CHARACTERISATION AND BIOACTIVITIES OF VOLATILE AND NON-VOLATILE CHEMICAL CONSTITUENTS FROM *Calophyllum inophyllum* LINN. (GUTTIFERAE) AND *Pterocarpus soyauxii* TAUB. (FABACEAE)

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CERTIFICATION

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DEDICATION

This work is dedicated to God Almighty, my creator, my solid rock, my source of divine inspiration, wisdom, knowledge and understanding. He has been the source of my strength throughout this program and on His wings only have I soared.

I also dedicate this to my beloved parents, mentors and loving siblings who encouraged me all the way and whose encouragement have made sure that I give it all it takes to finish strong!

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ABSTRACT

Diabetes and oxidative stress are responsible for an upsurge in the number of neuropathies, retinopathy, nephropathy, cancer, cardiovascular dysfunction and mortality world-wide. Traditionally, *Calophyllum inophyllum* and *Pterocarpus soyauxii* are used in the management of diabetes, skin diseases, haemorrhage and common cold in Nigeria. Despite their ethno-medicinal uses, there is sparse information on the chemical constituents responsible for their antidiabetic and antioxidant activities. Hence, this study was designed to isolate and evaluate the antidiabetic and antioxidant activities of volatile and non-volatile chemical constituents of both plants.

Calophyllum inophyllum and *Pterocarpus soyauxii* were collected and authenticated at University of Ibadan and Forest Research Institute, Ibadan. Essential oils (EOs) were extracted from leaf, leaf-stalk, flower, seed, pod, fruit-pulp, stem wood, stem bark, root wood and root bark of *C. inophyllum* and root parts of *P. soyauxii* by hydro-distillation using a Clevenger-type apparatus and analysed by gas chromatography-mass spectrometry. The ten parts of *C. inophyllum* and leaf, stem, root and root bark of *P. soyauxii* were extracted with methanol and subsequently partitioned with n-hexane and ethyl acetate (EtOAc) to obtain respective fractions. Bioactive fractions were purified using column chromatographic technique and isolated compounds were characterised using Fourier Transform Infrared (FTIR), Nuclear Magnetic Resonance (NMR) and Electron Ionisation Mass Spectrometer (EIMS). In vitro antidiabetic activity was evaluated using α-amylase and α-glucosidase methods, while antioxidant assay was done using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide (H_2O_2) radical methods. Data were evaluated using regression analysis.

A total of 102 and 95 compounds were identified in *C. inophyllum* and *P. soyauxii* Essential oils (EOs), respectively. The EOs were dominated by monoterpenes, sesquiterpene and their oxygenated derivatives. The predominant constituents in the EOs were hexadecanal, limonene and *cis*-cadina-1(6),4-diene. The pod and root bark EtOAc fractions of *C. inophyllum* and *P. soyauxii* had higher activities, respectively for both antidiabetic and antioxidant assays compared to hexane fractions. Friedelan-3-one, stigmasterol and 1,3,5-trihydroxy-2-methoxy-xanthone were isolated in the pod EtOAc fraction of *C. inophyllum*, while 3β-hydroxylup-20(29)-ene, 2,3-dihydroxyfriedelinol, stigmasterol and β-sitosterol, were isolated from *P. soyauxii* root bark. The most active compound was 1,3,5-trihydroxy-2-methoxy-xanthone and its structural elucidation showed absorption peaks (cm^{-1}) at 3459 (OH), 1653 (C=O) and 1611 (aromatic C=C). The molecular ion at *m/z* 274.2 [M^+] suggesting the molecular formula, $C_{14}H_{10}O_6$. The proton NMR gave signals (ppm) at δ 6.52 (1H, *s*), δ 6.98 (1H, *dd*) and δ 7.25 (1H, *dd*) for three aromatic protons, 12.52 (1H, *brs*-OH) and 3.45 (3H, *s*-OCH3). The carbon-13 NMR gave eight quaternary carbons, five methine carbons and one methyl carbon signals. Essential oils from both plants have antidiabetic and antioxidant activities with IC_{50} (mg/mL) ranging from 0.04 to 0.05 and 0.35 to 0.40, respectively. Isolated compounds had good antidiabetic and antioxidant activity with mean IC_{50} (mg/mL) ranging from 0.03 to 0.04 and 0.15 to 0.44, respectively.

Friedelan-3-one and 1,3,5-trihydroxy-2-methoxylxanthone from *Calophyllum inophyllum* pod as well as 3β-hydroxylup-20(29)-ene and 2,3-dihydroxy-

friedelinol from *Pterocarpus soyauxii* root bark exhibited antidiabetic and antioxidant activities.

Keywords: Essential oils, Terpenes, 2,3-dihydroxy-friedelinol, 1,3,5-trihydroxy-2-methoxy-xanthone, Diabetes, Oxidative stress

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TABLE OF CONTENTS

Title page		i
Certification		ii
Dedication		iii
Acknowledgements		iv
Abstract		vi
Table of contents		viii
List of Tables		xiv
List of Figures		xvi
List of scheme		xx
List of Abbreviations		xxi
Appendices		xxiii

CHAPTER ONE: INTRODUCTION

1.1	Background of the study	1
1.2.	Natural Products as Therapeutic Agents	2
1.3.	Drug discovery and medicinal plants	2
1.4.	Relevance of Medicinal Plants in Drug Discoveries	3
1.5.	Justification for the Study	4
1.6.	Research Objectives	5
1.6.1.	General objective	5
1.6.2.	Specific objectives	5

CHAPTER TWO: LITERATURE REVIEW

2.1.	Family Clusiacae	6
2.1.1.	Genus *Calophyllum*	6
2.1.2.	Uses of *C. inophyllum*	13
2.1.3.	Biological activities of isolated compounds from genus *Calophyllum*	13
2.1.4.	Previously isolated compounds from *C. inophyllum*	14
2.2.	Family Fabaceae	17
2.2.1.	Genus *Pterocarpus*	17
2.2.2.	Uses of *P. soyauxii*	18
2.2.3.	Biological activities of isolated compounds from *P. soyauxii*	19

2.2.4.	Previously isolated compounds from *P. soyauxii*	19
2.3.	Natural Products	26
2.3.1.	Alkaloids	26
2.3.2.	Flavonoids	27
2.3.3.	Anthocyanins	28
2.3.4.	Saponins	29
2.3.5.	Tannins	31
2.3.6.	Phenols	32
2.3.7.	Terpenoids	33
2.3.8.	Glycosides	35
2.3.9.	Cardiac glycosides	35
2.3.10.	Steroids	37
2.4.	Essential oils	39
2.5	Plant extraction methods	40
2.5.1.	Soxhlet Extraction	40
2.5.2.	Cold pressing	40
2.5.3.	Maceration	41
2.5.4.	Hydro-distillation	41
2.6.	Chromatographic purification of natural products	41
2.6.1.	Column chromatography	42
2.6.2.	Thin layer chromatography	42
2.6.3	High performance liquid chromatography	43
2.6.4.	Gas chromatography	43
2.6.5.	Gas chromatography-mass spectrometry (GC-MS)	44
2.7.	Spectroscopic analysis of natural products	45
2.7.1.	Ultraviolet spectroscopy	45
2.7.2.	Infrared spectroscopy	45
2.7.3	Mass spectroscopy	45
2.7.4.	Nuclear magnetic resonance	46
2.7.4.1	Distortionless Enhancement by Polarization Transfer (DEPT) NMR	46
2.7.4.2	Attached Proton Test (APT) NMR	47
2.7.4.3	Two-dimensional NMR in structural elucidation of Natural products	47

2.7.4.3.1	Correlation spectroscopy (COSY)	47
2.7.4.3.2	Heteronuclear single quantum correlation spectroscopy (HSQC)	47
2.7.5.2.	Heteronuclear multiple bond correlation (HMBC) Spectroscopy	48
2.8.	Biological assay techniques	48
2.8.1.	Anti-diabetic assay	48
2.8.2.	Antioxidant assay	49

CHAPTER THREE: MATERIALS AND METHODS

3.1	General experimental procedures	50
3.2	Collection and identification of plant Materials	51
3.3	Essential oil extraction from *C. inophyllum*	51
3.4	Extraction of plant materials from *C. inophyllum*	51
3.5	Essential oil extraction from *P. soyauxii*	52
3.6	Extraction of plant materials from *P. soyauxii*	52
3.7	Phytochemical screening	52
3.7.1	Test for saponins	52
3.7.2	Test for alkaloids	53
3.7.2.1	Mayer's test for alkaloids	53
3.7.2.2	Dragendorff's test for alkaloids	53
3.7.2.3	Wagner's test for alkaloids	52
3.7.3	Test for steroids	53
3.7.4	Test for tannins and phenolic compounds	53
3.7.5	Test for resins	53
3.7.6	Test for carbohydrates	54
3.7.7	Test for anthraquinones	54
3.7.8	Test for flavonoids	54
3.7.9	Test for terpenoids	54
3.7.9.1	Libermann-Burchard test for terpenoids	54
3.7.9.2	Salkowski test for terpenoids	54
3.7.10	Test for glycosides	55
3.8	Column chromatography of ethyl acetate fraction of *C. inophyllum*	55
3.9	Column chromatography of ethyl acetate fraction of *P. soyauxii*	55
3.9.1	Characterisation of isolated compounds	56

3.10	Biological activities	59
3.10.1	Antidiabetic activity	59
3.10.1.1	Evaluation of inhibition activity (α-amylase)	59
3.10.1.2	Evaluation of yeast inhibition activity (α-glucosidase)	59
3.10.2	Antioxidant activity of *C. inophyllum* and *P. soyauxii*	60
3.10.2.1	Evaluation of DPPH Antioxidant assay	60
3.10.2.2	Evaluation of Hydrogen peroxide (H_2O_2) scavenging activity	61

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1.	Characterisation of extracted volatile oil constituents from *C. inophyllum* and *P. soyauxii*	62
4.1.1	Characterisation of volatile oil constituents from *C. inophyllum*	62
4.1.1.1	Yield of essential oils from *C. inophyllum*	62
4.1.1.2	Chemical composition of essential oils from *C. inophyllum*	62
4.1.2	Characterisation of volatile oil constituents from *P. soyauxii*	74
4.1.2.1	Percentage yield of volatile oils from *P. soyauxii*	74
4.1.2.2	Chemical composition of volatile oils from *P. soyauxii*	74
4.2	Percentage yields and Qualitative phytochemical evaluation of extracts and fractions from *C. inophyllum* and *P. soyauxii*	80
4.2.1	Percentage yields of pod extract and fractions from *C. inophyllum*	80
4.2.2	Percentage yields of root bark fractionation from *P. soyauxii*	80
4.2.3	Phytochemical screening of extracts and fractions from *C. inophyllum* and *P. soyauxii*	80
4.2.3.1	Phytochemical screening of extracts and fractions from *C. inophyllum*	80
4.2.3.2	Phytochemical screening of extracts and fractions from *P. soyauxii*	86
4.3	Bioassay results	89
4.3.1	Antidiabetic activity of *C. inophyllum* and *P. soyauxii*	89
4.3.1.1	Antidiabetic activity of volatile oils from *C. inophyllum*	89
4.3.1.2	Antidiabetic activity of volatile oils from *P. soyauxii*	89
4.3.1.3	Antidiabetic activity of crude extracts from *C. inophyllum* and *P. soyauxii*	92
4.3.1.3.1	Antidiabetic activity of crude extracts from *C. inophyllum*	92
4.3.1.3.2	Antidiabetic activity of crude extracts from *P. soyauxii*	92

4.3.1.3.3	Antidiabetic activity of fractions from pod extract of *C. inophyllum*	95
4.3.1.3.4	Antidiabetic activity of fractions from root bark extract of *P. soyauxii*	95
4.3.1.3.5	Antidiabetic activity of isolated compounds from *C. inophyllum*	95
4.3.1.3.6	Antidiabetic activity of isolated compounds from *P. soyauxii*	96
4.3.2	Antioxidant activity of *C. inophyllum* and *P. soyauxii*	101
4.3.2.1	Antioxidant activity of essential oils from *C. inophyllum*	101
4.3.2.2	Antioxidant activity of essential oils from *P. soyauxii*	101
4.3.2.3	Antioxidant activity of crude extracts from *C. inophyllum* and *P. soyauxii*	104
4.3.2.3.1	Antioxidant activity of crude extracts from *C. inophyllum*	104
4.3.2.3.2	Antioxidant activity of crude extracts from *P. soyauxii*	104
4.3.2.3.3	Antioxidant activity of fractions from pod extract of *C. inophyllum*	107
4.3.2.3.4	Antioxidant activity of root bark fractions from *P. soyauxii*	107
4.3.2.3.5	Antioxidant activity of isolated compounds from *C. inophyllum*	110
4.3.2.3.6	Antioxidant activity of isolated compounds from *P. soyauxii*	110
4.4	Characterisation of non-volatile constituents from *C. inophyllum* and *P. soyauxii*	113
4.4.1	Isolation of secondary metabolites from pod ethyl acetate fraction of *C. inophyllum inophyllum*	113
4.4.1.1	Characterisation of CiPdE-1	113
4.4.1.2	Characterisation of CiPdE-2	128
4.4.1.3	Characterisation of CiPdE-3	143
4.4.2	Isolation from root bark ethyl acetate fraction of *P. soyauxii*	160
94.4.2.1	Characterisation of PsRbE-A	160
4.4.2.2	Characterisation of PsRbE-B	175
4.4.2.3	Characterisation of PsRbE-C	187
4.4.2.4	Characterisation of PsRbE-D	202
4.5	Antidiabetic and Antioxidant activities of isolated compounds	218

CHAPTER FIVE: CONCLUSION

5.1	Summary	220
5.2	Conclusion	221
5.3	Contribution to Knowledge	221

5.4 Recommendations 221
References 221
Appendices 241

LIST OF TABLES

Table 4.1.	Physicochemical properties of volatile oils from *C. inophyllum*	64
Table 4.2	Chemical composition of essential oils from ten parts of *C. inophyllum*	66
Table 4.3	Classes of compounds in essential oils from *C. inophyllum*	72
Table 4.4	Physicochemical properties of essential oils from *P. soyauxii*	74
Table 4.5	Chemical composition of volatile oils from root parts of *P. Soyauxii*	75
Table 4.6	Classes of compounds in essential oils essential oils from *P. soyauxii*	78
Table 4.7	Percentage yields obtained from Pod fractionation of *C. Inophyllum*	81
Table 4.8	Percentage yields obtained from root bark extraction and fractionation of *P. soyauxii*	82
Table 4.9	Phytochemical screening of ten (10) methanol extracts from *C. inophyllum*	83
Table 4.10	Phytochemical screening of pod methanol extract and fractions from *C. inophyllum*	84
Table 4.11	Phytochemical screening of methanol extracts from four parts of *P. soyauxii*	86
Table 4.12	Phytochemical screening of root bark methanol extract and Fractions from *P. soyauxii*	87
Table 4.13	IC_{50} data of α-amylase and α-glucosidase inhibition activity of Essential oils from *C. inophyllum*	89
Table 4.14	IC_{50} values of α-amylase and α-glucosidase inhibitory activity of Essential oils from *P. soyauxii*	90
Table 4.15	IC_{50} values of α-amylase and α-glucosidase inhibitory activity of rude extracts from *C. inophyllum*	92
Table 4.16	IC_{50} values of α-amylase and α-glucosidase inhibitory activity of crude extracts from *P. soyauxii*	93
Table 4.17	IC_{50} values of α-amylase and α-glucosidase inhibitory activity of fractions from pod extract *C. inophyllum*	96

