Phytochemical and Analytical Studies on Two Indigenous Medicinal Plants Found in KwaZulu-Natal, South Africa; Carissa macrocarpa and Harpephyllum caffrum

ROSHILA MOODLEY

2012

Phytochemical and Analytical Studies on Two Indigenous Medicinal Plants Found in KwaZulu-Natal; Carissa macrocarpa and Harpephyllum caffrum

ROSHILA MOODLEY

2012

A thesis submitted to the School of Chemistry and Physics, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville, for the degree of Doctor of Philosophy.

This thesis has been prepared according to **Format 4** as outlined in the guidelines from the College of Agriculture, Engineering and Science which states:

This is a thesis in which chapters are written as a set of discrete research papers, with an overall introduction and final discussion where one (or all) of the chapters have already been published. Typically, these chapters will have been published in internationally-recognized, peer- reviewed journals.

As the candidate's supervisor, I have approved this thesis for submission.

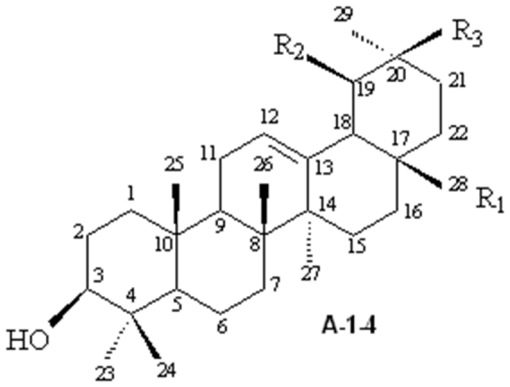
Supervisor:

Signed: ----------Name: ---------- Date: ----------

ABSTRACT

Vulnerable communities and rural households usually turn to natural resources for their nutritional and healthcare needs. However, very little is known about the nutritional and medicinal value of many plant species utilized by these communities despite widespread scientific research on medicinal plants. This is mainly due to the huge numbers of medicinal plants that are in use. This study aimed at investigating two plants species that are indigenous to KwaZulu-Natal and contain edible fruits namely *Carissa macrocarpa* and *Harpephyllum caffrum*, as a source of secondary metabolites and essential dietary elements, because of their claimed medicinal value and nutritional potential. The analytical results indicate that the fruits are a good source of essentiary dietary elements and can contribute to the recommended dietary allowances (RDAs) for most nutrients. Phytochemical analysis shows that the fruits of *C. macrocarpa* are rich in the pharmacologically active pentacyclic triterpenoids whose immune boosting properties are well-known. The fruits can therefore be consumed to boost the immune system in areas where immune boosting supplements are out of reach. The fruits of *H. caffrum* were found to be rich in the flavan-3-ol antioxidant, (+)-catechin. The fruits can therefore be used as a substitute for antioxidant supplements taken in Western and European countries. This study lends scientific credence and validity to the ethnomedicinal use of these plants and reveals the nutritional and medicinal benefits of consuming the indigenous edible fruits. It also adds to the growing body of research on indigenous medicinal plants.

SUMMARY OF ISOLATED COMPOUNDS

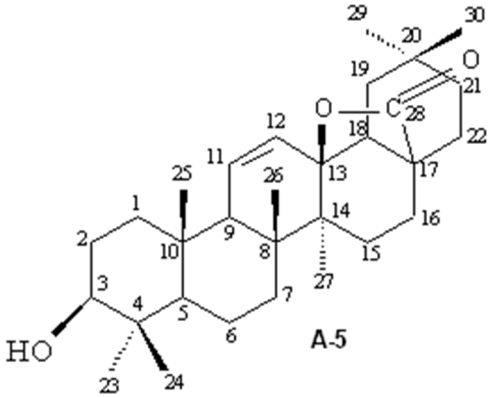


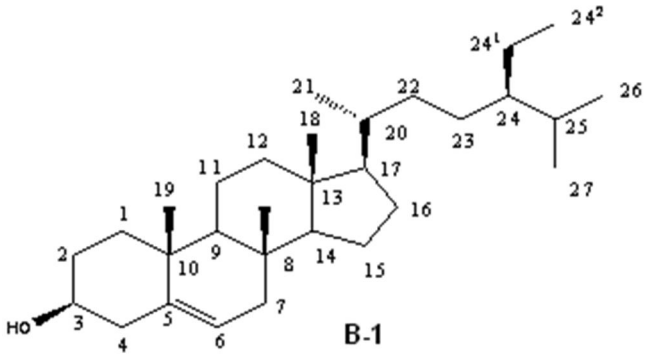
A-1: R_1=CH_3; R_2=H; R_3=CH_3

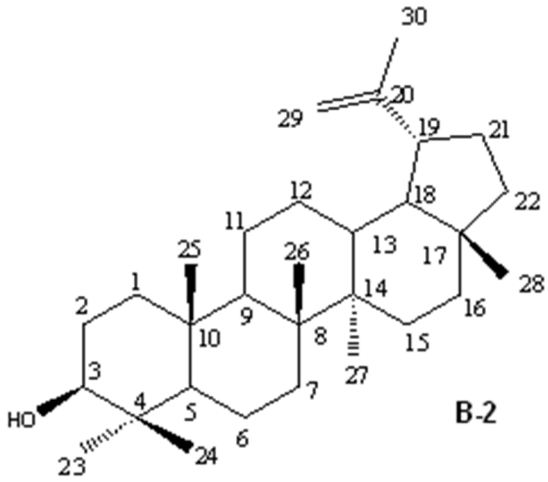
A-2: R_1=C(O)OCH_3; R_2=H; R_3=CH_3

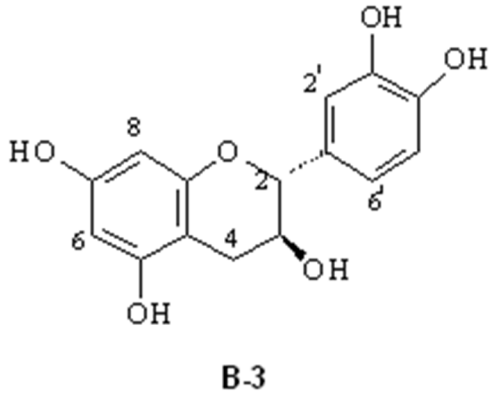
A-3: R_1=COOH; R_2=H; R_3=CH_3

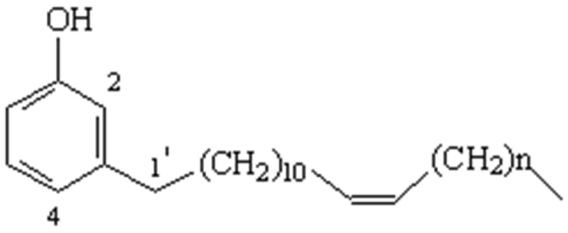
A-4: R_1=COOH; R_2=CH_3; R_3=H



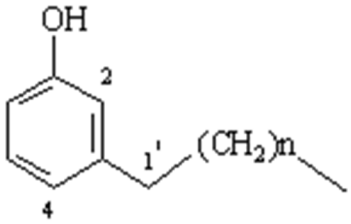




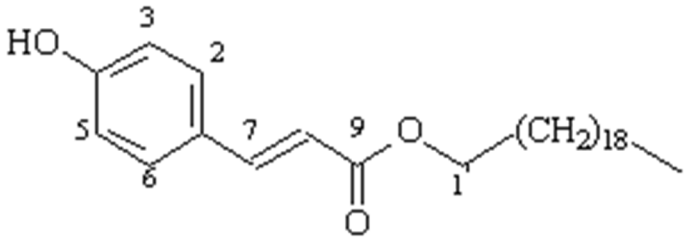




B-4a (n=5), B-4b, (n=3)



B-4c (n=15), B-4d (n=13)



B-5

ABBREVIATIONS

^{13}C NMR - C-^{13} nuclear magnetic resonance spectroscopy

^{1}H NMR - proton nuclear magnetic resonance spectroscopy

A - bioavailable

ANOVA - analysis of variance

BAF- bioaccumulation factor

CEC- cation exchange capacity

COSY - correlated spectroscopy

CRM- Certified Reference Material

d - doublet

dd - double doublet

DEPT - distortionless enhancement by polarization transfer

DM- dry mass

DPPH - 2,2-diphenyl-1-picrylhydrazyl

DRI - Dietary Reference Intake

EDTA- ethylenediamine tetraacetic acid

Ex - exchangeable

FRAP - ferric reducing antioxidant potential

GC-MS - gas chromatography-mass spectrometry

HMBC - heteronuclear multiple bond coherence

HSQC - heteronuclear single quantum coherence

Hz - Hertz

ICP-OES- Inductively Coupled Plasma-Optical Emission Spectrometry

IR - infrared

MUFA- monounsaturated fatty acid

NOESY - nuclear overhauser effect spectroscopy

PUFA- polyunsaturated fatty acid

r- correlation coefficient

RDA- recommended dietary allowance

SD - standard deviation

SOM - soil organic matter

T - total

TLC - thin-layer chromatography

UL- tolerable upper intake level

UV - ultraviolet

DECLARATIONS

Declaration 1 - Plagiarism

I, Roshila Moodley declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
   a. Their words have been re-written but the general information attributed to them has been referenced
   b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed ……

Declaration 2 – Publications

Publication 1

Title: Elemental composition and fatty acid profile of the edible fruits of *Amatungula* (*Carissa macrocarpa*) and impact of soil quality on chemical characteristics.

Authors: *Roshila Moodley*, Neil Koorbanally and Sreekanth B. Jonnalagadda.

Journal: Analytica Chimica Acta, **2012**, 730: 33-41.

Publication 2

Title: Antibacterial and anti-adhesion activity of the pentacyclic triterpenoids isolated from the leaves and edible fruits of *Carissa macrocarpa*.

Authors*: Roshila Moodley*, Hafizah Chenia, Sreekanth B. Jonnalagadda and Neil Koorbanally.

Journal*:* Journal of Medicinal Plants Research. **2011**, 5(19), 4851-4858. (Online).

Publication 3

Title: Antioxidant activity of Phenolic compounds isolated from the edible fruits and stem bark of *Harpephyllum caffrum*.

Authors*: Roshila Moodley*, Neil Koorbanally, Sreekanth B. Jonnalagadda and Md. Shahidul Islam.

Journal*:* manuscript submitted to *African Journal of Pharmacy and Pharmacology*.

Publication 4

Title: Elemental composition and nutritional value of the edible fruits of *Harpephyllum caffrum* and impact of soil quality on chemical characteristics

Authors*: Roshila Moodley*, Neil Koorbanally and Sreekanth B. Jonnalagadda.

Journal*:* manuscript accepted for publication: *Journal of Environmental Science and Health.*

In all of the publications I have performed all the experimental work and written the manuscripts. The co-authors were involved in discussion of the results and were responsible for verifying the scientific content and accuracy of the results as well as editing the manuscripts. I have been the corresponding author on two of the manuscripts contained in this thesis.

Signed: ……..

ACKNOWLEDGEMENTS

Firstly, I would like to acknowledge God, the Almighty, for giving me the power to believe in myself and pursue my dream and for endowing me with health and knowledge to realise this dream.

Secondly, my sincerest thanks and gratitude go to my supervisors, Prof. S.B. Jonnalagadda and Dr N. Koorbanally, who helped me conceptualize the topic and who inspired me to reach the successful completion of my PhD research. I am grateful to them both for sharing their expert knowledge and immense experience in research as well as assisting and guiding me with constructive criticism and invaluable suggestions. I would also like to acknowledge the College of Agriculture, Engineering and Science (UKZN), Dean and HOS, and DVC of Research (CAES) for affording me this opportunity.

I would also like to acknowledge with thanks:

- My friends, Shakira Shaik, Joyce Bett and Dorothy Okoth for their love and support.
- The technical team in the School of Chemistry at UKZN (Westville) for their assistance, in particular Anita Naidoo, Neil Broomhead and Dilip Jagjivan.
- The postgraduates in the Natural Products Research Group and my postgraduate students for their friendly faces and warm smiles.
- My colleagues in the School of Chemistry and Physics and Access Programme for their moral support.

- Hafizah Chenia (Microbiology) and Auwal M. Ibrahim (Biochemistry) for their advice and training.

My PhD journey would not have begun and surely not have ended without the continued love, support, and encouragement of my husband, Riza, and my mother who have been strong forces in all of my accomplishments. Thank you for always being there for me. Last but not least, I would like to thank my boys, Zia and Zubair, for their unconditional love, patience and understanding throughout my studies. I love you very much.

Contents

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

In many communities in sub-Saharan Africa, nutrient deficiencies are common and malnutrition and food insecurity are critical concerns as these communities do not have physical and economic access to foods that meets their dietary needs. A study of 150 rural households in South Africa reported that wild fruits are frequently consumed by the local population (Shackleton et al., 2005), whilst another study reported that gathered fruits comprise half of the fruits eaten by the locals, yearly (Herzog et al., 1993). Research has shown that vulnerable communities and rural households usually turn to natural resources for food security; purchased foods are being substituted with wild fruits and vegetables that are readily and freely available. Rural communities are generally predisposed to micronutrient deficiencies, childhood malnutrition, and protein-energy malnutrition (essential fatty acid deficiency) which are of concern in South Africa. If the nutritional quality and energy content of alternative foods like wild foods are assessed then consumption of those with high nutritional value can be promoted to attenuate malnutrition in these communities.

It is approximated that 80% of black South Africans use traditional medicine to meet their primary healthcare needs (Mbatha et al., 2012). The rural poor are also dependent on traditional medicine for treatment of HIV related infections such as venereal diseases, tuberculosis, diarrhea, and appetite loss (Bodeker et al., 2000; King, 2000). Traditional

healers utilise medicinal plants to treat these infections and other diseases or disorders. They have extensive knowledge on the use of plants and herbs for medicinal purposes and this empirical knowledge can provide fertile ground for research. This has been acknowledged and indigenous knowledge is being incorporated into scientific research by selecting plants for investigation that have claimed medicinal value. Intensive and extensive research has been done on medicinal plants, however due to the multitude of these plants that have been in use since time immemorial, many are to a large extent unresearched. Systematic research into these plants using ethnomedicine as a basis could unlock the door to a wide variety of lead compounds in the pharmaceutical industry.

Plants are intermediate reservoirs through which metals from the soil are transferred to humans. Although most elements are essential to plant growth for normal functioning, the amounts of these needed elements may vary. The ability of plants to take up and translocate these elements depends, to a large extent, on the soil matrix and plant genotype. Once metals are taken up and transferred through the agricultural food chain, they can threaten human health (whether essential or non-essential) if at elevated levels. On the other hand, intake levels that are too low may result in abnormal functioning of cells and deficiency diseases. Reliance on plants collected from the wild as a source of nutrition or medicine means that those consuming them are likely to be exposed to the influences of pollution especially from anthropogenic sources. Studies have shown that a high intake of certain trace elements may lead to abnormal cell functioning and are also linked to certain types of cancers (Mathee et al., 2002; Banas et al., 2010). The monitoring of plants that are used either nutritionally or medicinally and the associated soil to assess for heavy metal

contamination in the soil-plant-animal continuum is therefore vital. Awareness on safety of consuming wild plants needs to be generated since more and more industries are violating legislation and polluting the water body and soil on which these plants grow. Once areas of pollution are identified, possible associations between illnesses and trace element concentrations in soil can be made and measures can be taken to immobilize these metals and prevent further contamination. Furthermore, the analysis of plants for heavy and toxic metals should be routinely carried out and passed by a certified board before the plants are deemed to be safe for human consumption in order to prevent death and illness caused by toxic metals and related pollutants.

AIMS AND OBJECTIVES OF THE STUDY

The aim of the study was to phytochemically and analytically investigate two medicinal plant species indigenous to KwaZulu-Natal namely *Carissa macrocarpa* and *Harpephyllum caffrum*, which contain edible fruits, to determine their potential as nutraceuticals. The phytochemical investigation was done on plant parts that are used by traditional healers to determine if they contained any secondary metabolites that would validate their ethnomedicinal use, and on the fruits to determine their medicinal value. The analytical investigation was done primarily to determine the nutritional value of the fruits.

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 their biological activity thereby promoting further use of the plants or validating their ethnomedicinal use.
- To assess the plants for nutritional value by comparing elemental concentrations in the fruits to recommended dietary allowances (RDAs).
- To determine the elemental concentrations in the fruits as a function of geographic location and soil quality parameters aimed at determining their impact on elemental uptake and to assess the fruits for metal contamination.

LITERATURE REVIEW

Soil and Nutrients for Plants

Soil is a natural body consisting of layers of materials that cover the surface of the earth and serve as a natural medium for the growth of land plants. It is primarily composed of fine rock particles, minerals and elemental and biological material which differ from its parent material in texture, structure, consistency, colour, chemical, and biological characteristics (Birkeland, 1999). The parent material is the underlying geological material that contains consolidated or unconsolidated minerals that have undergone physical or chemical weathering influenced especially by climatic conditions. Trace metals occur naturally in soils usually at low concentrations as a result of weathering of rock from parent material (Kabata-Pendias & Pendias, 1992).

Soil is a complex, multi-component system of interacting materials, and the biological, physical and chemical properties of soil result from the net effect of all these interactions. Soil organic matter (SOM) is defined as the summation of plant and animal residues at various stages of microbial decomposition, which involves cells and tissues of soil organisms, and well-decomposed substances (Brady & Weil, 1999). SOM increases the nutrient holding capacity of the soil, and provides a pool of nutrients for plants. SOM also chelates nutrients thereby making them available to plants and is food for soil organisms like bacteria and worms that hold on to nutrients and release them in plant-available forms. It encourages root development and improves aggregation thereby preventing soil erosion

and reduces the negative environmental effects of pesticides and many other pollutants (Bot & Benites, 2005).

Inorganic and organic soil particles have negative surface charges. The negative surface charges of organic particles result from the dissociation of hydrogen ions present in the carboxylic acid and phenolic groups of the organic structures in soil (Taiz & Zeiger, 2002). Mineral cations adsorb to these negative surface charges and are not easily leached by water thereby providing a nutrient base for plant roots. Adsorbed cations can be replaced by other cations in the soil solution in a process known as cation exchange and the released cations thus become available for plant uptake (Rengasamy & Churchman, 1999). The cation exchange capacity (CEC) is the ability of the soil to hold onto nutrients and prevent them from leaching beyond the roots. It is simply a measure of the quantity of sites on soil surfaces that can retain positively charged ions by electrostatic forces.

Soil pH is a measure of the soil solutions acidity and alkalinity. Soil pH can impact plant growth based on its influence on the availability of essential plant nutrients and is one of the major aspects controlling the availability of heavy metals in soil (Smith, 1996).

Plant uptake of trace elements is generally the first step of their entry into the agricultural food chain (John & Leventhal, 1996). The limiting step for elemental entry to the food chain is usually from the soil to the root. Elemental concentrations in soil pore solutions and climatic conditions are two factors that largely control element mobility and availability (John & Leventhal, 1996). Water is essential for the solid-liquid partitioning in soil before uptake by organisms. Solubility of trace elements in the liquid determines its

mobility and bioavailability. For any trace element, only some of the total concentration of the element will be in soil solution whilst the rest would be adsorbed or bound to the soil matrix. The availability of the element for uptake by biota is determined by the fraction to which the element is complexed (Table 1). The dissolved fraction consists of metals in solution, including metal cation and anion complexes and hydrated ions; metals in this fraction are highly mobile and are readily available for plant uptake (Gunn, 1988).

Exchangeable fractions consist of metals bound to colloidal or particulate material and carbonate fractions consist of carbonate minerals in sedimentary rocks and soil; metals in these two fractions are highly mobile (Gunn, 1988). The Fe-Mn oxide fraction consists of metals adsorbed to Fe-Mn oxide particles or coatings and the organic fraction consists of metals bound to various forms of organic matter; metals here have medium mobility (Gunn, 1988). The sulfide fraction and the crystalline fraction (which consists of metals contained within the crystal structure of minerals) are normally not available to biota.

Table 1: Chemical forms of metals in solid phases.

	F R A C T I O N S	**MOBILITY**
T O T A L	DISSOLVED - IN PORE WATER	HIGH
	EXCHANGEABLE - WEAKLY ADSORBED	HIGH
	ASSOCIATED WITH CARBONATE	HIGH
	ASSOCIATED WITH Fe, Mn OXIDES	MEDIUM
	COMPLEXED BY ORGANICS	MEDIUM
	ASSOCIATED WITH SULPHIDE	LOW
	CRYSTALLINE - IN THE MINERAL LATTICE	LOW

Total metal concentration is an operationally defined metal concentration representing the total or pseudo-total amount of metal in soil after digestion in a strong acid. This concentration differs from the bioavailable or exchangeable amounts (Symeonides, 1977). The bioavailabile or exchangeable metal concentration is the amount that is potentially available to an organism for sorption with the potential for distribution, metabolism, elimination and bioaccumulation (McGreer, 2004). Bioavailable or exchangeable levels of trace elements in soil have been measured using a wide variety of empirical extraction techniques that simulate the action of nutrient uptake by plants and include neutral salts, chelating agents, and acids or bases.

According to Podlesakova (2001), ammonium acetate (1 M) is preferred for exchangeable metals because of its relatively high concentration and the metal complexing power of the acetate ion, which prevents readsorption or precipitation of released metal ions. The relatively high concentration of the ammonium ion results in the displacement of more of the metal cations adsorbed onto the soil and therefore provides a good indication of potential metal availability. Ure (1996) reported that acidic ammonium acetate is more effective in releasing the available metals from the soil as it releases the exchangeable metals and also those in the carbonate 'pool'. Ethylenediaminetetraacetic acid (EDTA) is a powerful chelating agent and is used extensively in soil science to determine the bioavailability of elements due to its non-selective nature. EDTA forms strong complexes with numerous heavy metals and is widely used to sequester di- and trivalent metal ions. It releases heavy metals from soil exchangeable and organically complexed 'pools' (Kabata-Pendias & Pendias, 1984).

The competition between the ions present in the soil also influences metal mobility and uptake by the plant. Synergism usually occurs when two metals compete for the same soil adsorption site. When the concentration of one metal increases in the soil, the soil retention capacity of the other metal is reduced (Prasad, 2006). This increases the bioavailability of the released metal. Thus, when both elements are present in the soil solution they exhibit enhanced bioavailability and reduced retention. Antagonism usually occurs when two metals are taken up by the plant by the same mechanism. Antagonism is more commonly associated with plant uptake as plant roots favour the uptake of the metal found in higher concentrations in the soil (Prasad, 2006).

An element is considered essential to plants if it is required for the completion of the life cycle of the plant, is not replaceable by another element, is directly involved in plant metabolism, and is required by a substantial number of plant species, not just a few. The quantity of a micronutrient that is available for plant uptake is dependent on many soil factors and parameters. According to Baker (1981) plants can be grouped into three categories: excluders, accumulators, and indicators. Excluders have low uptake of an element at quite high external concentrations of the element. Accumulators have high accumulation of an element at very low external concentrations of the element and if extremely high amounts of heavy metals are accumulated these plants are called hyperaccumulators. Excluders have some kind of barrier to avoid uptake whilst accumulators have certain detoxification mechanisms which allows for accumulation of such high amounts of metals (Baker, 2000). Indicators show poor control over metal uptake and transport processes, and the metal content in plants often reflects the

concentration in the soil. Different plant species and different genotypes of species have different efficiencies in taking up specific elements (Brooks & Robinson, 1989).

Nutrients for Humans

A nutrient is either a chemical element or compound used in an organism's metabolism or physiology. Macronutrients are nutrients needed in large amounts to provide calories or energy and include carbohydrates, proteins and fats (Walker, 1990). Micronutrients describe vitamins and minerals that are essential but only in very small amounts (McDowell, 2003). An element is essential to an organism if it cannot be synthesised by the organism and must be obtained from a food source. In the case of non-essential elements, the body has no need for the element at all (Harrison & de Mora, 1996). Some elements essential to man include Ca, Cr, Co, Cu, Fe, Mg, Mn, Ni, Se, and Zn and most of these elements are essential at low concentrations. Table 2 shows the recommended dietary allowances (RDAs) set to meet the needs of 97 to 98% of the individuals in a group.

The accumulation of trace metals usually due to anthropogenic causes are of concern due to adverse health effects which include chronic accumulation of metals in the kidney and liver of humans causing disruption of numerous biochemical processes (WHO, 1992), the development of cancer (Trichopoulos, 1997), and the development of abnormalities in children (Gibbes & Chen, 1989). The metals of concern are As, Cd, Cr, Cu, Hg, Pb, Ni, Se and Zn. Essential and non-essential elements are found in soil and at elevated concentrations can be toxic. These metals gain entry into human and animal food chains through crops grown on soils contaminated with them. Table 3 shows the Tolerable Upper

Intake Levels (ULs) which is the maximum level of daily nutrient intake that is likely to pose no risk of adverse effects.

Table 2: Dietary Reference Intakes (DRIs) - Recommended Intakes for Individuals (Food and Nutrition Board, Institute of Medicine, National Academies).

Life Stage	Ca	Cr	Cu	Fe	Mg	Mn	Se	Zn
Males	(mg d^{-1})	(µg d^{-1})	(µg d^{-1})	(mg d^{-1})	(mg d^{-1})	(mg d^{-1})	(µg d^{-1})	(mg d^{-1})
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	310	1.8	55	8
> 51 y	1,200	20	900	8	320	1.8	55	8

.

Table 3: Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels (ULs) (Food and Nutrition Board, Institute of Medicine, National Academies).

Males/ Females	As	Ca	Cr	Cu	Fe	Mg[a]	Mn	Ni	Se	Zn
(Life Stage)		(g d^{-1})		(µg d^{-1})	(mg d^{-1})	(mg d^{-1})	(mg d^{-1})	(mg d^{-1})	(µg d^{-1})	(mg d^{-1})
9 - 13 y	ND	2.5	ND	5,000	40	350	6	0.6	280	23
14 - 18 y	ND	2.5	ND	8,000	45	350	9	1	400	34
19 - 70 y	ND	2.5	ND	10,000	45	350	11	1	400	40
>70 y	ND	2.5	ND	10,000	45	350	11	1	400	40

ND = Not determinable.

[a] UL from Mg represents intake from supplements and fortified foods and do not include intake from food and water.

Plants in this Study

Two plants, *Carissa macrocarpa* and *Harpephyllum caffrum*, both found in KwaZulu-Natal and with reported medicinal use ((National Research Council, 2008; van Wyk, 2002; Pujol, 1996) were chosen for this study. Furthermore, these plants also contain fruits and can be easily collected and consumed should they contain compounds with beneficial properties.

Taxonomy of Carissa species

The plant family Apocynaceae (A. L. de Jussieu, Dogbane Family) comprises five subfamilies which include Rauvolfioideae, Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae. The subfamily Rauvolfioideae comprises eleven tribes, one of which is Carisseae Dumort. (1829), which is characterized by shrubs with spines in leaf axils that exudate milky latex and indehiscent fleshy fruit with a nonfibrous pericarp that contains less than twelve seeds (Bruyns, 2000). Two genera, *Acokanthera* G. Don (1838) and *Carissa* C. Linnaeus (1767) belong to this tribe. The genus *Carissa* contains species that are native to tropical and subtropical regions of Africa, Australia, and Asia. In Africa, they occur in two vast belts from Senegal to Sudan and from Ethiopia to South Africa. *Carissa macrocarpa* and *Carissa bispinosa* are two species that are indigenous to South Africa.

The lineage for *C. macrocarpa* is as follows:

Family: Apocynaceae

Subfamily: Rauvolfioideae

Tribe: Carisseae

Genus and species: *Carissa macrocarpa*

Carissa macrocarpa

Figure 1: *Carissa macrocarpa*

Carissa macrocarpa (Figure 1) is a common coastal species and hedge plant found on sand dunes and coastal regions in the Eastern Cape and KwaZulu-Natal, South Africa. It is a large twiggy, densely branched shrub with stems that contain long, strong, bifurcated spines. It bears a cluster of white fragrant flowers that develop into red, plum-like, fleshy fruit, the inside of which contains a white mottling and about twelve small, brown, flat seeds. All parts of the plant contain milky latex. The fruits usually grow in summer but many off-season fruits are also produced if conditions are favourable. In South Africa, the fruits are significant resources for informal/street traders and large quantities are sold along the roadside. The fruit can be eaten out of hand, added to deserts or made into a jam. Despite their widespread use and potential as a greater crop, this versatile foodstuff is not currently produced commercially.

An ethnomedicinal and phytochemical review of common Carissa species

An ethnomedicinal survey of *Carissa* species revealed widespread and diverse use of different morphological parts of the plants to treat a variety of medical conditions. Leaves of *C. macrocarpa* are used by the Zulu people to treat diarrhea in livestock and to treat coughs and venereal diseases (National Research Council, 2008). Other *Carissa* species are also used in traditional systems of medicine in different parts of the world. Of these, *Carissa edulis* is used in African traditional medicine (Ibrahim et al., 2007), *Carissa carandas* in Ayurvedic systems of medicine (Hegde et al., 2009) and *Carissa opaca* is used by the *hakims* in Pakistan (Abbasi et al., 2010). With the exception of *C. macrocarpa*, these other *Carissa* species have received widespread scientific attention. There has only been one phytochemical study on *C. macrocarpa* and only the common triterpenoids lupeol, β-sitosterol, and ursolic acid were identified using thin layer chromatography (Wehmeyer, 1986; Zaki et al., 1981). Compounds previously isolated from *Carissa* species, in general, include triterpenoids, lignans, sesquiterpenes, and flavonoids (Table 4). The isolation of tritepenoids from this genus is recorded to have begun as early as 1968, where the pentacyclic triterpenoids, lupeol and β-sitosterol were isolated from *C. edulis* and *C. carandas* (Pakrashi et al., 1968). In this study, triterpenoids were the main compounds isolated from the *Carissa* species investigated.

Table 4: Traditional medicinal uses and compounds isolated from *Carissa* species.

Plant species	***Traditional use***	***References***	***Isolated compounds***	***References***
C.edulis (roots)	antiepileptic, anti-inflammatory, aphrodisiac, anthelmintic, respiratory infections, anti-ulcer, antidiarrheal, antidiabetic, antibacterial (typhoid), anti-cancer, antimalarial, anti-rheumatoid, diuretic, antipyretic, anti-rabies, venereal diseases	Dalziel, 1937; Irvine, 1961; Watt & Breyer-Brandwijk, 1962; Bentley, 1984; Gelfand et al., 1985; Bazeeb, 1991; Addis et al., 2001; Giday, 2001; Nedi, 2004; Jeruto, 2008	lupeol, β-sitosterol, caffeic acid, nortrachelogenin, carinol, carissanol, carissone, naringin, cryptomeridiol, β-eudesmol, 2-hydroxyacetophenone, ursolic acid, carenone, oleuropein, carissol, 3'-(4''-methoxyphenyl)-3-oxo-ropionyl hexadecanoate, β-amyrin	Pakrashi et al., 1968; Raina et al., 1971; Achenbach et al., 1983; Bentley et al., 1984; Achenbach et al., 1985; Mathuram et al., 1998; Rao et al., 2005; Festus et al., 2009; Aliyu et al., 2011
C. carandas (fruits, leaves, roots)	antipyretic, astringent, aphrodisiac, treatment of eating disorders, analgesic, antipruritic, antiepileptic, antiscorbutic, antimalarial, dermatological infections, antibacterial, anthelmintic, stomachic, anti-rabies, anti-inflammatory, antihyperglycemic	Kirtikar & Basu, 1935; Chatterji & Roy, 1965; Jayaweera, 1981; Burkill, 1985; Pino, 2004; Rahmatullah, 2009; Itankar et al., 2011	lupeol, β-sitosterol, ursolic acid, methyl ursolate, carissone, carindone, carinol, carissol, carissic acid, carissin, des-*N*-methylnoracronycine, 3β -hydroxy-27-*E*-feruloyloxyurs-12-en-28-oic acid, pelargonidin-3-*O*-glucoside, chrysoeriol 7-*O*-glycoside, quercetin 3-*O*-methyl-7-*O*-glucoside, apigenin 6-*C*-rhamnosil-7-*O*-rhamnoside	Pakrashi et al., 1968; Pal et al., 1975; Naim et al., 1985; 1988, Siddiqui et al., 2003; Sulaiman et al., 2008
C. opaca (fruits, leaves, stems)	antipyretic, aphrodisiac, antiviral (hepatitis)	Ahmad et al., 2009; Abbasi et al., 2009	2-hydroxyacetophenone, vitexin, isoquercetin, hyperoside, carissone, myricetin, kaempherol, α-farnesene	Rai et al., 2005; Mallavarupu et al., 2009
C. macrocarpa (leaves)	venereal diseases, coughs	National Research Council, 2008	lupeol, β-sitosterol, ursolic acid	Wehmeyer, 1986; Zaki et al., 1981

Taxonomy of Harpephyllum caffrum

The plant family Anacardiaceae Lindl., the cashew family, comprises two subfamilies (Anacardioideae and Spondioideae) and five tribes (Rhoeae, Anacardieae, Semecarpeae, Dobineeae, Spondiadeae) with 82 genera and more than 700 species (Pell et al., 2011). The genus *Harpephyllum* belongs to the Spondiadeae and is a single species genus. The genera are native to Africa, Southern Europe, tropical and subtropical Asia, tropical and subtropical Australia, and most of the Pacific Islands with *Harpephyllum caffrum* being indigenous to South Africa. The Anacardiaceae comprises trees, shrubs, and lianas with resin canals that contain a clear to milky sap. This family is well known for its cultivated edible, indehesant, one-seeded fruits, seeds, and lacquer plants. In the Anacardiaceae, morphological fruit diversity is exceedingly high with a myriad of types found in the family.

The lineage of *Harphephyllum caffrum* is as follows:

Family: Anarcardiaceae

Subfamily: Spondiadoideae

Tribe: Spondiadeae

Genus and species: *Harpephyllum caffrum*

Harpephyllum caffrum

Figure 2: *Harpephyllum caffrum*

H. caffrum (Figure 2) is a common street tree distributed throughout the Eastern part of Southern Africa with distribution in South Africa ranging from the Eastern Cape to KwaZulu-Natal and the Limpopo Province. It is a large, single-stemmed, perennial, erect, evergreen, deciduous tree that grows up to 15 m in height. In South Africa, *H. Caffrum* is a common street tree that is known as 'wild plum' in English, 'umgwenya' in isiZulu, and 'mothêkêlê' in Sotho. The tree has dark green, shiny leaves that are divided into several leaflets and whitish green flowers that are borne near the ends of the branches with male and female flowers on separate trees (van Wyk et al., 1997). The plum-like fruits usually ripen in autumn but many off-season fruits are also produced if conditions are favourable. The fruit contains a single seed and is enjoyed by birds, animals, insects, and humans. Although members of the family are cultivated throughout the world for their edible fruits and seeds namely mango (*Mangifera indica* L.), pistachio (*Pistacia vera* L.), and cashew (*Anacardium occidentale* L.), this is not the case with *H. caffrum* and the fruits are only eaten by the native population.

An ethnomedicinal survey revealed *H. caffrum* to be commonly used in South African traditional medicine to treat, manage and control a variety of human ailments. These include being used as blood purifiers to treat skin conditions like acne and eczema, as analgesics, and to manage and control childhood convulsions and epilepsy (van Wyk, 2002; Pujol, 1996). The aqueous extract of the stem bark is reported to possess hypoglycaemic, hypotensive, anticonvulsant, and analgesic properties (Ojewole, 2006; 2007). Other species belonging to the Anacardiaceae are also used in traditional systems of medicine in different parts of the world. Of these, different morphological parts of *Mangifera indica* L. are used in traditional South Asian medicine and the root and root bark of *Ozoroa insignis* Del. and different morphological parts of *Anacardium occidentale L.* are used in African traditional medicine. The chemical constituents of *H. Caffrum* are not well known but ethanolic extracts from the leaves have been reported to contain protocatechuic acid, gallic acid, methyl gallate, quercetin, and kaempferol using relatively primitive techniques such as thin layer chromatography and UV spectroscopy (El Sherbeiny & El Ansari, 1976). Compounds isolated from other trees belonging to the same family include anacardic acid, anacardic acid methyl esters, cardols, cardanols, flavonoids, and triterpenoids (Table 5).

Table 5: Traditional medicinal uses and compounds isolated from plants belonging to the Anacardiaceae.

Plant species	***Traditional use***	***References***	***Isolated compounds***	***References***
Anacardium occidentale L. (shell, nut oil, fruits, bark, seeds)	antidiarrheal, antivenom, antimicrobial, astringent, anti-inflammatory, diuretic, hypoglycemic	Dahake et al., 2009	resorcinolic acid, anacardic acid, carotenoids, cardols, cardanols, flavonoids, kaempferol 3-O-glucoside, (3-O-galactoside, 3-O-glucoside, 3-O-xylopyranoside, 3-O-arabinopyranoside, 3-O-arabinofuranoside and 3-O-rhamnoside) of myricetin and quercetin	Assuncao & Mercadante, 2003; de Brito, 2007
Ozoroa insignis Del. (root, root bark)	antidiarrheal , venereal diseases, anthelmintic, kidney infections, analgesic, antimalarial, anthelmintic	Abreu & Liu, 2007, Liu & Abreu, 2006a	6-nonadecyl anacardic acid, ozoranone A, β-amyrin, betulinic acid, magnificol, betulonic acid, 6-tridecyl anacardic acid, 6-[8(*Z*)-pentadecenyl] anacardic acid, 6-[10(*Z*)-heptadecenyl] anacardic acid, anacardic acid methyl esters, cardanols, tirucallane triterpenes	Mwihaki et al., 2009; Liu & Abreu, 2006a; Liu & Abreu, 2006b
Mangifera indica (bark, fruits, leaves, peel)	anti-inflammatory, laxative, antibacterial, antiasthmatic, decongestant, antidiarrheal, anthelmintic, aphrodisiac	Singh, 1986; Sairam et al., 2003; Sharma et al., 1971	Mangiferin, homomangiferin, gallic acid, methyl gallate, methyl gallate ester, ellagic acid,isoquercitrin, 3,4-dihydroxy benzoic acid, quercetin pentoside,flavonoid glucosides	Nong et al., 2005; Barreto et al., 2008
Harpephyllum caffrum (fruits, leaves, stems)	dermatological infections, analgesic, antiepileptic, anticonvulsant	van Wyk, 2002; Pujol, 1996	protocatechuic acid, gallic acid, methyl gallate, quercetin, kaempferol	El Sherbeiny & El Ansari, 1976

REFERENCES

Abbasi, AM, Khan, MA, Ahmad, M, Zafar M, Khan, H, Muhammad N, Sultana S. 2009. Medicinal plants used for the treatment of jaundice and hepatitis based on socio-economic documentation. African Journal of Biotechnology, 8:1643-1650.

Abbasi, AM, Khan, MA, Ahmad, M, Zafar, M. 2010. Herbal medicines used to cure various ailments by the inhabitants of Abbottabad district, North West Frontier Province, Pakistan. Indian Journal of Traditional Knowledge, 9: 175-183.

Abreu, P, Liu, Y. 2007. Ozoroalide, a new orsellinic acid type macrolide from *Ozoroa insignis*. Fitoterapia, 78: 388-389.

Achenbach, H, Waibal, R, Addae-Mensah, I. 1983. Constituents of West African medicinal plants. Part 12. Lignins and other constituents from *Carissa edulis*. Phytochemistry, 22: 2325-2328.

Achenbach, H, Waibal, R, Addae-Mensah, I. 1985. Constituents of West African medicinal plants. Part 17. Sesquiterpenes from *Carissa edulis*. Phytochemistry, 24: 1056-1067.

Addis, G, Abebe D, Urga, K. 2001. A survey of traditional medicinal plants in Shirka district, Arsi zone, Ethiopia. Ethiopian Pharmaceutical Journal, 19: 30-47.

Ahmad, SS, Mahmood, F, Dogar, Z, Khan, ZI, Ahmad, K, Sher, M, Mustafa, I, Valeem, EE. 2009. Prioritization of medicinal plants of Margala Hills National Park, Islamabad, on the basis of available information. Pakistan Journal of Botany, 41: 2105-2114.

Assuncao, RB, Mercadante, AZ. 2003. Carotenoids and ascorbic acid from cashew apple (*Anacardium occidentale* L.): variety and geographic effects. Food Chemistry, 81: 495-502.

Baker, AJM, McGrath, SP, Reeves, RD, Smith, JA. 2000. Metal hyperaccumulator plants: A review of the ecology and physiology of a biological resource for phyto-remediation of metal polluted soil. *In* Phytoremediation. Lewis Publishers: Boca Raton, pp 85-108.

Baker, AJM. 1981. Accumulators and excluders – strategies in the response of plants to heavy metals. Journal of Plant Nutrition, 3: 643-654.

Banas, A, Kwiatek, WM, Banas, K, Gajda, M, Pawlicki, B, Cichocki, T. 2010. Journal of Biological Inorganic Chemistry, 15: 1147-1155.

Barreto, JC, Trevisan, MTS, Hull, WE, Erben, G, De Brito, ES, Pfundstein, B, Rtele, GW, Spiegelhalder, B, Owen, RW. 2008. Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (*Mangifera indica* L.). Journal of Agricultural and Food Chemistry, 56: 5599-5610.

Bazeeb, AS. 1991. The Medicinal Plants in Yemen. EL-Ershad Press: Sana'a, Yemen.

Bentley, MD, Brackett, SR, Chapya A. 1984. 2-hydroxyacetophenone: principal root volatile of the East African medicinal plant, *Carissa edulis*. Journal of Natural Products, 47: 1056-1057.

Birkeland, PW. 1999. Soils and Geomorphology, 3rd Edition. Oxford University Press: New York.

Bodeker, G, Kabatesi, D, King, R, Homsy, J. 2000. A regional task force on Traditional Medicine and AIDS. Lancet, 355: 1284.

Bot, A, Benites, J. 2005. The Importance of Soil Organic Matter. Key to drought-resistant soil and sustained food and production. Food and Agriculture Organization of the United Nations: Rome.

Brady, NC, Weil, RR. 1999. The Nature and Properties of Soils. Prentice-Hall: Upper Saddle River, New Jersey.

Brookes, KB, Dutton, MF. 2007. Bioactive compounds of the uteroactive medicinal plant, *Gunnera perpensa* (or Ugobo). South African Journal of Science, 3: 187-189.

Brooks, RR, Robinson, BH. 1989. Aquatic phytoremediation by accumulator plants. In Plants that Hyperaccumulate Heavy Metals, Their Role in Phytoremediation, Microbiology, Archaeology, Mineral Exploration and Phytomining. CAB International: Washington, DC, pp 119-131.

Burkill, HM. 1985. The Useful Plants of West Africa. Second Edition. Vol. 1. Families A-D. Royal Botanical Gardens: Kew, pp 145-146.

Chaney, RL. 1988. Metal Speciation and Interaction Among Elements Affect Trace Element Transfer in Agricultural and Environmental Food Chains. Lewis Publications: Boca Raton, Florida, pp 219-259.

Chatterji, ML, Roy, AR. 1965. Pharmacological action of *Carissa carandas* root. Bulletin of the Calcutta School of Tropical Medicine, 13:14-6.

Dahake, AP, Joshi, VD, Joshi, AB. 2009. Antimicrobial screening of different extract of *Anacardium occidentale* Linn. leaves. International Journal of ChemTech Research, 1: 856-858.

Dalziel, JM. 1937. The Useful Plants of West Tropical Africa. The Crown Agencies for the Colonies: London.

de Britoa, SE, de Arau´jo, MCP, Lin, L, Harnly, J. 2007. Determination of the flavonoid components of cashew apple (*Anacardium occidentale*) by LC-DAD-ESI/MS. Food Chemistry, 105: 1112-1118.

El Sherbeiny, AEA, El Ansari, MA. 1976. The polyphenolics and flavonoids of *Harpephyllum caffrum*. Planta Medica, 29: 129-132.

Endress, ME, Bruyns, PV. 2000. A revised classification of the Apocynaceae s.l. The Botanical Review, 66: 1-56.

Festus, TM, Geoffrey, RM, John, OM, Quang, N, Hashimoto, K, Asakawa, Y. 2009. The antiviral activity of compounds isolated from Kenyan *Carissa edulis* (Forssk.) Vahl. The Internet Journal of Alternative Medicine, 8: 12.

Food and Nutrition Board, Institute of Medicine, Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels, Elements, The National Academies Press, Washington, DC, 2001.

Gelfand, M, Mavi, S, Drummond, RB, Ndemera, B. 1985. The Traditional Medical Practitioner in Zimbabwe. Mambo Press: Zimbabwe.

Gibbes, H, Chenn, C. 1989. Evaluation of issues relating to carcinogens- risk assessment of chromium. Science of the Total Environment, 86: 181-186.

Giday, M. 2001. An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia, CBM: s Skriftserie 3, Uppsala 2001, 81-99.

Gunn, AM, Winnard, DA, Hunt, DTE. 1988. Trace Metal Speciation in Sediments and Soil. Lewis Publications: Boca Raton, Florida, pp 261-294.

Harrison, RM, De Mora, SJ. 1996. Introductory Chemistry for Environmental Sciences. Cambridge University Press: Cambridge, pp 194-195.

Hegde, K, Thakker, SP, Joshi, AB, Shastry, CS, Chandrashekhar KS. 2009. Anticonvulsant activity of *Carissa carandas* Linn. root extract in experimental mice. Tropical Journal of Pharmaceutical Research, 8: 117-125.

Herzog, F, Farah, Z, Amado, R. 1993. International Journal for Vitamin and Nutritional Research, 63: 234-238.

Ibrahim, H, Bolaji, RO, Abdurahman, EM, Shok, M, Ilyas, N, Habib, AG. 2005. Preliminary phytochemical and antimicrobial studies of the leaves of *Carissa edulis* VAHL. ChemClass Journal, 2: 15-18.

Irvine, FR. 1961. Woody Plants of Ghana. Oxford University Press: London, pp 616-618.

Itankar, PR, Lokhande, SJ, Verma, PR, Arora, SK, Sahu, RA, Patil, AT. 2011. Antidiabetic potential of unripe *Carissa carandas* Linn. fruit extract. Journal of Ethnopharmacology, 135: 430-433.

Jahan, R. 2009. A survey of medicinal plants in two areas of Dinajpur district, Bangladesh including plants which can be used as functional foods. American-Eurasian Journal of Sustainable Agriculture, 3: 862-876.

Jayaweera, DMA. 1981. Medicinal Plants used in Ceylon. The National Science Council of Sri Lanka: Colombo, pp 85.

Jeruto, P, Lukhoba, C, Ouma, G, Otieno, D, Mutai, C. 2008. An ethnobotanical study of medicinal plants used by the Nandi people in Kenya. Journal of Ethnopharmacology, 116: 370-6.

John, DA, Leventhal, JS. 1996. Bioavailability of metals. In Preliminary Compilation of Descriptive Geoenvironmental Mineral Deposit Models. U.S. Geological Survey Open-File Report 95-0831, pp 10-18.

Kabata-Pendias, A, Pendias, H. 1984. Trace Elements in Soils and Plants. CRC Press: Boca Raton, Florida.

King, R. 2000. Collaboration with Traditional Healers in HIV/AIDS Prevention and Care in Sub-Saharan Africa. UNAIDS: Geneva, Switzerland.

Kirtikar, KR, Basu, BD. 1935. Indian Medicinal Plants Vol II. Lalit Mohan Basu: Allahabad, India.

Liu, Y, Abreu, P. 2006a. Tirucallane triterpenes from the roots of *Ozoroa insignis*. Phytochemistry, 67: 1309-15.

Liu, Y, Abreu, P. 2006b. Long chain alkyl and alkenyl phenols from the roots of *Ozoroa insignis.* Journal of Brazilian Chemical Society, 17: 527-532.

Mallavarupu, GR, Mishra, RK, Chaudhary, S, Pandey, R, Gupta, S, Kumar, S, Kaul, VK, Pathania, V. 2009. 2-Hydroxyacetophenone, the main component of the essential oil of the roots of *Carissa opaca* Stapf ex Haines. Journal of Essential Oil Research, 21:385-387.

Mathee, A, von Schirnding, YER, Levine, J, Ismail, A, Huntley, R, Cantrell, A. 2002. A survey of blood lead levels among young Johannesburg school children. Environmental Research, 90: 181-184.

Mathuram, V, Brahmadhayalaselvam, A, Hussain, AJ, Rao, RB, Patra, A. 1998. Chemical constituents of *Carissa spinarum* and their antibacterial activity. Journal of Indian Chemical Society. 75: 262-264.

Mbatha, N, Street, RA, Ngcobo M, Gqaleni N. 2012. Sick certificates issued by South African Traditional Health Practitioners, current legislation, challenges and the way forward. South African Medical Journal, 102(3): 129-131.

McDowell, LR. 2003. Minerals in Animals and Human Nutrition. Elsevier Science B.V.: Amsterdam, The Netherlands, pp 1-4.

Mwihaki, NM, Hussain, H, Chhabra, S, Langat-Thoruwa, C, Krohn, K. 2009. Chemical constituents from the root bark of *Ozoroa insignis*. Biochemical Systematics and Ecology, 37: 116-119.

Naim, Z, Khan, MA, Nizami, SS. 1985. Isolation of a new triterpenic alcohol from *Carissa carandas.* Pakistan Journal of Scientific and Industrial Research, 28: 378-381.

Naim, Z, Khan, MA, Nizami, SS. 1988. Isolation of a new isomer of ursolic acid from fruits and leaves of *Carissa carandas*. Pakistan Journal of Scientific and Industrial Research, 31: 753-755.

National Research Council. 2008. Lost Crops of Africa: Volume III: Fruits. The National Academies Press: Washington, DC.

Nedi, T, Mekonnen, N, Urqa, K. 2004. Diuretic effect of the crude extract of *Carissa edulis* in rats. Journal of Ethnopharmacology, 95: 57-61.

Nong, C, He, W, Fleming, D, Pan, L, Huang, H. 2005. Capillary electrophoresis analysis of mangiferin extracted from *Mangifera indica* L. bark and *Mangifera persiciformis* C.Y. Wu et T.L. Ming leaves. Journal of Chromatography B, 826: 226-231.

Ojewole, JAO. 2006. Hypoglycemic and hypotensive effects of *Harpephyllum caffrum* Bernh. ex CF Krauss (Anacardiaceae) stem-bark aqueous extract in rats. Cardiovascular Journal of South Africa, 17: 67-72.

Ojewole, JAO. 2007. Anticonvulsant and analgesic effects of *Harpephyllum caffrum* Bernh. ex CF Krauss (Anacardiaceae) stem-bark aqueous extract in mice. International Journal of Pharmacology. 3: 241-247.

Pakrashi, SC, Datta, S, Ghosh-Dastidar, PP. 1968. Indian medicinal plants, XVII. Phytochemical examination of *Carissa* SPP. Phytochemistry, 7: 495-496.

Pal, R, Kulshreshtha, DK, Rastogi, RP. 1975. A new lignan from C*arissa carandas*. Phytochemistry, 14: 2302-2303.

Pell, SK, Mitchell, JD, Miller, AJ, Lobova, TA. 2011. Anacardiaceae. *In* The Families and Genera of Vascular Plants. Volume X. Flowering Plants Eudicots, Spindales, Cucurbitales, Myrtaceae, Kubitzki, K, (Ed.) Springer-Verlag: Berlin, Heidelberg.

Pino, JA, Marbor, R, Vazquez, C. 2004. Volatile flavour constituents of Caranda (*Carissa carandas* L.) fruit. Journal of Essential Oil Research, 16: 432-434.

