ISOLATION AND CHARACTERIZATION OF FOUR BIOACTIVE TRITERPENOIDS FROM *MUCUNA PRURIENS* (DC) (FABACEAE) AND *CANARIUM SCHWEINFURTHII* (ENGL) (BURSERACEAE)

By

BAMIDELE JOSEPH OKOLI

DEPARTMENT OF CHEMISTRY

FACULTY OF SCIENCE

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

NOVEMBER, 2015

ISOLATION AND CHARACTERIZATION OF FOUR BIOACTIVE TRITERPENOIDS FROM *MUCUNA PRURIENS* (DC) (FABACEAE) AND *CANARIUM SCHWEINFURTHII* (ENGL) (BURSERACEAE)

BY

Bamidele Joseph OKOLI, B.Sc (FUNAAB) M.Sc (UNIBANDAN)
PhD/SCI/1934/2011-2012

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN ORGANIC CHEMISTRY

DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

NOVEMBER, 2015

Declaration

I declare that the work in this thesis entitled **ISOLATION AND CHARACTERIZATION OF FOUR BIOACTIVE TRITERPENOIDS FROM *MUCUNA PRURIENS* (DC) (FABACEAE) AND *CANARIUM SCHWEINFURTHII* (ENGL) (BURSERACEAE)** has been performed by me in the Department of Chemistry, Ahmadu Bello University, Zaria, under the Supervision of Prof. G.I. Ndukwe, Prof. R.G. Ayo and Dr. J.D. Habila. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation has been previously presented for another degree or diploma at this or any institution.

…….	…….	…….
Name of Student	Signature	Date

Certification

This thesis entitled ISOLATION AND CHARACTERIZATION OF FOUR BIOACTIVE TRITERPENOIDS FROM *MUCUNA PRURIENS* (DC) (FABACEAE) AND *CANARIUM SCHWEINFURTHII* (ENGL) (BURSERACEAE) by Bamidele Joseph OKOLI, meets the regulations governing the award of the degree of Doctor of Philosophy (Ph.D) of Ahmadu Bello University, Zaria and is approved for its contributions to knowledge and literary presentation.

…… ……..

Prof. G. I. Ndukwe Date
Chairman, Supervisory Committeee

…… ……..

Prof. R. G. Ayo Date
Member, Supervisory Committee

…… ……..

Dr. J. D. Habila Date
Member, Supervisory Committee

…… ……..

Prof. V.O. Ajibola Date
Head of Department

…… ……..

Prof. K. Bala Date
Dean, School of Postgraduate Studies

Acknowledgment

I wish to appreciate the Almighty God, Jesus Christ, the supreme provider for making it possible for me to undertake this work. Professor G. I. Ndukwe, Professor R.G. Ayo and Dr. J. D. Habila for their scholarly contributions to this work. I acknowledge the encouragement of Professor J.Y. Olayemi (Former Dean PG School) ABU, Professor Peter Ogedebe (Dean, Faculty of Science, Baze University), Professor Ladeji Olushola (Dean, Faculty of Science, Bingham University) and Professor L. Fwa (Vice Chancellor, Bingham University). I also want to appreciate Dr. Adegbonro Peter Daso, Department of Environmental Water and Earth Sciences, Faculty of Science,Tshwane University of Technology, Pretoria, South Africa and Dilip of the School of Chemistry University of Kwazulu-Natal Westville campus, Durban, South African, for the use of their nuclear magnetic resonance machine for spectra analyse. To everyone who has made one contribution or the other in this work, I say thank you! To my beautiful wife, Theresa Okoli, for loving me and caring for me, and my two handsome sons; Divine and David for all they endured throughout the period of this research. Thank you my angels!

Abstract

The hexane, chloroform, ethylacetate and methanol extracts of *C. schweinfurthii* leaves showed high degree of ovicidal activity with 80 % inhibition at 10 µg/ml with chloroform crude extract being the most potent with a zone of inhibition of 24-26 mm against Gram-positive bacteria with a MIC of 12.5 mg/ml and MBC and MFC of 12.5 mg/ml. The chloroform and ethylacetate extracts of *C. schweinfurthii* stem bark showed high degree of larvicidal activity with 74 % inhibition at 10 µg/ml with ethylacetate crude extract showing a zone of inhibition of 22-33 mm against Gram-negative bacteria with a MIC of 12.5 mg/ml and, MBC and MFC of 25 mg/ml. The chloroform, ethylacetate and methanol extracts of *M. pruriens* showed potential ovicidal and larvicidal activities with 58 % inhibition at 10 µg/ml with chloroform crude extract having a zone of inhibition of 22-27 mm against Gram-negative bacteria with a MIC of 12.5 mg/ml and, MBC and MFC of 25 mg/ml. Four compounds were isolated – 3β-hydroxyllup-20(29)-en-28-oic acid (Betulinic acid) from the ethylacetate extract of *C. schweinfurthii* stem bark, a novel derivative of α-amyrin identified as 3β-hydroxyl olean-12,18-diene from the chloroform extract of *C. schweinfurthii* leaves, while stigmat-5-en-3β-ol (β-sitosterol) and naurol A were obtained from the chloroform extract of *M. pruriens*. 3β-hydroxyllup-20(29)-en-28-oic acid has the highest zone of inhibitions of 24 mm, 26 mm and 24 mm against *Staphylococcus aureus*, *Bacillus subtlis* and *Candida albicans* with MIC of 6.25 µg/ml against all tested organisms and MBC of 12.5 µg/ml and MFC of 25 µg/ml. 3β-hydroxyl olean-12,18-diene showed a comparative potency with zone of inhibitions of 29 mm, 32 mm and 26 mm against *Methicillin Resistance Staphylococcus aureus* , *Bacillus subtlis* and *Candida albicans* with MIC of 6.25 µg/ml and 12.5 µg/ml against the bacteria and *candida*s respectively, and MBC of 25 µg/ml and MFC of 25 µg/ml. The finding proved that 3β-hydroxyllup-20(29)-en-28-oic acid and 3β-hydroxyl olean-12,18-diene are the most potent isolated antimicrobial compounds.

The most ovicidal compounds were 3β-hydroxyl olean-12,18-diene and stigmat-5-en-3β-ol with percentage inhibition of 62-65 % and 55-62 % respectively while the most larvicidal compounds

were 3β-hydroxyllup-20(29)-en-28-oic acid ,3β-hydroxyl olean-12,18-diene and stigmat-5-en-3β-ol at 78-83 %, 76-81% and 64–70 % at a concentration of 10-20 µg/ml respectively compared to albendazole.

Table of Contents

Title Page i

Cover Page iii

Declaration iv

Certification v

Acknowledgment vi

Abstract vii

Table of Contents ix

List of Tables xv

List of Figures xvi

List of Scheme xviii

List of Plates xix

Abbreviations xx

CHAPTER ONE 1

1.0 INTRODUCTION 1

1.1 **Natural Products and the Earliest Known Medicines to Man** 1

1.2 **Medicinal Plants in Folklore** 2

1.3 **Historically Important Natural Products** 3

1.4 **Natural Products from Plants** 4

1.5 **Statement of the Problem** 7

1.6 **Justification of the Study** 7

1.7 **Aim and Objectives of the Research** 8

CHAPTER TWO 9

2.0 LITERATURE REVIEW 9

2.1 **Man and Parasite** 9

2.2 **Pathogenesis and Pathology of Ascariosis** 9

2.3 **Economical Importance of Ascariosis** 11

2.4 **Control of Ascariosis** 12

2.5 **Nematocidal Activity of Plants** 12

2.6 **Chemical Constituents** 13

2.6.1 Alkaloids 14

2.6.2 Flavonoids and polyphenols 16

2.6.3 Glycosides and saponins 19

2.6.4 Condensed tannins and sesquiterpene lactones 20

2.6.5 Triterpenes 25

2.6.5.1 Naturally-ocurring pentacyclic triterpenes 27

2.7 Fabaceae 28

2.7.1 *Mucuna* 29

2.7.2 Taxonomy and botanical descriptions 29

2.7.3 *Mucuna pruriens* 29

2.7.4 Species distribution 31

2.7.5 Ethno botanical description of *Mucuna pruriens* 31

2.7.6 Traditional uses of *Mucuna pruriens* 31

2.8 *In vitro* and *in vivo* Pharmacological Activities of *Mucuna pruriens* 31

2.8.1 Anti-parkinson's activity 31

2.8.2 Antiglycaemic effect 32

2.8.3 Hypoglycemic activity 32

2.8.4 Antioxidant activity 32

2.8.5 Antivenom activity 33

2.8.6 Aphrodisiac activity 33

2.8.7 Antimicrobial activity 33

2.8.8 Anthelmintic 34

2.9 Isolated Compounds from *Mucuna spp* 34

2.10 Burseraceae Kunth Family 40

2.11 The Genus *Canarium* L. 40

2.11.1 Species distribution 41

2.12 Botanical Features of *Canarium* L. 41

2.13 Traditional and Medicinal Uses of *Canarium* L. 42

2.14 *Canarium schweinfurthii* Engl. 42

2.15. *In vitro* and *In vivo* Pharmacological Activities of *Canarium* L 43

2.15.1 Antioxidant 43

2.15.2 Antibacterial and antifungal activities 44

2.15.3 Hepatoprotective activities 45

2.15.4 Anti-diabetic activity 45

2.15.5 Antitumor potential 45

2.15.6 Other biological activities 46

2.16 Isolated Compounds from Canarium spp 46

2.17. Microbial Agents 48

2.17.1 Infectious disease 48

2.17.2 Chemotherapeutic agents: Factors affecting their effectiveness 48

2.17.3 Antimicrobial drug resistance 49

CHAPTER THREE 50

3.0 MATERIALS AND METHODS 50

3.1 Chemicals/Reagents 50

3.1.1 Equipment/Materials 50

3.1.2 Parasite 50

3.1.3 Microorganisms 51

3.2 Plant Materials 51

3.3 Reference Drug 51

3.4 Extraction of Crude Plant Active Ingredients 55

3.5 Preliminary Phytochemical Screening 55

3.5.1 Detection of alkaloids 55

3.5.2 Detection of glycosides 55

3.5.3 Detection of saponins 55

3.5.4 Detection of phytosterols 55

3.5.5 Detection of flavonoids 55

3.5.6 Detection of phenols 55

3.5.7 Detection of tannins 55

3.6 Antimicrobial Evaluation 55

3.6.1 Preparation of inoculum 55

3.6.2 Antibacterial/fungal susceptibility test 57

3.6.3 Determination of minimum inhibitory concentration 57

3.6.4 Determination of minimum bactericidal/fungicidal concentration 58

3.7 Anthelmintic Activity of Crude Plant Extracts 58

3.7.1 Collection and identification of worms 58

3.7.2 Culture of *Ascaris suum* eggs 58

3.7.3 Egg hatch assay 59

3.7.4 Larval development assay 59

3.8 Column Chromatography 63

3.9 Isolation of Bioactive Compounds 63

3.10 Preparative Thin-Layer Chromatography 63

3.11 Statistical Analysis 68

CHAPTER FOUR 69

4.0 RESULTS 69

4.1 **Extraction** 69

4.2 **Column Chromatographic Separation** 69

4.3 **Phytochemical Screening of Extracts** 69

4.4 **Antimicrobial Screening** 69

4.5 **Minimum Inhibitory Concentration** 69

4.6 **Minimum Bactericial /Fungicidal Concentration** 79

4.7 ***In-vitro* Anthelmintic Activity** 79

4.7.1 Egg hatch assay 79

4.7.2 Larval hatch assay 79

4.8 **UV-Visible Spectrophotometer** 79

4.9 **Isolation of Bioactive Compounds and NMR Spectroscopy** 79

4.10 **Spectral Results** 92

CHAPTER FIVE 119

5.0 DISCUSSION 119

5.1 **Extraction** 119

5.2 **Phytochemical Screening of Crude Extracts** 119

5.3 **Antimicrobial Susceptibility Assasy of the Crude Extracts** 120

5.4 **Zone of Inhibition of Crude Extracts** 122

5.5 **Minimum Inhibitory Concentration of the Crude Extracts** 122

5.6 **Minimum Bactericial/Fungicidal Concentration of the Crude Extracts** 123

5.7 **Zone of Inhibition of the Isolated Compounds** 123

5.8 **Minimum Inhibitory Concentration of the Isolated Compounds** 124

5.9 **Minimum Bactericidal/Fungicidal Concentration of the Isolated Compounds** 125

5.10 **Effect of Extracts on the Eggs and Larval of *Ascaris suum*** 126

5.11 **Ovicidal Activity of the Isolated Compound** 128

5.12 **Larvicidal Activity of the Isolated Compound** 129

5.13 **Spectral Analysis** 130

5.13.1 Ultraviolet (UV) of CB 47/70 130

5.13.2 Characterization of compound CB 47/70 130

5.13.3 Ultraviolet (UV) of OB 45 135

5.13.4 Characterization of compound OB 45 135

5.13.1 Ultraviolet (UV) of OB 315 139

5.13.2 Characterization of compound OB 315 139

5.13.1 Ultraviolet (UV) of OB 312 143

5.13.2 Characterization of compound OB 312 143

CHAPTER SIX 146

6.0 CONCLUSION AND RECOMMENDATIONS 146

REFERENCES 148

List of Tables

Tables		Page
4.1:	Percentage Recovery Yield of Plant Extracts	70
4.2:	Extractive Values of Eluted Methanol Extracts	71
4.3:	Phytochemical Screening of Extracts	72
4.4:	Zone of Inhibition (mm) of the Stem Bark Extracts *C.schweinfurthii* against the Test Microorganisms	73
4.5:	Zone of Inhibition (mm) of the Leave Extracts *C. schweinfurthii* against the Test Microorganisms	74
4.6:	*Zone* of Inhibition *(mm)* of the *Mucuna pruriens* Extracts against the Test Microorganisms	75
4.7:	Zone of Inhibition of the Isolated Compounds against the Test Microorganism (mm)	76
4.8:	Minimum Inhibitory Concentration (mg/ml) of Extracts against the Test Microorganisms	77
4.9:	Minimum Inhibitory Concentration (µg/ml) of Isolated Compounds against the Test Microorganisms	78
4.10:	Minimum Bactericial (MBC)/Fungicidal Concentration (MFC) of the Extracts against the Test Microorganisms (mg/ml)	81
4.11:	Minimum Bactericial (MBC)/Fungicidal Concentration (MFC) of the Isolated Compounds against the Test Microorganisms (mg/ml)	82
5.1:	^{13}Carbon NMR Data of Compound CB 47/70	133
5.2:	^{13}Carbon NMR Data of Compound OB 45	137
5.3:	^{13}Carbon NMR Data of Compound OB 315	141
5.4:	^{13}Carbon NMR Data of Compound OB 312	144

List of Figures

Figures		Page
4.1:	% Inhibition of Eggs of *A.Suum* by *C. schweinfurthii* Leave Extracts	83
4.2:	% Inhibition of Eggs of *A.Suum* by *C. schweinfurthii* Stem Bark Extracts	84
4.3:	% Inhibition of Eggs of *A.Suum* by *M. pruriens* Extracts	85
4.4:	% Inhibition of Eggs of *A.Suum* by the Isolated Compounds	86
4.5:	% Inhibition of Larva Stage of *A.Suum* by *C. schweinfurthii* Leave Extracts	87
4.6:	% Inhibition of Larva Stage of *A.Suum* by *C. schweinfurthii* Stem Bark Extracts	88
4.7:	% Inhibition of Larva Stage of *A.Suum* by *Mucuna pruriens* Extracts	89
4.8:	% Inhibition of Larva of *A.Suum* by the Isolated Compounds	90
4.9:	UV-Visible Spectrophotometer of the Isolated Compounds	91
4.10:	Integrated Proton NMR of CB 47/70	93
4.11:	^{13}Carbon NMR Spectrum of CB 47/70	94
4.12:	Distortionless Enhancement Polarization Transfer (DEPT)	95
4.13:	Heteronuclear Single Quantum Correlation (HSQC) Spectrum of CB 47/70	96
4.14:	Heteronuclear Multiple Bond Correlation (HMBC) Spectrum of CB 47/70	97
4.15:	Correlation Spectroscopy (COSY) of CB 47/70	98
4.16:	Nuclear Overhauser Effect Spectroscopy (NOESY) of CB 47/70	99
4.17:	Integrated Proton NMR of OB 45	100
4.18:	^{13}Carbon NMR Spectrum of OB 45	101
4.19:	Distortionless Enhancement Polarization Transfer (DEPT) Spectrum of OB 45	102
4.20:	Heteronuclear Single Quantum Correlation (HSQC), Spectrum of OB 45	103
4.21:	Correlation Spectroscopy (COSY) of OB 45	104
4.22:	Heteronuclear Multiple Bond Correlation (HMBC) Spectrum of OB 45	105
4.23:	Nuclear Overhauser Effect Spectroscopy (NOESY) of OB 45	106
4.24:	Integrated Proton NMR of OB 315	107

4.25: ^{13}Carbon NMR Spectrum of OB 315 108

4.26: Heteronuclear Single Quantum Correlation (HSQC) Spectrum of OB 315 109

4.27: Correlation Spectroscopy (COSY) of OB 315 110

4.28: Heteronuclear Multiple Bond Correlation (HMBC) Spectrum of OB 315 111

4.29: Nuclear Overhauser Effect Spectroscopy (NOESY) of OB 315 112

4.30: Integrated Proton NMR of OB 312 113

4.31: ^{13}Carbon NMR Spectrum of OB 312 114

4.32: Correlation Spectroscopy (COSY) of OB 312 115

4.33: Nuclear Overhauser Effect Spectroscopy (NOESY) of OB 312 116

4.34: Heteronuclear Single Quantum Correlation (HSQC) Spectrum of OB 312 117

4.35: Heteronuclear Multiple Bond Correlation (HMBC) Spectrum of OB 312 118

List of Schemes

Schemes		Page
4.1:	Flow Chart for the Isolation of Compound CB 47/70	64
4.2:	Flow Chart for the Isolation of Compound OB 45	65
4.3:	Flow Chart for the Isolation of Compound OB 312	66
4.4:	Flow Chart for the Isolation of Compound OB 315	67

List of Plates

Plates		Pages
I:	*Mucuna pruriens* (L.) DC. var. *utilis* (Wall. ex Wight) Baker ex Burck	52
II:	*Canarium schweinfurthii* Engl Leave	53
III:	*Canarium schweinfurthii* Engl Stem Bark	54
IV:	Egg Stage of *Ascaris suum*	61
V:	Second Larvae Stage (L_2) of *Ascaris suum*	62

ABBREVIATIONS

^{1}H NMR- Proton nuclear magnetic resonance

^{13}C NMR- Carbon nuclear magnetic resonance

µg- Microgram

^{o}C- Celcius

CC- Column chromatrography

COSY- Correlation spectroscopy

d- Doublet

dd- Doublet of doublet

DMSO- Dimethyl sulfoxide

EHA- Egg hatch assay

g- Gram

HMBC- Heteronuclear multiple-bond correlation spectroscopy

kg- Kilogram

L- Litre

L_1- First stage larval

L_2- Second stage larval

L_3- Third stage larval

LDA- Larval development assay

M- Molar

m- Multiplet

MBC- Minimum bactericidal concentration

mg- Miligram

MHB- Mueller Hilton broth

MHA- Mueller Hilton agar

ml- Mililitre

MIC- Minimum inhibitory concentration

min- minutes

PTLC- Preparative thin layer chromatography

s- Singlet

t- Triplet

TLC- Thin layer chromatography

UV- Ultra-violet spectroscopy

CHAPTER ONE

1.0 INTRODUCTION

1.1 Natural Products and the Earliest Known Medicines to Man

Natural products have been the most successful sources of potential drug leads (Butler, 2004; Cragg and Newman, 2005; Haefner, 2003; Mishra and Tiwari, 2011; Rey-Ladino *et al*., 2011). However, their recent implementation in drug discovery and development efforts have somewhat demonstrated a decline in interest (Mishra and Tiwari, 2011). Nevertheless, natural products continue to provide unique structural diversity in comparison to standard combinatorial chemistry, which presents opportunities for discovering mainly novel low molecular weight lead compounds. Since less than 10% of the world's biodiversity has been evaluated for potential biological activity, many more useful natural lead compounds await discovery with the challenge being how to access this natural chemical diversity (Cragg and Newman, 2005). The earliest records of natural products were depicted on clay tablets in cuneiform from Mesopotamia (2600 B.C.) which documented oils from *Cupressus sempervirens* (cypress) and *Commiphora* species (myrrh) which are still used today to treat coughs, colds and inflammation (Cragg and Newman, 2005). The Ebers Papyrus (2900 B.C.) is an Egyptian pharmaceutical record, which documents over 700 plant-based drugs ranging from gargles, pills, infusions, to ointments. The Chinese Materia Medica (1100 B.C.) (Wu Shi Er Bing Fang, contains 52 prescriptions), Shennong Herbal (~100 B.C., 365 drugs) and the Tang Herbal (659 A.D., 850 drugs) are documented records of the uses of natural products (Cragg and Newman, 2005). The Greek physician, Dioscorides, (100 A.D.), recorded the collection, storage and the uses of medicinal herbs, whilst the Greek philosopher and natural scientist, Theophrastus (~300 B.C.) dealt with medicinal herbs. During the Dark and Middle Ages the monasteries in England, Ireland, France and Germany preserved this Western knowledge whilst the Arabs preserved the Greco-Roman knowledge and expanded the uses of their own resources, together with Chinese and Indian herbs unfamiliar to the Greco-Roman world

(Cragg and Newman, 2005). It was the Arabs who were the first to privately own pharmacies (8th century) with Avicenna, a Persian pharmacist, physician, philosopher and poet, contributing much to the sciences of pharmacy and medicine through works such as the Canon Medicinae (Cragg and Newman, 2005).

1.2. Medicinal Plants in Folklore

The use of natural products as medicines has been described throughout history in the form of traditional medicines, remedies, potions and oils with many of these bioactive natural products still being unidentified. The dominant source of knowledge of natural product uses from medicinal plants is as a result of man experimenting by trial and error for hundreds of centuries through palatability trials or untimely deaths, searching for available foods for the treatment of diseases (Kinghorn, *et al*., 2011). One example involves the plant genus *Salvia* which grows throughout the south-western region of the United States as well as north-western Mexico and which was used by Indian tribes of southern California as an aid in childbirth (Sam, 1989) Male new-born babies were "cooked" in the hot *Salvia* ashes as it was believed that these babies consistently grew to be the strongest and healthiest members of their respective tribes and are claimed to have been immune from all respiratory ailments for life (Sam, 1989). The plant, *Alhagi maurorum* Medik (Camels thorn) secretes a sweet, gummy material from the stems and leaves during hot days (Duke *et al*., 2008). This gummy sap is called "manna" and consists mostly of melezitose, sucrose and invert sugar and it has been documented and claimed by the Ayurvedic people that the plant aids in the treatment of anorexia, constipation, dermatosis, epistaxis, fever, leprosy, and obesity (Duke *et al.,* 2008). It was also used by the Israelis who boiled the roots and drank the extract as it stopped bloody diarrhea. The Konkani people smoked the plant for the treatment of asthma, whilst the Romans used the plant for nasal polyps (Duke *et al.,* 2008). The plant *Ligusticum scoticum* Linnaeus found in Northern Europe and Eastern North America was eaten raw first thing in the morning and was believed to protect a person from daily infection (Dillenius, 1724); the root was a cure for flatulence (Beith, 1999;

Lightfoot, and Scotica, 1977; Martin, 1934), an aphrodisiac (Beith, 1999) and was used as a sedative in the Faeroe Islands (Martin, 1934; Svabo, 1959). *Atropa belladonna* Linnaeus (deadly nightshade) is found in Central and Southern Europe, Western Asia, North Africa, North America and New Zealand. Its notoriously poisonous nature (three berries are sufficient to kill a child) firmly excluded it from the folk medicine compilation and seemed to have been accepted as dangerous to handle or to experiment with (Allen and Hatfield, 2004).

1.3. Historically Important Natural Products

Traditional medicinal practices have formed the basis of most of the early medicines followed by subsequent, clinical pharmacological and chemical studies (Butler, 2004). Probably the most famous and well known example to date would be the synthesis of the anti-inflammatory agent, acetylsalicyclic acid (aspirin) derived from the natural product, salicin isolated from the bark of the willow tree *Salix alba* L. (Der Marderosian and Beutler, 2002). Investigation of *Papaver somniferum* L. (opium poppy) resulted in the isolation of several alkaloids including morphine, a commercially important drug, first reported in 1803. It was in the 1870s that crude morphine derived from the plant *P. somniferum*, was boiled in acetic anhydride to yield diacetylmorphine (heroin) and found to be readily converted to codeine (painkiller). Historically, it is documented that the Sumerians and Ancient Greeks used poppy extracts medicinally, whilst the Arabs described opium to be addictive (Der Marderosian and Beutler, 2002). *Digitalis purpurea* L. (foxglove) had been traced back to Europe in the 10th century but it was not until the 1700s that the active constituent digitoxin, a cardiotonic glycoside was found to enhance cardiac conduction, thereby improving the strength of cardiac contractibility. Digitoxin and its analogues have long been used in the management of congestive heart failure and have possible long term detrimental effects and are being replaced by other medicines in the treatment of "heart deficiency" (Der Marderosian and Beutler, 2002). The anti-malarial drug quinine approved by the United States FDA in 2004, isolated from the bark of *Cinchona succirubra* Pav. ex Klotsch, had been used for centuries for the treatment of malaria, fever,

indigestion, mouth and throat diseases and cancer. Formal use of the bark to treat malaria was established in the mid-1800s when the British began the worldwide cultivation of the plant (Der Marderosian and Beutler, 2002). Pilocarpine found in *Pilocarpus jaborandi* (Rutaceae) is an L-histidine-derived alkaloid, which has been used as a clinical drug in the treatment of chronic open-angle glaucoma and acute angle-closure glaucoma for over 100 years. In 1994, an oral formulation of pilocarpine was approved by the FDA to treat dry mouth (xerostomia) which is a side effect of radiation therapy for head and neck cancers and also used to stimulate sweat glands to measure the concentrations of sodium and chloride (Aniszewski, 2007). In 1998, the oral preparation was approved for the management of Sjogren's syndrome, an autoimmune disease that damages the salivary and lacrimal glands.

1.4. Natural Products from Plants

Plants have been well documented for their medicinal uses for thousands of years. They have evolved and adapted over millions of years to withstand bacteria, insects, fungi and weather to produce unique, structurally diverse secondary metabolites. Their ethnopharmacological properties have been used as a primary source of medicines for early drug discovery (Fellows and Scofield, 1995; McRae, *et al.*, 2007). According to the World Health Organization (WHO), 80% of people still rely on plant-based traditional medicines for primary health care (Farnsworth *et al*.,1985) and 80% of 122 plant derived drugs were related to their original ethnopharmacological purpose (Fabricant and Farnsworth, 2001). The knowledge associated with traditional medicine (complementary or alternative herbal products) has promoted further investigations of medicinal plants as potential medicines and has led to the isolation of many natural products that have become well known pharmaceuticals. The most widely used breast cancer drug is paclitaxel (Taxol®), isolated from the bark of *Taxus brevifolia* (Pacific Yew). In 1962 the United States Department of Agriculture (USDA) first collected the bark as part of their exploratory plant screening program at the National Cancer Institute (NCI) (Cragg, 1998). The bark from about three mature 100 year old trees is required to provide 1 g of Paclitaxel

given that a course of treatment may need 2 g of the drug. Current demand for Paclitaxel is in the region of 100–200 kg per annum (i.e 50,000 treatments/year) and is now produced synthetically (Dewick, 2002). The first of several FDA approvals for various uses for Taxol® was announced in 1992 (Cseke, *et al.,* 2006). Taxol® is present in limited quantities from natural sources; its synthesis (though challenging and expensive) has been achieved (Nicolaou *et al*., 1994). Baccatin III present in much higher quantities and readily available from the needles of *T. brevifolia* and associated derivatives is an example of a structural analogue that can be efficiently transformed into Taxol®(Dewick, 2002).

Other examples of antitumor compounds currently in clinical trials include ingenol 3-O-angelate a derivative of the polyhydroxy diterpenoid ingenol isolated from the sap of *Euphorbia peplus* (known as "petty spurge" in England or "radium weed" in Australia) which is a potential chemotherapeutic agent for skin cancer is currently under clinical development by Peplin Biotech for the topical treatment of certain skin cancers (Kedei *et al*., 2004; Ogbourne *et al*., 2004). PG490-88 (14-succinyl triptolide sodium salt), a semisynthetic analogue of triptolide is a diterpene-diepoxide isolated from *Tripterygium wilfordii* which is used for autoimmune and inflammatory diseases in the People's Republic of China (Kiviharju *et al*., 2002 and Fidler *et al*., 2003). Combretastatin A-4 phosphate a stilbene derivative from the South African Bush Willow, *Combretum caffrum* acts as an anti-angiogenic agent causing vascular shutdowns in tumors (necrosis) and is currently in Phase II clinical trials (Cragg and Newman, 2005; Holwell *et al*., 2002).

The Acquired Immune Deficiency Syndrome (AIDS) pandemic in the 1980s forced the National Cancer Institute (NCI) and other organizations to explore natural products as sources of potential drug candidates for the treatment of AIDS. Over 60,000 extracts of plants and marine organisms were tested against lymphoblastic cells infected with HIV-1. The most important result of these tests is the class of compounds known as the calanolides. In particular the isolation of calanolide A and calanolide B from the *Calonphyllum* species, along with

prostratin from *Homalanthus nutans*, have now progressed into clinical and preclinical development (Cox, 2001; Gustafson *et al.,* 1992; Kashman *et al.,* 1992). Calanolide A was licensed and evaluated to Phase II clinical trials by Sarawak Medichem Pharmaceuticals, however there has been no subsequent announcement for further drug development. In 2010, Phase I human clinical trials of prostratin were carried out by the AIDS Research Alliance in Los Angeles, California

Arteether, introduced in 2000, as Artemotil derived from artemisinin (introduced in 1987 as Artemisin) which was first isolated from the plant *Artemisia annua* as approved antimalarial drugs (Cragg and Newman, 2007). The plant was originally used in traditional Chinese medicine as a remedy for chills and fevers. Other derivatives of artemisinin are in various stages of clinical development as antimalarial drugs in Europe (Cragg, 1998 and Dewick, 2002). To date, a synthetic trioxolane modeled on the artemisinin pharmacophore, is being assessed in combination with piperaquine (a synthetic bisquinoline antimalarial drug) in an effort to treat malaria (Davidson *et al.,* 2009).

Grandisines A and B are two indole alkaloids which were isolated from the leaves of the Australian rainforest tree, *Elaeocarpus grandis*. Grandisine A contains a unique tetracyclic skeleton, while Grandisine B possesses an unusual combination of isoquinuclidinone and indolizidine ring systems. Both Grandisine A and Grandisine B, exhibit binding affinity for the human δ-opioid receptor and are potential leads for analgesic agents (Carroll *et al.,* 2005). Galantamine hydrobromide is an Amaryllidaceae alkaloid obtained from the plant *Galanthus nivalis* and has been used traditionally in Turkey and Bulgaria for neurological conditions and is used for the treatment of Alzheimer's disease (Heinrich and Teoh, 2004; Howes *et al*., 2003). Apomorphine is a derivative of morphine isolated from the poppy (*Papaver somniferum*) and is a short-acting dopamine D1 and D2 receptor agonist, as well as a potent dopamine agonist, used to treat Parkinson's disease (Deleu *et al*., 2004). "Curare" is the arrow poison of the South American Indians and is prepared in the rain forests of the Amazon and Orinoco.

Tubocaurarine isolated from the climbing plant, *Chondrodendron tomentosum* (Menispermaceae) is one of the active constituents used as a muscle relaxant in surgical operations, reducing the need for deep anesthesia. The limited availability of tubocurarine has led to the development of a series of synthetic analogues which are now preferred over the natural product (Dewick, 2002).

1.5 Statement of the Problem

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. The majorities of infections due to helminthes are generally restricted to tropical regions and cause enormous hazards to health and contribute to the prevalence of malnourishment, anemia, eosinophilia and pneumonia (Bundy, 1994).

Gastrointestinal nematode infections of livestock, which are bred for the production of meat, milk, or wool all around the world, lead to enormous economic losses. The control of these parasites has relied on the use of chemical anthelmintics, resulting in development of drug-resistant strains.

Alternative control methods are biological control, vaccination, and traditional medicinal plants, which are the focus of examination over the world. The evidence of anthelmintic properties of plants is gained primarily from ethnoveterinary and ethnomedical knowledge. Novel approaches to use the plants for control of gastrointestinal nematodes in small ruminants are outlined in the excellent reviews of Githiori *et al*., (2006) and Hoste and Torres-Acosta (2011).

1.6 Justification of the Study

The gastrointestinal helminthes are becoming resistant to both the benzimidazoles and macrocyclic lactones which are the available anthelminthic (Gill *et al.,* 1991). Therefore, there is a foremost problem in treatment of helminthes diseases (Sondhi *et al*., 1994) and there is an increasing demand towards new anthelminthics, to treat drug-resistance helminth infections.

Anthelmintics of the macrocyclic lactone family (e.g. ivermectin, doramectin) have a

significantly longer residual effect in comparison with the other anthelmintics. Over time, questions have been raised concerning the long-term impact of the massive application of these highly efficacious, broad-spectrum anthelmintic compounds on the environment. Due to the bioavilability of some of these drugs, high percentages of these substances are being excreted unchanged after oral or systemic administration (Farkas *et al*., 2003; Plumb, 2008).

Mucuna pruriens (DC) and *Canarium schweinfurthii* (Engl) have a wide reputation among ancient India of being curative for helminthiases like elephantiasis (Oudhia, 2002), intestinal worms, genito-urinary diseases, black tongue, round worm, gonorrhea and stomach disorders (Warrier *et al*.,1996).

1.7 Aim and Objectives of the Research

The aim of this study is to isolate and characterise triterpenoids from *Mucuna pruriens* (DC) and *Canarium schweinfurthii* (Engl) and, evaluate the medicinal potentials.

The objectives of this work are to:

(i). extract the plants *M. pruriens* and *C. schweinfurthii,*

(ii) test the activities of the crude extracts against selected micro-organisms and *Ascaris suum,*

(iii) purify and isolate and the phytochemicals from *M. pruriens* and *C. schweinfurthii,*

(iv) characterise and structurally elucidate the isolated compounds using spectral techniques such as U.V, ^{1}HNMR, ^{13}CNMR, DEPT, COSY, NOESY, HSQC, HMBC) and

(v) test the activities of the pure compounds against on the selected micro-organisms and *A. suum*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Man and Parasite

Approximately 11,000 years ago, man started raising pigs as a source of meat to feed their families (Larson *et al*., 2000). Today, pig industry is the largest meat producing industry in the world with a yearly production exceeding 100 million tonnes, making pork the meat of choice worldwide (FAO). Since the early days of pig farming, farmers have seen the productivity of their animals compromised by the many parasitic infections affecting pigs. Fortunately, in the last couple of decades, the impact of these parasites on pigs and their productivity has been significantly reduced. The main reasons for this are the switch from smaller, open air, rural farming to more industrial-sized, high-intensity, indoor farming and the advent of pharmaceuticals directed against the parasites that affect pigs. Infections with, *Ascaris suum* and to a lesser extent, *Trichuris suis* and Oesophagostomum spp. have successfully survived the transition to conventional indoor conditions and are currently thriving in some farms (Joachim *et al.*, 2001; Roepstorff *et al.,* 2011; Roepstorff and Nansen, 1994). Infections with *A. suum* however, show the highest prevalence (Eijck and Borgsteede, 2005; Joachim *et al.*, 2001). Despite its high prevalence, and its economic impact in the pig industry, farmers mostly remain unaware of the 'worm-status' of their herd. A clear understanding of parasite presence and infection intensity is nevertheless essential for making informed decisions on how to control ascariosis on their farms.

2.2 Pathogenesis and Pathology of Ascariosis

Pigs that are infected with *A. suum* sometimes carry numerous adult worms in their intestine. Yet, these pigs do not show any disease specific symptoms and are undistinguishable from uninfected pigs. Clinical signs such as breathing difficulties and wheezing, and indirect signs like decreased growth rate and overall lower food conversion efficiency are not specific and therefore seldom associated with ascariosis by the farmer. Although the presence of adult

parasites in the small intestine might have an impact on the pig's productivity (Andersen, 1976; Bernardo *et al.,* 1990b), it does not appear to have a major impact on the pig's health. The larval stages of this parasite on the other hand, do cause significant damage to the internal organs of its host in their attempt to successfully complete their hepatopulmonary migration. In the liver, the inflammatory reaction to this damage is manifested as the so-called 'white spots' that are visible on the surface of the liver. Hepatic white spots are the most characteristic lesions caused by migrating *A. suum* larvae in pigs. After larvae migrated through the liver, the destroyed liver cells are replaced by interlobular depositions of fibrous tissue and cellular infiltrates, producing the typical white spots (Nakagawa *et al.,* 1983; Perez *et al.,* 2001). Three types of white spots have been described: compact and mesh-worked white spots both produced by eosinophilic interstitial hepatitis and the lymph nodular type spots caused by lymph follicular hyperplasia (Nakagawa *et al.,* 1983; Perez *et al.,* 2001). White spots appear as early as 3 days post infection and start to resolve after about 2-3 weeks post infection (Eriksen *et al.,* 1980; Roepstorff *et al.,* 1997). As a result of this, livers can appear normal about 35 days after primary inoculation with Ascaris eggs (Copeman and Gaafar, 1972). In order to complete their hepatopulmonary migration, larvae need to pass through the lungs as well. As these larvae move into the alveolar and bronchiolar air spaces they cause direct physical damage to the lungs. This can subsequently promote the development of pneumonia or pleurisy (also known as pleuritis). The damage to the respiratory system can be recorded at the slaughterhouse and has been associated with the presence of Ascaris infection (Bernardo *et al.,* 1990a; Flesja and Ulvesaeter, 1980; Nakagawa *et al.,* 1983). It is obvious that the severity of the damage will depend on the amount of larvae that migrate through the lung (Miskimins *et al.,* 1994) and that increased infection levels can easily cause respiratory distress and coughing. However, even after ingestion of high amounts of eggs, the *A. suum* infection itself very seldom causes lethal damage to the lung. It is rather the secondary, opportunistic, bacterial or viral infections that increase the chances of severe health problems.

2.3 Economical Importance of Ascariosis

Today, the economic pressure on the pig industry is of such extent that production efficiency is crucial and that no farmer can afford to neglect the treatment of a parasitic ailment like ascaridiosis. Infections with *A. suum*, and especially the larval migration phase, have shown to have a significant negative impact on the economic profitability of a pig farm (Kipper *et al.,* 2011; Van Meensel *et al.,* 2010). The presence of this parasite on a pig farm reduces its productivity in a number of direct or indirect ways. Perhaps the most obvious of which is the condemnation of the livers that are visibly affected by parasite migration. Whether the liver is trimmed or fully condemned evidently depends on the amount of white spots detected on the liver. At the level of the lung, the passage of ascarid larvae is associated with impaired pulmonary bacterial clearance (Curtis *et al.,* 1987) making it easier for secondary bacterial and viral infections to settle. Increased susceptibility to *Pasteurella multicoda, Escherichia coli* and *Salmonella spp.* have already been associated with ascariosis (Adedeji *et al.,* 1989; Smith *et al.,* 2011; Tjornehoj *et al.,* 1992). Moreover, (Steenhard *et al.,* 2009) recently observed a significant negative effect of *A. suum* infection on the seroconversion and antibody levels to a *Mycoplasma hyopneumoniae* vaccine. Not only the negative effects of these diseases on the productivity but also the cost of their treatment indirectly contributes to the impact of ascariosis. The decreased health status of pigs, as a consequence of roundworm infection, is reflected in general production parameters like daily weight gain, feed conversion efficiency and meat quality (Bernardo *et al.,* 1990a; Hale *et al.,* 1996; Kanora, 2009; Kipper *et al.,* 2011; Knecht *et al.,* 2012; Hale and Stewart, 1985). However, in farms with low infection levels, the impact of infection is expected to be minimal and the economical profitability of active Ascaris control can be questioned (Roepstorff, 1997).

It is difficult to determine the economic losses due to parasites that are not lethal. There are numerous interactions between the worm, the host, the presence of other diseases, the production system and many more. Recent evaluations of the economic performance of

infected pig farms has indicated that the improved feed conversion, increased daily weight gain, lower mortality and better carcass quality act in a synergetic way. Hence, strategic deworming will result in a win-win effect on economic and environmental performances with gross profit margin increases of 3 to 12 euro per average present finisher per year (Jourquin, 2007; Kanora, 2009; Van Meensel *et al.,* 2010). To distinguish between pig populations that suffer significant economic damage and those that are less affected, we depend on the availability of diagnostic tools to provide us with information on the presence and infection pressure of this parasite on a farm. If interpreted correctly, diagnostic tools can be of use to evaluate the applied control strategies and see whether efforts for parasite control need to be increased or changed.

2.4 Control of Ascariosis

Although, the aim of control programs against this parasite should be to eliminate the parasite completely, a significant reduction in transmission intensity will readily result in a marked decrease of adverse effects on the health and productivity of the pig herd. Although, anthelmintic treatment alone, without the support of good general farm management and increased hygienic standards will probably not suffice to effectively control parasitic infections on farms (Nilsson, 1982; Roepstorff and Jorsal, 1990), it is, at this moment, the only available method to clear pigs from roundworm infections.

2.5 Nematocidal Activity of Plants

Parasitic nematodes represent a serious threat to humans, animals, and plants. Gastrointestinal nematode infections of livestock, which are bred for the production of meat, milk, or wool all around the world, lead to enormous economic losses. The control of these parasites has relied on the use of chemical anthelmintics, resulting in development of drug-resistant strains. Alternative control methods are biological control, vaccination, and traditional medicinal plants which are the focus of examination over the world. The evidence of anthelmintic properties of plants is gained primarily from ethno-veterinary and ethno-medical knowledge. Novel

approaches to use the plants for control of gastrointestinal nematodes in small ruminants are outlined in the excellent reviews of Githiori *et al*., (2006) and Hoste and Torres-Acosta (2011). In parallel with exploring non-chemical methods of control to reduce the infective larvae in the field, the search for novel pharmacologically active compounds in plant extracts is necessary for developing future anthelmintics. Obviously, in vitro assays are applied to pre-screen the activity of plant extracts and isolated components on free-living and consequently on parasitic stages of nematodes. Data collected by numerous authors to date indicate that concentrations of potentially active substances used in vitro do not always correspond to in vivo bioavailability. Therefore, in vitro assays should always be followed by in vivo controlled studies. Recently, the potential of several plant extracts with antiparasitic activities with emphasis on gastrointestinal nematodes of livestock was reviewed by de Gives *et al.*, (2012) pointing to some other aspects. It should be taken into consideration that the different parts of higher plants (leaves, stems, roots, flowers, fruits) may contain different concentrations of the bioactive compounds that have to be determined early in the screening process. Moreover, the possible side effects on hosts should be established in vitro using cell lines or other sensitive tests to determined LC_{50} (lethal concentration at which 50 % of cells is killed), before carrying out in vivo assays.

Validation of antiparasitic activities of compounds obtained from plant extracts requires standardization of methodologies and this issue is discussed in the review of Athanasiadou *et al.*, (2001). The authors focussed on the strengths and weaknesses of the existing methodologies used in the controlled studies and also discussed issues like the seasonal variability of the plant composition and how this can affect their antiparasitic properties.

2.6 Chemical Constituents

Secondary metabolites found in plants are organic compounds that are not directly involved in the normal growth and development and reproduction of the plant. These compounds maybe part of the defence mechanism of the plant or facilitate the reproductive process.

The chemistry of known organic compounds such alkaloids, glycosides, sterols, saponins, flavonoids and tannins are interrelated with biological activities. Findings on the chemical constituents found in *Mucuna* and *Canarium* species can be used as template to the biological activities in *Mucuna pruriens* and *Canarium schweinfurthii* that possess similar chemical constituents

2.6.1 Alkaloids

Alkaloids represent a highly diverse group of compounds that are related only by the occurrence of a nitrogen atom in a heterocyclic ring. The nitrogen in the alkaloid molecule is derived from amino acid metabolism. Since the amino acid skeleton is often largely retained in the alkaloid structure, alkaloids originating from the same amino acid show similar structural features and can be classified according to their biosynthetic origin. Plants are estimated to produce approximately12, 000 different alkaloids, which can be organized into groups according to their carbon skeletal structures (Ziegler and Facchini, 2008).

Alkaloids often have pronounced bioactivities and are therefore thought to play an important role in the interaction of plants with their environment. Alkaloids and extracts of alkaloid-containing plants have been used throughout human history as remedies, poisons, and psychoactive drugs. Several alkaloids are used medicinally or provide lead structures for novel synthetic drugs (Fester, 2010).

A great number of alkaloids originating from marine organisms showed a potent activity on parasitic protozoa and on several helminth species *in vitro* and some specifically inhibit essential enzymatic systems in parasites. However there are few studies, in which alkaloids isolated from higher plants, were examined as anthelmintic agents.

Many known anthelmintics are effective against nematodes living in gastrointestinal system, but limited efficacy has been reported against larvae which migrate to human tissues where they may cause a severe pathology. This is the case for *Toxocara canis* and *Toxocara cati* larvae, both zoonotic nematode infections. Migration of larvae is associated with many non-

specific pathological syndromes, eosinophilia, and an allergic type of immune response. The seroprevalence of toxocariasis in the healthy human population was estimated to be 5-10 % and the disease ranks among the most frequent parasitic diseases in temperate climates. The search for plant phytochemicals effective against second stage larvae of *T. canis* were the focus of studies of several research groups. Satou *et al.,* (2002a) developed a specific test for the evaluation of nematocidal activity in vitro and introduced the concept of Relative Mobility (RM) of larvae. This shows the concentration of a compound at which RM equals 50 % of the control, the RM_{50}, value. Using this test, a set of isoquinoline alkaloids isolated from the plants *Macleaya cordata, Chelidonium majus,* and *Corydalis turtschaninovani*, grown in Japan for various purposes, were examined for inhibition of mobility of larvae and their cytotoxicity. Some of the isolated alkaloids were highly cytotoxic in HL_{60} tissue-culture cells, so only those with a high RM_{50},/IC_{50}, ratio (Selectivity index:SI>100) were proposed as potential anthelmintic alkaloid molecules, namely allocryptopine, dehydrocorydaline, and papaverine. However, the mechanism of inhibition of larval motility is so far unclear.

In an effort to identify a treatment for *Strongyloides stercoralis* larval in humans, the effects of isoquinoline alkaloids were examined by Satou *et al*., (2002b) using infective third-stage larvae of *Strongyloides ratti* and *S. venezuelensis* as model nematodes for *S. stercoralis*. The nematocidal activity of a set of isoquinoline alkaloids isolated from the same plants as in the previous study was further evaluated *in vitro* on L3 stage larvae. Total inhibition of mobility corresponding to the complete paralysis of larvae was test of choice for comparison of nematocidal activity of all alkaloids.

Three alkaloids: protopine, d-corydaline, and l-stylopine exhibited strong nematocidal activity at low concentrations (52/33 μM, 18/30 μM, 14/13μM for *S. ratti*, and *S. venezuelensis*, respectively.), and showed little cytotoxicity (SI>100) for HL_{60} tissue-culture cells. However, these concentrations were significantly higher than that found for ivermectin (2.2 and 2.3 μM, resp.), which was the most effective strongyloidosis treatment. β-carboline alkaloids are a large

group of natural and synthetic indole alkaloids with different degrees of aromaticity, some of which are widely distributed in nature, including various plants, foodstuffs, marine organisms, insects, mammals as well as human tissues, and body fluids. They possess diverse biological activities and can inhibit several enzymatic systems in mammals (Cao *et al.*, 2004).

A set of 17 different β-carboline alkaloids from the plants *Picrasma quassioides* and *Ailanthus altissima* which grow in Japan was examined using *T. canis* larvae in in vitro screening (Satou *et al.*, 2005). Inhibition of mobility (larval paralysis) and low cytotoxicity were found only for a few alkaloids and only one alkaloid with no cytotoxicity at 0.1 mg/ml was examined in vivo on infected mice.

In an attempt to slow-down metabolism of this alkaloid, it was entrapped in pegylated liposomal carriers and this drug formulation showed higher reduction of larval numbers in the brain of mice than reference drug albendazole, as well as decreased eosinophilia. No side effects in mice were observed. Alkaloid with reported high nematocidal potential on larvae migrating in the tissues of hosts which also showed very low cytotoxicity seems to be interesting molecules. Therefore, it is worth to elucidate their mode of action on larvae.

2.6.2 Flavonoids and Polyphenols

Flavonoids are plant pigments present in almost all terrestrial plants, where they provide UV protection and colour. They are synthesized from phenylalanine and their basic chemical structure (C6-C3-C6 skeleton) has a fused ring system consisting of an aromatic ring and an oxygen-containing heterocyclic benzopyran ring with a phenyl substituent. Flavonoids can be divided into different classes depending on their oxidative status and substituents. Most flavonoids, apart from cathecins, are usually present in plants as β-glycosides. The flavonoids appear to have an important role in the successful medical treatments of ancient times, and since the last century they are a subject of increasing interest of scientists working in various fields of medical research, including pharmacology of parasitic diseases. An excellent review on biochemistry and medical significance of the flavonoids has been written by Havsteen

(2002). His review deals also with a high spectrum of positive effects of various flavonoids on plant and mammalian cells in relation to therapeutical applications, their ability to inhibit specific enzymes, to simulate some hormones and neurotransmitters, and to scavenge free radicals. It is well known that some flavonoids can inhibit or kill many bacterial strains, inhibit important viral enzymes, such as reverse transcriptase and protease, and destroy some pathogenic protozoans.

In the many plant extracts which showed a high anthelmintic activity, chemical analyses revealed the presence of flavonoids, along with other classes of phytochemicals. Although toxicity of most isolated flavonoids to animal cells is very low (Middleton *et al.*, 2000), several ubiquitous flavonoids genistein, kaemferol, rutin, quercetin, etc, showed deleterious effects on selected species of parasitic helminths. It is possible, that different developmental stages (larvae, juvenile or adult) of helminths might possess different susceptibility to selected flavonoids. Phenolic diketone curcumin and the flavonoid kaempferol exerted a strong adulticidal effect on *Schistosoma mansoni*, but no activity against nematodes was demonstrated. Kaempferol (flavonol) and its three derivatives, were isolated from two plant species of *Styrax camporum* and *Styrax pohlii* (Braguine *et al.,* 2012), and were examined *in vitro*. Of these, kaempferol was the most effective in separating *S. mansoni* male and female couples and killing adult worms at a concentration of 100 μg/ml. The selective toxicity of the flavones quercetin, chrysin, and 3-hydroxyflavone toward the several cancer cell lines but not to normal mammalian cells is worthy of mention (Pilatova *et al.*, 2010).

