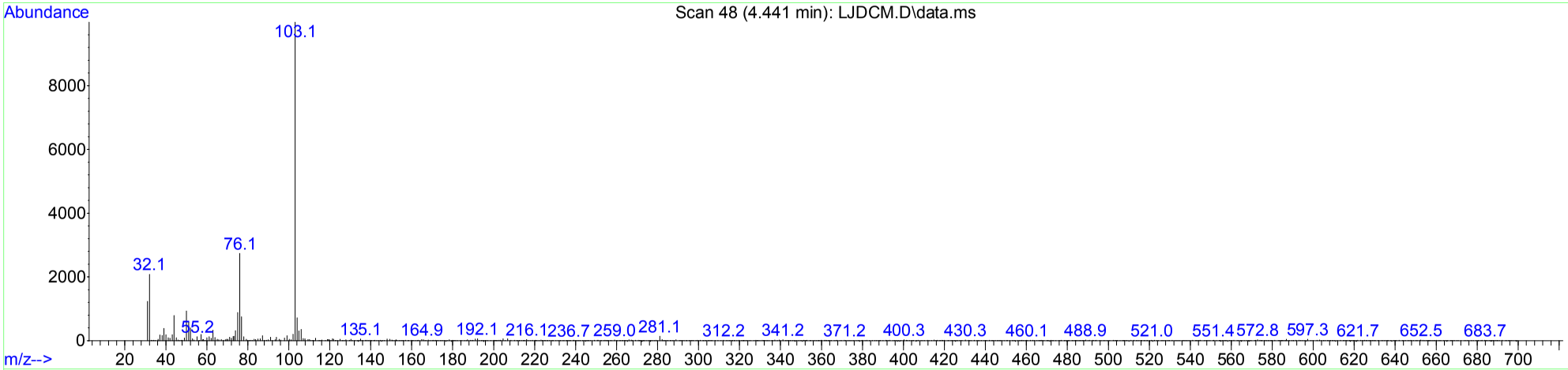
Library Searched : C:\Database\NIST05a.L
Quality : 83
ID : 3a,4,5,6,7,9a-Hexahydrocycloocta-1,3-dioxole-2-thione

