NELSON MANDELA UNIVERSITY

Evaluation of the toxicity of secondary metabolites in *Solanum incanum L.* to advance community knowledge

By

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Submitted in fulfilment of the requirements for the degree of Philosophiae Doctor in the Faculty of Sciences at the
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DEDICATION

I dedicate this thesis to my wife Patronella, and my two daughters Chengetayi and Rutendo. You are the inspiration for what I am today. May God bless you all.

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ABSTRACT

The effects of pests and the need to produce adequate food have influenced small-scale farmers in disadvantaged communities to adopt and utilise natural plant pesticides to improve harvests in many Southern African Development Communities. However, the phytochemistry associated with these indigenous plants' pesticide activity still needs to be explored. The lack of evidence of scientific knowledge of the plant species has caused a lot of health issues among the users of indigenous plant pesticides. *Solanum incanum* is among the plants utilised to control cabbage aphids in Mkoba village, Zimbabwe. *Solanum* species are known for their steroidal compounds which comprise glycoalkaloids and saponins. This study evaluated the knowledge, opinions, and attitudes of the vegetable peasant farming community in Gweru regarding their use of the indigenous plant (*S. incanum*) as a pesticide. The study also reported the phytochemical profiling, structural characterisation of the isolated compounds, and biological and pesticidal activity evaluation of phytochemicals isolated from *S. incanum.*

A descriptive survey was carried out to assess the knowledge, attitude, and practices of a conveniently sampled group of vegetable farmers in Mkoba village who use *S. incanum* as a pesticide. Forty-nine respondents comprised of 19 males and 30 females of ages ranging from 15 to above 60 years took part in the study by answering an open and closed-ended questionnaire. The survey revealed that parents and neighbours were instrumental in disseminating pesticidal information in the community. *Brassica napus* were the most grown vegetable and vulnerable to cabbage aphids. Mixed opinions amongst the respondents varied regarding the health and environmental impact of *S. incanum* as a pesticide. Seventy-five percent (75%) of the respondents supported the use of *S. incanum* as a pesticide whilst 25% claimed that the use of *S. incanum* was the source of the health problems experienced in the community. The survey demonstrated that (45)91% of the farmers displayed poor practices regarding the disposal of empty pesticide containers and the use of personal protective clothing. The most prevalent symptoms in the community were skin rash, nausea, headache, and poor vision and these symptoms were common in the age group 30 to 60 years.

The fruit (546 g) and root (148 g) biomass were extracted in sequence according to polarity using n-hexane, ethyl acetate, and methanol to afford crude extracts coded **HFE**, **EFE**, **MFE**, **Hroot**, **Eroot**, and **Mroot.** The phytochemical profiling was performed by employing a

qualitative and quantitative approach through high-performance thin-layer chromatography (HPTLC) and UV-Vis spectroscopy, respectively. *In vitro* biological screening involved the investigation of cytotoxicity, antioxidant, and pesticidal activities of the crude and isolated compounds using propidium iodide (PI), DPPH, and Leaf dip assay, respectively. Fractionation and purification of crude extracts were achieved through chromatographic techniques. The characterisation of single compounds was performed using NMR, HRMS, UV/Vis, FTIR, and XRD spectroscopic techniques.

Phytochemical profiling of crude extracts of *S incanum* using an HPTLC technique revealed the presence of alkaloids, tannins, saponins, glycosides, phenols, flavonoids, terpenoids, and steroids in the crude extracts. Quantitative estimation of the phytochemicals revealed that phenolics were the most abundant type of phytochemicals present in the methanol root crude extract at 3.72±1.28 GAE mg/100 mg sample. The highest content of alkaloids was found in the steroidal fruit extract at 0.14±0.06 mg ATP/100 mg sample whilst saponins were in abundance in the hexane root crude extract at 3.74±1.17 mg/100 mg sample. Terpenoids were abundant in the hexane root at 1.19±0.47 LIN mg/100 mg sample. The antioxidant activity was performed by the DPPH (1, 1diphenyl-2-picrylhydrazyl) radical scavenging method for different crude extracts of the root and fruit of *S. incanum*. The ethyl acetate fruit (EFE) and the ethyl acetate root (Eroot) crude extracts possessed the highest antioxidant potential with a DPPH radical scavenging activity ($IC_{50)}$ value of 35.8 µg·mL^{-1} and 39.5 µg· mL^{-1} respectively. The crude extracts were investigated for cytotoxicity activity against the monkey kidney cell line (Vero cells). Three crude extracts, namely methanol fruit, steroidal alkaloid, and ethyl acetate root showed significant cytotoxic potential to kill the Vero cells with activities of 80%, 70%, and 70%, respectively, at 100 µg· mL^{-1} in 48 hours. The efficacy of the fruit and root crude extracts and isolated compounds against the cabbage aphids was investigated using a leaf dip assay. The crude extracts generally exhibited a higher efficacy against the cabbage aphids than the isolated pure compounds, with the methanol fruit extract exhibiting the highest efficacy. The methanol fruit (MFE) extract had the highest lethal dose (LD_{50}) of 13.16±1.13 µg·mL^{-1}. From the isolated compounds, 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl6-O-(6-deoxy-α-L-manno pyranosyl)-β-D-glucopyranoside **6.20** exhibited the highest efficacy against the cabbage aphids at 54.44±5.44 µg·mL^{-1}.

Nine compounds were isolated from the fruit and root crude extracts of *S. incanum,* of which four were steroidal compounds, three phenolics, one ether, and a dihydrofuran. Two steroidal saponins namely (2S,2′R,4aR,4bS,5′R,6aS,6bR,7S,9aS,10aS,10bS)-4′,4a,6a,7-tetramethyl-,2,3,4,4a,4b,5,6,6a, 6b,7,9a,10, 10a, 10b,11-hexadecahydrospiro[naphtho[2′,1′:4,5]indeno[2,1-b]furan-8,2′-oxan]-2-ol (**6.15**) and 4-((2'*R*,5'*R*,6a*R*,8a*S*,9*R*)-4-hydroxy-6a,8a,9-trimethyl-1,3,3'4,4',5,5'6,6a,6b,6',7,8,8b,11a,12,12a,12b-icosahydrospiro[naphtho[2',1':4,5]indeno [2,1-b]furan-10,2'-pyran]-5'-ylbutan-2-one (**6.16**) were isolated from the root of *S. incanum*. Compound **6.16** is reported as a novel compound. Compound **6.15** has been detected in the aerial parts of *Solanum* species before but in this study, it is being reported for the first time from the root of *S. incanum.* Compound **6.17**, (2*S*,2'*R*,4a*R*,4b*S*,5'*R*,6a*S*,6b*R*,7*S*,9a*S*,10a*S*,10b*S*)-4a,5',6a,7-tetramethyl-1,2,3,4,4a,4b,5,6,6a,6b,7,9a,10,10a,10b,11-hexadecahydrospiro [naphtho[2',1':4,5]indeno[2,1-*b*]furan-8,2'-piperidin]-2-ol and (2*S*,2′*S*,3*R*,3′*R*,4*R*,4′*R*,5*R*,5′*R*, 6*S*,6′*S*)-2,2′-{[(2*R*,3*S*,4*S*,5*R*,6*R*)-4-hydroxy-2-(hydroxymethyl)-6-{[(2*S*,2′*R*,4a*R*,4b*S*,5′*R*,6a*S*, 6b*R*,7*S*,9a*S*,10a*S*,10b*S*)-4a,5′,6a,7-tetramethyl-1,2,3,4,4a,4b,5,6,6a, 6b,7,9a,10, 10a,10b,11-hexadecahydrospiro[naptho [2′,1′:4,5]indeno[2,1-*b*]furan-8,2′-piperidin]-2-yl]oxy}oxane-3,5-diyl]bis(oxy)}bis(6-methyloxane-3,4,5-triol) (**6.18**) are known steroidal alkaloids isolated from the fruit of *S. incanum*. Three phenolic compounds were detected from *S. incanum* namely, 2-3,4-(dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one (**6.19**), 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl6-O-(6-deoxy-α-L-manno pyranosyl)-β-D-glucopyranoside (**6.20**), 2,4 dihydroxy benzaldehyde (**6.22**). Previously, **6.20** was identified in the fruit of *S. anguvi, S. muricatum,* and *S. torvum,* the leaf part of *S. nigrum,* and the stem of *S. melongena* and it is detected for the first time in the fruit of *S. incanum*. Compounds **6.21** and **6.22** are novel in *Solanum* species. 3,4-dihydroxy-5-(1,2-dihydroxyethyl) furan-2(5H)-one (**6.23**) is a common dihydrofuran in *Solanum* species responsible for a wide range of biochemical processes in living species.

The findings in this study substantially contribute to understanding the indigenous knowledge the community hold regarding medicinal plants, and the chemistry associated with the pesticide activity of *S. incanum*. The isolated compounds are potential targets for developing lead compounds in the control of cabbage aphids. Furthermore, the isolation of the new compounds contributes to the gap in the literature regarding single compounds isolated from medicinal plants.

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List of Abbreviations

Abbreviation	Full name
ATP	Atropine
ALD	Aphid Leaf Dip
BCG	Bromocresol Green
BN	Brassica *napus*
CAF	Central Analytical Facilities
CAM	Cabbage aphid mortality
CC	Column Chromatography
CD	Circular Dichroism
CD_3OD	Deuterated Methanol
$CDC\ell_3$	Deuterated chloroform
CMV	Common method variance
H-H COSY	Correlated spectroscopy
DCM	Dichloromethane
DEPT 135	Distortionless enhancement by polarisation transfer
DMSO	Dimethyl sulphoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EFE	Ethyl acetate Fruit Extract
Eroot	Ethyl acetate root extract
ESIMS	Electrospray ionization mass spectrometer
EtoAc	Ethyl acetate
FCR	Folin–Ciocâlteu reagent
FTIR	Fourier transform infrared
fwt	Formula weight
GAE	Gallic acid
Gal	Galactose
GC-MS	Gas Chromatography-Mass Spectrometer
Glc	Glucose
Hex	n-Hexane
HFE	Hexane fruit extract

^{1}H NMR	Proton Nuclear Magnetic Resonance
^{13}C NMR	Carbon Nuclear Magnetic Resonance
HPLC	Higher Performance Liquid Chromatography
HPTLC	Higher Performance Thin Layer Chromatography
HMBC	Heteronuclear multiple bond correlation
HRMS	High-Resolution Mass Spectrometry
Hroot	Hexane root extract
HSQC	Heteronuclear Single Quantum Coherence
IC_{50}	Inhibition concentration at 50%
KAP	Knowledge, Attitudes, and Practices
LD	Leaf dip
LD_{50}	Lethal dose at 50%
LC_{50}	Minimum lethal concentration
LIN	Linalool
MeOH	Methanol
MFE	Methanol Fruit extract
Mroot	Methanol root extract
MS	Mass Spectrometer
MSH	Melanocyte Stimulating Hormone
NMR	Nuclear Magnetic Resonance
PC	Paper chromatography
PPC	Personal protective clothing
PTLC	Preparative Thin Layer Chromatography
QCT	Quercetin
Rha	Rhamnose
ROS	Reactive Oxygen Species
SGA	Steroidal glycoalkaloid
SAFE	Steroidal Alkaloid Fruit Extract
S. incanum	*Solanum incanum* L.
UV-VIS	Ultraviolet-Visible light spectrometer
VLC	Vacuum Liquid Chromatography
δ	Chemical shift (ppm)
λ	lambda

J	Spin-spin Coupling Constant
s	singlet
d	doublet
dd	doublet of doublet
q	quartet
m	multiplicity
ppm	Parts per million
°C	Degree Celsius
nm	nanometre
m/z	mass to charge ratio

Publications and Conferences

Conference output

Zivanayi W., Hlangothi B.G., Rasana N. 2022- Oral Presentation: "*Phytochemical study and biological evaluation of Solanum incanum extracts*", *24th Indigenous Plant Use Forum* 4-7 July 2022, University of Johannesburg, Auckland Park Kingsway Campus, Johannesburg, South Africa.

List of publications under review

Hlangothi B. G., Zivanayi W, Rasana N. *Knowledge, and perceptions of smallholder farmers regarding Solanum incanum L. use as a pesticide in Mkoba community in Gweru, Zimbabwe African Journal of Food, Agriculture, Nutrition and Development* (AJFAND), 2023:

CHAPTER 1

GENERAL INTRODUCTION AND THESIS OUTLINE

1.1 Introduction

The effects of pests and the need to produce adequate food have influenced small-scale farmers in disadvantaged communities to adopt and utilise natural plant pesticides to improve harvests in many African communities (Mujati, 2011). Although commercial pesticides are available, several studies showed that such pesticides are more reachable for large-scale, and commercial farmers where there are enough financial, technological, and human resources than for small-scale farmers (FAO, 2017; Özkara et al., 2016; Sharma et al., 2012). This leaves smallholder farmers with no other option except to use natural herbs as pesticides (Mfarrej and Rara, 2019). Most disadvantaged communities that practice subsistence and backyard farming practices use natural plants as pesticides because they cannot afford commercial ones (Chowański et al., 2016).

A pest is an insect or animal which damages crops or food supplies and a pesticide is a substance or mixture of substances that is mainly used in agriculture or public health protection programmes to protect plants from pests, weeds, or diseases, and human beings from vector-borne diseases, such as malaria, dengue fever, and schistosomiasis. Insecticides, fungicides, herbicides, rodenticides, and plant growth regulators are typical examples (Edmonds and Chweya, 1997). For instance, in Uganda, *Capsicum frutescens, Tagetes spp, Nicotiana tabacum, Cypressus spp., Tephrosia vogelii, Azadirachta indica, Musa spp, Moringa oleifera, Tithonia diversifolia, Lantana camara, Phytollacca dodecandra, Vernonia amygdalina, Aloe spp., Eucalyptus spp., Cannabis sativa, Cofea species, and Carica papaya* are used to control pests. The study findings revealed that the major field pests reported by farmers included banana weevil, bean fly, cereal stem borers, pod feeders, grain moths, rodents, moths, termites, birds, aphids, and cutworms (Mugisha-Kamatenesi et al., 2008). In Tanzania, indigenous plants are very popular as pesticides. Examples of these plans are *Azadirachta indica* (*Meliaceae*), *Tephrosia vogelii* (*Fabaceae*), *Tamarindus indica* (*Fabaceae*), *Aloe spp* (*Asphodelaceae*), *red pepper, Capsicum spp* (*Solanaceae*), *Nicotiana tabasum* (*Solanaceae*) (Mihale et al., 2009). In Mkoba villages, Gweru uses *S. incanum* as a pesticide. Although the use of *Solanum incanum*

as a pesticide has enabled the Mkoba community to protect their vegetables and produce enough for their families, there is a need to scientifically validate this plant pesticides to minimise negative health problems it may have on human beings (Mhazo, 2018). Besides, *Solanum* plants are assumed to be poisonous to animals (Haidan et al., 2016; Korpan et al., 2004; Thaiyah et al., 2010).

1.2 Natural Products

Natural products (secondary metabolites) have been the most successful source of potent chemicals for the pharmaceutical and agricultural industries. Early experimentation involving natural products against pests began as early as 1835, with Agostine Bassi's efficacy against lepidopteran pests (Li et al., 2017). Thereafter, the use of essential oils, phenolic compounds, and alkaloids began to gain momentum as plant protectants in experiments during the 19th century (O'Neal et al., 2018). The pesticidal power of natural products lies in phytochemical constituents such as phenols, flavonoids, quinones, tannins, essential oils, alkaloids, saponins, and sterols, which cause definite pesticidal actions on the pests (Adeyemi, 2010; Al Sinani and Eltayeb 2017; Chowański et al. 2016; Ibanez et al., 2012). Numerous efficacy studies have shown that *Solanum* species are bioactive to different types of pests such as the *Bombyx mori* L, *Spodoptera litura, Tribolium castaneum, Apis mellifera*, *Pseudoplusia* including *Leptinotarsa decemlineata,* and *Macrosiphum euphorbiae* (Chowański et al., 2016; Umar et al., 2015).

Natural products are being used today as pesticides and others as starting raw materials to synthesise new ones. Examples of these pesticides are shown in **Figure 1.1**. For instance, pyrethrum compounds, isolated from the *Chrysanthemum* flower have been used to synthesise pyrethrin I (**1.1**) and II (**1.2**) and cinerin I (**1.3**) and II (**1.4)** (Matsudaa, 2005). These pyrethrum-derived compounds are important in controlling different types of insects that attack vegetables (Khazaal and Shawkat, 2011). Rotenone (**1.5)** is a naturally occurring compound found in several plant species such as *Pachyrhizus erosus* and is a neurotoxin used extensively as an insecticide and fish poison (Soderstrom, 2009; Estrella-Parra et al, 2014).

Most terpenoids are biologically active as fungicides, herbicides, insecticides, antimicrobials, and antifeedants (Stephen, 1988). Among them are chamazulene (**1.6**) with an LD_{50} value of

8.2 μg·mL^{-1}, carvacrol (**1.7**) which showed an LD_{50} of 6.9 μg·mL^{-1}, and (−)-α-bisabolol (**1.8**) with LD_{50} of 10.3 μg·mL^{-1}.

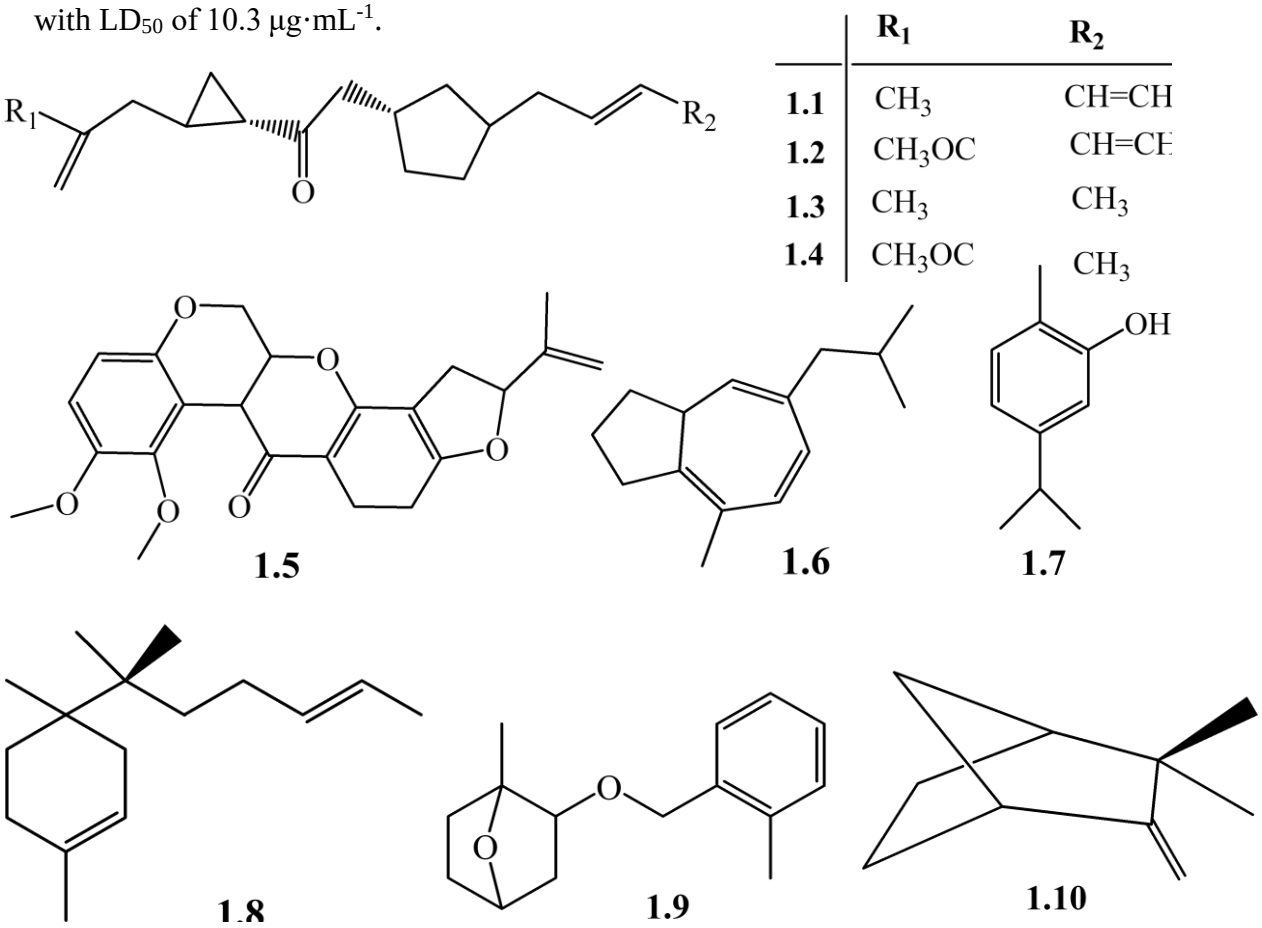

Figure 1.1: Examples of phytochemicals used as pesticides that have been isolated from plant species

Besides exhibiting pesticidal properties, terpenoids are used to synthesise herbicides as well. Exciting examples are toxaphene (**1.9**) which is synthesised from camphene and cinmethylin (**1.10**) a sesquiterpenoid lactone that is used as an herbicide (Hussain and Reigosa, 2014).

1.3 *Solanum incanum*

Solanum incanum L. *(S. incanum)* is a plant that belongs to the Solanaceae family and is widely spread in dry regions or rocky soils in Africa. *Solanaceae* plants have been used since the dawn of civilization to maintain health, treat diseases, and control pests in fields and granaries (Al Sinani and Eltayeb, 2017; Edmonds and Chweya, 1997; Gbile and Adesina, 1988; Meyer, et al, 2014; Sbhatu and Abraha, 2020). More recently, literature has emerged that offers contradictory findings about *S incanum*.

On one hand, literature has reported that *S. incanum* is a potential pesticide (Chowański et al., 2016; De Bon et al., 2014), but on the other hand, it has been reported as a poison to animals (Thaiyah et al, 2010). Madzimure *et al.* (2013) evaluated the efficacy of *S. incanum* aqueous fruit extracts against cattle ticks in on-station experiments and laboratory tick bioassays. The bioassays indicated that there was a high efficacy ratio for the lowest *S. incanum* fruit extract concentrations when ticks were exposed to acaricidal treatments for 48 hours.

In Tunisia, many investigations have been performed on local plants showing a toxicity effect on insects or modifying their behaviour. The insecticidal activity of methanolic extracts from leaves and seeds of *S. elaeagnifolium* against three pest species (*Myzus persicae, Phthorimaea operculella,* and *Tribolium castaneum*) was investigated. The seed extract had the greatest effect in causing mortality of 23.6% for peach potato aphids and 34% for the red flour beetle, and inhibiting oviposition (95.9%) and egg hatching (98.6%) for potato tuber moth (Hamouda et al, 2015). The present study cross-examines claims that: (i) the root and fruit of *S. incanum* (a family member from the *Solanum* genus) has insecticidal and repellent properties against several crop pests and could be employed as alternatives for chemical pesticides, and (ii) *S. incanum* negatively affects the environment and the health of the communities using it as a pesticide. Although a lot of bioactivity investigations of *Solanum* species have been carried out, little has been done to confirm scientifically the efficacy of the root and the fruit on the cabbage aphids.

Solanum incanum possesses a variety of biological activities as it contains glycoalkaloids, saponins, and phenolics (Al Sinani and Eltayeb, 2017). Saponins and glycoalkaloids play an important role in the pharmaceutical industry because their chemical structures are like steroidal

hormones. Therefore, they have been proposed for their use as important sources in the production of medicines, such as contraceptives and steroidal anti-inflammatory drugs (Almoulah, 2017; Feyera et al., 2017). Moreover, biological investigations of the fruit and roots showed significant cytotoxicity against several human cancer cell lines and skin tumours (Feyera et al., 2017).

S. incanum has been identified as a poison to animals. A study carried out by Thaiyah et al. (2010) revealed that the fruits of *S. incanum* are poisonous. The research allowed goats and sheep to feed on *S. incanum* fruits and were monitored over time. The animals showed symptoms of coughing, anorexia, depression with the head held low, staggering gait, continuous bleating, bloating, shivering, progressive weakness, lateral recumbency, and leg paddling movement (Thaiyah et al., 2010). Therefore, plant pesticides are not benign in being used to solve our pest problems, but should be taken with caution to avoid the dangers they bring in. More work must be done to improve the safety and knowledge regarding these natural plant pesticides. Many communities that use plant pesticides are in danger of poisoning due to a lack of adequate information which should help them to make informed decisions regarding the use or handling of plant pesticides.

1.4 Contextualisation of the study

Most rural households in Southern Africa have backyard gardens where they grow different types of vegetables to provide food that contains essential nutrients such as vitamins and minerals. For instance, *Brassica napus* (BN) (commonly known as chomolia, rape, or covo in Shona) is a vegetable that is used as a relish. The effort the households, backyard farmers, and peasant farmers put into producing this type of vegetable in abundance is thwarted by *Brevicoryne brassicae* (cabbage aphids) (Mhazo, 2018). The cabbage aphids attack *Brassica napus* leaves (the edible part of the vegetable), rendering the whole plant useless. Cabbage aphids are small insects that feed by sucking sap from plant leaves (Van Emden, 2017). Many of them can cause extensive damage to the plant, as illustrated below in **Figure 1.2**. Aphids can reproduce without mating and multiply into large numbers in a few days (Van Emden, 2017).

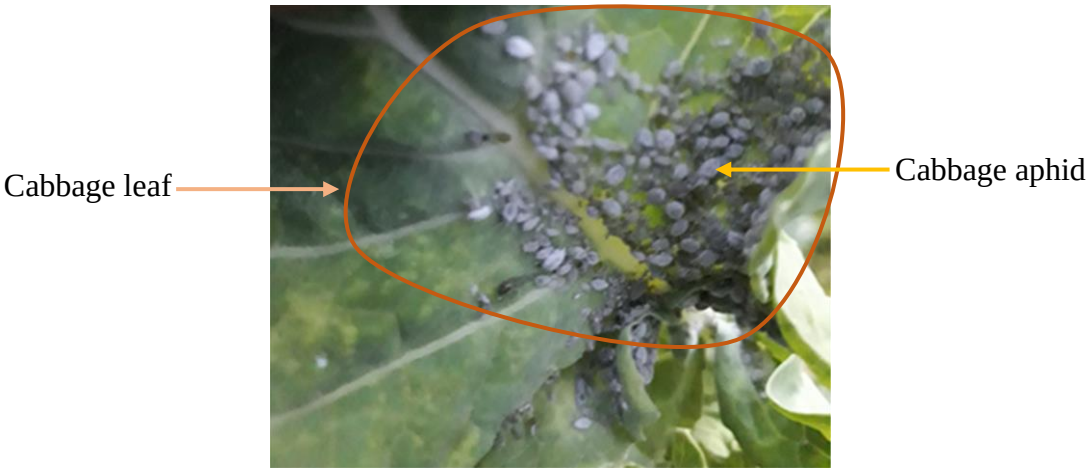

Figure 1. 2: A cabbage leaf infested with cabbage aphids

To counter the effects of these cabbage aphids, most households and backyard farmers use the fruits of a natural plant, *S. incanum*, as a pesticide. The plant is available in the local community in abundance and therefore provides a cheaper and more natural alternative to control pests (Makhatsa, 2007). Although *S. incanum* is poisonous (Madzimure et al., 2013), it has also been perceived to be very effective in controlling the effects of the cabbage aphids on BN (Madzimure et al., 2011; Makhatsa, 2007).

Therefore, the above-mentioned claim made it necessary to evaluate the phytochemicals and pesticide properties of, *S. incanum* based on community knowledge. This evaluation of the pesticide and cytotoxicity of the phytochemicals of *S. incanum* used by the community of Mkoba villages in Gweru, Zimbabwe will be the subject of laboratory investigations.

1.5 Area of study

The area of study was Mkoba Village located in the Gweru district of the Midlands Province of Zimbabwe (**Figure 1.3**). Midlands Province has an average population of 1,650,000 people and 5% of this population is in the Gweru district (Matsa et al., 2019). Gweru is the main city in the province and is located at latitude -19.4500008 and longitude 29.8166695 in the southern hemisphere (Matsa et al., 2019). The district receives an average annual rainfall of 660 mm and many people in the district rely on subsistence farming (Matsa et al., 2019).

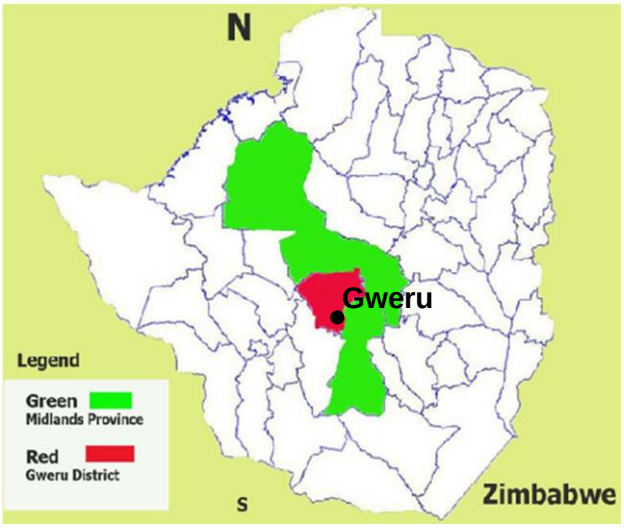

Figure 1. 3: The map of Zimbabwe showing the Gweru District

1.6 Problem Statement

Cabbage aphids destroy *Brassica napus*, a vegetable that is the essential staple relish for the people in Mkoba village, Zimbabwe. To avert the effects of the cabbage aphids, people in this community are using *S. incanum*, a natural plant, as a pesticide to control the cabbage aphids. A natural plant chemical is not necessarily a safe one. Indeed, some of the most toxic compounds known are of plant origin, such as aconitine, and have life-threatening consequences if ingested by mammals even in very small quantities (Stevenson et al., 2017). Thaiyah et al. (2011) suggest that all plant materials used as pesticides have a degree of poison in them (Thaiyah et al., 2011). This implies that the use of *S. incanum* as a pesticide could pose a health risk that the people in the community may not be aware of.

It has been observed that people in the community of Mkoba handle the *S. incanum* pesticide using bare hands, sometimes they eat food immediately after spraying it without washing their hands thoroughly. The chemicals from the pesticide might get into people's bodies through the ingestion of food, inhalation, and the lack of use of protective clothing. The lack of evidence-based knowledge about the toxicity of *S. incanum* might have resulted in cases of poisoning of the users and the fact that people handle the natural plant pesticide carelessly exacerbates the poisoning (Nicolopoulou-Stamati, et al., 2016). The people in the community are not aware

that the same poison targeting the pests may also accumulate in people's bodies and could even become fatal in the long run.

Exposure to *S. incanum* pesticide without careful precautional measures in place for the community requires in-depth research. The underlying scientific uncertainty and the experiences of this vulnerable community reveal the real complex character of the problem. Therefore, the determination of bioactive compounds, cytotoxicity, and the efficacy of the phytochemicals isolated from *S. incanum* may lead to the source of the real health effects experienced in the community. Despite a lot of information from previous studies about *S. incanum*, there is still a lack of information regarding the toxicity of its secondary metabolites and quantities in the crude extracts to guarantee safe use as a pesticide. These facts have prompted a comprehensive phytochemical investigation of the *S. incanum* plant indigenous to the Midlands Province in Gweru, Zimbabwe.

1.7 Purpose of the study

This study aimed to evaluate the toxicity of the phytoconstituents of crude extracts of *S. incanum* to conscientise the community about the possible health effects of this plant. The study also sought to determine the potent pesticide properties as well as document this pesticide knowledge as the community of Gweru narrates it.

1.8 General and Specific objectives

This research aimed to evaluate the pesticidal and toxicity of the phytochemical compounds of S. *incanum*

The research was guided by the following objectives:

i. To gather indigenous knowledge about *S. incanum* from the community through questionnaires

ii. To determine the health problems which may be associated with the use of *S. incanum* as a pesticide in Mkoba village using a survey

iii. To profile and screen crude extracts for cytotoxicity against mammalian cells (Vero cells) using the propidium iodide dye exclusion (PI) assay

iv. To determine the efficacy of the crude extracts against the cabbage aphids using the aphid and leaf dip method

v. To fractionate and isolate pure compounds from the crude extracts using chromatographic methods

vi. To perform structural elucidation of pure compounds by spectroscopic methods

1.9 Research design

The design for this study comprised two phases (**Figure 1.4**): Phase 1 - Evaluation of the community's knowledge and perceptions and Phase 2 - Laboratory experiments. The first phase (descriptive quantitative approach) was used to gather more information about general knowledge of the community on *S. incanum* as well as a detailed description of how it is prepared and applied to control pests together with the possible effects of the phytochemicals on the community. The laboratory experiments phase focused on the activities which enabled the isolation of phytochemicals from the root and fruit of *S. incanum*. The following processes were carried out: Extraction from the plant material, cytotoxicity determination, phytochemical analysis, isolation, purification, structural elucidation, antioxidant analysis, and the efficacy determination of the crude and the isolated compounds.

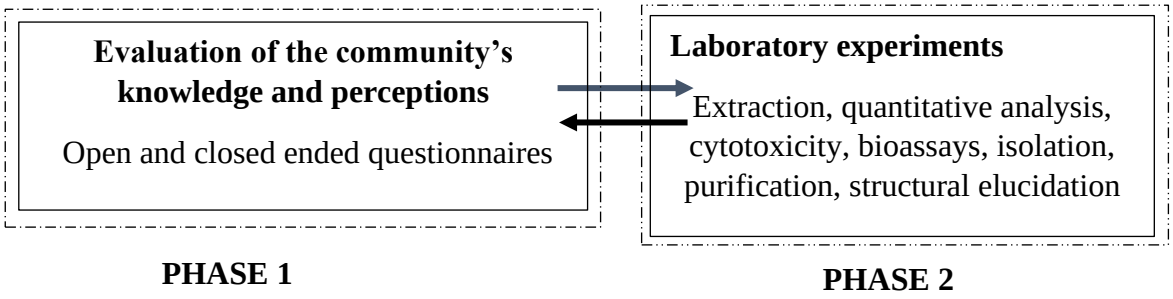

Figure 1. 4: Research design

1.10 Ethical Considerations

While endeavouring to achieve the objectives of the research, the researcher for this study ensured adherence to the guidelines for the ethical conduct of research as stipulated by the Human Research Ethics Committee of Nelson Mandela University. Ethical clearance was obtained from this Committee before the research commenced (**APPENDIX 1.1**).

Permission from the relevant gatekeepers, namely the councillor in Mkoba, Gweru District, was obtained (**APPENDIX 1.2**). The participants were given detailed information regarding the nature of the study, and their consent was sought before any data were elicited from them. Pseudonyms were used for each participant to ensure their anonymity and confidentiality. The data obtained from the community will be stored in an online drive with a secured password. The isolated compounds will be stored in a refrigerator of the natural product chemistry department in the Faculty of Science. The promoter (Dr. B. Hlangothi) was responsible for the security of all the material for this study for five years. The data collected would be used for research purposes, including possible future journal publication only.

1.11 Organization of the thesis

After Chapter **1**, this thesis contains six more chapters.

Chapter 2: A literature review.

In this chapter, a literature review on the *Solanum* species with regards to the traditional, pesticidal, and pharmacological uses was discussed, sighting some phytochemical compounds which were discovered. The chapter highlights the impact of the *Solanum* species particularly *S. incanum* in terms of cytotoxicity and toxicity of its phytochemical compounds.

Chapter 3: Evaluation of the community's knowledge of *S. incanum*'s pesticide properties – *Methodology*.

In this chapter, the methodology used to evaluate the knowledge the Mkoba community holds regarding *S. incanum* is presented. The methodology comprises the paradigm, research design, and methods used in the evaluation of the community's knowledge and perception of *S. incanum*'s pesticide properties.

Chapter 4: The results and discussion of the evaluation of the community's knowledge of the pesticide properties of *S. incanum*.

This chapter presents the key findings of the study on *S. incanum* through the descriptive survey methods from the community of Gweru.

Chapter 5: Chemical profiling, quantitative and qualitative analysis of crude extracts, and biological assays.

In this chapter, results, and discussion of the qualitative and quantitative analyses of crude extracts and the biological assays (Antioxidant, efficacy, and cytotoxicity) are given. The chapter includes an outline of the methods employed for the qualitative and quantitative analyses of crude extracts and the biological assays (antioxidant, efficacy, and cytotoxicity).

Chapter 6: Isolation of phytochemicals and structure elucidation

This chapter focuses isolation and structure elucidation of compounds isolated from *S. incanum*. The chapter presents and discusses the isolation and structure elucidation of the compounds first followed by the methods used in the isolations and structure determination of the phytochemicals.

Chapter 7: General conclusion

In this chapter, the main emerging ideas from this study are summarised and future recommendations will be discussed.

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CHAPTER 2

THE PHYTOCHEMISTRY OF *SOLANUM* SPECIES

2.1 Introduction

The *Solanaceae* family includes a sizable genus of plants called *Solanum* (Eskandari, Assadi, Shirzadian, and Mehregan, 2019). The world's temperate and tropical regions are home to this family's 102 genera and more than 1700 species (Eskandari et al., 2019). The nightshades (*Solanum nigrum, Solanum dulcamara, Solanum tuberosum*),, and *Solanum incanum*) are the most significant species to humans because they are used as food and medicine. Other important species include the *Solanum melongena* and *Solanum lycopersicum* (Wyk., 1997). There are about twenty (20) different species of *Solanum* known to exist in Arabia and seven are found in Africa and include *Solanum cordatum, Solanum incanum, Solanum melongena, Solanum nigrum, Solanum elachophyllum*, *Solanum tuberosum*, and *Solanum panduriforme* (Al Sinani, 2017).

The 102 *Solanum* species found in Africa and other temperate regions were the subject of a literature search, and many of the citations showed ethnopharmacological usage that had been documented through surveys (questionnaires and interviews). The utilisation of *S. incanum's* pesticidal qualities had received very little scientific validation. Furthermore, there aren't many published works on the medicinal and pesticidal characteristics of *S. incanum*, which is a plant that grows in Zimbabwe. Further research is, therefore, required to confirm the toxicity and efficacy of *S. incanum* as a pesticide.

The objectives of this chapter are to:

- Give a summary of the known traditional medicinal and pest control uses of *Solanum* species.
- Share the results of the phytochemical analyses of *S. incanum's* root and fruit.
- Provide a synopsis of the biological characteristics of *Solanum* species.

2.2 Ethno-pharmacological uses of *Solanum* species in Africa

Solanum species have been shown to have therapeutic effects all throughout tropical Africa and the Southern African Development Community (SADC). Extracts from *Solanum* species have been used to cure a variety of conditions, including sore throats, angina pectoris, stomachaches, colic, migraines, painful cutaneous mycotic infections, menstruation, and liver pain (Abebe and Haile, 2014; Dold, 2000; Kokwaro, 1996). Most medical conditions are treated orally with leaf, root, and/or fruit decoctions. To treat conditions like sore throats, some people chew roots and ingest sap. Muscle aches are treated with leaf juice. Muscle discomfort can be reduced using ashes made from the burned leaves of *S. incanum* (Madzimure et al., 2011). Additionally, skin conditions and venereal illnesses are treated with *Solanum* species.

Table 2. 1: Solanum species' traditional medical applications in Africa

Country	**Ethno-medicinal use**	**Country**	**Ethno-medicinal use**
Botswana	Skin diseases; abdominal pains; fever; stomach aches; Toothache (Mukami, 2016)	Rwanda	Arrow poison; fish poison (Abebe et al., 2014)
Ethiopia	Cures sore throat; angina; stomachache; colic; headache; Menstrual pains; liver pains; pain caused by pleurisy; Onchocerciasis; pneumonia and rheumatism; Controls cattle ticks (Abebe et al, 2014).	Zimbabwe	Snakebites; control cattle ticks; tooth decay; gonorrhoea; Skin diseases; Dandruff; control vegetable cabbage aphids (Abebe et al., 2014; Kambizi and Afolayan, 2001; Mukami, 2016)
Kenya	Skin diseases; abdominal pains; fever; pesticide, stomachache; Indigestion; tooth decay; toothache (Abebe et al, 2014).	South Africa	Benign tumours (Lin, 1990); chest pains; sore throat (Sundar, 2016); toothache (Abebe et al, 2014).
Botswana	Skin diseases; abdominal pains; fever; stomach aches; Toothache (Mukami, 2016)	Tanzania	Dandruff; sores; skin diseases; wounds (Baylor, 2015)
Mozambique	Arrow poison; fish poison (Sbhatu and Abraha, 2020)	Uganda	Snake bites (Abebe et al., 2014)

According to ethnobotanical studies. the root, leaf, stem, fruit, and seeds of the *Solanum* species are used as insecticides for field and stored crops as well as for livestock pests (Adeyemi, 2010; Mhazo, 2018; Mwine et al, 2011). For instance, Zimbabweans frequently use *S. incanum* to control cattle ticks. The effectiveness of *S. incanum*'s aqueous fruit extracts against cow ticks was assessed by Madzimure et al. (2011). They compared *Strychnos spinosa* aqueous fruit extract and Tick buster® (amitraz) spray, which was utilized as a positive control, at different concentrations of 5, 10, 20, and 40% (w/v). The treatment was allowed to stand for 48 hrs. A higher efficacy ratio was exhibited at a 5 % (m/v) concentration of the fruit of *S. incanum* treatment compared to the 5% (m/v) *Strychnos spinosa* aqueous extract and the Tick buster® (amitraz) spray against cattle ticks ($p < 0.05$).

According to Meragiaw et al (2016), Ethiopians employ native plants and spices like *Solanum* to fend off malaria-causing Anopheles mosquitoes. For pest management in fields and stored harvests, they also utilise *Solanum* spices such as *S. elaeagnifiolium* and *S. molengena* (Meragiaw et al., 2016). **Table 2.2** provides a review of the ethnic-pesticide traits of various *Solanum* species found in specific African nations.

More than half of all *Solanum* pesticide formulations in numerous African nations, including Uganda, Tanzania, Ghana, Nigeria, Tunisia, Zambia, Malawi, Kenya, and Zimbabwe, are water extracts, according to prior studies (Dari, Addo, and Dzisi, 2016; De Bon et al., 2014; Mhazo, 2018; Mwine et al., 2011). The use of the entire plant as an intercrop, a physical admixture of plant parts with produce, the use of crushed seed cake, the application of plant oil extracts, ash admixture, and the use of smoke from burning plant parts as a method of pest fumigation were some additional traditional pesticide preparation techniques reported (Indhumathi, 2014; Sallam, 2013).

Table 2.2: Reported pesticide properties of *Solanum* species in various African nations. (Adopted from Chowański et al., 2016)

Plant name	Part of the plant used	Insect genus	Activity
S. elaeagnifiolium	W	*Blattella germanica*	Repellence, antifeedants
S. incanum	W	*Amitermes message* *Microtermes najdenses*	Improves adult Mortality
S. jasminoides	W	*Phelobotomus papatasi*	Repellence, larvicidal
S. molengena L.	F	*Spodoptera litura* *Achaea Janata*	Antifeedants prevent larval growth, disturb the molting process, and prevent intestinal serine protease L
S. nigrum L.	L	*Sitophilusoryzae* *Tribolium Castaneum*	Catalysis of the adult mortality rate
		Anopheles cultisifacies	Improves the larvae mortality
		Culex quinquefasciatus	larvicidal
		Aedis aegypt, Culex pipiens	
	GF	*Culex quinquefasciatus* *Anopheles Stephens*	Larvicidal
	W	*Leptinotarsa*	high toxicity
S. psuedocapsicum L	L, F	*Agrotis ipsilon (Hufnagel)*	Antifeedants, larvicidal, deformations in the next instar larvae, pupae, and adults
		Anopheles stephensi	Larvicidal
S. torvum	L	*Culex quinquefasciatus*	L arvicidal
S. trilobatum	L	*Hippobosca maculate L*	Improves adult mortality
		Culex quinquefasciatus	larvicidal
		Anopheles stephensi	Arvicidal
S. tuberosum L	VW	*Zophobas atratus (Fab.)*	In vivo cardioinhibitory activity in pupae
		Leptinotarsa decemlineata	Improves mortality of larvae, pupae, and adults
	L	*Spodoptera exigua*	Disturbance of fecundity and fertility; generation of oxidative stress; decreased GST

		Culex quinquefasciatus	Affects the enzyme activities
		Anopheles subpictus	Larvicidal
S. xanthocarpum	YS	*Culex quinquefasciatus*	Contact toxicity, larvicidal, pupacidal activity

Part of the plant used for the formulation: **F**—fruit, **W**—whole plant, **L**—leaf, **S**—seed, **VW - vegetable** wash, **YS** — young shoot

The typical indigenous techniques used to administer crude extracts to afflicted crops include dusting, disseminating, fumigating, side-dressing, and baiting (Dari et al., 2016; Mwine et al., 2011). The type of crop, the pest's life cycle, and the nature of the plant extract influence the method selection. The most popular practice in African nations was found to be spraying. It is thought to be the most effective method for eliminating flying pests and larvae (Pavela, 2011). However, the pesticide also affects species that are not the target, which is a drawback of the broadcasting (spraying) strategy.

Although numerous ethnic-pesticide applications of *Solanum* species have been documented in Africa, particularly in the SADC region, they are still lacking scientific support (Nyirenda et al., 2011). These pesticide plants need to be properly evaluated scientifically. Furthermore, the importance of looking into how the users manage indigenous plant pesticides cannot be overstated. The community's perceptions regarding the methods they use in gathering, preparing, and using the pesticide without following the pesticide use regulations need to be explored (Thaiyah et al., 2011).

2.3 Secondary metabolites in *Solanum* plants

In most cases, secondary metabolites are not necessary for the growth, development, or reproduction of the plant. They are either created because of the plant adjusting to its surroundings or as a potential defence mechanism against predators to help the plant survive. The core activities of photosynthesis, glycolysis, and the Krebs cycles serve as the starting point for the lengthy production of secondary metabolites (Hakim and Kadarohman, 2012).

Terpenes, flavonoids, sterols, phenolic compounds, steroidal saponins, steroidal alkaloids, and terpenoids are only a few of the natural chemical substances that have been isolated from the

Solanum species. These compounds act as a plant's defence strategy against herbivorous animals, insects, and microbial predators (Esteves-Souza et al., 2002; Hakim and Kadarohman, 2012; Kaunda and Zhang, 2019). Terpenoids repel predators with their unpleasant smells and bitterness. Alkaloids are among the other natural products that deter, sterilize, and repel predators while also occasionally poisoning them (Chowaski et al., 2016). Additionally, medicinal uses for natural compounds derived from *Solanum* species have made significant contributions to the fight against diseases including viruses and bacteria (Al Sinani and Eltayeb, 2017).

2.3.1 Steroidal saponins

One of the main types of chemicals in *Solanum* species is saponins (Cui et al., 2016). According to Vincken et al. (2007), saponins are made up structurally of a hydrophobic aglycone with a steroidal (**2.1**) or triterpenoid skeleton (**2.2**) and a water-soluble moiety made up of sugar residues. The triterpenoid aglycone has a 22-carbon atom skeleton and generally pentacyclic structure.

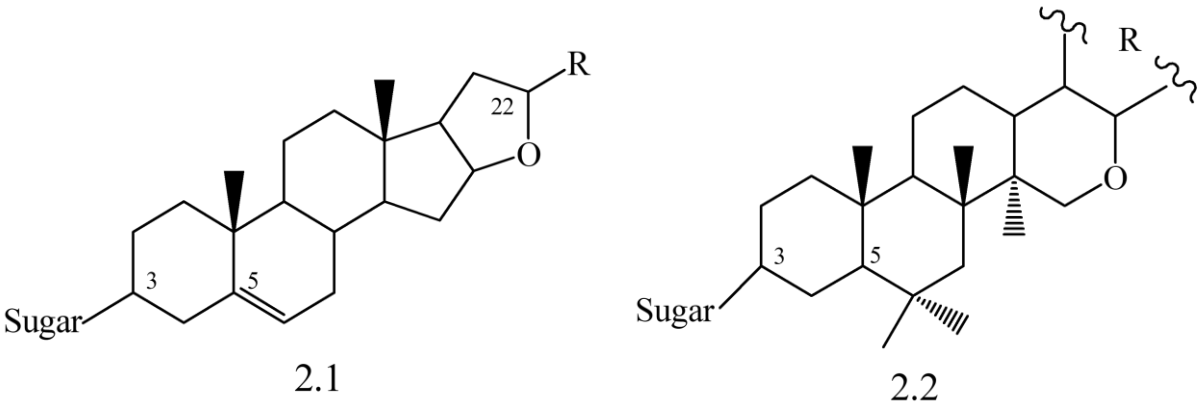


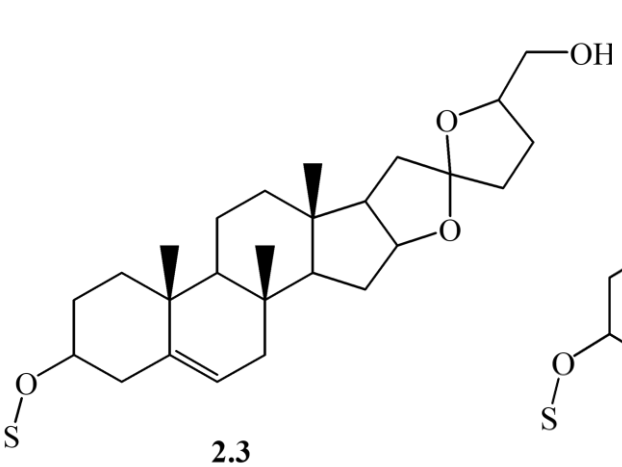
Figure 2. 1: Steroid and triterpenoid aglycone structures of saponins

The cholestane, furostane, and spirostane classes of steroid saponins were discovered in the *Solanum* species (Sadeghi et al., 2013). A spirostane has a cyclic **R**-group connected to C-22, whereas a furostane has an aliphatic **R**-group. Despite possessing a wide range of chemical properties, saponins share several common characteristics, such as a bitter taste, the ability to form stable forms in water, the hemolysis of red blood cells, and toxicity to cold-blooded creatures including fish, snails, and insects (Ozlem and Mazza, 2007; Thakur et al., 2011).

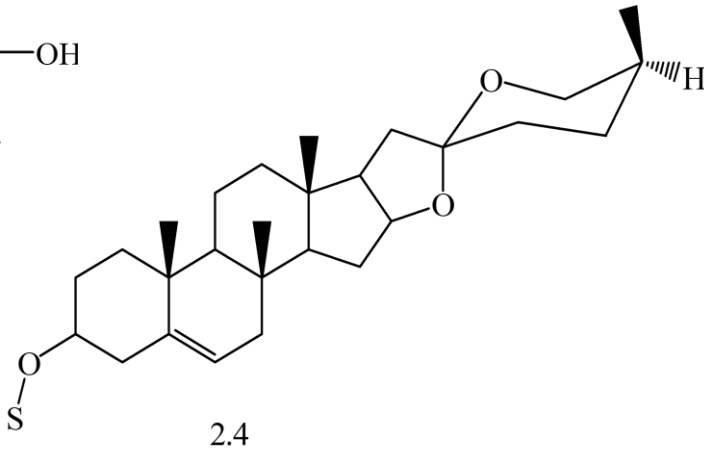
Some of the 134 steroidal saponins that have been identified thus far from various *Solanum* species are shown in **Figure 2.2** as compounds **2.3** to **2.8**. (Kaunda and Zhang, 2019). *S. 21emale* is the most investigated species of all those under study, and as a result, 32 saponins

have been isolated from it (Kaunda and Zhang, 2019). *Solanum* species are distinguished by steroidal saponins, which have a variety of biological functions. The majority of saponins have cytotoxic, anticancer, hepatoprotective, antihypertensive, antimelanogenic, antifungal, anti-inflammatory, anticonvulsant, and antiviral properties (Segal, 1977). For instance, nuatigenosido (**2.3**) isolated from *S. sisymbriifolium* roots reduces blood pressure in experimentally hypertensive rats (Kaunda and Zhang, 2019). Dioscin (**2.4**) has been isolated from the fruits of *S. indicum, S. melongena*, and *S. rostratum* (Segal, 1977).

Saponins from *S. nigrum, S. torvum*, and *S. molengena* have been isolated and separated using chromatographic techniques such as vacuum liquid and column chromatography (Kaunda and Zhang, 2019). The isolation of dioscin (**2.4**), protodioscin (**2.5**), and methyl-protodioscin from the methanol root extract of the *Solanum* species was made possible using a vacuum liquid chromatography (VLC) on reversed-phase RP-18, column chromatography (CC) on Sephadex



2.3



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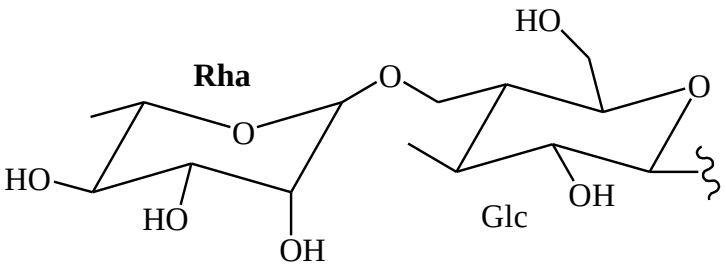
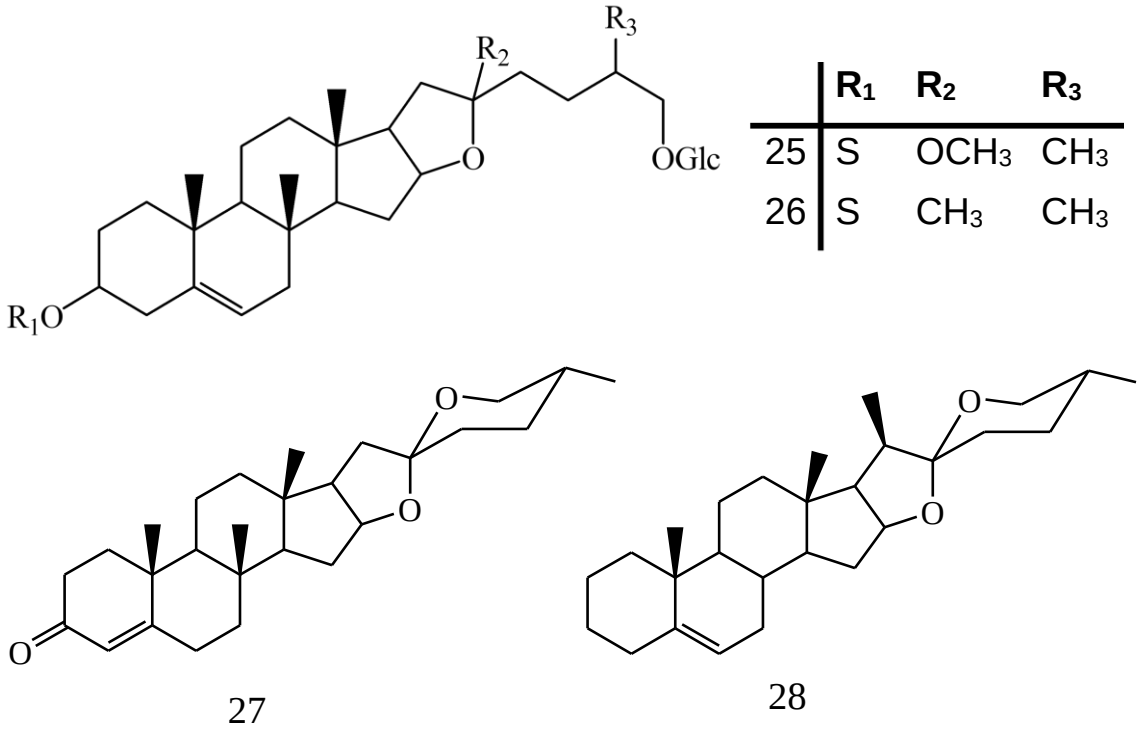


S: Sugar

Figure 2.2 continues



	R1	R2	R3
25	S	OCH_3	CH_3
26	S	CH_3	CH_3

Figure 2. 2: Examples of saponins isolated from the genus *Solanum*

LH-20, and higher-performance liquid chromatography (HPLC) on normal or reversed-phase RP-18 silica (**2.6**). With a minimum inhibitory concentration (MIC) ranging from 31.25 to 250 $g \cdot mL^{-1}$, dioscin (**2.4**) has an anti-melanogenesis action on -melanocyte-stimulating hormone (-MSH), which induces melanogenesis in B16 murine melanoma cells (Abhishek et al., 2015).

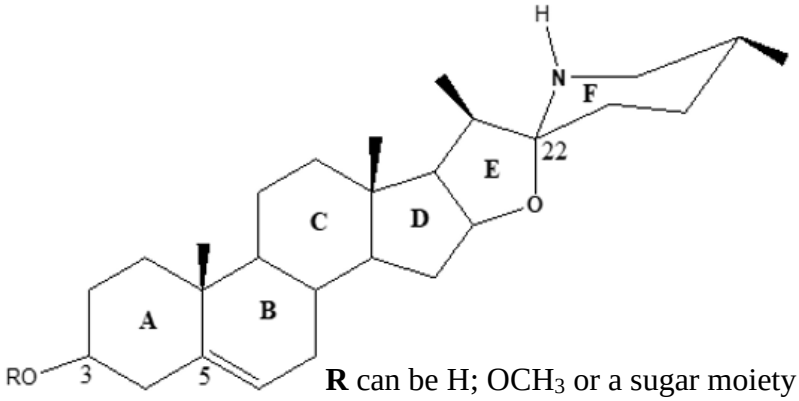
Diosgenin (**2.8**) was successfully isolated from the crude ethyl acetate extract of the aerial parts of *S. lycopersicum* and *S. melongena*, the fruits of *S. nigrum* and *S. Torvum*, and the stem of *S. tuberosum* by a Column Chromatography (CC), followed by repeated Preparative Thin Layer Chromatography (PTLC) (Kaunda and Zhang, 2019). Diosgenin has shown great promise in the treatment of a variety of disorders, including cancer, hypercholesterolemia, inflammation, and several infections. It is also used as a starting material for the creation of several steroidal drugs, such as family planning pills, in the pharmaceutical industry (Kaunda and Zhang, 2019).

In addition to serving as economically significant raw materials for a variety of steroid hormone medications, spirostane and furostane saponins and their glycosides are also crucial for anticancer medications (Al Sinani and Eltayeb, 2017; Kaunda and Zhang, 2019). Protodioscin (**2.6**) and methylprotodioscin (**2.5**), are two spirostane saponins derived from *S. heteracanthum* and *S. incanum* roots, which were tested for toxicity against human colon cancer cell lines (HT-29, HCT 116, and SW480), human prostate carcinoma cell line (DU145), and mouse mammary cancer cell line (mammary EMT6). On all cell lines, both substances **2.5** and **2.6** had some discernible cytotoxic effects (Mahenina, 2012). Therefore, it would be of much interest to investigate the potential cytotoxic activity and pesticide efficacy of saponins derived from *S. incanum* (the species found in Mkoba village, Gweru).

2.3.2 Alkaloids

The most prevalent and typical alkaloids in *Solanum* species are glycoalkaloids (Nepal and Stine, 2019; Kaunda and Zhang, 2019). Glycoalkaloids are a class of nitrogen-containing steroidal glycosides with a solasodine aglycone structure shown in **Figure 2.3**. Solasodine has been isolated from flowers, sprouts, immature berries, young leaves, or shoots of *Solanum* species and has been discovered in over 200 *Solanum* species (Cárdenas et al., 2015; Jayakumar and Murugan, 2016

Glycoalkaloids are made up of a solasodine aglycone unit with an **F** ring containing nitrogen and a hydrophobic 22-carbon cholestane skeleton (Milner et al., 2011). The numbered rings (**A-F**) on the aglycone backbone (seen in **Figure 2.3**) are typical characteristics of steroidal glycoalkaloids from other alkaloids.



R can be H; OCH_3 or a sugar moiety

Figure 2. 3: The aglycone structure of glycoalkaloids

The **F**-ring of the aglycone has an amino group between (C22-C26) and one double bond at (C5–6). Various kinds of aglycone structures exist in *Solanum* species, depending on the location and makeup of the **F** ring. Solanidanes (**2.9**), spirosolidanes (**2.10**), 3-aminosiorpstannes (**2.11**), 22,26-epiminocholstane (**2.12**), and epiminocyclohemiketal (**2.13**) shown in **Figure 2.4** are the variety of categories of aglycones of glycoalkaloids in S*olanum* species (Al Sinani and Eltayeb, 2017).

The aglycone combines with a mono or polysaccharide molecule using the C-O group at C-3. D-glucose (Glc), D-galactose (Gal), D-xylose (Xyl), and L-rhamnose (Rha) are common saccharides that are typically connected to the C-3-hydroxyl position of aglycones in a variety of combinations (Almoulah, 2017).

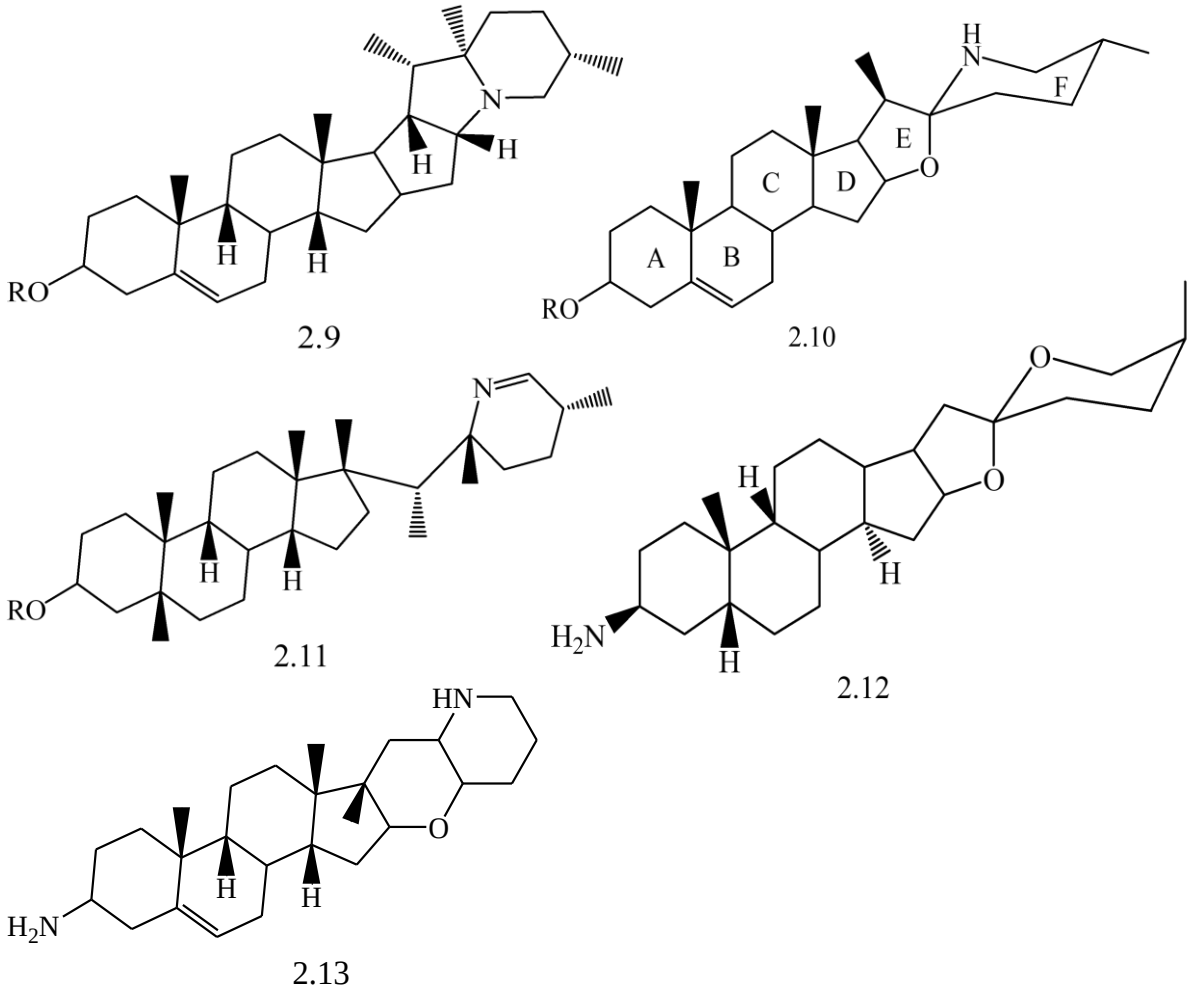
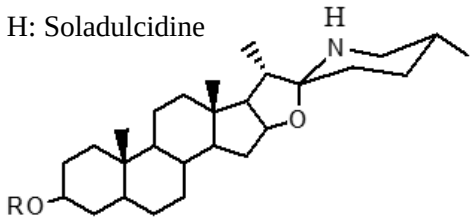
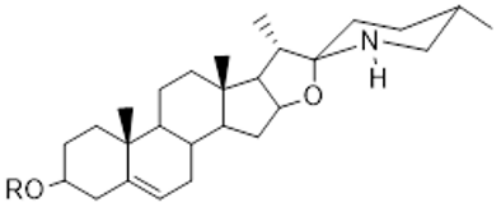
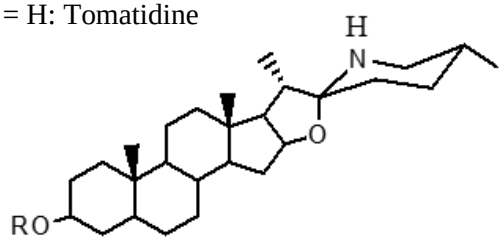


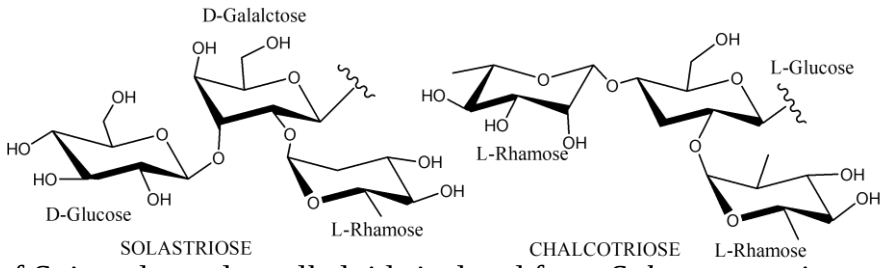
Figure 2. 4: Classes of glycoalkaloids aglycone structures found in *Solanum* species

The most common glycoalkaloids in the *Solanum* family are known to be solamargine and solasonine (Gürbüz et al., 2015). Solasonine, solamargine, solaverine, (23S)-23-hydroxy anguidine, solasodine, solarmine, and incanumine are a few spirosolane steroidal glycoalkaloids that have been identified from various *Solanum* species (**Figure 2.5**).

Chromatographic techniques are frequently used to isolate glycoalkaloids from *Solanum* species (Column chromatography, high-performance liquid chromatography, and preparative thin-layer chromatography). For instance, reversed-phase thin-layer chromatography (RP-TLC) was carried out by Gürbüz et al. (2015) utilising chloroform: methanol: water (61:32:7) (v/v/v) solvent solution, followed by silica gel column chromatography. Solamargine and solasonine were separated using the PTLC after combining fractions with the same retention duration. Incanumine **(Figure 2.5).**

CATEGORY OF THE AGLYCONE	AGLYCONE STRUCTURE	SUGAR MOEITY	COMPOUND NAME	SOLANUM PLANT	REF
Spirosolanes	**R** = H: Soladulcidine 	**R** = Solatriose	Solasonine (**2.14**)	*S. incanum* *S. berthauliti* *S. stoloniferum*	(Kaunda and Zhang, 2019)
		R = Chalcotriose	Solamargine (**2.15**)	*S. incanum* *S. panderforume* *S. ambosium*	
	R = H: Solasodine 	**R** = Lycotetraose	Dehydrotomatine (**2.16**)	*S. lycopersicum* *S. tuberosum,* *S. lycopersicon esculentum,* *S. brevidens*	(Kaunda and Zhang, 2019)
		R = Solatriose	α-Solamarine (**2.17**)	*S. brachycarum* *S. nigrum* *S. incanum*	
		R = Chalcotriose	β-Solamarine (**2.18**)	*S. tribolium* *S. brachycarum*	
	R = H: Tomatidine 	**R** = Lycotetraose	α-Tomatine (**2.19**)	***S. tuberosum,*** ***S. lycopersicon*** ***S. esculentum,*** ***S. brevidens*** ***S. demissium***	(Kaunda and Zhang, 2019)

Sugar moieties



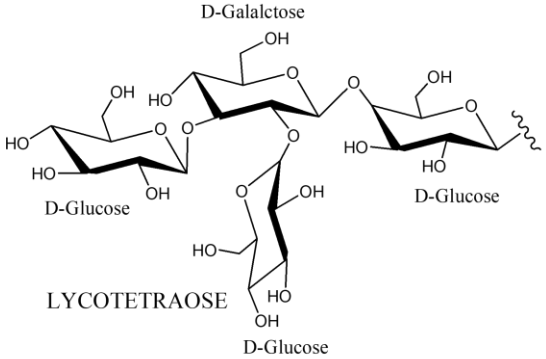


Figure 2. 5: Examples of Spirosolane glycoalkaloids isolated from *Solanum* species

On the other hand, more than 350 species of *Solanum* have been shown to contain solanidanes (Friedman et al., 1997; Milner et al., 2011). Like spirostan saponins, solanidanes differ in that, as seen in the aglycone structure in **Figure 2.6**, nitrogen joins the fused spirostan E and F hexa-cyclic rings (Munafo, 2015; Devkota et al.; 2015; Kaunda and Zhang, 2019). The main source of solanidines such -as solanine and -chaconine is *S. tuberosum*. Typically, acidified ethanol is used as the solvent to extract solanidanes from *Solanum* species. These compounds are subsequently separated and purified using ultra-performance chromatography (UPLC). For instance, solanidane has been extracted from the ethanolic extract of *Solanum Xanthocarpum's* unripe fruits. The various constituents were separated using an ethanol and toluene combination (Bhattacharya et al., 2013). Glycoalkaloids have been explored for their observed cytotoxic activity against certain cancer cell lines (Al Sinani and Eltayeb, 2017). For example, solamargine (**2.15**) causes the human hepatoma cells death (Hep3B) by apoptosis; α-solasonine (**2.14**) from *S. crinitum* and *S. jabrense* has a cytotoxic effect on the leukemia cells; chaconine (**2.28**), α-solanine (**2.29**), tomatine (**2.19**), and their derivatives inhibit the human colon (HT29) and liver (HepG2) cancer cells from growing; β-2-solamargine from *S. nigrum* has a toxic effect on the cell lines: HT-29 (colon), HCT-15 (colon), LN C P (prostate), PC-3 (prostate), T47D (breast and MDA-MB-231 (breast) (Almoulah, 2017; Sammani, 2014).

Some research has revealed that glycoalkaloids, such as -solanine and -chaconine, have lethal effects in contrast to their medicinal applications (Chowaski et al., 2016). By rupturing cell membranes and decreasing acetylcholinesterase function, -solanine and -chaconine can cause gastrointestinal and systemic effects *in vivo* and *in vitro* (Chowaski et al., 2016). Dimethylnitrosamine is a strong carcinogen found in the fruits of *S. nigrum* and *S. panduriforme* (Almoulah, 2017).

Similarly, it has been discovered that *S. incanum* fruit extracts can lead to skin cancer in mice (Thaiyah et al., 2010). Goats who ate unripe fruits of *S. incanum* were poisoned (Thaiyah et al., 2010). However, goats given doses of the extract up to 15,000 mg/kg orally did not exhibit symptoms of typical toxicity (Mwonjoria et al., 2014).

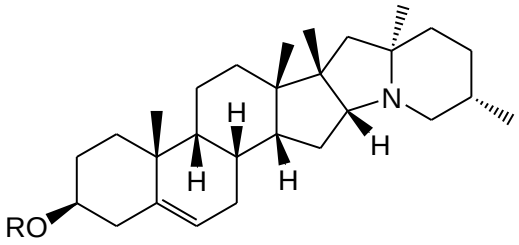
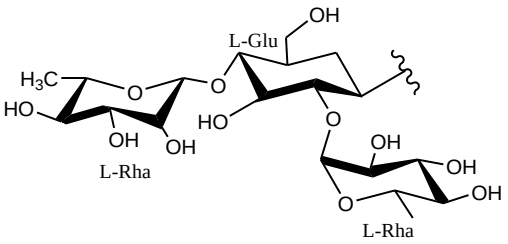
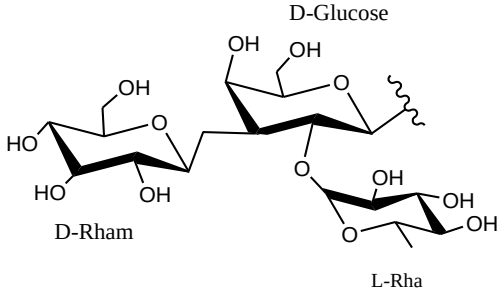
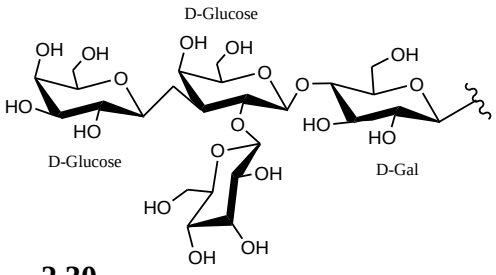
AGLYCONE STRUCTURE	SUGAR MOEITY (R-group)	SOLANUM PLANT	REF
 R = **H**: Solasodine (Milner et al., 2011) **R** = H: Solanidine	 **2.28**	*S. tuberosum tubers* *S incanum fruit* *S Torvum* *S nigrum*	(Tilahun et al., 2020)
	 **2.29**	*S. 29emale29num* *S. amygdalifolium* *S. asperum* *S. crinitum* *S. erianthum* *S. khasianum* *S. lycocarpum*	(Milner et al., 2011; Nguyen, et al., 2008; Cornelius et al., 2010)
	 **2.30**	*S. tuberosum*	(Distl and Wink., 2009; Milner et al., 2011)

Figure 2. 6: Examples of spirosolane glycoalkaloids isolated from different *Solanum* species

According to the literature, solanidanes are more poisonous than spirosolane alkaloids (Friedman, Henika, and Mackey, 2003). However, spirosolanes (solamargine and α-solasonine) are harmful when administered at a concentration above 2-3 $mg \cdot kg^{-1}$ body weight, in which they break cell membranes, block acetylcholinesterase, harm the liver, and harm the heart at this level according to human toxicological investigations.

About 20–60 mg per 100 g of total glycoalkaloid (TGA) are present in *S. tuberosum* (potato tubers). Concentrations greater than 20 mg per 100 g (fwt) of *S. tuberosum* may result in gastrointestinal symptoms, coma, and even death (Omayio, Abong, and Okoth, 2016). The toxic dose of S. tuberosum is approximately 3-6 $mg \cdot kg^{-1}$ (bw). A rupture of the cell occurs when glycoalkaloids form compounds with the membrane structure of 3-hydroxy sterols, such as cholesterol (Nepal and Stine, 2019). *S. incanum*, which is used as a pesticide in Mkoba village (the study region), is likely to include similar types of phytochemicals that are harmful to humans given the type of alkaloids mentioned above that are found in *Solanum* species.

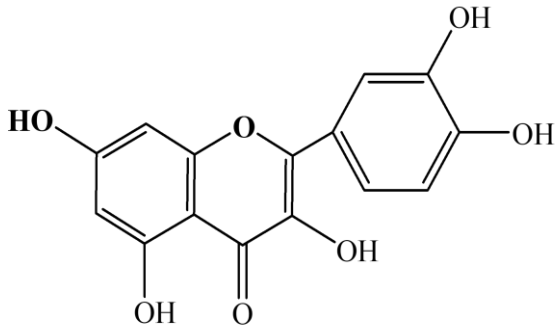
2.4 Pesticidal properties of *Solanum* compounds

Secondary plant metabolites, including alkaloids, glycoalkaloids, terpenoids, organic acids, alcohols, and phenolic compounds, have been identified as promising sources of plant-pesticidal activity in a variety of studies on *Solanum* plants (Adeyemi, 2010; Al Sinani and Eltayeb, 2017; Chowaski et al., 2016; Ibanez et al., 2012).

Numerous species of the *Solanum* family have been found to contain phenolic compounds which have pesticide properties. Examples are quercetin, kaempferol, and myricetin glycosides (Nino-Medina et al., 2017). Some examples of these phenol glycosides are shown in **Figure 2.7**. The most prevalent flavonol aglycone found in *S. nigrum* is quercetin. Sugar moieties attach to it to form derivatives which include quercitrin, isoquercitrin, and rutoside (Zagrean-Tuza et al., 2020). Quercetin (**2.24**) has been isolated from the aerial parts of *S. incanum*, *S. anguvi*, *S. elaeagnifolium*, and *S. anguvi* fruit (Kaunda and Zhang, 2019).