Selected flavones stimulated mechanisms leading to apoptosis of cancer cells and inhibited functions of important cell signalling molecules. The polyphenol curcumin (phenolic diketone) is the major constituent in the rhizome of *Curcuma longa* (Zingiberaceae), which is responsible for its characteristic yellow pigment. Curcumin is well known to exhibit several biological activities, including anti-inflammatory, antioxidant, antiviral, anti-infectious, and anti-carcinogenic activities (Maheshwari *et al.*, 2006). Angiogenesis is a key step in tumour growth

and invasion and, in the recent review of Varinska *et al.*, (2010), the anti-angiogenic potential of polyphenols including curcumin was discussed. Curcumin was shown to cause the death of all worms of *S. mansoni* at 50 and 100 μM concentrations due to the decreasing of their viability (Magalhães *et al*., 2009). All pairs of coupled adult worms were separated into individual male and female by the action of curcumin at the doses of 20-100 μM and it also reduced egg production by 50 %. An important issue in the drug development process is drug solubility in water and pharmacokinetic properties *in vivo*, which can be manipulated after their incorporation into drug delivery systems (DDS).

Interestingly, *in vivo* studies with curcumin showed a lack of significant toxicity (Perkins *et al.*, 2002). The mechanism by which curcumin exerts its *in vitro* schistosomicidal effect is not clear. However, it has been reported that it has a direct action involving parasite biochemical processes. One of the possible targets in schistosomes for the curcumin action is the ubiquitin-proteasome pathway. In the study of Allam (2009), a total dose of 400 mg/kg BW of curcumin was given to mice with S. mansoni infection (in 16 injections) and treatment was effective in reducing worm burden by 44.4 % and tissue-egg burden by 30.9 %. Moreover, modulation of cellular and humoral immune responses was observed as well as a decrease of hepatic granuloma volume and overall collagenesis.

Genistein (4',5,7-hydroxyisoflavone), a major component of soya, is a well-known phytoestrogen, which was found also in the ethanol extracts of higher plants *Flemingia vestita*, *Accacia spp., Stephania glabra,* and possibly others. Extensive studies have indicated beneficial effect of genistein on a multitude of disorders, including cancer, cardiovascular diseases, osteoporosis, and postmenopausal symptoms. It has strong anti-inflammatory and antibacterial properties *in vitro* and *in vivo* (Verdrengh *et al.,* 2004), however, in mammals frequent administration of the higher doses either as therapeutical or dietary supply might cause undesirable side effects (Klein and King 2007).

The anthelmintic activity of genistein or genistein-rich extracts from roots; of the plant

Flemingia vestita has been proven on the cestodes *Raillietina echinobothrida, Echinococcus multilocularis* and *Echinococcus granulosus* and the trematode, *Fasciolopsis buski in vitro*. *Flemingia vestita* is an indigenous medical plant of north-east India and, according to the ethno-medical experiences; the crude extract of the root-tuber peel was effective against trematodes and cestodes, but not nematodes. A high concentration of isoflavones was found in the extract from this plant (Rao and Reddy, 1991). The anthelmintic activity of genistein is mediated by its action on several cellular/molecular targets, and such a complex mode of action of this flavonoid was also found for mammalian cells. In parasitic flatworms, the primary or vital targets of genistein were suggested to be the tegumental enzymes, and their inhibition probably resulted in paralysis and worm death (Pal and Tandon, 1998). In vitro exposure to this compound resulted in tetanic contractions, flaccid paralysis, and disruption of tegument in *R. echinobothrida* (Tandon *et al.*, 1997).

2.6.3 Glycosides and Saponins

Saponins and glycosides are naturally occurring chemical compounds found in a wide variety of higher plants, in lucerne (*Medicago sativa*), sisal (*Agave sisalama*), and seeds like soyabeans (*Glycine max*) (Francis *et al*., 2002). Glycoside is a molecule in which a sugar is bound to a non-carbohydrate moiety, usually small organic molecules. They play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides, which are activated by enzyme hydrolysis. Saponins are chemically glycosides, which are composed of a lipid-soluble aglycon consisting of either a sterol or more commonly a triterpenoid with different, water-soluble, sugar residues. Saponins also have surfactant properties and sterol-group-containing saponins can particularly affect eukaryotic organisms that contain steroids in their membrane. They also demonstrate hemolytic action toward red blood cells, and can be toxic if given intravenously (Osbourn *et al.*, 2011). Moreover, consumption of food with a high saponin concentration by humans and animals can be potentially harmful (Milgate and Roberts, 1995). Glycosides and saponins were detected in

many higher plants used in traditional ethnomedicine at various concentrations. So far, only several of these molecules isolated from the plants have been examined for their anthelmintic activity under experimental conditions or in *in vivo* controlled studies with promising results (Makkar *et al.*, 2007).

In vivo studies have been conducted on rats with *H. diminuta* infection. Saponins extracted from *Acacia auriculiform* were administered orally at the dose of 200 mg/kg for several days. Adult worms were expelled within several days from intestines indicating that saponins induced tegumental damage followed by the worm paralysis, which usually precedes expulsion. At such relatively higher dose of crude extracts no side effects on the host were observed (Ghosh *et al.*, 1996). Chemical analysis of extract from the funicles of *A. auriculiformis* revealed the presence of triterpenoid saponins acaciaside A and B. They are unique molecules because they contain a conjugated unsaturated system, which is highly susceptible to peroxidation (Ghosh *et al.*, 1993). Besides cestocidal effect, these saponins showed also strong antifilarial activity, when concentration of 4 mg/ml killed 97 % of microfilaria of *Setaria cervi* and 100 % of adults within 100 min (Ghosh *et al.*, 1993). Significant reduction of infection was found *in vivo* after repeated doses of 100 mg/kg without seeing any toxic effects in rats. Drugs probably caused a very high physiological stress on adult worms, resulting in their death and expulsion which is opposite to the low efficacy of antifilarial drugs toward adults. Sinha Babu *et al.*, (1997) showed experimentally that these saponins enhance the cell membrane lipid peroxidation. They and others (Nandi *et al.*, 2004) suggested that the conjugated unsaturated system of selected saponins is involved in the formations of free radicals, which induce membrane damage through peroxidation of membranes in helminths.

2.6.4 Condensed tannins and sesquiterpene lactones

Tannins are group of secondary plant metabolites formed by water-soluble phenolic compounds with a great diversity, which can be divided into hydrolyzable and condensed tannins (Waterman, 1999). Condensed tannins are polyphenols (=proanthocyanidins) with high

molecular weight from 500 to 20,000 kDa, which consist mainly of oligomers or polymers of monomeric units of flavan-3-ols (catechin, epicatechin, and so on). Depending on the chemical structure of the monomeric unit, in particular the number of hydroxyl radicals, they are classified into four sub-classes: the prodelphinidins (PDs), procyanidins (PCs), prorobetinidins (PRs), and profisetinidins (PSs). In legume forages, PCs and PDs are mainly found, in various proportions. The ratio between PDs/PCs differs widely between plant species and/or varieties (Mueller-Harvey, 2006). Condensed tannins have a high affinity for proteins and polysaccharides, which they can precipitate from the aqueous solutions. The affinity of condensed tannins for proteins is determined by the molecular mass and the molecular configuration of both the tannin and the proteins. Tannin-protein binding is usually reversible in acid or alkaline pH or after treatment with detergents (surfactants).

Condensed tannins (CTs) have high relevance for livestock production as tannin-rich plants have a long tradition of use not only as forages but also as "green" control of gastrointestinal nematode infections. Several excellent reviews deal with the various aspects of feeding of small ruminants with forages containing tannin-rich plants or even fodder trees (Diaz *et al*., 2010; Hoste *et al*., 2005, 2006, 2012). They pointed that bioactive tanniniferous plants represent a valuable option as an alternative to commercial drugs for the control of gastro-intestinal nematodes (GINs) as consumption of these plants has been associated with antiparasitic effects: reductions in nematode numbers, worm fecundity, and nematode eggs excretion. The main threat to the use of solely chemical drugs is the rapid development of resistance to any anthelmintic drug in worm populations after commercialization (Waller, 2006) and the spread of anthelmintic resistance within worm populations (Kaplan, 2004).

These problems have stimulated research on plant-based phytochemicals with anthelmintic activity against gastrointestinal nematodes. Although consumption of high concentrations of condensed tannins (7 % of DM) had a number of detrimental effects on ruminants, such as reduction in food intake, growth inhibition and interference with the morphology and the

proteolytic activity of microbes in the rumen (Min and Hart, 2003; Waghorn and McNabb 2003), low or moderate concentrations of condensed tannins (6 % of DM) have resulted in a positive effects on animals. The pertinent use of tannin-rich fodders as nutraceuticals supposes a clear understanding of the mode of action against the worms. The term "nutraceutical" results from a contraction of nutrition and pharmaceutical. It is defined as "any substance that may be considered a food or part of a food which provides health benefits, including the prevention and treatment of disease" (Andlauer and Furst 2002).

Several aspects of direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infections have been analysed in detail in the recent review of Hoste *et al.*, (2012). The other group of the biochemical compounds with anthelmintic activity found in plants used as forage, in chicory, are sesquiterpene lactones (Foster *et al.*, 2006). Within the last decade a number of studies focused on isolation of condensed tannins and sesquiterpene lactones from various legume forages and plants with the aim to reveal their effects *in vitro* and *in vivo* on various species and developmental stages of nematodes. Differentiated action of condensed tannins on parasite stages was observed by Athanasiadou *et al.*, (2001), which were more effective against larvae than adults. This can also be explained by the difference between the cuticular components of the pre-parasitic stages (eggs to L3) and the parasitic stages (L4 and adults), as demonstrated by the study of Stepek *et al*., (2007a, b). CTs do have significant negative effects upon egg hatching and larval hatching *in vitro* as was demonstrated for example by Molan *et al*., (2002) and Novobilsky´ *et al.*, (2011).

Forage CTs are polymers of flavan-3-ol units, with a considerable range of structural variations. The constituent flavan-3-ol units in procyanidin (PC) polymers are either catechin (C) or epicatechin (EC), while prodelphinidin (PD) polymers contain either gallocatechin (GC) or epigallocatechin (EGC). *Trichostrongylus colubriform* is infective larvae were exposed to flavan-3-ols and their galloyl derivatives under in vitro conditions to compare their effects on the viability of eggs, development of first stage (L_1) larvae, and the viability of the infective

larvae determined by their mobility (Molan *et al.,* 2003a). The flavan-3-ol gallates were effective in all three tests, with epigallocatechin gallate being the most effective in the egg hatch test (100 % inhibition at 1 mg/ml), also in inhibition of viability of larvae at 500 µg/ml. There was complete inhibition of development by all compounds at 100-200 µg/ml and 50 % inhibition between 42 and 59 µg/ml for flavan-3-ol, while values between 32 and 48 µg/ml were observed for flavan-3-ol gallates. Flavan-3-ols caused some inhibition of viability, but were not effective on other developmental stages (Molan *et al.*, 2003a). The active CT extracts from forage legumes have epigallocatechin as the dominant flavan-3-ol extender unit, and epigallocatechin was the most active flavan-3-ol in both the EHT and LDA assays. Results may indicate that the anti-parasite properties of CTs are not significantly dependent on their structure in terms of 2,3- stereochemistry of the heterocylic C-rings (2,3-cis or 2,3-trans), but rather on the number of hydroxy groups in the B-ring (PD:PC ratio). The degree of polymerization and the inter-flavanoid linkages may play a significant role in the effects of CTs. *Onobrychis vicifolia* (sainfoin) is other leguminous forage rich in condensed tannins, which significantly inhibited migration ability of *H. contortus* L3 stage of larvae *in vitro* (Barrau *et al*., 2005). Inhibition was seen also for other bioactive compounds flavonol glycosides (rutin, nicotiflorin, and narcissin) present in low molecular weight fraction (up to 2 kDa) implying that both classes of phytochemicals contributed to the anthelmintic activity of this plant *in vivo*.

Chicory (*Cichorium intybus*) is a widespread herb with high nutritional value, that contains several secondary compounds, with sesquiterpene lactones and the major phenolics (condensed tannins) being the most abundant. The levels of the sesquiterpene lactones (lactucin, lactupicrin, and 8-deoxylactucin) and the hydroxyl coumarin chicorin were found to be highest in the most actively growing regions of the plant (Rees and Harborne, 1985). CT and crude sesquiterpene lactones (CSL) extracted from chicory have been shown to have direct effects on the motility of first-stage (L_1) and third-stage (L_3) larvae of deer lungworm (*Dictyocaulus*

viviparus) and L_3 larvae of gastrointestinal nematodes *in vitro* using the larval migration inhibition (LMI) assay (Molan *et al*,. 2003b). Condensed tannins appeared to be more effective than CSLs at inactivating L_1 and L_3 lungworm and L_3 gastrointestinal larvae in rumen fluid, but CSLs were particularly effective against L3 lungworm larvae in abomasal fluid. Condensed tannins were also effective at reducing the motility of infective larvae of gastrointestinal nematodes. Moreover, the L_3 larvae of gastrointestinal nematodes were more sensitive to CT than L_1 and L_3 larvae of lungworms. This may be attributed to the fact that gastrointestinal L_3 larvae were exsheathed, while the lungworm larvae were not. The protein surface of the sheath may interact with the CT and protect the larvae, whilst the absence of a sheath brings the larvae into direct contact with the CT and thus exposes them to a greater paralyzing effect. This was confirmed in the study of Molan *et al*., (2000a) who found that exsheathed larvae of deer gastrointestinal nematodes were more susceptible to the actions of CT than larvae with protecting layers. Indeed, Brunet and Hoste (2006) showed that monomers of condensed tannins affect directly the larval exsheathment of parasitic nematodes of ruminants. CSLs were effective at reducing the motility of lungworm and gastrointestinal nematode larvae, but their inhibitory activity in rumen fluid was lower than found for CT. This finding may be of practical value in controlling infection of the abomasum and intestine. In contrast to CT, pH probably does not affect the reactivity of CSL. The addition of PEG did not affect the biological activity of CSL, which may indicate that CSLs inhibit these larvae by a different mechanism than do CT.

The proportions of the sesquiterpene lactones (lactucin = LAC, lactupicrin= LPIC and 8-deoxylactucin = DOL) vary among forage chicory cultivars, that could modify the final anthelmintic activity of CSL (Foster *et al.*, 2011). After using the combined LAC, DOL, and LPIC concentrations in the range from 0 to 5.0 mg/ml, egg hatching of *Haemonchus contortus* decreased sharply in a linear fashion. Concentrations of sesquiterpene lactones required for 50 % lethality were 2.6 mg/ml for cultivar containing higher amount of DOL and 6.4 mg/ml for

cultivar with higher concentration of LAC, suggesting that LAC has minimal effect on egg hatching and that DOL or other constituent(s) in the CSLs are inhibitory (Foster *et al.*, 2011). Metabolism or breakdown of free and bound sesquiterpene lactones in the rumen of animals is likely, and degradation rates of the individual chemical structures may vary, influencing what compounds and amounts reach the relevant section of the gastrointestinal track. Further studies are needed to determine the extent to which sesquiterpene lactone glycosides might influence the anthelmintic potential of chicory herbage.

Variations in the concentration and biochemical composition of condensed tannins extracted from several legume forages (Lotus spp., *Hedysarum coronarium, Onobrychis viciifolia, Dorycnium spp*.) were probably directly responsible for altered behavior of *Trichostrongylus columbriformis* exsheathed L3 stage larvae in a migration inhibition assay (Molan *et al.*, 2000b, b). At 100 µg/ml, purified CTs from Dorycnium spp. were more effective in larval inhibition than CT from other forages. Similarly, at concentration of 1,000 µg/ml CT from Dorycnium spp. had the highest inhibitory activity (63 %) also on L1 stage of larvae, followed by CT from *Onobrychis viciifolia* (59 %). Data from selected studies briefly indicate the complexity of actions of condensed tannins from various sources on different developmental stages of GIN. The specific composition of CT and the stage specific protein antigenic array of nematodes, which interact together, may explain the observed variability. It is possible that CT can interfere with proteins involved in glucose metabolism as CT from *Chicorium intybus* inhibited glucose uptake in mammalian cells (Muthusamya *et al.*, 2008).

2.6.5 Triterpenes

Natural compounds have been used extensively in the treatment of many diseases and are of interest to researchers both in their natural forms and as templates for synthetic modification. Natural compounds currently used in medicine exhibit a very wide chemical diversity, and together with their analogues and several other natural products, they demonstrate the importance of compounds from natural sources in modern drug discovery efforts. Evidence

from tissue culture, animal, and clinical studies suggests that more than 20,000 triterpenoid-rich fruits are found in nature and have the potential ability to limit the development and severity of certain cancers and inflammatory diseases (Sporn and Suh, 2000). These triterpenoids, along with their close chemical relatives the steroids, are members of a larger family of related structures called cyclosqualenoids. Triterpenoids, synthesized in many plants by the cyclization of squalene (Phillips *et al*., 2006), are widely used in Asian medicine. More than 100 prescribed drugs in the United States are obtained from natural sources and represent one fourth of the total drugs used. Apart from these drugs that originate from natural sources, other phytochemicals also serve as potential drugs after structural modification (Kinghorn and Balandrin, 1993). Triterpenoids are metabolites of isopentenyl pyrophosphate oligomers that are chemically related to squalene, which is a large group of compounds having 30 carbon atoms arranged in five rings with several oxygen atoms attached. Triterpenoids are part of the largest group of plant products, Saponins can be chemically biosynthesized when one or more sugar moieties attach to aglycone. There are two types of saponins, steroidal aglycone and triterpenoid aglycone. Both steroid and triterpenoid systems are found to be biosynthesized from a common precursor such as squalene (Liu, 1995). Triterpenoids are synthesized from isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP). For this cyclization, three prenyltransferases synthesize the linear prenyl pyrophosphates geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP), and geranylgeranyl pyrophosphate (GGPP). Squalene is in turn derived biosynthetically by the cyclization of a number of units of isoprene, $(C_5H_8)n$, which undergo folding through 20 different patterns in the presence of prenyl pyrophosphates to form monocyclic, dicyclic, tricyclic, tetracyclic, or pentacyclic derivatives (Bouvier *et al*., 2005). A family of oxidosqualene cyclases may produce only a single product, such as lupeol cyclases, but there are also multifunctional oxidosqualene cyclases that use dammarenyl cation intermediates to produce many products. Once squalene undergoes cyclization, it goes through the cytosolic mevalonate pathway to make a proximate

tetracyclic C30 compound, lanosterol, which further undergoes oxidation and catabolic metabolism to form cholesterol

Terpenes and their metabolites are widely distributed in various plant systems that depend on various biotic and abiotic environmental factors. Terpenes and their metabolites are used in several developmental and physiological functions on the basis of the differential expression profiles of terpene synthase genes. Terpenes and their metabolites play a very important role in a plant's defense mechanism. They protect the plants from both constitutive and induced defensive responses against insects and environmental stress (Keeling and Bohlmann, 2006). Hence, triterpenoids provide a very good protection shield for plants, indicating their potential for use in the prevention of various inflammatory diseases in humans. Triterpenoids affect multiple signalling pathways, and the clinical properties of triterpenoids, particularly those of pentacyclic triterpenoids, have been shown in various studies. The structure activity relationships indicate that the presence of αβ-unsaturated carbonyl moieties significantly enhance the potency of these pentacyclic triterpenoids

2.6.5.1 Naturally-ocurring pentacyclic triterpenes

Several pentacyclic triterpenes have been isolated and reported as biologically active compounds (Vivek *et al*., 2010).

Betulinic Acid: the reported biological activities of betulinic acid include the following; anticancer (Amico *et al*., 2006; Huang *et al*., 2007), HIV-1 maturation inhibitor (Fukoja *et al*., 1994), antibacterial activity with inhibitory effects against *Bacillus subtilis* (Chandramu *et al*., 2003).

Oleanolic Acid: occurs in numerous varieties of plants and possesses many biological properties including anti-inflammatory, trypanocidal, anti-HIV and cytotoxic activities (Saleem *et al*., 2005; Sultana and Ata, 2008).

Maslinic Acid: Maslinic Acid is reported to have biological activities which include the following; antitumor, antioxidant (Jagers *et al*., 2009), anti-inflammatory (Vivek *et al*., 2010),

gastroprotective (Tati *et al*., 2008) effects among others.

Eugenol: is one of the major components of cloves. It is an essential oil reported to have biological activities such as; antioxidant (Abdulrahim *et al*., 2011), cytotoxic, apoptotic, antiangiogenic (Zheng *et al.*, 1992), antimicrobial (Himejima and Kubo, 1992) activities.

2.7 Fabaceae

The family *Fabaceae or Leguminosae*, commonly known as the legume, pea, or bean family, is a large and economically important family of flowering plants. It includes trees, shrubs, and herbaceous perennials or annuals plants, which are easily recognized by their fruit (legume) and their compound, stipulated leaves. The group is widely distributed and is the third-largest land plant family in terms of number of species, behind only the *Orchidaceae* and *Asteraceae*, with 630 genera and over 18,860 species (Judd *et al.,* 2002; Magallón *et al.,* 2001). The five largest of the 630 legume genera are *Astragalus* (over 2,000 species), *Acacia* (over 1000 species), *Indigofera* (around 700 species), *Crotalaria* (around 700 species), and *Mimosa* (around 500 species), which constitute about a quarter of all legume species. About 18,000 legume species are known, amounting to about 7% of flowering plant species (Judd *et al.,* 2002; Magallón *et al.,* 2001). *Fabaceae* is the most common family found in tropical rainforests and in dry forests in the Americas and Africa (Burnham and Johnson, 2004).

Recent molecular and morphological evidence supports the fact that the *Fabaceae* are a single monophyletic family (Lewis *et al.,* 2005). This point of view has been supported not only by the degree of interrelation shown by different groups within the family compared with that found among the *Leguminosae* and their closest relations, but also by all the recent phylogenetic studies based on DNA sequences (Doyle *et al.,* 2000; Kajita *et al.,* 2001; Wojciechowski *et al.,*2004). These studies confirm that the *Leguminosae* are a monophyletic group that is closely related with the *Polygalaceae, Surianaceae and Quillajaceae* families and that they belong to the order *Fabales* .

The Faboideae are a subfamily of the flowering plant family *Fabaceae* or *Leguminosae*. An acceptable alternative name for the subfamily is *Papilionoideae* or *Papilionaceae* when this group of plants is treated as a family (McNeill *et al.,* 2006). *Faboideae* may be trees, shrubs, or *herbaceous* plants.

2.7.1 *Mucuna*

Mucuna isa genus ofaround100accepted species ofclimbing vines and shrubs ofthe family *Faba ceae*, found worldwide in the woodlands of tropical areas.

The leaves are tripalmate, alternate, or spiraled, and the flowers are pea-like but larger, with distinctive curved petals, and occurring in racemes. Like other legumes, *Mucuna* plants bear pods. They are generally bat-pollinated and produce seeds that are buoyant sea-beans. These have a characteristic three-layered appearance, appearing like the eyes of a large mammal in some species and like a hamburger in others (most notably *M. sloanei*) and giving rise to common names like deer-eye beans, donkey-eye beans, ox-eye beans, or hamburger seed. The name of the genus is derived from *mucunã*, a Tupi–Guarani word for these species (Quattrocchi, 2000).

2.7.2 Taxonomy and Botanical descriptions

It is popularly known as “Magic bean” in Indian System of Medicine. The other vernacular names of this plant are Cowhage, Kiwanch or Konch (Hindi), Velvet bean or Cowitch (English), Atmagupta or Kapikacchu (Sanskrit), Poonaikkaali (Tamil), Alkushi (Bengali), Khaajkuiri (Marathi)(http://en.wikipedia.org/wiki/Mucunapruriens)

2.7.3 *Mucuna pruriens* (DC)

Mucuna pruriens, commonly known as Kiwanch or konch in Hindi, Cowitch in English and Kapikacho in Sanskrit and also known as *M. prurita* Hook, is an annual, climbing shrub with long vines that can reach over 15m in length (Alo *et al.,* 2012; Hussain and Manyam, 1997; Katzenschlager *et al.,* 2004). It is the most popular drug in the Ayurvedic and Unani system of medicine (Mishra and Tiwari, 2011). When the plant is young, it is almost completely covered

with fuzzy hairs, but when older, it is almost completely free of hairs (Sahaji, 2011).

This plant is cultivated in Bangladesh, India, Pakistan, Srilanka, South East Asia and Malaysia (Chopra *et al.,* 1956; Khare, 2004). Preparations of different parts like roots, leaves and seeds of *Mucuna pruriens* are commonly used for the treatment of several free radical mediated diseases such as ageing, rheumatoid arthritis, diabetes, atherosclerosis, impotence, snake bite, diabetes, cancer, male infertility and nervous disorders. It is also used as an aphrodisiac and management of Parkinsonism disease, as it's having L-dopamine (Vaidya *et al.,* 1978). The analgesic, anti-inflammatory, anti-epileptic and anti-neoplastic activities of methanol extract of *Mucuna pruriens* has been reported (Gupta *et al.,* 1997). The endocarp of *Mucuna pruriens* is non-toxic and 2-3 times more potent than L-dopa in controlling hyperprolactinemia motor symptoms of Parkinson's disease in animal models (Hussain and Manyam, 1997). *Mucuna pruriens* has also been shown to exhibit neuroprotective effects by increased brain mitochondrial complex-I activity and significantly restoring dopamine and norepinephrine levels in Parkinsonism (Manyam *et al.*, 2004).

The reported literature of *Mucuna pruriens* revealed that it contains a lot of valuable phytoconstituents and has a high impact in the medicinal plants category for the management of disease (Kumar *et al.*, 2014).

The use of *Mucuna pruriens* in treating several diseases gives it more edge over other traditional herbs and its safety and these are main reasons for chosing it for further study. The most important bioactive compounds of plants are alkaloids, flavonoids, tannins, phenolic compounds and sterol (Duke, 1995).

Botanical Classification:

Family: Fabaceae

Subfamily: Faboideae

Genus: Mucuna

Species: pruriens (http://en.wikipedia.org/wiki/*Mucunapruriens*)

2.7.4 Species distribution

Mucuna pruriens is widely distributed in South East Asia. Largely found in Bangladesh, India, Sri Lanka and Malaysia. It is also found in Asia, America and Africa (Suresh *et al*., 2010). It found in bushes and hedges at damp places, ravines and scrub jungles throughout the plains of India. It is cultivated for its pods as vegetable and young leaves as fodder.

2.7.5 Ethno botanical Description of *Mucuna pruriens*

Mucuna pruriens is an annual, climbing shrub with 15 m length. The fuzzy hairs are available in young state but it is completely hairless during old age. The leaves are ovular in shape and leaflets are 2-3 mm long. The flower heads are take form of axially arrayed panicles with 15-32 mm long and have 2-3 or many flowers with lavender, white or purple colures. In the fruit ripening stage about 4 to 13 cm long and 1 to 2 cm wide leguminous fruit develops. The husk is very hairy and carries up to seven seeds. The seeds are flattened uniform ellipsoid, 1 to 1.9 cm long, 0.8 to 1.3 cm wide and 4 to 6.5 cm thick. Its seed pods are about 10 cm long and are covered in loose orange hairs that cause a severe itch if they come in contact with skin. The chemical compounds responsible for the itch are a protein mucunain and serotonin (Chopra *et al.,* 1956; Hishika *et al.,* 1981; Kumar *et al.*, 2014).

2.7.6 Traditional uses of *Mucuna pruriens*

The root is used for the treatment of nervous disorders. Traditionally, the decoction of root is used as blood purifier and diuretics (Kumar *et al.*, 2014). Seed are used as CNS stimulant like coffee bean. It has several functions like to treat Parkinson's disease, impotence and as a diuretic. Sometimes it has antidepressant activity (Kumar*et al.*, 2014). Aerial parts in Ayurvedic and Unani system of Medicine, the whole plant has been used for diabetes, gout and rheumatoid (http://www.iloveindia.com/indianherb/mucuna-pruriens.html.).

2.8 *In Vitro* and *in vivo* Pharmacological Activities of *Mucuna Pruriens*

2.8.1 Anti-Parkinson's activity

The clinical syndrome of Parkinsonism was identified in ancient India even before the period of

Christ and was treated methodically. According to “Bhasava rajyam" Parkinsonism was treated by the administration of powdered seed of *M. pruriens* containing 4 to 6 % of L-DOPA (Ovallath and Deepa, 2013). Hussain and Manyam (1997) indicated that for the same dose, *M. pruriens* showed twice the anti-Parkinsonian activity of synthetic L-DOPA. In a clinical study, Nagashayana *et al*. (2000) revealed the contribution of L-DOPA in the recovery of PD followed by Ayurveda medication. Katzenschlager *et al*. (2004) revealed that 30 g Mucuna seed powder preparation has considerable faster action in treating PD patients than conventional standard drugs, namely, L-DOPA or Carbidopa and suggested that natural source of L-DOPA might possess advantages over conventional drugs in long term management of PD.

2.8.2 Antiglycaemic effect

Using a combination of chromatographic and NMR techniques, the presence of D-chiro-inositol and its two galacto-derivatives having antiglycaemic effect was demonstrated in *M. pruriens* seeds (Donati *et al*., 2010).

2.8.3 Hypoglycemic activity

The hypoglycemic effect of the aqueous extract of the seeds of *M. pruriens* was investigated in normal, glucose load conditions and streptozotocin (STZ)-induced diabetic rats. In normal and STZ diabetic rats, the aqueous extract of the seeds of *M. pruriens* (100 and 200 mg/kg body weight) significantly reduced the blood glucose levels 2 hrs after oral administration of seed extract. It also significantly lowered the blood glucose in STZ diabetic rats after 21 days of daily oral administration of the extract. Thus, it was clearly depicted that *M. pruriens* could be a source of hypoglycemic compounds (Bhaskar *et al*., 2011).

2.8.4 Antioxidant activity

In vitro assays indicated that the whole plant ethyl acetate and methanolic extract of *M. pruriens*, containing large amounts of phenolic compounds, exhibited high antioxidant and free radical scavenging activities. These plant extracts served as a significant source of natural

antioxidant, which might be helpful in preventing the progress of various oxidative stresses (Satheesh *et al.*, 2010; Kumar and Muthu, 2010).

2.8.5 Antivenom activity

Research on its effects against Naja species (Tan *et al*., 2009) has shown it has potential use in the prophylactic treatment of snakebites. Aqueous extracts of *M. pruriens* seeds were tested for their activity on various pharmacological effects like lethality, phospholipase activity, edema forming activity, fibrinolytic activity and haemorrhagic activity of cobra and krait venoms. About 0.16 and 0.19 mg of *M. pruriens* seed extracts were able to completely neutralize the lethal activity of LD_{50} of cobra and krait venom, respectively, thus suggesting that aqueous extracts of M. pruriens seeds possess compounds, which inhibit the activity of cobra and krait venoms (Meenatchisundaram and Michael, 2010). According to Fung and Tan (2012), rats pretreated with *M. pruriens* seed extract showed protective effect against the lethal and cardiovascular depressant effects of *Naja sputatrix* venoms by neutralization of the venom toxins.

2.8.6 Aphrodisiac activity

Shukla and Mahdi (2010) demonstrated that oral administration of 5 g of *Mucuna* seed powder once in a day for men with decreased sperm count and motility ameliorated psychological stress and seminal plasma liquid peroxide levels along with improved sperm count and motility. The study also concluded that *M. pruriens* not only reactivates the antioxidant defense mechanism, but also helps in the management of stress and improves semen quality.

2.8.7 Antimicrobial activity

M. pruriens is also used for antimicrobial properties for extracting plant metabolites against plant pathogenic bacteria and fungi. The methanolic extract showed high antibacterial activity against *Erwinia carotovora, Pseudomonas syringae, Pseudomonas marginalis, Pseudomonas acruginosa, Xanthomonas campestris* and high antifungal activity against *Curvularia lunata, Fusarium oxysporum, Pencillium expansum, Rhizoctonia solani, Tiarosporella phaseolina,*

Ustilago pomaydis (Rayavarapu and Kaladhar, 2011).

2.8.8 Anthelmintic

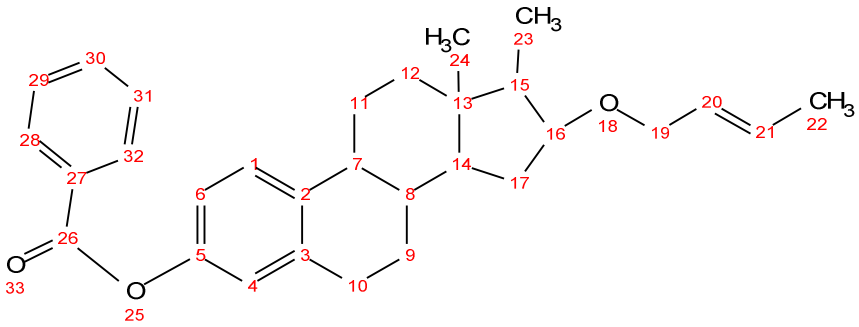
The spicules have in the past been (and may still be) sold commercially as "itching powder". Up until the 1950s they were also used as an oral vermifuge (anthelmintic) (retrieved from http://www.cdc.gov). [T]he medicinal part of the plant is the hair of the pods, which are generally imported into this country attached to the pod, and from which they are carefully removed, so that they do not fasten to the operator's hands." They report that a decoction or tincture of the spicules has no anthelmintic properties, suggesting that the action is physical: the worms are irritated and expelled alive. Administration was in syrup or molasses, followed a few hours later by a purgative. This treatment was reportedly used in the Caribbean and Central America.

2.9 Isolated Compounds from *Mucuna spp*

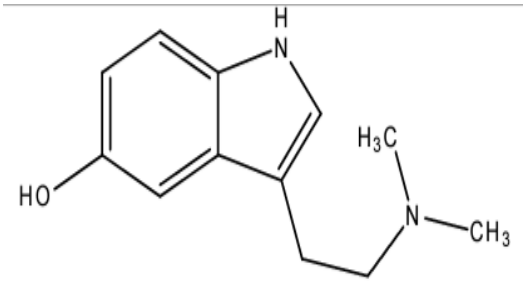
Compound (1) was eluted with methanol and chloroform at the ratio of 70:30. It was isolated as yellow oil (0.6g). Based on combined analysis of HREIMS (m/z 445.41), 1HNMR, 13CNMR and IR spectra, compound (1) was proposed as Estra–2 *ll* –en –17–ol, 3yl benzoate (1) with the molecular formula $C_{29}H_{34}O_4$, m/z 446 calculated. The isolated compound has the skeleton of Estradiol, a sex hormone. The proton in the second hydroxyl is replaced by phenyl oxide. Estradiol protects against brain injury, neurodegeneration and cognitive decline. It protects against stroke injury (Dubal *et al*., 2000)**.** Thus, this compound isolated from *M. pruriens* has biological and physiological activities. The presence of this compound in *M. pruriens* seed may be the reason why the natives use this plant in the treatment of disorders of the male and female reproductive systems, parkinson disease and increase libido in both men and women (Ezueh, 1997). Estradiol has been found to reduce anxiety and depression – like behavior of aged female mice (Walf and Frye, 2005). The isolated compound may also be the reason *M. pruriens* seeds are used by the herbalists as anti- depressant.

Seeds of velvet beans are known to produce the unusual non protein amino acid 3-(3, 4-

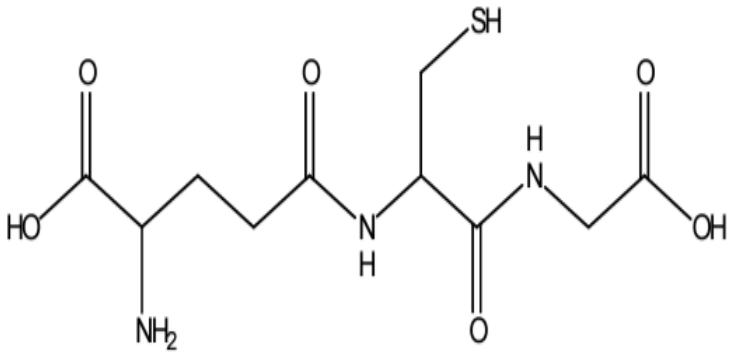
dihydroxyphenyl)-l-alanine (2) (Lorenzetti *et al*., 1998). It also contains glutathione (3), gallic acid (4) and beta sitosterol (5). It has unidentified bases like mucunine, mucunadine, prurienine, prurieninine. Other bases isolated from the pods, seeds, leaves and roots include indole -3- alkyl amines -N,N- dimethyl tryptamine (6). Serotonin (7) is present only in pods (Lorenzetti *et al*., 1998) and 6-methoxyharman (8) in the leaves. The seed also contains oils including palmitic, stearic, oleic and linoleic acids (Mishra, 2006). GC-MS analysis showed the presence of phytochemicals like n-hexadecanoic acid (48.21 %), squalene (7.87 %), oleic acid (7.62 %), ascorbic acid (3.80 %) and octadecanoic acid (6.21 %) were present in the extract (Bhaskar *et al*., 2011). The seed also contains two tetrahydroquinoline alkaloids namely 3-methoxy-1,1-dimethyl-6,7-dihydroxy-1,2,3,4–tetra hydroquinoline (9) and 3-methoxy-1, 1-dimethyl-7, 8-dihydroxy-1, 2, 3, 4- tetrahydroquinoline (10) (Misra and Wagner, 2004). It also contains serotonin, 5-hydorxy tryptophan (11), nicotine (12), N, N-dimethyl tryptamine (13), bufotenine, and N, N-dimethyl tryptamine (14), 5-imethoxy-N, N-dimethyl tryptamine-noxide. The mature seeds of the plant contain about 3.1-6.1 % L-DOPA with trace amounts of serotonin, nicotine, bufotenine, N-dimethyl tryptamine-noxide, and betacarboline (15).



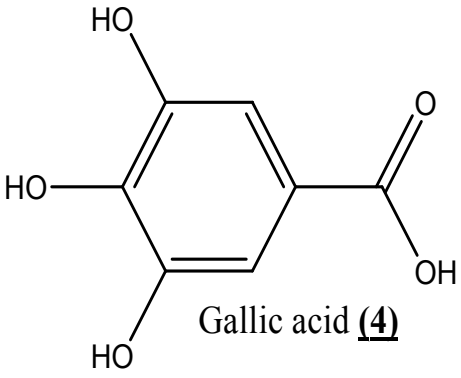
Estra–2 ll –en –17–ol, 3yl benzoate (1)



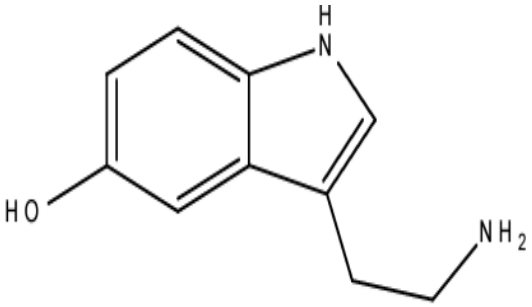
Bufotenine **(2)**



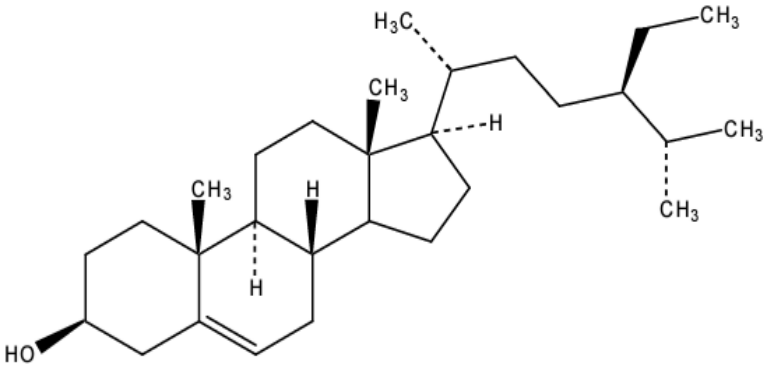
Glutathione **(3)**



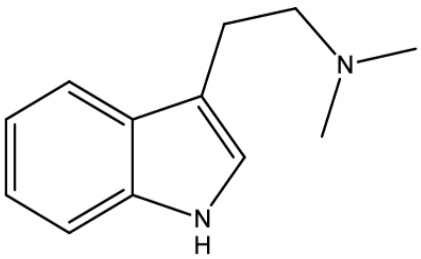
Gallic acid **(4)**



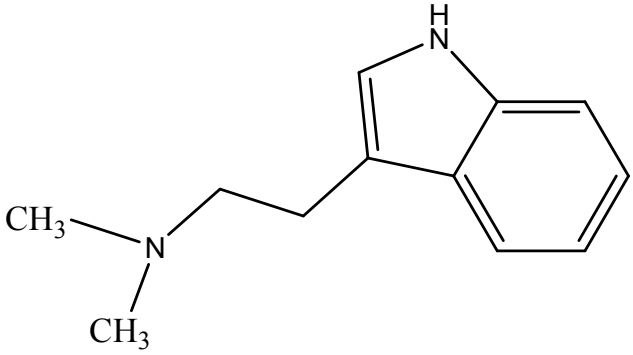
Serotonin **(3)**



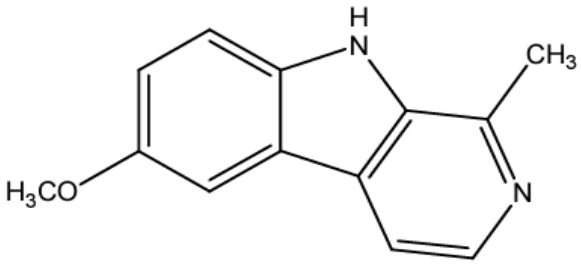
Beta sitosterol **(5)**



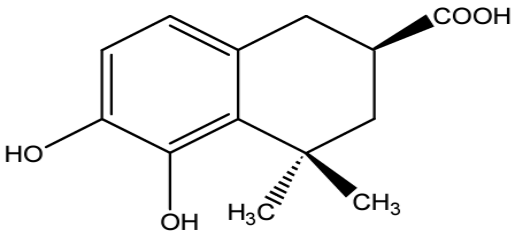
indole -3- alkyl amines -N,N- dimethyl tryptamine **(6)**



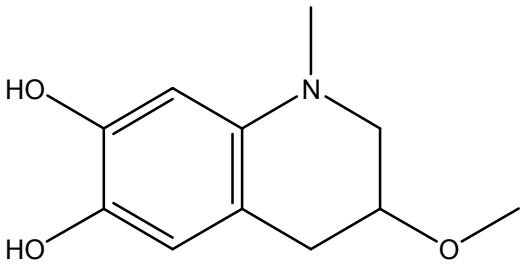
Serotonin **(7)**



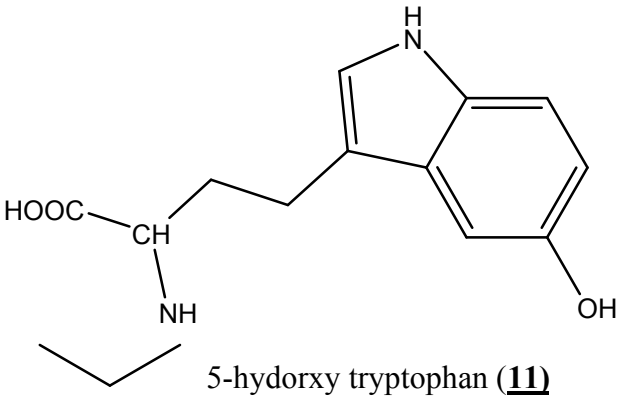
6-methoxyharman **(8)**



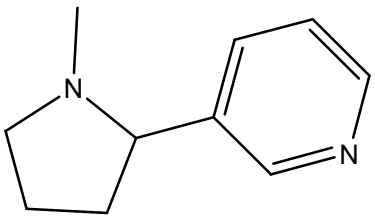
Dimethyl-6,7-dihydroxy-1,2,3,4–tetra hydroquinoline **(9)**



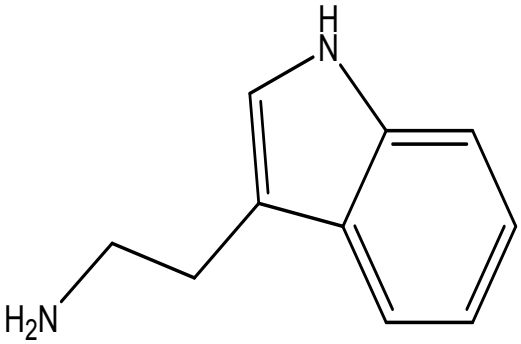
3-methoxy-1, 1-dimethyl-7, 8-dihydroxy-1, 2, 3, 4- tetrahydroquinoline **(10)**



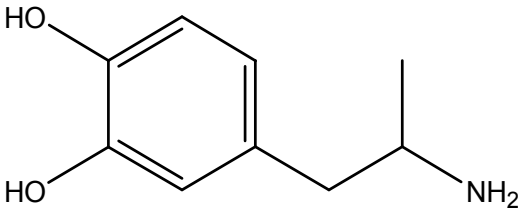
5-hydorxy tryptophan (**11)**



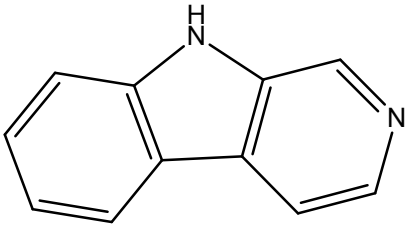
Nicotine **(12)**



N, N-dimethyl tryptamine **(13)**



3-(3, 4- dihydroxyphenyl)-l-alanine **(14)**



Betacarboline **(15)**

2.10 Burseraceae Kunth Family

The Burseraceae Kunth is one of the nine flowering plant families belonging to the order Sapindales Juss. ex Bercht. & J. Pearl that comprise the monophyletic group (5700 species), whose first known fossils appear in Europe 65 million years ago.

The Burseraceae Kunth consists of approximately 18 genera and 700 species. This family is divided into 3 tribes namely Canariceae, Protieae which are determined to be monophyletic and Burserae which was shown to be polyphyletic. The list of genera in the Burseraceae Kunth., approximate number of species, tribal and subtribal in the Burseraceae Kunth., approximate number of species, tribal and subtribal placement.The family is distributed pantropically across a broad range of low-elevation, frost-free habitats including rainforest, dry deciduous forest and deserts (Weeks *et al*., 2005).The Burseraceae Kunth. are best known for producing resins of economic, medicinal, and cultural values such as frankincense, myrrh and copal (Langenheim, 2003).

2.11 The Genus *Canarium* L.

The genus *Canarium* L. probably originated from the North American continent, not Gondwanaland (Weeks *et al.,* 2005). This embraces 75 species of trees which are mainly found in tropical Asia and the Pacific, and a few species in tropical Africa (Leenhouts, 1956), about 9 species were found in the Philippines (Leenhouts, 1956). The geographical centre of their genetic diversity is the Molucca Islands of eastern Indonesia, but their centre of cultivated diversity is undoubtedly western Melanesia. Twenty to 25 species are found in the South Pacific, of which 21 are in Papua New Guinea (Leenhouts ,1956), eight in the Solomon Islands (Evans ,1993) and 3 or 4 in Vanuatu (Wheatley, 1992).

The word *Canarium L.* (Leenhouts, 1956). *Canarium* L. species often produce edible kernels, called canarium nut which are of commercial interest: *Canarium indicum* L., *Canarium solomonense* B.L. Burtt, *Canarium harveyi* Seem, *Canarium odontophyllum* Miq. and *Canarium album* L. Another economical interest of *Canarium* L. species, is the

production of resins used in foods (*Canarium luzonicum* Miq.), in the making of incense and varnishing. In spite of these commercial potentials of *Canarium* L., little attention has been given to the collection and conservation of *Canarium* L. species (Wiart, 2006). The genetic diversity thus derogates at an alarming rate.

2.11.1 Species distribution

It does neither suggest that the species can be planted in every ecological zone within that country, nor that the species can not be planted in other countries than those depicted. Since some tree species are invasive, you need to follow biosafety procedures that apply to your planting site (Orwa *et al*., 2009).

Native range: Angola, Cameroon, Ethiopia, Ghana, Guinea-Bissau, Liberia, Mali, Nigeria, Senegal, Sierra Leone, Sudan, Tanzania, Togo, Uganda, Zambia and Exotic range: Indonesia

2.12. Botanical Features of *Canarium* L.

The members of the genus *Canarium* L. consist of medium to large buttressed trees up to 40-50m tall, or rarely a shrub. The barks are greenish grey, fawn or light yellow brown that are usually smooth, scaly or dippled with many small lenticels. Outer bark are thin while the inner barks are pinkish brown or reddish brown, laminated, soft and aromatic with a clear sticky or rarely oily exudate. The stems are usually terete. The leaves are pinnate, spiral and stipulated. The rachis is terete flattened to channel swollen at base, and bears 5-21 folioles. The folioles are oblique at base, entire, dentate or serrate at margin, often thick and acuminiate at apex. The secondary nerves are arching and joined near margin. The tertiary nerves are reticulate. The infloresence is an axillary or terminal panicle. The calyx is cupular. The corolla includes 3 creamy petals. The androecium comprises a whorl of 6 stamens. The disc within the stamens is 6 lobed. The gynaecium consist of 3 carpels united into a 3 lobular ovary. The drupes are seated on a persistent enlarged calyx and enclose a woody stone.

2.13. Traditional and Medicinal Uses of *Canarium* L.

Elemi (British Pharmaceutical Codex, 1934) is an oleoresin exuded through the bark of *Canarium luzonicum* Miq. or *Canarium commune* L. which has been used in the form of an ointment as a stomach stimulant and as an expectorant (Pernet, 1972). The barks of *Canarium indicum* L. has been used for chest pains whereas the oil has been patented for treatment of arthritis pain and the oleoresin of the tree is applied as a poultice for ulcerated wounds. The resin of *Canarium tonkinense* Engl. has been used as a stimulant, rubefacient and anti-rheumatic when applied externally. The oleoresin has been applied as ointment for ulcers (Jules and Paull, 2006). The dried fruit of Chinese olive or *Canarium album* (Lour.) Raeusch. is used to treat bacterial and viral infections, inflammation, poisoning and for detoxification (Zhiyong *et al*., 2008).

In Chinese folk medicine, the dried fruits of *Canarium album* (Lour.) Raeusch have been used for treatment of angina, dysentery, snake bites, cough-hematemesis, enteritis, diarrhoea, toxicosis from swellfish and alcohol (Zhao *et al*., 2010). *Canarium schweinfurthii* Engl. is used by traditional healers as a remedy for diabetes mellitus in southern Senegal (Kamtchouing *et al*., 2006) while in Congo and Central African Republic the plant is used in reducing fever, as stimulant, emollient, in post-partum pain, constipation, malaria, diarrhoea, sexual infections and rheumatism (Koudou *et al.*, 2005). In Indonesia, the bark of *Canarium littorale* Bl. is used to make a decoction taken to heal haemorrhoids (Leenhouts, 1956).

2.14 *Canarium schweinfurthii* Engl.

Canarium schweinfurthii Engl. (Burceraceae) is a forest region plant, wide spread over all Africa, from Senegal to Democratic Republic of Congo, Sudan and Ethiopia. *C. schweinfurthii* locally known as “Bete” is a big tree up to 40 m high and 1.5 m diameter with cylindrical bole scaly bark exuding resin, with buttresses not much developed (Adjanohoun *et al*., 1996). The stem bark decoction of C. *schweinfurthii* is used as a remedy for roundworms, colic, stomach

pains, after child birth, dysentery and gonorrhoea (Berhaut, 1974). Many plant species are known in folk medicine of different cultures to be used for their hypoglycaemic properties and therefore used for treatments of diabetes mellitus (Kamtchouing *et al*., 1998; Kamtchouing *et al*., 2006).