Podlesakova, E, Nemecek, J, Vacha, R. 2001. Mobility and bioavailability of trace metals in soils. In Trace Elements in Soils: Bioavailability, Flux and Transfer. Lewis Publishers: Boca Raton, Florida.

Prasad, MNV, Sajwan, KS, Naidu, R. 2006. Trace Elements in the Environment: Biogeochemistry, Biotechnology, Bioremediation. CRC Press: Boca Raton, Florida.

Pujol, J. 1996. Naturafrica- The Herbalist Handbook. Jean Pujol Natural Healers' Foundation: Durban.

Rahmatullah, M, Noman, A, Hossan, MS, Harun-Or-Rashid, M, Rahman, T, Chowdhury, MH,

Rai, SK, Mallavarapu, GR, Rai, SP, Srivastara, S, Sing, D, Mishra, R, Kuma S. 2005. Constituents of the flower oil of *Carissa opaca* growing in the Aravalli mountain range at New Delhi. Flavour and Fragrance Journal, 21: 304-305.

Raina, MK, Bhatnagar, JK, Atal, CK. 1971. Isolation of caffeic-acid from the roots of *Carissa spinarum*-D. Indian Journal of Pharmacy, 33: 76-7.

Rao, RJ, Kumar, US, Reddy, SV, Tiwari, AK, Rao, JM. 2005. Antioxidants and a new germacrane sesquiteroene from *Carissa spinarum*. Natural Products Research, 19: 763-769.

Rengasamy, P, Churchman, GJ. 1999. Cation exchange capacity, exchangeable cations and sodicity. In Soil Analysis: an Interpretation Manual. CSIRO Publishing: Collingwood, pp 147-157.

Sairam, K, Hemalatha, S, Kumar, A, Srinivasan, T, Ganesh, J, Shankar, M, Venkataraman, S. 2003. Evaluation of anti-diarrhoeal activity in seed extracts of *Mangifera indica*. Journal of Ethnopharmacology, 84: 11-15.

Shackleton, SE, Shackleton, CM, Netshiluvhi, TR, Geach, BS, Ballance, A, Fairbanks, DHK. 2005. Use patterns and value of savanna resources in three rural villages in South Africa. Economic Botany, 56: 130-146.

Sharma, LD, Bahga, HS, Srivastava, PS. 1971. *In vitro* anthelmintic screening of indigenous medicinal plants against *Haemonchus contortus* of sheep and goats. Indian Journal of Animal Research, 5: 33-38.

Siddiqui, BS, Ghani, U, Ali, ST, Usmani, SB, Begum, S. 2003. Triterpenoidal constituents of the leaves of *Carissa carandas*. Natural Product Research, 17: 153-158.

Singh, YN. 1986. Traditional medicine in Fiji. Some herbal folk cures used by Fiji Indians. Journal of Ethnopharmacology, 15: 57-88.

Smith, SR. 1996. Agricultural Recycling of Sewage Sludge and the Environment. Biddles Ltd: Guilford.

Sulaiman, SF, Shuang, KH, Yusof, SR. 2008. The antioxidant effect of *Carissa carandas* unripe fruit extracts and fractions. Project Report: USM.

Symeonides, A, McRae, SS. 1977. The assessment of plant available cadmium in soils. Journal of Environmental Quality, 6: 120-122.

Taiz, L, Zeiger, E. 2002. Plant Physiology. Sinauer Associates: Sunderland.

Trichopoulos, D. 1997. Epidemiology of cancer. *In*: Cancer, Principles and Practice of Oncology. Lippincott Company: Philadelphia, pp 231-258.

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

Vvn Wyk, BE, van Oudtshoorn, B, Gericke, N. 2002. Medicinal Plants of South Africa. Briza Publications: Pretoria, South Africa, pp 146-147.

Walker, AF. 1990. Human Nutrition. Cambridge University press: Cambridge, pp 3-19.

Watt, JM, Breyer-Brandwijk, MG. 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. E & S Livingstone Ltd.: Edinburgh, pp 609-670.

Wehmeyer, AS. 1986. Edible Wild Plants of Southern Africa: Data on Nutrition Contents of Over 300 Species. Council for Scientific and Industrial Research: Pretoria.

WHO. 1992. Cadmium- Environmental Health Criteria. World Health Organisation: Geneva, Switzerland, pp 34.

WHO. 2002. Traditional medicine strategy 2002-2005. World Health Organisation: Geneva, Switzerland.

Zaki, AY, El-Tohamy, SF, El-Fattah, SA. 1981. Study of lipid content and volatile oil of the different organs of *Carissa carandas* Lin. and *Carissa grandiflora* Dc. growing in Egypt. Egyptian Journal of Pharmaceutical Science, 22: 127-141.

CHAPTER 2

Antibacterial and anti-adhesion activity of the Pentacyclic Triterpenoids isolated from the leaves and edible fruits of Carissa macrocarpa

ABSTRACT

Four pentacyclic oleanane triterpenes (β-amyrin, methyl oleanolate, oleanolic acid and 3β-hydroxyolean-11-en-28,13β-olide) were isolated from the fruits of C*arissa macrocarpa* whilst the ursane triterpene, ursolic acid, was isolated from the leaves. 3β-hydroxyolean-11-en-28,13β-olide has only been found once previously (in the Lamiaceae) and its lactone ring, which contributes significantly to the expression of antibacterial activity in oleanane triterpenes, makes it an interesting molecule for synthetic and biological studies. The immune boosting properties of the triterpene rich edible fruits of *C. macrocarpa* are important, especially in South Africa, due to high incidences of HIV and hepatitis in this country.

Keywords: *Carissa macrocarpa*, *Amatungula*, oleanane triterpenes, ursolic acid.

INTRODUCTION

The dependence on uncultivated resources in sub-Saharan Africa, where purchased food is substituted with indigenous or wild fruit and vegetable, is well-documented (UNAIDS, 1999). The dependence on traditional medicine and medicinal plants by the rural poor for the treatment of human immunodeficiency virus (HIV) related infections such as venereal diseases, tuberculosis, diarrhea and appetite loss have also been reported (Bodeker, 2000; King, 2000). The reliance on wild foods and medicinal plants for nutrition, treatment and care is primarily a function of economic and physical accessibility. This can result in the decline of natural resources of commonly used medicinal plants as indicated by traditional healers at the 13th International AIDS Conference in Durban, South Africa, in 2000. This necessitates the identification of other natural resources, with the same nutritional and medicinal benefits. One such plant is *Carissa macrocarpa* 'Tomlinson' (Ecklon) A. DC, of the family Apocynaceae, which is indigenous to KwaZulu-Natal (KZN), South Africa (Botha & Botha, 1997). *C. macrocarpa* is a small, evergreen, twiggy shrub, found in abundance in KZN, with star-shaped white scented flowers, Y-shaped thorns and tasty red fruit (known as the *Amatungula* by the Zulu people of South Africa) that are enjoyed by both birds and children (Botha & Botha, 1997). The fruit is reputed to be rich in vitamin C, Ca, Mg, and P (Wehmeyer, 1966). Leaves of *C. macrocarpa* are used by the Zulu people to treat diarrhea in livestock. Different morphological parts are used in South African folk medicine to treat coughs and venereal diseases (National Research Council, 2008).

Other *Carissa* species are also used in traditional systems of medicine in different parts of the world. Of these, *C. edulis* is used in African traditional medicine (Ibrahim et al., 2005); *C.*

carandas in Ayurvedic systems of medicine (Hegde et al., 2009); and *C. lanceolata* by the Aboriginal communities of Western Australia (Lindsay et al., 2000). These *Carissa* species have received widespread scientific attention (Nedi et al., 2004; Siddiqui et al., 2002) whilst no phytochemical studies have been done on *C. macrocarpa* despite its role in South African traditional medicine. Furthermore, since the fruits are eaten by children, a phytochemical study to determine the types of compounds present in the fruit is essential.

Thus far, a preliminary phytochemical analysis and pharmacological screening has been done on the crude extracts of *C. macrocarpa* and some ubiquitous triterpenoids (lupeol, β-sitosterol and ursolic acid) were identified using thin layer chromatography, a technique that is neither quantitative nor absolute and enables only a tentative identification of the compound (Wehmeyer, 1986; Zaki et al., 1981). In this study, the isolation, characterization and identification of the phytocompounds from the edible fruits and leaves of *C. macrocarpa* was undertaken. Compounds isolated from other *Carissa* species include *inter alia* cardiac glycosides (Rastogi et al., 1969), lignans (Achenbach et al., 1983), sesquiterpenes (Achenbach et al., 1985) and triterpenes (Siddiqui et al., 2002). The antimicrobial activities of selected isolated compounds were also determined to evaluate the plant's potential to control microbial manifestations on biotic or abiotic surfaces.

MATERIALS AND METHODS

General experimental procedure

IR spectra were obtained using a Perkin Elmer Spectrum 100 FT-IR spectrometer with Universal ATR sampling accessory. NMR spectra were recorded in deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO) at room temperature on a Bruker Avance^{III} 400 spectrometer with tetramethylsilane (TMS) as internal standard. The ^{13}C-NMR spectral assignments were made by comparison of chemical shifts with literature data (Mahato & Kundu, 1994; Pereda-Miranda & Delgado, 1990), by analysis of DEPT spectra for the determination of primary, secondary and tertiary carbons and by use of 2D techniques (COSY, HSQC and HMBC). GC-MS data were recorded 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. He (2 mL min^{-1}) was used as a carrier gas and acetonitrile (ACN) was used to dissolve the sample. The injector was kept at 250 °C whilst the transfer line was at 280 °C. The column temperature was held at 50 °C for 2 min, and then ramped to 280 °C at 20 °C min^{-1} where it was held for 15 min. The MS was operated in the EI mode at 70 eV. Melting points were recorded on an Ernst Leitz Wetzlar micro-hot stage melting point apparatus and are uncorrected. All chemicals used were supplied by Merck and Sigma Chemical Companies and were of analytical-reagent grade.

Plant materials

The fruits and leaves from *C. macrocarpa* were collected from the University of KwaZulu-Natal (UKZN), Westville campus, South Africa, in May 2009. These were identified by

taxonomist, Prof. A. Nicholas, from the School of Biological and Conservation Sciences, UKZN, Westville and a voucher specimen (Moodley, R1) was deposited in the ward herbarium at UKZN.

Extraction and isolation

Ground fruit (900 g) and ground leaves (450 g) were subjected to exhaustive extraction with hexane, dichloromethane (DCM) and methanol (MeOH) by maceration and continuous shaking on an orbital shaker at room temperature for 48 h. The solvent extracts were concentrated by use of a rotary evaporator and the crude extracts were stored at 4 ºC for further analysis. The crude extracts were subjected to column chromatography (Merck Kieselgel 60, 0.063-0.200 mm, 70-230 mesh ASTM) on suitably sized columns and the fractions monitored by TLC (Merck silica gel 60, 20 x 20 cm F254 aluminium sheets) which was visualized using anisaldehyde spray reagent (97: 2: 1; MeOH: conc. H_2SO_4: anisaldehyde) and analyzed under UV (254 nm).

For the crude DCM extract from the fruits (3.0 g), a hexane: ethyl acetate step gradient was used on a 4 cm diameter column starting with 10% ethyl acetate in hexane. This was increased to 20%, then 50% ethyl acetate and finally 100% ethyl acetate. Twenty five fractions of 40 mL each were collected for each solvent system. Fractions 26-44 were combined to yield fraction A and fractions 71-82 were combined to yield fraction B. Fraction A was rechromatographed over silica gel in a 2 cm column with 100% hexane to yield compound **A-1** (12.65 mg) which started to elut after 20 mL. Fraction B was rechromatographed over silica gel in a 2 cm column with 100% DCM (50 mL) followed by 10% EtOAc in DCM (50 mL) and 20% EtOAc in DCM (50 mL). Fifteen fractions of 10 mL

each were collected, with fractions 7-10 yielding compound **A-5** (12.61 mg). Fractions 12-15 were combined and recrystallisation with $CHCl_3$: MeOH (1:1) afforded compound **A-2** (40.52 g).

The crude MeOH extract (10.0 g) from the fruits was dissolved in water and subjected to partitioning with an equal volume of EtOAc. The EtOAc fraction was then dried with anhydrous Na_2SO_4 and subjected to column chromatography on a 4 cm diameter column with 100% hexane (50 mL) followed by 100% DCM (100 mL). Ten fractions of 10 mL each were collected. Compound **A-3** (50.35 mg) eluted in fractions 6-10 yielding white crystals upon evaporation.

The crude DCM extract from leaves (8.85 g) was subjected to column chromatography on a 4 cm diameter column. For elution, a mobile phase consisting of a hexane:DCM and DCM:ethyl acetate gradient was used, starting with 100% hexane stepped to 50% and 100% DCM, which was further stepped to 20% and 40% ethyl acetate. Ten fractions of 50 mL each were collected in each step. Fractions 36-38 with similar TLC profiles were combined and purified on a 1.5 cm diameter column using 100% DCM (3 x 40 mL), stepped to 50% ethyl acetate (3 x 40 mL) and finally 100% ethyl acetate (3 x 40 mL). Compound **A-4** (1.78 g) was obtained in fractions 6-9.

The physical and spectroscopic data for compounds **A-1-4** matched those of Mahato and Kundu (Mahato & Kundu, 1994). The NMR data for compound **A-5** matched those of Pereda-Miranda and Delgado for most, but not all resonances, therefore NMR data is provided (Pereda-Miranda & Delgado, 1990).

3β-hydroxyolean-11-en-28,13β-olide (**A-5**), colourless crystals, melting point 260-262ºC; EI-MS *m/z* : 454 [M^+], 345, 281, 207, 55, 43; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.76 (3H, s, Me-25), 0.86 (3H, s, Me-24), 0.89 (3H, s, Me-26), 0.95 (3H, s, Me-27), 0.96 (3H, s, Me-30), 1.03 (3H, s, Me-23), 1.22 (3H, s, Me-29), 3.20 (1H, dd, *J*=11.55, 4.77 Hz, H-3), 5.39 (1H, dd, *J*=10.35, 3.03 Hz, H-11), 6.02 (1H, d, *J*=10.35 Hz, H-12); ^{13}C-NMR ($CDCl_3$, 400 MHz): δ 180.00 (C-28), 135.83 (C-12), 126.92 (C-11), 89.80 (C-13), 78.83 (C-3), 54.78 (C-5), 53.21 (C-9), 50.54 (C-18), 44.03 (C-17), 41.60 (C-14), 41.41 (C-8), 38.92 (C-4), 38.26 (C-1), 37.34 (C-19), 36.35 (C-10), 34.37 (C-7), 33.26 (C-29), 31.42 (C-20), 31.16 (C-21), 29.68 (C-22), 27.75 (C-23), 27.16 (C-2), 27.01 (C-16), 25.39 (C-15), 23.54 (C-30), 18.96 (C-27), 18.27 (C-26), 17.93 (C-24), 17.65 (C-6), 14.90 (C-25).

Determination of minimum inhibitory concentrations (MICs)

MICs were determined by broth microdilution method (Andrews, 2001). Three Gram-negative strains (*Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 35032 and *Escherichia coli* ATCC 25922) and four Gram-positive strains (*Staphylococcus saprophyticus* ATCC 35552, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 43300 and *Enterococcus faecium* ATCC 19434) were selected for study. Bacterial strains used as stock cultures were grown on Tryptic soy agar (TSA) and kept at 4ºC throughout the study. For MIC determinations, cultures were inoculated by suspending one isolated colony from TSA plates in 3 mL Triptone soy broth (TSB). After 18 h of growth at 37ºC, the suspensions were centrifuged and pellets were resuspended in sterile distilled water to obtain final inoculums of 5×10^8 cfu mL^{-1}, equivalent to a 0.5 McFarland standard (Andrews, 2001). Stock solutions of the isolated phytocompounds were at 10 mg mL^{-1} in

100% DMSO. The 96-well microtitre plates were prepared by dispensing 90 µL of Mueller-Hinton (M-H) broth into each well after which bacterial inoculum (10 µL) was added to the wells. Thereafter, appropriate volumes of the test compound stock solutions were dispensed into the wells to obtain two-fold dilutions of test concentrations ranging from 0.001 mg mL^{-1} to 2 mg mL^{-1} . This was done in triplicate for each test concentration. Tetracycline, at the concentration range of 0.04 - 32 µg mL^{-1}, was used as the standard antimicrobial agent for comparison. All plates contained a medium control to test for sterility as well as growth control wells which contained only medium and bacterial inoculum without any test compound. All plates were incubated at 37ºC for 24 h. The MIC for each test bacterium was considered as the lowest concentration of the test compound that prevented visible growth.

Bacterial adhesion

The anti-adhesion effect of oleanolic acid, ursolic acid and methyl oleanolate against *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 35032), *K. pneumoniae* (ATCC 700603), *E. faecium* (ATCC 19434), *S. aureus* (ATCC 25923), *S. aureus* (ATCC 43300) and *S. saprophyticus* (ATCC 35552) was determined using a modified microtitre plate protocol (Basson et al., 2008). Due to insufficient sample, β-amyrin and 3β-hydroxyolean-11-en-28,13β-olide could not be tested.

Bacterial strains were cultured overnight in TSB, then washed and resuspended in sterile distilled water to a turbidity equivalent to a 0.5 McFarland standard. Each well of a sterile 96-well U-bottomed microtiter plate was filled with 90 µL Luria Bertani broth (LB) and 10 µL of the selected cultures. Based on pre-determined MICs for each test compound, the effect of MIC, sub-MIC (0.5×MIC) and supra-MIC (2×MIC) of oleanolic acid, ursolic acid

and methyl oleanolate on bacterial adhesion was investigated. Plates were incubated aerobically at 37˚C for 24 h with shaking on an Orbit P4 microtiter plate shaker (Labnet).

The supernatant in each well was aspirated and then washed three times with 250 µL of sterile distilled water to remove planktonic bacteria. Adherent bacteria were fixed with 200 µL of 99% methanol for 15 min. Methanol was removed and plates were left to dry. Subsequently, 150 µL of 2% Hucker crystal violet was added and left to stand for 5 min to effect staining. Excess stain was rinsed off by thorough washing under running water and plates were left to air dry (Basson et al., 2008). Bound stain in each well was resolubilised with 150 µL of 33% (v/v) glacial acetic acid and the concentration of crystal violet was determined by measuring the optical density (OD) of destaining solution at 595 nm using a Fluoroskan Ascent F1 spectrophotometer (Thermolabsystems).

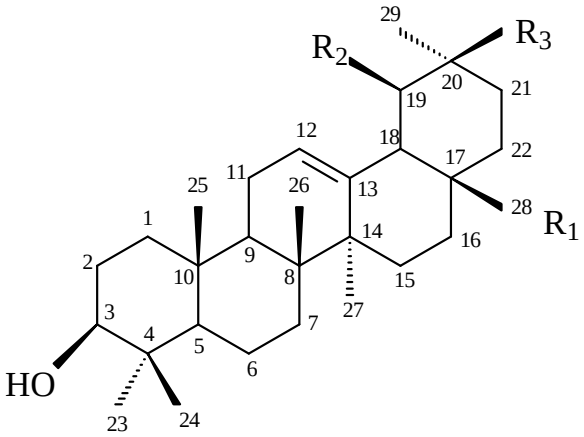
All experiments were performed in triplicate, repeated twice and the data was averaged to produce final results (Stepanović et al., 2000). The negative control for both assays was un-inoculated LB to test for sterility and non-specific binding of media, while the positive control was LB with respective cell suspensions without oleanolic acid, ursolic acid or methyl oleanolate. OD_{595nm} values of treated cells were compared to untreated cells to investigate the increase or decrease in adhesion as a result of antimicrobial agent exposure. Treated and untreated samples were compared statistically using paired t-tests and Wilcoxon signed rank tests if normality failed (SigmaStat V3.5, Systat Software, Inc).

RESULTS AND DISCUSSION

Structure elucidation

The dichloromethane (DCM) extract from the fruits of *C. macrocarpa* yielded three compounds, β-amyrin (**A-1**), methyl oleanolate (**A-2**) and 3β-hydroxyolean-11-en-28,13β-olide (**A-5**) whilst the methanol extract yielded one compound, oleanolic acid (**A-3**). The DCM extract from the leaves of *C. macrocarpa* yielded ursolic acid (**A-4**) only. EI-MS, IR spectroscopy, 1D-NMR (^{1}H, ^{13}C-NMR and DEPT) and 2D-NMR spectroscopy (COSY, HSQC and HMBC) together with data published in the literature were used to identify the compounds. Compounds **A-1-4** (Figure 3) are common triterpenes found widely in many plant species; however compound **A-5** (Figure 4) has only been isolated previously from *Hyptis albida* in the Lamiaceae family (Pereda-Miranda & Delgado, 1990).

For compounds **A-1-3**, the ^{1}H-NMR spectrum showed characteristic resonances at δ_H 2.81 (H-18), a triplet at δ_H 5.26 (H-12) and a doublet at δ_H 3.21 (H-3). The only structural difference in these compounds occurred at C-28 and therefore the resonances of H-3, H-12 and H-18 are similar. The H-3 and H-12 resonances of **A-4** are also similar; however the H-18 resonance occurred at δ_H 2.25, consistent with that of ursolic acid and the methyl group at C-19 instead of the tertiary carbon atom with two methyl groups at C-20. The ^{13}C-NMR and DEPT spectra for compounds **A-1-4** had the required methyl, methylene, methine and quaternary carbon resonances for β-amyrin, methyl oleanolate, oleanolic acid, and ursolic acid and the molecular ion peaks were observed at *m/z* 426 for **A-1**, 470 for **A-2** and 456 for **A-3** and **A-4,** respectively. The NMR data compared well with the data in the literature for these compounds (Mahato & Kundu, 1994).



β-amyrin (**A-1**): R_1=CH_3; R_2=H; R_3=CH_3

methyl oleanolate (**A-2**): R_1=$COOCH_3$; R_2=H; R_3=CH_3

oleanolic acid (**A-3**): R_1=COOH; R_2=H; R_3=CH_3

ursolic acid (**A-4**): R_1=COOH; R_2=CH_3; R_3=H

Figure 3. Chemical structures of compounds A-1-4.

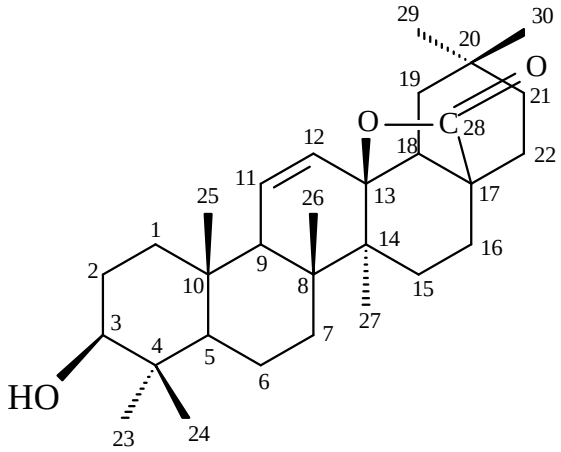


Figure 4. Chemical structure of 3β-hydroxyolean-11-en-28,13β-olide (A-5).

The ^{1}H-NMR spectrum for compound **A-5** showed a resonance at δ_H 3.21, for H-3, similar to the other four compounds. Two olefinic resonances at δ_H 5.39 (1H, dd, *J*=10.35, 3.03 Hz, H-11) and δ_H 6.02 (1H, d, *J*=10.35 Hz, H-12) were also observed. The ^{13}C-NMR spectrum for compound **A-5** resolved 30 carbon resonances comprising seven methyl, nine methylene, six methine, seven quaternary and one carbonyl resonance, identified using the DEPT spectrum. The carbon resonances in the A and B rings were similar to compounds **A-1**, **A-2** and **A-3**. This is typical of oleanane triterpenes where the geometry of the D/E ring junction does not cause significant alterations in the shielding of carbons in the A/B rings (Mahato & Kundu, 1994). The C-11 and C-12 resonances occurred at δ_C 126.90 and δ_C 135.80, respectively and the carbonyl resonance at δ_C 180.00 was assigned to C-28 which correlated to H-18 in the HMBC spectrum. The C-13 resonance occurred at δ_C 89.80. The data above compared well with the data in literature for 3β-hydroxyolean-11-en-28,13β-olide (Pereda-Miranda & Delgado, 1990) as confirmed by the molecular ion peak, M^+ at *m/z* 454, corresponding to the formula, $C_{30}H_{46}O_3$.

Antimicrobial activity

The pentacyclic triterpenoids, oleanolic acid and ursolic acid, and their derivatives show appreciable antibacterial activity (Krystyna et al., 2010) with the position of the hydroxyl group influencing the activity of the compound (Djoukeng et al., 2005). The present study shows that the pentacyclic triterpenes, β-amyrin **(A-1)**, methyl oleanolate **(A-2**), oleanolic acid **(A-3)** and ursolic acid **(A-4),** have moderate antibacterial activity (MICs in the range 0.12 – 1.0 mg mL^{-1}) whilst the oleanolic acid derivative, 3β-hydroxyolean-11-en-28,13β-olide **(A-5)**, has good antibacterial activity (MICs in the range 0.06 – 0.12 mg mL^{-1}) for all

bacterial strains studied (Table 6). The addition of a lactone ring contributes significantly to the expression of antibacterial activity in oleanane triterpenes. No difference in the extent of activity between Gram-positive and Gram-negative bacteria was observed indicating that the mode of antibacterial activity of these pentacyclic triterpenes is unaffected by the cell wall structural differences between these organisms. Most of the isolated compounds have a bacteriostatic (bacteria-inhibiting) effect on the studied microorganisms which is favoured in some cases especially when treating immuno-competent patients with urinary tract infections (Gleckman, 1975).

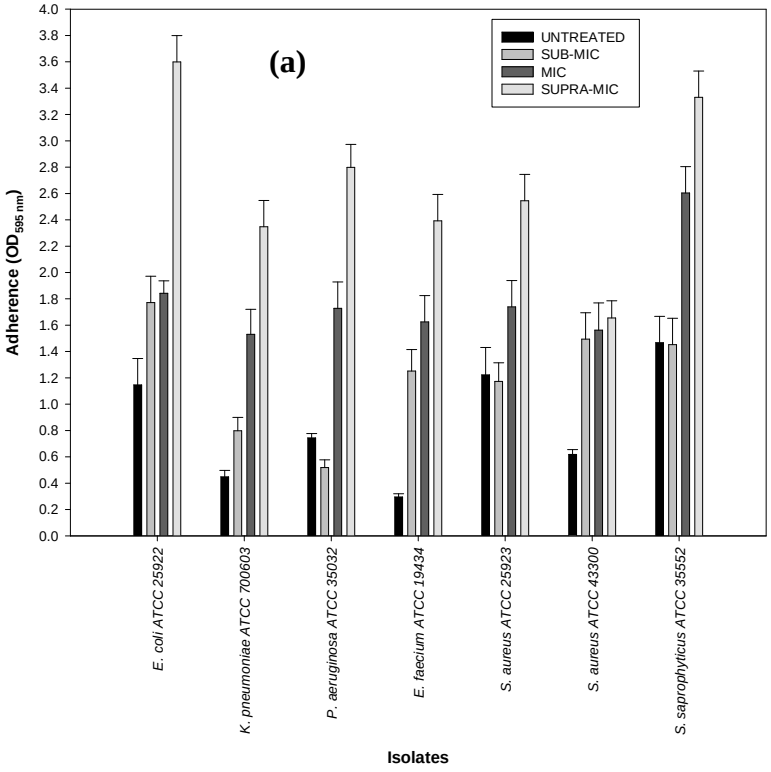
Table 6: Minimum inhibitory concentrations (MICs), in mg mL^{-1} , of compounds A-1-5 isolated from *Carissa macrocarpa*, against Gram-positive and Gram-negative bacteria.

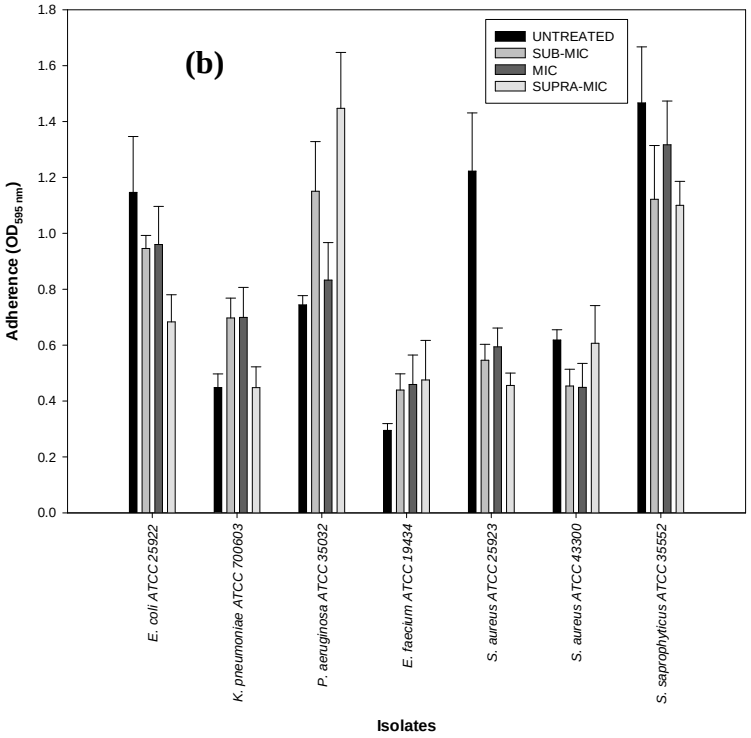
	MIC (mg mL^{-1})					
	A-1	**A-2**	**A-3**	**A-4**	**A-5**	**T[a]**
S. aureus (ATCC 25923)	0.25	0.25	0.25	0.25	0.06	1
S. aureus (ATCC 43300)	0.25	0.25	0.25	0.25	0.06	2
E. faecium (ATCC 19434)	0.25	0.25	0.25	0.5	0.12	8
S. saprophyticus (ATCC 35552)	0.25	0.25	1.0	0.25	0.12	4
E. coli (ATCC 25922)	0.12	0.25	1.0	0.25	0.06	2
K. pneumonia (ATCC 700603)	0.5	0.5	1.0	0.5	0.12	8
P. aeruginosa (ATCC 35032)	1.0	1.0	0.5	0.5	0.06	32

[a] Reference antibiotic in µg mL^{-1}; **T**: Tetracycline; **A-1**: β-amyrin; **A-2**: methyl oleanolate; **A-3**: oleanolic acid; **A-4**: ursolic acid; **A-5**: 3β-hydroxyolean-11-en-28,13β-olide

Increased adhesion to abiotic surfaces was observed for 100% of bacterial strains following all three oleanolic acid **(A-3)** exposures (Figure 5a), of which the increases observed following MIC and Supra-MIC exposures were statistically significant. Oleanolic acid **(A-3**) which increases the adhesion of Gram-positive and Gram-negative bacteria may be used in applications that need to limit the migration of pathogenic bacteria like in groundwater aquifers (Li & Logan, 1999). For methyl oleanolate **(A-2)**, variable effects were observed (Figure 5b), with 57% of bacterial strains displaying decreased adhesion. The decreased adherence for *E. coli, S. aureus* and *S. saprophyticus* indicated that methyl oleanolate **(A-2)** interfered with the ability of these microorganisms' to adhere to polystyrene surfaces. Increased adhesion was observed for the remaining three microorganisms demonstrating the ability of this compound to promote their biofilm formation.

Decreased adherence was also observed for *E. coli* and *S. aureus* bacterial strains with all three concentrations of ursolic acid **(A-4)** (Figure 5c). For *P. aeruginosa*, decreased adhesion was observed only with sub-MIC and MIC exposures, whilst increased adhesion was observed at supra-MIC exposure. In contrast, *S. saprophyticus* adhesion was increased at sub-MIC exposure but decreased following MIC and supra-MIC exposures. Of the three compounds tested, ursolic acid **(A-4)** demonstrated the greatest ability to prevent bacterial colonization, with 71% of bacterial strains showing decreased adhesion at most ursolic acid **(A-4)** exposures. It was interesting to note that the adhesion of *K. pneumonia,* the gram-negative opportunistic pathogen most frequently implicated in nosocomial infections, was increased following exposure to all three compounds at all concentrations. The reason for this is unclear.


(a)
UNTREATED
SUB-MIC
MIC
SUPRA-MIC
Adherence (OD595 nm)
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
E. coli ATCC 25922
K. pneumoniae ATCC 700603
P. aeruginosa ATCC 35032
E. faecium ATCC 19434
S. aureus ATCC 25923
S. aureus ATCC 43300
S. saprophyticus ATCC 35552
Isolates


(b)
UNTREATED
SUB-MIC
MIC
SUPRA-MIC
Adherence (OD595 nm)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
E. coli ATCC 25922
K. pneumoniae ATCC 700603
P. aeruginosa ATCC 35032
E. faecium ATCC 19434
S. aureus ATCC 25923
S. aureus ATCC 43300
S. saprophyticus ATCC 35552
Isolates

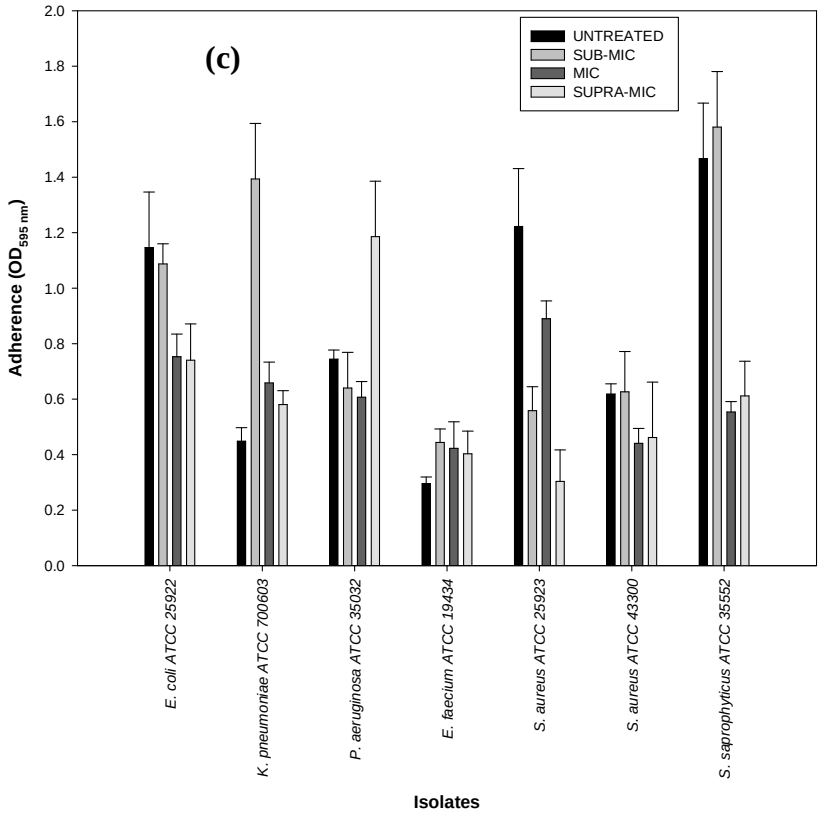


Figure 5. Alterations in adhesion profiles of Gram-positive and Gram-negative bacteria following sub-MIC, MIC and supra-MIC exposures of (a) oleanolic acid (b) methyl oleanolate and (c) ursolic acid.

Importance of the phytochemical constituents in the edible fruits

Oleanolic acid is known for its hepatoprotective effects (Oliveira et al., 2005). Consequently, it is used to treat acute and chronic hepatitis in China (Liu, 1995). In South Africa, due to the number of people living in informal settlements, the spread and prevalence of the hepaptitis virus is high (Tucker et al., 1996). Consumption of the easily accessible *Amatungula* fruit that has hepatoprotective effects should therefore be encouraged. Oleanolic acid is known for

its anti-inflammatory effects without causing ulcerations, unlike Aspirin (Singh et al., 1992); β-amyrin is more potent than Aspirin in inhibiting platelet aggregation (Ching et al., 2010) therefore consumption of the *Amatungula* fruit that is rich in oleanolic acid and β-amyrin can be a natural alternative to Aspirin, which is not readily available in the rural parts of South Africa, and may promote the health and well-being of the local people. Children eating the *Amatungula* fruit also benefit from the anti-cariogenic properties of the compounds that it holds (Hada et al., 1990). Oleanolic acid also exhibits hypoglycemic (Hao et al., 1989), anti-lipidemic (Liu et al., 1987) and anti-cancer activities (Li et al., 2002) so the benefits of ingestion of the *Amatungula* fruit are numerous.

CONCLUSION

Five pentacyclic triterpenes were isolated from the leaves and fruits of *C. macrocarpa*. All four of the triterpenes isolated from the fruits of *C. macrocarpa* had the oleanane skeleton and appear to be derived biosynthetically from oleanolic acid whilst only the ursane triterpene, ursolic acid, was isolated from the leaves. MICs show that the addition of a lactone ring to the oleanane backbone contributes significantly to the expression of antibacterial activity in pentacyclic oleanane triterpenes. For most microorganisms studied, oleanolic acid tended to promote adhesion to abiotic surfaces whilst methyl oleanolate and ursolic acid tended to inhibit it. The anti-adhesion effect of the three studied compounds were in the increasing order of oleanolic acid < methyl oleanolate < ursolic acid. The immune boosting properties of the compounds contained in the fruits of *C. macrocarpa* are important, especially for children in KZN, due to high incidences of HIV and hepatitis in this area. The cost of immune boosting supplements is out of reach for the indigenous people of South

Africa therefore freely available fruit such as the *Amatungula* is a welcome source of these compounds. This study lends scientific credence and validity to the ethnomedicinal use of *C. macrocarpa* and highlights the medicinal benefits of consuming the indigenous edible fruit.

Acknowledgements

The authors are thankful to UKZN for financial support.

REFERENCES

Achenbach H, Waibel R, Addae-Mensah I. 1983. Lignins and other constituents from *Carissa edulis*. Phytochemistry. 22: 749-753.

Achenbach H, Waibel R, Addae-Mensah, I. 1985. Sesquiterpenes from *Carissa edulis*. Phytochemistry. 24: 2325-2328.

Andrews JM. 2001. Determination of inhibitory concentrations. J. Antimicrob. Chemoth. 48: 5-16.

Basson A, Flemming LA, Chenia HY. 2008. Evaluation of adherence, hydrophobicity, aggregation and biofilm development of Flavobacterium johnsoniae-like isolates. Microbial Ecol. 55: 1-14.

Bodeker G, Kabatesi D, King R, Homsy J. 2000. A regional task force on Traditional Medicine and AIDS. Lancet. 355: 1284.

Botha C, Botha J. 1997. Bring Nature back to your garden. Durban: Natal Region of the Wildlife and Environment Society.

Ching J, Chua TK, Chin LC, Lau AJ, Pang YK, Jaya JM, Tan CH, Koh HL. 2010. β-amyrin from *Ardisia elliptica* Thunb. is more potent than aspirin in inhibiting collagen-induced platelet aggregation. Indian J. Exp. Biol. 48: 275-279.

Djoukeng JD, Abou-Mansour E, Tabacchi R, Tapondjou AL, Bouda H, Lontsi D. 2005. J. Ethnopharmacol. 101: 283-286.

Gleckman RA. 1975. Trimethoprim-sulfamethoxazole vs. ampicillin in chronic urinary tract infections: a double-blind multicentre cooperative controlled study. J. Am. Med. Assoc. 233: 427-431.

Hada S, Hattori T, Namba T. 1990. Dental caries prevention by traditional medicines. Effect of components of *Ganoderma lucidum* on glucosyltransferase from Streptococcus mutans. Chem. Abstracts. 113: 91423f.

Hao Z, Hang B, Wang Y. 1989. Hypoglycemic effect of oleanolic acid. Zhougguo Yaoke Daxue Xuebao. 22: 210-212.

Hegde K, Thakker SP, Joshi AB, Shastry CS, Chandrashekhar KS. 2009. Anticonvulsant activity of *Carissa carandas* Linn. root extract in experimental mice. Trop. J. Pharmaceut. Res. 8: 117-125.

Ibrahim H, Bolaji RO, Abdurahman EM, Shok M, Ilyasand N, Habib AG. 2005. Preliminary phytochemical and antimicrobial studies of the leaves of *Carissa edulis* VAHL. ChemClass J., 2: 15-18.

King R. 2000. Collaboration with traditional healers in HIV/AIDS prevention and care in sub-Saharan Africa. Geneva, Switzerland: UNAIDS.

Krystyna IW, Grudniak AM, Fiecek B, Kraczkiewicz-Dowjat A, Kurek A. 2010. Antibacterial activity of oleanolic and ursolic acids and their derivatives. Cent. Eur. J. Biol. 5: 543-553.

Li J, Guo WJ, Yang QY. 2002. Effects of ursolic acid and oleanolic acid on human colon carcinoma cell line HCT15. World J. Gastroentero. 8: 493-495.

Li Q, Logan BE. 1999. Enhancing bacterial transport for bioaugmentation of aquifers using low ionic strength solution and surfactants. Water Resour. 33: 1090-1100.

Lindsay EA, Berry Y, Jamie FJ. 2000. Antibacterial compounds from *Carissa lanceolata* R.Br. Phytochemistry. 55:403-406.

Liu J. 1995. Pharmacology of oleanolic acid and ursolic acid. J. Etnopharmacol. 49: 57-68.

Liu J, Chen XF, Xia L, Geng XZ, Li ZS. 1987. Effect of oleanolic acid on serum glyceride cholesterol and β-lipo-proteins in normal and experimental hyperlipedermia rats. Chinese Pharmacol. Bull. 4: 14-15.

Mahato SB, Kundu AP. 1994. ^{13}C NMR spectra of pentacyclic triterpenoids – A compilation and some salient features. Phytochemistry. 37: 1517-1575.

National Research Council. 2008. Lost Crops of Africa, Vol III: Fruits. Washington, D.C.: The National Academies Press.

Nedi T, Mekonnen N, Urqa K. 2004. Diuretic effect of the crude extract of *Carissa edulis* in rats. J. Ethnopharmacol. 95: 57–61.

Oliveira FA, Chaves MH, Almeida FRC, Lima RCP, Silva RM, Maia JL, Brito GA, Santos FA, Rao VS. 2005. Protective effect of α- and β-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March trunk wood resin, against acetaminophen-induced liver injury in mice. J. Ethnopharmacol. 98: 103-108.

Pereda-Miranda R, Delagdo R. 1990. Triterpenoids and flavonoids from *Hyptis albida*. J. Nat. Prod. 53: 182-185.

Rastogi RC, Kulshreshtha DK, Rastogi RP. 1969. Cardioactive constituents from *Carissa spinarum*. Indian J. Chem. 7: 1102-1104.

Siddiqui BS, Ghani U, Ali ST, Usmani SB, Begum S. 2002. Triterpenoidal constituents of the leaves of *Carissa carandas*. Nat. Prod. Res. 17: 153-158.

Singh GB, Singh S, Bani S, Gupta BD, Banerjee SK. 1992. Anti-inflammatory activity of oleanolic acid in rats and mice. J. Pharm. Pharmacol. 44: 456-458.

Stepanović S, Vuković D, Dević I, Savić B, Švabić-Vlahović M. 2000. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J. Microbiol. Meth. 40: 175-179.

Tucker TJ, Kirsch RE, Louw SJ, Isaacs S, Kannemeyer J, Robson SC. 1996. Hepatitis E in South Africa: evidence for sporadic spread and increased seroprevalence in rural areas. J. Med. Virol. 50: 117-119.

UNAIDS. 1999. A review of household and community responses to the HIV/AIDS epidemic in the rural areas of sub-Saharan Africa. UNAIDS, Geneva, Switzerland.

Wehmeyer AS. 1986. Edible wild plants of Southern Africa: data on nutrition contents of over 300 species. Council for Scientific and Industrial Research, Pretoria.

Wehmeyer AS. 1966. The nutrient composition of some edible wild fruits found in the Transvaal. S. Afr. Med. J. 40: 1102-1104.

Zaki AY, El-Tohamy SF, El-Fattah SA. 1981. Study of lipid content and volatile oil of the different organs of *Carissa carandas* Lin. and *Carissa grandiflora* Dc. growing in Egypt. Egypt J. Pharm. Sci. 22: 127-41.

CHAPTER 3

Elemental composition and fatty acid profile of the edible fruits of Amatungula (Carissa macrocarpa) and impact of soil quality on chemical characteristics

---

ABSTRACT

The *Amatungula* fruit, from *Carissa macrocarpa*, is frequently consumed by the local people of KwaZulu-Natal (KZN), South Africa. Levels of elements in the fruit were determined to assess if they conform to recommended dietary allowances (RDAs) and to assess for potential toxicities. Lipid profiling was done to determine the fruits potential as a source of essential fatty acids. Levels of elements in the plant as a function of geographic location and soil composition was assessed and the plants control on elemental uptake evaluated. Soils and fruit samples from nine sites in eastern KZN were investigated. Concentrations of elements in the fruit were found to be in the increasing order of Ni<Cr<Se<Mn≈Cu≈Pb<Zn<Fe<Mg<Ca. For the elements in focus, except for Pb, all of the elements found in the fruit contribute significantly towards the RDAs for most individuals. The fruit was rich in monounsaturated and essential fatty acids with the linoleic acid to α-linolenic acid ratio conforming to the recommended range for cardiac health. Concentrations of elements in soil had no significant effect on plant concentrations but competition between elements in soil influenced availability. Lead availability was significantly correlated to total soil concentrations of most metals studied showing the effect of these metals on Pb solubility. The plant showed ability to

accumulate Pb, with Pb levels in fruit at all sites being toxic to human health. Site location had a major effect on plant concentrations however uptake and distribution was primarily dependent on the plants inherent controls, as evidenced by the accumulation and exclusion of elements, to meet its physiological requirements.

Keywords: indigenous fruit, elemental composition, nutrition, soil quality, bioaccumulation, *Carissa macrocarpa*

INTRODUCTION

In sub-Saharan Africa, where nutrient deficiencies are common, and where malnutrition and food insecurity are critical concerns amongst HIV/AIDS afflicted households, providing foods which are not readily available is extremely difficult. A study of 150 rural households in South Africa reported that wild fruits were frequently consumed by the local people, whilst another study reported that gathered fruits make up half of the fruits eaten by the locals, yearly (Shackleton et al., 2004; Herzog et al., 1993). Research has shown that vulnerable communities and rural households usually turn to natural resources for food security. A low intake of the right type of food predisposes these communities to micronutrient deficiencies, childhood malnutrition, and protein-energy malnutrition (essential fatty acid deficiency) which are of concern in South Africa. To address the dietary concerns associated with malnutrition, the nutritional quality and energy content of the diet must be assessed.

Plants are intermediate reservoirs through which metals from soil are transferred to humans. Although most metals are essential to plant growth, the ability of plants to take up and translocate these metals depends, to a large extent, on the soil matrix and plant genotype. Once metals are taken up and transferred through the agricultural food chain, they can threaten human health (whether essential or non-essential) if at elevated levels. The consumption of medicinal plants in South Africa by the local people is common practice. Consumption of the organic constituents from medicinal plants is essential for therapeutic effectiveness but does not preclude intake of the inorganic constituents. Reliance on plants collected from the wild by both traditional healers and the local people warrants regulations

on its safety as industrial infringements can lead to contamination. Studies on the elemental composition of these plants to monitor the levels of trace metals ingested, in case of toxic effects needs to be undertaken. Several epidemiological studies undertaken in South Africa have indicated that a large percentage of South Africans have unacceptably high blood Pb levels; other studies have shown considerable levels of trace metals that have been associated with diseases like cancer (Mathee et al., 2002; Banas et al., 2010). The role of the diet as a determinant in chronic and terminal diseases needs to be evaluated by the quantitative determination of elements in edible plants to identify potential risks of exposure.

Carissa macrocarpa, of the plant family Apocynaceae, is an indigenous medicinal plant in KwaZulu-Natal, South Africa. The fruit, known as the *Amatungula* by the Zulu people, is reputed to be rich in vitamin C and is enjoyed by the locals especially children (Wehmeyer, 1996). The potential of the fruit to boost food security has been identified and the leaves are used by the Zulu people to treat diarrhea in livestock (National Research Council, 2008). Despite the role of the *Amatungula* fruit in South Africa, information on its nutritional quality is lacking. This study was undertaken to establish the contribution of the fruit to the nutritional needs of the local people by comparing to recommended dietary allowances (RDAs) for most individuals. The lipid fraction of the fruit was also profiled to determine its potential as a source of essential fatty acids.

The need to monitor medicinal plants and associated soil to assess for heavy metal contamination in the soil-plant-animal continuum is vital due to accessibility and reliance on these plants. The natural variability of heavy metal content in soils, which is heightened by pollution through anthropogenic activities, is high. Therefore, to assess for contamination,

soil analysis should be done by comparing with approved threshold levels. There is no information available concerning the elemental composition of the *Amatungula* fruit and the impact of soil quality on its elemental uptake. This study is aimed at investigating the elemental concentrations in the fruit as a function of geographic location and soil quality parameters to determine their impact on elemental uptake and to assess for metal contamination. The 12 elements selectively investigated from nine sampling sites were As, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Se, and Zn.

MATERIALS AND METHODS

Sample collection

Amatungula fruit and soil samples were collected from nine different sites in the KwaZulu-Natal east coast region which is part of the Savanna Biome during the month of March. Sites were flat and the climate humid and subtropical. Soils were generally sandy or loamy sand in texture. The chosen sites were: Site 1–Berea, Site 2–Canelands, Site 3–Park Rynie, Site 4–Pinetown, Site 5–Port Edward, Site 6–Port Shepstone, Site 7–Shelley Beach, Site 8–Uvongo, and Site 9–Westville (Fig. 6). Samples of tree-ripened fruit were picked from trees and placed into plastic bags. Thereafter, fruit samples were cut into halves and oven dried at 60°C for 24 h to ensure complete removal of moisture. Dried fruit samples were ground in a food processor (Braun range) to obtain a powder, which were stored in a refrigerator in polyethylene bags until analyzed. Soil samples were systematically collected from six points around the tree at a depth of 15 cm with the use of a plastic spade. Representative soil samples were composited in a clean plastic bucket to achieve homogeneity and reduced to

500 g by quartering. Soil samples from each site were passed through a 2 mm mesh sieve to remove gravel then dried overnight in an oven at 40°C, after which time all moisture was removed. Afterwards, the soil was crushed to reduce particle size with a mortar and pestle. Samples were stored in polyethylene bags and kept at 4°C in a refrigerator until analyzed.

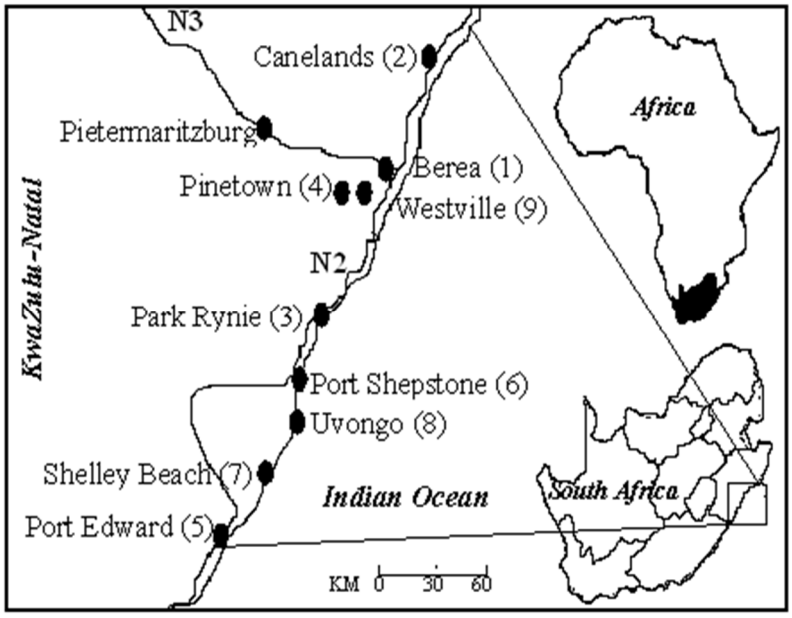


Figure 6. Map of sampling sites.