Rutin (**2.22**) has been found to have several beneficial qualities in addition to its pesticidal effects. These include anti-inflammatory, oestrogenic, enzyme inhibition, antibacterial, antiallergic, antioxidant activity, cytotoxic, and anticancer activity (Almoulah, 2017; Kaunda and Zhang, 2019). The stem of *S. indicum*, *S. nienkui*, *S. sessiliflorum*, and *S. surattense*, as

well as the fruit of *S. anguvi*, have all been investigated for their rutin content (Patel, 2012) *S. anguvi* (fruit), S. guaraniticum (leaf), *S. lycocarpum* (fruit), *S. lyratum* (whole plant),



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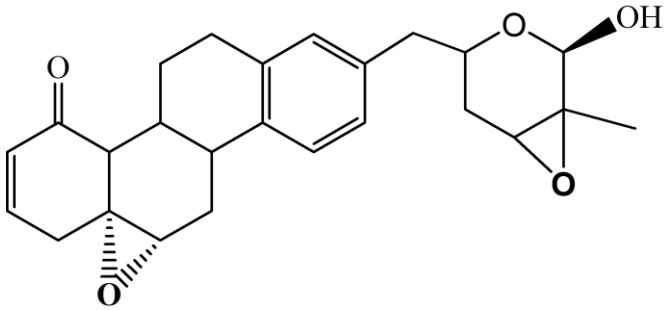

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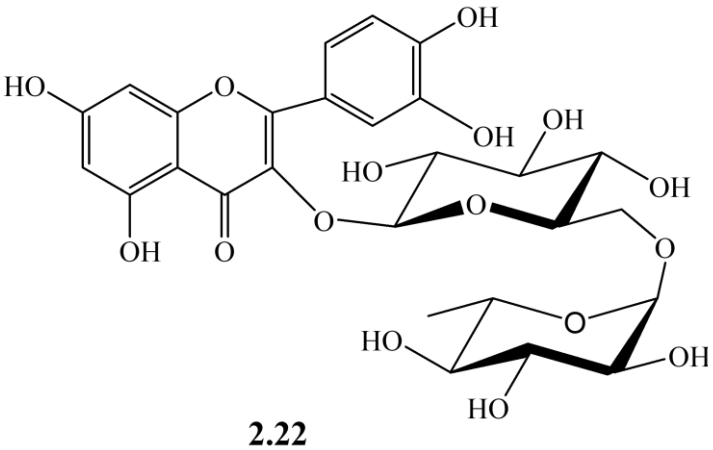

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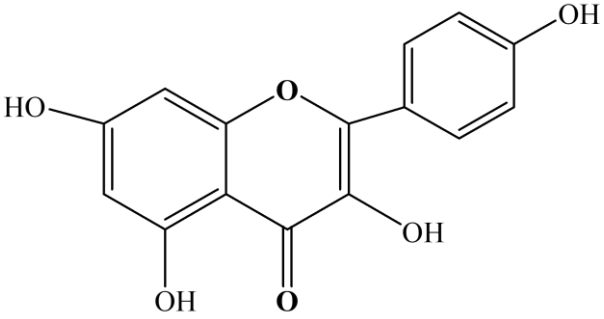

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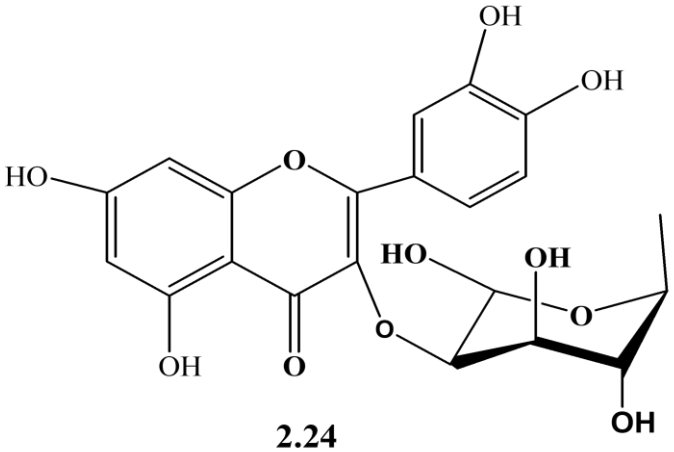

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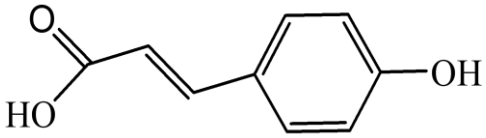

2.25

Figure 2.7 continues

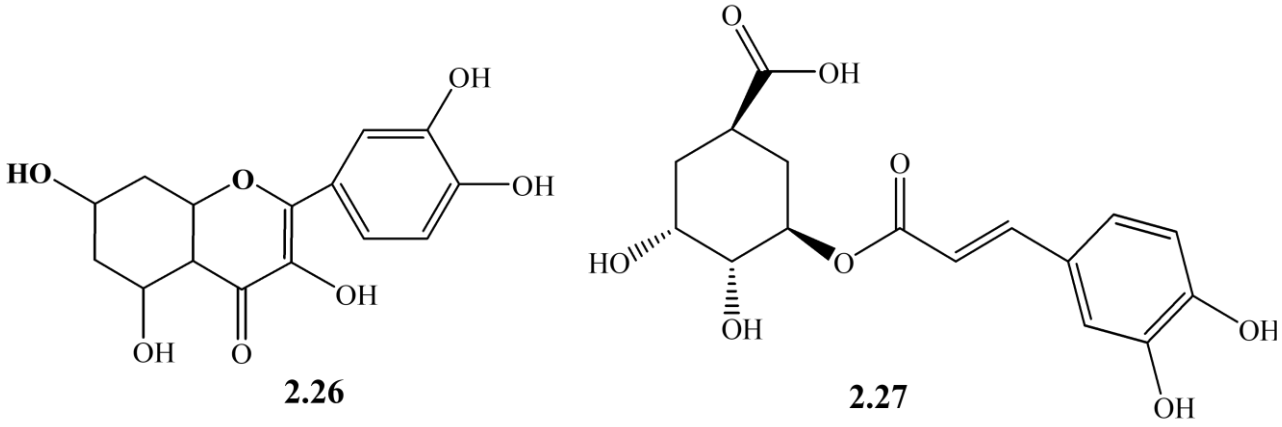

Figure 2.7: Examples of phenolic compounds associated with pesticidal properties which have been isolated from different *Solanum* species

S. melongena (stem, and leaf), and *S. surattense* (whole plant) have all been found to contain chlorogenic acid (**2.27**). (Elekofehinti, 2013). The literature claims that chlorogenic acid has anticancer effects (Kaunda and Zhang, 2019).

In *Solanum* species, terpenes and terpenoids make up most of the sterols and essential oils. Essential oils typically serve as insect repellents. The following isolated terpenes from the *Solanum* plants are 2-undecanone, 2-dodecanone, 2-tridecanone, and 2-pentadocanone (Chowaski et al., 2016). Terpenes increase the death rate of *Aphis cracavora* in addition to being repellents (Ofori et al., 2014; Rakhshani et al., 2005). Terpenes also prevent pests from ovipositing.

These secondary metabolites target pests' organelles, metabolic processes, and macromolecules like proteins and nucleic acids as part of their mechanism of action (Mwine et al., 2011). According to Al Sinani and Eltayeb (2017) and Chowaski et al. (2016), some natural products render plants indigestible to insects, attract insects to poison them, and kill pests immediately upon contact. Additionally, one of the phytochemicals' most significant qualities is that they have a short half-life in the environment (Mhazo, 2018), and because of their wide range of physiological activity, pests find it difficult to adapt resistance to them (Friedman et al., 2003). Since phytochemicals affect pests in a variety of ways, the likelihood that insects will develop resistance to any one of them is decreased (Ibanez et al., 2012).

The toxicity of the phytochemicals obtained from *Solanum* species is displayed at all levels of biological processes in various insects, having both lethal and sub-lethal effects (Chowaski et al., 2016). Alkaloids disrupt biological membranes, interfere with metabolism, and cause acute toxicity by preventing the acetylcholinesterase enzyme (AchE) from transmitting impulses (Adeyemi, 2010; Chowaski et al., 2016; Elgorashi et al., 2004). Terpenes disrupt metabolism, cause the mid-gut to malfunction, and keep insects away from the crop (Sánchez et al., 2009). Phenolic chemicals typically interfere with biological membranes and change how pests feed (Pavela, 2011). **Figure 2.8** (Chowaski et al., 2016) illustrates a variety of harmful effects of *Solanum* species on many levels of biological organisation.

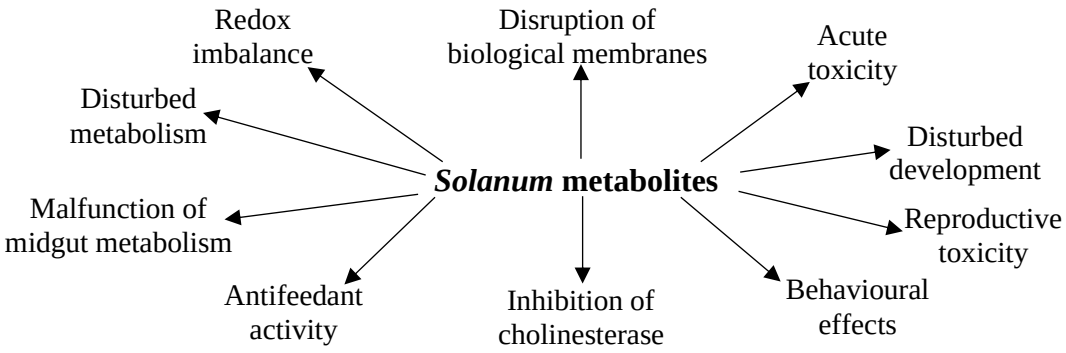

Figure 2. 8: Range of toxic effects of *Solanaceae* metabolites on various levels of biological organisation

The biological activity of glycoalkaloids, tropane alkaloids, and steroidal alkaloids isolated from *Solanum* species against pests is based on (a) inhibition of membrane 3-hydroxy sterols, which leads to membrane disruption and loss of its integrity, and (b) complexation with membrane acetylcholinesterase (AchE), which is responsible for terminating cholinergic transmission at the neuromuscular junction and the central nervous system (Milner et al., 2011).

Potential antifeedants include alkaloids such -as chaconine (**2.28**) and -solanine (Chowaski et al., 2016). Insects that consume leaves include leaf miners, cabbage aphids, thrips, cutworms, armyworms, cabbage loopers, beetles, earworms, hornworms, green vegetable bugs, spider mites, stem borers, root-knot nematodes, snails, and slugs. A-Solanine (**2.29**) has promised to target these pests (Anu, et al., 2011; Martin et al., 2010). A-Tomatine (**2.19**) exhibits retarded growth and delayed development of the Colorado potato beetle.

Glycoalkaloids are effective against rodents, animal-sucking pests like mosquitoes and cockroaches, and other pests that consume human waste and stored product pests like *Sitophilus oryzae, S. granaries, Corcyra cephalonica,* and *Sitophilus oryzae* (Sorensen, Baker, Carter, and Stephan, 1994). Demissine (**2.30**) increases synaptic transmission, which has unfavourable behavioural consequences and demonstrates insecticidal action via imitating acetylcholine. The antifeedant Demissine (**2.30**) is also used (Cohen, 2015). According to Lopez-Carretero et al., (2005), *Tribolium castaneum*, -solanine (2.29), chaconine (**2.28**), tomatine (**2.19**), solamargine (**2.15**), and solasonine (**2.14**) interfere with insect reproductive systems by lowering egg production and impeding the insect's oocytes' capacity to mature.

These phenolics' various pesticidal properties depend on their chemical compositions and physicochemical settings (such as pH, redox potential, and oxidase, oxidant, and antioxidant concentrations) (Aberoumand et al., 2008; Poiatti et al., 2009). According to Chowaski et al. (2016), oxidation processes brought on by phenolic compounds typically have cytotoxic effects on an insect's internal tissues, causing damage to the midguts of herbivorous insects and producing repulsive odours and an unpleasant taste. Trans-coumaric acid (**2.20**), salpichrolide (**2.21**), chlorogenic acid (**2.22**), and rutoside are significant pesticidal phenolic compounds identified from *Solanum* species (**2.24**).

Rutin and chlorogenic acid are bioactive towards *Heliothis virescens F., Manduca sexta* L., and *Pseudoplusia. These compounds* reduce the adult pest survival period affecting the normal growth of *Spodoptera frugipedrda* respectively (Anku, Mamo, and Govender, 2017; Chowański et al, 2010).

Table 2.2 is a summary of the pesticidal phytochemicals isolated from different *Solanum* species. From the reviewed literature, there is no doubt that *Solanum* species possess pesticide properties, but it is worthwhile to identify which compounds in *S incanum*'s fruit and root are bioactive against the cabbage aphids, something which has not been investigated.

Table 2.3: Isolated compounds from different Solanum species. Which have been identified as having pesticide properties (Chowański et al, 2016)

Compound	Class of Compound	Insect Genus	Feeding*	Activity
(2S,4R)-4-hydroxy-1-methyl-2-pyrrolidine carboxylic acid	Alkaloid	*Liriomyza trifoli Burg*	C	refrain oviposition
2-undecanone	Terpene	*Aphis craccivora Koch*	C	improves adult mortality
2-dodecanone	Terpene	*Aphis craccivora Koch*	*C*	enhances adult mortality
2- tridecanone	Terpene	*Aphis craccivora Koch*	C	improves adult mortality
2 – pentadecanone	Terpene	*Aphis craccivora Koch*	C	improves adult mortality
Scopolamine	Alkaloid	Apis mellifera	O	deterrence
		Myzus *persicae*	C	decreases fecundity and feeding habit
		Pseudoplusia includes	C	decreases body weight
		Tribolium castaneum	S	poisonous
		Zophobus atratus		reduces heart activity
∝-chalconine	Alkaloid	*Leptinotarsa decemlineata*	C	stimulates agitating and restless behaviour
		Plutella xylostella L.	C	avicidal, highly toxic to eggs
		Ceratitis capitate	C	reduces larval survival prolongs; the maturation of eggs
		Myzus persicae	C	reduces fecundity, feeding adults
∝-solanine	Alkaloid	*Tribolium castaneum*	C	acute toxicity; improves mortality of nymphs
		Zophobus atratus	C	decreased heart activity
Chlorogenic acid	Phenol	*Heliothis virescens F., Manduca Spodoptera frugipedrda*	P	Lowers development, growth
Leptine	Alkaloid	*Leptinotarsa decemlineata*	C	reduces feeding

Luciamin	Glycoside	Schizaphis graminum	C	antifeedants decrease adult survival
		Podisus maculiventris	P	Lowers development, growth
Rutin	Phenol	*Heliothis virescens F., Manduca*		Lowers development, growth
		Spodoptera frugipedrda		decreased adult survival
		Macrosiphum euphorbine	*C*	deterrence prolongs in ovulation
		Tribolium confusum	S	malformations of all insects
Solasodine	Alkaloid			development stages slowed
				rate of pupations slowed
				metamorphosis
calystegine B4 (1∝,2β,3∝-4∝,-tetrahydroxy-nor-tropane	Alkaloid	*Bombyx mori* L	T	midgut trehalose prevented
Solamargine	Alkaloid	*Tribolium castaneum*	S	prevents larval growth
∝-tomatine	Alkaloid	*Heliothis zea (Boddie*)	C	prevents larvae growth
		Spodoptera exigua	C	antifeedance
		Zophobus atratus	C	lowers heart activity
		Tribolium castaneum	C	prevents larvae growth
		Hyposter exigme	C	Prolonged larvae development

Key: Insects were classified as **C**—crop pests, **T**—tree pests, **S**—stored product pests, **P**—parasitoids and predators, **O**—others (incl. mites, termites, rats, and snails)

16.D **Antioxidant activity**

An imbalance between the creation and build-up of reactive oxygen species (ROS) in cells and tissues and a biological system's capacity to detoxify these reactive products results in the phenomena known as oxidative stress (Pizzino et al., 2017). Because of this, the generation of ROS, such as hydroxyl radicals (OH), causes oxidative stress, which may impair the normal operation of physiologically significant molecules in cells, such as DNA, proteins, and lipids (Chowaski et al., 2016; Okmen et al., 2009). Numerous human diseases, including cancer, rheumatoid arthritis, atherosclerosis, and aging-related degenerative processes, are brought on by the unchecked creation of free radicals (Pizzino et al., 2017

According to Lin, (2010), an antioxidant is a substrate that stops molecules inside cells from oxidizing. Free radicals can be taken out of a substrate through a chemical procedure. Antioxidant substances can therefore stop a chain reaction by getting rid of its intermediate free radicals. The strong reactivity of polyphenols, saponins, terpenoids, and glycoalkaloids as hydrogen or electron donors, which can stabilize and delocalize the unpaired electron, is what gives these substances their antioxidative effects (Muruhan et al., 2013; Thitilertdecha et al., 2010). The phytochemicals can scavenge nearly all known ROS depending on their structure.

From various plant components, including oilseeds, cereal crops, vegetables, fruits, leaves, roots, spices, and herbs, several natural antioxidants have been identified. Additionally, numerous studies have shown that medicinal plants include a variety of naturally occurring antioxidants, including phenolic chemicals, which have antioxidant activity (Lattanzio, 2013).

By scavenging for radicals, natural antioxidants prevent the creation of a single oxygen radical (Lobo et al., 2010). Vitamin E, cinnamic acid derivatives, curcumin, caffeine, catechins, gallic acid derivatives, salicylic acid derivatives, chlorogenic acid, resveratrol, folate, anthocyanins, and tannins are a few examples of polyphenol natural antioxidants obtained from plant sources. In DPPH tests, *S. nigrum* has been discovered as a source of potent natural antioxidants (Pizzino et al., 2017). The DPPH assay is used to measure a substance's capacity to function as a hydrogen donor or free radical scavenger, as well as to gauge how effective an antioxidant is (Gaber et al., 2021).

Many *Solanaceae* species have been reported as oxidative substances. For example, *S. fastigiatum* exhibited strong antioxidant activity in the DPPH assay (Muthuvel et al., 2014). Almaouh (2017) studied the effect of the methanolic and ethyl acetate fruit extracts of *S. nigrum*, *S. melongena*, and *S. panduriform* as antioxidant agents. All extracts displayed strong DPPH radical scavenging inhibition

16.D **Cabbage aphids**

In this work, the effectiveness of *S. incanum* extracts as a pesticide is examined using cabbage aphids. Small, soft-bodied insects known as cabbage aphids can survive by sucking plant sap. Aphids are named after the plant species they attack. For instance, melon cabbage aphids attack watermelons whereas potato cabbage aphids feed on green peaches, bean cabbage aphids attack

bean harvests, and so on (Opfer, 2013). The genus *Brevicoryne* contains the cabbage aphid, which feeds on cabbages (Griffin, 2012). Many crops in the Brassicaceae family suffer significant yield losses because of cabbage aphids (cabbage family). For instance, severe damage to many plants in the family *Brassicaceae* has been reported in many areas including Canada, South Africa, India, China, and Zimbabwe (Gill et al., 2013). Cabbage aphids attack the crop at any stage, causing it to wither and ultimately die (Carter, 2013).

Sap from their host plants is sucked by cabbage aphids to get food. Ants consume honeydew, a sugary waste product that they create. In exchange, the ants shield the cabbage aphids from their natural predators. Plants become yellow, wilted, and stunted when cabbage aphids continue to feed on them (Opfer, 2013). Due to honeydew secretions, severely infected plants develop a thick layer of tiny sticky cabbage aphids, which can eventually cause leaf loss and degradation. The centre of the cabbage head and the underside of the leaves are where cabbage aphids feed (Hines and Hutchison 2013). They frequently delve deep into the crowns of cabbage and Brussels sprouts, preferring to eat the young leaves and blossoms.

Cabbage aphids attack a variety of vegetables including cauliflower (*Brassica olearacea* L. var. *botrytis L.*), brussels sprouts (*Brassica olearacea* L. var. *gemmifera* DC), broccoli (*Brassica olearacea* L. var. italic Plenck), cabbage (*Brassica olearacea* L.), oilseed rape (*Brassica napus* L.), and other members of (*Brassicarapa* L.). Additionally, it infects kale (*Brassica alboglabra*), Chinese broccoli, Chinese cabbage (*Brassica rapa*, subspecies pekinensis, and chinensis), and radish. Raphanus and Griffin, (2012) and Baidoo and Adam (2012), posit that the cabbage aphids are a vector of more than 20 pathogens. Both the wingless (apterae) and winged (alate) aphids can transmit viruses with the wingless being the most transmitter of pathogens (Gill et al., 2013). The cabbage aphid's mode of pathogen transmission is non-persistent: the cabbage aphid picks up the virus by feeding on infected plants and transfers the pathogen to healthy plants by probing with its mouthparts or feeding (Kibrom, 2012). Cabbage aphids cause major losses to broccoli by reducing yield, with real damage being contamination of harvested heads of broccoli (Gill et al., 2013).

In Zimbabwe, the cabbage aphids attack the vegetable *Brassica olecerea*, a vegetable considered to be a staple food for most communities in Zimbabwe. The vegetable aphid habitats on the leaves of *Brassica olecerea* where they suck the sap stopping the leaves to get adequate nutrients resulting in the plant wilting. Great losses of the staple vegetable have been realised

hence the community decided to use the indigenous plant pesticide as a way of controlling the spread of the vegetable aphid. One such indigenous plant used as a pesticide is *S. incanum*. The study aims to compare the effectiveness of the crude and isolated compounds which has not been done yet. Most of the past studies have concentrated on the efficacy of the crude extracts only.

2.7 Extraction and chemical structure determination

Careful plant selection is necessary for success in natural product research from plant sources. This selection should be based on a variety of methods, including (a) chemotaxonomic data, (b) indigenous knowledge, (c) field observations, (d) random selection by chemical screening, and I follow-up of biological activity reports (Jones and Kinghorn, 2012). The indigenous knowledge includes using databases, herbalism, and identifying plants utilized in organized traditional medicine and pest control systems.

One principal approach in the isolation of the bioactive constituents from extracts is toxicity profiling which involves the detailed analysis of an extract's chemical composition by chromatographic-spectroscopic techniques and a subsequent activity or toxicity evaluation (Amin et al., 2017). Another effective approach to the isolation of new lead compounds is bioactivity-guided isolation, in which biological assays are used to target the isolation of bioactive constituents (Amin et al., 2017). Details of the extraction, separation, isolation, and structure elucidation of the bioactive components are explained in Chapters **5** and **6**.

2.8 Conclusion

In conclusion, further research into the insecticidal properties of plant extracts is required. To develop the communities that utilise the plant as a pesticide, the environmental effects (such as on soil fauna or aquatic species) and the health effects the crude extracts of *S. incanum* may cause in Mkoba village need to be thoroughly examined. Applying plant-derived compounds carefully may become a crucial method to reduce the risks that the plant pesticide may pose to those utilising it in this area. A vital stage in the hunt for novel pesticides is extract research because *Solanum* species contain a variety of compounds that could serve as sources for commercially synthesised insecticides. The research found that the most effective *Solanum*

species to use for extracts are *S. molengena* L, *S. tuberosum* L, *S. nigrum* L, and *S. Torvum*. These plants have been described for their deadly and varied sublethal effects on pests. The purpose of the study is to determine whether the fruits and roots of *S. incanum* have any impact on cabbage aphids. For the community to make educated judgments about the potential negative effects *S. incanum* may have on the environment and human health, it is crucial to determine the pesticidal qualities and toxicity of insecticides generated from *S. incanum*.

The next chapter will describe the methodology used to evaluate the knowledge and the perceptions the community of Mkoba holds regarding the pesticidal properties of *S incanum*. Chapter 4 will then present the results and discussion of the community's knowledge and perception regarding *S. incanum*.

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CHAPTER 3

METHODOLOGY – EVALUATION OF THE COMMUNITY'S PESTICIDAL KNOWLEDGE AND PERCEPTION ABOUT *SOLANUM INCANUM* L.

3.1 Introduction

It is a noble idea that the scientific evaluation of *S. incanum* starts with highlighting the community's perceptions and knowledge regarding its pesticidal properties. Zimbabwe is one of the most biocultural countries in the Southern African region (SADC) (Mutasa et al, 2015). The communities in Zimbabwe have strong smallholder farming practices in which a variety of crops are produced. Mkoba village in the Gweru district is no exception to these practices. The proceeds of the community gardening activities are used as the source of their food and income. However, the quality and the quantity of the harvest are usually low due to the insurgence of pests such as cabbage aphids. To improve the harvest, the community resort to the use of indigenous plant pesticides (*S. incanum*) because the majority cannot afford the commercial ones. S. *incanum* belongs to the *Solanaceae* family and a literature search reveals that most of the *Solanaceae* species are poisons (Thaiyah et al, 2010). There is a claim in the Mkoba community that the prevalent symptoms of diseases are because of the use of *S. incanum* as a pesticide.

In the next two chapters, an attempt to understand the community of Mkoba's knowledge level and perceptions regarding *S. incanum* is presented in a study before the plant is scientifically evaluated. Therefore, the objective of chapter **3** is to outline the methodology used to document and assess the knowledge, perceptions, and attitudes of the community of Mkoba regarding the use of *S. incanum* as a pesticide. Details of the research philosophy, research design, sample and sampling techniques, and the method utilised are described in this chapter.

3.2 Research Methodology

According to Creswell (2009), research designs are plans and procedures for research that span the decisions from broad assumptions to detailed methods of data collection and analysis. Eventually, the decision on which design and specific methods of data collection, analysis, and

interpretation to be used involves the worldview assumptions the researcher brings to the study (paradigm). In this study, the research questions and objectives had to do with describing the phenomenon using numbers, and where words were used to clarify and elaborate a point, the words were coded to create descriptive statistics. Therefore, the study utilised closed and open-ended questions and not numbers (Creswell and Creswell, 2017). Hence, it was appropriate to locate the study in the pragmatic paradigm of the quantitative research approach (Cram and Mertens, 2015; Creswell, 2013). In this study, the appropriate worldview upon which the methodology used pragmatism because the study sought to identify the community's knowledge and perceptions about a common phenomenon (health impact of *S. incanum*) within the community. The details of the pragmatic paradigm are described next.

3.2.1 The research paradigm

In social research, the term "paradigm" is used to refer to the philosophical assumptions or to the basic set of beliefs that guide the actions and define the worldview of the researcher (Lincoln, Lynham, and Guba, 2011). When it was introduced by Thomas Kuhn, the term "paradigm" was used to discuss the shared generalisations, beliefs, and values of a community of specialists regarding the nature of reality and knowledge. A paradigm is described as "a way of thinking about and making sense of the complexities of the real world" (Creswell et al., 2011; Denzin and Lincoln, 2011; Rossman and Rallis, 2003). Although there are several paradigms or worldview structures in modern social research work (post-positivism, constructivism, participatory action frameworks, and pragmatism), they are all essentially philosophical and encompass the following common elements: axiology—beliefs about the role of values and morals in research; ontology—assumptions about the nature of reality; epistemology—assumptions about how we know the world, how we gain knowledge, the relationship between the knower and the known; methodology—a shared understanding of best means for gaining knowledge about the world; and rhetoric—shared understanding of the language of research (Creswell, 2009; Lincoln et al., 2011).

Paradigms are conceptual and practical "tools" that are used to solve specific research problems; in other words, paradigms function as heuristics in social research (Skinner, Edwards, and Smith, 2020). Each paradigm has a different perspective on the axiology, ontology, epistemology, methodology, and rhetoric of research. For instance, post-positivism,

one of the older approaches to social research, is often associated with quantitative methods and highly formal rhetoric which focuses on precision, generalisability, reliability, and replicability (Katz, 2015), while postpositivist researchers view inquiry as a series of logically related steps and make claims of knowledge based on objectivity, standardisation, deductive reasoning, and control (Katz, 2015; Shah and Corley, 2006). A brief description of the pragmatism paradigm is given below.

3.2.2 Pragmatism

Pragmatism as a research paradigm finds its philosophical foundation in the historical contributions of the philosophy of pragmatism and, as such, embraces a plurality of methods. As a research paradigm, pragmatism is based on the proposition that researchers should use the philosophical and/or methodological approach that works best for the research problem that is being investigated (Tashakkori, Teddlie, and Teddlie, 1998). It is often associated with mixed methods or multiple methods (Johnson, 2004; Kaushik and Walsh, 2019; Yilmaz, 2013), where the focus is on the consequences of research and the research questions rather than on the methods.

The word pragmatism is originally derived from the Greek word "pragma," which means action, and is the central concept of pragmatism (Cain, 2012; Carter, 2016; Creswell and Tashakkori, 2007). Pragmatist philosophy holds that human actions can never be separated from past experiences and from the beliefs that have originated from those experiences (Kaushik and Walsh, 2019). Human thoughts are thus intrinsically linked to action. People take actions based on the possible consequences of their actions, and they use the results of their actions to predict the consequences of similar actions in the future. A major contention of pragmatist philosophy is that meaning of human actions and beliefs is found in what they value most (Morgan, 2014). Pragmatists believe that reality is not static—it changes at every turn of events (Hildebrand, 2005). Similarly, the world is also not static—it is in a constant state of mutation or change. The world changes through actions—action is the way to change existence. Actions have the role of an intermediary; therefore, they are pivotal in pragmatism (Morgan, 2014).

(Morgan, 2014) identifies three widely shared ideas of pragmatism that focus on the nature of experience, unlike other philosophies that emphasize the nature of reality. The pragmatic

concepts according to (Morgan, 2014) are: (a) Actions cannot be separated from the situations and contexts in which they occur. This implies that the world is a world of unique human experiences in which, instead of universal truths, there are warranted beliefs, which take shape as we repeatedly take actions in similar situations and experience the outcomes. Our warranted beliefs are produced by the repeated experiences of predictable outcomes (Morgan, 2014). (b) Actions are linked to consequences in ways that are open to change. Meaning that, if the situations of the action change, their consequences would also change, despite the actions being the same. Pragmatist philosophy maintains that it is not possible to experience the same situation twice, so our warranted beliefs about the possible outcome are also provisional, which means that our beliefs about how to act in a situation are inherently provisional (Schwartz, 2015) and (c) Actions depend on worldviews that are socially shared sets of beliefs. Pragmatists believe that no two people have identical experiences, so their worldviews can also not be identical (Milner et al., 2020). However, there are always varying degrees of shared experiences between any two people that lead to different degrees of shared beliefs (Kaushik and Walsh, 2019). The likelihood of acting in the same way in a similar situation and assigning similar meanings to the consequences of those actions depends on the extent of shared belief about that situation. Therefore, worldviews can be both individually unique and socially shared (Kaushik and Walsh, 2019). Pragmatists generally agree that all knowledge in this world is socially constructed, but some versions of those social constructions match individuals' experiences more than others (Kaushik and Walsh, 2019).

A major underpinning of pragmatist epistemology is that knowledge is always based on experience. Social experiences influence a person's perceptions of the world. Each person's knowledge is unique as her/his unique experiences create it. Nevertheless, much of this knowledge is socially shared as it is created from socially shared experiences. Therefore, all knowledge is social knowledge (Morgan, 2014). Pragmatist epistemology does not view knowledge as reality but as constructed for better managing one's existence and taking part in the world (Kaushik and Walsh, 2019).

According to Dewey's framework, as cited in (Kaushik and Walsh, 2019), living organisms, can establish and maintain dynamic coordination with the environment they live in. The process of establishing and upholding the coordination results in habit formation and these traits become more consistent with the organism's ever-changing environment. This is a learning process, basically a process of trial and error, through which we acquire a complex,

yet the flexible set of habits for action (Cameron and Green, 2019). Practices can adequately handle human beings' demand for action in many situations and these actions become reflexes to any given situation (Cameron and Green, 2019).

Unlike positivistic researchers, who assert objective knowledge is acquired by examining empirical pieces of evidence and hypothesis testing, and constructivists, who propose that knowledge is relative, and reality is too complex, pragmatists believe that the process of acquiring knowledge is a continuum rather than two opposing and mutually exclusive poles of either objectivity or subjectivity (Creswell and Creswell, 2017; Creswell et al., 2011). Thus, pragmatism is situated somewhere in the centre of the paradigm continuum in terms of the mode of inquiry. Post-positivism supports quantitative methods and deductive reasoning, whereas constructivism emphasizes qualitative approaches and inductive reasoning; however, pragmatism embraces the two extremes and offers a flexible and more reflexive approach to research design (Johnson, 2004; Tashakkori et al., 1998) with abductive reasoning that moves back and forth between deduction and induction. In this way, the researcher is actively involved in creating data as well as theories (Kaushik and Walsh, 2019).

Another important consideration is the aspect of pragmatism that is part of a researcher's worldview and therefore can influence the way researchers conduct their projects. In pragmatism, it is the researcher who makes the choices and decides which question is essential and what methodology is appropriate, and those choices are certainly influenced by the aspects of the socio-political location of the researcher, his/her personal history, and his/her belief system (Morgan, 2014).

3.2.3 Research design

Three main research approaches are common in social science studies which include qualitative, quantitative, and mixed methods. According to (Creswell and Creswell, 2017) qualitative and quantitative research approaches should not be considered as distinct rigid and polarised designs but should be viewed as a continuum whereby the two approaches can be used in one study. The difference is that one approach can be more quantitative than qualitative or vice versa.

Qualitative designs are framed in terms of using words rather than numbers. Qualitative researchers typically gather multiple forms of data, such as interviews, observations, and documents, rather than rely on a single data source. Then, they review all the data and make sense of it and organize it into categories or themes that cut across all the data sources from the bottom up (Creswell, 2013).

(Creswell, 2013) has outlined five approaches to qualitative inquiry. They are narrative, phenomenology, ethnography, case study, and grounded theory. While the five methods generally use similar data collection techniques (observation, interviews, documents, and audio-visual materials), the purpose of the study differentiates them. Also, the data collection varies in emphasis and extent; even the types of data one would collect and analyse would differ considerably (Creswell, 2013). Stories about an individual's life comprise narrative research. A description of the essence of the experience of the phenomenon becomes phenomenology. Theory, often portrayed in a visual model, emerges in grounded theory, and a holistic view of how a culture-sharing group works results in an ethnography. An in-depth study of a bounded case system or a case (or several cases) becomes a case study in terms of using numbers to describe the results of the study. The numbers are usually derived from the experiments.

On the other hand, the quantitative research design is framed on gathering information that focuses on describing a phenomenon across a more significant number of participants thereby providing the possibility of summarizing characteristics across groups or relationships (Brazzeal, 2006). This approach surveys many individuals and applies statistical techniques to recognize overall patterns in the relations of processes (Creswell and Creswell, 2017). Notably, the use of surveys can be done across groups of females and males, different age groups, or different levels of education. It is then possible to compare these two groups on outcomes of interest and determine their differences. It is also relatively easy to survey people several times, thereby concluding that a certain feature (like influences) influences specific outcomes (well-being or achievement later).

The main characteristics of the quantitative approach include the following:

- *The data is usually gathered using structured research instruments.*
- *The results are based on larger sample sizes that are representative of the population.*

- *The research study can usually be replicated or repeated, given its high reliability.*

- *The researcher has a clearly defined research question to which objective answers are sought.*
- *All aspects of the study are carefully designed before data is collected.*

- *Data are numbers and statistics, often arranged in tables, charts, figures, or other non-textual forms.*
- *The project can generalize concepts more widely, predict future results, or investigate causal relationships.*

- *The researcher uses tools, such as questionnaires or computer software to collect numerical data.*

This study described as **PHASE 1** of the research utilised a quantitative approach within a descriptive survey. Quantitative data can come from experiments, research surveys with ratings or closed questions, and controlled observations (Treiman, 2014). Researchers are mostly primarily concerned with accurate numerical data which can be interpreted using descriptive and inferential statistics (Mishra et al., 2019). An opened ended questionnaire was used which enabled both qualitative and quantitative data to be collected regarding the knowledge about how the community process *S. incanum* for use as a pesticide, toxicity knowledge, and perceptions about the effectiveness of *S. incanum* in the community of Mkoba. The open-ended questionnaire responses were coded, and descriptive statistics were used.

3.3 Methods

3.3.1 Descriptive survey

This study utilised a descriptive survey research method. The descriptive survey method was chosen so that the respondents would be allowed to describe in words their knowledge and perceptions in the designed questionnaire. According to Dulock (1993), a descriptive survey describes the facts and characteristics of a given population of interest systematically (Dulock,

1993). It also aims to provide an accurate portrayal or account of the characteristics of an individual, situation, or group, as a way of discovering new meanings (Jagtap and Jagtap, 2015; Siedlecki, 2020). A descriptive survey research method lays a foundation for describing what exists, determining the frequency of its occurrence, and categorising the findings (Johnson and Onwuegbuzie 2004). Sometimes, the association or relationships emerging between or among selected variables are used to answer the research questions or hypothesis based on current events (Lakusa and Astuti, 2021).

Descriptive research can utilise quantitative or qualitative approaches in a study (Siedlecki, 2020). It can involve collections of quantitative information that can be tabulated along a continuum in numerical forms, such as scores on a test or the number of times a person chooses to use a specific feature highlighted. It also involves the description of categories of information such as gender or patterns of interaction or usage of a phenomenon (Gorard, 2010; Kowalczyk, 2015). Descriptive research involves gathering data describing events and then organising, tabulating, depicting, and describing the data collection (Dulock, 1993; Thomlison, 2001). It often uses visual aids such as graphs and charts to aid the reader in understanding the data distribution. Because the human mind cannot fully import a large mass of raw data, descriptive statistics are vital in reducing the data to a manageable form. Therefore, summary data such as measures of central tendency, including the mean, median, mode, deviance from the mean, variation, percentage, and correlation, are vital in descriptive studies (Lakusa and Astuti, 2021). In addition, when in-depth, narrative descriptions of small numbers of cases are involved, the research uses description as a tool to organize data into patterns that emerge during analysis. Those patterns aid the mind in comprehending a qualitative study and its implications.

To gather descriptive data, observational and survey methods are widely utilised because most descriptive studies aim to answer the questions “what is, how, why, and when” (Siedlecki, 2020). This type of measurement is frequently used in survey research, but the inference-drawing process frequently goes beyond descriptive statistics because qualitative studies, case studies, observational studies, interviews, and portfolio evaluations can also produce in-depth, rich descriptions of phenomena. The three basic purposes of descriptive research are to describe, explain, and assess results in a context (Moser and Kalton, 2017; Steckler et al., 1992). Creative exploration leads to the emergence of the description, which organizes the facts to fit them with explanations before testing or validating those explanations (Moser and Kalton, 2017).

3.3.2 Research site and population

The research was carried out at a communal garden in Mkoba villages, Gweru in Zimbabwe (See **Figure 1.3**). The population for this study was derived from the community members of Mkoba villages who practised community and backyard vegetable gardens. There are approximately two hundred (200) people comprised of both men and women in Mkoba who are using *S. incanum* as a pesticide.

3.3.3 Sampling

Two sampling methods which included, convenience and systematic sampling were used. In convenience sampling, the respondents were selected based on their availability and willingness to participate at the garden site, and in systematic sampling, the research assistant would select the participants periodically since respondents' movements to the gardens were regulated due to COVID-19 pandemic protocols. From a convenient sampling strategy, valid results can be obtained, but the results are prone to significant bias because those who volunteer to take part may be different from those who choose not to (volunteer bias), and the sample may not be representative of other characteristics, such as age or sex since volunteer bias is a risk of all non-probability sampling methods (Elfil and Negida, 2016; Onwuegbuzie and Collins, 2007). The other bias which arises from convenience sampling would be that the researcher may be getting information from respondents who are already 'baptised', where the researcher will get praise all through the status quo. It was convenient for the researcher to meet in the communal garden to avoid a lot of movement during the COVID-19 pandemic period. It was assumed that anyone who visited the garden was able to supply the information the researcher was looking for, therefore, the respondents were picked at the working site.

In this study, a convenient sample of 57 respondents was selected from Mkoba village. The sample included 35 females and 22 males. Besides convenience, a systematic approach whereby only 3 respondents were selected every 2 hours was used. The systematic approach was utilised to reduce bias that emanated from the convenience sampling strategy.

3.3.4 Sample composition

The study sample was composed of men and women aged 16 years and upwards, who resided in one of the villages in Mkoba. A total of 35 females and 22 males were selected. The study targeted community members who practised community and backyard gardens using the *S. incanum* formulation.

3.4 The research instrument

The main instrument used in data collection was a closed and open-ended questionnaire written in both English and Shona. A questionnaire ensured a high response rate as the questionnaires were distributed to respondents to complete and were collected personally by the researcher. They required less time and energy to administer. They offered the possibility of anonymity because subjects' names were not required on the completed questionnaires. There was less opportunity for bias as they were presented invariably. Besides, the questionnaire was appropriate at that time due to the COVID-19 regulations where face-to-face interaction was not allowed. Apart from the advantages that have been listed above, questionnaires have their weaknesses; for example, there is the question of validity and accuracy. For instance, the subjects might not reflect their true opinions. Still, they might answer what they think will please the researcher, and valuable information may be lost as answers are usually brief.

3.4.1 The design and structure of the instrument

The main ideas to formulate items for the questionnaire for this research were adapted from various sources, including articles and theses. For instance, the demographical items were adopted from articles of (Khan et al., 2018). The items about the indigenous knowledge of pesticide plants, effectiveness, and toxicity were adopted from (Abang et al., 2014; Bwambale et al., 2015; Nicol, 2003). Modifications were done to the adopted items to suit the aims and objectives of this study. The perceptions, attitudes, and practices items were adopted from the knowledge, attitudes, and practices (KAP) survey model (Qutob and Awartani, 2021). The selected items were modified to suit the aims and objectives of this study.

The Questionnaire used for this research consisted of two parts. **Part A** contained items soliciting basic demographic data from the respondents. The information included data such as

age, level of education, size of family, and gender was solicited in this part using closed-ended dichotomous questions. **Part B** dealt with closed and open-ended questions that sought information about respondents' beliefs and perceptions about *S. incanum.* The data collected from the questionnaire included the harvesting, preparation, and spraying methods practised in the Mkoba community. The prec'utionary measures, pre-and post-harvesting period, and the concerns regarding the environmental and health impact of *S. incanum* together with perceptions and beliefs about *S. incanum*'s pesticidal efficacy were also gained from the questionnaire.

Open-ended questions were mainly used to follow up on multiple-choice questions and closed-ended types of questions to allow respondents to provide more diverse detail. For instance, questions 11, 17, 20, 22, and 25 were closed-ended, but follow-up questions 12, 18, 21, 23, and 26 were used to solicit more details from the respondents. In this case, the respondent was required to respond in writing, whereas closed-ended questions had options the researcher determined. Questions 24, 29, and 30 were Likert scale multiple choice type of questions, whilst question 32 was a checklist of multiple-choice questions. The respondent could choose as many responses as possible according to his/her experience. The questionnaire is attached as **APPENDIX 3.1**.

3.5 Reliability and validity of the instrument

The design of the questionnaire involved putting up together questions from different sources to reduce potential common method variance (CMV). Questionnaire validity and reliability were catered for at the design stage by reducing CMV biases. For instance, the Knowledge, Attitudes, and Practices (KAP) survey model was used to guide the researcher to develop items that dealt with knowledge, attitudes, and practices. Items that involved the indigenous knowledge, toxicity, and effectiveness of a pesticide were adopted from Abang et al. (2014). Some modifications were done to the items to suit the current study. Construct validity was done with the supervisor's assistance (a specialist in traditional medicinal plants), who advised on the construction and placed the items in chronological order so that the items could measure what they were supposed to measure. The questionnaire was then piloted with sixteen randomly selected respondents who do not reside in the study area. Language corrections, rephrasing of the items, and rearrangement of items were made to improve the quality of the questionnaire.

To check the reliability and internal consistency in the closed-ended and the items which used the Likert scale system, for instance in the demographical data and item 24, a Cronbach's Alpha coefficient method was conducted using Statistical Package for Social Science (SPSS) Version 27.0.1.0.

3.6 Questionnaire translation process

The questionnaire was translated into the local language (Shona) to cater for the community members who could not understand English well or preferred to use a Shona version of the questionnaire. A specialist in the Shona language translated the questionnaire protocol. The translator was a holder of a Bachelor of Arts in languages, and a diploma in Education specialising in English and Shona and has more than twenty-five years of experience in teaching Shona at both Ordinary and advanced levels. She was also engaged with the Midlands State University as a part-time translator of English questionnaires to Shona for students.

3.7 Selection of the research assistant

A research assistant (a member of the community) was appointed to assist with the questionnaire administration. The research assistant was a University of Zimbabwe Honours graduate who had majored in Geography and Environmental Sciences and was a resident of Mkoba village. The assistant would explain the purpose of the study, provide the respondents with the consent forms, and administer and collect the completed questionnaires from the respondents.

3.8 Letters requesting permission to undertake research in Zimbabwe

Ethics are a set of moral principles that are widely accepted, which guided the research assistant in observing the rules. Ethical considerations required special attention during the study. Throughout the study period, the research assistant was strongly advised to abide by the code of ethics of Nelson Mandela University (NMU). The response letter from the councillor was then used to seek ethical clearance from the Ethics committee at NMU. The letter presented to the councillor is **APPENDIX 1.1**. Permission from the councillor (gatekeeper) was very important as the councillor was the political figure responsible for regulating any activities being held in Mkoba. The councillor also coordinates all activities which happen in Mkoba

village. The research assistant prepared and presented documentation about himself, consent letters, and the project and submitted them to the Ethics Committee of Nelson Mandela University to seek approval to do fieldwork.

3.9 The piloting study

After constructing the first draft of the questionnaire, a pilot study was conducted. A pilot study represents a full-scale study in a snapshot in preparation for the actual study. It can also be termed a 'feasibility' study. Therefore, the purpose of the pilot study was to:

- *Mimic the actual data collection procedure before the study*
- *Identify and correct ambiguous items* (In, 2017).
- *Identify practical problems of the research procedure since the data collection was carried out during the COVID-19 period when the community was undergoing many restrictions.*
- *Inform the researchers about questionnaire reliability and validity.*
- *Try out all research techniques and methods, which the researcher has in mind to see how well they will work in practice* (Ornstein, 2013; Van Teijlingen and Hundley, 2002). *Measure the validity of the questionnaire* (Maqableh et al., 2015).
- *Measure the validity of the questionnaire* (Maqableh et al., 2015).

3.9.1 The piloting process

The pilot study was carried out before the actual study. The pilot study took place over two months in June and July 2020, and it was conducted in Mambo Village in Gweru District. Mambo village is about 6 km on the South-Eastern side of Mkoba. The people in Mambo do community gardening and own backyard gardens just like the residents of Mkoba village. The research assistant identified community members in this village who had similar characteristics (owned a vegetable garden and used *S. incanum* formulation as a pesticide) to the Mkoba residents.

Due to the travel restrictions during the COVID-19 period, the researcher assigned an assistant to the administration of the questionnaires on behalf of the researcher. Before undertaking the questionnaire for the piloting, the gatekeeper was informed about the researcher's intention to

carry out a pilot study in an adjacent community to the one in which the actual research was to be carried out.

3.9.2 Pilot study and main study procedure

Due to the restrictions of movement and gathering in large numbers because of the COVID-19 pandemic, the assistant researcher used people who came to attend to their gardens as respondents. Upon accepting to take part in the pilot study, a consent letter was issued and issues of privacy, anonymity, and freedom for the subject to withdraw from participation were explained to the participant in Shona before he/she could sign the consent form.

3.9.3 Data collection procedure

Due to the restrictions of movement and gathering in large numbers because of the COVID-19 pandemic, the assistant researcher used people who came to attend to their gardens as respondents in the study. The respondents were not coming in large numbers due to the COVID-19 restrictions, so the research assistant would wait for hours at the communal garden site to be able to find people willing to take part in the study. Upon accepting to take part in the study, a consent letter was issued and issues of privacy, anonymity, and freedom for the subject to withdraw from participation were explained to the participant in Shona before he/she could sign the consent form. Questionnaires were personally distributed by the research assistant to respondents at the garden site who waited for the respondent to complete and submit the questionnaire.

3.9.4 Data processing, coding, and analysis

The responses for the open-ended items were expressed in frequency distribution tables. The types of statistical analysis that were conducted on the collected response data using the questionnaire depended on the measurement levels of the variables which are outlined in **Table 3.1.**

Table 3.1: Measurement of Levels of variation in the Questionnaire

Section	Items	Variable	Measurement Level
Demographic data	1-7	Age	Interval
		Gender	Nominal
		Relationship	Nominal
		Highest level of Education	Ordinal
		Family size	Ordinal
Knowledge and perceptions	8-16: Open-ended items	Knowledge and perceptions	Nominal
	17-19	Knowledge and perceptions	Nominal
	21-24		Ordinal
	25-31	Environmental and health issues	ordinal

The questionnaires collected from the fieldwork were checked to find out whether the respondents had attempted all items in the questionnaire. The incomplete questionnaires were not used for the analysis of the results. Both open and closed-ended questions were allocated codes and the code for each question was captured in an excel spreadsheet. Where multiple responses were given, the responses were grouped into themes and allocated a code (**APPENDIX 3.1**). The spreadsheet was then transferred to SPSS software for further processing and analysis of the results.

The data obtained were summarised using frequency distribution tables, pie charts, and bar graphs. Descriptive statistics which included mean and standard deviation was also used to summarise a given data set. In addition, the inferential statistical analysis was taken into consideration where necessary.

3.10 Conclusion

This chapter covered a description of the final intervention programme, highlighting the methodology utilised in the study. The research design, the paradigm, population, sampling techniques, and research instruments used in the first phase (Evaluation of the Mkoba community's knowledge and perceptions regarding pesticidal properties of *S. incanum* of the

study were explained. The data collection and analysis procedures were also presented in this chapter. The current research study is a quantitative study where an open-ended questionnaire was utilised. The pilot study procedure and the rationale behind it were outlined. The chapter which comes next will cover the results and discussion of both the pilot study and the actual study.

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CHAPTER 4

THE EVALUATION OF THE COMMUNITY'S KNOWLEDGE OF THE PESTICIDE PROPERTIES OF *SOLANUM INCANUM* – RESULTS AND DISCUSSION

4.1 Introduction

The survey's results are reported in this chapter. First, the findings of the pilot study are reported. Open-ended responses were coded and presented using descriptive statistics, which included frequency distribution tables, graphs, and inferential statistics, which included Chi-square and independent t-tests. The primary research findings were presented item by item. The results of the actual major study are reported after the findings of the pilot study.

4.2 Results and discussion of the pilot study

4.2.1 Socio-demographics of the respondents in the pilot study

A total of sixteen (16) respondents were recruited for the pilot study in Mambo village. There were more female (75%) respondents compared to males (25%) (**Table 4.1**). The majority (75%) of respondents were in the age group of 31–50 years of which 62.5% of them were married. Half (50%) of the male respondents attained a primary level of education and whilst 40% of the female respondents attained a primary education level.

Table 4. 1: Socio-demographics of the respondents in the pilot study

Gender	Percentage frequency										
	Total %	Age			Marital status				Education level		
		15-30	31-50	51-60	S	M	D	W	1º	2º	3º
Male	25%	0%	75 %	25%	0%	50%	50%	0%	25%	50%	25%
Female	75%	17%	50%	33%	17%	58%	17%	8%	42%	42%	16%

S-single; **M** – married; **D** – divorced; **W** – widow; **1º** – primary: **2º** – secondary; **3º** – tertiary
*The percentages were calculated according to gender

4.2.2 Adjustments of the items in the questionnaire

The items in **Table 4.2** were adjusted to remove the ambiguity in the items after the pilot study.

Table 4. 2: The items which were adjusted after the pilot study had been carried out

Item	Adjustment
Item 6	It was adjusted to cater to larger family sizes. 6 and 7 which had been excluded from the preliminary questionnaire were added
Item 7	The item was rephrased to: "Do you practice vegetable gardens in the community?"
Item 15	The options which were in the preliminary were removed in the final questionnaire to allow respondents to give their options
Item 19	Another option of "other" was added
Item 27	Another option of "other" was added

A pilot study always gives the researcher areas to improve in the methodology. In this study, ambiguous items were identified in the questionnaire which was adjusted to suit the language and the information they intended to obtain from the participants. The pilot study enabled the researcher to adjust the questionnaire items to refine technicalities that were associated with the data collection processes. The questionnaire, therefore, was analysed and presented according to the specifications in **Table 4.3**. The questionnaire is in **APPENDIX 3.1**.

Table 4. 3: A summary of the final structure of the questionnaire according to the objective, items, and the variable measured

Objective	Item(s)	Variable
Gather indigenous knowledge of *S. incanum (Nhundurwa)* from the community.	8; 9; 10; 11; 13; 12; 15; 17; 18; 23; 24; 26; 27; 14	Indigenous knowledge
To describe how the community prepares and applies *S. incanum (Nhundurwa)* as a pesticide	12; 13; 14; 15	Indigenous knowledge
To assess the effectiveness of *S. incanum ('Nhundurwa)* as used as a pesticide.	24	Indigenous knowledge
To determine the toxicity of the extracts from the fruit of *S. incanum*	16; 23; 24; 28	Toxicity/poison nature
To identify the health problems which may be associated with the use of *S. incanum* as a pesticide.	9; 10; 16; 18; 19; 20; 24; 25; 24; 26; 28; 29; 30; 31; 32	Health issues

4.3 Ethical considerations

Permission was granted by the Ethics committee of Nelson Mandela University (**APPENDIX 1.1**) reference number-(H19-SCI-CHE-001) allowing the researcher to proceed with the process of data gathering. The following steps were taken into consideration to ensure that the research was conducted ethically.

4.3.1 Entry into the research site

Negotiating entry into the research setting requires the researcher to understand the language and culture of the research respondents for easy interaction with the respondents (Reeves, 2010). In this study, this requirement was met because the research assistant was Shona. The research assistant resided in the same community and had the same culture and spoke the same language, which made interaction with the respondents much easier. This made it easier to identify the respondents and eliminate bias experienced through the employment of an interpreter.

4.3.2 Informed consent

During the research process, the research assistant ensured that all respondents were properly briefed about the aim of the study and their rights and roles in the study. The respondents were made aware that participation in the study was voluntary, and that they were free to withdraw from the project at any time. The respondents were asked to sign the consent form if they agreed to take part in the study.

4.3.3 Freedom from harm

The research assistant did not exploit the respondents as they were free to participate in the study. No harm was inflicted on the respondents, nor were they exposed to unnecessary risks because only questionnaires were used for gathering data. Cases of outright refusal to participate in the study were not encountered. All the respondents were willing to share their experiences in aspects of their knowledge about *S. incanum* pesticide except for six respondents who chose to leave the questionnaire partially complete.

4.3.4 Right to self-determination

The right to self-determination is based on respect for persons and indicates that people can control their destinies. The respondents should be treated as autonomous agents, who have the freedom to conduct their lives as they choose without external control (Rankoana, 2012). During the study, the respondents were informed that participation was voluntary and that they could withdraw at any time from the research process. However, none of the respondents withdrew from participation in the study.

4.3.5 Right to full disclosure

The principle of respect for human dignity encompasses the respondents' right to make informed choices, and voluntary decisions about study participation, which requires full disclosure (Reeves, 2010). Before data collection, the respondents were provided with full information about the study and the value of their participation in the study.

4.3.6 Right to fair treatment

The respondents have the right to fair and equitable treatment before, during, and after they participated in the study (Thomas et al., 2007). During the study, the respondents were fairly treated to participate freely in the study, and their cultural preferences were taken into consideration.

4.3.7 Right to privacy

The identities of the respondents were protected and not revealed in the research findings. The respondents' privacy was protected by obtaining their informed consent and assuring them that there would be no invasion of their privacy. The research assistant ensured the respondents that the information collected would only be used for the research purpose.

4.3.8 Anonymity and confidentiality

The respondents' anonymity was ensured by not asking them to provide their names on the questionnaire. Instead, the research assistant numbered the research instruments according to the order in which the respondents returned the questionnaire. The research assistant ensured that all information gathered during the study was treated as strictly confidential and only available to the research assistant and those directly involved with the study. In the research report, statistics were used without individual names mentioned.

4.4 Results and discussion of the major study

Quantitative data were processed using Statistical Package for Social Sciences(SPSS) using descriptive statistics which included frequency distribution tables, central tendency, and dispersion. The data from open-ended questions were analysed and the emerging themes were coded and displayed using frequency distribution tables. The Chi-square and Independent t-tests were used to test the hypothesises.

4.4.1 Administration and return rate of questionnaires

Fifty-seven questionnaires were distributed to the community members who practised community gardens. The questionnaire return rate was 86%. According to Adeniran (2019), a response rate of 50% is adequate for data analysis and reporting; a rate of 60% is good and a response rate of 70% and over is excellent. This implies that an 86% response rate for this study was excellent for data analysis and reporting (**Table 4.4**).

Table 4. 4: A response rate of questionnaire distribution

Questionnaire	Frequency	Percentage
Returned completed	49	86%
Returned incomplete	8	14%
Total	**57**	**100**

4.4.2 Demographical data

4.4.2.1 Items 1-7**:**

Forty-nine questionnaires were analysed. The respondents were community members in different villages in Mkoba which included Mkoba North (comprised of villages 17-22), Mkoba Thoyo (villages 13-16), Mkoba South (villages 9-12), and Old Mkoba (1-7). In **Table 4.5** the village distribution of the sample is reported. The Villages' respondents were distributed as follows: Mkoba North (55%); Mkoba South (14%), Thoyo (14%), and Old Mkoba (16%) as indicated in **Table 4.5**. As reported in **Table 4.5**, the majority (61%) of the sample were female.

Table 4. 5: A summary of the respondents who participated in this study

	Females N (%)	Males N (%)	Total N (%)
Mkoba North	21(42%)	6(13%)	27(55%)
Thoyo	2(4%)	5(10%)	7(14%)
Mkoba South	2(4%)	5(10%)	7(14%)
Old Mkoba	5(10%)	3(6%)	8(16%)
Total	**30(61%)**	**19(39%)**	**49(100%)**

4.4.2.2 Age distribution

In **Table 4.6** the gender distribution of the sample is reported. The age distribution of the respondents ranged from 16 to 70 years (**Table 4.6**). The age group which visited the vegetable gardens most was 30-50 which made up 37% of the total number of respondents. Out of this age group, 60% were females and 40% were males. Approximately 22% who participated in the study were in the age group 51-60 years of age and 16% of the participants were above 60 years of age.

Table 4.6: Frequency distribution – Age

Age Distribution	Frequency	Percentage frequency(%)	Cumulative	
16-30	12	24%		
31-50	18	37%	30	61%
51-60	11	22%	41	84%
>60	8	16%	49	100%
Total	**49**	**100%**		

Mean = 43 years; SD = 14.2 years

4.4.2.3 Marital status

In **Table 4.7**, the marital status distribution of the sample is reported. Most of the respondents 23(47%) were married, followed by the widowed – 16(33%), the single respondents constituted 7(14%) and the divorced represented the lowest percentage of 6. From the results, most of the respondents were not married -26(53%).

Table 4.7: Frequency distribution – Marital status

Marital status	Frequency	Percentage frequency(%)
Married	23	47%
Single	7	14%
Widow	16	33%
Divorced	3	6%
Total	49	100%

Mean = 12.25; SD = 8.99

4.4.2.4 Education level

In **Table 4.8**, the education level distribution of the sample is reported. As reported in **Table 4.8**, most of the sample attained the secondary level of education 21(43%), and those who attained the primary level had the lowest percentage of 20%. At least 63% of the respondent had done up to the secondary level.

Table 4.8: Frequency distribution – Education level

Education level	Frequency	Percentage frequency(%)	Cumulative	
Primary	10	20%	31	63%
Secondary	21	43%	49	100%
Tertiary	18	37%		
Total	49	100%		

4.4.2.5 Size of the family

In **Table 4.9**, the family size distribution of the respondents is reported. The size of the families of those who participated in the study ranged from 1 to 9 (Mean = 6.13; SD = 4.39). Many of the community members who participated in the study fell into the family size range of 4-6 and they constituted about 61% of the total number of respondents.

Table 4.9: Frequency distribution – Family size

Size of family	Frequency	Percentage frequency(%)		
Two	3	6%	Cumulative	
Three	3	6%	6	12%
Four	10	20%	16	33%
Five	14	29%	30	61%
Six	7	14%	37	76%
Seven	8	16%	45	92%
Eight	2	4%	47	96%
Nine	2	4%	49	100%
Total	49	100%		

Mean = 6.13; SD = 4.39

4.5 Information regarding vegetables grown in the community

4.5.1 Item 8 – Which type of vegetables do you grow?

In **Table 4.10(a),** the type of vegetables commonly grown in the communal gardens distribution of the sample is reported. As reported in **Table 4.10(a),** the most common type of vegetables grown by the community was: Brassica napus (BN) (86%) followed by *Brassica oleracea var. sabellica* (69%) and the least grown vegetable was *Cucurbita* (4%).

Table 4.10(a) Frequency Distributions: Vegetable Types (N = 49)

	English Name	**Scientific Name**	**(Local name) Shona**	**No**	**(%)**	**Yes**	**(%)**
1	Rape	*Brassica napus*	Muriwo	7	14%	42	86%
2	African kale	*Brassica oleracea var. sabellica*	Rugare	15	31%	34	69%
3	Tomato	*Solanum lycopersicum L*	Domasi	33	67%	16	33%
4	Cabbage	*Brassica oleracea var. capitate f. alba*	Khabheji	36	73%	13	27%
5	Mustad green	*Brassica juncea (L)*	Tsunga	36	73%	13	27%
6	Spinach	*Spinacia oleracea*	Mowa	37	76%	12	24%
7	Beetroot	*Beta vulgaris*	Bheturutu	39	80%	10	20%
8	Carrots	*Daucus carota*	Karoti	44	90%	5	10%
9	Onion	*Allium cepa*	Hanyanisi	45	92%	4	8%
10	Pumpkins	*Cucurbita*	Nhanga	47	96%	2	4%

Table 4.10(b) shows the frequency distribution of vegetable varieties grown by respondents in the communal garden. As reported in **Table 4.10(b)**, most of the farmers grew at least three or four varieties of vegetables (Mean = 3.22; SD = 1.03) as indicated in **Table 4.10(b).**

Table 4.10(b): Frequency distribution – Variety of Vegetable Types

A variety of vegetables cited	**Respondents**	**Percentage**	**Cumulative**	
One	2	4%		
Two	9	18%		
Three	18	37%	11	22%
Four	18	37%	29	59%
Six	2	4%	47	96%
Total	49	100%	49	100%

4.5.2 Item 9 – What challenges do you experience growing your vegetable in your garden?

In **Table 4.11**, the frequency distribution of the challenges experienced by the respondents is reported. As reported in **Table 4.11**, the biggest challenge was the pests that attacked the vegetables in the garden. All respondents cited the cabbage aphids as the biggest challenge.

Table 4.11: Frequency Distributions: Challenges for Growing Vegetables (N = 49)

Challenge	No	(%)	Yes	(%)
Aphids	0	0%	49	100%
Red spider	32	65%	17	35%
Birds	35	71%	14	29%
Locust	44	90%	5	10%
Other	41	84%	8	16%

Mean = 1.90; SD = 0.87.

4.5.3 Item 10: How do you resolve these challenges?

In **Table 4.12**, the frequency distribution of the mentioned solutions to the challenges of pests in the gardens is reported. As reported in **Table 4.12**, the use of the indigenous plant pesticide (fruit and root of *Solanum incanum*) was mentioned by most of the respondents 39(80%). The least popular pesticide was ashes, mentioned by 18% of the respondents.

Table 4.12: Frequency Distributions: Solutions to Challenges for Growing Vegetables (N = 49)

Solutions	No	(%)	Yes	(%)
Apply *S. incanum*	10	20%	39	80%
Apply commercial pesticide (roga)	29	59%	20	41%
Apply ashes	40	82%	9	18%
‘Other solution	44	90%	5	10%

4.6 The Knowledge about *S. incanum* (Nhundurwa)

4.6.1 Item 11: Do you know *S. incanum* (Nhundurwa)

The items were used to find out whether respondents know the source and the uses of *S. incanum* in the community of Mkoba. All 49 respondents were able to state at least one additional use of *S. incanum* in the community besides it being used as a pesticide

4.6.2 Item 12: For what purpose do you use Nhundurwa?

In **Table 4.13**, the frequency distribution of the uses of *S. incanum* fruit and root is reported. As reported in **Table 4.13**, respondents 40(87%) use *S. incanum* as a pesticide. Some respondents mentioned more than one use of *S. incanum*.

Table 4.13: Frequency Distributions: The Uses of Nhundurwa (N = 46)

	Uses	No	(%)	Yes	(%)
1	Pesticide	6	13%	40	87%
2	Wound healing	25	54%	21	46%
3	Chicken diseases	31	67%	15	33%
4	Ear infection	40	87%	6	13%
5	Toothache	40	87%	6	13%
6	Stomach-ache	44	96%	2	4%

Mean = 1.96; SD = 0.82

4.7 Practices related to *S. incanum*

4.7.1 Item 13: From where do you get Nhundurwa (*S. incanum*)?

In **Table 4.14**, the frequency distribution of the sources of *S. incanum* fruit and root is reported. As reported in **Table 4.14**, most respondents 35(74%) get *S. incanum* from the bushes around the villages.

Table 4.14: Frequency distribution – Source of Nhundurwa

Source	Respondents (N)	% Frequency (%)
Bushes	35	74%
Field	12	26%
Total	47	100%

4.7.2 Item 14: Who informed you about Nhundurwa?

In **Table 4.15**, the frequency distribution of the sources of information about *S. incanum* fruit and root is reported. As reported in **Table 4.15**, most of the information regarding *S. incanum* came from the parents 17(36%).

Table 4.15: Frequency distribution – Source of information about Nhundurwa (*S. incanum*)

Source of information	Respondents (N)	Frequency (%)
Parent	17	36%
Friend	11	23%
Granny	14	30%
Neighbour	5	11%
Total	47	100%

4.7.3 Item 15: Who prepares the formulation?

In **Table 4.16**, the frequency distribution of the participants' responses on who prepares the formulation used as the pesticide is reported as 28 (57%). As reported in **Table 4.16**, most of the respondents prepared the formulation themselves. The preparation of the formulation is also left to the adults instead 21(43%).

Table 4.16: Frequency distribution – Who prepares the Nhundurwa formulation?

Source of information	Respondents (N)	Frequency (%)
Myself	28	57%
Any adult	21	43%
Total	49	100%

4.7.4 **Item 16**: Motivation on item 15 responses

The item was an open-ended question where the participants described the way the formulation was described. All respondents gave a similar account of how the formulation is prepared, that is, about 5-7 ripe fruits of *S. incanum* were cut into pieces and mixed with water, and allowed to stand overnight before the juice was used as a pesticide. The roots were also pounded first before they were mixed with water and allowed to stand for at least 12 hours before the juice was used as a pesticide.

4.7.5 **Item 17**: Storage of the formulation

Table 4.17 below, shows the frequency distribution of how the prepared formulation is stored. The participants reported that the prepared formulation is kept at the garden 27(55%). Some mentioned the rooftop 6%. As reported in **Table 4.17**, most of the respondents prepared the formulation themselves.

Table 4.17: Frequency distribution – Storage of the formulation

Storage	Respondents (N)	Frequency (%)
Garden	27	55%
Storeroom	16	33%
Roof top	6	12%
Total	49	100%

4.7.6 **Item 18: Reason for formulation storage site**

In item 18, the respondents were asked to justify the cited storage places. **Table 4.18** below shows that the majority (37-76%) reported that the formulation is kept in the garden as a precautionary measure.

Table 4.18: Frequency distribution – Reason for formulation storage site

Reason for storage	Respondents (N)	Frequency (%)
Precautional	37	76%
Sharing	12	24%
Total	49	100%

4.7.7 **Item 19**: What is done with unused formulation?

Table 4.19 below, shows the frequency distribution of respondents' description of how they dispose of the extra formulation. Methods of the disposal of the extra how the prepared formulation is stored. As reported in **Table 4.19**, 24(49%) of the respondents dispose of the extra formulation in the garden, 15(31%) disposed of it in the nearby pits, 7(14%) disposed of it in the nearby bushes whilst a few 3(6%) stored it for future use.

Table 4.19: Frequency distribution – What is done with unused formulation?

Reason for storage	Respondents (N)	Frequency (%)
Dispose of it in garden	24	49%
Dispose of it in the pit	15	31%
Dispose of it in surrounding bushes	7	14%
Store it for future use	3	6%
Total	49	100%

4.8 Perceptions regarding Nhundurwa formulation

The questionnaire probed perceptions regarding the effect of *S. incanum* on health and the environment. The responses to the perception were guided by items 20 to 31 in the questionnaire as outlined below.

4.8.1 **Items 20** and **21:** Do you think that nhundurwa (S. incanum) affects human health?

The evaluation tools are based on a five-point Likert scale (Strongly agree = 5, Agree = 4, Disagree = 3, Strongly disagree = 2 and I don't know =1) were used to pursue the opinion of the respondents on whether the formulations of *S. incanum* affect human health or not. In **Table 4.20**, the frequency distribution of the respondents' perceptions regarding the effect of *S. incanum* on human health is reported. As reported in **Table 4.20**, 26(53%) said that *S. incanum* had no impact on human health in the community whilst 17(34%) reported that *S. incanum* had health issues in the community.

Table 4.20: Does the formulation affect human health? And – and motivation

Scale	Does the formulation affect human health?		Motivation							
			No evidence of effects		People feel sick sometimes		It is poisonous		It decomposes fast into harmless substances	
Don't know	6	16%	8	16%	0	0%	0	0%	8	16%
Strongly disagree	17	35%	11	22%	0	0%	0	0%	12	25%
Disagree	9	18%	5	10%	0	0%	0	0%	5	10%
Agree	12	24%	0	0%	8	16%	7	14%	0	0%
Strongly agree	5	10%	0	0%	5	10%	4	9%	0	0%
Total	49	100%	24	48%	14	28%	11	23%	20	41%

In item 21, the respondents were asked to motivate their response to item 20. The participants' responses were coded into three categories; no evidence, people feel sick, it's poisonous and it decomposes quickly. As reported in **Table 4.20**, 24(48%) of the respondents stated that there was no evidence to link *S. incanum* to the health issues in the community. Twenty (20) 41% of the respondents claimed that the formulations quickly decompose to safe chemicals after a while. A much smaller percentage of the respondents 11(23%) mentioned that *S. incanum* was a health hazard because the formulation is made up of poisonous chemicals which affected the community.

4.8.2 Item 22 – Do you think that Nhundurwa formulation can affect the environment?

In **Table 4.21**, the frequency distribution of the respondents' opinions is reported. As reported in **table 4.21**, 24(49%) said that *S. incanum* had an impact on human health in the community whilst 17(34%) mentioned that *S. incanum* was poisonous.

Table 4.21: Does the formulation affect human health? – and motivation

Scale			Motivation					
			Poisonous chemicals		**Decompose**		**I don't know**	
	N	%	N	%	N	%	N	%
Don't know	7	14%	0	0%	0	0%	7	100%
Strongly disagree	16	33%	0	0%	12	75%	0%	0%
Disagree	9	18%	0	0%	8	89%	0%	0%
Agree	10	21%	8	80%	2	20%	0%	0%
Strongly agree	7	14%	5	71%	0	0%	0%	0%
Total	49	100%	13	26%	22	45%	8	23%

The study sought to find out the community's perceptions of environmental pollution caused by *S. incanum* pesticides. Item 22 was based on a five-point Likert scale (Strongly agree = 5, Agree = 4, Disagree = 3, Strongly disagree = 2 and I don't know =1). In **Table 4.21**, the frequency distribution of the respondents' perceptions regarding the environmental effect of S. incanum was reported. As reported in **Table 4.21**, 25(51%) of the respondents believed that *S. incanum* is harmless to the environment whilst 17(35%) reported that the formulations were an environmental hazard.

Item 23, the respondents were asked to motivate their responses to item 22. The participants' responses were coded into three categories: poisonous. Decompose and I don't know. As reported in **Table 4.21**, 22(45%) of the respondents stated that the *S. incanum* formulations decomposed quickly, therefore, were not healthy whilst 13(26%) of the respondents indicated that the formulation was poison and would affect the species in the environment.

4.8.3 Item 24 –Perceptions regarding Nhundurwa formulation (N = 49)

Respondents were asked to give their perceptions regarding *S. incanum*. Six items were given, and respondents rated each item based on a five-point Likert scale (Very Much = 5, Much = 4,

A little = 3, Not at all = 2 and I don't know =1). In **Table 4.22**, the frequency distribution of the respondents' perceptions regarding *S. incanum* is reported. As reported in **Table 4.22**, for item Q24.1 – 28(57%) the respondents reported that using *S. incanum* was a waste of time. For item Q24.2 – 24(49%) of the respondents reported that *S. incanum* has helped the community to control cabbage aphids, for item Q24.3 – 16(33%) of the respondents reported that *S. incanum* was poisonous, for item Q24.4 – 18(39%) thought that *S. incanum* was creating health problems in the community; for item Q24.5 – 27(55%) of the respondents agreed that the use of s. incanum as a pesticide is helping them to save money and for the item, Q24.6 – 29(59%) of the respondents reported that the *S. incanum* formulation was effective on the cabbage aphids

Table 4.22: Frequency Distributions: Perceptions regarding *S. incanum* formulation – Q24 items (N = 49)

No.	Item	Responses				
		Don't Know N (%)	**Not At All N (%)**	**A Little N (%)**	**Much N (%)**	**Very Much N (%)**
24.1	The community wastes time in applying *S. incanum*, it does not work on the vegetables	28(57%)	19(39%)	2(4%)	0(0%)	0(0%)
24.2	The use of *S. incanum* as a pesticide has helped the community to produce vegetables in abundance	0(0%)	5(10%)	3(6%)	17(35%)	24(49%)
24.3	*S. incanum* is poisonous	8(16%)	4(8%)	16(33%)	13(27%)	8(16%)
24.4	The use of *S. incanum* as a pesticide has created health problems in the community	8(16%)	2(4%)	16(33%)	19(39%)	4(8%)
24.5	The use of *S. incanum* as a pesticide has helped the community to save money, which they could have used to buy pesticides in shops.	10(20%)	2(4%)	1(2%)	9(18%)	27(55%)
24.6	*S. incanum* kills the aphids	0(0%)	0(0%)	5(10%)	29(59%)	15(31%)

In **Table 4.23(a)**, the frequency distribution of the perception regarding *S. incanum* for the Negative impact on the Environment and Health (PNF-NEH) and efficacy (PNF-E) are reported. As reported in **table 4.23(a)**, the PNF-NEH was at 35% moderate whilst the PNF-E was at 49% high.

Table 4.23(a): Frequency Distributions: PNF Factors (N = 49)

	Very Low 1.00 to 1.79		**Low** 1.80 to 2.59		**Middle** 2.60 to 3.40		**High** 3.41 to 4.20		**Very High** 4.21 to 5.00	
PNF-NEH	3	6%	10	20%	17	35%	14	29%	5	10%
PNF-Eff	0	0%	1	2%	4	8%	20	41%	24	49%

In **table 4.23(b)**, the results of an independent-sample t-test conducted to compare PNF NEH scores in the primary – secondary level and the tertiary respondents are presented. There was no significant difference in the perception scores for the primary-secondary group (M = 3.30, SD = 0.84), and tertiary (M = 2.94, SD = 0.74); t (47) = 1.51, p = 0.138). These results suggest that education level did not influence the respondent's perceptions of the environmental and health impact. The magnitude of the difference in the means was small (Cohen's d-test = 0.045).

An independent-sample t-test was conducted to compare PNF Eff scores for primary – secondary level respondents and tertiary respondents. The results are indicated in **Table 4.23(b)**. There was no significant difference in perception scores for the primary-secondary respondents (M = 3.99, SD = 0.68), and tertiary (M = 4.13, SD = 0.50; t (47) = -0.72, p = 0.476). These results suggest that education level did not influence the respondent's perceptions of the efficacy of *S. incanum*. The magnitude of the difference in the means was small (Cohen's d-test = 0.021).

Table 4.23(b): An independent t-Tests to determine any significant difference between PNF Factors (NEH) and (EFF) with the Education Level of the respondents

Variable	Education level	N	Mean	S.D.	Difference	t	*p*	Cohen's d
PNF-NEH	Primary-Secondary	31	3.30	0.84	0.35	1.51	.138	0.045
	Tertiary	18	2.94	0.70				Small
PNF- Eff	Primary-Secondary	31	3.99	0.68	-0.13	-0.72	.476	0.021
	Tertiary	18	4.13	0.50				Small
				(*d.f.*=47)				

4.9 Practices related to *S. incanum* (nhundurwa) formulation

4.9.1 Item 25 – Do you have any special clothes you use when working with nhundurwa?

In **Table 4.24**, the frequency distribution of the respondents according to the type of PPC (personal protective clothing) they use and their motivation for not using PPC is reported. As reported in **table 4.24**; - 33(67%) of the respondents did not use PPCs whilst 16(33%) used PPCs. Out of those who did not use the PPC, 22(45%) could not afford the PPC. The majority of those who used PPC (63%) used old clothes.

Table 4.24 (a): Frequency distribution – Special clothes used when working with Nhundurwa? And suggested forms of PPCs. N = 49

	Frequency N (%)	**Special clothes used when working with Nhundurwa – More about**				
		It is not poisonous N (%)	**Old clothes used** N (%)	**Overall and gloves** N (%)	**Can't afford PPC** N (%)	**Total** N (%)
Yes	16(33%)	0(0%)	10(63%)	3(9%)	3(9%)	16(100%)
No	33(67%)	14(42%)	0(0%)	0(0%)	19(57%)	33(100%)
Total	49(100%)	14(29%)	11(22%)	4(8%)	22(45%)	49(100%)

Table 4.24(b) reports the distribution of the respondents according to education level and the choice of PPC. A chi-square was conducted to determine if the decision of the respondent to use PPC was influenced by his/her level of education.

Table 4.24(b): Contingency Table – Education level and Special clothes used when working with *S. incanum* (Nhundurwa)

Education level	Special clothes used when working with Nhundurwa?					
	Yes		No		Total	
Primary/Secondary	12	39%	19	61%	31	100%
Tertiary	4	22%	14	78%	18	100%
Total	16	33%	33	67%	49	100%

Chi^2(d.f. = 1, N = 49) = 1.41; p = 0.235

The proportion of the primary-secondary education level respondents who used special clothing was not significantly different from the proportion of the tertiary education level respondents who used special clothing as they worked with the formulation χ^2 ($d.f.$ = 1, N = 49) = 1.41; p = 0.235. Education level did not influence the respondent on the choice of whether they used PPC or not.