2.15. *In vitro* and *In vivo* Pharmacological Activities of *Canarium* L.

Only 12 % of 75 species have been studied for their pharmacological activities. Extracts and pure compounds derived from *Canarium* L. were reported to have a variety of pharmacological activities such as antioxidant, antibacterial, antifungal, antitumor, anti-inflammatory, hepatoprotective, analgesic and anti-diabetic

2.15.1 Antioxidant

Antioxidant activities were reported on *Canarium album* (Lour.) Raeusch, *Canarium odontophyllum* Miq. and *Canarium schweinfurthii* Engl. A tonic soup made of *Canarium album* (Lour.) Raeusch used mainly in China displayed significant antioxidant activity by 1,1,-diphenyl-2-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assay (Guo *et al*., 2008). Tannins extracted from the leaves, twigs and stem barks of *Canarium album* (Lour.) Raeusch showed potent antioxidant activity in the DPPH radical scavenging activity with IC_{50} values of 56.86 µg/ml, 62.31 µg/ml and 54.80 µg/ml, respectively and ferric reducing power of 4.28, 3.74 and 4.49 mmol equivalent of dried tannin (Zhang and Lin, 2008). Pure compounds from this species include brevifolin (16) and quercetin-3-galactoside (17) which showed free radical scavenging activity in DPPH assay (Guo *et al*., 2008).

The essential oil of *Canarium schweinfurthii* Engl. was tested for the antioxidant activity with the DPPH assay and by ß-carotene bleaching test. It exhibited highest antioxidant activity at 150 µg/ml activities in both assay (Obame *et al.,* 2007).The ethyl acetate fraction of peel of *Canarium odontophyllum* Miq exhibited 95±1.00% scavenging activity at the concentration of 40µg/ml (Anand *et al*., 1992; Obame *et al*, 2007). The bark and leaf ethanol extracts of

Canarium patentinervium Miq exhibited excellent antioxidant activities in the DPPH assay with EC_{50} value of 2.33 µg/mL and 2.93µg/ml. It exhibited 77.80±0.01 and 65.80±0.01 % DPPH radical scavenging activity at the concentration of 10µg/ml (Mogana *et al*, 2011).

2.15.2 Antibacterial and antifungal activities

Antibacterial activities were reported in *Canarium schweinfurthii* Engl. and *Canarium patentinervium* Miq. Dichloromethane extract of *Canarium schweinfurthii* Engl. had bactericidal activity against Gram-negative *Vibrio cholerae* with minimum inhibitory concentration (MIC) of 0.62 mg/ml while the ethylacetate extract was active against Gram-positive and Gram-negative bacteria namely *Staphylococcus aureus* and *Proteus vulgaris* with MIC values of 10mg/ml and 5mg/ml respectively. Ethanol extract was active against Gram-negative *Vibrio cholerae* and *Proteus vulgaris* with MIC values of 0.62 mg/ml and 10 mg/ml respectively (Moshi *et* al., 2009).

In a separate disc diffusion assay, the essential oil of *Canarium schweinfurthii* Engl. abrogated the survival of Gram-negative *Salmonella enterica*, Gram-positive *Streptococcus pyogens* and *Staphylococcus aureus* with an inhibition zone of 27 mm, 25 mm and 18 mm respectively. However the author did not inform on the concentration of the extract per disc. The oil was also fungicidal against Candida albicans with an inhibition zone of 23 mm (Obame *et al*, 2007).

The ethanol extract of leaves and barks and hexane extract of barks of *Canarium patentinervium* Miq, exhibited significant antimicrobial activity against Gram-positive bacteria *Staphylococcus aureus, Bacillus cereus, methicillin-resistant Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa*. The highest sensitivity obtained was with the ethanol extract of the leaves which inhibited the growth of Gram-positive *Staphylococcus aureus, Bacillus cereus, methicillin-resistant Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* with an inhibition zone of 14 mm, 13 mm, 13 mm and 15 mm respectively. Disc diffusion assay was done at a concentration of 2 mg/disc and the extracts were more active than ampicillin and streptomycin.

2.15.3 Hepatoprotective activities

Hepatoprotective activity was exhibited in *Canarium manii* King and *Canarium album* (Lour.) Raeusch. The biflavanoid agathis flavone from *Canarium manii* King preserved the integrity of the liver cells membrane of rodents as evidenced by the decrease in the CCl_4-induced rise of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels. GPT which is predominantly found in the liver showed a dose-dependent and significant reduction (Anand *et al*., 1992).

The triterpenes urs-12-ene-3α,16ß-diol (18) and olean-12-ene-3 α,16ß-diol (19) from *Canarium album* (Lour.) Raeusch markedly reduced the amount of alanine aminotransferase leakage from the primary cultured hepatocytes intoxicated with 0.2 mM of D-galactosamine (GaIN). The phenols brevifolin protected rat hepatocytes against GaIN-induced injury (Ito *et al*., 1990).

2.15.4 Anti-diabetic activity

Anti-diabetic activity was reported in the methanol/methylene chloride extract of stem bark of *Canarium schweinfurthii* Engl. At a dose of 300 mg/kg there was 67.1 % reduction in blood glucose levels after a once daily subcutaneous injection on streptozotocin-induced diabetic male rats over 14 days, versus insulin that had 76.8 % reduction. Weight gain was only 6.6 % as opposed to untreated rats that had lost 14.1 % of body weight. There was also significant reduction in food and fluid consumption by 68.5 % and 79.7 %. These results showed the extract could reverse hyperglycemia, polyphagia and polydipsia provoked by streptozotocin, thus having anti-diabetic activity (Kamtchouing *et al*., 2006).

2.15.5 Antitumor potential

Antitumor potential was demonstrated by the chloroform and ethanol extract of bark of *Canarium patentinervium* Miq significant with GI_{50} value of 23.44 µg/ml and 34.40 µg/ml respectively via the MTT biological assay. Extracts were most sensitive to the breast cancer cell line MDA 468 (Mogana *et al*., 2011).

2.15.6 Other Biological Activities

The essential oil of *Canarium schweinfurthii* Engl. collected from the region of Cameroon inhibited the enzymatic activity of lipoxygenase with an IC50 value of 62.6ppm (Donati *et al*., 2010). However, in a separate study the same species collected from central African region did not show any activity in the cotton pellet induced granuloma formation experiment (Koudou *et al*., 2005). This may suggest possible evidence of variation in secondary metabolites according to locations.

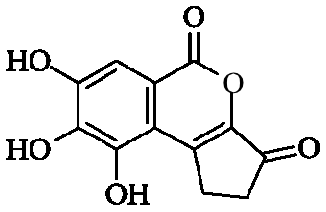
The essential oil of *Canarium schweinfurthii* Engl. at a dose of 1, 2 and 3 mg/ kg i.p. displayed potent analgesic effect in the acetic acid-induced writhing and hot plate experiments (Koudou *et al*., 2005).

2.16 Isolated Compounds from Canarium spp

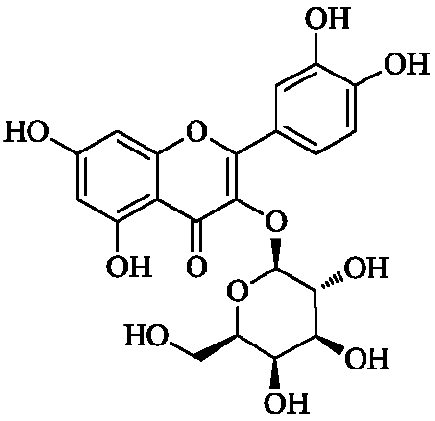
Isolation and structure elucidations of secondary metabolites in Canarium L. has been carried out since the 50s (Bhuvanendran *et al.,* 1950). Majority of investigations include the resin and the fruit of the species.

To date about 99 compounds have been isolated from 9 species, including *Canarium schweinfurthii* Engl*., Canarium boivinii* Engl*., Canarium odontophllum* Miq*., Canarium manii* King*., Canarium album* (Lour) *Raeusch, Canarium zeylanicum* (Retz.) Blume*, Canarium commune L., Canarium muelleri* F.M. Bailey and *Canarium bengalense* Roxb. The extensively researched species are *Canarium boivinii* Engl and *Canarium album* (Lour.) Raeusch (Zhiyong *et al*., 2008; Zhao *et al.,* 2010; Zhang and Lin, 2008; Zhiyong *et al.,* 2009).

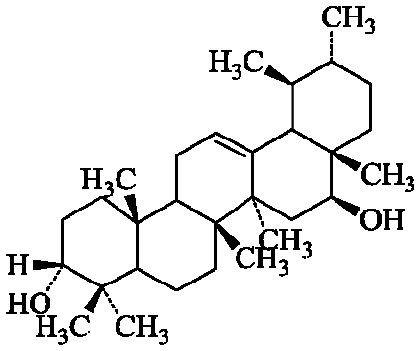
Among the secondary metabolites isolated from members of the genus *Canarium* L. are terpenes (monoterpenes, triterpenes, tetraterpenes like carotenoids, sesquiterpenes, cyclohexane and sterols), carboxylic acids, coumarins, furans, lipids and phenols (flavonoids, tannins, phenolic acids). The main secondary metabolites isolated so far from the genus *Canarium* L. consists of 58 terpenes and 11 flavonoids.



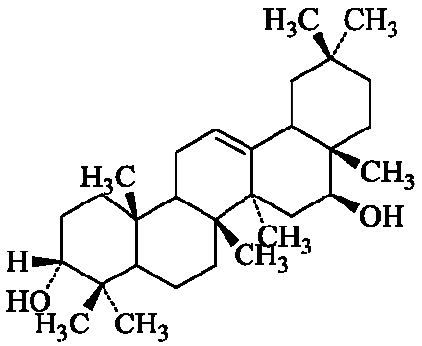
Brevifolin **(16)**



Quercetin-3-galactoside **(17)**



urs-12-ene-3α, 16ß-diol (**18)**



olean-12-ene-3α, 16ß diol (**19)**

2.17. Microbial Agents

2.17.1 Infectious disease

Since their introduction, antimicrobials (antibiotics) have played an essential role in decreasing morbidity due to infectious diseases. Antimicrobials are used for treatment of infections and for prophylaxis against infections in humans and animals, for growth promotion in food animal rearing and in agriculture. However infectious diseases remain the leading cause of death worldwide and bacteria have become more resistant to conventional antibiotic in recent years. The widespread use of these compounds is thought to further encourage the emergence of antimicrobial resistance (WHO, 1999). The number of resistant pathogenic bacteria grows at an alarming rate worldwide and the search for novel antimicrobial agents from medicinal plants to combat such pathogens has become crucial for avoiding the emergence of untreatable bacterial infections (Pfaller *et al*., 1998).

2.17.2 Chemotherapeutic agents: Factors affecting their effectiveness

The ideal chemotherapeutic agent has a high therapeutic index with selective toxicity, thereby resulting in lethal damage to pathogens through the inhibition of cell wall synthesis, protein synthesis or nucleic acid synthesis, as well as through the disruption of the cell membrane and the inhibition of certain essential enzymes. This results in selective disruption of the specific structure and/or function essential to bacterial growth and survival, without causing similar effects to its eukaryotic host (Prescott, 1996).

The efficacy of antimicrobial agents is influenced by a number of factors. Firstly, it is of obvious importance that the antimicrobial agent reaches the site of the infection.

This greatly depends on the stability of the drug, its lipophilic or hydrophilic nature, its absorption from a specific site and the presence of blood clots or necrotic tissue, the latter of which may protect the pathogen against the antibiotic. Secondly, the susceptibility of the pathogen to the particular chemotherapeutic agent is of utmost importance, as well as the specific growth phase in which the pathogen is in at that particular stage (Prescott, 1996).

2.17.3 Antimicrobial drug resistance

Bacteria have evolved many different mechanisms of resistance. These can be classified as: alteration in, or addition of, the target site of antimicrobial binding; alteration in access to the target site for instance the decreased permeability of cell wall or efflux mechanisms; and inactivation of the antimicrobial binding. Furthermore resistance may arise through mutation or by acquisition of resistance genes by horizontal transmission from another bacterial species (WHO, 1999).

On the other hand, drug resistance may be brought about by the limited drug diffusion into the biofilm matrix, enzyme-mediated resistance, genetic adaptation, efflux pumps, as well as through the adaptation of the outer microbial membrane, the latter occurring either through the lack of or through the overexpression of certain membrane proteins (Cloete, 2003).

This phenomenon of increased drug resistance, combined with the multiplicity of side effects by existing agents and the emergence of diseases for which no treatment yet exists, makes the search for new antimicrobial agents a highly relevant and important subject for research. For centuries, plants have been used in the traditional treatment of microbial infections. This assembly of knowledge by indigenous peoples about plants and their products continue to play an essential role in health care of a great proportion of the population (Iwu *et al*., 1999).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Chemicals/Reagents

i. Dimethylsulphoxide (BDH)

ii. Lugol's iodine

iii. Acetic anhydride (BDH)

iv. Hydrochloric acid (BDH)

v. Ethylacetate (BDH)

vi. Hexane (BDH)

vii. EthanoI(BDH)

viii. Chloroform (BDH)

ix. Dragendroff's reagent

x. Mayer's reagent

xi. Potassium hydroxide (BDH)

xii. Albendazole (Supharma Chem)

xiii. Sodium hydroxide (BDH)

xiv. Ferric Chloride

3.1.1 Equipment/Materials

i. Magnetic stirrer/Heater (Thermolyne Nouva II)

ii. Rotatory Evaporator (Buchi R-210)

iii. Bruker-Advance 400MHz NMR Spectrometer

iv. UV-Visible Spectrophotometer(UV 752 PEC Medical USA)

3.1.2 Parasite

Ascaris suum

3.1.3 Microorganisms

Methicillin Resistant staphylococcus aureus, *Vancomycin Resistant Enterococci*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillcus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Shigella dysenteriae*, *Candida albicans*, *Candida Stellatoidea*, *Candida tropicalis* and *Candida krusei*

3.2 Plant Materials

The fresh leaves of *Mucuna pruriens* (Plate I), *Canarium schweinfurthii Engl* leave (Plate II) and *Canarium schweinfurthii Engl* stem bark (Plate III) were collected from Shika, Zaria and Shendan Local Government Area of Pleateau state, in September 2011 and were identified and authenticated by a taxonomist; Mallam Musa Mohammed of the Herbarium of the Department of Biological Sciences, Ahmadu Bello University. Voucher specimens number 392 and 7232 for *M. pruriens* and *C. schweinfurthii* respectively were deposited at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria, Kaduna State, Nigeria.

3.3 Reference Drug

The reference drug (albendazole) used in this study was bought from a local Veterinary Clinic. This drug was chosen due to its broad spectrum activity in the prevention and treatment of gastrointestinal helminthes in domestic animals (Tagbota and Townson, 2008). This drug was diluted in distilled water to obtain a stock solution with a concentration of 10 mg/ml, which was serially diluted to obtain the same concentrations as with the organic extracts. About 1.25 % DMSO in distilled water was used as controls.

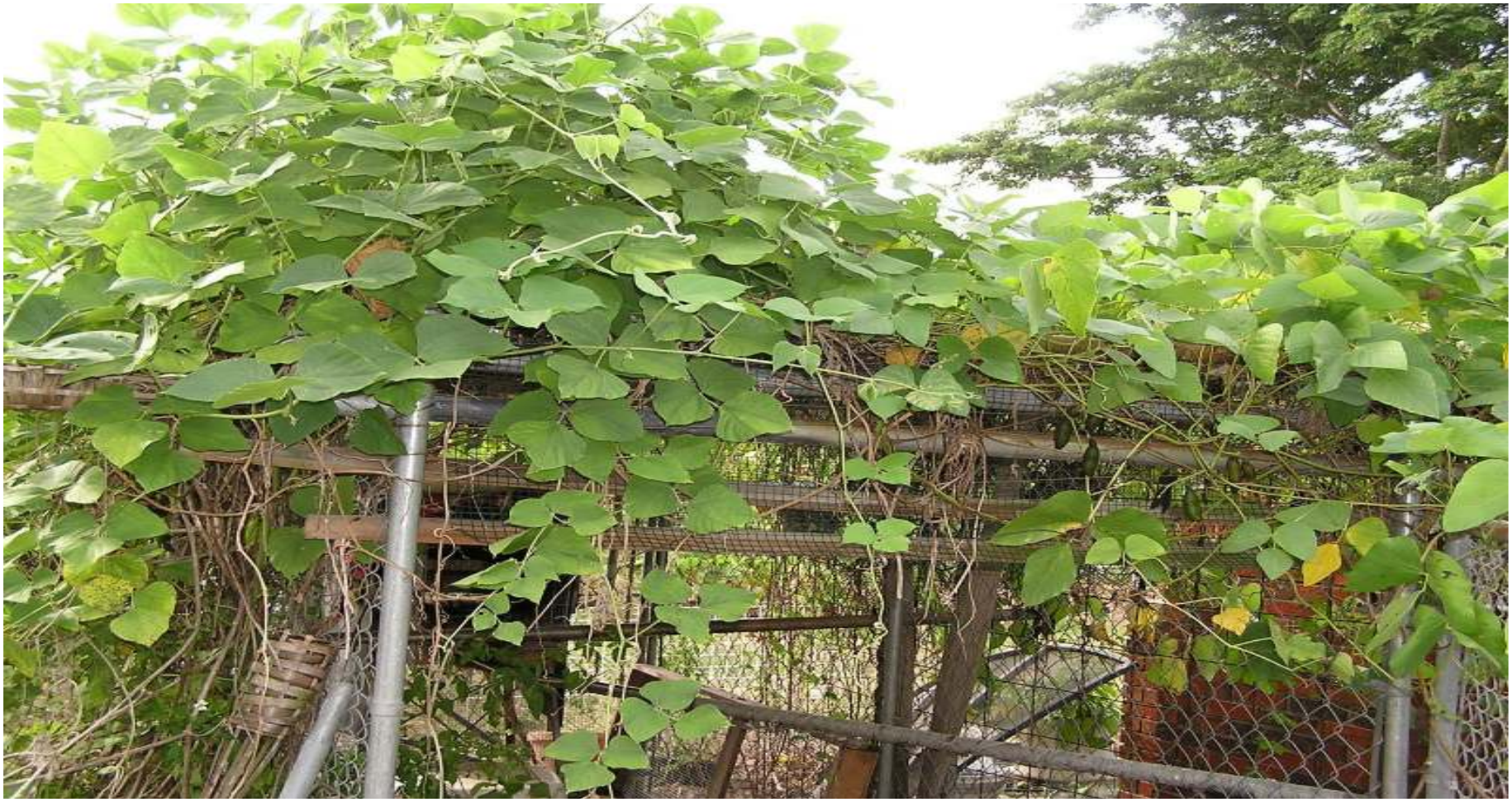


Plate I: The Leaves of *Mucuna pruriens* (L.) DC. var. *utilis* (Wall. ex Wight) Baker ex Burck



Plate II: *Canarium schweinfurthii* Engl Leaves



Plate III: *Canarium schweinfurthii* Engl Stem Bark

3.4 Extraction of Materials

The plant materials were air dried at ambient temperature, pulverized, and stored a in air tight containers and for exhaustive extraction.

The respective materials (1.5 kg) were extracted successively with methanol (2L x 3). The filtered extracts were combined, and the solvent evaporated *in vacuo* to yield the crude extracts. The percentage yields of the crude methanol extracts were determined and recorded using the equation below (Huie, 2002).

$$\%\, recovery\ yield = \left(\frac{mass\ of\ extract\ (g)}{mass\ of\ dried\ plant\ material\ (g)}\right)100$$

3.5 Preliminary Phytochemical Screening

The methanol extracts were analyzed for phytochemicals using standard methods as described by Ciulei (1964); Harbone (1973); Mojab *et al*., (2003) and Tchamadeu *et al*., (2010) for the presence of alkaloids (Meyer's and Drangedorffs test), tannins (Gelatin test), saponins (foaming test), flavonoids (Alkaline reagent test), glycosides and phytosterol and phenols .

3.5.1 Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of a dirty yellow coloured precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.

3.5.2 Detection of glycosides

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution.

Formation of pink colour in the ammonical layer indicates the presence of anthranol glycosides.

3.5.3 Detection of saponins

Froth Test: Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes foam was produced indicating the presence of saponins.

3.5.4 Detection of phytosterols

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken and allowed to stand. Golden yellow colour indicated the presence of triterpenes.

3.5.5 Detection of flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. An intense yellow colour, which becomes colourless on addition of dilute acid, was observed and this indicates the presence of flavonoids

3.5.6 Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. The presence of phenols, was confirmed by the formation of bluish black colour .

3.5.7 Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. A white precipitate formed confirms the presence of tannins

3.6 Antimicrobial Evaluation

3.6.1 Preparation of inoculum

The test organisms *Methicillin Rest staph aureus* (MRSA)*, Vancomycin Rest Enterococci* (VRE), *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillcus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Shigella dysenteria*, *Candida albicans*, *Candida krusei*, *Candida tropicalis* and *Candida*

Stellatoidea were obtained from the Department of Medical Microbiology, A.B.U Teaching Hospital, Zaria, Kaduna State, Nigeria. Stock cultures were maintained at 5 ^{o}C on slants of nutrient agar. Active stock culture was inoculated in fresh tubes of Muller–Hinton broth medium (MHB) and the bacteria and fungi were incubated for 24 h at 37 ^{o}C for bacteria and 7 days for fungi respectively. Fifteen fresh subculture Muller–Hinton agar slants were prepared and stored in refrigerator at 5 ^{o}C for future use.

3.6.2 Antibacterial/fungal susceptibility test

Spectrum of antibacterial activity was studied by using the technique described by Bauer *et al.*(1966).The crude methanol extracts of the *M. pruriens* and *C. schweinfurthii* (leave and stem bark) were investigated for their antibacterial and antifungal activities against eleven bacteria and four Candida strains. Ciprofloxacin, erythromycin and fluconazole sensitivity disc were used as a positive antibacterial and antifungal controls while DMSO was taken as negative control. At the end of incubation, inhibition zones formed around the well were measured with transparent ruler in millimeter.

3.6.3 Determination of minimum inhibitory concentration

The Broth Dilution method (Thornsberry and Donald, 1975) was employed in this determination. Mueller Hinton broth was prepared, 10mL was dispensed into test tubes and sterilized at 121 ^{o}C for 15 mins, and the broth was allowed to cool. McFarlands turbidity scale number 0.5 was prepared to give turbid solution. 10 mL of normal saline was dispensed into sterile test tubes and the test microorganisms were inoculated into the different test tubes containing the normal saline. Incubation of the normal saline was done at 37 ^{o}C for 6h. Dilution of the test microorganisms in the normal saline was done continuously until the turbidity marched that of the McFarland's scale by visual comparison. At this point the test microbe has a concentration of about 1.5×10^{8} cfu/ml. Two-fold serial dilution of the extracts in the sterile broth was made to obtain the concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml

and 6.25 mg/ml. The initial concentration was obtained by dissolving 0.5 g of the extracts in 10 ml of the sterile broth. Having obtained the different concentrations of the extracts in the broth, 0.1 ml of the test microorganism in the normal saline was then inoculated into the different concentrations; incubation was done at 37 ^{o}C for 24 h after which the broth was observed for turbidity (growth).

3.6.4 Determination of minimum bactericidal/fungicidal concentration

Mueller Hinton agar was prepared, sterilized at 121 ^{o}C for 15 min, 20 ml was poured into sterile petri dishes and was allowed to cool and solidify. The contents of the MIC in the serial dilution were subcultured into the prepared medium. Incubation was done at 37 ^{o}C for 24 h, after which the plates were observed for colony growth

3.7 Anthelmintic Activity of Crude Plant Extracts

3.7.1 Collection and identification of worms

The eggs recovery method as described by Jabbar *et al*. (2007) was used in this study. Eggs for this experiment were obtained from live adult females of *Ascaris suum* collected from the abomasum of naturally infected pig at the slaughter slab in Sabon-Gari, Zaria. The intestines were collected and transported to Helminthology laboratory of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University Zaria, and processed according to the method described by Jabbar *et al*., (2007). In the laboratory, the intestines were opened up and the ascarid worms removed then the adult worms were put in a bottle containing cool normal saline (pH: 7.2, 4°C) (Fairbairn, 1970), and were used for the in-*vitro* anthelminthic study. The *A. suum* was identified by Mallam Yusuf Magagi of the Department of Parasitology and Entomology of Veterinary Medicine Faculty, Ahmadu Bello University Zaria.

3.7.2 Culture of *Ascaris suum* eggs

The eggs (Plate IV) were washed with 0.5 M KOH solution into a beaker. The eggs were then

agitated gently in the 0.5 M KOH solution for 30 minutes in order to dissolve the sticky albuminous layer and allow for uniform sampling (Fairbairn, 1970). The preparation were placed in centrifuge tubes and centrifuged at 1500 rpm for 3 minutes to recover the eggs. The supernatant was decanted and the eggs washed three times with distilled water and also with embryonating fluid (0.1M sulphuric acid) for the same period. The eggs collected were suspended in the embryonating fluid, and transferred to the petric dishes and incubated for 21 days in an oven at 25 ^{o}C for the egg hatch assay (EHA).

3.7.3 Egg hatch assay

The egg hatch assay (EHA) was conducted as reported by McGaw *et al*., (2007) and Bizimenyera *et al*., (2006). The counted number of eggs in a 0.5 ml of egg suspension was pipetted into test tubes. In addition, the plant extract (0.5 ml) were added at different concentrations of 10, 20, 40 and 80 µg/mL to the different test tube. A commercial anthelmintic drug (albendazole®) was used as the positive control at the same concentrations and DMSO as a negative control. All tests were carried out in triplicate.

The test tubes were incubated under humidified conditions at 25 ºC for 48 hours and thereafter a drop of Lugol's iodine solution was added to each well to stop further hatching. All unhatched eggs and L_1 larvae were then counted. Inhibition percentages were calculated using the method of Cala *et al*., (2012).

$$\% \text{ inibition} = \frac{(\text{Eggs} + L_1) - L_1}{(\text{Eggs} + L_1)} \times 100$$

3.7.4 Larval development assay

The larval development assay (LDA) was conducted as described by Bizimenyera *et al*., (2006). The counted number of eggs in an 0.5 mL of the egg suspension was put into each test tube with a 100 µl of lyophilized penicillin-streptomycin to combat fungal growth. The contents of the tubes were then mixed, and placed in an incubator under humidified

conditions at ambient temperature of 28 ^{o}C for 48 hours for incubation of the eggs. After 48 hours, 0.5 mL of the extracts of *M. pruriens* and *C. schweinfurthii* (leave and stem bark) as well as albendazole® as a positive control at 10, 20, 40 and 80 µg/mL were added to respective plates. The negative control plates received 0.5 mL of DMSO. All experiments were replicated three times. Incubation of the tubes was continued for 21 days, after which all the tubes were examined to determine the survival of larvae at different concentrations. All the L_1, L_2 and L_3 stage larvae in each well were counted and a percentage inhibition of larval development was calculated using the formula (Cala *et al*., 2012):

$$\% \text{ inhibition} = \frac{(L_1 + L_2 + L_3) - L_3}{L_1 + L_2 + L_3} \times 100$$

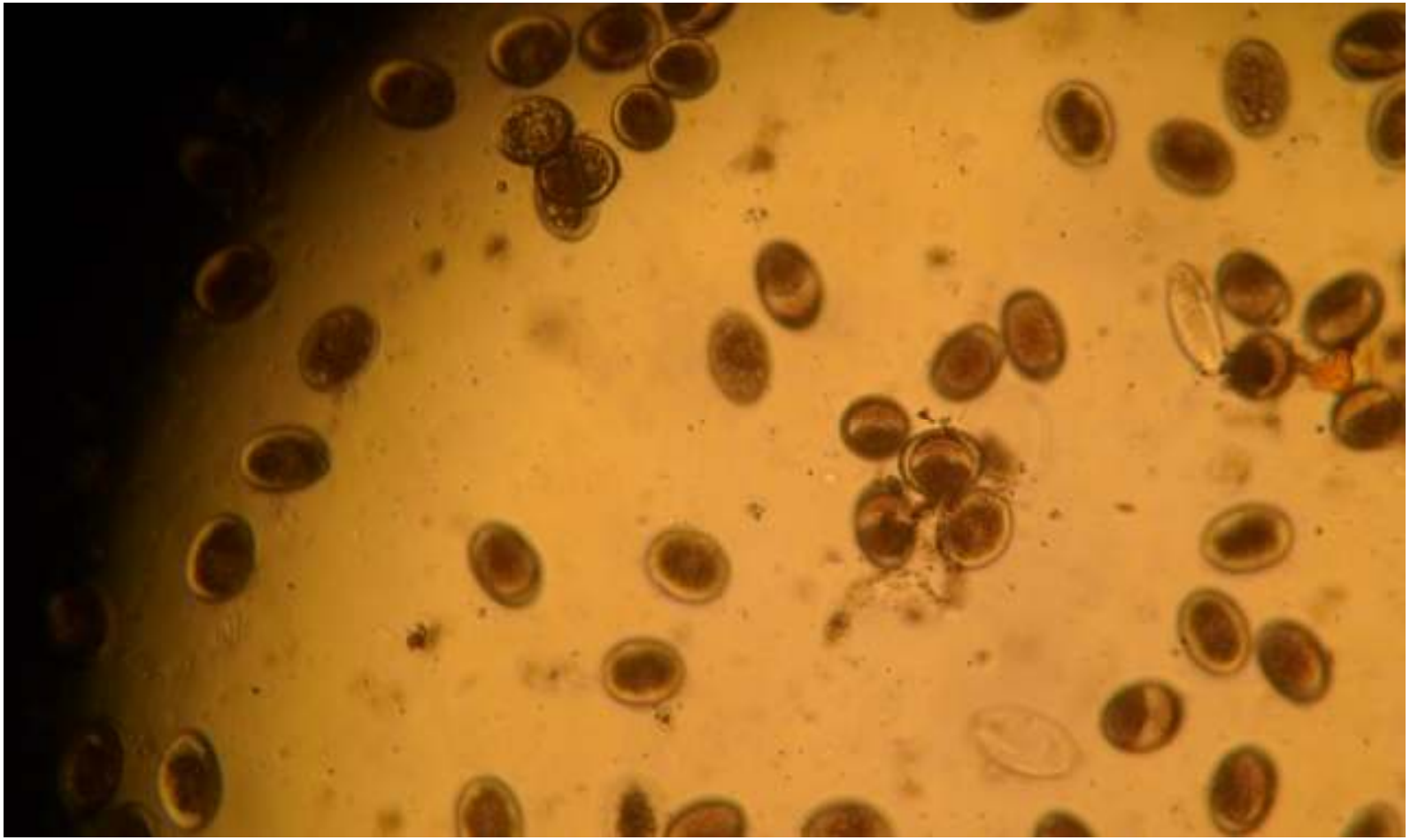


Plate IV: Eggs of *Ascaris suum*



Plate V: Second Larval Stage (L_2) of *Ascaris suum*

3.8 Column Chromatography

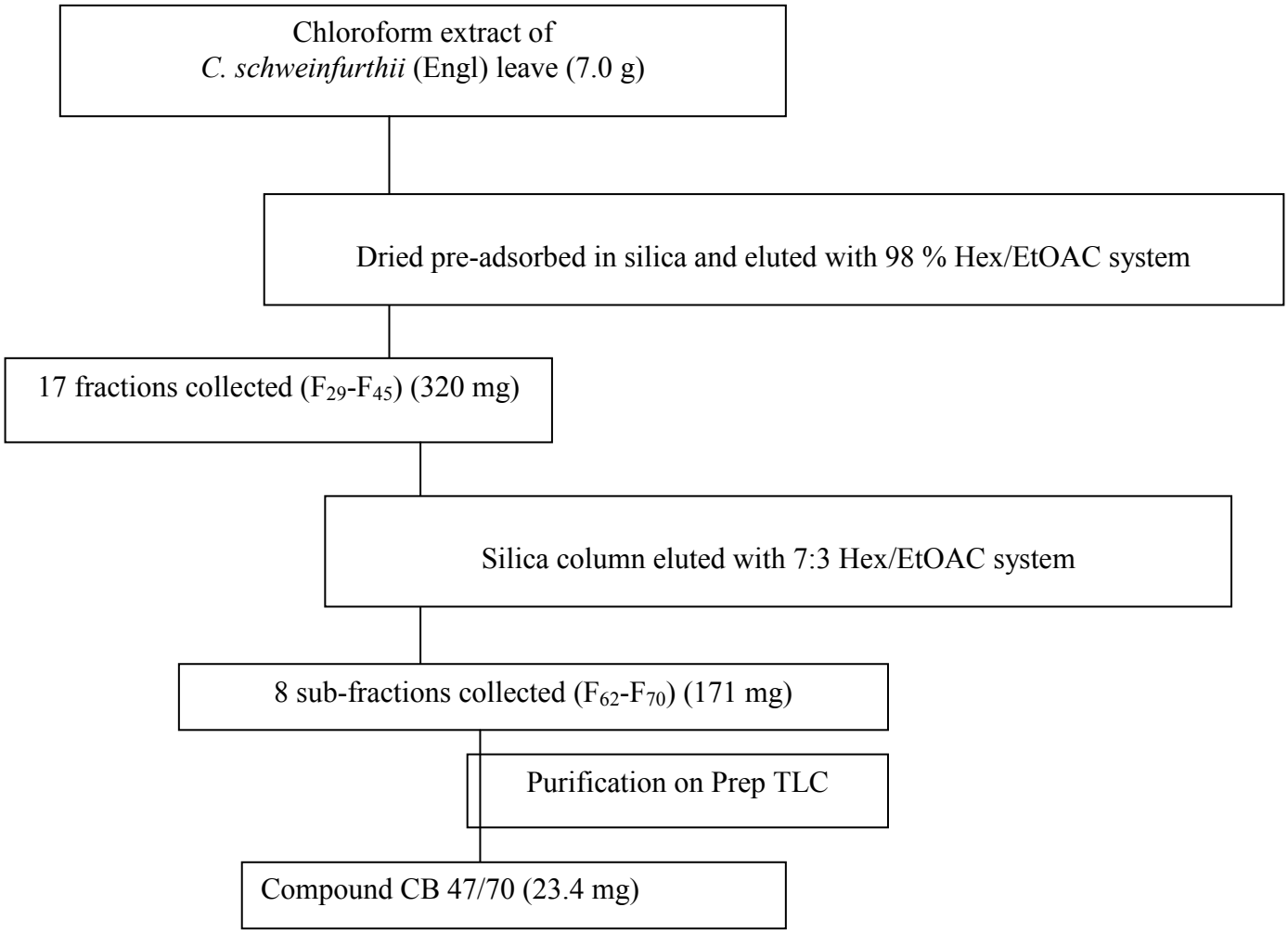
The crude methanol extracts was loaded in a 1 m length open column loaded with silica gel and eluted with neat 1.5 L n-hexane, chloroform ethyl acetate and methanol respectively. The most potent crude fractions (about 5-7 g) of the various extracts were subjected to column chromatography (CC) on silica gel eluted with EtOAc-hexane solvent system and monitored by a thin-layer chromatographic plate and the fractions of similar R_f values were pooled together.

3.9 Isolation of Bioactive Compounds

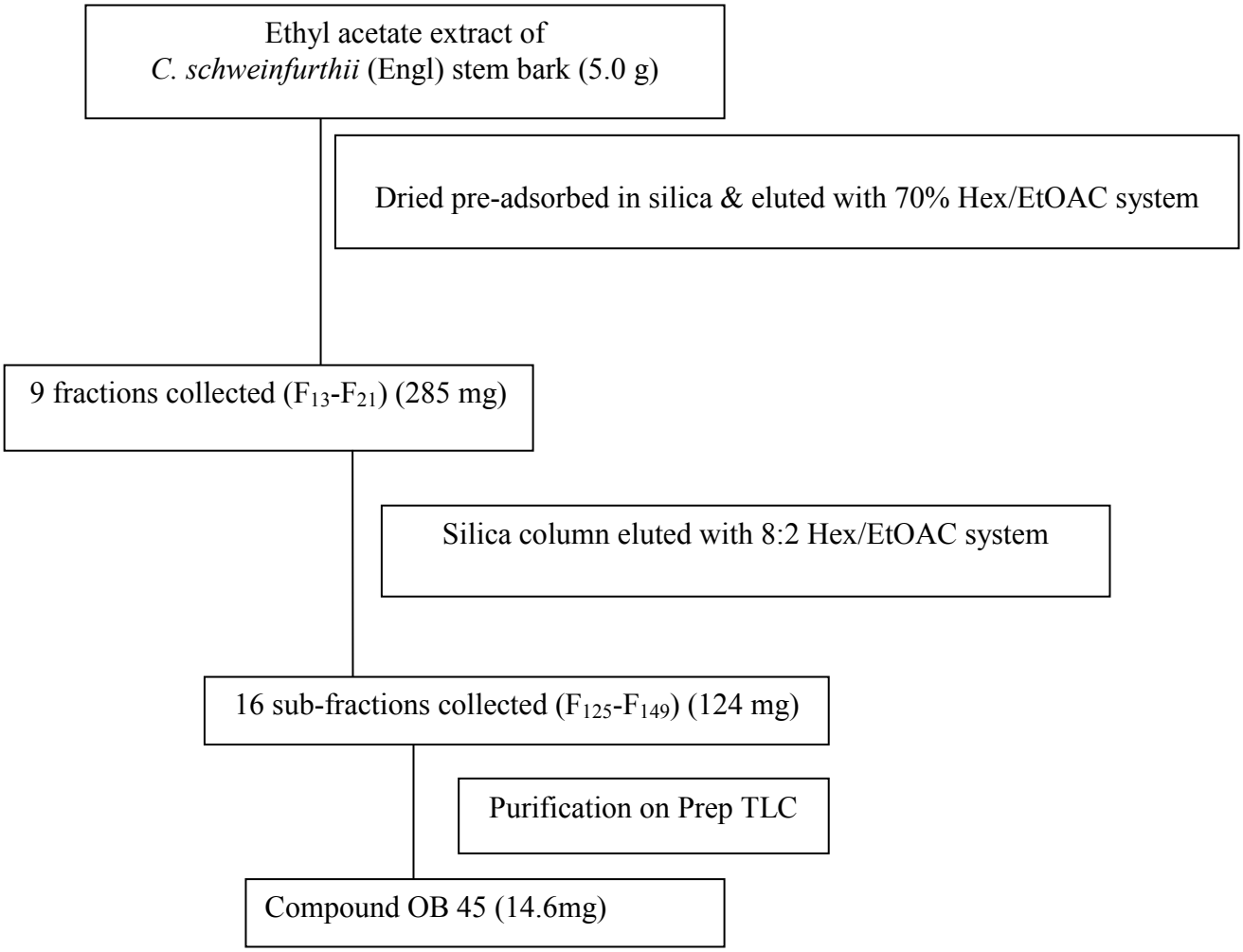
The most sensitive crude extracts from the antimicrobial and anthelemintic screening were subjected to fractionation by column chromatography (CC). Not less than five grams (5 g) of the extract was dissolved in the appropriate solvent and pre-adsorbed on 5.0 g silica gel (qualikens 60-120 mesh). The dried pre-adsorbed extract was transferred to a mortar and ground to give a fine powder and was added as a uniform layer on top of the column. The hexane descended on a horizontal line indicating that the column was well packed. The isolation schemes for the different compounds are presented in scheme 3.1 – 3.4.

3.10 Preparative Thin-Layer Chromatography

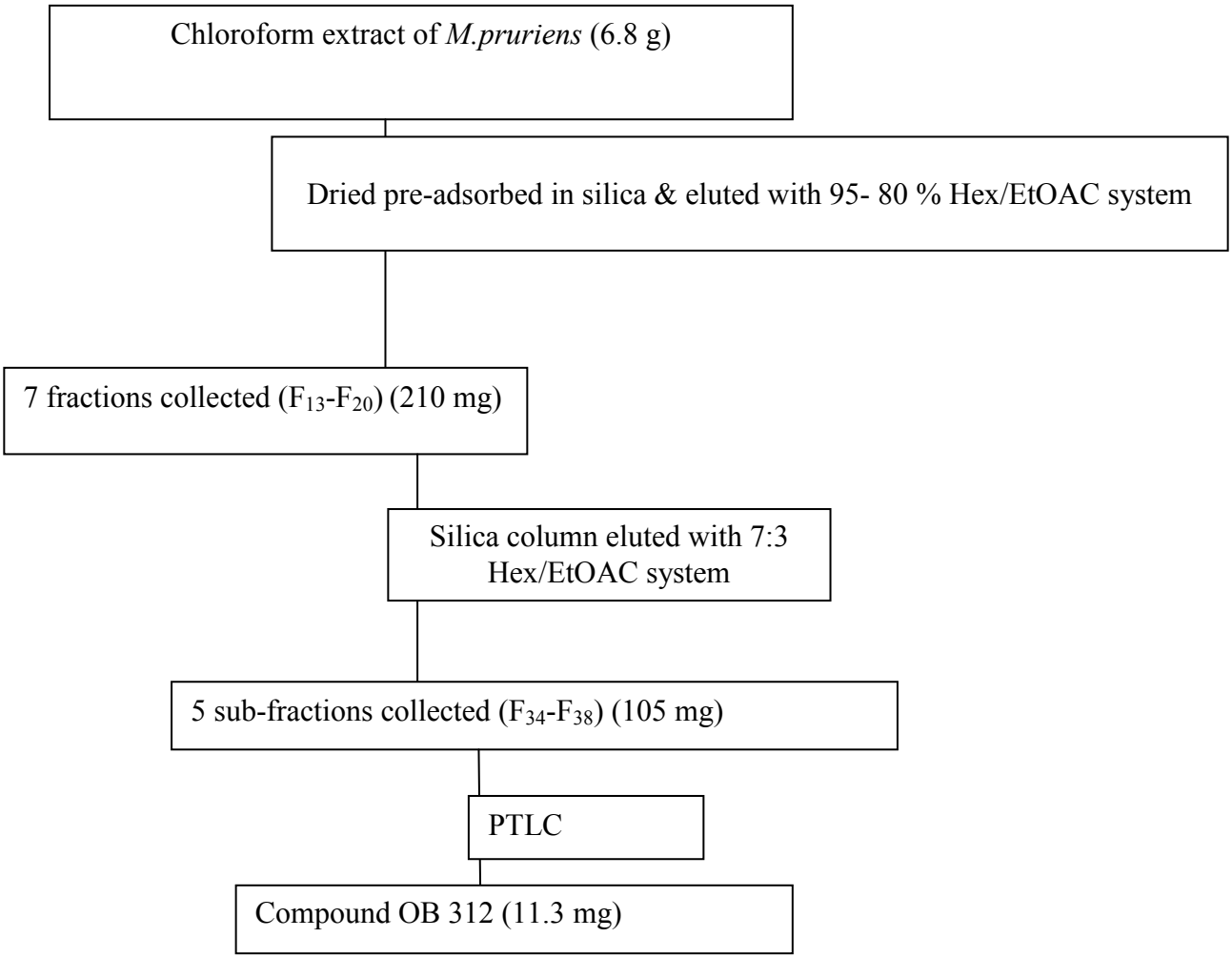
The isolated compounds were further purified by preparative thin-layer chromatography (PTLC). The glass plates were lined with the sample, 1.5 cm from the bottom of the plate. The samples were loaded onto the plates by using a Pasteur pipette from one end of the plate to the other end. The plates were developed in a chromatography tank with hexane:EtOAc solvent system and the compounds of interest on the extreme end detected under UV light and in some cases by the use of 20 % sulphuric acid. The bands of the isolates were marked-out with a ruler, scrapped and dissolved in the appropriate hexane:EtOAc solvent system and then filtered. The filtrates were allowed to dry and packed into glass bottles for spectroscopic analysis.



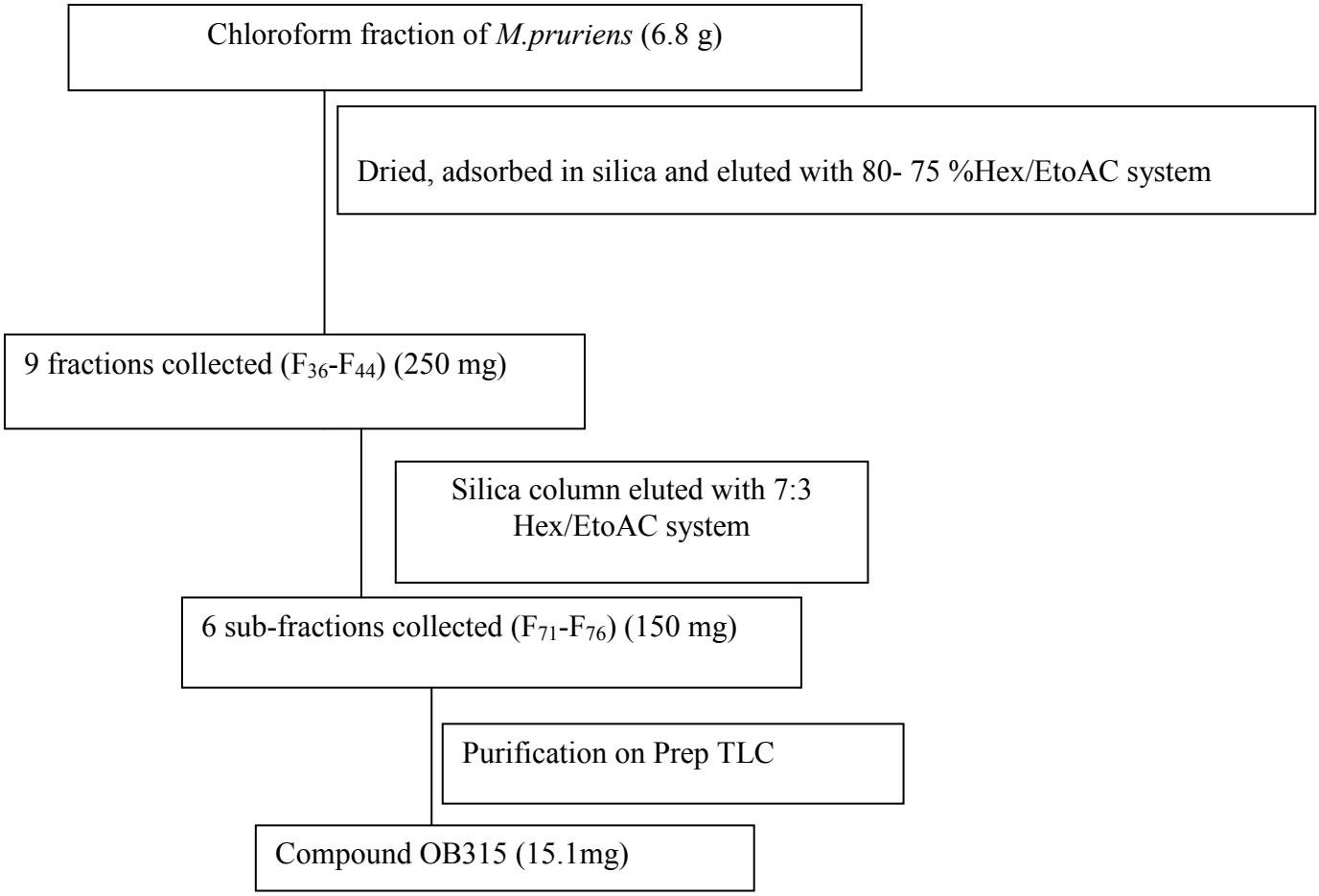
Scheme 3.1: Flow Chart for the Isolation of Compound CB 47/70



Scheme 3.2: Flow Chart for the Isolation of Compound OB 45



Scheme 3.3: Flow Chart for the Isolation of Compound OB 312



Scheme 3.4: Flow Chart for the Isolation of Compound OB 315

3.11 Statistical Analysis

Values are expressed as Mean ± S.E.M. Randomized Complete Block Design Analysis of Variance was used for statistical analysis. P Values less than 0.05 were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1. Extraction

M. pruriens and *C. schweinfurthii* (leaves and stem bark) (1.5 kg) were exhaustively extracted with methanol and percentage recovery yield is presented in Table 4.1.

4.2. Column Chromatographic Separation

The crude methanol extracts were eluted with neat hexane, chloroform, ethyl acetate and methanol and the percentage extractive values of eluted methanol extracts as presented Table 4.2.

4.3 Phytochemical Screening of Extracts

The extracts of *M. pruriens* and *C. schweinfurthii* (leave and stem bark) were subjected to phytochemical screening and the results are presented in Table 4.3. Preliminary phytochemical screening of the plant extracts showed the presence of various phytochemical constituents such as tannins, saponins, flavonoids, phytosterols, alkaloids and cardiacglycosides.

4.4 Antimicrobial Screening

The test micro-organisms were tested for their sensitivity/resistance towards the extracts, isolates, antibactrerial and anticandida positive controls. The zone of inhibition (mm) of the extracts and isolates were recorded in Table 4.4-4.6 and Table 4.7 respectively.

4.5. Minimum Inhibitory Concentration (mg/ml)

The minimum inhibitory concentration of the extract and isolates were carried out to establish the minimum inhibition concentration (MIC) required to inhibit the growth of the test organism and are presented in Table 4.8 and Table 4.9.