Table 4.18	IC_{50} values of α-amylase and α-glucosidase inhibitory activity of fractions from from root bark extract of *P. soyauxii*	97
Table 4.19	IC_{50} values of α-amylase and α-glucosidase inhibitory activity of Isolated compounds from *C. inophyllum*	98
Table 4.20	IC_{50} values of α-amylase and α-glucosidase inhibitory activity of Isolated compounds from *P. soyauxii*	99
Table 4.21	IC_{50} values from DPPH and H_2O_2 scavenging activity of essential Oils from of essential oils from *C. inophyllum*	101
Table 4.22	IC_{50} values from DPPH and H_2O_2 scavenging activity of essential oils from *P. soyauxii*	102
Table 4.23	IC_{50} values from DPPH and H_2O_2 scavenging activity of crude methanol extracts from *C. inophyllum*	104
Table 4.24	IC_{50} values from DPPH and H_2O_2 scavenging activity of crude methanol extracts from *P. soyauxii*	105
Table 4.25	IC_{50} values from DPPH and H_2O_2 scavenging activity of fractions from Pod extract of *C. inophyllum*	107
Table 4.26	IC_{50} values from DPPH and H_2O_2 scavenging activity of fractions from Root bark extract of *P. soyauxii*	108
Table 4.27	IC_{50} values from DPPH and H_2O_2 scavenging activity of isolated compounds from *C. inophyllum*	110
Table 4.28	IC_{50} values from DPPH and H_2O_2 scavenging activity of isolated compounds from *P. soyauxii*	111
Table 4.29	^{1}H and ^{13}C NMR spectral data of CiPdE-1 in $CDCl_3$	115
Table 4.30	^{1}H and ^{13}C NMR spectral data of CiPdE-2 in $CDCl_3$	130
Table 4.31	^{1}H and ^{13}C NMR spectral data of CiPdE-3 in DMSO	145
Table 4.32	^{1}H and ^{13}C NMR spectral data of PsRbE-A in $CDCl_3$	162
Table 4.33	^{1}H and ^{13}C NMR spectral data of PsRbE-B in $CDCl_3$	177
Table 4.34	^{1}H and ^{13}C NMR spectral data of PsRbE-C in $CDCl_3$	190
Table 4.35	^{1}H and ^{13}C NMR spectral data of PsRbE-D in $CDCl_3$	205

LIST OF FIGURES

Fig 2.1:	Hypericin	7
Fig 2.2:	Pseudohypericin	7
Fig 2.3:	Aerial part of *C. inophyllum*	8
Fig 2.4	Fruit part of *C. inophyllum*	9
Fig 2.5:	Pod of *C. inophyllum*	10
Fig 2.6:	Root wood of *C. inophyllum*	11
Fig 2.7:	Root bark of *C. inophyllum*	12
Fig 2.8:	Inoxanthone	14
Fig 2.9:	3,23-epoxy-friedelan-28-oic acid	15
Fig 2.10:	Canophyllol	15
Fig 2.11:	Oxo-3-friedelan-28-oic acid	15
Fig 2.12:	Friedelin	15
Fig 2.13:	Canophyllic acid	15
Fig 2.14:	Epifriedelanol	15
Fig 2.15:	Brasilixanthone B	16
Fig 2.16:	Pyranojacareubin	16
Fig 2.17:	Caloxanthone A	16
Fig 2.18:	Caloxanthone B	17
Fig 2.19:	Flavonoid	19
Fig 2.20:	Pterostilbene	20
Fig 2.21:	Ascorbic acid	20
Fig 2.22:	Santalin A	21
Fig 2.23:	Santarubin A	21
Fig 2.24:	Santarubin B	22
Fig 2.25:	Pterocarpin	22
Fig 2.26:	Aerial part of *P. soyauxii*	23
Fig 2.27:	Leaves of *P. soyauxii*	24
Fig 2.28	Stems of *P. soyauxii*	25
Fig 2.29	Morphine	26
Fig 2.30	Nicotine	27
Fig 2.31	Flavan-3,4-diol	27
Fig 2.32	Geniste	28

Fig 2.33	Cyanidin	28
Fig 2.34	Peonidin	29
Fig 2.35	Petunidin	29
Fig 2.36	∝-Amyrin	30
Fig 2.37	Digitonin	30
Fig 2.38	Ellagic acid	30
Fig 2.39	Phenol	32
Fig 2.40	Phenolphthalein	32
Fig 2.41	*p*-cymene	33
Fig 2.42	Myrcene	33
Fig 2.43	γ-Bisabolene	33
Fig 2.44	Geraniol	33
Fig 2.45	Pinane	33
Fig 2.46	Cholestone	34
Fig 2.47	Digitoxigenin	35
Fig 2.48	Uzarigenin	35
Fig 2.49	Strophanthidin	36
Fig 2.50	Scillaren A	36
Fig 2.51	Cholesterol	37
Fig 2.52	Ergosterol	37
Fig 2.53	Androsterone	38
Fig 4.1:	Mass spectrum for non-identified compound with retention time 36.84 in leaf oil (15.85%) of *C. inophyllum*	65
Fig 4.2	Structure of **CiPdE-1** (Friedelin)	114
Fig 4.3	^{1}H NMR Spectrum (500MHz) of CiPdE-1 in $CDCl_3$	117
Fig 4.4	^{13}C NMR Spectrum (500MHz) of CiPdE-1 in $CDCl_3$	118
Fig 4.5	DEPT 135 Spectrum of CiPdE-1 in $CDCl_3$	119
Fig 4.6	DEPT 90 Spectrum of CiPdE-1 in $CDCl_3$	120
Fig 4.7	HSQC Spectrum of CiPdE-1 in $CDCl_3$	121
Fig 4.8	COSY Spectrum of CiPdE-1 in $CDCl_3$	122
Fig 4.9	HMBC Spectrum of CiPdE-1 in $CDCl_3$	123
Fig 4.10	NOESY Spectrum of CiPdE-1 in $CDCl_3$	124
Fig 4.11	IR Spectrum of CiPdE-1	125
Fig 4.12	High Resolution Mass Spectrum (TOF MS ES+) of CiPdE-1	126

Fig 4.13	Structure of CiPdE-2 (Stigmasterol)	129
Fig 4.14	^{1}H NMR Spectrum (500MHz) of CiPdE-2 in $CDCl_3$	132
Fig 4.15	^{13}C NMR Spectrum (500MHz) of CiPdE-2 in $CDCl_3$	133
Fig 4.16	DEPT 135 Spectrum of CiPdE-2 in $CDCl_3$	134
Fig 4.17	DEPT 90 Spectrum of CiPdE-2 in $CDCl_3$	135
Fig 4.18	HSQC Spectrum of CiPdE-2 in $CDCl_3$	136
Fig 4.19	COSY Spectrum of CiPdE-2 in $CDCl_3$	137
Fig 4.20	HMBC Spectrum of CiPdE-2 in $CDCl_3$	138
Fig 4.21	NOESY Spectrum of CiPdE-2 in $CDCl_3$	139
Fig 4.22	IR Spectrum of CiPdE-2	140
Fig 4.23	Mass Spectrum of CiPdE-2	141
Fig 4.24	Structure of CiPdE-3 (1,3,5-trihydroxy-2-methoxyxanthone)	144
Fig 4.25	^{1}H NMR Spectrum (500MHz) of CiPdE-3 in DMSO	146
Fig 4.26	^{1}H NMR Spectrum (500MHz) of CiPdE-3 in DMSO (expanded)	147
Fig 4.27	^{13}C NMR Spectrum (500MHz) of CiPdE-3 in DMSO	148
Fig 4.28	DEPT 135 Spectrum of CiPdE-3 in DMSO	149
Fig 4.29	DEPT 90 Spectrum of CiPdE-3 in DMSO	150
Fig 4.30	Stacked spectra of DEPT experiments of CiPdE-3 in DMSO	151
Fig 4.31	HSQC Spectrum of CiPdE-3 in DMSO	152
Fig 4.32	COSY Spectrum of CiPdE-3 in DMSO	153
Fig 4.33	HMBC Spectrum of CiPdE-3 in DMSO	154
Fig 4.34	NOESY Spectrum of CiPdE-3 in DMSO	155
Fig 4.35	IR Spectrum of CiPdE-3	156
Fig 4.36	Mass Spectrum of CiPdE-3	157
Fig 4.37	Mass Spectrum (TOF ES+) of CiPdE-3	158
Fig 4.38	Structure of PsRbE-A (2,3-dihydroxyfriedelin)	161
Fig 4.39	^{1}H NMR Spectrum (500MHz) of PsRbE-A in $CDCl_3$	164
Fig 4.40	^{13}C NMR Spectrum (500MHz) of PsRbE-A in $CDCl_3$	165
Fig 4.41	DEPT 135 Spectrum of PsRbE-A in $CDCl_3$	166
Fig 4.42	DEPT 90 Spectrum of PsRbE-A in $CDCl_3$	167
Fig 4.43	HSQC Spectrum of PsRbE-A in $CDCl_3$	168
Fig 4.44	COSY Spectrum of PsRbE- in $CDCl_3$	169
Fig 4.45	HMBC Spectrum of PsRbE-A in $CDCl_3$	170
Fig 4.46	NOESY Spectrum of PsRbE-A in $CDCl_3$	171

Fig 4.47	IR Spectrum of PsRbE-A	172
Fig 4.48	Mass Spectrum of PsRbE-A	173
Fig 4.49	Structure of PsRbE-B (3β-hydroxylup-20(29)-ene)	176
Fig 4.50	^{1}H NMR Spectrum (500MHz) of PsRbE-B in $CDCl_3$	178
Fig 4.51	^{13}C NMR Spectrum (500MHz) of PsRbE-B in $CDCl_3$	179
Fig 4.52	DEPT 135 Spectrum of PsRbE-B in $CDCl_3$	180
Fig 4.53	HSQC DEPT 90 Spectrum of PsRbE-B in $CDCl_3$	181
Fig 4.54	HMBC Spectrum of PsRbE-B in $CDCl_3$	182
Fig 4.55	COSY Spectrum of PsRbE-B in $CDCl_3$	183
Fig 4.56	NOESY Spectrum of PsRbE-B in $CDCl_3$	184
Fig 4.57	IR Spectrum of PsRbE-B	185
Fig 4.58	Mass Spectrum of PsRbE-B	186
Fig 4.59	Structure of PsRbE-C (Stigmasterol)	189
Fig 4.60	^{1}H NMR Spectrum (500MHz) of PsRbE-C in $CDCl_3$	192
Fig 4.61	^{13}C NMR Spectrum (500MHz) of PsRbE-C in $CDCl_3$	193
Fig 4.62	DEPT 135 Spectrum of PsRbE-C in $CDCl_3$	194
Fig 4.63	DEPT 90 Spectrum of PsRbE-C in $CDCl_3$	195
Fig 4.64	HSQC Spectrum of PsRbE-C in $CDCl_3$	196
Fig 4.65	COSY Spectrum of PsRbE-C in $CDCl_3$	197
Fig 4.66	HMBC Spectrum of PsRbE-C in $CDCl_3$	198
Fig 4.67	NOESY Spectrum of PsRbE-C in $CDCl_3$	199
Fig 4.68	IR Spectrum of PsRbE-C	200
Fig 4.69	Mass Spectrum of PsRbE-C	201
Fig 4.70	Structure of PsRbE-D **(**β-sitosterol**)**	204
Fig 4.71	^{1}H NMR Spectrum (500MHz) of PsRbE-D in $CDCl_3$	207
Fig 4.72	^{13}C NMR Spectrum (500MHz) of PsRbE-D in $CDCl_3$	208
Fig 4.73	DEPT 135 Spectrum of PsRbE-D in $CDCl_3$	209
Fig 4.74	DEPT 90 Spectrum of PsRbE-D in $CDCl_3$	210
Fig 4.75	HSQC Spectrum of PsRbE-D in $CDCl_3$	211
Fig 4.76	COSY Spectrum of PsRbE-D in $CDCl_3$	212
Fig 4.77	HMBC Spectrum of PsRbE-D in $CDCl_3$	213
Fig 4.78	IR Spectrum of PsRbE-D	215
Fig 4.79	Mass Spectrum of PsRbE-D	216
Fig 4.80	Mass Spectrum of PsRbE-D	217

LIST OF SCHEME

Scheme 3.1: Isolation scheme of compounds from *C. inophyllum* 57

Scheme 3.2: Isolation scheme of compounds from *P. soyauxii* 58

LIST OF ABREVIATIONS

α	Alpha
APT	Attached Proton Test
β	Beta
BHA	Butylated Hydroxyl Anisole
d	doublet
dd	doublet of doublets
Brs	Broad singlet
^{13}C	Carbon-13
δ_C	Carbon chemical shift in the carbon NMR spectra
CC	Column chromatography
IC_{50}	Concentration that causes 50% inhibition activity
COSY	Correlation spectroscopy
J	Coupling constant in Hertz
^{2}J	Coupling Coupling over two bonds
^{3}J	Coupling Coupling over three bonds
^{o}C	Degree Celcius
1D	1-Dimensional
2D	2-Dimensional
DEPT	Distortionless enhancement by polarization transfer
DPPH	2,2-diphenyl-1-picrylhydrazyl
EI-MS	Electron ionization mass spectrometry
FTIR	Fourier transform infra-red spectroscopy
γ	Gamma
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
HSQC	Heteronuclear single quatum coherence
Hz	Hertz
HPLC	High Performance Liquid-Chromatography
LC_{50}	Lethal concentration that causes 50% inhibition activity
m	Multiplet

MS	Mass spectroscopy
m/z	Mass-to-charge ratio
μL	Microliter
μM	Micro Molar
μg	microgram
mg	milligram
nm	nanometer
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser effect
NOESY	Nuclear Overhauser effect spectroscopy
ORAC	Oxygen Radical Absorption Capacity
ppm	Parts per million
KBr	Potassium Bromide
^{1}H	Proton
δ_H	Proton chemical shift in the proton NMR spectra
R_f	Retention Factor
TLC	Thin layer chromatography
TOF-EIMS	Time of Flight Electron Impact Mass Spectrometry
t	triplet
UV-Vis	Ultraviolet-visible

Appendices

Appx 1.1	Chromatogram of volatile oil from leaves of *C. inophyllum*	240
Appx 1.2	Chromatogram of volatile oil from leaf stalk of *C. inophyllum*	241
Appx 1.3	Chromatogram of volatile oil from flower of *C. inophyllum*	242
Appx 1.4	Chromatogram of volatile oil seed essential oil from *C. inophyllum*	243
Appx 1.5	Chromatogram of pod volatile oil from *C. inophyllum*	244
Appx 1.6	Chromatogram of fruit-pulp volatile oil from *C. inophyllum*	245
Appx 1.7	Chromatogram of stem wood volatile oil from *C. inophyllum*	246
Appx 1.8	Chromatogram of stem bark volatile oil from *C. inophyllum*	247
Appx 1.9	Chromatogram of root wood volatile oil from *C. inophyllum*	248
Appx 1.10	Chromatogram of root bark volatile oil from *C. inophyllum*	249
Appx 1.11	Chromatogram of root wood essential oil from *P. soyauxii*	250
Appx 1.12	Chromatogram of root bark of essential oil from *P. soyauxii*	251

CHAPTER ONE

INTRODUCTION

1.1. Background of the Study

Plants are gifts from God, and they occupy a strategic place in the social, cultural, and economic definition of societies. Plants also offer essential materials for food, drugs, shelter, clothing, furniture, weapons, flavours and fragrances (Balick and Cox, 1996; Da Silva *et al*., 2022). During man's interaction with plants for these purposes, he was able to identify plants that have physiological effects on his system. This knowledge was transferred from generation to generation either orally or mystically, and effective plants have been selected by trial and error. In Africa and other developing countries relevance is increasingly associated with the use of herbal medicine as a means of ameliorating diseases thereby eroding the utilization of expensive imported drugs with deleterious side effects (Sofowora, 1993; Padhee, 2001). Ethnomedicinal system based on herbal therapies is very pivotal in defining the health status of many emerging, and underdeveloped nations. Comtemporarily, herbal medicine has also gained broad applications due to their biomedical benefits (Harvey, 2008). The mode of preparation and plant part utilised differs depending on the state and location considered. Ethnomedicinal information and plant origin are important to pharmaceuticals where plants provide raw materials (Tabuti *et al.,* 2003).

Conventional medications and comtemporary drugs were originally derived from medicinal plants. A good number of commercial medications used today for disease control such as diabetes, cancer, fever, tuberculosis, viral diseases, cardiovascular diseases, asthma and many other diseases are derived from compounds of plant origin. Medicinal plants are the richest, most reliable and safest source of medicinal compounds. Screening of plants is conducted either to discover a new drug or a lead structure. Chemical modifications or transformations can be carried out on natural product lead structures in order to arrive at relevant therapeutic molecular fragment referred to as the pharmacophore (Manuchair, 2002; Da Silva *et al*., 2022).