4.9.2 Item 26 – Motivation of the response to item 25

In item 26, the respondents were asked to motivate their responses in item 25. Out of the 67% of the respondents who did not use PPC, 19(57%) of them mentioned that they could not afford the PPC whilst others stuck to their perception that s. incanum was not poisonous therefore there was no need for PPCs.

4.9.3 Items 27 and 28 – What method(s) do you use to apply the nhundurwa solution to the vegetable?

In **Table 4.25**, the frequency distribution of the type of method the respondents used in the garden is reported. As reported in **Table 4.25**, the popular method was broadcasting with a frequency of 28(57%). Very few mentioned the use of aerosol or nozzle spray methods with frequencies of 9(18%) and 6(12%) respectively. Item 4.28 requested the respondents to motivate their responses in item 4.27. As reported in **Table 4.25**, most of the respondents, 12(43%) mentioned that the broom method was easier to use, 3(50%) of the six who mentioned the nozzle spray said it because it was available at home, and those who reported the use of the spongy (33%) mentioned that it was the only item available to use at home.

Table 4.25: Contingency Table – Method used to apply Nhundurwa and Motivation for the method

Method	Frequency	Easier to use	Covers more area	The only equipment available	I have it at home	Designed for spraying pests	Directed to the plant
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Use of Broom	28(57%)	12(43%)	9(32%)	1(4%)	6(21%)	0(0%)	0(%)
Nozzle spray	6(12%)	1(17%)	0(0%)	3(50%)	0(0%)	2(33%)	0(%)
Aerosol spray	9(18%)	6(67%)	0(0%)	0(0%)	3(33%)	0(0%)	0(0%)
Spongy	6(12%)	1(17%)	0(0%)	1(17%)	2(33%)	1(17%)	1(17%)
Total	49(100%)	20(41%)	9(18%)	5(10%)	11(22%)	3(6%)	1(2%)

4.9.4 Item 29 – After how long do you notice the effect of the nhundurwa formulation on the aphids?

In **Table 4.26**, the frequency distribution of the respondents according to the period they see the effects of the formulation after spraying is reported. As reported in table 4.26; - the majority, 26(53%) of the respondents reported that they see the effects of the formulation after two (2) days after spraying.

Table 4.26: Frequency distribution – After how long do you notice the effect of the Nhundurwa formulation on the aphids?

	Period of the effect	Frequency N (%)
1	After 2 weeks	1(2%)
2	After 5 days	13(27%)
3	In 2 days	26(53%)
4	Immediately	9(18%)

4.9.5 Items 30 and 31 -After how long do you harvest your vegetables when treated with nhundurwa formulation? And motivation.

In **Table 4.27**, the frequency distribution of the respondents according to the period they wait before harvesting the vegetable and the motivations for their responses are reported. As reported in **Table 4.27**, most of the respondents, 41% reported that they wait for 2 days before they harvest the vegetables whilst 8% of the respondents mentioned that they harvest immediately after spraying.

Table 4. 27: Contingency Table – After how long do you harvest your vegetables when treated with Nhundurwa formulation? And the motivation for the responses

Method	**Waiting period before harvesting**	**It is not poisonous or harmful to people**	**Its effects disappear fast**	**The formulation is effective after aphids**	**I never asked**
After 2 weeks	8(16%)	3(38%)	1(13%)	2(25%)	2(25%)
After 5 days	17(35%)	2(12%)	3(18%)	7(41%)	5(29%)
In 2 days	20(41%)	4(20%)	6(30%)	9(45%)	1(5%)
Immediately	4(8%)	3(20%)	3(20%)	0(0%)	0(0%)
Total	49(100%)	13(27%)	10(20%)	18(37%)	8(16%)

When the respondents were asked to explain their responses in item 30, different reasons were given by the respondents. Most of those who mentioned that they harvest the vegetables the same day (20%) stated that the formulation was not poisonous so there was no need to wait for long periods. Ten respondents reported that the effects of *S. incanum* disappear quickly whilst some stated that they did not have an alternative to the relish if they had to wait for a longer period. Probably, the respondents who harvest the vegetables immediately after spraying believe that the formulation works differently from the commercial pesticides. Noticeably, those who mentioned that they take about two weeks before harvesting. Their reasons were that they believed that the *S. incanum* formulation was toxic.

4.10 *S. incanum* (nhundurwa) symptoms

4.10.1 Item 32 – Which ONE (s) are the common symptoms or illnesses common with people who work in the garden?

In **Table 4.28**, the frequency distribution of the respondents regarding the common symptoms common in the village is reported. As reported in **Table 4.28**, at least 29(63%) of the respondents reported skin irritation as a common symptom in the village. The other common symptoms included stomach-ache 16(35%), vomiting 16(35%), poor vision 8(17%), dizziness 7(15%), fatigue 2(4%), and coughing 3(7%).

Table 4. 28: Frequency Distributions: Nhundurwa symptoms (N = 46)

Symptom	Frequency	
	N	%
Skin irritation	29	63%
Stomach-ache_	16	35%
Vomiting	16	35%
Headache	14	30%
Nausea	13	28%
Poor vision	8	17%
Dizziness	7	15%
Coughing	3	7%
Fatigue	2	4%
Sweating	2	4%

Table 4.29: Central Tendency and Dispersion: No. of Nhundurwa Symptoms (N = 46)

	Mean	S.D.	Min	Quartile 1	Median	Quartile 3	Max
No. of Nhundurwa Symptoms	2.39	1.77	0.00	1.00	3.00	3.00	6.00

Nine symptoms were mentioned by respondents of the age groups categorised as below 50 and above 50 as indicated in **Table 4.30.** In **table 4.30**, an analysis is reported to determine whether the reported symptoms were associated with age. A Chi-square was used. As reported in **Table 4.30**, most of the symptoms were not statistically associated with a particular age group except for nausea. The proportion of the respondents of the age group < 50 years of age who experienced poor vision symptoms in the community was significantly different from the

proportion of the respondents of the age group > 50 years of age who experienced poor vision symptoms in the community, χ^2 (*d.f.* = 1, *N* = 46) = 8.52; *p = 0.004*; V = 0.43 Medium. Thirty-seven percent (37%) of the age group above 50 experienced poor vision compared to the 4% of the age group below 50 years of age. Older people above 50 years of age were more likely to experience poor vision than younger ones. Further investigation is required to ascertain whether the symptoms were influencing poor vision, or it was just a coincidence since literature also reveals that poor vision is more prevalent in old age (above 50). The proportion of the respondents of the age group < 50 years of age who experienced nausea symptoms in the community was significantly different from the proportion of the respondents of the age group > 50 years of age who experienced nausea symptoms in the community, χ^2 (*d.f.* = 1, *N* = 46) = 5.02; *p = 0.025*; *V* = 0.33 Medium. Younger people were more likely affected by nausea symptoms than older ones.

Table 4.30: Contingency Tables – Age and *S. incanum* Symptoms

	Age					
	16-50		>50		Total	
Headache	10	37%	4	21%	14	30%
χ^2 (*d.f.* = 1, *N* = 46) = 1.35; *p = 0.246*						
Dizziness	4	15%	3	16%	7	15%
χ^2 (*d.f.* = 1, *N* = 46) = 0.01; *p = 0 .928*						
Skin irritation	16	59%	13	68%	29	63%
χ^2(*d.f.* = 1, *N* = 46) = 0.40; *p = 0 .526*						
Nausea	11	41%	2	11%	13	28%
χ^2(*d.f.* = 1, *N* = 46) = 5.02; *p = 0 .025*; V = 0.33 Medium						
Vomiting	7	26%	9	47%	16	35%
χ^2 (*d.f.* = 1, *N* = 46) = 2.26; *p = 0 .133*						
Coughing	3	11%	0	0%	3	7%
χ^2 (*d.f.* = 1, *N* = 46) = 2.26; *p = 0 .133*						
Poor vision	1	4%	7	37%	8	17%
χ^2(*d.f.* = 1, *N* = 46) = 8.52; *p = 0 .004*; V = 0.43 Medium						
Sweating	1	4%	1	5%	2	4%
χ^2(*d.f.* = 1, *N* = 46) = 0.07; *p = 0.798*						
Fatigue	2	7%	0	0%	2	4%
χ^2(*d.f.* = 1, *N* = 46) = 1.47; *p = 0.225*						
Stomach-ache	11	41%	5	26%	16	35%
χ^2(*d.f.* = 1, *N* = 46) = 1.02; *p = 0.312*						

Nine symptoms were mentioned by both female and male respondents as indicated in **Table 4.31.** Therefore, it was also important to determine whether the reported symptoms were

statistically associated with a particular gender. A Chi-square was used. As reported in **Table 4.31**, most of the symptoms were not statistically associated with a particular gender except for vomiting. The proportion of females that experienced vomiting was significantly different from the proportion of the males who experienced vomiting, $\chi^2(d.f. = 1, N = 46) = 5.15$; $p = .023$; V = 0.33 Medium. The proportion of males that experienced poor vision was significantly different from the proportion of the females who were affected by poor vision, $\chi^2((d.f. = 1), N = 46) = 6.81$; $p = .009$; $V = 0.38$ Medium.

Table 4.31: Contingency Tables - Gender and *S. incanum* Symptoms

	Gender					
	Male		Female		Total	
Headache	6	32%	8	30%	14	30%
$\chi^2(d.f. = 1, N = 46) = 0.02$; $p = .887$						
Dizziness	2	11%	5	19%	7	15%
$\chi^2(d.f. = 1, N = 46) = 0.55$; $p = .457$						
Skin irritation	10	53%	19	70%	29	63%
$\chi^2(d.f. = 1, N = 46) = 1.51$; $p = .220$						
Nausea	4	21%	9	33%	13	28%
$\chi^2(d.f. = 1, N = 46) = 0.83$; $p = .362$						
Vomiting	3	16%	13	48%	16	35%
$\chi^2(d.f. = 1, N = 46) = 5.15$; $p = .023$; $V = 0.33$ Medium						
Coughing	2	11%	1	4%	3	7%
$\chi^2(d.f. = 1, N = 46) = 0.85$; $p = .356$						
Poor vision	0	0%	8	30%	8	17%
$\chi^2(d.f.. = 1, N = 46) = 6.81$; $p = .009$; $V = 0.38$ Medium						
Sweating	1	5%	1	4%	2	4%
$\chi^2(d.f. = 1, N = 46) = 0.07$; $p = .798$						
Fatigue	1	5%	1	4%	2	4%
$\chi^2(d.f. = 1, N = 46) = 0.07$; $p = .798$						
Stomach ache	8	42%	8	30%	16	35%
$\chi^2(d.f. = 1, N = 46) = 0.77$; $p = .382$						

4.10.2 Item 33: What do you do to relieve yourself of the illness?

In **Table 4.29**, the frequency distribution of the respondents regarding the remedies they use when affected by the formulation is reported. As reported in **Table 4.29**; - 13(36%) of the respondents use milk or water to wash the affected part of the body. Only 3(8%) of the respondents mentioned that they go to the clinic to seek medication.

Table 4.32: Frequency Distributions: Remedies for Nhundurwa symptoms (N = 36)

Remedy	Frequency	
	N	%
Fresh milk	13	36%
Wash with soap	13	36%
Go to clinic	3	8%
None	7	20%

4.11 Discussion

In this study, we surveyed 49 community members who use *S. incanum* as a pesticide in their gardens in the Gweru district of Zimbabwe and evaluated their indigenous knowledge and perception of the environmental and health issues linked to the use of *S. incanum* exposure. Thirty females and 19 males were the respondents. Overall, the results showed that the community indeed used *S. incanum* as a pesticide and was quite knowledgeable about how the formulations are prepared and administered as a pesticide. There were mixed feelings from the respondents regarding the efficacy and the poisonous nature of *S. incanum.* It was evident from the responses given by the participants that the symptoms experienced (assumed to be linked to the use of *S. incanum* as a pesticide) were not enough to deter the community from using *S. incanum* as a pesticide, probably due to a lack of alternative cheaper resources.

4.11.1 Knowledge of the community regarding *S. incanum*

4.11.1.1 Vegetables grown

The study revealed that the community grew a variety of vegetables in their communal gardens that included *Brassica napus, Brassica oleracea var sabellica, Solanum lycopersicum L, Brassica oleracea var. capitate f alba, Brassica juncea L, Spinacia oleracea, Beta vulgaris, Daucus carota, Allium cepa,* and *Cucurbita.* The study confirmed that *Brassica napus* were the most grown vegetable in the village (mentioned by all the respondents) and the most vulnerable to the cabbage aphids as well. In reviewing the literature, no data was found to explain why the *Brassica napus* are susceptible to cabbage aphids. *Brassica napus* are the staple relish in Zimbabwe. It is confirmed in previous studies that countries in the SADC region grow similar types of vegetables. According to (Nyirenda et al., 2011), the most common vegetables in Zambia are mustard (35%), rape (31%), tomato (13%), Chinese cabbage (8%), and cabbage

(6%). It is reported also that the most valuable vegetables were tomato, rape, and cabbage, with about 38%, 23%, and 12% share of the total value of sales, respectively.

4.11.1.2 The respondents' knowledge regarding *S. incanum*

The respondents' knowledge was examined based on their processing methods of the *S. incanum* fruit and root in to be used as a pesticide and the general uses of *S. incanum* in the community. The responses summarised in section 4.8.3 item 15 revealed that most of the respondents described the processing of the fruit and the root of *S. incanum* in the same way. This shows that there was a common way the community used to process the formulation which was passed from one generation to the other.

Item 9 requested the respondents to list down the challenges encountered in the community gardens. As reported in **Table 4.11**, the challenges included the attack on their vegetables by pests such as cabbage aphids, red spiders, birds, and locusts. Cabbage aphids were mentioned by almost all respondents as the most problematic pest which attacked *Brassica napus*. The red spider was mentioned by the respondents especially those who grew tomatoes in their gardens. When probed further to explain what the community was doing to avert the challenges caused by pests; most of the respondents mentioned that they used *S. incanum* pesticides. *S. incanum* formulation was mentioned by 39(80%), followed by synthetic pesticides such as dimethoate (rogor) and Bulalazonke 20(41%) of the respondents. The other method the respondents use to minimise the challenge of pests was the use of plastics to cover part of the vegetable which was commonly attacked by birds and locusts.

These results reflect those of Stevenson et al (2012) who also found that rural communities in Malawi and Zambia struggled with the African bollworm, cabbage moth, cabbage webworm, caterpillars, cutworms, diamondback moth, flea beetle, leaf miner, spider mites, semi loopers, thrips, whitefly, and white grubs in their community gardens (Stevenson et al., 2017). Stevenson et al (2012) also cited cabbages as the most susceptible to attack by aphids (Stevenson et al., 2012).

Diverse uses of *S. incanum* were also reported which included medicinal and food. These findings were consistent with the results in the previous studies conducted by (Chowański et al., 2016), (Abebe, Gebre, and Haile, 2014; Kambizi and Afolayan, 2001; Sundar and Pillai, 2016), who reported that *S. incanum* is used in the food, agricultural, and pharmaceutical

industries. For instance, in Uganda, *S. incanum* is mixed with fresh milk to make it curdle faster (Sundar and Pillai, 2016). In Ethiopia and Tanzania, it is used for curing headaches and muscle pain (Kambizi and Afolayan, 2001). In South Africa, the fruit is used for toothache and stomachache (Mhazo, 2018).

One interesting and obvious finding was that the knowledge the community held was passed from one person to the other through parents, grandparents, and friends. The community was the source of knowledge about *S. incanum*. The respondents' responses confirm what had been said in literature that indigenous knowledge is passed from one to the next person through community interactions. The way indigenous knowledge is acquired is confirmed by (Senanayake, 2006) when he posited that indigenous knowledge is passed from generation to generation, usually by word of mouth and cultural rituals, and has been the basis for agriculture, food preparation, and conservation, health care, education, and the wide range of other activities that sustain a society and its environment in many parts of the world for many centuries.

4.11.2 Perceptions of the respondents regarding *S. incanum's* effectiveness as a pesticide

Items 20 to 25 were used to obtain the community's perceptions regarding the use of *S. incanum* as a pesticide. Items 20 and 21 asked respondents to state their perception regarding the effect of *S. incanum* on health and the environment respectively. As reported in **table 4.20**, above, 26(53%) said that *S. incanum* had no impact on human health in the community whilst 17(34%) reported that *S. incanum* had health issues in the community. A Chi-square test conducted indicated that there was no statistically significant difference between respondents who said that *S. incanum* was a health hazard and those who said it was safe to use as a pesticide χ^2 ($d.f = 1$, $N = 49$) = 0.67; $p = 0.413$. The proportion of the respondents who perceived that *S. incanum* had an environmental impact was not statistically significantly different from the proportion of the respondents who perceived it to be safe χ^2 ($d.f = 1$, $N = 49$) = 0.87; $p = 0.254$.

An independent t-test was conducted to determine if education level influences the perceptions of the respondents regarding the health and environmental impact of *S. incanum*. As reported in **Table 4.24(b)**, the t-test results for primary-secondary ($M = 3.30$, $SD = 0.84$) and tertiary

(M = 2.94, SD = 0.74); t (47) = 1.51, $p = 0.138$ suggested that education level did not influence the respondent's perceptions on the environmental and health impact.

The perception that *S. incanum* was not a health hazard or environmental problem was echoed in several responses as reported in **Table 4.24**. Most of the respondents (41%) as indicated in **Table 4. 27** reported a maximum of 2 days waiting period before harvesting which alluded to and supported the connotation that *S. incanum* was non-poisonous. A Chi-square was conducted to determine if there was an association between the pre-harvesting period and the education level of the respondents. From the Chi-square conducted, it was concluded that education level was not statistically associated with the pre-harvesting period choice of the respondents $\chi^2(d.f = 3, N = 49) = 5.68$; $p = 0\ .128$. Probably, the waiting period depended on the cultural information in the community. Another possible explanation for this might be that the community imitated the behaviour of their predecessors or elders without putting much thought into the results of their actions.

About 28(55%) of the respondents in this study reported that they were not aware of the harmful environmental effects of *S. incanum* and as a result, the majority in the community ignored the use of PPCs or/and the waiting period before harvesting time. For those who assumed that *S. incanum* had chemicals that were dangerous to the community's health 10(19%), the knowledge they hold did not significantly change their practices or attitudes towards the safe use of *S. incanum*. Many in the community adopted risky behaviours because of a lack of knowledge and understanding of the contents of the *S. incanum* formulation.

4.11.3 Personal protective clothing (PPC)

The use of appropriate PPC, such as coveralls, goggles, and gloves while handling pesticides, (organic or synthetic) is a good practice to reduce pesticide poisoning (Adewunmi and Fapohunda, 2018). A Chi-square test was therefore conducted to find out if a relationship existed between the level of education and the decision to use PPC by the respondents at a 5% significance level. There was a statistically significant relationship between the education level and choice to use PPC, χ^2 (2, N = 51) = 5.11, $p = 0.04$. Therefore, the education level attained by the respondent influenced the decision taken on whether to use the PPC or not.

It was confirmed from previous studies that farmers from disadvantaged communities do not put too much importance on PPC. For instance, low usage of protective clothing was observed in Zimbabwe and South Africa where Magauzi et al., (2011) and Rother, Hall, and London (2008) revealed that the zeal amongst smallholder farmers to use protective clothing was very low, 14% and 17.3% respectively irrespective to whether they were using synthetic or natural pesticides (Magauzi et al., 2011; Rother et al., 2008) due to the cost of purchasing the PPCs. An interview carried out using smallholder farmers in Cape Town (De Bon et al., 2014) revealed that due to the cost of buying the PPC, 12.7% of the farmers never wore gloves, 16.8% of them never used masks, 2.5% of them never wore boots, and 11.0% of them never wore protective clothing. A similar study in West Kenya unearthed that 88.9% of the farmers in disadvantaged communities applied pesticides without taking any personal precautions, and only 11.1% of the farmers wore boots while preparing and applying pesticides (Midega et al., 2012).

4.11.4 Symptoms experienced in the community

Several symptoms of ill health reported by the respondents included skin irritation, vomiting, stomachache, headache, nausea, dizziness, coughing, sweating, and fatigue. About 80% of the respondents linked the symptoms to the use of *S. incanum* and 20% to the use of commercial pesticides. This finding was contrary to the responses given on the previous items (items 20-24) that asked about the environmental and health impact of *S. incanum* and the responses given suggested that *S. incanum* was non-poisonous. This rather contradictory result showed that there is no adequate knowledge in the community regarding the safe use of *S. incanum*. As reported in **Table 4.30**, most of the symptoms were not associated with a particular age group except for nausea and poor vision which were linked to those above 50 years of age. There was no association between gender and the *S. incanum* symptoms highlighted by the respondents in most of the symptoms which included skin irritation, vomiting, stomachache, headache, nausea, dizziness, coughing, sweating, and fatigue except for vomiting and poor vision. The literature revealed that *S. incanum* fruit contains alkaloids, therefore, the symptoms experienced by the respondents may be linked to alkaloid exposure. Alkaloids are known to cause symptoms that include nausea, vomiting, stomach, and abdominal cramps, and diarrhoea (Omayio et al., Abong, 2016).

A similar study was carried out by (Abang et al., 2014), who investigated farmers' knowledge and perceptions regarding the effect of plant pesticides. Interviews were used to collect data and it was reported that 37.5% of the farmers experienced itchy skin, 37.0% of them had a headache, 24.9% of them experienced excessive sweating, and 21.3% of them had diarrhoea (Abang et al., 2014). (Akter, Fan, Rahman, Geissen, and Ritsema, 2018) used a cross-sectional survey in Bangladesh to find out the type of symptoms experienced by smallholder farmers who practiced market gardens and were using commercial pesticides. They reported similar symptoms as reported in this study. The farmers mentioned irritation in the eyes and face, dizziness, chest pain, skin irritation, headache, abdominal pain, and fever as common symptoms amongst the farmers (Akter et al., 2018).

Several studies found that plant compounds derived from crude extracts that are potent in pest control, such as *nicotine* and r*otenone*, have a relatively high acute mammalian toxicity (El-Wakeil, 2013). However, in practice, the acute human health risk of these compounds, as used in pest control, is mitigated by the low concentrations of the active substances typically used in crude preparations. For example, the oral lethal dose of rotenone is reported to be between 300 and 500 mg/kg in humans (Sola et al., 2014). The maximum concentration of rotenoids in *Tephrosia* dry leaf is reported to be around 0.1 % by weight (Stevenson et al., 2017). Therefore, a 70 kg person would need to consume more than 20 kg of dry *Tephrosia* leaf material to get a lethal dose (Nyahangare et al., 2012). Several phytochemical studies have stated that *Solanum* species possess glycoalkaloids (Al Sinani and Eltayeb, 2017; Omayio et al., 2016). Glycoalkaloid levels above 14 mg/100 g result in bitterness of taste while varieties having more than 20 mg/100 g led to a burning sensation in the throat and mouth (Omayio et al., 2016).

According to the study, farmers in the community dispose of empty pesticide containers, unwanted pesticides or left-over spray solutions, and the water used for washing spraying equipment in unsafe ways, including disposal near water bodies and nearby bushes. This represents a pollution problem for those who drink directly from these water sources as well as aquatic systems which are sources of livelihood for some communities (Syafrudin et al., 2021). It also shows that the community has little knowledge of the toxicity effects of *S. incanum*. Disposal of chemical containers, left-over spray solutions, and wastewater from sprayer equipment also play important roles in contracting diseases caused by pesticides (Khan et al., 2020).

4.12 Limitations of the study

A limitation of a study such as this was that it was based mainly on self-reported data (questionnaires), relying on the honesty of respondents which was subjected to bias (Demetriouert al, 2014). As a self-report, there may be some inaccurate data such as respondents wanting to report socially desirable behaviours. For example, self-report of PPC, use and safe disposal of leftovers, and the adoption of other safety practices may have been influenced by the respondents' desire to indicate that they comply with protective measures.

Another limitation relates to the inability to directly link health symptoms experienced by respondents to *S. incanum* pesticide exposure alone. The health symptoms experienced by respondents, such as headaches and fatigue, were not specific, and in some of the cases, these symptoms might have been due to causes other than exposure to pesticides. Long exposure to the sun, especially if no head protection is worn, or/and hunger is examples of these factors. Finally, based on the number of respondents 51, the researcher cannot claim that the results are representative of all community members in Mkoba.

4.13 Conclusion

Despite its limitation, this study provided an overview of pesticide knowledge and practices among farmers in Mkoba villages that can contribute to educational and policy recommendations that aim at preventing or reducing the hazards associated with indigenous pesticides. The prevalence of symptoms of some diseases possibly due to *S. incanum* poisoning in the community was revealed (80%). The smallholder garden farmers are being exposed to undesirable levels of pesticides (that is. those levels that caused symptoms). The findings indicate that health and safety regarding the use of *S. incanum* in the community were inadequate. The symptoms highlighted by the community and how they handled the symptoms expose a lack of knowledge on the effects of *S. incanum* pesticides. Many of the respondents ignored the symptoms without taking any medication until the symptoms faded out. The formulation or leftover formulations were stored at places that were out of reach of children. This was an indication that the adults were aware of the dangers the formulation may cause. These results indicate that *S. incanum* may have deleterious health implications on humans and

hence, advice on their safety should accompany the promotion of its use and the progress will depend upon the phytochemistry study of this plant.

The study also identified some challenges that the community in Mkoba villages is experiencing. Such challenges include a lack of a mechanism for measuring accurate doses when using fruit and root as the pesticide; limited scientific research to establish the effectiveness and efficacy. There is minimal scientific research on *S. incanum* pesticide effects in Mkoba village to establish whether the formulations contain the poison ingredients which are having adverse effects on human beings in the community. Understanding farmers' level of knowledge and practices regarding the safe use of *S. incanum* is vital for limiting the health and environmental hazards caused in the community (Jallow et al., 2017). Therefore, the next two chapters sought to extract and isolate the phytochemical compounds present in the fruit and root of *S. incanum* to find explanations about pesticides to support the community.

4.14 References

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CHAPTER 5

QUALITATIVE AND QUANTITATIVE ANALYSIS AND THE BIOLOGICAL ACTIVITIES OF THE FRUIT AND ROOT OF *SOLANUM INCANUM* L.

5.1 Introduction

The preparation of plant material for experimental purposes is the first and most important step if quality experimental results are to be achieved (Abubakar and Haque, 2020). The preparation involves the correct and timely collection of the plant, authentication by an expert, and adequate drying and pulverisation (Pandey and Tripathi, 2014) before proceeding with the isolation of the phytochemicals from the plant material (Zhang et al., 2018; Kavitha and Indira, 2016). Therefore, the plant material comprising fruits and roots of *S. incanum* was collected on the 5th of May 2018 from the bushes surrounding Mkoba village, Gweru, Zimbabwe based on the ethno-pesticidal and/ or ethnomedicinal information provided by the local herbalist in the village. The herbalist accompanied us to identify the plant species. The plant material was then dried and pulverised before it was transported to Nelson Mandela University as biomass for extraction.

This chapter presents and discusses the results of the plant material preparation, profiling of the crude extracts, and biological activities. The experimental procedures which were carried out are also described.

5.2 Results and discussion

5.2.1 Crude extraction

The fruit (546 g) and root (148 g) biomass were extracted sequentially in the order of increasing polarity from *n*-hexane, ethyl acetate, and methanol to afford crude extracts coded HFE, EFE, MFE, Hroot, Eroot, and Mroot as indicated in **Table 5.1**. The Steroidal Alkaloid Fruit extraction (SAFE) yielded 2.61 g of crude from 112 g of fruit biomass. The yield and the physical characteristics of extracted crudes are reported in **Table 5.1**. Polar solvents achieved a higher number of crude extracts than non-polar solvents. As reported

in **Table 5.1**, the ethyl acetate fruit (EFE) crude extract produced a black soft solid with the highest yield of 17.8 g and the hexane fruit (HFE) crude extract was a dark green solid had the lowest yield of 10.4 g.

Table 5. 1: Yields and physical characteristics of crude extracts of *S. incanum*

Crude Name	Code	Mass of biomass (g)	Mass of crude (g)	Colour	The texture of the crude extract
Methanol fruit	MFE	546	13.7	dark green solid	Oily Sticky
Ethyl acetate fruit	EFE	546	17.8	Black solid	Soft
Hexane fruit	HFE	546	11.2	Dark green solid	Oily
Methanol root	Mroot	148	10.4	dark brown solid	Hard solid
Hexane root	Hroot	148	14,2	Brown solid	Hard
Ethyl acetate root	Eroot	148	12.9	Brown solid	Hard
Steroidal alkaloids	SAFE	112	2.61	Brown solid	Sticky

5.2.2 Phytochemical profiling of the crude extracts of *S. incanum* using HPTLC

The phytochemical profile of *Solanum* species has been described as consisting of a wide variety of phytochemicals which comprises steroidal alkaloids, steroidal saponins, phenolic compounds, and terpenoids (Al Sinani and Eltayeb, 2017; Singh and Singh, 2010). Phenolic compounds and steroidal compounds have been identified as the predominant type of phytochemicals in *Solanum* species (Kaunda and Zhang, 2019). This section investigated the profile of the classes of phytochemicals that may be present in the different crude extracts of the fruit and root of *S. incanum*, particularly in search of those associated with pesticide activity. The profile of the classes of phytochemicals contained in *S. incanum* 0was investigated after being aware that steroidal alkaloids, terpenoids, and phenolic compounds from *Solanum* species are linked to pesticidal activity (Chowaski et al., 2016).

Quercetin and solasodine were used as standards to profile phenols and steroidal glycoalkaloids compounds present in the crude extracts of the fruit and root of *S. incanum*. HPTLC profile of the crude extracts of the fruit and root was generated in two solvent systems of different polarities to ascertain the total number of chemical components - a lower polarity that comprised toluene: ethyl acetate: formic acid (80:19.5:0.5 v/v/v) and a more polar solvent system that consisted of dichloromethane: methanol (95:5 v/v). A mixture of 10% concentrated

sulphuric in methanol was used as a universal derivatisation spray reagent. Concentrated sulphuric acid in methanol is considered a universal spraying reagent and is normally used to detect, in general, the number of spots (components) available in a sample (Ramakrishna and Bhat, 1987).

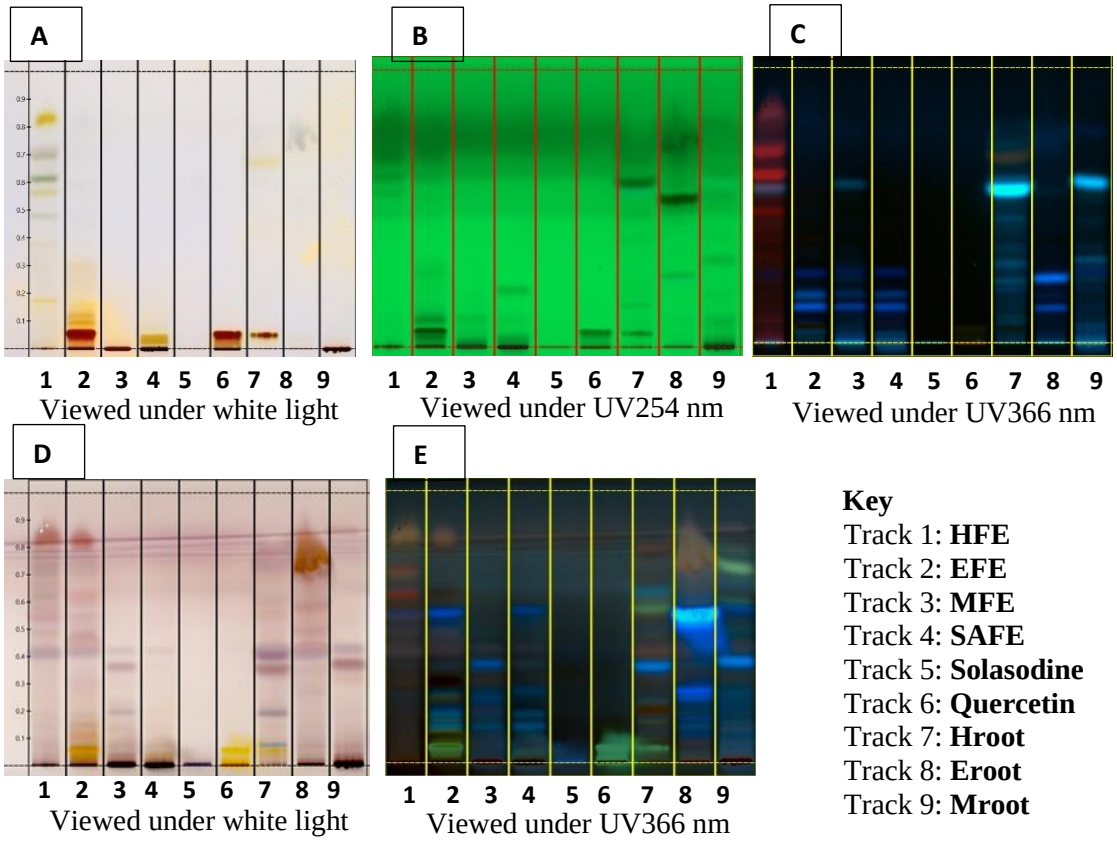


Figure 5.1: HPTLC chromatograms of the fruit and root of *S. incanum.* Plates A, B, and C were viewed under white light, UV 254, and UV 365 nm, respectively, and plates D and E were viewed under 254 nm and 366 nm respectively after derivatisation by 10% sulphuric acid in methanol. Tracks 1-4 and 7-9, were compared to reference standards solasodine and quercetin (Tracks 5 and 6). The HPTLC solvent system used was toluene: ethyl acetate: formic acid (80:19.5:0.5 v/v/v).

The HPTLC fingerprint profiling of the hexane fruit (HFE), ethyl acetate fruit (EFE), methanol fruit (MFE), hexane root (Hroot), ethyl acetate root (Eroot), methanol root, and the steroidal alkaloid fruit (SAFE) extracts of *S. incanum* was carried out using toluene: ethyl acetate: formic acid (80:19.5:0.5 v/v/v). The chromatograms before and after derivatisation were visualised under white light, UV 254 nm, and 366 nm as shown in **Figure 5.1** above.

Many bands were observed under white light after derivatisation. The ethyl acetate fruit extract-EFE (Tracks **2)** and the hexane root - Hroot (track **7)** revealed the highest number of bands. At least eight different types of phytoconstituents were observed from the derivatised ethyl acetate fruit crude extract under white light. The observed dark spots against the green background under a short wavelength (chromatogram **B**) suggested the presence of phenolic compounds (Brahmi et al., 2015). Compounds containing aromatic rings such as phenols absorb UV light at 254 nm, quenching the fluorescence of the pigment present in the silica gel (Brahmi et al., 2015; Jug et al., 2018).

The green bands viewed under UV 366 nm after derivatisation indicated the presence of flavonoids (Chromatogram **E**) (Thenmozhi, 2015). The ethyl acetate fruit (EFE), the hexane root (Hroot), and the methanol root (Mroot) crude extracts showed green bands on the derivatised chromatogram **E** when viewed under UV 366 nm and these observations were used as a confirmation of the presence of flavonoids in the hexane root (Hroot) and the methanol root crude extracts. Quercetin was used as a reference for the detection of flavonoids.

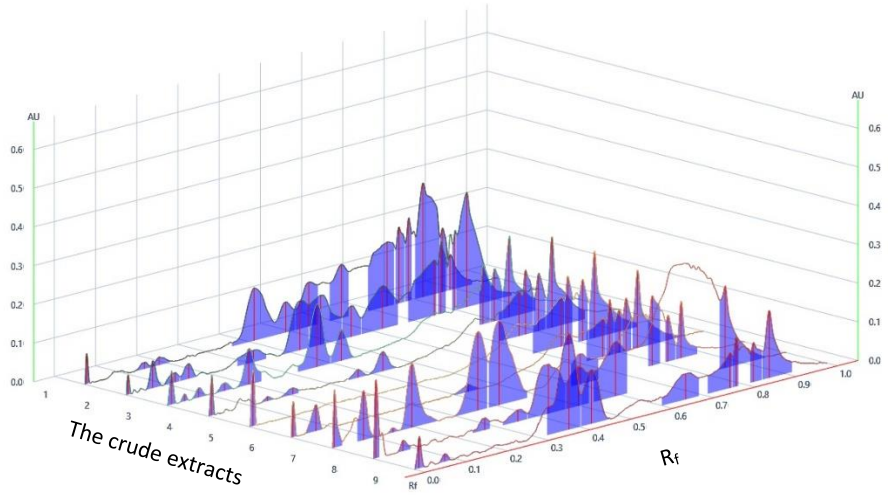


Figure 5.2: HPTLC densitogram of the crude extracts of fruit and root of *S. incanum* under white after derivatisation showing different peaks of phytoconstituents. Tracks 1 to 4 and 7 to 9 represent the crude extracts. Tracks 5 and 6 represent solasodine and quercetin standards.

Quercetin, a common phenolic compound (track **6**) in *Solanum* species was used as a standard for the detection of phenols (fingerprinting). The presence of quercetin was detected in EFE **(**track **2,** and Eroot (track 8) under UV 254 nm in chromatogram **A** (as a brown band) as well

as in chromatogram **B** (as a dark band). The bands have the same R_f value of 0.05 as that of standard quercetin observed on track 6 in **Figure 5.2.**

Terpenoids can be observed under daylight, UV 254 nm, and UV 366 nm before derivatisation and after derivatization, blue, or bluish violet colour under visible light confirm the presence of terpenoids in the sample (Karthika and Paulsamy, 2015). The blue-coloured bands observed in chromatogram **C** under UV 366 nm before derivatisation on tracks 2-4 and 5-7 confirmed the presence of terpenoids and/or saponins (Karthika and Paulsamy, 2015). The R_f values for the blue bands ranged between 0.15-0.60 as shown in the densitogram in **Figure 5.2**. The EFE, SAFE, MFE, and Eroot had a blue band at the same R_f value of 0.15 as observed in chromatogram **C** which indicated that these crude extracts had the same type of phytochemicals.

Solasodine is a steroidal alkaloid that is commonly isolated in *Solanum* species (Fan et al., 2021). Solasodine was used as the standard compound for fingerprinting solasodine and identifying steroidal alkaloids in fruit and root crude extracts of *S. incanum*. While no significant solasodine or steroidal glycoalkaloid-related band was observed on track **5**, where solasodine was spotted when a low polar solvent system (toluene: ethyl acetate: formic acid (80:19.5:0.5 v/v/v)) was used for HPTLC profiling, a more polar solvent system (5% methanol in dichloromethane) was then utilised to develop the plates for the crude extracts. The chromatogram was derivatised with 10% sulphuric acid in methanol.

The HPTLC chromatograms of the crude extracts before and after derivatisation are shown in **Figure 5**.3. The 5% methanol in the dichloromethane solvent system allowed the detection of solasodine on track **5** at an R_f value of 0.03 as indicated in chromatogram **I**. Solasodine was also detected in SAFE and MFE. Similar grey colour bands under the white light after derivatisation were also observed in track 3 (R_f values of 0.21; 0.39 and 0.50), track 4 (Rf value of 0.15), and track 7 (R_f values of 0.35 and 0.52) observed under white light after derivatisation (chromatogram **I**). These grey-coloured bands may be linked to the presence of glycoalkaloids (Milner et al., 2011).

Figure 5.3 of the crude extract before derivatisation under UV 254 nm chromatogram **G**, displayed more dark bands compared to the bands detected in chromatogram **B** when toluene:

ethyl acetate: formic acid (80:19.5:0.5 v/v/v) solvent system was used. According to Brahmi et al, (2015), simple phenolic compounds such as phenolic acids do not fluoresce under UV light but quench to give dark absorbing spots against a green TLC background when observed under UV light 254 nm (Jug et al., 2018). This indicated that more phenolic could be detected in a

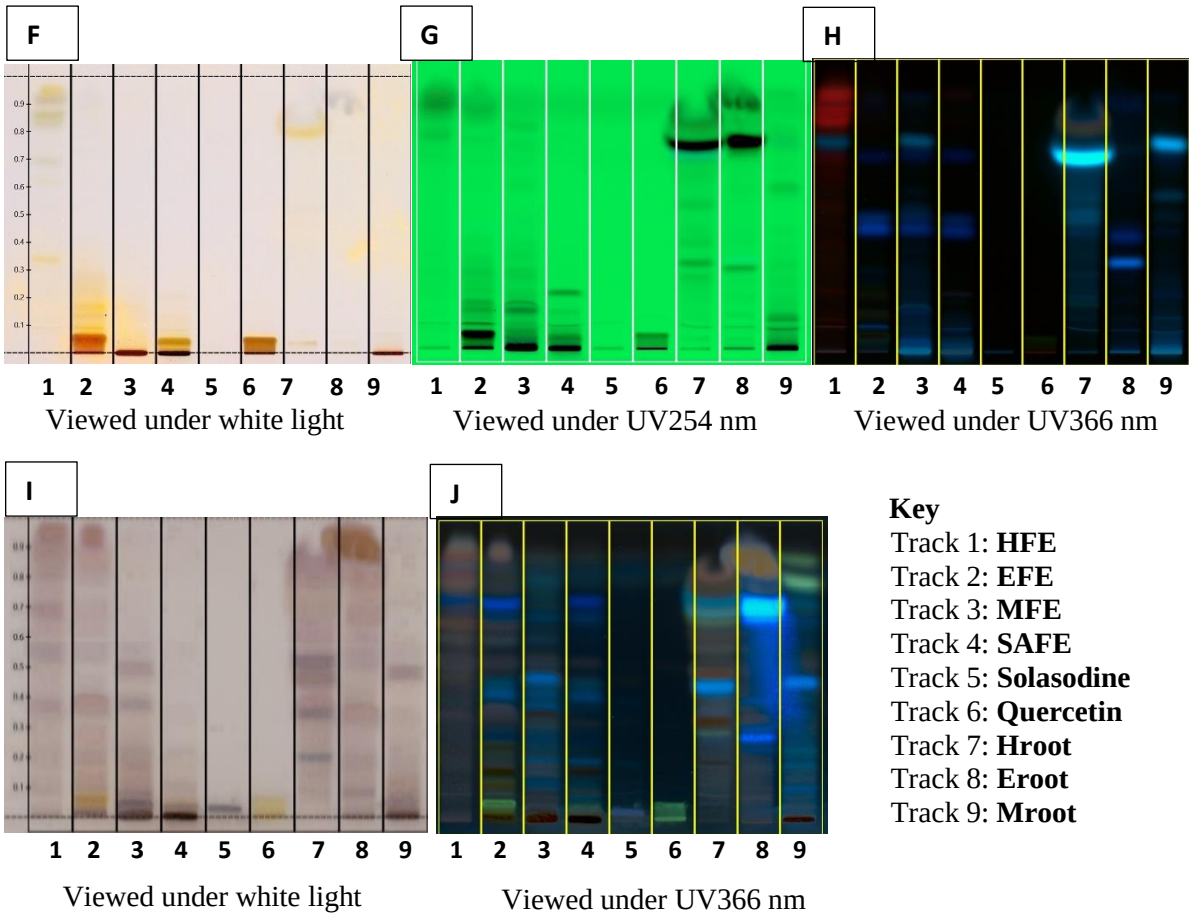


Figure 5.3: HPTLC profiling of the phytoconstituents in the crude extracts of the fruit and root of *S. incanum* using 5% methanol in dichloromethane as the solvent system. Tracks 5 and 6 contained solasodine and quercetin respectively that were used as standards The plates viewed under Plates F to G were viewed under white light, UV 254, and UV 365 nm before derivatisation, and plates I and J were viewed after derivatisation in 10% sulphuric acid in methanol.

more polar solvent system. Under UV 366 nm in chromatogram **K**, dark blue spots at various Rf values indicated phenolic acids (Brahmi et al., 2015). Flavonoids were picked up in chromatogram **J** at various R_f values. The flavonoids depicted a green colour like the colour shown by quercetin which was used as a reference in track **6**. Therefore, the green bands observed in tracks **2**, **7,** and **9** in the derivatised chromatogram **J** under UV 254 nm confirmed the presence of flavonoids in EFE, Hroot, and Mroot respectively.

The results from HPTLC profiling revealed that the fruit and the root of *S. incanum* contained several phytochemicals that included phenolics, glycoalkaloids, saponins, and terpenoids. The observed phytochemical screening results are comparable to those obtained by Babajide et al (2010), who detected alkaloids, and phenolics compounds from the methanolic and ethyl acetate extracts of the fruit of *S. incanum* (Babajide et al, 2010).

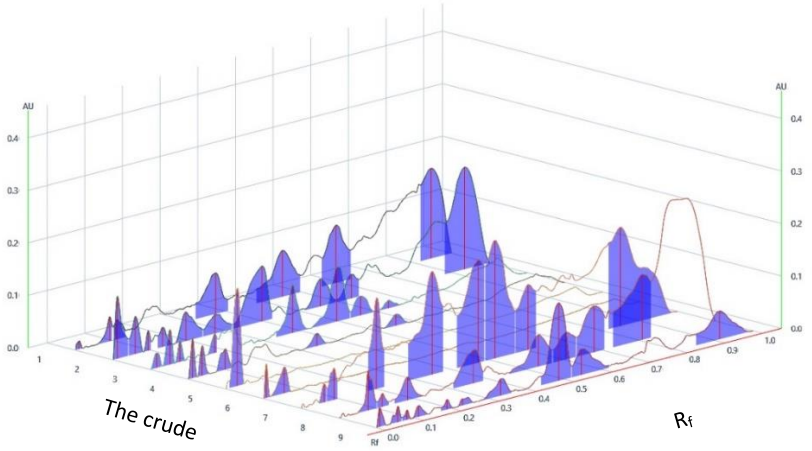


Figure 5.4: HPTLC densitogram of the crude extracts of *fruit and root of S. incanum* under white after derivatisation showing different peaks of phytoconstituents. Tracks 1 to 4 and 7 to 9 represent the crude extracts. Tracks 5 and 6 represent solasodine and quercetin standards

Alkaloids, phenols, saponins, and terpenoids are widely distributed in *Solanum species* and have been reported to exhibit a diverse range of biological activity including antibacterial, anti-cancer, anti-inflammatory, antidiabetic, anticancer, and pesticidal properties (Dasgupta et al, 1979; Charles et al, 1987). Previous studies also suggest that alkaloids are responsible for the toxic effect of the fruit of *S. incanum* (Thaiyah et al, 2011; Chowański et al. 2016), hence the need to carry out the bioactivity assays on the crude extracts. The phytochemical profiling alone is not sufficient, to conclude the phytoconstituents of crude extracts, further quantitative analysis is required to confirm the total content of these types of phytochemicals.

5.2.3 Quantitative analysis

The total phytochemical content varies in plant species according to the region and weather pattern (Kumar et al, 2017). Therefore, there was a need to report quantity estimates of the phytochemicals in *S. incanum* obtained from Mkoba village in Gweru. The UV-vis Spectrophotometry was used to estimate the total phytochemical content of alkaloids, phenolics, flavonoids, saponins, and terpenoids in different crude extracts of the fruit and root of *S. incanum*.

Table 5. 2: The validation parameters obtained for the different standard curves of atropine, quercetin, Gallic acid, and linalool

Parameter	Standard			
	Atropine	**Quercetin**	**Gallic acid**	**Linalool**
Recovery (%)	99.24	99.9	97.9	99.8
SD	3.88	8.438	7.85	6.11
SE of intercept ($x10^{-2}$)	3.32	15.5	19.4	0.082
SD intercept ($x10^{-2}$)	8.14	37.9	43.3	0.184
Mean	6	6	5	6
LOD (x 10^{-2}) mg/mL	1.32*	0.072*	0.000268*	13.8*
LOQ (x 10^{-2}) mg/mL	0.03996*	0.22*	8.08*	42*
Accuracy mg/mL	99.24 ±3.88	99.9±8.44	97.89 ±7.85	99.8±6.11
Gradient	0.055	0.017	0.02	0.45
Intercept	0	0	0.0024	0.113
Linearity range/ mL	0.2-1.2	0.5-3	1-5	0.012-0.062
Correlation (R)	0.996	0.990	0.99	0.99
Variance (R^2)	0.992	0.980	0.98	0.98
Linear equation	y = 0.0556x	y = 0.0173x	y = 0.0214x + 0.0024	y = 0.0196x+ 0.0032

* At 95% confidence level.

The estimated total phytochemical content (mg/ 100 mg crude material) was based on the atropine, gallic acid, quercetin, and linalool standard curves the quantities were equivalence to known standards.

The validation parameters for estimating the quantities of the types of phytochemicals in the extracts are reported in **Table 5.2.** Atropine, quercetin, linalool, and gallic acid were used as standards in the validation process and the parameters which included recovery percentage, Limit of Quantitation **(**LOQ), Limit of Demand (LOD), calibration curves, linearity range and

variance of the analytes were calculated using the Microsoft Excel (2016) version. The (LOQ) values for all three standard curves (**Table 5.2**) showed that the method presented for the different total content estimates was detectable and quantifiable under conditions used as low as 8.08×10^{-4} mg GAE, without suffering an alteration of equipment's intrinsic factors. As reported in table 2, the recovery rate for the quantitative analysis ranged between 99.2 and 99.9% for all the experiments. Standard calibration curves for the standards - atropine, quercetin, linalool, and Gallic acid were plotted (**Appendix 5.1)**.

The total alkaloid content estimation was based on the formation of a yellow alkaloid-bromocresol green (BCG) complex and the absorbance of this complex is proportional to the concentration of alkaloids present (Gupta et al, 2013; Ushir et al, 2011). The total alkaloidal estimation was then calculated using the atropine (ATP) standard calibration curve, indicated in **Table 5.2**. The estimation of the total phenolic content of the extracts was because, in an alkaline medium, phenols reduce the mixture of phosphotungstic and phosphomolybdic acid present in the Folin-Ciocalteu reagent to a blue-coloured tungsten and molybdenum oxide chromophore which is proportional to the concentrations of phenolic compounds present in the extracts (Ushir et al, 2011). The quantity of the phenolics was estimated using the Gallic calibration curve (**Table 5.2**).

The total flavonoids content of the extracts was measured based on the formation of a yellow flavonoid - Al^{3+} whose intensity is proportional to the concentration of the flavonoids in the sample, which was calculated from the Quercetin calibration curve (**Table 5.2**) (Gupta et al, 2013). The total terpenoids in the crude extract were based on the reaction of the terpenoid with a mixture of chloroform and concentrated sulphuric acid to form a red brick precipitate (Chowański et al, 2016). The concentration of the terpenoid is directly proportional to the intensity of the brick red colour on the UV-spectrophotometer. The total phenolics were calculated using the linalool calibration curve indicated in **Table 5.2**.

The estimation of the saponins was based on the gravitational analysis in which the saponin in the crude extract was dissolved in *n*-butanol and the solution was precipitated to recover the saponin (El Aziz et al, 2019; Harris et al, 20 14).

The quantity estimates of the phytochemicals in the crude extracts are reported in **Table 5.3**. As reported in **Table 5.3**, the alkaloid content was highest in the steroidal crude extract, phenolics were highest in the methanol root, flavonoids in the hexane fruit, terpenoids and saponins in the hexane root at 0.14±0.06 mg ATP /100 mg crude; 3.72±1.28 mg GAE /100 mg crude; 3.62±1.23 mg QCT /100 mg crude; 1.71±0.47mg LIN /100 mg crude and 0.3.74±1.17 mg /100 mg crude respectively The results of quantitative analysis showing the phytochemical content of each plant part are summarised in **Table 5.3.**

Table 5. 3: The results of the total estimates of the phytochemicals in different crude extracts of *S. incanum*

Crude	Alkaloids mg ATP /100 mg sample	Phenolics mg GAE /100 mg sample	Flavonoids mg QCT /100 mg sample	Terpenoids mg LIN /100 mg sample	Saponins mg/100 mg sample
MFE	0.11±0.56	3.49±1.16	1.18±0.93	1.16±0.79	0.79±0.15
Mroot	0.03±0.44	3.72±1.28	0,99±0.07	0.64±0.08	2.82±0.68
EFE	0.05±0.11	3.53±1.05	0.34±0.16	1.03±1.03	3.3±1.15
Eroot	0.08±0.24	3.14±1.22	0.41±0.10	0.36±0,14	2.67±1.01
HFE	0.03±0.06	2.52±1.09	3.62±1.23	1.06±0.44	2.02±0.27
Hroot	0.06±0.03	2.84±0.87	2.35±1,01	1.19±0.47	3.74±1.17
SAFE	0.14±0.06	0.19±0.11	0.33±0.15	0.1±0.07	0.42±0.17

*Each experiment was carried out in triplicate and results were expressed as Mean ± SD (N=3) at 95% confidence level.

HFE: Hexane fruit extract
EFE: Ethyl acetate fruit extract
Mroot: Methanol root
SAFE Steroidal alkaloid fruit extract
MFE: Methanol fruit extract
Hroot: Hexane root
Eroot: Ethyl acetate root

The LOQ values for the standard curve for atropine, quercetin, and Gallic acid were ATP, 0.00219 mg QCT, 0.000644 mg LIN, and 0.000808 mg GAE respectively. The LOQ values for all three standard curves showed that the method presented for the different total content estimates was detectable and quantifiable under conditions used as low as 8.08×10^{-4} mg GAE, without suffering an alteration of equipment's intrinsic factors.

The calibration curve ($y = 0.0556x$; $R^2 = 0.993$) for atropine (Graph 1 in **Appendix 5.1**) was used for the calculation of the total alkaloid content in the crude extracts. The highest alkaloid content of 0.14±0.06 mg ATP /100 mg sample was obtained in the steroidal alkaloid extract (SAFE). The highest flavonoid content was obtained in HFE (3.62±1.23 mg QCT/100 mg

sample), when a standard plot (y = 0.0173x; R^2 = 0.981) for the determination of the total flavonoid content (**Graph 2** in **Appendix 5.1**) was used. It was found that phenolic compounds were most abundant in the methyl root crude extract (Mroot) at 3.72±1.28 mg GAE/100 mg sample. The standard curve used for the total phenolic content estimation was y = 0.0214x + 0.0024 (**Graph 3** in **Appendix 5.1**). Saponins were highest in the hexane root extract (Hroot) with a concentration of 3.74±1.17 mg/100 mg sample. The linalool standard curve (y = 0.0196x + 0.0032; R^2 = 0.9983) for the estimation of the total terpenoid content was used. HFE indicated a total terpenoid quantity of 1.71±0.44 mg LIN/100 mg sample.

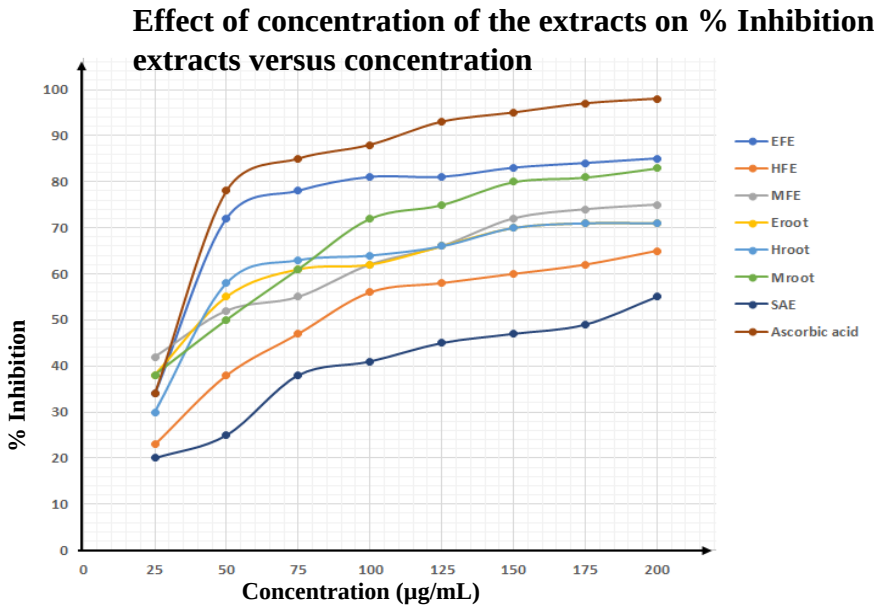
This study's results revealed that phenolic compounds are the most abundant secondary metabolites in *S. incanum* at 33.5%. The high phenolic content probably explains why *S. incanum* fruit is considered one of the strongest antioxidants (Pizzino et al., 2017). Phenolic compounds are composed of hydroxyl groups which are an important part of the compounds for free radical scavenging activities (Maharana et al, 2010). The parentage abundance of saponins was at 16.7% and that of alkaloids was at 0.96%. Alkaloids are responsible for the bitter taste and the toxicity effect of the *S. incanum* fruit although they are found in smaller amounts compared to the other phytochemicals such as phenols and saponins (Thaiyah et al., 2011).

The total phytochemical quantities in *S. incanum* crude extracts were compared with the other crude extracts of other *Solanum* species. The estimated quantity of phenolic compounds in the Mroot of *S. incanum* (3.72±1.28 mg GAE/100 mg sample) was comparable to the total content of phenolic compounds found in the roots of *S. nigrum* (3.01±1.11 mg GAE/100 mg sample and *S. Torvum* (2.94±0.86 mg GAE/100 mg sample) when a similar standard was used

The total alkaloid content was the lowest compared to other phytochemicals in other crude extracts for *S. incanum*. A similar trend in quantities of alkaloids was observed in an investigation in which the total content of alkaloids, flavonoids, and terpenoids in a methanol crude extract of the fruit of *S. incanum* was estimated (Janarthanam and Sumathi, 2015). Their investigation revealed that alkaloids had a concentration of 0.07 mg ATP/100 mg, 1.09 mg QCT /100 mg, a sample of flavonoid, and 1.14 mg LIN/100 mg sample of the terpenoids (Janarthanam and Sumathi, 2015).

5.2.4 The Antioxidant assay

The anti-oxidative properties of the crude extracts (MFE, Mroot, EFE, Eroot, HFE, Hroot, and SAFE) were investigated using 1.0×10^{-5} $\text{mol} \cdot \text{dm}^{-3}$ 1,1-Diphenyl-1-picrylhydrazyl (DPPH). The scavenged amount of the DPPH by each crude extract expressed as a percentage inhibition is presented in **Figure 5.5**. Ascorbic acid was used as the control in the assays.



HFE: Hexane fruit extract
EFE: Ethyl acetate fruit extract
Mroot: Methanol root
SAFE Steroidal alkaloid fruit extract

MFE: Methanol fruit extract
Hroot: Hexane root
Eroot: Ethyl acetate root

Figure 5. 5: Free radical-scavenging activity of extracts from *S. incanum* measured by DPPH assay. Results are mean ± SD (n=3) ($p<0.05$).

At $200\mu\text{g} \cdot \text{mL}^{-1}$, the percentage inhibition of the following crudes: EFE and Mroot were as high as 85% and 83%, respectively. The scavenging activity of these three extracts was highly comparable to that of the positive control-ascorbic acid, which was at 98% at a concentration of $200\mu\text{g} \cdot \text{mL}^{-1}$. The lowest scavenging activity was realised in steroidal crude extract (SAFE), which had a 55% inhibition at a concentration of $200\mu\text{g} \cdot \text{mL}^{-1}$.

The ant-oxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions such as iron and copper, and inhibition of

enzymes responsible for free radical generation (Muruhan et al, 2013). Depending on their structure, flavonoids can scavenge practically all known reactive oxygen species (ROS) (Salerno et al., 2014).

The results revealed that the phytochemicals in all seven crude extracts of *S. incanum* had significant antioxidant potential. According to Gulcin (2020), antioxidants play a vital role in reducing oxidative processes and harmful effects of ROS in the human body (Gulcin, 2020). The high antioxidant activity of the fruit of *S. incanum* explains why some communities in Africa and India use some of the *Solanum* species as food (Pizzino et al., 2017). The inhibitory concentration (IC_{50}) of the fruit and root crude extracts of *S. incanum* is summarised in **Table 5.4**.

Table 5.4: The inhibitory concentration (IC_{50}) of the fruit and root crude extracts of *S. incanum*

The Crude Extract	Code	IC_{50} (µg·mL^{-1})
Ethyl acetate fruit	EFE	35.53±0.53
Hexane fruit	HFE	149.93±1.19
Methanol fruit	MFE	44.7±0.37
Ethyl acetate root	Eroot	39.63±0.54
Hexane root	Hroot	39.93±0.21
Methanol root	Mroot	59.67±0.37
Steroidal alkaloid fruit	SAFE	193.77±0.45
-	Ascorbic acid	34.86±0.25

*Each experiment was carried out in triplicate and results were expressed as Mean ± SD (n=3) at a 95% confidence level

HFE: Hexane fruit extract
EFE: Ethyl acetate fruit extract
Mroot: Methanol root
S**AFE** Steroidal alkaloid fruit extract
MFE: Methanol fruit extract
Hroot: Hexane root
Eroot: Ethyl acetate root

The IC_{50} values of the crudes ranged from 35.8 to 193.7 $\mu g \cdot mL^{-1}$. A lower IC_{50} value implied that the sample's potency in scavenging the DPPH free radicals was high. EFE, Eroot, and Hroot had low IC_{50} values of 35.8, 39.5 and 39.9 $\mu g \cdot mL^{-1}$ respectively and these values were highly comparable to the IC_{50} value of the positive control which was ascorbic acid which recorded an IC_{50} of 34.9 $\mu g \cdot mL^{-1}$.

The results showed that the root and fruit of *S. incanum* have antioxidant properties. Overall, the root extracts of the plant exhibited higher DPPH radical scavenging activity than the fruit extracts and the potency of the extract depends on the extraction solvent.

5.2.5 Efficacy of the crude extracts

A leaf dip assay was used to investigate the efficacy of the crude extracts against the cabbage aphids. **Table 5.5** reports the percentage of aphid mortality rates at different concentrations which were obtained after 24 hours. All seven crude extracts indicated some pesticidal activity against the cabbage aphids. Generally, the efficacy of the extracts increased with an increase in the concentration. MFE and SAFE extracts indicated the highest aphid mortality of 52.3±6.33% and 60.6±8.67 % at 25 $\mu g \cdot mL^{-1}$.

Table 5.5: The percentage of aphid mortality rate at different concentrations measured after 24 hours.

	Concentration ($\mu g \cdot mL^{-1}$)	**25**	**100**	**200**	**300**
		% Aphid mortality			
Crude extract	MFE	52.3±6.33a	71.7±7.99a	85.3±11.33a	90.3±9.01a
	Mroot	32.3±4.12c	48.7±8.24c	52.3±4.44b	53.3±9.82c
	EFE	40.7±3.33a	86.6±9.20a	94.3±10.66a	98.7±12.56a
	Eroot	35.3±6.67c	58.3±7.75b	70.7±7.63a	73.3±9.63b
	HFE	22.3±5.67c	40.7±6.78c	48.3±8.78b	56.7±7.56cc
	Hroot	28.3±4.33c	42.3±5.14c	49.3±5.92b	57.7±8.66
	SAFE	60.6±8.67b	83.3±8.86a	87.7±6.54a	91.7±10.78a
	Control*	70.7±10.64b	90.3±10.65d	85.7±12.33a	90.3±10.54a

- Each value in the mean of 3 replications with ±SD
- In a column showing mortalities, values denoted by the same letter are not significantly different by LSD at $p \leq 0.05$
- In a row showing mortalities, values denoted by different letters are significantly different by LSD at $p \leq 0.05$

**Positive control

A Probit transformed technique was used to transform the percentage of aphid mortalities into regression curves (**Figure 5.6**) to enable the determination of the LD_{50} as indicated in **Table 5.5.** The LD_{50} of the MFE and Mroot was comparable to that of the control used (Bambazonke) which had an LD_{50} of 10.19±1.15 $\mu g \cdot mL^{-1}$.

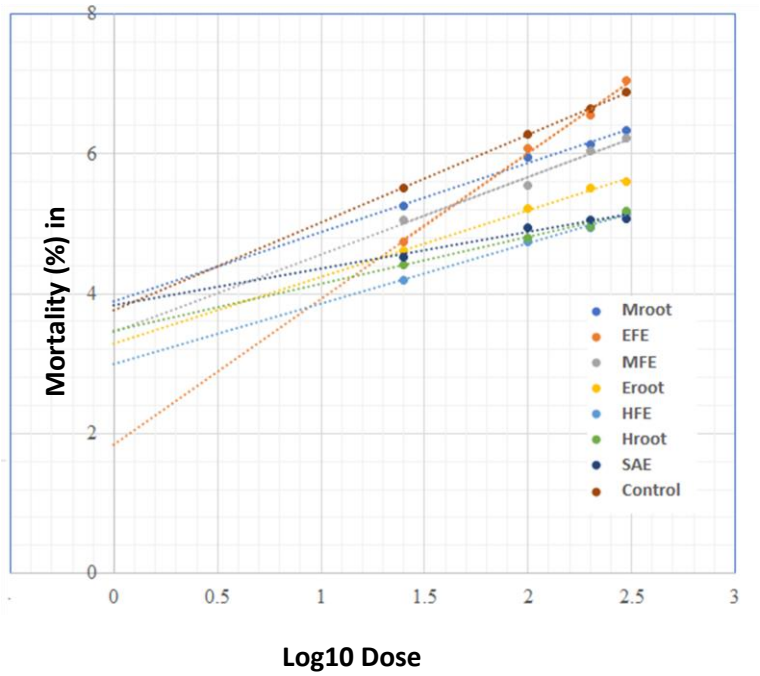


Figure 5.6: The Probit transformed mortality

As reported in **Table 5.6**, the MFE indicated a high lethal dose against the cabbage aphids followed by the steroidal extract with a lethal dose (LD_{50}) of 13.16±3.78 µg·mL^{-1} and 24.82±1.13 µg·mL^{-1}.

Table 5.6: The Lethal dose LD_{50} values of the *S. incanum* crude extracts against cabbage aphids using an aphid-leaf dip method

Crude extract	**Code**	***LD_{50}** (µg·mL^{-1})
Steroidal alkaloid fruit	SAFE	24.82±1.13a
Methanol fruit	MFE	13.16±3.78b
Ethyl acetate	EFE	32.64±5.44a
Ethyl acetate root	Eroot	62.3±11.68c
Hexane fruit	HFE	95.92±14.21d
Hexane root	Hroot	169.04±21.25e
Methanol root	Mroot	194.9±16.84e
Bambazonke pesticide	Control	10.19±1.55e

*The results are expressed as (mean ± SD), N = 3

**If the letters next to the LD_{50} are different, it shows that the values are significantly different by LSD ($p \leq 0.05$)

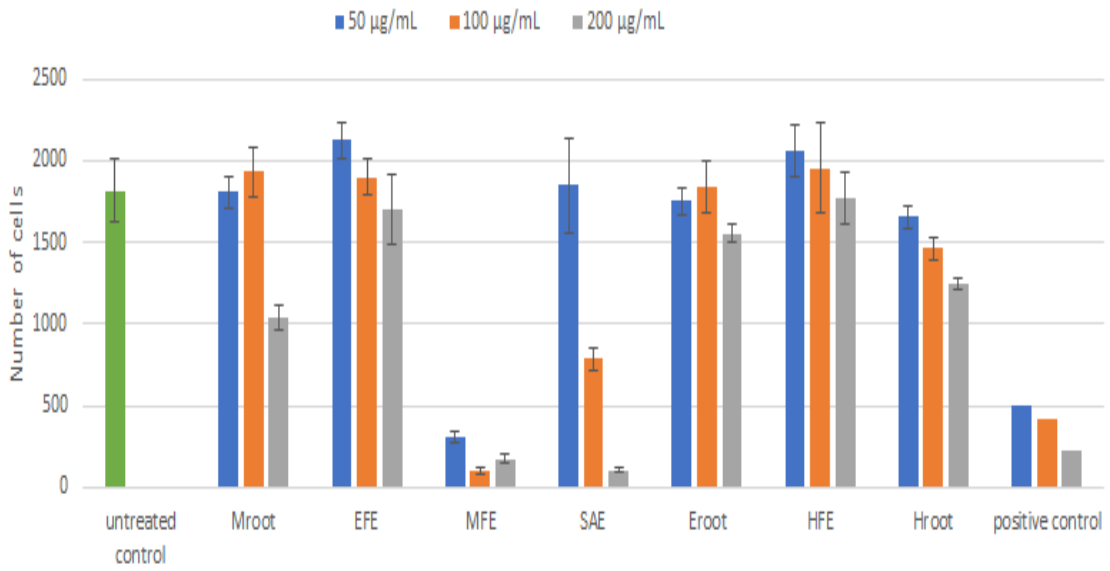
The findings agree with that of (Mhazo, 2018; Belmain et al., 2001), in which the investigated methanol crude extracts of several *Solanaceae* species exhibited an aphid mortality rate above 70%. (Belmain et al., 2001) deductions were encored by (Madzimure et al., 2013), who investigated the effect of *S incanum* fruit against cattle ticks. The results indicated that the fruit had biological activity against the cattle ticks, with a tick mortality percent of 58.8 compared to that of the positive control (tickbuster®) which recorded 100% tick mortality in 48 hours.

In another study conducted by Koul and Walia, 95% ethanol extract of *S. incanum* significantly affected the mortality rate of ticks in a dose-dependent manner ranging from 24% to 93±5% with an additional effect on the reproductive physiology of ticks by inhibiting 36.2% (Koul and Walia, 2009).

Based on the results of the analysis effect of the crude extracts on the mortality rate of the cabbage aphids, it can be concluded that the extracts of *S. incanum* from the fruits and the roots can be used to control the cabbage aphids. It was realised also that the higher the dosage and the more the crude extracts become more effective. Therefore, the fruits and the roots of *S. incanum* contain toxins that can control the cabbage aphids that brassica olecerea. The next sections present the results of the efficacy of the isolated compounds and the cytotoxicity of the crude extracts.

5.2.6 Cytotoxicity test

Cytotoxicity was evaluated in Vero cells using the propidium iodide (PI) assay. Seven crude samples (MFE, Mroot, Eroot, EFE, HFE, SAFE, and Hroot) were screened for cytotoxicity after 48 hours. The monkey kidney cell lines were used for the cytotoxicity investigation. The results obtained are shown in **Figure 5.7**. In general, after 48 hours, all crude extracts exhibited cell viability above 50% at 50 to 200 $\mu g \cdot mL^{-1}$. The cytotoxicity of the MFE and the SAFE were comparable to that of the melphalan at concentrations lower than 200 $\mu g \cdot mL^{-1}$.



Key:

Crude extract	Code	Crude extract	Code
Steroidal alkaloid fruit	SAFE	Hexane fruit	HFE
Methanol fruit	MFE	Hexane root	Hroot
Ethyl acetate	EFE	Methanol root	Mroot
Ethyl acetate root	Eroot	Bulalazonke pesticide	Control

Figure 5.7: Cytotoxicity of *S. incanum* crude extracts against Vero cells. Melphalan was used as a positive control at 10, 20, and 40 µM. Data points represent the mean ± SD of quadruplicate (4) determinations. $p<0.05$

The Steroidal crude extract (SAFE) and the methanol fruit (MFE) possessed strong cytotoxic activity against Vero cells, killing approximately 95% of the Vero cells at less than 100 $\mu g \cdot mL^{-1}$ of the concentration. Eroot and Mroot showed toxicity at a higher concentration of 200 $\mu g \cdot mL^{-1}$ while the EFE and HFE exhibited the lowest cytotoxicity. Therefore, the four samples MFE, SAFE, Mroot, Eroot, and Hroot which displayed high cytotoxicity against the Vero cells are possible candidates for further anti-proliferative testing.

The results obtained in this study agreed with the previous cytotoxicity tests carried out on crude extracts of other *Solanum* species. For instance, an *In vitro* study carried out by Chun-Nan et al (1990) revealed that the fruit of the *Solanaceae* plants possesses cytotoxicity activity against human PLUPRF/S cells in vitro (Chun-Nan et al., 1990). Another *in vitro* study performed by Assefa et al (2006) reported several glycoalkaloids as cytotoxic which include solamargine, solasodine, and solanine (Assefa et al., 2006). Solamargine and solasonine possess

inhibitory effects against JTC-26 cells in vitro (Vijayan et al., 2002). A study was carried out to determine the toxicity of unripe fruits of *S. incanum* in sheep and revealed that the fruit was highly toxic to sheep. The animals exhibited signs of blood clots, coughing, cerebellar hyperplasia, pneumonia, froth in the bronchi, lung emphysema, and ulcers (Thaiyah et al., 2011). Based on this, a study of *S. incanum* fruit and roots is more likely to result in the discovery of more effects associated with *S. incanum* and not only on the fruit but on the roots as well. By exhibiting the most potent cytotoxic activity against Vero cells, *S. incanum* roots have the greatest potential for the discovery of cytotoxic compounds.

5.3 Conclusion

This chapter aimed to carry out a profile of the phytochemicals of the fruit and root extracts and to investigate the antioxidant, cytotoxicity, and efficacy of *S. incanum* against the cabbage aphids. The HPTLC profiling of the methanol, ethyl acetate, and hexane crude extracts revealed that the root and fruit of *S. incanum* have different types of phytochemicals that include alkaloids, phenolic compounds, terpenoids, flavonoids, and saponins. The quantitative screening of the methanol, ethyl acetate, and hexane crude extracts of the root and fruit of *S. incanum* indicated significant amounts of alkaloids, flavonoids, saponins, terpenoids, and phenolic compounds

The crude extracts from the root and the fruit of *S. incanum* possess biological activities that included antioxidant, cytotoxicity, and pesticidal properties. The ethyl acetate fruit extract (EFE) could be a promising source of antioxidant phytochemicals because of its high antioxidant activity. The fruit extracts performed better than the root extracts confirming their importance in their traditional medicinal use. The high scavenging activity in the EFE and the MFE is attributed to the high estimation of the phenolic and alkaloid content detected in the fruit of *S. incanum* (Almoulah, 2017). The high antioxidant activity in the fruit, therefore, confirms and supports the use of *S. incanum* fruit as an antioxidant in the Gweru community in Mkoba. The root exhibited less potent inhibition compared to the root.

The methanol fruit (MFE) had a significant cytotoxic value of less than 20% cell viability at less than 100 $\mu g \cdot mL^{-1}$ in concentration, indicating that it may be valuable as a source of cytotoxic phytochemicals that can be used in manufacturing anticancer drugs. The presence of

alkaloids in the fruit and root crude extracts supports the exhibited cytotoxicity properties. Literature also supports that the presence of alkaloids (glycoalkaloids in particular) in *S. incanum* contributes immensely to the cytotoxicity properties exhibited in the fruit.

The community uses crude extracts as the pesticide and due to the pre-harvesting period stated by the respondents, the community likely eat vegetable which still has some crude extract residue. Therefore, there is a higher chance that the crude extracts used as the pesticide from the root and fruit of *S. incanum* contribute to the symptoms realised by the community. There is no information about the half-life of the crude extracts of *S. incanum* fruit used as pesticides, but based on other botanical pesticides' half-lives, the probability that the Gweru community harvest and consume vegetables that contain chemical residues is high. According to Thaiya and co-workers, the chemicals from these residues accumulate in consumers' bodies until they become poisons (Thaiya et al, 2011).

In general, the fruit crude extracts exhibited a highly potent efficacy against cabbage aphids compared to the root extracts. The efficacy against the cabbage aphids was high in the methanol fruit (MFE) and the steroidal alkaloid (SAFE) crude extracts although the crude efficacies were significantly lower than the positive control.

From the findings observed in this chapter on the efficacy screening of the fruit and root, we can deduce preliminary confirmation of their pesticidal use in the management of cabbage aphids in Gweru. The isolated phenolic and alkaloids exhibited favourable efficacies against the cabbage aphids. However, further analysis is required to ascertain the complete mechanisms of pesticidal activity of the fruit and root of *S. incanum* and their isolated compounds.

5.4 Experimental

5.4.1 Solvents and reagents

Unless otherwise stated, all chemicals and reagents were purchased from Sigma Aldrich (South Africa). These were hexane, ethyl acetate, toluene, petroleum ether, *n*-butanol, methanol, sodium hydroxide (NaOH), hydrochloric acid, (HCl), phosphotungstic, $H_3PW_{12}O_{40}$ and phosphomolybdic, $H_3PMo_{12}O_{40}$ acids, Folin-Ciocalteu reagent (FCR), quercetin, sodium carbonate (Na_2CO_3), gallic acid, ($C_7H_6O_5$ aluminum (III) chloride ($AlCl_3$), and quercetin ($C_{15}H_{10}O_7$). Vero cells were purchased from Cellonex, South Africa. Dulbecco's Modified Eagle Medium (DMEM) and Foetal Bovine Serum (FBS) were purchased from GE Healthcare Life Sciences (Logan, UT, USA). PBS with and without Ca^{2+} and Mg^{2+} and trypsin were purchased from Lonza (Walkersviıle, MD, USA). Bis-benzamide H 33342 trihydrochloride (Hoechst 33342) and propidium iodide (PI) were purchased from Sigma (St. Louis, MO, USA).

5.4.2 Plant material

The plant material comprised fruits and roots of *S. incanum* collected from bushes surrounding Mkoba village (latitude -19.4500008 and longitude 29.8166695 in the southern hemisphere (Matsa et al., 2019), based on the ethno-pesticidal/medicinal information provided by the local community who were using the plant for various medicinal and pesticidal activities. Mr Chirume a herbalist in the community accompanied me to identify and harvest the *S. incanum* specimen. The collection of the specimen was done on the 5th of May 2018. The plant material was processed in Zimbabwe and then transported to Nelson Mandela University, South Africa, as biomass. Plant authentication was performed by Tony Dold, a curator, and taxonomist at Selmar Schonland Herbarium (GRA) in Makhanda, Eastern Cape, South Africa, where a specimen of *S. incanum L.* with voucher number Zivanayi012 (GRA) was deposited.