Table 4.1: Percentage Recovery Yield of Plant Extracts

Plant sample	Colour	Mass of extracts(g)	Percentage recovery yield (%)
M. pruriens	Green	279	18.6
C. schweinfurthii leave	Green	235.5	15.7
C. schweinfurthii stem bark	Reddish Brown	333	22.2

Table 4.2: Percentage Extractive Values of Eluted Methanol Extracts

	C.schweinfurthii stem bark			*C.schweinfurthii* leave			*M. pruriens*		
Fraction	Colour	Weight (g)	Recovery yield (%)	Colour	Weight (g)	Recovery yield (%)	Colour	Weight (g)	Recovery yield (%)
Hexane	Yellow	1.5	13.5	Orange	3.5	17.8	Orange	0.5	2.3
Chloroform	White	8.0	30.8	Green	4.2	21.3	Green	7.4	33.3
Ethyl acetate	Green	5.6	21.5	Green	5.6	28.4	Green	4.8	21.6
Methanol	Reddish Brown	10.7	41.1	Dirty green	4.8	24.4	Brown	8.6	38.7

Table 4.3: Phytochemical Screening of Extracts

	C. schweinfurthii **leave extracts**				***C. schweinfurthii*** **stem bark extracts**				***M. pruriens*** **extracts**			
Phytochemical	I	II	III	IV	I	II	III	IV	I	II	III	IV
Alkaloid	-	-	+	+	-	-	-	-	-	+	+	-
Cardiac glycoside	-	-	+	+	-	-	+	+	-	+	+	+
Saponins	-	-	-	+	-	-	+	+	-	-	-	-
Phytosterols	+	+	+	+	+	+	+	-	+	+	+	-
Phenols	-	-	-	+	-	-	+	+	+	+	-	+
Flavonoids	-	+	+	+	+	+	+	+	+	-	+	+
Tannins	-	-	+	-	-	-	+	+	-	-	+	+

Key: I-Hexane extract, II- Chloroform extract, III- Ethyl acetate extract and IV- Methanol extract
[+]-Present and [-]- Absent

Table 4.4: Zone of Inhibition (mm) of the Stem Bark Extracts *C. Schweinfurthii* against the Test Microorganisms

		C. schweinfurthii stem bark				Control		
S/N	**TEST ORGANISM**	**Hexane**	**Chloroform**	**Ethylacetate**	**Methanol**	**Ciprofloxacin**	**Erythromycin**	**Fluconazole**
1	*Methicillin Rest staph aureas*	20.2	26	22	29	35	0	-
2	*Vancomycin Rest Enteriococci*	21.0	24	22	0	0	32	-
3	*Staphylococcus aureus*	23.1	28	24	27	37	35	-
4	*Streptococcus pyogenes*	0	0	0	0	35	32	-
5	*Bacillcus subtlis*	23	25	33	27	40	37	-
6	*Escherichia coli*	20	0	27	25	35	35	-
7	*Salmonella typhi*	20	23	25	24	42	0	-
8	*Kebsiella pneumoniae*	21	20	28	26	40	37	-
9	*Pseudomonas aeruginosa*	0	0	0	0	0	32	-
10	*Proteus mirabilis*	0	0	0	0	0	37	-
11	*Shigella dysenteriae*	28	26	22	28	37	38	-
12	*Candida albicans*	26	23	25	24	-	-	35
13	*Candida krusei*	17	18	24	20	-	-	32
14	*Candida tropicalis*	0	0	0	0	-	-	37
15	*Candida stellatoidea*	0	0	0	0	-	-	32

Table 4.5: Zone of Inhibition (mm) of the Leave Extracts *C. Schweinfurthii* against the Test Microorganisms

S/N	TEST ORGANISM	*C. schweinfurthii* leave extract				Control		
		Hexane	Chloroform	Ethyl acetate	Methanol	Ciprofloxacin	Erythromycin	Fluconazole
1	*Methicillin Rest staph aureas*	20	26	22	29	35	0	-
2	*Vancomycin Rest Enteriococci*	21	24	22	0	0	32	-
3	*Staphylococcus aureus*	23	28	24	27	37	35	-
4	*Streptococcus pyogenes*	0	0	0	0	35	32	-
5	*Bacillcus subtlis*	23	25	33	27	40	37	-
6	*Escherichia coli*	20	0	27	25	35	35	-
7	*Salmonella typhi*	20	23	25	24	42	0	-
8	*Kebsiella pneumoniae*	21	20	28	26	40	37	-
9	*Pseudomonas aeruginosa*	0	0	0	0	0	32	-
10	*Proteus mirabilis*	0	0	0	0	0	37	-
11	*Shigella dysenteriae*	28	26	22	28	37	38	-
12	*Candida albicans*	18	23	26	22	-	-	35
13	*Candida krusei*	0	0	0	0	-	-	32
14	*Candida tropicalis*	14	18	24	22	-	-	37
15	*Candida stellatoidea*	20	22	26	21	-	-	32

Table 4.6: Zone of Inhibition (mm) of the *M. pruriens* Extracts against the Test Microorganisms

S/N	TEST ORGANISM	*Mucuna pruriens*				Control		
		Hexane	Chloroform	Ethyl acetate	Methanol	Ciprofloxacin	Erythromycin	Fluconazole
1	*Methicillin Rest staph aureas*	0	0	0	35	35	0	-
2	*Vancomycin Rest Enteriococci*	28	25	22	0	0	32	-
3	*Staphylococcus aureus*	0	0	0	37	37	35	-
4	*Streptococcus pyogenes*	27	22	22	35	35	32	-
5	*Bacillcus subtlis*	30	27	25	40	40	37	-
6	*Escherichia coli*	26	23	21	35	35	35	-
7	*Salmonella typhi*	27	23	20	42	42	0	-
8	*Kebsiella pneumoniae*	0	0	0	40	40	37	-
9	*Pseudomonas aeruginosa*	0	0	0	0	0	32	-
10	*Proteus mirabilis*	25	23	21	0	0	37	-
11	*Shigella dysenteriae*	29	25	22	37	37	38	-
12	*Candida albicans*	26	22	26	20	-	-	35
13	*Candida krusei*	0	0	0	0	-	-	32
14	*Candida tropicalis*	0	25	27	22	-	-	37
15	*Candida stellatoidea*	0	0	0	0	-	-	32

Table 4.7: Zone of Inhibition of the Isolated Compounds against the Test Microorganism (mm)

		Isolated Compounds				Control		
S/N	TEST ORGANISM	CB 47/70	OB 315	OB 45	OB 312	Ciprofloxacin	Erythromycin	Fluconazole
1	*Methicillin Rest staph aureas*	29	0	22	18	35	0	0
2	*Vancomycin Rest Enteriococci*	0	24	22	0	0	32	0
3	*Staphylococcus aureus*	27	28	24	20	37	35	0
4	*Streptococcus pyogenes*	0	0	0	0	35	32	0
5	*Bacillcus subtlis*	32	0	26	22	40	37	0
6	*Escherichia coli*	0	14	0	16	35	35	0
7	*Salmonella typhi*	25	0	22	23	42	0	0
8	*Kebsiella pneumoniae*	29	27	21	0	40	37	0
9	*Pseudomonas aeruginosa*	0	26	0	22	0	32	0
10	*Proteus mirabilis*	0	21	0	18	0	37	0
11	*Shigella dysenteriae*	28	26	22	0	37	38	0
12	*Candida albicans*	26	0	24	0	0	0	35
13	*Candida krusei*	0	21	0	0	0	0	32
14	*Candida tropicalis*	24	18	18	20	0	0	37
15	*Candida stellatoidea*	26	21	20	22	0	0	32

Table 4. 8: Minimum Inhibitory Concentration (mg/ml) of Extracts against the Test Microorganisms

	TEST ORGANISMS	*C. schweinfurthii* leave extracts				*C. schweinfurthii* stem bark extracts				*M. pruriens* extracts			
S/N		I	II	III	IV	I	II	III	IV	I	II	III	IV
1	*Methicillin Rest staph aureus*	25	12.5	6.25	12.5	12.5	12.5	12.5	12.5				
2	*Vancomycin Rest Enterococci*					12.5	12.5	12.5	12.5	12.5	12.5	6.25	12.5
3	*Staphylococcus aureus*	25	12.5	6.25	12.5	6.25	6.25	6.25	12.5				
4	*Streptococcus pyogenes*									12.5	12.5	12.5	12.5
5	*Bacillcus subtilis*	25	12.5	6.25	−	6.25	6.25	6.25	12.5	12.5	6.25	6.25	12.5
6	*Escherichia coli*	25	12.5	6.25	12.5					12.5	12.5	12.5	12.5
7	*Salmonella typhi*	25	12.5	12.5	12.5	6.25	12.5	12.5	12.5	12.5	12.5	6.25	12.5
8	*Klebsiella pneumoniae*	25	12.5	6.25	12.5	6.25	6.25	6.25	12.5				
9	*Pseudomonas aeruginosa*					12.5	12.5	12.5	12.5				
10	*Proteus mirabilis*									12.5	12.5	12.5	12.5
11	*Shigella dysenteriae*	25	12.5	6.25	12.5	12.5	12.5	12.5	12.5	12.5	12.5	6.25	12.5
12	*Candida albicans*	50	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
13	*Candida krusei*					12.5	12.5	12.5	12.5				
14	*Candida tropicalis*	50	12.5	12.5	12.5					12.5	12.5	6.25	12.5
15	*Candida stellatoidea*	25	12.5	12.5	12.5								

Table 4. 9: Minimum Inhibitory Concentration (µg/ml) of the Isolated Compounds against the Test Microorganisms

S/N	TEST ORGANISMS	Isolated compounds			
		CB47/70	OB315	OB45	OB312
1	*Methicillin Rest staph aureus*	6.25		6.25	12.5
2	*Vancomycin Rest Enterococci*	12.5	6.25	6.25	
3	*Staphylococcus aureus*	6.25	6.25	6.25	6.25
4	*Streptococcus pyogenes*				
5	*Bacillcus subtilis*	6.25		6.25	12.5
6	*Escherichia coli*		12.5		12.5
7	*Salmonella typhi*	12.5		6.25	6.25
8	*Klebsiella pneumoniae*	6.25	12.5	6.25	
9	*Pseudomonas aeruginosa*		6.25		12.5
10	*Proteus mirabilis*		12.5		12.5
11	*Shigella dysenteriae*	6.25	6.25	6.25	
12	*Candida albicans*	12.5		6.25	
13	*Candida krusei*		12.5		
14	*Candida tropicalis*	12.5	6.25	12.5	12.5
15	*Candida stellatoidea*	6.25	12.5	6.25	12.5

4.6 Minimum Bactericidal/Fungicidal Concentration (mg/ml)

Minimum bactericidal (MBC)/fungicidal concentration (MFC) was carried out to determine whether the test microbes were killed or only their growths were inhibited. The presence of the colonies on agar plates was an indication that the plant extracts/isolates only inhibited the growth of the organism without killing them and the absence indicated that the plant extracts/isolates were able to kill the organisms. The smallest concentration of the plant extract/isolate that was able to kill the microorganisms was considered as the minimum bactericidal/fungicidal concentration (Table 4.10 and Table 4.11).

4.7 *In-vitro* Anthelmintic Activity

4.7.1 Egg hatch assay

The *In-vitro* anthelmintic assay was carried out on the non-infective stage (egg) of the parasite (*A. suum*). The effect of the extracts and isolates against the eggs of *A. suum* is presented as the percentage inhibition of the eggs into the pre-infective stage (L_1 stage larvae) and the results are presented in Fig 4.1-4.3 and Fig 4.4

4.7.2 Larval development assay

The *In-vitro* anthelmintic assay was then carried out on the pre-infective stage of the parasite (*A.suum*). The effect of the extracts and isolates on the L_1 stage of *A. suum* is presented as the percentage inhibition. The results are presented in Fig 4.5-4.7 and Fig 4.8 respectively.

4.8. UV-Visible Spectroscopy

The isolates were subjected to UV-Visible spectroscopic analysis to determine the wavelength maximum (λ_{max}) which is an indication of the presence of a chromorphore. The results are presented graphically in Fig 4.9

4.9. Isolation of Bioactive Compounds and NMR Spectroscopy

Four compounds were isolated from *C. schweinfurthii* and *M. pruriens* using various isolation techniques which include TLC, CC and characterized with NMR method. The results of the

NMR studies are presented in Fig 4.10-4.35.

Table 4.10: Minimum Bactericial (MBC)/Fungicidal Concentration (MFC) of the Extracts against the Test Microorganisms (mg/ml)

S/N	TEST ORGANISMS	*C. schweinfurthii* leave				*C. schweinfurthii* stem bark				*M.pruriens*			
		I	II	III	IV	I	II	III	IV	I	II	III	IV
1	*Methicillin Rest staph aureus*	50	12.5	12.5	25	50	50	25	50				
2	*Vancomycin Rest Enterococci*					50	50	25	50	25	25	12.5	50
3	*Staphylococcus aureus*	50	12.5	25	25	25	25	12.5	25				
4	*Streptococcus pyogenes*									50	25	25	50
5	*Bacillcus subtilis*	25	12.5	12.5	25	50	25	12.5	25	25	25	12.5	25
6	*Escherichia coli*	50	12.5	25	25					50	25	25	50
7	*Salmonella typhi*	50	12.5	25	25	50	50	25	50	50	50	25	50
8	*Klebsiella pneumoniae*	50	12.5	12.5	25	25	50	25	50				
9	*Pseudomonas aeruginosa*					50	25	25	25				
10	*Proteus mirabilis*									50	25	25	50
11	*Shigella dysenteriae*	50	12.5	12.5	25	50	50	25	50	25	25	12.5	50
12	*Candida albicans*	50	12.5	25	50	25	25	25	25	50	50	25	50
13	*Candida krusei*					50	50	25	50				
14	*Candida tropicalis*	50	12.5	25	50					25	25	25	50
15	*Candida stellatoidea*	50	12.5	25	50								

Key: I-Hexane extract, II- Chloroform extract, III- Ethyl acetate extract and IV- Methanol extract

Table 4.11: Minimum Bactericidal (MBC)/Fungicidal Concentration (MFC) of the Isolated Compounds against the Test Microorganisms (mg/ml)

S/N	TEST ORGANISMS	Isolates			
		CB47/70	OB315	OB45	OB312
1	*Methicillin Rest staph aureus*	12.5		25	25
2	*Vancomycin Rest Enterococci*	25	12.5	12.5	
3	*Staphylococcus aureus*	12.5	12.5	12.5	25
4	*Streptococcus pyogenes*				
5	*Bacillcus subtilis*	12.5	0	12.5	25
6	*Escherichia coli*		50		25
7	*Salmonella typhi*	50	0	25	25
8	*Klebsiella pneumonia*	25	12.5	12.5	
9	*Pseudomonas aeruginosa*		25		25
10	*Proteus mirabilis*		12.5		50
11	*Shigella dysenteriae*	25	25	12.5	
12	*Candida albicans*	25	0	25	
13	*Candida krusei*		50		
14	*Candida tropicalis*	50	25	25	25
15	*Candida stellatoidea*	25	25	25	25

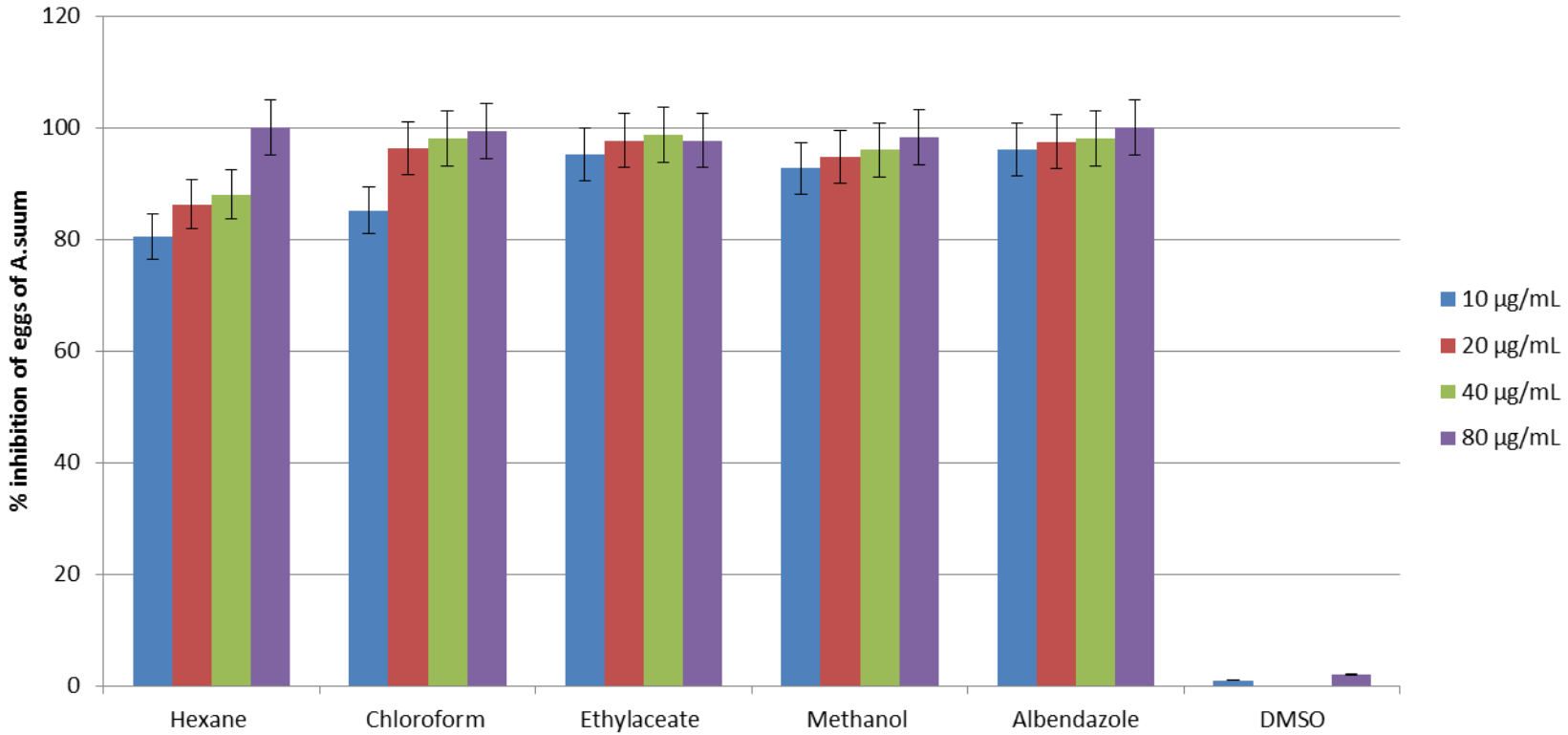


Figure 4.1: % Inhibition of Eggs of *A. suum* by *C. schweinfurthii* Leave Extracts

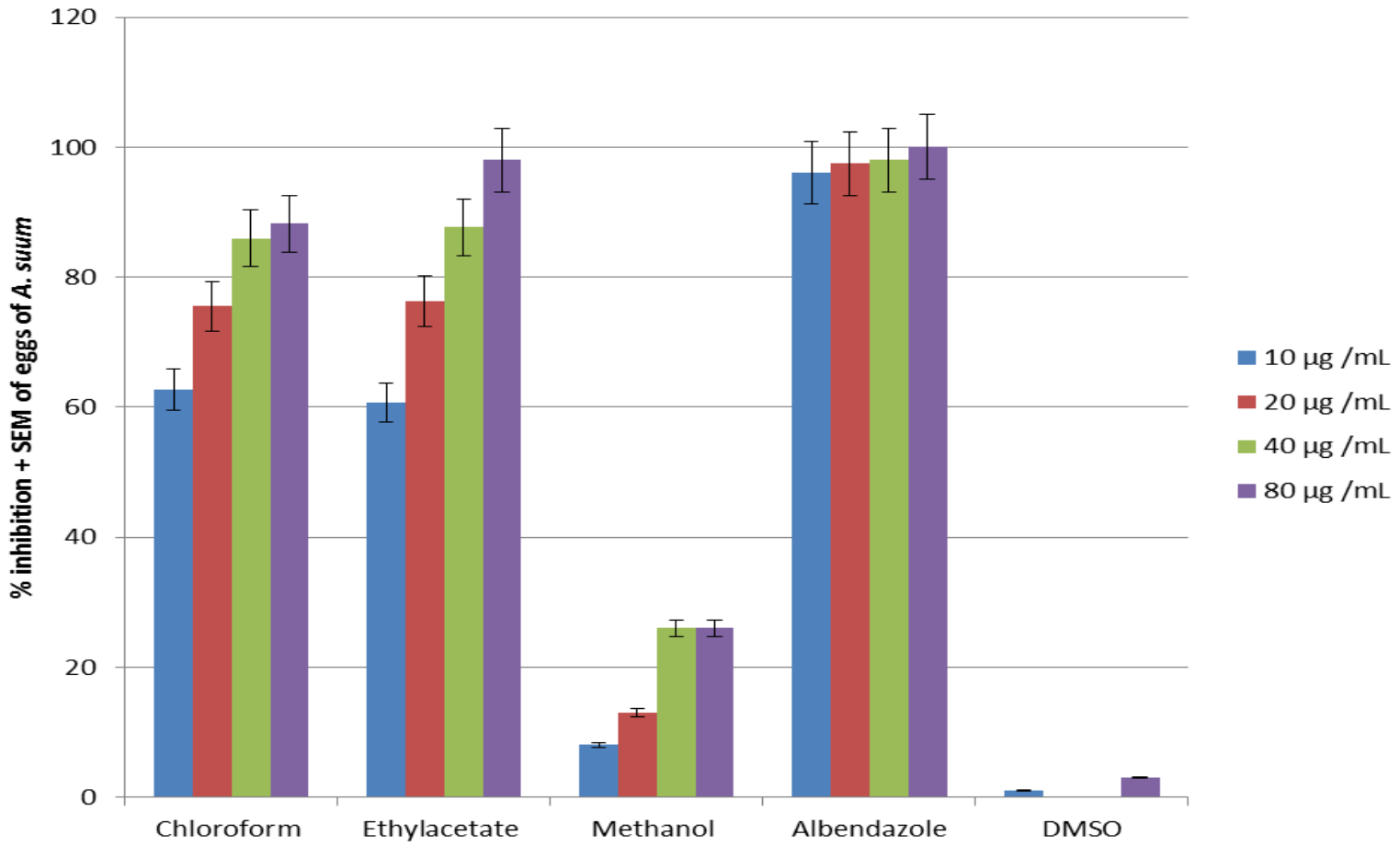


Figure 4.2: **% Inhibition of Eggs of *A. suum* by *C. schweinfurthii* Stem Bark Extracts**

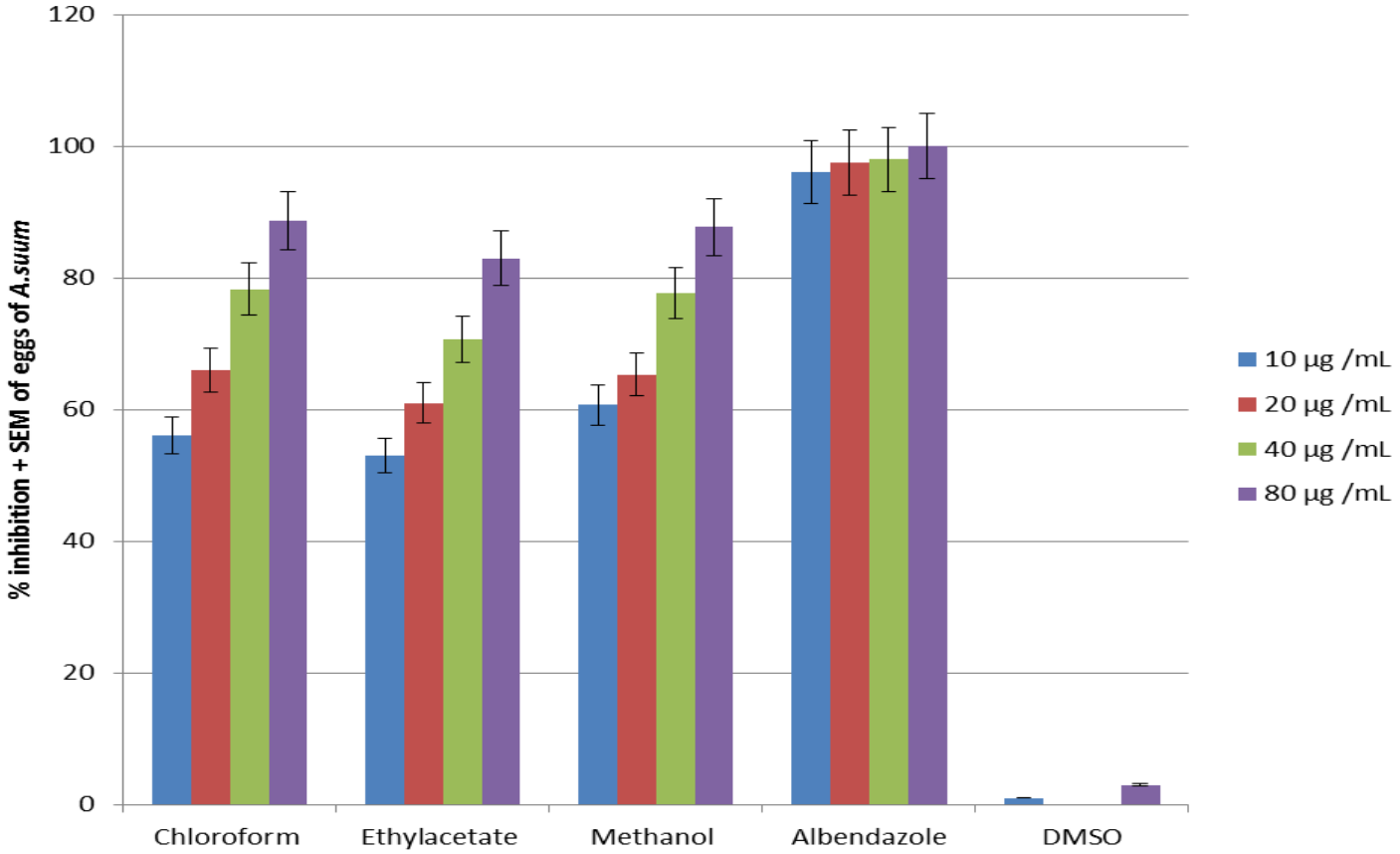


Figure 4.3: **% Inhibition of Eggs of *A. suum* by *M. pruriens* Extracts**

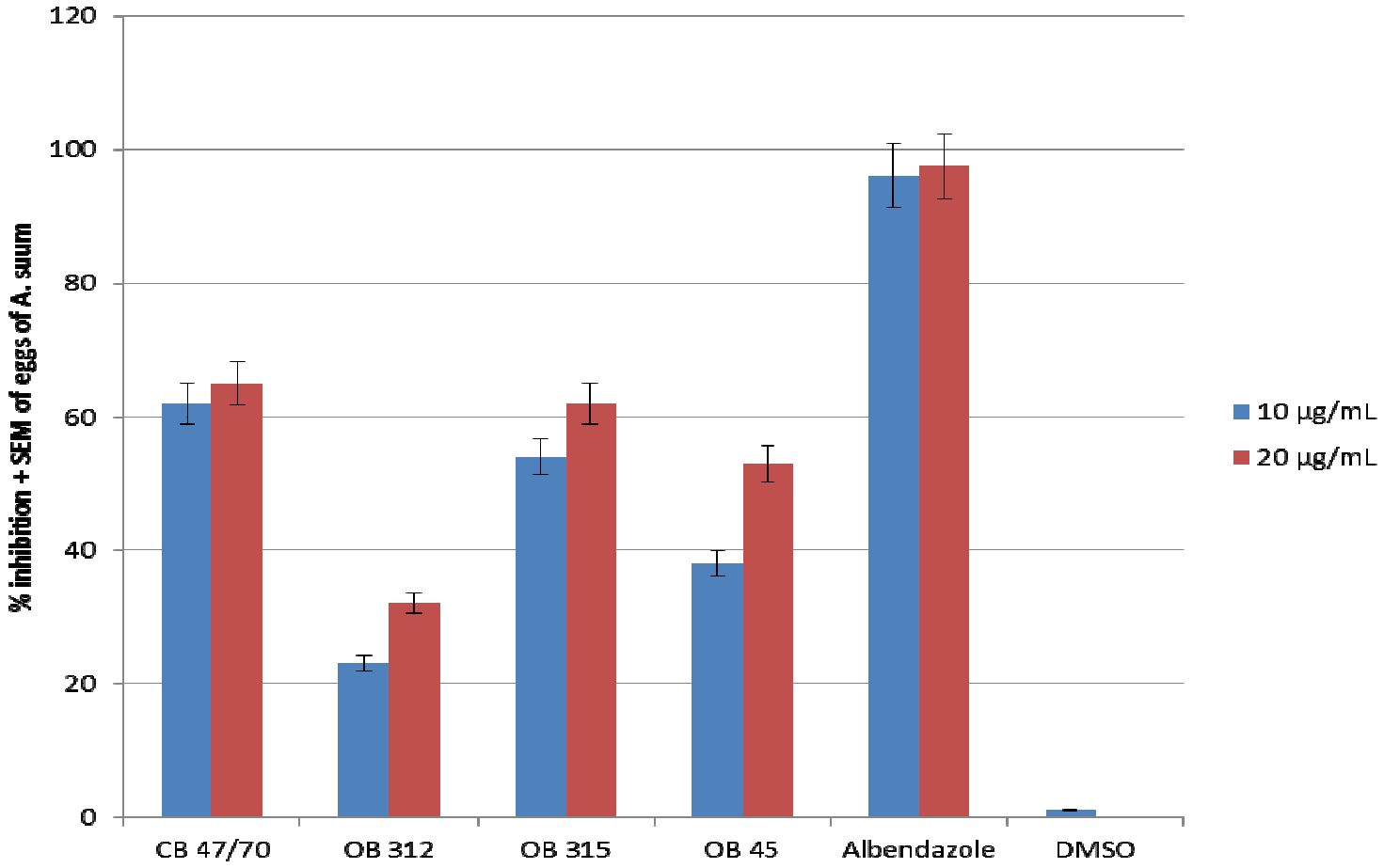


Figure 4.4: % Inhibition of Eggs of *A. suum* by the Isolated Compounds

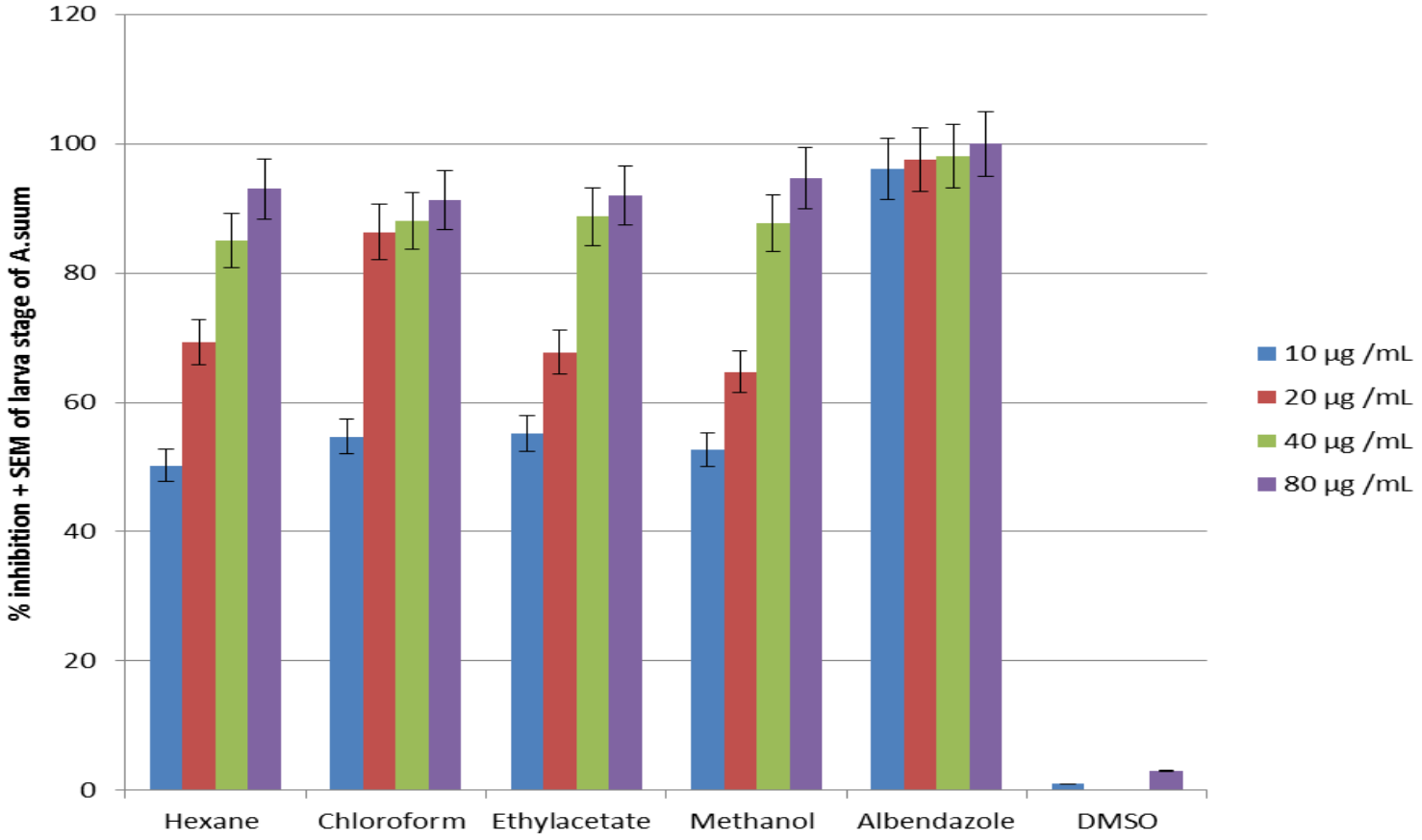


Figure 4.5: **% Inhibition of Larval Stage of *A. suum* by *C. schweinfurthii* Leave Extracts**

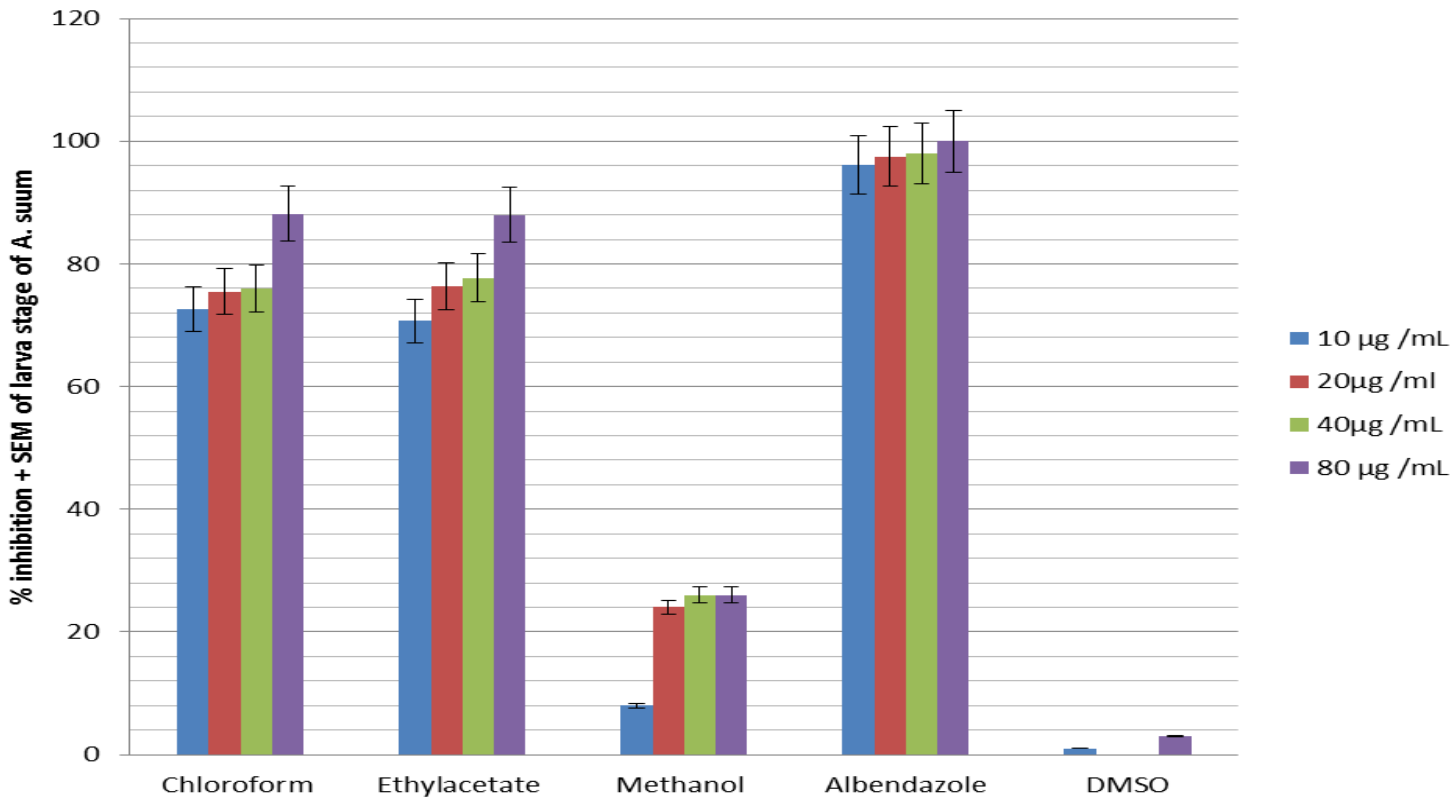


Figure 4.6: **% Inhibition of Larval Stage of *A. suum* by *C. schweinfurthii* Stem Bark Extracts**

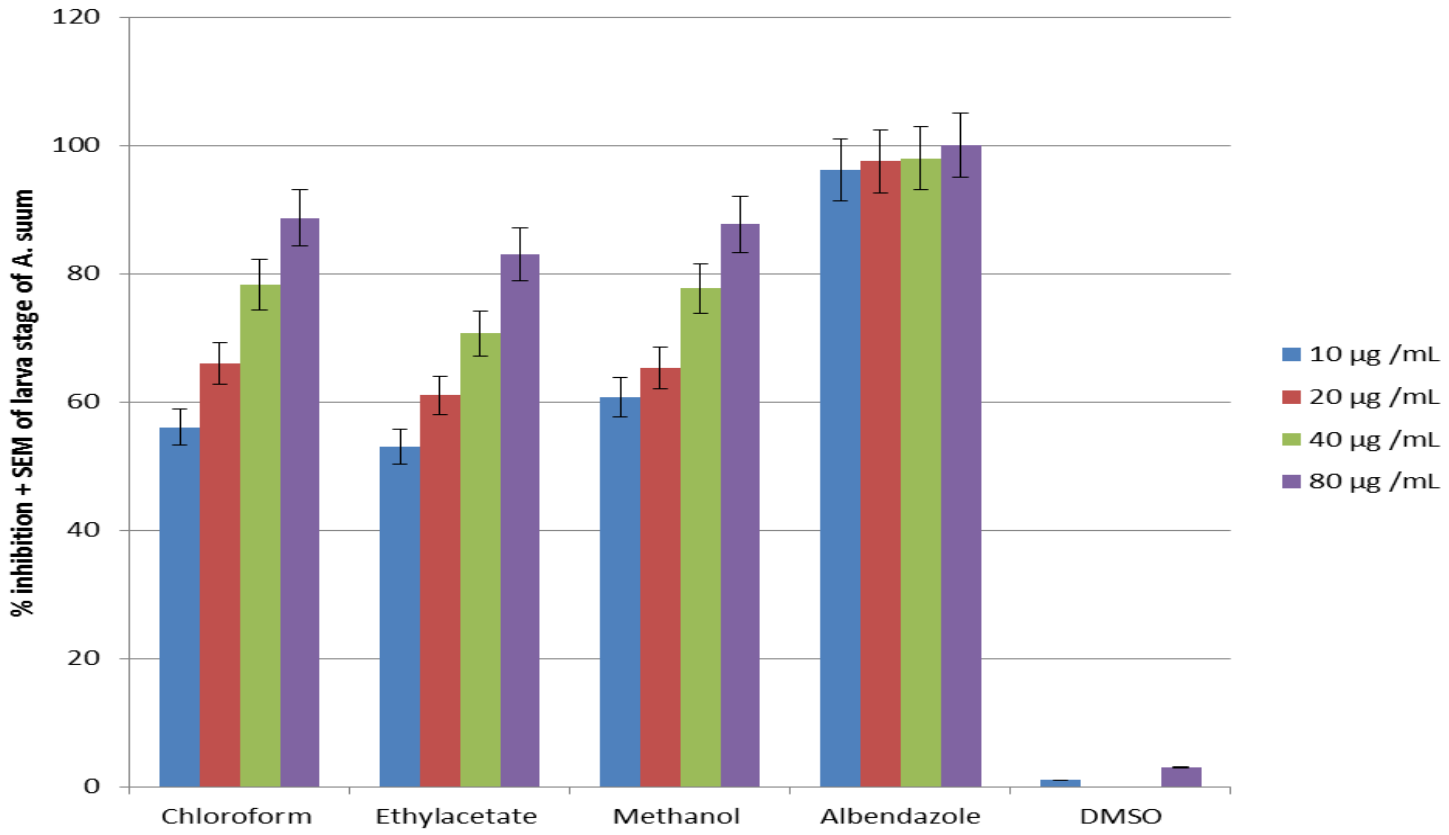


Figure 4.7: % Inhibition of Larval Stage of *A. suum* by *M. pruriens* Extracts

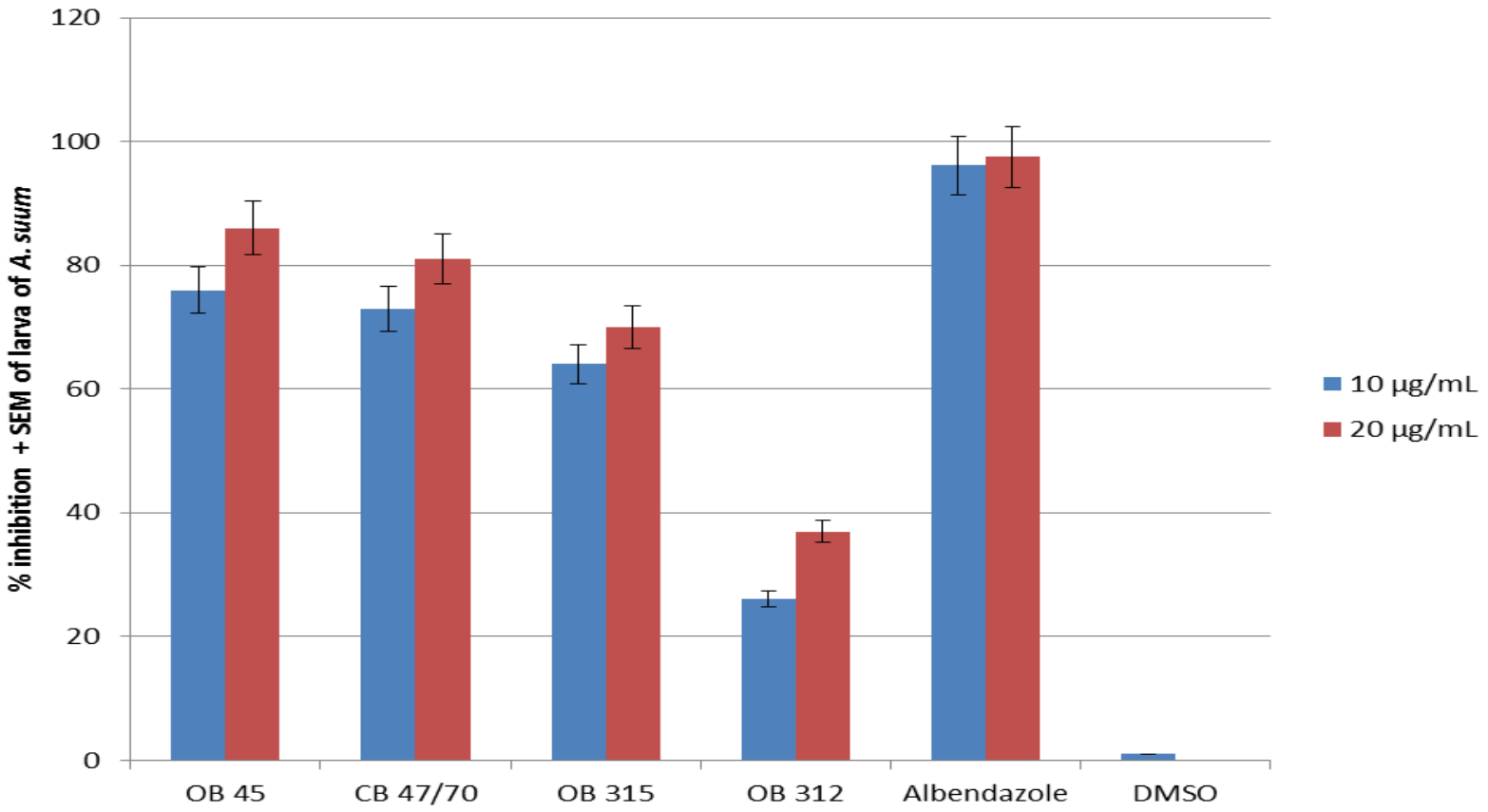


Figure 4.8: % Inhibition of Larval of *A.suum* by the Isolated Compounds

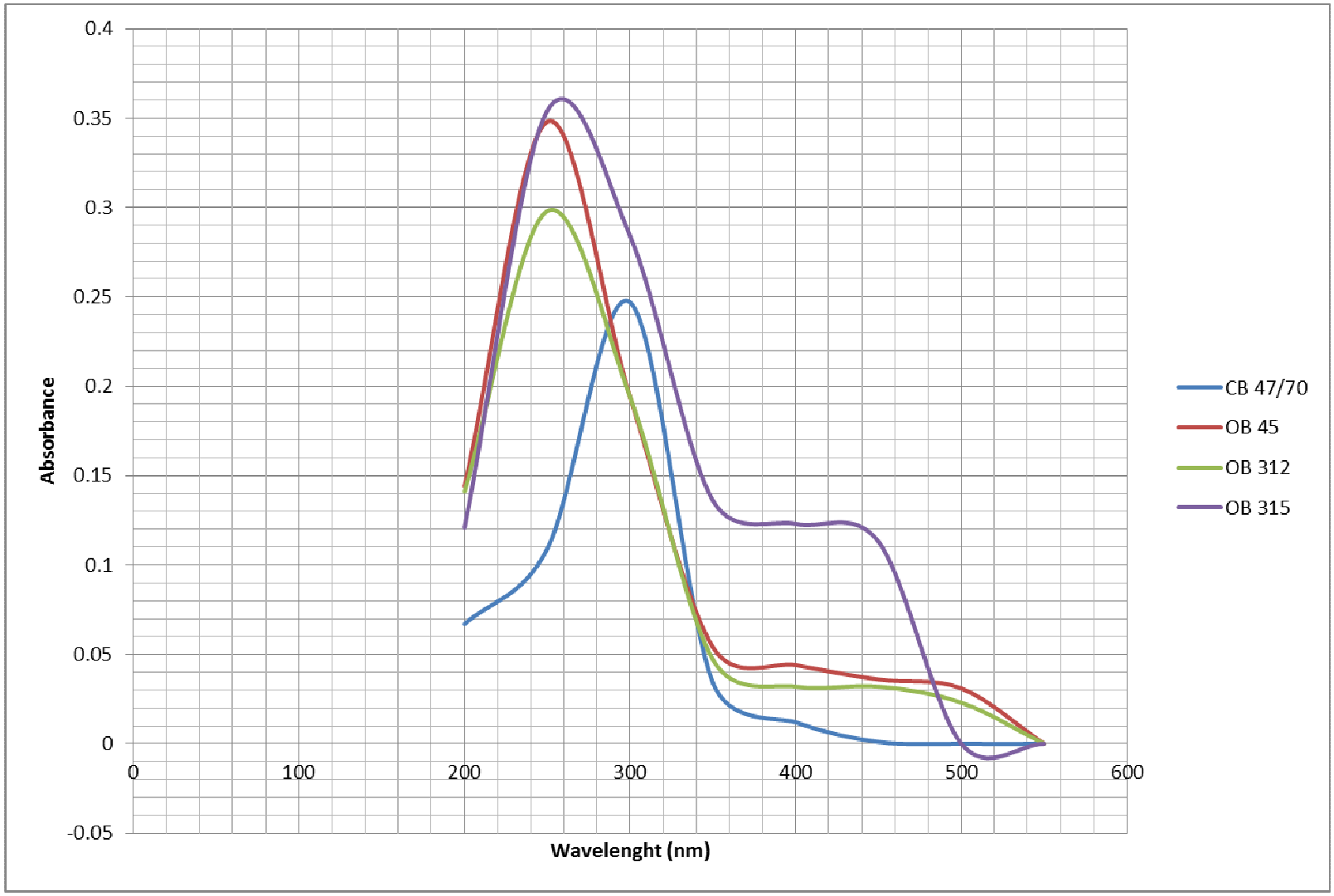


Figure 4.9: UV-Visible Spectrophotometer of the Isolated Compounds

4.10 Spectral Result

Compound CB 47/70 was obtained as a white amorphous solid (23.5 mg). ^{1}H NMR (400 MHz, CD_3Cl) indicates protons resonating at δ 3.40, 5.20, 5.35 and 2.25 (Fig 4.10). The ^{13}C-NMR (400 MHz, CD_3Cl) spectrum showed carbon atoms at δ 64.10, 55.18,121.73, 139.30, 124.42, 38.79, 27.24, 18.38, 34.74, 22.60, 29.71, 39.62, 33.35, 37.15 ,δ 29.37, 21.41, 15.63, 16.81, 23.70, 26.00, 30.09 and 28.77 (Fig 4.11).

The compound OB 45 was obtained as white crystalline material (14.7 mg). The ^{1}H NMR (400 MHz, DMSO) with protons at δ 2.79 , 4.71 , 4.81 , 1.56, δ 1.20, δ 1.14, 0.56 , 0.37 , 1.31 and 4.35 (Fig 4.17). The ^{13}C NMR data (400 MHz, DMSO) spectrum showed carbon atoms at δ 110.06, δ 150.82, 77.29 , 177.70, 38.75, 27.63, 18.44, 19.43, 25.58, 30.51, 32.21, 29.68, 38.76 , 55.39, 50.43, 38.09, 47.09 and 49.06 ,27.53, 15.22, 16.41, 16.44 and 19.43 (Fig 4.18)

Compound OB 315 was obtained as a white powder (15.1 mg). The ^{1}H NMR spectrum of OB 315 showed the presence of signals at δ 0.68, 1.01, 0.81, 0.83, 0.93, 0.84, 5.36 and 3.53 (Fig 2.24). The ^{13}C NMR data (400 MHz, CD_3Cl) spectrum showed the presence carbon atoms at δ 141.32, 121.98, 71.41, 32.15, 55.45, 56.06, 55.57, 33.99, 15.59, 29.58 ,37.39, 31.50, 42.33, 32.73, 21.62, 39.01, 26.22, 28.34, 47.50, 18.61 , 37.15, 18.61, 26.22, 23.51, 21.63, 18.63 and 15.72 (Fig 4.25).

Compound OB 312 was obtained as a red amorphous solid (11.3 mg). The ^{1}H NMR (400 MHz, CD_3Cl) spectrum showed the presence of protons at δ 6.03, 5.89, 4.10, 4.10, 4.02 and 2.50 (Fig 4.30). The ^{13}C NMR data (400 MHz, CD_3Cl) showed the presence carbons atoms at δ 129.05, 127.88, 131.12, 128.78, 76.31, 64.10, 37.15, 24.97, 34.29, 37.39, 17.32, 22.91, 22.91 and 24.97 (Fig 4.31).