Reagents and standards

All chemicals used were supplied by Merck and Sigma Chemical Companies and were of analytical-reagent grade. Standard fatty acids (Gas chromatography (GC) grade) were supplied by Fluka. Double distilled water was used throughout the experiments. Working elemental standards for calibrations were prepared from spectroscopic grade stock standard solutions (1000 mg L^{-1}). Glassware and other equipment were cleaned with 6.0 M HNO_3 and rinsed off with double distilled water to prevent contamination.

Fatty acid extraction, methylation and analysis

Solvent extractions were performed on 10.0 g of dried fruit samples using hexane and a Soxhlet extractor for 8 h thereafter solvent was removed by use of a rotary evaporator. For methylation of oils, approximately 0.50 g of extracted oil was accurately weighed into PTFE lined screw-capped glass bottles with 2 mL of 1 mg mL^{-1} internal standard (pentadecanoic acid) solution prepared in toluene and 3 mL of fresh solution of 10% methanolic HCl, using a micropipette. Bottles were sealed and placed in a water bath at 70ºC for 2 h. Thereafter, 5 mL of 6% K_2CO_3 solution and 1 mL of toluene was added and thoroughly vortexed for 1 min. The organic phase was separated from aqueous phase by centrifugation at 1100 rpm for 5 min after which the organic phase was dried with anhydrous Na_2SO_4 and filtered using a Millipore 0.45 µm filter membrane. The volume injected into the GC was 0.1 µl. Peaks were identified by comparison with standard fatty acids that were prepared similar to sample. All samples were stored in an atmosphere of N_2. An Agilent Cerity-6820 GC apparatus equipped with DB-wax fused silica capillary column (30 m x 320 µm, 0.25 mm i.d., 0.25 µm film thickness) was used with He as front inlet carrier gas and N_2 as front detector makeup gas. The injector (split mode) and flame ionization detector were at 280ºC and oven was at 150ºC. The column temperature was held at 150ºC for 1 min, ramped to 200ºC at 25ºC min^{-1} where it was held for 1 min, and finally increased to 240ºC at a rate of 5ºC min^{-1} where it was held for 4 min. Unidentified peaks were identified by gas chromatography–mass spectrometry (GC-MS) using 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. He (2 mL min^{-1}) was used as a carrier gas and toluene was used to dissolve the sample. The injector was kept at 250ºC

whilst the transfer line was at 280ºC. The column temperature was held at 50ºC for 2 min, and then ramped to 280ºC at 20ºC min^{-1} where it was held for 15 min. The MS was operated in the EI mode at 70 eV. Mass spectra of all detected compounds were compared to spectra in the NIST 98 library for identification.

Extraction of bioavailable metals

A solution containing a combination of chemical extractants was prepared by diluting 38.542 g ammonium acetate ($NH_4CO_2CH_3$), 25 mL acetic acid (CH_3COOH) (96%) and 37.225 g ethylenediaminetetraacetic acid (EDTA) to 1L. Approximately 5.0 g of dry soil samples were accurately weighed into 250 mL polyethylene bottles, to which 50 mL of extractant solution was added and shaken in a laboratory shaker for 2 h. Thereafter, this solution was centrifuged for 10 min at 6000 rpm and then filtered on Millipore 0.45 µm filter membranes to permit analysis of extracted metals. All samples were stored in polyethylene bottles.

Elemental analysis

The microwave-assisted closed vessel digestion technique was used for digestion of fruit and soil samples due to its superior digestion capability and sample throughput. Digestions were performed using the Anton Paar Multiwave Microwave Sample Preparation System (1000 W) with six high-pressure Teflon (TFM) lined ceramic vessels (HF 50). To ensure improved precision, three sub-samples of each (both fruit and soil) were digested. Fruit (0.3 g) samples were weighed into the ceramic vessels; thereafter 5 mL of 69% HNO_3 was added to each vessel and sealed. The power was ramped to 500 W for the first 5 min, where it remained for the next 5 min, then ramped to 650 W for 15 min during which complete digestion occurred.

The microwave power was reduced and the bombs cooled by forced ventilation for 15 min. The same procedure applied to the soil samples (0.2 g) except with a harsher digestion program. The power was ramped from 100 W to 600 W for 10 min then ramped from 600 W to 900 W for the next 12 min. The microwave power was reduced and the bombs cooled by forced ventilation for 15 min. Fruit and soil digests were transferred to 50 mL volumetric flasks, diluted to the mark with double distilled water and stored in polyethylene bottles for elemental analysis.

All extracted and digested samples were analyzed for As, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Se, and Zn by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) due to its multi-element determination capability, high dynamic linear range and sensitivity. All measurements were performed using the Perkin Elmer ICP-OES with radial plasma observation. Analytical wavelengths were selected based on minimum spectral interferences and maximum analytical performance. Initially, the three most sensitive lines were chosen. From these lines, the lines with no interfering elements were selected. Method validation for elemental analysis was done by concurrent analysis of certified reference material (CRM), lyophilized brown bread (BCR 191), from the Community Bureau of Reference of the Commission of the European Communities. Values for Ca and Mg are indicative since these were additional tests, to those required for certification, determined by several laboratories.

Soil organic matter (SOM), cation exchange capacity (CEC) and soil pH

The pH of soil was determined by measuring a 1:1 soil / water suspension using a pH meter fitted with a glass electrode. SOM was estimated using the wet chemistry extraction technique known as the Walkley-Black Method (Walkley & Black, 1934). The pH 7.0

ammonium acetate method was used for the determination of CEC in the soil (Chapman, 1965).

Bioaccumulation factors

The relative accumulation of metals taken up by plants can be calculated by dividing the concentration of the metal in the plant by the concentration in the soil. This relative accumulation is known as the bioaccumulation factor (BAF).

$$BAF = \frac{[Metal]_{plant}}{[Metal]_{soil}}$$

The BAF can be obtained for both total and bioavailable amounts of metals found in soil.

Statistical analysis

An analysis of covariance was performed with concentration in fruit as response variable, site and element as factors, and total and available soil concentrations as covariates. Analysis of covariance (ANCOVA) allows to compare one variable in 2 or more groups taking into account (or to correct for) variability of other variables, called covariates. Significance of plant-soil relationships and soil competition effects were established by obtaining Pearson's product-moment correlation coefficients. All statistical analyses were done using the Statistical Package for the Social Sciences (PASW Statistics 18, IBM Corporation, Cornell, New York).

RESULTS AND DISCUSSION

Fatty acid content

The oil content of the *Amatungula* fruit was 90 mg g^{-1} dry mass (DM), which was much higher than that observed in Carissa edulis fruits (30 mg g^{-1} DM) grown in Burkina Faso (Glew et al., 1997). The fatty acid profile of the lipid fraction of fruit is shown in Table 7.

Table 7: Fatty acid profile (in mg g^{-1} dry mass (DM), ± standard deviation; 95% confidence interval, n=3) in *Amatungula* fruit.

Fatty Acids	***Concentration (mg g^{-1} DM)***
Saturated	
C14:0 (myristic acid)	1.89 ± 0.05
C16:0 (palmitic acid)	0.89 ± 0.02
C18:0 (stearic acid)	0.76 ± 0.02
C20:0 (arachidic acid)	0.02 ± 0.01
C22:0 (behenic acid)	0.10 ± 0.01
C24:0 (lignoceric acid)	0.04 ± 0.01
Monounsaturated	
C16:1, n-7 (palmitoleic acid)	7.65 ± 0.30
C18:1, n-9 (oleic acid)	0.16 ± 0.01
C20:1, n-9 (gondoic acid)	0.02 ± 0.01
Polyunsaturated	
C18:2, n-6 (linoleic acid)	0.35 ± 0.01
C18:3, n-3 (α-linolenic acid)	0.18 ± 0.01

Saturated fatty acids (SFAs) constitute 30.7% of the fatty acid of the fruit with myristic acid (C14:0) at 1.89 ± 0.05 mg g^{-1} DM making up most of the SFAs. Palmitoleic acid (C16:1, n-7) was the dominant monounsaturated fatty acid at 7.65 ± 0.3 mg g^{-1} DM and also contributed the most (63.4%) towards the total fatty acid content. Two polyunsaturated fatty acids, linoleic acid (C18:2, n-6) and α-linolenic acid (C18:3, n-3), contributing 2.9% and 1.5% towards the total fatty acid content, respectively were detected. Linoleic acid and α-linolenic acid are essential fatty acids. Clinical studies have demonstrated that the ingested ratio of linoleic acid to α-linolenic acid should range from 1:1 to 4:1 to prevent the risk of cardiovascular disease (Griffin, 2008). In this study, the linoleic acid to α-linolenic acid ratio is 1.9:1, which is within the recommended range for the maintenance of cardiac health. Monounsaturated and polyunsaturated fatty acids contribute 69.3% towards the total fatty acid content of the *Amatungula* fruit. Other compounds detected in the lipid fraction of the fruit by GC-MS analysis were the non-aromatic hydrocarbons namely nonacosane, tricosane, and eicosane and the pentacyclic triterpenoids, β-amyrin and oleanolic acid, that have previously been isolated from this fruit (Moodley et al., 2011).

Elemental analysis

The values obtained for the CRM (n = 6, p = 0.05) were 2.34 ± 0.31 μg g^{-1}, 41.18 ± 0.52 μg g^{-1}, 19.67 ± 0.18 μg g^{-1}, and 20.33 ± 0.84 μg g^{-1} for Cu, Fe, Mn, and Zn; compared to certified values 2.6 ± 0.1 μg g^{-1}, 40.7 ± 2.3 μg g^{-1}, 20.3 ± 0.7 μg g^{-1}, and 19.5 ± 0.5 μg g^{-1}, respectively. Recorded values for Ca and Mg were 0.42 ± 4.72 mg $g^{-1}$and 0.48 ± 0.02 mg g^{-1}, which compared well to indicative values of 0.41 mg $g^{-1}$and 0.50 mg g^{-1}.

If present, As and Cd in all fruit samples were found to be in insignificant concentrations (below the instrument detection limits). Selenium was found in all fruit samples (2.89-6.95 μg g^{-1}) despite total soil Se being low and available concentrations being below the instrument detection limit. The uptake of Se, which is not known to be essential to plant growth, is probably due to its chemical similarity to S which is readily taken up by plants. An intake of 0.050-0.200 mg of Se per day is recommended (National Research Council, 1983). The consumption of two fruit (approximately 10.0 g) per day, may contribute approximately 0.0453 mg towards this RDA. Selenium toxicity in humans through the agricultural food chain is at plant tissue concentrations that are not generally phytotoxic. This study shows Se concentrations in the fruit to be safe and adequate.

Table 8: Elemental concentrations in μg g^{-1} (mean (SD), n=3) and bioaccumulation factors for selected elements in *Amatungula* fruit and corresponding soil (Total (T) and Available (A)) samples.

		Concentration (μg g^{-1})			*Bioaccumulation factors*		$[Soil]_A/[Soil]_T$
Sites[a]		*Fruit*	*Soil (T)*	*Soil (A)*	$[Fruit]/[Soil]_T$	$[Fruit]/[Soil]_A$	*%*
1	Ca	1011(6)	4513(15)	1945(45)	0.22	0.52	43.11
2		2843(23)	5634(31)	2052(43)	0.50	1.39	36.42
3		1199(4)	1693(14)	758(16)	0.71	1.58	44.8
4		1419(17)	2811(7)	1349(11)	0.49	1.05	46.87
5		1819(16)	1801(4)	877(33)	1.01	2.08	48.66
6		1659(5)	9454(34)	2749(50)	0.18	0.60	29.09
7		1358(4)	1916(21)	591(6)	0.71	2.30	30.86
8		1270(6)	1664(8)	1057(9)	0.76	1.20	63.52
9		1858(16)	6155(42)	3240(115)	0.31	0.57	53.21
1	Cr	2.22(0.03)	105.4(0.9)	0.66(0.02)	0.02	3.37	0.63
2		0.78(0.01)	60.3(0.3)	0.65(0.01)	0.01	1.20	1.09
3		0.44(0.04)	22.0(0.2)	0.30(0.01)	0.02	1.48	1.36
4		0.89(0.04)	60.3(0.5)	0.68(0.14)	0.01	1.31	1.14
5		1.11(0.02)	41.75(0.09)	0.46(0.02)	0.03	2.42	1.10

6		0.33(0.04)	157(1)	0.53(0.09)	0.00	0.63	0.34
7		0.39(0.04)	36.1(0.1)	0.82(0.01)	0.01	0.47	2.28
8		1.56(0.03)	67.6(0.4)	0.78(0.01)	0.02	1.99	1.15
9		2.67(0.04)	32.5(0.1)	0.83(0.02)	0.08	3.21	2.55
1	Cu	14.5(0.1)	8.5(0.2)	6.2(0.0)	1.71	2.33	73.41
2		14.2(0.2)	22.8(0.5)	8.7(0.1)	0.62	1.63	38.16
3		10.7(0.1)	0.4(0.0)	0.3(0.0)	26.16	42.9	60.98
4		9.8(0.1)	30.1(0.2)	15.4(0.1)	0.33	0.64	51.15
5		10.2(0.3)	0.5(0.0)	0.2(0.0)	20.34	50.84	40.00
6		6.8(0.1)	23.4(0.1)	10.0(0.5)	0.29	0.68	42.92
7		6.4(0.1)	1.8(0.3)	0.6(0.1)	3.65	11.02	33.14
8		12.3(0.2)	24.5(0.5)	10.3(0.1)	0.50	1.20	42.04
9		9.8(0.1)	15.9(0.2)	7.9(0.2)	0.62	1.24	49.85
1	Fe	46.6(0.3)	18582(69)	290(5)	0	0.16	1.57
2		52.5(1.0)	31475(82)	534(7)	0	0.10	1.70
3		40.9(0.3)	14058(125)	208(2)	0	0.20	1.49
4		38.6(0.3)	8428(23)	470(3)	0	0.08	5.58
5		31.7(0.2)	7123(24)	316(4)	0	0.10	4.45
6		40.1(0.3)	27137(156)	214(38)	0	0.19	0.79
7		35.2(0.2)	6543(51)	469(2)	0.01	0.07	7.17
8		50.7(0.4)	7532(65)	206(1)	0.01	0.25	2.74
9		37.9(0.5)	13610(82)	375(11)	0	0.1	2.76
1	Mg	768(3)	1421(10)	243(4)	0.54	3.16	17.08
2		905(8)	2482(20)	309(4)	0.36	2.92	12.48
3		1075(7)	1055(14)	257(3)	1.02	4.18	24.37
4		896(8)	904(6)	193(2)	0.99	4.64	21.36
5		1314(8)	436(3)	226(1)	3.02	5.81	51.97
6		863(4)	4403(24)	257(23)	0.20	3.36	5.84
7		1052(3)	379(4)	94(1)	2.77	11.16	24.86
8		637(6)	325(6)	124(1)	1.96	5.11	38.46
9		893(7)	987(10)	223(6)	0.90	3.99	22.67
1	Mn	8.72(0.06)	273.5(2.2)	133.8(2.3)	0.03	0.07	48.92
2		19.17(0.18)	714.1(4.1)	305.1(3.7)	0.03	0.06	42.72
3		21.28(0.08)	199.2(3.0)	87.4(1.1)	0.11	0.24	43.9
4		6.7(0.1)	106.3(0.5)	40.2(0.3)	0.06	0.17	37.8
5		10.5(0.1)	68.3(0.2)	38.4(1.1)	0.15	0.27	56.16

6		6.95(0.04)	613.1(3.2)	175.6(24.5)	0.01	0.04	28.64
7		26.67(0.03)	108.1(1.3)	47.5(0.3)	0.25	0.56	43.98
8		8.22(0.04)	112(1)	59.6(0.2)	0.07	0.14	53.23
9		7.45(0.05)	233.2(1.3)	95.2(2.5)	0.03	0.08	40.82
1	Ni	0.61(0.03)	13.88(0.3)	1.19(0.03)	0.04	0.51	8.61
2		2.11(0.05)	14.13(0.10)	1.82(0.03)	0.15	1.16	12.91
3		0.33(0.08)	1.25(0.17)	0.16(0.01)	0.27	2.06	12.96
4		0.22(0.05)	11.88(0.14)	3.17(0.05)	0.02	0.07	26.68
5		0.78(0.08)	2.50(0.12)	0.45(0.02)	0.31	1.73	18.0
6		0.33(0.06)	34.1(0.1)	1.69(0.24)	0.01	0.20	4.96
7		0.67(0.10)	1.75(0.15)	0.24(0.01)	0.38	2.78	13.71
8		1.44(0.06)	3.13(0.06)	0.28(0.01)	0.46	5.23	8.83
9		1.61(0.10)	7.25(0.16)	0.60(0.02)	0.22	2.69	8.28
1	Pb	10.7(0.7)	66.75(2.13)	14.19(0.07)	0.16	0.75	21.26
2		11.3(0.9)	98.50(3.10)	15.23(0.03)	0.12	0.74	15.48
3		9.17(0.52)	49.75(2.91)	4.45(0.03)	0.18	2.06	8.94
4		8.50(0.32)	85.25(2.22)	22.93(0.07)	0.10	0.37	26.89
5		8.84(0.58)	33.0(0.7)	3.50(0.06)	0.27	2.53	10.6
6		13.5(0.4)	71.75(2.95)	8.30(0.06)	0.19	1.63	11.57
7		10.67(0.33)	36.0(0.6)	2.54(0.09)	0.3	4.19	7.07
8		9.0(0.9)	46.25(2.41)	10.99(0.05)	0.19	0.82	23.75
9		9.17(0.67)	49.0(1.9)	6.58(0.02)	0.19	1.39	13.43
1	Zn	16.06(0.16)	108.25(1.05)	28.22(0.64)	0.15	0.57	26.07
2		32.84(0.20)	166.13(1.67)	49.53(0.94)	0.20	0.66	29.81
3		23.12(0.09)	76.5(1.5)	28.49(0.19)	0.30	0.81	37.24
4		11.28(0.06)	211.1(1.7)	75.18(0.89)	0.05	0.15	35.61
5		11.95(0.17)	40.75(0.04)	2.26(0.14)	0.29	5.29	5.55
6		13.34(0.08)	109.38(1.27)	34.30(4.74)	0.12	0.39	31.36
7		13.39(0.07)	54.25(0.91)	5.15(0.02)	0.25	2.60	9.49
8		13.11(0.13)	60.75(1.12)	18.97(0.06)	0.22	0.69	31.23
9		29.34(0.16)	84.0(0.3)	29.81(0.81)	0.35	0.98	35.49

[a] Sites: 1–Berea, 2–Canelands, 3–Park Rynie, 4–Pinetown, 5–Port Edward, 6–Port Shepstone, 7–Shelley Beach, 8–Uvongo and 9–Westville.

Total soil Fe was extremely high at all sites (Table 8). High concentrations of available Fe taken up by plants may be toxic and may inhibit their growth (Chaney et al., 1980). Although total soil Fe is high, availability is low. At site 2, total soil Fe is highest (31475 µg g^{-1}); 1.7% is available but 10% of this is taken up by the plant. The variability in total soil Fe is large (6543-31475 µg g^{-1}) whilst the variability in fruits is marginal (52.50-31.73 µg g^{-1}); Fe was excluded by the plant with only 7-25% of available Fe being taken up. This indicates that the plant controls uptake.

The plant tended to accumulate Ca and Mg, to a larger extent. At site 7, fruit Mg was more than eleven times that of available Mg (Table 8). The minimum RDA for Ca and Mg is 1000 mg day^{-1} and 310 mg day^{-1}, respectively (Food and Nutrition Board, 2001). Consumption of 10.0 g of fruit contributes about 1.6% and 3% towards the RDA for Ca and Mg, respectively for most adults.

The accumulation of trace metals is a normal and essential process for the growth and nurturing of plants. The trace metals, Ni, Cu, Zn, and Mn are readily taken up by plants but kills plants at levels below those associated with adverse health effects (McLaughlin et al., 1999). Phytotoxicity therefore prevents transfer of these metals from soil at toxic levels through the food chain. Both soil and fruit Ni concentrations were low with concentrations in fruit ranging from 0.33-2.11 µg g^{-1} and available Ni ranging from 0.16-3.17 µg g^{-1}, which is below the maximum permissible extractable level of 50 µg g^{-1} set for South African soils (van der Waals & Snyman, 2004). Although total and available soil Cu was low, the plant tended to accumulate the metal in the range 6.4-14.5 µg g^{-1}, with bioaccumulation factors as high as 50.84. Plants are quite sensitive to Zn, with phytotoxicity of Zn being one of the

primary concerns of excess Zn in soils (Brown et al., 2004). At site 4, available Zn was high therefore uptake was low (bioaccumulation factor, BAF = 0.15) as opposed to site 5 that had low available Zn with a high BAF (5.29). The maximum level for Cu and Zn in foodstuff set by the Department of Health in South Africa is 30 mg kg^{-1} and 40 mg kg^{-1}, respectively (Department of Health, 2004). The plant exhibited safe levels of Cu and Zn. Manganese levels in plant and soil were beneath the maximum limits of 2000 µg g^{-1} and 500 µg g^{-1}, respectively (Kabata-Pendias & Pendias, 1992).

Given the health effects associated with high blood Pb concentrations, the maximum level of Pb in small fruits and berries in South Africa is set at 0.2 mg kg^{-1} (Department of Health, 2004). All sites exceed this level. Lead is known to be more mobile in organic rich soils since they form complexes with organic ligands in the soil solution and are less likely to be sorbed by soil (Farrah & Pickering, 1977). Although Pb is toxic to plants, plants usually show ability to accumulate Pb, sometimes even several hundred times the threshold level for humans, without visible changes in their appearance or yield (Bigdeli & Seilsepour, 2008). The plant showed ability to accumulate Pb with BAFs as high as 4.19. To assess for enrichment (contamination) of metals in soils, total metal concentrations are usually compared to background concentrations. The background concentration for Pb in South African soils is 65.8 µg g^{-1} (Herselman et al., 2005). Total soil concentrations of Pb between 200 to 400 µg g^{-1} would indicate moderate contamination (Herselman et al., 2005). In this study, soils from all sites showed no evidence of Pb contamination and are within the suggested threshold value of 100 mg kg^{-1} set for NH_4-EDTA extractable Pb in South African soils (van der Waals & Snyman, 2004).

The maximum permissible level of extractable Cr in South African soil is set at 80 mg kg^{-1} (van der Waals & Snyman, 2004). All sites considered in the current research conformed to this limit. The environmental risks of Cr are not considered high since most Cr in soil occurs as Cr^{3+} which is relatively inert due to it being strongly sorbed by soil (Gray & McLaren, 2006). In this study, an average of 1.3% of the total metal content was available with plant Cr ranging from 0.33-2.67 µg g^{-1}. The minimum RDA for Cr is 0.024 mg day^{-1} for most adults (Food and Nutrition Board, 2001). Consumption of 10.0 g of *Amatungula* fruit contributes about 50% towards this RDA. Although the consumption of too much of fruit may result in exceeding the RDA for Cr, the risk of adverse effects is not likely. Research has shown that excessive Cr intake is not associated with any adverse effects therefore no tolerable upper intake level was established for this metal (Food and Nutrition Board, 2001).

The data suggests that the plant controls the concentrations of elements in the *Amatungula* fruit with the elements being in the increasing order of Ni < Cr < Se < Mn ≈ Cu ≈ Pb < Zn < Fe < Mg < Ca.

For the essential elements Ca, Cu, Fe, Mn, Ni, and Zn, the plant tended to accumulate or exclude the elements to meet its physiological requirement levels (Epstein, 1994). When available concentrations were below this level the plant accumulated the element and when it was above then it excluded it (Fig. 7). This trend was not observed for Cr, which is not essential to plant growth, although it was taken up by the plant. A previous study reported that plants take up Cr for various metabolic pathways for the production of biomolecules (Misra et al., 2010).

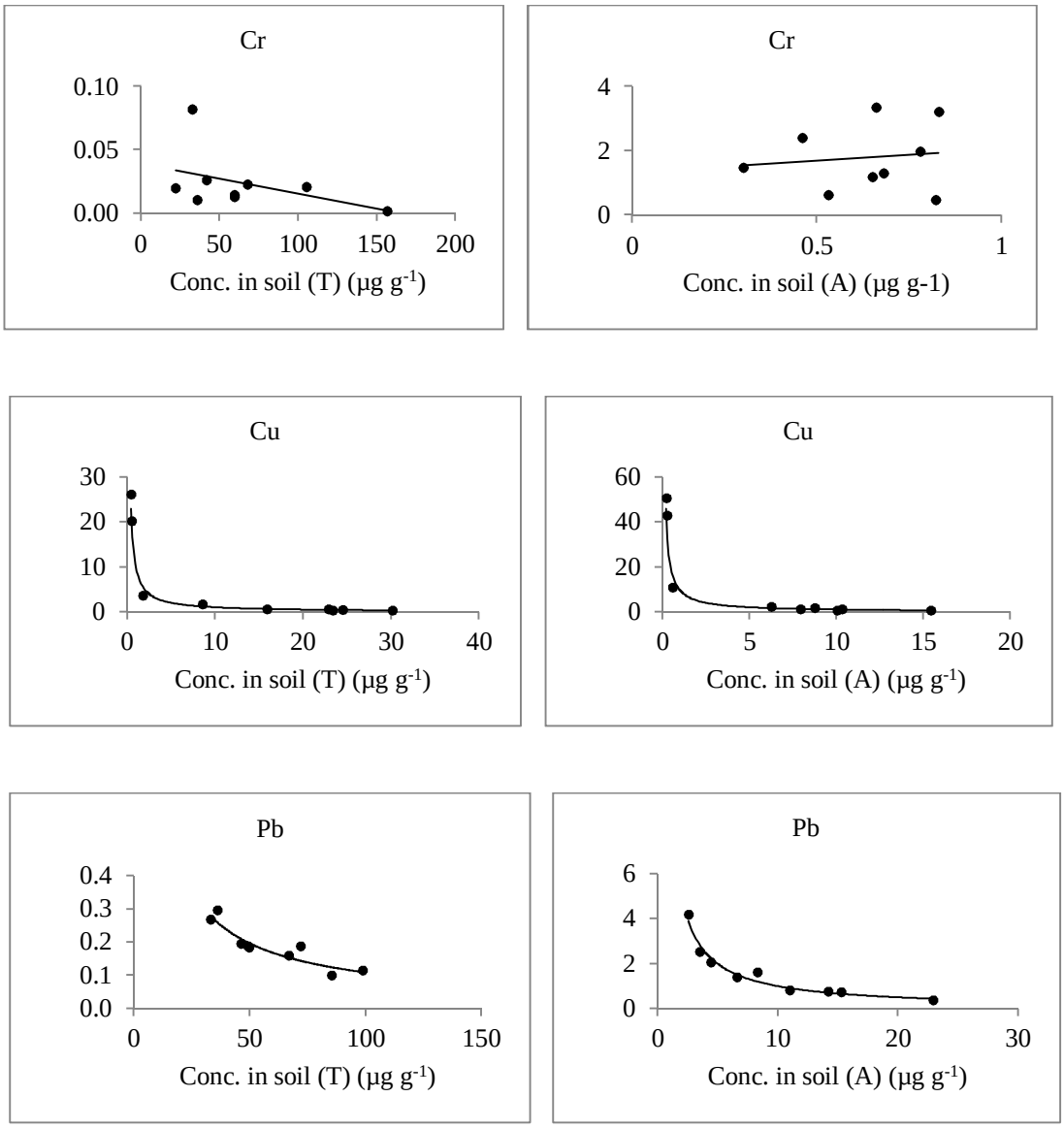


Figure 7. BAF: $[P]/[S]_{T \text{ or } A}$ = Bioaccumulation factor: concentration in plant/concentration in soil (Total or Available) vs. concentration in soil (T or A) in µg g^{-1} for the elements Cr (non-essential), Cu (essential), and Pb (toxic).

Lead, which is non-essential and toxic to plants, displayed the trends of essential elements; accumulation and exclusion at low and high soil concentrations, respectively (Fig. 7). Lead concentrations in plant were however restricted to the narrow range 8.5-13.5 µg g^{-1}. A well

recognized competition in plant uptake occurs between Ca and Pb where plants take up Pb (which mimics the physiological behavior of Ca) instead of Ca. This could explain, in part, why plant uptake of Pb is similar to that of essential elements.

The BAFs obtained for the essential elements indicated that when soil concentrations (total and available) were below the plants physiological requirements, accumulation occurred until the required level was reached. Conversely, if soil concentrations exceeded the physiological requirements of the plant, uptake was inhibited thereby partially excluding the element. A plot of relative accumulation as a function of total soil content indicated essentiality of the element if a rectangular hyperbola was produced. A similar trend was observed in a previous study where also, a linear graphical plot indicated non-essentiality as with Cr in this study (Timperley et al., 1970). In the case of some non-essential elements like Pb in this study, a non-linear graphical plot may indicate the biological similarity in uptake and translocation between non-essential and some essential elements, rather than the true essentiality of the element.

Statistical analysis

The concentrations of elements present in the soil (total and exchangeable) and in the fruits were determined. This was done for each of a number of elements, sites and for the fruits. The analysis of covariance was done to determine whether there is a relationship between the presence of an element in the soil and in the fruit. A summary of the results is given in Table 9.

Table 9: Analysis of covariance for all sites and elements regarding the presence of elements in *Amatungula* fruit.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8.033E7	82	979585.784	968.158	.000
Intercept	199955.864	1	199955.864	197.623	.000
Soil (T)[a]	161.818	1	161.818	.160	.690
Soil (A)[b]	205.297	1	205.297	.203	.653
element	1.415E7	8	1768655.885	1748.023	.000
site	851921.350	8	106490.169	105.248	.000
element * site	6181954.815	64	96593.044	95.466	.000
Error	161888.596	160	1011.804		
Total	1.013E8	243			
Corrected Total	8.049E7	242			

r squared = 1.000 (Adjusted r squared = 1.000)
ns, *** - not significant or significant at $P \leq 0.001$, respectively.
[a]T = Total; [b]A = Available.

Table 10: Analysis of covariance for all sites regarding the presence of each element in *Amatungula* fruit.

	Elements								
Source	**Ca**	**Cr**	**Cu**	**Fe**	**Mg**	**Mn**	**Ni**	**Pb**	**Zn**
Soil (T)[a]	ns	ns	ns	ns	ns	ns	ns	ns	ns
Soil (A)[b]	ns	ns	ns	ns	*	ns	ns	ns	ns
Site	***	***	***	***	***	***	***	***	***

Ns - not significant.
*, *** - significant at $P \leq 0.05$ and $P \leq 0.001$, respectively.
[a]T = Total; [b]A = Available.

The results show that site, element and the interaction affected element concentrations in the fruits. For this reason, the elements in the fruit were analyzed separately (Table 10). For all elements analyzed, total and exchangeable soil concentrations alone had no effect on fruit

concentrations but there were significant differences between the concentrations found at the different sites (significant at $P \leq 0.001$) (Table 10). A number of factors influence the concentration of elements on and within plants at different sites. These include climate; atmospheric depositions on aerial parts of plant as influenced by environmental factors such as temperature, moisture, and wind; the physical and chemical nature of soil on which the plant grows; and irrigation (Zurera et al., 1989).

Concentrations of elements in fruit, soil, pH, SOM, and CEC were to varying degrees correlated with each other (Table 11). The information extracted from the correlation matrix was used to discuss the positive and negative relationships that exist between the cations in the soil and the fruit. Relationships with correlation coefficients > 0.7 are synergistic and < -0.7 are antagonistic. It should be noted that the results obtained in this study are indicative and are limited to the number of samples and sites that were analysed.

In an earlier work, an intercorrelation between Cu, Cr, Fe, and Mg was found in the soil with a corresponding positive inter-correlation of the same elements in nuts, illustrating the value of total soil micronutrients in determining phytoavailability in nuts (Moodley et al., 2007). In this study, a positive intercorrelation between Ca, Cr, Fe, Mg, Mn, Ni, and Pb was found in the soil only (Table 11). This illustrates the limited value of total soil micronutrients in determining phytoavailability in this plant however it confirmed that these elements were linked to the same geological parent material.

Competition between ions present in soil influences metal mobility and uptake by the plant. A synergistic relationship occurs when an increase in total soil concentration of one element reduces the soil retention capacity of another, indicating that the elements are competing for

the same soil adsorption site, as for Cu and Zn. In contrast, other studies on interactions in soil have indicated antagonistic effects between Zn and Cu (Chaudry et al., 1973). A three-way synergy between Pb, Mg, and Mn was also observed (Table 11).

An antagonistic relationship occurs when the plant takes up two different elements by the same mechanism, where uptake of the element found in higher concentrations is favored, as for Cu and Mg ($r = -0.7$).

Since available Ca, Cu, Mn, and Zn are positively correlated to their total soil concentrations, total soil concentration of these elements can be used to predict availability. The importance of total metal concentration in predicting metal availability has been reported in a number of other studies (Sauvĭe et al., 2000).

Nickel availability correlated significantly to total soil Zn ($r = 0.9$) and Cu ($r = 0.7$) and correlated negatively to SOM and CEC. Interestingly, soil-water pH was not a significant parameter for controlling Ni availability in this study, compared to other studies (Sauvĭe et al., 2000). This may have been because the range in soil-water pH (4.95-6.81) was relatively small with 56% of the samples having soil-water pH values between 6 and 7. Lead availability was significantly correlated to total soil concentrations of all metals studied, except Cu and Zn. This shows that with increase in concentration of most metals in soil, solubility of Pb increases thereby facilitating absorption by the plant, which can explain the accumulation of this metal. The significance of SOM and CEC as a predictor of availability of the studied metals was not evident.

Table 11: Inter-item correlation matrix for concentrations of elements in *Amatungula* fruit (F) and soil (Total (T) & Available (A)).

	CaF	CaT	CaA	CrF	CrT	CrA	CuF	CuT	CuA	FeF	FeT	FeA	MgF	MgT	MgA	MnF	MnT	MnA	NiF	NiT	NiA	PbF	PbT	PbA	ZnF	ZnT	ZnA	SOM	CEC	pH
CaF[a]	**1.0**																													
CaT[b]	0.4	**1.0**																												
CaA[c]	0.4	**0.9**	**1.0**																											
CrF	-0.1	0.1	0.5	**1.0**																										
CrT	-0.1	**0.7**	0.5	-0.1	**1.0**																									
CrA	0.1	0.1	0.3	0.5	0.0	**1.0**																								
CuF	0.2	-0.1	0.1	0.5	0.0	0.0	**1.0**																							
CuT	0.3	0.4	0.5	0.1	0.4	0.4	0.1	**1.0**																						
CuA	0.1	0.4	0.5	0.2	0.4	0.4	0.2	**1.0**	**1.0**																					
FeF	0.2	0.2	0.2	0.2	0.3	0.2	**0.7**	0.5	0.4	**1.0**																				
FeT	0.6	**0.8**	0.6	-0.1	0.5	-0.2	0.3	0.3	0.2	0.5	**1.0**																			
FeA	0.6	0.0	0.0	-0.1	-0.3	0.5	0.0	0.2	0.2	0.0	0.1	**1.0**																		
MgF	0.2	-0.3	-0.4	-0.4	-0.5	-0.5	-0.4	-0.7	-0.7	-0.7	-0.2	0.2	**1.0**																	
MgT	0.4	**0.9**	0.6	-0.3	**0.8**	-0.2	-0.1	0.4	0.3	0.2	**0.9**	-0.1	-0.2	**1.0**																
MgA	0.5	0.5	0.5	0.0	0.2	-0.6	0.4	0.1	0.1	0.3	**0.8**	0.0	0.1	0.6	**1.0**															
MnF	0.1	-0.4	-0.5	-0.5	-0.5	-0.1	-0.2	-0.6	-0.6	0.0	0.0	0.3	0.4	-0.2	-0.1	**1.0**														
MnT	**0.7**	**0.8**	0.6	-0.2	0.5	-0.1	0.2	0.4	0.3	0.5	**1.0**	0.1	-0.2	**0.9**	**0.7**	0.0	**1.0**													
MnA	**0.7**	0.6	0.5	-0.1	0.4	-0.1	0.4	0.3	0.2	0.6	**1.0**	0.3	-0.3	**0.7**	**0.7**	0.1	**1.0**	**1.0**												
NiF	**0.7**	0.1	0.3	0.4	-0.2	0.5	0.5	0.2	0.1	0.5	0.3	0.4	-0.3	-0.1	0.2	0.1	0.4	0.5	**1.0**											
NiT	0.2	**0.9**	**0.7**	-0.2	**0.9**	-0.1	-0.1	0.5	0.5	0.2	**0.7**	-0.1	-0.3	**0.9**	0.5	-0.4	**0.7**	0.6	-0.2	**1.0**										
NiA	0.2	0.4	0.3	-0.1	0.4	0.1	0.1	**0.7**	**0.8**	0.1	0.4	0.4	-0.2	0.4	0.3	-0.4	0.4	0.3	-0.2	0.6	**1.0**									
PbF	0.4	-0.3	-0.3	0.0	-0.3	-0.1	0.2	-0.5	-0.5	-0.2	-0.1	0.3	0.6	-0.3	0.1	0.2	-0.1	0.0	0.2	-0.3	-0.2	**1.0**								
PbT	**0.7**	0.6	0.5	-0.1	0.6	-0.1	0.3	0.3	0.3	0.3	**0.8**	0.2	0.0	**0.7**	**0.7**	-0.2	**0.8**	**0.8**	0.3	**0.7**	0.4	0.4	**1.0**							
PbA	0.3	**0.8**	0.6	0.0	**0.9**	-0.1	0.2	0.4	0.4	0.4	**0.8**	-0.3	-0.4	**0.8**	0.5	-0.4	**0.8**	**0.7**	0.1	**0.8**	0.3	-0.1	**0.8**	**1.0**						
ZnF	0.6	0.3	0.5	0.2	-0.3	0.0	0.4	0.0	0.0	0.4	0.6	0.3	-0.1	0.2	0.6	0.3	0.5	**0.7**	**0.7**	0.0	-0.1	0.0	0.2	0.0	**1.0**					
ZnT	0.3	0.3	0.3	-0.1	0.2	0.1	0.2	**0.7**	**0.8**	0.3	0.4	0.5	-0.3	0.4	0.4	-0.2	0.4	0.4	0.0	0.4	**0.9**	-0.2	0.3	0.2	0.2	**1.0**				
ZnA	0.2	0.3	0.3	-0.1	0.2	0.0	0.2	**0.7**	**0.8**	0.3	0.4	0.4	-0.4	0.3	0.4	-0.3	0.3	0.3	0.0	0.4	**0.9**	-0.4	0.2	0.1	0.2	**1.0**	**1.0**			
SOM	0.1	-0.3	0.0	0.5	-0.4	0.2	**0.8**	0.0	0.1	0.4	0.1	0.5	-0.2	-0.4	0.2	0.1	0.0	0.2	0.3	-0.3	0.2	0.2	0.0	-0.3	0.5	0.4	0.3	**1.0**		
CEC	0.0	-0.2	0.0	0.4	-0.2	0.1	**0.8**	-0.2	-0.1	0.4	0.2	0.3	-0.2	-0.3	0.3	0.2	0.0	0.3	0.2	-0.3	0.1	0.2	0.0	-0.2	0.4	0.3	0.2	**1.0**	**1.0**	
pH	0.3	0.2	0.4	0.6	0.2	0.5	**0.7**	0.6	0.6	0.6	0.3	0.4	-0.6	0.0	0.3	-0.5	0.2	0.4	0.5	0.2	0.5	0.1	0.4	0.2	0.3	0.6	0.6	**0.7**	0.6	**1.0**

[a] XF – $[X]_{Fruit}$ where X=the various elements;
[b] XT – [soil $X]_{Total}$ where X=the various elements;
[c] XA – $[X]_{Available}$ where X=the various elements;
SOM – Soil organic matter; CEC – Cation exchange capacity.

CONCLUSION

The *Amatungula* fruit was found to be rich in monounsaturated and essential fatty acids that are dietary requirements for the prevention of protein-energy malnutrition. Elemental analysis indicated that the fruit conforms to the RDAs for the elements in focus and can contribute significantly to the diet. Picking and buying fruits from streets and high vehicular areas should however be avoided due to the risk of Pb toxicity. Concentration of elements in soil had no significant effect on plant concentrations but their competition with each other in soil influenced availability, as with Pb. Lead availability was significantly correlated to total soil concentrations of most metals studied showing the effect of these metals on the solubility of Pb which influences uptake and accumulation. The plant showed ability to accumulate Pb in the fruit at levels above those associated with toxic effects. Site location had an effect on plant concentrations nevertheless uptake by the plant was controlled to meet its physiological requirement levels as evidenced by the accumulation and exclusion of elements.

Acknowledgements

The authors would like to acknowledge the University of KwaZulu-Natal (UKZN) for financial support and the staff and post graduate students in the School of Chemistry at UKZN.

REFERENCES

Banas, A, Kwiatek, WM, Banas, K, Gajda, M, Pawlicki, B, Cichocki, T. 2010. J Biol. Inorg. Chem. 15: 1147-1155.

Bigdeli, M, Seilsepour, M. 2008. American-Eurasian J. Agric. & Environ. Sci. 4: 86-92.

Brown, S, Chaney, RL, Hallfrisch, J, Ryan, JA, Berti, WR. 2004. J. Environ. Qual. 33: 522-531.

Chaney, RL. 1980. *In*: G. Bitton, B.L. Damro, G.T. Davidson, J.M. Davidson (Eds.), Sludge - Health Risks of Land Application, Ann Arbor Sci. Publ., Ann Arbor, MI, USA, pp. 59-83.

Chapman, HD. 1965. *In*: CA. Black (Ed.), Methods of Soil Analysis Part 2 - Chemical and Microbiological Properties, American Society of Agronomy, Madison, Wisconsin, pp. 891-901.

Chaudry, FM, Sharif, M, Latif, A. 1973. Plant Soil. 38: 573-580.

Department of Health. 2004. Government Notices, Foodstuffs, cosmetics and disinfectants Act, (Act No. 54 of 1972), Government Gazette, South Africa.

Epstein, E. 1994. Proc. Natl. Acad. Sci. U.S.A. 91: 11-17.

Farrah, H, Pickering, WF. 1977. Water Air Soil Poll. 8: 189-197.

Food and Nutrition Board. 2001. Institute of Medicine, Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels, Elements, The National Academies Press, Washington, DC.

Glew, RH, VanderJagt, DJ, Lockett, C, Grivetti, LE, Smith, GC, Pastuszyn, A, Millson, M. 1997. J. Food Compos. Anal. 10: 205-217.

Gray, CW, McLaren, RDG. 2006. Water Air Soil Poll. 175: 3-4.

Griffin, BA. 2008. Curr. Opin. Lipidol. 19: 57-62.

Herselman, JE, Steyn, CE, Fey, MV. 2005. S. Afr. J. Sci. 101: 509-512.

Herzog, F, Farah, Z, Amado, R. 1993. Int. J. Vitam. Nutr. Res. 63: 234-238.

Kabata-Pendias, A, Pendias, H. 1992. Trace Elements in Soils and Plants, CRC Press, Baton Raton, FL.

Mathee, A, von Schirnding, YER, Levine, J, Ismail, A, Huntley, R, Cantrell, A. 2002. Environ. Res. 90: 181-184.

McLaughlin, MJ, Parker, DR, Clarke, JM. 1999. Field Crop Res. 60: 143-163.

Misra, A, Srivastava, AK, Srivastava, NK. 2010. Int. Res. J. Plant Sci. 1: 14-20.

Moodley, R, Chenia, H, Jonnalagadda, SB, Koorbanally, N. 2011. J. Med. Plants Res. 5: 4851-4858.

Moodley, R, Kindness, A, Jonnalagadda, SB. 2007. J. Environ. Sci. Heal. B. 42: 2097-2104.

National Research Council. 1983. Selenium in Nutrition, The National Academies Press, Washington, DC.

National Research Council. 2008. Lost Crops of Africa: Volume III: Fruits, The National Academies Press, Washington, DC.

Sauv´e, S, Hendershot, W, Allen, HE. 2000. Environ. Sci. Tech. 34: 1125-1131.

Shackleton, SE, Shackleton, CM, Netshiluvhi, TR, Geach, BS, Ballance, A, Fairbanks, DHK. 2004. Econ. Bot. 56: 130-146.

Timperley, MH, Brooks, RR, Peterson, PJ. 1970. J. Appl. Ecol. 7: 429-439.

van der Waals, JH, Snyman, HG. 2004. *In*: Proceedings of the 2004 Water Institute of Southern Africa (WISA) Biennial Conference 2, Document Transformation Technologies, Cape Town, South Africa.

Walkley, A, Black, IA. 1934. Soil. Sc. 37: 29-38.

Wehmeyer, AS. 1996. S. Afr. Med. J. 40: 1102-1104.

Zurera, GR, Salmeron, J, Pozo, R. 1989. J. Sci. Food Agric. 49: 307-314.

CHAPTER 4

Antioxidant activity of Phenolic compounds isolated from the edible fruits and stem bark of Harpephyllum caffrum

ABSTRACT

Two pharmacologically active triterpenoids, β-sitosterol and lupeol, and the powerful flavan-3-ol antioxidant, (+)-catechin, were isolated from the edible fruits of *Harpephyllum caffrum* whilst a mixture of cardanols (1-hydroxy-3-[(Z)-12'-nonadecenyl] benzene, 1-hydroxy-3-[(Z)-12'-heptadecenyl] benzene, 1-hydroxy-3-heptadecanyl benzene and 1-hydroxy-3-pentadecanyl benzene), the alkyl *p*-coumaric acid ester (eicosanyl-*trans*-*p*-coumarate), and (+)-catechin were isolated from the stem bark. This is the first report of these compounds being isolated from this plant. The antioxidant capacity of (+)-catechin was higher than the other isolated compounds as well as the known antioxidant, ascorbic acid. These findings highlight the medicinal benefits of the fruits of *H. caffrum* that can serve the same purpose in South Africa as vitamin cocktails containing antioxidants and sterols consumed in Western and European countries, with the added benefit of them being in a natural and inexpensive form.

Keywords: *Harpephyllum caffrum*, (+)-catechin, cardanol, eicosanyl-*trans*-*p*-coumarate, antioxidant activity.

INTRODUCTION

Harpephyllum caffrum Bernh. belongs to the Anacardiaceae, the fourth largest tree family in southern Africa. It is a large, evergreen, deciduous tree that grows up to 15 m in height. The natural distribution of *H. Caffrum* is restricted to southern Africa (Palmer & Pitman, 1972) with distribution ranging from the Eastern Cape to KwaZulu-Natal and Limpopo Province. In South Africa, *H. Caffrum* is a common street tree that is known as 'wild plum' in English, 'umgwenya' in isiZulu, and 'mothêkêlê' in Sotho. The plum-like fruit contains a single seed and is enjoyed by birds, animals, insects, and humans. Extracts of the stem bark are used in South African traditional medicine to treat or control a variety of human ailments such as blood purifiers to treat skin conditions like acne and eczema, analgesics, and to manage and control childhood convulsions and epilepsy (van Wyk, 2002; Pujol, 1990). The aqueous extract of the stem bark is reported to possess hypoglycaemic, hypotensive, anticonvulsant, and analgesic properties (Ojewole, 2006; 2007), scientifically supporting ethno-medicinal use of the plant for the treatment of these conditions.

The chemical constituents of *H. Caffrum* are not well known but ethanolic extracts from the leaves have been reported to contain protocatechuic acid, gallic acid, methyl gallate, quercetin, and kaempferol using relatively primitive techniques such as thin layer chromatography and UV spectroscopy (El Sherbeiny & El Ansari, 1976). Other trees belonging to the same family include mango (*Mangifera indica*), African resin tree (*Ozoroa insignis),* M'Peku (*Lannea Velutina*) and cashew nut (*Anacardium occidentale*). Compounds isolated from these trees include anacardic acid, cardols, cardanols, flavonoids,

and triterpenoids (Himejima & Kubo, 1991; Scartezzini & Speroni, 2000; Liu & Abreu, 2006).

Oxygen is essential to the survival of higher eukaryotes yet it can, at the same time, be inherently dangerous to their existence. A large body of evidence indicates that oxidative stress is involved in a wide variety of degenerative disorders and diseases (Esposito et al., 2002). Some of these oxidation-linked diseases or disorders are initiated or exacerbated by numerous environmental pro-oxidants and pro-oxidant drugs and foods. On the other hand, compounds found in certain foods, support biological resistance to oxidants. There is growing interest in the possible protective value of plant-derived antioxidants as people the world over are moving away from synthetically derived drugs to the more natural forms of pharmaceutical therapy, such as fruits and vegetables containing beneficial classes of organic compounds, flavonoids being a prime example. This is especially due to the mutagenic and carcinogenic effects of synthetic antioxidants (Ramanathan & Das, 1993) and the uncertainty of the fate of the drugs when ingested.

In this study, the isolation, characterization, and identification of the phytocompounds from the edible fruits and stem bark of *H. caffrum* was undertaken. The antioxidant capacity of the isolated compounds was determined using the ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-β-picrylhydrazyl (DPPH) methods, using ascorbic acid as the standard antioxidant for comparison, to evaluate the plant for its antioxidant value.

MATERIALS AND METHODS

General experimental procedure

IR spectra were obtained using a Perkin Elmer Spectrum 100 FT-IR spectrometer with Universal ATR sampling accessory. ^{1}H and ^{13}C NMR spectra were recorded in deuterated chloroform ($CDCl_3$) or methanol (CD_3OD) at room temperature on a Bruker Avance^{III} 400 spectrometer at 400 MHz and 100MHz, respectively with tetramethylsilane (TMS) as internal standard. GC-MS data were recorded on an Agilent GC-MSD apparatus equipped with an HP-5MS (5% phenyl)-methylpolysiloxane (30 m x 0.25 mm i.d., 0.25 µm film thickness) column. He (2 mL min^{-1}) was used as a carrier gas and acetone or methanol (MeOH) was used to dissolve the sample. The injector was kept at 250^{o}C whilst the transfer line was at 280^{o}C. The column temperature was held at 50^{o}C for 2 min, and then ramped to 280^{o}C at 20^{o}C min^{-1} where it was held for 15 min. The MS was operated in the EI mode at 70 eV. Crude extracts were subjected to column chromatography with silica gel (Merck, 70-230 mesh) on suitably sized columns and fractions were monitored by TLC (Merck silica gel 60, F254 aluminium sheets), analyzed under UV (254 nm) and visualized using anisaldehyde spray reagent (97: 2: 1; MeOH: conc. H_2SO_4: anisaldehyde). All chemicals used were supplied by Merck and Sigma Chemical Companies and were of analytical-reagent grade.

Plant material

The fruits and stem bark from *H.caffrum* were collected from the grounds within the University of KwaZulu-Natal (UKZN), Westville campus, South Africa, in August 2010.

These were identified by taxonomist, Prof. A. Nicholas, from the School of Life Sciences, UKZN, Westville and a voucher specimen (Moodley, R2) deposited in the ward herbarium at UKZN.