5.4.3 Extraction and quantitative analysis

The methods of solvent extraction described by Gupta *et al* and Ushir *et al* were utilised in this study (Gupta et al, 2013; Ushir et al, 2011). The fruit biomass (546 g) and the root biomass (148 g) were extracted sequentially with hexane, ethyl acetate, and biomass using the maceration method for 72 hours, to obtain crude extracts of fruit and root labelled as, hexane

fruit, HFE, ethyl acetate fruit crude, EFE, methanol fruit, MFE, hexane root, Hroot, ethyl acetate root, Eroot, and methanol root, Mroot. Each extract was filtered using a Buchner funnel under vacuum, and the solvent was concentrated *in vacuo* using rotary evaporation (Buchi, Switzerland) at 50 °C. All extracts were stored in a refrigerator at 4 °C until the time needed for the analysis. The experimental procedure for the steroidal alkaloid extraction (SAFE) was as follows: The coarse powdered dry fruit (200 g) of *S. incanum*, was transferred to a 5 L round bottom flask and was defatted with 300 mL petroleum ether to yield a greenish-yellow oil. The oil was filtered off and the residue was then refluxed with 32% v/v conc. HCl, water, and toluene in 1:2:3 ratios for 5 hours. The reaction mixture was subsequently made alkaline with 1M NaOH, to a pH of 10, then refluxed again for 2 hours. After refluxing, the upper pale-yellow toluene layer was siphoned out and the lower aqueous layer was extracted with toluene three times. The toluene layer was acidified with 25% v/v acetic acid and the mixture was allowed to stand for an hour. The toluene layer was then filtered off and the acetic acid layer was made alkaline with 25% v/v NH_4OH to a pH of 10. The mixture was briefly heated and then cooled at room temperature to precipitate steroidal glycoalkaloids. The precipitate was filtered off and washed with cold water and dried in air. The SAFE was stored at 4 °C until the time needed for the analysis.

5.4.4 High-Performance Thin Layer Chromatography (HPTLC)

HPTLC profiles of different crude extracts were developed using glass TLC plates precoated with silica gel 60 F_{254}. A CAMAG instrument, Version 2.5.18262.1 was used for the profiling using suitable solvent systems. A similar procedure for the HPTLC was used for each of the crude extracts except in the variation of the mobile phase and the staining reagents.

5 mg of each test sample (MFE, EFE, HFE, Mroot, Hroot, Eroot, and SAFE) was dissolved in respective solvents. The solutions were filtered into a vial. The TLC glass plate (200 × 100 mm) was set into a CAMAG Linomat V Automatic Sample Spotter (Camag Muttenz, Switzerland). A volume of 6 µL for each test sample, was loaded as 8 mm bands using a CAMAG microliter syringe on the glass TLC plate. The plate was developed using toluene: ethyl acetate: formic acid (80:19.5:0.5 v/v/v) mobile phase. The chromatogram was developed in the twin trough glass chamber (200 x 100 mm) pre-saturated with 25 mL appropriate mobile phase for 8 minutes. The chromatogram was developed up to the length of 70 mm. The plate was allowed

to dry at room temperature in the air before scanning them at UV254 nm, 366 nm, and white light.

The air-dried plates were kept in a photo documentation chamber (CAMAG REPROSTAR 3) and captured the images in visible light, UV 366 nm, and UV 254 nm before spraying with detection reagent (10% concentrated sulphuric acid in methanol). After derivatisation, the plates were then heated at 110 °C for 3 minutes and then scanned to capture the images at ordinary light, UV 254 nm, and 366 nm. The R_f values and colour of the resolved bands were recorded by WIN CATS (1.3.4 version) software.

For the second experiment, a more polar solvent system of 5% methanol in dichloromethane was used. The plate development process was repeated as in the first experiment until the chromatograms were visualised and the images were scanned and captured at ordinary light, UV 254 nm and 366 nm.

5.4.5 Quantitative analysis

Spectrophotometric analysis was used to estimate the quantitative analysis of the extracts via the use of calibration curves of selected standards of the major phytochemicals under this study. The total alkaloid content was assessed using the bromocresol green (BCG) method with atropine standard as described by Gupta *et al* and Ushir *et al* with little modification (Gupta et al, 2013; Ushir et al, 2011). Atropine was used as the reference standard to generate the calibration curve, as indicated in **Table 5.1**. The absorbance of the yellow BCG- atropine complex in chloroform as well as the extracts was measured in triplicates at 470 nm against a blank- chloroform, using SHIMADZU 3100 UV-VIS spectrophotometer. The total alkaloid content was estimated and expressed in milligram atropine equivalent per 100 mg of extract (mg ATP/100 mg extract).

The total phenolic content of each extract was assessed using the single electron transfer mechanism of the Folin-Ciocalteau reagent as described by Ushir et al with minor modifications (Gupta et al, 2013). The standard gallic acid (GAE) was used to generate the calibration curve, as indicated in **Table 5.2**. The absorbance of the standard was measured at 760 nm and recorded against reagent blank- methanol. The total phenolic content was estimated and expressed in milligrams of gallic acid equivalent per 100 milligrams of the extract (mg GAE/100 mg extract).

The total flavonoid content of each extract was estimated using the aluminum-chloride colorimetric assay described by Gupta et al with slight modification (Gupta et al, 2013). Quercetin was used as the reference standard to generate the calibration curve as indicated in **Table 5.2**. The total flavonoid content was estimated and expressed in milligram quercetin equivalent per 100 milligrams of extract (mg GAE/100 mg extract).

The total terpenoid content method was adopted from Ghora *et al* with minor modifications (Ghora et al, 2012). The total terpenoid content of the extracts was estimated using a linalool calibration curve as indicated in **Table 5.2**. The absorbance was measured against a blank at 538 nm. The total terpenoid content of each sample was estimated and expressed in milligram linalool equivalent per 100 milligrams of extract (mg LIN/100 mg extract).

For the estimation of saponins, El Aziz et al's method was adopted (El Aziz et al, 2019). An estimated quantity of the saponins was carried out by dissolving 2.0 g of each crude extract sample in 50 mL of 20% aqueous ethanol. The solution was heated under reflux for 4 hours at 55 °C. The resulting mixture was filtered and then concentrated to 50% of the original volume, extracted with diethyl ether. The aqueous layer was recovered while the ether layer was discarded. The aqueous extract was purified by adding 60 mL n-butanol and then washed with 10 mL of 5% aqueous NaCl (aq). The solution was dried and the saponin content was calculated as follows:

$$\% \text{ Saponins} = \frac{\text{Mass of saponin}}{\text{Mass of the sample}} \times 100 \qquad (1)$$

5.4.6 Antioxidant activity

The antioxidant activities of the *S. incanum* crude extracts were validated using the 1,1-diphenyl-2picrylhydrazyl (DPPH) assay. The DPPH radical scavenging activity was estimated based on the method used by Veerapagu et al (2018). Ascorbic acid was used as a standard. Test samples were dissolved separately in appropriate solvents to a concentration of $1.0\ \text{mg} \cdot \text{mL}^{-1}$. Series of crude sample dilutions to make concentrations of 25; 50; 75; 100; 125; 150; 175 and $200.0\ \mu\text{g} \cdot \text{mL}^{-1}$ were prepared. Assays were performed in vials by adding 2 mL of $1.0 \times 10^{-6}\ \text{mol} \cdot \text{dm}^{-3}$ DPPH solution. The mixture was shaken gently and left to stand for 30 min in the dark at room temperature. The absorbance was measured

spectrophotometrically at 517 nm using a SHIMADZU UV-3100 UV-VIS spectrophotometer. Methanol was used as the blank. Ascorbic acid was used as a reference (control) antioxidant compound and was prepared the same way as the above crude samples. Each analysis was done in triplicate. The percentage of the DPPH scavenging effect was calculated using the equation below.

$$\text{DPPH \% scavenging effect} = \frac{A_c - A_S}{A_C} \times 100 \qquad (2)$$

A_s – Absorbance of the sample.
A_c - Absorbance of the DPPH solution without any antioxidant

Results were expressed as mean ± SD. The IC_{50} values were calculated using Microsoft Excel 2016 version.

5.4.7 Cytotoxicity

(a) Sample preparation

Test extracts were reconstituted in dimethyl sulfoxide (DMSO) to give a final concentration of 100 $mg \cdot mL^{-1}$. Samples were sonicated if solubility was a problem. Samples were stored at 4°C until required.

(b) Treatment protocol

The African green monkey kidney cell line, Vero cells, was used for cytotoxicity screening. They were maintained at 37°C in a humidified incubator with 5% CO_2 in 10 cm culture dishes. The complete growth medium consisted of DMEM supplemented with 10% FBS. Cells were seeded into 96 well microtiter plates at a density of 3000 cells/well using a volume of 100 µl in each well. The microtiter plates were incubated at 37°C, 5% CO_2, and 100% relative humidity for 24 hours before the addition of test samples to allow for cell attachment.

Cells were treated with 50, 100, and 200 µg/mL of each extract and 10, 20, and 40 µM melphalan as the positive control, diluted in a culture medium. One hundred microliter aliquots of the diluted extract in the fresh medium were used to treat cells. Cells were incubated for a further 48 hours.

The treatment medium was aspirated from all wells and replaced with 100 µL of Hoechst 33342 nuclear dye (5 $\mu g \cdot mL^{-1}$) and incubated for 20 minutes at room temperature. Thereafter, cells were stained with propidium iodide (PI) at 100 $\mu g \cdot mL^{-1}$ to enumerate the proportion of dead cells within the population. Cells were imaged immediately after the addition of Pyridium iodide (PI) using the ImageXpress Micro XLS Widefield Microscope (Molecular Devices) with a 10x Plan Fluor objective and DAPI and Texas Red filter cubes. Nine image sites were acquired per well which is representative of roughly 75% of the surface area of the well.

(b) Data Quantification

Quantification of live and dead cells for the screening assay was performed using the ImageXpress Micro XLS Widefield Microscope (Molecular Devices) and acquired images were analysed using the MetaXpress software and Multi-Wavelength Cell Scoring Application Module. Acquired data were transferred to an EXCEL spreadsheet and data was analysed and processed.

5.4.8 Efficacy of the extracts (Leaf dip bioassays) (Adopted from (Immaraju, Morse, and Brawner, 1990)

(a) Sample preparation

An aphid leaf dip (ALD) method adopted by Immaraju, Morse, and Brawer (1990) was used to determine the efficacy of the extracts against the cabbage aphids. The cabbage aphids were collected as part of the infested leaf of the Brassica olecerea vegetable from a local garden in Gqeberha Central. The infested leaves were plucked off from the main plant and transported to Nelson Mandela University -Post Graduate Chemistry laboratory for assay.

Figure 5. 8: Cabbage aphid-infested Brassica olecerea leaf[1]

(b) Treatment protocol

A Series of crude sample dilutions of concentrations of 25; 100; 200 and 300 $\mu g \cdot mL^{-1}$ were prepared for each crude extract and isolated compound. The number of live cells was magnified and counted on each infested leaf cutting. Assays were performed by dipping the leaf cutting in the differently prepared aliquots for 5 seconds before placing it on a piece of tissue paper and then placed in a 5.5 cm petri dish with moistened cotton. The assessment was carried out using a magnifying lens by counting the dead cabbage aphids after 24 hours. Those cabbage aphids which had no leg movement were considered dead. Each experiment was carried out in triplicate and results were expressed as Mean ± SD (N = 3) at a 95% confidence level. The cabbage aphid mortality in the treatments was corrected with that in the negative control according to Abbott's formula (Fleming and Retnakaran, 1985).

$$\text{Corrected \% cabbage aphid mortality (CAM)} = \frac{\%\text{MT} - \%\text{MC}}{100 - \%\text{MC}} \text{ x } 100$$

- %MT is the percentage mortality of the treatment
- %MC is the percentage of mortality due to the solvent (negative control)
- %MT is the percentage mortality of the treatment

A Probit transformation technique was used to determine the lethal doses (LD_{50}).

[1] The infested leaf of Brassica olecerea vegetable. Picture was taken by W. Zivanayi in Gqeberha

5.5 References

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CHAPTER 6

ISOLATION AND CHARACTERISATION OF SINGLE COMPOUNDS

6.1 Introduction

Solanum incanum **(***S. incanum*) is a herb or soft wooded shrub that is between 1.0 and 1.8 m in height with spines on the stem, stalks, and calyces and has velvet hairs on the leaves (Abebe et al, 2014; Dold and Cocks, 2000). It is abundant and common as a weed in overgrazed savannah grasslands, on roadsides, and in low-fertile soils such as sandy and rocky areas (Taye, 2011). Flowers range from pale to deep blue, mauve, and purple. The leaves are alternate, egg-shaped in outline with the broad end at the base (ovate) with slightly wavy margins (especially on young leaves), with a grey-green upper surface and a green-white lower surface (Abebe et al., 2014; Beaman-Mbaya and Muhammed, 1976).

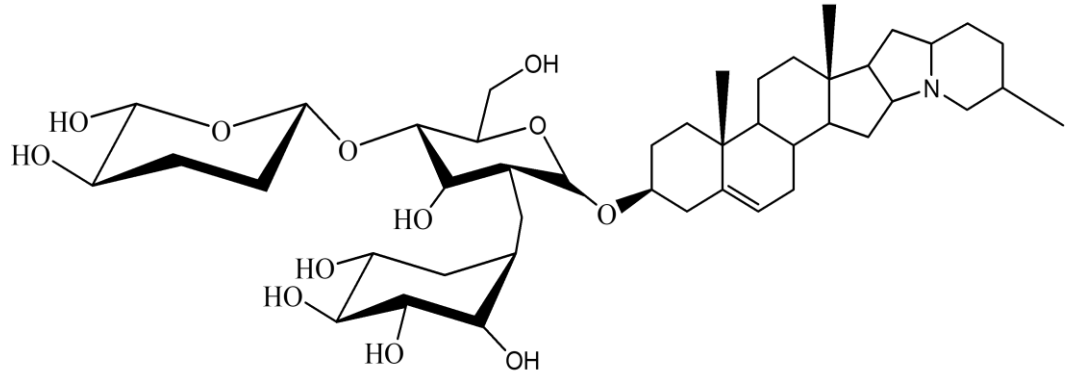
Figure 6. 1: *Solanum incanum*: (I), stem and fruit (II), dried roots (III) dried ripe fruit (IV) the inside of the fruit of *S. incanum*

6.2 Phytochemistry and biological activity of *S. incanum*

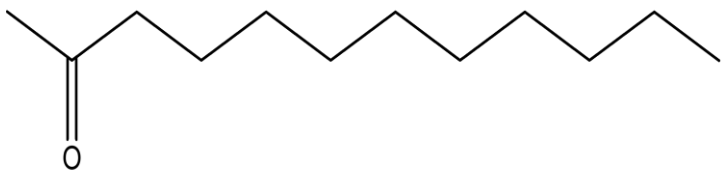
Previous studies on *S. incanum* identified secondary plant metabolites, such as alkaloids, glycoalkaloids, terpenoids, organic acids, alcohols, and phenolic compounds as promising sources of plant-pesticidal activity (Adeyemi, 2010; Al Sinani and Eltayeb, 2017; Chowański et al., 2016; Ibanez et al, 2012). For instance, 2-undecanone (**6.2**), tridecanone (**6.3**), and dodecanone (**6.4**) are terpenes that are bioactive to *Aphis craccivora Koch* and *Musca domestica* L (Chowański et al., 2016).

The most abundant phytochemicals which have been isolated from *S. incanum* are alkaloids. According to Al Sinani and Eltayeb (2017), solamargine (**6.8**) and solasonine (**6.7**) extracted from the ethanolic extract of *S. incanum* interfere with insect reproductive systems by inhibiting oocyte maturation and decreasing egg production (Al Sinani and Eltayeb, 2017). Besides being pesticidal, compound (**6.7**) causes human hepatoma cells death (Hep3B) by apoptosis. Almoulah, (2017) claimed that compound **6.7** has a toxic effect on the cell lines: HT-29 (colon), HCT-15 (colon), LN C P (prostate), PC-3 (prostate), T47D (breast and MDA-MB-231 (breast) (Almoulah, 2017). α-Solanine (**6.6**) has been isolated from the peels of *S. tuberosum*. There are claims that (**6.8**) causes gastroenteritis symptoms, coma, and even death at concentrations greater when more than 20 mg per 100 g fwt is ingested (Nepal and Stine, 2019). The antioxidant properties of α-chaconine (**6.1**) were documented by Elekofehinti et al (2013).

Steroidal saponins have been isolated from *S. incanum* (Kaunda and Zhang, 2019). For instance, diosgenin (**6.9**) is an anticancer, antitumor, anti-inflammatory, antioxidant, antiviral, antifungal, antimicrobial, molluscidal, and anti-hypercholesteraemic drug (Kaunda and Zhang, 2019). Saponins have some common characteristic properties such as having a bitter taste, formation of stable forms in aqueous solution, haemolysis of red blood cells, and toxicity to cold-blooded animals such as fish, snails, insects (Ozlem and Mazza, 2007; Thakur et al, 2011). Steroidal saponins are prominent characteristic components in *Solanum* species and have diverse biological activities. Most of the saponins have presented cytotoxic, anticancer, hepatoprotective, antihypertensive, antmelanogenesis, antifungal, anti-inflammatory, anticonvulsant, and antiviral activities (Segal, 1977)



6.1



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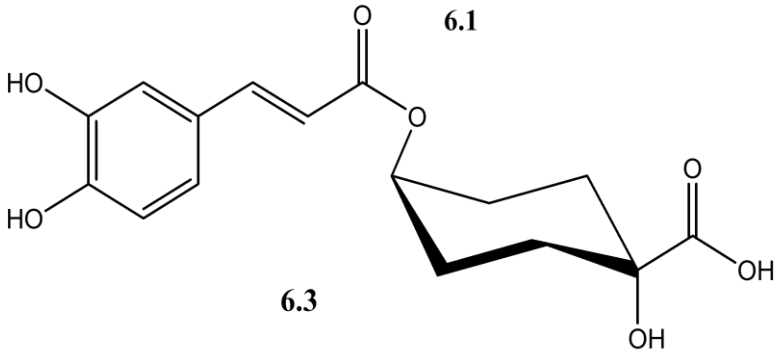

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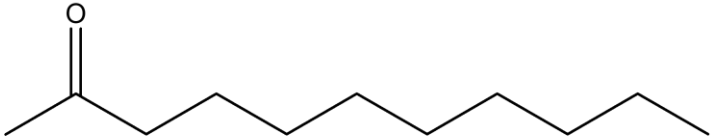

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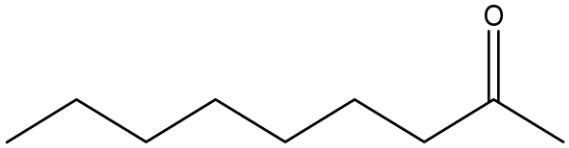

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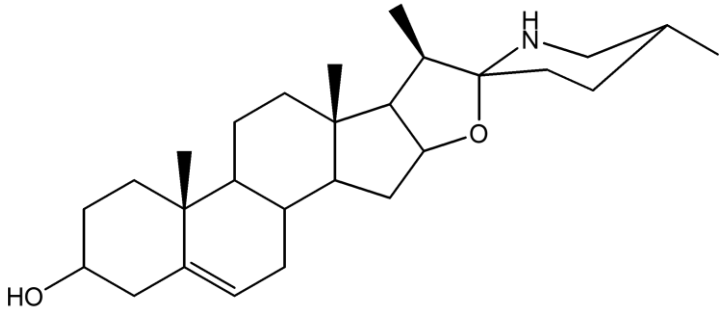


6.6

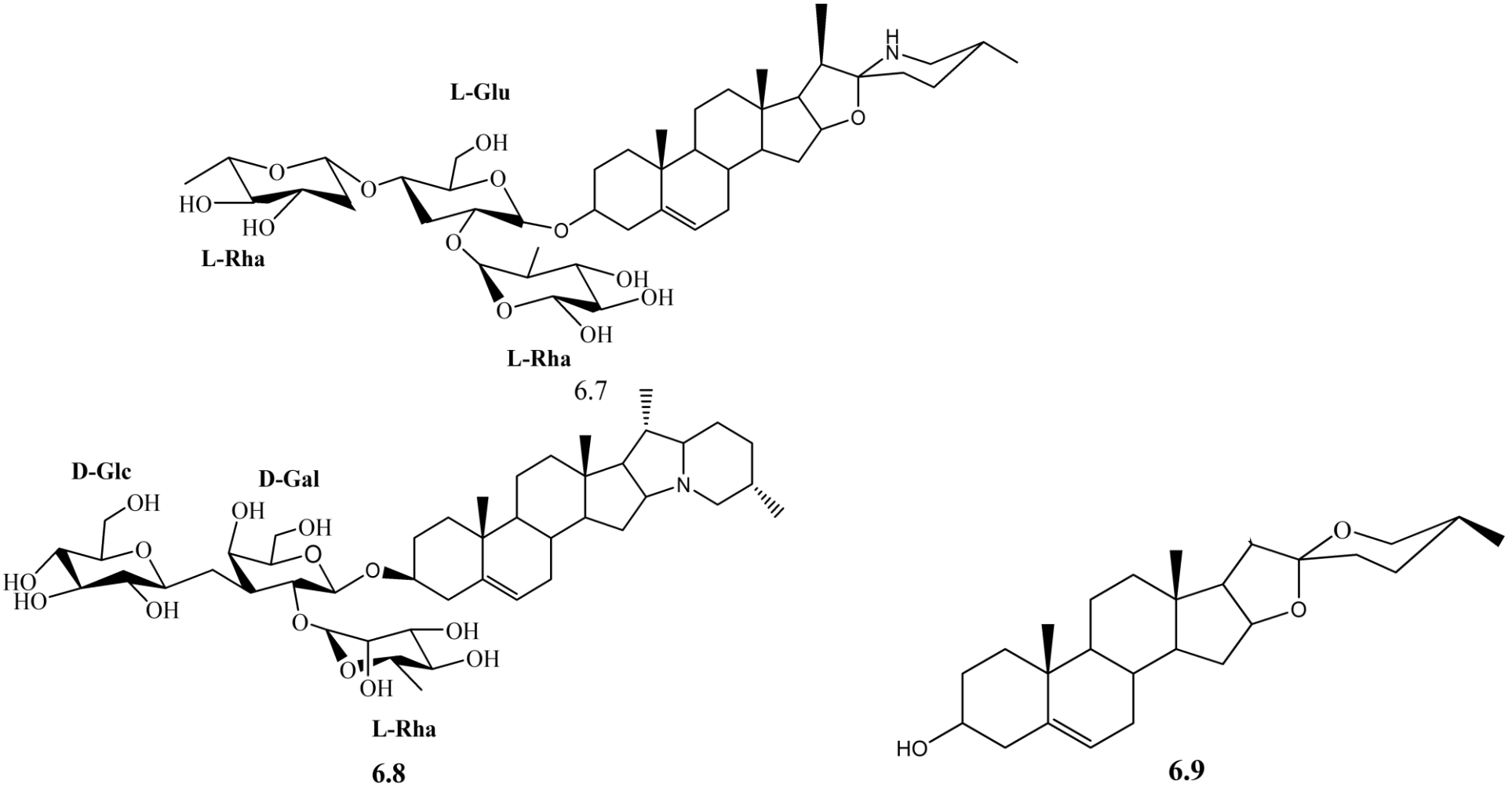


Figure 6.2: Some bioactive compounds isolated from different *Solanum* species.

Many polyphenolics and glycosides of quercetin (**6.10**), kaempferol (**6.11**), and myricetin (**6.12**) had been reported from various species of *Solanum* species. Compound **6.10** is the most commonly occurring flavonol aglycone isolated from the fruit of S. *nigrum* (Ud-Din et al, 2009).

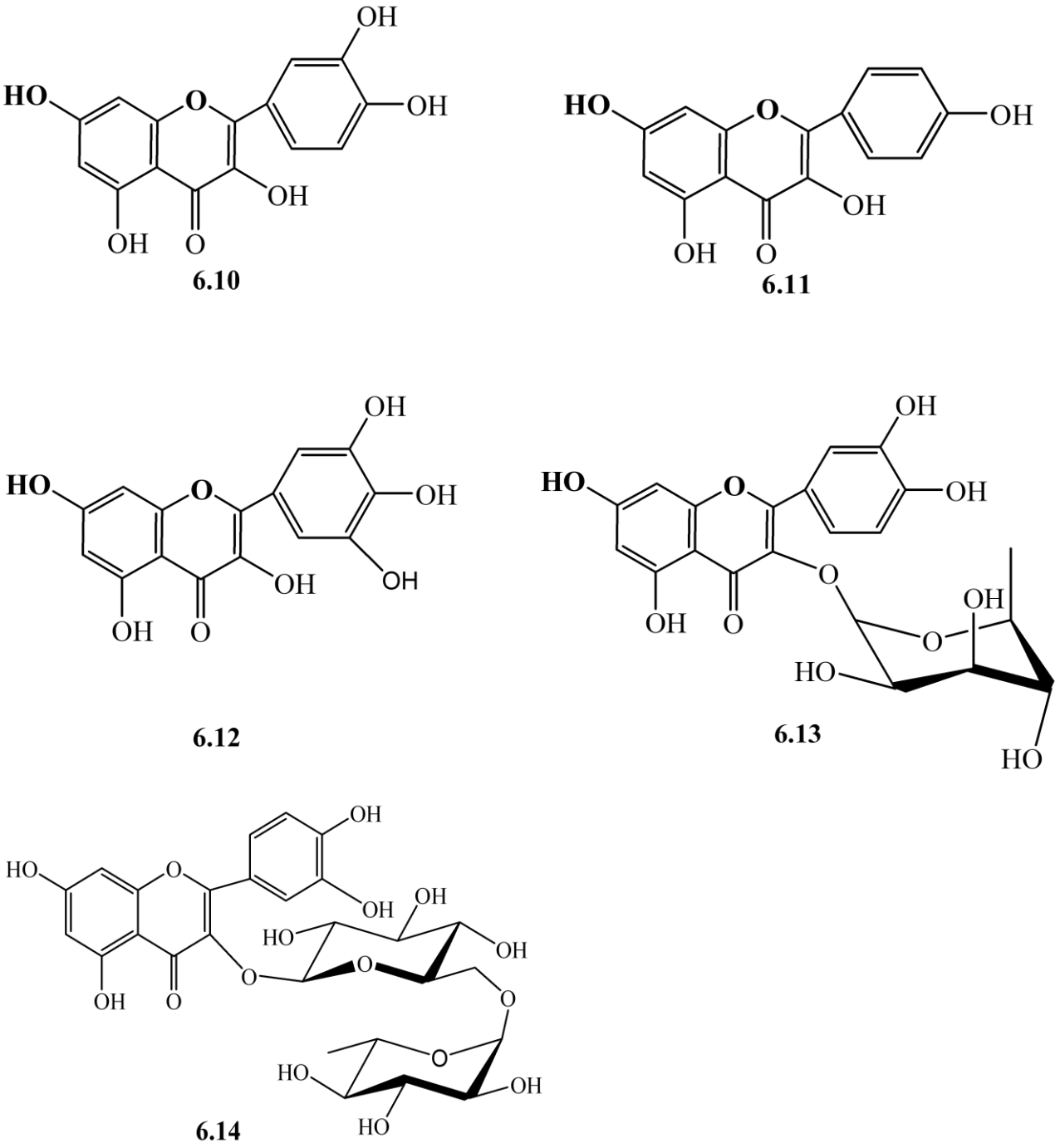


Figure 6.3: Some bioactive compounds isolated from *Solanum* species

Compound (**6.11**) possesses anti-inflammatory activity, oestrogenic activity, enzyme inhibition, and antimicrobial properties (Poonam et al, 2019). Derivatives of compound **6.10** that include myricetin (**6.13)** and rutin **(6.14)** were isolated from an aqueous ethanolic extract of *S. nigrum.* Rutin has high antioxidant properties (Silva et al, 2022). Compound **6.12** has been isolated from *S. incanum* (Lin et al., 2000). Like many polyphenolic compounds, quercetin and rutin are strong antioxidant compounds (Lin et al., 2000).

6.3 Results and discussion

6.3.1 LC-MS analysis of the fruit and root crude extracts of *S. incanum*

LC-MS was used to tentatively identify the chemical composition of the crude extracts of *S. incanum*. The molecular ion peaks of the LC-MS chromatograms of the **MFE**, **EFE**, **SAFE**, **HFE**, **Hroot**, **Eroot,** and **Mroot** crude extracts of *S. incanum* were compared with the standard mass spectra in the NIST library, ChemSpider, PubChem, and MassBank for the tentative identity of the compounds in the extracts. **Figure 6.4** shows a representative LC-MS chromatogram of the Ethyl acetate fruit (**Eroot**) crude extract. The ESIMS of the positive mode displayed six major peaks with molecular ion peaks at *m/z* 264.128, 304.300, 312.123, 314.139, 414.337, and 722.447. The ion molecular peaks numbered **1** to **3** in **Figure 6.4** show some of the identified compounds previously from *S. incanum*. The search from literature and chemical databases such as the ChemSpider, PubChem, and MassBank suggested that Peak **1** at (Rt = 6.85; *m/z* 414.336) with a molecular formula of $C_{27}H_{42}O_3$ represented (25(*R*)-spirost-5-en-3β-ol), peak **2** at Rt = 9.42; *m/z* 303.306 with a molecular formula $C_{15}H_{10}O_7$ was proposed for quercetin and peak **3** at (Rt = 7.07; *m/z* 412.322 with a molecular formula $C_{29}H_{48}O$ or $C_{27}H_{43}NO_2$ represented stigmasterol or solasodine.

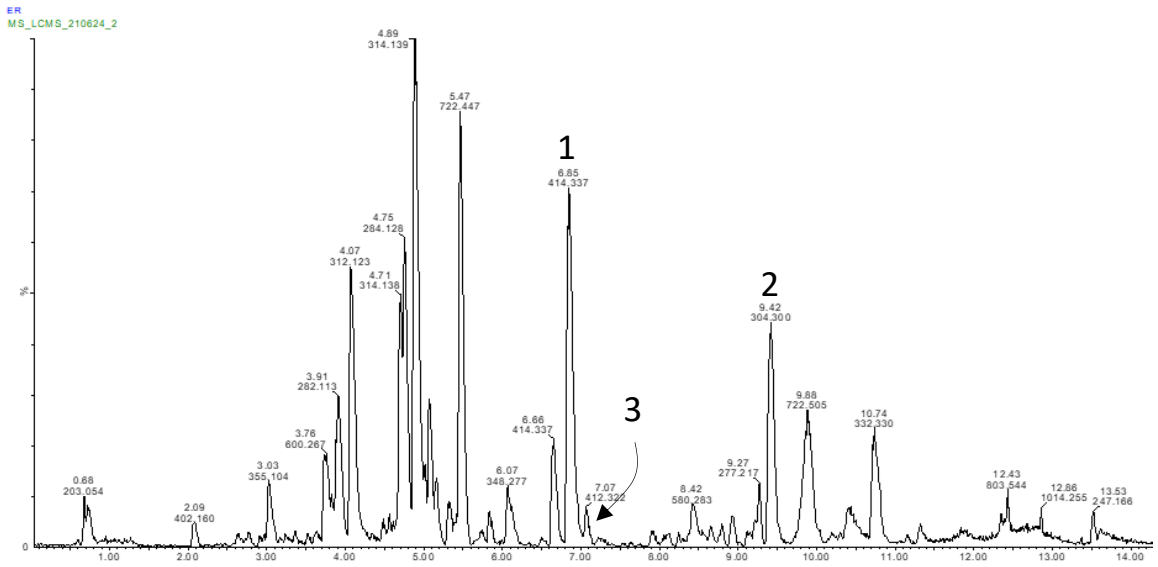


Figure 6. 4: The LC-MS spectrum of the ethyl acetate fruit (Eroot) crude extract. Column: Waters BEH C18, 2.1x100mm, both solvent lines contained 0.1% formic acid

The LC-MS chromatogram of the **EFE** extract (**Figure 6.5**) revealed major ion molecular peaks at *m/z* 165.055, 304.300, 355.103, 412.322, and 868.504. The data obtained from the positive ionisation mode of the LC-MS (**Figure 6.5**) revealed that **EFE** extract contained sapogenins and alkaloidal steroids, and phenolic compounds. The ESIMS spectrum of the peak at retention time 6.64 min displayed an ion at *m/z* 412.322 with a fragmentation of 413 $[M-H]^-$ corresponding to molecular formula $C_{27}H_{43}NO_2$. The molecular formula $C_{27}H_{43}NO_2$ represented solasodine. Solasodine (**6.14**) is a steroidal alkaloid that has been reported in *Solanum* species. The ESIMS spectrum of the peak at retention time 4.94 min displayed ions at m/z 867.504 with a peak of 868.436 $[M+H]^+$ corresponding to molecular formula $C_{45}H_{73}NO_{15}$. The molecular formula $C_{45}H_{73}NO_{15}$ represented solamargine (Avula et al, 2014). Solamargine (**6.6**) is a steroidal glycoalkaloid that has been reported in the leaves of *Solanum* species. solamargine has been reported as a poison to grazing animals (Thaiyah et al, 2010).

The ESIMS spectrum of the peak at retention time 9.44 min displayed ions at m/z 304.300 with a fragmentation of 304 $[M+2H]^+$corresponding to molecular formula $C_{15}H_{10}O_7$. The spectra data banks confirmed that the molecular formula $C_{15}H_{10}O_7$ represented a phenolic compound quercetin (**6.10**) (Chen et al, 2016).

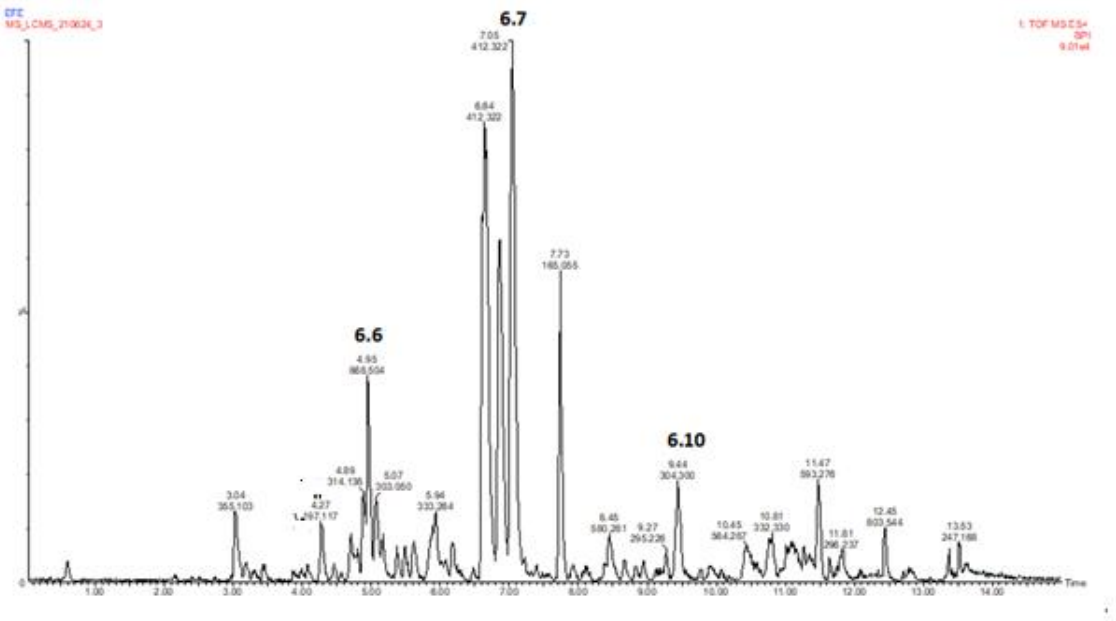


Figure 6. 5: The LC-MS spectrum of the ethyl acetate fruit (EFE) crude extract. Column: Waters BEH C18, 2.1x100mm, both solvent lines contained 0.1% formic acid

The identification of phenolic and alkaloid compounds in both the **Eroot** and **EFE** and a sterol in **Eroot** also reaffirms the abundance of different phytochemicals in *S. incanum*. The MS spectrum with ion fragmentation peaks of compound **6.7** ($C_{27}H_{43}NO_3$), compound **6.6** ($C_{45}H_{73}NO_{15}$), and compound **6.10** ($C_{15}H_{10}O_7$) are indicated in **Figures 6.6** and **6.7**. Several other compounds were also detected in the LC-MS analysis and are summarised in **Table 6.1**.

It should be noted that LC-MS analysis delivered a preliminary result of the presence of known phytochemicals. Isolation and characterisation are required to confirm the structure of the identified compound, as the observed mass signal could potentially be that of an isomeric structure.

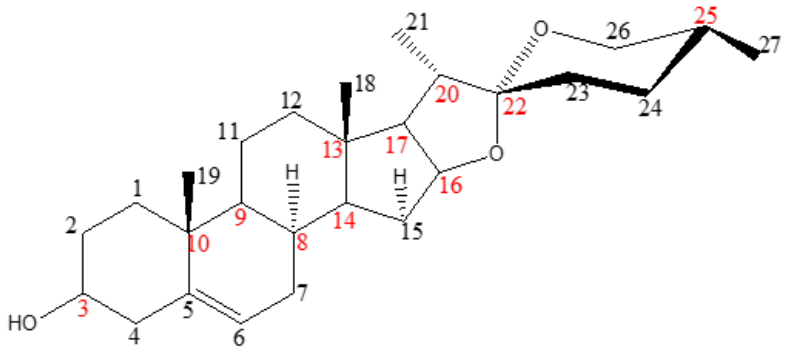
Table: 6.1: Some tentatively identified compounds from the EFE, Eroot, MFE, Hroot, SAFE, HFE, and Mroot extracts from the LC-MS analysis

Compound No	Name	TR (min)	Elemental composition	$[M + H]^+$ ion detected	Molecular weight
1	Stigmasterol	6.64	$C_{29}H_{48}O$	413.322	412,7
2	Phytol	11.81	$C_{20}H_{40}O$	297.228	296.5
3	N-coumaroyl tyramine	4.75	$C_{17}H_{17}NO_3$	284.128	283.3
4	N-sinapoyl putrescine	5.09	$C_{15}H_{22}N_2O_4$	295.165	***294.4***
5	N-feruloyl tyramine	4.71	$C_{18}H_{19}NO_4$	314.139	313.3
6	Diosgenin	6.68	$C_{27}H_{42}O_3$	415.337	414.6
7	Solasodine	5.12	$C_{27}H_{41}NO_2$	414.405	413,6
8	2-(4-Hydroxy-3-methoxphenyl	10.81	$C_{18}H_{16}O_6$	200.010	198.1
9	Indolin-2-one, 1-methyl-3-t-butyl	0.98	$C_{13}H_{17}NO$	203.054	203.3
10	Heptadecane, 2,6,10,14-tetramethyl	1.81	$C_{21}H_{44}$	298.226	296.6
11	Quercetin	9.44	$C_{15}H_{10}O_7$	303.300	302.2
12	Solamargine	4.95	$C_{45}H_{73}NO_{15}$	868.504	867.5
13	2-Phenoxyethanol	2.34	$C_8H_{10}O_2$	138.067	138.2

6.4 Isolation and structural characterisation of single compounds

Herein, we present the characterisation of the isolated and purified compounds from the fruit and root crude extracts of *S. incanum*. The following nine pure compounds were isolated from different crude extracts of *S. incanum:* diosgenin (**6.15**), 4-((2'*R*,5'*R*,6a*R*,8a*S*,9*R*)-4-hydroxy-6a,8a,9-trimethyl-1,3,3'4,4',5,5'6,6a,6b,6',7,8,8b,11a,12,12a,12b-icosahydrospiro[naphtho[2',1':4,5]indeno[2,1-b]furan-10,2'-pyran]-5'-ylbutan-2-one (**6.16**), solasodine (**6.17**), solamargine (**6.18**), quercitin **(6.19)**, rutin (**6.20**), phenoxy-2-ethan-1-ol (**6.21**), 2,4-Dihydroxybenzaldehyde (**6.22**), and 3,4-dihydroxy-5-(1,2-dihydroxyethyl) furan-2(5H)-one (**6.23)**.

6.4.1 Characterisation of diosgenin (6.15):



Compound **6.15** was isolated from the roots of *S incanum*. The Hroot extract was subjected to column chromatography using silica gel as the stationary phase and a mixture of *n*-hexane and ethyl acetate in the ratio 8:2 (v/v) as the mobile phase. Upon monitoring the fractions using Thin Layer Chromatography (TLC), the fractions were regrouped into 4 sub-fractions labelled Hroot **A**-**D**. Sub-fraction **B** was subjected to a preparative thin layer chromatography (PTLC) using the mobile phase Hex: EtOAc - (8.5:1.5 v/v) to enable the isolation of compound **6.15** (26.86 mg) at R_f 0.4 and compound **6.16** (15.32 mg) at R_f 0.25.

Compound **6.15** was a white crystalline substance and was analysed using the single-crystal X-ray diffraction technique. The formula of **6.15** was $C_{27}H_{42}O_3$ and the structure showed the absolute configuration of 3*S*, 8*S*, 9*S*, 10*R*, 13*S*, 14*S*, 16*S*, 17*R*, 20*S*, and 22*R* on the carbon atoms C-3, C-8, C-9, C-10, C-13, C-14, C-16, C-17, C-20, C-22, and C-25 respectively. The X-ray absolute configurations agreed with those reported for **6.15** (Segal, 1977) since this compound had been isolated before from other plant species and other *Solanum* species. The observed positions of methyl groups at C-18, C-19, C-21, and C-27 and the oxygenated methine and oxygenated quaternary carbons at C-3, C-16, and C-22 in the X-ray structure are also confirmed by the NMR spectral data of **6.15** in **APPENDIX 8.7.1.** A summary of the spectral data is given in **Table 6.2**.

Table 6.2: ^{13}C and ^{1}H NMR chemical shifts (δ, ppm) of diosgenin (**6.15**)

Position	Diosgenin (6.15) in $CDCl_3$	
	δ_C/ppm	δ_H (ppm) (*m*, *J* in Hz)
1	37.2	1.83;1.00, *(*2H, *t, J* =7.2*)*
2	31.5	2.08;1.98, (2H, *m*)
3	71.7	3.451, (1H, *m*)
4	42.3	2.20;2.21, (2H, *d, J*=16)
5	140.8	-
6	121.4	5.28, (1H, *s*)
7	32	1.21;1.72, (2H, *m*)
8	31.4	1.73, (1H, *m*)
9	50.1	0.90, (1H, *d*, *J* = 7.02)
10	36.6	-
11	20.9	1.43; 1.43, (2H, *m*)
12	39.8	1.67;1.10, (2H, *s*)
13	40.3	-
14	56.5	1.01, (1H, *m*)
15	31.8	2.05;1.19, (1H, *m*)
16	80.8	4.34, (1H, *q*, *J*=7.60, 7.60, 7.84)
17	62.1	1.69, (1H, *m*)
18	16.2	0.71; 0.71; 0.71 (1H, *d, J*=4.33)
19	19.4	1.05, (3H, s)
20	41.6	1.86, (1H, *m)*
21	14.5	0.88; 0.88; 0.88, (3H, *d, J*=4.33)
22	109.3	-
23	31.6	1.75;2.05, (2H, *m*)
24	29.6	1.16; 1.16, (2H, *m*)
25	30.9	1.53, (1H, *m*)
26	66.9	2.88, 3.39, (2H, *m*)
27	17.1	0.72; 0.72; 0.72, (3H, *d, J*=4.33)

The bond length of the C=C (1.339 Å) and C-O (1.445 Å) bond recorded from the X-ray agreed with the bond lengths of C=O and C-O bonds reported in the literature (Wang, 2016). The melting point of **6.15** is 205.5 °C. The rings **A**, **B**, **C**, **D**, and **E** are fused. The rigid conformation displayed by **6.15** is like that reported for diosgenin where the **A** and **B**, **B** and **C**, and **C** and **D** ring junctions are trans, while the cyclopentane D-ring is cis fused to the tetrahydrofuran **E**-ring (**Figure 6.4**).

Table 6. 3: Comparison of the XRD experimental and literature data for compound **6.15**

Property	**Compound 6.15**	**Literature data (**Wang et al, 2016)
Crystal system	Acentric, monoclinic, multiplicity	Acentric, monoclinic, multiplicity
Space group	$P2_1$	$P2_1$
Symmetry	x, y, z; -x, 1/2+y, -z	x, y, z; -x, 1/2+y, -z
Unit cell dimensions	a = 10.2843(6), b = 7.2022(4), c = 16.6921(10)	a = 10.2843(6), b = 7.2022(4), c = 16.6921(10)
Unit cell angles, Å	$\alpha = \gamma$ = 90, β = 98.341(2)	$\alpha = \gamma$ = 90, β = 98.341(2)
Volume, $Å^3$	2452.2(3)	2452.2(3)
Z	2	2

The six-membered rings **A**, **C**, and **F** have chair conformation, while **B**-ring is a half-chair conformation due to the existence of a carbon−carbon double bond, and the **E**-ring exhibits an envelope conformation. The NMR spectral data (**APPENDIX 8.7.1**) for **6.15** agreed with a (25*R*)-spirostan steroidal type skeleton, given in **Figure 6.4.** The crystal had been deposited in an X-ray resource data bank.

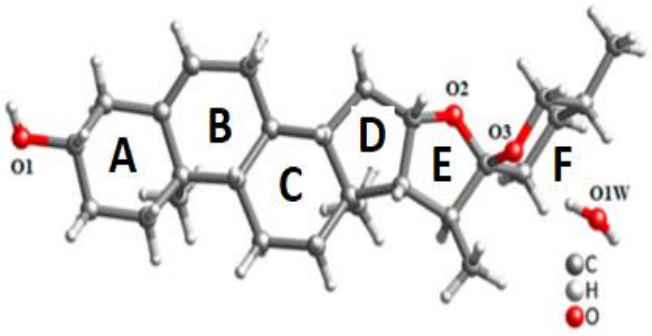
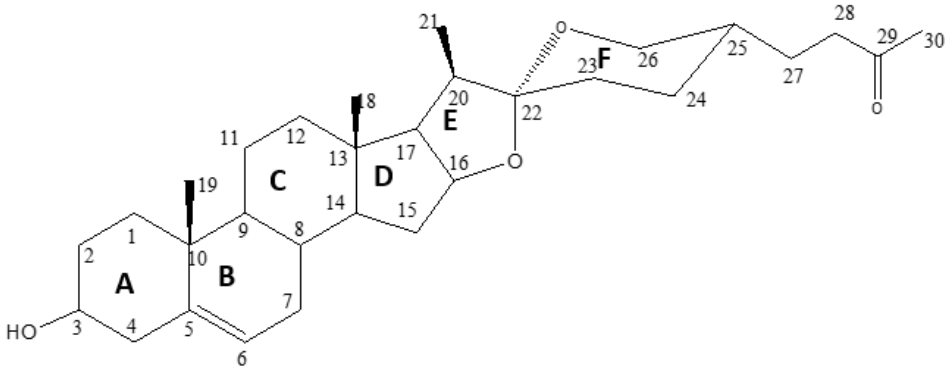


Figure 6. 6: A partially labelled thermal ellipsoid plot of compound 6.15 showing 50% probability surfaces. All hydrogen atoms are shown as small spheres of arbitrary radius.

Based on this compound **6.15** was characterised as (2S,2′R,4aR,4bS,5′R,6aS,6bR,7S,9aS, 10aS,10bS)-4′,4a,6a,7-tetramethyl-,2,3,4,4a,4b,5,6, 6a,6b,7,9a,10, 10a, 10b, 11-hexadecahydrospiro [naphtho[2′,1′:4,5]indeno[2,1-b]furan-8,2′-oxan]-2-ol. This compound is commonly known as diosgenin (**6.15**), a spirostan saponin that has been isolated and reported from the fruit and leaves of *S. Lycopersicum*, *S. Melongena*, and *S. tuberosum*, as well as from the stem part of *S. violaceum*. This is, however, the first report of diosgenin (**6.15**) in S. *incanum*. Diosgenin is used as a precursor for making several hormones, which include the synthesis of progesterone and contraceptive pills (Al Sinani and Eltayeb, 2017; Kaunda and Zhang, 2019).

6.4.2 Characterisation of solanuminone (6.16)



Compound **6.16** was isolated from the same preparative thin layer chromatography (PTLC) plate used for the isolation of compound **6.15**. The characterisation of **6.16** was done based on the MS and comparison of the NMR data of compound **6.16** to that of **6.15** (a saponin that had previously published data) (Huang et al, 2021). Compound **6.16** is a novel compound named for which the trivial name solanuminone is suggested. A positive-ion mode, HRESIMS generated a pseudo-molecular ion peak $[M+H]^+$ at *m/z* 470.3401 (100%) which corresponded to the molecular formula $C_{30}H_{46}O_4$ for compound **6.16.** The base molecular ion fragmentation peaks confirmed the molecular formula at m/z 471.3430 $[C_{30}H_{46}O_4+H]^+$(87.5%), 445.2930$[C_{29}H_{43}O_4 – CH_3]^+$(15%), 414,3589$[C_{27}H_{42}O_3]^+$, 413.2930$[C_{27}H_{29}O_2 – C_3H_7O + H]^+$ (PLATE **6.16G**). The FTIR spectrum showed major absorption bands at 3380 cm^{-1} for the stretching vibrations of the OH group, 2934 cm^{-1} (C-H), 1669 cm^{-1} (C=C), and 1720 cm^{-1} (C=O) (**PLATE 6.16H**). NMR (^1H, ^{13}C, COSY, HMBC, and HSQC) were recorded to elucidate the structure of **6.16**. The UV profile of **6.16** evidenced λ_{max} at 202 and 248 nm in

PLATE 6.16.I. The maximum absorption of UV at 206 nm indicated the presence of -C=C- in C-5 (δ_C 140.7), whereas λ_{max} at 248 nm evident the presence of a carbonyl group–C=O at C-29 (δ_C 206.8) (Liu et al., 2012). The absolute configuration of 3*S*, 8*S*, 9*S*, 10*R*, 13*S*, 14*S*, 16*S*, 17*R*, 20*S*, and 22*R* on the carbon atoms C-3, C-8, C-9, C-10, C-13, C-14, C-16, C-17, C-20, C-22, and C-25 respectively were established from the CD analysis. The melting point of **6.16** is 212.2 °C.

Table 6.4: ^{13}C and ^{1}H NMR chemical shifts (δ, ppm) comparison of diosgenin (**6.15**), and compound **6.16 (**The differences in the spectra are highlighted in green).

Position	Diosgenin (6.15) in $CDCl_3$		Compound 6.16 in $CDCl_3$	
	δ_C/ppm	δ_H (ppm) (*m*, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J*, Hz)
1	37.2	1.83;1.00, *(*2H, *t*, *J* =7.2*)*	30.2	1.85, 1.05 (2H, *m*)
2	31.5	2.08;1.98, (2H, *m*)	31.6	1.50; 1.50 (2H, *m*)
3	71.7	3.451, (1H, *m*)	71.7	3.55 (1H, *m*)
4	42.3	2.20;2.21, (2H, *d*, *J*=16)	37.1	2.27, 2.46 (2H, *m*)
5	140.8	-	140.7	-
6	121.4	5.28, (1H, *s*)	121.3	5.39 (1H, *br s*, *J* = 4.5)
7	32	1.21;1.72, (2H, *m*)	31.9	1.3; 1.3 (2H, *m*)
8	31.4	1.73, (1H, *m*)	31.4	1.69 (1H, *m*)
9	50.1	0.90, (1H, *d*, *J* = 7.02)	49.8	0.97 (1H, *d*, *J* = 6.3)
10	36.6	-	36.6	-
11	20.9	1.43; 1.43, (2H, *m*)	20.8	1.58 (2H, *m*,)
12	39.8	1.67;1.10, (2H, *s*)	39.7	1.17; 1.71 (2H, *m*)
13	40.3	-	40.2	-
14	56.5	1.01, (1H, *m*)	56.5	1.1 (1H, *m*)
15	31.8	2.05;1.19, (1H, *m*)	31.8	1.85, 1.89 (2H, *m*)
16	80.8	4.34, (1H, *q*, *J*=7.60, 7.60, 7.84)	80.7	4.41 (1H, *dd*, *J* = 7.5, 7.5)
17	62.1	1.69, (1H, *m*)	62.0	1.78 (1H, *m*)
18	16.2	0.71; 0.71; 0.71 (1H, *d*, *J*=4.33)	16.2	0.79 (3H, *s*,)
19	19.4	1.05, (3H, s)	19.3	1.05 (3H, *s*)
20	41.6	1.86, (1H, *m)*	41.5	1.80 (1H, d, J = 7.5)
21	14.5	0.88; 0.88; 0.88, (3H, *d*, *J*=4.33)	14.4	1.15 (3H, *d*, *J* = 7.2)
22	109.3	-	109.2	-
23	31.6	1.75;2.05, (2H, *m*)	33.3	1.59; 1.59 (2H, *m*)
24	29.6	1.16; 1.16, (2H, *m*)	17.0	1.71- 1.77 (2H, *m*)
25	30.9	1.53, (1H, *m*)	31.3	1.88 (1H, *m*)
26	66.9	2.88, 3.39, (2H, *m*)	66.8	3.37; 3.46 (2H, *m*)
27	17.1	0.72; 0.72; 0.72, (3H, *d*, *J*=4.33)	28.7	1.20 (2H, *m*)
28	-	-	42.2	2.21(2H, *t*, *J* = 14.4, 15.6)
29	-	-	206.8	-
30	-	-	29.6	2.10 (3H, *s*)

The structure of the compound was elucidated based on the results of the NMR spectroscopic experiments including ^{1}H and ^{13}C NMR, DEPT135, 2D experiments (COSY, HSQC, and HMBC), IR data (**APPENDIX 8.7.2**). An analysis and comparison of ^{13}C and the ^{1}H data show that the NMR spectral data obtained for **6.16** agreed with a (25*R*)-spirostan steroidal type skeleton, (Al Sinani and Eltayeb, 2017) given in **Figure 6.6** obtained for compound **6.15**.

Thirty signals from the ^{13}C NMR data for compound **6.16** were observed of which twenty-six of the signals indicated the steroidal aglycone structure. Through 2D NMR data, the position of the carbonyl group was confirmed. HMBC correlation of H-24 (δ_H 1.71) with methylene carbon C-27(δ_C 28.7), H-24a (δ_H 1.71) had a long-range correlation with C-28 (δ_C 42.2 and H-30 (δ_H 2.10) to C-28 (δ_C 42.2) were observed. The observed COSY correlation Ha-27 (δ_H 1.20) to Ha-28 (δ_H 2.21) and Ha-25 correlated to H- 27a (δ_H 1.20) further confirmed the positions of C-27 (δc 28.7); C-28 (δc 42.2) and C-30 (δc 29.6). The HMBC and the COSY correlations are shown in **Figure 6.7.**

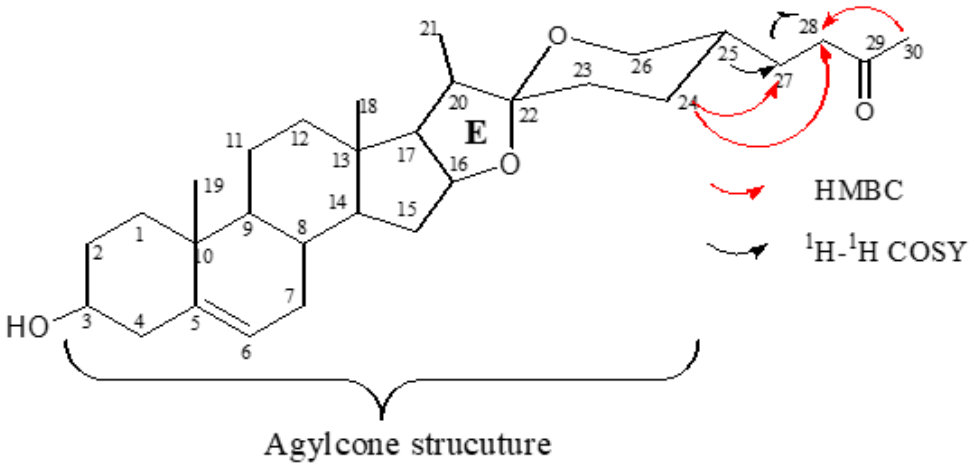
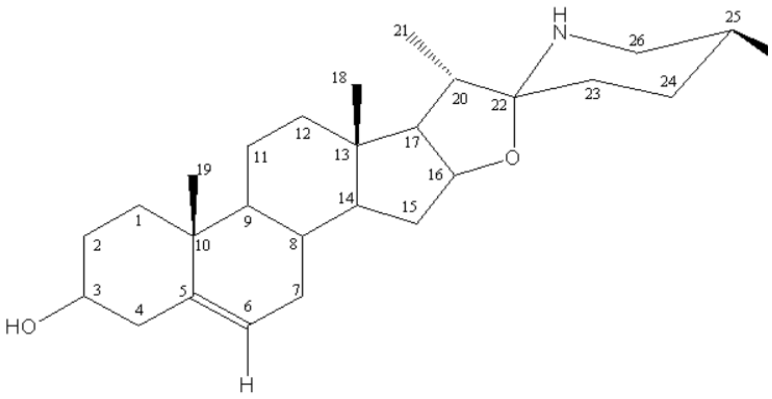


Figure 6.7: Selected HMBC (red arrows) and COSY (black arrows) correlations of compound **6.16**

Moreover, the ^{1}H NMR spectrum (**PLATE 6.16A**) showed a three-proton singlet at δ_H 2.10 for the methyl group at C-30 connected to a carbonyl carbon C-29, a 2-proton triplet at δ_H 2.21 for the methylene group at C-28 indicating 2 neighbouring protons at C-27 ((δ_C 28.7). A multiplet at H-27 (δ_H 1.20) for the 2 protons connected to C-27 (δ_C 28.7) was observed. Therefore, compound **6.16** was identified as 4-((2'*R*,5'*R*)-4-hydroxy-6a,8a,9-trimethyl-1,3,3',4,4',5,5',6,6a,6b,6',7,8,8a,8b,9,11a,12,12a,12b-icosahydrospiro[naphtho[2',1':4,5] indeno[2,1-b]furan-10,2'-pyran]-5'-yl)butan-2-one.

6.4.3 Characterisation of solasodine (6.17)



Based on the findings in Chapter 5 section 5.2.2, the presence of solasodine (used as a reference) at an R_f value of 0.03, was also confirmed in SAFE and MFE. To isolate **6.17** from MFE, several techniques which comprised column chromatography (CC), thin layer chromatography (TLC), and preparative thin layer chromatography (PTLC) were employed to afford the isolation of a pure single compound **6.17** using $CHCl_3$: MeOH (4:1 v/v) as the solvent system. Compound **6.17** is a steroidal alkaloid isolated as an amorphous white powder from the fruit crude extract of *S. incanum*.

The characterisation of **6.17** was done based on the MS and its NMR data. A pseudo molecular ionic peak at *m/z* 412.3364 $[M+H]^+$ (at 100%), and base peaks at *m/z* 98.096 $[C_6H_{13}N+H]^+$], 126.1778 $[C_8H_{15}N + H]^+$, 253.1954$[C_{19}H_{24}-H_2O+H]^+$, 271.2062 $[C_{19}H_{24}+H]^+$, 397.3345 $[C_{27}H_{43}NO-H_2O+H]^+$ and 413.3364 $[M]^+$ (100%) were observed in the HRESIMS spectrum and these fragments agreed with the molecular formula assignment $C_{27}H_{43}NO_2$ for **6.17** (**PLATE 6.17G** The UV spectrum displayed λmax at 236 and 211 nm. The maximum absorption at 211 nm indicated the presence of a double bond (C=C) between C5 and C6 while λ_{max} at 236 nm indicated the presence of stretching of the alcoholic amine group-NH in the ring E (**PLATE 6.17.I.**). The FTIR spectrum of **6.17** showed major absorption bands at 3410 cm^{-1} (stretching of the phenolic hydroxyl group -NH), 2923.4 cm^{-1} (the flipping of the C-H), 1454 cm^{-1}, 1669 cm^{-1} (stretching of the C=C) - **PLATE 6.17H**.

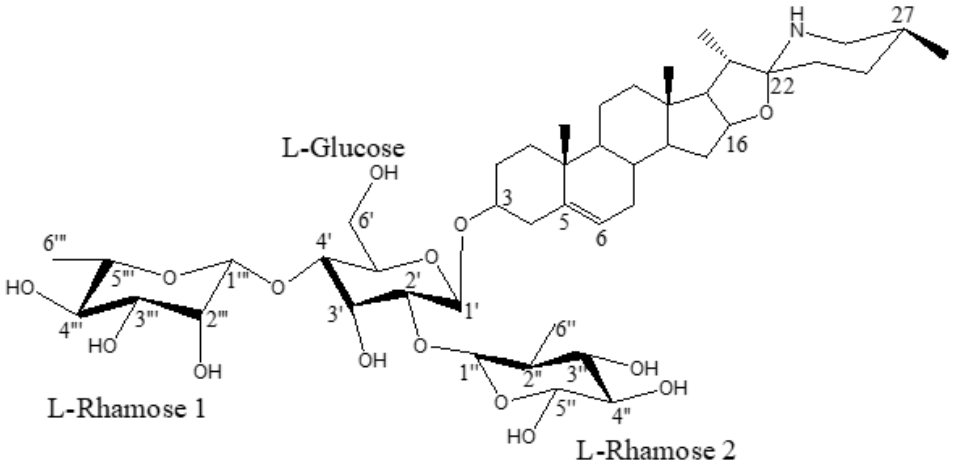
The NMR (^{1}H, ^{13}C, COSY, HSQC, and HMBC) experiments were conducted to elucidate and confirm the structure of **6.17**. Twenty-seven ^{13}C NMR signals were observed. The four ^{13}C NMR signals at positions C-3 (δ_C 71.7), C-5 (δ_C 141.5), C-16 (δ_C 80.8), C-22 (δ_C 100.2), and C-26 (δ_C 47.5) of **6.17** are diagnostic signals for a steroidal alkaloid backbone (Avula et al, 2014). The aglycone attachments of a steroidal saponin and a steroidal alkaloid differ from each other at the tertiary C-22. Steroidal alkaloids have a C22-N-C26 bond whereas steroidal saponin has a C22-O-C26 bond in the **F**-ring. This difference gives rise to signals in the ^{13}C NMR used to differentiate the steroidal saponin [compound **6.15** (diosgenin)] to a steroidal alkaloid [compound **6.17** (solasodine)] at C-22 (δ_C 100.2), and C-26 (δ_C 47.5). The presence of the amino group (NH) between C-22 (δ_C 100.2), and C-26 (δ_C 47.5) gives rise to a ^{13}C NMR signal δ_C (100.2) ppm at C-22 whereas in diosgenin the signal at C-22 is downfield at δ_C (109.6) ppm. The signal at C-26 for solasodine (a steroidal alkaloid) is observed up-field at δ_C 44.5 ppm, whereas that of diosgenin (a steroidal saponin) appears at δ_C 71.9 ppm (Wanyonyi et al., 2003).

Four methyl proton groups at δ_H 0.90 (3H, *s*, H_3-18), 2.10 and 1.05 (3H, *s*, H_3-19), 0.97 (3H, *d*, *J=6.3* Hz, H_3-21), 1.15 (3H, *d*, *J=7.2* Hz, H_3-27) are observed in the ^{1}H NMR spectrum [**PLATE 6.17A**]. The ^{13}C NMR and the DEPT 135 spectra **PLATE 6.17B** and **6.17E** were used to identify the signals attributed to four quaternary carbons, nine methines (8 sp^3) and (1 sp^2) hybridised, including two oxygenated methine groups at C-3 and C-16 and one with an amino (NH) group between C-22 (δ_C 100.2), and C-26 (δ_C 47.5). Ten methylene (CH_2) groups: all sp^3 were identified and the four methyl (CH_3) groups were recognised as well. These ten methylene proton signals are attributed to the aglycone as the chemical shifts for them were evident in the up-field region. One olefinic proton signal at (δ_H 5.39 ppm (*d*, 1H, *J* = 4.5 Hz) was indicative of a single unsaturation unit on ring-B of the aglycone. The amino (NH) group gives rise to a singlet at δ_H (2.79) ppm in the ^{1}H NMR which is not observed in diosgenin.

Based on the HREISMS, FTIR, and of **6.17** and comparison with NMR data of diosgenin (**6.15**) and **6.17**, the structure of **6.17** was assigned and confirmed (2*S*,2'*R*,4a*R*,4b*S*,5'*R*,6a*S*,6b*R*,7*S*,9a*S*,10a*S*,10b*S*)-4a,5',6a,7-tetramethyl-1,2,3,4,4a,4b,5,6,6a,6b,7,9a,10,10a,10b,11-hexadecahydrospiro[naphtho[2',1':4,5]indeno[2,1-*b*]furan-8,2'-piperidin]-2-ol (solasodine (Akter, 2013; Kaunda and Zhang, 2019; Silva et al., 2005; Wanyonyi et al., 2003). Compound **6.17** has been previously isolated from several plants of

the *Solanum* species. Examples from where compound **6.17** has been isolated are the fruit of *S. incanum, S. melongena,* and *S. sisymbriifolium,* and the whole plant of *S. Torvum, S. trilobatum,* and *S. nigrum.* Solasodine belongs to the class of steroidal alkaloids called spirosolanes (Beaman-Mbaya and Muhammed, 1976; Chowański et al., 2016).

6.4.4 Characterisation of solamargine (6.18)



Compound **6.18** was isolated from the methanol fruit extract (MFE) of *S. incanum.* Chromatographic techniques that included thin-layer chromatography (TLC) and Preparatory Thin-Layer Chromatography (PTLC) were employed to enable the isolation and purification (**6.18)**. The NMR and high-resolution mass (HRESIMS) spectrometric analysis were used to identify compound **6.18**. Compound **6.18** is identified and separated from the fruit of *S. incanum* for the first time although it has been isolated from the fruit of *S. nigrum* before (Chun-Nan et al., 1990)

An HRESIMS generated a molecular ion $[M+H]^+$ at *m/z* 867.4582. of **6.18** agreed with a molecular formula $C_{45}H_{73}NO_{15}$. The HREISMS fragmentations of solamargine (**6.18)** showed molecular ion peaks at *m/z* 704.4374$[C_{27}H_{43}NO\text{-}Rha\text{-}H_2O+H]^+$, 414.3372$[C_{27}H_{43}NO\text{-}2Rha\text{-}Glc+H]^+$, 397.3345$[C_{27}H_{43}NO\text{-}2Rha\text{-}Glc\text{-}2H_2O+H]^+$, 271.2062$[C_{19}H_{24}N+H]^+$, and 98.096$[C_6H_{13}N+H]^+$ which are characteristic features of a solasodine aglycone fragmentations (Lelario, Labella, Napolitano, Scrano, and Bufo, 2016). The molecular ion peaks fragmentation patterns in the spectrum at *m/z* 704.4374 $[M\text{-}Rha\ 1\text{-}H_2O+H]^+$, 544.4116 $[M\text{-}Rha\ 1\ \text{-}Rha\ 2\ \text{-}2H_2O + H]^+$ and 397.3268 $[M\text{-}Rha\text{-}2Rha\text{-}Glc\text{-}2H_2O + H]^+$(**PLATE 6.18G**), was consistent with the sequential loss of three sugars from a solasodine aglycone – the rhamnosyl and

rhamnosyl-glucosyl side attachment to an aglycone moiety. Compound **6.18** was suggested to be solamargine, a glycoalkaloid (a derivative of solasodine) composed of solasodine aglycone and the three sugar moieties. The fragmentation pattern from the HREIMS spectrum of compound **6.18** is indicated in **Figure 6.8.**

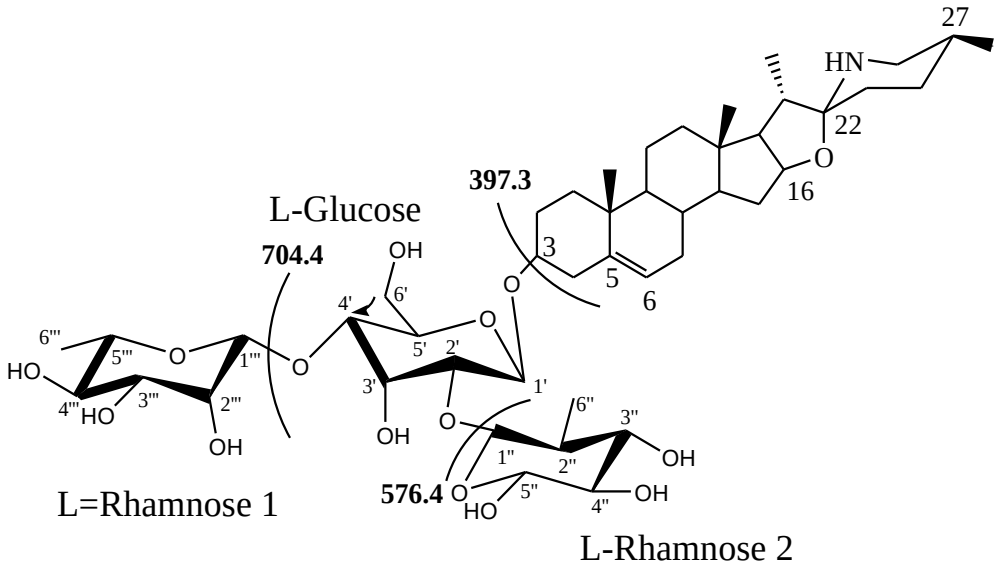


Figure 6. 8: The fragmentation pattern from the HREIMS spectrum of solamargine (**6.18**). The fragments are observed as molecular ion peaks in the HRMS spectrum in **APPENDIX 6.18H**.

The solasodine aglycone attached to the three sugar moieties (glucose and two rhamnoses) suggested that the compound was likely to be solamargine (**6.18**). The maximum absorption at 205 nm indicated the presence of a double bond (C=C) at C5 and λ_{max} at 238 nm indicated the presence of -C-NH-C in the ring E.

The NMR spectral assignment of compound **6.18** was performed through comparison with (**6.17**) as shown in Table 6.6. The ^{1}H NMR and ^{13}C NMR of solarmargine showed signals of a steroidal aglycone like those in compound **6.17**. The ^{1}H NMR spectrum of **6.18** (**PLATE 6.18A**) showed signals at δ_H 5.39 (1H, *br s*, *J* = 4.5 Hz, H-6), 0.87 (3H, d, *J* = 6.0 Hz, H-27), 0.72 (3H, s) and 0.95 (3H, s) are prominent features of a steroid alkaloidal aglycone. The ^{13}C NMR spectra (**PLATE 6.18B**) displayed signals of a vinyl group at δ_C 142.5 ppm (C5-C6), a quaternary carbon at δ_C 100.3 ppm (C22) which linked **E** and **F** rings, and the signal at δ_C 78.8 ppm (C-16) representing a methoxy group in **E** ring confirmed the steroidal aglycone structure.

The ^{13}C NMR spectrum signals appearing between 65-78 ppm confirm the presence of the sugar moieties in compound solamargine (**6.18).**

The ^{1}H NMR spectrum of solarmargine showed three anomeric proton signals at δ_H 4.54 (1H, d, *J* = 7.5 Hz), 4.86 (1H, d, *J* = 7 Hz), and 5.22 (1H, br s) and the ^{I3}C NMR spectrum of solarmargine showed three signals due to the anomeric carbons at δ_C 100.8, 104.8, and 105.2 ppm (**PLATE 6.18 A**).

The ^{13}C NMR and the DEPT 135 spectra of **6.18** in **APPENDIX 8.74 (PLATE 6.18B** and **C**) respectively revealed six oxymethine carbon signals at C-1' (δ_C 100.1), C-2' (δ_C 74.1), C-3' (δ_C 85.3), C-4' (δ_C 78.2), C-5' (δ_C 76.5), and C-6' (δ_C 61.5) ppm reasonably assigned to glucosyl pyranose carbons by comparison with the chemical shift values of glucosyl carbons in the literature of a previously isolated glycoalkaloids (Akter, 2013; Silva et al., 2005). Five more oxymethine carbon signals at C-1''(δ_C 101.5), C-2''(δ_C 72.2), C-3''(δ_C 73.3), C-4'' (δ_C 83.3), C-5'' (δ_C 71.1), and one methyl group at C-6''(δ_C 18.0) were assigned to the rhamnosyl pyranose carbons by comparison with the chemical shift values of rhamnosyl carbons of a glycoalkaloid found in literature and the last five methine carbon signals at C-1''' (δ_C 104.8), C-2''' (δ_C 76.7), C-3''' (δ_C 77.5), C-4''' (δ_C 71.3), C-5'''(δ_C 69.3) and one methyl group at C-6'''(δ_C 18.6).

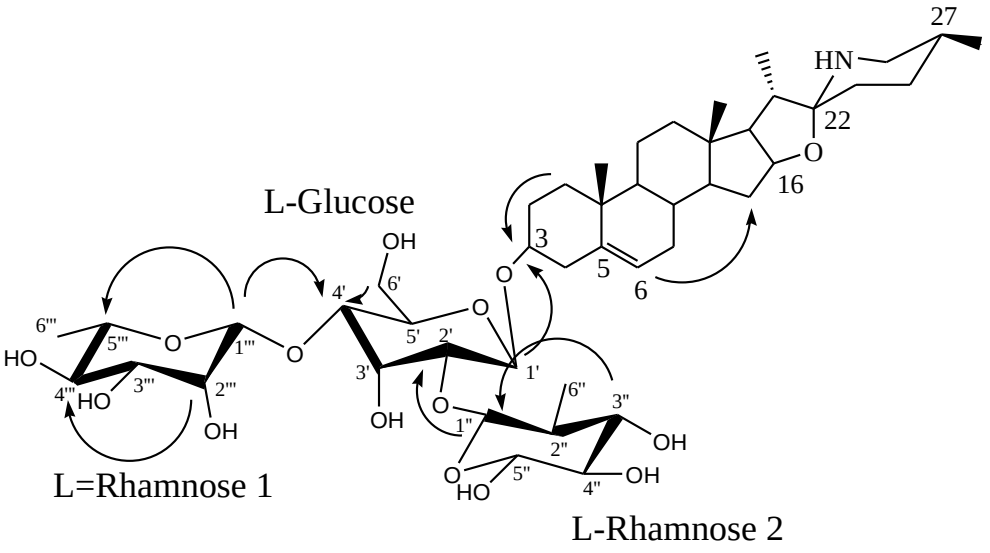


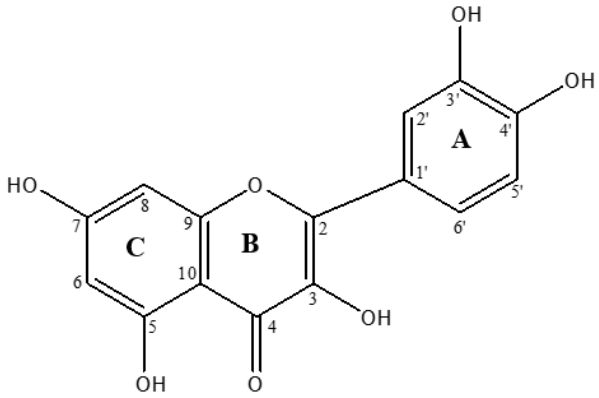
Figure 6. 9: The HMBC proton to carbon correlations observed for solamargine

Table 6.5: ^{1}H and ^{13}C NMR chemical shifts for solamargine (**6.18**) (in MeOD, 400 MHz) compared to the literature NMR data obtained of solamargine form literature (in $CDOD_3$, 500 MHz, respectively)

	Aglycone structures						Sugar moieties					
	Compound 6.18 in MeOD			Literature data of solamargine in $CDOD_3$			Compound 6.18			Literature data of solamargine		
Position	**δ_C /ppm**	**Dept 135**	**δ_H (multi, *J* in Hz)**	**δ_C /ppm**	**δ_H (multi, *J* in Hz)**	**Position**	**δ_C /ppm**	**Dept 135**	**δ_H (multi, *J* in Hz)**			
1	37.6	CH_2	1.27 (s), 1.8.0 (d)	37.9	1.73(d), 1.00(s)	**Glucose moiety**						
2	30.1	CH_2	1.50 (m), 1.82 (m)	30.5	2.10(m, 1.87(m)	1'	100.8	CH	4.82 (d, *J* = 1.2 Hz)	103.3	5.86 (s)	
3	78.1	CH-OH	3.50 (m)	78.5	3.89 (m)	2'	79.5	CH	3.77 (m)	72.9	4.69 (sl)	
4	38.9	CH_2	2.43 (m), 2.29 (m)	39.3	2.82(m), 2.75(m)	3'	77.7	CH	3.40 (m)	73.1	4.55 (dd, *J*= 9.1; 2.9)	
5	140.8	-		141.2		4'	76.7	CH	3.38 (m)	74.3	4.38	
6	121.6	CH	5.30 (br s)	122.2	5.32 (br s)	5'	79.0	CH	3.91 (m)	70.8	4.92	
7	32.5	CH_2	1.50 (m), 2.00 (m)	32.1	1.90(m), 160(m)	6'	61.4	CH	1.23 (d, *J* = 7 Hz)	18.9	1.64 (d, *J* = 5.9)	
8	32.4	CH	1.61 (m)	32.0	1.48(m)	**Rhamnose moiety**						
9	50.1	CH	0.88 (d)	50.7	0.90(d)	1"	101.5	CH	4.82 (d, *J* = 1.2 Hz)	103.3	5.86 (s)	
10	37.4	-		37.5		2"	71.8	CH	3.77 (m)	72.9	4.69 (sl)	
11	21.5	CH_2	1.50 (m)	21.5	1.45(m)	3"	72.2	CH	3.40 (m)	73.1	4.55 (dd, *J* 9.1; 2.9)	
12	40.6	CH_2	1.88 (m)	40.5	1.63/1.05(m)	4"	73.4	CH	3.38 (m)	74.3	4.38	
13	39.9	-		41.0		5"	69.9	CH	3.91 (m)	70.8	4.92	
14	56.9	CH	0.95 (m)	57.0	1.10(m)	6"	18.3	CH	1.23 (d, *J* = 7 Hz)	18.9	1.64 (d, *J* = 5.9)	
15	31.5	CH_2	1.87 (m), 1.31 (m)	32.9	2.08/1.45	**Rhamnose moiety**						
16	80.0	CH	4.30 (m, *J* = 7 Hz)	79.2	1.73/1.00	1'"	104.8	CH	5.20 (d, *J* = 1.2 Hz)	102.4	6.41 (s)	
17	62.5	CH	1.55 (m)	63.9	1.87(m)	2'"	71.9	CH	3.78(m)	72.9	4.84 (br s)	
18	16.7	CH_3	0.88 (s)	16.9	0.88 (s)	3'"	72.1	CH	3.59 (m)	73.1	4.64 (dd, *J* = 9.1; 2.9	
19	19.7	CH_3	0.95 (s)	19.8	1.07 (s)	4'"	73.5	CH	3.36 (m)	74.5	4.34	
20	42.0	CH	1.98 (m)	42.0	1.98(m)	5'"	69.3	CH	4.10 (m)	69.9	4.96	
21	15.6	CH_3	0.99 (d, *J* = 7 Hz)	16.1	1.09 (d, *J* 6.9)	6'"	18.5	CH	1.25 (d, *J* = 7 Hz)	19.0	1.78 (d, *J* 6.0)	
22	98.9	-		98.7								
23	31.2	CH_2	1.50 (m)	35.0	1.52(m)							
24	31.1	CH_2	2.20 (m)	31.4	2.78(m)							
25	32.1	CH	1.52 (m)	32.1	1.65(m)							
26	48.0	CH_2	2.72 (d, *J* = 7 Hz), 2.60 (t, *J* = 7 Hz)	48.2	0.83 (d, *J* = 4.8 Hz)							
27	19.5	CH_2	0.87 (d, *J* = 6.4 Hz)	20.1	0.98(m), 0.90(m)							

A comparison of the spectral data (**Table 6.5**) with the literature revealed that compound **6.18** is
(2*S*,2'*S*,3'*R*,4'*R*,5*R*,5'*R*,6'*S*)-2,2'-{[2*R*,3*S*,4*S*,5*R*,6*R*)-4-Hydroxymethyl)-6-{[2*S*,2'*R*,4a*R*, 4b*S*,5'*R*,6a*R*,7*S*,9a*S*,10a*S*,10b*S*)-4a,5',6a,7-tetrmethyl-1,2,3,4,4a,4b,5,6,6a,6b,7,9a,10,10a, 10b,11-hexadecahydrospiro[napho[2',1',4,5]indeno[2,1-b]furan-8,2'-piperidin]-2-yl]oxy} oxane-3,5-diyl]bis(6-methyloxane-3,4,5-triol), commonly known as solamargine. It has been reported in the fruit of *S. nigrum* before an it is being reported in the fruit of *S. incanum* for the first time (Ding et al, 2012; Kaunda and Zhang, 2019.