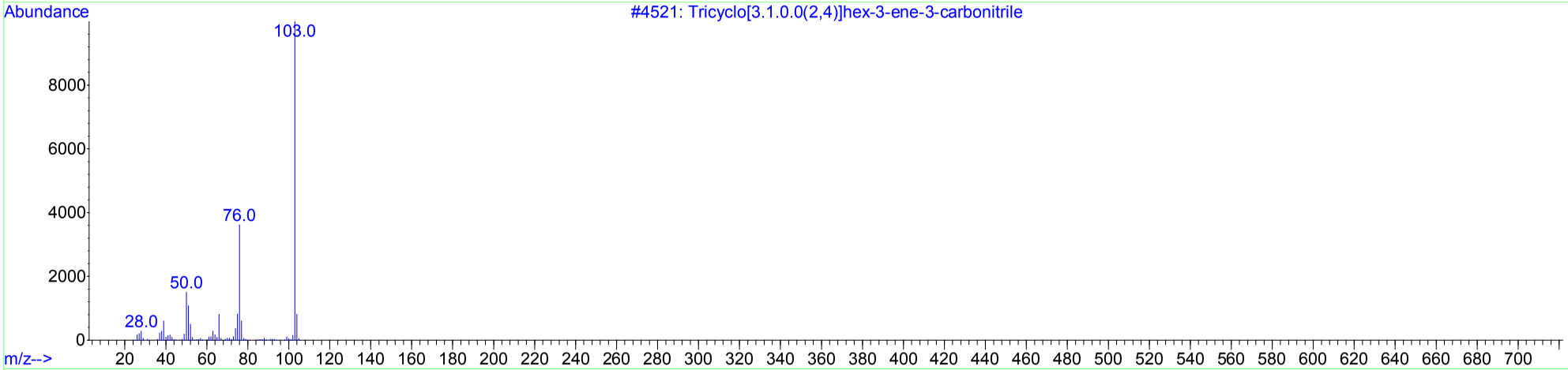


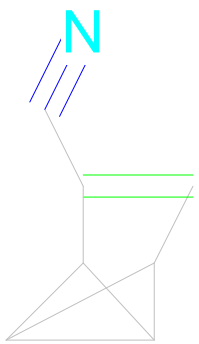




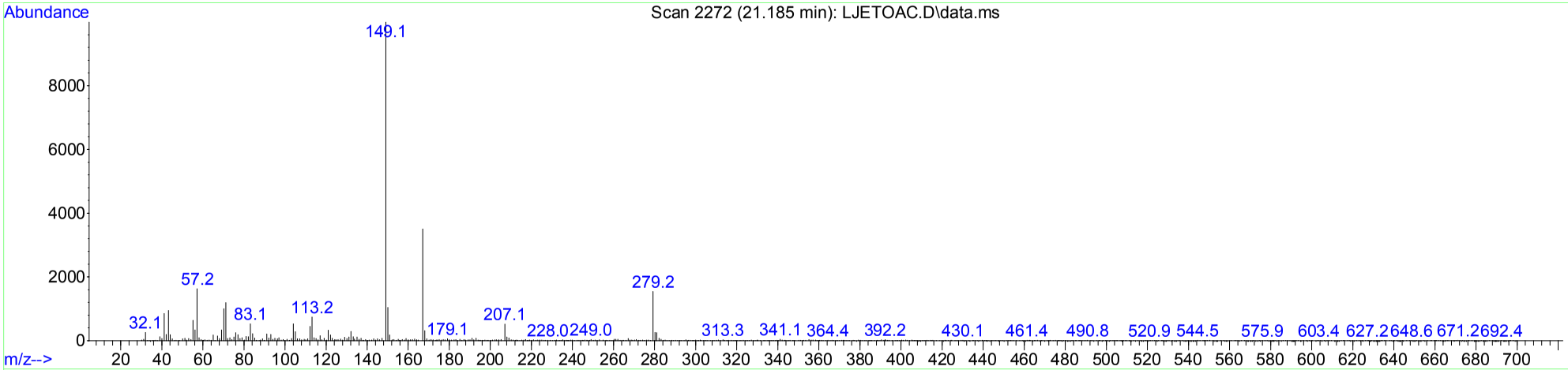
Library Searched : C:\Database\NIST05a.L
Quality : 87
ID : Tricyclo[3.1.0.0(2,4)]hex-3-ene-3-carbonitrile