1.2 Natural Products as Therapeutic Agents

Chemical constituents or compounds formed by living organisms such as animals, plants, insects and other organisms that possess a broad spectrum of distinctive biological, pharmacological and pharmaceutical relevance are known as natural products. Mankind has completely relied on medicinal plants for disease control and amelioration before the availability of comtemporary synthetic drugs (Farnsworth *et al*., 1985; Da Silva *et al*., 2022). Primary health care has gained significance in most part of the world due to the availability, application and utilization of suitable drugs. Medicinal plants in the form of herbal formulations or as active compounds have always been a common source of medication (Akerele, 1993). Scientific research has developed drugs and active ingredients around the world for the mitigation of old and emerging diseases. Recent findings revealed that out of about 120 drugs isolated from plant fashion, about 75% are utilised in conventional-medicine in ways that completely relates with their traditional applications by different cultures in the World. The therapeutic effect of some secondary metabolites identified in some medicinal plants with several pharmaceutical, biological and pharmacological relevance has been the subject of continual investigations involving the prospection of novel therapies (Mohammadhosseini *et al.*, 2016; Aidi-Wannes *et al.*, 2017; Mohammadhosseini, 2017).

1.3 Drug Discovery and Medicinal Plants

Plants of medicinal values have been used as therapeutic agents from time immemorial due to their potency in the mitigation of several disease conditions such as common cold, asthma, diabetes, oxidative stress, malaria, typhoid fever, schistosomiasis, onchocerciasis, lymphatic filariasis, African dengue and trypanosomiasis (Harbone, 1993; Uddin MS *et al*., 2023). These medications were originally in diverse and unique forms of crude formulations such as powders and tinctures. The application of therapeutically relevant medicinal plants in more recent times as medications has involved the purification of active secondary metabolites from impurities, beginning with the isolation, identification and structural elucidation of morphine from *opium* in the mid 19th century. Medicinal plants were also strategic in the purification and structural elucidation of other early therapeutic agents such as codeine, cocaine, digitoxin and quinine. (Kinghorn, 2001; Samuelsson, 2004).

The discovery of therapies and active ingredients from plants has translated to involve other areas of study and several methods of analysis. Drug discovery comtemporarily include fields such as botany, ethnobotany, pharmacognosy, pharmacology, ethno-pharmacology and ecology. The methodology usually begins with an ethnobotanist who identifies and authenticates the plant of interest. Plant selection and authentication may include taxa collected at random for a broad screening study or species with established pharmacological or biological relevance. Protection of the intellectual privileges of a location where plant samples of interest are obtained is very crucial and an exclusive duty of the individual collecting the samples. Phytochemists, prepare extracts from plant materials, screen them biologically in pharmacologically important assays, and then begin isolating and characterising the active components through chromatographic purifications (Bruhn and Bohlin, 1997). Through the careful implementation of suitable assays tailored toward specific molecular targets, emerging science disciplines such as molecular biology have become critical to plant-based drug discovery. Pharmacognosy is an essential discipline that combines all of the aforementioned areas into a single discipline. (Kinghorn, 2001; Samuelsson, 2004). Pharmacognosy is the physico-chemical and bio-organic properties of drug substances. This field involves the science and applications of natural substances from plant and animal substances including insects, bacteria, fungi and even marine organisms. Recently the field has been extended to include the study of dietary supplements (Bruhn and Bohlin, 1997; Tyler, 1999; Cardellina, 2002).

1.4. Relevance of Medicinal Plants in Drug Discoveries

The detection of possible drug candidates relies heavily on medicinal plants. Several drugs have been isolated and derivatised from natural sources using one or a combination of synthetic approaches, combinatorial techniques and molecular models (Lombardino and Lowe, 2004; Uddin MS *et al*., 2023). A report in 2001 and 2002 by the World Health Organization revealed that a good number of the world bestselling drugs originated from ecofriendly and readily available natural sources such as plants, insects, fungi and many other sources. In the United States for example, several drugs and medications obtained from medicinally relevant plants have been recently introduced for pharmaceutical uses. A good example of such drug is Arteether which is a good, reliable and highly potent antimalarial drug derived from artemisinin-based moiety, a sesquiterpene type lactone purified from the plant *Artemisia annua* L. This

plant was exhaustively utilised in traditional Chinese medicine (TCM) for the treatment and prevention of several disease condition (vanAgtmael *et al.,* 1999). In Europian countries, other artemisinin-based compounds are in different stages of development or clinical trials as antimalarial therapies. (vanAgtmael *et al.,* 1999). In Russian early traditional medicine (1950) wide ethnobotanical survey led to the discovery of therapeutically important Galantamine, a natural productwhich was first isolated from family Amaryllidaceae known as *Galanthus woronowii* Losinsk. (Heinrich and Teoh, 2004). Drug research has benefited greatly from emerging science fields such as molecular biology. Galantamine is an Alzheimer's disease medication that has been approved by the FDA. It functions by inhibiting acetylcholinesterase and binding to the nicotinic receptor (acetylcholine), which it modulates. (Heinrich and Teoh, 2004).

1.5 Justification for the Study

Plants have been widely utilised in the amelioration of a variety of diseases, especially in emerging states where health-care are scarce or unavailable. (Nickavar and Yousefian, 2009). Medicinal plants contain several volatile and non-volatile chemical constituents that are prospective agents of combating existing, emerging and re-emerging diseases. This has been the scope and focal point of research-works in recent times with the goal of exploring potent drugs (Aidi Wannes *et al.*, 2017). The rise in disease incidence, especially in developing countries like Nigeria, has fueled a global demand for disease prevention and treatment alternatives that are safe, dependable, efficient, environmentally sustainable and cost-effective. (Mohammadhosseini, 2017). Several chemical agents are commercially available as anti-diabetic, and antioxidant drugs, but some have several undesirable side-effects. Furthermore, orthodox medicine has had only limited exploit in the mitigation of hyperglycemia, diabetes mellitus, oxidative stress, and related diseases. Hence, the need for alternative natural products from plant fashion used in traditional medicine for several therapeutic interventions (Kahn *et al*., 2014; Luksamee *et al*., 2023).

Traditionally, *Calophyllum inophyllum a*nd *Pterocarpus soyauxii* are reportedly used as antidiabetic and antioxidant agents in ethnomedicine (Saha *et al.,* 2013; Zushang *et al.*, 2013; Christophe *et al*., 2000; Janki *et al*., 2012). Despite, the importance of both plants in African ethnomedicine, there is paucity of information in literature on the

antidiabetic and antioxidant of the chemical constituents responsible for their wide ethnomedicinal uses, hence this study.

1.6 Research Objectives

1.6.1 General Objective

To identify, isolate and characterize the volatile and non-volatile constituents of *Calophyllum inophyllum* and *Pterocarpus soyauxii* responsible for their biological activities.

1.6.2 Specific Objectives

The specific objectives of this research are to;

1. Extract and characterise essential oils from *Calophyllum inophyllum* and *Pterocarpus soyauxii*
2. Screen phytochemicals in extracts from *Calophyllum inophyllum* and *Pterocarpus soyauxii.*
3. isolate and characterize secondary metabolites from *Calophyllum inophyllum* and *Pterocarpus soyauxii*
4. Assess the anti-diabetic potential of essential oils and chromatographically purified compounds from *Calophyllum inophyllum* and *Pterocarpus soyauxii* using α-amylase and α-glucosidase inhibitory activities.
5. Determine the antioxidant activities of essential oils and purified compounds from *Calophyllum inophyllum* and *Pterocarpus soyauxii* using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Hydrogen peroxide (H_2O_2) radical methods.

CHAPTER TWO

LITERATURE REVIEW

2.1 Family Clusiaceae

Clusiaceae is flora-family with 40 genera and 1200 species. The plant morphology among its genera and species shows a large number of variations. In schizogenous spaces or canals, they emerge as herbs, shrubs, or trees that usually carry essential oils (Li *et al.,* 1990; Elnaz *et al*., 2023). Hypericin **[2.1]** or pseudohypericin **[2.2]** are common components of some clusiaceae genera which are characterised by red or black coloured glands. The leaf part of family clusiaceae are usually plain and gland fringed entirely. Their fruits are typically succulent, fleshy or non-fleshy, and come in the form of drupes, berries, or capsules with one-to-many seeds (Li *et al.,* 1990; Luksamee *et al*., 2023). Endosperms may or may not exist in the seeds of this family. The flowers are often actinomorphic. The flower's bracteoles are usually hidden under the calyx and are not visible from the sepals. The sepals are completely fused, decussated, or imbricated in the bud. The flowers are normally made up of four to twelve imbricate petals. When the petals are grouped together, they are called cymes or panicles. This unique family is very common in tropics such as South-Eastern Africa, Latin America, and Southeast Asia. They are made up of plants with coloured or milky sap (Taher *et al.*, 2010; Elnaz *et al*., 2023).

2.1.1 Genus *Calophyllum*

Genus *Calophyllum* are evergreen tropical plants with approximately 200 different species (Dweck and Meadowst, 2002). *Calophyllum linnaeus* is the full name of this genus, and the name *Calophyllum* comes from a Greek term that means beautiful leaf. This genus can be found all over the world, but it is most commonly found in tropical areas like West Africa, Australia, Madagascar, China, and tropical America. In a variety of ecosystems, including coastal areas, flat forests, coral cays, and mountain edges, the genus grows up as shrubs or trees (Ong *et al.*, 2011). Poon in Myanmar and

bintagor tree in Malaysia, are among the many local names for the genus (Taher *et al.*, 2010; Luksamee *et al*., 2023).

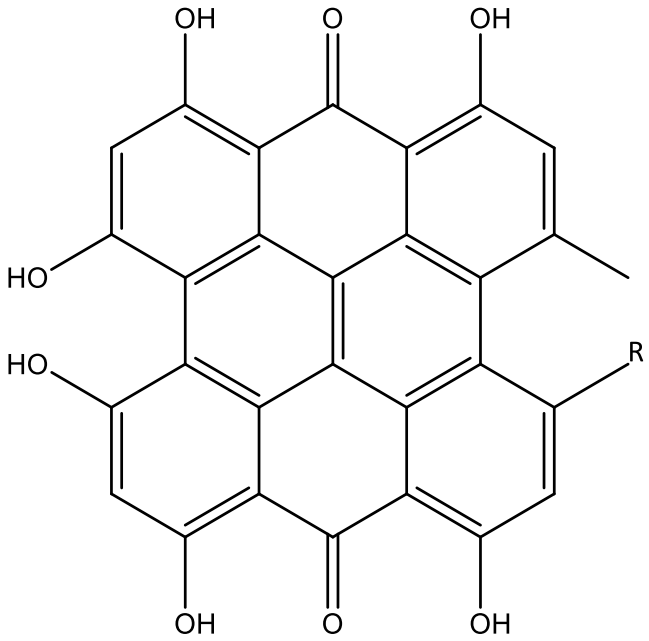


Fig. [2.1] R=CH_3 Hypericin

Fig. [2.2] R=CH_2OH Pseudohypericin

Fig. 2.3. Aerial part of *C. inophyllum*

Fig. 2.4. Fruit part of *C. inophyllum*

Fig. 2.5. Pod of *Calophyllum inophyllum*

Fig. 2.6. Root wood of *Calophyllum inophyllum*.

Fig. 2.7. Root bark of *Calophyllum inophyllum*

2.1.2 Uses of *Calophyllum inophyllum*

Traditional medicine, medicinal intervention and industrial utility are some of the known applications of *Calophyllum inophyllum* (Luksamee *et al*., 2023). It has been extensively utilised to treat several ailments, including hyperglycemia, hemorrhoids, tumors, inflammation, gastric ulcers, rheumatism, infections and more. The extracted oil from the aerial part of this plant is used to treat dermatitis, neuritis and ulcers due to its ethnomedicinal properties (Luksamee *et al*., 2023). Rheumatism, skin infections, cuts, burns and sores are all treated with a decoction made from the leaf (Uma *et al.*, 2012). A combined decoction of leaf and stem bark exhibited reduced the glucose and lipid properties. Its fruit extracts have been beneficial in the treatment of dermatitis; Stem bark extracts are utilised in mitigating vaginal disorders following abortion, gonorrhea, and internal haemorrhages in the local area (Burkhil, 1994; Elnaz *et al*., 2023). The plant has had recently been described as a prospective repository of biodiesel. Seed oil has been described as a good raw material for vegetable oil processing. The parts of this genus have high economic value in addition to biological value. This genus plants are known for their wood, which is used in plywood and boat construction (Hathurusingha and Ashwath, 2007).

2.1.3 Biological activities of isolated constituents from genus *Calophyllum*

Calophyllum species contain biologically active secondary metabolites including xanthones, flavonoids, triterpenes and coumarins, according to studies. These compounds are responsible for *Calophyllum* species' molluscicidal, anti-microbial and cytotoxic properties (Kashman *et al*., 1992).

Coumarins (Pengsuparp *et al.*,1996), triterpenoids (Hang *et al.*, 2006) and flavonoids have all been obtained from genus *Calophyllum* (Li *et al.*, 2007). *Calophyllum* species also contain some coumarins that exhibit cytophaticity (Spinor *et al.*, 1998). A xanthone derivative extracted from *Calophyllum inophyllum* root bark has been discovered to be a potential antimicrobial and cytotoxic agent (Marie *et al.*, 2004). Five bioactive compounds in *Calophyllum inophyllum* leaves showed good lipid inhibition in *in-vivo* tests, including some calophyllic acids (Janki *et al.*, 2012). Calophyllolide also, has been found to be an anticoagulant (Bhalla *et al*., 1980). The

plant has also been discovered to be an effective anticancer agent (Itoigawa *et al.*, 2001).

2.1.4 Previously isolated compounds from *Calophyllum inophyllum*

The phytochemical investigation of *Calophyllum inophyllum* was published in 2004 by Yimdjo and coworkers. The compound inoxanthone **[2.8]** was isolated from the root part using a mixture of CH_2Cl_2 and MeOH. The isolate had yellow-coloured flakes and a ferric chloride test ($FeCl_3$) revealed the presence of phenolics. Caloxanthones B, 1,5-dihydroxyxanthone, inophylloidic acid, macluraxanthone, calophynic acid, friedelan-3-one, caloxanthones A, and brasiliensic acid were also isolated from this extract. After repeated column chromatographic purifications using the same solvent system the plant's nuts yielded four compounds: inophyllums C, calaustralin, inophyllums E, and calophyllolide. (Yimdjo *et al.*, 2016). Li and colleagues isolated a unique triterpenoid from the ethanolic fraction of the stem and leaf sections, along with seven other recognized triterpenoids. HREIMS was used to determine the structure of the new compound, which was 3,23-epoxy-friedelan-28-oic acid **[2.9].** Seven other known compounds were elucidated by comparing them to literature data: canophyllol **[2.10]**, oxo-3-friedelan-28-oic acid **[2.11]**, canophyllol **[2.12]**, canophyllic acid **[2.13]**, epifriedelanol **[2.14]** and friedelin **[2.15]** by comparing data from the literature, we were able to validate our findings. Compounds **[2.8]**, **[2.10]**, **[2.13]**, and **[2.15]** were screened for anti-leukemia activity to determine their function and compound **[2.11]** was found to effectively inhibit leukemia cells in the human body (Li *et al.*, 2010).