6.4.5 Characterisation of Quercetin (6.19)



Based on the findings in Chapter 5 section 5.2.2, the presence of quercetin (used as a reference) at an R_f value of 0.05, was also confirmed in EFE and Eroot. Compound **6.19** was isolated from the EFE extracts of *S. incanum as a* yellow powder. The HRESIMS positive mode spectrum depicted a molecular ion peak $[M+H]^+$ at *m/z* 303.0498 [**PLATE 6.19G**], thus, the molecular formula of **6.19** was determined as a flavonoid of formula $C_{15}H_{10}O_7$. Compound **6.19** has been reported in the leaves of *S. nigrum* (Schilling, 1984). It is usually isolated as a flavonoid glycoside in *Solanum* species (Silva et al, 2022), but in this study, it has been detected with no sugar molecules attached to it.

The UV spectrum of **6.19** exhibited a typical two maxima flavonoid absorption pattern with a pronounced band **II** at λ_{max} 207 and 248 nm indicating the presence of hydroxylation on the A-ring at positions C-5 and C-7 and band **I** at λ_{max} at 288 nm. The smaller absorption peak observed at 280 nm confirmed the presence of the -OH at position C-3 on the C-ring (Biler et al, 2017). Compound **6.19** had no chiral centres.

The FTIR spectrum of **6.19** (**PLATE 6.19H**) exhibited a broad peak at 3399, 3279 cm^{-1} due to the stretching of the phenolic hydroxyl groups (O-H) while the band at 1664 cm^{-1} indicating the presence of the carbonyl (C = O) stretching frequency and the C-O-H deformation was observed at 1304 cm^{-1}.

The NMR (^{1}H, ^{13}C, COSY, HSQC, and HMBC) experiments were conducted to elucidate the structure of **6.19**. The ^{1}H NMR spectrum (**PLATE 6.19A** in the appendix) disclosed the presence of two types of aromatic doublet protons at δ_H 7.54 and 6.88 ppm with the respectively constant coupling of *J* = 8.4 Hz. This is typical meta-coupling in the aromatic system of a flavone ring structure (Akter, 2013; Liu, Di, and Shi, 2008). The ^{1}H NMR spectrum also showed protons at δ_H 7.68 (1H, *d, J* =2.2 Hz, H-2′), 7.54 (1H, *dd, J* =2.2 Hz, 8.4 Hz, H-6′) and 7.54 (1H, *d, J* =2.16 Hz, H-5′) which had a constant coupling constant of 2.2 Hz. These coupling constants are typical of the 1', 3', and 4' trisubstituted aromatic ring which is likely to be the B ring of the flavone aglycone of **6.19** (Ganbaatar et al., 2015). A total of 5 protons were observed at δ_H 6.21, (1H, *s*, H-6), 6.41, (1H, *s*, H-8), 6.88 (1H, *d, J* = 2.16Hz, H-5'), 7.69 (1H, d, *J* = 8.4Hz, H-2'), 7.56 (1H, dd, *J* = 2.2, 8.4 Hz, H-6') from the aromatic rings.

The ^{13}C NMR spectrum [**PLATE 6.19B**], indicated a total of 15 carbon signals. The DEPT 135 NMR spectrum [**PLATE 6.19C**] showed a deshielded carbon signal at δ_C 179.6 ppm which was assigned to a carbonyl carbon (C-4), five methine carbons at C-6 (δ_C 92.3), and C-8 (δ_C 98.7, on **A**-ring and C-2' (δ_C 113.6), C-5' (δ_C 117.2) and C-6' (δ_C 120.4) on **B** ring were also assigned. The carbon atoms linked to hydroxyl groups appeared at C-7 (δ_C 164.2), C-2 (δ_C 161.1), C-3' (δ_C 147.4), C-4' (δ_C 146.6), and C-2 (δ_C 135.8) as indicated in **PLATE 6.19B.**

For further validation, the HMBC NMR spectrum [**PLATE 6.19E**], correlations were observed between the protons at H-6'(δ_H 7.54) to C4', H-2' δ_C 7.68 to C2; C6'; C8 and H-6 (δ_C 6.19) to C8; and C4 - **Figure 6.10**. The analysis of the HRMS, UV, FTIR, and NMR data led to the assignment of (2-3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one.

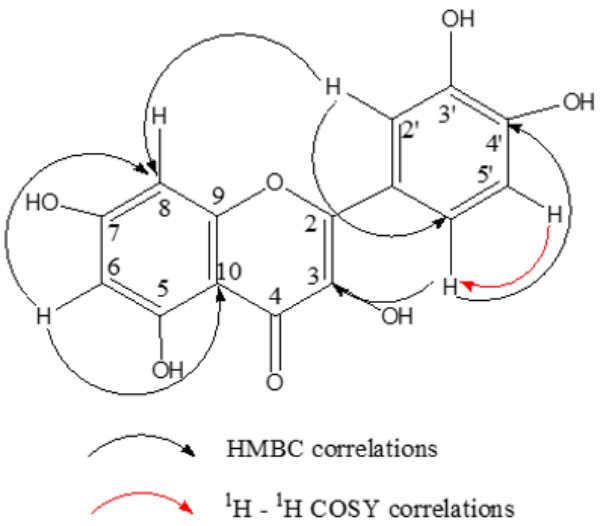
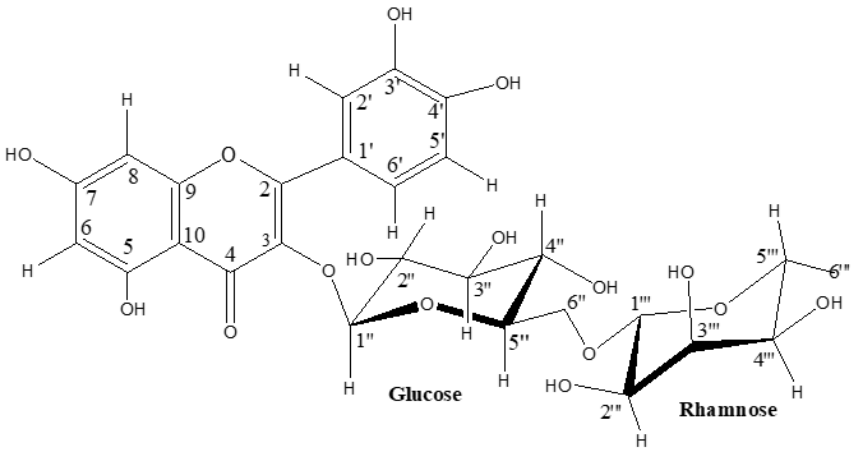


Figure 6. 10: The HMBC and COSY correlations observed in (2-3,4-dihydroxyphenyl)-3,5,7- trihydroxy-4H-chromen-4-one

6.4.6 Characterisation of rutinoside (6.20)



The presence of phenolics was confirmed in the methanol fruit MFE, ethyl acetate EFE, and ethyl root **Eroot** extracts by the appearance of green colour on the derivatised chromatogram when the crude extracts were compared to quercetin used as the reference when viewed under UV 366 nm as indicated in chapter 5 section 5.2.2. Herein we reported the isolation and structural elucidation of **6.20** a flavonoid glycoside isolated in the MFE crude of *S. incanum.*

The DCM: EtOAc:MeOH (8.0:1.5:0.5 v/v/v) mobile phase enabled the isolation of **6.20** (25.46 mg) at R_f 0.14 as a fine yellow powder from a methanol fruit extract (MFE). A molecular ion peak at *m/z* 611.1567 $[M+H]^+$ and the base molecular ion peaks due to the fragmentation of the sugar moieties at m/z 303.0349[M – rutoside + H]$^+$, 147.3402 [$C_6H_{11}O_4^+$], and

163.0623[$C_6H_{11}O_5^+$] (**PLATE 6.20G**) confirmed the molecular formula of (**6.20**) as $C_{27}H_{30}O_{16}$. The fragments 147.3402 [$C_6H_{11}O_4^+$], and 163.0623[$C_6H_{11}O_5^+$] represent the rhamnose and the glucose sugar ion peaks respectively.

The FTIR spectrum (**PLATE 6.20H**) showed major absorption bands at 3409 and 3324 cm^{-1} (stretching effect of the phenolic hydroxyl groups and the hydroxyl groups from the sugar moieties respectively), 2907.87 cm^{-1} (stretching of the C-H), 1595.76 cm^{-1} (C=C), 1650.99 cm^{-1} (C=O), 1362 cm^{-1} for a C-C, 1294 cm^{-1} for C-O stretching, and O-H in-plane deformation coupling at 1204 cm^{-1}, while the vinyl ether (C—O—C) bonds exhibited stretching vibrations at 1064 cm^{-1}. The phenolic fingerprint at 799.80 to 476.55 cm^{-1} was also observed. The UV λ_{max} ranged from (230-380) nm which is in the λ_{max} region of the aromatic group.

Similar ^{13}C NMR and ^{1}H NMR data to that observed for compound **6.19** were also detected for compound **6.20** in which two types of aromatic protons two doublets at δ_H 7.54 and 6.88 ppm were displayed in the ^{1}H NMR spectrum (**PLATE 6.20A**) with both having a constant coupling of *J* = 8.4 Hz each. The ^{1}H NMR spectrum also displayed protons at δ_H 7.68 (1H, d, *J* =2.2 Hz, H-2′), 7.54 (1H, dd, *J* =2.2 Hz, 8.4 Hz, H-6′) and 7.54 (1H, d, *J* =2.16 Hz, H-5′) with the same constant coupling of 2.2 Hz. This was an indication of the presence of a flavone quercetin aglycone in **6.20**. The noticeable differences in the ^{1}H NMR of **6.20** compared to that of **6.19** are the proton signals at δ_H 5.15 and H-4.95 and the overlapping signals between 3.25 to 3.75 ppm. The chemical shift H-1' (δ_H 5.15) and H-1'' (δ_H 4.95), indicated the anomeric protons in **6.20**. The signals for the sugar moieties are also observed in the ^{13}C NMR spectrum of 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl-6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside (**6.20**) at the δ_C range of 65-78 ppm as indicated in the ^{1}H NMR **PLATE 6.20A**.

The six signals from the ^{13}C NMR spectrum (**PLATE 6.20B**) at C-1' (δ_C 101.0), C-2' (δ_C 73.9), C-3' (δ_C 75.6), C-4' (δ_C 76.3), C-5' (δ_C 71.7), and C-6' (δ_C 66.9) ppm represented an β-D-glucose moiety and the other six represented C-1''' (δ_C 100.6), C-2''' (δ_C 70.1), C-3''' (δ_C 68.2), C-4''' (δ_C 70.4), C-5''' (δ_C 69.5) and C-6''' (δ_C 17.6) ppm an ∝-L-rhamnose sugar moiety. The coupling constants for non-anomeric protons on sugars could not easily be ascertained from ^{1}H NMR signals due to superimposition (Ghani et al, 2021). However, the splitting patterns of the anomeric sugar moieties were recognised. Therefore, the anomeric linkages for glucose and rhamnose were established from the coupling constants of the

anomeric protons which were at δ_H 5.12, (1H, d, *J*=7.6 Hz (β, H-1")) and 4.56, d, *J*=1.5 Hz (α, H-1") respectively. The data was compared with that from literature (Georgeta, Pana, Tunde, and Sanda, 2016).

The oxymethine (the carbon of the glucopyranose at C-1' (δ_C 101.0) was an anomeric alpha (α) carbon (Georgeta et al., 2016; Won, 1990). The DEPT NMR spectrum (**PLATE 6.20C**) was used to determine the oxymethine groups of the glucopyranose as C-2' (δ_C 73.9), C-3' (δ_C 75.6), C-4' (δ_C 76.3), and C-5' (δ_C 71.7) ppm and a methylene carbon at and C-6' (δ_C 66.9). The methylene carbon of glucopyranose formed a glycosidic bond with the rhamnopyranose moiety at C-1''' (δ_C 100.6) (Akter, 2013). The DEPT spectrum revealed the oxymethine groups of the rhamnopyranose (δ_C 70.1), C-3''' (δ_C 68.2), C-4''' (δ_C 70.4), and C-5''' (δ_C 69.5) with the methyl carbon signal at δ_C 17.8 ppm (C-6"'). The HMBC spectrum (**PLATE 6.20E**) correlated the proton at δ_H = 5.11 (H – 1'') to C – 3; 4.54 (H – 6'') to C – 3''; 3.84 (H -5''') to C – 1'''and C -4''' (**Figure 6.11**). There is a long-range correlation between the proton signal at δ_H 5.11(the anomeric proton of the glucose) and the carbon atom at δ_c 133, belonging to the C-3 on ring **C**, which allowed the placement of the glycosidic bond at position 3. The anomeric proton of the rhamnose sugar occurs at δ_H 4.54 and was observed in the HMBC spectrum to have a long-range correlation with the oxymethylene carbon at δc 70.0 for the CH_2OH group on the glycosidic sugar.

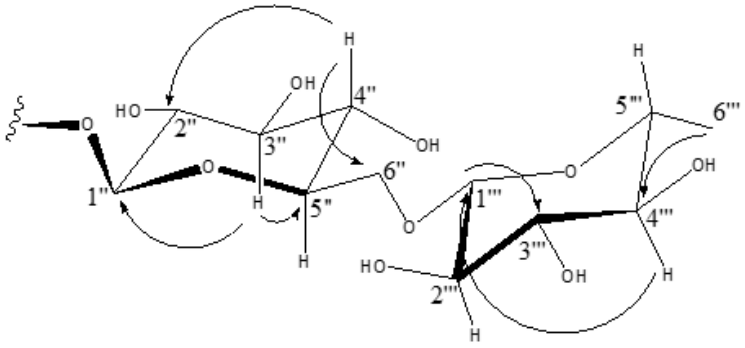
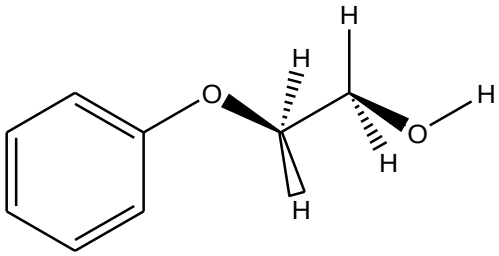


Figure 6. 11: The HMBC correlations observed for the disaccharide of compound 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl-6-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside

The analysis of the HRMS, UV-vis, and the NMR data led to the assignment of **6.20** to 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside (rutinoside). Compound **6.20** has been isolated in many other *Solanum* species which include the fruit of *S. anguvi, S. muricatum,* and *S. torvum,*

the leaf part of *S. nigrum,* and the stem of *S. melongena* and it is isolated for the first time from the fruit of S. incanum (Kaunda and Zhang, 2020). According to Kaunda and Zhang, (2020) and (Chua, 2013), **6.20** exhibit significant pharmacological activities that include anti-oxidation, anti-inflammation, anti-diabetic, anti-audiogenic, neuroprotective, and hormone therapy.

6.4.7 Characterisation of phenoxy-2-ethan-1-ol (6.21)



Phenoxy-2-ethan-1-ol (**6.21**) was isolated for the first time from the fruit of *S. incanum* although it is reported in extracted from *Muscari armeniacum* and *Erysimum allionii* flowers (Suburg et al, 1993) and peals of unripe tomatoes (Ron and Louisa, 1993). Phenoxy-2-ethan-1-ol (**6.21**) also known as glycol ether was obtained as a colourless oily liquid from the methanol fruit crude extract (MFE). Due to its high volatility, it was subjected to a GC-MS to determine its fragmentations and molecular mass.

The MS (**APPENDIX 6.21G**) showed peaks at m/z 137.0781, corresponding to the molecular ion $C_8H_9O_2^+$, and fragments at m/z 107.14 $(C_7H_7O)^+$, 93.11 $(C_6H_5O)^+$, 44.52 $(C_2H_4O)^+$ and 45.07 $(C_2H_5O)^+$ confirmed the molecular formula of **6.21** as $C_8H_{10}O_2$. Strong UV absorption peaks were observed at λ_{max} 230 and 280 nm. The λ_{max} of 280 nm exhibited by phenoxy-2-ethan-1-ol (**6.21**) was because of the π - π^* transitions of the delocalised electrons (**APPENDIX UV 6.21**) of the phenyl group. The relative retention time (R_t) and mass spectra of compound **6.21** were compared with the standard mass spectra in the NIST 21 Library and with the NMR data to identify it as phenoxy-2-ethan-1-ol. Major FTIR absorption bands were observed at 3336 cm^{-1}(OH), 2923 cm^{-1}(CH stretching), 1051 (C-O ether), and 2051 cm^{-1} (CH aromatic stretching). Compound **6.21** had no chiral carbons, therefore could not yield any cotton effects in their CD spectra.

The NMR data were used to confirm the identity of **6.21**. In the ^{1}H NMR spectrum (**PLATE 6.21A**) of phenoxy-2-ethan-1-ol (**6.21**) a downfield signal was observed at δ_H 7.26 (2H, m)

integrating for two protons where one proton is at position H-3 and the other at H-5 a characteristic of protons at meta positions on a non-substituted benzene ring. The ^{1}H NMR spectrum also indicated a signal at (δ_H 6.92) ppm integrating for two protons that are in the same environment at positions H-2(δ_H) and H-6(δ_H) shielded by the substituent on the benzene ring. The third signal at δ_H and 6.90 ppm assigned to the deshielded group integrating for one proton is at a para position to the benzene substituent and substituent has little effect on this proton hence having almost the same chemical shift as protons on the meta positions to it. A downfield triplet proton at δ_H 4.01 (2H, *t*, *J* = 3.88, 5.16 Hz) was assigned to the methylene group next to the phenyl group at C-7 (δ_C 68.3) and a triplet up field at δ_H 3.85 (2H, *t*, *J* = 3.52, 4.88 Hz) was assigned to the other methylene group at C-8 (δ_C 59.5). A singlet proton at δ_H at δ_H 4.85 ppm was assigned to the hydroxyl proton. The ^{13}C NMR spectrum (**PLATE 6.21B**) showed eight signals at C-1 (δ_C 159.3), C-2 (δ_C 113.9), C-3 (δ_C 128.9), C-4 (δ_C 120.6), C-5 (δ_C 128.9), and C-6 (δ_C 113.9), C-7 (δ_C 68.3) and C-7 (δ_C 59.5). The carbon signal at C-2 (δ_C 113.9) overlapped with the signal at C-6 and the signal at C-3 (δ_C 128.9) overlapped with the signal at C-5 (δ_C typical characteristic of a monosubstituted benzene ring. The DEPT 135 NMR spectrum (**PLATE 6.21C**) revealed 5 methine carbons at δ_C 113.9 (C-2)2, 128.9 (C-3)2, and 120.6 (C-4) and 2 methylene carbons at δ_C 68.3 and 59.5 ppm. Therefore, the proposed name of **6.21** was phenoxy-2-ethan-1-ol (**6.21**)

Further structural confirmation was achieved by analysing the HMBC and the ^{1}H-^{1}H COSY spectra (**PLATES 6.21E** and **F**). The HMBC correlations were observed between the proton at H-2 (δ_H 7.26) and carbons atoms C-3 and C-1, H-5 (δ_H 6.92), carbon atoms C-3, C-6 and C-1, δ_C H-7 (δ_H 4.01), and carbon atoms C-1 and C-8. A ^{1}H-^{1}H COSY coupling was observed between protons at H-2 (δ_H 7.26) and H-3 (δ_H 6.92); and the proton at δ_H H-7 (δ_H. 401) and H-8 (δ_H 3.85) as indicated in **Figure 6.12.**

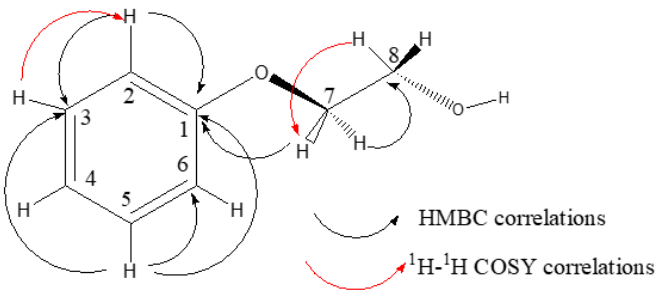
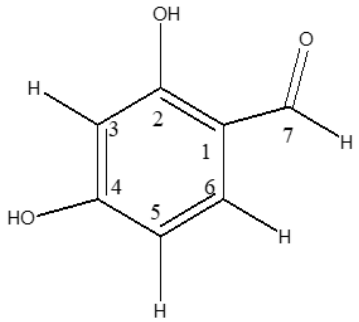


Figure 6. 12: The HMBC and ^{1}H-^{1}H COSY correlations observed for phenoxy-2-ethan-1-ol

Therefore, compound **6.21** was confirmed to be phenoxy-2-ethan-1-ol. The isolation of **6.21** is reported for the first time from *S. incanum*. It is an aromatic glycol ether which is has been extracted from *Muscari armeniacum* and Erysimum *allionii* flowers (Suburg et al, 1993) and peals of unripe tomatoes (Ron and Louisa, 1993). Compound **6.21** is synthesised in the industry as an ingredient for cosmetic products such as moisturizers, hand disinfectants, soaps, sunscreen creams, mascara, and perfumes because of its pure chemical form, pleasant smell, and colourless appearance. Other uses of phenoxy-2-ethan-1-ol (**6.21**) include insect repellents, antiseptics, solvents, anaesthetics, cellulose acetate solvents, and dyes. At higher concentrations, it is effective in controlling microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, and fungal infections caused by *Candida* (Minko Essono, Mvondo, Ngadjui, Kemka Nguimatio, and Watcho, 2020). Besides the benefits phenoxy-2-ethan-1-ol (**6.21**) holds, it has toxic effects if inhaled or ingested in high doses. phenoxy-2-ethan-1-ol may cause dehydration, vomiting, central nervous system problems, and diarrhoea when inhaled (Akgündüz et al, 2020).

6.4.8 Characterisation of 2, 4-dihydroxybenzaldehyde (6.22)



Compound **6.22** was isolated for the first time in *S. incanum*. Several techniques which comprised column chromatography (CC), thin layer chromatography (TLC), and preparatory thin layer chromatography (PTLC) were employed to enable the isolation of **6.22** form the ethyl acetate root crude extract. Repeated PTLC using a mixture of hexane and ethyl acetate in the ratio (7.3: 2.7 v/v) as mobile phase enabled the purification of 2, 4-dihydroxybenzaldehyde (**6.22**) at R_f 0.69. Compound **6.22** had not been reported in *S. incanum* before, although literature confirms its presence in Pterocarpus *santalinus*, *Adenanthera pavonine (*Abdullah, Salim, and Ahmad, 2016), and in the leaves and stems of *Anthyllis sericea and Uncaria cordata var. ferruginea* (Khadem and Marles, 2010), this is the first time its **6.22** has been isolated from *S. incanum* roots. Strong UV absorption peaks were observed at λ_{max} 238 for the carbonyl

carbon and 320 nm for the benzene ring (**APPENDIX 6.22.I**). Major FTIR absorption bands were observed at 3336 cm^{-1} (OH), 2923 cm^{-1} (CH-stretching), 2051 cm^{-1} (CH aromatic stretching), and 1711 cm^{-1} (C=O). Compound **6.22** had no chiral carbons, therefore could not yield any cotton effects in their CD spectra.

The seven ^{13}C NMR chemical shifts observed suggested that **6.22** was a small molecule, therefore, a GC-MS was used to determine the molecular mass of **6.22** as indicated on the MS spectrum (**PLATE 6.22G**). The MS fragmentation molecular ion at m/z [M^+] 138.035 confirmed the molecular formula as $C_7H_6O_3$. The relative retention time (R_t) and mass spectra of compound **6.22** were compared with the standard mass spectra in the NIST 21 Library and with the NMR data to identify it as 2, 4-dihydroxybenzaldehyde.

The NMR data further confirmed the structure of **6.22**. In the ^1H NMR spectrum (**PLATE 6.22A**) of 2, 4-dihydroxybenzaldehyde (**6.22)**, two ortho coupled doublets were observed at δ_H 7.50 (1H, d, J = 8.5 Hz, H-6) and 6.45 (1H, d, J = 8.5 Hz, H-5) a characteristic of protons attached adjacent to each other on a benzene ring. A singlet appearing at δ_H 6.30 (1H, s) was assigned to C-3 (δ_C 102.1) ppm of the benzene ring. This suggested that the benzene ring was tri-substituted 1, 2, and 4. According to the literature, the deshielded proton at δ_H 9.72 ppm represented an aldehyde proton (Akter, 2013; Kaunda and Zhang, 2019). From the ^{13}C NMR spectrum **PLATE 6.22B**), seven signals (C-1 (δ_C 114.7), C-2 (δ_C 164.2), C-3 (δ_C 102.1), C-4 (δ_C 165.9) C-5 (δ_C 108.5), C-6 (δ_C 135.4) and C-7 (δ_C 194.1) were observed suggesting that **6.22** had seven carbons. The DEPT 135 revealed that **6.22** had 4 methine groups, though three were displayed as part of the benzene ring at C-3 (δ_C 102.1), C-5 (δ_C 108.5), C-6 (δ_C 135.4), and C-7 (δ_C 194.0). Therefore, three of the carbons were unprotonated at C-4 (δ_C 165.9) C-2 (δ_C 164.2), and C-1 (δ_C 114.7). The deshielded proton at δ_H 9.71 ppm was assigned to a carbonyl carbon at δ_C 194.0 (C-7). The oxygen-linked carbons were at C-4 (δ_C 165.9) and C-2 (δ_C 164.2).

Further structural confirmation of **6.22** was achieved by analysing the HMBC and the ^1H-^1H COSY spectra (**PLATES 6.22E** and **6.22F**). HMBC correlations were observed between the proton at H-7 (δ_H 9.71) and the carbon atoms at C-3 (δ_C 102.1), C-6 (δ_C 135.4), and C-2 (δ_C 164.17). The proton at δ_H 7.51 (H-6) correlated with carbon atoms at C-7 (δ_C 194.0), C-4 (δ_C 165.9), and the proton at H-5 (δ_H 6.45) correlated with carbon atoms at C-1 (δ_C 114.7), C-3 (δ_C 102.1). The ^1H-^1H COSY spectrum showed coupling between protons H-5 and H-6. The

correlations are summarised in **Figure 6.13.** Two protons from hydroxyl groups which gave a singlet signal at δ_H 3.27 ppm were assigned to the hydroxyl protons (H-2) and (H-4).

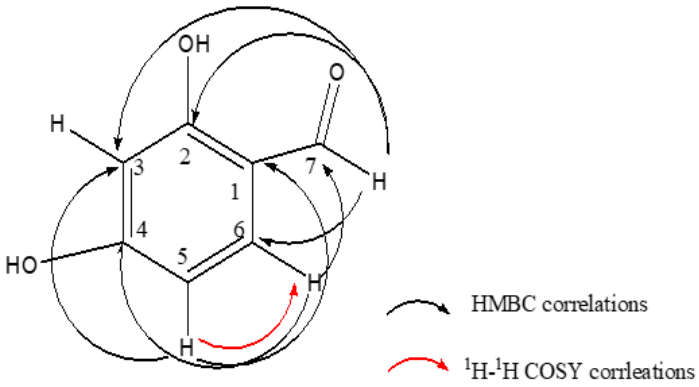
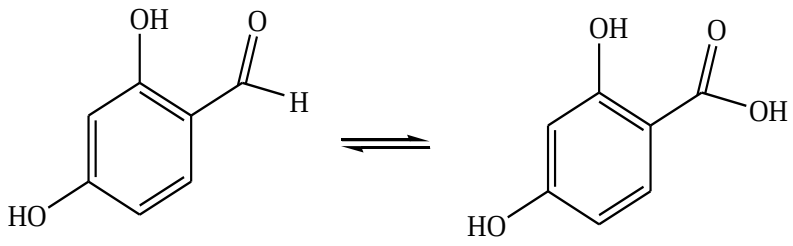


Figure 6. 13: The HMBC and ^{1}H-^{1}H COSY correlations observed in 2, 4-dihydroxybenzaldehyde

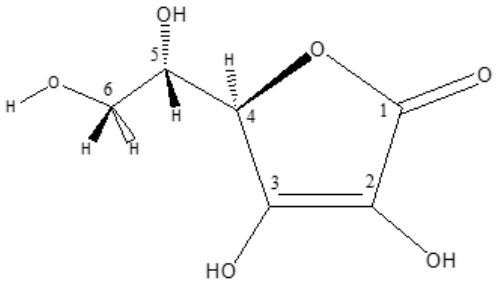
Based on experimental NMR data and literature data, compound **6.22** was assigned as 2, 4-dihydroxybenzaldehyde (**6.22**), This is the first report of 2,4-dihydroxypheylaldehyde in the genus of *Solanum*. 2,4-dihydroxypheylaldehyde has previously been isolated from cranberry (*Vaccinium oxycoccos*) (Blumberg et al., 2013). 2, 4-Dihydroxybenzaldehyde (**6.22**) is converted (oxidised) into β-Resorcylic acid (2,4-dihydroxybenzoic acid) (**Figure 6.14**.).



2,4-Dihydroxyphenylaldehyde β-Resorcyclic acid

Figure 6.14: The conversion of 2,4-dihydrobenzaldehyde to 2,4-dihydrobenzoic acid

6.4.9 Characterisation of vitamin C (6.23)



Compound 6.23 has been isolated from *S. incanum* before. In this study, it was isolated from the MFE extract of the fruit of *S. incanum* using chromatographic methods that include column chromatography and PTLC. The HRESIMS positive mode spectrum of **6.23** produced a molecular ion peak $[M+H]^+$ (100%) at *m/z* 177.0354 [**PLATE 6.23G**]. The molecular formula of 3,4-dihydroxy-5-(1,2-dihydroxyethyl)furan-2(5H)-one **(6.23)** was determined as $C_6H_8O_6$ after comparing this EISMS value with those of previously isolated compounds from the *S. incanum* plant.

The UV spectrum displayed a strong absorption peak at λ_{max} 267 nm and (in methanol). The absorption peak is due to a galactose ring in which the ene-diol portion and the oxygen of the lactone moiety are conjugated to the carbonyl group (Wittine et al., 2004).

A negative Cotton effect is observed at λ_{max} ($\Delta\varepsilon$)227 nm, and a positive CD-band at λ_{max} ($\Delta\varepsilon$)247 nm ascribed to the cyclic conjugated enones which are negative at UV 265 nm and positive around 240 nm giving rise to the *S* configuration at the chiral carbon at C-5 (δ_C 69.3) confirms the assignment of 6.23 to 3,4-dihydroxy-5-(1,2-dihydroxyethyl)furan-2(5H)-one**.**

A major FTIR absorption band at a range of 3320 cm^{-1} was observed which represented the stretching of the (O-H) (**PLATE 6.23H**). The band at 1614.49 cm^{-1} represented the C=C stretching, and the band located at 1720 cm^{-1} represented the C=O stretching. According to the literature, the stretching mode of the O-H for **6.23** is observed at a range of 3320 cm^{-1}, and the stretching mode for the C=O and C=C at 1614 cm^{-1}at 1753 cm^{-1} respectively (Dabbagh, Azami, and Farrokhpour, 2014). The bands 1062 and 1471 cm^{-1} are linked to the stretching mode of the C-O and C-C bonds for compound **6.23** (Dabbagh et al., 2014).

From the ^{1}H NMR (**PLATE 6.23A**), a singlet signal at H-6 (δ_H 3.70) integrating into two protons and a multiplet signal at H-5 (δ_H 3.92) integrating into a doublet signal at H-4 (δ_H 3.38) integrating into a proton are observed (**PLATE 6.23A**). The fourth signal downfield at δ_H 4.82 integrating into 4 protons indicated the 4 protons. The four protons form the hydroxyl groups at H-6; H-5; H-3 and H-2. The ^{13}C NMR spectrum (**PLATE 6.23B**) revealed six carbon signals, a carbonyl carbon at C-1 (δ_C 172.1); quaternary carbons at C-2 (δ_C 153.4) and C–3 (118.5); methine carbons at C-4 (δ_C 75.6) and C-5 (δ_C 69.3), and a methylene carbon at C-6 (δ_C 62.2. The NMR data above led to the assignment of **6.23** to 3,4-dihydroxy-5-(1,2-dihydroxyethyl)furan-2(5H)-one**.**

Further structural confirmation was achieved by analysing the HMBC and ^{1}H-^{1}H COSY spectra [**PLATES 6.23E** and **PLATES 6.23F**] respectively. The HMBC spectrum showed the presence of cross peaks between the proton at H-5 (δ_H 3.92) and carbons at C-6 (δ_C 62.19), and C-4 (δ_C 75.62). The proton at δ_H 3.38 (H-4) had a long-range correlation with carbons at δ_C C-6 (δ_C 62.2), C-1 (δ_C 172.2), C-2 (δ_C 153.4), and C-3(δ_C 118.50. The ^{1}H-^{1}H COSY spectrum revealed a correlation of protons at δ_H 3.94 (H-5) with H-6 and H-4.

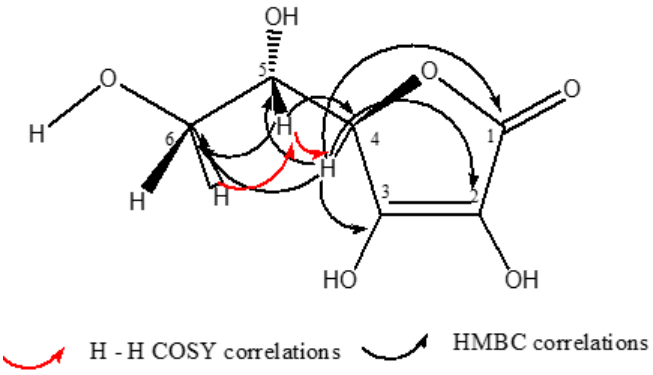


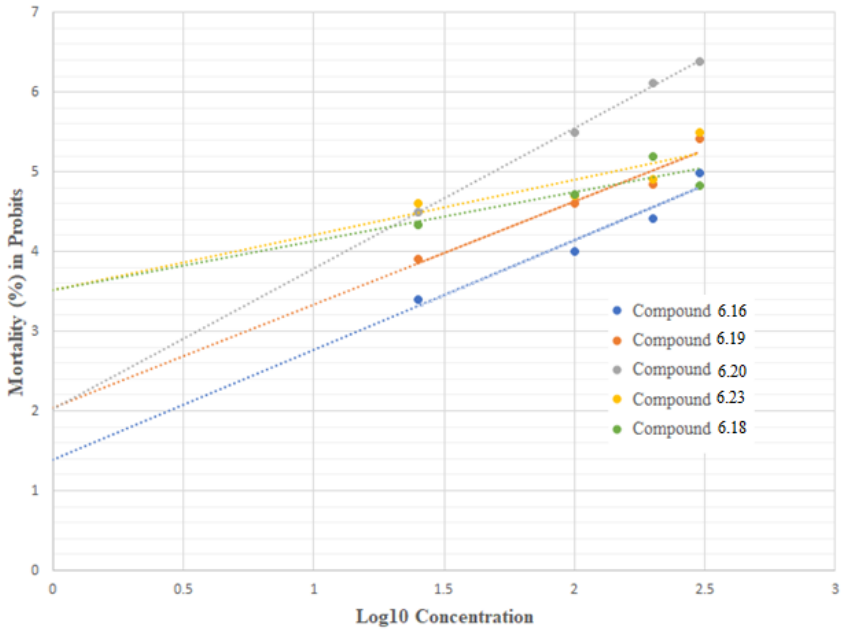
Figure 6. 15: The HMBC correlations of 3,4-dihydroxy-5-[(*S*)-1,2-dihydroxyethyl] furan-2(5H)-one

Based on the experimental NMR data of 3,4-*d*ihydroxy-5-[(S)-1,2-dihydroxyethyl] furan-2(5H)-one **(6.23)** and that from literature, compound **6.23** was assigned 3,4-dihydroxy-5-(1,2-dihydroxyethyl) furan-2(5H)-one (Dabbagh et al, 2014). Compound **6.23** has been isolated from other Solanum species which include *S. nigrum, S. tuberosum,* and *S. incanum* (Kaunda and Zhang, 2019).

Compound **6.23** 6.23 is ascorbic acid popularly known as vitamin C. It is involved in a wide variety of biochemical processes of living species (plants, animals, and human beings) serving multifunctional roles as an antioxidant or scavenger of radicals, enzyme cofactor, *etc* (Dabbagh et al, 2014). As an antioxidant, it behaves as a two-electron donor through hydrogen atom (electron + proton) transfer, giving rise to the ascorbate radical ion called "semi-dehydro-ascorbic acid" and then dehydroascorbic acid (DHA). It is, therefore, capable of scavenging various oxidizing reactive oxygen (hydroxyl radical $^{\cdot}OH$, superoxide radical $^{\cdot}O_2^-$, hydrogen peroxide H_2O_2, etc.) and nitrogen species, which are usually related to the oxidation process of various components (Dabbagh et al, 2014; Wittine et al, 2004)

6.5 Efficacy of the isolated compounds towards cabbage aphids

The efficacy of the isolated compounds was performed using the Aphid Leaf dip method as explained in Chapter 5, section **5.4.8**. The dose variation effect of the isolated compounds of *S. incanum* on the cabbage aphids was evaluated using a Probit transformation analysis (**Figure 6.16**). The data of the five compounds (**6.16**, **6.19**, **6.20**, **6.23,** and **6.18**) which showed activity against the cabbage aphids processed using the Probit transformation technique to calculate the lethal dose (LD_{50}) of the isolated compounds is displayed. The efficacy of two compounds isolated from the root and three compounds isolated from the fruit of *S. incanum* were determined. The mortality rates of the compounds were Probit transformed to determine the lethal dose at 50% of the population of the cabbage aphids investigated. The Probit transformed results are indicated in **Figure 6.16**.



Key:

	Name of the isolated compound		Name of the isolated compound
Compound **6.16**	(2*S*,2′*R*,4a*R*,4b*S*,5′*R*,6a*S*,6b*R*,7*S*,9a*S*,10a*S*,10b*S*)-4a,5′,6a,7-tetramethyl-1,2,3,4,4a,4b,5,6,6a,6b,7,9a,10,10a,10b,11-hexadecahydrospiro [naptho 2′,1′:4,5] indeno[2,1-*b*]furan-8,2′-piperidin]-2-ol	Compound **6.23**	3,4-*d*ihydroxy-5-[(S)-1,2-dihydroxyethyl]furan-2(5H)-one
Compound **6.19**	2-3,4-(dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one	Compound **6.18**	Solarmargine
Compound **6.20**	phenoxy-2-ethan-1-ol	Control	Bambazonke

Figure 6.16: The Probit transformed the analysis of the isolated compounds extracts

Table 6.6 shows the results of the lethal doses of the five isolated compounds. The phytochemicals isolated from the roots and fruits of *S. incanum* showed different levels of efficacy against cabbage aphids. Solamargine (6.**18**) displayed the highest aphid mortality rate with an LD_{50} of 54.44±6.23 $\mu g \cdot mL^{-1}$, followed by (**6.23**) at 89.62±11.68 $\mu g \cdot mL^{-1}$ exhibited the lowest aphid mortality rate with an LD_{50} of 115.24±15.78 $\mu g \cdot mL^{-1}$. The crude extracts exhibited higher efficacy than those from the isolated compounds. Probably, the phytochemicals in the crude extracts work in synergy for them to be more effective.

Table 6.6: The data of the evaluation of the isolated compounds from *S. incanum* fruit and root crude extracts

	Name of the isolated compound	**LD_{50}** (μg $\cdot mL^{-1}$)	**Source**
Compound **6.16**	(2*S*,2′*R*,4a*R*,4b*S*,5′*R*,6a*S*,6b*R*,7*S*,9a*S*,10a*S*,10b*S*)-4a,5′,6a,7-tetramethyl-1,2,3,4,4a,4b,5,6,6a,6b,7,9a,10,10a,10b,11-hexadecahydrospiro [naptho 2′,1′:4,5] indeno[2,1-*b*]furan-8,2′-piperidin]-2-ol	115.24±15.78a	Root
Compound **6.19**	2-3,4-(dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one	103.15±18.10a	Root
Compound **6.20**	Phenoxy-2-ethan-1-ol	54.44±5.44b	Fruit
Compound **6.23**	3,4-*d*ihydroxy-5-[(S)-1,2-dihydroxyethyl] furan-2(5H)-one	89.62±11.68c	Fruit
Compound **6.18**	Solarmargine	95.92±14.21c	Fruit
control	Bulalazonke	10.19±1.55e	-

*The results are expressed as (mean ± SD), N = 3
**Means followed by a different letter(s) are significantly different at the 5 percent level, the LSD test

6.6 Conclusion

The extraction and isolation of phytochemicals from the crude extracts of the root and fruit of solanuminone **(6.16)** and diosgenin (**6.15**). Three phenols that included quercetin (**6.19**), rutin (**6.20**) and 2,4-dihydroxypheylaldehyde (**6.22**) were isolated form the methanol fruit extract of *S. incanum*. Compound **6.21** is a phenyl ether. Compounds **6.21** and **6.22** are being reported for the first time from *S. incanum* although a literature search revealed that **6.21** had been isolated from the *Muscari armeniacum* and Erysimum *allionii* flowers (Suburg et al, 1993) and from peals of unripe tomatoes (Ron and Louisa, 1993) and **6.22** has been isolated from *Vaccinium oxycoccos* (Blumberg et al., 2013). 3,4-*d*ihydroxy-5-[(S)-1,2-dihydroxyethyl] furan-2(5H)-one **(6.23)** is a dihydrofuran isolated from the ethyl acetate fruit extract. According to Mwonjoria et al, (2014), compound **6.23** has been isolated from the aerial parts of *S. incanum* before. Compound **6.23** is an antioxidant.

The study demonstrated that *S. incanum* has the potential to be used as a pesticide. Furthermore, the use of indigenous plants as pesticides by small-scale farmers provides cheaper and easily accessible pesticides. A recent review by Stevenson et al (2017). reported a huge diversity of pesticidal plants in Africa and prospects for developing plant-based pesticides.

6.7 Experimental

6.7.1 Chromatographic techniques

Liquid Chromatography Mass Spectrometry (LC-MS) was used to detect the elementary constituent compounds in the crude samples by providing the possible m/z. The LC-MS results were obtained on a Waters Synapt G2 quadrupole time-of-flight mass spectrometer (detected by an ESI-positive source with a 15 V cone voltage). Identification of isolates present was based on computer matching with the ChemSpider, PubChem, and MassBank as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature.

Column chromatography (CC) was performed on open glass columns packed with silica gel of particle size (silica-Amorphous, precipitated; size 60A, 35-60 mesh. Separation of compounds was achieved through the varying absorption on and interaction between the stationary and mobile phase. Vacuum Liquid Chromatography (VLC) was performed on a Buchner funnel column packed with silica gel of particle size (silica-Amorphous, precipitated; size 60A, 35-60 mesh) connected to a vacuum pump. Preparative thin-layer chromatography (PTLC), was used to separate, isolate, and purify fractions. A PTLC was carried out using an aluminium plate (200 × 100 mm, precoated with silica gel 60 F_{254}). The plates were run in the appropriate solvent system determined by the TLC process. The products (separation of the compounds in the fraction) were located by UV visualisation, at UV 254, 366 nm, and marked with a pencil.

The MS spectrum of the isolated compounds **6.21** and **6.22** was obtained using a GC-MS, model (Agilent GC- 17 A/ MS QP5050A. Agilent 6890N GC, 5973) connected to an SLB®-5ms medium polarity column (30 m x 0.25 mm × 0.25 µm film thickness) which was directly coupled to the mass spectrometer. The carrier gas was helium (40 $mL \cdot min^{-1}$), with a flow rate in 50:1 split mode. The injection volume was 1 µL. The initial oven temperature was 50 ºC and

was increased by 10 °C·min^{-1} to 340 °C. The detector temperature was 300 ºC, while the column temperature was programmed at 360 ºC maximum. The flow rate was 1 µL·min^{-1}, with 27 min run time. Identification of components present in extracts was based on computer matching with the Wiley 229, NIST 107, and NIST 21 Library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature.

6.7.2 Isolation of compounds

6.7.2.1 Methanol fruit extract (MFE)

Sixteen (16) grams of MFE crude sample was dissolved in 5% aqueous acetic acid at room temperature and filtered off using a Buchner funnel. The resulting solution was then basified with NH_4OH (pH=11) and extracted with water-saturated butan-1-ol to obtain a 2,8 g crude sample labelled **MFE**. The crude extract (2.0 g) was then subjected to vacuum liquid chromatography (VLC) eluting with varying DCM: MeOH. From the VLC, 5 fractions (MF1-MF5) were collected. Three bands were obtained when viewed under UV light (short and long). The band at R_f 0.42 was scrapped out, dissolved in methanol, filtered, and allowed to dry to obtain the compound MF22 (W1) (8.34 mg). Based on the TLC profile obtained using DCM: MeOH (93:0.7 v/v) solvent system, fraction MF4 (0.2 g) was subjected to a repeated preparative thin layer chromatography (PTLC), using silica gel [(20 x 20) cm, Kieselgel 60 F_{245}] eluted with chloroform/DCM/methanol (70:20:10) generated 28 sub-fractions. Based on the TLC profiles, the fractions were grouped into four (MF41-MF44). Sub-fraction MF42 was then subjected to another CC on silica gel (silica-Amorphous, precipitated; size 60A, 35-60 mesh) eluted with DCM: MeOH (93:0.7) to give 24 sub-fractions which were re-grouped to four sub-fractions MF421 to MF424. Sub-fraction MF422 (21,3 mg) was further purified by subjecting it to a preparative thin layer chromatography (PTLC) to yield 9.8 mg of compound (W2) (**6.19**) at R_f 0.16 and 11.6 mg of compound (W3) (**6.22**) at R_f 0.14.

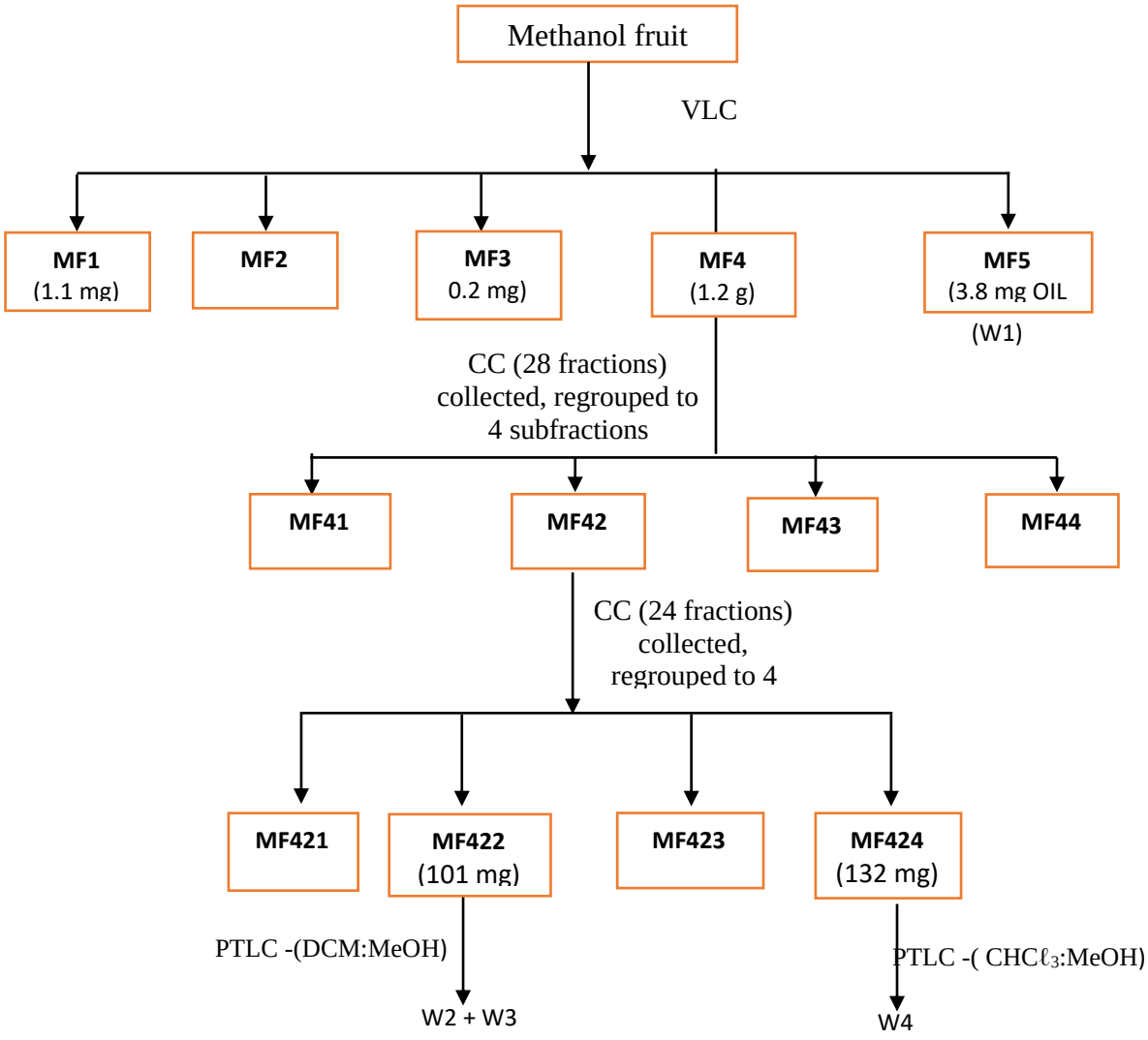


Figure 6. 17: Flow diagram for the isolation of compounds from the methanol crude extract

6.7.2.2 Ethyl acetate root extract (Eroot)

Based on the TLC profiling using Hex: EtOAc (9.1 v/v) solvent system, 1.3 g of the Eroot crude was subjected to a CC (43 x 3cm) prepared using silica gel (silica-Amorphous, precipitated; size 60A, 35-60 mesh) eluted with Hex: EtOAc (8:2 v/v) and the fractions were monitored by TLC. Those fractions that had similar retention factors were combined as indicated in Table 6.7.

Table 6.7: The combined fractions from an open CC run

Fractions combined	Code	No. of spots observed on the TLC plate	Mass of fraction /mg
f1 - f15	ER1	2	235.4
F15 - f20	ER2	4	1,01
F21 - f23	ER3	7	0,5
F23 - f32	ER4	8	0,4

Using a Chloroform-ethyl acetate (8:2 v/v) solvent system, fraction ER1 (235,4 mg) was subjected to a preparative thin layer chromatography (PTLC) repeatedly using an aluminium plate coated with silica gel [(20 x 20) cm, Kieselgel 60 F_{245}] to obtain four sub-fractions ER11 (0.45 mg), ER12 (0.92 mg), ER13 (1.02 mg) and ER14 (16.1 mg). Based on the TLC profiling, ER14 was subjected to a repeated PTLC eluted with Hex: EtOAc (8:2 v/v) to obtain 3 sub-fractions ER14A to ER14C. A repeat of the PTLC on sub-fraction ER14A enabled the isolation of 8.34 mg of a white precipitate named ER14A1 (**6.23**).

6.7.2.3 Hexane root crude extract (Hroot)

A 1.5 g sample of Hroot crude fraction was subjected to open CC (43 X 3cm) eluted with Hexane: EtOAc solvent system (8:2 v/v) under gradient and the 23 fractions collected were monitored by TLC. Those fractions that had similar retention factors were combined to make four fractions labelled HR1 to HR4. Fraction HR4 with the most amount in mass was subjected to repeated PTLC using the Hex: EtOAc (8:2 v/v) solvent system to enable isolation of compound HRC43 (**6.15**) at R_f 0.4 and HRC41 (**6.16**) at R_f 0.25. HRC43 was a colourless needle-like crystal that was analysed and identified using X-ray diffraction (XRD) whereas

HRC41 was a white precipitate that was analysed using spectroscopic techniques to determine its structure.

6.7.2.4 Steroidal alkaloid extract (SAE)

A Lancaster and Mann methods were adopted for the isolation of steroidal alkaloids as described in chapter 5 (Section **5.4.3**). After a TLC profiling of the precipitate obtained from the Lancaster and Mann method, a PTLC was then run using $CHCl_3$:MeOH (4:1) to enable the isolation of one steroidal alkaloid compound (**6.17)**, from the SAFE at R_f 0.17.

6.8 Spectroscopy techniques

6.8.1 Hight Resolution-Mass spectra (HRMS)

High-resolution mass spectrometry (HRMS) was a useful tool for analysing isolated compounds and generate fragmentation patterns, in chemical formula prediction. The mass analyser, TOF, was used to generate the fragmentation data. Identification of the molecular mass of the compound, and the molecular ionic fragments present was based on computer matching with the ChemSpider, PubChem, and MassBank as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature. The HRMS spectroscopy was also carried out at the Central Analytical Facilities (CAF), Stellenbosch University, South Africa.

6.8.2 Nuclear magnetic resonance (NMR) spectroscopy)

All NMR experiments (1D and 2D) were performed with a Bruker Avance DPX-400 instrument (Bruker Spectro spin, Rheinstetten, Switzerland) operating at a proton frequency of 400 MHz. This spectrometer was equipped with a 5 mm broadband inverse detection z-gradient probe tuned to ^{13}C (100.61 MHz). For all 1D and 2D NMR experiments pulse sequences provided by the spectrometer, and manufacturer were used. Samples were run either in deuterated chloroform ($CDCl_3$), deuterated dimethyl sulphoxide (DMSO-d), or deuterated methanol (CD_3OD). The chemical shifts were in ppm relative to the residual solvent signal.

The coupling constants were measured in Hz and the following abbreviations are adopted: **s** (singlet); **d** (doublet); **t** (triplet) m (multiplet); **dd** (doublet of doublet); **dt** (doublet of triplets).

6.8.3 Fourier-transform infrared spectroscopy (FTIR)

Infrared spectra were collected by a Digi Lab FTS 3100 Excalibur HE Series, controlled by Digi Lab Resolution 4.0 Opus software. Fourier transmission IR spectrometry (FT-IR) was used to determine the various functional groups in the isolated compounds recorded in the range 4000-500 cm^{-1} using OPUS – software (version 6.5.6) on a Bruker Platinum Tensor 27 ATR-IR spectrophotometer and analysis using OPUS data collection program.

6.8.4 Single X-ray Crystallography

Crystals obtained from the isolations were analysed and identified using X-ray diffraction studies performed using a Bruker Kappa Apex II was used for data collection, and SAINT for cell refinement and data reduction, SHELTXT-2014 was used to elucidate the structure whereas refinement was done by least-squares procedures using SHELXL-2017/1 with SHELXLE as a graphical interface. The numerical method implemented in SADABS was used to correct data for absorption effects.

6.8.5 Circular Dichroism spectroscopy (CD)

The samples were dissolved in 1ml Methanol and diluted into a 10 mm quartz cuvette for analysis. The concentration of each sample was adjusted within 80% of the maximum absorbance allowed of around 2.0-2.5AU. Each sample was diluted with methanol until the total absorbance window from 220-450nm just fit below this maximum value. This ensured the optimum concentration for the CD spectra of each sample.

The absorbance spectra are included for each sample and plotted against the Methanol blank for comparison. The CD spectra are processed by subtracting the Methanol blank spectra and then smoothing the subtracted spectra with up to a 6nm window. The UV cut-off for methanol is 205nm as all are absorbed and no light can pass through the solvent below or close to that wavelength.

Compounds with no chiral carbon would not yield any cotton effects in their CD spectra and compounds with multiple chiral centres produced very complex CD spectra.

6.9 Physical data of isolated compounds

6.9.1 (2*S*,2′*R*,4a*R*,4b*S*,5′*R*,6a*S*,6b*R*,7*S*,9a*S*,10a*S*,10b*S*)-4′,4a,6a,7-tetramethyl-,2,3,4,4a,4b,5,6, 6a, 6b,7,9a,10, 10a, 10b, 11-hexadecahydrospiro [naphtho[2′,1′:4,5] indeno[2,1-b]furan-8,2′-oxan]-2-ol (6.15)

Compound 6.15: Isolated as white crystals; m.p. 205.5 °C: ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.79 (s, H-18 methyl), 0.79 (d, *J* = 6.2 Hz; H-27 methyl), 0.79 (*J* = 7.1 Hz; H-21 methyl), 1.03 (s, C-19 methyl), 3.38 (t, *J* = 10.6 Hz; H-26a), 3.47 (d, dd, *J* = 10.5; 4 Hz; H-26,), 3.49 (broad, H-3), 4.41 (q, *J* = 7.l Hz; H-16), 5.35 (broad d, *J* = 5.35 Hz; H-6); ^{13}C NMR (100 MHz, $CDCI_3$); δ_C 37.2 (C-1), 31.8 (C-2), 71.7 (C-3), 42.3 (C-4), 140.8 (C-5), 121.4 C-6), 31.4 (C-7), 31.4 (C-8), 50.0 (C-9), 36.6 (C-10), 20.9 (C-11), 39.8 (C-12), 41.6 (C-13), 56.5 (C-14), 31.6 (C-15), 80.8 (C-16), 62.8 (C-17), 16.3 (C-18), 19.4 (C-19), 41.6 (C-20), 14.5 (C-21), 109.3 (C-22), 31.4 (C-23), 28.8 (C-24), 30.3 (C-25), 66.8 (C-26), 17.1 (C-27). UV (MeOH) λ_{max}: 208 nm, *m/z* 416.4233 $[M+H]^+$ (calculated for $C_{27}H_{42}O_3$). FTIR spectrum (KBr, *v*max, cm^{-1}): 3429.45, 2974.59, 1600.36, 1361.27, 1050.74.

6.9.2 4-((2'*R*,5'*R*,6a*R*,8a*S*,9*R*)-4-hydroxy-6a,8a,9-trimethyl-1,3,3'4,4',5,5'6,6a,6b,6', 7,8,8b,11a,12,12a,12b-icosahydrospiro [naphtho[2',1':4,5]indeno[2,1-b]furan-10,2'-pyran]-5'-yl0butan-2-one (6.16)

Compound 6.16: Isolated as a white precipitate, m.p. 212.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.79 (s, H-18 methyl), 0.79 (d, *J* = 6.2 Hz; C-27 methyl), 0.79 (*J* = 7.1 Hz; C-21 methyl), 1.03 (s, H-19 methyl), 3.38 (t, *J* = 10.6 Hz; H-26a), 3.47 (d, dd, J = 10.5 Hz and *J* approx. 4 Hz; H-26), 3.49 (broad, H-3a), 4.41 (q, *J* = 7.l Hz; H-16), 5.35 (broad d, *J* = 5.35Hz; H-6); ^{13}C NMR (100 MHz, $CDCl_3$); δ_C 37.2 (C-1), 31.8 (C-2), 71.7 (C-3), 38.3 (C-4), 140.8 (C-5), 121.4 (C-6), 31.4 (C-7), 31.4 (C-8), 50.0 (C-9), 36.6 (C-10), 20.8 (C-11), 39.8 (C-12), 40.2 (C-13), 56.5(C-14), 31.6 (C-15), 80.8 (C-16), 62.8 (C-17), 16.3 (C-18), 19.4 (C-19), 41.6 (C-20), 14.5 (C-21), 109.8 (C-22), 31.4 (C-23), 28.8 (C-24), 30.3 (C-25), 66.8 (C-26), 30.8 (C-27), 42.2 (C-28), 206.8 (C-29), 30.8 (C-30); UV (MeOH) λ_{max}: 206 and 248 nm; *m/z* 471.3401$[M+H]^+$ (calculated for $C_{30}H_{46}O_4$); FTIR spectrum (KBr, *v*max, cm^{-1}): 3491.7 (OH), 2931.9 (sp^3-H), 2955.0 (CH_3), 1722.3 (C=O), 1600.36 (C=C), 1055.2 (C–O)

6.9.3 (2*S*,2′*R*,4a*R*,4b*S*,5′*R*,6a*S*,6b*R*,7*S*,9a*S*,10a*S*,10b*S*)-4a,5′,6a,7-tetramethyl-1,2,3,4,4a,4b,5,6,6a,6b,7,9a,10,10a,10b,11-hexadecahydrospiro[naptho[2′,1′:4,5]indeno[2,1-*b*]furan-8,2′-piperidin]-2-ol (6.17)

Compound 6.17: Isolated as a white precipitate. m.p. 201.8 °C. ^{1}H NMR (400 MHz, MeOD): δ_H 4.58 (1H, s), 4.39 (1H, q, *J* = 7.4 Hz), 3.41 (8H, tt, *J* = 10.6, 5.0, 3.2 Hz), 2.71 (1H, d, *J* = 11.2 Hz), 2.61 (1H, t, *J* = 11.2 Hz), 2.32 – 2.20 (2H, m), 2.17 (2H, s), 2.08 – 1.98 (3H, m), 1.94 – 1.77 (4H, m), 1.74 – 1.51 (7H, m), 1.51 – 0.94 (14H, m), 0.94 – 0.84 (6H, m). ^{13}C NMR (101 MHz, MeOD) δ_C 140.2 (C-5), 120.0 (C-6), 100.2 (C-22), 80.9 (C-16), 70.3 (C-3), 62.5 (C-17), 55.6 (C-14), 49.6 (C-9), 44.3 (C-26), 43.2 (C-4), 41.4 (C-20), 40.9 (C-13), 40.6 (C-12), 39.6 (C-10), 36.4 (C-1), 35.7 (C-7), 33.2 (C-23), 32.1 (C-2), 31.0 (C-8), 30.7 (C-15), 30.7 (C-25), 30.2 (C-24), 21.5 (C-11), 19.8 (C-19), 17.7 (C-27), 17.3 (C-18), 13.2 (C-21); UV (MeOH) λ_{max}: 211 and 236 nm; *m/z* 414.3364 $[M+H]^+$ (calculated for $C_{27}H_{42}NO_2$); FTIR spectrum (KBr, *ν*max, cm^{-1}): 3410.8, 2923.0 (CH_3), 2856.9 (CH_3), 974.59, 1735.7, 1454.5, 1050.7

6.9.4 (2*S*,2'*S*,3'*R*,4R,4'*R*,5*R*,5'*R*,6*S*)-{[2*R*,3*S*,4*S*,5*R*,6*R*)-4-hydroxy-2-(hydroxy methyl)-6-{[2*S*,2'*R*,4a*R*,4b*S*,5'*R*,6a*S*,6b*R*,7*S*,9a*S*,10a*S*,10b*S*)-4a,5',6a,7-tetramethyl-1,2,3,4a,4b,5,6,6a,6b,7,9a,10,10a,10b,11-hexadechydrospiro[naptho[2',1',4,5] indeno[2,1-b]furan-8,2'-piperidin]-2-yl]oxy}oxane=3,5-diyl]bis(6-methyloxane-3,4,5-triol)

Compound 6.18: Isolated as a white precipitate; m.p. 301.1 °C: ^{1}H NMR (400 MHz, MeOD) δ_H 5.29 (1H, s), 5.11 (1H, s), 4.40 (1H, d, *J* = 7.6 Hz), 4.02 (1H, d, *J* = 9.2 Hz), 3.93 (1H, d, *J* = 14.3 Hz), 3.83 (2H, s), 3.77 – 3.66 (4H, m), 3.60 – 3.37 (14H, m), 3.29 (3H, t, *J* = 7.9 Hz), 3.21 (2H, s,), 2.95 (1H, d, *J* = 11.9 Hz), 2.75 (1H, t, *J* = 12.2 Hz), 2.36 (1H, d, *J* = 15.3 Hz), 2.26 – 2.14 (2H, m), 2.06 (3H, s), 1.84 – 1.67 (5H, m), 1.62 (1H, d, *J* = 13.7 Hz), 1.51 (2H, d, *J* = 11.1 Hz), 1.42 (3H, d, *J* = 13.1 Hz), 1.21 – 1.11 (8H, m), 1.07 (3H, d, *J* = 7.5 Hz), 0.95 (3H, s), 0.88 (4H, d, *J* = 6.7 Hz), 0.76 (6H, s). ^{13}C NMR (101 MHz, MeOD) δ_C 37.6 (C-1), 30.1 (C-2), 78.1 (C-3), 38.9 (C-4), 140.9 (C-5), 121.6 (C-6), 32.5 (C-7), 32.4 (C-8), 50.1 (C-9), 37.4 (C-10), 21.5 (C-11), 40.6 (C-12), 39.9 (C-13), 56.9 (C-14), 31.5 (C-15), 80.0 (C-16), 62.5 (C-17), 16.7 (C-18), 19.7 (C-19), 42.0 (C-20), 15.6 (C-21), 98.9 (C-22), 31.2 (C-23), 31.1 (C-24), 32.1 (C-25), 48.0 (C-26), 19.5 (C-27), 100.8 (C-1'), 79.5 (C-2'), 77.7 (C-3'), 76.7 (C-4'), 79.0 (C-5'), 61.4 (C-6'), 101.5 (C-1''), 71.8 (C-2''), 72.2 (C-3''), 73.4 (C-4''), 69.9 (C-5''), 18.3 (C-6''), 104.8 (C-1'''), 71.9 (C-2'''), 72.1 (C-3'''), 73.5 (C-4'''), 69.3 (C-5'''), 18.5

(C-6’’’); UV (MeOH) λ_{max}: 205, 238 nm; *m/z* 867.4582 $[M+H]^+$ (calculated for $C_{45}H_{73}NO_{15}$); FTIR spectrum (KBr, *v*max, cm^{-1}): 3374.5, 2925.6, 2856.4, 1492.0, 1059.1

6.9.5 (2-3,4-dihydroxy phenyl)-3,5,7-trihydroxy-4H-chromen-4-one (6.19)

Compound 6.19: Isolated as a yellow precipitate; m.p. 316.4 °C: ^{1}H NMR (400 MHz, MeOD) δ_H 12.5 (1H, s), 7.68 (1H, s), 7.55 (1H, dd, *J* = 8.4, 2.3 Hz), 6.89 (1H, d, *J* = 8.3 Hz), 6.41 (1H, d, *J* = 2.2 Hz), 6.21 (1H, s), 3.41 (2H, s), 2.52 (1H, s). ^{13}C NMR (400 MHz, MeOD) δ_C 175.9 (C-4), 164.2 (C-7), 161.1 (C-5), 156.8 (C-9), 147.4 (C-2), 146.6 (C-1’), 144.8 (C-4’), 135.8 (C-3), 122.8 (C-1’), 120.3 (C-6’), 114.8 (C-5’), 114.6 (C-2’), 103.1 (C-10), 97.8 (C-6), 93.0 (C-8); UV (MeOH) λ_{max}: 207, 248; 288 nm; *m/z* 303.0498 $[M+H]^+$ (calculated for $C_{15}H_{10}O_7$); FTIR (KBr) νmax/cm^{-1}: 3399.3, 3279.9, 1664.9, 1608.3, 1304.12, and 931.27;

6.9.6 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4h-chromen-3-yl-6-o-(6-deoxy-α-l-mannopyranosyl)-β-d-glucopyranoside-2-3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one (6.20)

Compound 6.20: Isolated as a yellow precipitate; m.p. 241.8 °C: ^{1}H NMR (400 MHz, MeOD) δ_H 7.71 (4H, m), 6.89 (2H, d, *J* = 8.4 Hz), 6.40 (2H, d, *J* = 2.2 Hz), 6.22 (2H, d, *J* = 2.2 Hz), 5.12 (2H, d, *J* = 7.6 Hz), 4.59 (1H, s), 4.54 (2H, d, *J* = 1.7 Hz), 3.86 – 3.79 (2H, m), 3.67 (2H, dd, *J* = 3.5, 1.6 Hz), 3.57 (2H, dd, *J* = 9.5, 3.4 Hz), 3.53 – 3.47 (3H, m), 3.47 – 3.25 (14H, m), 2.17 (7H, s), 1.14 (6H, d, *J* = 6.2 Hz). ^{13}C NMR (101 MHz, MeOD) δ_C 177.3 (C-4), 163.8 (C-7), 160.8 (C-9), 157.2 (C-5), 156.4 (C-2), 147.7 (C-3’), 143.7 (C-4’), 133.5 (C-3), 121.5 (C-1’), 121.0 (C-6’), 115.6 (C-5’), 113.9 (C-2’), 103.5 (C-10), 101.0 (C-1’’), 100.6 (C-1’’’), 97.9 (C-6), 92.78 (C-8), 73.9 (C-2’’), 75.6 C-3’’), 76.3 (C-4’’), 73.6 (C-4’’’), 71.7 (C-5’’), 70.4 (C-3’’’), 70.1 (C-2’’’), 69.5 (C-5’’’), 66.9 (C-6’’), 17.6 (C-6’’’): UV (MeOH) λmax; 230 - 380; nm; *m/z* 611.157 $[M+H]^+$ (calculated for $C_{27}H_{30}O_{16}$): FTIR (KBr) νmax/cm^{-1} : 3409.4, 13324.7, 1304.12 and 931.27.

6.9.7 Phenoxy-2-ethan-1-ol (6.21)

Compound 6.21: Isolated as a colourless oil: ^{1}H NMR (400 MHz, MeOD) δ_H 7.30 (2H, m), 6.94 (3H, m), 4.01 (2H, t, *J* = 4.6; 4.48 Hz), 3.87 (2H, t, *J*= 6.12, 4.88 Hz), 2.11(1H, s). ^{13}C NMR (101 MHz, MeOD) δ_C 158.6 (C-1), 128.2 (C-3), 119.8 (C-4), 113.2 (C-2 and 6), 68.3 (C-7), 59.4 (C-8): UV (MeOH) λ_{max}: 230, 280 nm; *m/z* 138.1451 $[M+H]^+$ (Calculated for $C_8H_{10}O_2$): FTIR (KBr) νmax/cm^{-1} : 3336.4, 2923.1, 2855.5, 2051.2, 1711.3

6.9.8 2, 4-dihydroxy benzaldehyde (6.22)

Compound 6.22: Isolated as a white solid; m.p. 135.6 °C: ^{1}H NMR (400 MHz, MeOD) δ_H 9.72 (1H, s), 7.50 (1H, d, *J* = 8.5 Hz), 6.46 (1H, d, *J* = 8.5 Hz), 6.30 (1H, s), 4.88 (2H, s). ^{13}C NMR (101 MHz, MeOD) δ_C 194.0 (C-7), 165.8 (C-4), 164.1 (C-2), 135.3 (C-6), 114.6 (C-1), 108.5 (C-5), 102.1 (C-3): UV (MeOH) λ_{max}: 238 and 320 nm; *m/z* 138.6324 $[M+H]^+$ (Calculated for $C_7H_6O_3$); FTIR (KBr) νmax/cm^{-1} 3320.6, 2923.1 and 1711.3.

6.9.9 (5R)-[(1S)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one (6.23)

Compound 6.23: Isolated as a colourless solid; m.p. 190.8 °C: ^{1}H NMR (400 MHz, MeOD) δ_H 4.83 (4H, s), 3.96 – 3.88 (1H, m), 3.70 (2H, d, *J* = 6.8 Hz), 3.35 (1H, d, *J* = 15.0 Hz).^{13}C NMR (101 MHz, MeOD) δ_C 172.12 (C-1), 153.37 (C-2), 118.50 (C-3), 75.54 (C-4), 69.25 (C-5), 62.19 (C-6). ($C_6H_8O_6$): UV (MeOH) λ_{max}: 267 nm: *m/z* 177.0158 $[M+H]^+$, 159 $[M-H_20]^+$ FTIR (KBr) νmax/cm^{-1}: 3320.6, 3217.9, 1614.5, 1471.7, 1062.1

6.10 References

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CHAPTER 7: General conclusion

The goal of this study was to further our knowledge and comprehension of *S. incanum*'s phytochemistry about its use as a pesticide in the Mkoba community. As a result, community-based pesticide-use data was compiled Characterised from the investigation was one novel chemical and three new compounds described for the first time in *S. incanum*. The new substance (**6.16**) had strong effectiveness against cabbage aphids. Previously identified substances (**6.18**, **6.21**; and **6.23**) also demonstrated notable effectiveness against cabbage aphids.

The results of a descriptive survey done in Mkoba village to assess the knowledge and perceptions of the locals indicated conflicting views on the effects of *S. incanum* insecticide on the environment and public health. It was discovered that perceptions and attitudes toward the usage of *S. incanum* pesticides were influenced by gender, age, and educational attainment. Thus, the lack of scientific evidence of the pesticidal properties of *S. incanum* makes the community of Mkoba carelessly use *S. incanum* pesticides. The respondents noted several symptoms, (skin irritation, vomiting, stomach-ache, headache, nausea, dizziness, coughing, sweating, and fatigue) but it was impossible to determine if these symptoms were brought on by *S. incanum* because many pre-survey variables such as controlling the use of commercial pesticides were uncontrolled.

The phytochemical constituents associated with the pesticidal activity in plant species are saponins, alkaloids, phenols, flavonoids, and terpenes (Almouh, 2017; Kaunda and Zhang, 2019). The phytochemical analysis of extracts revealed that *S. incanum* retained high concentrations of phytochemicals, especially the phenols, alkaloids, and saponins which contributed to the toxicity of the fruit and root of *S. incanum* against cabbage aphids. Besides controlling aphids, the antioxidant and cytotoxicity tests carried out on the fruit and root crude extracts showed promising antioxidant and cytotoxicity properties. The presence of phenolics in high concentration in the fruit supports the literature in which some communities use the fruit as food to provide the needed antioxidant properties. Glycoalkaloids and saponins were detected in the different fruit and root crude extracts of *S. incanum*. The presence of glycoalkaloids such as solasodine (**6.17**) confirmed the pesticidal properties of *S. incanum* (Jayakumar and Murugan, 2016; Chowański et al, 2016). Toxic pesticides are required to

manage pests, but if handled improperly, these organic substances can also poison people (Thaiya et al, 2010).

The crude extracts exhibited a higher efficacy against the cabbage aphids than the isolated pure compounds with the methanol fruit extract exhibiting the highest efficacy. Based on the analysis of the effects of the crude extracts on the mortality rate of cabbage aphids, it is possible to conclude that *S. incanum's* fruit and root extracts have pesticide qualities that can be used as precursors in the synthesis of new pesticide compounds

Four steroidal compounds were isolated from *S. incanum*, namely diosgenin (**6.15**), solanuminone (**6.16**), solasodine (**6.17**) and solamargine (**6.18**). Diosgenin and solanuminone were steroidal saponins isolated from the hexane root crude extract with solanuminone reported for the first time. The literature search revealed that compound **6.15** has been detected in the aerial parts of *Solanum* species before (Kaunda and Zhang, 2019) but in this study, it is being reported for the first time from the root of *S. incanum.* No information was found regarding the pesticidal activity of saponins toward cabbage aphids from literature, however, the study revealed that **6.16** is a potent efficacy towards cabbage aphids.