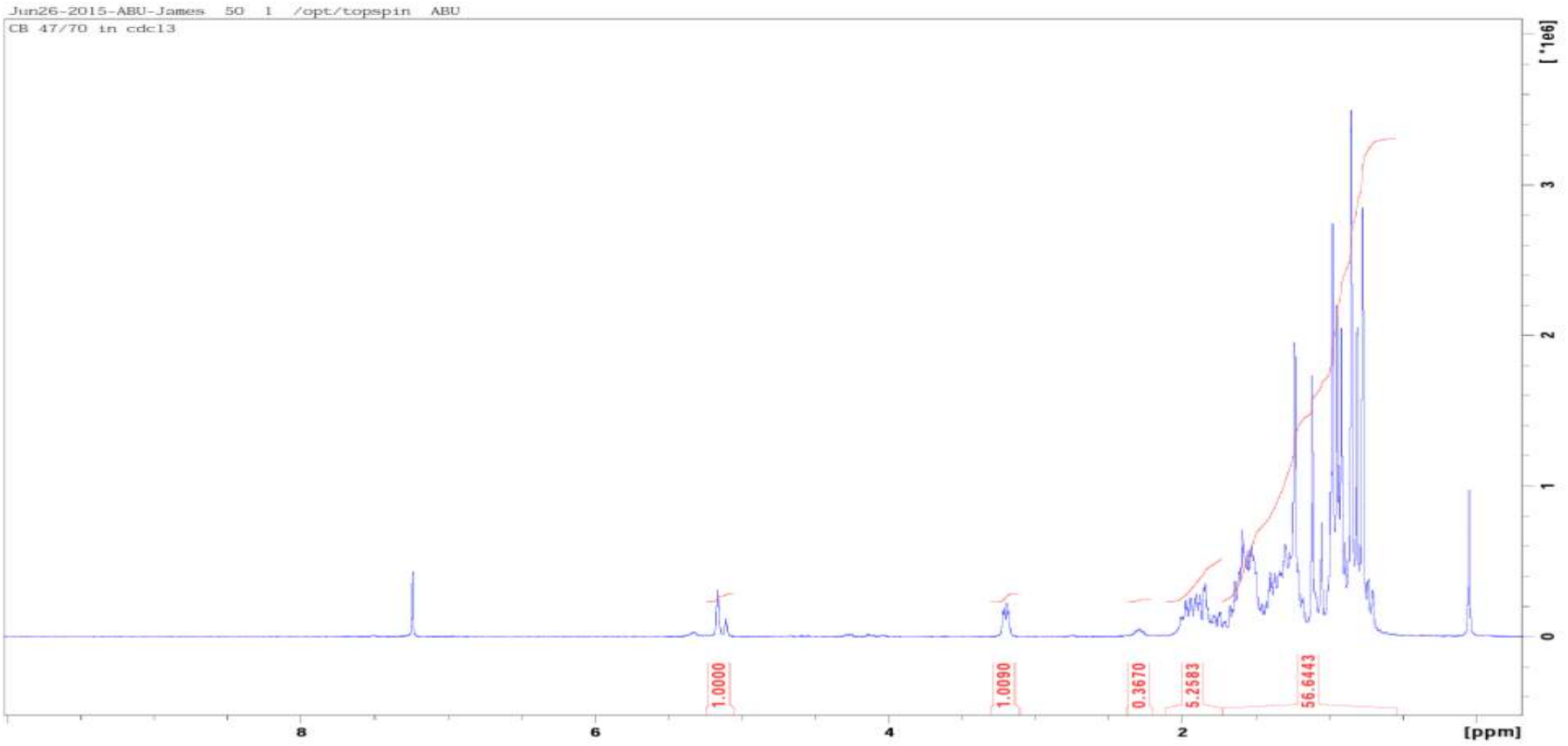


Figure 4.10: Integrated Proton NMR of CB 47/70

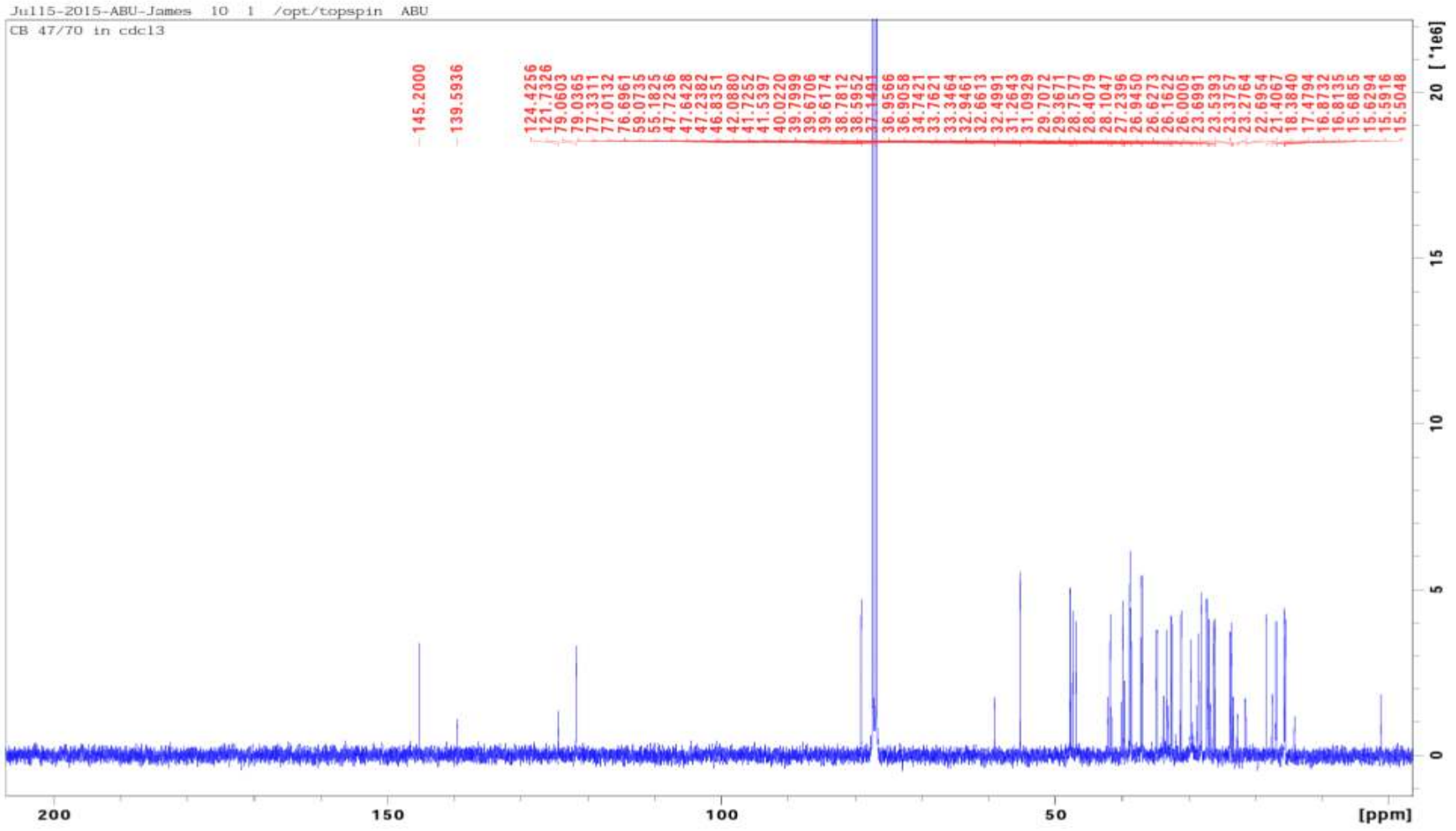


Figure 4.11: ^{13}Carbon NMR Spectrum of CB 47/70

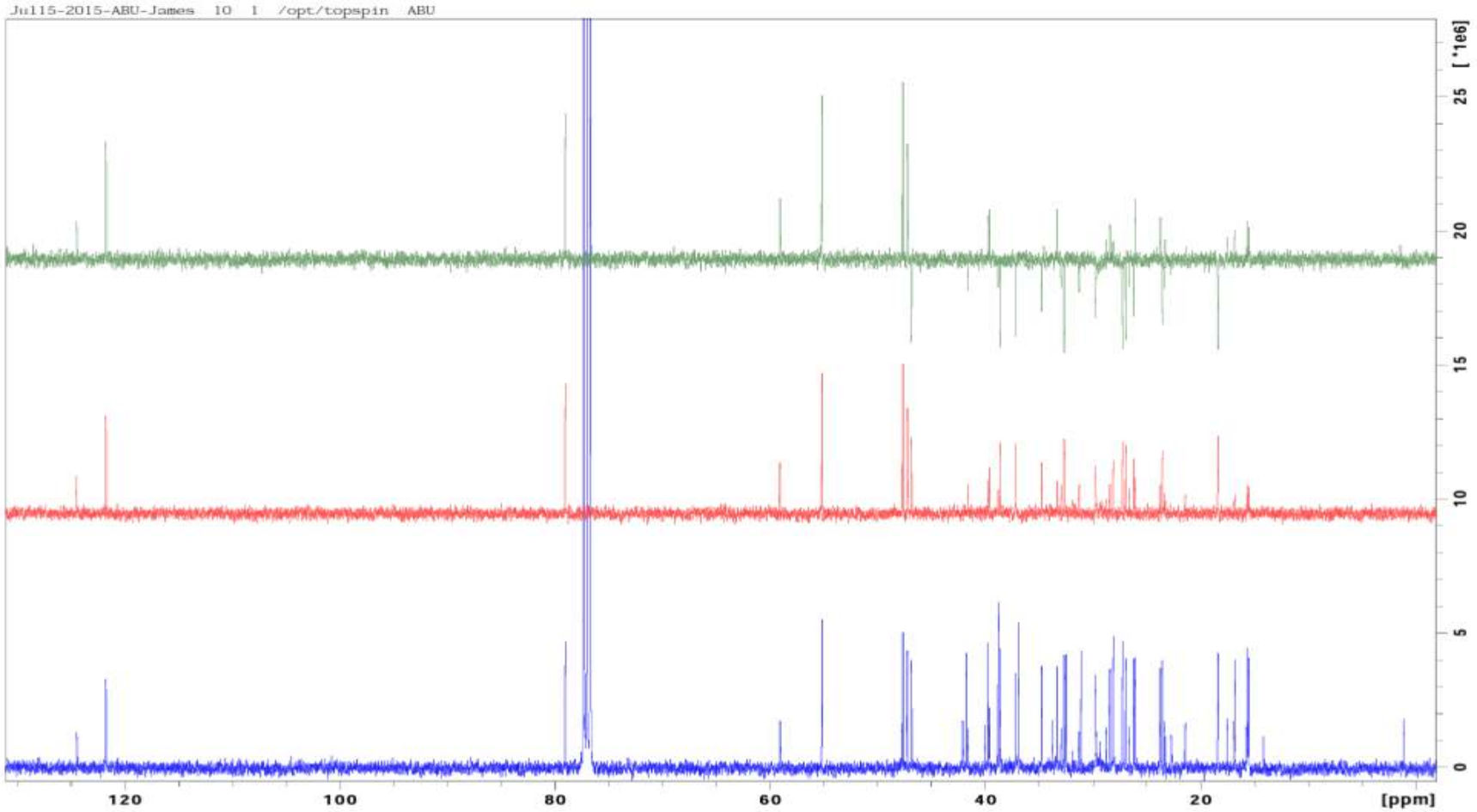


Figure 4. 12: Distortionless Enhancement Polarization Transfer (DEPT)

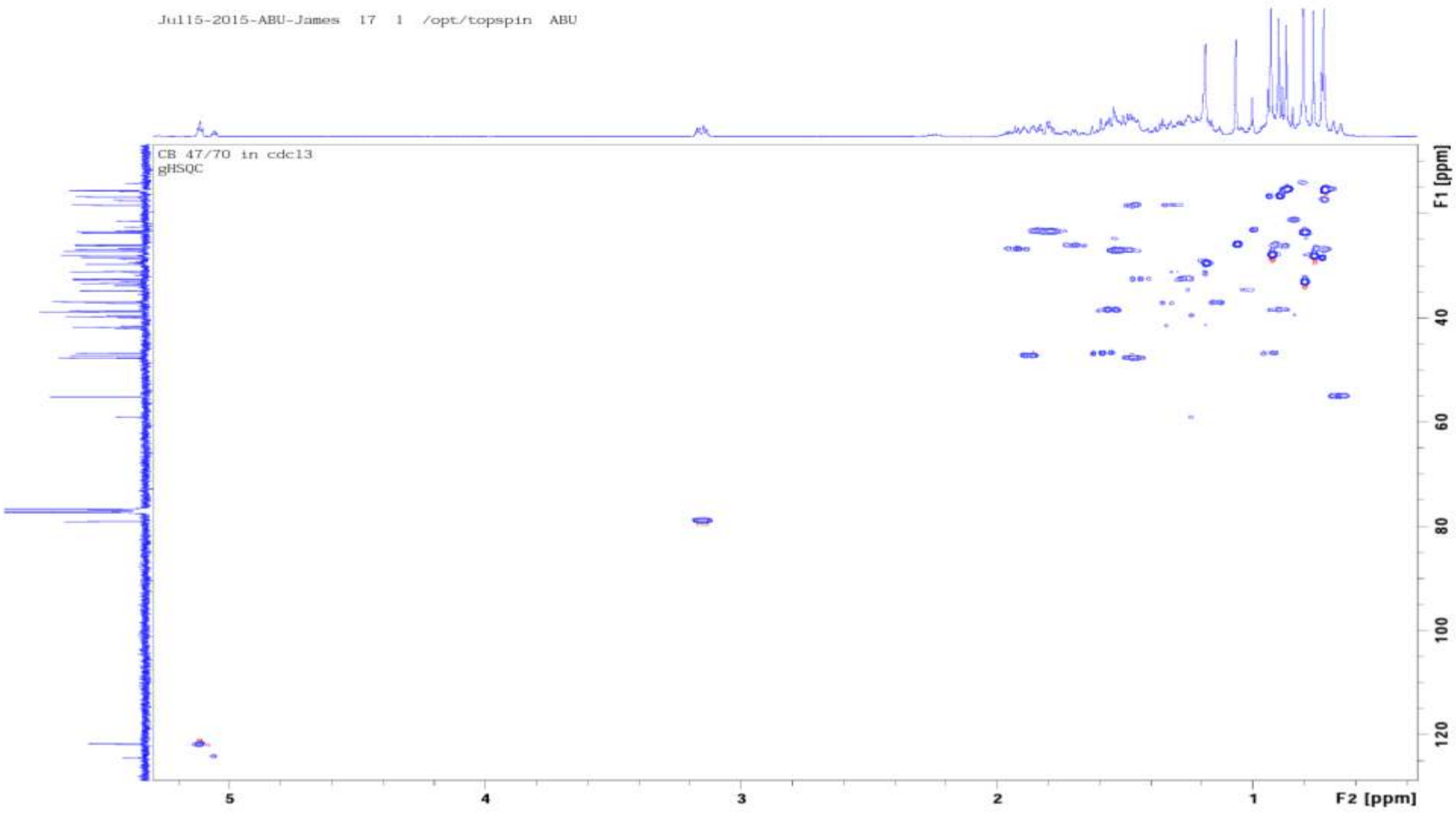


Figure 4.13: Heteronuclear Single Quantum Correlation (HSQC) Spectrum of CB 47/70

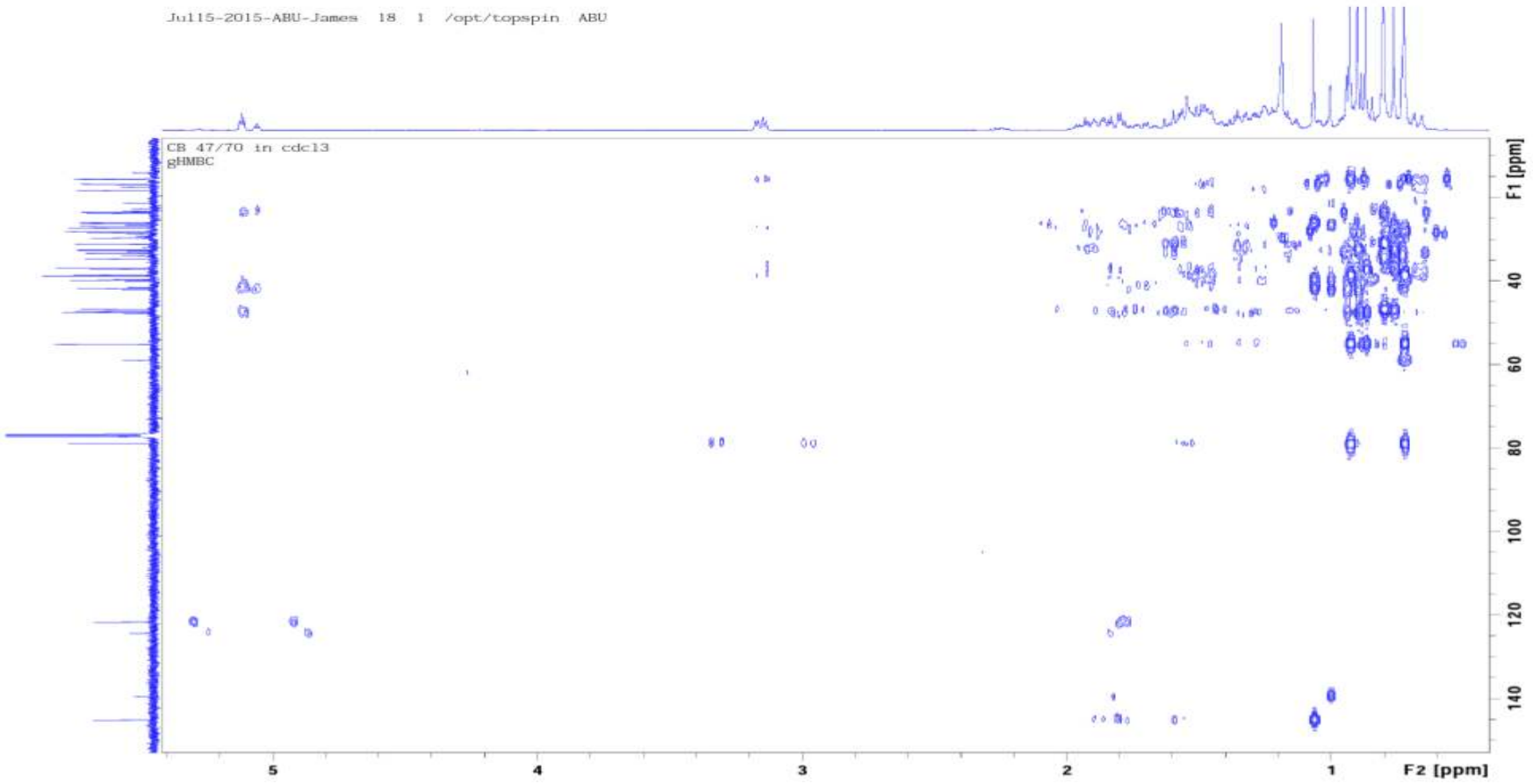


Figure 4.14: Heteronuclear Multiple Bond Correlation (HMBC) Spectrum of CB 47/70

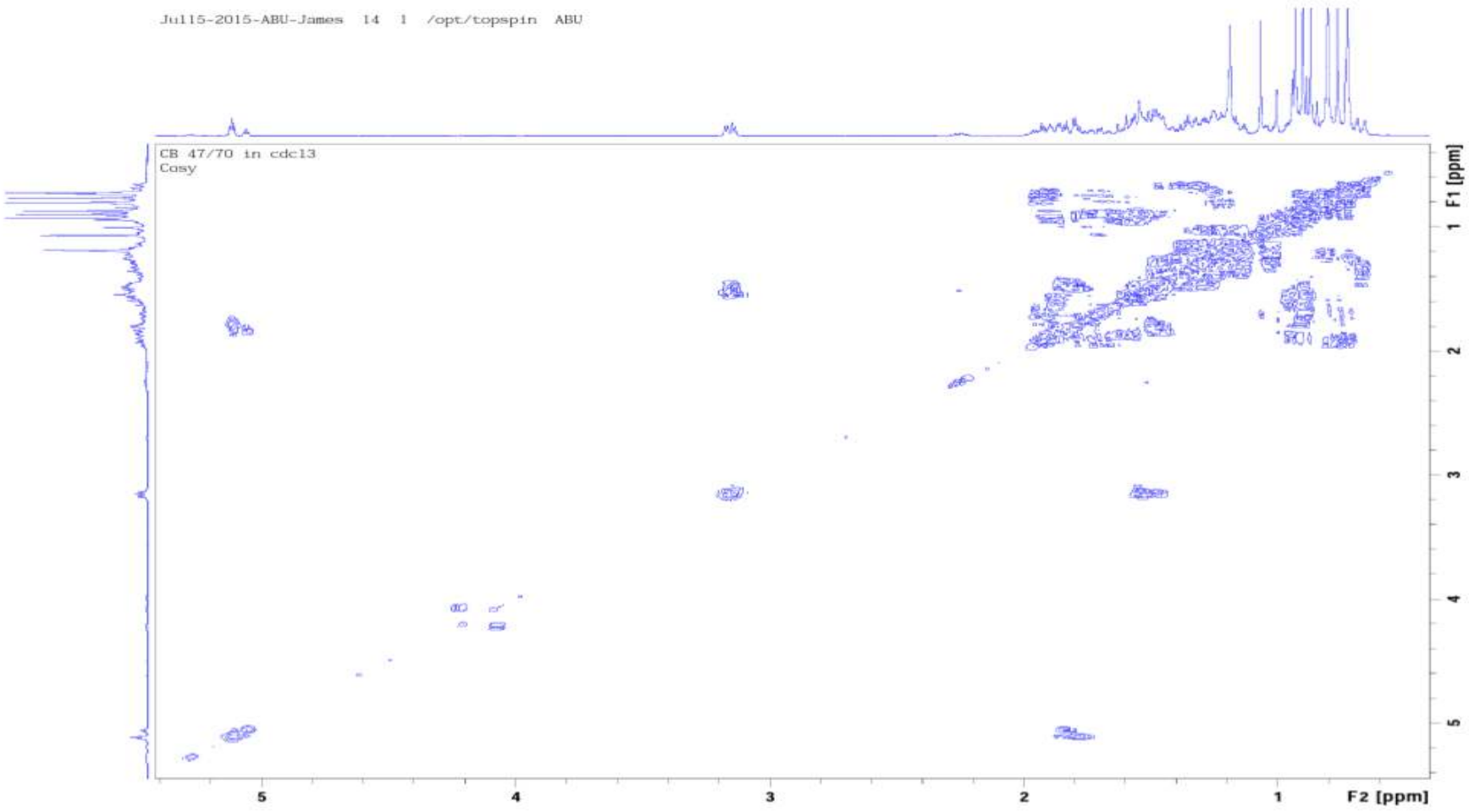


Figure 4.15: Correlation Spectroscopy (COSY) of CB 47/70

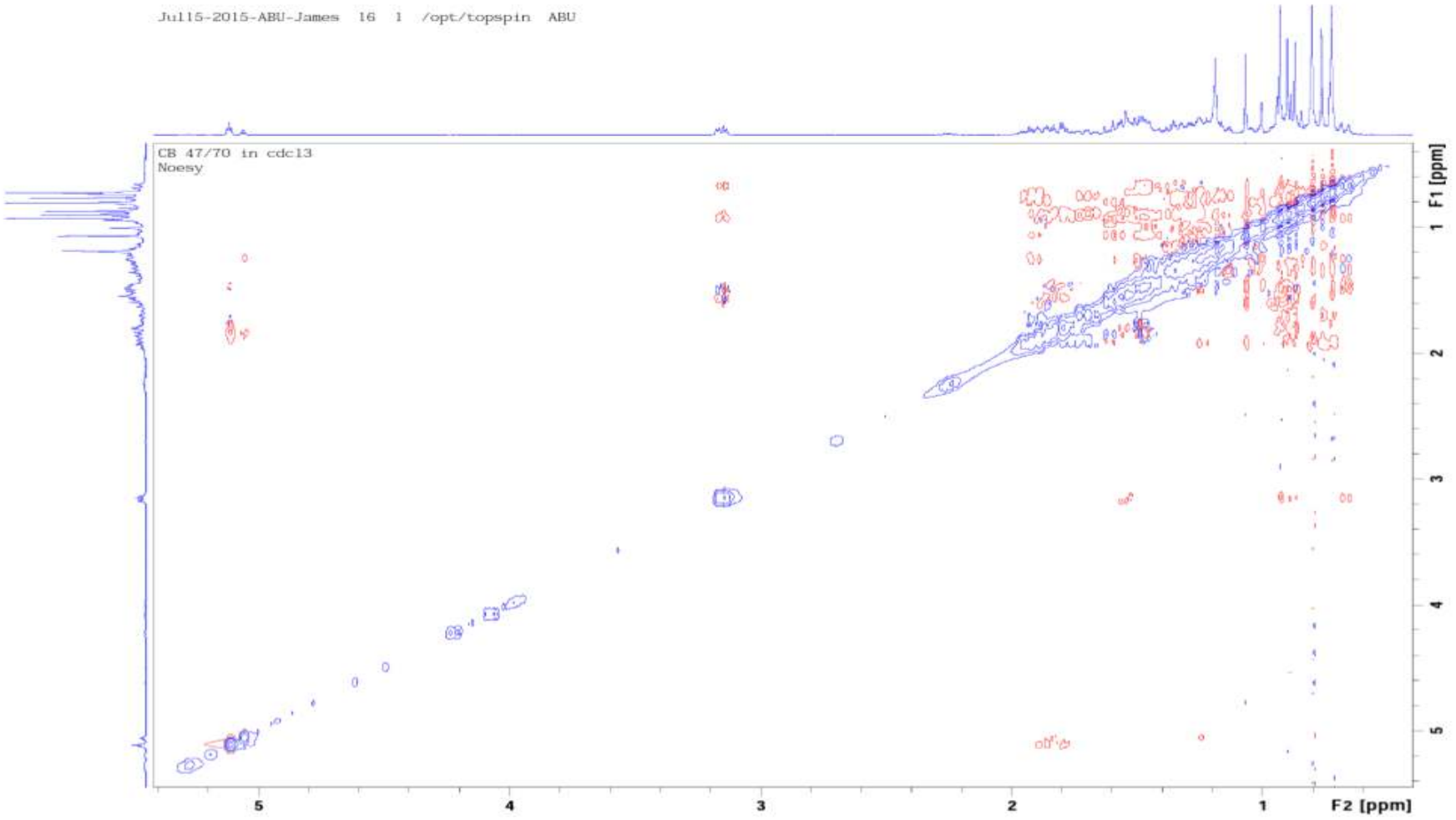


Figure 4.16: **Nuclear Overhauser Effect Spectroscopy (NOESY) of CB 47/70**

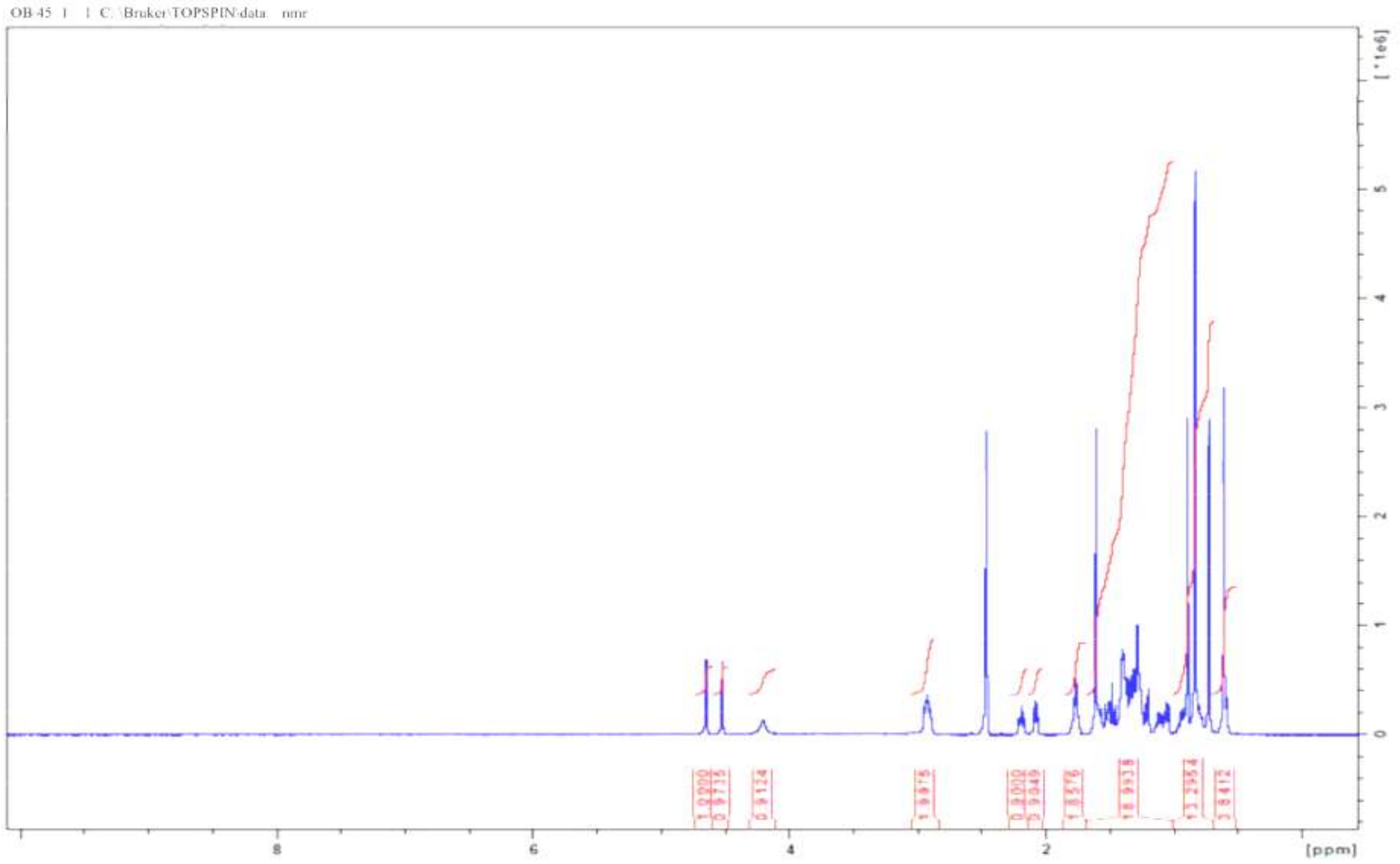


Figure 4.17: Integrated Proton NMR of OB 45

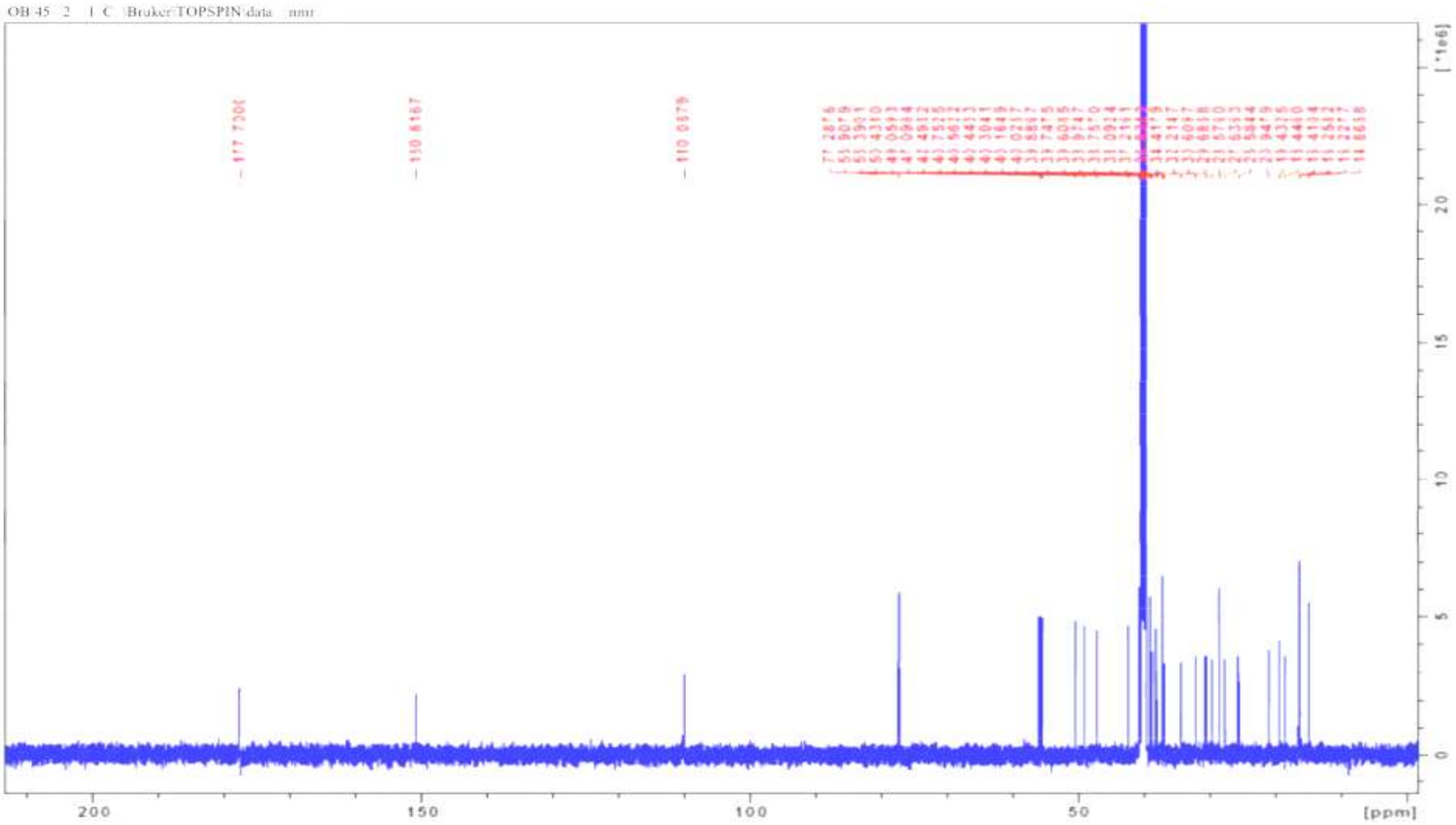


Figure 4.18: ^{13}Carbon NMR spectrum of OB 45

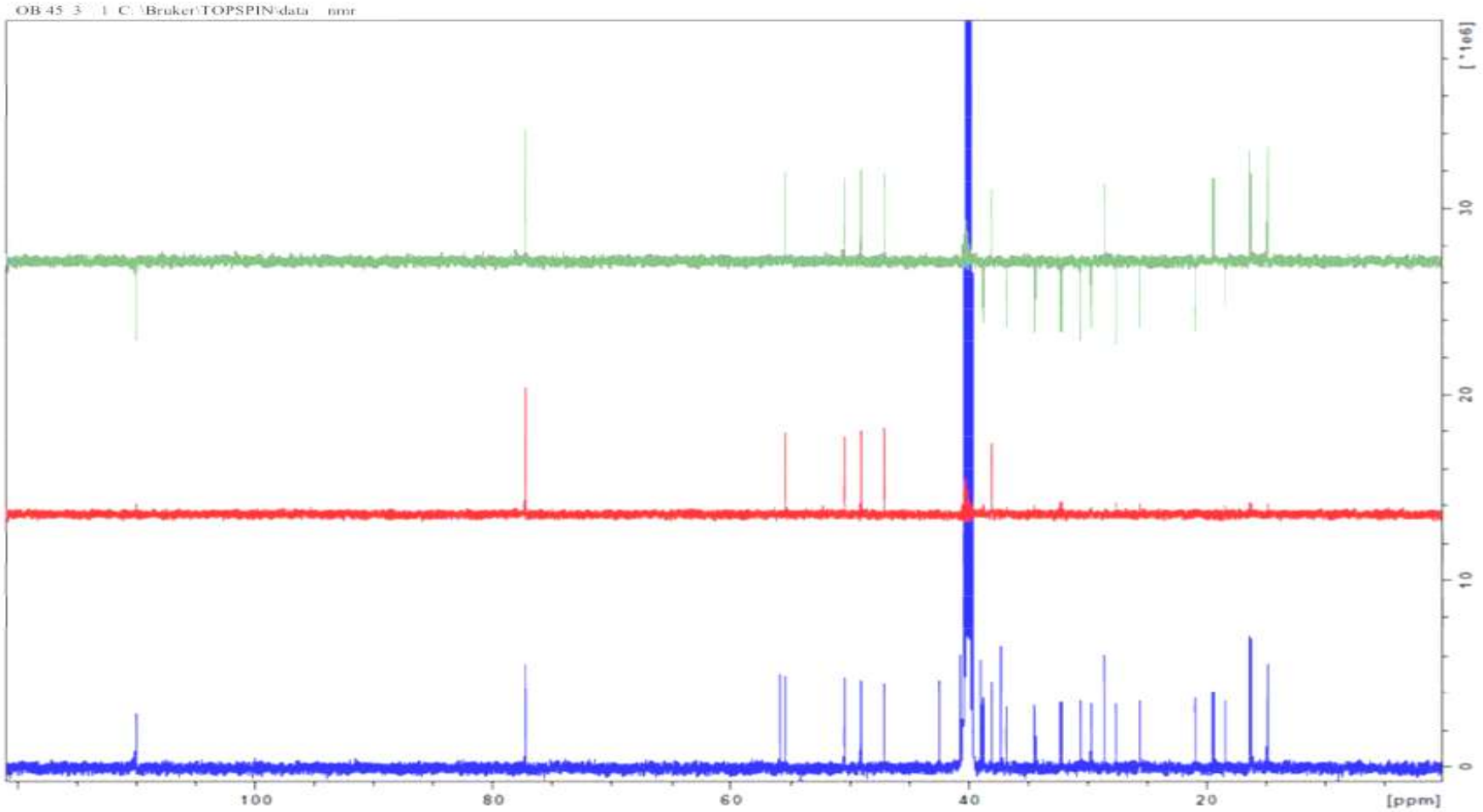


Figure 4.19: Distortionless Enhancement Polarization Transfer (DEPT) Spectrum of OB 45