Extraction and isolation

Ground fruit (1.00 kg) and chopped stem bark (1.45 kg) were extracted with hexane, dichloromethane (DCM) and MeOH by continuous shaking on an orbital shaker at room temperature for 48 h. After filtration, the extracts were concentrated and stored in a refrigerator until analysed.

For the crude DCM extract from the fruits (16.35 g), a hexane: ethyl acetate step gradient was used on a 4 cm diameter column starting with 100% hexane which was stepped to 60% ethyl acetate by 10% increments and finally to 100% ethyl acetate. Five fractions of 40 mL each were collected for each solvent system. Fractions 9-12 were combined to yield fraction A and fractions 16-18 were combined to yield Fraction B. Fraction A was rechromatographed over silica gel in a 2 cm diameter column with 100% hexane to yield compound **B-1** (6.65 mg) which eluted after 25 mL. Fraction B was recrystallised with $CHCl_3$: MeOH (1:1) affording compound **B-2** (5.02 mg).

The crude MeOH extract (10.0 g) from the fruits was dissolved in water and subjected to partitioning with an equal volume of DCM. The DCM fraction was then dried with anhydrous Na_2SO_4, concentrated, and the resultant extract subjected to column chromatography on a 4 cm diameter column with 100% DCM. Ten fractions of 40 mL

each were collected. Compound **B-3** (35.4 mg) eluted in fractions 7-10 yielded yellow crystals upon evaporation.

Approximately 20.85 g of the crude DCM extract from stem bark was subjected to column chromatography on a 4 cm diameter column. For elution, a mobile phase consisting of a hexane: ethyl acetate step gradient was used, starting with 100% hexane and stepped to 100% ethyl acetate. Ten fractions of 80 mL each were collected in each step. Fractions 23-25 yielded compound **B-4** (21.10 mg) and fractions 26-32 were combined and purified on a 1.5 cm diameter column using 100% DCM. Fractions 5-7 yielded compound **B-5** (1.36 g) as a white powder after 12 mL. The crude MeOH extract (80.0 g) from stem bark was treated similar to that of fruit and also yielded compound **B-3** after elution with 100% DCM.

Antioxidant Activity

Determination of the reducing potential using the Ferric Reducing Antioxidant Power (FRAP) assay

The total reducing power of the isolated compounds from *H. caffrum* was determined according to the Ferric Reducing Antioxidant Power (FRAP) method as described by Ferreira et al. (2007). Working solutions of the isolated compounds were prepared in DCM or MeOH in 10 mL volumetric flasks from 10 mg mL^{-1} stock solutions to obtain two-fold dilutions ranging from 250.00 μg mL^{-1} to 15.63 μg mL^{-1}. Exactly 2.50 mL of the sample solutions were mixed with 2.50 mL of phosphate buffer solution (0.20 M, pH = 6.6) and 2.50 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution in test tubes and placed in a

water bath at 50^{0}C for 20 min. Thereafter, 2.50 mL of 10% trichloroacetic acid (TCA) was added to the mixture, of which 2.50 mL was pipetted into a flask containing 2.50 mL distilled water and 0.50 mL of 0.1% $FeCl_3$ solution and allowed to stand for 10 min. The absorbance of the mixture was measured at 700 nm using a UV-VIS spectrophotometer (UV mini 1240, Shimadzu Corporation, Kyoto, Japan). A higher absorbance of the reaction mixture indicated greater reducing power. Ascorbic acid was used as the standard antioxidant for this assay and all procedures were performed in triplicate.

Measurement of free radical scavenging activity using the DPPH assay

The free radical scavenging activity (antioxidant capacity) of isolated compounds from *H. caffrum* on the stable radical, DPPH•, was evaluated by the method established by Shirwakar et al. (2006). In this assay, 1.50 mL of the sample solution which was prepared in DCM or MeOH at different concentrations ranging from 250.00 µg mL^{-1} to 15.63 µg mL^{-1} was mixed with 0.50 mL of a 0.10 mM solution of DPPH in MeOH. An equal amount of MeOHl and DPPH without sample served as a control. The reaction mixture was kept in the dark at room temperature for 30 min. Thereafter, the absorbance was measured at 517 nm against MeOH or DCM as a blank using a UV-Vis spectrophotometer. The proton transfer to the DPPH• free radical by a scavenger causes a decrease in absorbance at this wavelength. A lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percentage free radical scavenging activity was calculated according to the following equation:

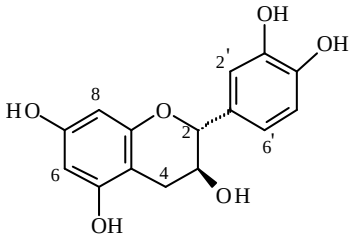
% Scavenging activity = [(Ac-As) / Ac] × 100

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

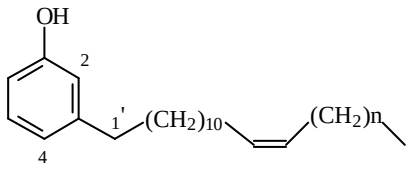
RESULTS AND DISCUSSION

Structure elucidation

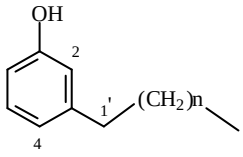
The DCM extract from the fruits of *H. caffrum* yielded two compounds, β-sitosterol (**B-1**) and lupeol (**B-2**) whilst the MeOH extract yielded one compound, (+)-catechin (**B-3**) (Fig. 8). The DCM extract from the stem bark of *H. caffrum* yielded a mixture of cardanols (**B-4**) identified as 1-hydroxy-3-[(Z)-12'-nonadecenyl] benzene (**B-4a**), 1-hydroxy-3-[(Z)-12'-heptadecenyl] benzene (**B-4b),** 1-hydroxy-3-heptadecanyl benzene (**B-4c**) and 1-hydroxy-3-pentadecanyl benzene (**B-4d**), and eicosanyl-*trans-p*-coumarate (**B-5**). Similar to the fruit, the MeOH extract of stem bark yielded (+)-catechin (**B-3**) (Fig. 8). GC-MS, IR spectroscopy, 1D-NMR (^{1}H, ^{13}C-NMR and DEPT), 2D-NMR (COSY, HSQC and HMBC) and data published in literature were used to identify the compounds. Compounds **B-1-2** are ubiquitous triterpenes in plant species. The physical and spectroscopic data for compounds **B-1** and **B-2** matched those reported by Mahato and Kundu (1994).

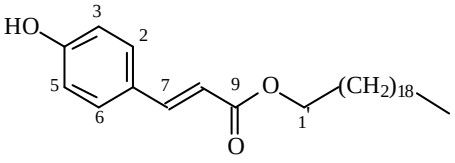


B-3



B-4a (n=5), B-4b (n=3)





B-4c (n=15), B-4d (n=13) **B-5**

Figure 8. Compounds isolated from *H. caffrum*.

The ^{1}H-NMR spectrum for compound **B-3** showed characteristic resonances for flavonoids at δ_H 6.74 (H-2', d, *J*=1.60 Hz), δ_H 6.66 (H-5', d, *J*=8.2 Hz) and δ_H 6.61 (H-6', dd, *J*=1.60 Hz, 8.2 Hz) from the B-ring catechol moiety as well as at δ_H 5.83 (H-6, d, *J*=2.24 Hz) and δ_H 5.77 (H-8, *J*=2.24 Hz) from the meta-coupled protons of the A-ring resorcinol moiety. Resonances at δ_H 4.47 (H-2, d, $J_{(H2ax\text{-}H3ax)}$=7.48 Hz), δ_H 3.88 (H-3, ddd, $J_{(H3ax\text{-}4eq)}$=5.60 Hz, $J_{(H3ax\text{-}2ax,\ H3ax\text{-}4ax)}$=7.80 Hz), δ_H 2.74 (H-4ax, dd, $J_{(H4ax\text{-}3ax)}$=5.42 Hz, $J_{(H4ax\text{-}4eq)}$=16.11 Hz) and δ_H 2.41 (H-4eq, dd, $J_{(H4eq\text{-}3ax)}$=8.10 Hz, $J_{(H4eq\text{-}4ax)}$=16.11 Hz) indicated that compound 3 was a flavan-3-ol. According to Es-Safi et al. (2006), the isomers catechin and epicatechin may be differentiated by the chemical shift of C-2 in the ^{13}C-NMR spectrum which is approximately δ_C 76.0 for epicatechin and δ_C 84.0 for catechin, and correlations between H-2 and H-3 in the COSY experiment which is strong for catechin and weak for epicatechin because of the difference in the dihedral angle. Based on the resonance for C-2 at δ_C 82.9, strong H-2/H-3 correlations in the COSY experiment, ^{1}H-NMR, ^{13}C-NMR, and data in literature (Hye et al., 2009) compound **B-3** was identified as (+)-catechin. This was confirmed by GC-MS, IR and UV-Vis spectroscopy. The $[M]^+$ ion at *m/z* 290 is in agreement with the molecular formula of $C_{15}H_{14}O_6$ for (+)-catechin. The IR spectrum showed characteristic absorption bands for the O-H group (3400-3100 cm^{-1}), C=C group (1615 cm^{-1}) and C–O group (1150-1010 cm^{-1}). The UV-Vis spectrum showed two absorption bands, a strong one at 238 nm and a weaker one at 281 nm originating from the A and B rings (Maoela et al., 2009). (+)-Catechin has previously been isolated from the Anacardiaceae (Scartezzini & Speroni, 2000; Maiga et al., 2007).

The cardanols were isolated as a mixture of 4 compounds (**B-4a-d**). The UV-Vis spectrum showed an absorption band at 290 nm for phenolic compounds. The IR spectrum showed absorption bands at 3370 cm^{-1}, 2922 and 2852 cm^{-1}, 1589 cm^{-1} and 1456 cm^{-1} confirming the presence of an OH group, C-H branch chain, double bonds and aromatic ring, respectively. Its ^{1}H-NMR spectrum presented 4 signals in the aromatic region; a triplet at δ_H 7.16 (H-5, *J*=7.68Hz), a doublet at δ_H 6.77 (H-4, *J*=7.56 Hz), a singlet at δ_H 6.67 (H-2) and a doublet at δ_H 6.61 (H-6, *J*=2.36 Hz) similar to that reported by Silva et al. (2008). The resonance at δ_H 2.58 (H-1', t, *J*=7.6 Hz) was assigned to the benzylic protons. The ^{13}C-NMR spectrum showed several methylene resonances and one methyl resonance indicating the presence of a hydrocarbon side chain. The resonance at δ_H 5.39 was that of two olefinic protons that coalesced into a triplet with *J*=4.76 Hz which correlated to two olefinic carbon resonances at δ_C 129.9 and δ_C 129.8, confirming the presence of the double bond in the alkyl side chain. The small coupling constant indicated a Z stereochemistry.

The four compounds were identified by GC-MS where the peaks in the chromatogram correlated to masses 358 (**B-4a**), 330 (**B-4b**), 332 (**B-4c**) and 304 (**B-4d**) a.m.u. This is in agreement with the molecular formulae $C_{25}H_{42}O$, $C_{23}H_{38}O$, $C_{23}H_{40}O$ and $C_{21}H_{36}O$, respectively. All compounds gave a base peak at *m/z* 108, characteristic of cardanols that fragment at the β position of the benzyl ring to form the stable tropolium ions. For $C_{25}H_{42}O$ (**B-4a**), fragmentation at *m/z* 288 formed by elimination of pentene through McLafferty rearrangement (Kapche et al., 2007) confirmed the position of the double bond at $\Delta^{12'}$. For $C_{23}H_{38}O$ (**B-4b**), the characteristic ions at *m/z* 57 $[(CH_2)_3CH_3]^+$, 83 $[CH_3(CH_2)_3CH_2]^+$ and 97 $[CH_3(CH_2)_4(CH)_2]^+$ confirmed the presence of the double bond in

the hydrocarbon side chain at $\Delta^{12'}$ (Kapche et al., 2007). Thus, the mixture was identified as being two monoene cardanols, 1-hydroxy-3-[(Z)-12'-nonadecenyl] benzene (**B-4a**) (tentative structure) and 1-hydroxy-3-[(Z)-12'-heptadecenyl] benzene (**B-4b)** and two cardanols with saturated side chains, 1-hydroxy-3-heptadecanyl benzene (**B-4c**) and 1-hydroxy-3-pentadecanyl benzene (**B-4d**). Compounds **B-4b-c** have previously been isolated from the roots of *Ozoroa insignis* (Anacardiaceae) (Liu & Abreu, 2006) however, to the best of our knowledge, compound **B-4a** was not isolated before.

The ^{1}H-NMR spectrum for compound **B-5** indicated the presence of a *p*-coumaric acid moiety attached to a large hydrocarbon chain. Resonances at δ_H 7.41 (H-3/5, *J*=8.6 Hz) and δ_H 6.82 (H-2/6, *J*=8.6 Hz) suggested a *para*-substituted benzene ring. The olefinic resonances at δ_H 7.60 (H-7, d, *J*=16.0 Hz) and δ_H 6.28 (H-8, d, *J*=16.0 Hz) confirmed a *trans* geometry. The ^{13}C-NMR spectrum showed a methylene resonance at δ_C 64.60 (C-1'), identified using the DEPT spectrum, corresponding to δ_H 4.16 in the HSQC spectrum and which correlated to the carbonyl (C-9) in the HMBC spectrum. This together with a methyl group at δ_H 14.10 (C-20') and several other methylene resonances indicated the presence of a hydrocarbon moiety attached to the *p*-coumaric acid moiety.

The UV-Vis spectrum of compound **B-5** showed an absorption band at 290 nm comparable to that of cinnamic acid (NIST Chemistry Webbook, National Institute of Standards and Technology, 2008). The IR spectrum displayed an ester carbonyl at 1711 cm^{-1} indicating an α, β-unsaturated ester. The $[M]^+$ ion at *m/z* 444 is in agreement with the molecular formula $C_{29}H_{48}O_3$. The strong peak at *m/z* 163 corresponds to the hydroxyl cinnamic acid ion thus indicating a *p*-coumaric acid moiety. Based on mass spectral data (Mahmood et

al., 2003; Usama, 2011), the length of the hydrocarbon chain was determined as 20 carbons (*m/z* 281), thereby identifying compound **5** as eicosanyl-*trans*-*p*-coumarate. This compound was previously isolated from the roots of *Tanacetum longifolium* (Asteraceae) (Mahmood et al., 2003).

The presence of these compounds in *H. caffrum* has not previously been reported. The flavonoids, quercetin and kaempferol, that were previously identified by TLC in this plant by El Sherbeiny & El Ansari (1976) were not found in this study. However, the flavan-3-ol, (+)-catechin, was found as one of the major compounds in the plant.

The isolated compounds, (+)-catechin (**B-3**), the mixture of cardanols (**B-4a-d**) and eicosanyl-*trans*-*p*-coumarate (**B-5**), along with lupeol and sitosterol, were evaluated for their antioxidant potentials using the FRAP and DPPH assays. Lupeol and β-sitosterol did not show any antioxidant activity and is therefore omitted from the results.

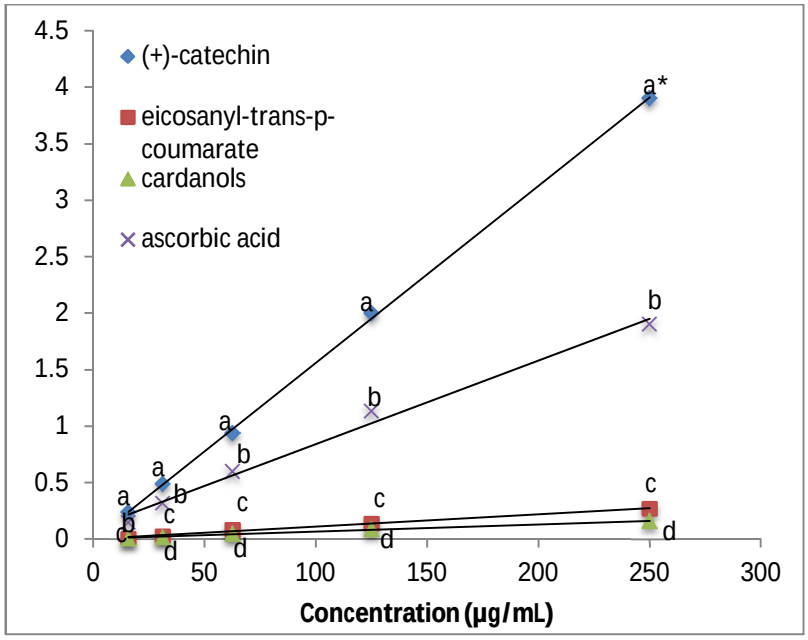


Figure 9. Reducing power of compounds from *H. caffrum* by FRAP method.
* Mean separation by Duncan's Multiple Range test at 5% level (*n* = 3).

Fig. 9 shows the concentration vs absorbance curves for the three samples and ascorbic acid in the FRAP assay. Each point represents the mean of three absorbance readings from three separate runs. Readings were highly reproducible with standard deviations in each case being ≤ 0.02. The concentration vs absorbance characteristics for different compounds showed different activities, but the reducing power for each compound was essentially linear implying that the antioxidant potential is concentration dependent. The results showed the reducing power (transformation of Fe^{3+} to Fe^{2+}) of all compounds to increase with increasing concentration. The reducing power of eicosanyl-*trans*-*p*-coumarate and the cardanols was significantly lower than that of the standard ascorbic acid but that of (+)-catechin was significantly higher than the standard ascorbic acid at all concentrations tested. The steeper slope for (+)-catechin compared to that of ascorbic acid indicated that the difference in reducing power between (+)-catechin and ascorbic acid got proportionately greater with concentration. The reducing power of the tested compounds in decreasing order was found to be: (+)-catechin > ascorbic acid > eicosanyl-*trans*-*p*-coumarate > cardanols.

Table 12 shows the percentage scavenging activity (donation of hydrogen to the DPPH radical) of the tested compounds at different concentrations. At 15.6 µg mL^{-1}, the scavenging ability of (+)-catechin was considerably higher (89.2%) than that of ascorbic acid (37.7%), indicating its ability to act as a scavenger at low concentrations. At 250 µg/mL, the scavenging ability of both compounds, although significantly different, was more comparable with a 5.1% difference in scavenging ability and residual DPPH percentage being 2.1 and 7.9 in (+)-catechin and ascorbic acid, respectively. The radical

scavenging reaction of (+)-catechin with DPPH was instantaneous showing greater antioxidant power than ascorbic acid. For all concentrations tested, the cardanols and eicosanyl-*trans*-*p*-coumarate gave significantly higher values of residual DPPH than ascorbic acid.

Table 12: Results of the antioxidant activity of compounds isolated from *H. caffrum* as measured by the DPPH method.

	Percentage scavenging activity				
Concentration µg mL^{-1}	**15.6**	**31.1**	**62.5**	**125**	**250**
(+)-catechin	89.2±0.1^{a*}	91.9±0.4^{a}	93.8±0.2^{a}	95.1±0.4^{a}	97.9±0.0^{a}
Eicosanyl-*trans*-*p*-coumarate	2.6±0.2^{c}	5.0±0.2^{c}	8.4±0.3^{c}	15.5±0.4^{c}	29.7±1.4^{c}
Cardanols	1.1±0.2^{d}	2.4±0.3^{d}	4.1±0.2^{d}	8.3±0.2^{d}	15.3±0.5^{d}
Ascorbic Acid	37.7±0.2^{b}	78.0±0.4^{b}	79.7±0.5^{b}	90.8±0.8^{b}	92.1±0.3^{b}

* Mean separation by Duncan's Multiple Range test at 5% level (n = 3).

According to Halliwell and Gutteridge (1999), mechanisms of antioxidant action can include inter alia suppression of radical formation by chelating trace metals involved in free radical production, scavenging of reactive oxygen species, and protecting antioxidant defences. The high antioxidant potential of the flavan-3-ol compared to the other compounds was expected due to the number and arrangement of the phenolic hydroxyl groups. The low redox potential (strong reducing ability) of (+)-catechin was demonstrated by the FRAP and DPPH assay, however its ability to reduce free radicals will depend on the redox potential of the radicals (Jovanovic, 1998). Highly oxidising free radicals such as the superoxide, peroxyl, alkoxyl, and hydroxyl radicals have high redox potentials (strong

oxidising agents) which would make it thermodynamically possible for (+)-catechin to reduce them by hydrogen atom donation. The major structural characteristics which makes (+)-catechin a good antioxidant is the cathecol moeity in the B-ring and the hydroxyl group in the C-ring. Loss of the two hydrogen atoms from the B-ring hydroxyl groups or one from the B-ring hydroxyl group and one from the C-ring hydroxyl group to free radicals can result in the formation of a stable quinone structure or 3-chromanone structure, respectively due to stabilisation of the B-ring rich in delocalised pi-electrons. Lupeol and sitosterol showed no antioxidant activity which may be explained by the lack of the phenolic moiety in the structure.

Importance of the phytochemical constituents in the edible fruits

Pasten et al. (2007), in a study on the cardioprotective benefits of polyphenols, suggested that (+)-catechin has a profibrinolytic effect on cultured human coronary artery endothelial cells which contributes to cardioprotection. Chaudhuri et al. (2012) showed that insulin has a profibrinolytic effect in patients with acute myocardial infarction. Other similarities between insulin and the extracts of *H. caffrum* are reported by Ojewole (2006) which could be explained, in part, by the presence of (+)-catechin in the plant. In countries like South Africa, with high levels of poverty, use of natural resources for food and health care are critical. The fruit of *H. caffrum*, which is a natural and inexpensive source of the antioxidant (+)-catechin, can be an alternative to the inaccessible green tea (*Camellia sinensis*) and can also be used as a substitute to insulin by type 2 diabetes sufferers. The fruit also contains the pharmacologically active triterpenoids, lupeol and β-sitosterol, that are known principally for their anti-inflammatory activity and cholesterol-lowering effects,

respectively (Geetha & Varalakshmi, 2001; Yokoyama, 2004). Aside from its medicinal benefits, the nutritional benefits, on consumption of the fruit, can only contribute positively to the health and well-being of the local people.

CONCLUSION

Two pharmacologically active triterpenoids, β-sitosterol and lupeol, and the powerful flavan-3-ol antioxidant, (+)-catechin, were isolated from the edible fruits of *H. caffrum* whilst a mixture of cardanols, an alkyl *p*-coumaric acid ester and (+)-catechin were isolated from the stem bark. The presence of these compounds in *H. caffrum* has not been previously reported. The reducing power of (+)-catechin by the FRAP method and its antioxidant activity as evaluated by the DPPH method was higher than the other isolated compounds including ascorbic acid. These findings highlight the medicinal benefits associated with consumption of the edible fruits of *H. caffrum*. This is especially important in South Africa where reliance on wild foods and medicinal plants for nutrition, treatment, and care is high due to economic and physical accessibility. This study lends scientific credence and validity to the ethnomedicinal use of *H. caffrum* whilst underpinning the benefits of consuming the indigenous edible fruit. These fruits my serve the same purpose as vitamin cocktails containing antioxidants and sterols taken in Western and European countries, with the added benefit of them being in a natural and inexpensive form.

Acknowledgements

The authors are thankful to UKZN for financial support.

REFERENCES

Chaudhuri, A, Janicke, D, Wilson, MF, Tripathy, D, Garg, R, Bandyopadhyay, A, Calieri, J, Hoffmeyer, D, Syed, T, Ghanim, H, Aljada, A, Dandona, P. 2004. Myocardial Infarction Anti-Inflammatory and Profibrinolytic Effect of Insulin in Acute ST-Segment-Elevation Circulation. 109: 849-854.

El Sherbeiny, AEA, El Ansari, MA. 1976. The polyphenolics and flavonoids of *Harpephyllum caffrum*. Planta Medica 29: 129-132.

Esposito, E, Rotilio, D, Di Mattoe, V, Di Giulio, C, Cacchio, M, Algeri, S. 2002. A review of specific dietary antioxidants and the effects on biochemical machanisms related to neurodegenerative processes. Neurobiol. Aging. 23: 719-735.

Es-Safi, N, Guyot, S, Ducrot, P. 2006. NMR, ESI/MS, and MALDI-TOF/MS Analysis of Pear Juice Polymeric Proanthocyanidins with Potent Free Radical Scavenging Activity. J. Agric. Food Chem. 54: 6969-6977.

Ferreira, ICFR, Baptista, P, Vilas-Baos, M, Barros L. 2007. Free radical scavenging capacity and reducing capacity of wild edible mushrooms from northeast Portugal: individual cap and stipe activity. Food Chem. 100: 1511-1516.

Geetha, T, Varalakshmi, P. 2001. Anti-inflammatory activity of lupeol and lupeol linoleate in rats. J. Ethnopharmacol. 76: 77-80.

Halliwell, B, Gutteridge, JMC. 1999. The chemistry of free radicals and related 'reactive species'. In: Free Radicals in Biology and Medicine, Oxford University Press, Oxford, pp 36-104.

Himejima, M, Kubo, I. 1991. Antibacterial Agents from the Cashew *Anacardium occidentale* (Anacardiaceae) Nut Shell Oil. J. Agric. Food Chem. 39: 418-421.

Hye, MA, Taher, MA, Ali, MY, Ali, MU, Zaman, S. 2009. Isolation of (+)-catechin from *Acacia catechu* (Cutch Tree) by a convenient method. J. Sci. Res. 1(2): 300-305.

Jovanovic, S, Steenken, S, Simic, MG, Hara, Y. 1998. Antioxidant properties of flavonoids: reduction potentials and electron transfer reactions of flavonoid radicals. In: Rice-Evans CA, Packer L (eds) Flavonoids in Health and Disease, Marcel Dekker Inc., New York, pp 137-161.

Kapche, GDWF, Laatsch, H, Fotso, S, Kouam, SF, Wafo, P, Ngadjui, BT, Abegaz, BM. 2007. Lanneanol: A new cytotoxic duhydroalkylcyclohexenol and phenolic compounds from *Lannea nigritana* (Sc. Ell.) Keay. Biochem. Syst. Ecol. 35: 539-543.

Liu, Y, Abreu, PJM. 2006. Long Chain Alkyl and Alkenyl Phenols from the Roots of *Ozoroa insignis.* J. Braz. Chem. Soc. 17(3): 527-532.

Mahato, SB, Kundu, AP. 1994. ^{13}C NMR spectra of pentacyclic triterpenoids. A Compilation and some salient features. Phytochemistry. 37: 1517-1575.

Mahmood, U, Kaul, VK, Acharya, R, Jirovetz, L. 2003. *p*-Coumaric acid esters from *Tanacetum longifolium*. Phytochemistry. 64: 851-853.

Maiga, A, Malterud, KE, Mathisen, GH, Paulsen, RE, Thomas-Oates, J, Bergström, E, Reubsaet, L, Diallo, D, Paulsen, BS. 2007. Cell protective antioxidants from the root bark of *Lannea velutina* A. Rich., a Malian medicinal plant. J. Med. Plant. Res. 1(4): 066-079.

Maoela, MS, Arotiba, OA, Baker, PGL, Mabusela, WT, Jahed, N, Songa, EA, Iwuoha, EI. 2009. Electroanalytical determination of catechin flavonoid in ethyl acetate extracts of medicinal plants. Int. J. Electrochem. Sci. 4: 1497-1510.

National Institute of Standards and Technology, web version. 2008. NIST Chemistry Webbook. http://webbook.nist.gov/chemistry. Accessed 11 June 2012.

Ojewole, JAO. 2006. Hypoglycaemic and hypotensive effects of *Harpephyllum caffrum* Bernh ex CF Krauss (Anacardiaceae) stem-bark aqueous extract in rats. Cardiovas. J. S. Afr. 17: 67-72.

Ojewole, JAO. 2007. Anticonvulsant and analgesic effects of *Harpephyllum caffrum* Bernh ex CF Krauss (Anacardiaceae) stem-bark aqueous extract in mice. Int. J. Pharmacol. 3(3): 241-247.

Palmer, E, Pitman, J. 1972. Trees of Southern Africa. Balkema, Cape Town.

Pasten, C, Olave, NC, Zhou, L, Tabengwa, EM, Wolkowicz, PE, Grenett, HE. 2007. Polyphenols downregulate PAI-1 gene expression in cultured human coronary artery endothelial cells: Molecular contributor to cardiovascular protection. Thromb. Res. 121: 59-65.

Pujol, J. 1996. Naturafrica-the herbalist handbook. Jean Pujol Natural Healers' Foundation, Durban.

Ramanathan, L, Das, NP. 1993. Natural products inhibit oxidative rancidity in salted, cooked, groundfish. J. Food Sci. 58: 318-320.

Scartezzini, P, Speroni, E. 2000. Review on some plants of Indian traditional medicine with antioxidant activity. J Ethnopharmacol. 71: 23-43.

Shirwakar, A, Shirwakar, AR, Rajendran, K, Punitha, IRS. 2006. *In vitro* antioxidant studies on the benzyltetraisoquinoline alkaloid berberine. Biol. Pharm. Bull. 29: 1906-1910.

Silva, MSS, De Lima, SG, Oliveira, EH, Lopes, JAD, Chaves, MH, Reis, FAM, Citó, GL. 2008. Anacardic acid derivatives from Brazalian propolis and their antibacterial activity. Eclet. Quim. 33(3): 53-58.

Usama, YS. 2001. *p*-Coumaric acid ester with potential antioxidant activity from the genus Salvia. Free Radicals Antioxid. 1(1): 23-27.

van Wyk, BE, van Oudtshoorn, B, Gericke, N. 2002. Medicinal Plants of South Africa. Briza Publications, Pretoria, South Africa, pp 146-147.

Yokoyama, WH. 2004. Plasma LDL cholesterol lowering by plant phytosterols in a hamster model. Trends Food Sci. Tech. 15: 528-531.

CHAPTER 5

Elemental composition and nutritional value of the edible fruits of Harpephyllum caffrum and impact of soil quality on chemical characteristics

---

ABSTRACT

Harpephyllum caffrum is a medicinal plant and common street tree distributed throughout the eastern part of Southern Africa. The elemental concentration in the edible fruit of *H. caffrum* was determined to assess for nutritional value. Concentrations of metals in the fruit and growth soil were determined from samples acquired from eight different sites in eastern KwaZulu-Natal, South Africa, to evaluate the impact of soil parameters on elemental distribution in the fruit. Typical elemental concentrations (μg g^{-1}, dry mass) in soil (Exchangeable/Total) and fruit samples, at Umhlanga, north of Durban, were Ca (1221/696 and 3333), Co (2.5/2.1 and 0.16), Cr (35/0.8 and 5.8), Cu (14/9 and 21), Fe (9424/394 and 116), Mg (199/139 and 915), Mn (268/187 and 13), Ni (2.8/0.51 and 3.4), Pb (36/32 and 1.2), and Zn (26/21 and 15). The results showed that metal interactions in soil influenced their availability, but uptake was to a greater extent controlled by the plant. The concentrations of elements in the fruits were found to be in the order of Ca > Mg > Fe > Cu > Zn > Mn > Cr > Ni > Pb > Co. The concentrations of toxic metals, As and Pb investigated were low. The fruits can contribute to the health and nutritional needs of people and has potential to improve the Fe status.

Keywords: indigenous fruit, elemental composition, nutritional value, soil quality.

INTRODUCTION

The low income people, in particular the rural poor, tend to subsist on staple crops such as maize and wheat, as they cannot afford to cultivate fruits and vegetables and purchased foods are not accessible or economically affordable. This predisposes them to malnutrition which, especially in children, can adversely affect their health, physical and mental capacity, educability, and productivity later on in life (Swart et al., 2008). People subsisting on staple crops only are prone to recurrent starvation due to the 'hunger gap', when crops are out of season and they lose their primary source of nourishment. In such instances, emergency foods are critical to their survival. Many people in rural communities live near wild fruit-bearing trees and bushes that produce food at times when crops do not; these are potential sources of emergency foods. Exploitation of these wild foods can help attenuate malnutrition and starvation. Therefore, raising awareness on the use of wild fruits primarily as a source of nourishment and also to contribute to a diverse diet, and promoting its use for agriculture can help many people who periodically struggle against starvation. Also, these foods usually contain adequate nutrition which is essential for optimal growth and development.

Plants are an important link between the environment and humans. Soil is the primary source of elements to the food chain since plants take up elements from the soil, the quality of which is affected by the surrounding environment. Trace elements in plants and the food

chain therefore depend on soil quality. Once elements are taken up and transferred through the agricultural food chain, they can threaten human health, whether essential or non-essential, if at elevated levels. In recent years, the assessment of trace elements in soil has become increasingly important due to increased public awareness on the impact of soil on concentrations of trace elements in food. Pollution of the environment by metals is a problem since metals are not biodegradable and most of them are toxic to living organisms if at elevated levels (Singh, 2005). Studies have shown that high intake of certain trace elements may lead to abnormal cell functioning and are also linked to certain types of cancers (Mathee et al., 2002; Banas et al., 2010). High concentrations of trace elements in soil are usually due to anthropogenic sources. Reliance on plants collected from the wild means that those consuming them are likely to be exposed to the influences of pollution. To assess for pollution and potential toxicities to human health, the quality of soil needs to be determined and its influence on plant uptake evaluated.

Harpephyllum caffrum, Bernh belongs to the Anacardiaceae, the fourth largest tree family in Southern Africa. It is a large, evergreen, deciduous tree that grows up to 15 m in height. *H. caffrum* is a common street tree distributed throughout the eastern part of Southern Africa with distribution in South Africa ranging from the Eastern Cape to KwaZulu--Natal and Limpopo Province (Palmer & Pitman, 1972). In South Africa, *H. Caffrum* is known as ‘wild plum’ in English, ‘umgwenya’ in isiZulu, and ‘mothêkêlê’ in Sotho and its plum--like fruit are enjoyed by birds, animals, insects, and humans. Although members of the family are cultivated throughout the world for their edible fruits and seeds namely mango (*Mangifera indica* L.), pistachio (*Pistacia vera* L.), and cashew (*Anacardium occidentale*

L.), this is not the case with *H. caffrum* which is restricted to localized consumption. This plant is also used in South African traditional medicine to treat a variety of human ailments (van Wyk et al., 2002; Pujol, 1996). Consumption of the fruit is therefore for nutritional or medicinal value where consumption for medicinal value depends on the organic constituents but does not preclude intake of the inorganic constituents.

There is little or no literature information on the nutritional value of the fruit of *H. caffrum* and the impact of soil quality on its elemental uptake. This study investigated the elemental concentrations in the fruit to assess for nutritional value. Fruits and soil samples from different geographic locations were investigated to determine the impact of soil quality parameters on elemental uptake and to assess for metal contamination. From eight sampling sites, the 12 elements selectively investigated were As, Ca, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Se, and Zn.

MATERIALS AND METHODS

Sample Collection

H. caffrum fruit and soil samples were collected from eight different sites in the KwaZulu-Natal east coast region which is part of the Savanna Biome in the month of September. Sites were flat and the climate humid and subtropical. Soils were generally sandy or loamy sand in texture. The chosen sites were: 1-Southbroom, 2-Port Edward, 3-Port Shepstone, 4-Springfield, 5-Reservoir Hills, 6-Umhlanga, 7-Umkomaas, and 8-Westville (Fig. 10).

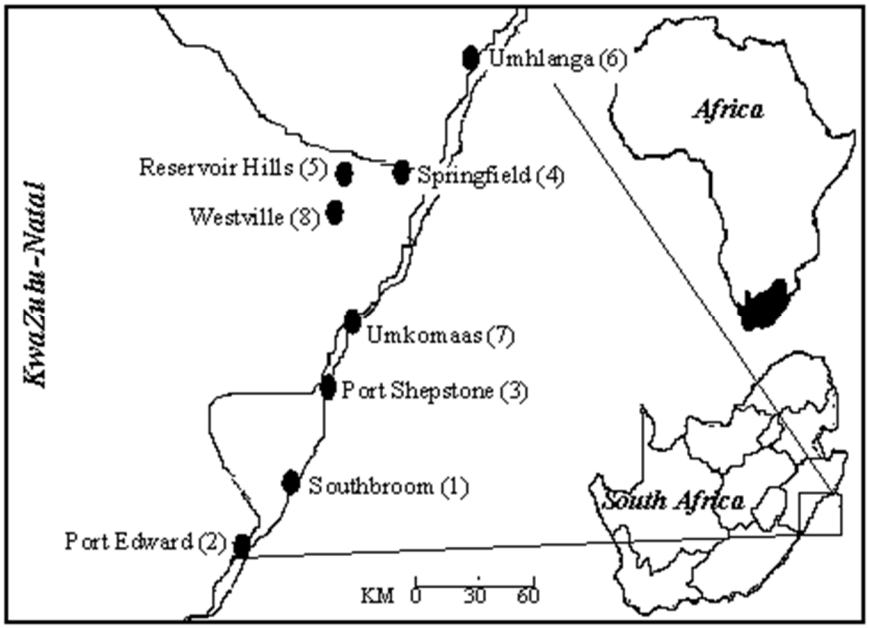


Figure 10. Map of sampling sites.

Samples of tree-ripened fruit were picked from trees and placed into plastic bags. Thereafter, fruit samples were cut into halves and oven dried at 60°C for 24 h to ensure complete removal of moisture. These were ground in a food processor (Braun range) to obtain a powder, which was stored in a refrigerator in polyethylene bags until analyzed. Soil samples were collected from six points around the tree, along the drip line, at a depth of 15 cm, with the use of a plastic spade. These were composited in a clean plastic bucket to achieve homogeneity and reduced to 500 g by quartering. Soil samples from each site were dried overnight in an oven at 40°C then passed through a 2 mm mesh sieve to remove organic matter and gravel. Some of this soil (10 g) was crushed to reduce particle size with a mortar and pestle for microwave digestion. Samples were stored in polyethylene bags and kept in a refrigerator until analyzed.

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 for all the experiments. Working elemental standards for calibrations were prepared from spectroscopic grade stock standard solutions (1000 mg L^{-1}). Glassware and other equipment were cleaned with 6.0 M HNO_3 and rinsed off with double distilled water to prevent contamination.

Extraction of Bioavailable Metals

A solution containing a combination of chemical extractants was prepared by diluting 38.542 g ammonium acetate ($NH_4CO_2CH_3$), 25 mL acetic acid (CH_3COOH, 96%) and 37.225 g ethylenediaminetetraacetic acid (EDTA) to 1L in double distilled water. Exactly 50 mL of extractant solution was added to 5.0 g of dry soil samples in 250 mL polyethylene bottles and shaken in a laboratory shaker for 2 h. Thereafter, solutions were filtered on Whatman No. 1 filter papers and then Millipore 0.45 µm filter membranes to permit analysis of extracted metals. All samples were stored in polyethylene bottles.

Elemental Analysis

The microwave-assisted closed vessel digestion technique was used for digestion of fruit and soil samples. Digestions were performed using the CEM MARS 5 Microwave Accelerated Reaction System (CEM Corporation, USA) with patented Xpress technology that consists of MARSXpress™ vessels and IR temperature sensors that instantaneously measure the temperature of each individual vessel designed for temperatures up to 260°C. Each vessel comprises liners (Teflon® PFA), caps, and composite sleeves that have a self-

regulating pressure control. Samples (0.5 g for fruit and 0.25 g for soil) were accurately weighed into the 50 mL liners and 10 mL of HNO_3 was added into each liner. Liners were capped, placed into the sleeves, loaded onto the 40-place carousel, and placed into the microwave. The appropriate method was loaded and the system started. For fruit samples, the power was set to 100% at 1600 W and temperature was ramped to 180°C (ramp time 15 min) where it was held for 15 min. For soil samples, the power was set to 100% at 1600 W and temperature was ramped to 200°C (ramp time 15 min) where it was held for 15 min. Fruit and soil digests were transferred to 50 mL volumetric flasks, diluted to the mark with double distilled water and stored in polyethylene bottles for elemental analysis.

All extracted and digested samples were analyzed for As, Ca, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Se, and Zn by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) due to its multi-element determination capability, high dynamic linear range and sensitivity. All measurements were performed using the Perkin Elmer ICP-OES with radial plasma observation. Analytical wavelengths were selected based on minimum spectral interferences and maximum analytical performance. Initially, the three most sensitive lines were chosen. From these lines, the lines with no interfering elements were selected. Method validation for elemental analysis was done by concurrent analysis of certified reference material (CRM), lyophilized brown bread (BCR 191), from the Community Bureau of Reference of the Commission of the European Communities. Values for Ca and Mg are indicative since these were additional tests, to those required for certification, determined by only a few laboratories.

Soil Organic Matter (SOM), Cation Exchange Capacity (CEC) and soil pH

The pH of soil was determined by measuring a 1:1 (m/m) soil / water suspension using a pH meter fitted with a glass electrode. Soil organic matter (SOM) was estimated using the Walkley-Black Method (Walkley & Black, 1934). Cation exchange capacity (CEC) of soil was determined using the pH 7.0 ammonium acetate method (Chapman, 1965).

Statistical Analysis

Significance of plant-soil relationships and soil competition effects were established by obtaining Pearson's product-moment correlation coefficients for elemental concentrations in soil (total and exchangeable) and fruits. All statistical analyses were done using the Statistical Package for the Social Sciences (PASW Statistics 19, IBM Corporation, Cornell, New York).

RESULTS AND DISCUSSION

Elemental Analysis

The accuracy of the method for elemental analysis was measured by comparing results obtained with certified results. Values for Ca, As and Mg are indicative so no standard deviations were provided. The values obtained for the CRM (n = 6, p = 0.05) were 20 ± 4 ng g^{-1}**,** 2.6 ± 0.1 μg g^{-1}, 40.6 ± 0.2 μg g^{-1}, 20.5 ± 0.2 μg g^{-1}, and 19.9 ± 0.5 μg g^{-1} for As, Cu, Fe, Mn, and Zn, respectively; compared to certified values 23 ng g^{-1}, 2.6 ± 0.1 μg g^{-1}, 40.7 ± 2.3 μg g^{-1}, 20.3 ± 0.7 μg g^{-1}, and 19.5 ± 0.5 μg g^{-1}, respectively. Recorded values for

Ca and Mg were 0.45 ± 0.5 mg g^{-1} and 0.5 ± 0.1 mg g^{-1}, which compared well to indicative values of 0.41 mg g^{-1} and 0.50 mg g^{-1}.

Table 13: Dietary Reference Intake (DRI) – Recommended Dietary Allowance (RDA) and Tolerable Upper Intake Levels (UL) of elements for most individuals – compared to average concentration of elements (n=5) in fruit.

Element	Average Concentration (mg 20 g^{-1}, DM)	DRI (mg day^{-1})		Estimated Contribution to RDA (%)
		RDA	UL	
Ca	115.8	1000-1300	2500	9-12
Cr	0.12	0.024-0.035	ND	>300
Co	0.00325	ND	ND	ND
Cu	0.37	0.9	8	41
Fe	2.9	8-18	45	16-36
Mg	26.4	310-320	350	8.5
Mn	0.20	1.6-2.3	9	8.7-12.5
Ni	0.076	ND	1	ND
Zn	0.32	8-11	34	2.9-4

ND- Not determined.

The average concentration (in mg) of the various elements studied that is found in 20 g of fruit (DM) is given in Table 13. These are compared to Dietary Reference Intakes (DRIs), (Institute of Medicine, 2001), specifically RDAs, and the estimated contribution to the RDAs is calculated (Table 13). The fruit of *H. caffrum* can contribute to the health and nutritional needs of most individuals for most elements. Severely malnourished children are often deficient in Zn, Fe, Cu, and Se (Maharaj et al., 2003). Iron and Cu are essential to human health. Iron deficiency can lead to anemia and increased vulnerability to infections (Labadarios, 2007). Copper deficiency can lead to anemia, pancreatic atrophy, and glucose intolerance (Davis et al., 1987). The Fe status in South Africa in 2005 was 13% for children aged 1-9 years who lived in rural areas and 6% for those who lived in informal, urban areas (Labadarios, 2007). Although fortification of wheat and maize flour has

improved the Fe status of children in South Africa, this is not a far-reaching initiative as it only benefits those who have access to these foods (Faber et al., 2005). About 20 g of *H. caffrum* fruit contributes more than 41% towards the RDA for Cu and 16-36% towards the RDA for Fe in most individuals (Table 13). Chromium is essential for normal carbohydrate, lipid, and protein metabolisms and its deficiency interferes with these metabolisms and can even lead to diabetes mellitus and cardiovascular abnormalities (Kobla & Volpe, 2000; Khan & Safdar, 2003). Consumption of 20 g of *H. caffrum* fruit contributes more than 300% towards the RDA for Cr in most individuals (Table 13). Chromium is considered a safe nutrient as there are no observed adverse effects even with intakes 300 times the acceptable limit and vomiting is likely to expel Cr before any toxicity damage can occur (Dourson, 1994). It has been reported that higher intake of Cr can improve glucose tolerance in malnourished children and elderly diabetics (Hopkins, 1968; Levine, 1968). Cobalt is an essential trace element because it is an integral component of vitamin B_{12} which is essential to human health (Domingo, 1989). For the general population, the diet is the main source of exposure to Co. The Total Diet Study done in the UK estimated the population average intake of Co to be 0.012 mg day^{-1} (Food Surveillance Information Sheet, 1997). Consumption of 20 g of *H. caffrum* fruit per day would provide an average intake of 0.00325 mg of Co (Table 13).

In South Africa, many households rely on natural resources for food therefore nutrition interventions should include information regarding the nutritive value of edible wild foods such as the fruits of *H. caffrum*. These fruits are readily and freely available and can help with the fight against malnutrition and hunger if consumed regularly or under adversity.

Table 14: Concentrations of the elements (µg g^{-1} DM; mean (SD), n = 5) in soil (Total-T and Exchangeable-E) and fruit and bioaccumulation factors.

					Soil(T)/Soil(E)	*BAF*	
Site[a]	***Element***	***Soil(T)***	***Soil(E)***	***Fruit***	***%***	***Fruit/Soil(T)***	***Fruit/Soil(E)***
1	Ca	1423(201)	796(13)	8175(81)	55.7	5.7	10.3
2		3426(200)	136(30)	7746(285)	39.6	2.3	5.7
3		8357(35)	533(5)	6987(59)	6.4	0.8	13.1
4		4444(258)	3783(32)	4465(73)	85.1	1.0	1.2
5		3314(122)	2652(66)	8554(143)	80.0	2.6	3.2
6		1221(35)	696(10)	3333(50)	57.1	2.7	4.8
7		1455(146)	895(13)	4704(50)	61.5	3.2	5.3
8		6256(135)	3642(75)	2345(28)	58.2	0.4	0.6
1	Co	2.6(0.3)	0.99(0.09)	0.20(0.01)	33.4	0.1	0.2
2		5.3(0.6)	1.9(0.09)	0.10(0.01)	24.5	0.0	0.1
3		1.4(0)	0.46(0.04)	0.16(0.05)	32.6	0.1	0.4
4		11(1)	8.5(0.4)	0.10(0.01)	77.5	0.0	0.0
5		5.2(0.4)	2.6(0.3)	0.10(0.01)	49.7	0.0	0.0
6		2.5(0.1)	2.1(0.2)	0.16(0.09)	82.7	0.1	0.1
7		5.4(0.3)	3.3(0.1)	0.36(0.05)	62.6	0.1	0.1
8		2.3(0.1)	0.58(0.04)	0.12(0.04)	25.4	0.1	0.2
1	Cr	37(3)	1.1(0.1)	8.3(0.5)	2.7	0.2	8.2
2		37(4)	1.2(0.0)	5.3(0.3)	3.4	0.1	4.3
3		24(1)	1.6(0.1)	5.6(0.2)	6.7	0.2	3.5
4		77(9)	7.0(0.2)	5.6(0.3)	9.1	0.1	0.8
5		39(5)	0.8(0.0)	5.7(0.1)	2.0	0.1	7.4
6		35(3)	0.8(0.1)	5.8(0.5)	2.4	0.2	7.1
7		80(7)	1.3(0.1)	5.9(0.4)	1.6	0.1	4.5
8		36(3)	1.5(0.0)	5.9(0.2)	4.0	0.2	4.0
1	Cu	3(0.2)	2(0.2)	18(2)	49.2	5.5	11.1
2		5(1)	2(0.3)	11(2)	30.8	2.1	6.9
3		7(1)	4(0.5)	28(2)	59.2	4.1	6.9
4		27(3)	17(0.5)	11(2)	65.1	0.4	0.6
5		8(1)	5(0.2)	5(1)	56.2	0.7	1.2
6		14(1)	9(0.6)	21(4)	63.2	1.5	2.4
7		15(0.6)	10(0.5)	30(6)	69.5	2.0	2.9
8		13(1)	4(0.1)	25(3)	31.5	1.9	6.0

1	Fe	7640(59)	229(4)	272(4)	3.0	0.0	1.2
2		17179(55)	567(7)	64(2)	3.3	0.0	0.1
3		4994(58)	356(6)	143(3)	7.1	0.0	0.4
4		24763(417)	3033(25)	43(2)	12.2	0.0	0.0
5		16753(165)	493(11)	127(3)	2.9	0.0	0.3
6		9424(161)	394(4)	116(3)	4.2	0.0	0.3
7		15751(112)	355(6)	329(5)	2.3	0.0	0.9
8		15899(139)	432(7)	69(2)	2.7	0.0	0.2
1	Mg	199(18)	124(5)	1524(7)	124.5	15.3	12.3
2		2235(108)	175(13)	1898(39)	7.9	0.8	10.8
3		179(22)	123(3)	1734(29)	68.9	9.7	14.1
4		2085(105)	276(11)	925(21)	13.2	0.4	3.4
5		2172(107)	275(14)	1139(22)	12.7	0.5	4.1
6		199(15)	139(4)	915(19)	69.9	4.6	6.6
7		729(41)	215(6)	1572(15)	29.5	2.2	7.3
8		785(45)	394(13)	834(19)	50.2	1.1	2.1
1	Mn	90(5)	40(2)	15(3)	44.5	0.2	0.4
2		184(7)	106(4)	11(1)	57.4	0.1	0.1
3		46(3)	27(2)	13(0.4)	57.1	0.3	0.5
4		804(50)	7071(13)	7(0.1)	87.9	0.0	0.0
5		261(11)	199(8)	7(0.3)	76.0	0.0	0.0
6		268(10)	187(9)	13(2)	69.9	0.1	0.1
7		241(14)	177(6)	10(1)	74.2	0.0	0.1
8		90(5)	62(3)	4(0.1)	68.9	0.0	0.1
1	Ni	5.0(2.5)	0.26(0.02)	5.0(0.41)	5.2	1.0	19.2
2		4.8(1.3)	0.34(0.02)	2.9(0.44)	7.0	0.6	8.6
3		1.8(0.52)	0.33(0.02)	3.8(0.40)	17.9	2.1	11.5
4		21(5.4)	3.6(0.14)	2.8(0.53)	17.6	0.1	0.8
5		9.7(0.5)	1.1(0.03)	2.6(0.19)	11.1	0.3	2.4
6		2.8(1.5)	0.51(0.05)	3.4(0.34)	18.1	1.2	6.7
7		8.0(0.78)	0.89(0.04)	6.4(1.07)	11.2	0.8	7.2
8		4.6(1.4)	0.75(0.03)	3.5(0.41)	16.2	0.8	4.7
1	Pb	4(0.5)	2(0.3)	2.0(0.1)	57.9	0.4	0.7
2		15(1)	6(0.2)	0.84(0.2)	36.1	0.1	0.2
3		5(0.3)	4(0.3)	2.3(0.6)	85.3	0.5	0.6
4		64(3)	57(2)	0.84(0.3)	89.4	0.0	0.0
5		22(1)	12(1)	0.66(0.1)	57.8	0.0	0.1
6		36(1)	32(2)	1.2(0.4)	88.3	0.0	0.0
7		30(1)	26(0.5)	1.1(0.3)	85.3	0.0	0.0
8		23(1)	16(0.3)	0.88(0.3)	72.0	0.0	0.1

1	Zn	10(2)	5(0.3)	22(2)	51.6	2.2	4.3
2		55(5)	9(0.8)	11(1)	16.3	0.2	1.3
3		11(1)	7(0.2)	24(4)	60.9	2.2	3.5
4		127(8)	66(3)	8(2)	52.1	0.1	0.1
5		48(5)	28(1)	15(4)	58.3	0.3	0.5
6		26(2)	21(1)	15(4)	82.4	0.6	0.7
7		53(5)	19(1)	16(3)	35.0	0.3	0.8
8		30(1)	15(0.3)	17(4)	47.8	0.6	1.2

[a] Sites: 1-Southbroom, 2-Port Edward, 3-Port Shepstone, 4-Springfield, 5-Reservoir Hills, 6-Umhlanga, 7-Umkomaas, and 8-Westville.