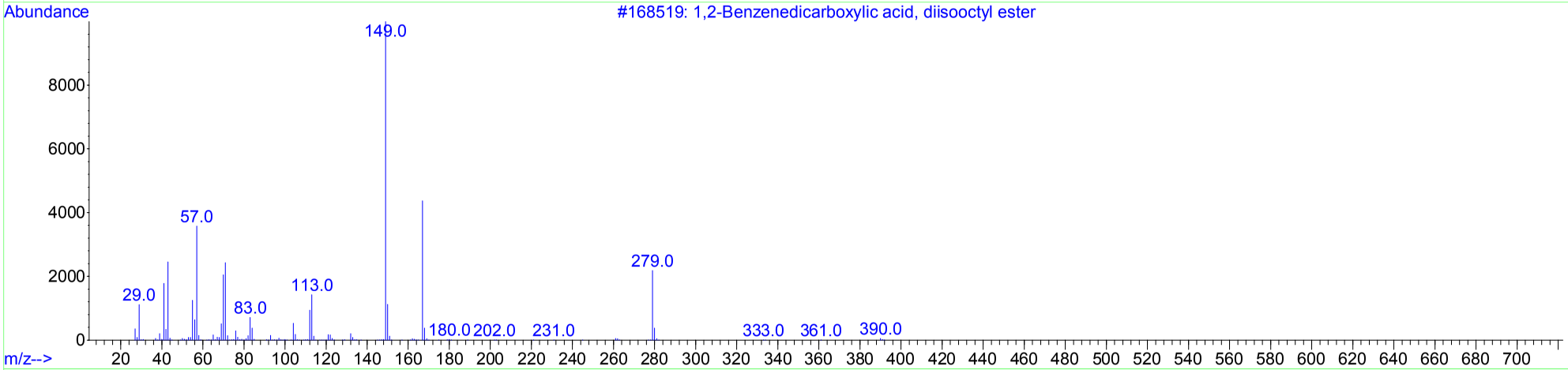


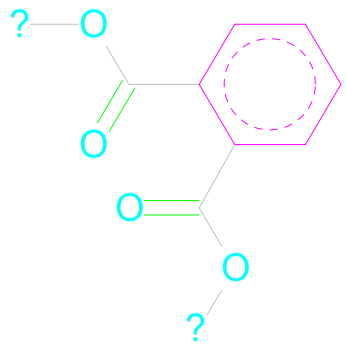




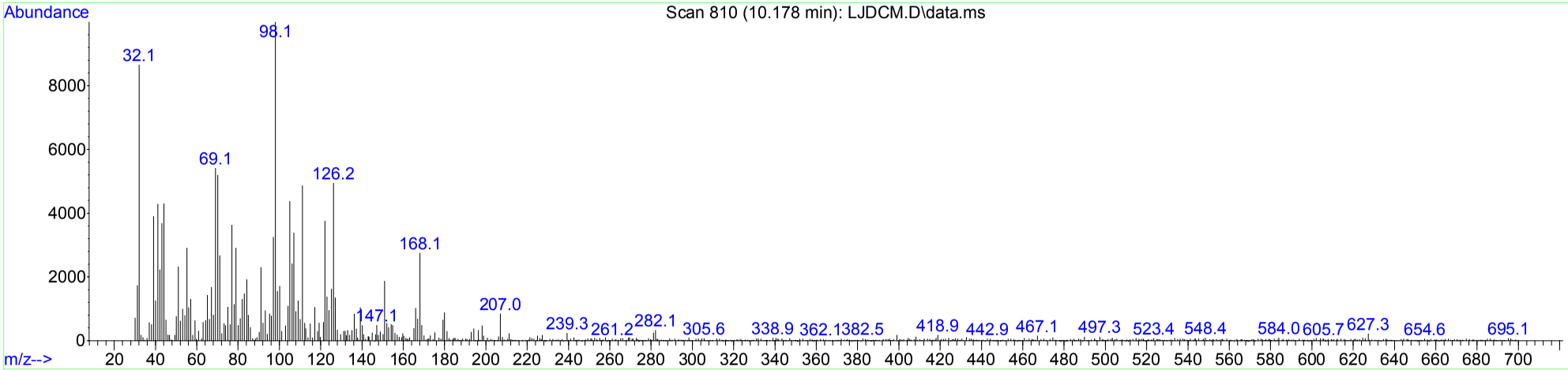
Library Searched : C:\Database\NIST05a.L
Quality : 91
ID : 1,2-Benzenedicarboxylic acid, diisooctyl ester