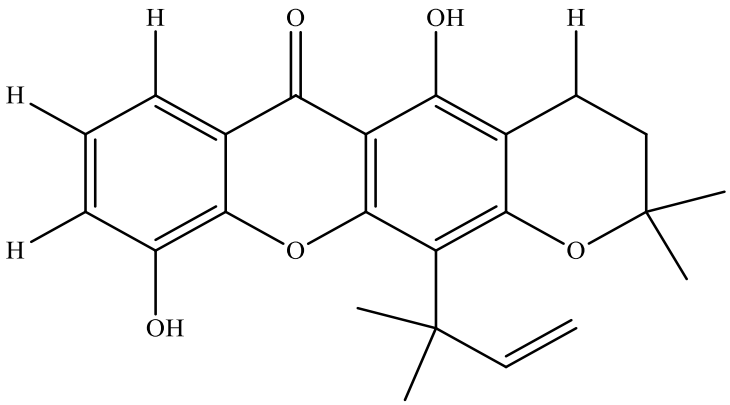


Fig. [2.8] Inoxanthone

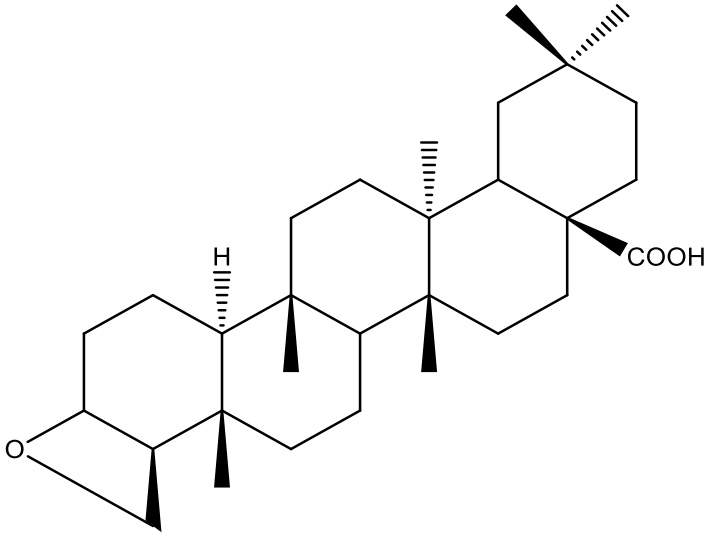


Fig. [2.9] 3,23-epoxy-friedelan-28-oic acid

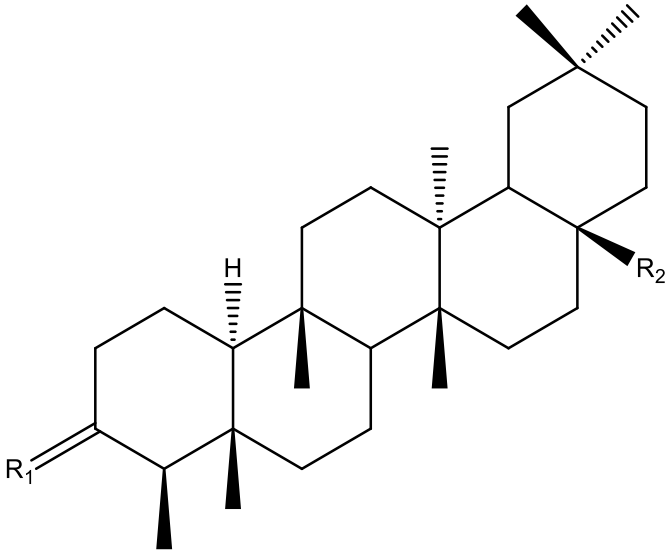


Fig. [2.10] $\mathbf{R_1}$=O $\mathbf{R_2}$=CH_2OH Canophyllol

Fig. [2.11] $\mathbf{R_1}$= βOH $\mathbf{R_2}$=CH_3 Oxo-3-friedelan-28-oic acid

Fig. [2.12] $\mathbf{R_1}$=O $\mathbf{R_2}$=CHO (Friedelin)

Fig. [2.13] $\mathbf{R_1}$=βOH $\mathbf{R_2}$=COOH (Canophyllic acid)

Fig. [2.14] $\mathbf{R_1}$=O $\mathbf{R_2}$=COOH **(**Epifriedelanol)

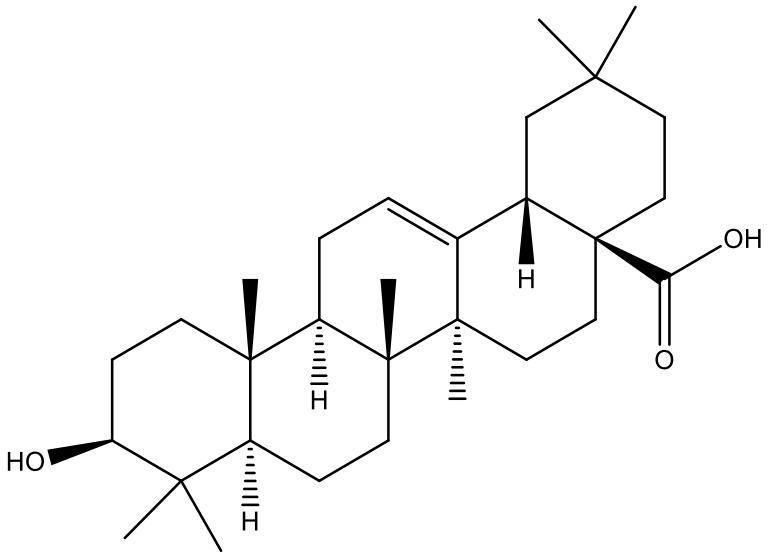


Fig. [2.15] Brasilixanthone B

The root part contains important xanthones such as brasilixanthone B **[2.15]**, pyranojacareubin **[2.16]**, caloxanthone A **[2.17]** and caloxanthone B **[2.18]** (Kijjoa *et al.*, 2000; Ee *et al.*, 2009).

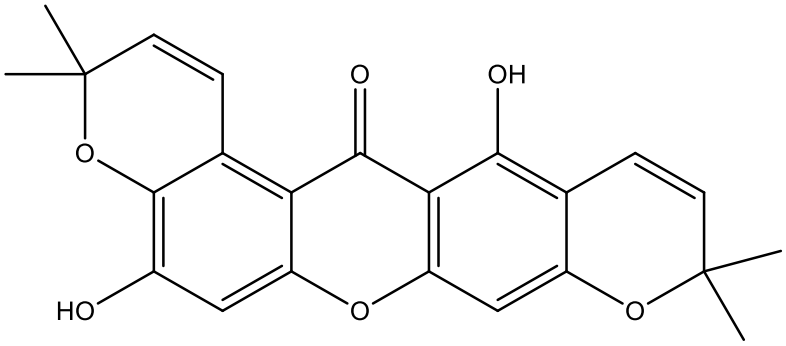


Fig. [2.16] Pyranojacareubin

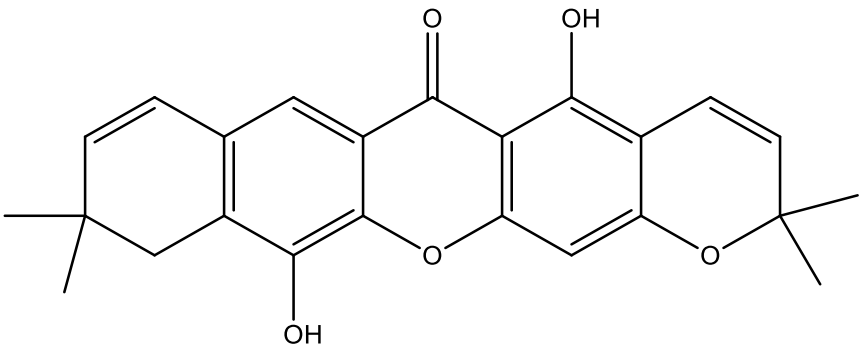


Fig. [2.17] Caloxanthone A

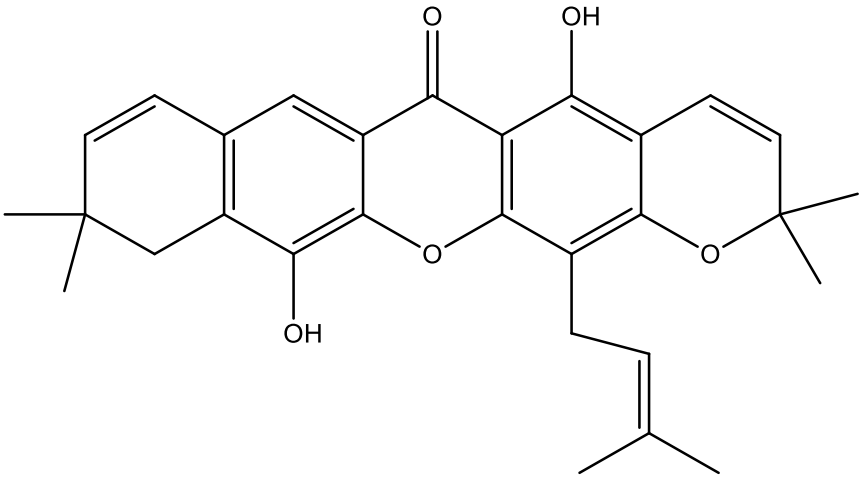


Fig. [2.18] Caloxanthone B

2.2 Family Fabaceae

The Fabaceae is a very large group of flower-bearing plants. The group is also known as the legume family (Christenhusz and Byng, 2016). They are herbaceous plants that grow as trees, shrubs and herbaceous plants with leguminous fruit and stipulate leaves. The family cutting across seven hundred and fifty-one genera and over nineteen thousand known species is one of the widest land-plant families considering the large number of species (Judd *et al.,* 2002; Christenhusz and Byng, 2016). Reports estimated that over 19,000 legume species make up about 7% of all angiosperms (Magallón and Sanderson, 2001; Judd *et al.,* 2002). The Fabaceae family is distributed in tropical rainforests in Africa (Burnham and Johnson, 2004).

2.2.1 Genus *Pterocarpus*

Genus *Pterocarpus* is a pantropical woody Fabaceae family. *Pterocarpus* has recently been assigned to the informal monophyletic *Pterocarpus* clade and most *Pterocarpus* species produce valuable padauk timber. *Pterocarpus santalinus,* a member of the *Pterocarpus* genus, produces Zitan, China's most valuable rosewood. *Pterocarpus* is a genus of woody trees native to the tropics. When the wood from the narra tree (*Pterocarpus indicus*) and the Burmese padauk tree (*Pterocarpus macrocarpus*) has evolved into burls, it is marketed as amboyna. The genus possesses unique seed pod shape (Lavin *et al*., 2001; Lavin and Pennigton, 2001).

Padauk wood comes from a variety of *Pterocarpus* plants. Padauks come from either Africa or Asia. Padauks are known for their resilience, durability, and decorative

qualities, with the majority of them having a reddish wood. *Pterocarpus* woods may be used as dyes because they contain water or alcohol-soluble substances. The most common padauk found is African padauk from *Pterocarpus soyauxii*, which is bright red or orange when freshly cut but fades to a warm brown over time when exposed to sunlight. It is common among woodworkers because of its colour. *Pterocarpus macrocarpus* grows in Burma, while *Pterocarpus dalbergioides* grows in the Andaman Islands. Padauks may be confused with true rosewoods, to which they are similar, but padauks are coarser and have a less decorative figure. Padauk, like rosewood, is used to produce xylophone, organ, and marimba keys, as well as guitars. It's a popular option for traditional Chinese furniture (Assanta and Robert, 2011; Cardoso *et al*., 2013).

2.2.2 Uses of *Pterocarpus soyauxii*

Hyperglycemia, hypertension, intestinal parasites, renal infections, chronic anemia, skin diseases, and fungal infections are all treated with *Pterocarpus soyauxii*. The sap of the plant has the potential for mitigating ear-aches as well as jaundice by indigenous peoples in the Brazilian Amazon. The fruit of this plant is taken internally in Solomon Islands to encourage fertility. Colombian tribes claim that the fruits and leaves have anti-inflammatory properties and can be used to treat skin ailments. (Oteng-Gang and Mbachu, 1990; Bremaud *et al*., 2011). Even after frying, the leaves of *Pterocarpus soyauxii* is an outstanding source of antioxidants and possess high nutritional value. The bark of the plant has demonstrated antifungal activity and thus can be used to treat skin infections. The bark is used in ethno-veterinary medicine to ward off skin parasites. Dysmenorrhoea, uterine haemorrhage, dysentery, and haemorrhoids can be ameliorated using the root parts in the Congo and Central African Republic. (Lainé *et al.,* 1985). Inflammations, oedemas, incipient hernia and whitlow are treated with the bark-pulp of the plant. Broncho-pulmonary infections are treated with vapour baths of leaves and bark (Nzokou and Kamdem, 2003).

Pterocarpus soyauxii is a versatile tree that is used to make high-quality wood, dye, and a variety of industrial products. It is a prominent source of barwood dye, which is currently used to colour fabrics and fibers (Burkil, 1994).

2.2.3 Biological activities of isolated compounds from *Pterocarpus soyauxii*

Pterocarpus soyauxii phytochemical constituents are responsible for the activities expressed by the plant. Flavonoids are strong free radical scavengers with anti-cancer properties. The presence of saponins in *Pterocarpus soyauxii* leaf extracts finds application in the mitigation of hormonal disorders (Francis *et al.*, 2002). Saponins' antifungal properties have been studied extensively, especially for pharmaceutical and agricultural applications. Obute and Osuji in (2002), recorded that many saponin-rich extracts in the plant had antifungal properties. The presence of tannins in *Pterocarpus soyauxii* supports its role in mitigating haemorrhoids, frost and ulcers. (Villegas *et al.*, 1997).

2.2.4 Previously isolated compounds from *Pterocarpus soyauxii*

Flavone and its derivatives **[2.19],** pterostilbene **[2.20]**, and ascorbic acid **[2.21]** were discovered in this plant as major phytochemicals. (Arnone *et al*., 1977; Barend and Brandt, 1987; Oteng-Gyang and Mbachu, 1990; Tchamadeu *et al*., 2011).

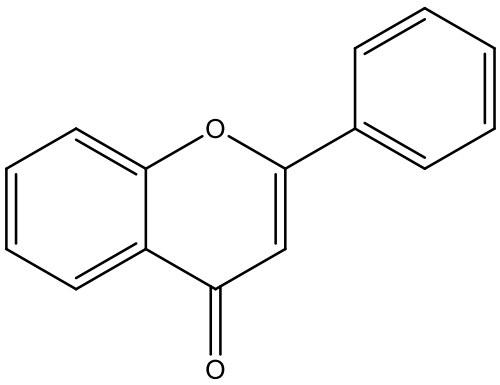


Fig. [2.19] Flavonoid

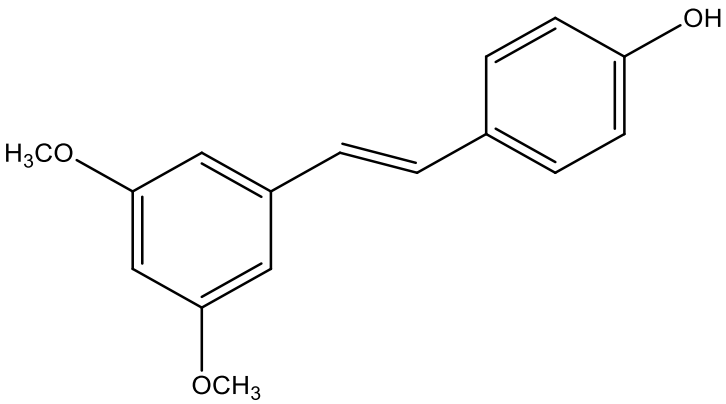


Fig. [2.20] Pterostilbene

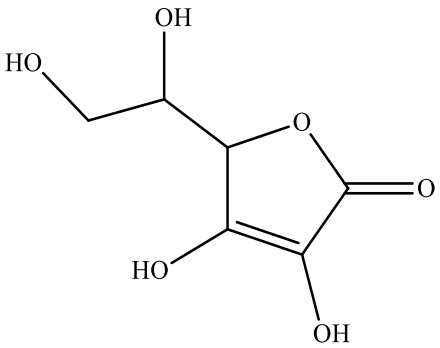


Fig. [2.21] Ascorbic acid

Santalin A **[2.22]**, santarubin A **[2.23]**, and santarubin B **[2.24]** are found in the heartwood of *Pterocarpus soyauxii*. Pterocarpin **[2.25]**, formononetin, and prunetin are some of the isoflavonoids isolated from it (Arnone *et al*., 1977). The tannin content of the wood contributes to its widespread use in the dyeing process (Rojo, 1972).

Flavonoids, terpenoids and alkaloids were identified as major constituents in the leaf ethanolic extract of this plant, while glycosides were also identified in different solvent extracts of the plant. Antioxidants are abundant in *Pterocarpus soyauxii*. These findings revealed that *Pterocarpus soyauxii* contains micronutrients, minerals and secondary metabolites, all of which are essential for healthy development (Maduka *et al.,* 2018).