The effect of soil on elemental composition in fruit was determined (Table 14). If present, As and Se in all samples were found to be in insignificant concentrations (below the instrument detection limits). The plant tended to accumulate Ca, Mg, and Ni. Bioaccumulation factors (BFs) with exchangeable soil concentrations were 0.6-13.1 for Ca, 2.1-14.1 for Mg and 0.8-19.2 for Ni. For Ca and Mg, when exchangeable concentrations were low, accumulation was high as at site 1 and when exchangeable concentrations were high accumulation was low as at site 8.

Total soil Fe was high at all sites (4994-24763 µg g^{-1}) but exchangeable concentrations were low. The plant tended to accumulate Fe when exchangeable concentrations were low as at Site 3 and exclude it when exchangeable concentrations were high as at Site 4. Certain plants excrete metal chelates into the rhizosphere that mobilize Fe thereby providing a ready supply for plant uptake (Fan et al., 1997). Under Fe deficient conditions, plants tend to absorb Cu, Mn, and Zn instead, via this pathway (Fan et al., 1997). This phenomenon was observed at site 3, where total soil Fe was relatively low and Cu, Mn, and Zn in fruit was relatively high. Under conditions of excess Cu and Zn, xylem loading by these metals

is restricted to prevent uptake; uptake is only allowed when metal concentrations become low. This could also explain the higher uptake of these metals at site 3 where soil concentrations were low, and lower uptake at site 4 where soil concentrations were high.

Plant roots contain a high number of metal specific binding sites that restrict uptake of bound metal ions (Liao et al., 2000). Because the binding capacity of Mn is low, plants that grow under conditions of excess Mn tend to accumulate this metal; this occurs in shoots and not fruits (Chino & Baba, 1981; McBride, 1982; Kabata-Pendias & Pendias, 1989). This study affirms this low concentration of Mn in fruits (4-15 µg g^{-1}) even when soil concentrations were higher (Site 4).

Lead is a major soil contaminant. Phytoremediation studies on Pb-contaminated soil have shown that some plants can accumulate Pb at high concentrations (Huang & Cunningham, 1996). To assess for Pb contamination in soils, total metal concentrations were compared to background concentrations for Pb in South African soils (Herselman et al., 2005). Total soil Pb > 200 µg g^{-1} would indicate moderate contamination. In this study, soils from all sites showed no evidence of Pb contamination (4-64 µg g^{-1}). Differential absorbing capacity and growth responses of various plant species to Pb suggest that different plant species have different mechanisms to potentiate its uptake and accumulation (Navaroja & Kanchana, 2012). Lead concentrations in fruit was restricted to the narrow range (0.66-2.3 µg g^{-1}); this exceeds the limit for small fruits and berries set in South Africa (0.2 µg g^{-1}) (Department of Health, 2004). This limit would therefore appear to be unrealistic as there was no evidence of Pb accumulation (BAFs < 0.7) or contamination.

The distribution of Co in plants is entirely species-dependent and uptake is controlled by different mechanisms in different species (Hua-Fen et al., 2009). Cobalt has proven to be beneficial to at least some plants, but it is still not clear whether Co has direct effect on higher plants. The requirement of Co for N_2 fixation in legumes and non-legumes has been documented clearly. Total soil Co was low (< 11 µg g^{-1}) but seemed to be quite mobile; 24.5-82.7% was exchangeable. The concentration of Co in the fruit was found to be in the narrow range 0.1-0.36 µg g^{-1}. Plants tend to take up very low concentrations of Co as low concentrations can stimulate plant growth but higher concentrations are toxic (Palit et al., 1994). Total soil Cr ranged from 24-80 µg g^{-1} and < 9.1% was mobile. Except for site 1, Cr concentration in fruit seemed to be relatively constant and around 5 µg g^{-1}, irrespective of soil concentrations. Chromium distribution in plants generally has a stable character and does not depend on soil properties and concentrations (Golovatyj & Bogatyreva, 1999). In this study, the concentrations of elements in the fruits were found to be in the order of Ca > Mg > Fe > Cu > Zn > Mn > Cr > Ni > Pb > Co.

Plants have evolved highly specific mechanisms to take up, translocate and store nutrients and to maintain intracellular concentrations of these nutrients within their physiological ranges. This study showed that for the essential elements, when soil concentrations were high, the plant tended to exclude the element and when soil concentrations were low it accumulated it according to metabolic needs. This trend is not observed for the elements non-essential to plants like Co and Cr. This could be because in deficiency and sufficiency conditions, plants develop strategies to maximize and minimize uptake of essential elements, respectively (Welch, 1995). This is not true for not non-essential elements.

Concentrations of elements in fruit, soil, pH, SOM, and CEC were to varying degrees correlated with each other (Table 15). In general, trace elements in soil were strongly correlated with each other suggesting their common geochemical characteristics. Competition between ions present in soil influences metal mobility and uptake by the plant. Competition by elements for the same soil adsorption site would be synergistic as an increase in total soil concentration of one element would reduce the soil retention capacity of the other which increases its exchangeability. Synergistic relationships were observed for the metals Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn ($r \geq 0.7$) (Table 15).

Competition by elements for uptake by the plant via the same mechanism would be antagonistic. Generally, uptake of the element found in higher concentrations in the soil solution is favored. The exchangeable soil concentrations of Co, Fe, Mn, Ni, and Pb negatively influenced uptake of Zn ($r = -0.7$) (Table 15). For Fe this was expected since plants tend to absorb Zn under Fe deficient condition and vice versa; the negative correlation between exchangeable Fe and plant Cu and Mn is also prevalent but to a lesser extent. The only notable correlations with soil parameters pH, SOM and CEC were a positive correlation between CEC and total soil Zn ($r = 0.7$) and a negative correlation between SOM and Pb in fruit ($r = -0.8$) (Table 15). Lead in soil is usually tightly bound to organic material or in precipitated form so the strong negative relationship between SOM and plant Pb is expected.

CONCLUSION

The study showed that the elemental concentrations of the studied elements were well controlled in the fruit as their concentrations were restricted to a small range of variation. This was achieved by accumulation or exclusion of essential elements, characteristic of most plants to help prevent phytotoxicities and deficiencies. For most elements in soil, competition between elements present influenced exchangeability but uptake and distribution was primarily dependent on the plants metabolic needs. The concentrations of elements in the fruits were found to be in the order of Ca > Mg > Fe > Cu > Zn > Mn > Cr > Ni > Pb > Co. The fruits of *H. caffrum* can contribute to the nutritional needs of most individuals for most elements and were low in the toxic metals investigated.

Acknowledgements

The authors would like to acknowledge the University of KwaZulu-Natal (UKZN) for financial support and the staff and post graduate students in the School of Chemistry at UKZN.

Table 15: Inter-item correlation matrix for concentrations of elements in *H. caffrum* fruits (F) and soil (Total (T) & Exchangeable (E)).

	CaE	CaF	CoT	CoE	CoF	CrT	CrE	CrF	CuT	CuE	CuF	FeT	FeE	FeF	MgT	MgE	MgF	MnT	MnE	MnF	NiT	NiE	NiF	PbT	PbE	PbF	ZnT	ZnE	ZnF	pH	SOM	CEC
CaT	0.3	-0.1	-0.2	-0.1	-0.4	-0.3	0.2	-0.4	0.0	-0.1	0.2	-0.1	0.1	-0.5	0.0	0.3	0.0	-0.1	-0.1	-0.4	-0.1	0.1	-0.4	-0.2	-0.2	0.4	0.0	0.0	0.3	0.2	-0.3	0.1
CaE		-0.3	0.6	0.5	-0.5	0.3	0.6	-0.3	0.6	0.4	-0.4	0.8	0.6	-0.6	0.6	0.9	-0.7	0.5	0.6	-0.9	0.7	0.7	-0.5	0.6	0.5	-0.6	0.6	0.7	-0.6	-0.2	0.3	0.2
CaF			0.0	-0.2	-0.1	-0.3	-0.3	0.3	-0.7	-0.5	-0.5	-0.2	-0.2	0.2	0.3	-0.5	0.7	-0.2	-0.3	0.4	-0.1	-0.3	-0.1	-0.6	-0.6	0.2	-0.2	-0.3	0.2	0.6	-0.4	-0.1
CoT				0.9	-0.2	0.8	0.8	-0.3	0.7	0.7	-0.5	0.9	0.9	-0.3	0.7	0.3	-0.2	0.9	0.9	-0.4	1.0	0.9	-0.2	0.8	0.7	-0.6	1.0	0.9	-0.9	0.2	0.0	0.6
CoE					-0.1	0.8	0.9	-0.3	0.9	0.9	-0.4	0.8	0.9	-0.2	0.5	0.3	-0.4	1.0	1.0	-0.3	1.0	1.0	-0.2	0.9	0.9	-0.4	0.9	1.0	-0.7	0.0	0.0	0.5
CoF						0.5	-0.3	0.3	0.0	0.1	0.7	-0.3	-0.3	0.9	-0.5	-0.3	0.3	-0.2	-0.2	0.3	-0.2	-0.2	1.0	-0.1	0.0	0.2	-0.2	-0.3	0.3	-0.4	-0.2	0.2
CrT							0.6	-0.1	0.7	0.8	0.0	0.7	0.6	0.3	0.3	0.3	-0.2	0.7	0.7	-0.3	0.8	0.7	0.4	0.7	0.7	-0.4	0.8	0.7	-0.6	-0.2	0.0	0.6
CrE								-0.2	0.8	0.8	-0.3	0.7	1.0	-0.4	0.4	0.3	-0.3	0.9	0.9	-0.4	0.9	0.9	-0.3	0.8	0.8	-0.2	0.9	0.9	-0.6	0.1	-0.3	0.5
CrF									-0.4	-0.3	0.1	-0.4	-0.3	0.6	-0.5	-0.3	0.1	-0.3	-0.3	0.5	-0.2	-0.3	0.5	-0.4	-0.3	0.3	-0.4	-0.3	0.5	-0.3	-0.5	-0.5
CuT										1.0	0.0	0.7	0.8	-0.3	0.2	0.5	-0.6	0.9	0.9	-0.5	0.8	0.9	-0.1	1.0	1.0	-0.4	0.8	0.9	-0.6	-0.3	0.1	0.4
CuE											0.0	0.6	0.8	-0.1	0.2	0.2	-0.5	0.9	0.9	-0.3	0.8	0.9	0.0	0.9	1.0	-0.3	0.8	0.9	-0.6	-0.2	0.0	0.4
CuF												-0.5	-0.4	0.5	-0.8	-0.1	0.1	-0.5	-0.4	0.2	-0.5	-0.4	0.7	-0.2	-0.1	0.6	-0.5	-0.4	0.6	-0.4	-0.3	0.0
FeT													0.7	-0.4	0.8	0.7	-0.4	0.8	0.8	-0.7	0.8	0.8	-0.3	0.8	0.7	-0.8	0.9	0.8	-0.9	0.1	0.4	0.6
FeE														-0.5	0.5	0.3	-0.4	0.9	1.0	-0.4	0.9	1.0	-0.4	0.8	0.8	-0.3	0.9	0.9	-0.7	0.1	-0.1	0.5
FeF															-0.5	-0.4	0.4	-0.4	-0.4	0.5	-0.2	-0.4	0.9	-0.4	-0.3	0.3	-0.4	-0.4	0.5	-0.3	-0.4	-0.1
MgT																0.4	0.0	0.5	0.5	-0.5	0.6	0.5	-0.6	0.4	0.3	-0.7	0.7	0.5	-0.8	0.5	0.4	0.5
MgE																	-0.6	0.3	0.3	-1.0	0.4	0.4	-0.3	0.4	0.3	-0.6	0.4	0.4	-0.4	-0.4	0.5	0.1
MgF																		-0.4	-0.5	0.6	-0.4	-0.5	0.3	-0.6	-0.6	0.4	-0.3	-0.6	0.3	0.7	-0.5	0.3
MnT																			1.0	-0.3	0.9	1.0	-0.3	0.9	0.9	-0.5	0.9	1.0	-0.8	0.0	0.1	0.5
MnE																				-0.4	0.9	1.0	-0.3	0.9	0.9	-0.4	0.9	1.0	-0.8	0.0	0.0	0.4
MnF																					-0.5	-0.5	0.3	-0.4	-0.4	0.6	-0.5	-0.5	0.4	0.3	-0.4	-0.3
NiT																						1.0	-0.2	0.8	0.8	-0.5	0.9	0.9	-0.7	0.0	0.0	0.5
NiE																							-0.3	0.9	0.9	-0.4	0.9	1.0	-0.7	0.0	0.0	0.4
NiF																								-0.2	-0.1	0.3	-0.3	-0.4	0.4	-0.4	-0.4	0.1
PbT																									1.0	-0.5	0.9	0.9	-0.8	-0.2	0.3	0.4
PbE																										-0.4	0.8	0.9	-0.7	-0.3	0.2	0.4
PbF																											-0.6	-0.5	0.8	0.1	-0.8	-0.2
ZnT																												0.9	-0.9	0.2	0.1	0.7
ZnE																													-0.7	-0.1	0.1	0.4
ZnF																														-0.2	-0.5	-0.5
pH																															-0.2	0.5
SOM																																-0.1

[a] XF – $[X]_{Fruit}$ where X=the various elements;
[b] XT – $[\text{soil X}]_{Total}$ where X=the various elements;
[c] XE – $[X]_{Exchangeable}$ where X=the various elements;
SOM – Soil organic matter; CEC – Cation exchange capacity.

REFERENCES

Banas, A, Kwiatek, WM, Banas, K, Gajda, M, Pawlicki, B, Cichocki, T. 2010. Correlation of concentrations of selected trace elements with Gleason grade of prostate tissues. J. Biol. Inorg. Chem. 15: 1147-1155.

Chapman, HD. 1965. Cation Exchange capacity. *In* Methods of Soil Analysis Part 2 - Chemical and Microbiological Properties; Black, C.A., Ed.; American Society of Agronomy, Madison, Wisconsin, pp. 891-901.

Chino, M, Baba, A. 1981. The effects of some environmental factors on the partitioning of zinc and cadmium between roots and tops of rice plants. J. Plant Nutr. 3: 203-214.

Davis, GK, Mertz, W. 1987. Copper. *In* Trace Elements in Human and Animal Nutrition; Mertz, W., Ed.; Academic Press, Orlando, pp. 301-364.

Domingo, JL. 1989. Cobalt in the environment and its toxicological implications. In Reviews of Environmental Contamination and Toxicology, Springer-Verlag, New York, pp. 105-132.

Dourson, ML. 1994. The chromium reference doses (RfD). *In* Risk Assessment of Essential Elements; Mertz, W., Abernathy, C.O., Olin, S.S., Eds; ILSI Press, Washington, DC, pp. 207-212.

Faber, M, Kvalsvig, JD, Lombard, CJ, Benade, AJ. 2005. Effect of a fortified maize-meal porridge on anemia, micronutrient status, and motor development of infants. Am. J. Clin. Nutr. 82: 1032-39.

Fan, TWM, Lane, AN, Pedler, J, Crowley, D, Higashi, RM. 1997. Comprehensive analysis of organic ligands in whole root exudates using nuclear magnetic resonance and gas chromatography mass spectrometry. Anal. Biochem. 251: 57-68.

Food Surveillance Information Sheet. 1997. No.131. 1994 Total Diet Study: Metals and Other Elements; Ministry of Agriculture, Fisheries and Food, United Kingdom.

Golovatyj, SE, Bogatyreva, EN. 1999. Effect of levels of chromium content in a soil on its distribution in organs of corn plants. *In* Soil Research and Use of Fertilisers; Bodevich, I.M.' Lapa, V.V., Levitan, T.V.' Eds.; BRISSA, Minsk, Belarus, pp. 197-204.

Department of Health. 2004. Government Notices, Foodstuffs, cosmetics and disinfectants Act, (Act No. 54 of 1972). Government Gazette, South Africa.

Herselman, JE,Steyn, CE, Fey, MV. 2005. Baseline concentration of Cd, Co, Cr, Cu, Pb, Ni and Zn in surface soils of South Africa. S. Afr. J. Sci. 101: 509-512.

Hopkins, LL, Ransome-Kuti, O, Majaj, AS. 1968. Improvement of impaired carbohydrate metabolism by chromium(III) in malnourished infants. Am. J. Clin. Nutr. 21: 203-211.

Hua-Fen, L, Gray, C, Mico, C, Zhao, F, McGrath, SP. 2009. Phytotoxicity and bioavailability of cobalt to plants in a range of soils. Chemosphere. 75: 979-986.

Huang, JW, Cunningham, SD. 1996. Lead phytoextrction: species variation in lead uptake and translocation. New Phytol. 134: 25-84.

Institute of Medicine, Food and Nutrition Board. 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc; National Academy Press, Washington, DC.

Kabata-Pendias, A, Pendias, H. 1989. Trace Elements in the Soil and Plants. CRC Press, Boca Raton, Florida.

Khan, A, Safdar, M. 2003. Role of diet, nutrients, spices and natural products in diabetes mellitus. Pak. J. Nutr. 2: 1-12.

Kobla, HV, Volpe, SL. 2000. Chromium, exercise, and body composition. Crit Rev Food Sci Nutr. 40: 291-308.

Labadarios, D. 2007. The National Food Consumption Survey – Fortification Baseline (NFCS-FB): The Knowledge, Attitude, Behaviour and Procurement Regarding Fortified Foods, a Measure of Hunger and the Anthropometric and Selected Micronutrient Status of Children Aged 1-9 years and Women of Child Bearing Age: South Africa, 2005. Department of Health, Nutrition Directorate, Pretoria, South Africa.

Levine, RA, Streeten, DPH, Doisy, RJ. 1968. Effects of oral chromium supplementation on the glucose tolerance of elderly subjects. Metabolism. 17: 114-125.

Liao, MT, Hedley, MJ, Woolley, DJ, Brooks, RR, Nichols, MA. 2000. Copper uptake and translocation in chicory (Cichorium intybus L. cv Grasslands Puna) and tomato (Lycopersicon esculentum Mill. cv Rondy) plants grown in NFT system. II. The role of nicotianamine and histidine in xylem sap copper transport. Plant Soil. 223: 243-252.

Maharaj, KB, Bhandari, N, Bahl, R. 2003. Management of the severely malnourished child: perspective from developing countries. Br. Med. J. 326: 146.

Mathee, A, von Schirnding, YER, Levine, J, Ismail, A, Huntley, R, Cantrell, A. 2002. A survey of blood lead levels amongst young Johannesburg school children. Environ. Res. 90: 181-84.

McBride, MB. 1982. Electron spin resonance investigation of Mn^{2+} complexation in natural and synthetic organics. Soil Sci. Soc. Am. J. 46: 1137-1143.

Navaroja, DR, Kanchana. M. 2012. Comparative lead uptake and responses of five different plant species grown on lead contaminated soil. Bioscan. 7: 225-227.

Palit, S, Sharma, A, Talukder, G. 1994. Effects of cobalt on plants. Bot. Rev. 60: 149-181.

Palmer E, Pitman, N. 1972. Trees for Southern Africa Covering all known Indigenous Species in Republic of South Africa, South West Africa, Botswana, Lesotho and Swaziland; A.A. Balkema, Cape Town, South Africa.

Pujol, J. 1996. Naturafrica-The Herbalist Handbook; Jean Pujol Natural Healers' Foundation: Durban, South Africa.

Singh,VP. 2005. Toxic Metals and Environmental Issues; Sarup and Sons, New Delhi, India, pp. 121-276.

Swart, R, Sanders, D, McLachlan, M. 2008. Nutrition: A primary healthcare perspective. In South African Health Review; Barron, P., Roma-Reardon, J., Eds.; Health Systems Trust, Durban, South Africa.

van Wyk, BE, van Oudtshoorn B, Gericke, N. 2002. Medicinal Plants of South Africa; Briza Publications, Pretoria, South Africa, pp. 146-147.

Walkley, A, Black, I.A. 1934. An Examination of the Degjareff Method for Determining SOM and a Proposed Modification of the Chromic Acid Titration Method. Soil. Sc. 37: 29-38.

Welch, RM. 1995. Micronutrient nutrition of plants. Crit. Rev. Plant Sci. 14: 49-82.

OVERALL SUMMARY

This study focused on two plants species WHICH are indigenous to KwaZulu-Natal and contain edible fruits namely *C. macrocarpa* and *H. caffrum*. Both of these plants are commonly used in South African traditional medicine to treat, manage or control a variety of human ailments such as coughs, venereal diseases, skin conditions, pain, convulsions and epilepsy. *H. caffrum* is popularly planted as a street tree whereas *C. macrocarpa* is usually used as a hedge plant in a number of South African towns and cities. The fruit of *C. macrocarpa* usually grow in summer and those of *H. caffrum* usually ripen in autumn but off-season fruits are also produced if conditions are favourable. The fruits are enjoyed by the local people, especially children and they are also sold along the roadside in KwaZulu-Natal by hawkers. In South Africa, the potential of *C. macrocarpa* and *H. caffrum* has been identified as commercial crops and to boost food security yet not much else has been done to promote these plants.

Because of the claimed medicinal value and nutritional potential of these two plants, this study aimed at investigating them as a source of secondary metabolites and essential dietary elements. Firstly, the isolation and characterisation of the secondary metabolites was undertaken. This was done primarily to determine what phytocompounds were present in these plants and to verify the plants medicinal value. In so doing, the ethnomedicinal use of these plants would be validated and a scientific basis for the traditional use of these plants would be provided. In addition, bioassays were identified to test the biological activities of isolated compounds. This was done to augment the findings and to highlight any additional medicinal potential.

The edible fruits of these plants were of most interest in this investigation. Whilst the phytochemical analysis elucidated the medicinal benefits associated with consuming the fruits, the analytical analysis revealed the nutritional benefits. The concentrations of essential elements in the fruits were determined and these were compared to RDAs to assess for nutritional value. The impact of soil quality on elemental uptake by the plants was determined and the plants control on elemental uptake was evaluated. This was done to determine if the plant had the ability to accumulate metals especially toxic ones that are harmful to human health.

Findings from C. macrocarpa

The *Amatungula* fruit was found to be rich in monounsaturated and essential fatty acids that are dietary requirements for the prevention of protein-energy malnutrition. Also, the linoleic acid to α-linolenic acid ratio in the fruits conformed to the recommended range for cardiac health. Elemental analysis indicated that the fruits conform to the RDAs for most elements and if consumed, may contribute significantly to the diet. The results showed that site location had a major effect on fruit concentrations therefore picking and buying fruits from high vehicular areas should be avoided due to the risk of Pb toxicity. The plant however had good control on elemental uptake and the fruits were low in the toxic metals investigated.

Five pentacyclic triterpenes were isolated from the leaves of the plant and *Amatungula* fruit. All four of the triterpenes isolated from the fruits had the oleanane skeleton and appear to be derived biosynthetically from oleanolic acid while only an ursane triterpene was isolated from the leaves. The four pentacyclic oleanane triterpenes were β-amyrin,

methyl oleanolate, oleanolic acid and 3β-hydroxyolean-11-en-28,13β-olide and the ursane triterpene was ursolic acid. MICs showed that addition of the lactone ring to oleanane triterpenes contribute significantly to the expression of antibacterial activity, which makes it an interesting molecule for synthetic and biological studies. The study highlighted the immune boosting properties of the triterpene rich edible fruits which are especially important in certain areas of South Africa where high incidences of HIV and hepatitis are prevalent and where immune boosting supplements are out of reach. These fruits that are freely and readily available, if consumed, can provide some of the benefits of these supplements.

Findings from H. caffrum

The analytical results showed that for most elements in the soil, competition between elements present influenced exchangeability but uptake and distribution was primarily dependent on the plants metabolic needs. This resulted in elemental concentrations in the fruits being restricted to a small range of variation. The concentrations of toxic metals like As and Pb investigated were low. The fruits can contribute to the health and nutritional needs of most individuals for most elements. It has the potential to improve the Fe status and contribute towards a balanced diet.

Two pharmacologically active triterpenoids, β-sitosterol and lupeol, and the powerful flavan-3-ol antioxidant, (+)-catechin, were isolated from the edible fruits of *H. caffrum* whilst a mixture of cardanols (1-hydroxy-3-[(*Z*)-12'-nonadecenyl] benzene, 1-hydroxy-3-[(*Z*)-12'-heptadecenyl] benzene, 1-hydroxy-3-heptadecanyl benzene and 1-hydroxy-3-pentadecanyl benzene), the alkyl *p*-coumaric acid ester (eicosanyl-*trans*-*p*-coumarate), and

(+)-catechin were isolated from the stem bark. The reducing power of (+)-catechin by the FRAP method and its antioxidant activity as evaluated by the DPPH method was higher than the other isolated compounds including ascorbic acid. These findings highlight the medicinal benefits associated with consumption of the fruits which are especially important in a third world country like South Africa. They can serve the same purpose as vitamin cocktails containing antioxidants and sterols taken in Western and European countries, with the added benefit of them being in a natural and inexpensive form.

CONCLUSION

The aim of the study was to phytochemically and analytically investigate two medicinal plant species indigenous to KwaZulu-Natal namely *C. macrocarpa* and *H. caffrum*, which contain edible fruits with a view to determining their potential as nutraceuticals. The phytochemical investigation was done on plant parts that are used by traditional healers to determine if they contained any secondary metabolites that would validate their ethnomedicinal use, and on the fruits to determine their medicinal value. The analytical investigation was done primarily to determine the nutritional value of the fruits.

The two plant species studied in this work produced a range of secondary metabolites in the plant parts investigated. These phytocompounds have been shown to have biological activity, both in the literature and by our own present bioassays. This knowledge is important to Indigenous Knowledge Systems as it provides a scientific basis for the use of these plants by traditional healers. The fruits, in particular, have been shown to be rich in secondary metabolites and essential dietary elements. As a result they provide an

inexpensive alternative to the pharmaceuticals or supplements available on the market but inaccessible to many communities in South Africa. They can also contribute to human nutrition and health as they are a source of essential elements and can contribute to a balanced diet.

This study lends scientific credence and validity to the ethnomedicinal use of the plant and highlights the nutritional and medicinal benefits of consuming the indigenous edible fruits.

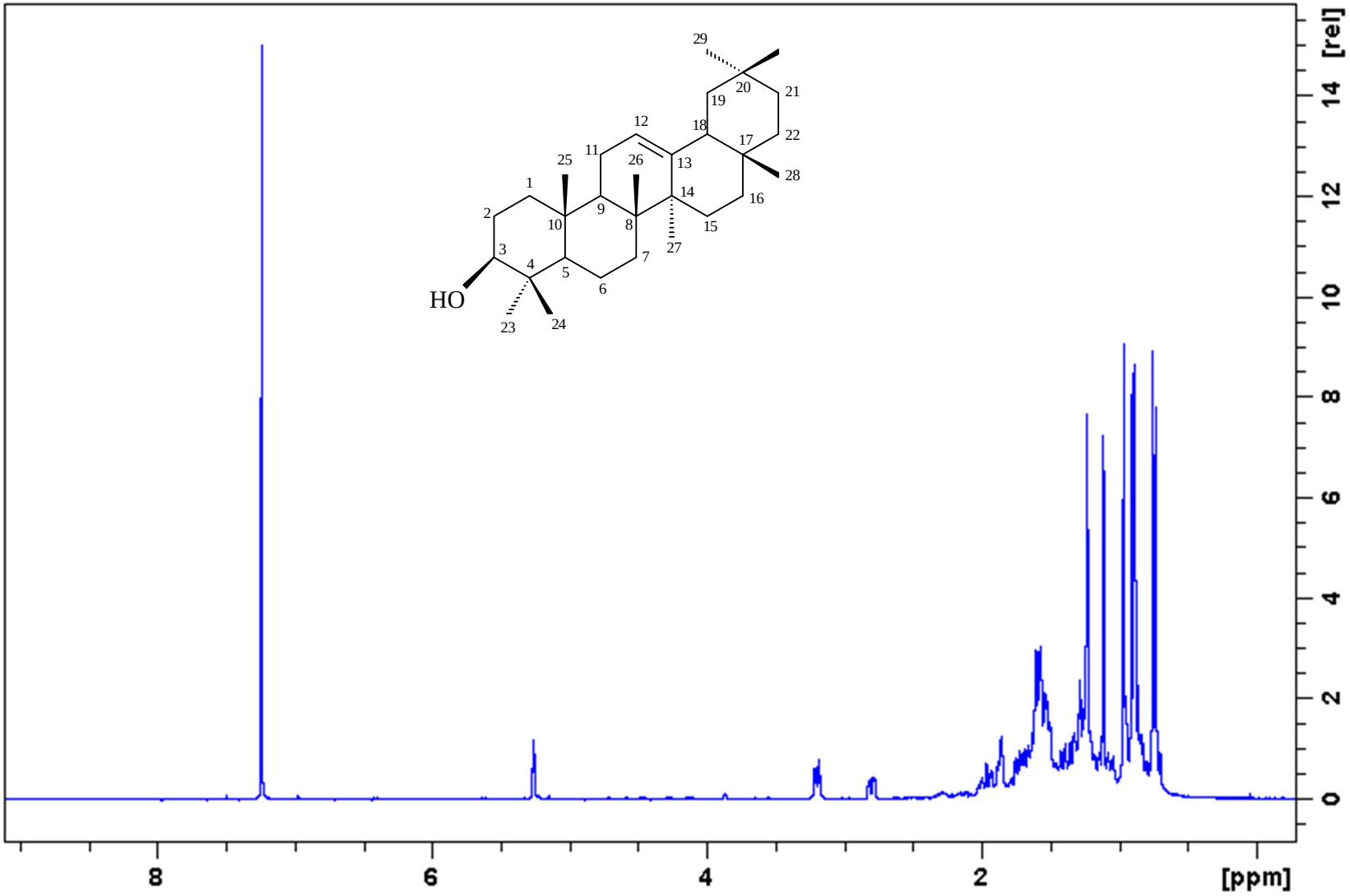
RECOMMENDATION FOR FURTHER WORK

- An investigation into the reasons why the adhesion of *K. pneumonia,* the gram-negative opportunistic pathogen, was increased following exposure to test compounds.
- Isolation and identification of the phytocompounds in the other parts of the investigated plants.

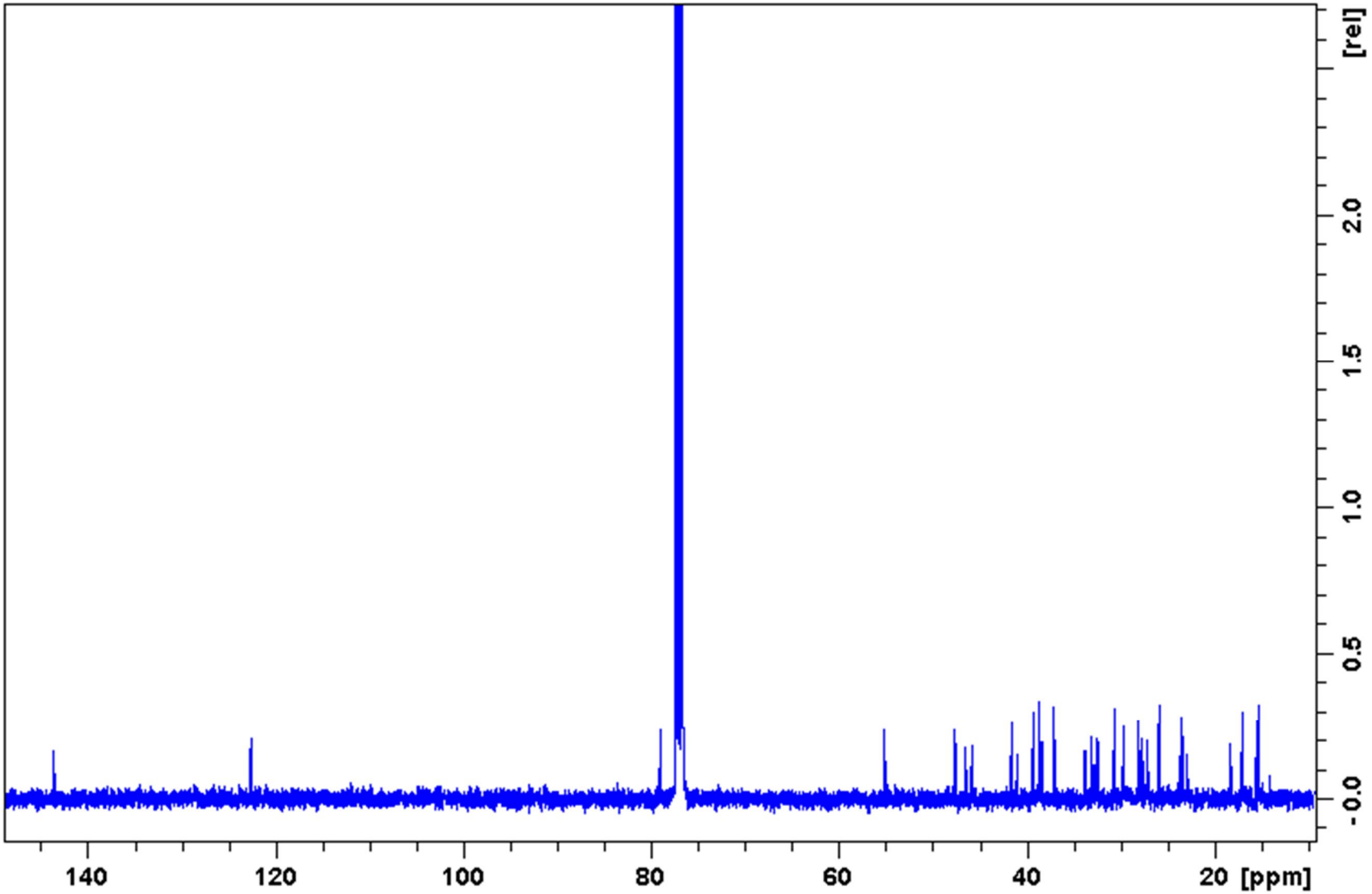
APPENDIX

SUPPORTING INFORMATION

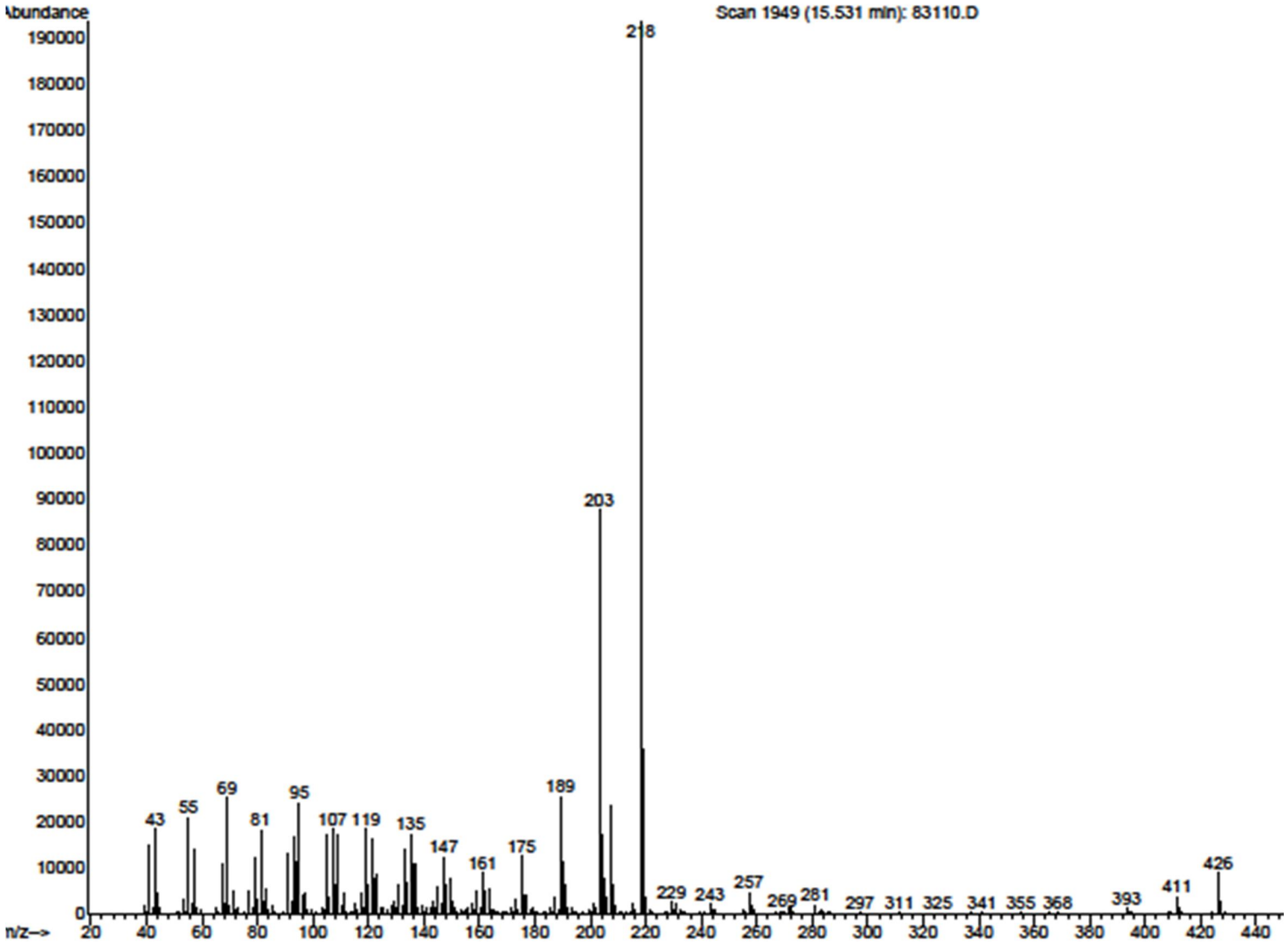
Supporting information includes NMR, IR, UV, and MS data.



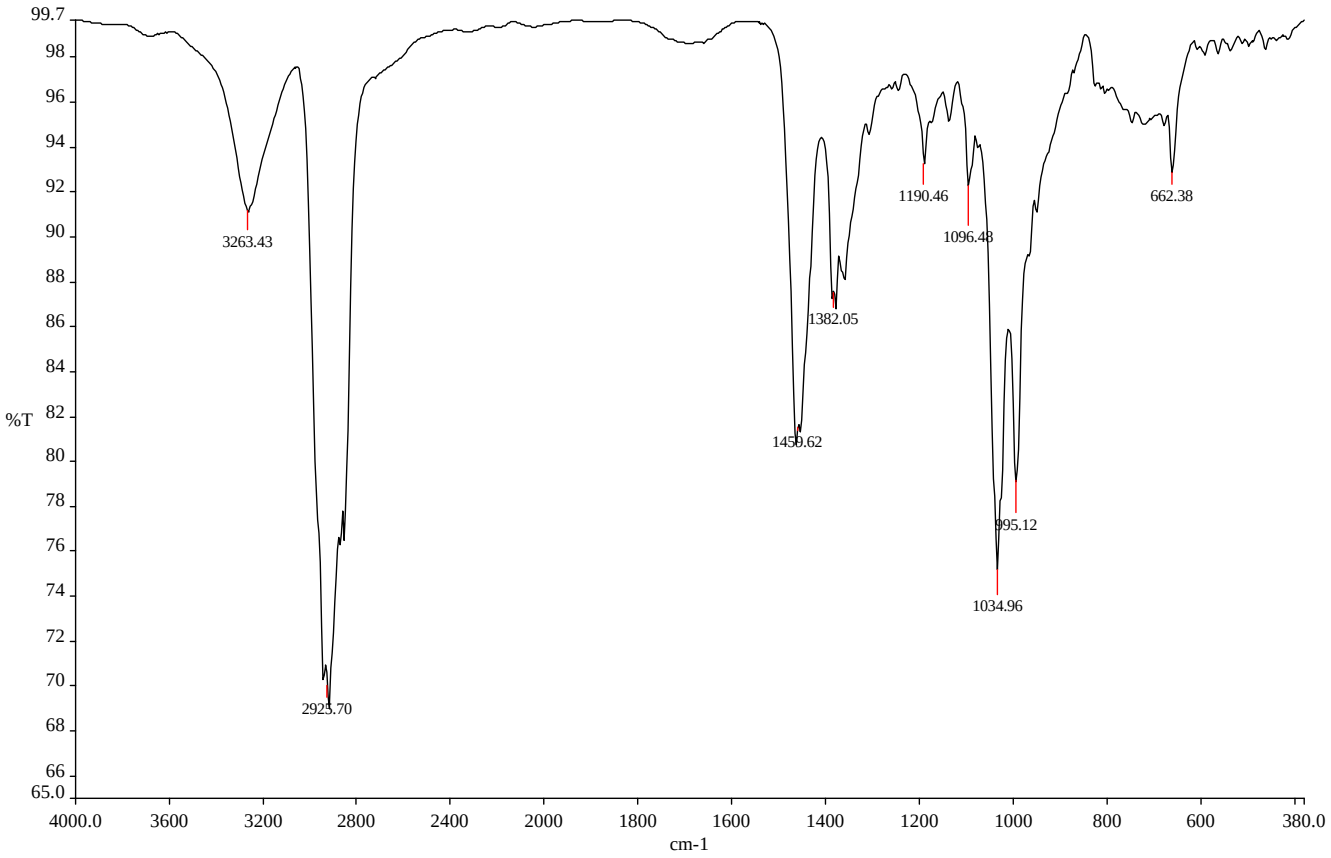
^{1}H-NMR for A-1 (β-amyrin)

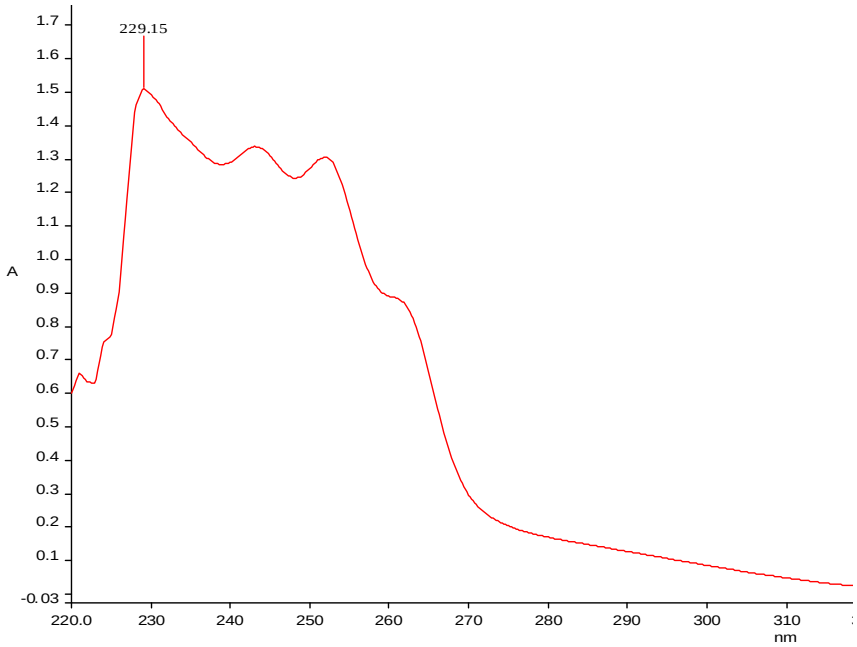


^{13}C-NMR for A-1 (β-amyrin)

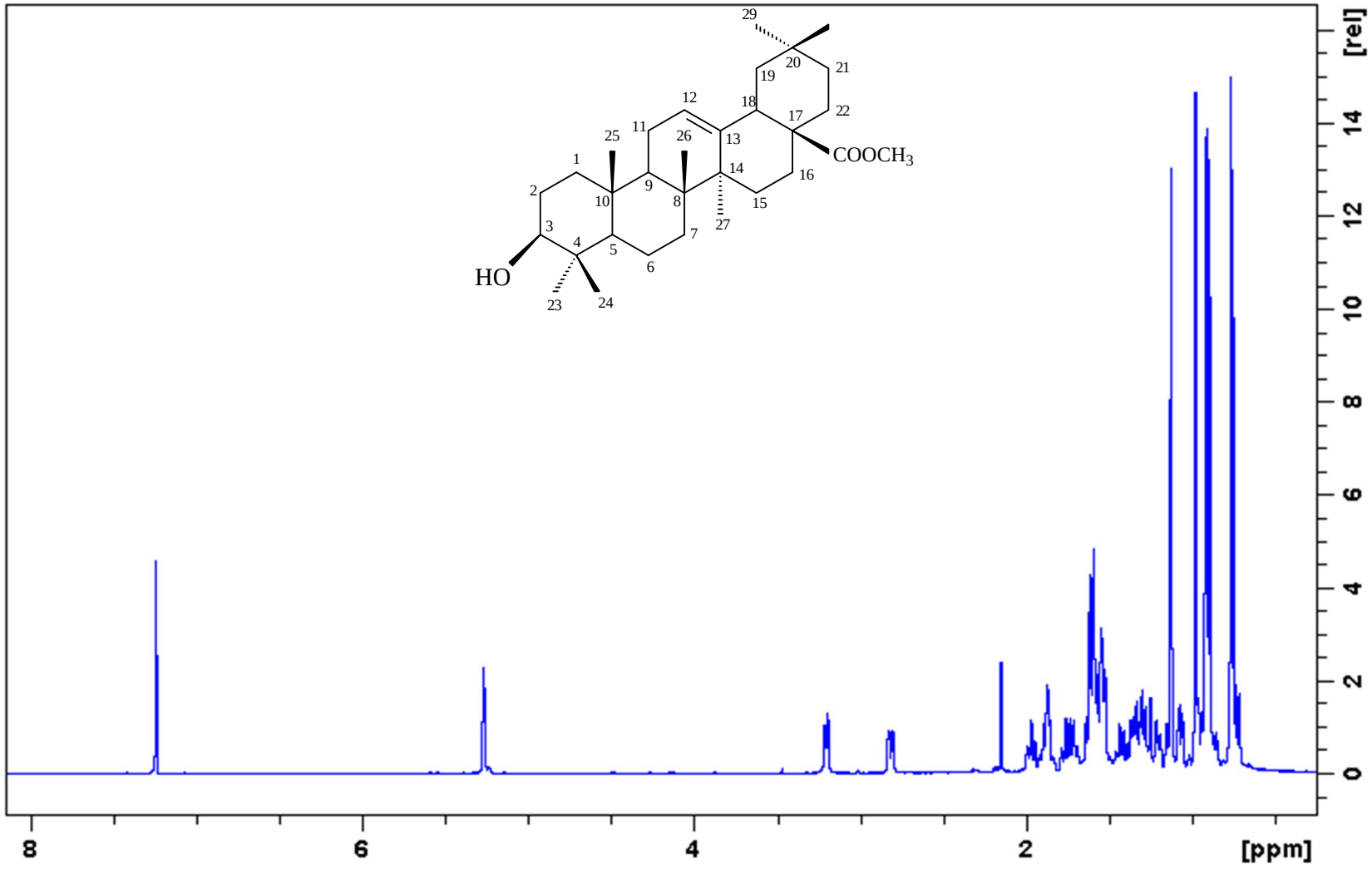


GC-MS spectrum for A-1 (β-amyrin)

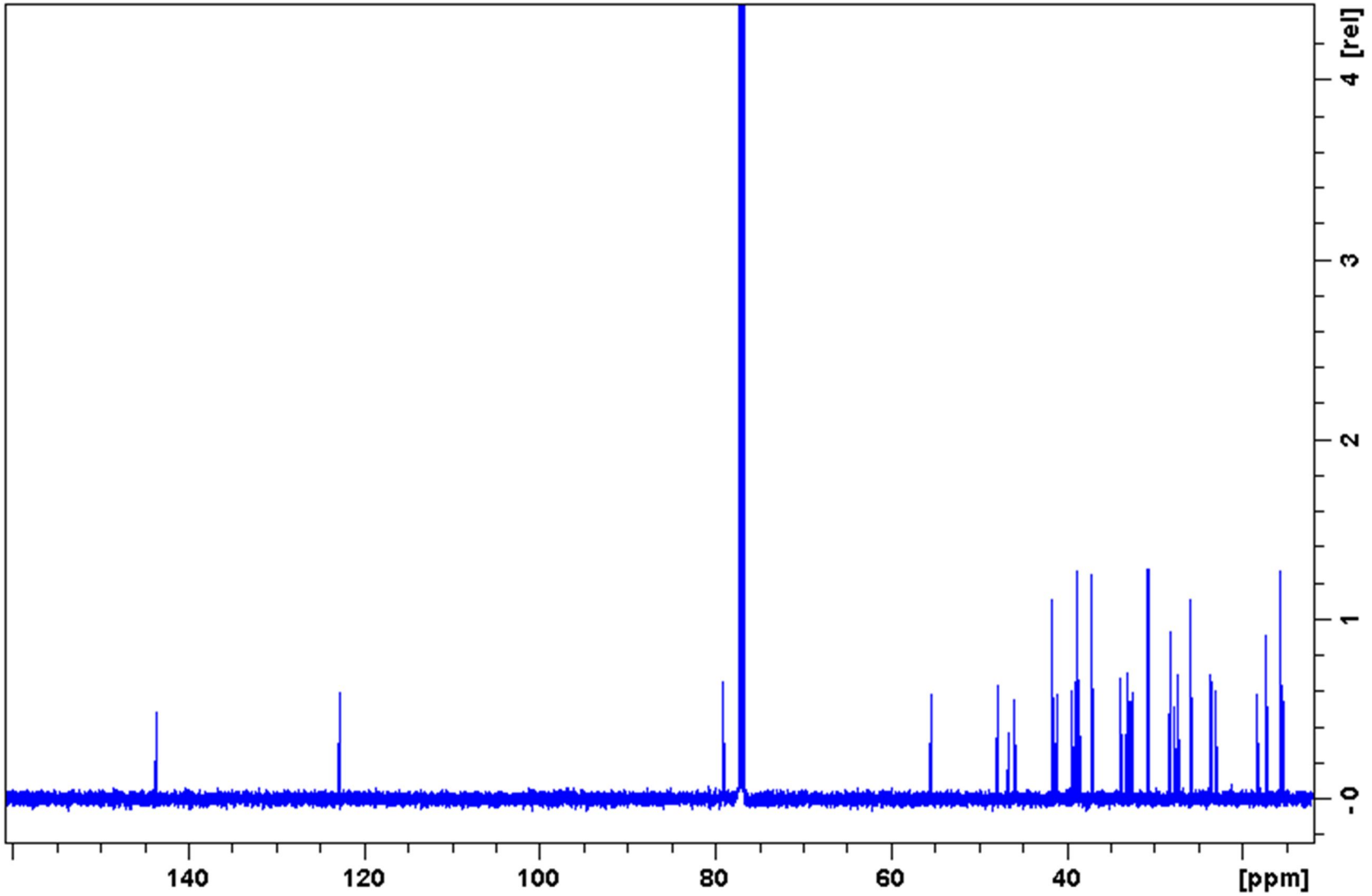




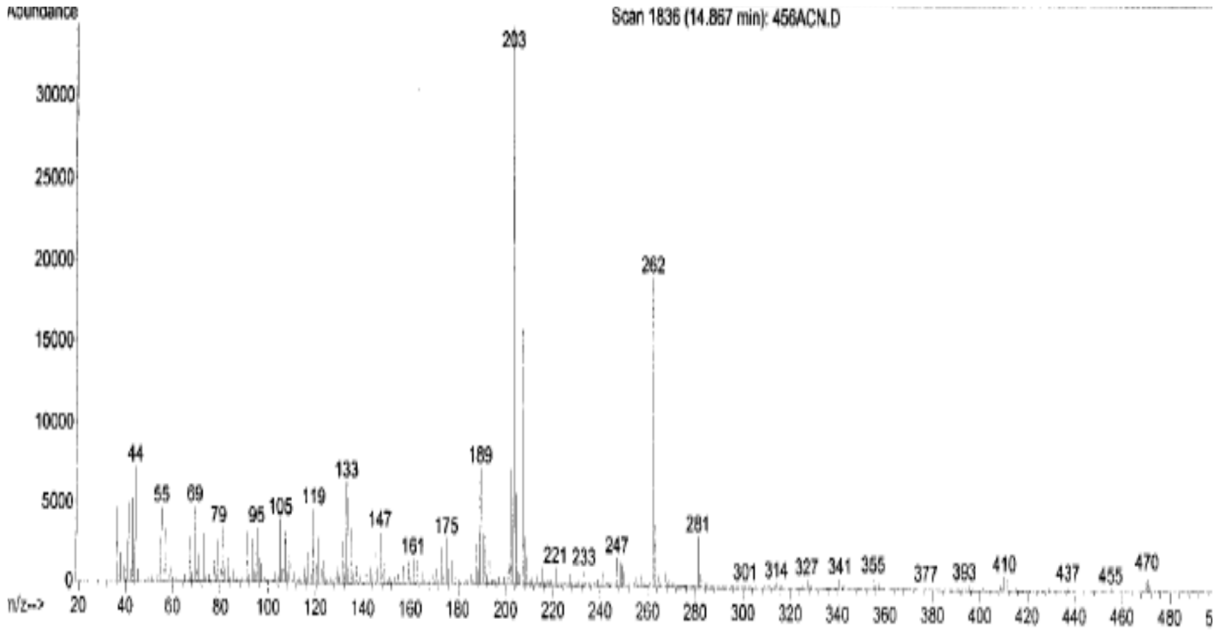
IR and UV spectra for A-1 (β-amyrin)