Three phenolic compounds were isolated from *S. incanum* namely, 2-3,4-(dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one (**6.19**), 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl6-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside (**6.20**), 2,4 dihydroxy benzaldehyde (**6.22**). Compounds **6.19** and **6.20** are common in the inn fruit of *Solanum* species and **6.22** is being reported for the first time in the root of *S. incanum*. Compound **6.20** is a flavonoid with a sugar attachment. Previously, **6.20** was identified in the fruit of *S. anguvi, S. muricatum*, and *S. torvum*, in the leaf part of *S. nigrum,* and in the stem of *S. melongena* (Kaunda and Zhang, 2019); it is detected for the first time in *S. incanum*. The phenolic compounds are the main contributors to the antioxidant capacity of the plant, and this is confirmed by the antioxidant screening results indicated in Chapter 5 section **5.2.4.**

3,4-dihydroxy-5-(1,2-dihydroxyethyl) furan-2(5H)-one (**6.23**) is a dihydrofuran has been reported in other *Solanum* species that include *S. nigrum, S. tuberosum,* and *S. incanum* (Kaunda and Zhang, 2019). Compound **6.23** is a natural nutrient. It is involved in a wide variety of biochemical processes of living species (plants, animals, and human beings)

serving multifunctional roles as an antioxidant or scavenger of radicals, enzyme cofactor, *etc* (Dabbagh *et al.*, 2014).

This study has made an important contribution to understanding the community's perceptions of the traditional use of *S. incanum* as a pesticide. The identification of new compounds for the first time in *S. incanum* shows that *S. incanum* has an abundance of different phytochemicals. Therefore, *Solanum* species are an exciting source of interesting bioactive molecules and are of future promise for an ongoing search for pesticides and other medicinal drugs.

Based on the above conclusion, the following recommendations are thereof suggested:

- In-depth studies are needed to address the community's perceptions, attitudes, beliefs, views, and opinions and to develop strategies for scientific evidence for the community. Understanding the chemistry of the plant pesticide is critical for agricultural, environmental, and health purposes in society.

- It would be particularly interesting to evaluate the effect of harvesting *S. incanum* to use as a pesticide has to the ecosystem.

- Further experiments should be done particularly on the cytotoxicity and efficacy of the isolated compounds on pests to confirm their biological (pesticidal and cytotoxic) activity to advance the community of Mkoba.

- The mass detections from the LC-MS suggested that there are a lot of unexplored phytochemical constituents of *S. incanum*. More isolations should be carried out to find more compounds.

- More investigations are required to determine the pesticide mode of action and the pesticide evaluation of these novel compounds in the management of cabbage aphids to advance the community in Mkoba villages and the world at large.

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8. APPENDICES

8.1 Appendix 1. 1: The ethics approval letter

Chairperson: Research Ethics
Committee (Human) Tel: +27 (0)41
504 2235
charmain.cilliers@mandela.ac.za

NHREC registration nr: REC-042508-025 Ref: [H19-SCI-CHE-001] / Approval]

9 December 2019

Dr B Hlangothi
Faculty: Science

Dear Dr Hlangothi

EVALUATION OF THE TOXICITY OF SECONDARY METABOLITE ALKALOIDS IN *SOLANUM INCANUM L.* TO ADVANCE COMMUNITY KNOWLEDGE

PRP: Dr B Hlangothi
PI: Mr W Zivanayi

The above-entitled application served at the Research Ethics Committee (Human) (*meeting on 31 July 2019*) for approval. The study is classified as a medium-risk study. The ethics clearance reference number remains **H19SCI-CHE-001** and approval is subject to the following conditions:

1. The immediate completion and return of the attached acknowledgment to Imtiaz.Khan@mandela.ac.za, the date of receipt of such returned acknowledgment determining the final date of approval for the study where data collection may commence.
2. Approval for data collection is for 1 calendar year from the date of receipt of the above-mentioned acknowledgement.
3. The submission of an annual progress report by the PRP on the data collection activities of the study (form RECH-004 to be made available shortly on the Research Ethics Committee (Human) portal) by 15 November this year for studies approved/extended in the period October of the previous year up to and including September of this year, or 15 November next year for studies approved/extended after September this year.
4. In the event of a requirement to extend the period of data collection (i.e. for a period above 1 calendar year from the date of approval), completion of an extension request is required (form RECH-005 to be made available shortly on Research Ethics Committee (Human) portal)
5. In the event of any changes made to the study (excluding extension of the study), completion of an amendments form is required (form RECH-006 to be made available shortly on Research Ethics Committee (Human) portal).
6. Immediate submission (and possible discontinuation of the study in the case of serious events) of the relevant report to RECH (form RECH-007 to be made available shortly on the Research Ethics Committee (Human) portal) in the event of any unanticipated problems, serious incidents or adverse events observed during the study.

7. Immediate submission of a Study Termination Report to RECH (form RECH-008 to be made available shortly on the Research Ethics Committee (Human) portal) upon unexpected closure/termination of the study.
8. Immediate submission of a Study Exception Report of RECH (form RECH-009 to be made available shortly on the Research Ethics Committee (Human) portal) in the event of any study deviations, violations, and/or exceptions.
9. Acknowledgement that the study could be subjected to passive and/or active monitoring without prior notice at the discretion of the Research Ethics Committee (Human).

Please quote the ethics clearance reference number in all correspondence and enquiries related to the study. For speedy processing of email queries (to be directed to Imtiaz.Khan@mandela.ac.za), it is recommended that the ethics clearance reference number together with an indication of the query appear in the subject line of the email.
We wish you well with the study.

Yours sincerely

Prof C Cilliers
Chairperson: Research Ethics Committee (Human)

Cc: Department of Research Capacity Development Faculty Officer: Science

8.2 Appendix 1. 2The letter sent to the gatekeeper for permission to carry out the study in Mkoba village

Gweru City Council

Box 278 Civic centre Gweru
Telephone 054 227 079

DATE: 28 April 2019

William Zivanayi
Nelson Mandela University
Faculty of Education
Building 6, South Campus
Summerstrand
6021
Port Elizabeth.
South Africa.

Dear William Zivanayi

Ref: Permission to engage community members in your study.

It is my understanding that you want to conduct an academic research in Mkoba 17 through interviews and questionnaires in which you want to engage our community members during the period of august 2019 to December 2020. I have also read the design of the study as well as the targeted population and the benefits this research may bring to the community.

I support and agree that you conduct the research using our community members as participants in Mkoba 17. We will provide any assistance necessary for the successful implementation of your study. If you have any questions, please do not hesitate to contact us.

Best wishes.

Councillor.

T. CHINENI

CITY OF GWERU
COMMISSIONER OF OATHS
28 APR 2019
COUNCILLOR

8.3 Appendix 3 1: The Questionnaire

Dear Participant,

We are carrying out an educational study about nhundurwa (S. incanum) so that we can get information regarding how you use it on your vegetables and find the community's perception of its effectiveness as a pesticide and the possible effects it may pose on the users. The study will help us to document valuable knowledge the community holds regarding nhundurwa. It will take you approximately an hour to complete the questionnaire. Thank you for taking the time to fill in this questionnaire. Please return your completed questionnaire to the questionnaire administrator. Your answers will be treated with complete confidentiality.

Please complete each item using your best knowledge and first impression. There are no right or wrong answers.

Section A: Demographical data.

(Please **tick** ✓ the appropriate box).

1. **My age range (in years) is:**

16 -30	☐	**31-50**	☐
51-60	☐	**Above 60**	☐

2. **Gender**: Female ☐ Male ☐

3. **My marital status**: Married ☐ Single ☐ Divorced ☐ Widow ☐

4. **What is the highest level of schooling have you attained?**

Primary	
Secondary	
Tertiary	

5. **How many are you in the family?**

1		**2**		**3**		4		5		6	
Other											

6. **In which Mkoba Village do you live?** ……..……

7. **Do you practice backyard vegetable gardening? Yes** ☐ **No** ☐

If **YES** to item **7** above proceed to **Section B**. If **NO** please return the questionnaire to the administrator. Thank you for participating.

Section B: ***Please attempt to answer all questions***

8. Which type of vegetables do you grow?

……

…….....

9. What challenges do you experience growing your vegetable in your garden?

……

……

..……..

10. How do you resolve these challenges?

……

……

……..…

11. Do you know nhundurwa? Yes ☐ **No** ☐

If **YES** to item **11** above proceed to **item 12**. If **NO,** please return the questionnaire to the administrator. Thank you for participating.

12. For what do you use Nhundurwa?

……

……

……..…

13. From where do you get Nhundurwa (S. incanum)?

……

……..

……..

14 Who informed you about Nhundurwa?

……..……

15 Who prepares the nhundurwa formulations for your garden?

……..…

16. How is the nhundurwa formulation prepared for your garden? *(Please briefly describe on the space given below).*

……..……

……..……

……

17. **Where do you store the nhundurwa formulation?** (Please **tick** ✓ the appropriate box)

Open shed just for pesticides	
Refrigerator, with other items	
In the open field	
Locked chemical store	
Living area	
Other (specify)…….. ……...	

18. **Why**?

.....

19. **What do you do with the unused (mixed, diluted) nhundurwa formulation?** (Please **tick** ✓ the appropriate box)

Dispose of in the garden	
I just throw it away	
I apply on other vegetables	
Keep it for use next time	
Dispose of in the nearby dam where we collect water for the garden.	
Dispose of in the drainage designed for leftovers	
Other response: …….. …….	

20. **Do you think that nhundurwa (S. incanum) affects human health?** (Please **tick** ✓ the appropriate box)

Strongly agree		Agree		Disagree		Strongly disagree		I don't know	

21. Please tell me more about your response in Question 20 above

……

……

……

Do you think that Nhundurwa formulation can affect the environment? (Please **tick** ✓ the appropriate box)

Strongly agree		Agree		Disagree		Strongly disagree		I don't know	

22. Please tell me more about your response in Question 22 above

……

……

23. Choose the answer from the options below by placing a tick ✓in the appropriate box.

	Very much	Much	A little	Not at all	I don't know
The use of 'Nhundurwa' as a pesticide has helped the community to save money, which they could have used to buy pesticides in shops.	5	4	3	2	1
Nhundurwa kill the aphids	5	4	3	2	1
The use of 'Nhundurwa' as a pesticide has created health problems in the community	5	4	3	2	1
The use of 'Nhundurwa' as a pesticide has helped the community to produce vegetables in abundance	5	4	3	2	1
The community wastes time in applying nhundurwa, it does not work on the vegetables	5	4	3	2	1
Nhundurwa is poisonous	5	4	3	2	1

24. **Do you have any special clothes you use when working with nhundurwa?** (Please **tick** ✓ the appropriate box)

Yes ☐ **No** ☐

25. Please tell me more about your response in Question 25 above

……

……

……..……..

26. What method(s) do you use to apply the nhundurwa juice to the vegetable? (Please **tick** the appropriate boxes)

Broad casting using brooms ☐ Nozzle Sprayer ☐

Aerosol sprayers ☐ Other (Please specify)……

27. **Please tell me more about your response in Question 27 above**

……..……

……

28. After how long do you notice the effect of the nhundurwa formulation on the aphids?

Just after spraying ☐ In 2 days ☐ After 5 days ☐ In 2 weeks ☐

29. **After how long do you harvest your vegetables when treated with nhundurwa formulation?** (Please **tick** ✓the appropriate box)

Just after using the nhundurwa formulation ☐ In 2 days ☐ After 5 days ☐ In 2 weeks ☐

30. Why?

……

……

……

31. **Which ONE (s) are the common symptoms or illnesses common with people who work in the garden? (**Please **tick** ✓ the appropriate boxes)

Symptoms	***Tick the appropriate box**	**Symptoms**	***Tick the appropriate box**
Headaches		Shortness of breath	
Dizziness		Excessive sweating	
Skin irritation		Fatigue	
Nausea		Stomach-ache	
Itchy eyes		Poor vision	
Vomiting		Other	
Coughing		No health impairment	

*you may **tick more than one** symptom

32. What do you do to relieve yourself of the illness?

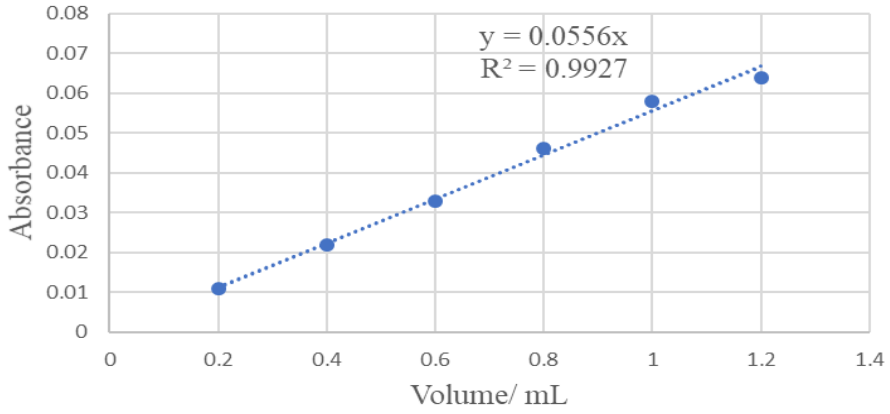
……

…… ……

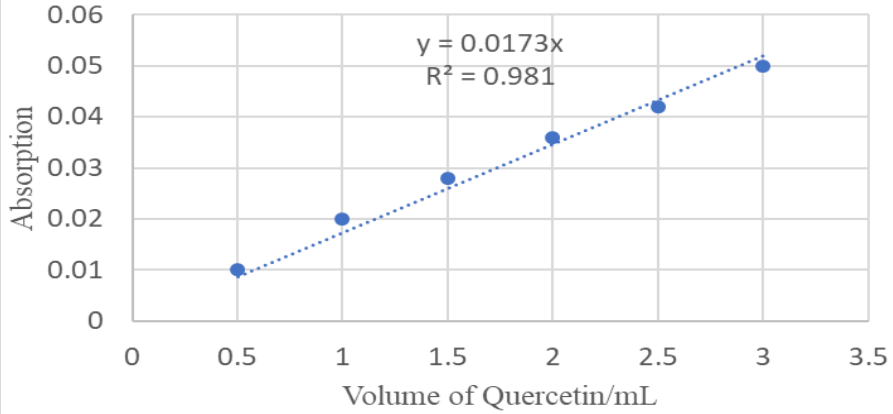
Thank you for participating in this study. May you return the questionnaire to the research administrator?

8.4 Appendix 5. 1: The calibration curves for the determination of the total quantity of alkaloids, phenols, flavonoids, and saponins respectively

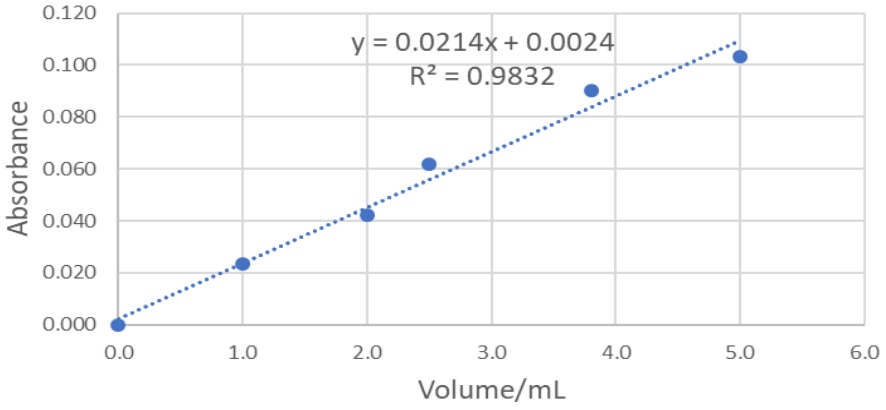
Graph 1.



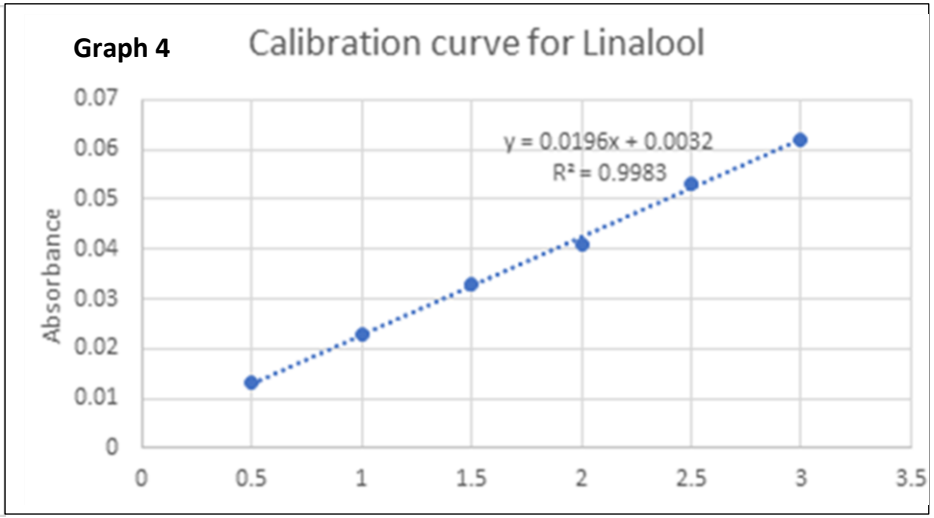
Graph 2.



Graph 3.

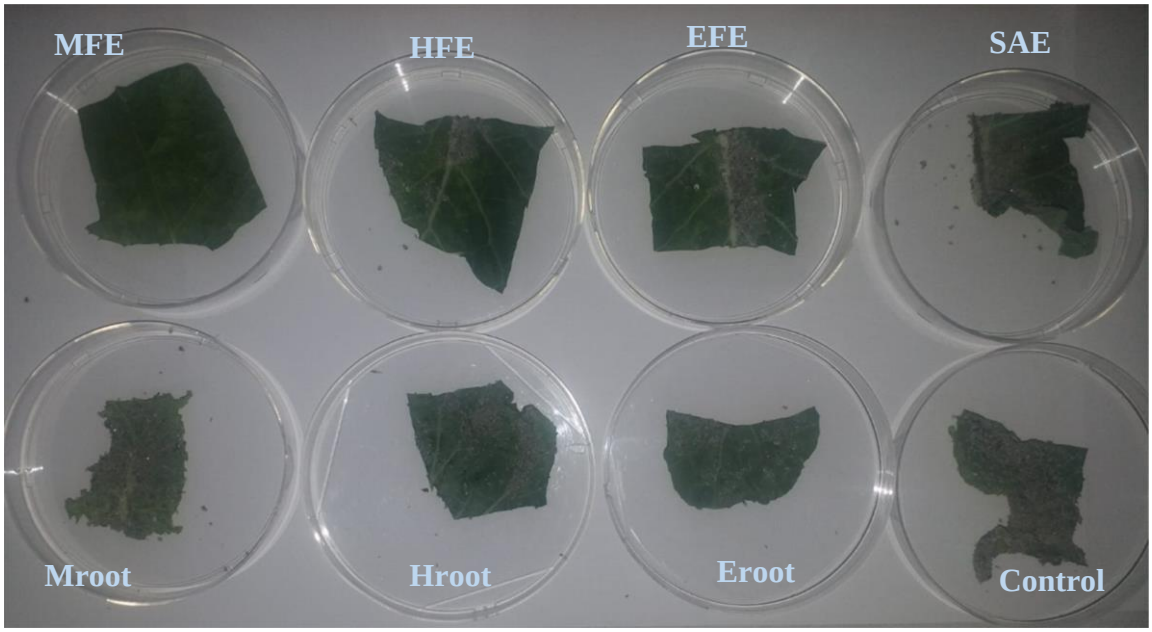


Graph 4



8.5 Appendix 5. 2: The pictures of the efficacy experimental setup

5.1 A[2]



5.2 B[3]

5.3 C[4]

[2] The efficacy experiment set up – **Pic 5.1A**
[3] The photograph showing the manual counting of the aphids – **Pic 5.2B**
[4] The *brassica napus*vegetables where the cabbage aphids were harvested – **Pic 5.3C**

8.6 Spectra for Isolated compounds

8.6.1 Spectra for (2*S*,2′*R*,4a*R*,4b*S*,5′*R*,6a*S*,6b*R*,7*S*,9a*S*,10aS,10b*S*)-4′,4a,6a,7-tetramethyl-,2,3,4,4a,4b,5,6, 6a, 6b,7,9a,10, 10a, 10b, 11-hexadecahydrospiro [naphtho[2′,1′:4,5] indeno[2,1-b]furan-8,2′-oxan]-2-ol

PLATE 6.15A: ^{1}H NMR spectrum of compound **6.15** in $CDCl_3$

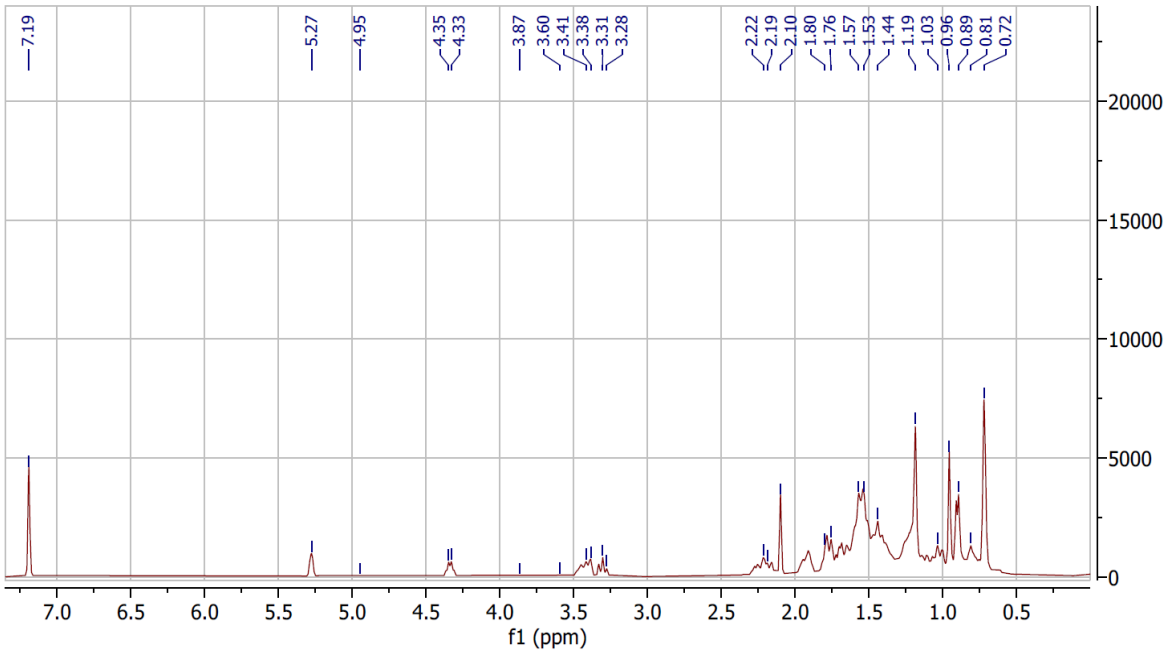


PLATE 6.15B: ^{13}C NMR spectrum of compound **6.15** in $CDCl_3$

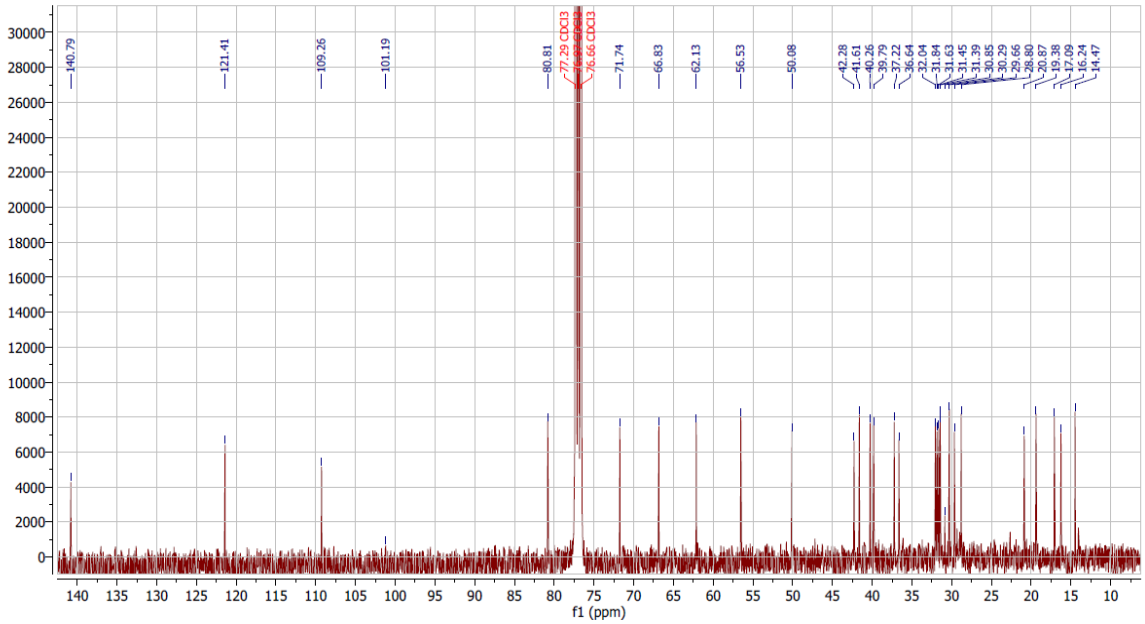


PLATE 6.15C: DEPT 135 NMR spectrum of compound **6.15** in $CDCl_3$

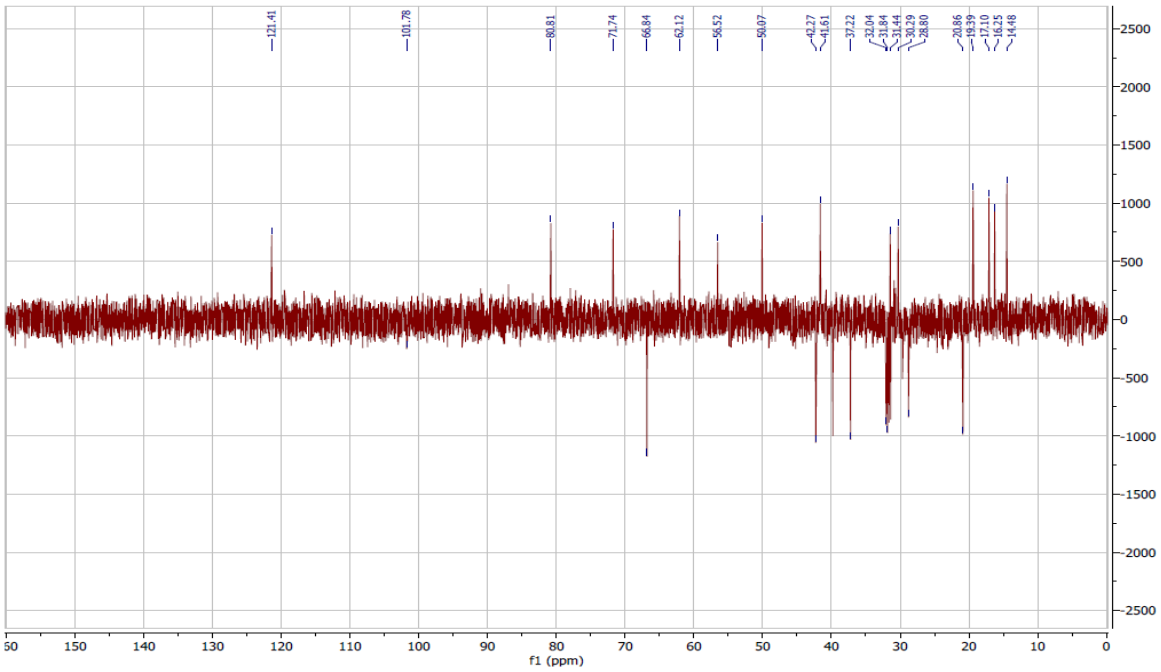


PLATE 6.15D: HSQC NMR spectrum of compound **6.15** in $CDCl_3$

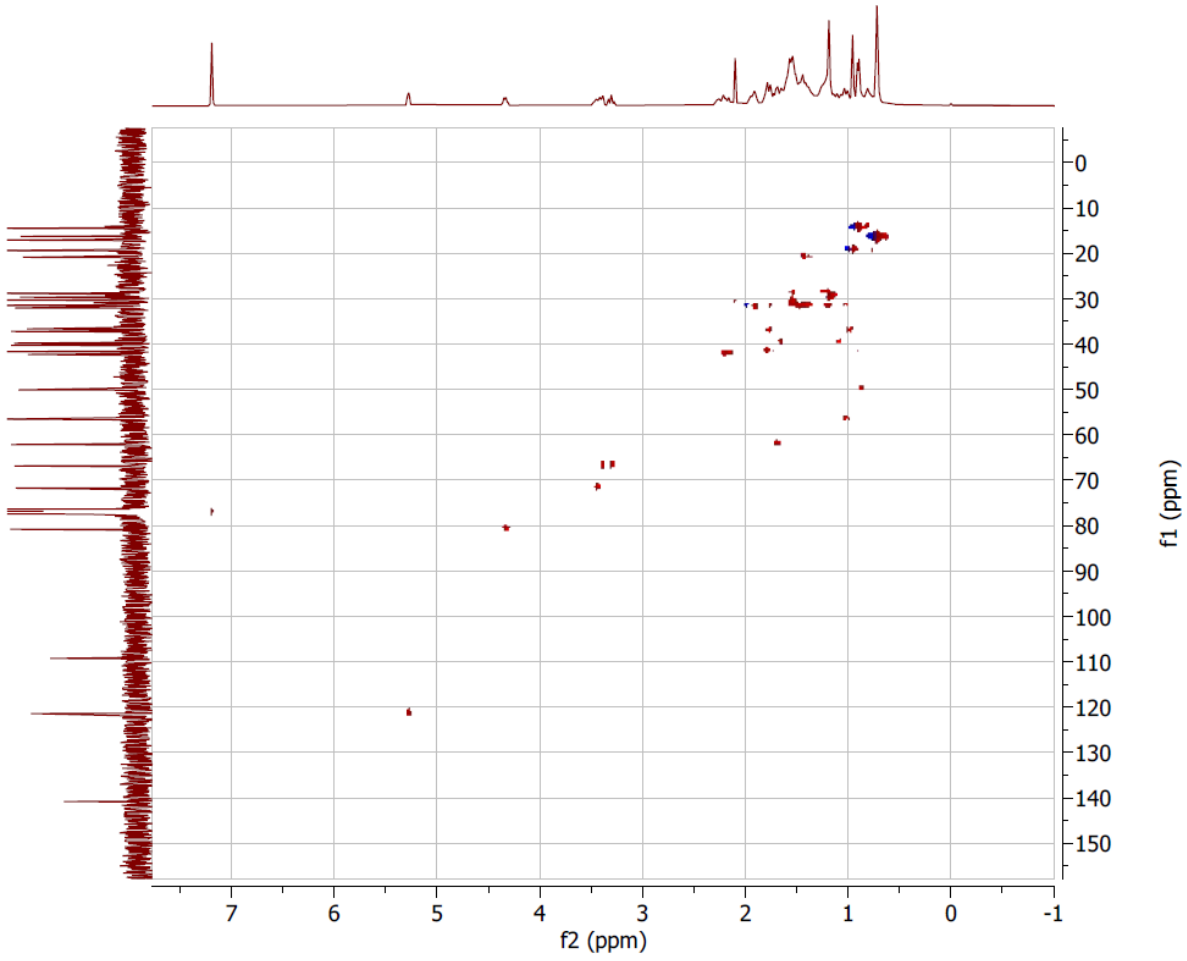


PLATE 6.15E: HMBC NMR spectrum of compound **6.15** in $CDCl_3$

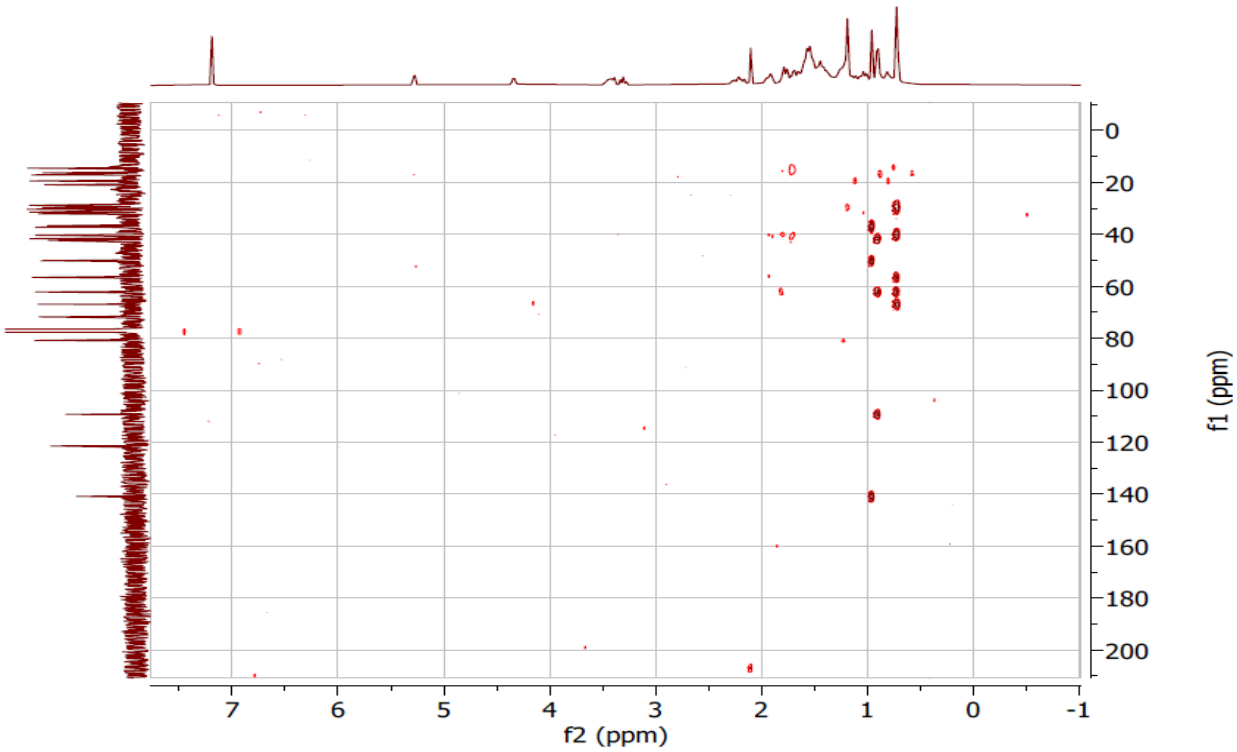


PLATE 6.15F: COSY NMR spectrum of compound **6.15** in $CDCl_3$

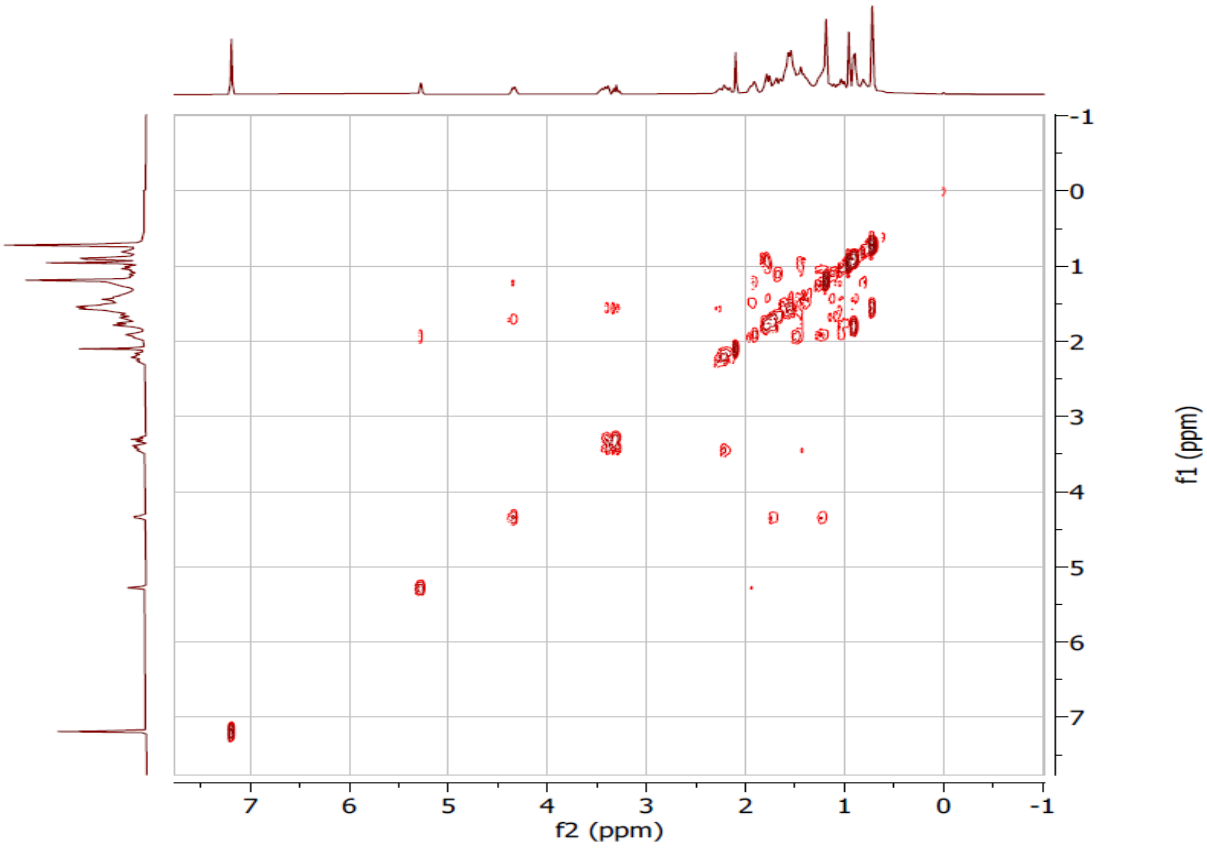


PLATE 6.15G: The HREIMS spectrum of compound **6.15**

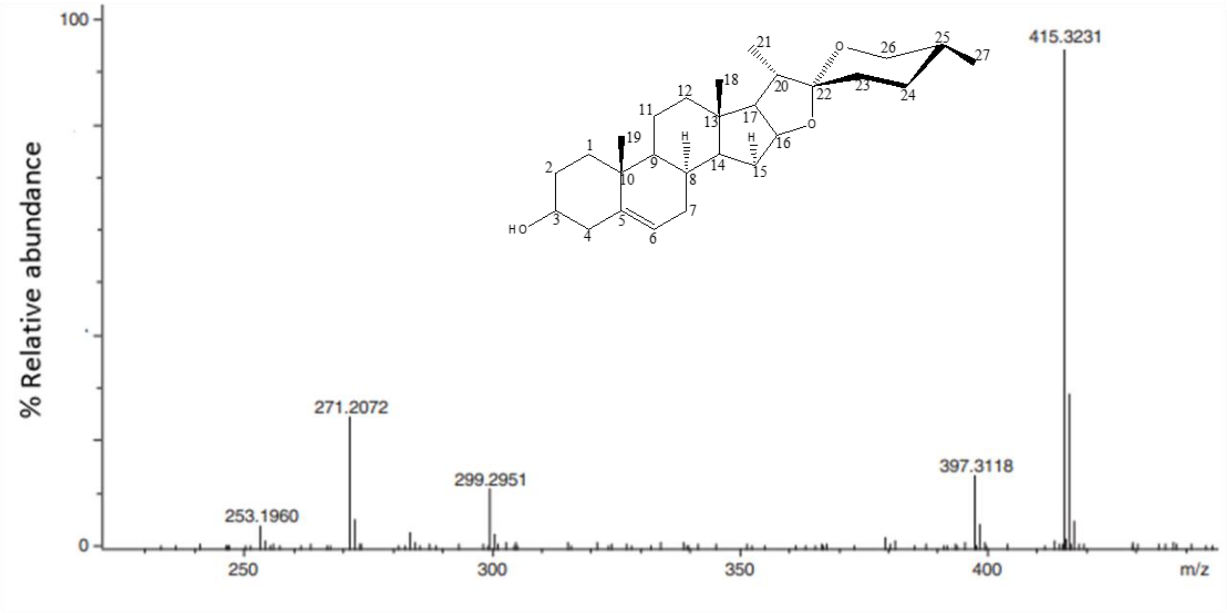
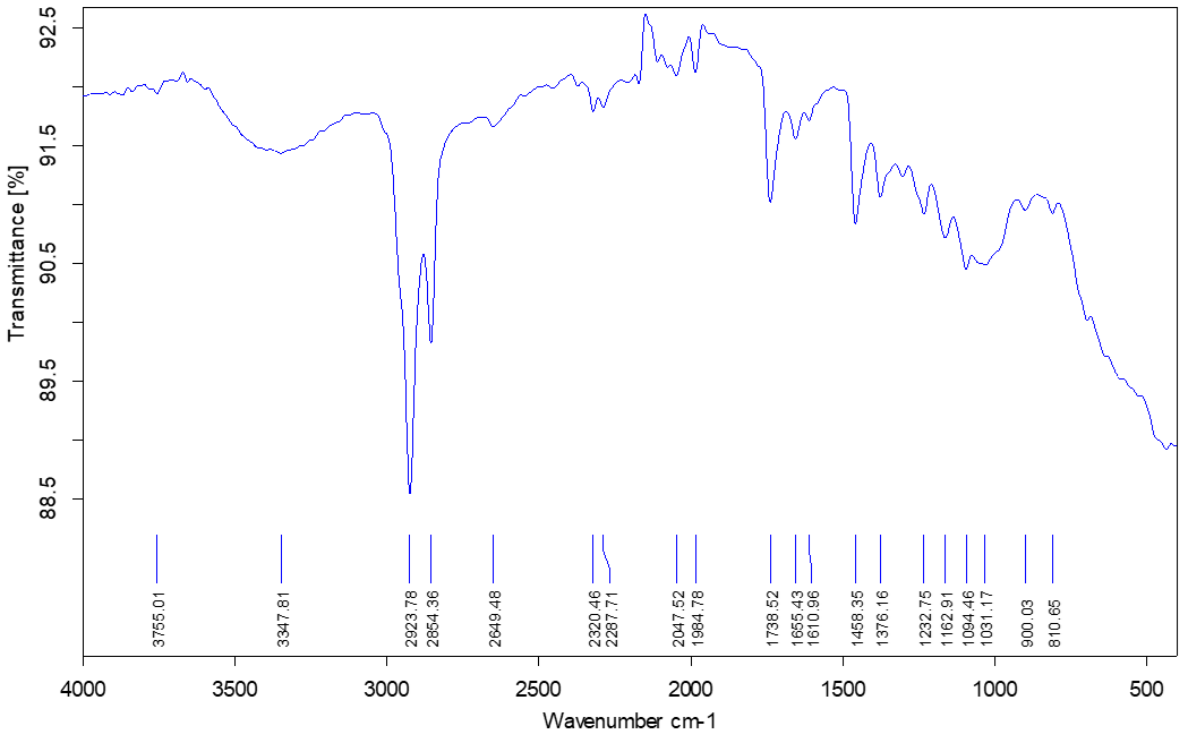


PLATE 6.15H FTIR spectrum of compound **6.15**



8.6.2 Spectra of 4-((2'*R*,5'*R*,6a*R*,8a*S*,9*R*)-4-hydroxy-6a,8a,9-trimethyl-1,3,3'4,4',5,5'6,6a,6b,6',7,8,8b,11a, 12,12a,12b-icosahydrospiro [naphtho[2',1':4,5]indeno [2,1-b]furan-10,2'-pyran]-5'-ylbutan-2-one (6.16)

PLATE 6.16A: ^{1}H NMR spectrum of compound **6.16** in $CDCl_3$

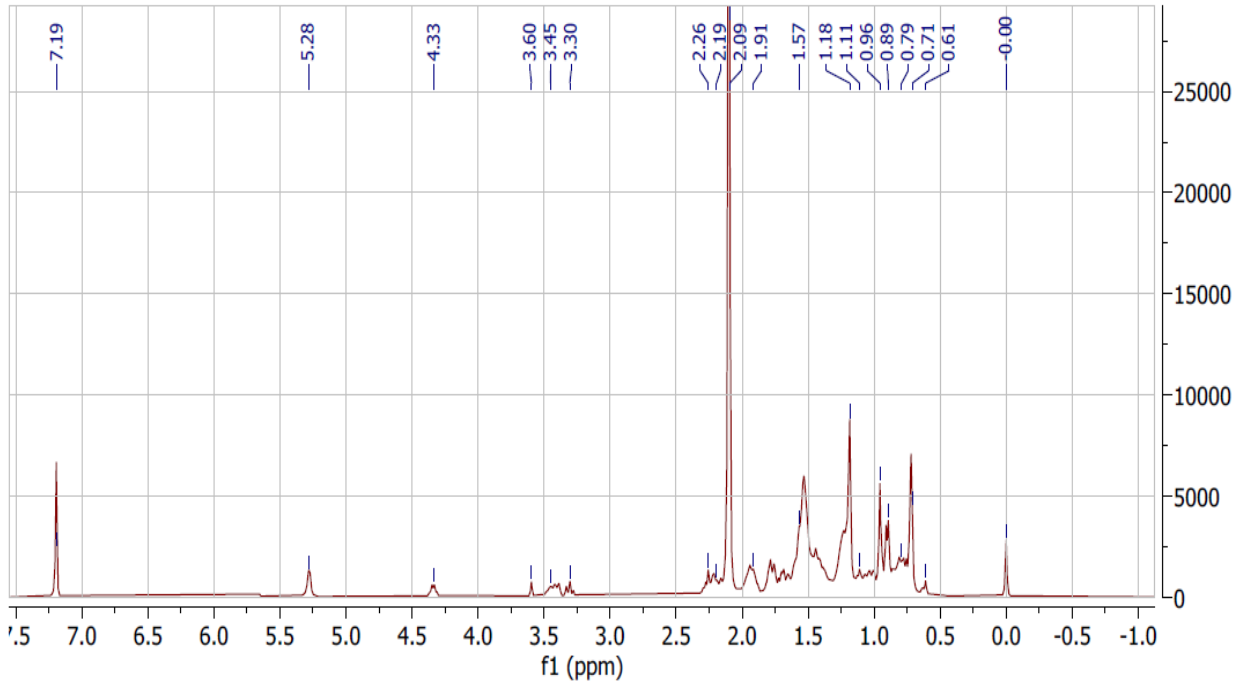


PLATE 6.16B: ^{13}C NMR spectrum of compound **6.16** in $CDCl_3$

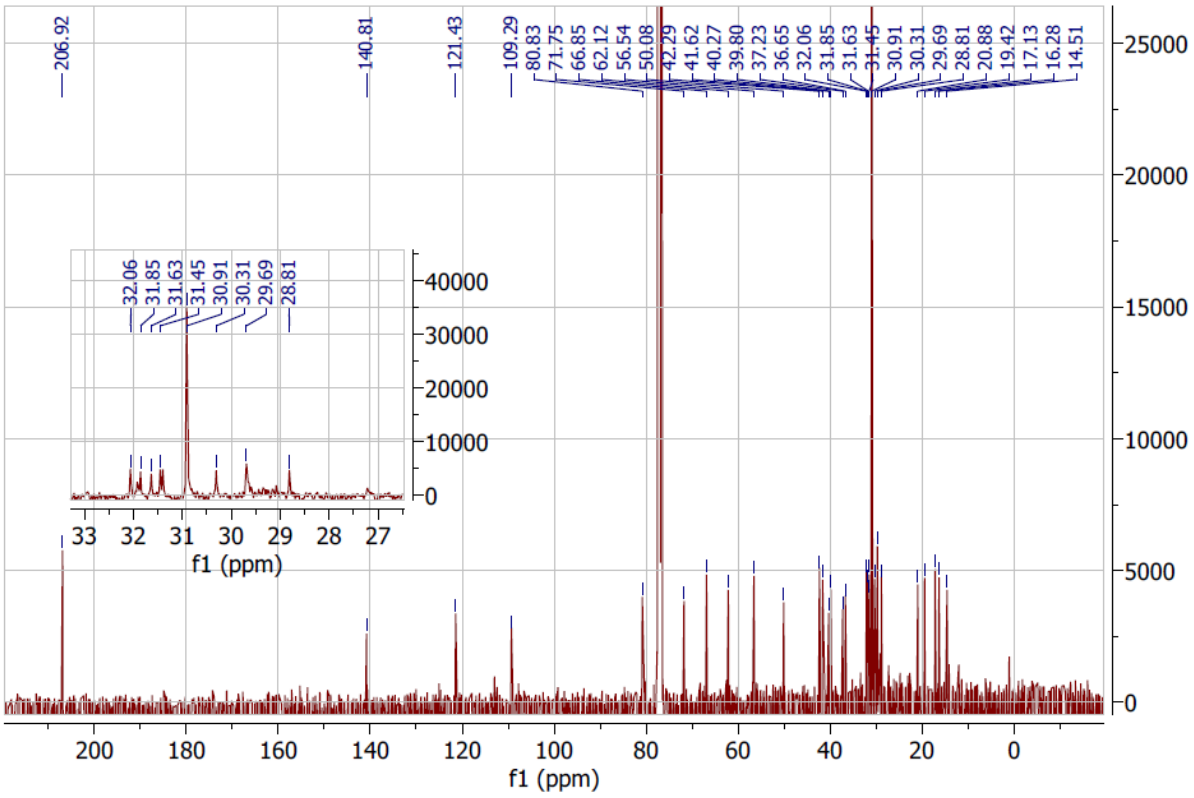


PLATE 6.16C: DEPT 135 NMR spectrum of compound **6.16** in $CDCl_3$

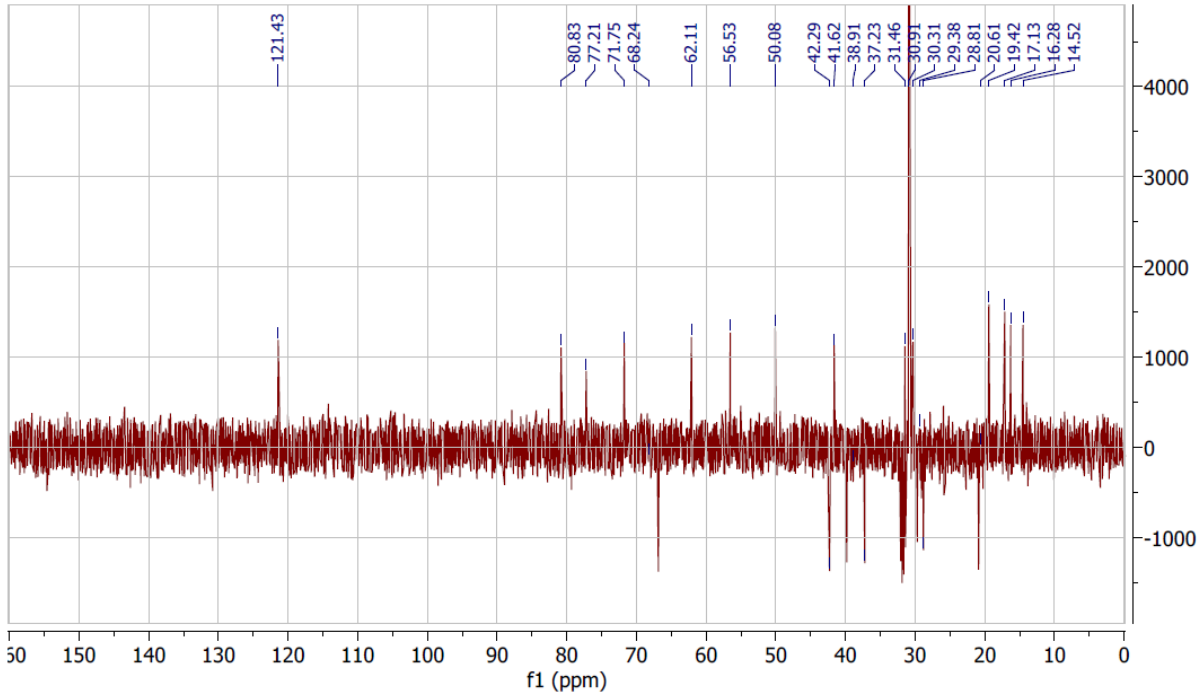


PLATE 6.16D: HSQC NMR spectrum of compound **6.16** in $CDCl_3$

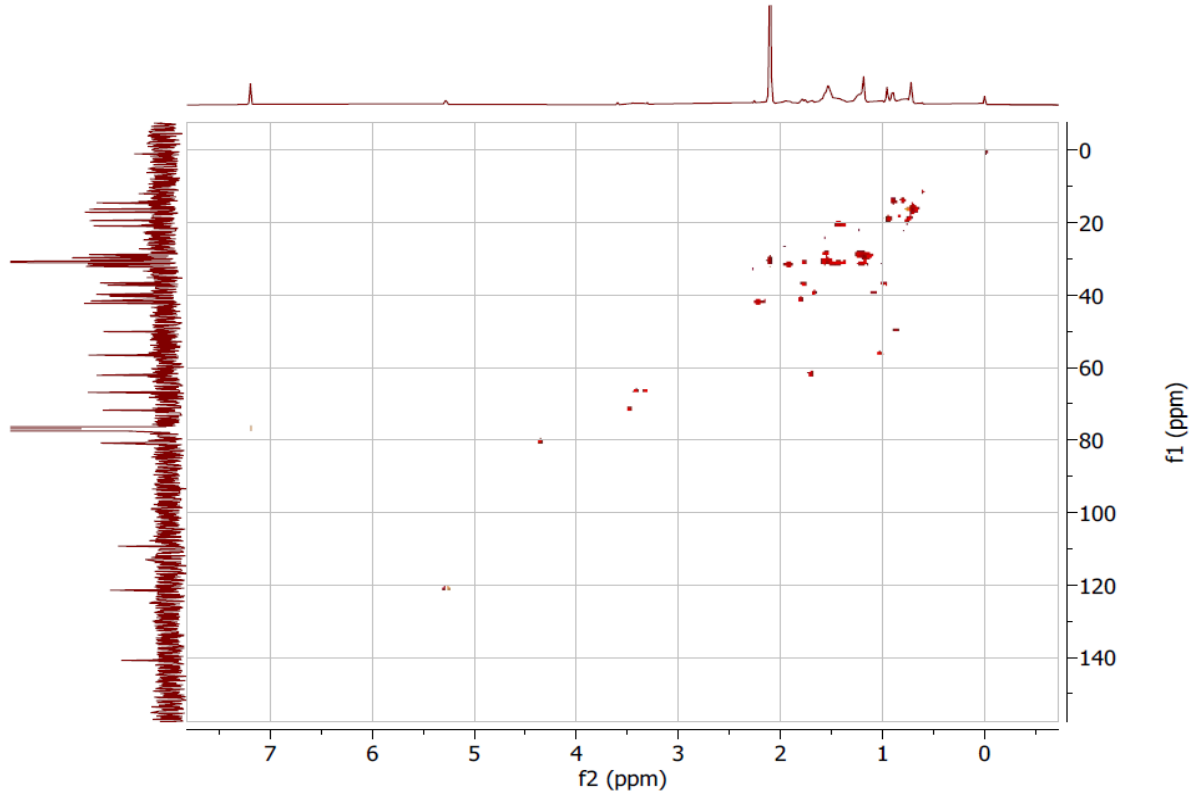


PLATE 6.16E: ^{1}HMBC NMR spectrum of compound **6.16** in $CDCl_3$

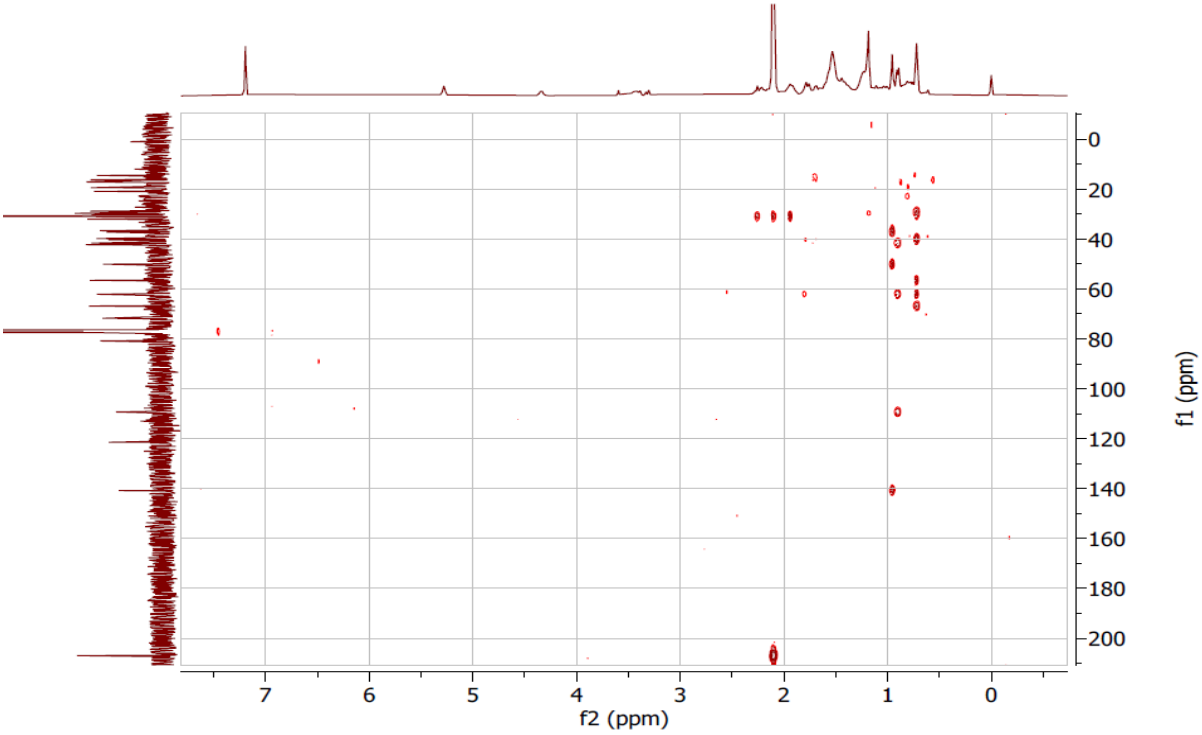


PLATE 6.16F: ^{1}H-^{1}H COSY NMR spectrum of compound **6.16** in $CDCl_3$

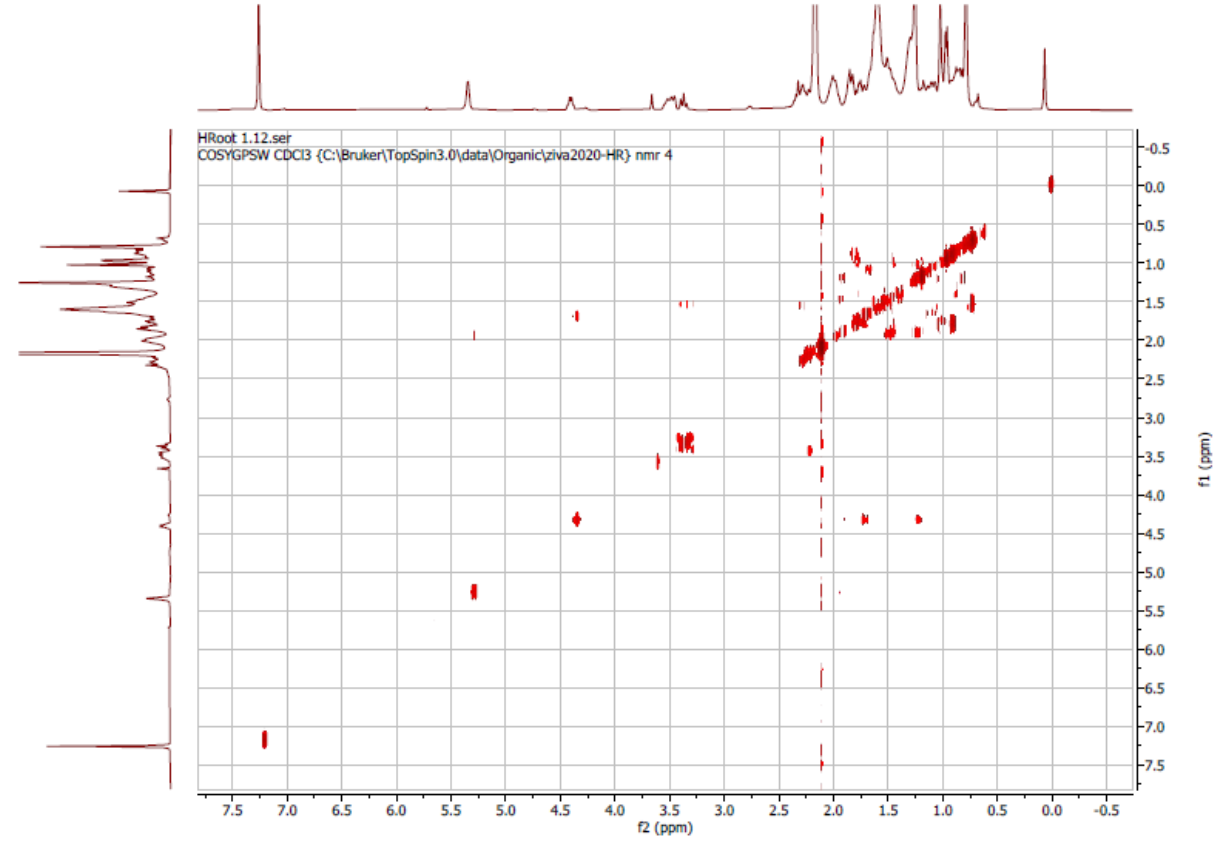


PLATE 6.16G: The HREIMS spectrum of compound **6.16**

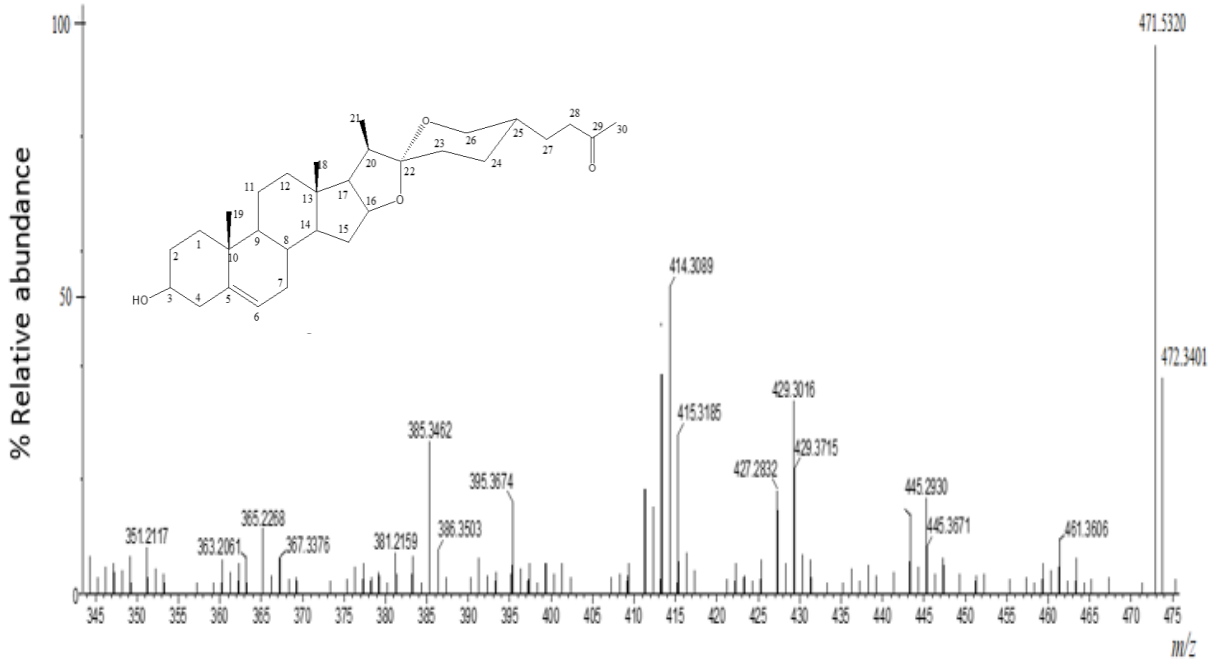
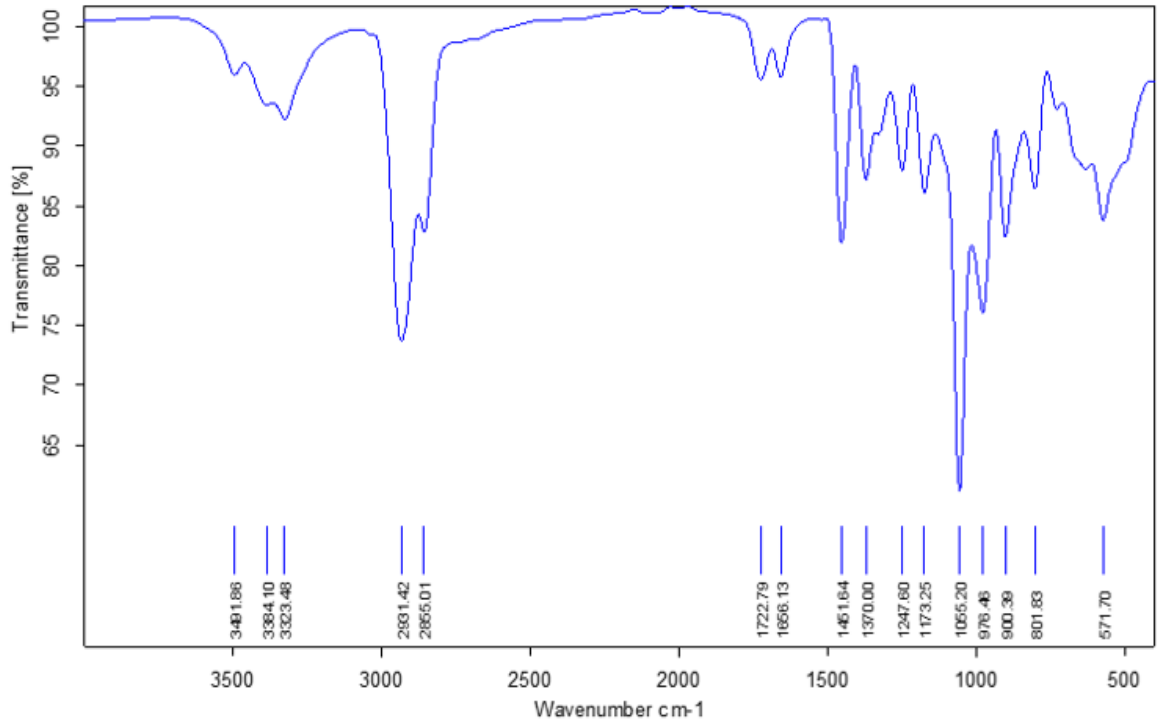


PLATE 6.16H FTIR spectrum of compound **6.16**



8.6.3 Spectra of 2*S*,2′*R*,4a*R*,4b*S*,5′*R*,6a*S*,6b*R*,7*S*,9a*S*,10a*S*,10b*S*)-4a,5′,6a,7-Tetramethyl-1,2,3,4,4a,4b,5,6,6a,6b,7,9a,10,10a,10b,11-hexadecahydrospiro [naptho[2′,1′:4,5]indeno[2,1-*b*]furan-8,2′-piperidin]-2-ol (6.17)

PLATE 6.17A: -^{1}H NMR spectrum of compound **6.17** in MeOD

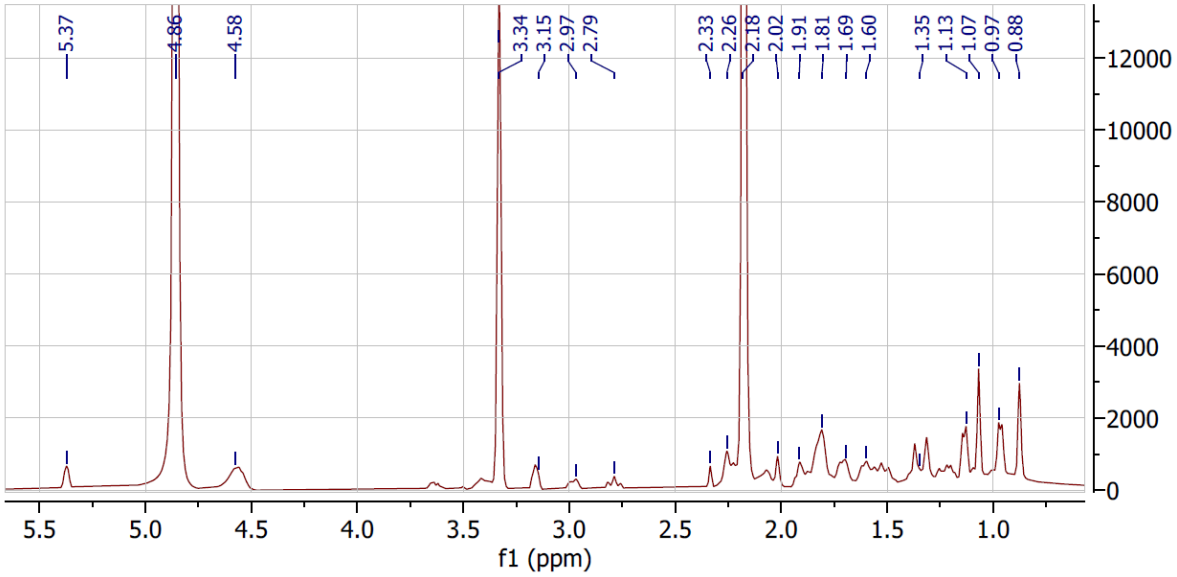


PLATE 6.17B: ^{13}C NMR spectrum of compound **6.17** in MeOD

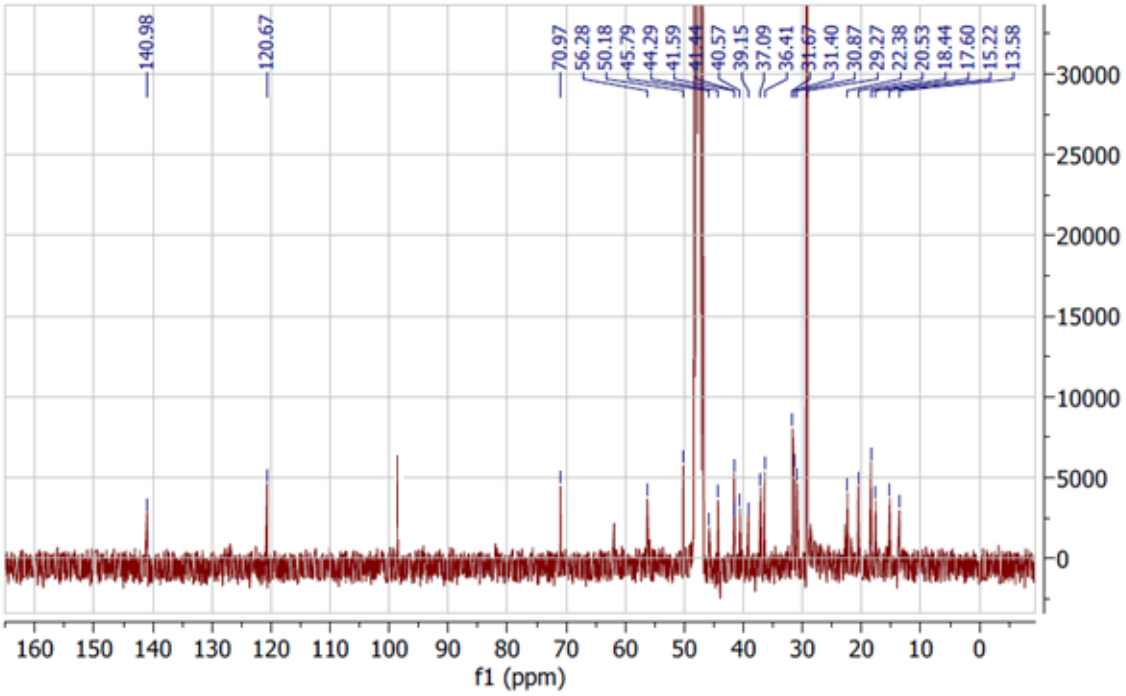


PLATE 6.17C: DEPT 135 NMR spectrum of compound **6.17** in MeOD

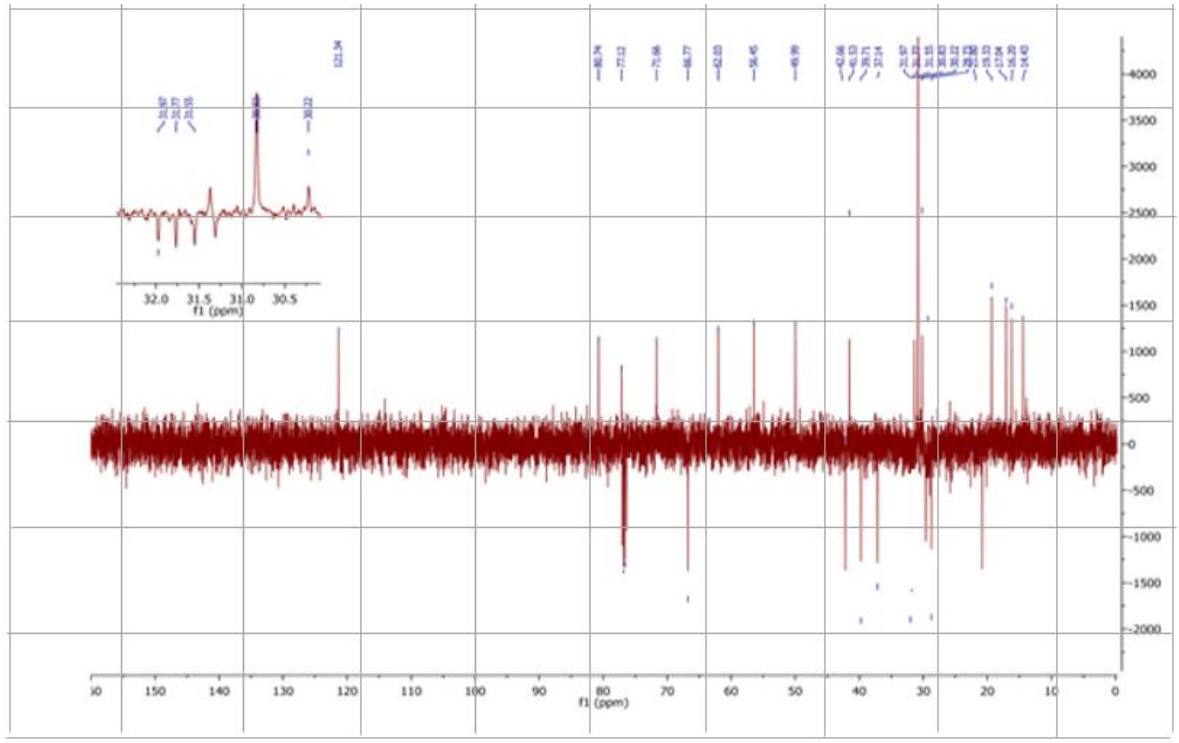


PLATE 6.17D: HSQC NMR spectrum of compound **6.17** in MeOD

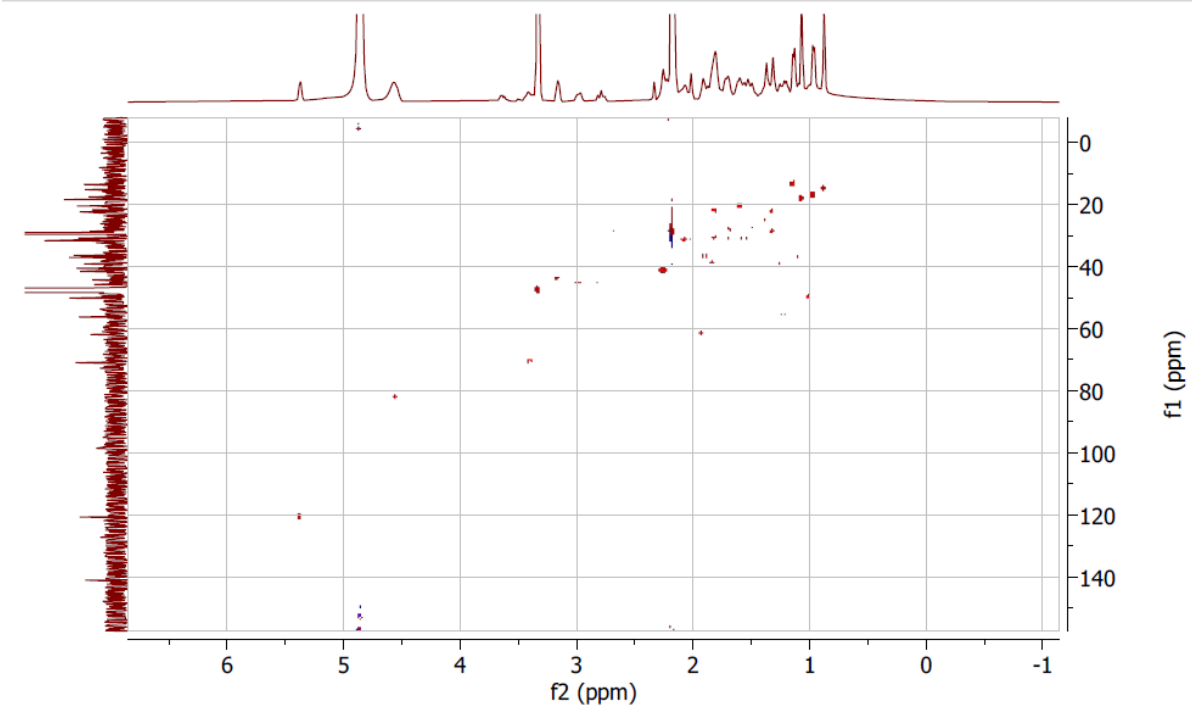


PLATE 6.17E: HMBC NMR spectrum of compound **6.17** in MeOD

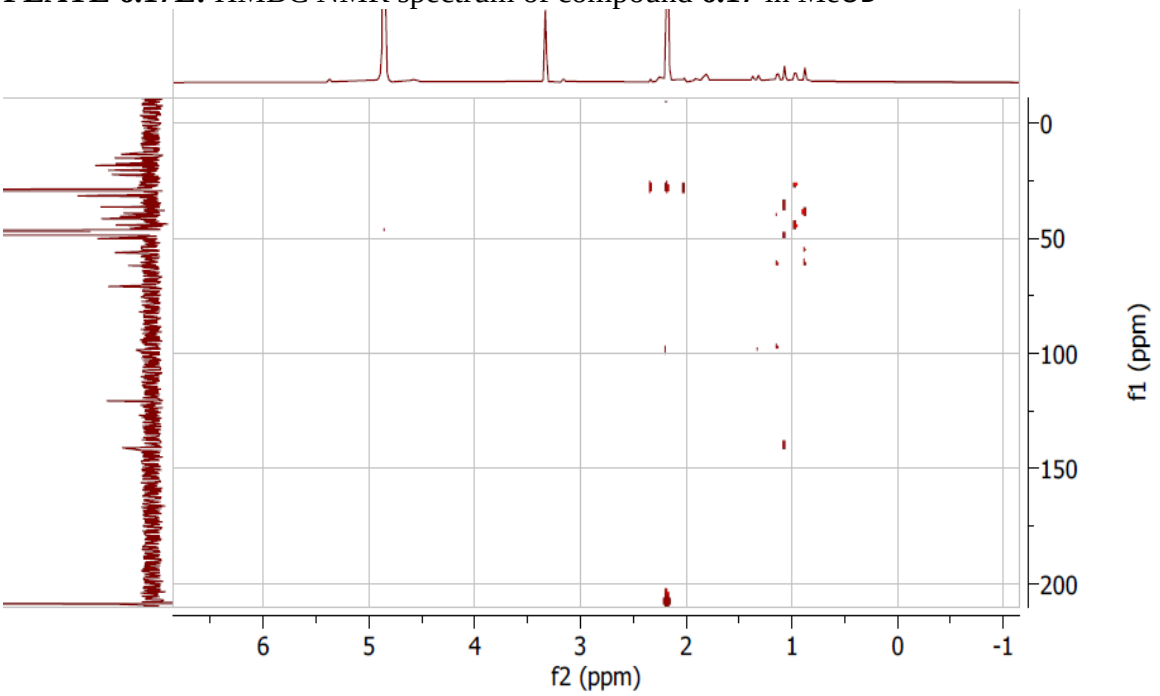


PLATE 6.17F: ^{1}H-^{1}H COSY NMR spectrum of compound **6.17** in MeOD

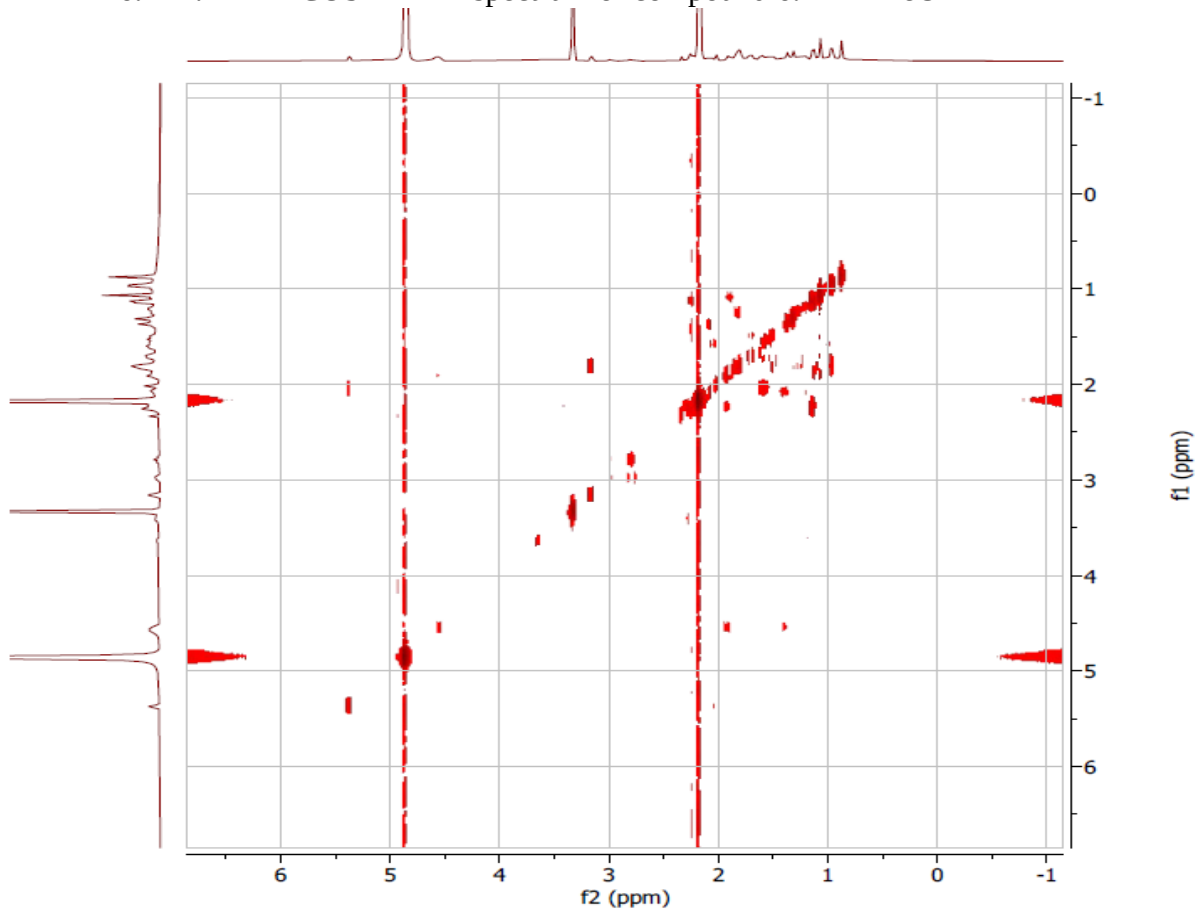


PLATE 6.17G: The HREIMS spectrum of compound **6.17**

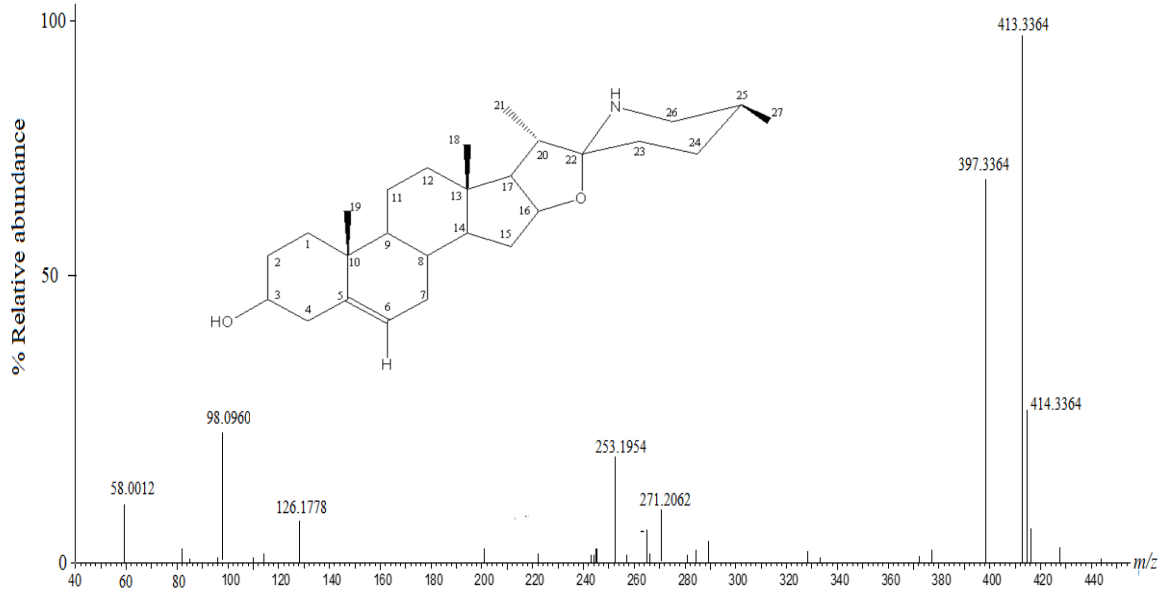
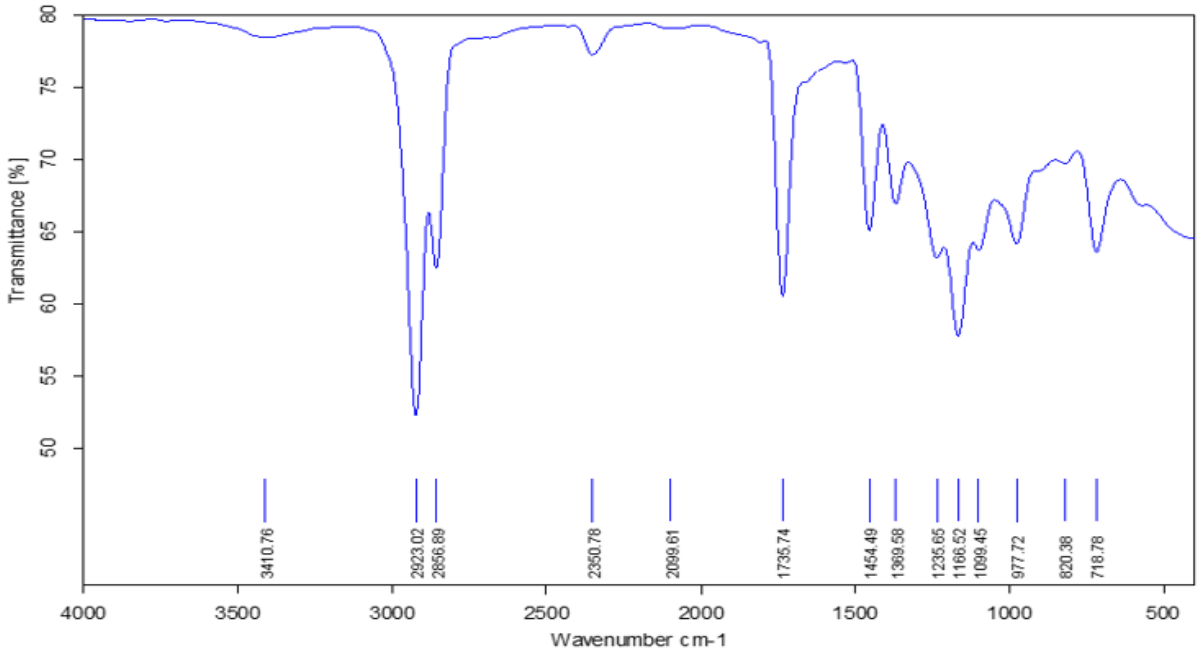