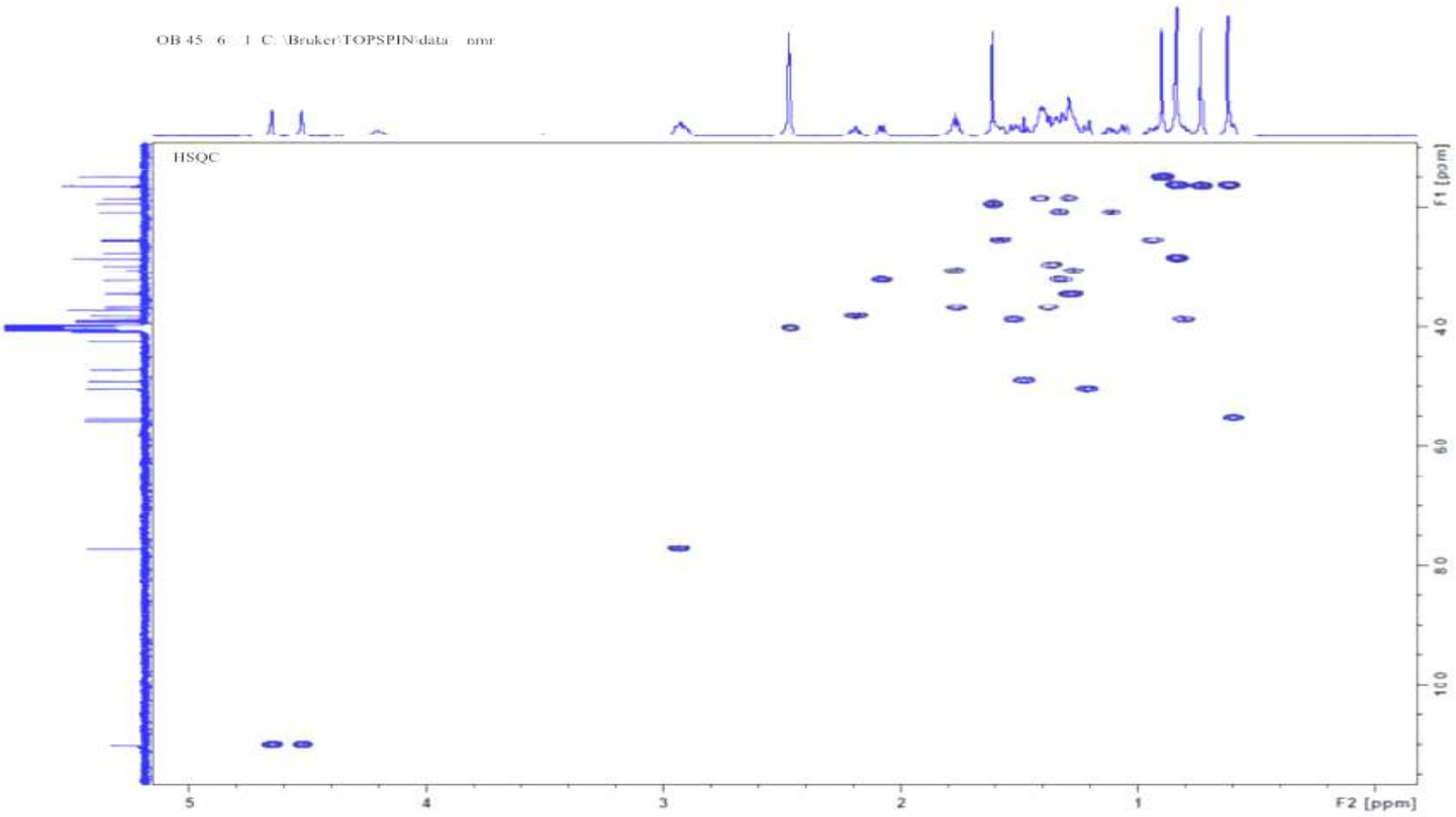


Figure 4.20: Heteronuclear Single Quantum Correlation (HSQC) Spectrum of OB 45

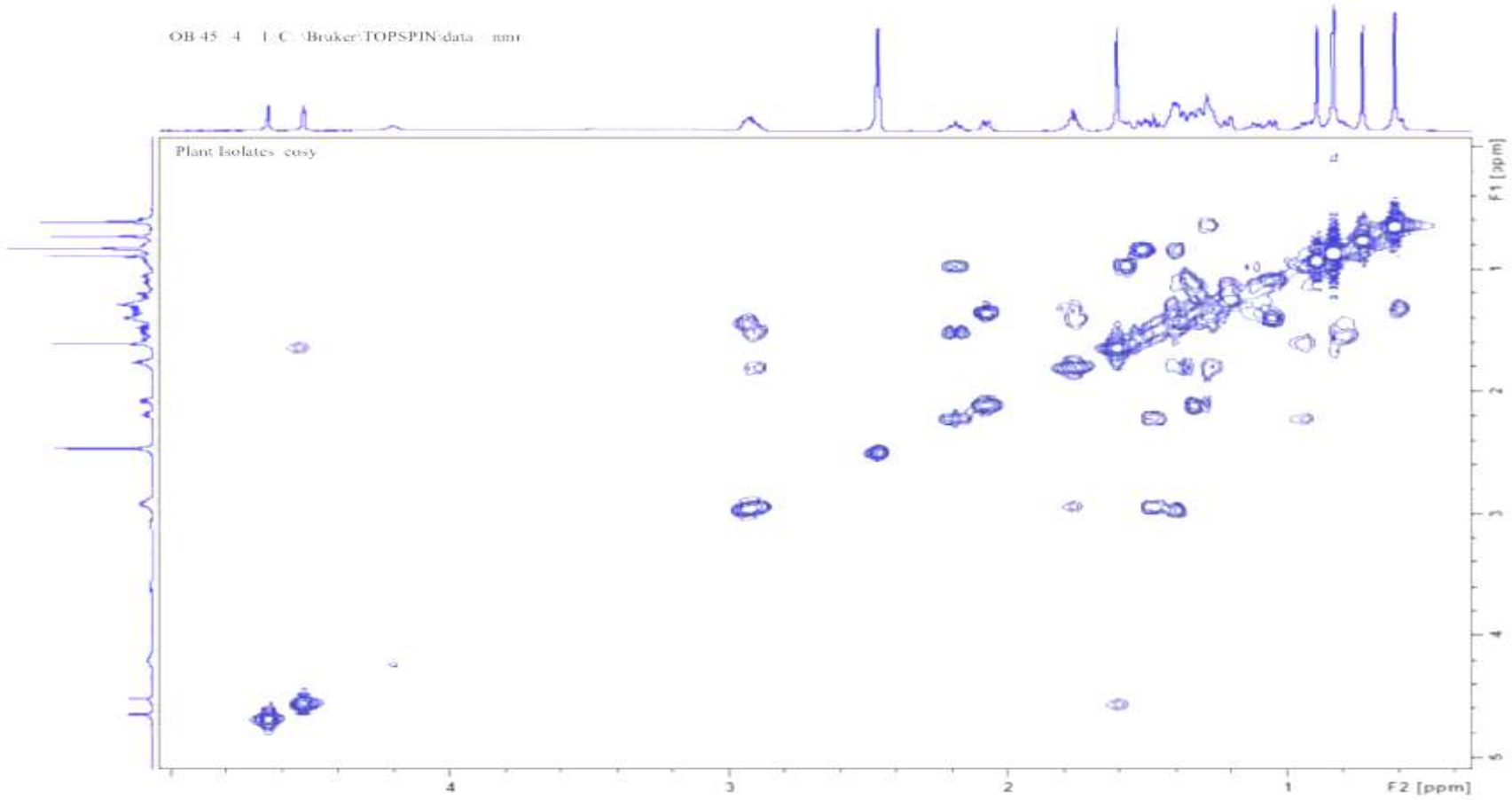


Figure 4.21: Correlation Spectroscopy (COSY) of OB 45

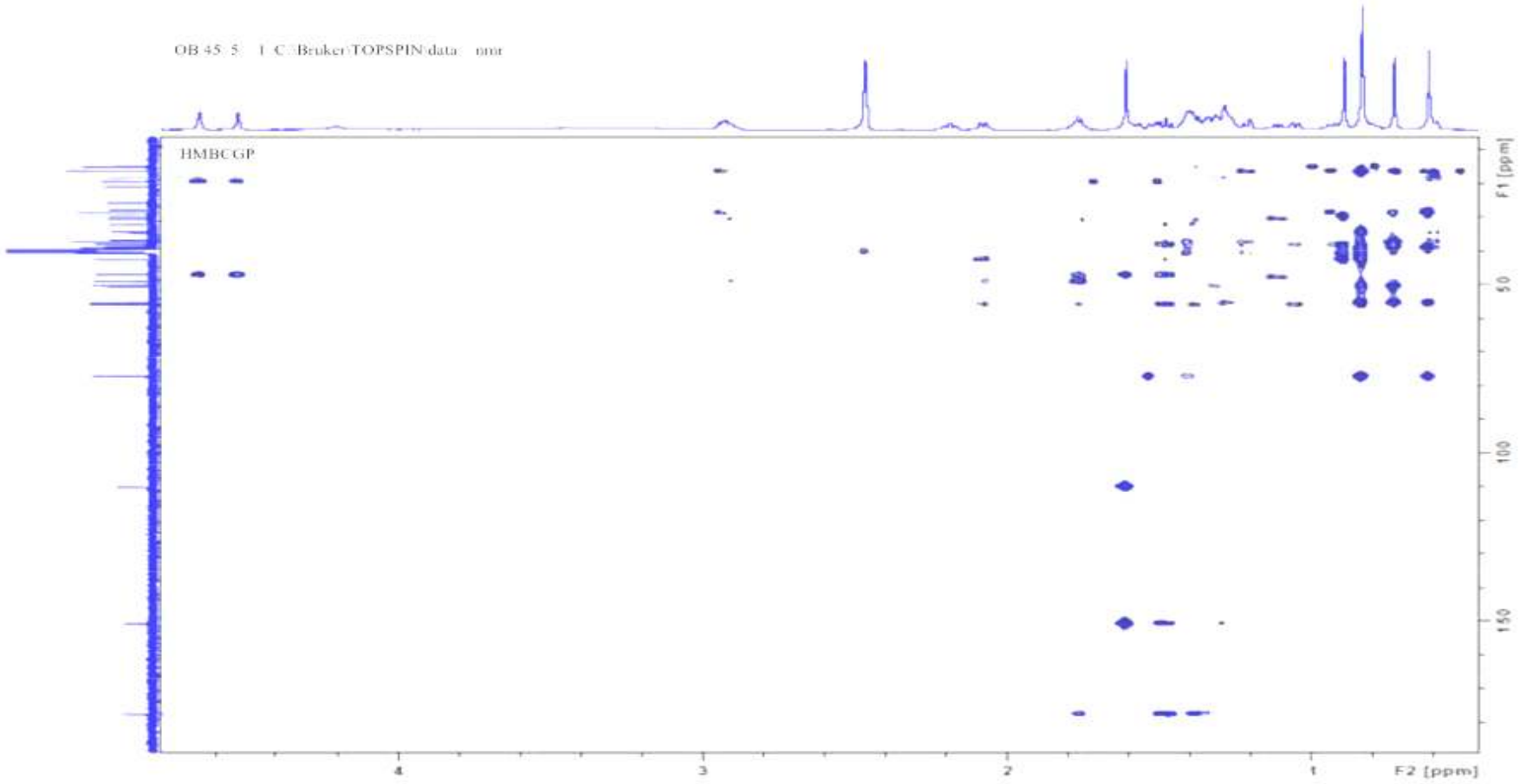


Figure 4.22: Heteronuclear Multiple Bond Correlation (HMBC) Spectrum of OB 45

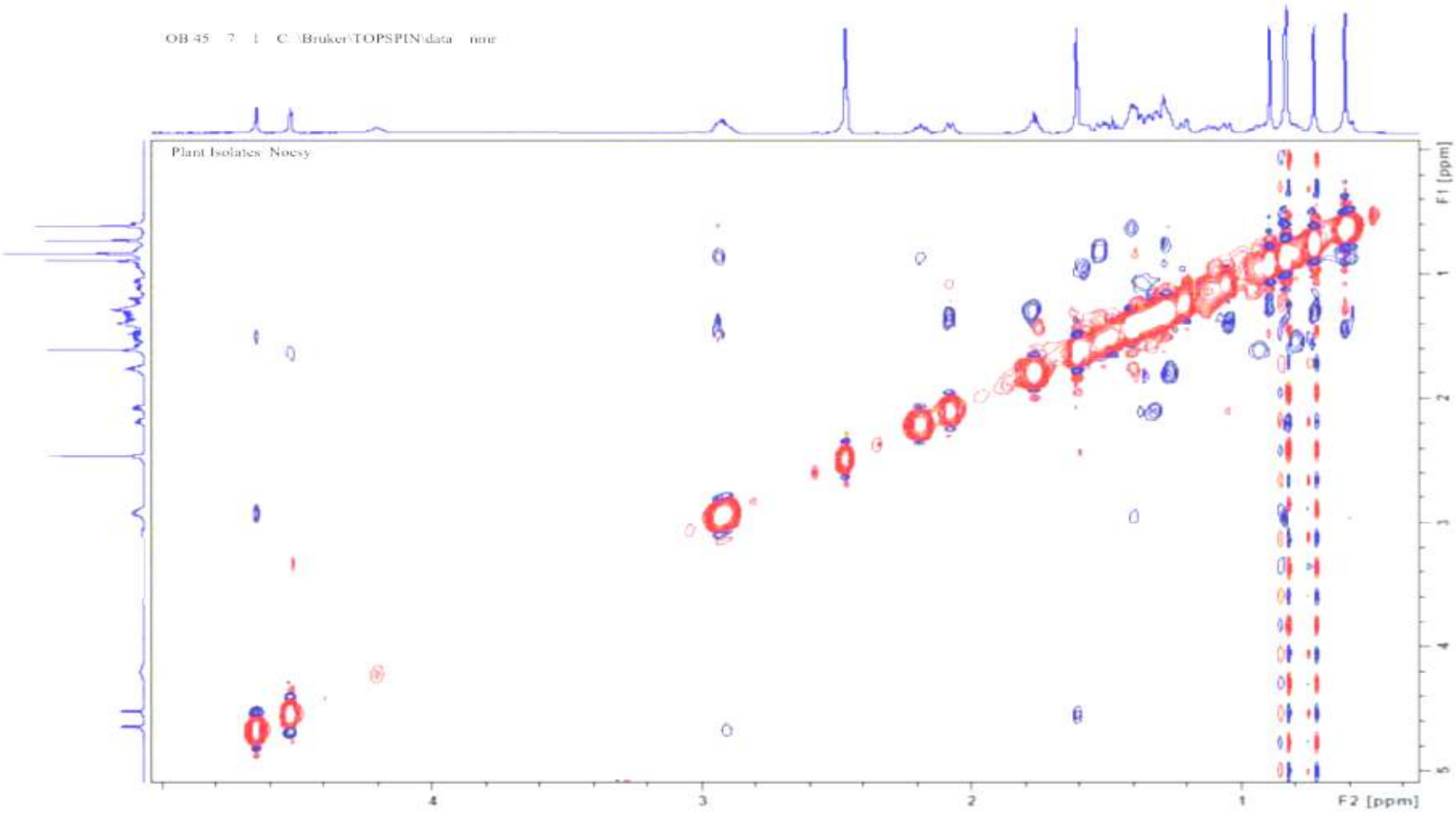


Figure 4.23: Nuclear Overhauser Effect Spectroscopy (NOESY) of OB 45

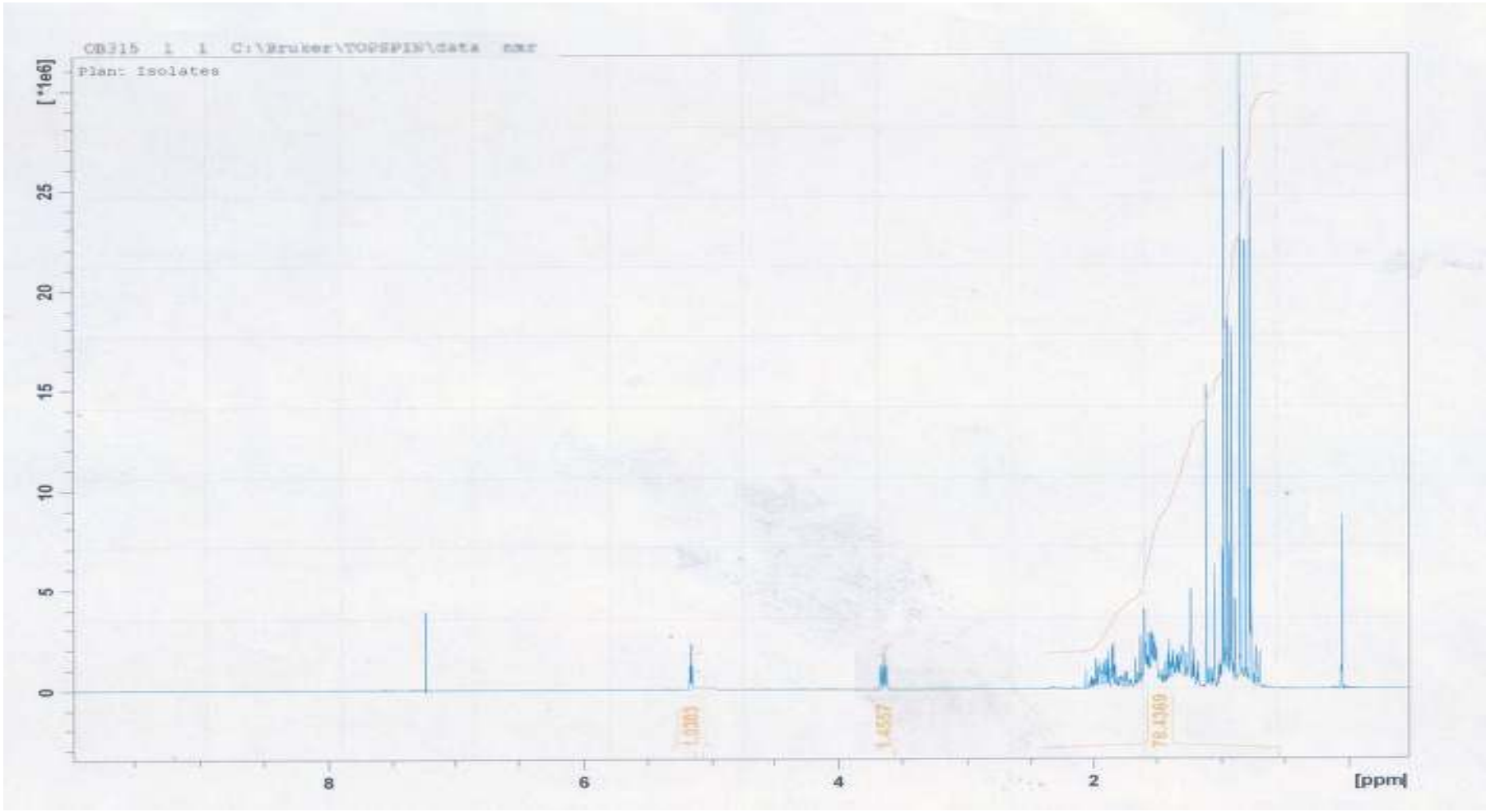


Figure 4.24: Integrated Proton (^{1}H) NMR of OB 315

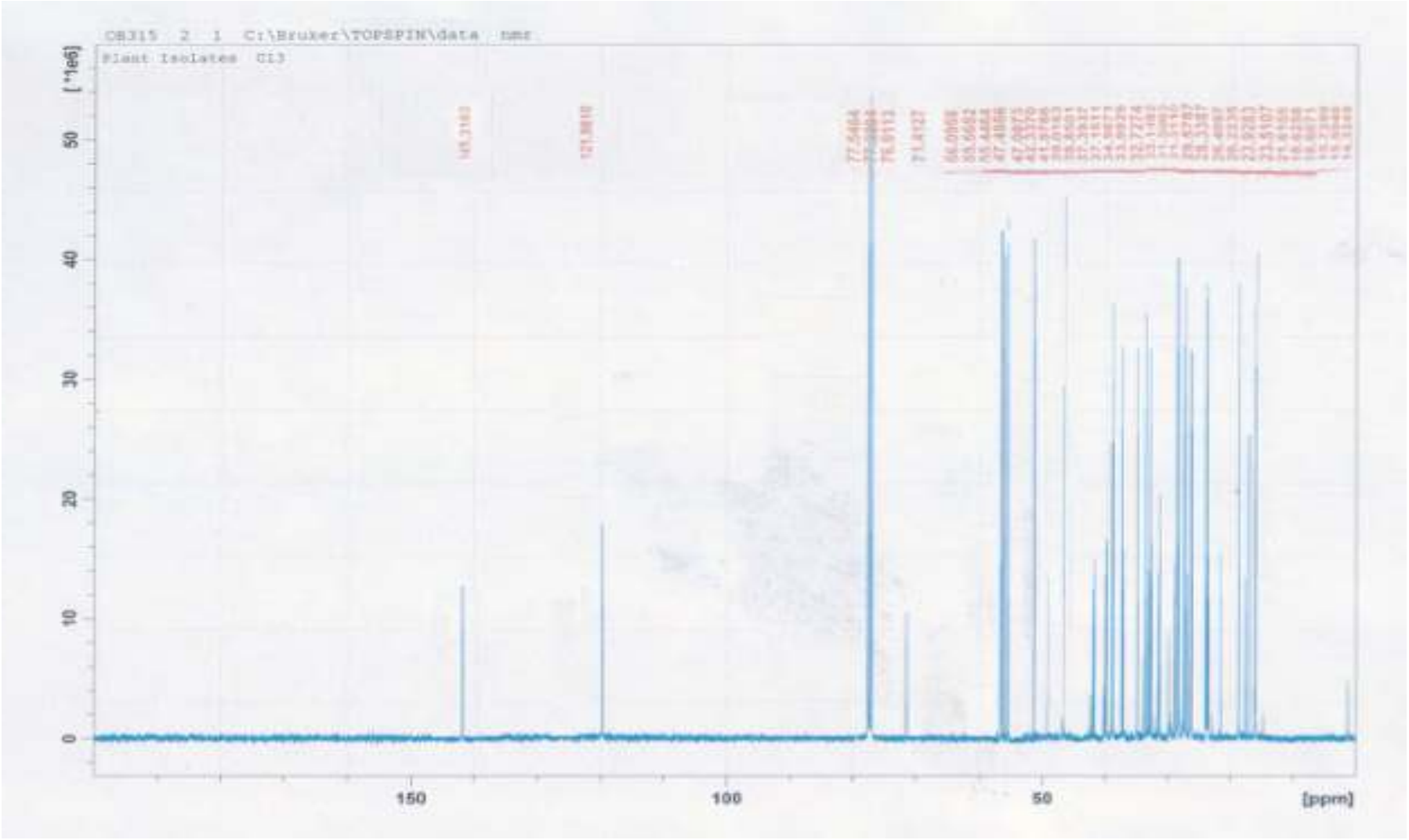


Figure 4.25: ^{13}Carbon (^{13}C) NMR Spectrum of OB 315

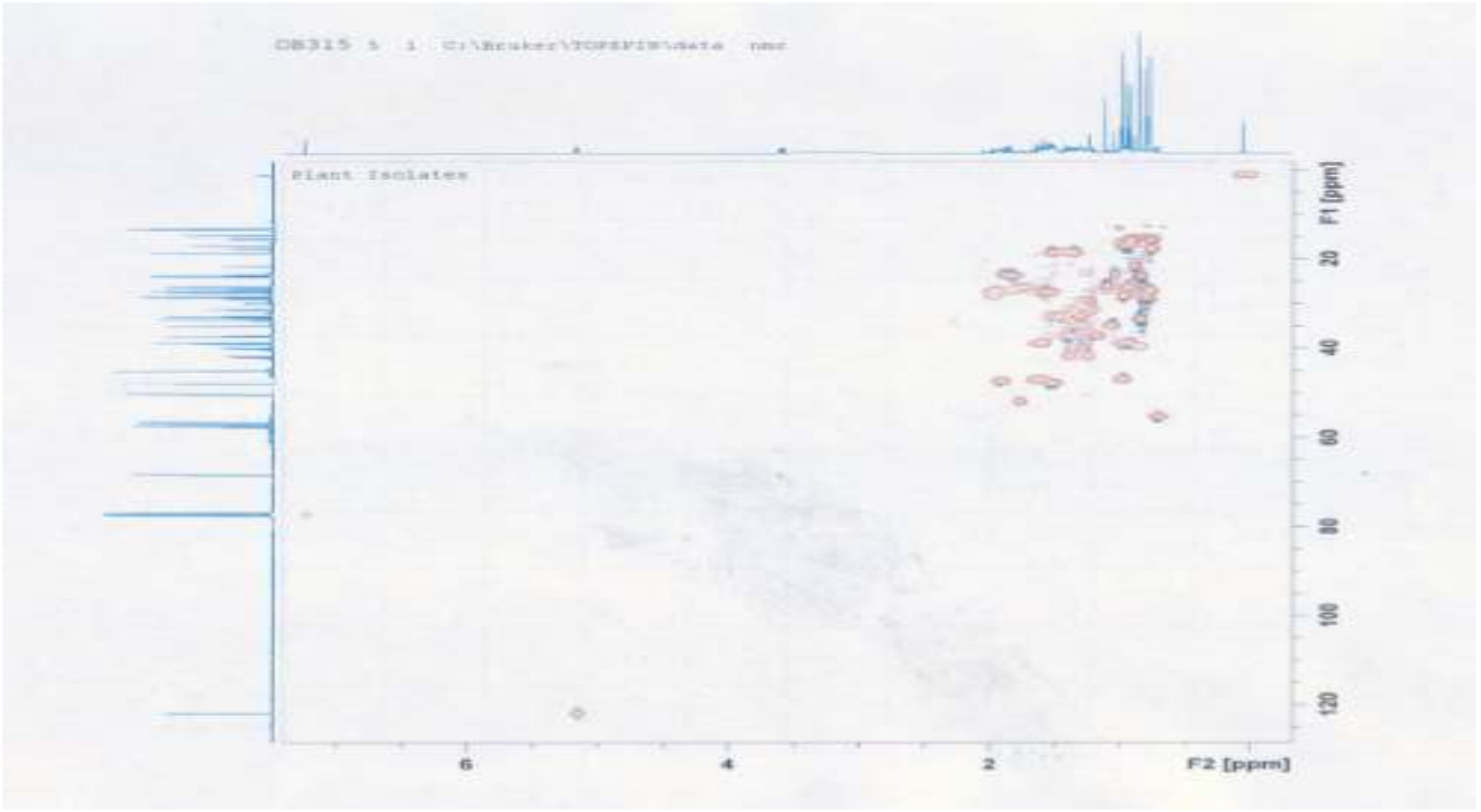


Figure 4.26: Heteronuclear Single Quantum Correlation (HSQC) Spectrum of OB 315

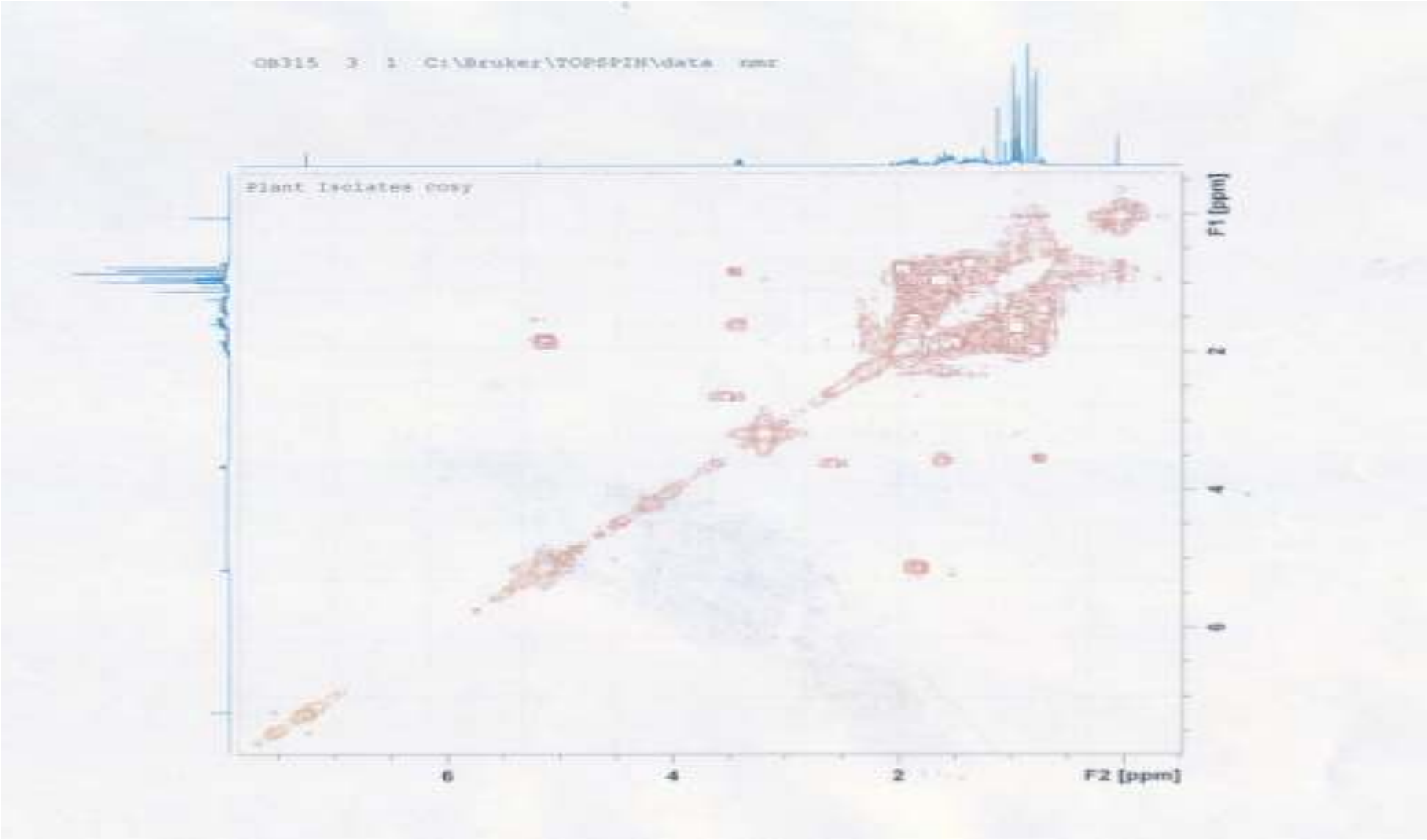


Figure 4.27: Correlation Spectroscopy (COSY) of OB 315

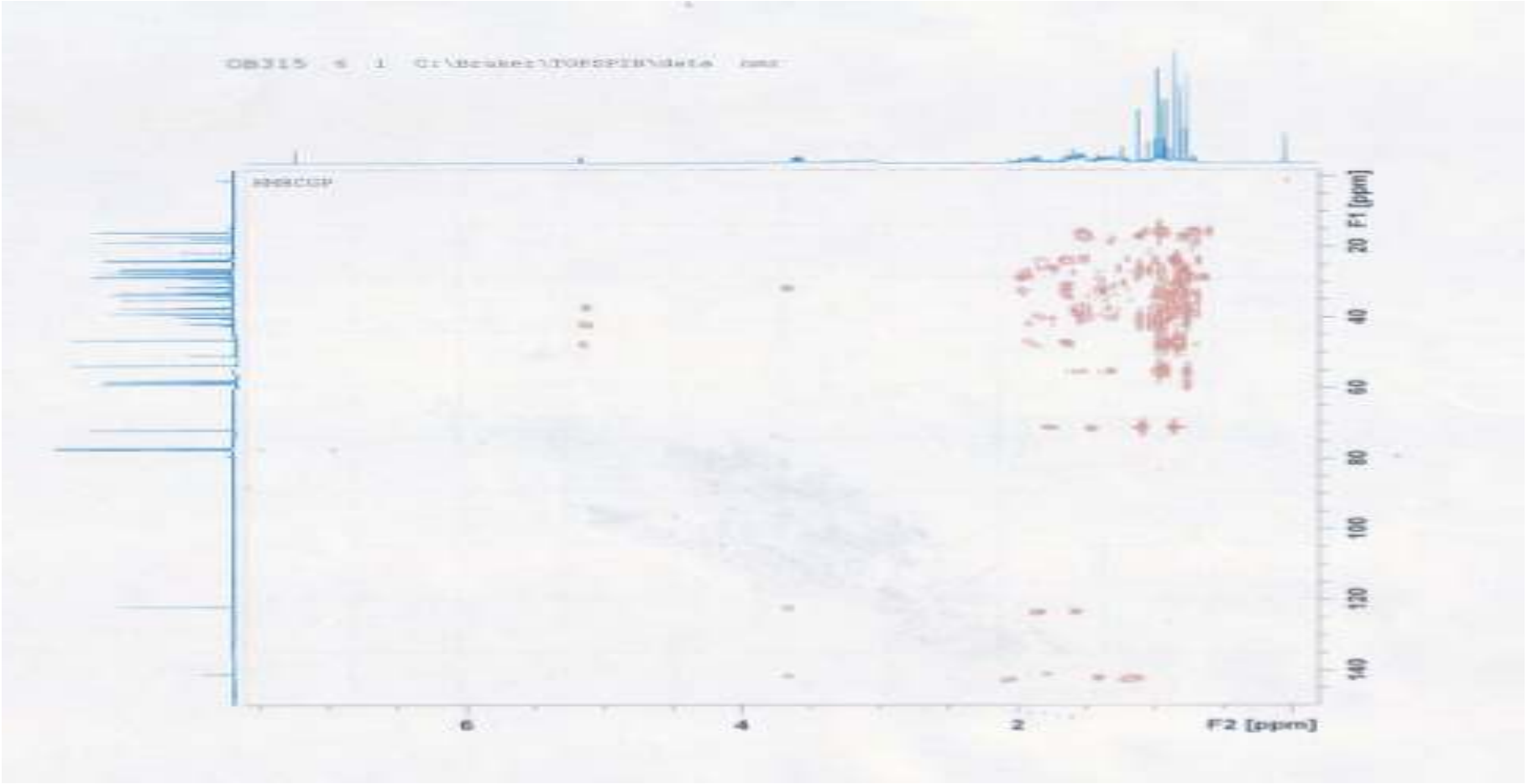


Figure 4.28: Heteronuclear Multiple Bond Correlation (HMBC) Spectrum of OB 315

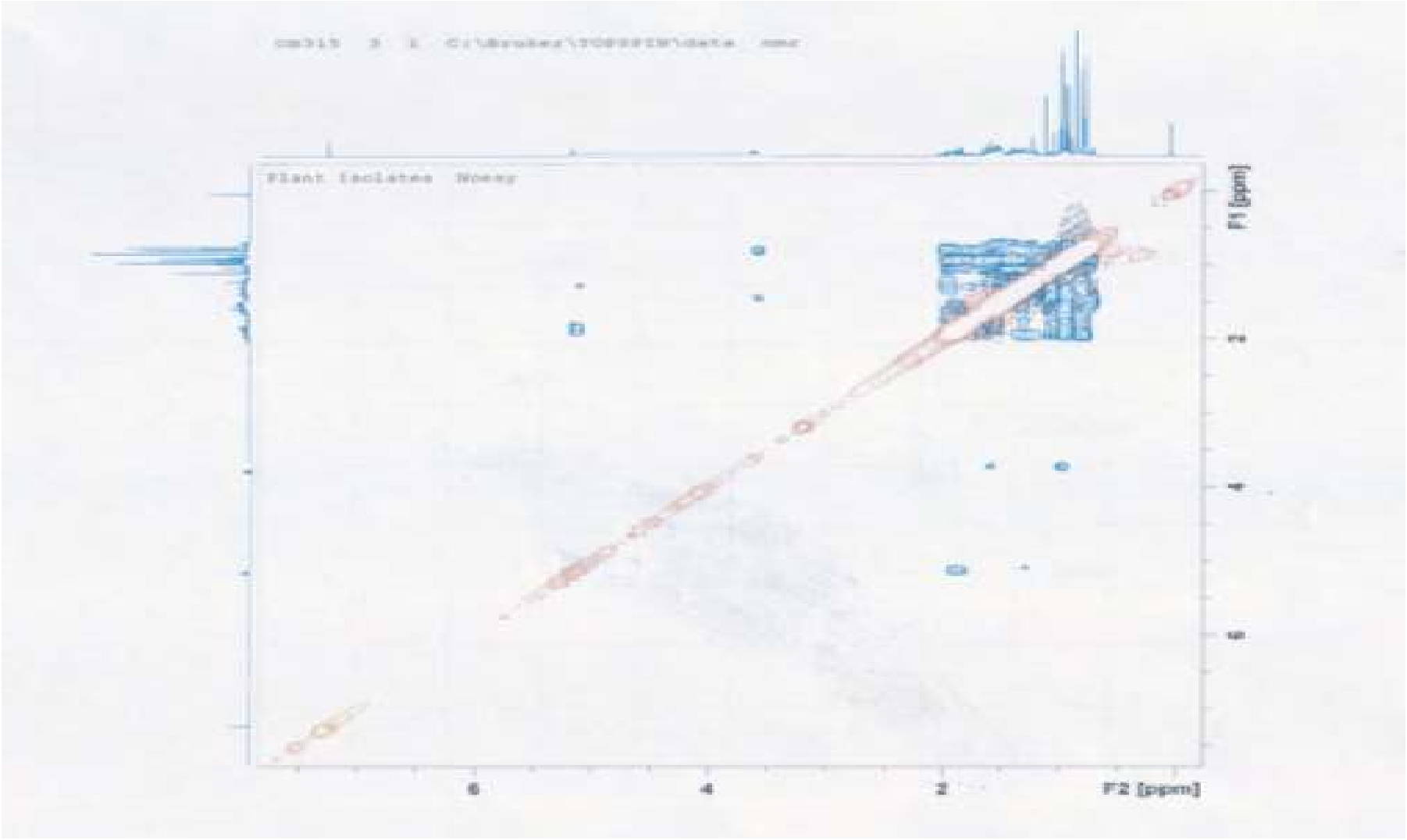


Figure 4.29: Nuclear Overhauser Effect Spectroscopy (NOESY) of OB 315

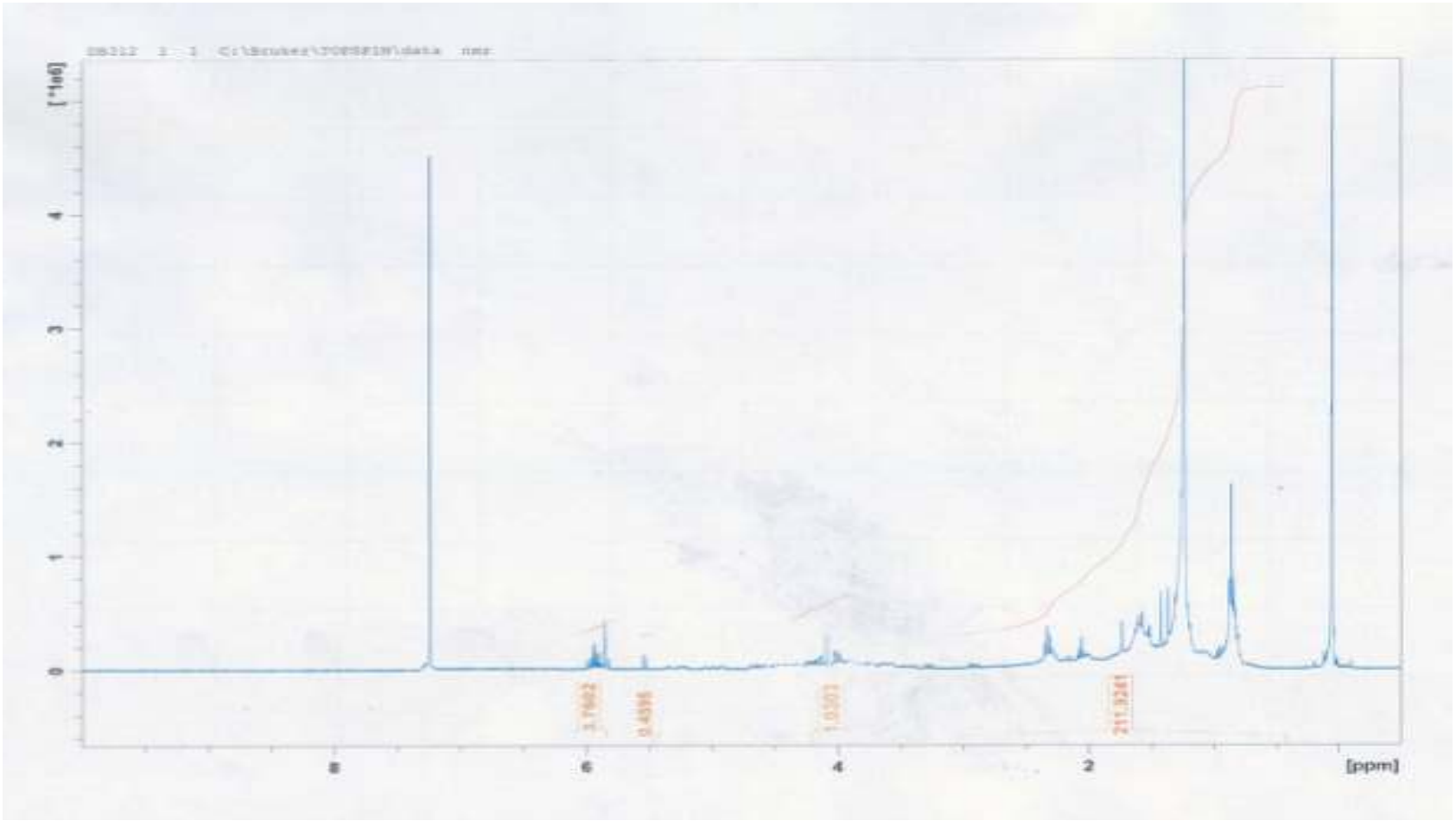


Figure 4.30: Integrated Proton (^{1}H) NMR of OB 312

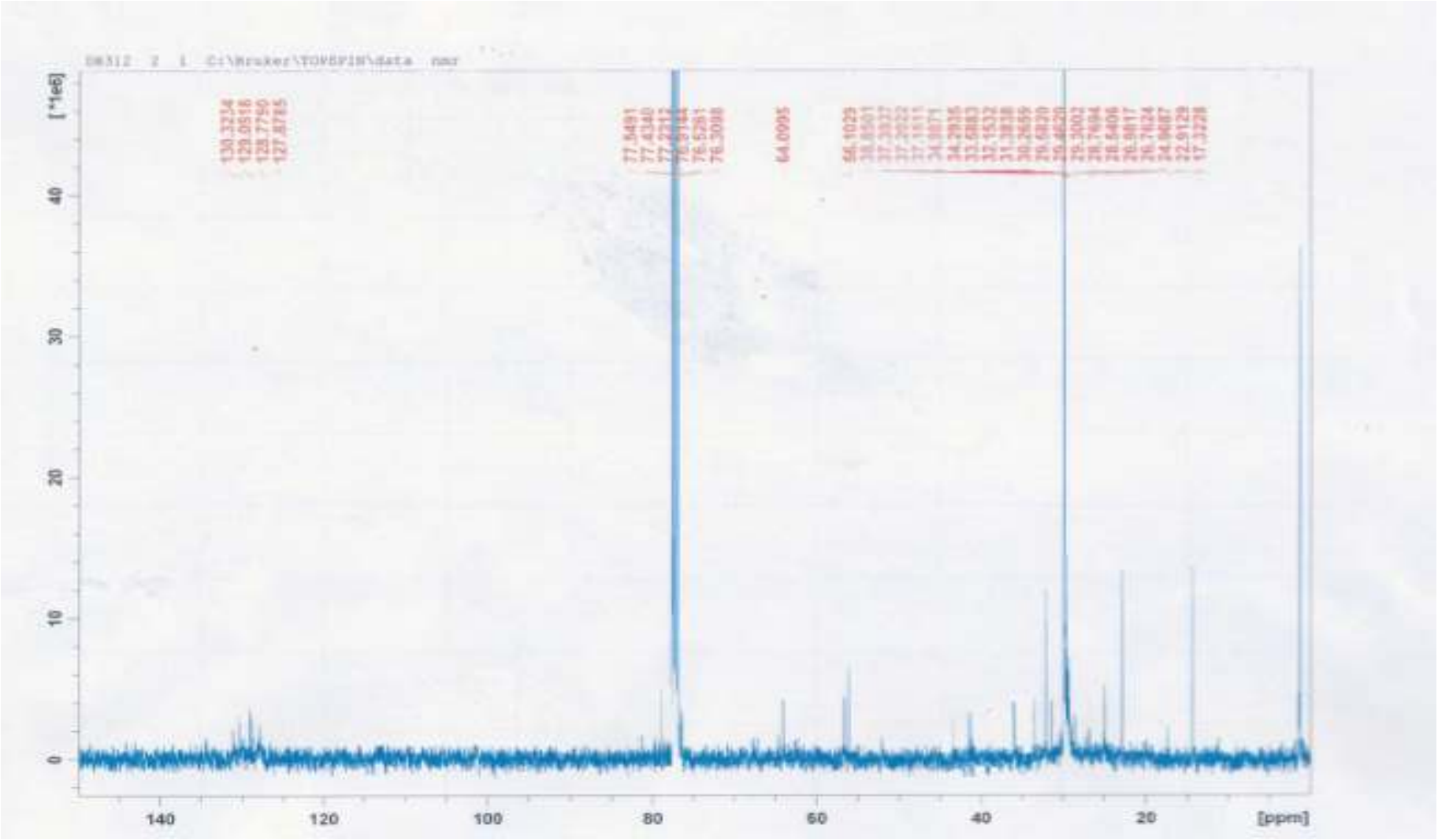


Figure 4.31: ^{13}Carbon NMR Spectrum of OB 312

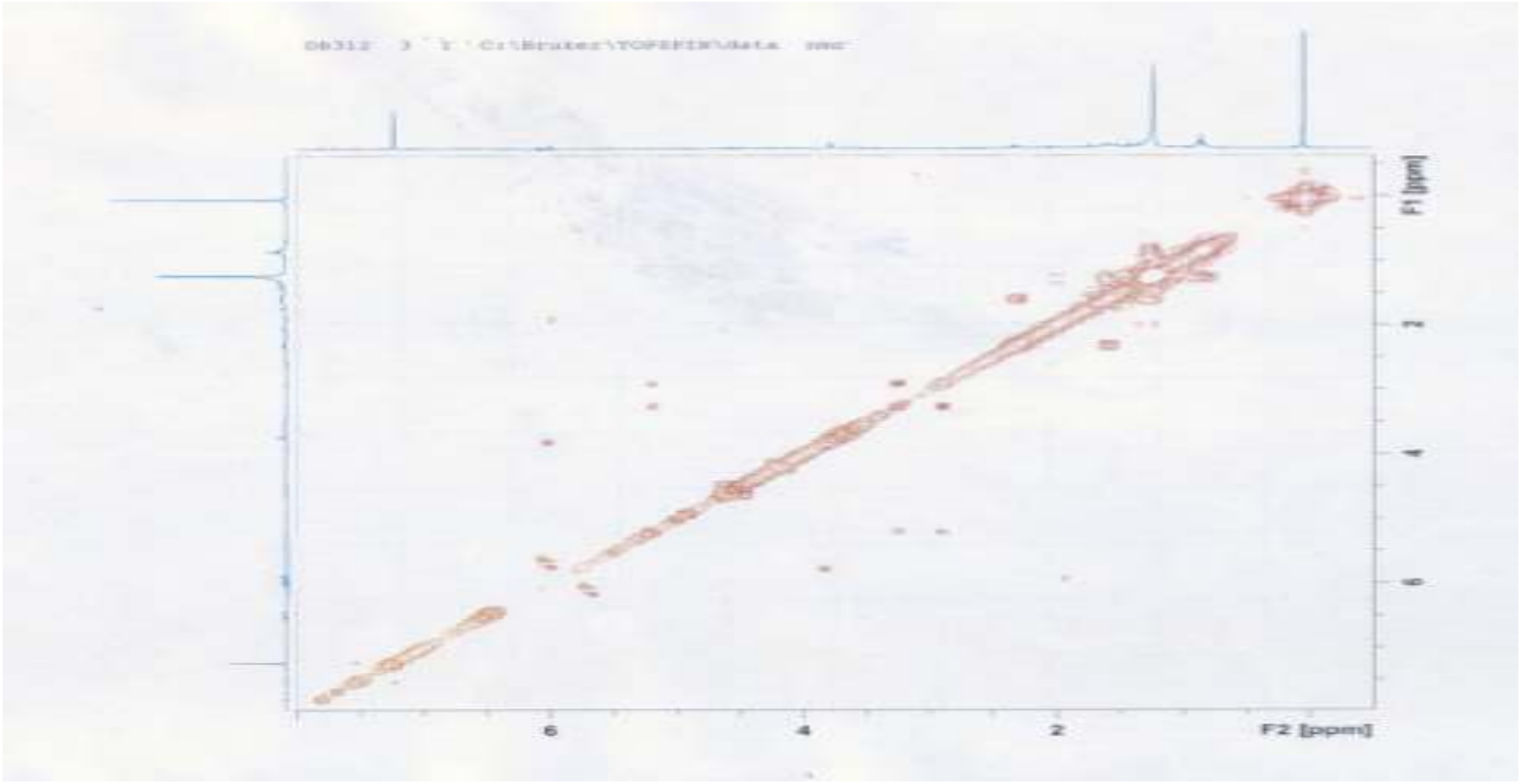


Figure 4.32: Correlation Spectroscopy (COSY) of OB 312

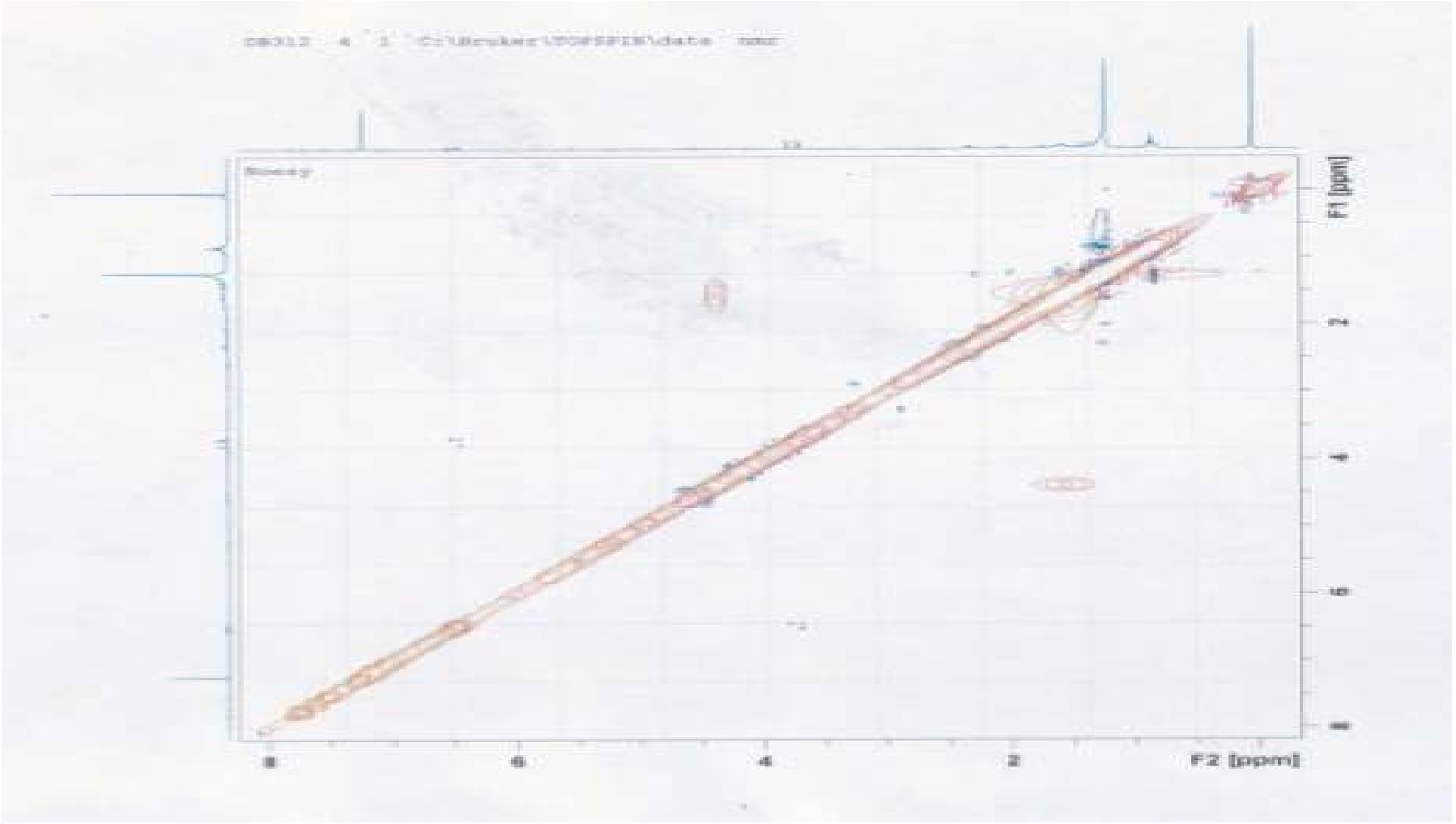


Figure 4.33: Nuclear Overhauser Effect Spectroscopy (NOESY) of OB 312

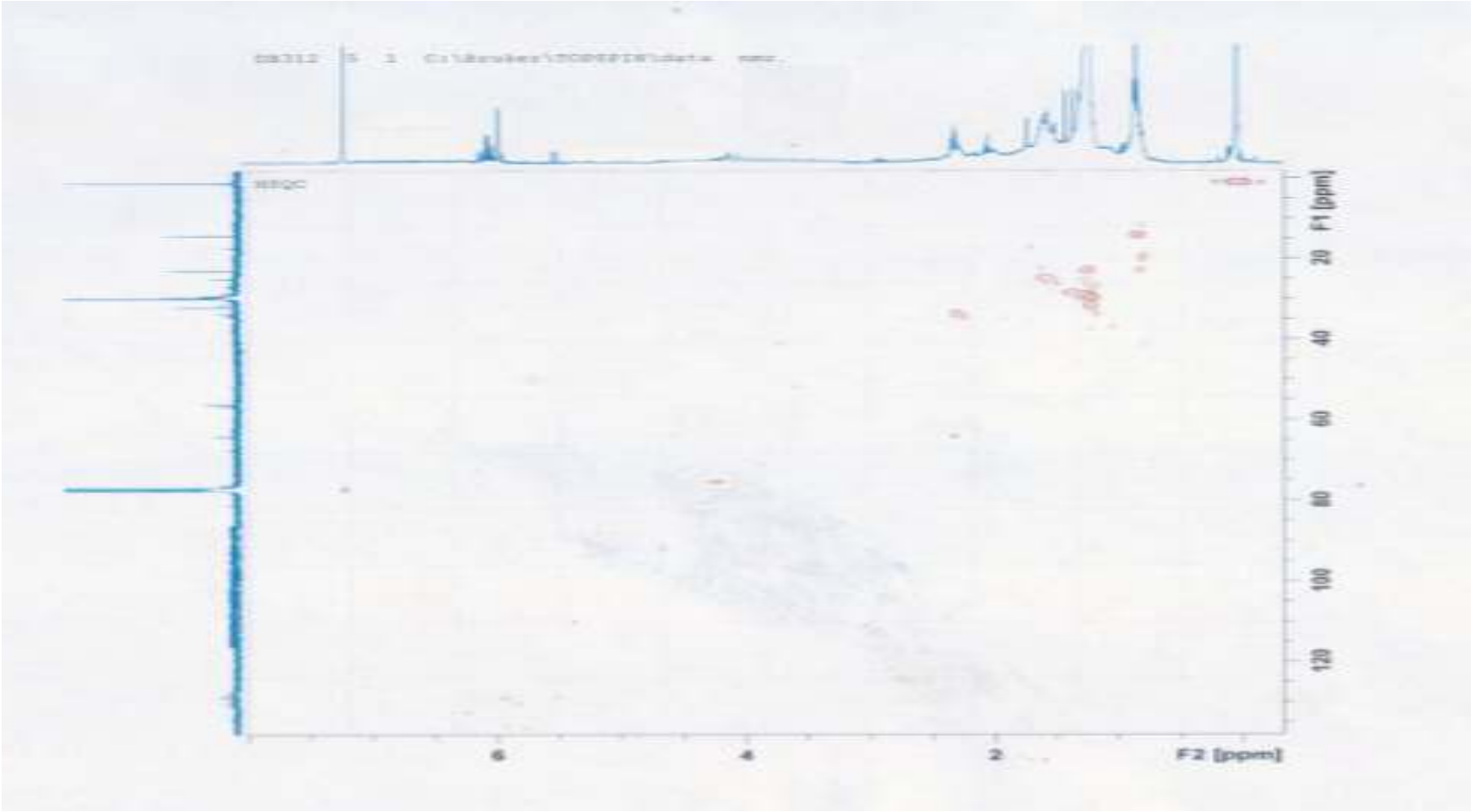


Figure 4.34: Heteronuclear Single Quantum Correlation (HSQC) Spectrum of OB 312

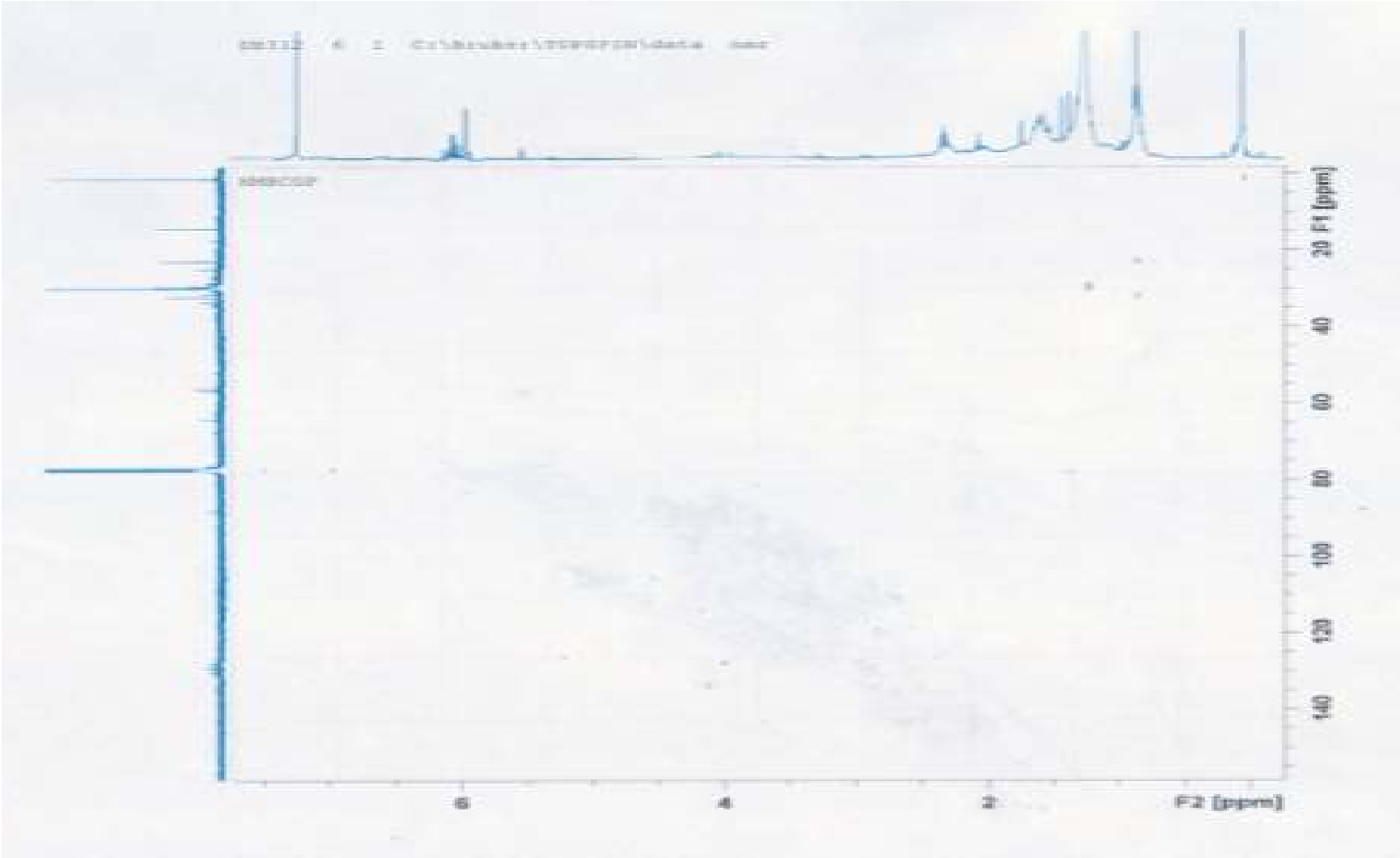


Figure 4.35: Heteronuclear Multiple Bond Correlation (HMBC) Spectrum of OB 312

CHAPTER FIVE

5.0 DISCUSSION

5.1. Extraction

The percentage recovery yield of *M. pruriens* and *C. schweinfurthii* (leaves and stem bark) when 1.5 kg of powdered plant materials were exhaustively extracted with methanol, gave 18.6 %, 15.7 %, and 22.2 % extracts respectively (Table 4.1). This indicates that the stem bark of *C. schweinfurthii* conatins more secondary metabolite than *C. schweinfurthii* leaves and *M. pruriens.*

5.2 Phytochemical Screening of Crude Extracts

Presence of phenolic compounds in medicinal plants and dietary herbs enhances the antimicrobial activity of these plants. Thus, the medicinal potential of medicinal plants lies in these chemical compounds that produce a definite physiological action in the human body (Akinpelu *et al.,* 2011). These phenolic compounds include phenolic acids, flavonoids, tannins, saponins, cardiac glycosides among others. In this study, the phytochemical screening of crude methanol extracts revealed the presence of on the plant crude methanol extracts revealed the presence tannins, cardiac glycosides, phytosterols, phenolic compounds, flavonoids and alkaloids (Table 4.3). The stem bark of *C. schweinfurthii* and *M. pruriens* did not show the presence of alkaloids, and saponins respectively. These phytochemical constituents are known to exhibit medicinal as well as physiological activities (Jain and Bari, 2010).

Flavonoids and phytosterols were found in all hexane, chloroform, ethyl acetate and methanol extract of *C. schweinfurthii* (leaves and stem bark) and *M. pruriens*. The phytochemical screening result obtained for *C. schweinfurthii* (leaves and stem bark) extracts is consistent with the report of Ngbede *et al.,* (2008).

5.3 Antimicrobial Susceptibility Assasy of the Crude Extracts

The *M. pruriens* and *C. schweinfurthii* leaves and stem bark extracts were screened against eleven bacteria and four *candida species* with ciprofloxacin, erythromycin and fluconazole as positive controls (Tables 4.4-4.6).

The extracts of *C. schweinfurthii* leaves and stem bark showed no activity against *S. pyogenes, P. mirabili* while *C. stellatoidea, P. aeruginosa*, *C. krusei,* MRSA and *K. pneumoniae* showed no sensitivty to *M. pruriens* extracts. The resistance of the *candida species* toward the extracts is due to the presence of plasmid which is a primary source of resistance genes (Hudson *et al.,* 2014).

The resistance of Gram-negative bacteria against the extract can be explained by the formation of biofilms by this class of bacteria which protect them from adverse environmental factors. Gram-positive bacteria are sensitive due to their inability to inhibit the synthesis of peptidoglycan, but unlike β-lactam antibiotics, glycopeptide antibiotics which target and bind to amino acids in the cell wall, preventing peptidoglycan cross-linkages from forming which is the basis of their activity (Nathisuwan *et al*., 2001).

The methanol extract of *M. pruriens* had zones of inhibition (ZI) which ranged from 35–42 mm with maximum and minimum inhibition for *S. typhi* and *MRSA,* respectively. Hexane extract exhibited maximum antibacterial inhibition against *MRSA* (30 mm) followed by *VRE* (28 mm) and least for *P. mirabilis* (25 mm). Ethyl acetate extract gave lower ZI ranging from 20 mm for *S. typhi,* to 25 mm for *B. subtilis*, while no activity was observed for *S. aureus, K. pneumoniae* and *P. aeruginosa*.

Generally, the extracts showed greater antibacterial activity towards Gram-positive as compared to Gram-negative bacteria. Methanol extract showed greater inhibition than hexane and chloroform extracts with significantly lower inhibition observed for the ethyl acetate extract.

The same trend had been reported by other workers on the antibacterial properties of methanol extracts of *M. puriens* against the pathogens selected in the study (Rayavarapu and Kaladhar, 2011). A lower antimicrobial action against Gram-positive bacteria as compared to Gram-negative organisms could be due to the presence of additional lipopolysaccharide layer that prevents the passive transport of the bioactive compounds (Martinez *et al.,* 1996).

Jain and Bari (2010) reported that the presence of these phytochemicals could account for high antimicrobial activity demonstrated by the plant. Tanins possess physiological, astringent and haemostatics properties which hasten wound healing and ameliorate inflamed mucus membrane and also inhibit the growth of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them. They also form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. In general, their non-specific activity has been ascribed to their ability to complex with metal ions, scavange radicals and reduces active oxygen species and form tight complexes with a wide array of proteins and polysaccharides (Finar, 1989).