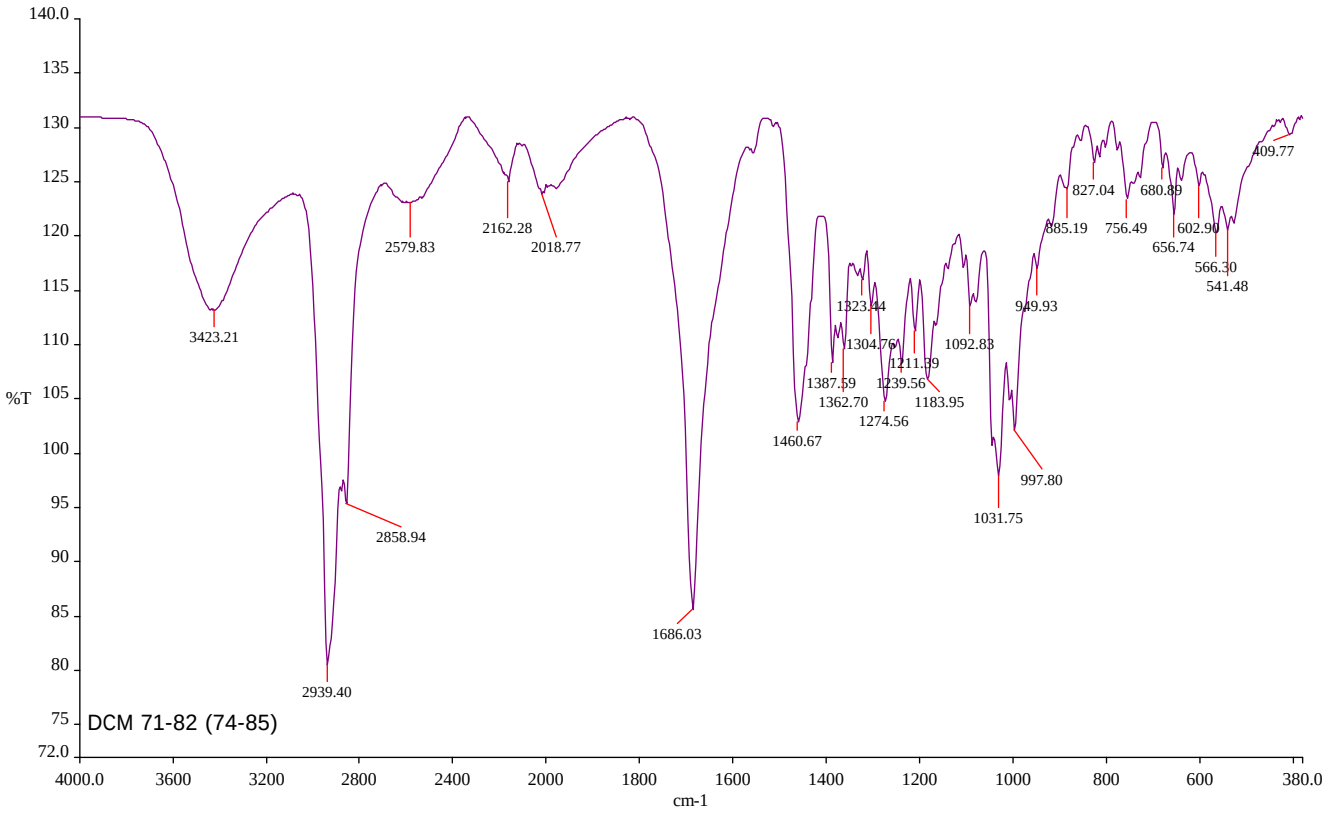
^{1}H-NMR for A-2 (methyl oleanolate)

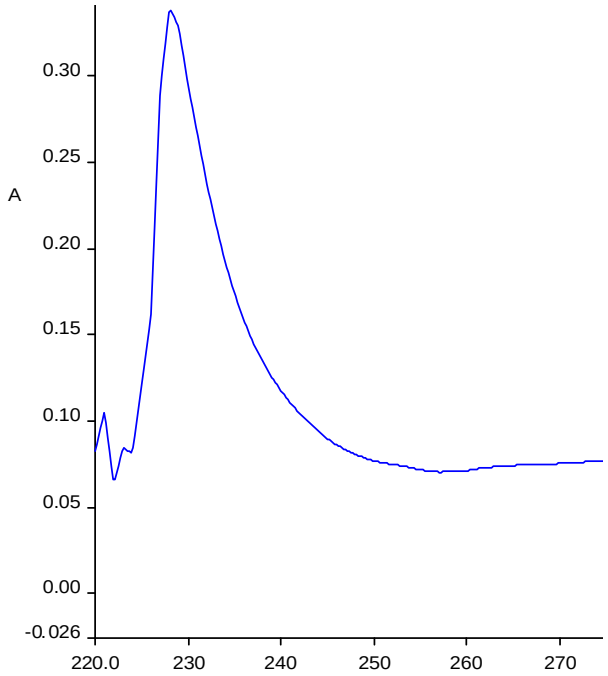


^{13}C-NMR for A-2 (methyl oleanolate)

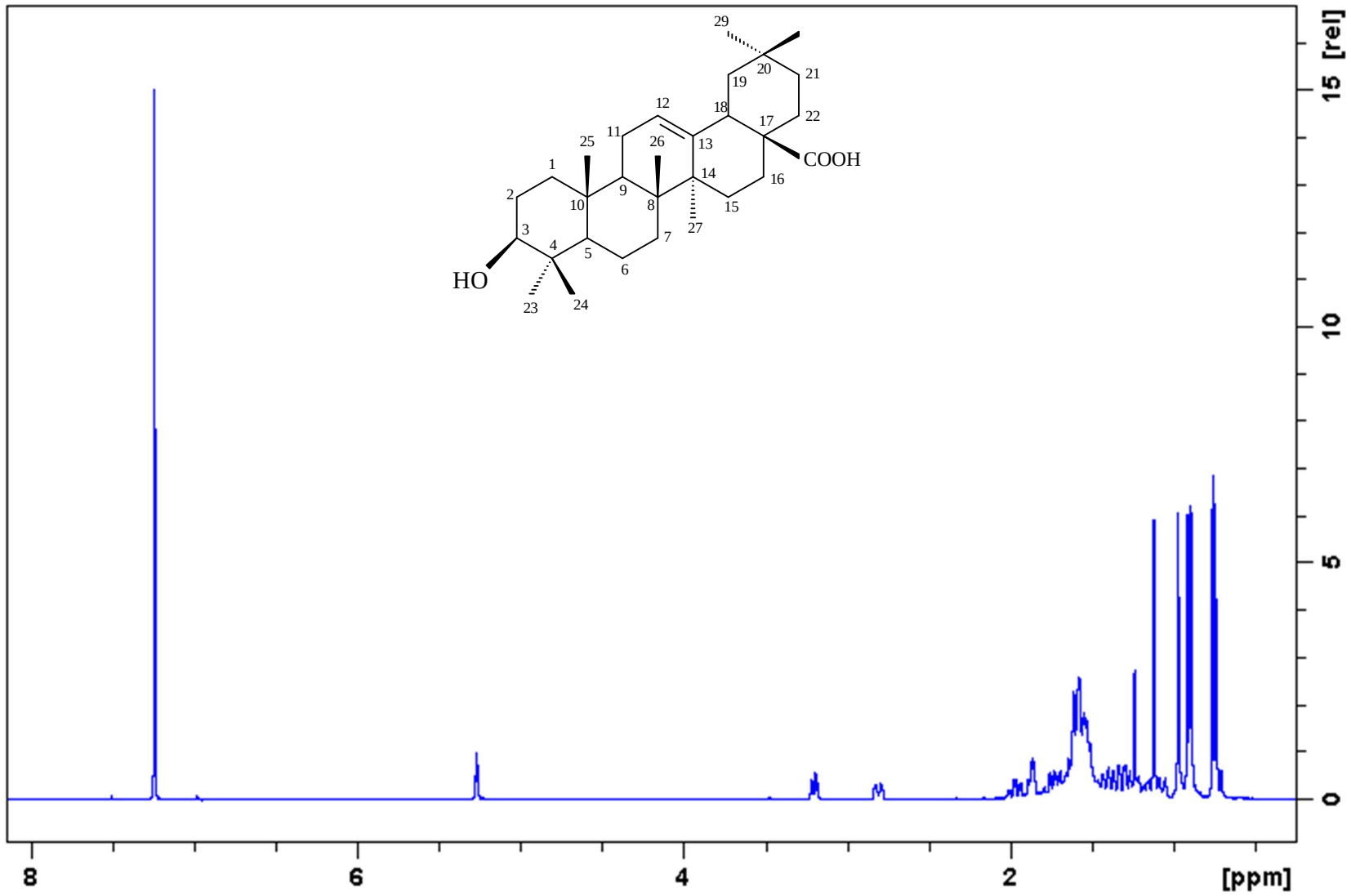


GC-MS spectrum for A-2 (methyl oleanolate)

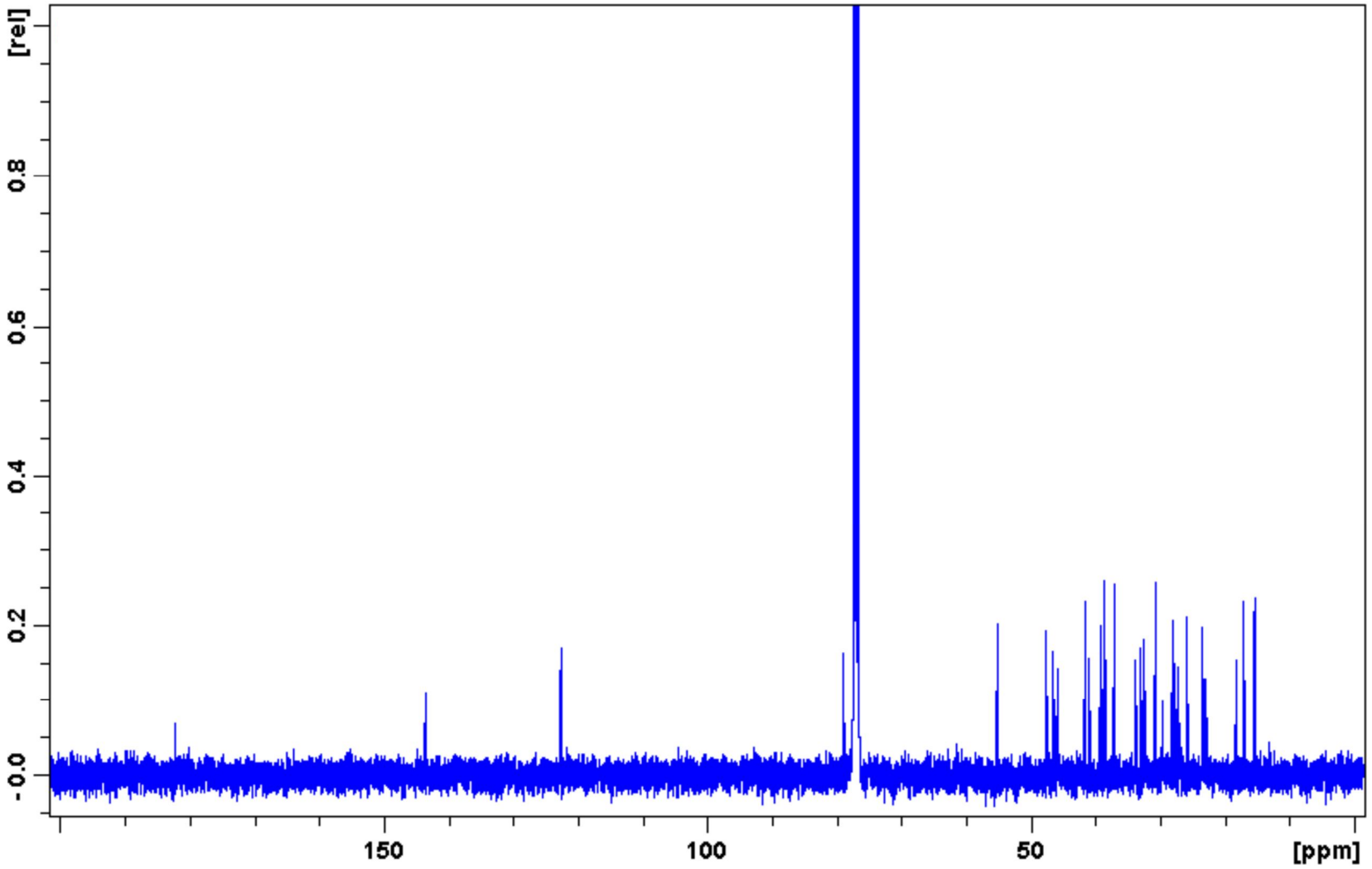




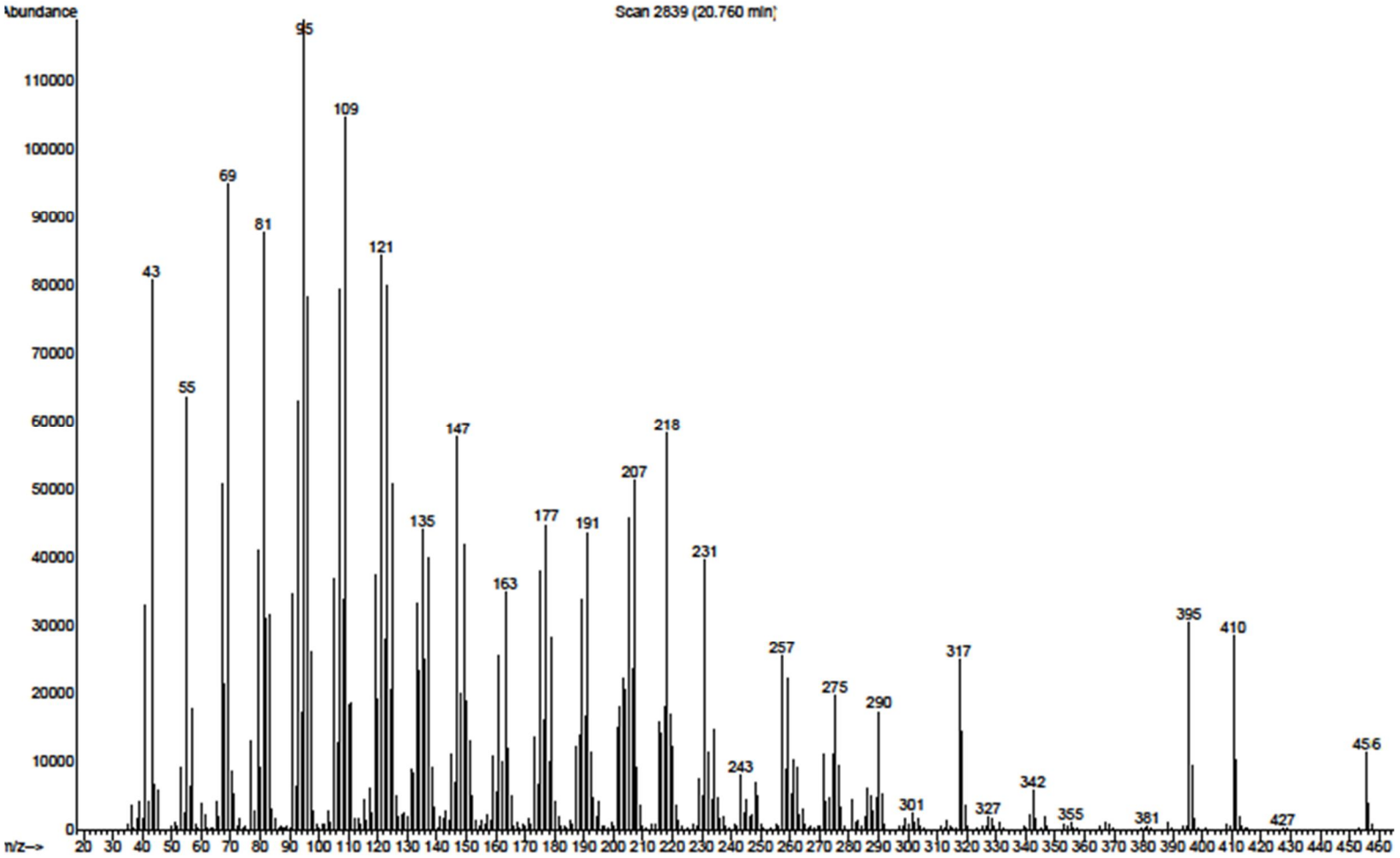
IR and UV spectra for A-2 (methyl oleanolate)



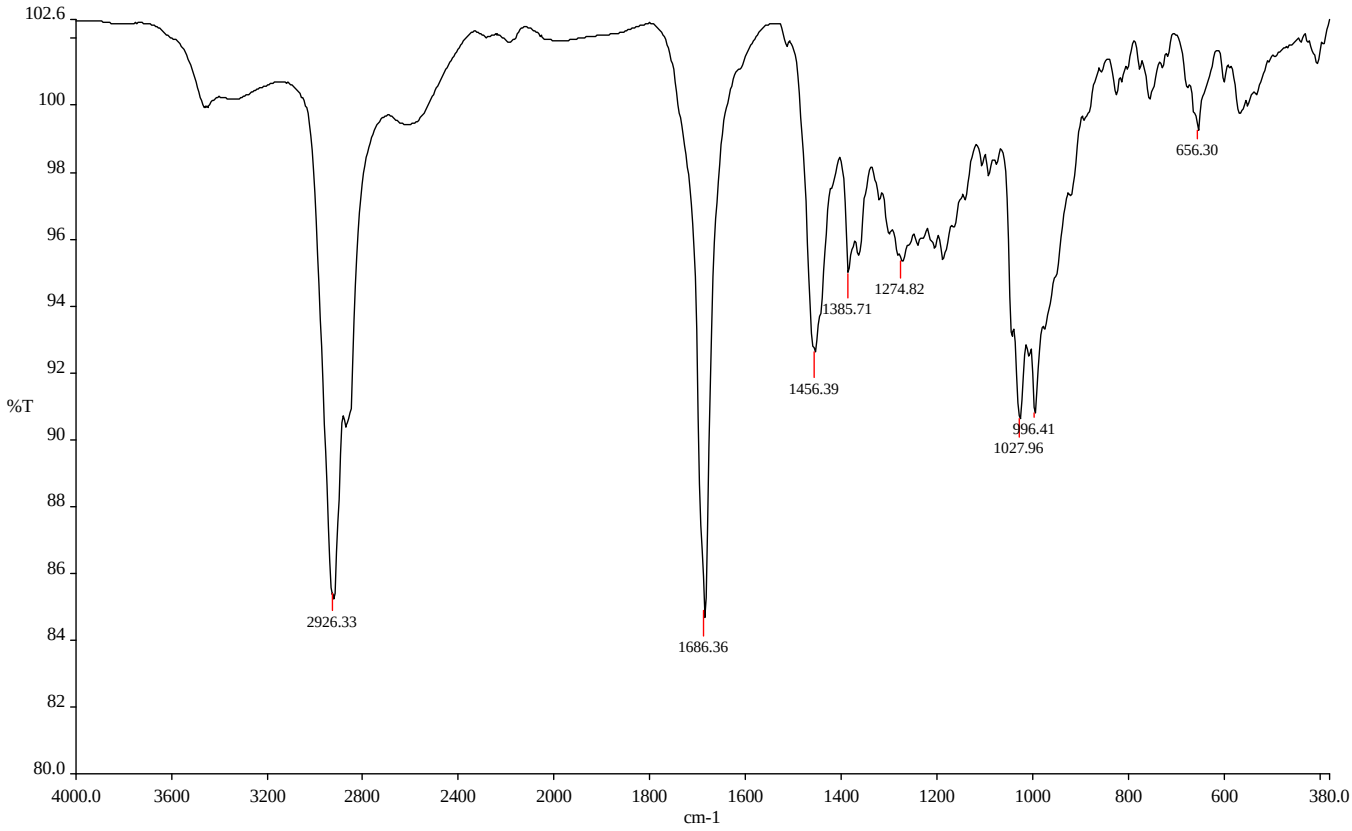
^{1}H-NMR for A-3 (oleanolic acid)

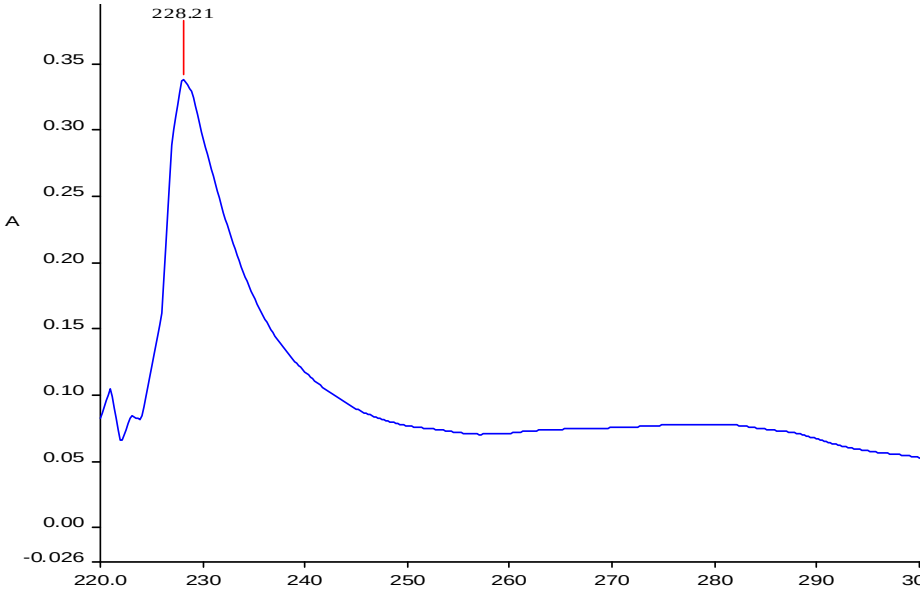


^{13}C-NMR for A-3 (oleanolic acid)

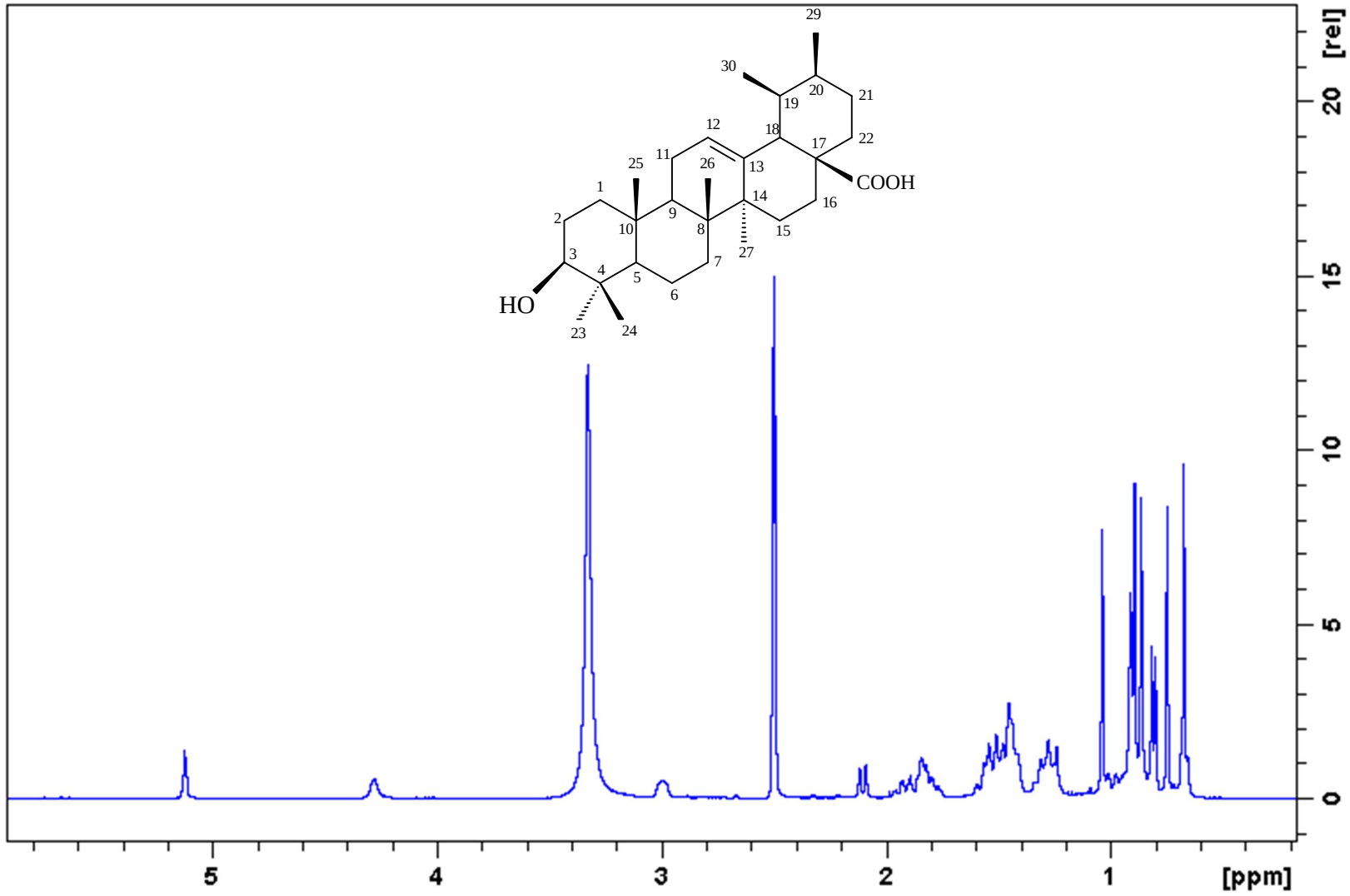


GC-MS spectrum for A-3 (oleanolic acid)

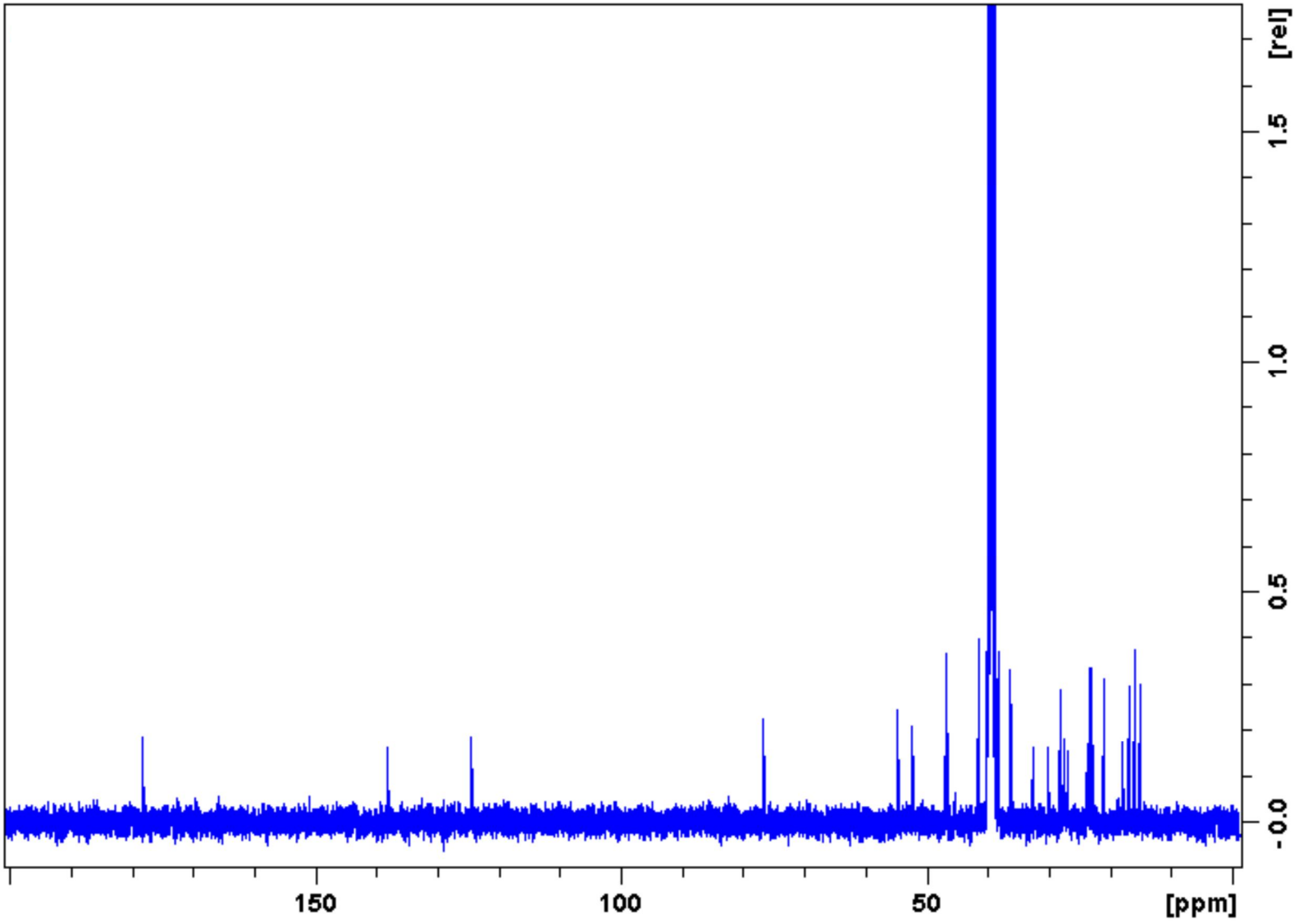




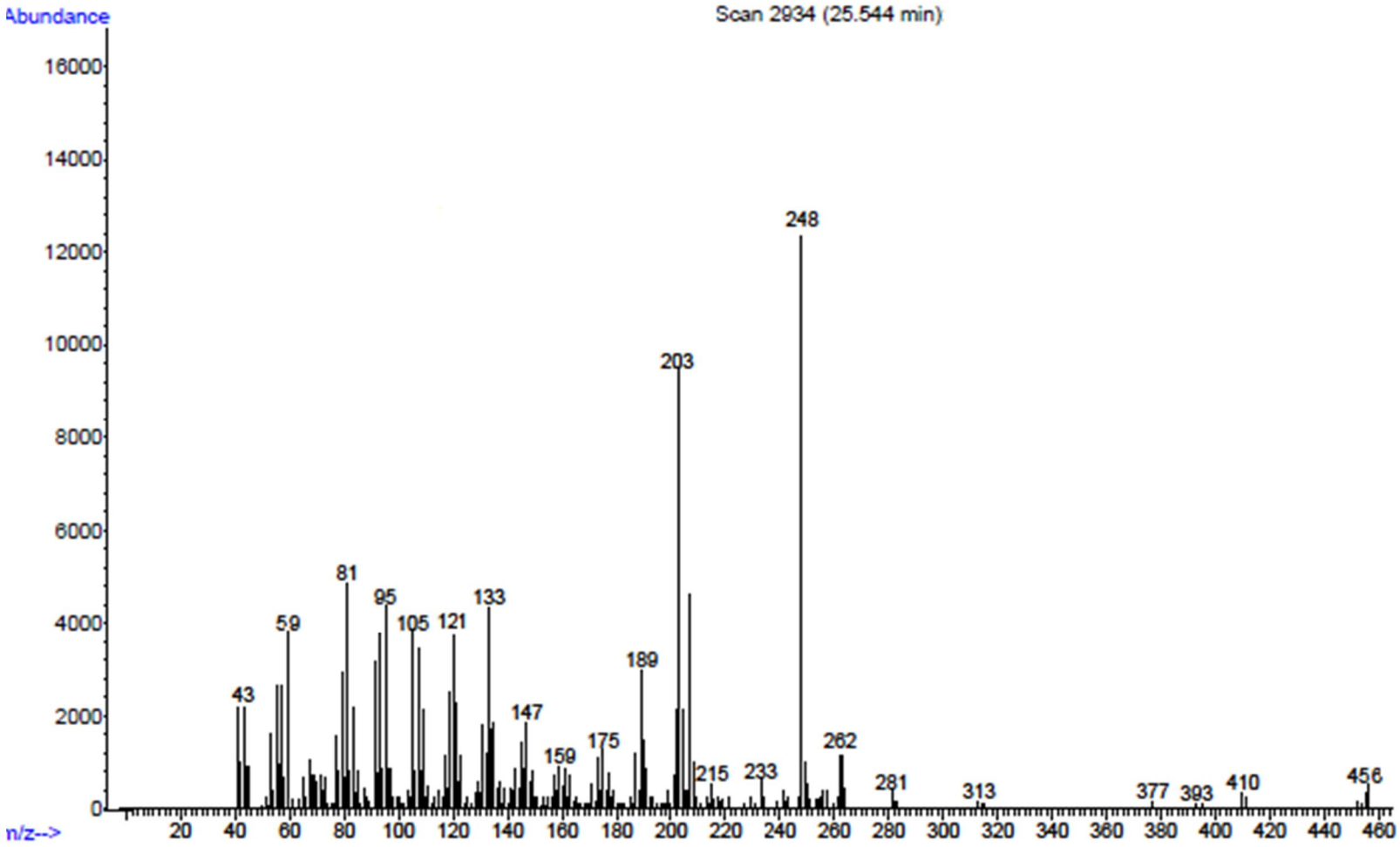
IR and UV spectra for A-3 (oleanolic acid)



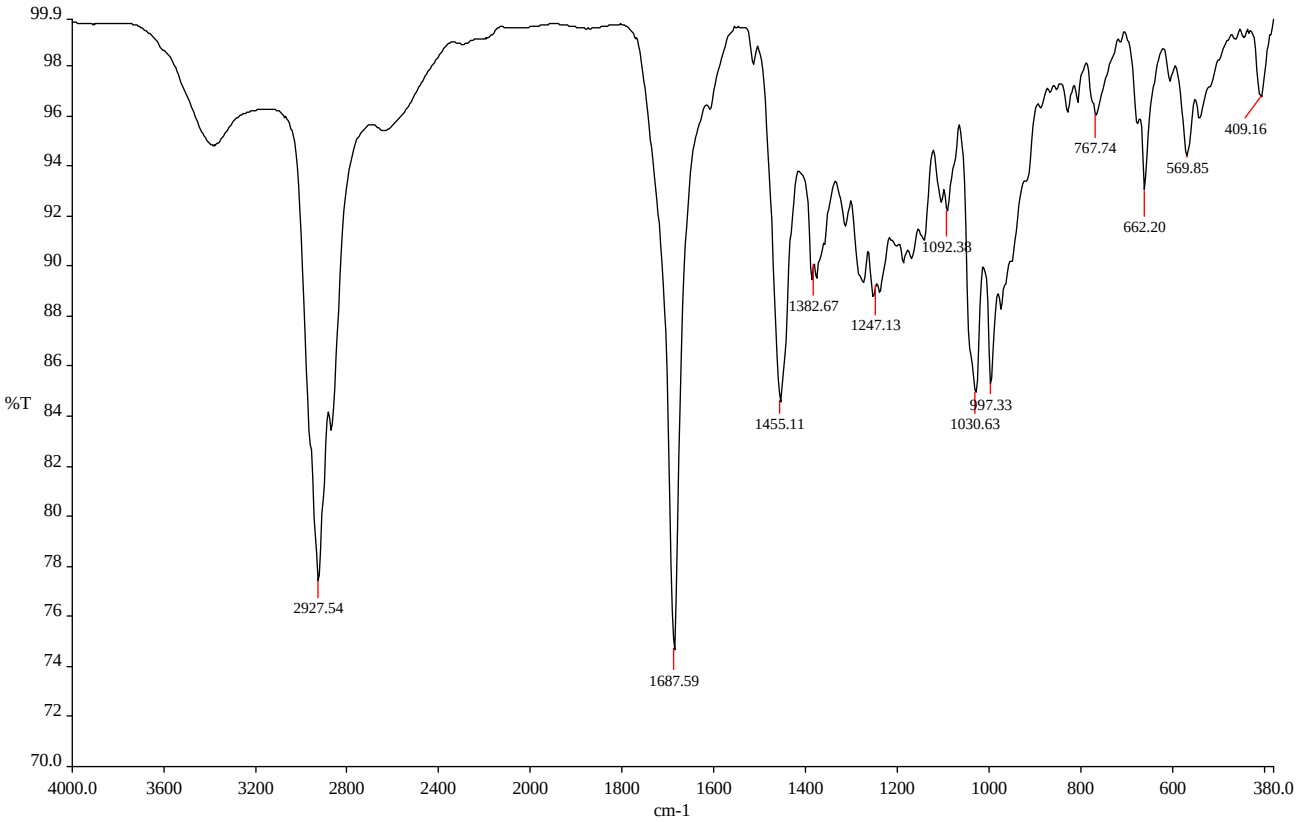
^{1}H-NMR for A-4 (ursolic acid)

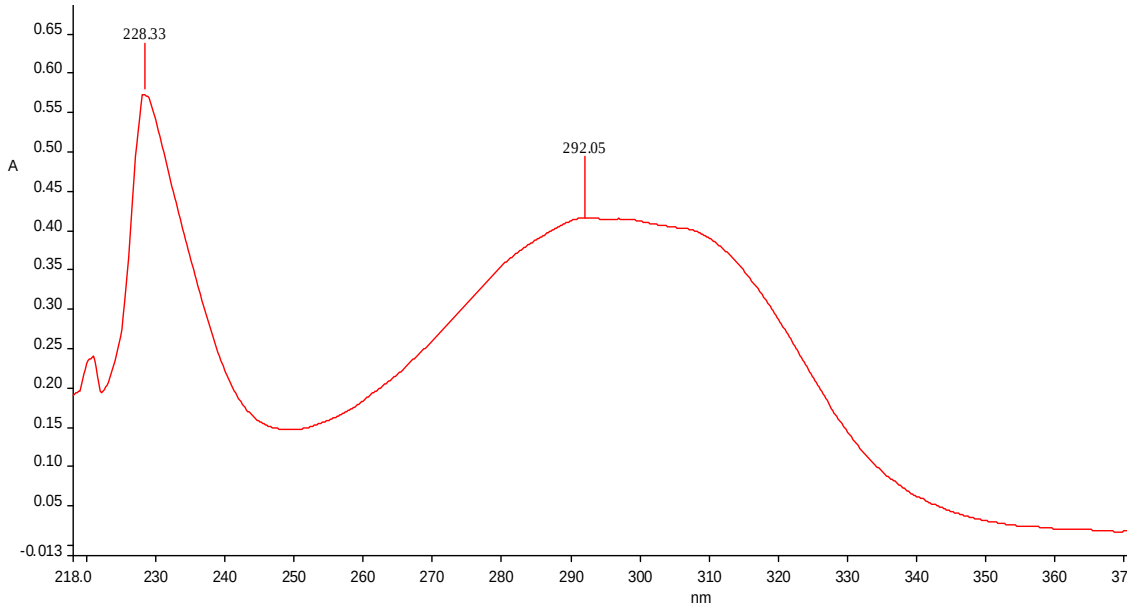


^{13}C-NMR for A-4 (ursolic acid)

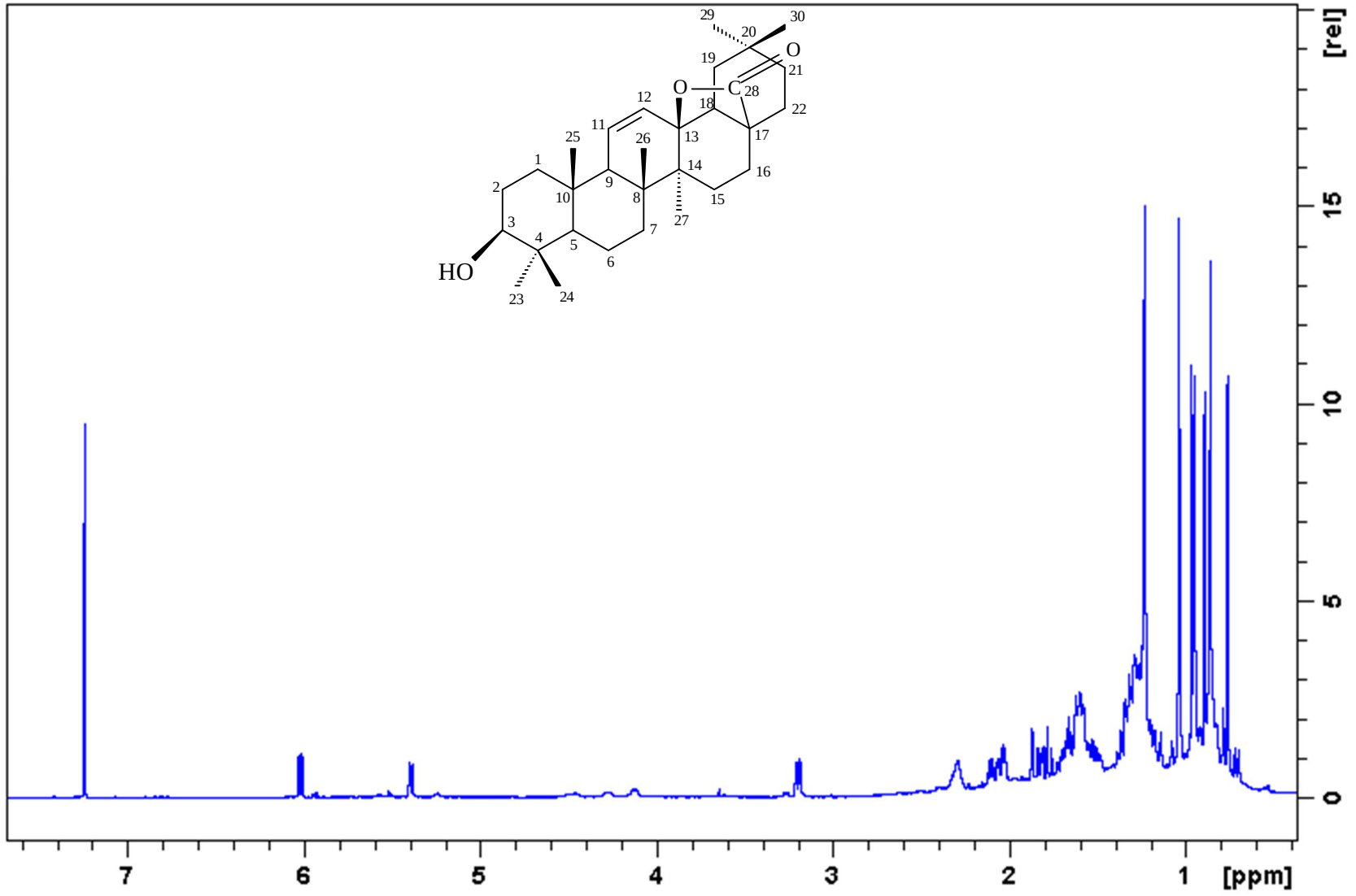


GC-MS spectrum for A-4 (ursolic acid)

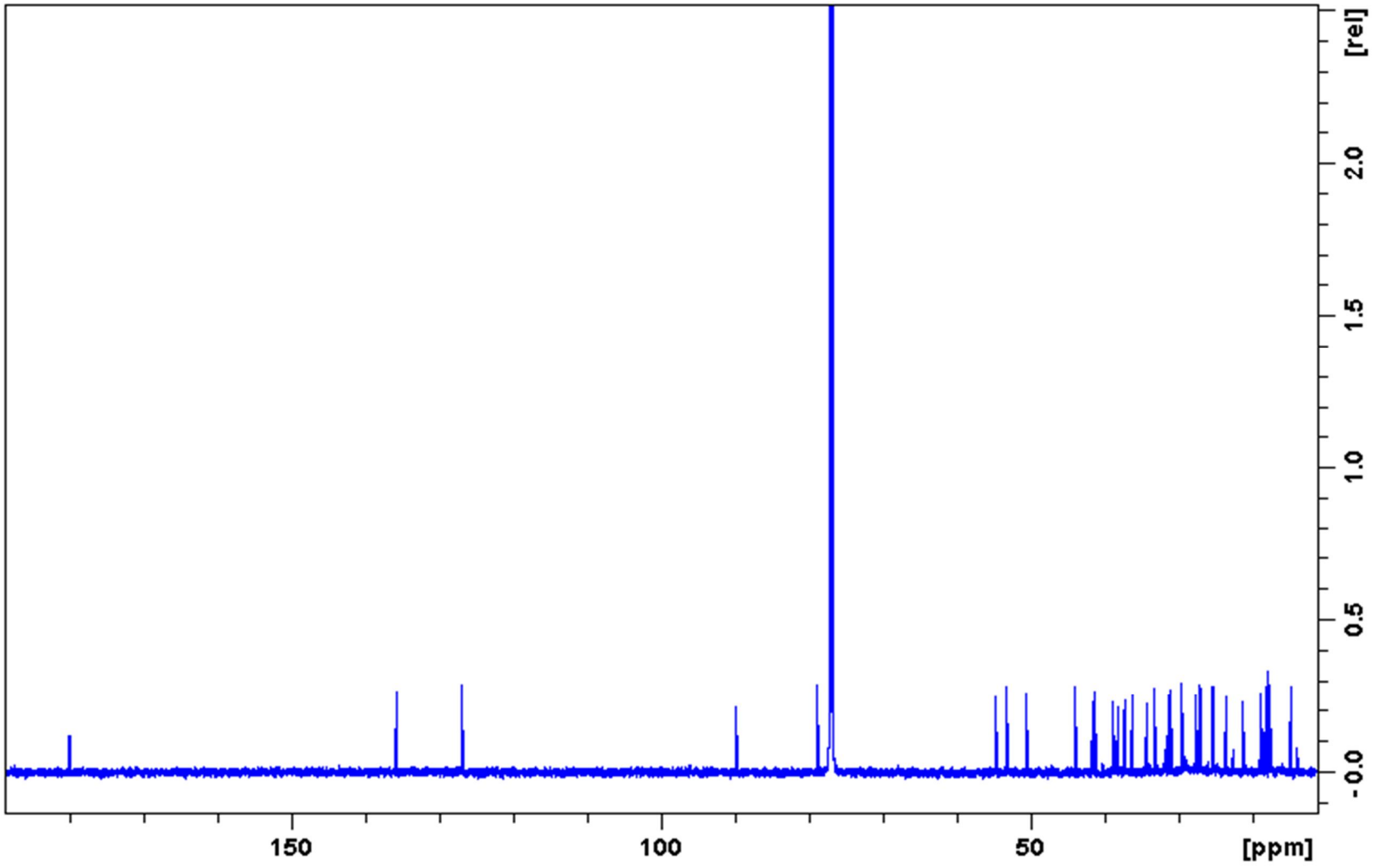




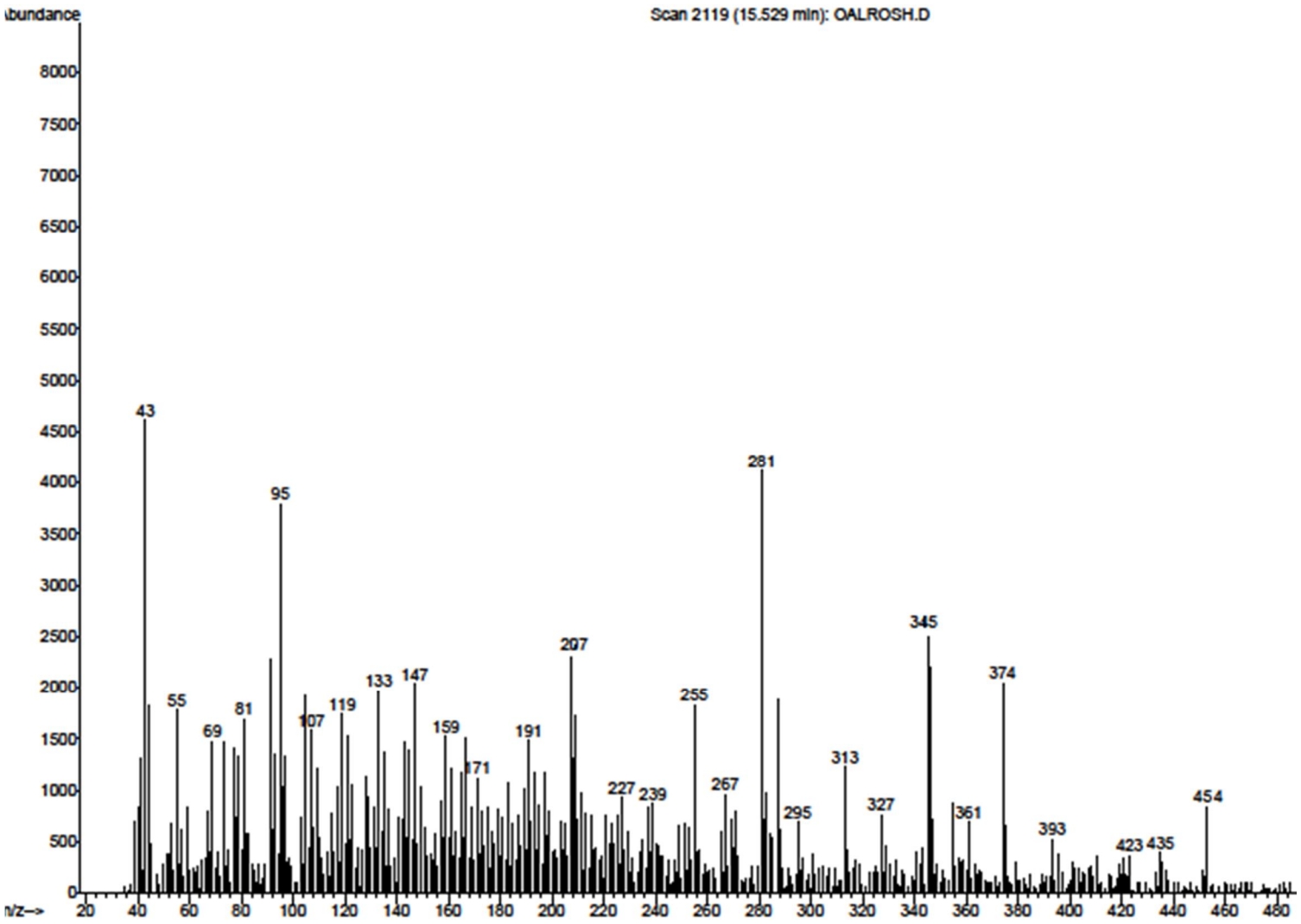
IR and UV spectra for A-4 (ursolic acid)