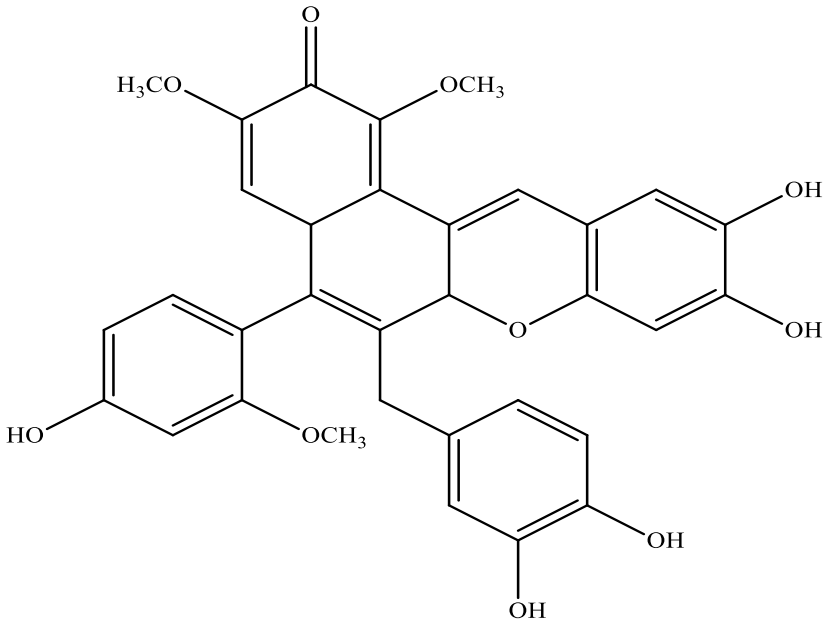


Fig. [2.22] Santalin A

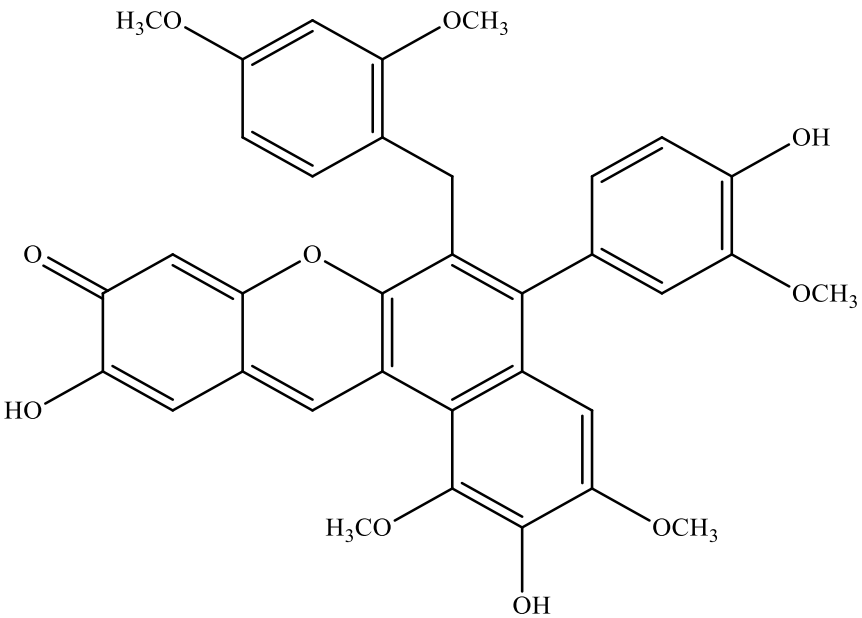


Fig. [2.23] Santarubin A

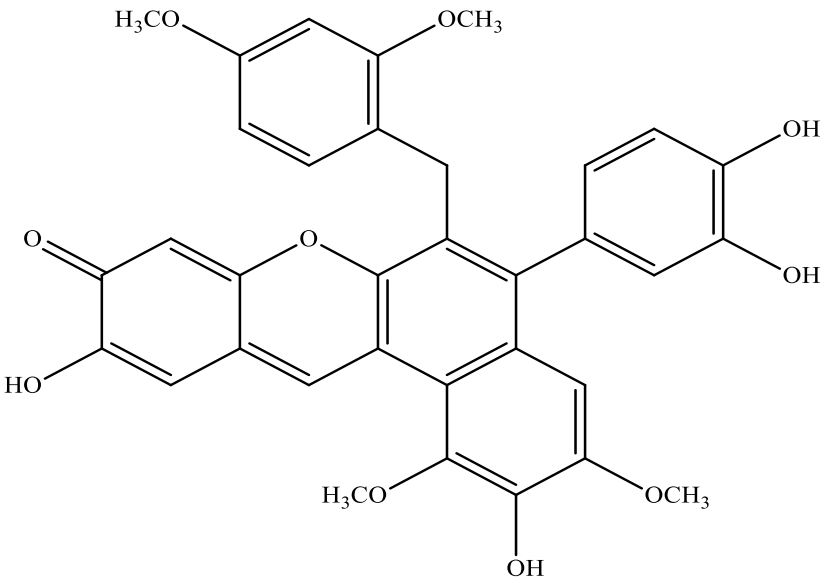
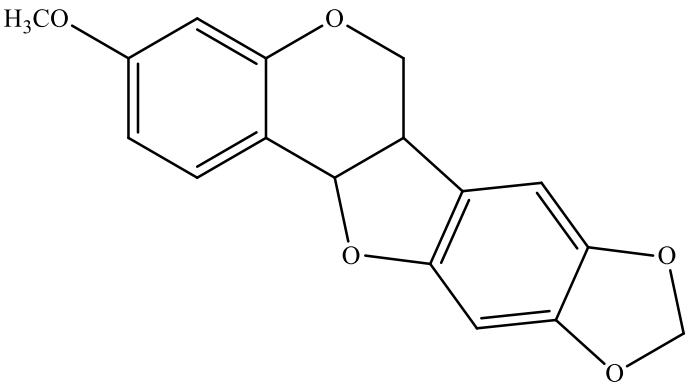


Fig. [2.24] Santarubin B



[2.25] Pterocarpin

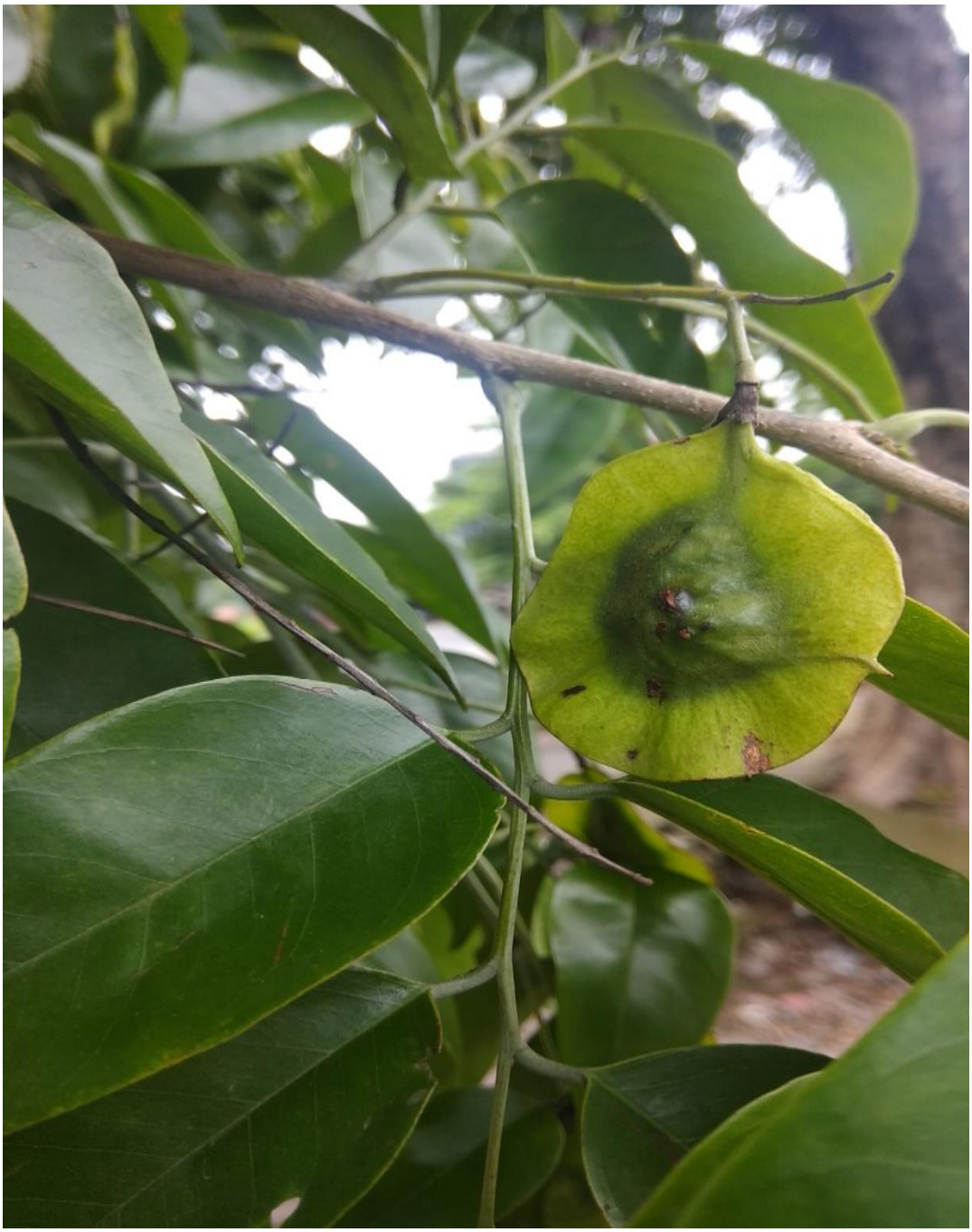


Fig 2.26*:* Aerial part of *Pterocarpus soyauxii*.

Fig 2.27 Leaves of *Pterocarpus soyauxii*

Fig 2.28 Stems of *Pterocarpus soyauxii*

2.3 Natural Products

A living organism produces a chemical compound or material known as a natural product. The cells of living organisms are the site of intricate and complex synthetic activities that result in the formation of a variety of compounds, many of which are of great importance to man (Finar, 1973; Elnaz *et al*., 2023; Song *et al*., 2023). Metabolites are small molecules that play intermediary roles in natural metabolic processes (Cooper and George, 2015). Examples of natural products include alkaloids, flavonoids, anthocyanins, saponins, terpenoids, and essential oils, cardiac glycosides and steroids.

2.3.1 Alkaloids

Alkaloids are essential compounds that, in most cases, contain at least one nitrogen atom as part of a cyclic mechanism (Song *et al*., 2023). They are very essential for plant protection from deleterious microorganisms thus exhibiting antifungal and antibacterial functions (Molyneux *et al*., 1996). These plant constituents are extremely valuable in terms of economics (Wink *et al.,* 1998). Some alkaloids such as morphine [**2.29**] and nicotine [**2.30**] have stimulant property which can be utilized as pain-relieving and quinine in drugs for anti-malaria (Chapman, 1997; Song *et al*., 2023).

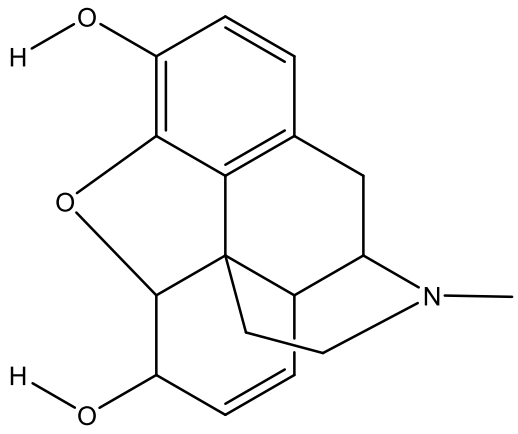


Fig. [**2.29**] Morphine

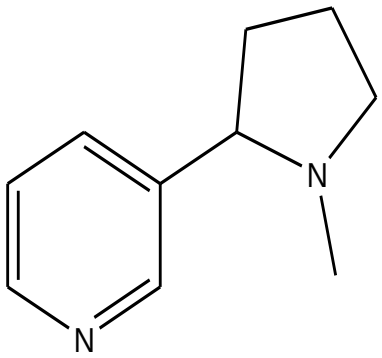


Fig. [**2.30**] Nicotine

2.3.2 Flavonoids

Flavonoids are structurally related to flavone-2-phenyl chromone, the parent product. They are polyphenolic substances present in plants and found mostly in seeds, fruits skin, pollen, bark or flowers (Pridham, 1960; Xi *et al*., 2022). Flavonoids are characteristically plant products found in all groups except algae, bacteria and fungi. Flavonoids are pharmacologically active, largely through their inhibitory effects on many mammalian enzymes or cellular systems (Finar, 1975). They are good cytotoxic, antimicrobial, anti-inflammatory and anti-cancer agents (Xi *et al*., 2022). However, their ability to act as effective anti-oxidants is the most well-known. (Mamta *et al.,* 2013). Usually, the molecular structure of flavonoids determines their antioxidant ability. The radical scavenging power of OH groups are evaluated by their location in the chemical structure. Flavonoids like luteolin and cathechins are also stronger antioxidants than established ones (Mamta *et al.,* 2013). See figures **[2.31]** and **[2.32]** below for structures of flavan-3,4-diol and Geniste respectively.

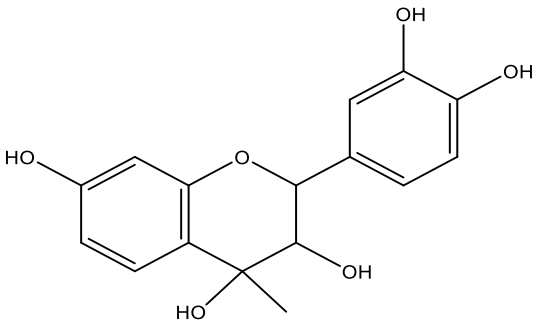


Fig. [2.31] Flavan-3,4-diol

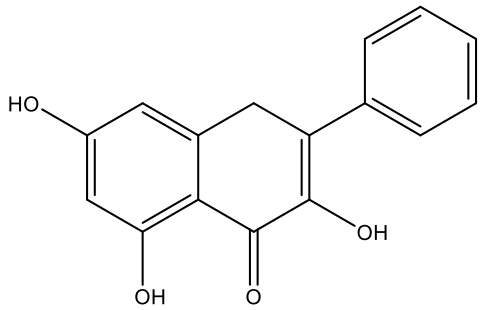


Fig. [2.32] Geniste

2.3.3 Anthocyanins

Anthocyanins are pigments found in plants. They are glycosides and these classes of compounds are responsible for the colours of flowers. Furthermore, these compounds all have the same carbon skeleton, with the only difference being the nature of the substituent group (Harbone, 1993). Anthocyanin pigments are amphoteric, with acid salts that are typically red and metallic salts that are typically blue. Anthocyanins are violet in neutral solution. Anthocyanins have been identified as potential inhibitor of type II Diabetes Mellitus (Denise *et al*., 2023). They include: Cyanidin **[2.33],** Peonidin **[2.34]** and Petunidin [**2.35]** (Harbone, 1993; Denise et al., 2023).

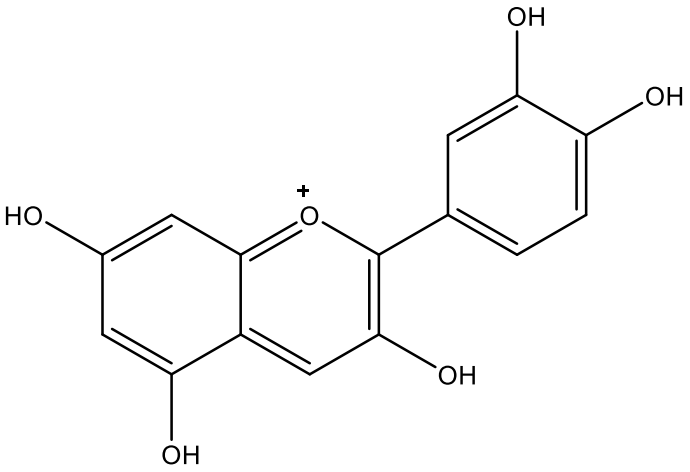


Fig. [**2.33]** Cyanidin

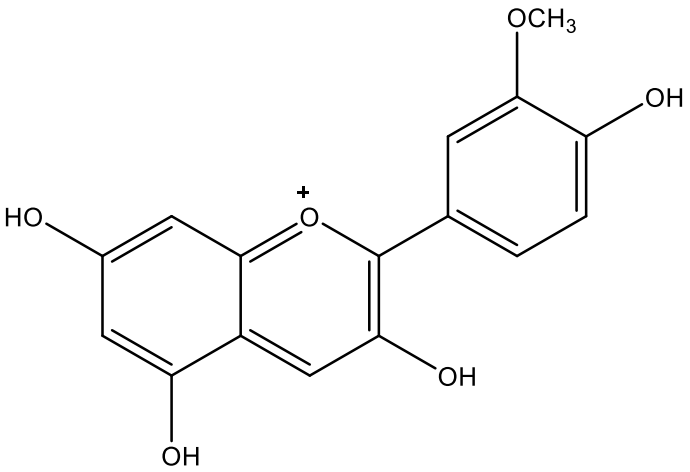


Fig. [**2.34]** Peonidin

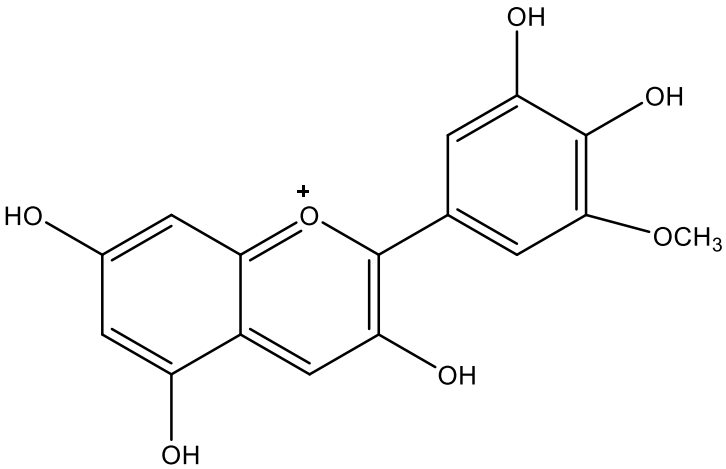


Fig. [**2.35]** Petunidin

2.3.4 Saponins

Saponins are found in abundance in nature. They exhibit surface active and soap-like behavior in aqueous solutions. This behaviour is due to their protic and aprotic nature. The presence of saponins can be determined by the formation of tenacious froth during plant preparation. Plants rich in saponins generally have good anti-inflammatory, antimicrobial and antithrombotic properties (Mieres-Castro *et al*., 2023). Oleanane is a common aglycone. The sugar component comprises of at least one sugar group glycosidically connected to asapogenin (aglycon). Monodesmoside-saponins have one sugar atom joined at Carbon-3’ position, whereas bidesmoside saponins have at least

two sugars, one at Carbon-3’ position and the other at the Carbon-22’ position. (Lasztity *et al*., 1998).