PLATE 6.17H FTIR spectrum of compound **6.17**



8.6.4 Spectra of (2*S*,2'*S*,3'*R*,4R,4'*R*,5*R*,5'*R*,6*S*)-{[2*R*,3*S*,4*S*,5*R*,6*R*)-4-hydroxy-2-(hydroxy methyl)-6-{[2*S*,2'*R*,4a*R*,4b*S*,5'*R*,6a*S*,6b*R*,7*S*,9a*S*,10a*S*,10b*S*)-4a,5',6a,7-tetramethyl-1,2,3,4a,4b,5,6,6a,6b,7,9a,10,10a,10b,11-hexadechydrospiro [naptho[2',1',4,5] indeno[2,1-b]furan-8,2'-piperidin]-2-yl]oxy}oxane=3,5-diyl]bis(6-methyloxane-3,4,5-triol) 6.18

PLATE 6.18A: ^{1}H NMR spectrum of compound **6.18** in MeOD

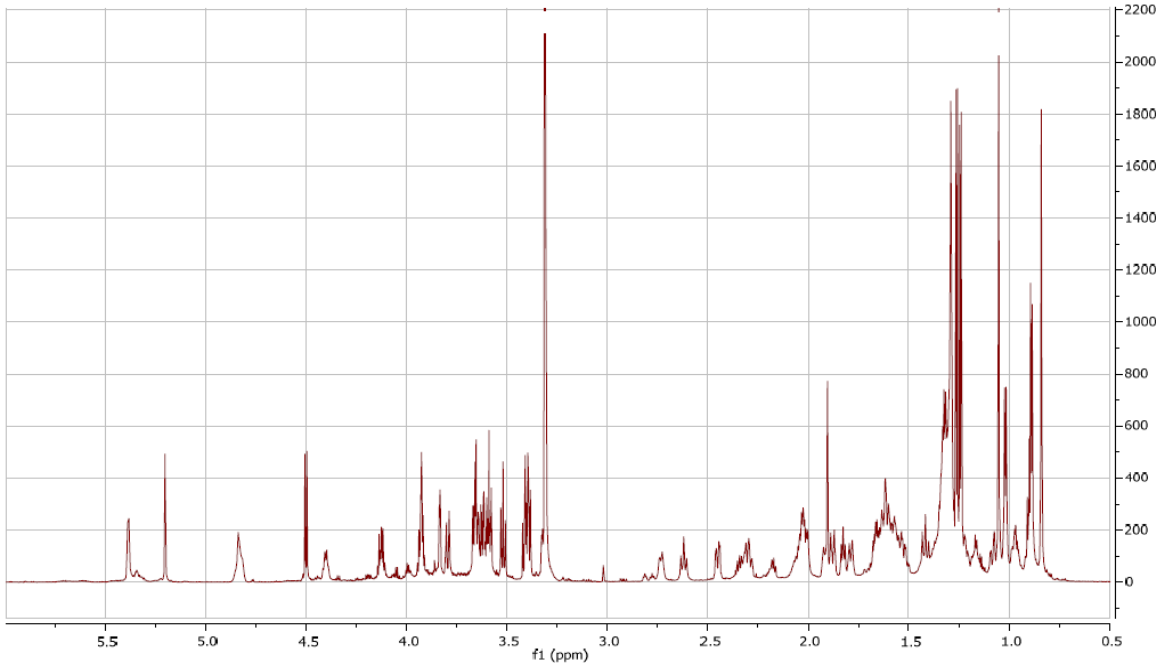


PLATE 6.18B: ^{13}C NMR spectrum of compound **6.18** in MeOD

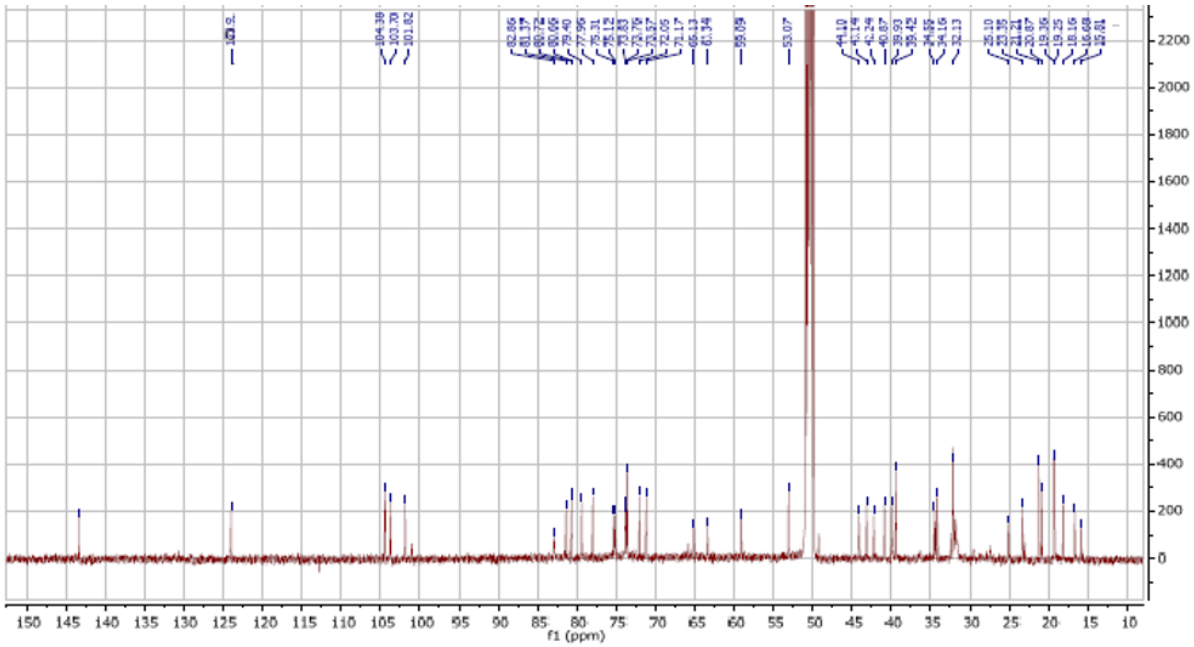


PLATE 6.18C: DEPT 135 NMR spectrum of compound **6.18** in MeOD

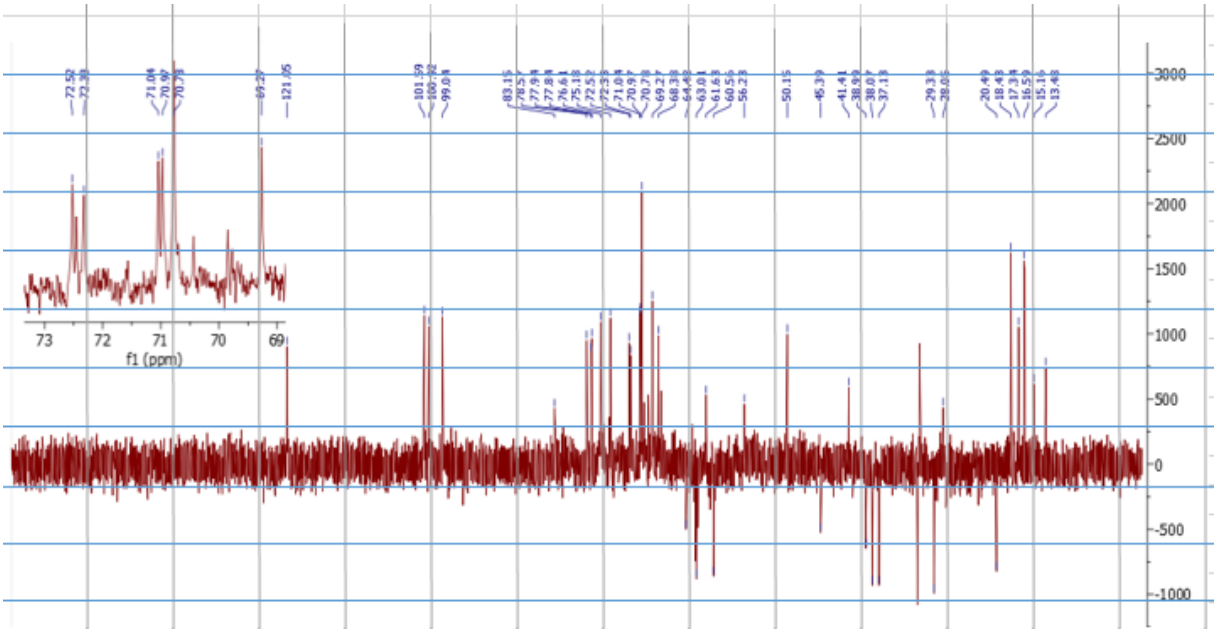


PLATE 6.18D: HSQC NMR spectrum of compound **6.18** in (MeOD

PLATE 6.18E: ^{1}HMBC NMR spectrum of compound **6.18** in MeOD

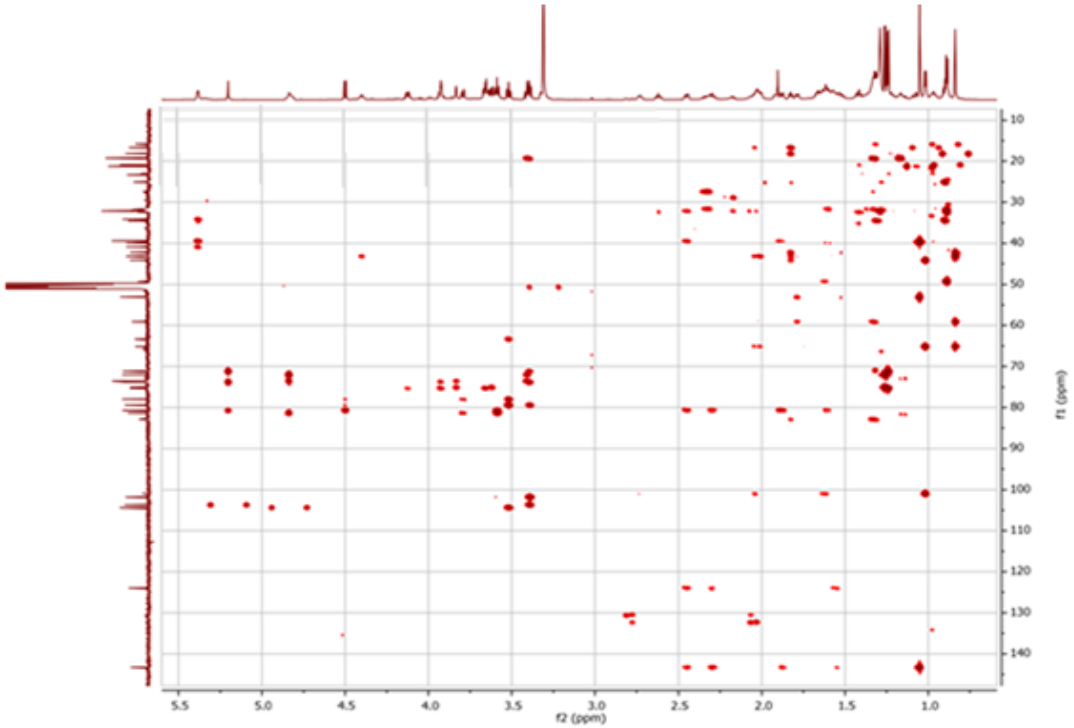


PLATE 6.18F: ^{1}H-^{1}H COSY NMR spectrum of compound **6.18** in MeOD

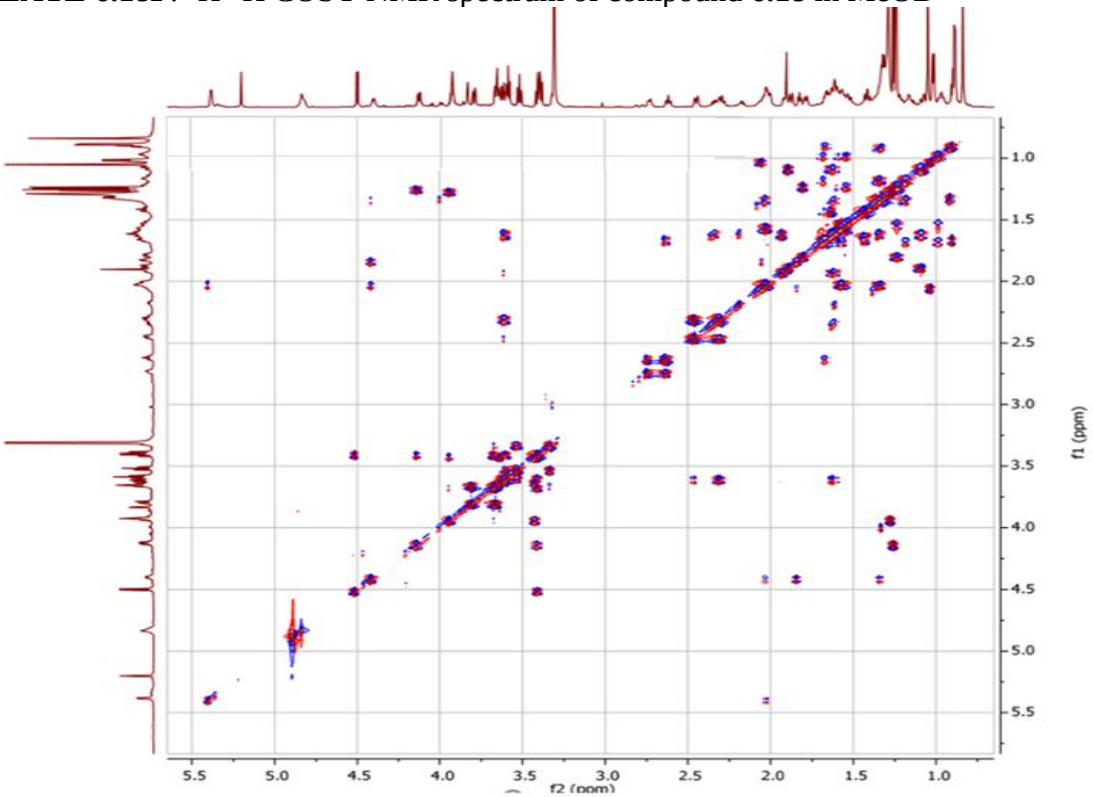


PLATE 6.18G: The HREIMS spectrum of compound **6.18**

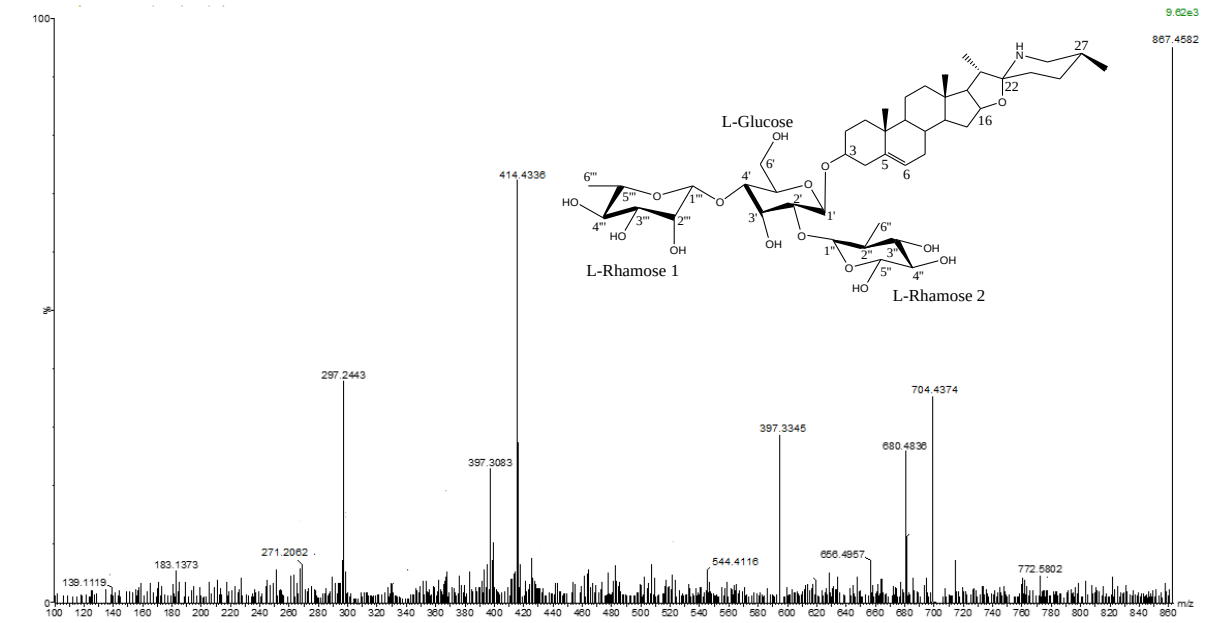
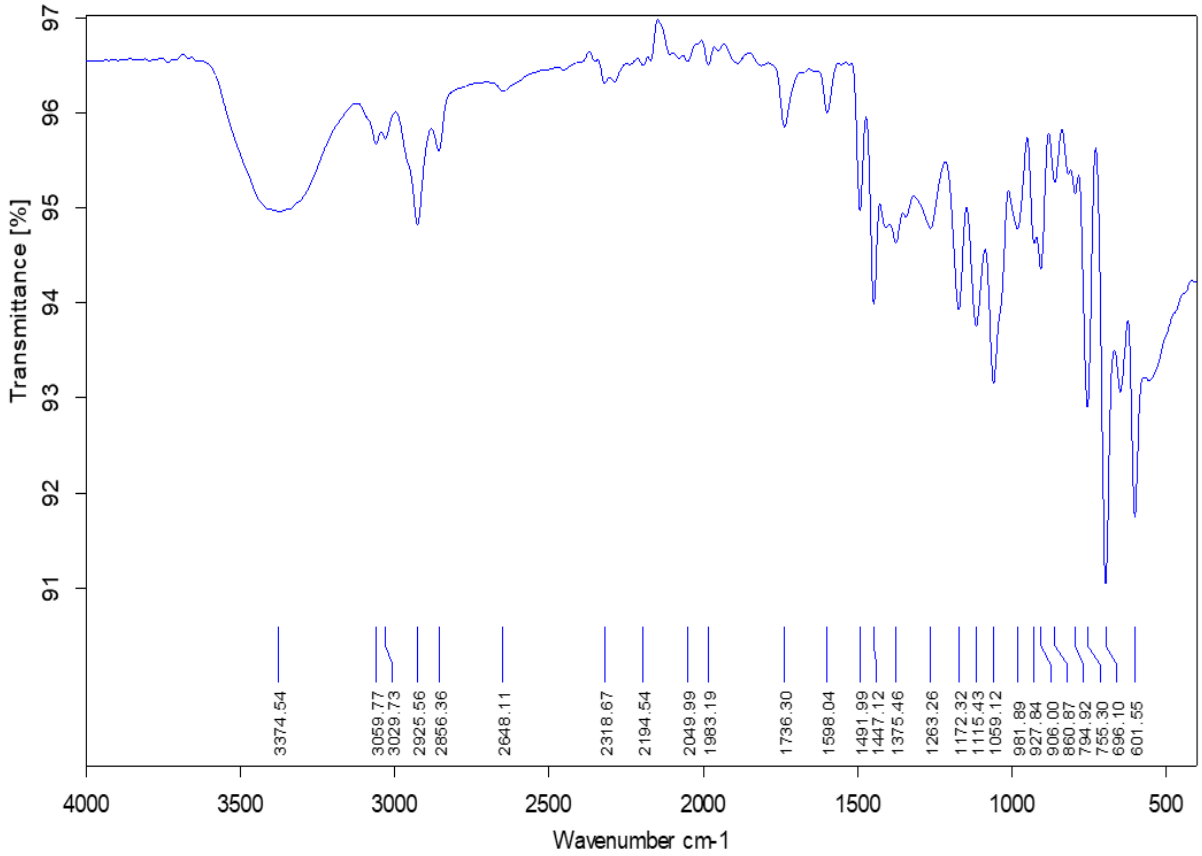


PLATE 6.18H FTIR spectrum of compound **6.18**



8.6.5 Spectra of 2-3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one

PLATE 6.19A: ^{1}H NMR spectrum of **6.19** in MeOD

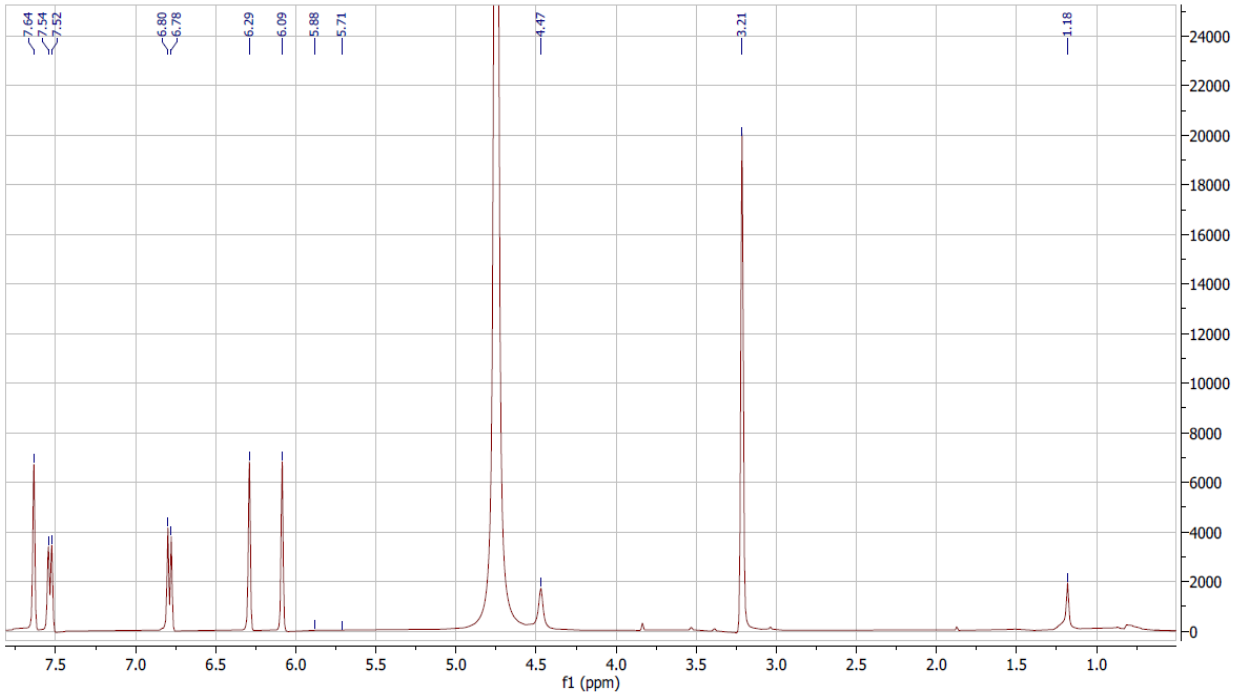


PLATE 6.19B: ^{13}C NMR spectrum of **6.19** in MeOD

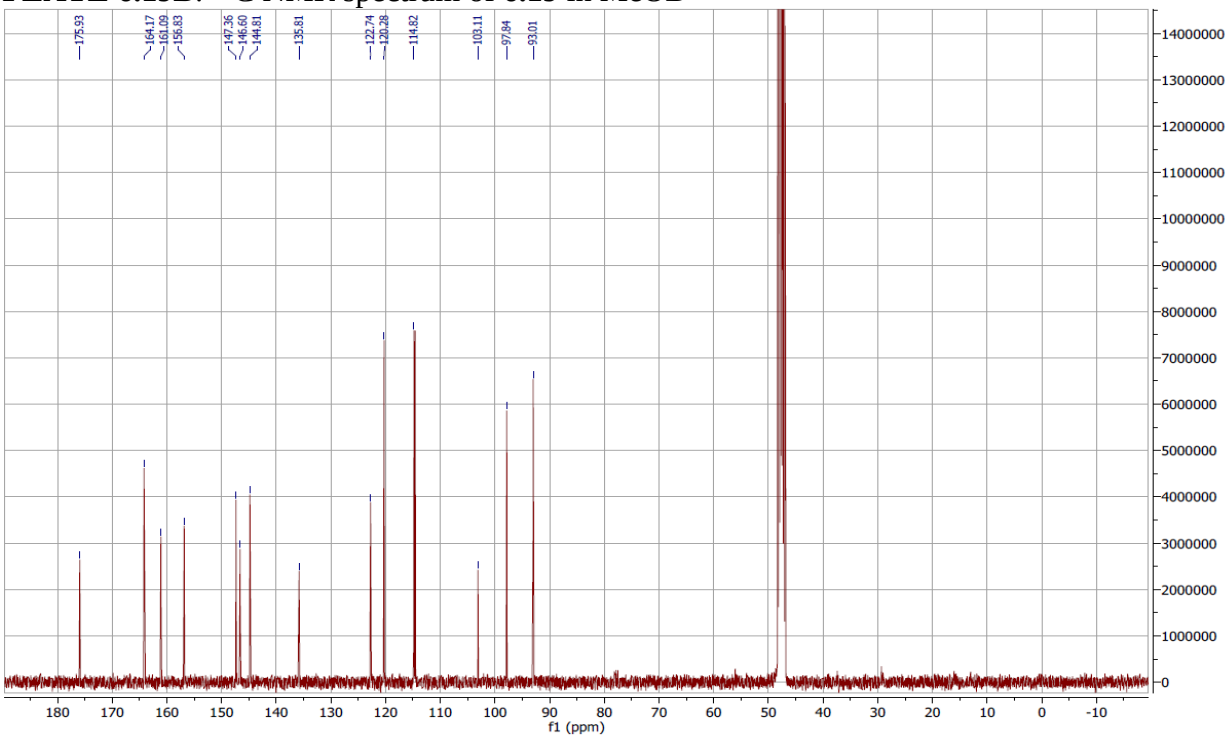


PLATE 6.19C: DEPT 135 NMR spectrum of **6.19** in MeOD

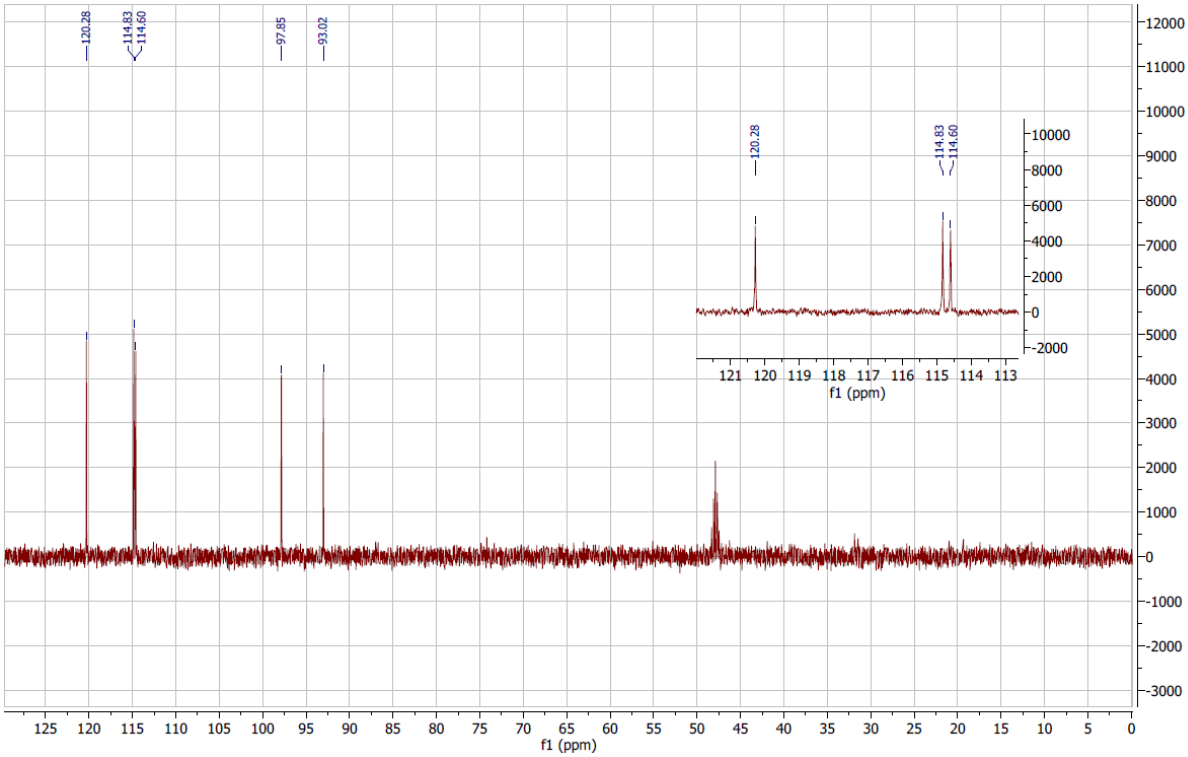


PLATE 6.19D: HSQC NMR spectrum of **6.19** in MeOD

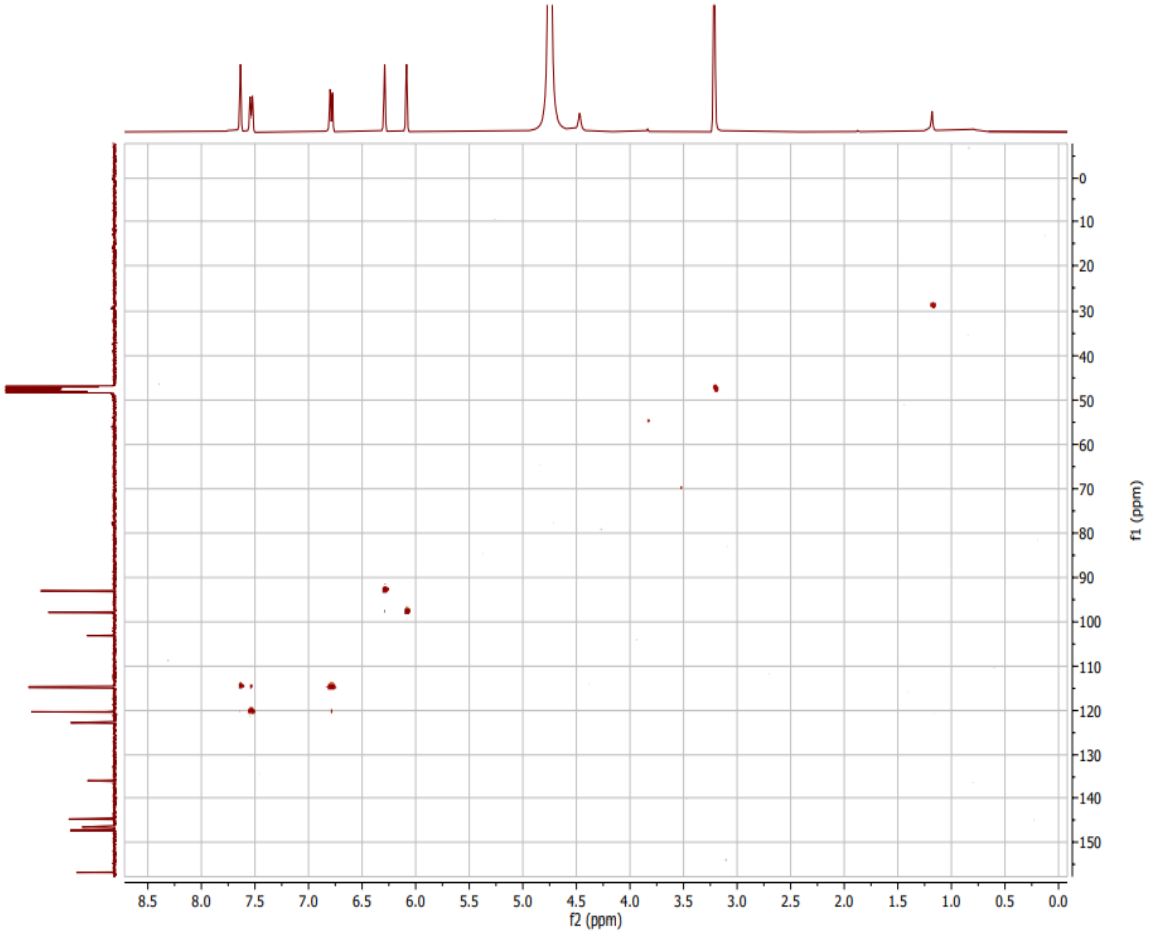


PLATE 6.19E: HMBC NMR spectrum of **6.19** in MeOD

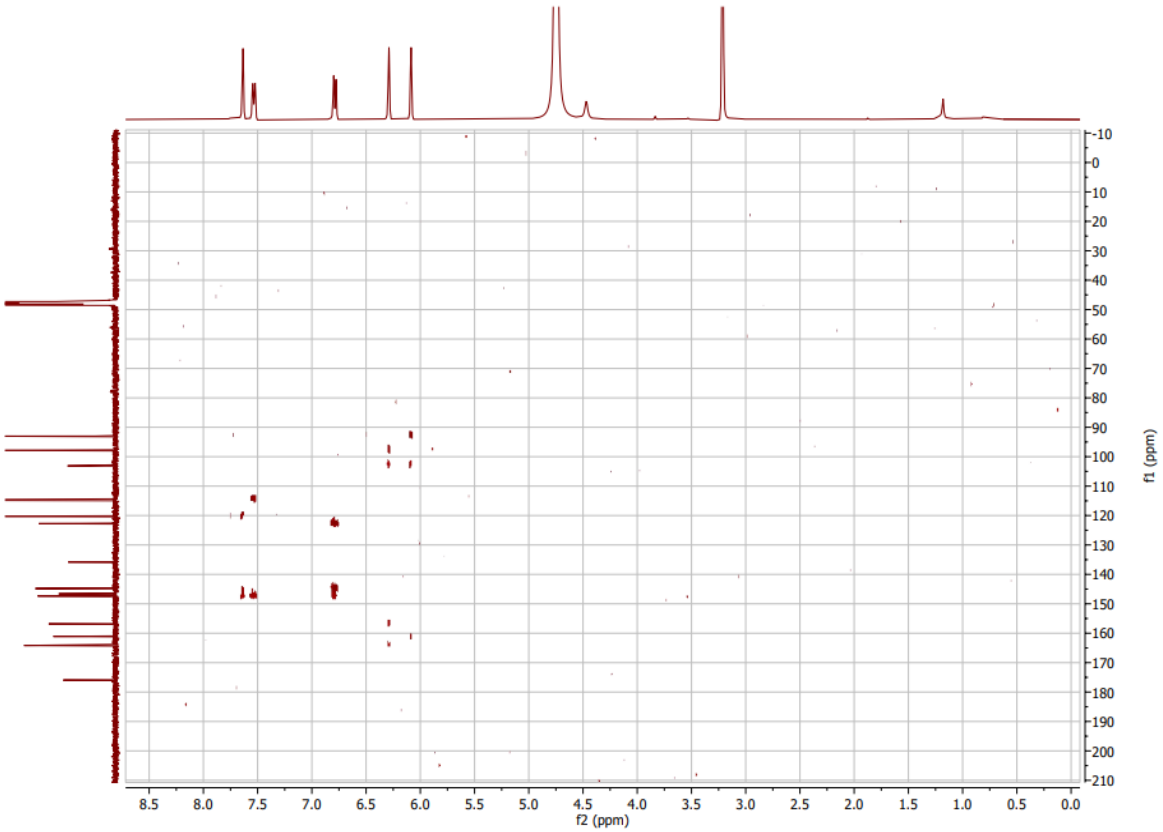


PLATE 6.19F: ^{1}H-^{1}H COSY NMR spectrum of **6.19** in MeOD

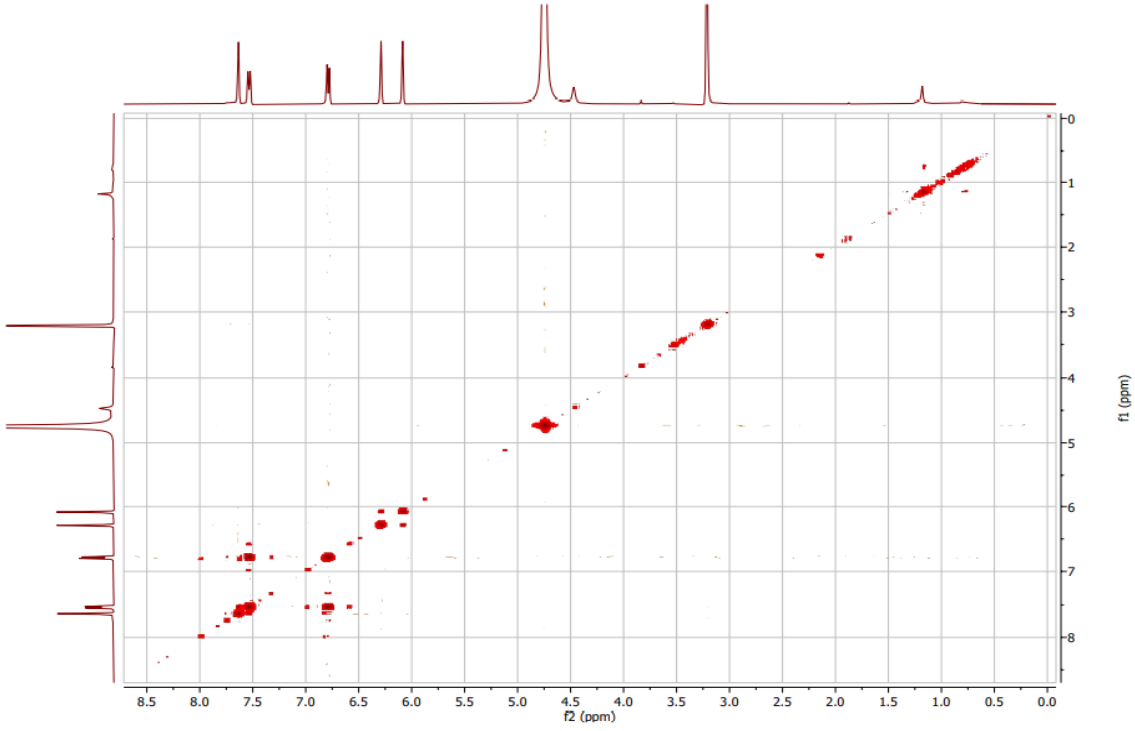


PLATE 6.19G: The HREIMS spectrum of compound **6.19**

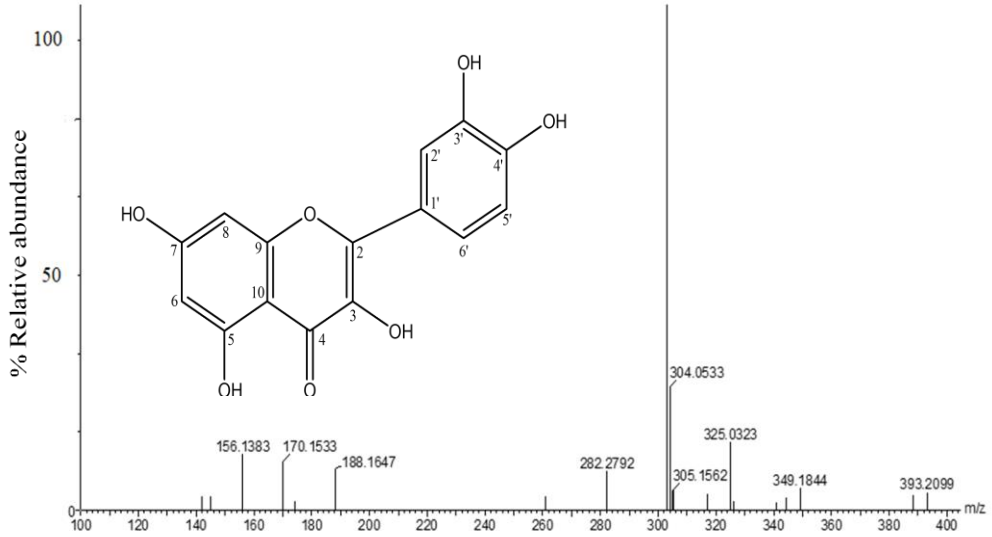
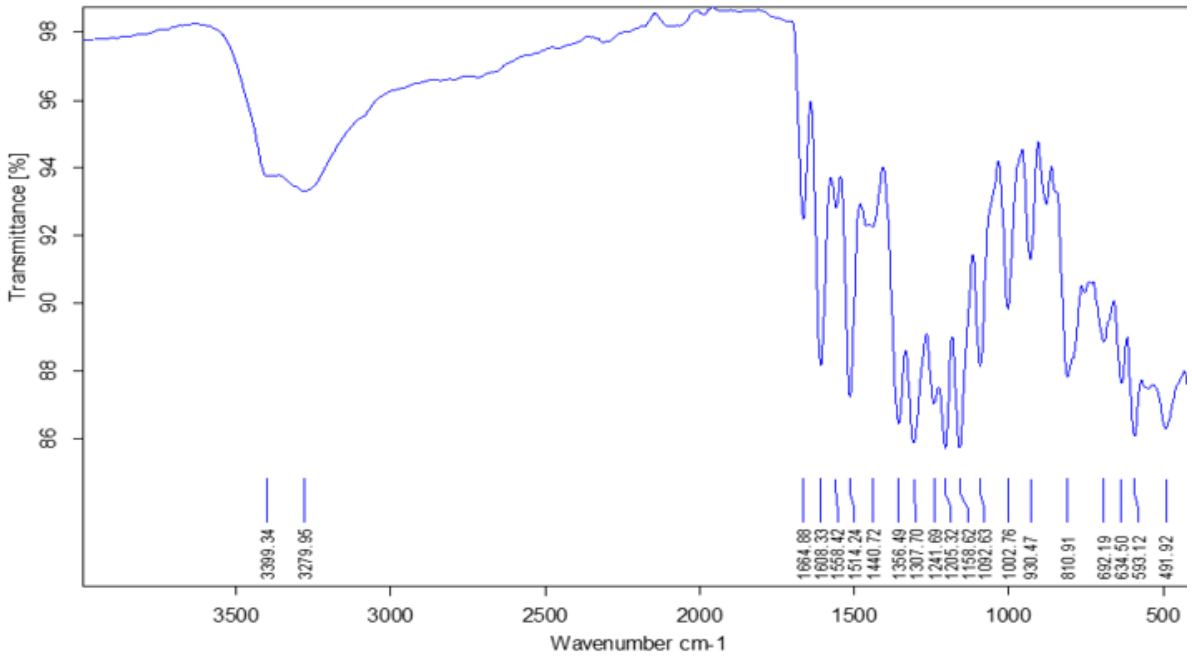


PLATE 6.19H FTIR spectrum of compound **6.19**



8.6.6 Spectra of 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl-6-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside2-3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one

PLATE 6.20A: ^{1}H NMR spectrum of **6.20** in DMSO

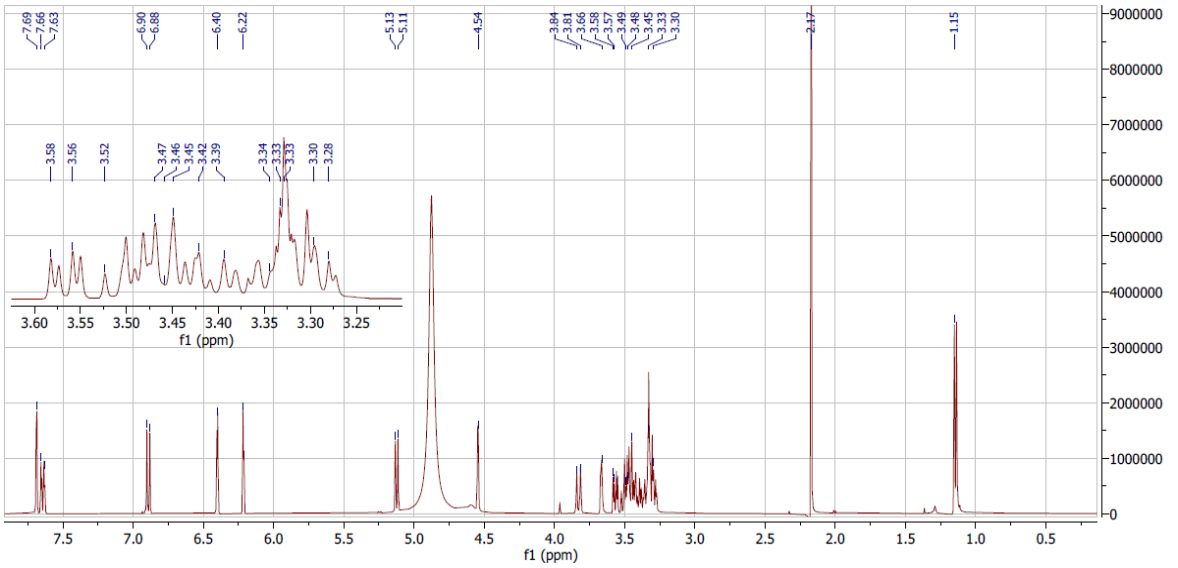


PLATE 6.20B: ^{13}C NMR spectrum of **6.20** in DMSO

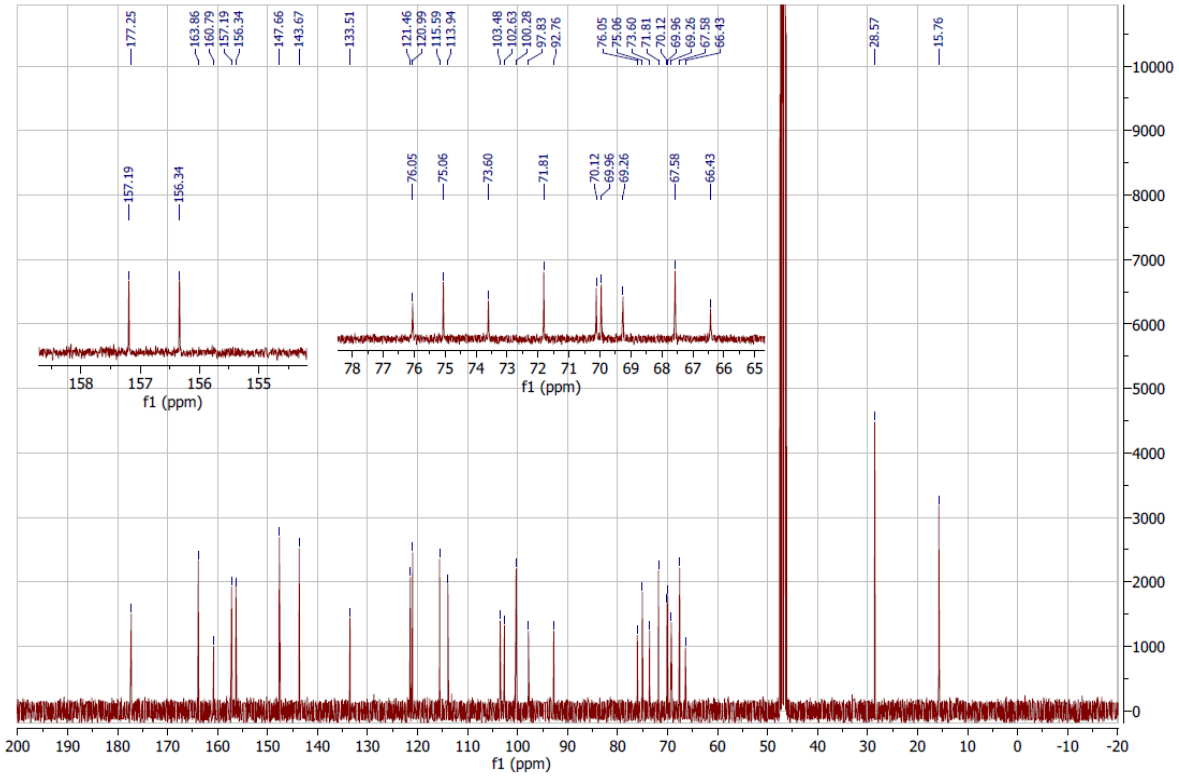


PLATE 6.20C: DEPT 135 NMR of compound **6.20** in DMSO

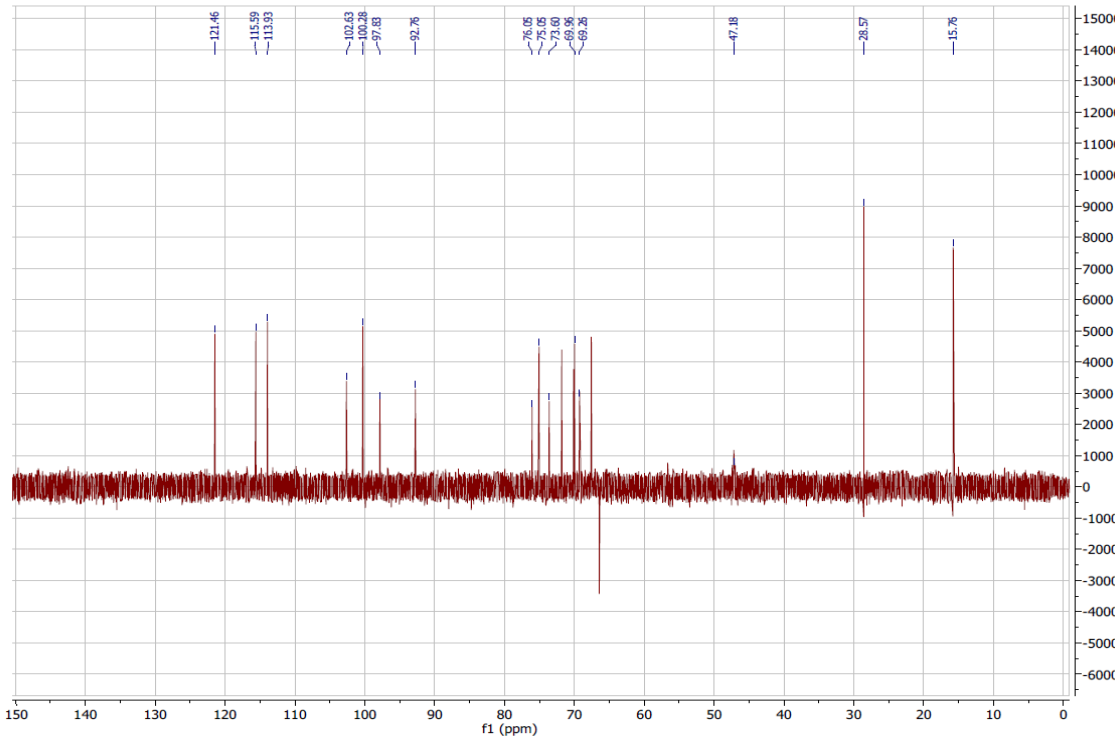


PLATE 6.20D: HSQC NMR of compound **6.20** in DMSO

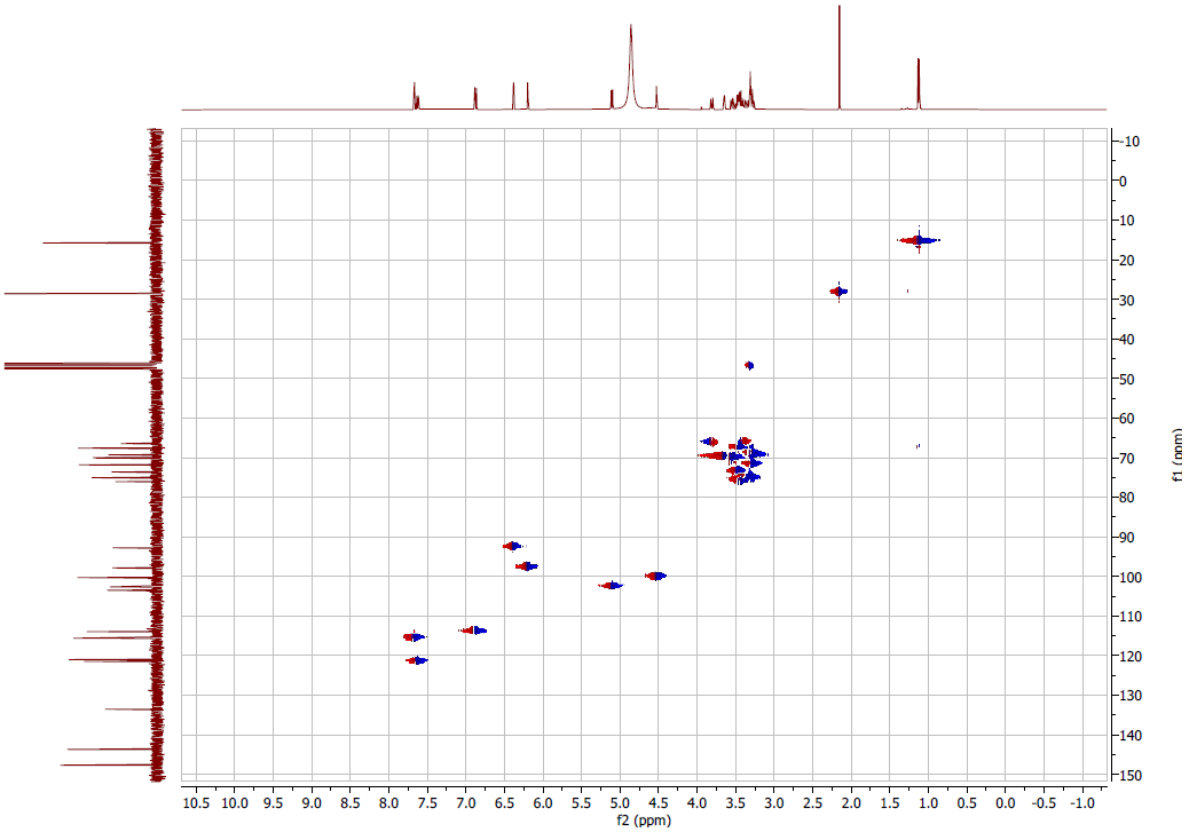


PLATE 6.20E: HMBC NMR of compound **6.20** in DMSO

PLATE 6.20F: ^{1}H-^{1}H COSY NMR of compound **6.20** in DMSO

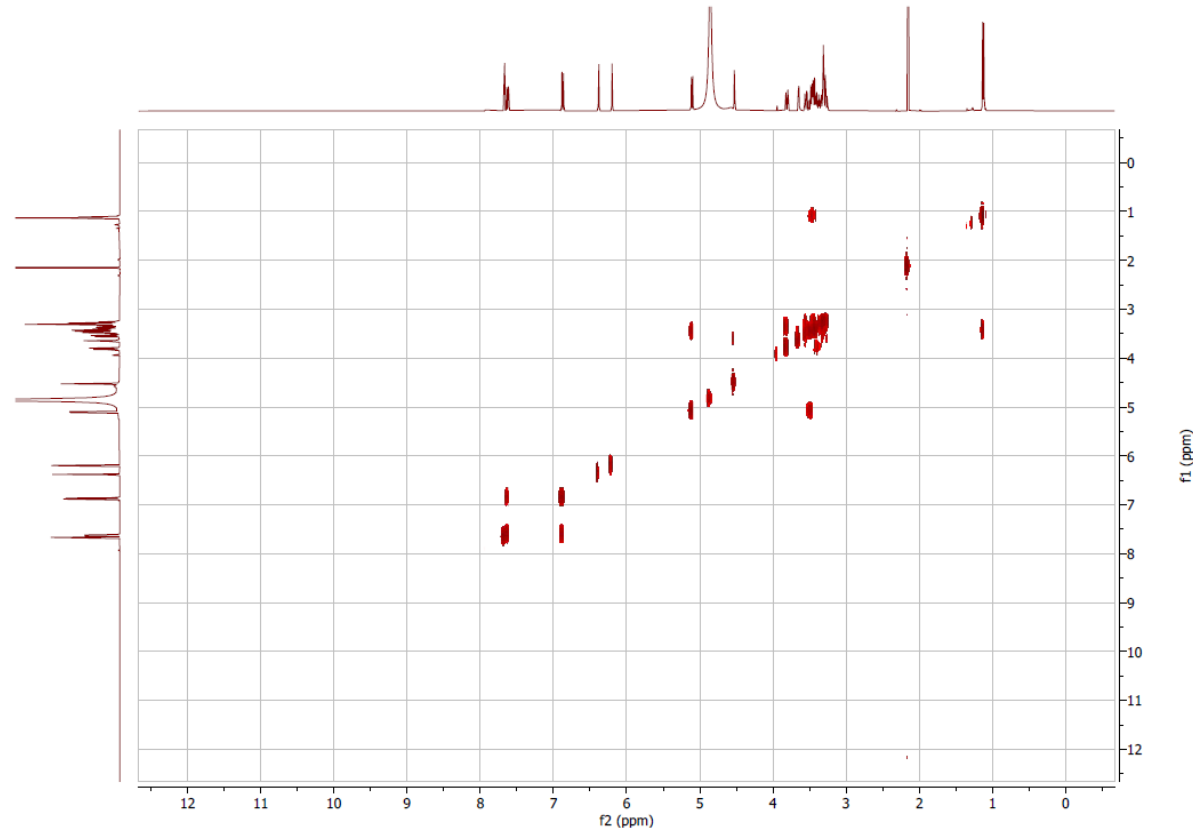


PLATE 6.20G: The HREIMIS spectrum of compound **6.20**

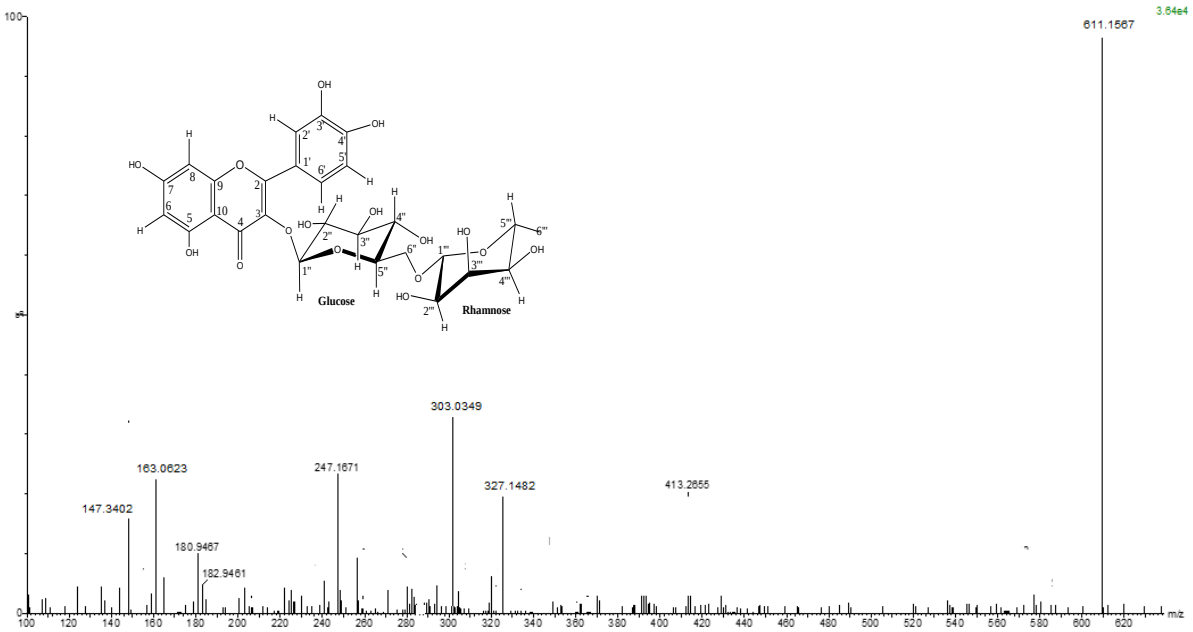
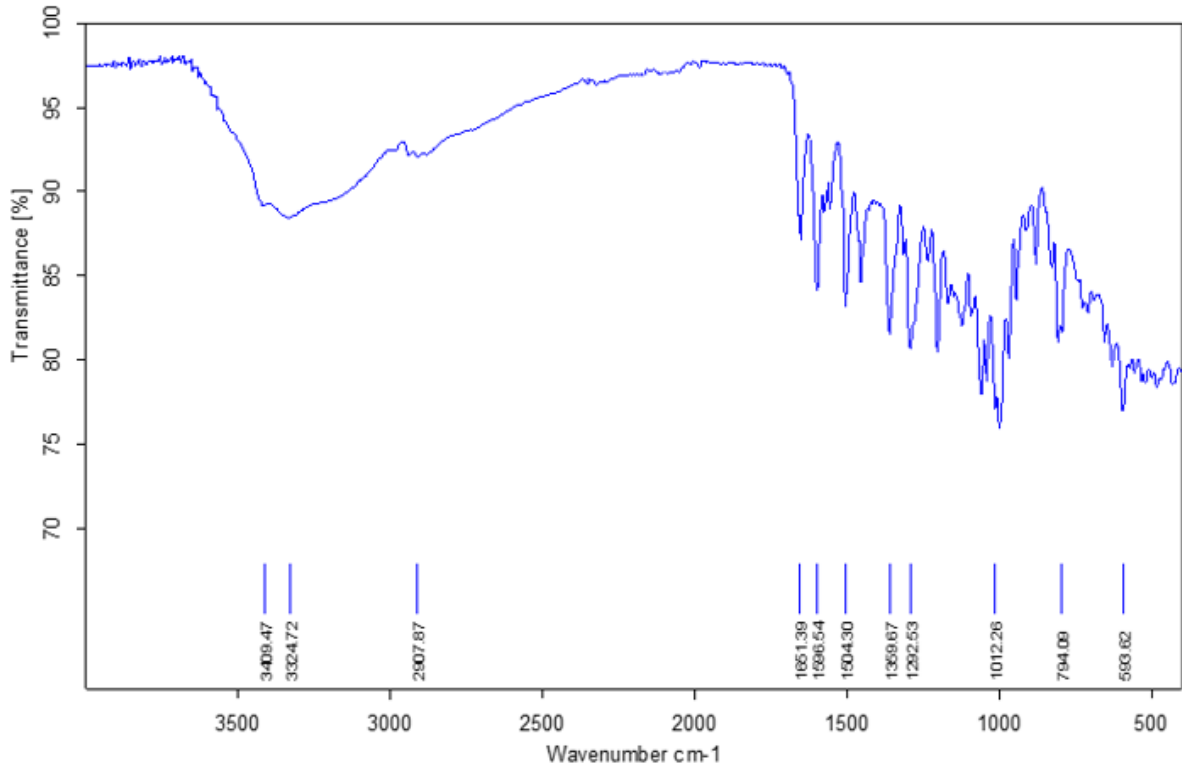