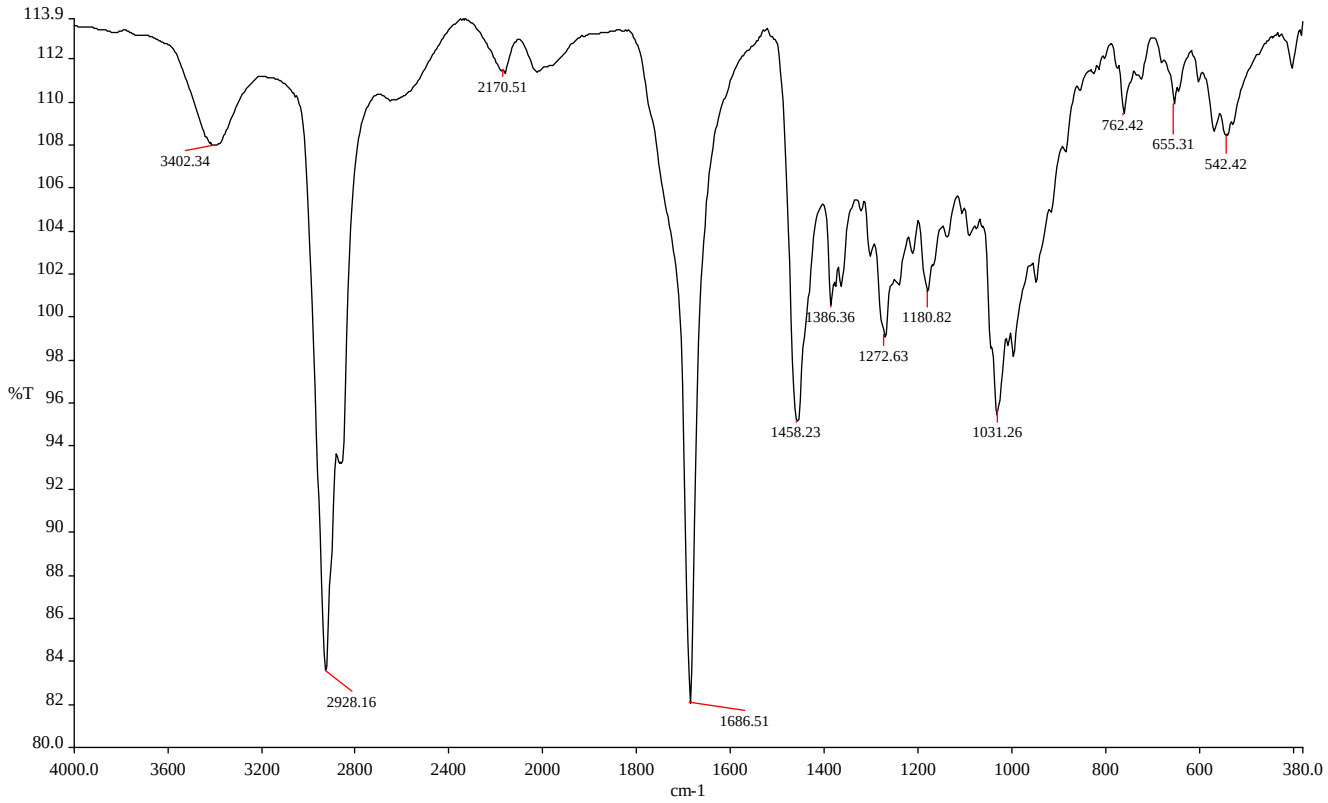
^{1}H-NMR for A-5 (3β-hydroxyolean-11-en-28,13β-olide)

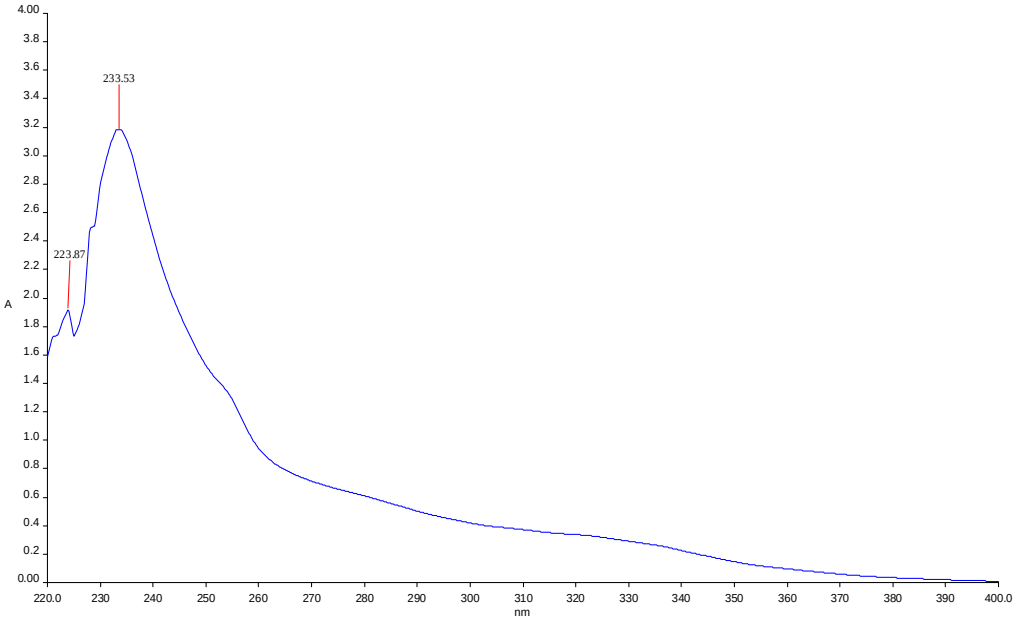


^{13}C-NMR for A-5 (3β-hydroxyolean-11-en-28,13β-olide)

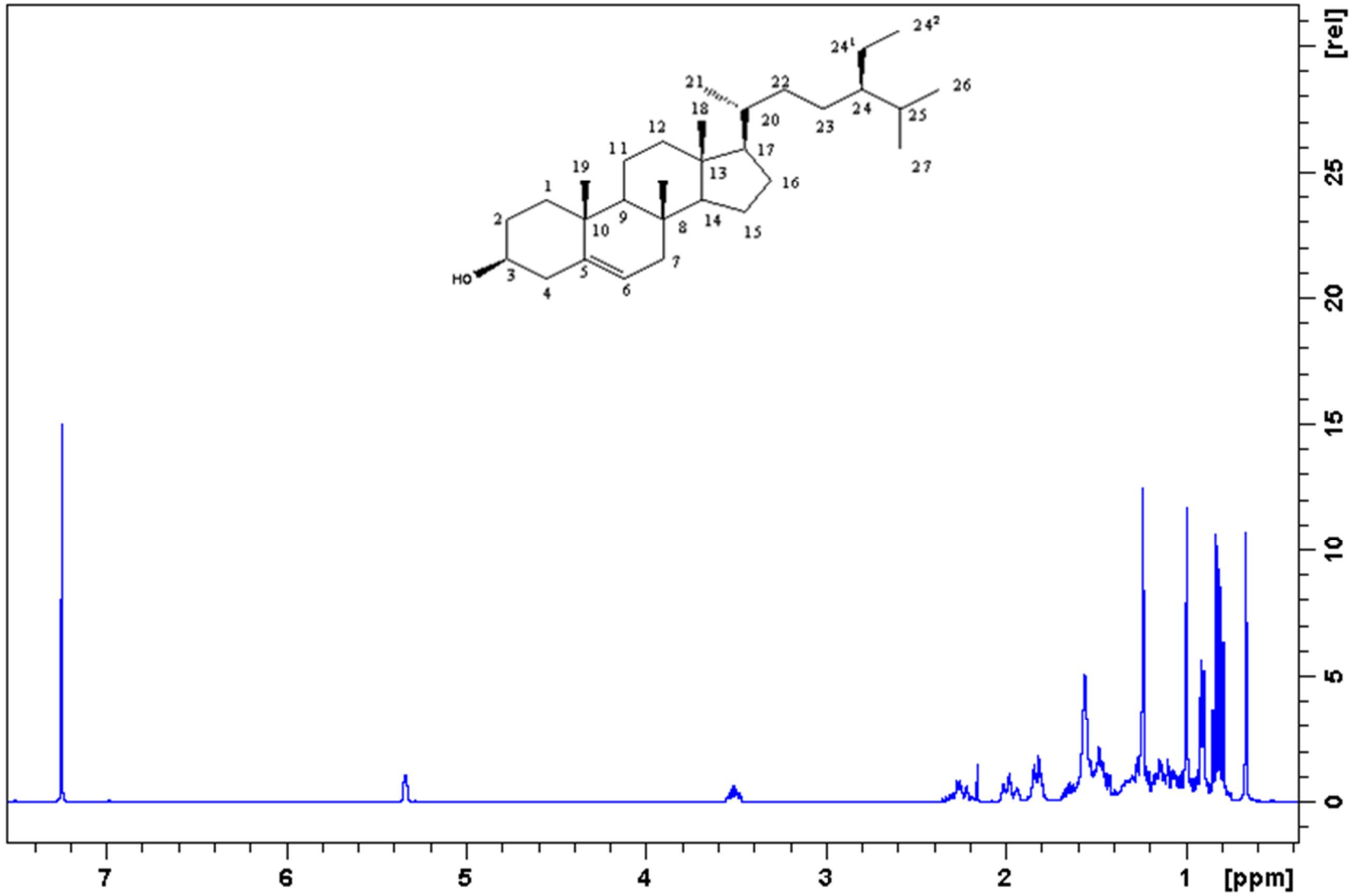


GC-MS spectrum for A-5 (3β-hydroxyolean-11-en-28,13β-olide)

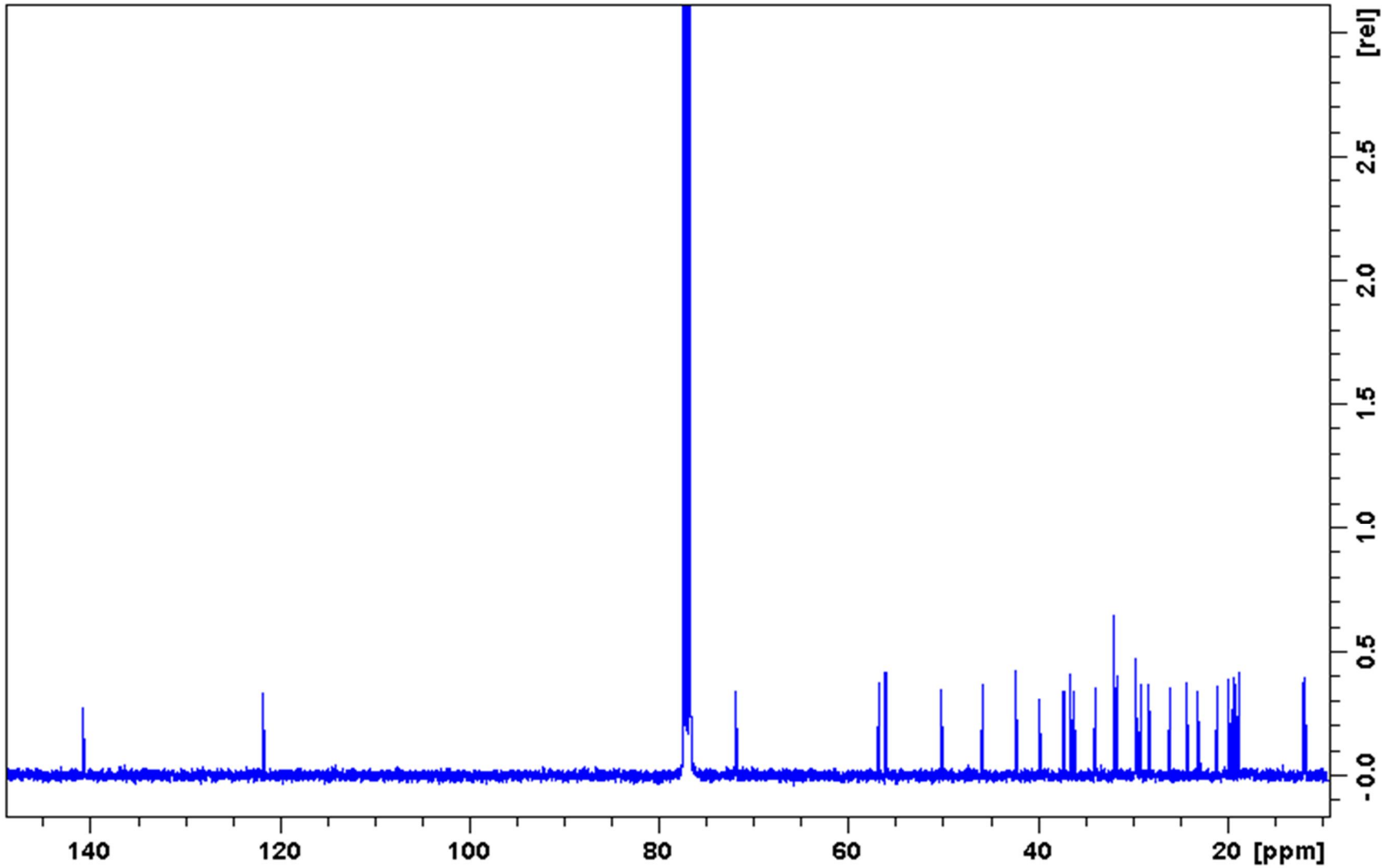




IR and UVspecta for A-5 (3β-hydroxyolean-11-en-28,13β-olide)

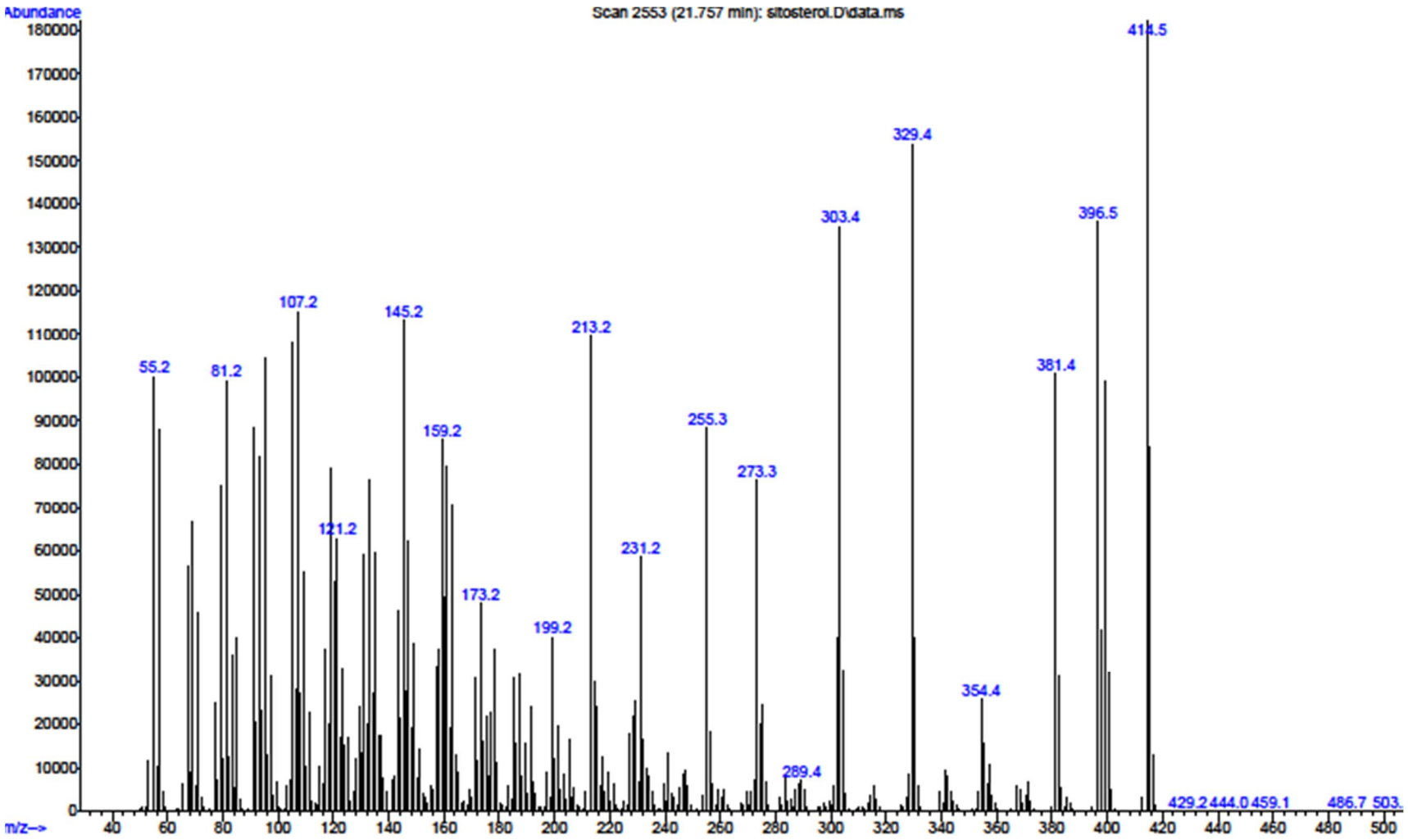


^{1}H-NMR for B-1 (sitosterol)

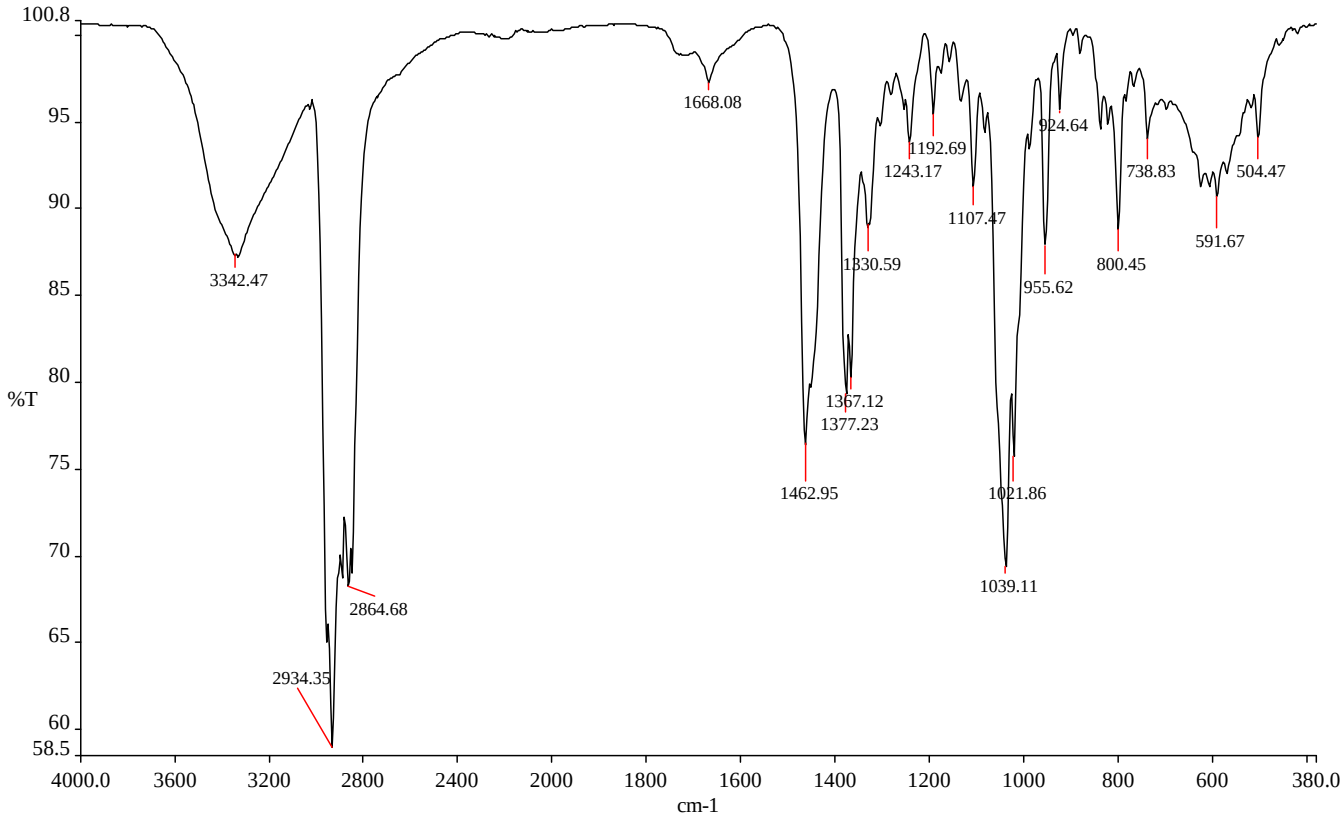


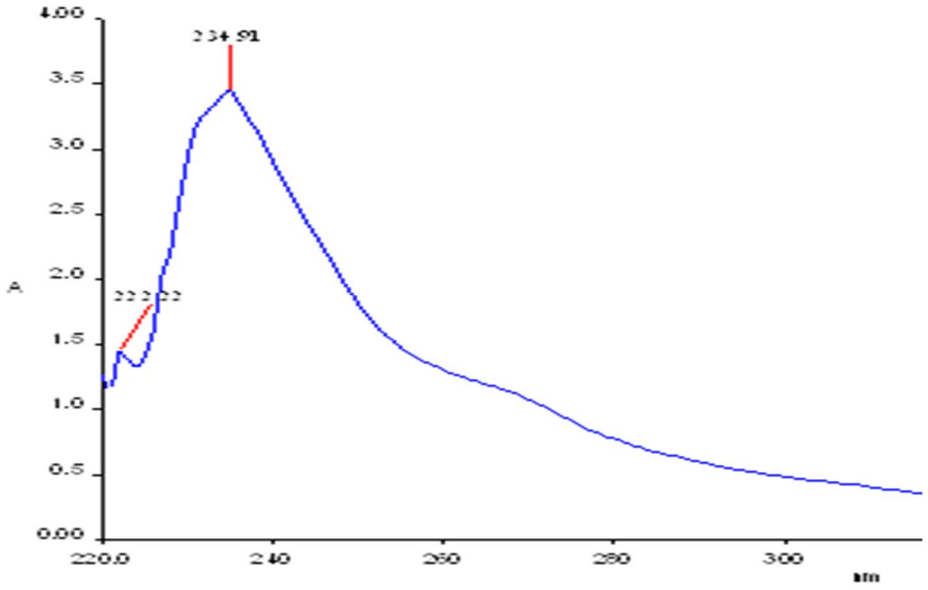
153

^{13}C-NMR for B-1 (sitosterol)

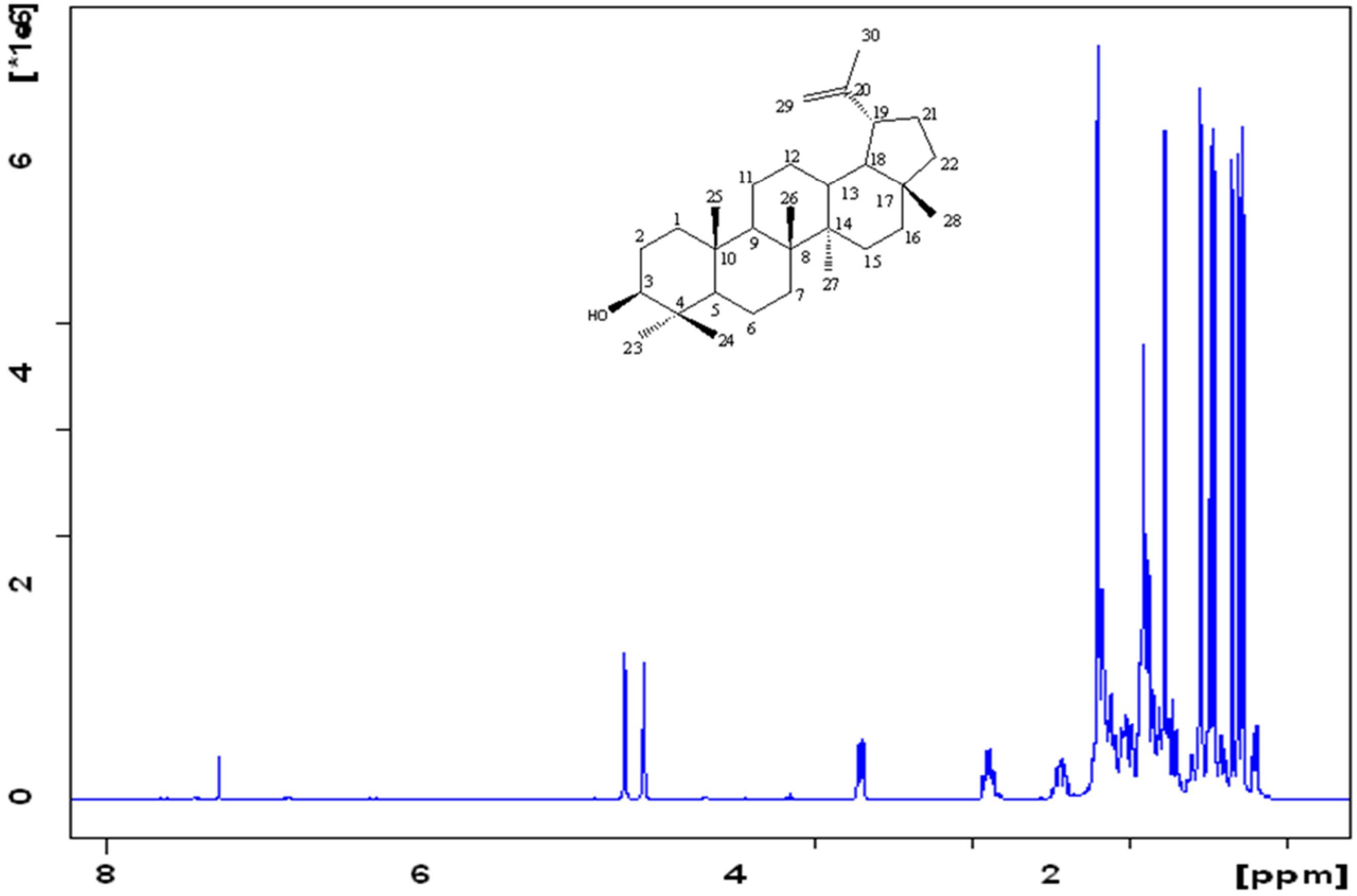


GC-MS spectrum for B-1 (sitosterol)



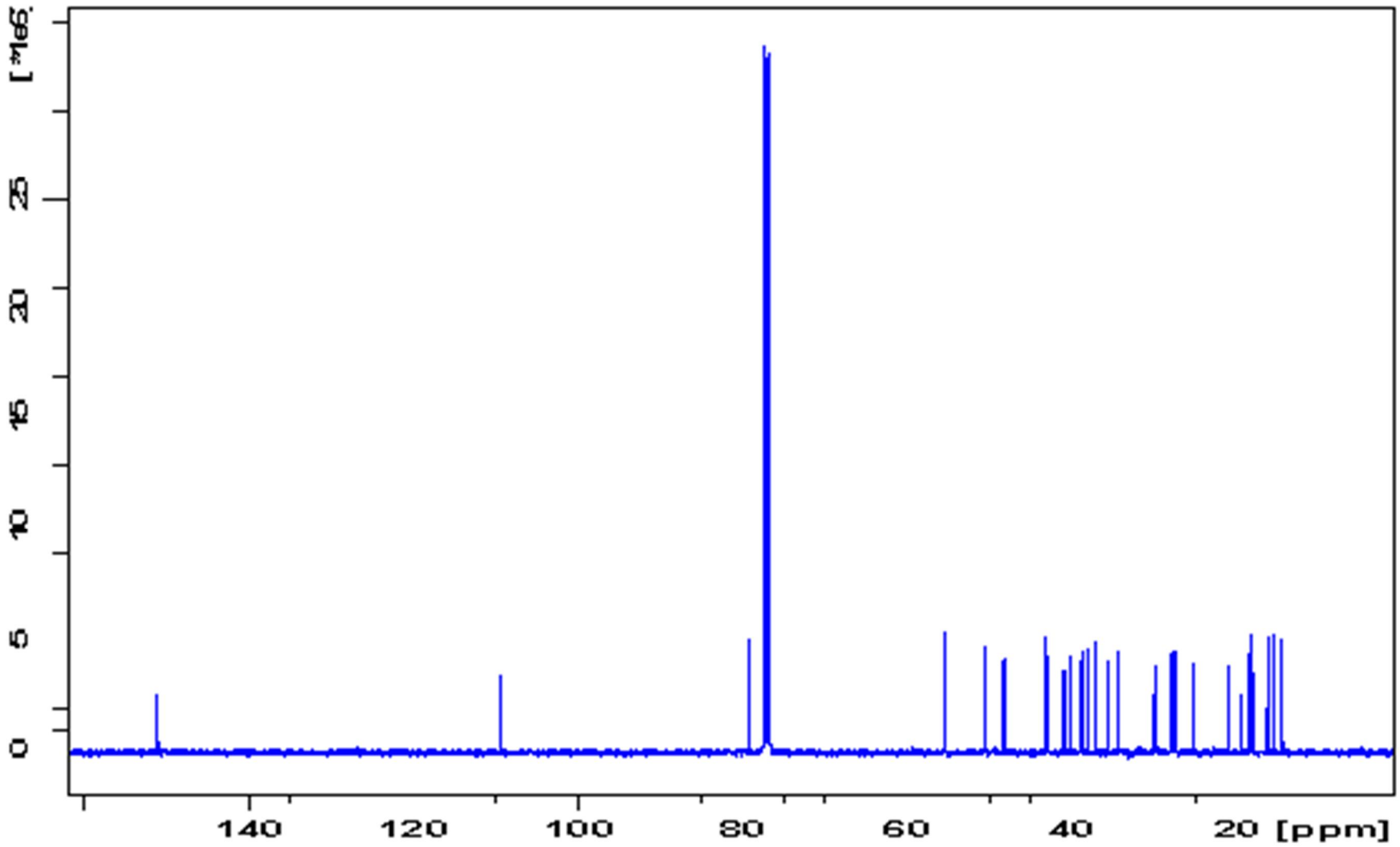


IR and UV spectra for B-1 (sitosterol)

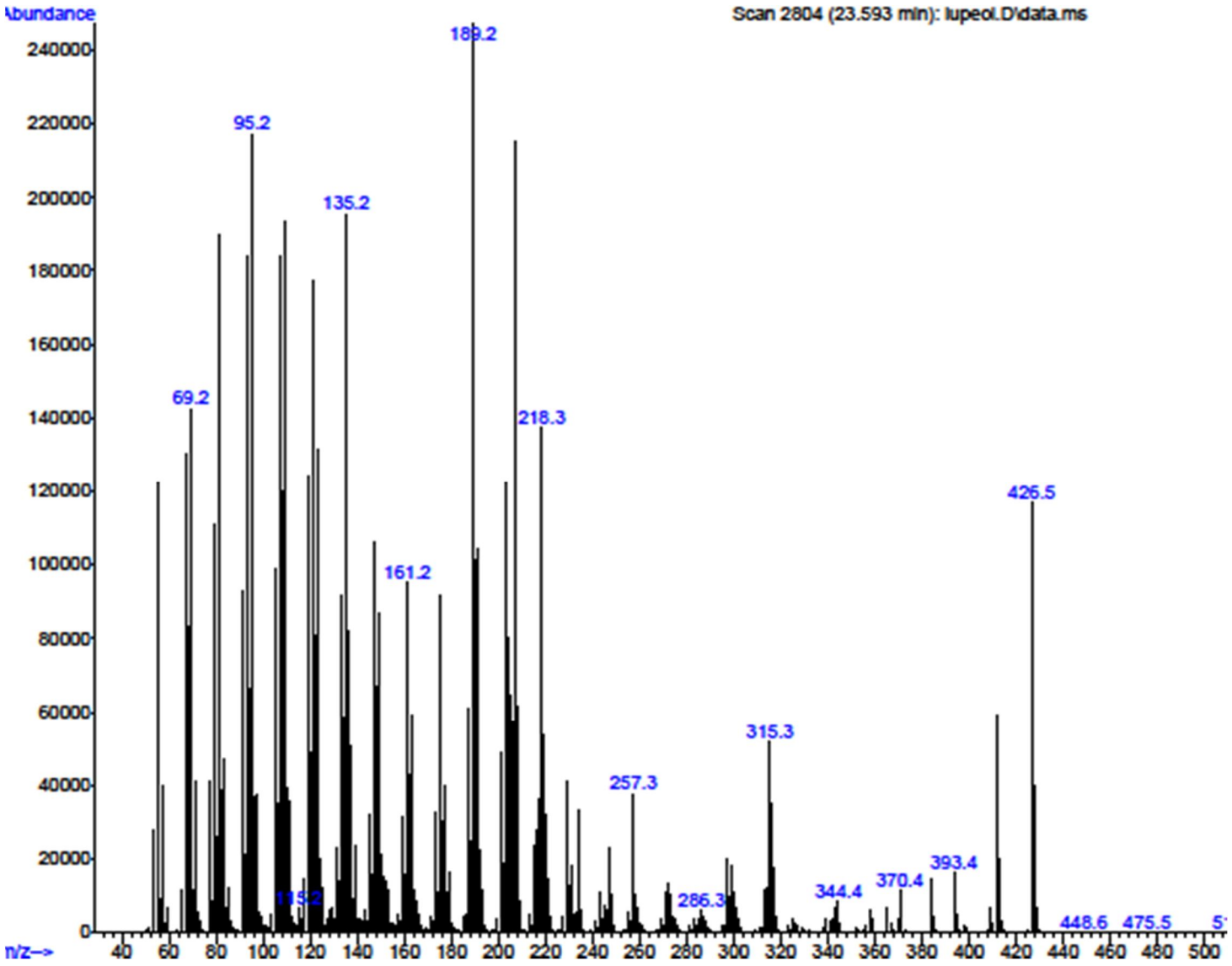


156

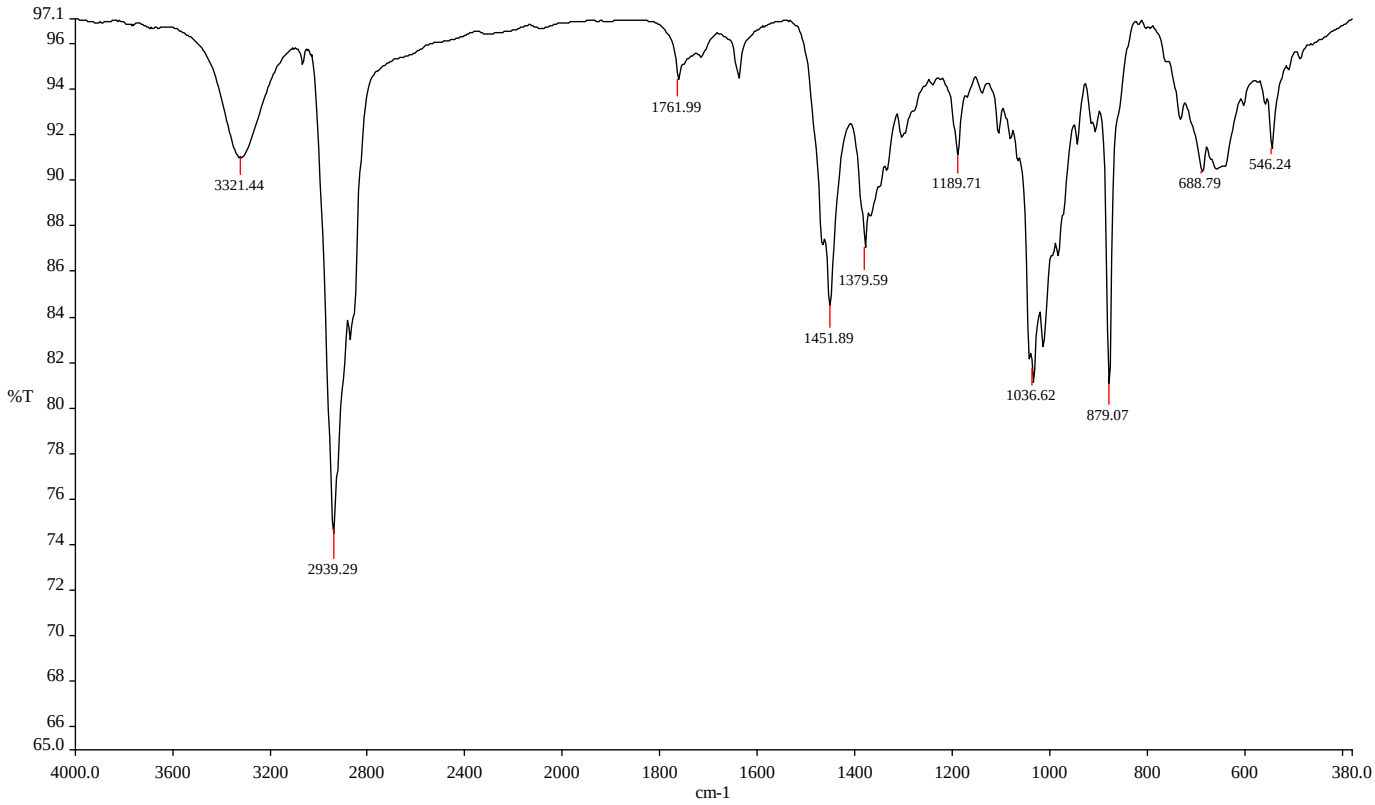
^{1}H-NMR for B-2 (lupeol)

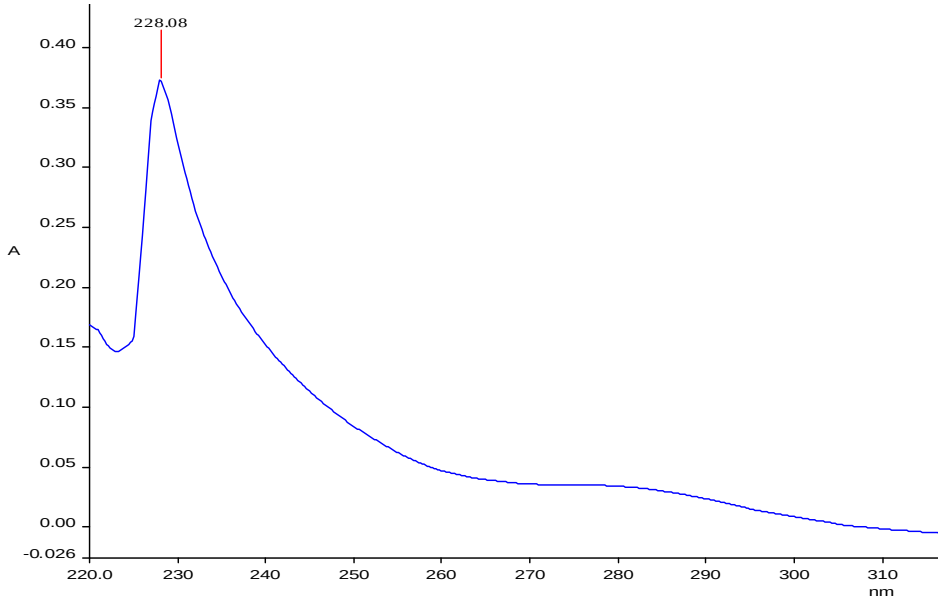


^{13}C-NMR for B-2 (lupeol)

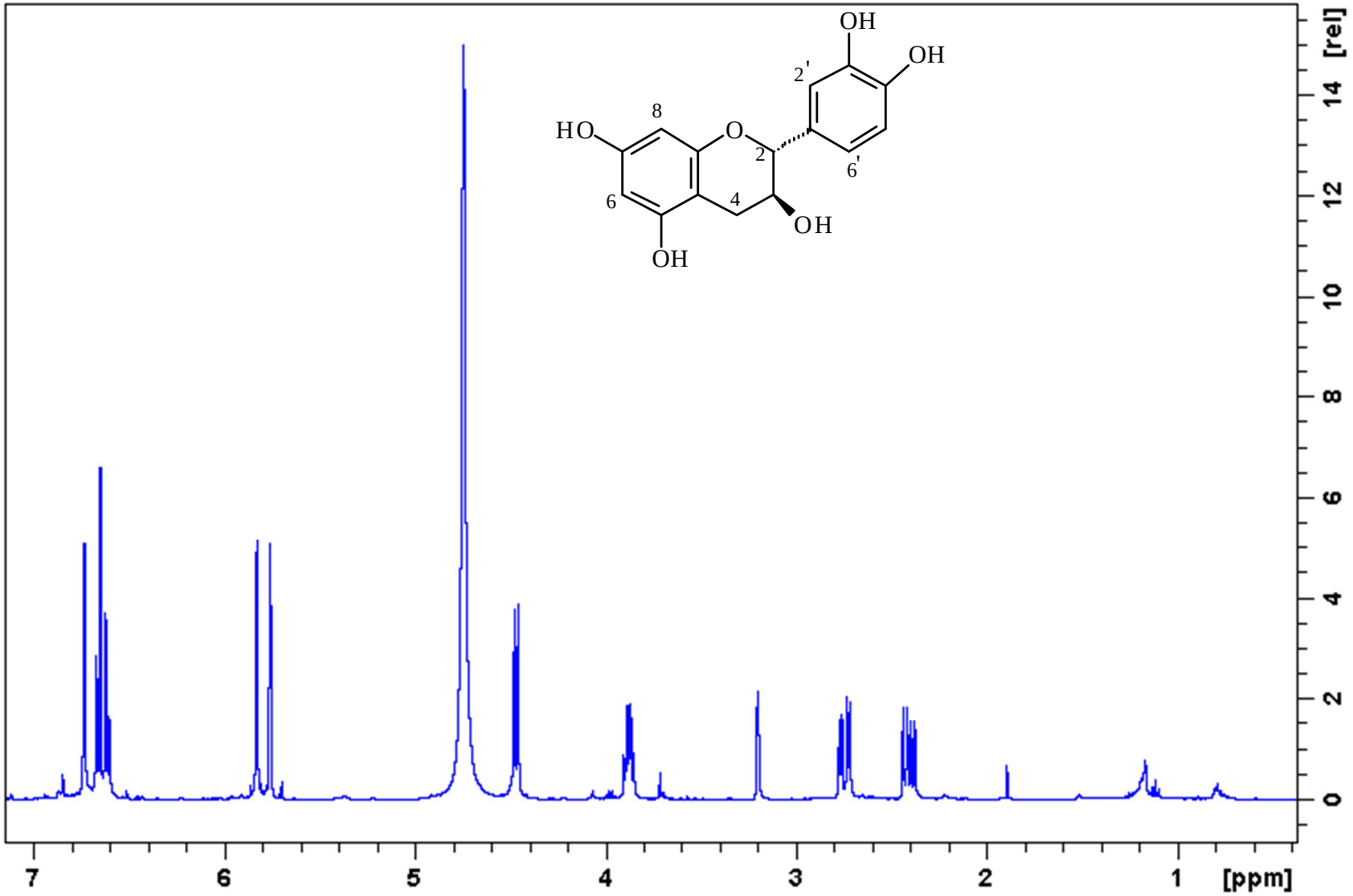


GC-MS spectrum for B-2 (lupeol)



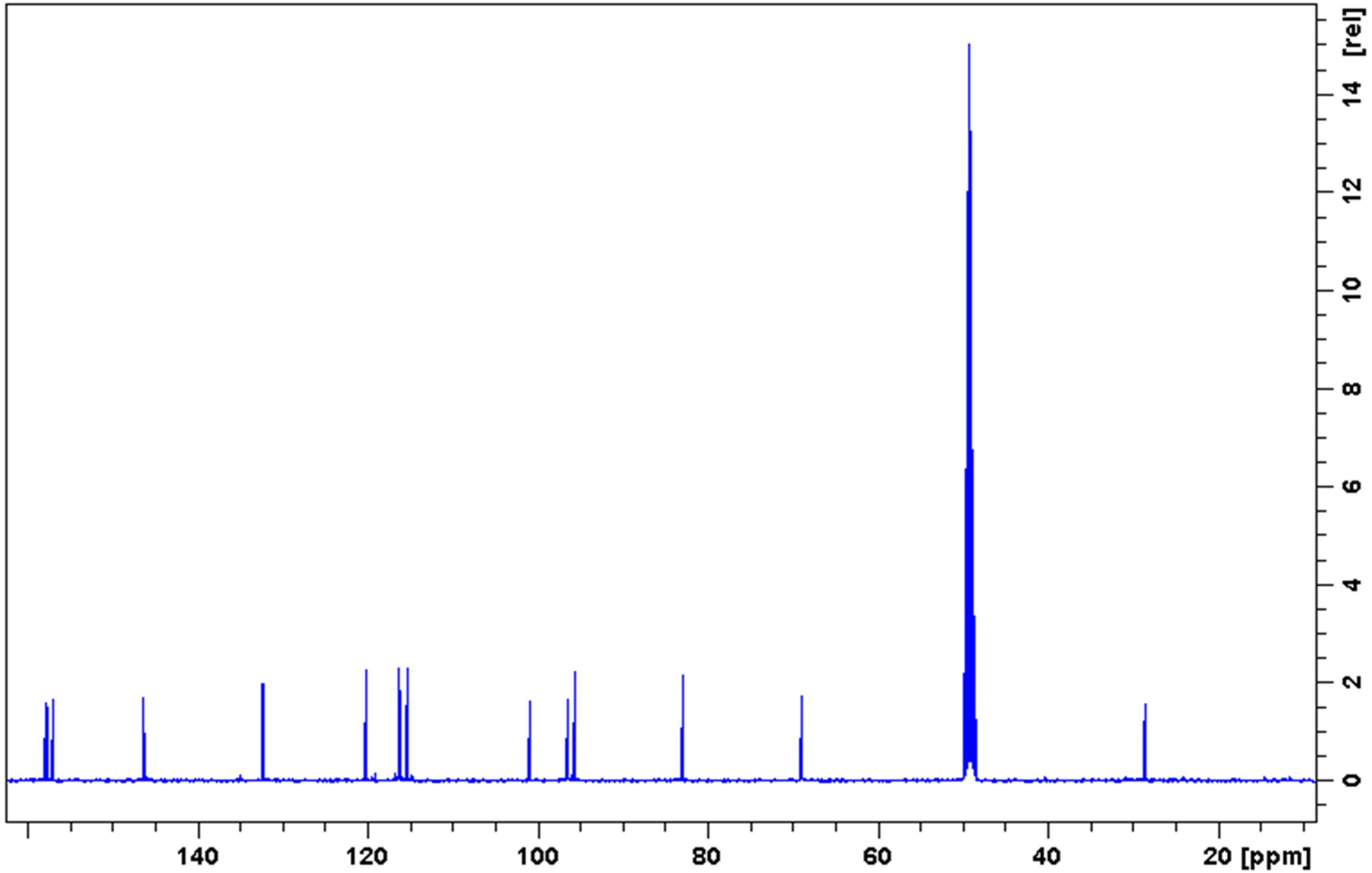


GC-MS spectrum for B-2 (lupeol)



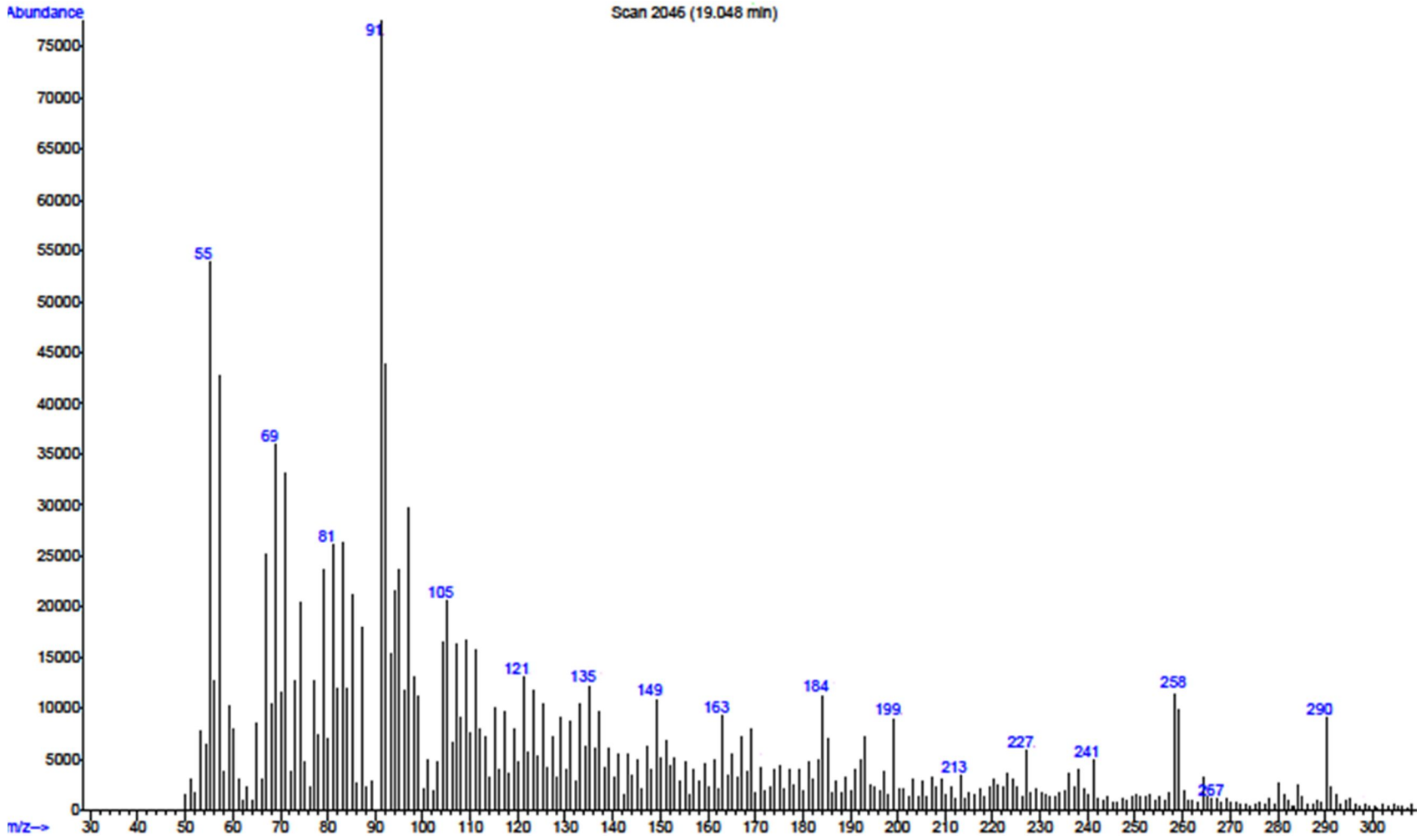
160

^{1}H-NMR for B-3 ((+)-catechin)