Saponin blends found in plants have a variety of biological effects on organisms. They act as cancer preventatives, impair protein processing and nutrient and mineral absorption in the gut, induce hypoglycemia, and act as antiviral agents. (Morrissey and Osbourn, 1999; Traore *et al*., 2000). ∝-amyrin **[2.36]** is a typical example of saponin aglycon

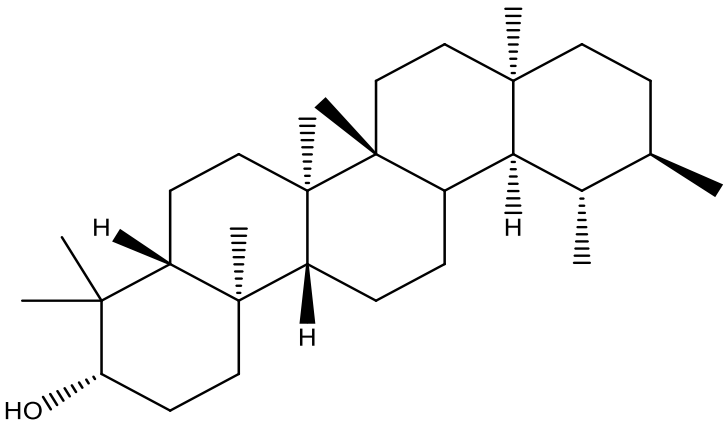


Fig. [2.36] ∝-Amyrin

Saponins are extremely effective emulsifiers from a pharmacological standpoint. They have been shown to alter the permeability of the small intestine, which can aid drug absorption (Mieres-Castro *et al*., 2023). Digitonin is a good example of a saponin [**2.37]**

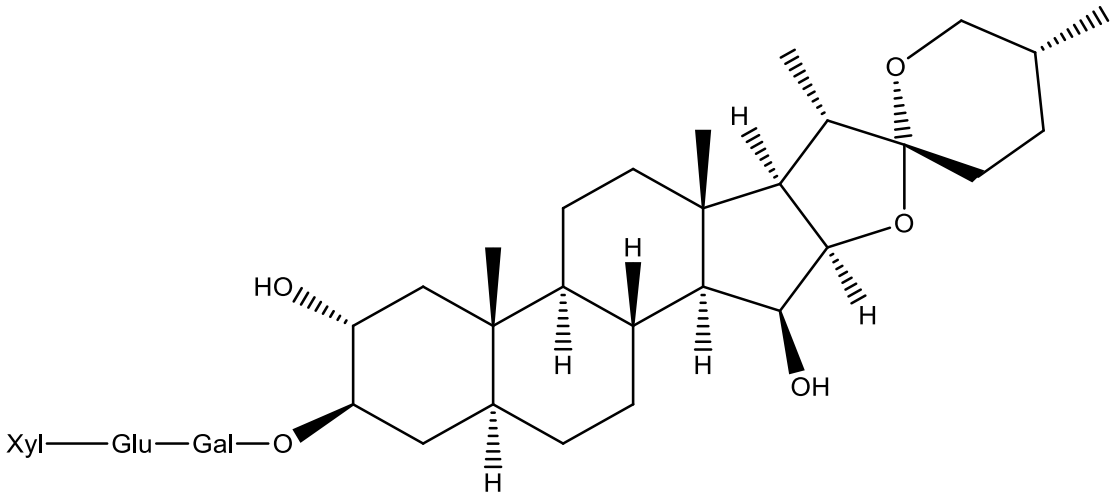


Fig. [**2.37]** Digitonin

2.3.5 Tannins

Tannins are difficult to classify chemically since the term encompasses a wide range of oligomers and polymers. They are polyphenolic compounds with a high atomic weight (Harborne, 1999; Tong *et al*., 2022). They usually form dynamic complexes with sugars and other molecules (Vansoest, 1994; Schofield *et al*., 2001). It has been possible to classify tannins into four distinct classes based on their auxillary properties: complex tannins, condensed tannins, ellagitannins, and gallotannins (Mole and Waterman, 1987; Mangan., 1988). In Asian traditional healing, they are used as anti-inflammatory, cancer prevention and antiseptic agent. Tannins find relevance in several industrial applications such as dye and ink production. They are also used for brewing wine (Tong *et al*., 2022). Ellagic acid [**2.38**] is an example of tannins.

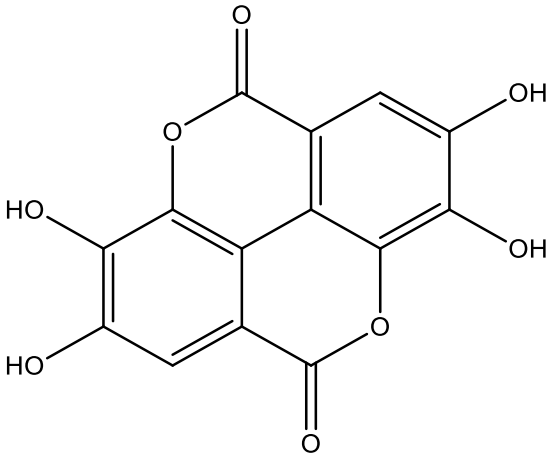


Fig. [**2.38**] Ellagic acid

2.3.6 Phenols

Phenols are described by the hydroxyl group bonded to an aromatic system [**2.39**]. Phenols (C_6H_5OH) are the most straightforward natural products with unlimited pharmacological applications (Walton *et al*., 2003). They make the largest group of secondary metabolites. Most phenolics incorporate the polyphenolic (hydrolysable and condensed tannins) and monomers, for example, ferulic and catechol (Harbone, 1993; Bianca *et al*., 2023). Some important members of this class include: flavonoids, phenolic acids, and polyphenols (Mamta *et al.*, 2013). Phenolic acids have been the focal point of agrarian, organic and therapeutic examinations (Mamta *et al*., 2013). Phenolic compounds are common in nature as polymerized molecules, like the proanthocyanidins and lignins. Various bioactivities of phenolics have been accounted obtained including an increase in bile emission and antimicrobial potential (Gryglewski *et al*., 1987; Mamta *et al*., 2013). Phenolic acids have cell reinforcement, antiulcer, anti-inflammatory, cytotoxic, antispasmodic, and antidepressant activities (Ghasemzadeh *et al*., 2010; Bianca *et al*., 2023). Phenolphthalein [**2.40**] is a good example of phenols.

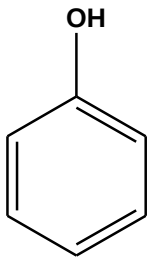


Fig. [**2.39**] Phenol

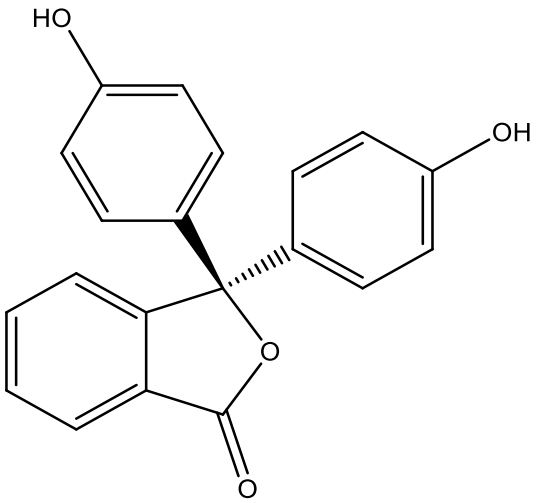


Fig. [**2.40**] Phenolphthalein

2.3.7 Terpenoids

Terpenoids are basic skeleton organic compounds and products of the mevalonic acid pathway. They are derivatives of basic isoprene units bound in various fashions (Ayu *et al*., 2022). Essential oils, which are volatile oils extracted from the sap and tissues of some plants and trees, are primarily composed of mono and sesquiterpenoids. Plant tree gums and resins contain diterpenoids and triterpenoids that are not steam volatile. Fragrance from plants is generated by the essential oil fraction. (Chapman, 1997). Examples include *p*-cymene [**2.41],** myrcene [**2.42]**, γ-bisabolene [**2.43]**, geraniol [**2.44]**, pinane [**2.45]** (Edeoga *et al.*, 2005; Ayu *et al*., 2022).

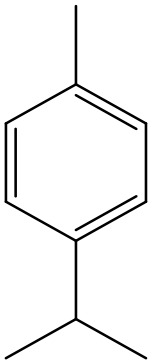


Fig. [**2.41]** *p*-cymene

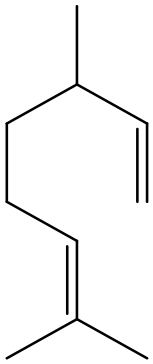


Fig. [**2.42]** Myrcene

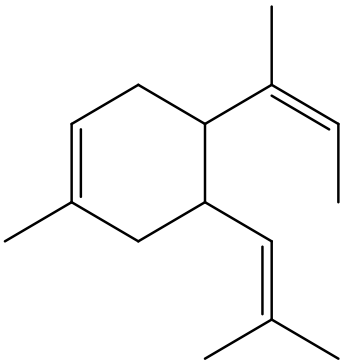


Fig. [**2.43]** γ-Bisabolene

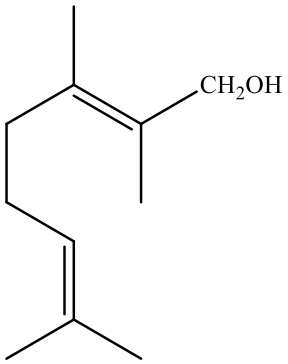


Fig. [**2.44]** Geraniol

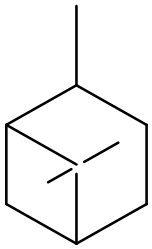


Fig. [**2.45]** Pinane

2.3.8 Glycosides

This is a wide range of phytochemicals with conjugation to sugar unit(s). A glycosidic bond connects a sugar molecule to a non-sugar moiety, called an aglycone (He *et al*., 2023). Anthocyanins are glycosides of natural plant pigments and anthocyanidins are their non-sugar counterparts. The water-soluble anthocyanins are responsible for the property of stimulating heart muscle and have been used as stimulants to increase heart muscle contraction in the case of heart failure. Glycosides assume different roles in living organisms, for instance; numerous plants store chemicals as stationary glycosides. They are enacted by chemical hydrolysis, causing the sugar part to be severed, making the aglycone accessible for use. As part of poisons elimination from the body, they are often bound to sugar molecules in animals and humans (Finar, 1975; He *et al*., 2023). Cholestone **[2.46]** is an example of a glycoside.

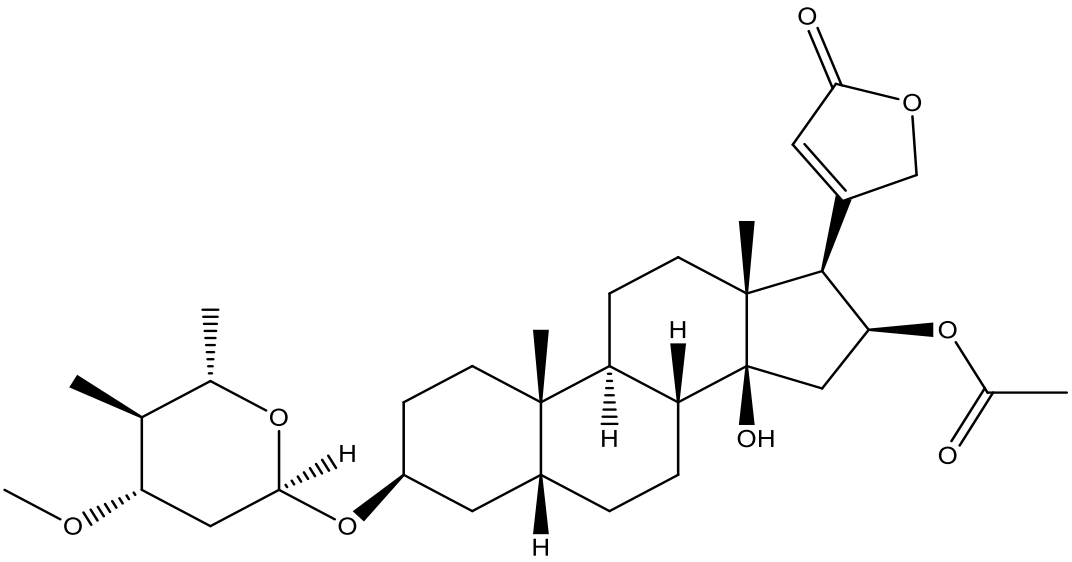


Fig. [2.46] Cholestone

2.3.9 Cardiac glycosides

Cardiac glycosides are plant steroids that come in the form of glycosides which have the ability to stimulate heart muscle contractions. A dehydration product of the aglycon is formed in some cases. These aglycons are of two types: the more common type contains α, β– unsaturated-γ-lactone ring which are known as the cardenolides. The less common type contains a δ–ring which has a conjugated diene system and are known as the bufadienolides. The bufadienolides occur as glycosides in plants of the squill family and as esters of suberyl arginine in the venom from the skin secretions of poisonous toads (e.g., bufotoxin.) (Edeoga *et al.*, 2005; He *et al*., 2023). Cardenolides include digitoxigenin [**2.47]**, uzarigenin [**2.48]**, and strophanthidin [**2.49]**. Scillaren A [**2.50]** is a bufadienolide