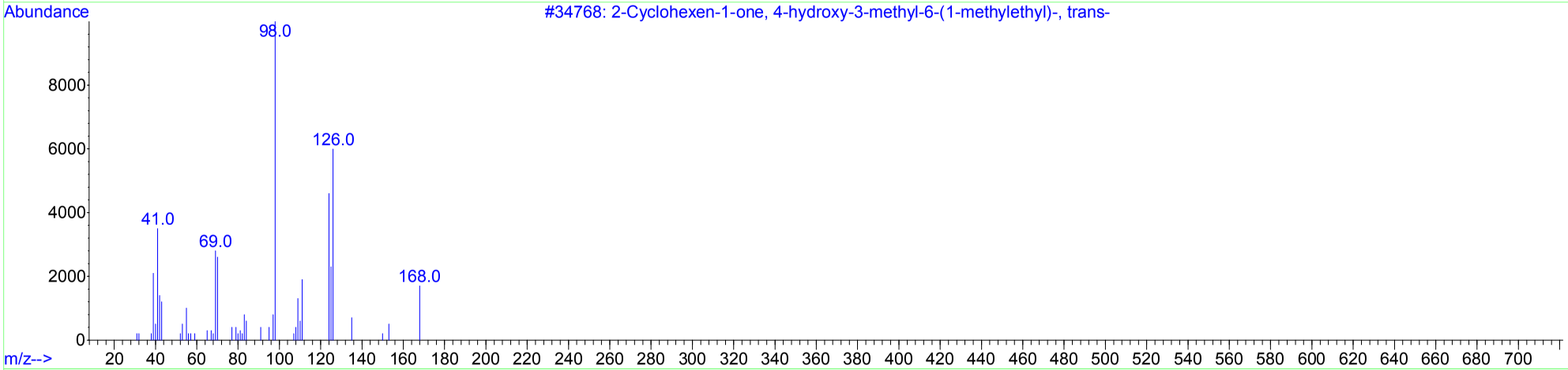


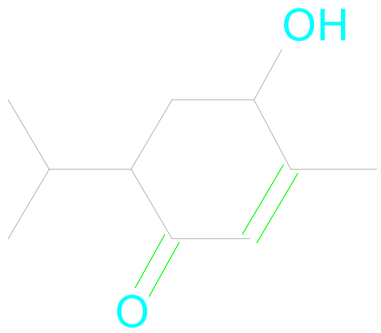




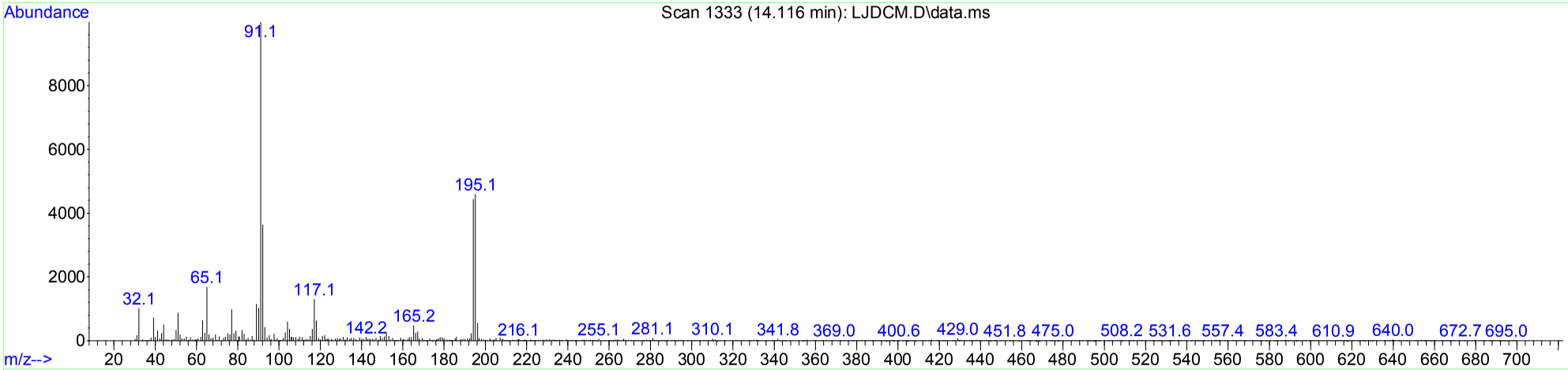
Library Searched : C:\Database\NIST05a.L
Quality : 58
ID : 2-Cyclohexen-1-one, 4-hydroxy-3-methyl-6-(1-methylethyl)-, trans-