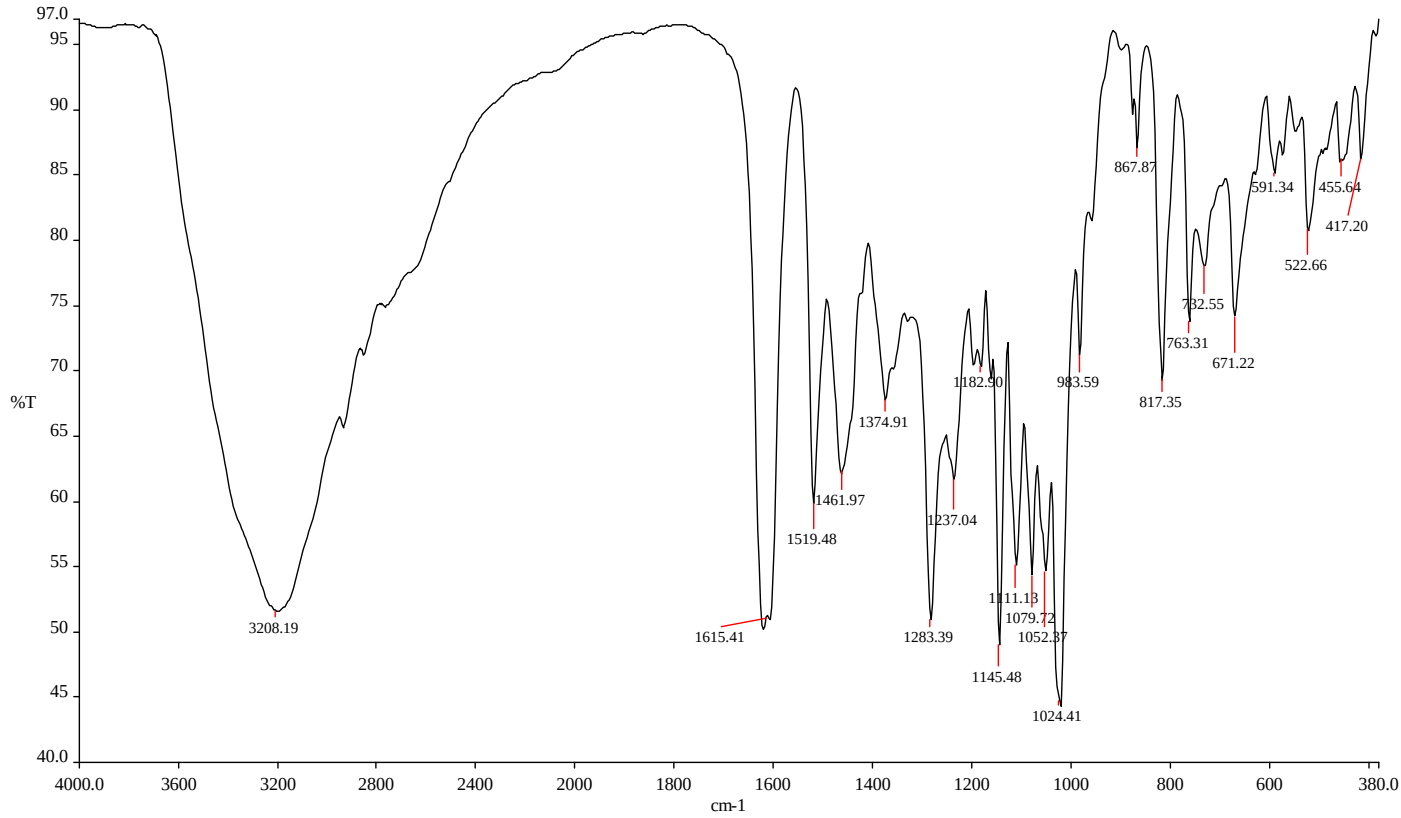


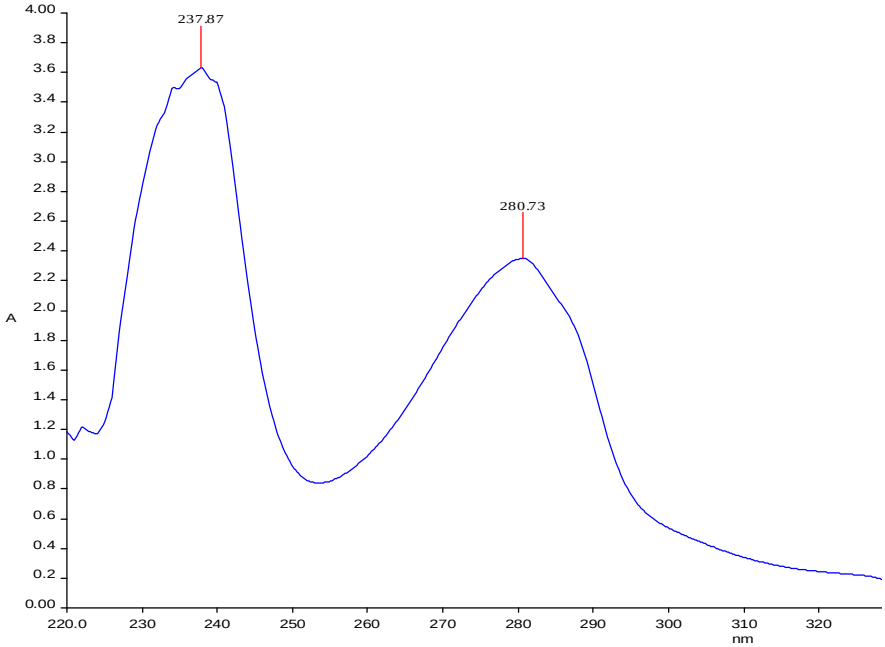
161

^{13}C-NMR for B-3 ((+)-catechin)

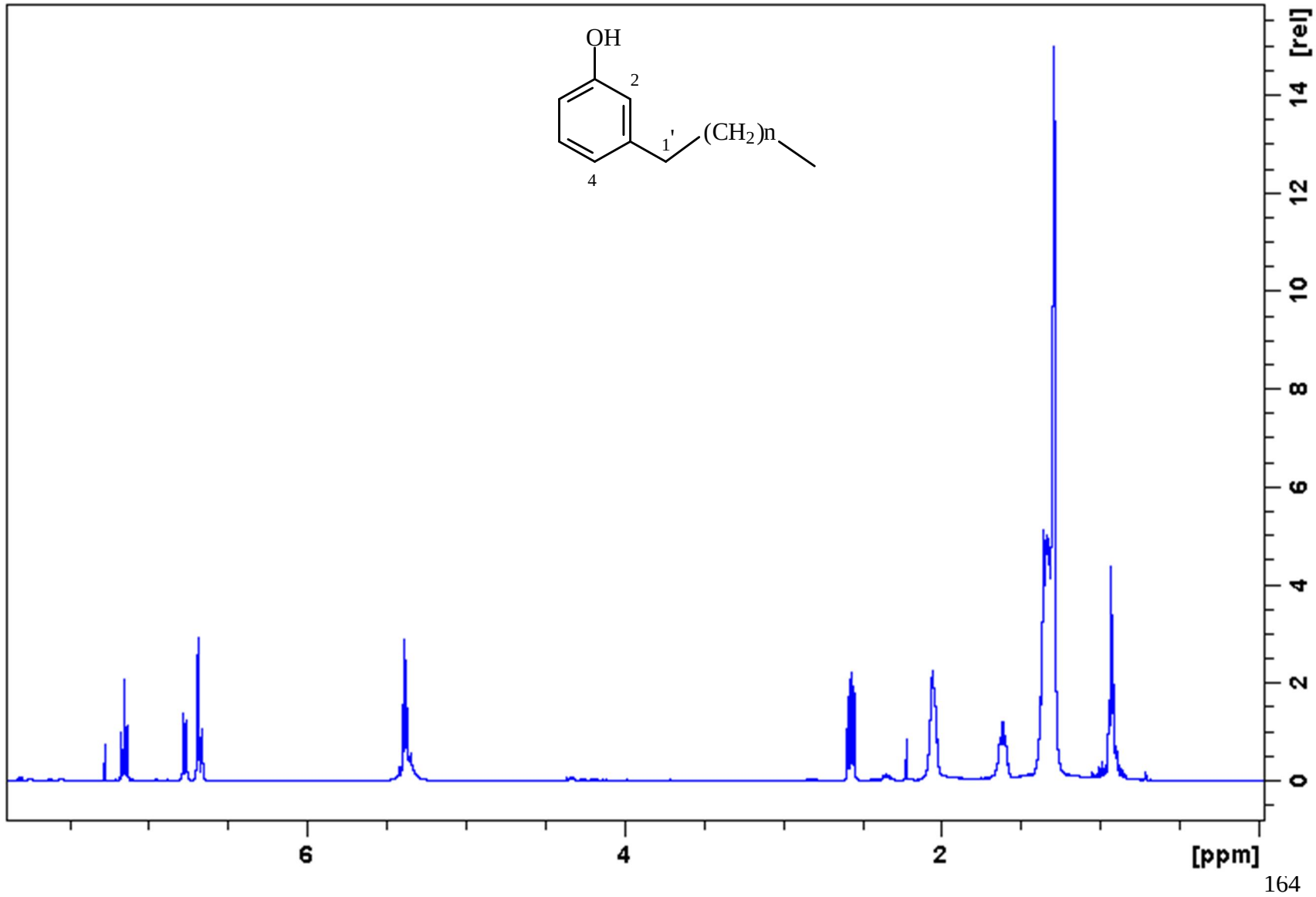


GC-MS spectrum for B-3 ((+)-catechin)



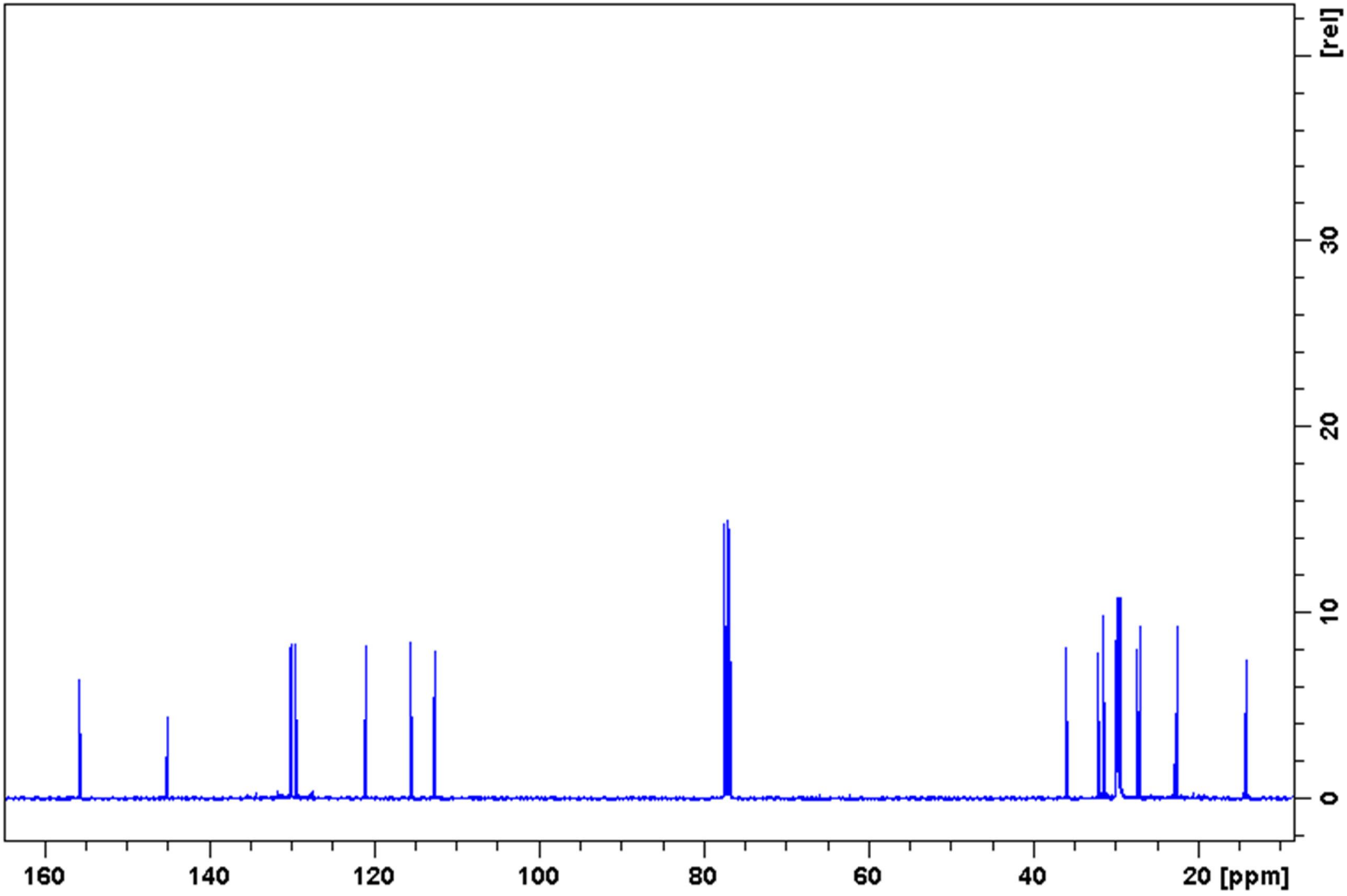


IR and UV spectra for B-3 ((+)-catechin)

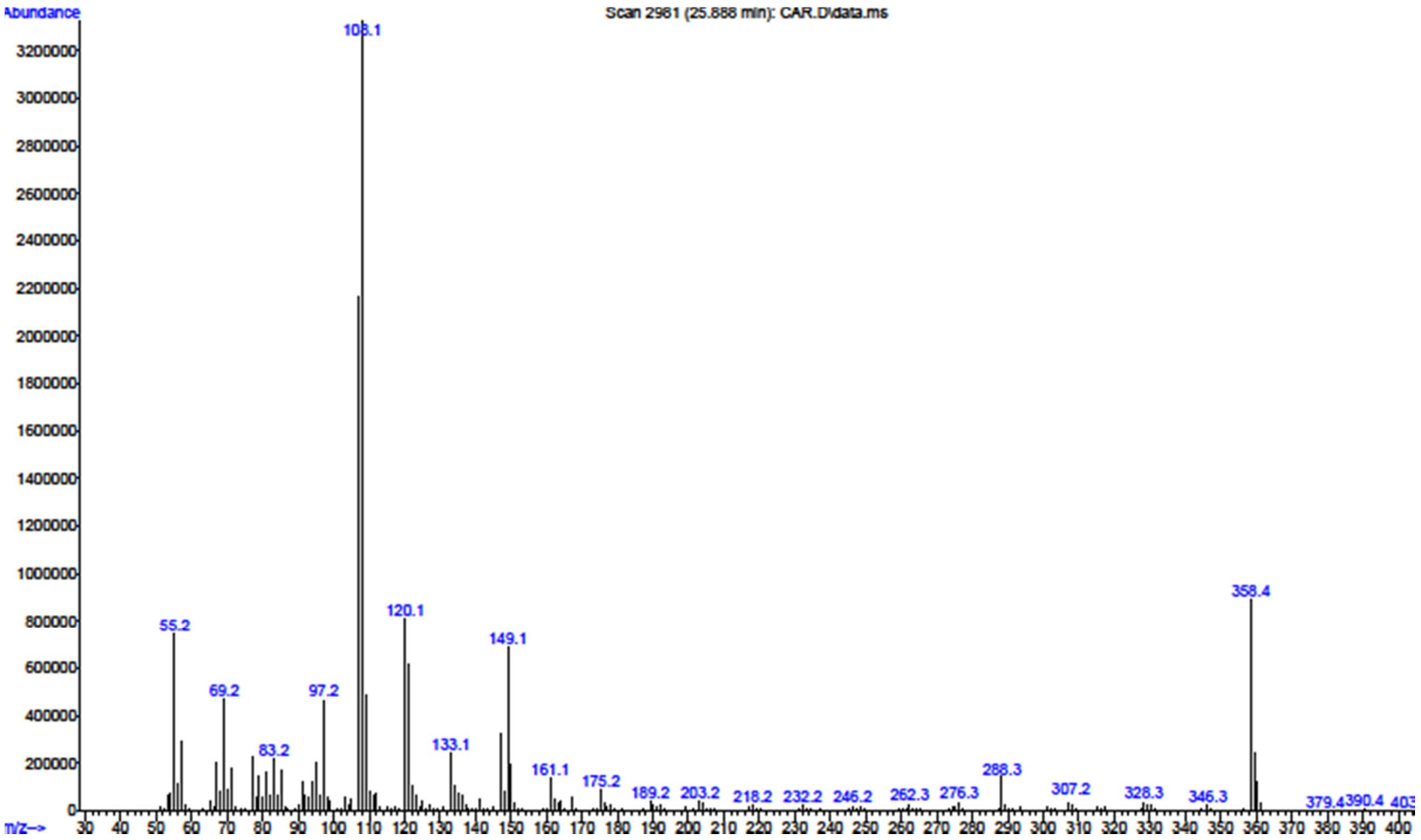


164

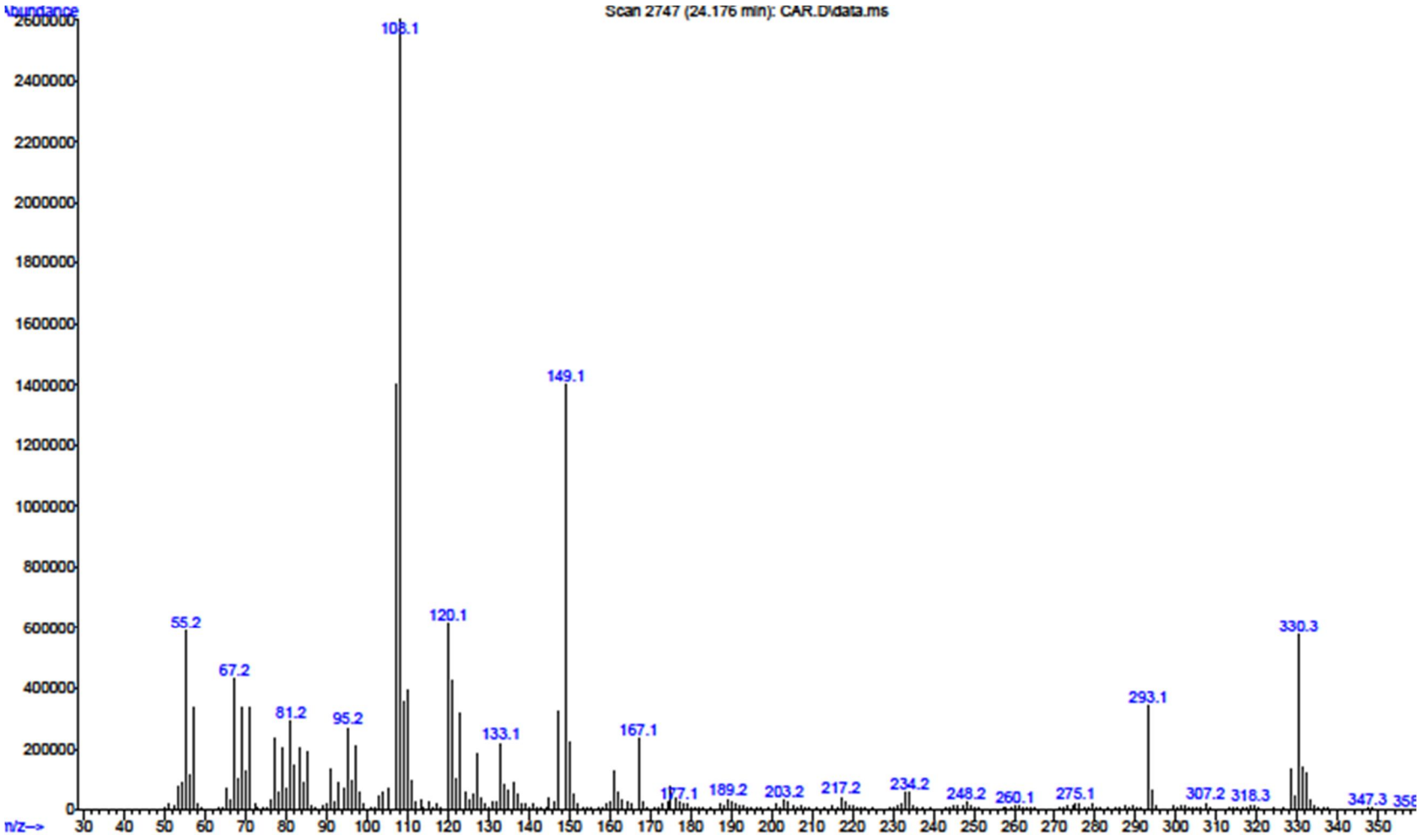
^{1}H-NMR for B-4 (cardanols)



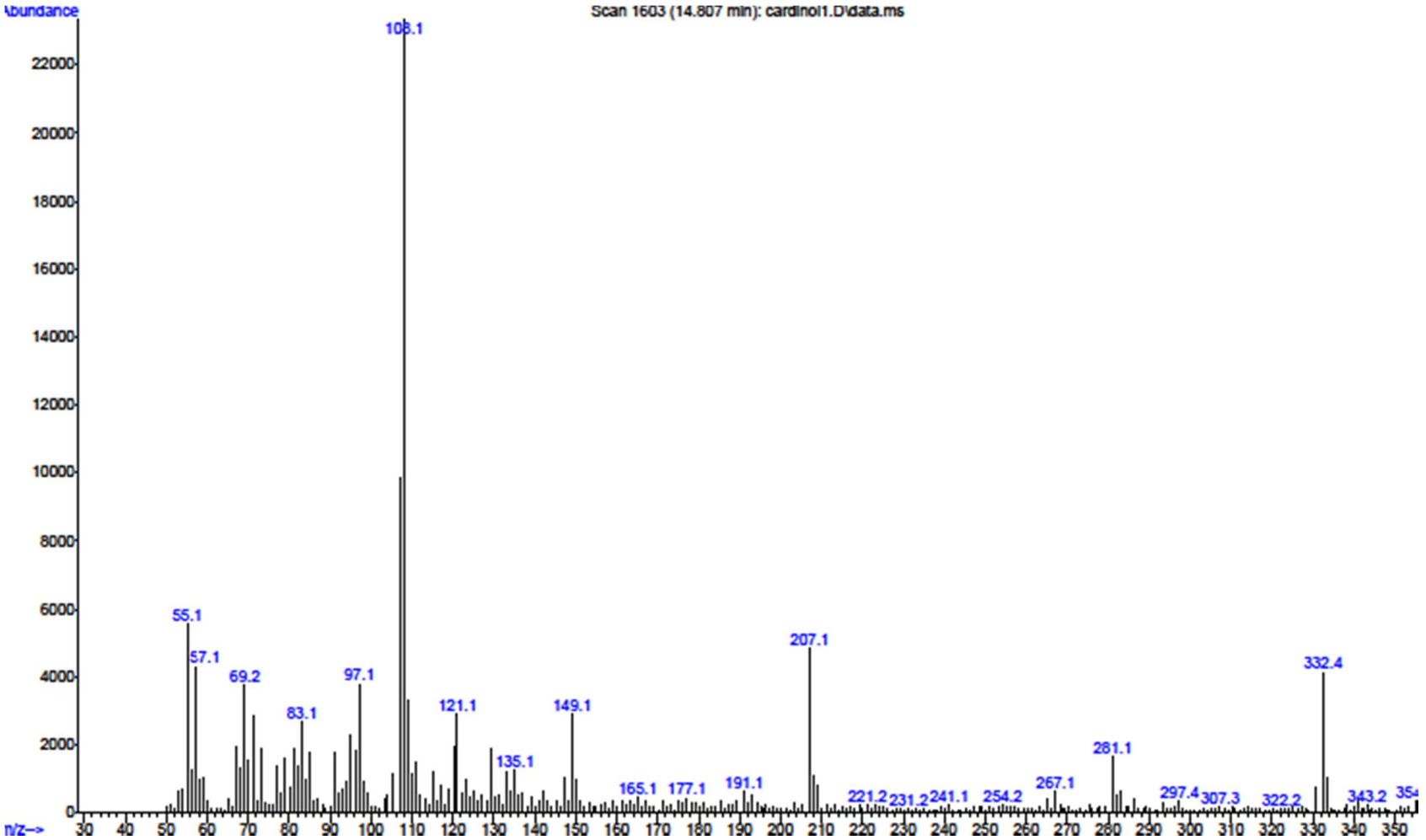
^{13}C-NMR for B-4 (cardanols)



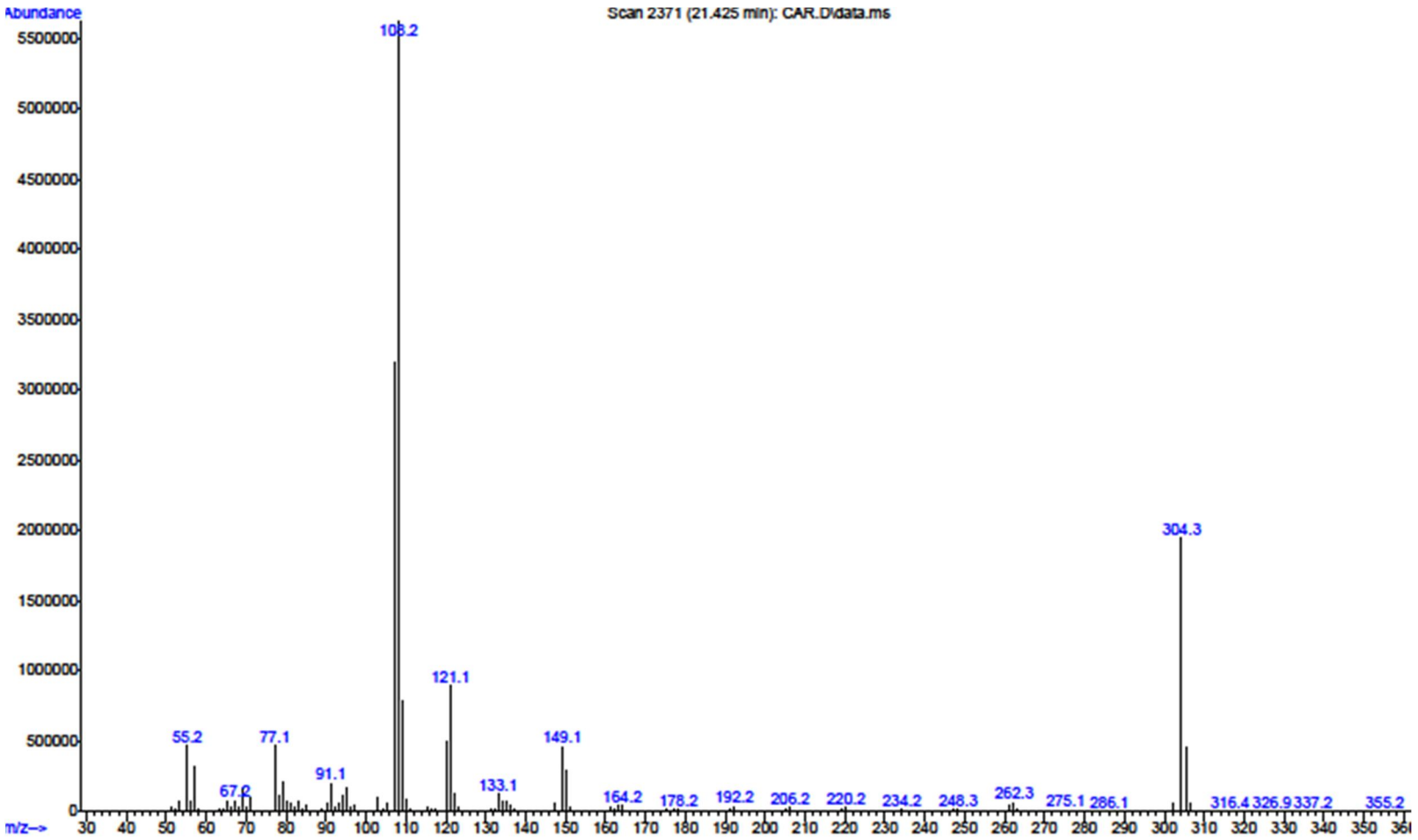
GC-MS spectrum for B-4a (1-hydroxy-3-[(Z)-12'-nonadecenyl] benzene)



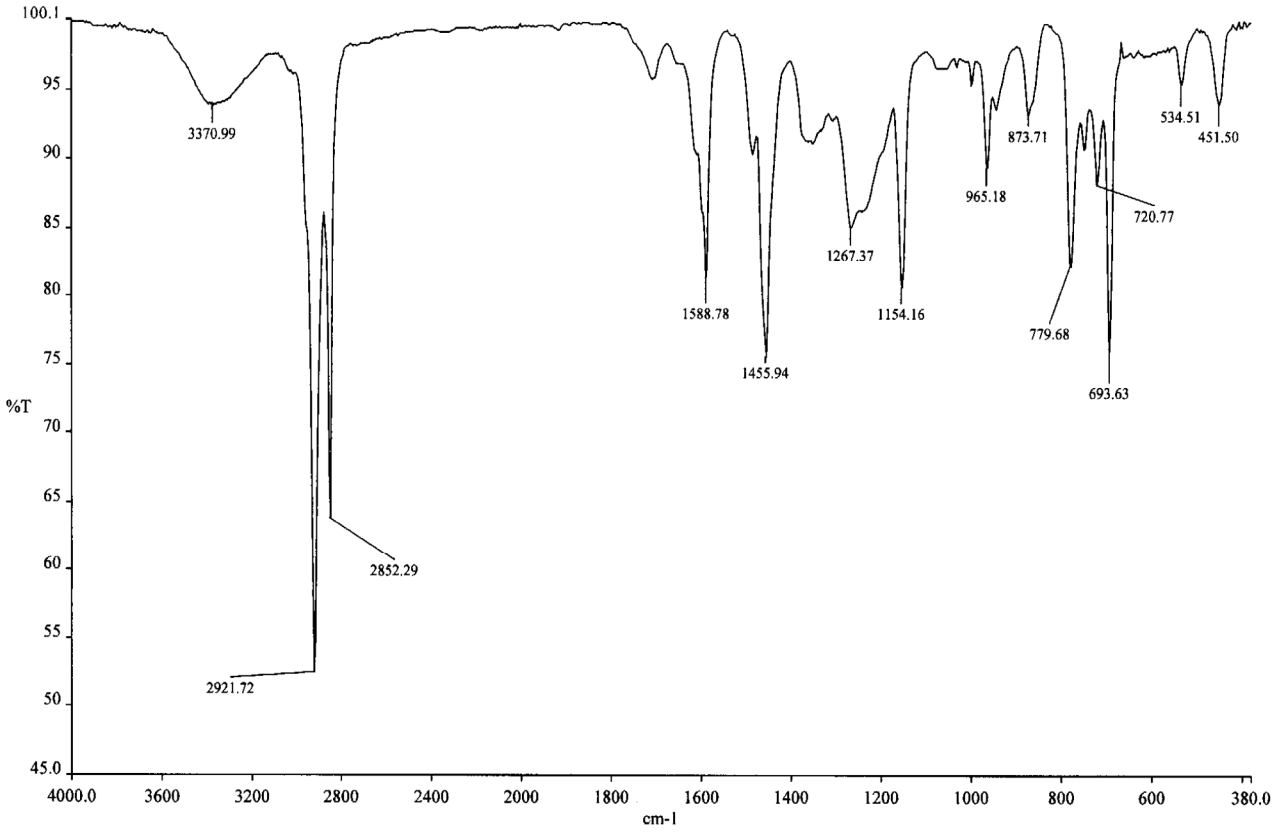
GC-MS spectrum for B-4b (1-hydroxy-3-[(Z)-12'-heptadecenyl] benzene)

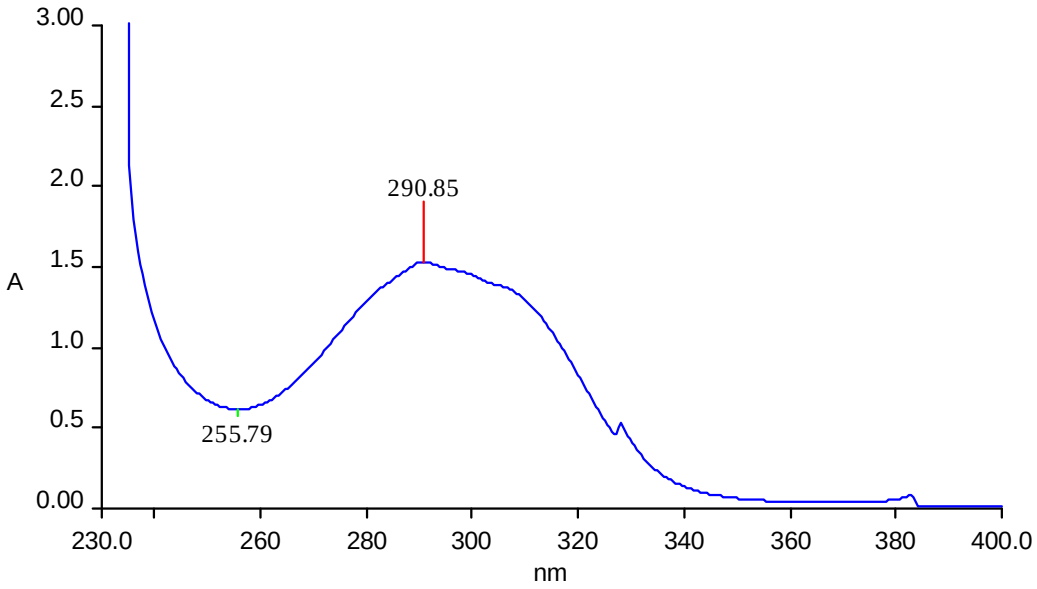


GC-MS spectrum for B-4c (1-hydroxy-3-heptadecanyl benzene)

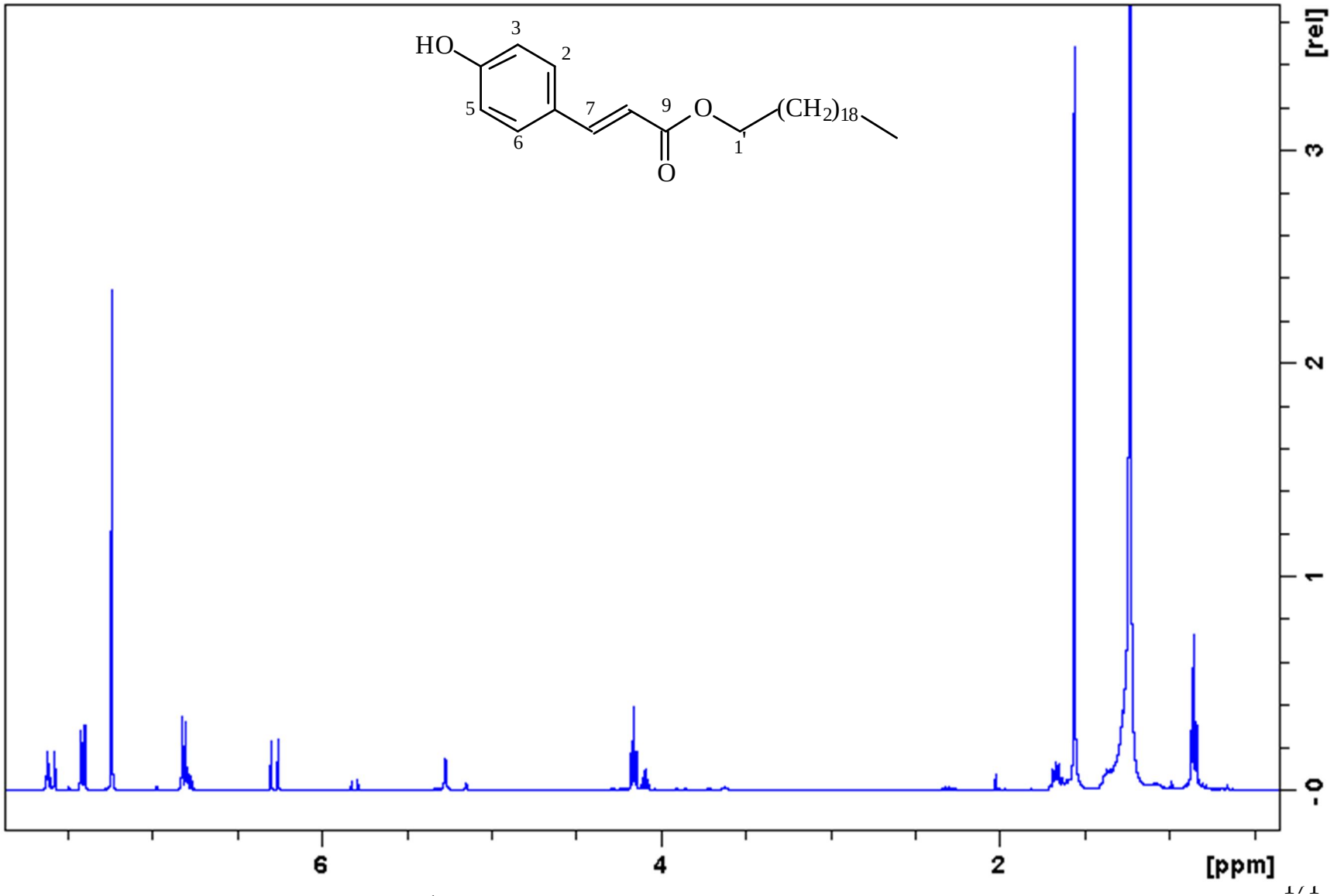


GC-MS spectrum for B-4d (1-hydroxy-3-pentadecanyl benzene)

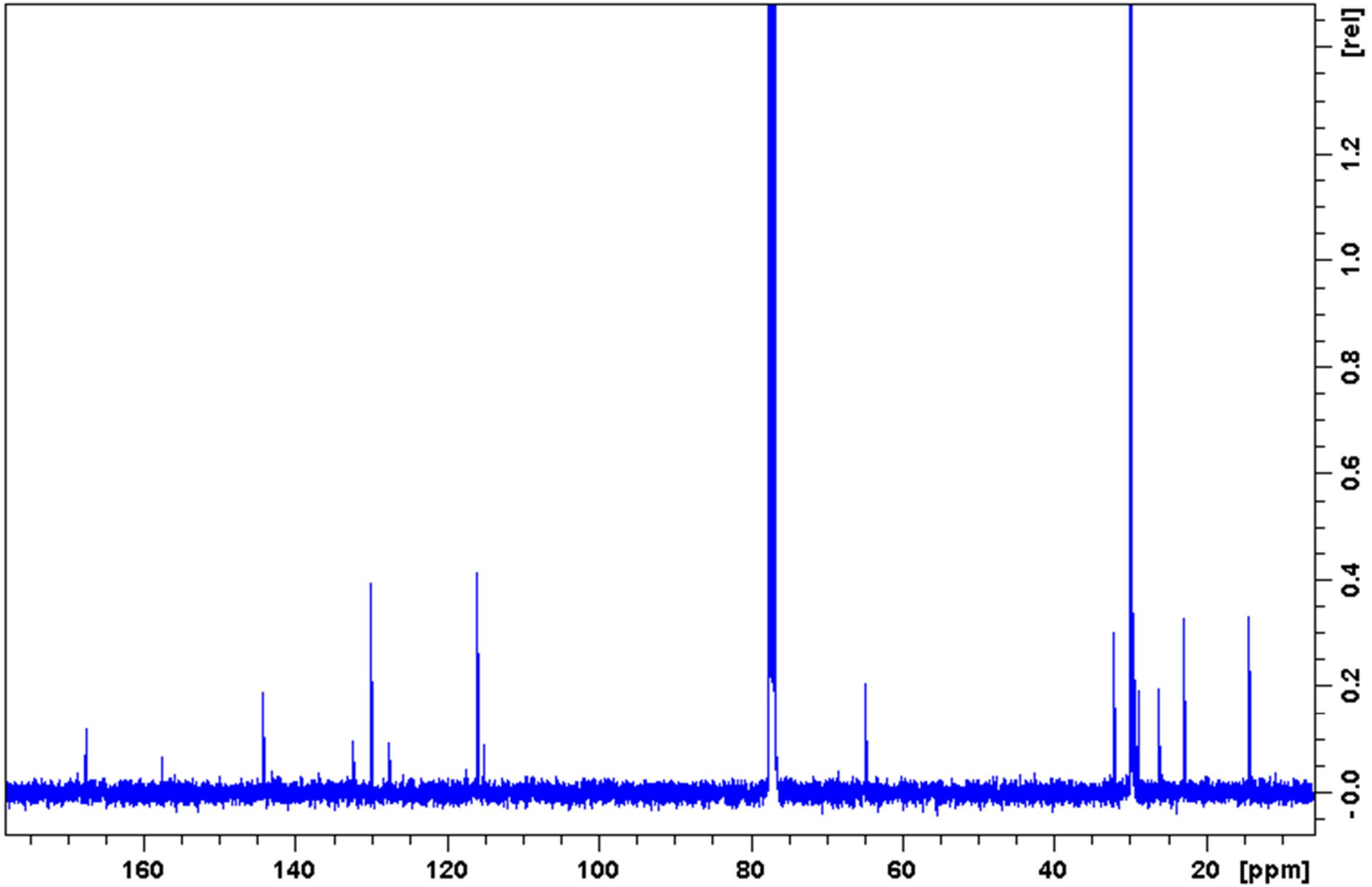




IR and UV spectra for B-4 (cardanols)

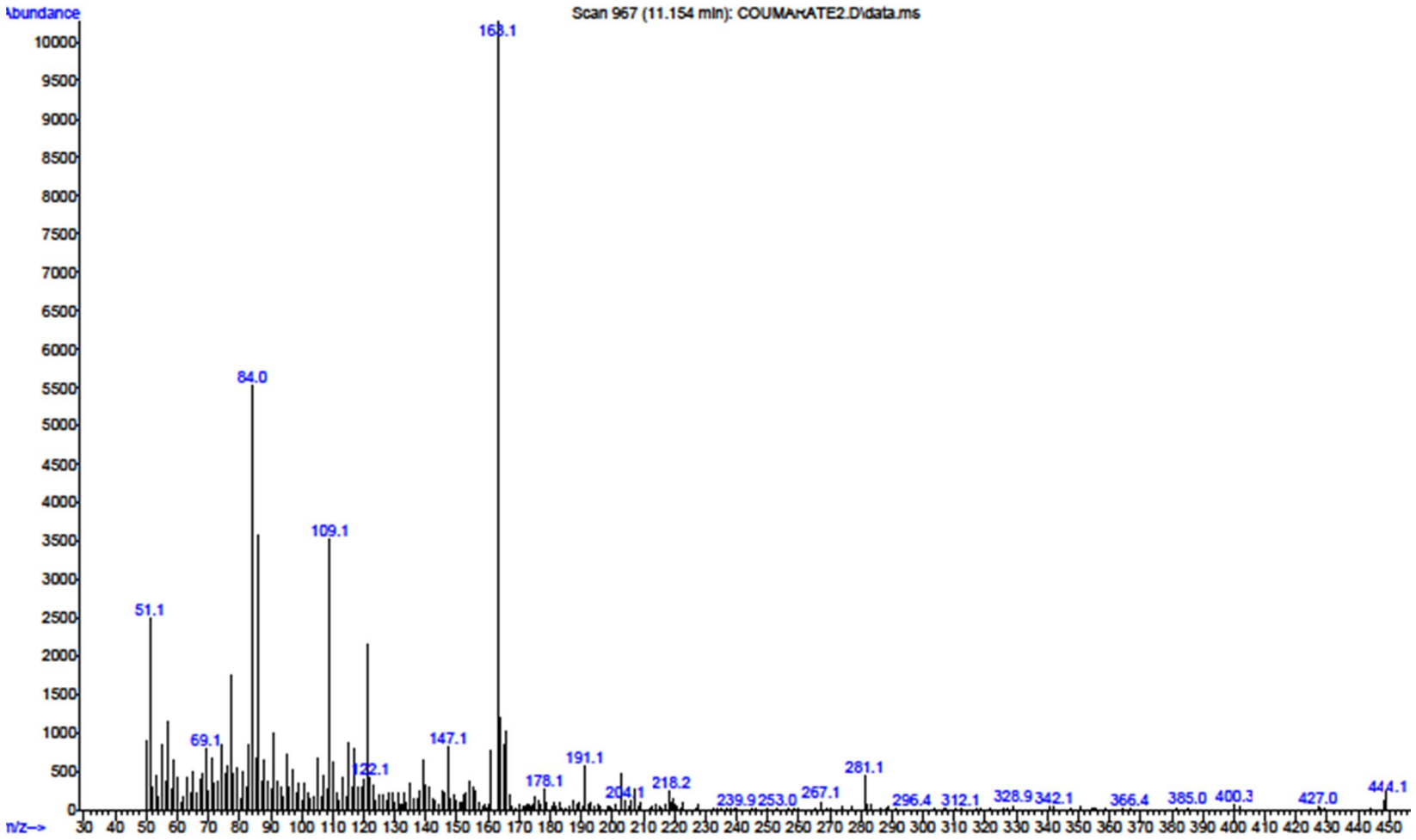


^{1}H-NMR for B-5 (eicosanyl-*trans*-*p*-coumarate)

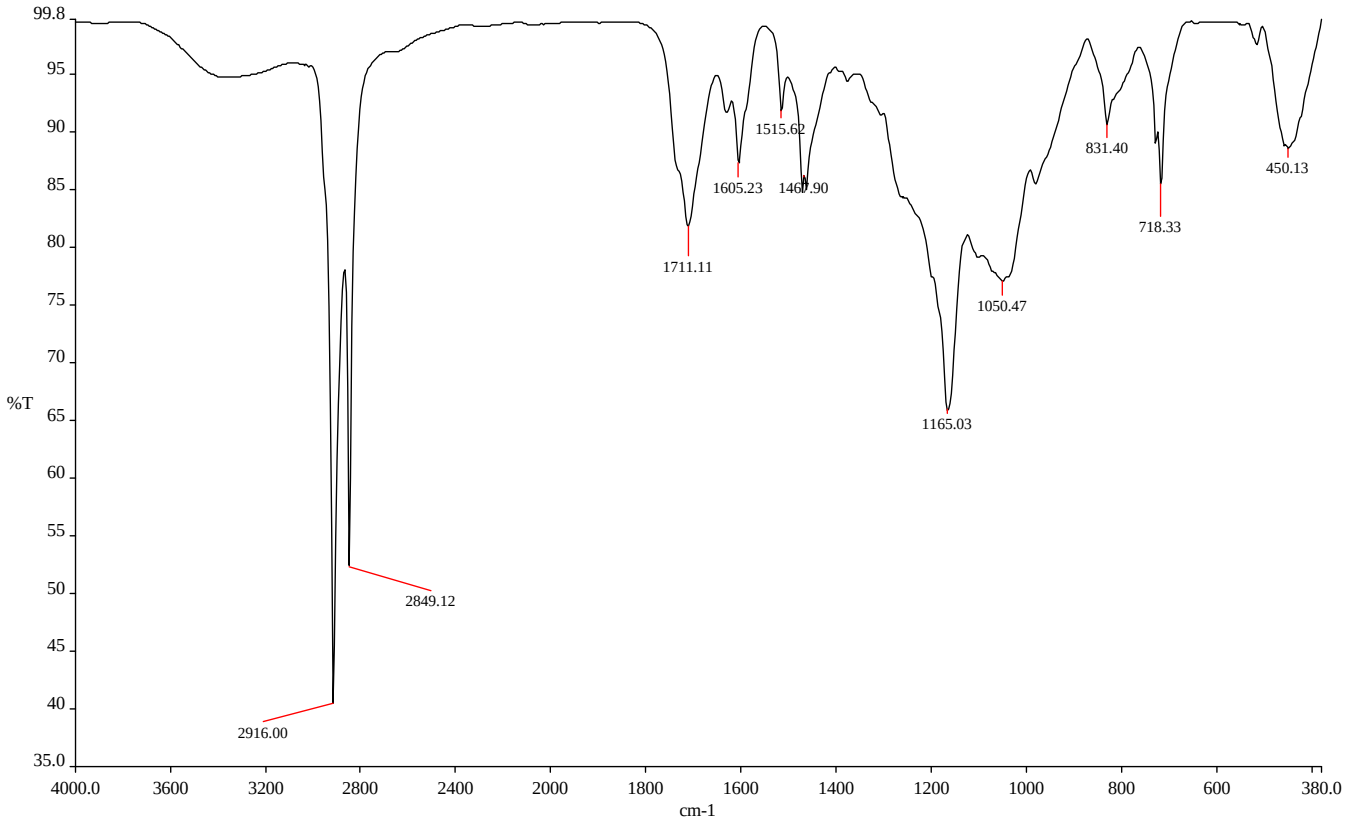


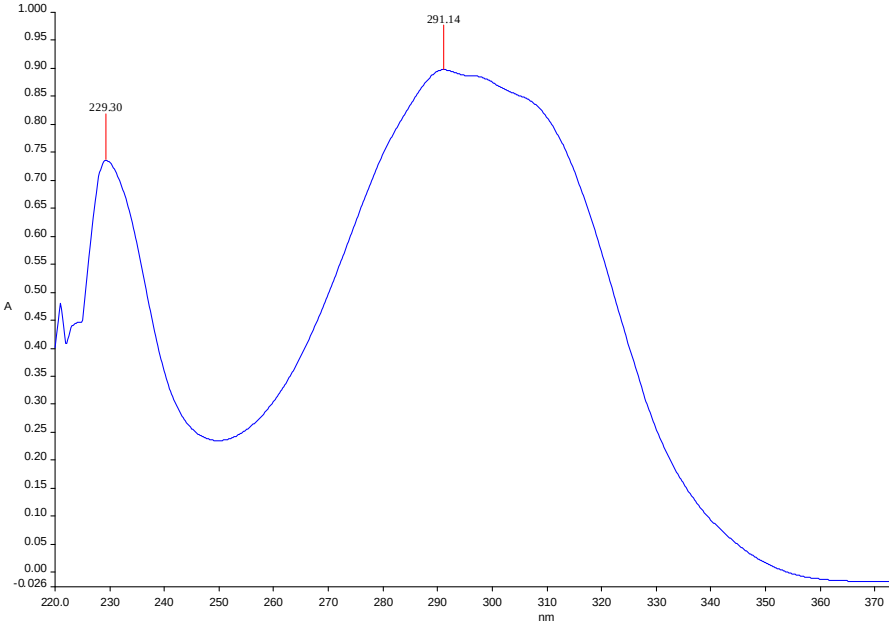
172

^{13}C-NMR for B-5 (eicosanyl-*trans*-*p*-coumarate)



GC-MS spectrum for B-5 (eicosanyl-*trans*-*p*-coumarate)





IR and UV specta for B-5 (eicosanyl-*trans*-*p*-coumarate) 174