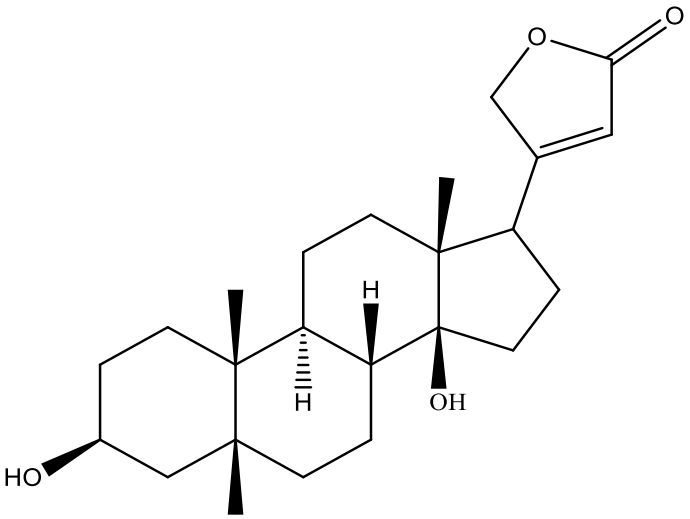


Fig. [**2.47]** Digitoxigenin

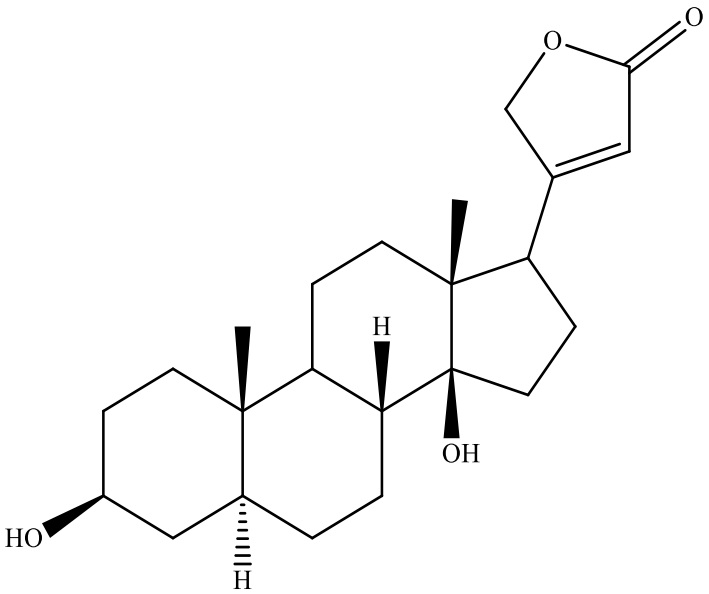


Fig. [**2.48]** Uzarigenin

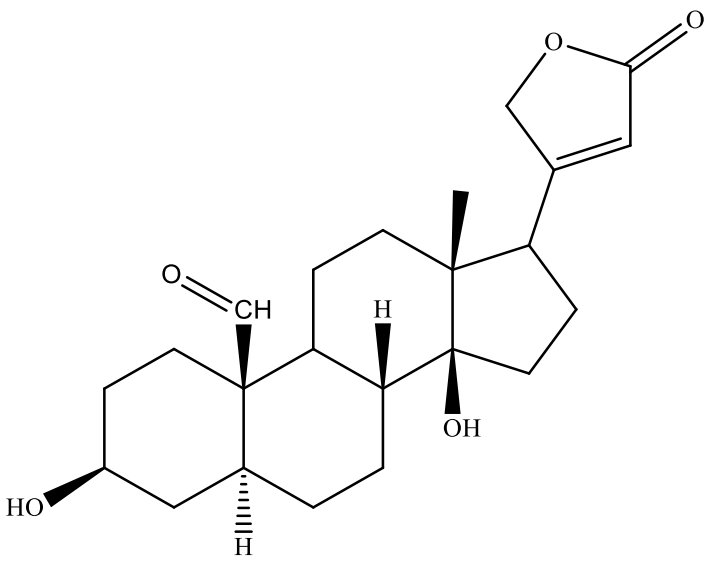


Fig. [**2.49]** Strophanthidin

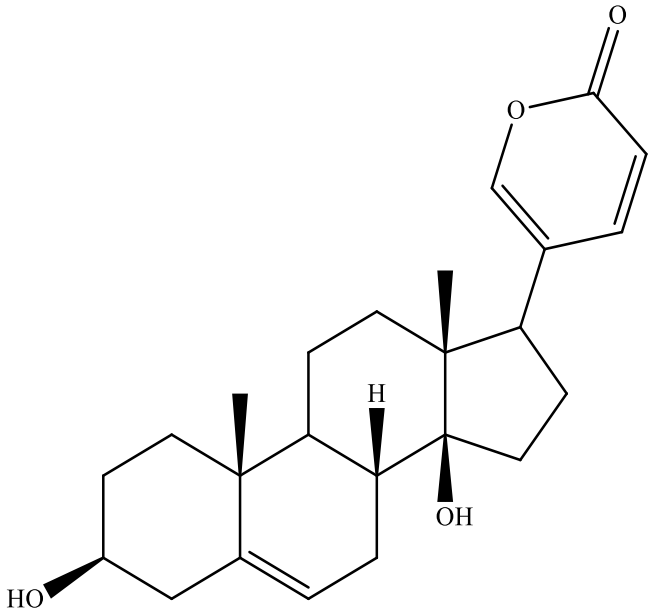


Fig. [**2.50]** Scillaren A

2.3.10 Steroids

Steroids are found both in the free and combined states as esters or glycosides. Very often, they occur in the form of complex mixtures, the components of which are difficult to separate. Cholesterol [**2.51]** is one of the most common compounds in

vertebrates, invertebrates, and plants. Upon dehydrogenation with Selenium at 360^{o}C, all steroids produce diels hydrocarbon, among other things. The structures of steroids are based on the 1,2-cyclopentenophenanthrene skeleton. Certain steroids exert a specific and powerful effect on the cardiac muscles and thus are called cardiac active steroids. Ergosterol [**2.52]** and androsterone [**2.53]** are good examples of steroids (Chapman, 1997).

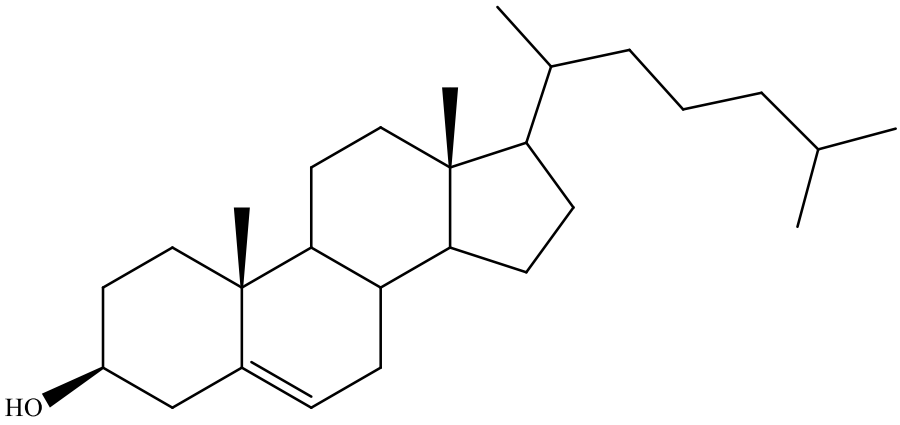


Fig. [**2.51]** Cholesterol

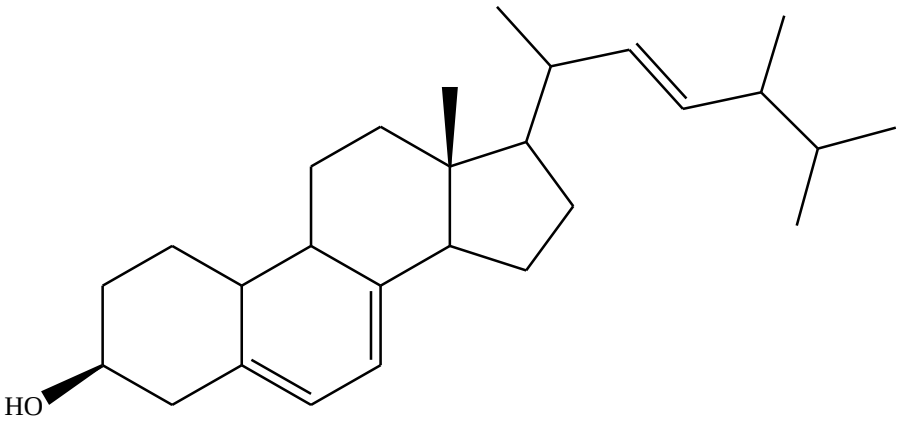


Fig. [**2.52]** Ergosterol

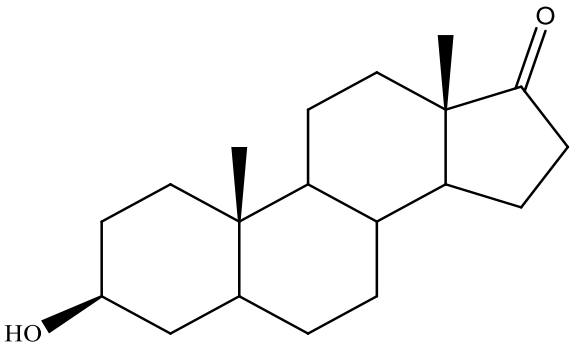


Fig. [**2.53]** Androsterone

2.4 Essential Oils

Essential oils are naturally occurring, complex odouriferous ethereal suspensions and distillable exudates obtained from plants with wide pharmacological activities. The compounds are responsible for the aroma and flavour of various parts of a plant (Hussain *et al*., 2010; Mohamed and Alotaibi, 2023). The odourific volatile oils are liquid mixtures of organic compounds with a heavy odour, and because of the bioactive components they contain, they are also promising sources of natural medicinal products (Buchbauer, 2000). Pharmacologically, essential oils have been used as insect repellent, insecticide, antimicrobial, antioxidant, pesticide, and deodorants; therefore, the possibility of using them as plant-based drugs is important (Tongnuanchan and Benjakul, 2014). They vary in different parts of a plant depending on the action of endogenous and exogenous secretory plant tissues. Owing to their smell, fragrance and numerous helpful impacts, they are broadly used in cosmetics, perfumes, nutrition, and aromatherapy (Jelena *et al.*, 2015). Essential oils have been shown in studies to be efficient antioxidants that can generate an unfavourable environment for the destruction of free radicals, thereby preventing mutations, oxidants in cells, and their scavenging action with the anticipation of complementary radicals (Onocha *et al.*, 2011). Antioxidant products can stop lipids from oxidizing by terminating the propagation of the oxidation process (Irene *et al*., 2016). The synergy of the multitude of compounds found in essential oils results in the general upsurge in the antioxidant activity than the movement of each variable separately (Singh *et al.*, 2005a; Mohamed and Alotaibi, 2023). Various researches revealed that essential oils have antimicrobial and cytotoxic actions against different bacteria (Hussain *et al*., 2010).

2.5 Plant Extraction Methods

Plant extractions are procedures used for isolating organic components of plant materials using standard methods. The goal of this process is to expand a plant material's dimension, which builds the exchange pace of soluble parts from the plant to the dissolution solvent. Following that, selective solvents are used to isolate the ideal components, followed by concentration to remove the non essential mass materials. The extract is finally dried in order to remove the dissolvable elements, yielding a dried rough concentrate (Sarker *et al.*, 2006). Before the extraction step, treatment of plant material is significant at the beginning stage of common item separation. Plant tissues ought to be organized properly in order to lessen the danger of contamination and loss the important constituents (Harborne, 1998).

2.5.1 Soxhlet extraction

Oil, fat, and other phytochemicals from solid materials are extracted using special glassware called a soxhlet extractor (Zygler *et al*., 2012). In using this method, the sample material is pulverized and configured in a thimble. During extraction, the glass-thimble is positioned above a flask containing the desired solvent and underneath a condenser. The solvent evaporates from the flask and condenses into a liquid in the condenser, where it drips into the extraction chamber, which contains the sample. (Harbone, 1998).

Soxhlet extraction technique is needed when the component of interest has sufficient dissolvability in a solvent. The fact that only one clump of dissolvable is reused rather than vast amounts of warm dissolvable is a substantial bit of leeway in this framework. This method is not advisable for extracting labile components since repeated heating will cause the compounds to break down. (Sutar *et al*., 1999).

2.5.2 Cold pressing

Cold pressing method is often applied to extract citrus fruits like oranges, lemons, and grapefruits. After being stripped from the fruit, citrus rinds are ground or chopped and then pressed. As a result, a watery essential oil and liquid mixture is obtained that separates over time (Harbone, 1993).

2.5.3 Maceration

The pulverized sample is immersed in a particular solvent and periodic mixing is used during the extraction process to enhance the extraction process. The components of the sample diffuse into the advanced concentrate until they reach a state of equilibrium. The depleted extract is then isolated from the marc by filtration. A major drawback of maceration technique is that it is tedious and requires an enormous quantity of dissolvable. (Seidel, 2006). This process, also known as batch extraction, entails macerating the tissue with the appropriate solvent in a wring blender, soaking for a short time, filtering through a buchner funnel of appropriate size, and then returning the residue to fresh solvent for further extraction. Following that, the combined solvent extracts are evaporated and the residue is subjected to suitable fractionation procedures. (Harbone, 1993).

2.5.4 Hydrodistillation

Hydrodistillation is the easiest and oldest method of extracting volatile oils from plant products, and it has become the standard used in many industries. It is also utilised in separating non-water-soluble samples with a wide range of boiling points. The method entails fully immersing macerated plant sample in water, followed by heating at a sufficiently high temperature. The oil extracted is protected by this process because the surrounding water prevents it from overheating. (Zarith *et al.,* 2018). This extraction method is a one-of-a-kind technique that is commonly used for extracting hydrophobic natural plant material. The distinct feature of this approach is that the necessary materials can be distilled at a low temperature, avoiding their loss and unwanted chemical changes. (El-Asbahani *et al*., 2009).

2.6 Chromatographic Purification of Natural Products

The separation of mixtures when molecules are moved through a stationary phase is referred to as chromatography. In this separation method, the principle is based on unique characteristics of molecules such as: adsorption (liquid-solid), partitioning (liquid-solid), and affinity (Porath, 1997; Usama *et al*., 2022). As a result of molecular differences, certain molecules of the mixture are left in the non-mobile phase and are propelled gradually through the chromatography system. The rate of elution through a

chromatographic system is dependent on the adsorptive properties of the molecules (Harris, 2004). Common chromatographic techniques include the following:

2.6.1 Column chromatography

In Column chromatography, a solvent serves as the mobile phase, while a finely divided solid surface with appreciable surface area serves as the non-mobile phase. The non-mobile phase takes the form of a tightly packed column from which the mobile phase will flow. A glass wool-filled tube is clamped and filled with a clean, dry, long, narrow tube. A silica gel slurry is made, poured into the column, and allowed to settle naturally. The sample is either injected as a liquid or as a dry sample into the column, and acidified sand is used to cover the sample layer (Harbone, 1993; Das *et al*., 1998; Gurib-Fakim, 2006; Usama *et al*., 2022).

The stationary phase is absorbed to varying degrees by the components of the mixture. As the mobile step passes over the adsorbent surface, the components are dispersed between the solvents and the adsorbent surface. The solvent and sample compete for positions in the solid adsorbent, with the solvent reversibly and constantly displacing the sample in the direction of the solvent flow. As a consequence, a poorly adsorbed compound is eluted first because it spends less time in the adsorbent (Gelosa *et al.,* 2009). As the solvent travels past the stationary support, compounds with a strong affinity for it are taken along. Compounds with a higher preference for the solid pass slowly and are the last to be eluted. This method entails filling a glass column with a strong support, adding up to several grams of sample to the column's top, and slowly collecting eluates from the column (Usama *et al*., 2022).

2.6.2. Thin Layer Chromatography (TLC)

This method involves a solid adsorbent on a solid support as a thin layer with thickness of about 0.25 mm. The separated sample is eluted in a desired solvent system and the solvent system is spotted on the thin layer chromatographic plate. The technique absorbs a portion of each of the mixture's components, leaving the rest in solution. A strongly adsorbed compound spends more time on the adsorbent and has a greater fraction of its molecule adsorbed, while a weakly adsorbed compound spends less time on the adsorbent and has a smaller fraction of its molecule adsorbed. (Gelosa *et al.,* 2009). Molecules with polar functional groups or hydrogen bonding capabilities can

bind strongly to the adsorbent surface and be retained. There are discrete adsorption sites on the adsorbent surface. Silica-gel and alumina are the two most common adsorbents for thin layer chromatography. Slurry of micro granular absorbent with water is spread as evenly as possible on a plate by means of special applicator. The application of the sample is called spotting usually with a capillary tube. Development of the chromatography can be by ascending or descending. Development usually discontinued when the solvent front has advanced 75% toward the end of plate (Donald *et al*., 2006).

2.6.3. High Performance Liquid-Chromatography (HPLC)

This analytical protocol is similar to Gas chromatography (GC) the difference is that the non-mobile phase is held by a narrow bore stainless steel column bonded to a porous polymer, while the mobile phase is pushed to move under high pressure. The mobile phase is usually an isochratic or non-isochratic solvent system that mixes sufficient quantities of two different substances to achieve the desired gradient. (Meyer, 1988). HPLC is primarily used for non-volatile compounds and works best for compounds revelaed in the ultraviolet or visible region (Harbone, 1984). A HPLC instrument consists of solvent supply system feeding a pumping device which is coupled to a column through a sample introduction system. The eluate from the column then passes through a detector and the response of the detector is measured by digital output device. The mobile phase consists of pure organic solvent in mixtures with aqueous solutions of salts or buffers. The column is made up of stainless-steel tubes with internal diameters of 2 to 4.6 mm and lengths of 10 to 100 cm. High performance liquid chromatography allows researchers to study molecules that are difficult to separate by other means (Bohm, 1994).