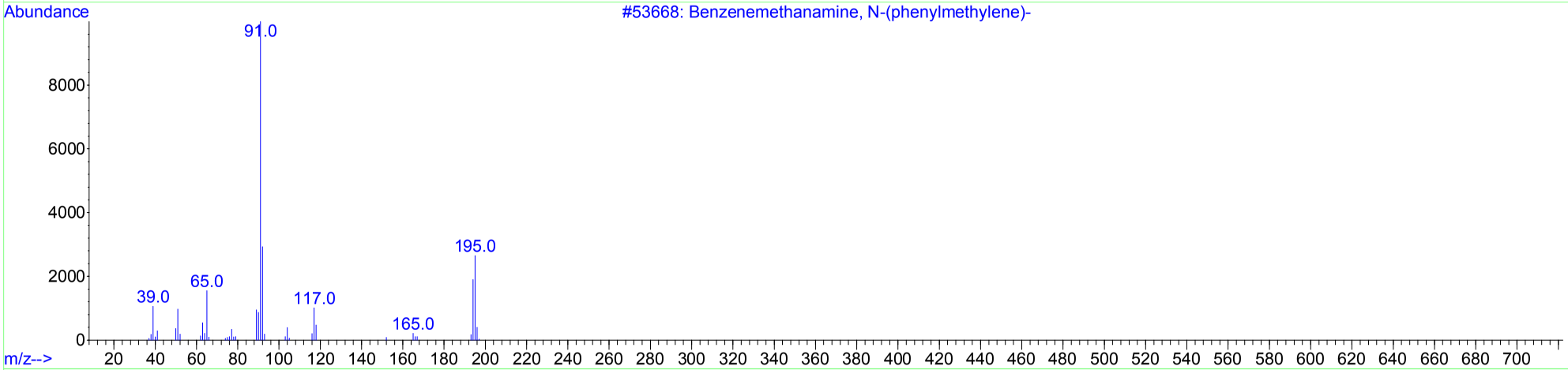


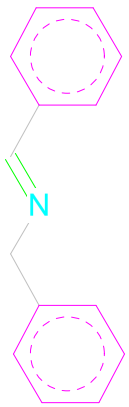




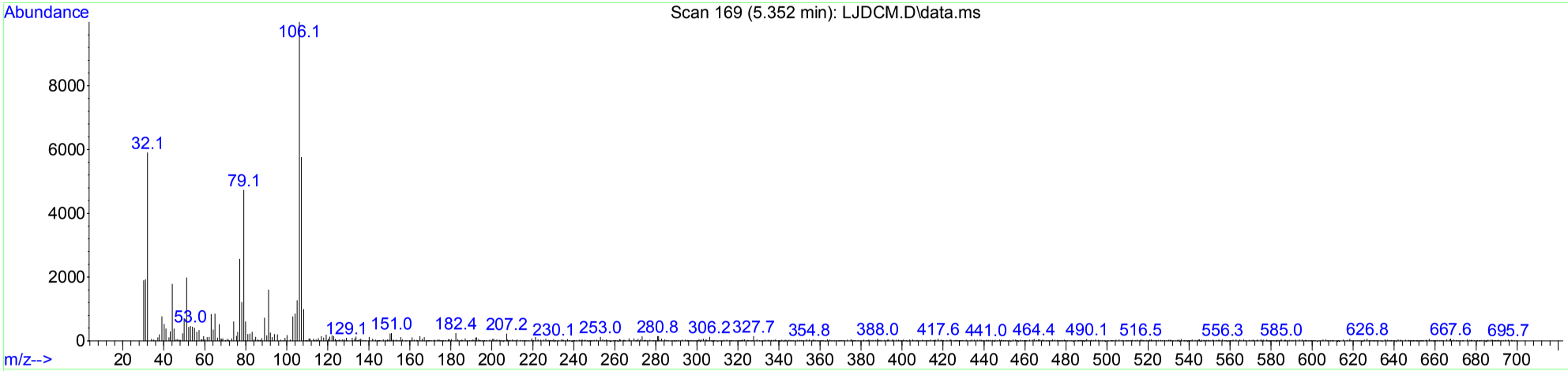
Library Searched : C:\Database\NIST05a.L
Quality : 94
ID : Benzenemethanamine, N-(phenylmethylene)-