The presence of saponins and alkaloids in the leaves and stem bark of *C. schweinfurthii* influences the relatively high activity toward Gram-negative bacteria and Candida. Saponins, a special class of glycosides, have expectorant action and are reported to have anti-diabetic and anti-fungal properties (Kamel, 1991; Just *et al.,* 1998). Saponins are known to produce inhibitory effects on inflammation (Okwu, 2004) and have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, heamolytic activity, cholesterol binding properties and bitterness (Sodipo *et al*., 2000; Raquel, 2007).

Steroids have been reported to have antibacterial properties (Okwu, 2001), and they are very important compounds especially due to their activities as sex hormones (Cowan, 1999).

Alkaloids are reported to produce some highly efficacious antimicrobials which bind to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption and metal ion complexation (Nobori, 1994). Alkaloids have been associated with medicinal uses for centuries and one of their commonest biological properties is their cytotoxicity (Nyarko and Addy, 1990). Several workers have reported the analgesic, antispasmodic and antibacterial (Sodipo, 2000) properties of alkaloids.

The presence of these phytochemicals in *M. prurien and C. schweinfurthii* extracts suggests that the plant is pharmacologically active; supporting the claim by the traditional healers and proving the plant to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. This supports the claim by herbal healers for the use of the plant in the treatment of diarrhoea, dysentery and wounds (Iwu, 1993 and Timperio *et al*., 2012).

5.4 Zone of Inhibition (mm) of Crude Extracts

The presence of alkaloid in the ethyl acetate and methanol extracts is likely responsible for the observed high zone of inhibition against Gram-positive bacteria (25-32 mm) as compared with other leaves and stem bark extracts.The stem bark extract of *C. Schweinfuthii* (Tables 4.4 and 4.5) showed a comparable antibacterial activity against Gram-negative bacteria as observed in the leaves extract (Table 4.4) but lower anti-candida activity (Table 4.4) which is in agreement with Cowan (1999) in the report on the mechanism of action of alkaloids.

The chloroform and ethyl acetate extracts of the stem bark of *C. schweinfurthii* gave high zones of inhibition of 30 mm and 26 mm (Table 4.4) against *B. subtilis* and *C. albicans* respectively, the high activity is due to the ability of the phytochemicals to hydrolyse the cell walls which are made up of an inner layer of chitin and an outer layer of mannoproteins which differ in hydrophobicity.

5.5 Minimum Inhibitory Concentration of the Crude Extracts

The hexane extracts of *C. schweinfurthii* leaves and stem bark and *M. pruriens* have a MIC

of 25, 6.25 and 12.5 mg/ml respectively, the chloroform extracts of *C. schweinfurthii* leaves and stem bark and *Mucuna pruriens* have a MIC of 12.5, 6.25 and 12.5 mg/ml respectively, the ethyl acetate of *C. schweinfurthii* leaves and stem bark, and *M. pruriens* have a MIC of 6.25 mg/ml, 12.5 and 6.25 mg/ml respectively and the methanol extract of *C. schweinfurthii* leaves and stem bark and *M. pruriens* have this same MIC of 12.5 mg/ml (Table 4.6).The most active of the menstrum of the *C. schweinfurthii* leaves and stem bark and *M. pruriens* is the ethyl acetyate and chloroform with MIC of 6.25 mg/ml.

5.6 Minimum Bacterialcidal and Fungicidal Concentrations of the Crude Extracts

The concentrations of the extract required by the hexane extracts of *C. schweinfurthii* leaves and stem bark and *M. pruriens* to kill the majority of the tested bacteria and candida is 50 mg/ml and 25 mg/ml, choloroform extracts of *C. schweinfurthii* leaves and stem bark, and *M. pruriens* requires 12.5 mg/ml, 50 mg/ml, ethyl acetate extracts of *C. schweinfurthii* leaves and stem bark and *M. pruriens* 25 mg/ml and methanol extracts of *C. schweinfurthii* leaves and stem bark and *M. pruriens* requires 25mg/ml, 50mg/ml and 50mg/ml respectively (Table 4.10).

The most potent extract of *C. schweinfurthii* leaves and stem bark and *M. pruriens* are the chloroform ethyl acetate and chloroform extracts with MBC/MBF of 12.5 mg/ml, 25 and 25 mg/ml respectively.

5.7 Zone of Inhibition (mm) of the Isolated Compounds

Compound CB 47/70 was sensitive towards three Gram-postive bacteria (*MRSA, S. aureus* and *B. subtilis*), three Gram-negative bacteria (*K. pneumoniae, S. dysenteriae* and *S. typhi*) and three candida species (*C. albicans, C. tropicalis* and *C. Stellatoidea*). The zone of inhibition of the Gram-positive bacteria were between 27-32 mm, Gram-negative 16-29 mm compared with positive control ciprofloxcian 35-40 mm while the zone of inhibition of the candida speices were measured at 24-26 mm compared to fluconazole 32-37 mm (Table 4.7).

Compound OB 45 was sensitive to four Gram-postive bacteria (*MRSA, S. aureus, VRE* and *B. subtilis*), three Gram-negative bacteria (*K. pneumoniae, S. dysenteriae* and *S. typhi*) and three candida spicies (*C. albicans C. tropicalis* and *C. stellatoidea*). The zones of inhibition of Gram-positive bacteria were between 22-26 mm, Gram-negative 22-27 mm compared with positive control ciprofloxcian 35–40 mm while the inhibition zone of the *Candidas* were measured as 21-24 mm compared to fluconazole 32-37 mm (Table 4.7). Compound OB 315 was active on two Gram-postive bacteria (*S. aureus* and *VRE*), five Gram-negative bacteria (*S. dysenteriae, K. pneumoniae, E. coli, P. aeruginosa* and *P. mirabilis*) and three candida species (*C. tropicalis, C. krusei, C. stellatoidea*). The zones of inhibition for Gram-positive bacteria were between 18-24 mm, Gram-negative 21-27 mm compared with positive control ciprofloxcian 35-40 mm while the inhibition zone of the Candida species was 18 mm compared to fluconazole 32-37 mm (Table 4.12). Compound OB 312 was active against two Gram-postive bacteria (MRSA, *B. subtilis*), four Gram-negative bacteria (*P. aeruginosa*, *S.typhi*, *E. coli and P. mirabilis*) and two candidas (*C. tropicalis* and *C. stellatoidea*). The zones of inhibition for the Gram-positive bacteria was 22 mm, Gram-negative 21-27 mm compared with positive control ciprofloxcian 35-40 mm while the inhibition zones of the *Candidas* was at 16-20 mm compared to fluconazole 32-37 mm (Table 4.7).

Compound CB 47/70 and OB 45 possesses both antibacterial and antifungal properties and tends to show strong anticandida activity. Compound OB 315 possesses moderate antibacterial and poor anticandida properties while compound OB 312 showed poor antimicrobial and moderate anticandida properties. The micro-organism lipohillic or hydrophillic cell wall is responsible for the difference in the activities of the isolates; it allows the passive transport of most drugs accross cell membranes.

5.8 Minimum Inhibitory Concentration (µg/ml) of the Isolated Compounds

Compound CB 47/70 inhibited the growth of four Gram-postive bacteria (*MRSA, VRE, S.*

aureus and *B. subtilis*) at 6.25 µg/ml, three Gram-negative bacteria (*K. pneumoniae, S. dysenteriae* and *S. typhi*) 6.25 µg/ml and three candida species (*C. albicans, C. tropicalis* and *C. stellatoidea*) at 12.5 µg/ml (Table 4.9). Compound OB 45 inhibited the growth of four Gram-postive bacteria (*MRSA, S. aureus, VRE* and *B. subtilis*) at 6.25 µg/ml, three Gram-negative bacteria (*S. dysenteriae, E. coli* and *S. typhi*) at 6.25 µg/ml and three candida species (*C. albicans, C. tropicalis* and *C. stellatoidea*) at 6.25 µg/ml (Table 4.9).Compound OB 315 inhibited the growth of two Gram-postive bacteria (*S. pyogenes* and *VRE*) at 6.25 µg/ml, three Gram-negative bacteria (*S. dysenteriae, E. coli* and *P. mirabilis*) 12.5 µg/ml and three candida species (*C. tropicalis, C. stellatoidea* and *C. krusei*) at 6.25 µg/ml (Table 4.12). Compound OB 312 inhibited the growth of three Gram-postive bacteria (*MRSA, S.aureus* and *B.subtilis*), three Gram-negative bacteria (*K. pneumoniae*, *S. dysenteriae and P. mirabilis*) and two *candida species* (*C. tropicalis* and *C. albicans*) 12.5 µg/ml **(**Table 4.9).

Compounds OB 45, OB 315 and CB 47/70 inhibit the growth of both Gram-postive and Gram-negative bacteria with a moderate inhibition against candida except for the poor antibacterial activity of OB 315. Generally, the observed activities of the compounds were comparatively high towards the bacteria and moderate towards candida species.

5.9 Minimum Bactericidal and Fungicidal Concentration of the Isolated Compounds

The minimum concentration required to kill the microorganism varies from one isolate to the other depending on the transmembrane exclusion resistance of the organism. Compound CB 47/70 was bactericidal towards Gram-postive bacteria at 12.5 µg/ml, Gram-negative bacteria at 25 µg/ml and fungicidal against candida species at 25-50 µg/ml (Table 4.11). Compound OB 45 was bactericidal towards four Gram-postive bacteria at 12.5 µg/ml, Gram-negative bacteria at 25 µg/ml and fungicidal towards candida species at 25 µg/ml (Table 4.11). The bactericidal concentration of compound OB 315 against Gram-postive bacteria is 12.5 µg/ml, three Gram-negative bacteria at 50 µg/ml and fungicidal concentration of 25 µg/ml (Table

4.11). Compound OB 312 totally stopped the growth of Gram-postive bacteria, Gram-negative bacteria and candidas at 12.5 µg/ml, 25 µg/ml and 50 µg/ml (Table 4.11).

Compounds OB 45 and CB 47/70 were bactericidal at very low concentration of 12.5 µg/ml but OB 315 and OB 312 were bactericidal at high concentration of 50 µg/ml. Generally, the observed fungicidal concentration of the compound OB 312 towards the test organism is comparatively high.

5.10 Effect of Extracts on the Eggs and Larvae of *Ascaris Suum*

The rate of development and longevity of nematode eggs and larvae vary with temperature, rainfall and humidity in different geo-ecological regions (Tembely, 1998). According to Hounzangbe-Adote (2005), the egg is the stage of dissemination into the environment hence is protected by a thick wall making it resistant to unfavourable conditions such as the climatic and environmental conditions and can therefore remain infective for a long period of time, several years to be exact.

Results obtained from the present study showed high level of egg hatch relative to the larval inhibition, which could be attributed to the reported ability of nematode eggs to withstand adverse conditions (Salih, 2003). The reason eggs are not susceptible to medicinal components as compared to the larval development is because during the experiment larvae are exposed to the medicinal components when compared to eggs (Molan *et al.,* 2003). L_1 and L_2 stage larvae are the feeding stages and therefore they could even ingest or absorb medicinal components (Ferhat *et al*., 2011).

The results from the present study showed that chloroform and ethyl aceate extracts led to high egg hatch inhibition percentages than methanol extracts of the two plants. This indicates that the chloroform and ethyl aceate are more effective in extracting the anthelmintic compounds in the two plant materials. In the present study, there was less than 10 % larval development from L_1 to L_3 for all *C. schweinfurthii* leave and stem bark extracts as about 90

% died as first stage larvae without developing to the infective stage (L_3 larvae) at 80 µg/ml. The first stage larvae are the more sensitive ones to medicinal components than eggs (Cala *et al*., 2012). The infective larvae, L_3 larvae, of *A. suum* have high capacity to survive even in adverse conditions; high temperatures with high rainfall favour the development of the larvae from the pre-infective stage (L_1 stage larvae) to infective stage (L_3 larvae) throughout the year (Bizimenyera *et al*., 2006). The results from our study showed that the chloroform and ethylaceate extracts from both *C. schweinfurthii* leave and stem bark and *M. pruriens* were more effective than the methanol extracts against the egg viability (Fig. 4.1-4.3). Eguale *et al*., (2011) stated that the difference in effectivity of the extract solvent might be due to the variation in solubility of active constituents. Chloroform and ethyl aceate are suitable extractants as they extracts compounds of a wide variety of polarity (Bizimenyera *et al*, 2006). However, nematodes have been documented with the ability to quickly adapt to their environments, therefore it is either that the methanol extracted components provided a suitable medium for the survival of the larvae or the larvae quickly adapted to the extract itself. The *C. schweinfurthii* leave and stem bark have been documented with essential oils of resins (monoterpenes hydrocarbon) (Dongmo *et al.*, 2010) while *M. pruriens* was documented with n-hexadecanoic acid, squalene, oleic acid, ascorbic acid and octadecanoic acid, (-)-3-methoxy-1,1-dimethyl-6,7-dihydroxy-1,2,3.4-tetrahydroquinoline and (-)-3-methoxy-1,1-dimethyl-7,8-dihydroxy-1,2,3.4-tetrahydro quinolone, serotonin, 5-hydroxyl tryptophane, nicotine, N,N-dimethyl tryptamine, bufotenine which actually imposes bactericidal, fungicidal, antioxidant and significant anti-inflammatory activity via lipooxygenase method with IC_{50} of 62.6ppm (Duke, 1995). The anthelmintic effectiveness of medicinal extracts from both plants is due to their phytochemical composition which might be from single or combined effect of the compounds or chemical groups. Ferhat *et al*., (2011) stated that medicinal compounds might interfere with the osmotic balance of the worm hence

death will result. There are two mechanisms of nematode uptake of drugs, it can either be through/by diffusion of the drug through the eggshell or through the larval cuticle or by diffusing through the intestinal cells (Suteky and Dwatmadji, 2011; Eguale *et al*., 2011). The presence of tannins in the plants (Table 4.3) is characterized with the connection to free proteins or connection to the larval cuticle reducing the availability of nutrients therefore resulting in death of the larvae due to starvation; this is because tannins are rich in glycoproteins (Cala *et al*., 2012). The anthelmintic activity exhibited by *C. schweinfurthii* leaves and stem bark and *M. pruriens* crude extracts against gastrointestinal nematodes of pig (*A. suum*) in this study has a significant importance considering the worldwide emergence of anthelmintic resistance.

5.11 Ovicidal Activity of the Isolated Compound

The eggs of *A. suum* are protected by a thick wall making it resistant to unfavourable conditions (Hounzangbe–Adote *et al*., 2005) which are made up of P-glycoproteins, which have a significant role in transmembrane exclusion of anthelmintics from the CNS, gut, and hepatobiliary tract of hosts, which could account for reduced oral bioavailability of some anthelmintic compounds (Prichard *et al*., 2012). The present study was to measure the percentage eggs that metamorphorsize into the pre-infective stage. The ovicidal activity of the isolates was measured at two different concentrations. At 10 µg/ml the percentage inhibitions were 62, 58, 38, 23 % and at 20 µg/ml; 68, 62, 50, 30 % (Fig 4.4) for CB 47/70, OB 315, OB 45 and OB 312 respectively (Fig 4.4). The most potent ovicidal isolate relative to the control drug (albendazole) was CB 47/70. The activities of the isolates were concentration dependent (Fig 4.4). Much has not being reported about the anthelmintic potency of triterpenoids and steroids like the tannins and saponins (Eguale *et al*., 2011). The structure of the isolates may explain why the isolates inhibited embryonation and egg hatching. The five carbocyclic rings of the triterpenes CB 47/70, OB 315 and OB 312 will

increase the lipophilic nature of the isolates and increase the diffusion of the isolates into the egg shell, which will interfere with the osmotic balance and hence leading to death (Ferhat *et al*., 2011). The mechanism of action of albendazole (benzimidazole drug) is by blocking glucose uptake in the larval and adult stages of susceptible parasites, and also depleting their glycogen reserves, thus decreasing ATP formation (Martin, 1997). Clearly, the isolated compounds do not have a benzimidazole frame work hence, another mechanism of action may be proposed. A natural product anthelmintic compound; having a receptor-mediated effect on glutamate-gated chloride (GluCl) ion channels, which has been directly correlated to nematocidal activity and which is now considered as their major mode of action (Martin and Pennington, 1988) maybe proposed as the mechanism of action of the isolates.

5.12 Larvicidal Activity of the Isolated Compounds

The larvicidal activities of the isolates are high compared to the ovicidal activities. The larvacidal activities ranges from 25-78 % at 10 µg/ml to 38-84 % at 20 µg/ml (Fig 4.8); which is an indication that the P-glycoproteins does not play any significant role in transmembrane exclusion of the isolates. The high larvicidal activities may also be as a result of the free-feeding stage of the larvae which allows the isolates to penetrate the gut and access the CNS and hepatobiliary tract of the host. Saponins are closely related members of the triterpenes have also been reported to have nematoicidal activities and are said to interact with the cell membrane causing changes in cell wall permeability (Ademola and Eloff, 2010). They also interact with collagen proteins from the cuticle of nematodes and this interaction may be responsible for the nematotoxic effects (Argentieri *et al.,* 2008). At high concentrations, the isolates significantly inhibited the development of L1 and L2 larvae. These substances are able to penetrate the cuticle of the nematode and to prevent the absorption of the glucose, or block post synaptic receptors thus paralyzing the larvae. The most larvicidal isolates is OB 45 with percentage inhibitions of 75 % and 84% at 10 and 20

µg/mL respectively; while the least is OB 312 with percentage inhibitions of 25 % and 38% at 10 and 20 µg/mL (Fig 4.8) respectively. The activity of OB 45 above can be attributed to the carboxylic acid group which has the ability of hydrolysing the P-gylcoprotein gateway, while the low activity of OB 312 might be as a result of the epoxide group undergoing hydroxylation in the aqueous acid in the embroyating fluid to give glycols which increases its transmembrane exclusion and hence, its low larvicidal activity. Terpenoids most likely undergo lipid peroxidation which results in damage to the cell membrane, mostly the end product of lipid peroxidation may be mutagenic and carcinogenic (Marnett, 1999).

5.13 Spectral Analysis

5.13.1 Ultraviolet (UV) of CB 47/70

In CB 47/70, the UV absorption band is indicative of weak auxochromes that are pH dependent like OH and a conjugated olefinic group with an UV absorption maximum at about 290 nm (Fig: 4.9).

5.13.2 Characterization of compound CB 47/70

Compound CB 47/70 was obtained as a white amorphous solid. The ^{13}C-NMR spectrum showed 30 carbon atoms. Distortionless Enhancement Polarization Transfer (DEPT) subspectrum indicated five methine (CH) carbons at δ 64.10, 55.18, 121.73, 139.30, 124.42, nine methylene (CH_2) at δ 38.79, 27.24, 18.38, 34.74, 22.60, 29.71, 39.62, 33.35, 37.15 and eight methyl (CH_3) groups at δ 29.37, 21.41, 15.63, 16.81, 23.70, 26.00, 30.09 and 28.77. Quartenary carbon atoms do not contain attached protons hence do not appear in DEPT subspectrum. They may be identified as the signals which are additionally in the proton broadband decoupled ^{13}C-NMR spectra, therefore, eight quaternary carbons were identified (Fig 4.11).

The ^{1}H NMR (400 MHz, CD_3Cl) spectrum showed the presence of an oxymethine proton resonating at δ 3.40 (1H), two olefinic protons at δ 5.20 (1H), δ 5.35 (1H) and hydroxyl

proton at δ 2.25 (1H). The signal from δ 0.70-1.98 (multiplet) are due to the presence of overlapping methyl, methylene and methine protons (Fig 4.10).

The ^{13}C NMR data (400 MHz, CD_3Cl) spectrum showed the presence of four olefinic carbon atoms resonating at δ 124.43, δ 139.59, δ 121.73 and δ 145.20. The oxymethine carbon peaked at δ 79.1 while the signals at δ 47.72, δ 55.18, are methine groups. The signals from δ 37.20, 27.66, 23.70, 28.10, 34.74, 31.26 and 38.60 are probably due to methylene groups, while the methyl groups are indicated by the following signals δ 21.41, 17.48, 18.38, 22.70, 21.41, 23.53, 23.70 and 16.87 respectively (Fig 4.11).

The COSY data indicates that the olefinic protons at δ 5.20 (1H (t)) and δ 5.35 (1H(s)) show correlation with methine protons at δ 1.97and 1.88. The oxymethine proton at δ 3.19 shows correlation with 1.41 methine, 1.51, 1.52 methylene and 0.95, 0.94 methyl protons (Fig 4.15).

The HSQC was used in assigning the ^{1}H signals to the corresponding ^{13}C signals, the oxymethine proton at δ 3.40 (1H (t)) shows direct correlation to the ^{13}C signals at δ 79.1, olefinic protons at δ 5.20 (1H (d)) and δ 5.35 (1H (d)) show correlation to the ^{13}C signals δ 124.43 and δ 121.43 respectively. The ^{13}C signals at δ 18.38 - 46.83 show correlation with the methyl protons around δ 1.50-1.98 (Fig 4.13)

The oxymethine protons peaked at δ 3.40 shows correlation with 39.62 methine, 37.15 and 27.24 methylene and 29.39 and 21.41 methyl protons. The olefinic protons at δ 5.20 (1H (t)) and δ 5.35 (1H (s)) show multiple bond correlations with 32.92, 33.25, 37.15, 28.77 and 42.09 while the angular methyl protons at δ 1.01 and δ 0.99 show correlation to δ 40.04 and δ 139.8 The COSY data indicates that the olefinic protons at δ 5.20 (1H (t)) and δ 5.35 (1H(s)) show correlation with methine protons at δ 1.97and 1.88. The oxymethine proton at δ 3.19 shows correlation with 1.41 methine, 1.51, 1.52 methylene and 0.95, 0.94 methyl protons (Fig 4.15).

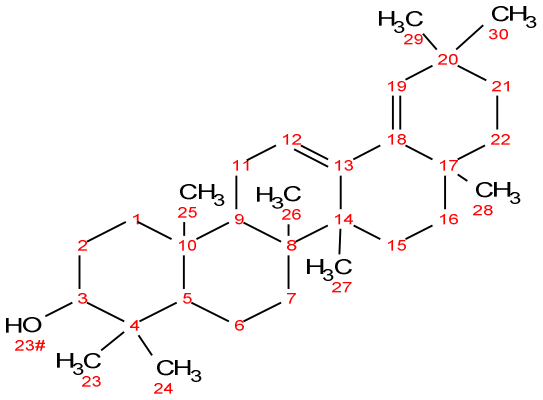
Based on these information from the the 1 D and on the COSY, HMBC, and NOESY values,

CB 47/70 was deduced to be a 3β-hydroxyl olean-12,18-diene (β -amyrin) **(20).** The complete ^{13}C NMR spectroscopic assignments are consistent with those reported in literature (Table 5.1) (Mahato and Kundu, 1994; Gonzalez *et al.,* 1981).

Table 5. 1: ^{13}C NMR Data of Compound CB 47/70

Carbon atoms	Experimental δc(ppm)	*Literature δc(ppm)	DEPT
C1	38.79	38.51	CH_2
C2	27.24	27.43	CH_2
C3	79.06	79.20	CH
C4	39.62	39.02	C
C5	55.18	55.75	CH
C6	18.38	18.31	CH_2
C7	34.74	34.75	CH_2
C8	40.02	40.81	C
C9	47.72	51.31	C
C10	38.60	37.31	C
C11	22.60	22.20	CH_2
C12	121.73	123.88	CH
C13	139.30	141.50	CH
C14	42.09	42.84	C
C15	29.71	30.85	CH_2
C16	39.62	39.01	CH_2
C17	34.74	35.65	C
C18	145.20	142.25	C
C19	124.42	129.76	CH
C20	32.95	32.92	C
C21	33.35	33.22	CH_2
C22	37.15	37.52	CH_2
C23	29.37	28.81	CH_3
C24	21.41	20.81	CH_3
C25	15.63	15.62	CH_3
C26	16.81	16.40	CH_3
C27	23.70	23.73	CH_3
C28	26.00	24.12	CH_3
C29	30.09	30.71	CH_3
C30	28.77	28.71	CH_3

*(Mahato and Kundu, 1994; Gonzalez *et al.,* 1981)



3β-hydroxyl olean-12,18-diene (β-amyrin)**(20)**

5.13.3 Ultraviolet (UV) of OB 45

In OB 45, the UV absorption band is indicative of weak auxochromes and an isolated olefinic group showed UV absorption maximium at 255 nm (Fig 4.9)

5.13.4 Characterization of compound OB 45

The compound OB 45 was obtained as white crystalline compound. ^{13}C NMR (Fig 4.18) and DEPT (Fig 4.17) showed 30 carbon atoms in the molecule consisting of one carboxyl, seven quaternary, five methine, eleven methylene and six methyl groups. The proton and carbon chemical shifts are characteristic spectral features of triterpenoids (Bross-Walch *et al.*, 2005). The ^{1}H NMR (400 MHz, DMSO) spectrum showed the presence of an oxymethine proton peaked at δ 2.79 (1H (t)), two olefinic protons at δ 4.71 (1H (s)) and δ 4.81 (1H (s)).The signal at δ 1.56, δ 1.20, δ 1.14, δ 0.56 and δ 0.37 (3H m) are likely due to the presence of overlapping methine and methylene and methyl while the signals at δ 1.31 and δ 4.35 (1H,s) are due to isopropyl protons (Fig 4.17)

The ^{13}C NMR data (400 MHz, DMSO) spectrum showed the presence of two olefinic carbon atoms at δ 110.06, δ 150.82. The oxymethine carbon peaked at δ 77.29 and carboxyl carbon at δ 177.70. The signals at δ 38.75, 27.63, 18.44, 19.43, 25.58, 30.51, 32.21, 29.68, 38.76 are methylene group. The signals from δ 55.39, 50.43, 38.09, 47.09 and 49.06 are due to methine groups, while the CH_3 methyl groups are indicated by the following signals δ 27.53, 15.22, 16.41, 16.44, 19.43 (Fig 4.18).

The sp^2 hybridized protons at δ 4.71 and 4.81 show direct correlation to the same carbon at δ 110.06; this implies that the two protons are attached to the same carbon.The oxymethine proton at δ 2.79 is showed a direct correlation to the signal at δ 77.29. The ^{13}C signals at δ 38.75, 30.51, 32.21 and 38.76 showed direct correlation to methylene protons around δ 1.5 – 2.0 ppm (Fig 4.21).

The olefinic proton at δ 4.71 shows a three bond correlation with the proton signal at δ 1.50. The methyl protons are usally found resonating in this region hence, it is a methyl proton.

Another key cross peak is the oxymethine protons showing correlation with methylene protons around δ 1.4–1.80, this locates the oxymethine proton two to three bonds away from a methylene group (Fig 4.21).

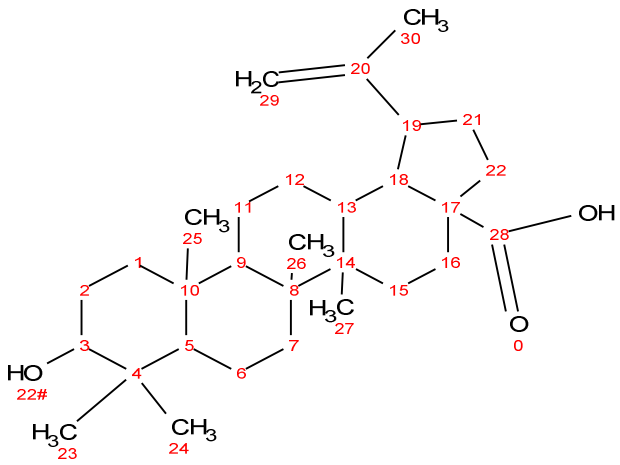
The olefinic protons showed correlation to the carbon resonating at δ 19.43 and 49.06. The oxymethine proton showed key correlations with the methylene and methyl carbon signals at 38.97 and 15.22 respectively (Fig 4.22)

Thus, the structure of OB 45 was considered to be a 3β-hydroxyllup-20(29)-en-28-oic acid (betulinic acid) **(21)**. This is consistent with those reported in literature (Table 5.2) (Okwute, and Isyaka, 2014; Habila *et al*., 2013).

Table 5. 2: ^{13}C NMR Data of Compound OB 45

Carbon Atoms	Experimental δc(ppm)	*Literature δc(ppm)	DEPT
C1	38.75	38.7	CH_2
C2	27.63	27.4	CH_2
C3	77.29	78.9	CH
C4	38.97	38.8	C
C5	55.39	55.3	CH
C6	18.44	18.3	CH_2
C7	34.41	34.3	CH_2
C8	40.03	40.7	C
C9	50.43	50.5	CH
C10	37.22	37.2	C
C11	19.43	20.8	CH_2
C12	25.58	25.5	CH_2
C13	38.09	38.4	CH
C14	42.49	42.4	C
C15	30.51	30.5	CH_2
C16	32.21	32.1	CH_2
C17	55.91	56.3	C
C18	47.09	46.8	CH
C19	49.06	49.2	CH
C20	150.82	150.3	C
C21	29.68	29.7	CH_2
C22	38.76	37.0	CH_2
C23	27.53	27.9	CH_3
C24	15.22	15.3	CH_3
C25	16.41	16.0	CH_3
C26	16.44	16.1	CH_3
C27	14.86	14.7	CH_3
C28	177.29	180.5	C
C29	110.06	109.6	CH_2
C30	19.43	19.4	CH_3

* (Okwute, and Isyaka, 2014 and Habila *et al*., 2013).



3β-hydroxyllup-20(29)-en-28-oic acid (Betulinic acid) **(21)**

5.13.5 Ultraviolet (UV) of OB 315

In OB 315, the UV absorption band is indicative of an isolated olefinic group showed UV absorption maximium at 260 nm (Fig: 4.9)

5.13.6 Characterization of compound OB 315

The ^{1}H (Fig 4.24) and ^{13}C NMR (Fig 4.25) spectra are typical of triterpenoids (Bross-Walch *et al.*, 2005). Compound OB 315 was obtained as a white powder (15.1 mg). The ^{13}C-NMR spectrum showed 29 carbon atoms (Fig 4.25).

The ^{1}H NMR spectrum of OB 315 showed the presence of six methyl signals that appeared as two methyl sinlgets at δ 0.68, and 1.01; three methyl doublets that appeared at δ 0.81, 0.83, and 0.93; and a methyl triplet at δ 0.84.It also showed one olefinic proton at δ 5.36 and this suggest presence of a trisubstituted double bond in its structure. The ^{1}H NMR spectrum of OB 315 showed oxymethine proton corresponding to the proton connected to the hydroxy group which appeared as a triplet of doublet of doublets at δ 3.53 (Fig 4.24).

The ^{13}C NMR data (400 MHz, CD_3Cl) spectrum showed the presence of two olefinic carbon atoms at δ 141.32 and 121.98. The oxymethine carbon peaked at δ 71.41 while the signals at δ 32.15, δ 55.45, 56.06, 55.57, 33.99, 15.59 and 29.58 are methine group. The signals from δ 37.39, 31.50, 42.33, 32.73, 21.62, 39.01, 26.22, 28.34, 47.50 and 18.61 are probably due to CH_2 groups, while the CH_3 methyl groups are indicated by the following signals δ 37.15, 18.61, 26.22, 23.51, 21.63, 18.63 and 15.72 are of methyl type (Fig 4.25).

Using the HSQC in assigning the ^{1}H signals to the corresponding ^{13}C signals, the oxymethine proton at δ 3.53 (1H (m)) showed direct correlation to the ^{13}C signals at δ 71.41, olefinic proton at δ 5.36 (1H (t)) showed correlation to the ^{13}C signals δ 121.98. The ^{13}C signals at δ 55.45, 55.57 and 56.06 show correlations with methylene protons at δ 0.50-1.98 (Fig 4.26).

The COSY data indicates that the olefinic protons at δ 5.36 (1H (d)) show correlation with methine protons at δ 1.98 and methyl protons at 1.50. The oxymethine proton δ 3.53 showed

correlations with 1.46 and 0.97 methylene protons (Fig 4.27).

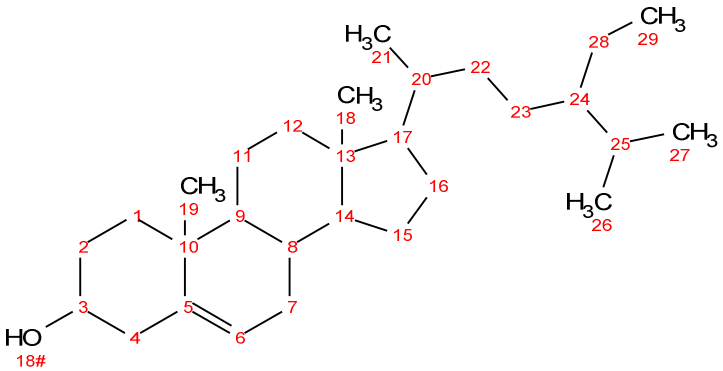
The oxymethine protons δ 3.53 showed correlation with δ 121.98 methine, 141.32 quartneary carbons, this placed it two or three bonds away from the olefinic bond and 31.50 methylene protons. The olefinic protons at δ 5.36 (1H (t)) showed multiple bond correlations with 55.45, 42.33 and 37.39 while the angular methyl protons at δ 1.09 and methyl proton at δ 1.52 showed correlation with the δ 141.32 and δ 121.98.

Thus, the structure of OB 315 was assigned as stigmat-5-en-3β-ol (β-sitosterol) **(22)** and this is consistent with those in the literature (Table 5.3) (Habib *et al*., 2007; Jamal *et al*., 2009).

Table 5. 3: ^{13}C NMR Data of Compound OB 315

Carbon atoms	Experimental δc(ppm)	*Literature δc(ppm)
C1	37.39	37.5
C2	31.50	31.9
C3	71.65	72.0
C4	42.33	42.5
C5	141.32	140.9
C6	121.56	121.9
C7	32.73	32.1
C8	32.15	32.1
C9	55.45	50.3
C10	37.39	36.7
C11	21.62	21.3
C12	39.01	39.9
C13	41.97	42.6
C14	56.06	56.9
C15	26.22	26.3
C16	28.34	28.5
C17	55.57	56.3
C18	37.15	36.3
C19	18.61	19.2
C20	33.99	34.2
C21	26.22	26.3
C22	47.50	46.1
C23	23.51	23.3
C24	15.59	12.2
C25	29.58	29.4
C26	21.63	20.1
C27	18.63	19.6
C28	18.61	19.0
C29	15.72	12.0

*(Habib *et al*., 2007; Jamal *et al*., 2009)



Stigmat-5-en-3β-ol (β-sitosterol) (22)

5.13.7 Ultraviolet (UV) of OB 312

In OB 312, the UV absorption band is indicative of an isolated olefinic group showed UV absorption maximium at 255 nm (Fig: 4.9).

5.13.8 Characterization of compound OB 312

Compound OB 312 was obtained as a red amorphous solid (11.1 mg). The ^{13}C-NMR spectrum showed 30 carbon atoms (Fig 4.31).

The ^{1}H NMR (400 MHz, CD_3Cl) spectrum showed the presence of six olefinic protons at δ 6.03 (1H (d)), δ 5.89 (1H (m)), 4.10 (1H (d)) and 3.85 (1H (d)). Two oxymethine proton peaked at δ 2.10 (1H (d)) and methine proton at δ 2.20 (1H (t)) (Fig 4.30).

The ^{13}C NMR data (400 MHz, CD_3Cl) showed the presence of six olefinic carbons atoms at δ 129.05, 127.88, 130.32, 128.78 and.The signals at δ 76.31 and 76.53 are oxymethine carbon while the signals at δ 64.10 and 64.10 are methine groups, while the quarternary carbon peaked at δ 56.65. The signals δ 24.97, 34.29 and 37.39 are methylene groups, while the methyl groups peaked at δ 17.32, 22.91, 22.91 and 24.97 respectively (Fig 4.31).

In correlating the ^{1}H signals to the corresponding ^{13}C signals, Olefinic protons at δ 6.03 (1H (d)) and δ 5.89 (1H (d)) showed correlation to the ^{13}C signals), δ 127.88 and δ 128.78 respectively (Fig 4.32).

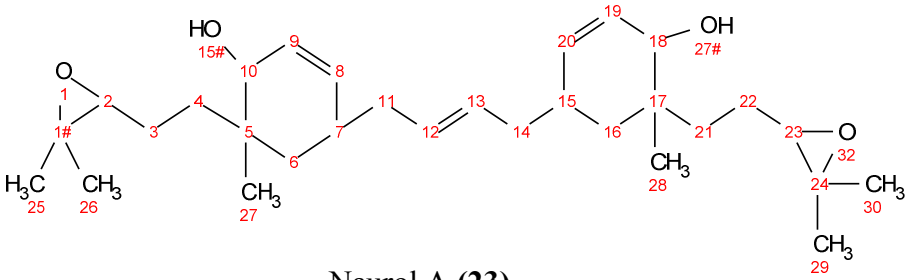
The olefinic protons at δ 6.03 (1H (d)) and δ 5.89 (1H (d)) showed correlation with methine protons at δ 1.97 and 1.88 (Fig 4.32).

The olefinic protons at δ 4.10 and 3.85 showed a long range correlation to the carbons of and respectively.Based on all these information and on the COSY and HMBC, experiments described. Compound OB 312 was assigned to be naurol A **(23)**; a triterpene alcohols with an uncommon symmetrical carbon skeleton centered about a linear conjugated tetraene moiety and having only two carbocyclic rings and this is consistent with literature value (Table 5.4) (Florecita *et al*., 1991).

Table 5. 4: ^{13}C NMR Data of Compound OB 312

Carbon atoms	Experimental δc(ppm)	*Literature δc(ppm)
C1	22.91	21.42
C2	56.65	58.55
C3	64.10	64.35
C4	24.97	24.12
C5	34.29	34.88
C6	37.15	37.56
C7	34.98	35.25
C8	38.85	38.62
C9	131.12	132.92
C10	128.78	128.48
C11	76.31	74.91
C12	37.39	38.00
C13	129.05	128.57
C14	127.88	127.07
C15	17.32	17.53
C16	22.91	21.42
C17	131.12	132.92
C18	128.78	128.48
C19	76.31	74.91
C20	37.15	37.56
C21	34.98	35.25
C22	34.29	34.88
C23	24.97	24.12
C24	64.10	64.35
C25	56.65	58.55
C26	21.63	20.1
C27	21.63	20.1
C28	18.61	19.0
C29	18.63	19.6
C30	22.91	21.42

(Florecita *et al*., 1991)


HO
15#
OH
27#
O
1
1#
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
O
32
24
25
26
27
28
29
30
H_3C
CH_3
CH_3
CH_3
CH_3

Naurol A **(23)**

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

This study demonstrates for the first time that 3β-hydroxylolean-12, 18-diene, 3β-hydroxyllup-20(29)-en-28-oic acid, stigmat-5-en-3β-ol and naurol A obtained from *C. schweinfurthii* leaves and, stem bark and *M.puriens* respectively possesses nematocidal activity. 3β-hydroxylolean-12, 18-diene, 3β-hydroxyllup-20(29)-en-28-oic acid and stigmat-5-en-3β-ol are therefore good lead compounds for the treatment of gastrointestinal infections. 3β-hydroxylolean-12, 18-diene and stigmat-5-en-3β-ol were the most ovicidal triterpenoids isolated with 65 % and 62 % percentage inhibition respectively while 3β-hydroxylolean-12, 18-diene, 3β-hydroxyllup-20(29)-en-28-oic acid and stigmat-5-en-3β-ol also showed potent larvicidal triterpenoids with 81 %, 85 % and 70 % percentage inhibition respectively against the *A. suum* larvae. 3β-hydroxylolean-12, 18-diene, 3β-hydroxyllup-20(29)-en-28-oic acid and stigmat-5-en-3β-ol showed a good antibacterial activity against *S. pyogenes, S. aureus*, *B. subtlis* and *S. typhi* with MIC of 6.25-12.5 µg/ml. The most potent antimicrobial agent were 3β-hydroxylolean-12, 18-diene and 3β-hydroxyllup-20(29)-en-28-oic acid with MBC of 12.5 µg/ml and MFC of 25-50 µg/ml respectively.

In conclusion, results from the present study reveal that extracts from *C. schweinfurthii* and *M. puriens* have some ovicidal, larvicidal and antimicrobial agents. Hence, the traditional uses of the plants are justified.

We wish to recommend that the compounds; 3β-hydroxylolean-12, 18-diene, 3β-hydroxyllup-20(29)-en-28-oic acid, stigmat-5-en-3β-ol and naurol A be further evaluated to confirm their *in-vivo* activity and toxicity on the host. Studies into structure-activity relationship (SAR) to gain a better understanding of the antimicrobial and nematocidal activities of the isolted triterpenoids are important to understanding the mechanism(s)

through which the compounds exert therapeutic effects should be clearly elucidated.

Other benefical potentials of the isolated triterpenoids can be evaluated and future work would be the isolation and characterization of potential compounds of other classes, than the triterpenes. For further investigation of the active compounds in *C. schweinfurthii* and *M. puriens* a better approach would be a bioassay-guided fractionation. This approach can reduce the time needed to isolate active pure compounds and also contributes to chemical profiling of the plant.

REFERENCES

Abdulrahim, F. A. A., Zeyad, D. N., Mohammad, J. S., Khalid, M. A., Salman, A. A., Zhari, I. and Amin, M. S. A. (2011). Evaluation of antiangiogenic, cytotoxic and antioxidant effects of *Syzigium aromaticum*. L. Extracts. *Asian Journal of Biological Sciences*, **4**(3): 282-290

Adedeji, S. O., Ogunba, E. O. and Dipeolu, O. O. (1989). Synergistic effect of migrating Ascaris larvae and *Escherichia coli* in piglets. *Journal of Helminthology,* **63**: 19-24.

Ademola, O. I. and Eloff, J. N. (2010). *In Vitro* Anthelmintic Activity of *Combretum molle* (R. Br. Ex G. Don) (Combretaceae) against *Heamonchus contortus* Ova and Larvae. *Veterinary Parasitology*, **169**: 198-203. http ://dx.doi.org /10.1016/j. vetpar. 2009. 12. 036

Adjanohoun, J. E., Aboubakar, N. and Dramane, K. (1996). Contribution to ethnobotanical and floristic studies in Cameroon, traditional medicine and pharmacopoeia (CSTR/OUA or OAU/STRC), Porto Novo Benin. pp. 117.

Akkari, H., Darghouth, M. A. and Ben Salem, H. (2008). Preliminary investigations of the anti-nematode activity of *Acacia cyanophylla* Lindl: excretion of gastrointestinal nematode eggs in lambs browsing A. cyanophyllawith and without PEG or grazing native grass. *Small Ruminant Research,* **74**: 78–83. doi:10.1016/j. small rumres.2007 .03.012

Akinpelu, A. D., Awotorebo, T., Agunibade, O. M., Aiyegoro, A. O. and Okoli, I. A. (2011). Anti-vibro and preliminary phytochemical characteristics of crude methanolic extracts of the leaves of *Diallum guineense*. *Journal of Medicinal Plant Research*. **5**(11): 2398-2404.

Allam, G. (2009). Immunomodulatory effects of curcumin treatment on *Murines chistosomiasis* mansoni. Immunobiology, 214: 712–727. doi:10. 1016/j.imbio .2008.11. 017

Allen, D. E. and Hatfield, G. (2004). Medicinal Plants in Folk Tradition: An Ethnobotany of Britain and Ireland; Timber Press: Cambridge, UK, pp. 431.

Alo, O. C., Okeh, C., Anyim, J. O. and Orji, J. (2012). Natural Product Plant Resource Scholars. *Research Library*, **2**(3): 449-455.

Amico, V., Barresi, V., Condorelli, D., Spatafora, C. and Tringali, C. (2006). Antiproliferative terpenoids from almond hulls (*Prunus dulcis*): Identification and structure-activity relationships. *Journal of Agricultural Food Chemistry,* **54**: 810814.

Anand, K., Gupta, V. N., Rangari, V., Singh, B. and Chandan, B. K. (1992). Structure and hepatoprotective activity of a Biflavonoid from *Canarium manii*. *Planta Medica*, **58**: 493-495.

Andersen, S. (1976). The influence of *Ascaris suum* infection upon growth rates in pigs.

Nordisk veterinaer medicine, **28**: 322-330.

Andlauer, W. and Furst, P. (2002). Nutraceuticals: a piece of history, present status and outlook. *Food Research International,* **35**: 171–176. doi:org/10.1016/S0963-9969 (01) 00179-X

Aniszewski, T. (2007). Alkaloids—Secrets of Life. In Alkaloid Chemistry, Biological Significance, Applications and Ecological Role; Elsevier Science: Amsterdam, The Netherlands. pp. 334.

Argentieri, P. M., D'Addabbe, T., Tava, A., Agostinelli, A., Jurzysta, M. and Anato, P. (2008). Evaluation of Nematicidal Properties of Saponins from *Medicago sativa* spp. *European Journal of Plant Pathology*, **120**: 89-197. http://dx.doi.org/10.1007/s10658-007-9207-8

Athanasiadou, S., Kyriazakis, I., Jackson, F. and Coop, R. L. (2001). Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Veterinary Parasitology,* **99**: 205–219. doi:org/10.1016/S0304-4017(01)00467-8

Barrau E., Fabre, N., Fouraste, I. and Hoste, H. (2005). Effect of bioactive compounds from Sainfoin (*Onobrychis viciifoliaScop*.) on the *in vitro* larval migration of *Haemonchus contortus*: role of tannins and flavonol glycosides. *Parasitology,* **131**: 531–538. doi:org /10.1017/S0031182005008024

Bauer, A. W., Kirby, E. and Sherris, E. M. (1966). Antibiotic by standardized single disk method, *American Journal Clinical Pathology,* **45**(4): 493–496.

Beith, M. (1999). Healing Threads: Traditional Medicines of the Highlands and Islands Edinburgh; Polygon: Edinburgh, UK.

Berhaut, J. (1974). Flore illustrée du Sénégal. Dicitylédones.Tome II, Balanophoracées. Dakar, 131-132, 414-416.

Bernardo, T. M., Dohoo, I. R. and Donald, A. (1990a). Effect of ascariasis and respiratory diseases on growth rates in swine. *Canadian journal of veterinary research,* **54**: 278-284.

Bernardo, T. M., Dohoo, I. R., Donald, A., Ogilvie, T. and Cawthorn, R (1990b). Ascariasis, respiratory diseases and production indices in selected Prince Edward Island swine herds. *Canadian journal of veterinary research,* **54**: 267-273.

Bhaskar, A., Nithya, V. and Vidhya, V. G. (2011). *Journal of Chemical and Pharmaceutical Research,* **3**(5): 689-696.

Bhuvanendran, R., Manson, W. and Spring, F.S. (1950). Triterpene Resinols and Related Acids: Isolation of a Triterpene Diol from *Canarium schweinfurthii* resin. *Journal of Chemical Society*, 3472-82

Bizimenyera, E. S., Githiori, J. B., Eloff, J. N. and Swan, G. E. (2006). *In vitro* activity of

Peltophorum africanum Sond. (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *Trichostrongylus colubriformis*. *Veterinary Parasitology*, **142**: 336–43.

Bouvier, F., Rahier, A. and Camara, B. (2005). Biogenesis, molecular regulation and function of plant isoprenoids. *Programme Lipid Resource*, **44**(6): 357–429

Braguine, C. G., Bertanha, C. S., Goncalves, U. O., Magalhaes, L. G., Rodrigues, V., Gimenez, V. M. M., Groppo, M., Silva, M. L. A. E., Cunha, W. R., Januario, A. H. and Pauletti, P. M. (2012). Schistoso micidal evaluation of flavonoids from two species of Styrax against *Schistoso mamansoni* adult worms. *Pharmaceutical Biology*, **50**: 925–929.doi: 10.3109 /13880209.2011.649857

Bross-Walch, N., Kühn, T., Moskau, D. and Zerbe, O. (2005). Strategies and tools for structure determination of natural products using modern methods of NMR spectroscopy, *Chemical Biodiversity*, **2**: 147.

Brunet, S. and Hoste, H. (2006). Monomers of condensed tannins affect the larval exsheathment of parasitic nematodes of ruminants. *Journal Agriculture Food Chem*istry **54**: 7481–7487.doi:10.1021/jf0610007

Bundy, D A. (1994). Immunoepidemiology of intestinal helminthic infection I: The global burden of intestinal nematode disease. *Trans Royal Society of Tropical Medical Hygiene,* **8**: 259-261.

Burnham, R. J. and Johnson, K. R. (2004). South American palaeobotany and the origins of neotropical rain forests. *Trans Royal Society London*, **359**: 1595-1610.

Butler, M. S. (2004). The role of natural product in chemistry in drug discovery. *Journal of Natural. Product*. **67**, 2141–2153.

Cala, A. C., Chagas, A. C. S., Oliveira, M. C. S, Matos, A. P., Borges, L. M .F., Sousa, L. A. D., Souza, F. A. and Oliveira, G. P. (2012). *In vitro* anthelmintic effect of *Melia azedarach* L. and *Trichilia classenii* C. against sheep gastrointestinal nematodes. *Experimental Parasitology*, **130**: 98-102.

Cao, S., Guza, R. C., Miller, J. S., Andriantsiferana, R., Rasamison, V. E and Kingston, D. G. I. (2004). Cytotoxic triterpenoids from *Acrido carpusvivy* from the Madagascar rainforest. *Journal Natural Product*; **67**(6): 9869.

Carroll, A. R., Arumugan, G., Quinn, R.J., Redburn, J., Guymer, G. and Grimshaw, P. (2005). Grandisine A and B, novel indolizidine alkaloids with-opioid receptor binding affinity from the leaves of the human Australian rainforest tree *Elaeocarpus grandis*. *Journal Organic Chemistry*, **70**: 1889–1892

Chandramu, C., Manohar, R. D., Krupadanam, D. G. and Dashavantha, R. V. (2003). Isolation, characterization and biological activity of betulinic acid and ursolic acid from Vitex negundo. *Phytotherapy Research*, **17**: 129

Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956). Glossary of Indian Medicinal plants,

CSIR, New Delhi.

Ciulei, J. (1964). Manual for analysis of vegetable drugs

Cloete, T. E. (2003). Biofouling: what we know and what we should know. *Material Correlation,* **54**: 520–526.

Copeman, D. B. and Gaafar, S. M. (1972). Sequential development of hepatic lesions of ascaridosis in colostrum-deprived pigs. *Australian Veterinary Journal,* **48**: 263-268.

Cowan M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, **12**(4):564-82

Cox, P. A. (2001). Saving the Ethnopharmacological Heritage of Samoa. *Pharmaceutical Biology*, **39**: 33–40.

Cragg, G.M. (1998). Paclitaxel (Taxol): A success story with valuable lessons for natural product drug discovery and development. *Medical Research Review*, **18**: 315–331.

Cragg, G. M. and Newman, D. J. (2005). Biodiversity: A continuing source of novel drug leads. *Pure Applied Chemistry,* **77**: 7–24.

Cragg, G. M. and Newman, D. J. (2007). Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products,* **70**: 461–477.