PLATE 6.20H FTIR spectrum of compound **6.20**



8.6.7 Spectra of phenoxy-2-ethan-1-ol (6.21)

PLATE 6.21A: ^{1}H NMR spectrum of compound **6.21** in MeOD

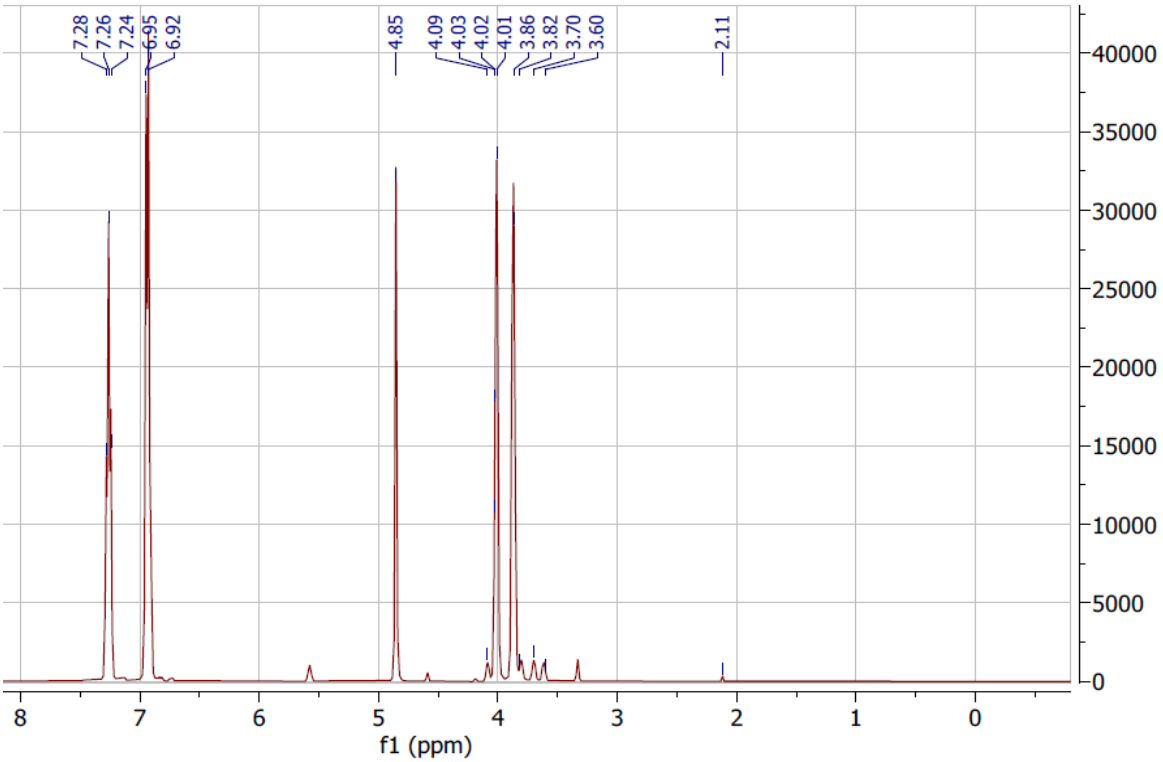


PLATE 6.21B: ^{13}C NMR spectrum of compound **6.21** in MeOD

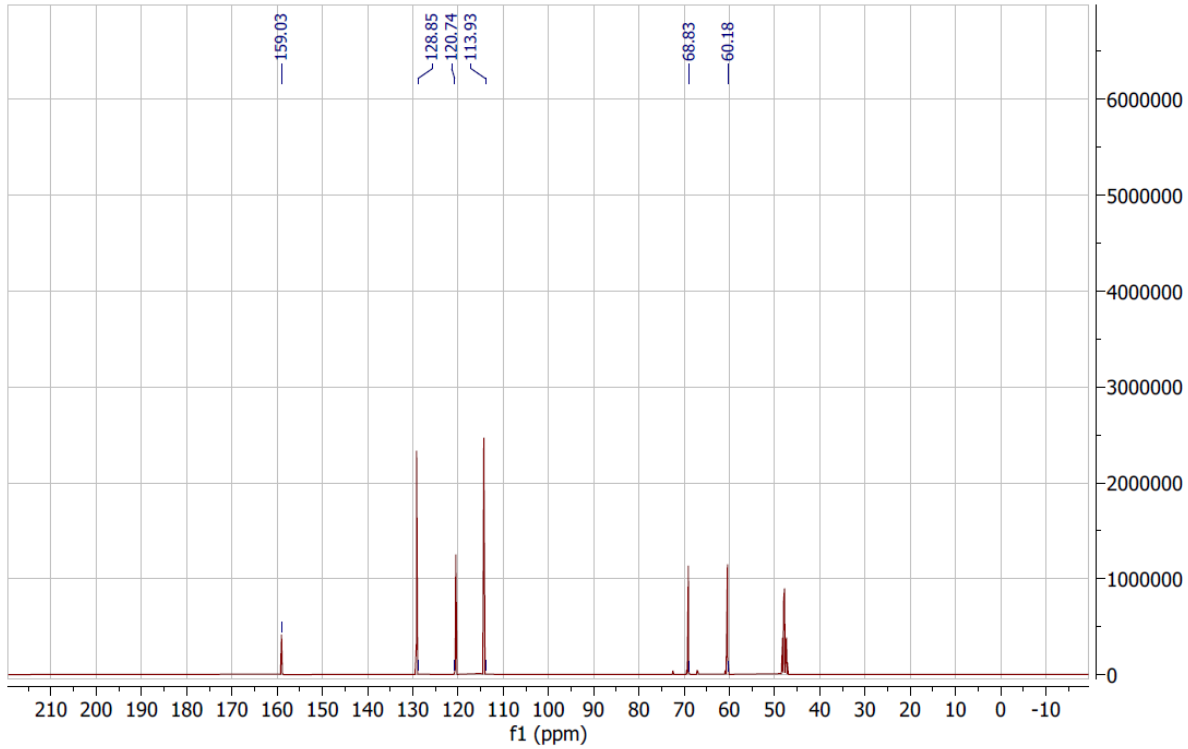


PLATE 6.21C: DEPT 135 NMR spectrum of compound **6.21** in MeOD

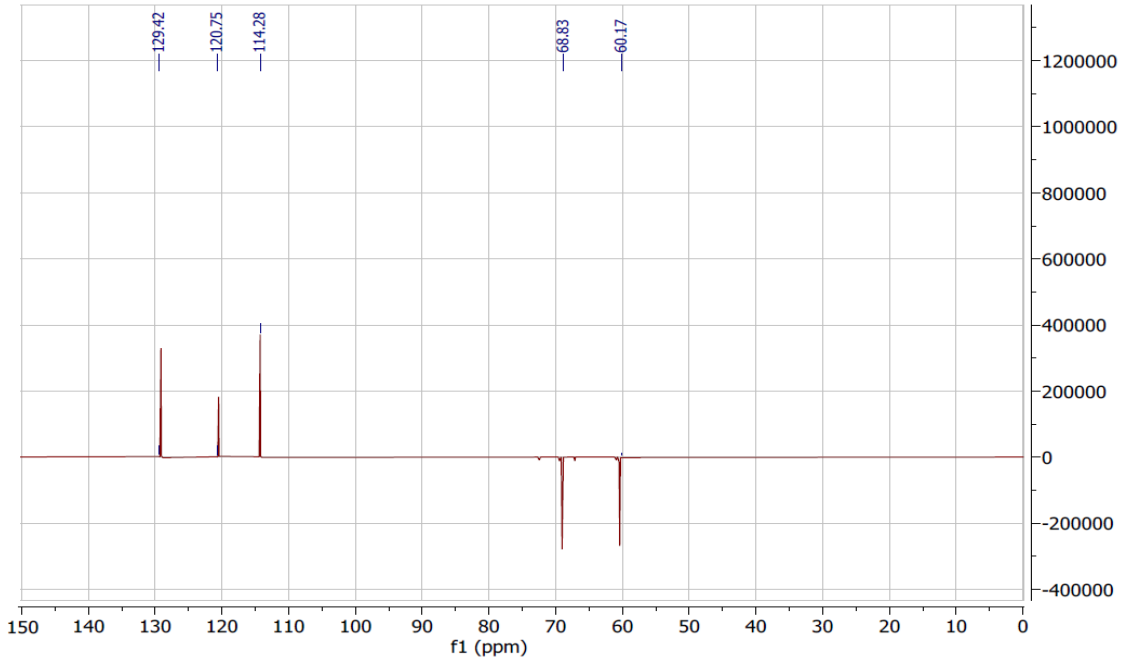


PLATE 6.21D: HSQC NMR spectrum of compound **6.21** in MeOD

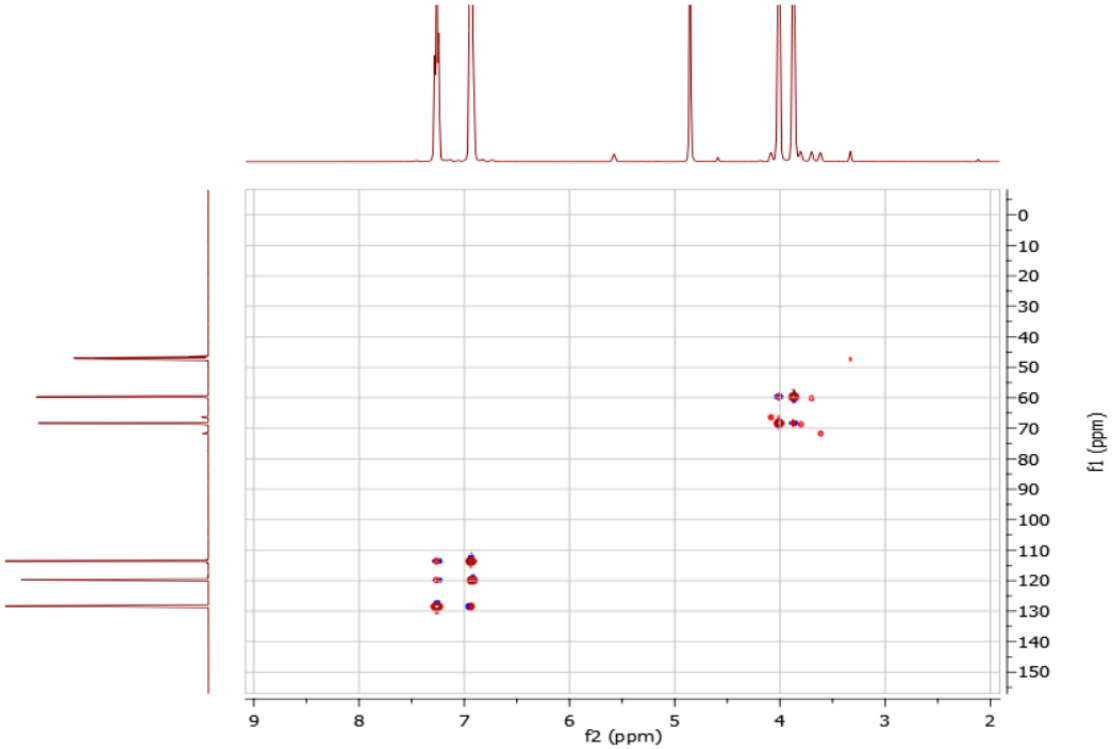


PLATE 6.21E: HMBC NMR spectrum of compound **6.21** in MeOD

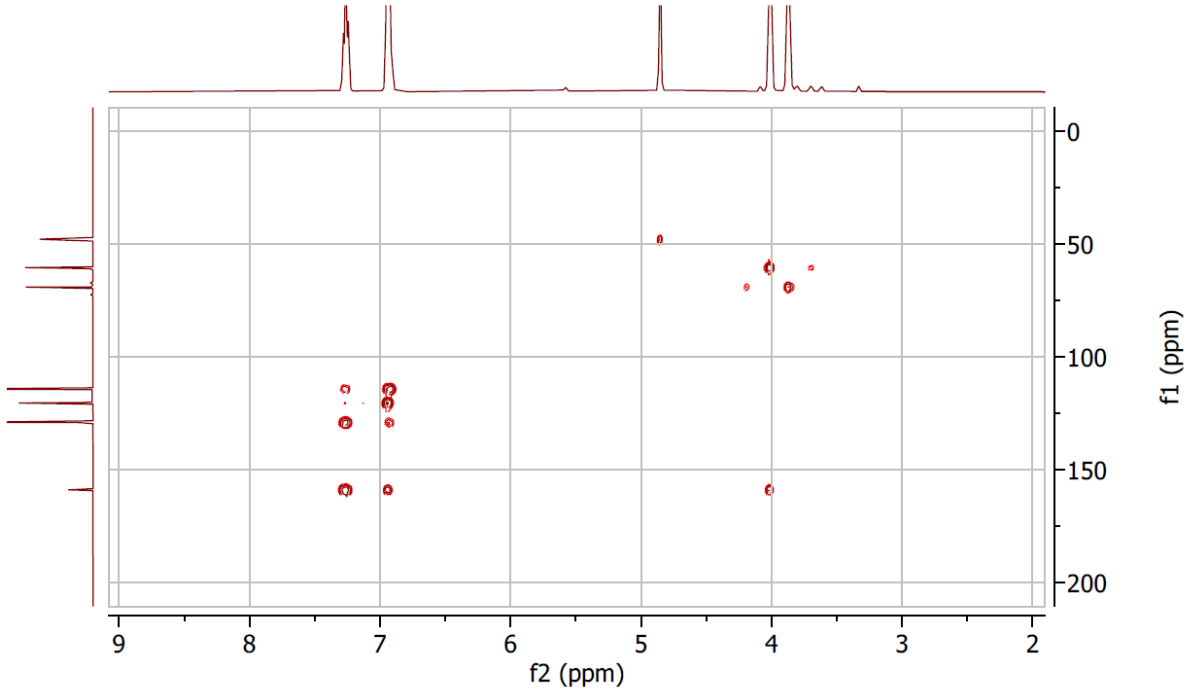


PLATE 6.21F: ^{1}H-^{1}H COSY NMR spectrum of compound **6.21** in MeOD

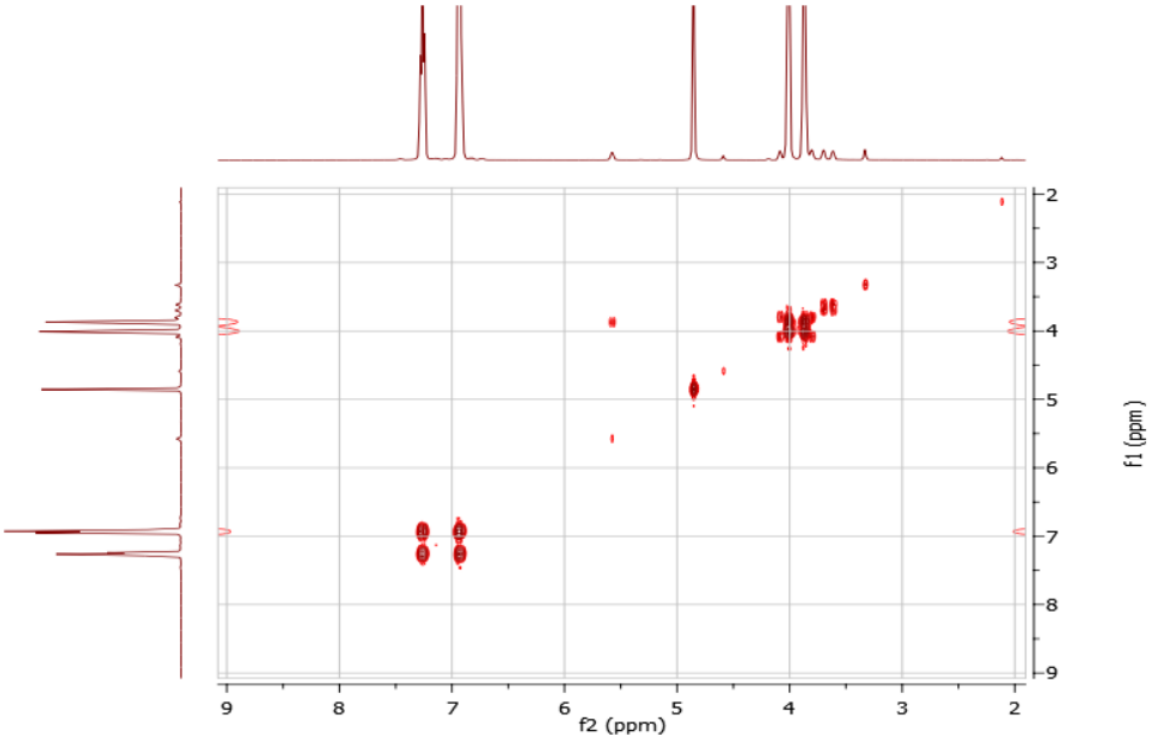


PLATE 6.21G: The GC-MS spectrum of compound **6.21**

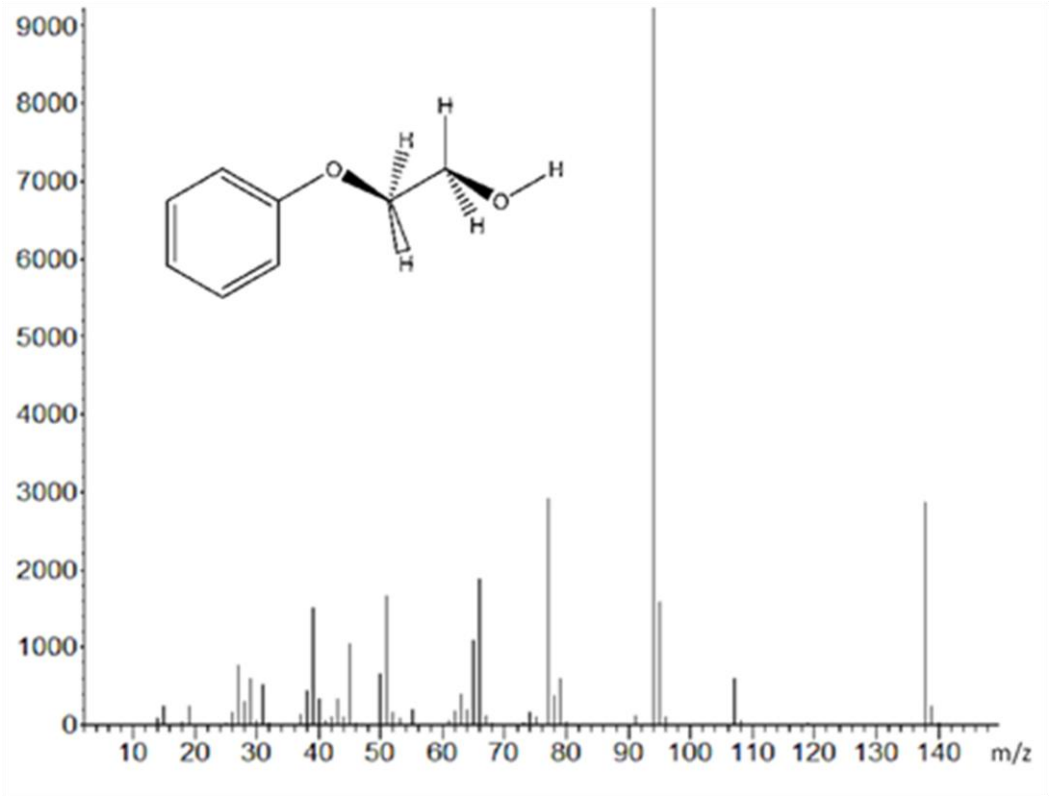
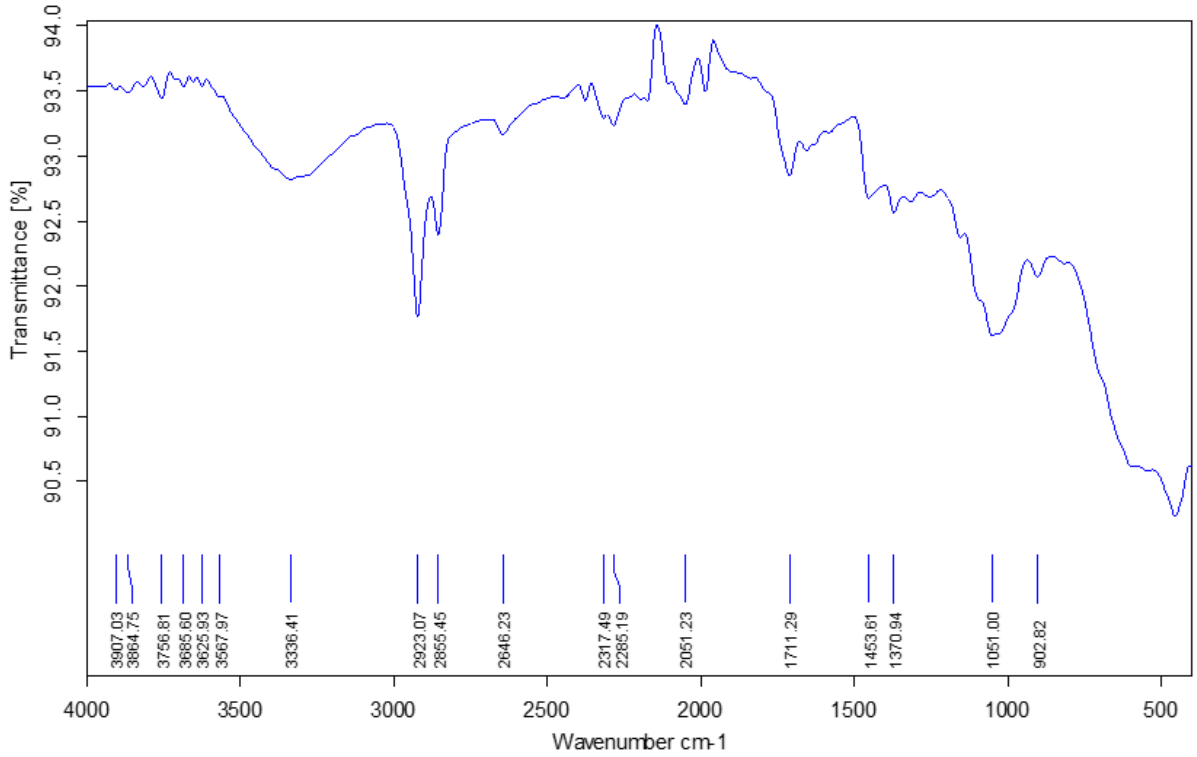


PLATE 6.21H FTIR spectrum of compound **6.21**



8.6.8 Spectrum of 2,4-dihydroxypheylaldehyde (6.22)

PLATE 6.22A: ^{1}H NMR spectrum of compound **6.22** in MeOD

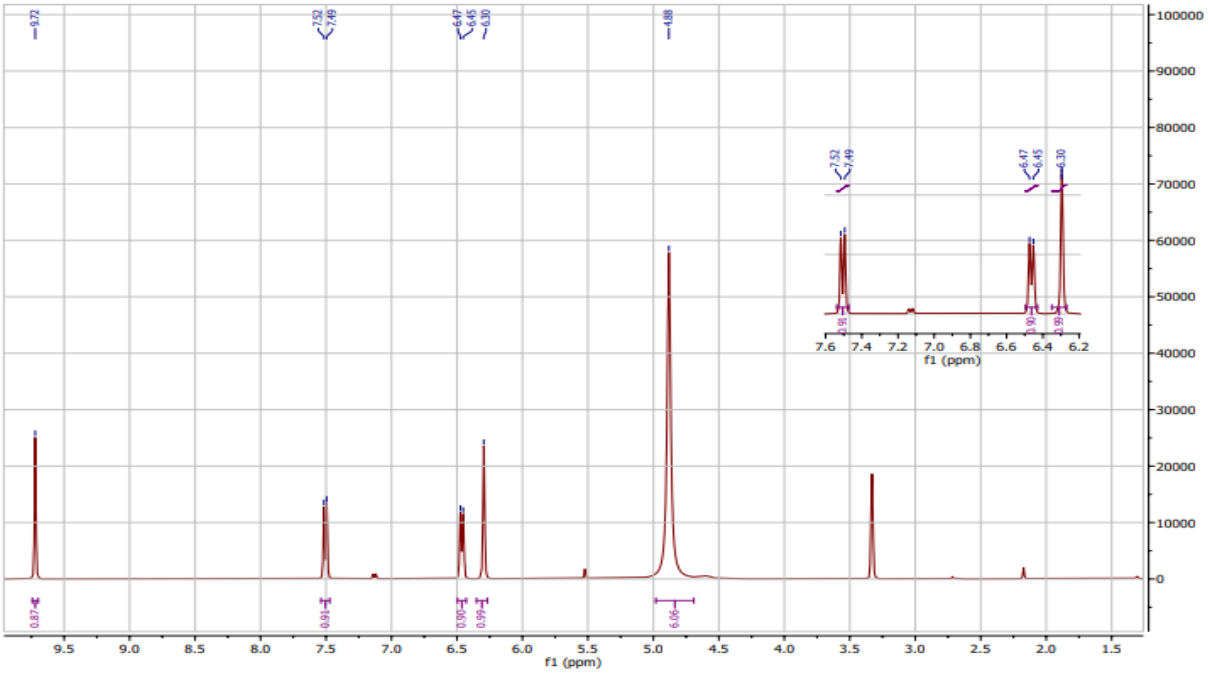


PLATE 6.22B: ^{13}C NMR spectrum of compound **6.22** in MeOD

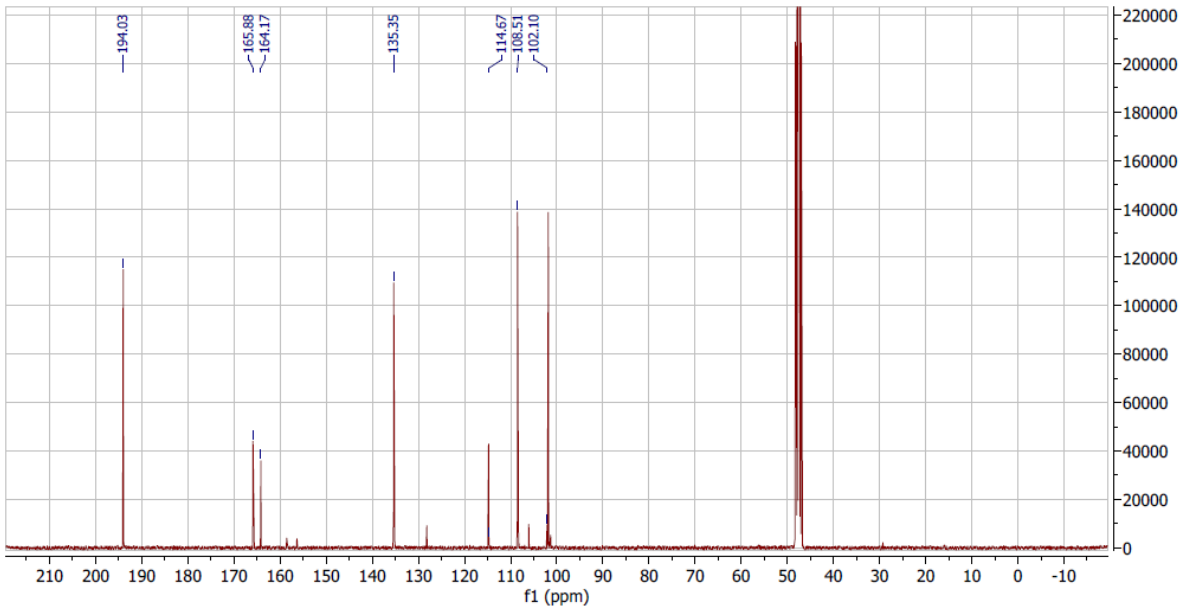


PLATE 6.22C: DEPT 135 NMR spectrum of compound **6.22** MeOD

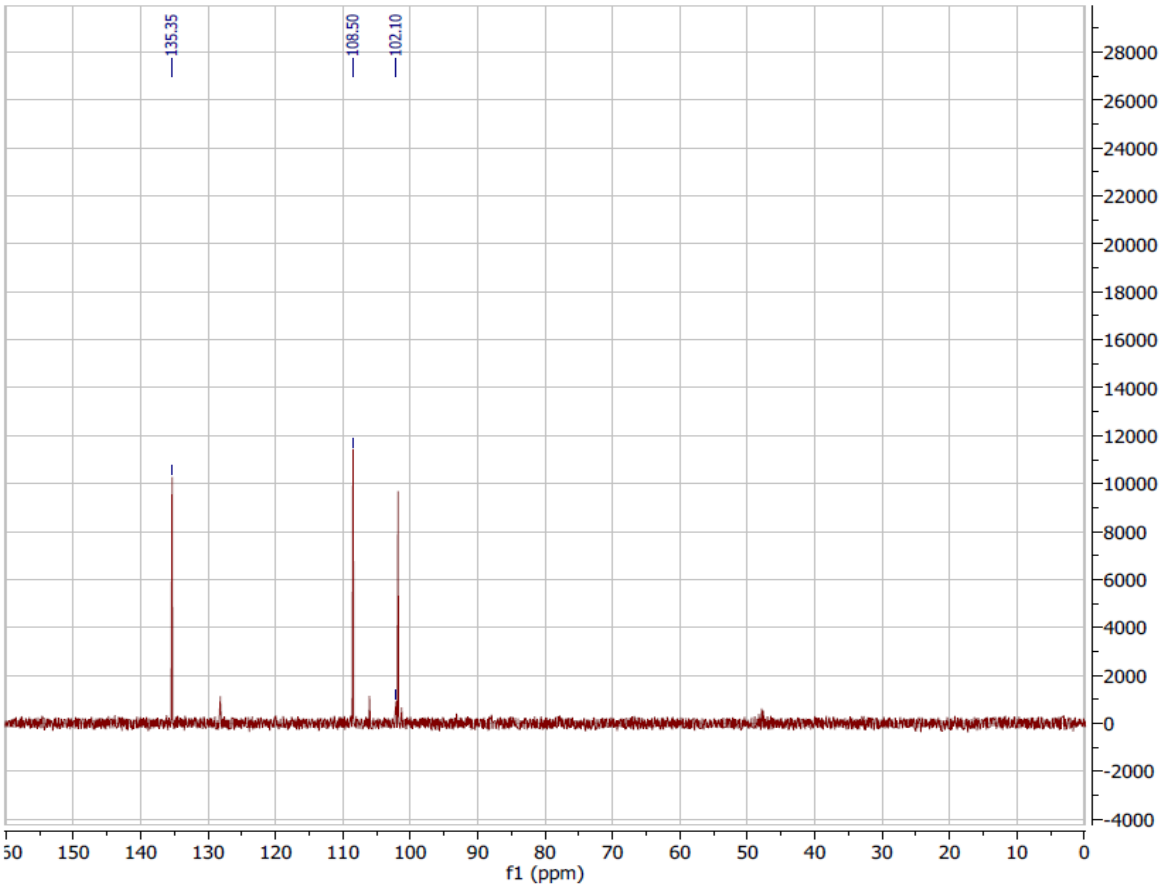


PLATE 6.22D: HSQC NMR spectrum of compound **6.22** MeOD

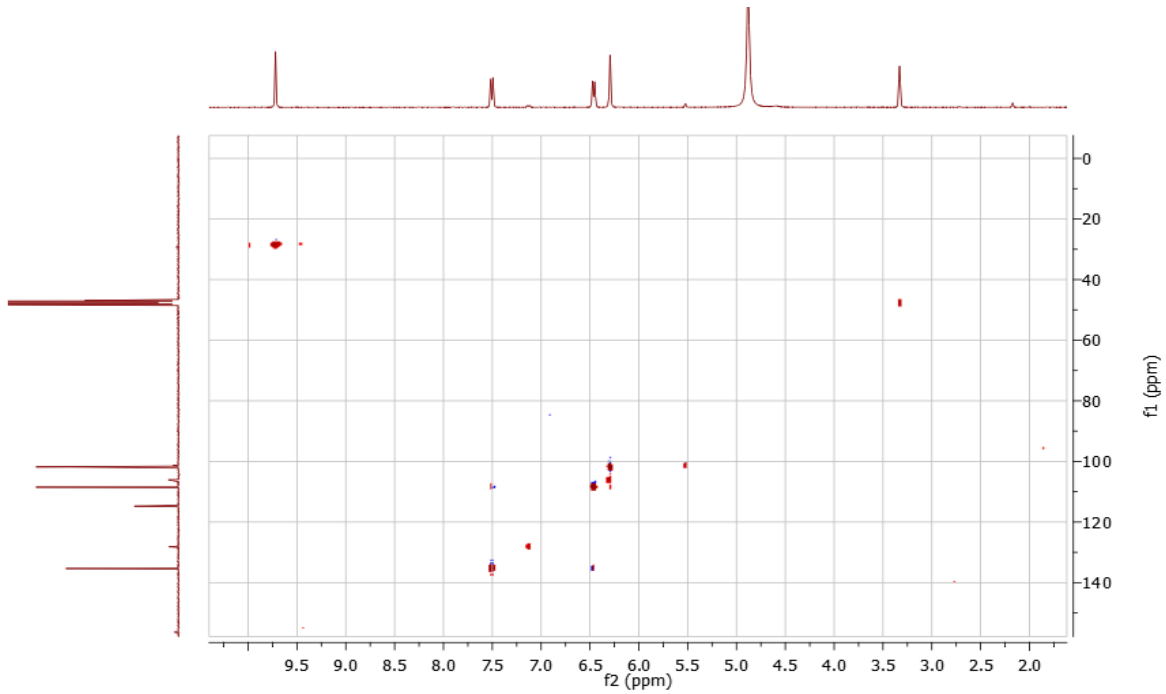


PLATE 6.22E: HMBC NMR spectrum of compound **6.22** in MeOD

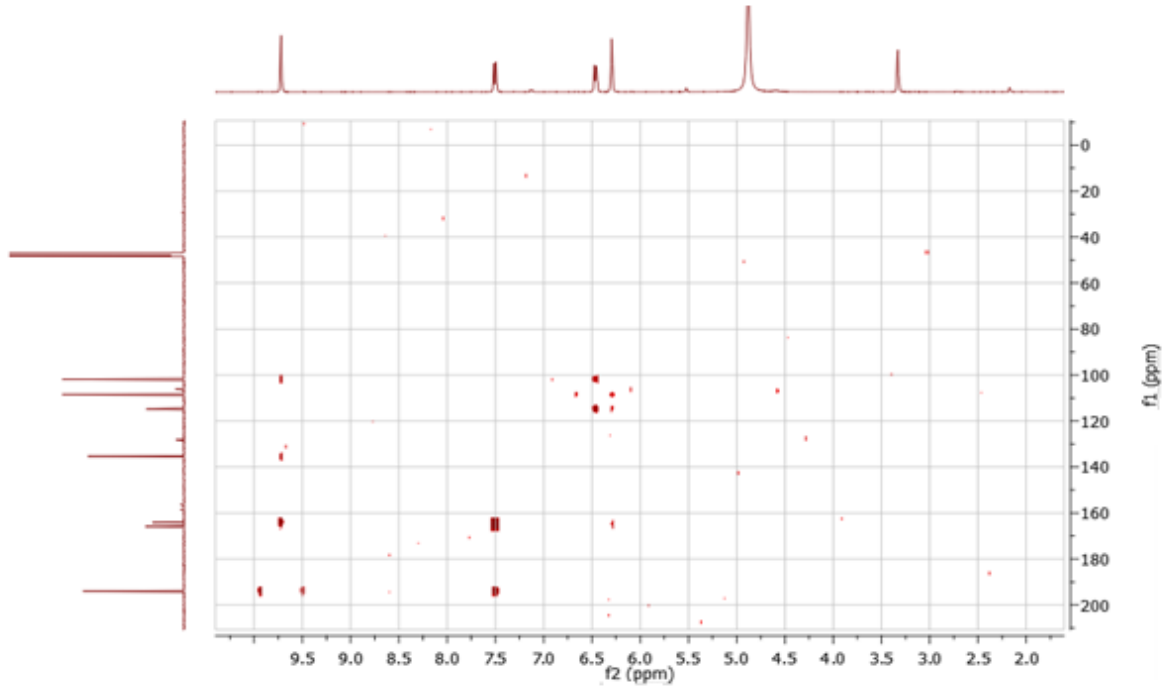


PLATE 6.22F: ^{1}H-^{1}H COSY NMR spectrum of compound **6.22** in MeOD

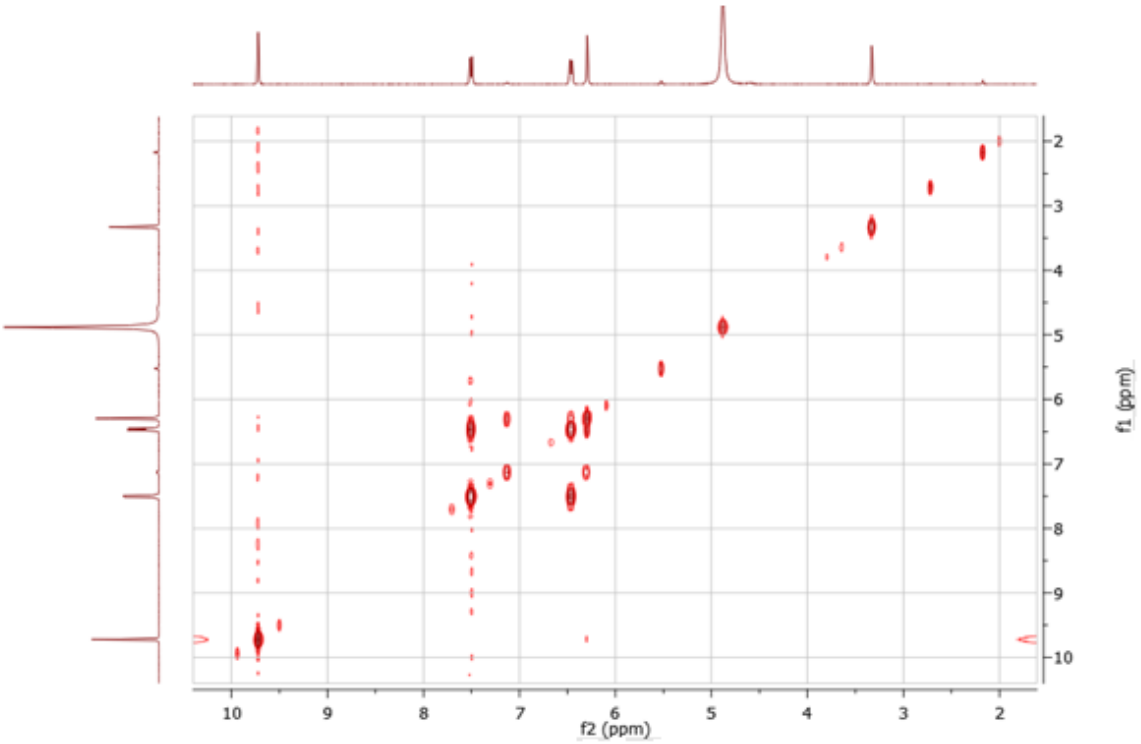


PLATE 6.22G: The GC-MS spectrum of compound **6.22**

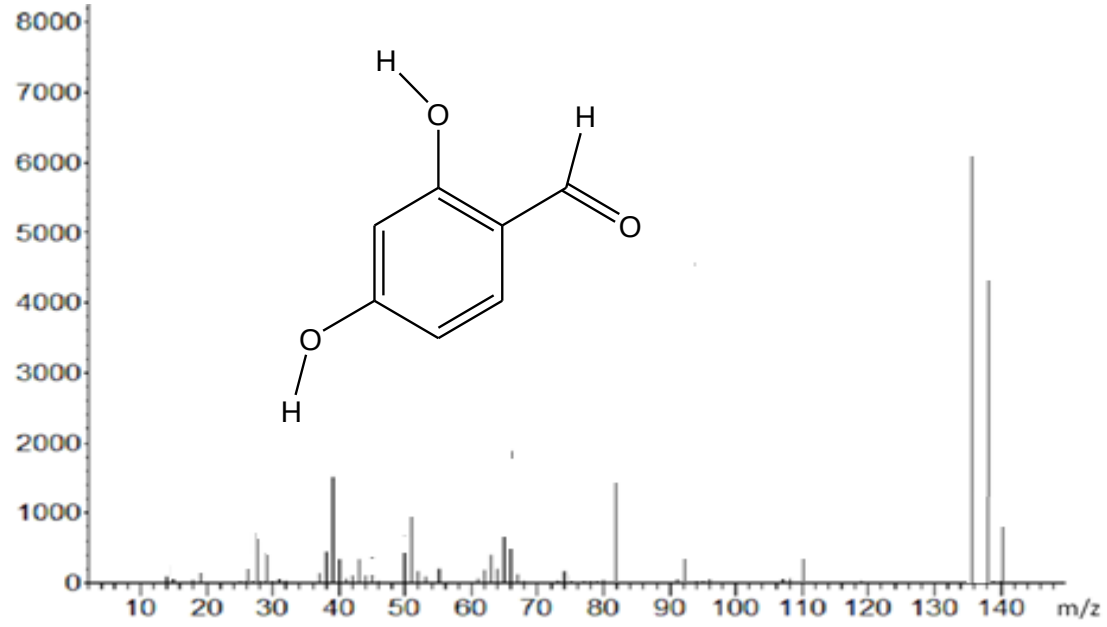
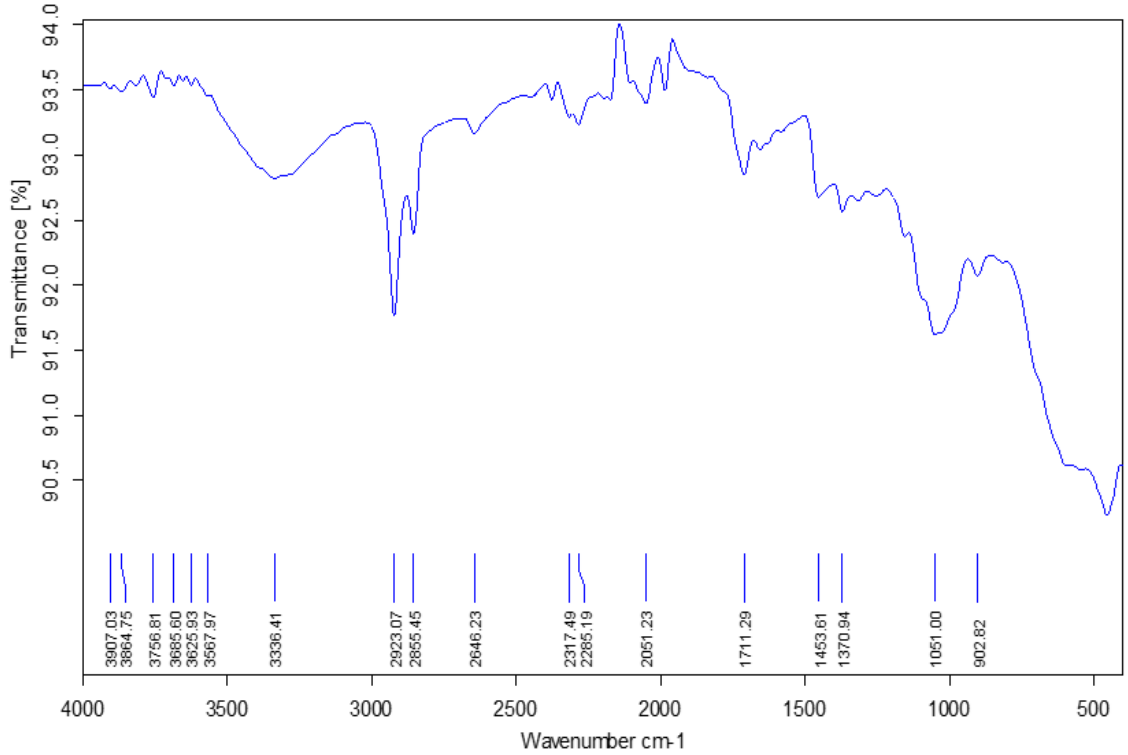


PLATE 6.22H FTIR spectrum of compound **6.22**



8.6.9 NMR of spectra of 3,4-dihydroxy-5-(1,2-dihydroxyethyl)furan-2(5H)-one (6.23)

PLATE 6.23A: ^{1}H NMR of compound **6.23** in MeOD

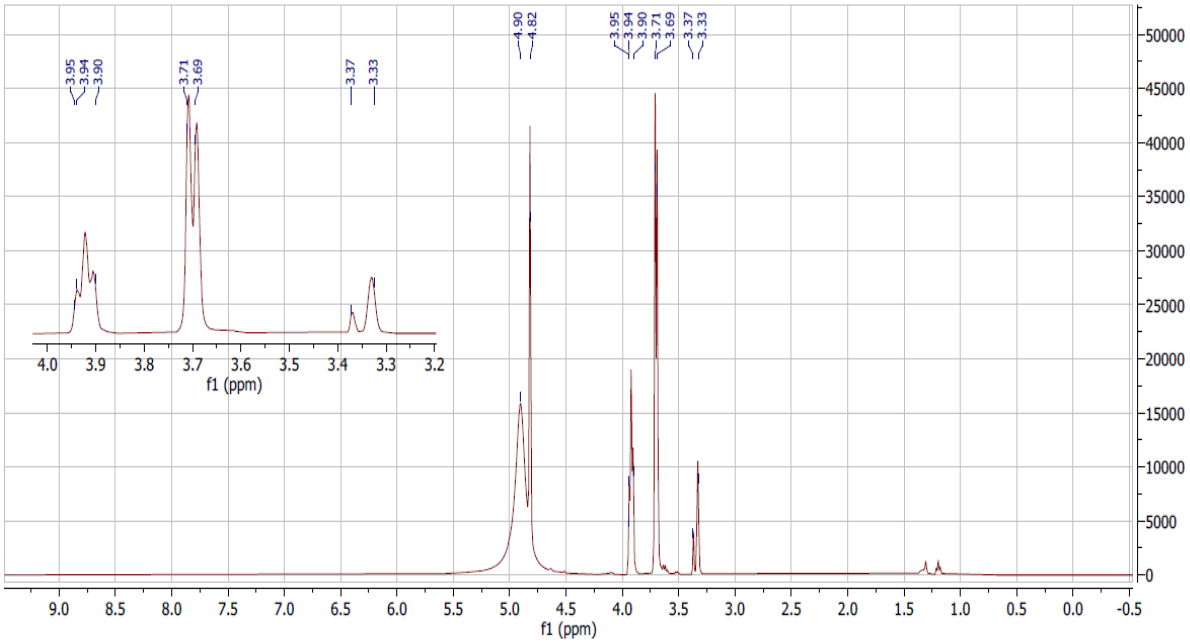


PLATE 6.23B: ^{13}C NMR of compound **6.23** in MeOD

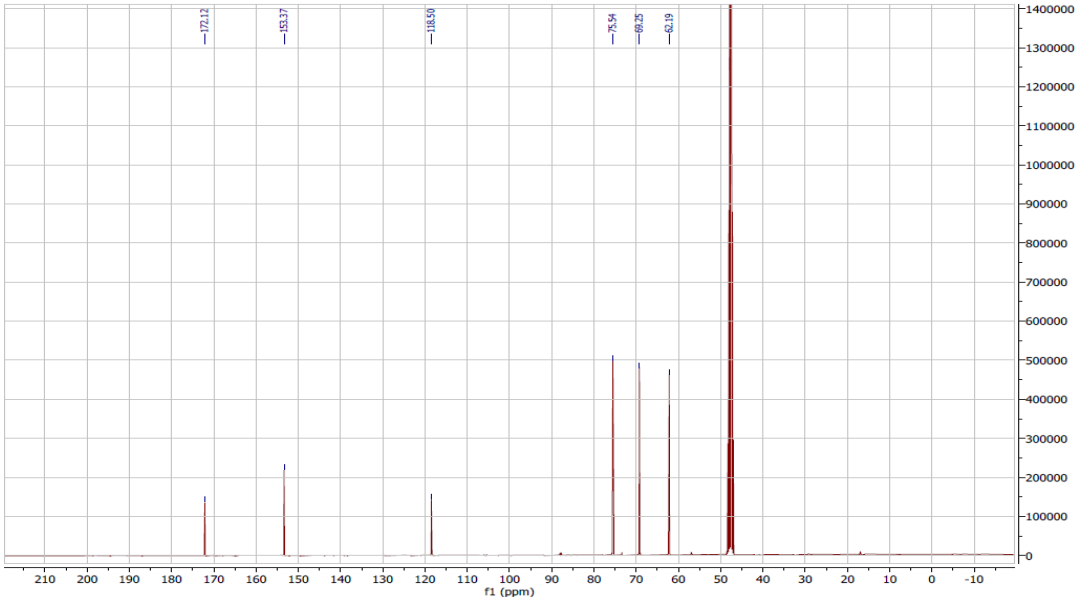


PLATE 6.23C: DEPT 135 NMR of compound **6.23** in MeOD

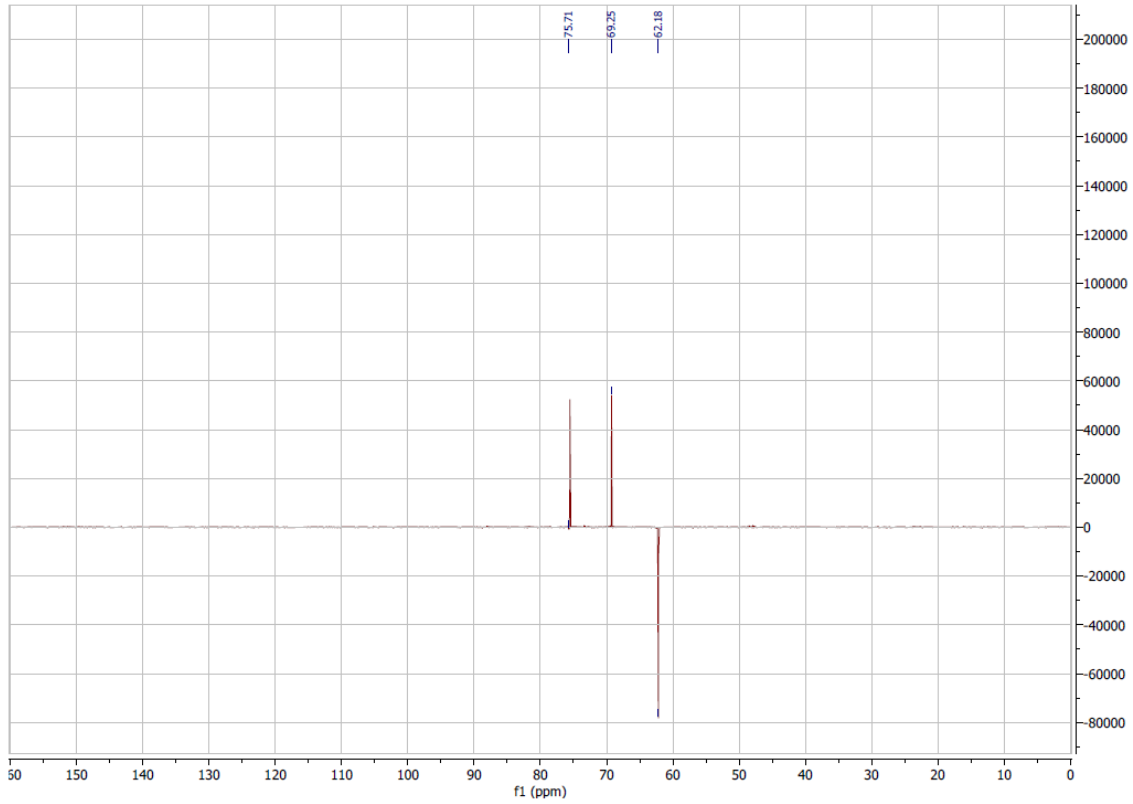


PLATE 6.23D HSQC NMR of compound **6.23** in MeOD

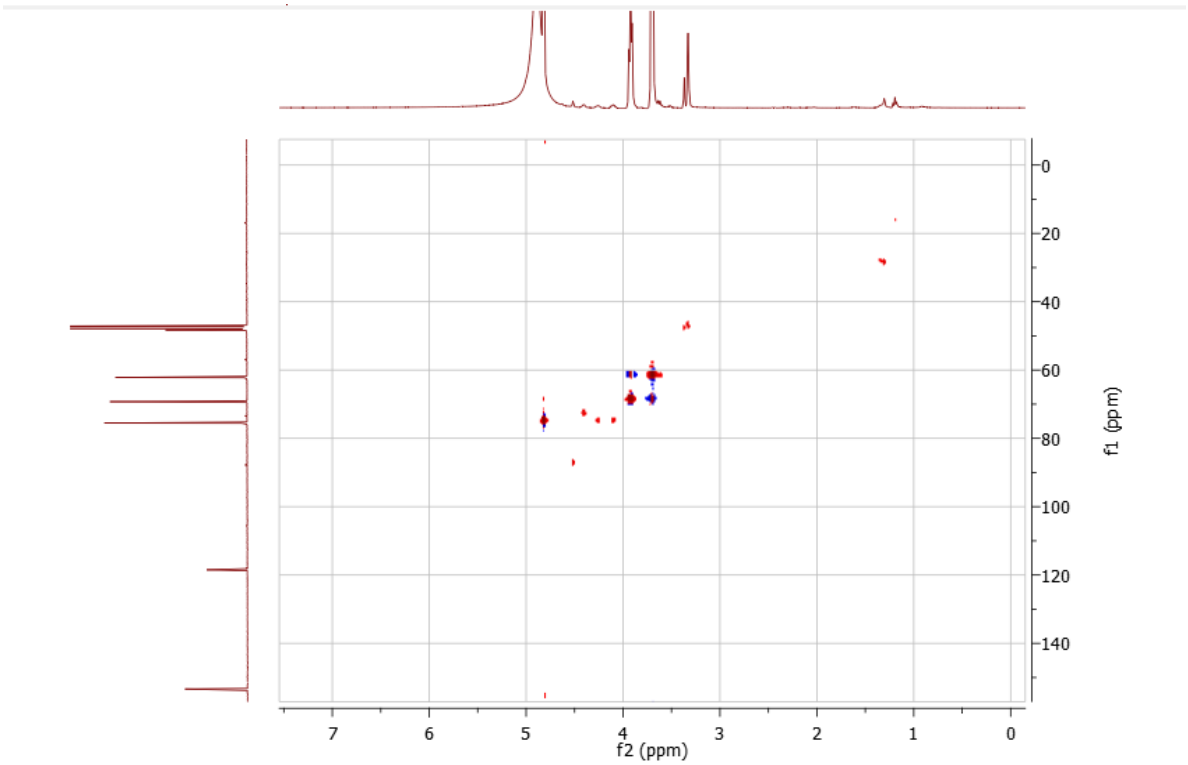


PLATE 6.23E: HMBC NMR of compound **6.23** in MeOD

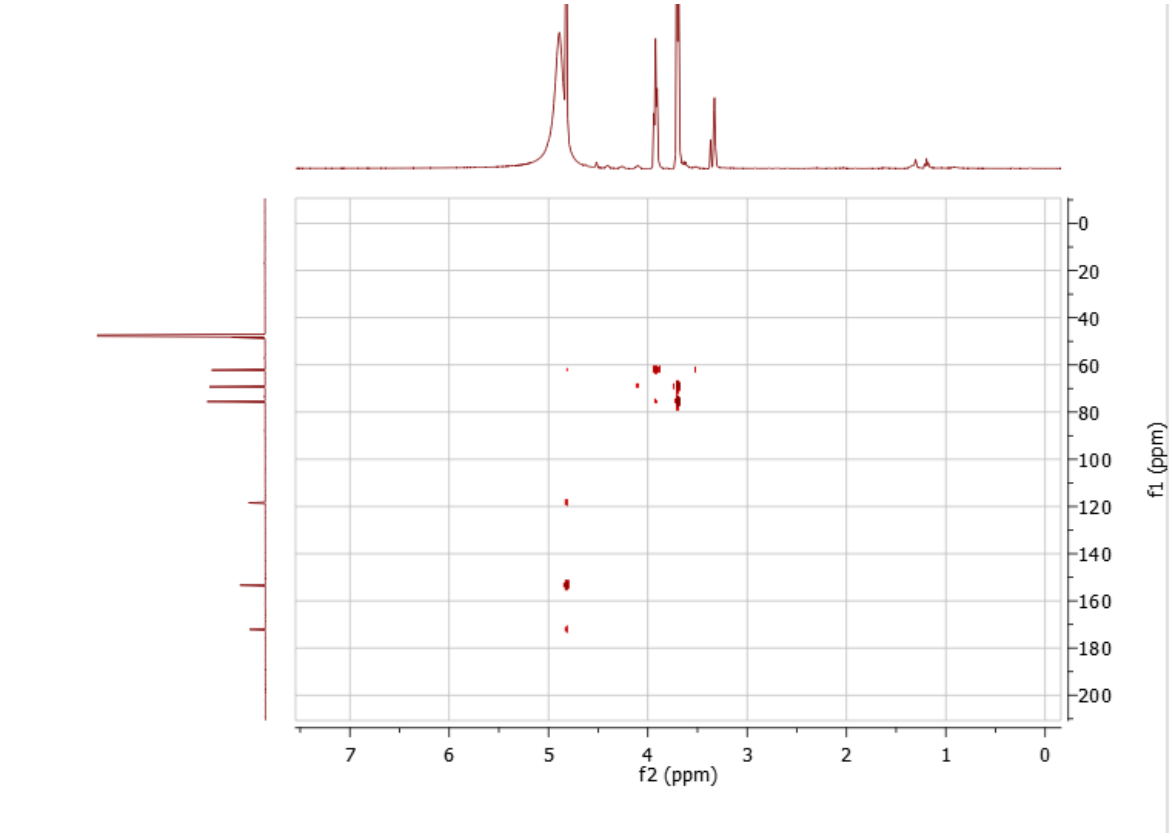


PLATE 6.23F: ^{1}H-^{1}H COSY NMR of compound **6.23** in MeOD

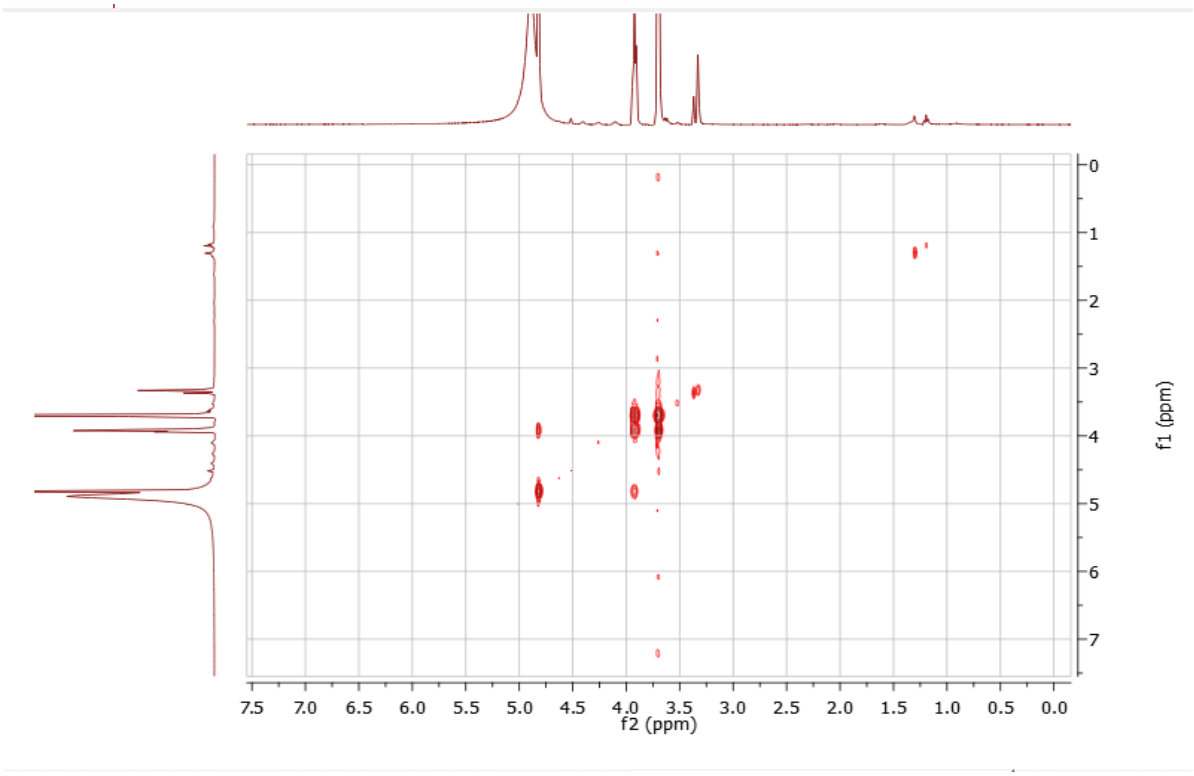


PLATE 6.23G The HREIMS of compound **6.23**

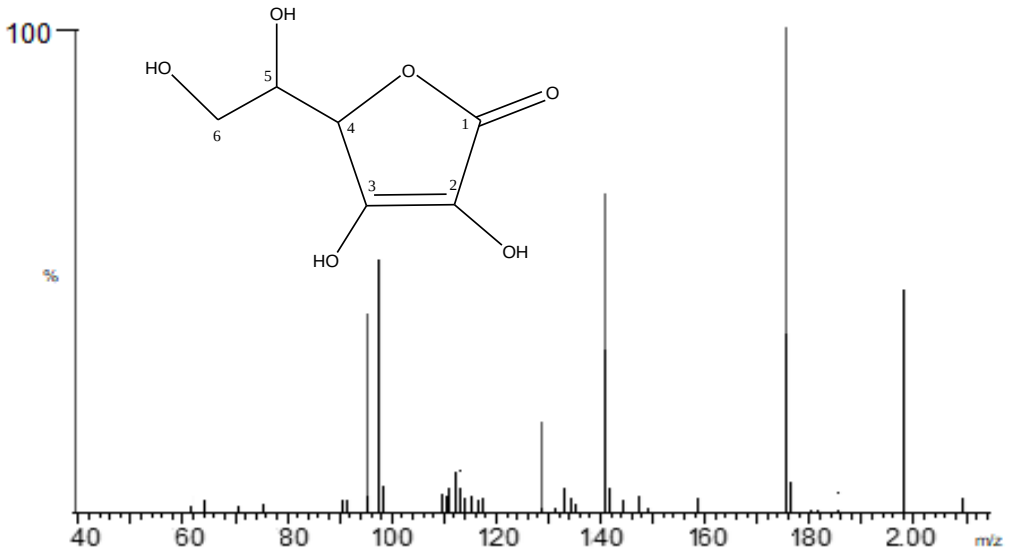
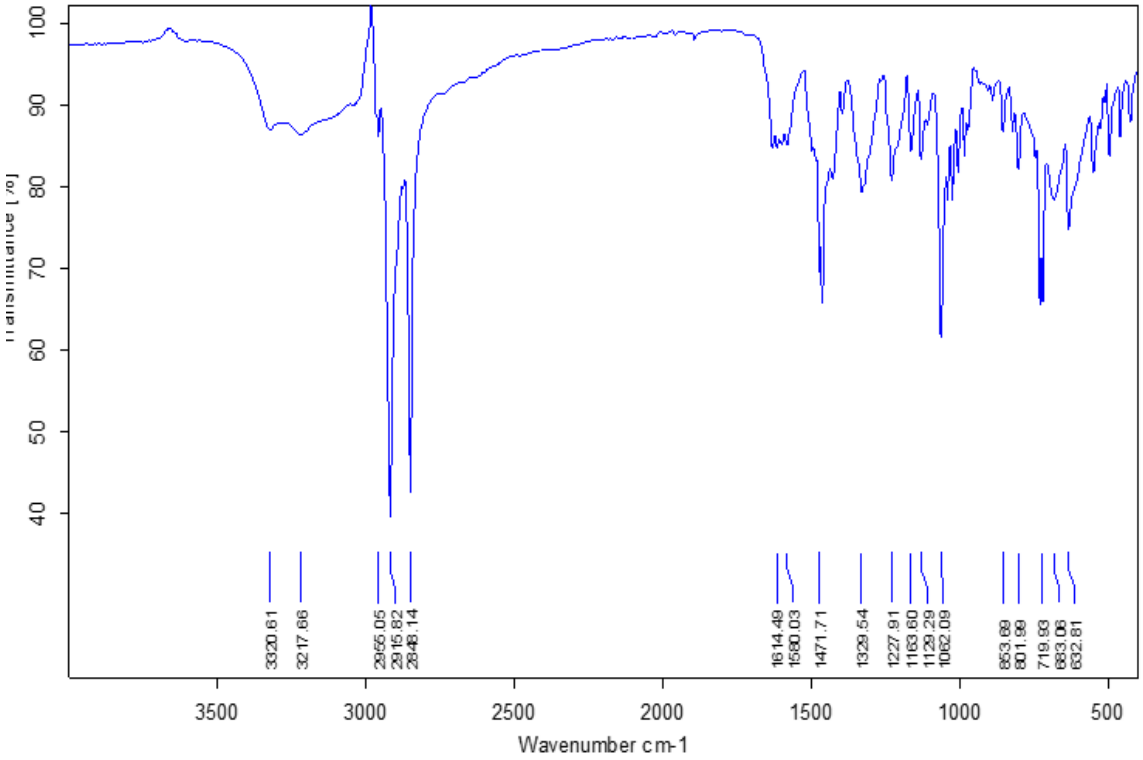
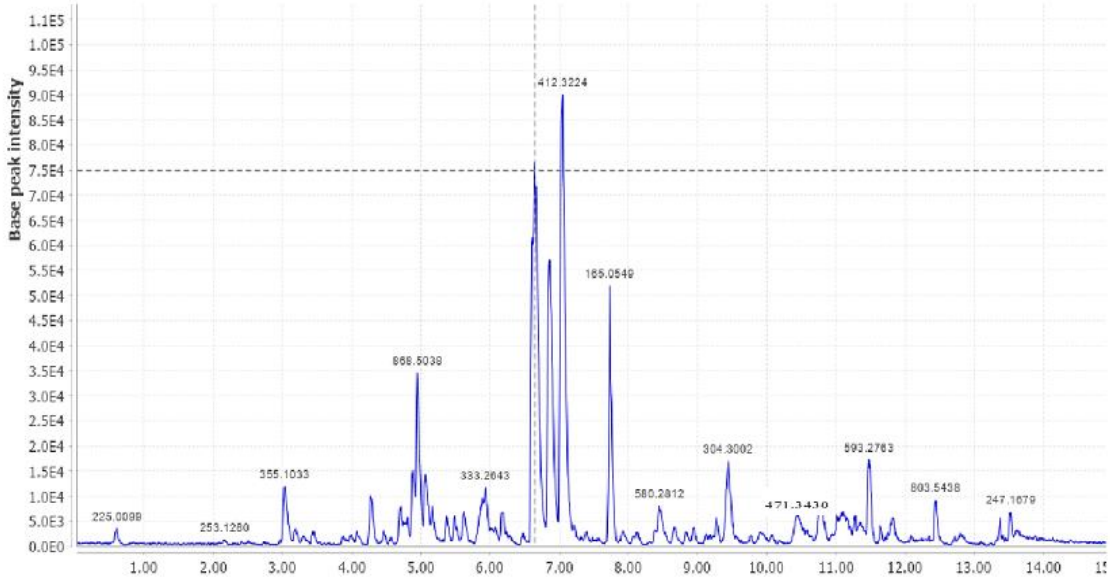


PLATE 6.23H The FTIR spectrum of compound **6.23**

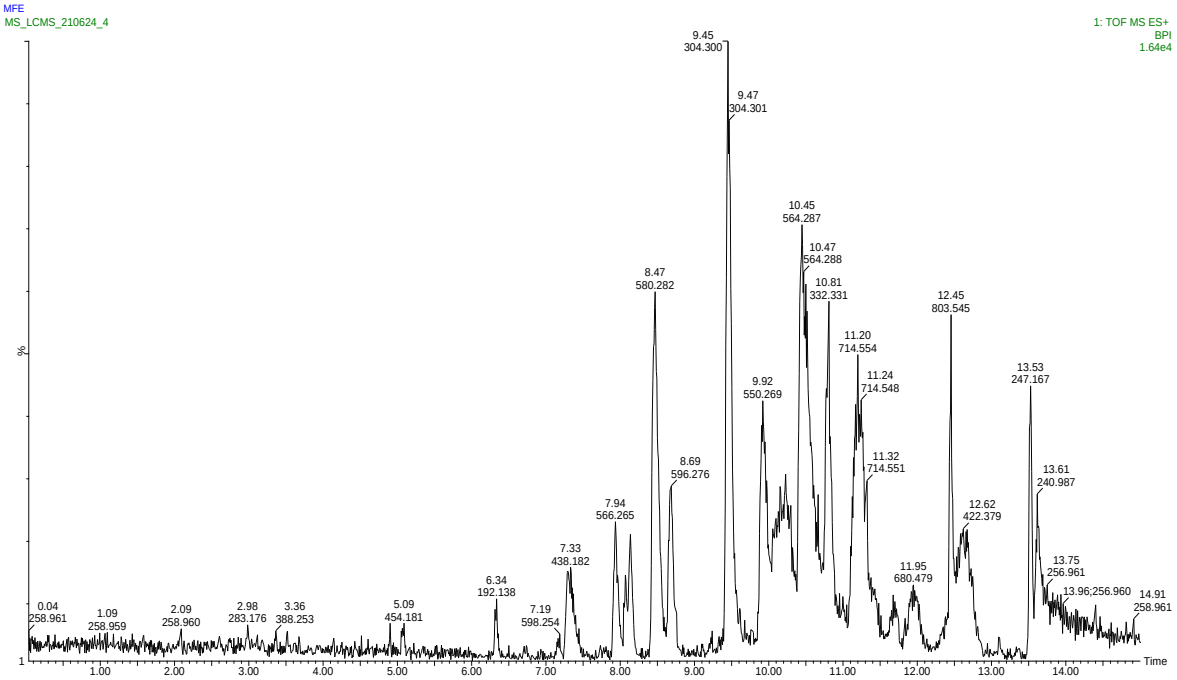


8.6.10LC-MS chromatograms for the crude extracts

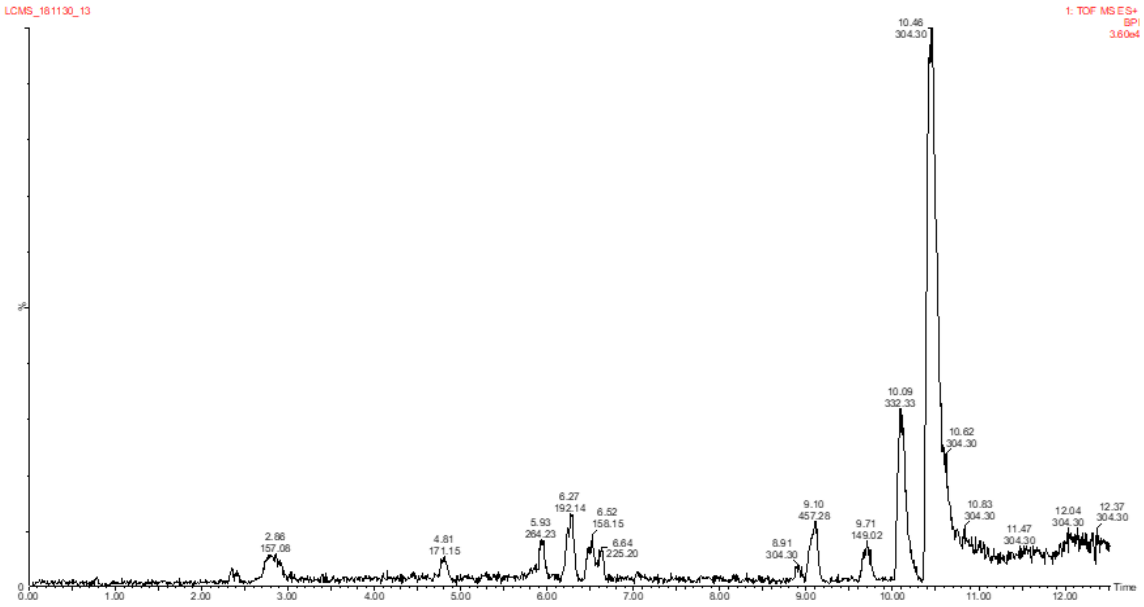
8.6.10.1: The LC-MS for the **Hroot** crude extract



8.6.10.2: The LC-MS for the **SAFE** crude extract. Waters BEH C18, 2.1x100mm, both solvent lines contained 0.1% formic acid



8.6.10.3: The LC-MS for the MFE crude extract. Waters BEH C18, 2.1x100mm, both solvent lines contained 0.1% formic acid



8.7: The UV-Vis spectra of the isolated compounds from the fruit and root of *S. incanum*

PLATE 6.15 I

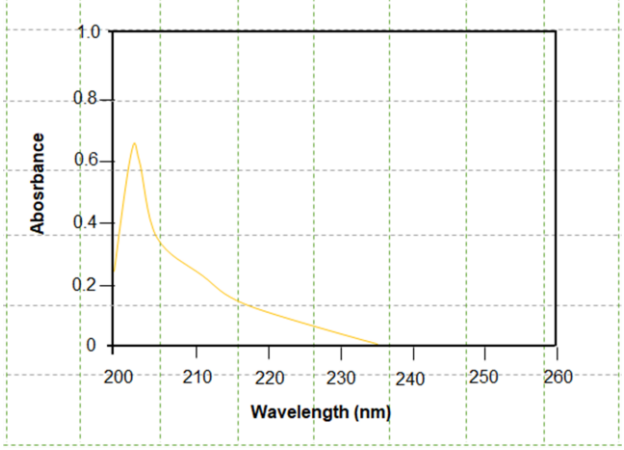


PLATE 6.16 I

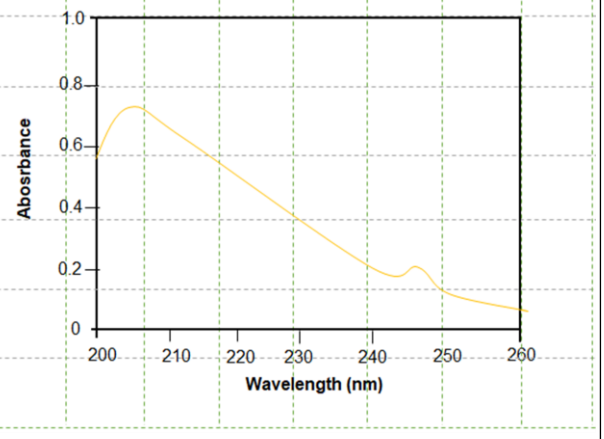


PLATE 6.17 I

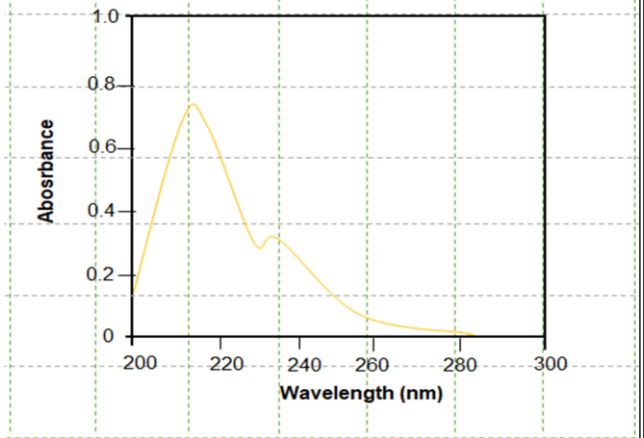


PLATE 6.18 I

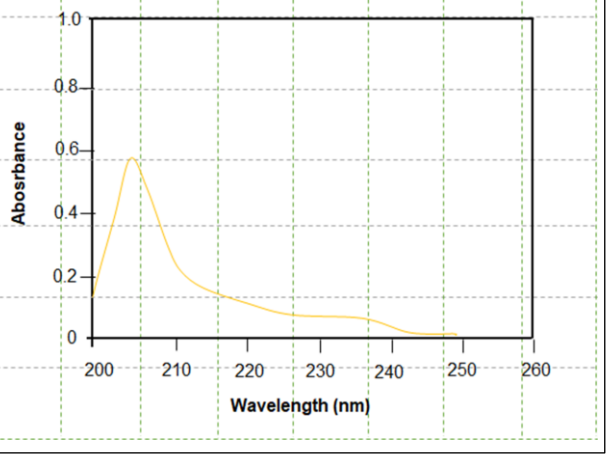


PLATE 6.19 I

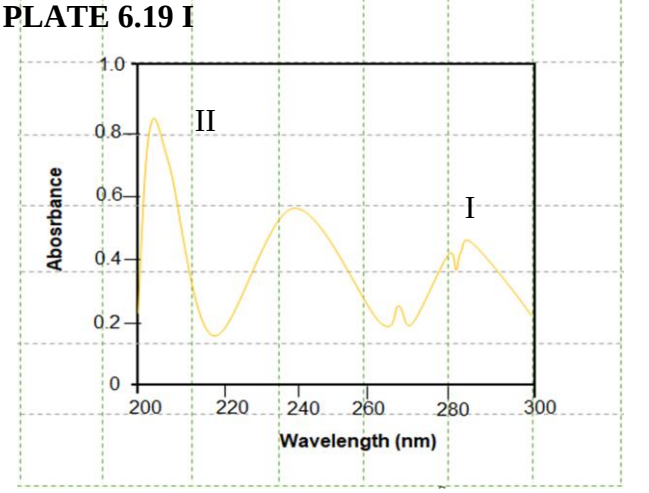


PLATE 6.20 I

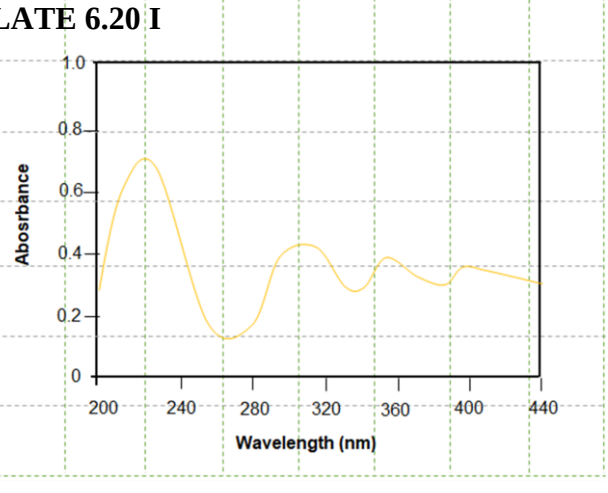


PLATE 6.21 I

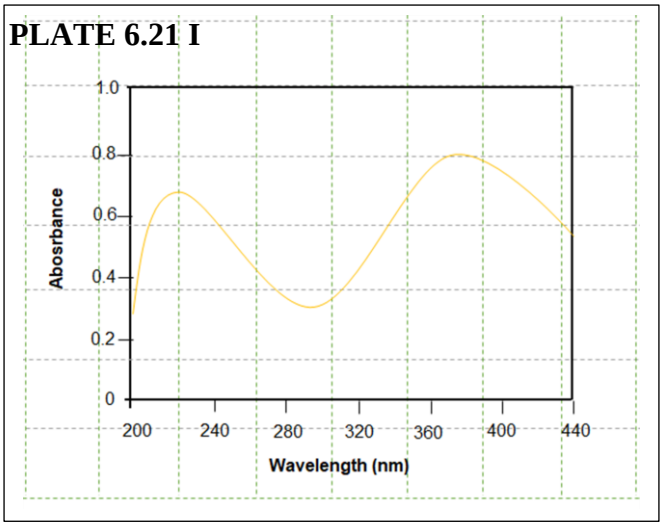


PLATE 6.22 I

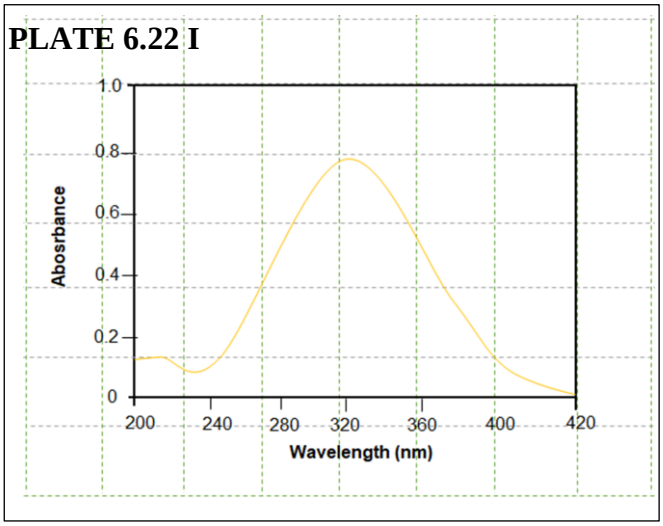
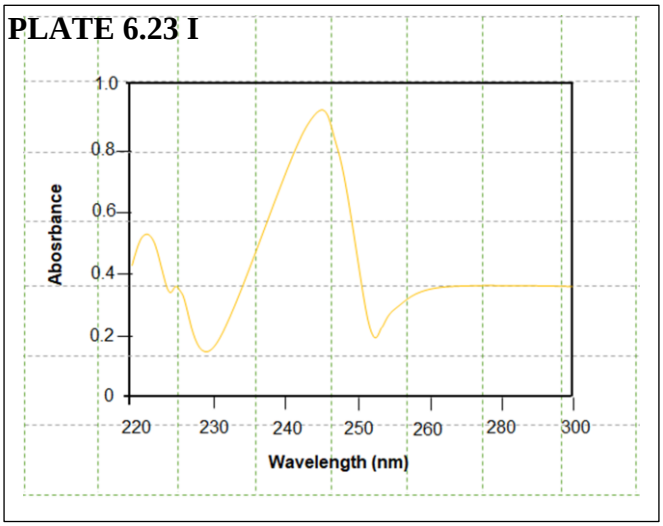
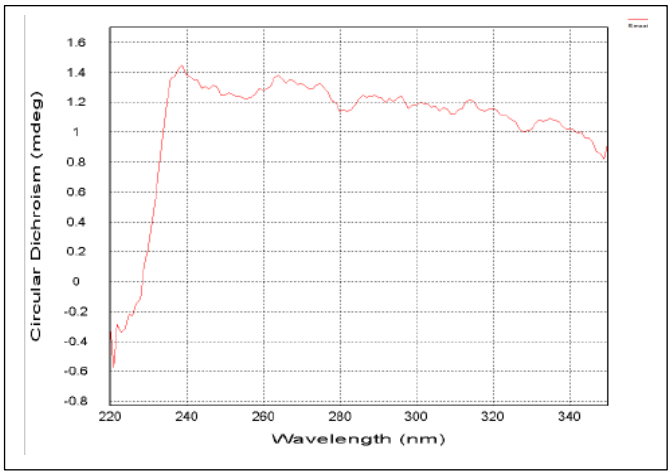


PLATE 6.23 I



8.8: The CD spectrum of:

8.8.1 The novel compound (solanuminone) isolated from the root of S. incanum



8.8.2 3,4-dihydroxy-5-(1,2-dihydroxyethyl)furan-2(5H)-one (6.23) isolated from the fruit of *S. incanum*