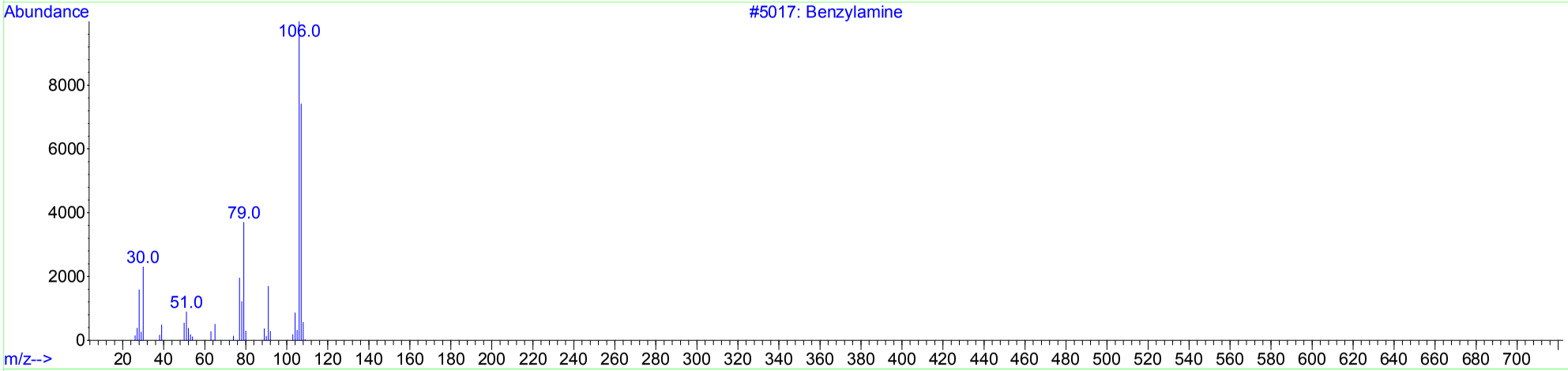


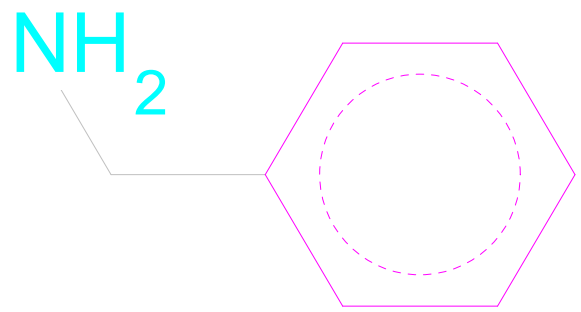




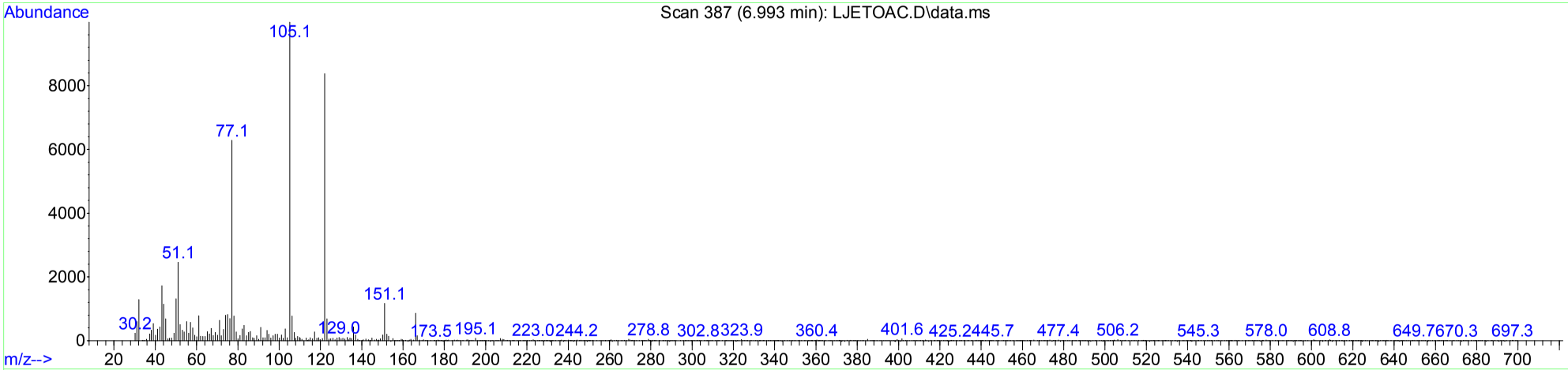
Library Searched : C:\Database\NIST05a.L
Quality : 93
ID : Benzylamine