Cseke, L. J., Kirakosyan, A., Kaufmann, P. B., Warber, S. L., Duke, J. A. and Brielmann, H. L. (2006). Natural Products from Plants, 2nd ed.; CRC, Taylor and Francis: Boca Raton, FL, USA, pp. 640.

Curtis, S. E., Tisch, D. A., Todd, K. S. and Simon, J. (1987). Pulmonary Bacterial Deposition and Clearance during Ascarid Larval Migration in Weanling Pigs. *Canadian Journal of Veterinary Research,* **51**: 525-527.

Davidson, R.N.; den Boer, M. and Ritmeijer, K. (2009). Paromomycin. *Trans. Royal Society. Tropical Medical Hygiene*, **103**: 653–660.

Der Marderosian, A. and Beutler, J. A. (2002). The Review of Natural Products, 2nd ed.; Facts and Comparisons; Seattle, WA, USA. pp. 13–43.

Diaz, A. M. A., Acosta, T. J. F. J., Castro, S. C. A., Hoste, H. (2010). Tannins in tropical tree fodders fed to small ruminants: a friendly foe. *Small Ruminant Research*, **89**: 164–173. doi:10.1016/j.smallrumres.2009.12.040

Dillenius, J. J. (1724). Synopsis Methodica Stirpium Britannicarum, 3rd ed.; *Journal of Innys*: London, UK, pp. 482.

Donati, D., Lampariello, L.R., Pagani, R., Guerranti, R., Cinci, G. and Marinello, E. (2005). Antidiabetic oligocyclitols in seeds of *Mucuna pruriens*. *Phytother Research*, **19**(12): 1057-1560.

Dongmo, P., François, T., Bernadin, N., Wilson, A., Bertrand, S., Paul, H. A. Z. and Chantal, M. (2010). Chemical characterization, antiradical, antioxidant and anti-inflammatory potential of the essential oils of *Canarium schweinfurthii* and *Aucoumea klaineana* (Burseraceae) growing in Cameroon. *Agricultural Biology Journal of Natural. American*, **1**: 606-611.

Doyle, J. J., Chappill, J. A., Bailey, C. D. and Kajita. T. (2000). Towards a comprehensive phylogeny of legumes: evidence from rbcL sequences and non-molecular data. pp. 1 - 20 in Advances in legume systematics, part 9, (P. S. Herendeen and A. Bruneau, eds.). Royal Botanic Gardens, Kew, UK.

Dubal, D. B., Zhu, H., Yu, J., Rau, S. W., Shughrue, P. J., Merchenthaler, I., Kindy, M. and Wise, P. M. (2000).“Estrogen receptor a, not b, is a critical link in estradiol-mediated protection against brain injury”. *Proceeding National Academic Science USA, 10,1073.*

Duke, A .T. (1995). Handbook of Medicinal Herbs, 3rd Edn, CRS, Press, London, pp. 220.

Duke, J. A., Duke, P. A. K and du Cellier, J. L. (2008). Duke's Handbook of Medicinal Plants of the Bible; CRC Press Taylor and Francis Group: Boca Raton, FL, USA, pp. 552.

Eguale, T., Tadesse, D. and Giday, M. (2011). *In Vitro* Anthelmintic Activity of Crude Extracts of Five Medicinal Plants against Egg-Hatching and Larval Development of *Haemonchus contortus*. *Journal of Ethnopharmacology,* **137**: 108-113. http:// dx. doi.org/10.1016/j.jep.2011.04.063

Eijck, I. A. and Borgsteede, F. H. (2005). A survey of gastrointestinal pig parasites on free-range, organic and conventional pig farms in The Netherlands. *Veterinary research communications,* **29**: 407-414.

Eriksen, L., Andersen, S., Nielsen, K., Pedersen, A. and Nielsen, J. (1980). Experimental *Ascaris suum* infection in pigs.Serological response, eosinophilia in peripheral blood, occurrence of white spots in the liver and worm recovery from the intestine. *Nord Veterinary Medcine*, **32**: 233-242.

Evans, B. (1993). Canarium nuts-a new cash crop for the Solomon Islands. *Tropical Forest Management*. Update, **3**:7-9.

Ezueh, M. I. (1997). Cultivation and Utilization of Minor Food Legumes in Nigeria. Tropical Grain legume Information Center, IITA, Ibadan, Nigeria

Fabricant, D.S. and Farnsworth, N.R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspective*, **109**: 69–75.

Fairbairn, D. (1970). “Physiological Hatching of Ascaris lumbricoides Eggs”. Experiments and Techniques in Parasitoolgy. A.I.Mclnnis and M.Vogue eds. Freeman: San Francisco, CA

FAO (1993). Expert Committee on Food Additives. Meeting (40th: Geneva Switzerland), Residues of some veterinary drugs in animals and foods : monographs prepared by the fortieth Meeting of the FAO Expert Committee on Food Additives, Geneva, 9-18 June 1992. Food and Agriculture Organization of the United Nations, Rome, xiii, pp. 177. FAO Food and Nutrition Paper.

Farkas, R., Gyurcso, A. and Borzsonyi, L. (2003). Fly larvicidal activity in the faeces of cattle and pigs treated with endectocide products. *Medicinal Veterinary Entomology*, **17**: 301-306.

Fellows, L. and Scofield, A. (1995). Chemical diversity in plants. In Intellectual Property Rights and Biodiversity Conservation—An Interdisciplinary Analysis of the Values of Medicinal Plants; University Press: Cambridge, UK.

Ferhat, M., Muhammad, Q., Zaheer-Ud-Din K., Nameen, I., Shahid, M., Muhammad, L. and Phool, S. (2011). *In vitro* evaluation anthelmintic activity of essential oils from different parts *Skimma laureola* (DC.) zucc.ex walp., ver. Nair. *Pakistan Journal Botany*, **43**: 2915-18

Fester, K. (2010). Plant alkaloids.Publishedonline.doi:10.1002/9780470015902.a0001914

Fidler, J. M., Li, K. and Chung, C. (2003). PG490-88, a derivative of triptolide, casues tumor regression and sensitizes tumors to chemotherapy. *Molecular Cancer Therapy*. **2**: 855–862.

Finar, I. L. (1989). Organic chemistry; stereochemistry and the chemistry of natural products. 5th Edition Vol. 2. Longman group, U.K. pp. 517605.

Flesja, K. I. and Ulvesaeter, H.O. (1980). Pathological lesions in swine at slaughter.III. (Inter- relationship between pathological lesions, and between pathological lesions and 1 carcass quality and 2) carcass weight. *Acta veterinaria Scandinavica*. Supplementum, pp. 1-22.

Florecita, S., De Guzman, Francis, J., Schmitz (1991). Naurol A and B, novel triterpene alcohols from a Pacific sponge. *Journal of Organic Chemistry*, **56**(1): 55–58 doi: 10.1021/jo00001a012

Foster, J. G., Clapham, W. M., Belesky, D. P., Labreveux, M., Hall, M. H. and Sanderson, M. A. (2006). Influence of cultivation site on sesquiterpene lactone composition of forage chicory (*Cichoriuminty busL.*). *Journal Agriculture Food Chemistry,* **54**: 1772–1778. doi: 10.1021/jf052546g.

Foster, J. G., Cassida, K. A. and Turner, K. E. (2011). *In vitro* analysis of the anthelmintic activity of forage chicory (*Cichorium intybus L.*) sesquiterpene lactones against a predominantly *Haemonchus contortus* egg population. *Veterinary Parasitology* **180**: 298–306. doi:10.1016/j.vetpar.2011.03.013.

Francis, G., Kerem, Z., Makkar, H. P. S. and Becker, K. (2002). The biological action of saponins in animal systems: a review. *Brazilian Journal of Nutrition,* **88**: 587–605. doi:

10.1079/BJN2002725

Farnsworth, N. R., O. Akerele, A. S., Bingel, D. D., Soejarto and Z. Guo (1985). *Medicinal plants in therapy*. Bull. World Health Organisation., **63**: 965-981.

Fukoja, T., Kashiwada, Y., Kilkushi, R. E., Consentino, L. M., Ballas, L. M., Jiang, J. B., Janzen, W. P., Chen, I. S. and Lee, K. H. (1994). Betulinic acid and platonic acid as anti-HIV principles from *Syzigium clarifbrum* and the anti-HIV activity of structurally related triterpenoids. *Journal of Natural Product*, **57**: 243

Fung, S.Y and Tan, N.H (2012). Effect of *Mucuna pruriens* seed extract pretreatment on the responses of spontaneously beating rat Atria and Aortic Ring to *Naja sputatrix* (Javan Spitting Cobra) Venom. *Journal Evidence. Based Complementary Alternative. Medcine*. 2012:486390.

Ghosh, M., Sinha Babu, S.P., Sukul, N.C and Mahato, S.B (1993). Antifilarial effect of two triterpenoid saponins isolated from *Acacia auriculiformis*. *Indian Journal Experiment Biology,* **31**:604–606

Ghosh, N. K., Babu, S. P., Sukul, N. C., Ito, A. (1996). Cestocidal activity of *Accacia auriculiformis*. *Journal Helminthology* **70**: 171–172.doi:10.1017/S0022149X00015340

Gill, J. H., Redwin, J. M., van Wyk, J. A. and Lacey, E. (1991). Detection of resistance to ivermectin in *Haemonchus contortus*. *International Journal of Parasitology*, **21**: 771–776

Githiori, J. B., Athanasiadou, S and Thamsborg, S. M. (2006). Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. *Veterinary Parasitology*, **139**(4): 308-320.

Gonzalez, A. G., Fraga, B. M., Gonzalez, P., Hernandez, M. G. and Ravelo, A. G. (1981). *Phytochemistry* **20**: 1919.

Guo, D. J., Cheng, H. L., Chan, S. W., Yu, P. H. F. (2008). Antioxidative activities and the total phenolic contents of tonic Chinese medicinal herbs. *Inflammo pharmacology*, **16**: 201-7.

Gupta, M. B., Nath, R., Srivastava, N, Shanker, K., Kishor, K. and Bhargava, K. P. (1997). Antiinflammatory and antipyretic activities of beta sitosterol. *Planta Medicine* **39**(2): 15763

Gustafson, K. R., Cardellin, J. H., II, McMahon, J. B., Gulakowski, R. J., Ishitoya, J., Szallasi, Z., Lewin, N. E., Blumberg, P. M., Weislow, O. S. and Beutler, J. A. (1992). A nonpromoting phorbol from the Samoan medicinal plant *Homalanthus nutans* inhibits cell killing by HIV-1. *Journal of Medical Chemistry* **35**: 1978–1986.

Habib, M. R., Nikkon, F., Rahman, M., Haque, M. E., Karim, M. R. (2007). Isolation of Stigmasterol and b-Sitosterol from Methanolic Extract of Root Bark of *Calotropis gigantean* (Linn), *Pakistan Journal of Biological Sciences*. **10**: 4174-4176

Habila, A. J., Habila, J. D., Shode, F. O., Opoku, A. R., Atawodi, S. E and Umar, I. A. (2013). Inhibitory effect of betulinic acid and 3βacetoxybetulinic acid on rat platelet aggregation. *African Journal of Pharmacy and Pharmacology*, **7**(43): 2881-2886. doi 10.5897/AJPP2013.

Haefner, B. (2003). Drugs from the deep: Marine natural products as drug candidates. *Drug Discovery*. **8**: 536–544.

Hale, O. M. and Stewart, T. B. (1985). Influence of an experimental infection of *Ascaris suum* on performance of pigs. *Journal of Animal Science*. **60**: 220–225.

Hale, T., Keusch, L. and Gerald, T. (1996). "Shigella: Structure, Classification, and Antigenic Types". In Baron, Samuel.Medical microbiology (4 ed.). Galveston, Texas: University of Texas Medical Branch. ISBN 978-0-9631172-1-2.Retrieved February 11, 2012.

Havsteen, B.H. (2002). The biochemistry and medical significance of the flavonoids. *Pharmacology Thererapy,* **96**: 67-202.doi:PII:S0163-7258(02)00298-X

Heinrich, M. and Teoh, H. L (2004). Galanthamine from snowdrop the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. *Journal Ethnopharmacology*. **92**: 147–162.

Himejima, A. and Kubo, I. (1992). Antimicrobial agents from *Licaria puchuri* major and their synagistic effect with polygodial. *Journal of Natural products*, **55**: 620-625

Hishika, R., Shastry, S., Shinde, S. and Guptal, S. S. (1981). *Indian Journal Pharmacology*, **13**(1): 97-98.

Holwell, S. E., Cooper, P. A., Grosios, J. W., Lippert, J. W. III., Pettit, G. R., Snyder, S. D. and Bibby, M. C. (2002) .Combretastatin A-1 phosphate, a novel tubulin-binding agent with *in vivo* anti-vascular effects in experimental tumors. *Anticancer Research*, **22**: 707–712.

Hoste, H., Torres-Acosta, J. F., Paolini, V., Aguilar-Caballero, A., Etter, E., Lefrileux, Y., Chartier, C and Broqua, C. (2005). Interactions between nutrition and gastrointestinal infections with parasitic nematodes in goats. *Small Ruminant Research,* **60**: 141–151. doi:10.1016/j.smallrumres.2005.06.008

Hoste, H., Jackson, F., Athanasiadou, S., Thamsbong, S. M. and Hoskin, S. O. (2006). The Effects of Tannin-Rich Plants on Parasitic Nematodes in Ruminants. *Trends in Parasitology*, **22**: 253-261. http://dx.doi.org/10.1016/j.pt.2006.04.004.

Hoste, H. and Torres-Acosta, J. F. J. (2011). Non chemical control of helminths in ruminants: adapting solutions for changing worms in a changing world. *Veterinary parasitology*, **180**(1): 144-154.

Hoste, H., Martinez-Ortiz-De-Montellano, C., Manolaraki, F., Brunet, S., Ojeda-Robertos, N., Fourquaux, I., Torres-Acosta, J. F. J and Sandoval-Castro, C. A. (2012). Direct and

indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infections. *Veterinary Parasitology,***186**: 18–27.doi:10.1016/j.vetpar. 2011.11.042 .

Hounzangbe-Adote, M. S., Paolini, V., Fouraste, I., Moutairou, K., Hoste, H. (2005). *In vitro* effects of four tropical plants on three lifecycle stages of the parasitic nematode, *Haemonchus contortus*. *Research Veterinary Sci*ence; **78**: 155–60

Howes, M. J. R., Perry, N. S. L. and Houghton, P. J. (2003). Plants with traditional uses and activitities, relevant to the management of Alzheimer's disease and other cognitive disorders. *Phytotherapy Research*, **17**: 1–18.

Huang, L., Ho, P. and Chen, C. H. (2007). Activation and Inhibition of the proteasome by betulinic acid and its derivatives. FEBS Letters, **581**: 4955-4959

Hudson, C., Bent, Z., Meagher, R. and Williams, K. (2014). "Resistance Determinants and Mobile Genetic Elements of an NDM-1-Encoding *Klebsiella pneumoniae* Strain". *PLOS ONE*9: e99209. doi:10.1371/journal.pone.0099209. PMID 24905728.

Huie, C. W. (2002). A review of modern sample preparation techniques for the extraction and analysis of medicinal plants, *Analytical Bioanalytical Chemistry*, **23**: 373.

Hussain, G. and Manyam, B. V. (1997). Is *Vernonia amygdalina* hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats. *Phytotherapy Research*, **11**: 419-423.

Iwu, M. M. (1993). Handbook of African Medicinal Plants. CRC Press inc, Florida

Iwu, M. M., Duncan, A. R. and Okunji, C. O. (1999). New Antimicrobials of plant origin. In Janick, J. (ed) Perspectives in New crops and New uses. ASHS Press, Alexandria, V.A. pp. 457-462

Jabbar, A., Zaman, M. A., Iqbal, Z., Yassen, M. and Shamim, A. (2007). Anthelmintic activity of *Chemopodium album* (L) and *Caesalpinia crista* (L) against trichostrongylid nematodes of sheep. *Journal of Ethnopharmacology*, **114**: 86–91

Jain, P. S. and Bari, S. B. (2010). Isolation of Lupeol, Stigmasterol and Campesterol from Petroleum Ether Extract of Woody Stem-Bark of *Wightia tinctoria*. *Asian Journal of Plant Sciences*, **9**(3): 163- 167

Jamal, A. K., Yaacob, W. A. and Din, L. B. (2009). A Chemical Study on *Phyllanthus columnaris*, *European Journal of Scientific Research*, **28**: 76-81

Joachim, A., Dulmer, N., Daugschies, A. and Roepstorff, A. (2001). Occurrence of helminths in pig fattening units with different management systems in Northern Germany. *Veterinary Parasitology*, **96**: 135-146

Jourquin, J. (2007). Strategic de-worming to boost performance. *International Pig Topics* **22**: 7-9.

Judd, W. S., Campbell, C. S., Kellogg, E. A., Stevens, P. F. and Donoghue, M. J. (2002). Plant systematics: a phylogenetic approach, Sinauer Axxoc, pp. 287-292. ISBN 0-87893-403-0.

Jules, J and Paull, R.E (2006). *The encyclopedia of fruit and nuts*, 205-207.

Just, M.J., Recio, M.C., Giner, R.M., Cueller, M.U., Manez, S, Billia, A.R and Rios, J.L (1998). Antiinflammatory activity of unusual lupine saponins from *Bupleurum fruticescens*, **64**: 404-407.

Kajita, T., Ohashi, H., Tateishi, Y., Bailey, C.D and Doyle, J.J (2001). Llegume phylogeny, with particular reference to Phaseoleae, Millettieae, and allies. *Systematic Botany,* **26**: 515-536.

Kamel, J. M. (1991). An extract of the mesocarps of fruits of *Balanite aegyptiaca* exhibited a prominent antidiabetic properties in Mice. *Chemical Pharmacology*. Bull. **39**: 1229-1233

Kamtchouing, P., Sokeng, D. S. and Moundipa, F. P. (1998). Protective role of *Anacardium occidentale* extract against streptozotocin induced diabetes in rats. *Journal of Ethnopharmacology,* **62:** 95-99.

Kamtchouing P., Kahpui, S. M., Dzeufiet, P. D., Djomeni, T., Asongalem, L and Dimo, T (2006). Anti-diabetic activity of methanol/methylene chloride stem bark extracts of *Terminalia superba* and *Canarium schweinfurthii* on streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology,* **104**: 306-309.

Kanora, A. (2009). Effect on productivity of treating fattening pigs every 5 weeks with flubendazole in feed. *Vlaams Diergeneeskundig Tijdschrift,* **78**: 170-175

Kaplan, R. M. (2004). Drug resistance in nematodes of veterinary importance: a status report. *Trends Parasitology*, **20**: 477–481. doi:org/10.1016/j.pt.2004.08.001

Kashman, Y., Gustafson, K. R., Fuller, R. W., Cardellin, J. H., McMahon, J. B., Currens, M. J., Buckheist, R. W., Hughes, S. H., Cragg, G. M. and Boyd, M. R. (1992). The Calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, calophyllum lanigerum. *Journal of Medicinal Chemistry*, **35**: 2735–2743.

Katzenschlager, A. Evans, A. and Manson, J. (2004). Neurology and Psychiatry , **74**: 1672-177.

Kedei, N.; Lundberg, D. J.; Toth, A.; Welburn, P.; Garfield, S. H. and Blumberg, P. M. (2004). Characterization of the interaction of ingenol 3-angelate with protein kinase C. *Cancer Research*, **64**: 3243–3255.

Keeling, C. I. and Bohlmann, J. (2006). Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. *New Phytology*, **170**: 657–675

Khare, C. P. (2004). Encyclopedia of Indian medicinal plants. Springerverley Berlin Heidelberg

Kinghorn, A. D. and Balandrin, M. F. (1993). Human Medicinal Agents from Plants (ACS Symposium Series); American Chemical Society: Washington, DC, USA, **534**: 2-12.

Kinghorn, A. D., Pan, L., Fletcher, J. N. and Chai, H. (2011). The relevance of higher plants in lead compound discovery programs. *Journal Natural Product* **74**: 1539–1555.

Kipper, M., Andretta, I., Monteiro, S. G., Lovatto, P. A. and Lehnen, C. R. (2011). Meta-analysis of the effects of endoparasites on pig performance. *Veterinary Parasitology*, **181**: 316-320.

Knecht, D., Jankowska, A. and Zalesny, G. (2012). The impact of gastrointestinal parasites infection on slaughter efficiency in pigs. *Veterinary Parasitology*, **184**: 291-297

Kiviharju, T. M., Lecane, P. S., Sellers, R. G. and Peehl, D. M. (2002). Antiproliferative and proapoptic of triptolide (PG490), a natural product entering clinical trials, on primary cultures of human prostatic epithelial cells. *Clinical Cancer Research*. **8**: 2666–2674.

Klein, C. B. and King, A. A. (2007). Genisteingeno toxicity: Critical considerations of *in vitro* exposure dose. *Toxicology Applied Pharmacology* **224**: 1–11.doi:10.1016/j.taap .2007.06.022

Koudou, J., Abena A. A., Ngaissona, P. and Bessiere, J. M. (2005). Chemical composition and pharmacological activity of essential oil of *Canarium schweinfurthii. Fitoterapia*, **76**: 700-703.

Kumar, D. S. and Muthu, A. K. (2010). Free radical scavenging activity of various extracts of whole plant of *Mucuna pruriens* (Linn): An *in vitro* evaluation. *Journal of Pharmacology Research* **3**(4): 718-721.

Kumar, A., Rajput, G., Dhatwalia, V. K. and Srivastav, G. (2014). Phytocontent Screening of Mucuna Seeds and Exploit in Opposition to Pathogenic Microbes. *Journal of Biological and Environmental Sciences*, **3**(9): 71-76

Langenheim, J. H. (2003). Plant Resins: Chemistry, Evolution, Ecology, and Ethnobotany. Timber Press, Portland,Oregon. pp. 586.ISBN0-881925-748

Larson, R. (2000).Toward a psychology of positive youth development. *American Psychologist*, **55**: 170−183

Larson, R., Hansen, D. and Moneta, G. (2007). Profiles of developmental experiences in organized youth activities. *Developmental Psychology*, **42**: 849−863

Leenhouts, P. W. (1956). Burseraceae. *Flora Malesiana*, **5**: 209-296.

Lewis, G., Schrire, B., Mackinder, B. and Lock, M. (2005). (eds.) Legumes of the world.

The Royal Botanic Gardens, Kew, Reino Unido, pp. 577. ISBN 1-900347-80-6.

Lightfoot (1977). Benjamin White: London, UK, *Journal of Flora Scotica* Volume 2.

Lorenzetti, E., MacIsaac, S., Arnason, J. T., Awang, D. V. C., Buckles, D. IDRC, Ottawa, (1998). Canada and IITA, Ibadan, Nigeria, **57**.

Magalhães, L. G., Machado, C. B., Morais, E. R., Buenode, Carvalho Moreira, E., Sossai Soares, C., Henriqueda Silva, S., DaSilva Filho, A. A. and Rodrigues V. (2009). *In vitro* schistosomicidal activity of curcumin against *Schistosom amansoni* adult worms. *Parasitology Research,* **104**: 1197–1201. doi:10.1007/s00436-008-1311-y

Magallón, S. A., Sanderson, M. J. and Sanderson. (2001). "Absolute diversification rates in angiosperm clades". *Evolution,* **55** (9): 1762–1780. doi:10.1111/j.0014-3820.2001.tb00826.x. PMID 11681732.

Mahato, S. B. and Kundu, A. P. (1994).^{13}C nmr spectra of pentacyclic triterpenoids and some salient features. *Phytochemistry*, **37**(6): 1517-1575.

Maheshwari, R. K., Singh, A. K., Gaddipati J and Srimal, R. C. (2006). Multiple biological activities of curcumin: a short review. *Life Science* **78**: 2081–2087 .doi:10 .1016 /j.lfs.2005.12.007

Makkar, H. P. S., Francis, G and Becker, K. (2007). Bioactivity of phytochernicals in some lesser known plants and their effects and potential applications in livestock and aquaculture production systems. *Animal*,**1**: 1371–139.doi:10.1017/S1751731107000298

Manyam, B. V., Dhanasekaran, M. and Hare, T. (2004). Effects of condensed tannins on established populations and on incoming larvae of Trichostrongylus colubriformis and Teladorsagia circumcincta in goats, *Phytotherapy Research*, **18**: 706-712.

Marnett, L. J. (1999). Lipid peroxidation _DNA damage by malondialdehyde.Theme medical publisher, Inc. New York, **8**: 82-95.

Martin, R. and Pennington, A. J. (1988). Effect of dihydroavermectin B1a on chloride-single-channel currents in *Ascari*s muscle. *Pesticide Science*, **24**: 90–91

Martin, R. J. (1997). Modes of action of anthelmintic drugs. *Veterinary Journal* **154**: 11–34. doi:1(190-0233/t)7/ 04(1011-24/S12.00/0

Martin, M. A. (1934). Description of the Western Isles of Scotland, 4th ed.; Macleod, D.J., Ed.; Stirling: Eneas Mackay: Cornhill, UK.

Martinez, M. H., Betancourt, J., Alonso–Gonzalez, N. and Jauregui, A. (1996). Screening of some Cuban medicinal plants for antimicrobial activity. *Journal of Ethnopharmacology*, **52**: 171-174.

McGaw, L. J., Rabe, T., Sparg, S. G., Jager, A. K., Eloff, J. N. and van Staden, J. (2000). An

investigation on the biological activity of Combretum species. *Journal of Ethnopharmacology*. **75**: 4550

McNeill, J., Barrie, F. R., Burdet, H. M. (2006). International code of botanical nomenclature (Vienna Code) adopted by the seventeenth International Botanical Congress, Vienna, Austria, July 2005(electronic ed.), Vienna: International Association for Plant Taxonomy, retrieved 2011-02-20 , Article 19.7

McRae, J., Yang, Q., Crawford, R., Palombo, W (2007). Review of the methods used for isolating pharmaceutical lead compounds from traditional medicinal plants. Environmentalist 27, 165–174.

Meenatchisundaram, S. and Michael, A. (2010). Antitoxin activity of *Mucuna pruriens* aqueous extracts against Cobra and Krait venom by *in vivo* and *in vitro* methods. Int. *Journal of Pharmaceutical Technology Research*, **2**(1): 870-874.

Middleton, E., Kandaswami, C. H. and Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacology Review*, **52**: 673–751

Milgate, J. and Roberts, D. C. K. (1995). The nutritional and biological significance of saponins. *Nutritional Research*, **15**: 1223–1249.doi:org/10.1016/0271-5317(95)00081-S

Mishra, B. B. and Tiwari, V. K. (2011). Natural products: An evolving role in future drug discovery. *European Journal Medical Chemistry,* **46**: 4769–4807.

Miskimins, D. W., Greve, J. H and Baker, J. R. (1994). The Serious Effects of Ascarid Larval Migration on a Group of Market-Weight Swine. *Veterinary Medicine*, **89**: 247-253.

Misra, L and Wagner, H (2004). Alkaloidal constituents of *Mucuna pruriens* seeds. *Phytochemistry,* **65**: 2565–2567

Mogana, R., Khoo, T. J. and Wiart, C (2011). *In vitro* antimicrobial, antioxidant activities and phytochemical analysis of *Canarium patentinervium* Miq. from Malaysia. *Biotechnology Research International*, **5**: 67-95

Mojab, F., Kamalinejad, M., Ghaderi, N. and Vahidipour, H. R (2003). Phytochemical Screening of Some Species of Iranian Plants. *Iranian Journal of Pharmaceutical Research,* 77-82.

Molan, A. L., Waghorn, G. C., Min, B. M. and McNabb, W. C. (2000a). The effect of condensed tannins from seven herbages on *Trichostrongylus colubriformis* larval migration *in vitro*. *Folia Parasitology* **47**:39–44. doi:10.1136/vr.150.3.65.

Molan, A. L., Hoskin, S. O., Barry, T. N. and McNabb, W.C. (2000b). Effect of condensed tannins extracted from four forages on the viability of the larvae of deer lungworms and gastrointestinal nematodes. Veterinary Research, **147**: 44–48. doi:0.1136/vr. 14 7.2.44

Molan, A. L., Waghorn, G. C. and McNabb, W. C. (2002) The impact of condensed tannins on egg hatching and larval development of *Trichostrongylus colubriformis in vitro*. *Veterinary Record*, **150**: 65–69

Molan, A. L., Meagher, L. P., Spencer. P.A, Sivakumaran, S. (2003a). Effect of flavan-3-ols on *in vitro* egg hatching, larval development and viability of infective larvae of *Trichostrongylus colubriformis*. *International Journal Parasitology* **33**: 1691–1698. doi:10.1016/S0020-7519(03)00207-8

Molan, E. L., Meagher, L.P. and Spencer, P. A. (2003). Effects of flavan-3-ols on the *in vitro* egg hatching, larval development and viability of infective larvae of *Trichostrongylus colubriformis*. *International Journal Parasitology*. **33**: 1691-98

Moshi, M., Innocent, E., Masimba, P. J., Otieno, D. F., Weisheit, A., Mbabazi, P., Lynes, M., Meachem, K., Hamilton, A. and Urassa, I. (2009). Antimicrobial and brine shrimp toxicity of some plants used in traditional medicine in Bukoba district, north-Western Tanzania. *Tanzania Journal of Health Research,* **11**: 23-28

Mueller-Harvey, I. (2006). Unravelling the conundrum of tannins in animal nutrition and health. *Journal Science Food Agriculture,* **86**: 2010–2037. doi:10.1002/jsfa.2577

Muthusamya,V. S.,Ananda, S., Sangeethaa, K. N.,Sujathaa, S., Arunb.,Balakrishnan and Lakshmi, B. S. (2008). Tannins present in *Cichorium intybus* enhance glucose uptake and inhibit adipogenesis in 3T3-L1 adipocytes through PTP1B inhibition. *Chemical Biology Interaction* **174**: 69–78.doi:10.1016/ j.cbi.2008.04.016

Nagashayana, N., Sankarankutty, P., Nampoothiri, M. R., Mohan, P. K. and Mohan, K. K. P. (2000). Association of L-DOPA with recovery following Ayurveda medication in parkinson's disease. *Journal Neurology Science,* **176**(2): 124-127.

Nakagawa, M., Yoshihara, S., Suda, H. and Ikeda, K. (1983). Pathological studies on white spots of the liver in fattening pigs. Natl Inst Anim Health Q (Tokyo) **23**: 138-149.

Nandi, B., Roy, S., Bhattacharya, S. and Babu, S. P. S. (2004). Free radicals mediated membrane damage by the *Saponins acaciaside Aandacaciaside* B. *Phytotherapy Research*, **18**: 191–194.doi:10.1002/ptr.1387

Nathisuwan, S., Burgess, D. S. and Lewis, J. S. (2001). "Extended-Spectrum β-Lactamases: Epidemiology, Detection, and Treatment". *Pharmacotherapy.* **21**(8): 920–928. doi:10.1592/phco.21.11.920.34529.

Ngbede, J., Yakubu, R. A. and Nyam, D. A. (2008). Phytochemical screening for active compounds in *Canarium schweinfurthii* (*Atili*) Leaves from Jos North, plateau State, Nigeria. Research *Journal of Biological Science*, **3**(9): 1076- 1078

Nicolaou, K. C.,Yang, Z., Liu, J. J., Ueno, H., Nantermet, P. G., Guy, R. K., Claiborne, C. F., Renaud, J., Couladouros, E. A., Paulvannan, K. and Sorensen, E. J. (1994). Total synthesis of taxol. *Nature*, **367**: 630–634.

Nilsson, O. (1982). *Ascariasis* in the pig.An epizootiological and clinical study.*Acta veterinaria Scandinavica*. Supplementum **79**: 1-108.

Nobori, T., Miurak, K., Wu, D. J., Takabayashik, L. A. and Carson, D. A. (1994). Deletion of cyclin-dependent kinase inhibitor gene in multiple human cancers. *Nature*, **46**: 753-756

Novobilsky,´ A., Mueller-Harvey, I. and Thamsborg, S. M. (2011). Condensed tannins act against cattle nematodes. *Veterinary Parasitology,* **182**: 213–220. doi:10.1016/j. vetpar. 2011.06.003.

Nyarko, A .A. and Addy, M. E. (1990). Effects of aqueous extract of *Adenia cissampeloides* on blood pressure and serum analyte of hypertensive patients. *Phytotherapy Research*, **4(**1**)**: 25-28.

Obame, L., Koudou, J., Kumulungui, B. S., Ismael, H. N., Prosper, E., Aboubakar, S. O and Alfred, S. T. (2007). Antioxidant and antimicrobial activities of *Canarium schweinfurthii* Engl. Essential oil from Central African Republic. *African Journal of Biotechnology,* **6**: 2319-2323.

Ogbourne, S. M., Suhrbier, A. and Jones, B. (2004). Antitumour activity of ingenol 3-angelate: Plasma membrane and mitochondrial disruption and necrotic cell death. *Cancer Research,* **64**: 2833–2839.

Okwute, S. K. and Isyaka, M. S. (2014). Betulinic Acid from Antimicrobial Root Wood Extract of *Dalbergia saxatilis* Hookf.(Fabaceae) *European Journal of Medicinal Plants,* **4**(6): 686-694

Okwu, D. E. (2001). Evaluation of the chemical composition of indigenous spices and flavoring agents. *Global .Journal of Pure and applied sciences,* 7**(3)**: 455-459

Okwu, D. E. (2004). Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. *Journal of Sustainable Agriculture and Environment*, **6**(1): 30-37

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Simons A (2009). Agroforest tree Database:a tree reference and selection guide version 4.0 (http://www.worldagroforestry.org/af/treedb

Osbourn, A., Goss, R. J. M. and Field, R. A. (2011). The saponins—polar isoprenoids with important and diverse biological activities. *Natural Production Report*, **28**: 1261–1268.doi:10.1039/c1np00015b

Oudhia, P. (2002). Kapikachu or Cowhage (*Mucuna pruriens*) Crop Fact Sheet. Version of 5-9-2002.

Ovallath, S. and Deepa, P. (2013). The history of parkinsonism: descriptions in ancient Indian medical literature. *Movement Disorder*. **28**(5): 566-568.

Pal, P. and Tandon, V (1998). Anthelmintic efficacy of *Flemingia vestita* (Leguminoceae): genistein induced alterations in the activity of tegumental enzymes in the cestode, *Raillietina echinobothrida*. *Parasitology International* **47**: 233–243. doi:org/10.1016/S

1383-5769(98)00025-7

Perez, J., Garcia, P. M., Mozos, E., Bautista, M. J., Carrasco, L., (2001). Immunohistochemical characterization of hepatic lesions associated with migrating larvae of *Ascaris suum* in pigs. *Journal Computational Pathology*, **124**: 200-206.

Perkins, S., Verschoyle, R. D., Hill, K., Parveen, I., Threadgill, M. D., Sharma, R. A., Williams, M. L., Steward, W. P. and Gescher, A. J. (2002). Chemopreventive efficacy and pharmacokinetics of curcuminin the min mouse, a model of familial adenomatous polyposis. *Cancer Epidemiology Biomarkers Preversion,* **11**: 535–540

Pernet, R. (1972). Phytochimie des Burseracees. *Lloydia*, **35**: 280.

Pfaller, M. A., Jones, R. N., Doern, G. V., Sader, H. S., Hollis, R. J. and Messer, S. A. (1998). International surveillance of bloodstream infections due to *Candida species*: frequency of occurrence and antifungal susceptibilities of isolates collected in 1997 in the United States, Canada and South America for the SENTRY Program. The SENTRY Participant Group. *Journal Clinical Microbiology* **36**: 1886-1889

Phillips, D. R., Rasbery, J. M., Bartel, B. and Matsuda, S. P. (2006). Biosynthetic diversity in plant triterpene cyclization. *Current Opinion Plant Biology*, **9**: 305–314

Pilatova, M., Stupakova, V., Varinska, L., Sarissky, M., Mirossay, L., Mirossay, A., Gal, P., Kraus, V., Dianiskova, K. and Mojzis, J (2010). Effect of selected flavones on cancer and endothelial cells. *General Physiology Biophysics* **29**: 134–143.doi:10.4149 /gpb. 2010.02.134

Plumb, D.C. (2008). Plumb's Veterinary Drug Handbook, 6 Edition. Blackwell Publishing.

Prescott, L. M., Harley, J. P. and Klein, D. A. (1996). Microbiology, 6th Ed., pp. 501-502. WCB/McGraw-Hill, USA

Prichard, R., Ménez, C. and Lespine, A. (2012). Moxidectin and avermectins: consanguinity but not identity. *International Journal of Parasitology Drugs* Drug Resistance, **2**: 134–153. http://dx.doi.org/10.1016/ j.jpddr.2012.04.001

Quattrocchi, Umberto (2000). CRC World Dictionary of Plant Names.3 M-Q.CRC Press. pp. 1738. ISBN 978-0-8493-2677-6.

Rao, H. S. P. and Reddy, K. S. (1991). Isoflavones from Flemingia vestita. *Fitoterapia* **63**: 485

Raquel, F. E. (2007). Bacterial lipid composition and antimicrobial efficacy of cationic steroid compounds. *Biochemica et Biophysica Acta*, 2500-2509

Rayavarapu, A. K. and Kaladhar, D. S. V. G. K. (2011). Evaluation of antimicrobial activity of *Mucuna pruriens* on plant pathogens. *Asian Journal Biochemistry Pharmaceutical Research*. **2**(1): 593-600.

Rees, S. B. and Harborne, J. B. (1985). The role of sequiterpene lactones and phenolics in the chemical defence of the chicory plant. *Phytochemistry*, **24**: 2225–2231. doi:0031-9422/85

Rey-Ladino, J., Ross, A. G., Cripps, A. W., McManus, D.P. and Quinn, R. (2011). Natural products and the search for novel vaccine adjuvants. *Vaccine,* **29**: 6464–6471.

Roepstorff, A. and Jorsal, S. E. (1990). Relationship of the Prevalence of Swine Helminths to Management- Practices and Anthelmintic Treatment in Danish Sow Herds. *Veterinary Parasitology*, **36**: 245- 257.

Roepstorff, A. and Nansen, P., (1994). Epidemiology and control of helminth infections in pigs under intensive and non-intensive production systems. *Veterinary Parasitology*, **54**: 69-85.

Roepstorff, A., Eriksen, L., Slotved, H. C. and Nansen, P. (1997). Experimental *Ascaris suum* infection in the pig: worm population kinetics following single inoculations with three doses of infective eggs. *Parasitology* ,**115**(4): 443-452.

Roepstorff, A. and Murrell, K. D. (1997). Transmission dynamics of helminth parasites of pigs on continuous pasture: *Ascaris suum* and *Trichuris suis*. *International Journal of Parasitology*, **27**: 563-572.

Roepstorff, A., Mejer, H., Nejsum, P. and Thamsborg, S. M. (2011). Helminth parasites in pigs: new challenges in pig production and current research highlights. *Veterinary parasitology* **180**: 7281

Sahaji, P. S. (2011). Acute oral toxicity of *Mucuna pruriens* in albino mice. *International Reserach Journal of Pharmacology*, **2**(5): 162-163

Saleem, M. Kaur, S. Kweon, M. Adbami, V. M. Afag, F. and Mukhtar, H. (2005). Lupeol a fruit and vegetable based triterpene induces apoptosis death of human paucreatic adeno carcinoma cells via inhibition of Ras Signaling pathway. *Carcinogenesis*, **26**: 1956-1964

Salih, N. E. (2003). A brief review on the development of strongylid nematode eggs and larvae under constant and changing temperature conditions egg development. *Journal Thermal Biology*. **6**: 287-95

Sam Hicks (1989). Desert Plants and People .The Southwest School of Botanical Medicine http://www.swsbm.com. **1**: 24-26

Satheesh, K. D., Kottai, M. A., Anton, S. A. and Manavalan, R. (2010). *In vitro* antioxidant activity of various extracts of whole plant of *Mucuna pruriens* (Linn). *International. Journal Pharmacology*. Technology Research. **2**(3): 2063-2070.

Satou, T., Akao, N., Matsuhashi, R., Koike, K., Fujita, K. and Nikaido, T. (2002a). Inhibitory effect of isoquinoline alkaloids on movement of second-stage larvae of *Toxocara canis*. *Biological Pharmacology* Bull **25**: 1651–1654

Satou, T., Koga, M., Matsuhashi, R., Koike, K., Tada, I. and Nikaido, T. (2002b). Assay of nematocidal activity of isoquinoline alkaloids using third-stage larvae of *Strongyloid esratti* and *S. venezuelensis*. *veterinary Parasitology,* **104**: 131–138.PII:S0304-4017 (01) 00619-7

Satou, T., Horiuchi, A., Akao, N., Koike, K., Fujita, K. and Nikaido, T. (2005). *Toxocara canis*: search fora potential drug amongst b-carboline alkaloids—*in vitro* and mouse studies. *Experimental Parasitology,* **110**: 134–139.doi:org/10.1016/j .exppara. 2005 .02.006

Shukla, K.K and Mahdi, A.A (2010). *Mucuna pruriens* reduces stress and improves the quality of semen in infertile men. Advance Access Publication **7**(1):137-144.

Sinha Babu, S. P., Sarkar, D., Ghosh, N. K., Saha, A., Sukul, N. C., Bhattacharya, S. (1997). Enhancement of membrane damage by saponins isolated from *Acacia auriculiformis*. *Journal of Pharmacology,* **75**: 451454

Smith, R. P., Sanchez-Vazquez, M. J., Cook, A. J., Edwards, S. A. (2011). Abattoir-based study investigating the association between gross pathological lesions and serological tests for Salmonella infection in pigs. *The Veterinary record,* **168**: 240

Sodipo, O. A., Akiniyi, J. A. and Ogunbamosu, J. U. (2000). Studies on certain characteristics of extracts of bark of *Pansinystalia macruceras* (K schemp) picrre Exbeille. *Globa*l *Journal of Pure and Applied Science*, **6**: 83-87

Sondhi, S.M., Shahu, R and Archana, M. (1994). Anti-Amoebic and Anthelmintic Evaluation of Heterocyclic Compounds Containing Nitrogen and/or Sulphur. *Indian Drugs*, **31**: 317-320

Sporn, M. B. and Suh, N. (2000). Chemoprevention of cancer. *Carcinogenesis*, **21**: 525530

Steenhard, N. R., Jungersen, G., Kokotovic, B., Beshah, E., Dawson, H. D., Urban, J. F., Jr., Roepstorff, A. and Thamsborg, S. M. (2009). *Ascaris suum* infection negatively affects the response to a *Mycoplasma hyopneumoniae* vaccination and subsequent challenge infection in pigs. *Vaccine* **27**: 5161-5169.

Stepek, G., Lowe, A. E., Buttle, D. J., Duce, I. R. and Behnke, J. M. (2007a). Anthelmintic action of plant cysteine proteinases against the rodent stomach nematode, *Protospirura muricola*, in vitro and *in vivo*. *Parasitology* **134**: 103–112. doi:10. 1017/ S0031182 006001302

Stepek G., Lowe, A. E., Buttle, D. J., Duce, I. R. and Behnke, J. M. (2007b). *In vitro* anthelmintic effects of cysteine proteinases from plants against intestinal helminths of rodents. *Journal Helminthology,* **81**: 353–360. doi:10.1017/S0022149X0786408X

Stewart, T.B and Hale, O.M. (1988). Losses to internal parasites in swine production. *Journal of Animal Science,* **66**:1548-1554.

Sultana, N. and Ata, A. (2008). Oleanolic acid and related derivatives as medicinally important compounds. *Journal of Enzyme Inhibition Medicinal Chemistry*, **23**: 739-756.

Suresh, C., Zhao, H., Gumbs, A., Chetty, C. S. and Bose, H. S. (2012). New ionic derivatives of betulinic acid as highly potent anti-cancer agents. *Bioorganic and medicinal chemistry letters*, **22**(4): 1734-8

Suteky, T., and Dwatmadji, T. (2011). Anthelmintic activity of *Melastoma malabatricum* extract of *Haemonchus contortus in vitro*. *Asian Journal Pharmaceutical Clinical Research*. **4**: 68-70

Svabo, J. C. (1959). Indberetninger fra en Reise I Færøe; Selskabet til Udgivelse af Færøske Kildeskrifter og Studier: Copenhagen, Denmark. 497.

Tagbota, S. and Townson, S. (2008). Antiparasitic properties of medicinal and other naturally occurring products, *Advance Parasitology*; **50**: 199-205

Tandon, V., Pal, P., Roy, B., Rao, H. S. P and Reddy, K. S. (1997). *In vitro* anthelmintic activity of root tuber extract of *Flemingia vestita*, an indigenous plant in India. *Parasitology Research*, **83**: 492–498

Tati, I. Donatini, R. D. S. Diaz, I. E. C. Yoshida, M. Bacchi, E. M. and Kato, E. T. M. (2008). Evaluation of Gastroprotective activity of *Plinia edulis* (vell.) Sobral (Myrtaceae) leaves in rats. *Journal of Ethnopharmacology*, **118**(3): 527-529

Tchamadeu, M. C., Dzeufiet, P. D. D., Kouambou, N. C. C., Azebaze, A. G. B., Allard, J., Girolami, J. P., Tack, I., Kamtchouing, P. and Dimo, T. (2010). Hypoglycaemic effects of *Mammea africana* (Guttiferae) in diabetic rats. *Journal of Ethnopharmacology*, **127**: 368–372. doi:10.1016/j.jep.2009.10.029

Tembely, S. (1998). Development and survival of infective larvae of nematode parasites of sheep on pasture in a cool tropical environment. *Veterinary Parasitology*. **79**: 81-87

Thornsberry, C. and Donald, R.S. (1975). Broth-Dilution Method for Determining the Antibiotic Susceptibility of Anaerobic Bacteria. *Antimicrobial Agents and Chemotherapy*, **7**(1): 15-21.

Timperio, A. M., d'Alessandro, A., Fagioni, M., Magro, P. and Zolla, L. (2012). Production of phytoalexins trans resveratrol and deltaviniferin in two economy relevant grape cultivars upon infection with *Botrytis cinerera* in field conditions. *Plant physiology and Biochemistry*, **50**(1): 65-71

Tjornehoj, K., Eriksen, L., Aalbaek, B. and Nansen, P. (1992). Interaction between *Ascaris suum* and *Pasteurella multocida* in the lungs of mice. *Parasitology Research*, **78**: 525-528.

Vaidya, R. A., Allorkar, S. D., Seth, A. R. and Panday, S. K. (1978). *Neurology*, **26**: 179-186.

Van Meensel, J., Kanora, A., Lauwers, L., Jourquin, J., Goossens, L. and Van Huylenbroeck, G., (2010). From research to farm: ex ante evaluation of strategic deworming in pig

finishing. *Veterinary Medicine*-Czech **55**: 483-493.

Varinska, L., Mirossay, L., Mojzisova, G. and Mojzis, J. (2010). Antiangogenic effect of selected phytochemicals. Pharmazie **65**:57–63.doi:10.1691/ph.2010.9667

Verdrengh, M., Collins, L.V., Bergin, P. and Tarkowski, A. (2004). Phytoestrogen genisteinas an antistaphylococcal agent. *Microbes Infection,* **6**: 86–92. doi: 10.1016/j. micinf. 2003.10.005

Vivek, R. Y., Sahdeo, P. and Bharat, B. A. (2010). Targeting inflammatory pathways of triterpenoids for prevention and treatment of cancer. Review. *Toxins*, **2**: 24282466

Waghorn, G. C. and McNabb, W. C. (2003). Consequences of plant phenolic compounds for productivity and health of ruminants. Proceeding of Nutrition Society **62**: 383–392. doi:org/10.1079/PNS2003245

Walf, A. A. and Frye, C.A (2005). Anxiety and anti- depressive behavior produced by Physiological estradiol regimen may be modulated by hypothalamic – pituitary adrenal axis activity.

Waller, P. J. (2006). From discovery to development: current industry perspectives for the development of novel methods of helminth control in livestock. *Veterinary Parasitology* **139**: 1–14.doi:10.1016/j.vetpar.2006.02.036

Warrier, P.K., Nambiar, V.P.K and Ramankutty C (1996). Indian medicinal plants. Orient Longman, Chennai, India. **4**: 68-72.

Waterman, P. G. (1999). The tannins-an overview. In Brooker JD (ed) Tannins in livestock and human nutrition. Proceedings of international workshop, Adelaide, Australia, *Australian Centre for International Agricultural Research*, pp 10–13. doi:10.1016/j.pt.2006.04.004

Weeks, A., Daly, D. C. and Simpson, B.B. (2005). The phylogenetic history and biogeography of the frankincense and myrrh family (Burseraceae) based on nuclear and chloroplast sequence data. *Molecular Phylogenetics and Evolution*, **35**: 85-101.

Wheatley, J. I. (1992). A Guide to the Common Trees of Vanuatu. Department of Forestry, Port Vila, Vanuatu

WHO (1999). Progress Report by the Director General, Document No. 44/20, 22 March 1991, World Health Organization, Geneva. Wichers, H.J,, Visser, J.F., Huizing, H.J., Niesko P (1993). Occurrence of L DOPA and dopamine in plants and cell cultures of *Mucuna pruriens* and effects of 2,4-D and NaCl on the compounds. Plant Cell Tissue Organic Culture, **33**: 259-264.

Wiart, C. (2006). Medicinal Plant of Asia Pacific-Drugs for the Future.World Scientific Publishing Co.Pte.Ltd, British Library Cataloguing in Publication Data 380.

Wojciechowski, M F., Lavin, M., Sanderson, M. J. and Sanderson, L. (2004). "A phylogeny

of legumes (*Leguminosae*) based on analysis of the plastid mat K gene resolves many well-supported sub clades within the family". *American Journal of Botany,* **91**(11): 1846–1862. doi:10.3732/ajb.91.11.1846. PMID 21652332.

Zhang, L. and Lin Y. M. (2008). Tannins from *Canarium album* with potent antioxidant activity. *Journal of Zhejiang University-Science*, **9**: 407-415.

Zhao, B., Chen, A. S., Yong, S. J., Guang, L.W., Xiang, L. X. and Chen, W. (2010). Phenolic Constituents of *Canarium album*. *Chemistry of Natural Compounds*, **46**.

Zheng, G. Q., Kenney, P. M. and Lam, L. K. T. (1992). Sesquiterpenes from Clove (*Eugenia caryophyllata*). *Journal of Natural Products*, **55**: 999-1003

Zhiyong, H., Wenshui, X., Qinghua, L. and Chen, J. (2009). Identification of a new phenolic compound from Chinese olive (*Canarium album* L.) fruit. *European Food Research Technology*, **228**: 339-343.

Ziegler, J. and Facchini, P. J. (2008). Alkaloid biosynthesis:metabolism and trafficking. *Annual Review Plant Biology*, **59**: 735–769.doi:10.1146/annurev.arplant.59.032607.092730

Zhiyong, H., Wenshui, X. and Chen, J. (2008). Isolation and structure elucidation of phenolic compounds in Chinese olive (*Canarium album* L.) fruit. *European Food Research Technology*, **226**: 1191-1196.

Ziegler, H. L., Staerk, D., Christensen, J., Hviid, L., Hägerstrand, H. and Jaroszewski, J. W . (2002). *In vitro Plasmodium falciparum* drug sensitivity assay: Inhibition of parasite growth by incorporation of *stomatocytogenic amphiphiles* into the erythrocyte membrane, *Antimicrobial Agents Chemotherapy*, **46**: 1441.