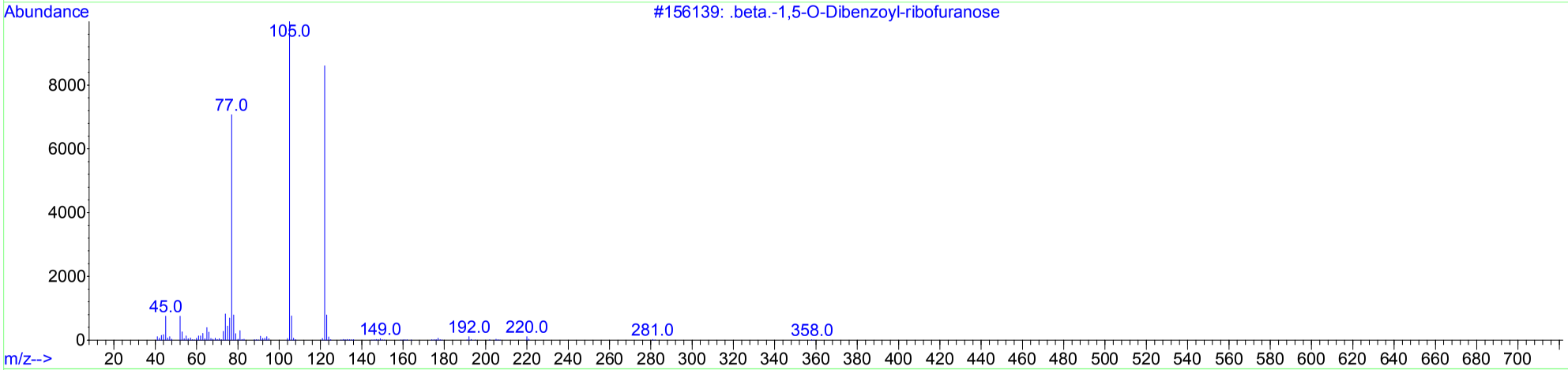


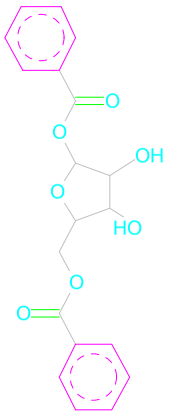




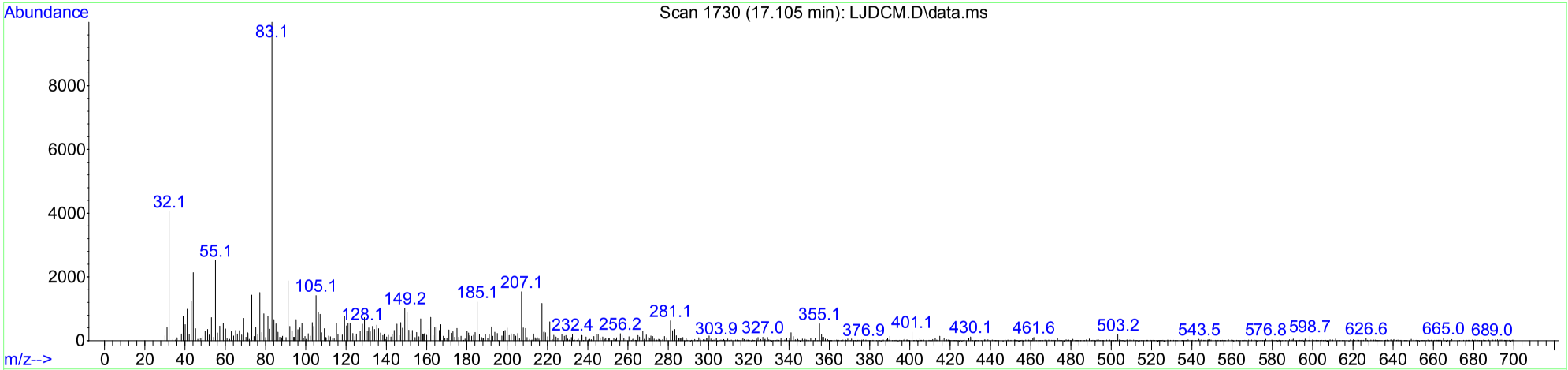
Library Searched : C:\Database\NIST05a.L
Quality : 96
ID : .beta.-1,5-O-Dibenzoyl-ribofuranose

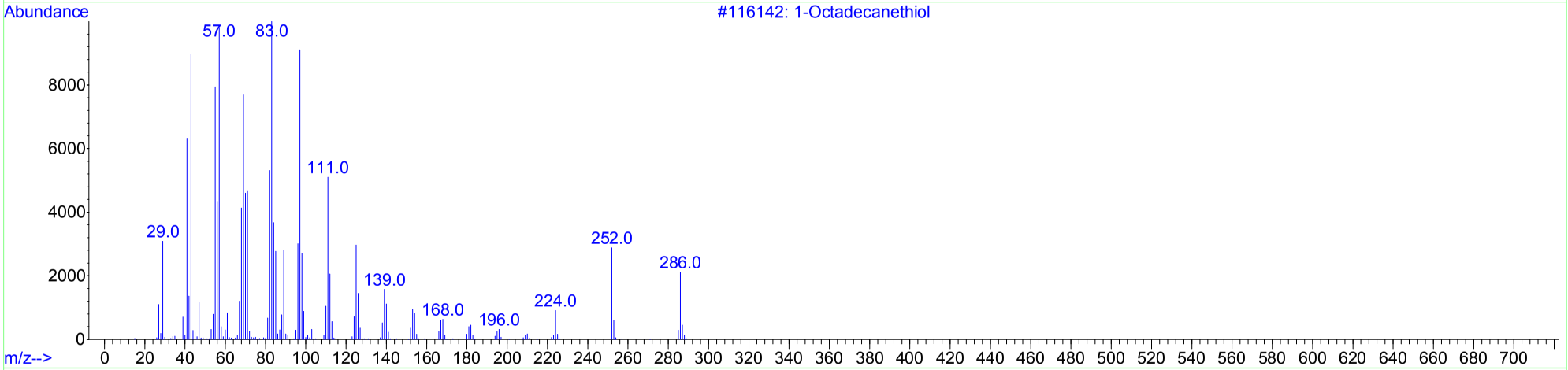


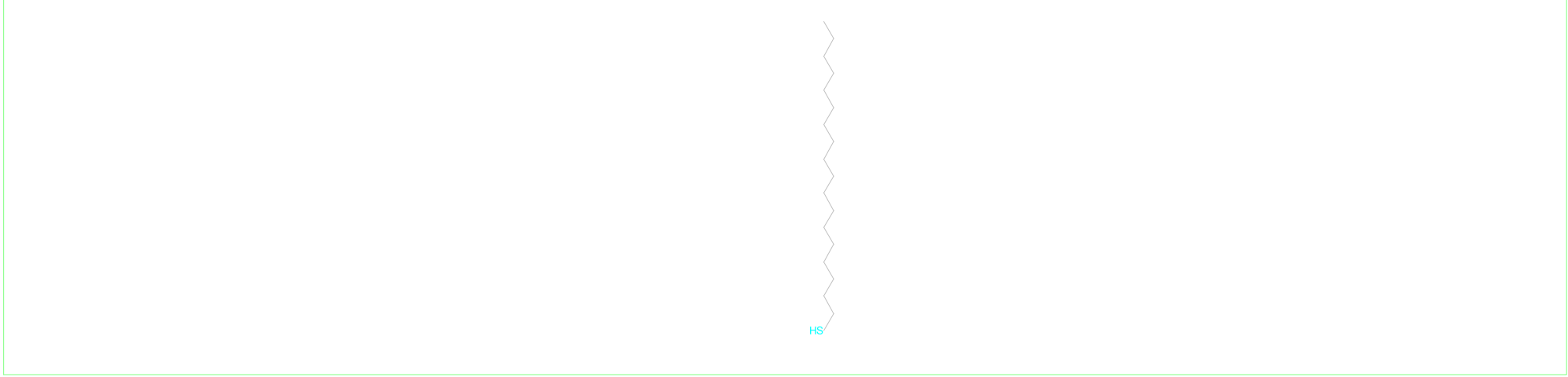




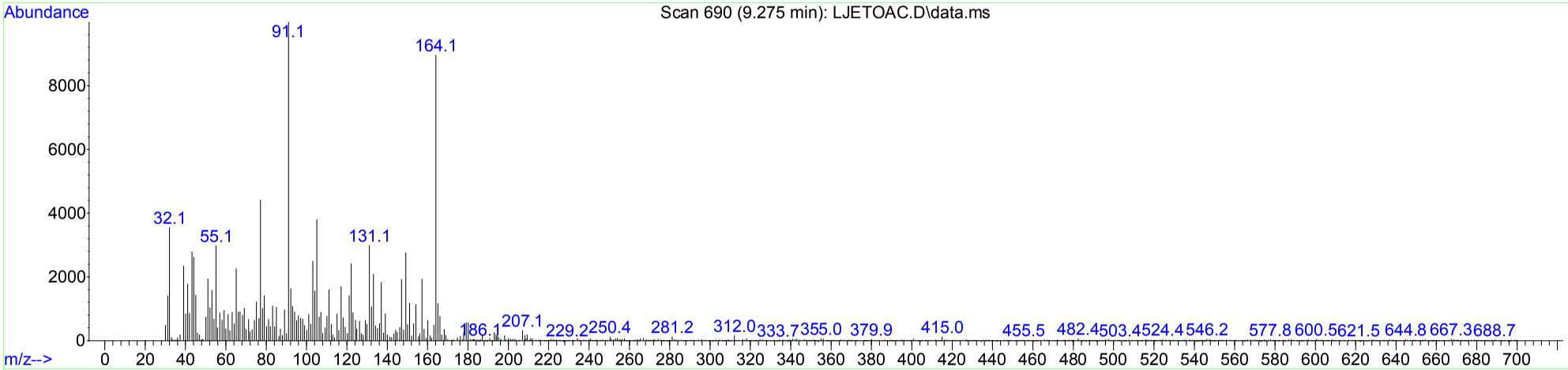
Library Searched : C:\Database\NIST05a.L
Quality : 95
ID : 1-Octadecanethiol

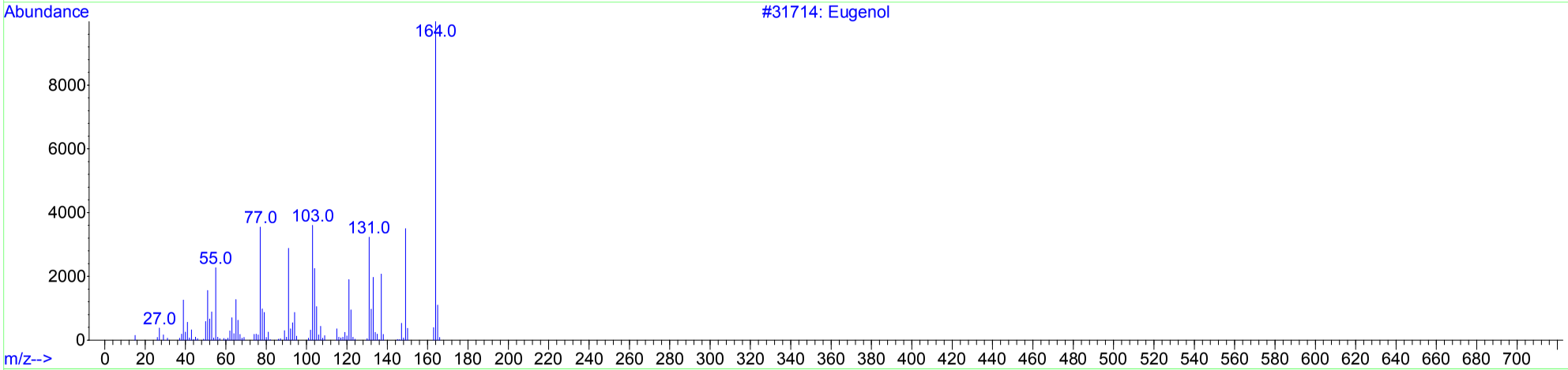


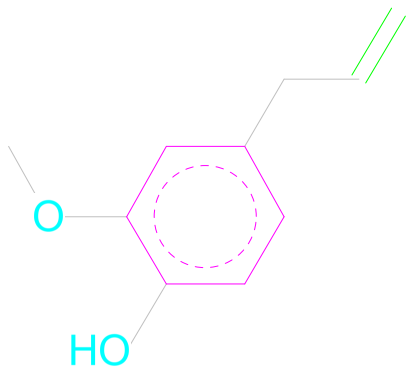




Library Searched : C:\Database\NIST05a.L
Quality : 92
ID : Eugenol







Library Searched : C:\Database\NIST05a.L
Quality : 94
ID : Benzenecarboxylic acid