2.6.4 Gas Chromatography (GC)

This analytical technique is gas-liquid chromatographic analysis of volatile or semi-volatile organic compounds. As a result of spending varying amounts of time in the non-mobile phase, the molecules in the mixture are separated. The vapour pressure (boiling point) determines the duration for a compound to elute and preference for the stationary phase, as compounds can only travel down the column while in the gaseous phase (solubility). The lower the vapour pressure of the component, the longer it will

take to elute because the proportion of time such compound spends in the mobile phase will be reduced. The sample is injected with a pneumatic syringe into the injection chamber in the gas chromatograph. The carrier gas sweeps the vapourized sample into the column, which is filled with a non-mobile phase. This column is being baked at a specific temperature. The sample is subjected to a variety of gas-liquid partitioning processes as it moves through the column, and the portion is removed. A detector senses the presence and quantity of each component as it leaves the column, generating a signal that is recorded on an outline recorder or integrator (Eiceman, 2000).

2.6.5 Gas Chromatography-Mass Spectrometry (GC-MS)

This technique is a hyphenated protocol that synergizes the principle of gas chromatographic methods and mass spectrometry to recognize various substances in an analyte. Gas chromatography can be utilized to separate volatile components, but the instrument cannot distinguish them. Mass spectrometry provides nitty-gritty basic data on most substances, allowing them to be reliably identified; however, it cannot distinguish them quickly. GC-MS is applied in the study of complex macromolecules. Components in a mixture are separated by passing an inert gas that bears the sample through a non-mobile phase. As the array of compounds exit a chromatographic-column, they are recieved by the mass spectrometer that identifies and quantifies the analyte in order of their mass-charge ratio (m/z). These spectra would then be able to be stored on the computer and analysed. In GC-MS the mobile-phase is a bearer gas, generally an unreactive gas, for example, helium or an un-reactive gas, such as, nitrogen and the stationary phase is a tiny layer of fluid or polymer on a solid support which is inert, inside glass or metal tubing, called a column. In the capillary column, there is a stationary phase; fine solid support covered with a nonvolatile liquid. By a stream of helium gas, the sample is moved through the column. Mass Spectrometry (MS) is the detector for the GC. As the sample leaves GC column end it is ionized and the fragments got after ionization are arranged by mass forming a pattern of fragmentation. The protocol is explicit to such an extent that it is frequently alluded to as the molecular fingerprint (Skoog *et al*., 2007).

2.7 Spectroscopic Analysis of Natural Products

2.7.1 Ultraviolet spectroscopy

Ultraviolet spectroscopy refers to absorption within the ultraviolet-visible region of the electromagnetic spectra. The method is widely applied in the quantitative determination of conjugated organic compounds. In structural elucidation using ultraviolet spectroscopy, the ultra violet region of wavelengths from 200 - 400 nm is very useful (Morrison and Bough, 2004).

2.7.2 Infrared spectroscopy

The infrared spectrum is used for identification of functional groups present in compounds. In addition, it can be used to establish the identity of a compound. A specific group of atoms produces characteristic bands, which means that a specific group absorbs light at specific frequencies that are almost identical from compound to compound. Compounds and molecules are excited to higher energy states as it absorbs an infrared photon, resulting in more energetic vibrational transitions. Some transitions are very localized, involving only two or a few atoms at most. Only vibrations that results in the change of dipole moment of the molecule are absorbed in the Infrared region (Harbone, 1993).

2.7.3 Mass spectroscopy (MS)

This protocol is usually used for determining the molecular weight of compounds. The structure of organic compounds is usually determined by splitting the molecule into smaller recognizable fragments (Aksenov *et al*., 2017). An electron beam is used to bombard a vapourized sample to start mass spectral analysis. This can cause an electron to be dislodged from the molecule, producing a positive molecular ion which can further fragment to give smaller daughter ions. The base peak is the most abundant peak in the mass spectrum, and it has a value of 100 percent. The molecular ion (M^+) and the corresponding peak as molecular ion peak result from the loss of one electron from the molecule. Fragment peak refers to any peak with a mass less than that of the molecular ion. Information derived from mass fragments can be used to decipher the molecular ion and formula of an organic compound (Dudley, 1980).

2.7.4. Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance **(**NMR) is usually applied in measuring the energy consumed as nuclei undergo nuclear spin transitions. Photons with much less energy are involved in these transitions than those involved in electronic transitions. Nuclear magnetic resonance is concerned with the nucleus of H-atoms (the proton) or the nucleus of carbon atoms (^{13}C). With the proton nuclear magnetic resonance, information on many different kinds of hydrogen environment is obtained (Kemp, 1993). Molecules are made up of protons with different frequencies of resonance based on the chemical environment. Since the frequency shift and the fundamental resonance frequency are proportional to the magnetic field power, consequently, the shift is converted to a field-independent parameter called the chemical shift which is expressed as a % of the NMR spectrometer's operating frequency. A molecule's atomic nuclei are like tiny magnets possessing unique magnetic fields. In the vicinity of each nucleus, there are also negatively charged electrons with magnetic properties (Dudley, 1980). Nuclear magnetic resonance is basically applied in the natural sciences for the determination of structures of molecules and to study their physical properties (Ernst and Anderson., 1966; Jeener, 1971; Holzgrabe *et al.,* 1999).

2.7.4.1 Distortionless Enhancement by Polarization Transfer (DEPT) NMR

Distortionless Enhancement by Polarization Transfer (DEPT) NMR are ^{13}C NMR experiments that reveal the number of protons that are attached to individual ^{13}C carbon atoms in a molecule (Kemp, 1993). It tells whether a peak represent a primary (methyl or $-CH_3$ group), secondary (methylene or $-CH_2$ group), tertiary (methine or -CH group), or quaternary carbon. Such knowledge is very important for structural elucidation, especially for more complex molecules. Running two or more DEPT experiments can help distinguish between CH and CH_3 more effectively than the Attached Proton Test. There are several DEPT variants: DEPT-45, DEPT-90 and DEPT-135 NMR. Only CH peaks show up in the DEPT-90, Both CH and CH_3 peaks point up in the DEPT-135 while CH_2 peaks point down in the DEPT-135 (Schmidt-Rohr *et al*., 2002).

2.7.4.2 Attached Proton Test (APT) NMR

The Attached Proton Test (APT) experiment is a simple method to assign C-H multiplicities in ^{13}C NMR spectra. It provides information on all carbon multiplicities within a single experiment. The APT experiment yields methine (CH) and methyl (CH_3) signals negative and quaternary (C) and methylene (CH_2) signals positive. It is slightly less sensitive than DEPT but a single experiment shows all carbon signals at once unlike DEPT which needs three different spectra. Even though this technique does not distinguish fully between CHn groups, it is so easy and reliable that it is frequently employed as a first attempt to assign peaks in the spectrum and elucidate the structure. It is sometimes possible that a CH and CH_2 signal have coincidentally equivalent chemical shifts resulting in signal cancelation in the APT spectrum due to the opposite phases (Schmidt-Rohr *et al*., 2002). For this reason the conventional $^{13}C\{^1H\}$ spectrum or HSQC are usually also acquired.

2.7.4.3 Two-dimensional (2D) NMR in structural elucidation of natural products

The application of 2D NMR spectroscopy to structural elucidation of organic molecules is very important. It helps in the measurement of properties that cannot be directly meaured in the one dimensional NMR experiment. Some of the useful 2D NMR techmiques used in modern structural elucidation are:

2.7.4.3.1 Correlation spectroscopy (COSY)

This is a 2D NMR technique that looks at proton-proton interactions. While coupling in 1D ^1H NMR experiments provides information on proton-proton interactions, the COSY experiment has a number of advantages: In general, COSY makes it easier to find coupling partners in complex structures than a regular proton 1D NMR spectrum. Furthermore, COSY spectra can frequently be used to assign couplings (Kemp, 1993).

2.7.4.3.2 Heteronuclear Single Quantum Correlation (HSQC) spectroscopy.

This experimental protocol compares the chemical variation of a proton to that of a directly bound carbon. One-bond couplings are used in this experiment. A proton spectrum is usually placed on the horizontal axis while, the carbon is on the vertical axis. Correlation between a given proton and carbon is determined by the cross peak (Schmidt-Rohr *et al*., 2002).

2.7.4.3.3 Heteronuclear Multiple Bond Correlation (HMBC) spectroscopy.

This important technique examines bond-couplings over two (2) or three (3) bonds (J=2 to 15) are used in this experiment. Cross peaks occur when protons (H) and carbons (C) are separated by two or three bonds. The peaks of direct one-bond away are non-detectable. In the same way that the proton-proton COSY experiment offers connectivity information over several bonds, this experiment does the same (Kemp, 1993).

2.8 Biological Assay Techniques

Biological assays (bioassays) encompass all scientific procedures employed for the monitoring and measurement of some desired effect of a biologically active substance on living organisms using an intermediate *in-vivo* or *in-vitro* tissue or cell model under controllable conditions. This is done by comparing the behaviour of organisms and/or their parts under regulated conditions to the conditions that are being studied. Bioassays are commonly used when there are multiple phases between the observed behavior and the drug exposure, which are typically poorly understood, or when the substance is a complex mixture of materials with unknown active components (Rojas *et al.*, 2003).

2.8.1 Antidiabetic assay

Diabetes is a severe health condition affecting millions of people globally. Diabetes in form of mellitus is a serious health challenge identical with improper blood glucose levels (DeFronzo, 1999; Ayat *et al*., 2015; Hind *et al*., 2007; Ali *et al*., 2023). The etiologies of the disease are characterized by abnormalities in glucose functions based on gross inadequacy of insulin secretion (Shaw *et al*., 2010). Postprandial hyperglycemia is a chronic complication for diabetes-related diseases. (Hanefeld *et al.*, 1996). It may result to long-term complications, such as micro and macrovascular conditions like neuropathy, cardiovascular disease and cerebrovascular disease (Boutati and Raptis, 2004). Regulating postprandial plasma glucose levels is crucial for managing diabetes mellitus complications. (Ortiz-Andrade *et al.*, 2007). Alpha-amylase and alpha-glucosidase inhibition are effective pathways for controlling type II diabetes. These inhibitors slow the metabolism of postprandial blood glucose (Kwon *et al.*, 2007). There have been several studies on the implication of engineered enzyme

inhibitors on the degree of glucose after food intake. (Shihabudeen *et al*., 2011). One of the most common antidiabetic drugs is acarbose. The drug has a significant benefit and are appropriate for treating diabetes mellitus, but they often cause gastrointestinal side effects, limiting their effectiveness as a preventive strategy. (de Sales *et al*., 2012). Several researchers are investigating and designing dietary approaches to fully treat postprandial hyperglycemia without causing adverse effects in the digestive tract, which is a good thing (Santhakumari *et al*., 2006). The ability to screen for enzyme inhibitors from plants has improved over time, and the inhibitory activities of these enzymes have recently been extensively investigated using natural products (Matsuda *et al.*, 2002; Ali *et al*., 2023).

2.8.2 Antioxidant assay

An antioxidant is a molecule that slows or prevents other molecules from oxidizing. Antioxidants avoid chain reactions by inhibiting other oxidative reactions and removing free radical intermediates (Djafarous *et al*., 2023). When chain reactions occur in a cell, the cell is damaged; antioxidants stop chain reactions by inhibiting other oxidative reactions and eliminating free radical intermediates. Natural antioxidant agents are gaining popularity among scientists. Epidemiological survey revealed that daily consumption of antioxidants helps in mitigating the deleterious effect of cardiovascular diseases (Ames *et al*., 1993; Sies, 1997).

Plant antioxidant capacities have been estimated using a variety of assays. Examples include 2,2 diphenyl-1-picryhydrazyl (DPPH), Hydrogen peroxide and oxygen radical absorption capacity (ORAC). Antioxidants are thought to shield the body system against deleterious oxygen species, which are byproducts of natural aerobic cell respiration. Increased dietary antioxidant intake can aid in maintaining an adequate antioxidant status, allowing a living system's normal physiological role to be achieved (Shenoy and Shirwaiker, 2002; Sagar *et al.*, 2011; Djafarous *et al*., 2023).

CHAPTER THREE

MATERIALS AND METHODS

3.1 General experimental procedures

Solvents used for the research were obtained from Sigma Aldrich Science Chemical Company and double-distilled before use. Thin film (pre-coated) with silica-gel 60 HF_{254} was used for thin layer chromatography (TLC) while column chromatographic separation was carried out on silica-gel (Merck 100-200 mesh; gypsum binder). Ultraviolet light at wavelength 350 nm, Vanillin Sulphuric acid spray was used for spots visualization on the TLC. All glassware were washed, rinsed with water several times, oven dried overnight at 125°C and allowed to cool before use.

The proton (^{1}H) spectroscopic information was determined at 400, 500 and 600 MHz while the carbon 13 (^{13}C) was at 100, 125 and 150 MHz respectively on a "Bruker" NMR instrument in specific deuterated solvents. Chemical shifts are measured in parts-per million (ppm) downfield of Trimethylsilane (TMS), as an internal guide. The Perkin Elemer Spectrum 8400S (Shi-madzu) spectrometer was used for the measurement of all infrared (IR) spectra. Mass spectroscopic data were recorded using a Hewlett Packard system (G1800A GCD) and Time-of-Flight (TOF) mass spectrometer. The Ultra-violet/visible spectra were obtained using a SHIMADZU UV-3101 PC spectrophotometer and quartz cuvettes with a path length of 1 cm. All melting point determinations were carried out on an electrothermal melting point stand.

The volatile constituents were identified using Agilent-7.8.9.0-B GC-MS (Clara-Santa) device in the electron ionization (EI) mode at seventy (70) electron Volt with an HP-5 MS capillary.column (5 percent n-phenylmethyl-polysiloxane, thirty (30) meter, zero point five (0.25) millimeter internal diameter, 0.1 meter film-thickness) that was systematically-programmed under the following condition: Sixty degree celcius (60°Celcius/four minutes), followed by four (4°Celcius/min to 160°Celcius), then eleven (11°Celcius/min to 280°Celcius), and finally fifteen degree celcius per minutes up to three hundred degree celcius. The helium-based carrier-gas was set at a flow rate

of 1.2 milliliters/minute; the temperature of injection was 280^{o}Celcius, while the temperature of transfer was 300^{o}C; the volume for injection was one (1) microliter; the ratio of splitting was one ratio one hundred (1:100); the time utilized for running was fifty-seven (57) minutes; and the mass range applied for acquisition was twenty-nine to four hundred (29-400) atomic mass unit. The volatile oil constitution was classified using their various retention index factors (which were measured experimentally using a homologous sequence of C-8 to C-30) and computer matching of their mass spectral fragmentation patterns against established libraries. Adams and NIST libraries' retention indexes (Adams 2007; NIST 2017). Peak area normalization was used to achieve relative peak area percentages, which were the average of three determinations with an RSD percent of less than 10% in all situations.

3.2. Collection and identification of plant materials

Calophyllum inophyllum was collected by Mr. D. Esimekhuai from Ibadan (University of Ibadan, main campus, Ibadan, Oyo State) and assigned voucher number at the University-herbarium section of Botany Department, University of Ibadan, Nigeria (UIH 22659). Mr. T. Odewo collected *Pterocarpus soyauxii* from the Jericho region of Ibadan, Nigeria, and had it authenticated in the “Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria” (FIH 112031). Plant materials were washed to remove humus, and then dried at room temperature. Prior to extraction, the plant materials were pulverized and stored in airtight polythene bags.

3.3. Essential oil extraction from *Calophyllum inophyllum*

Each separated part of this plant was dried at room temperature, ground and subjected to hydro-distillation for three hours. The essential oils were stored in a refrigerator before analysis. All essential oils from *Calophyllum inophyllum* were subjected to antidiabetic, and antioxidant assays.

3.4. Extraction of plant materials from *Calophyllum inophyllum*

The air-dried, powdered pod (1.5 kg) from *Calophyllum inophyllum* (CiPd) was extracted using methanol. The methanol extract obtained was successively fractionated with ethyl acetate and hexane. The fractions were concentrated separately on a temperature regulated rotatory-type evaporator at 40°Celcius to about 50 milliliters

before being freeze dried to eliminate residual solvents. The hexane fraction yielded a dark green solid (20 g) coded CiPdH. Fractionation with ethyl acetate yielded a brown extract (20 g) coded CiPdE while methanol the methanol fraction was obtained as a dark brown extract (35 g), coded CiPdM. All the extracts were subjected to anti-diabetic, and antioxidant assay.

3.5 Essential oil extraction from *Pterocarpus soyauxii*

In a Clevenger-type apparatus configured to British-based Pharmacopeia requirements, root and root bark of *Pterocarpus soyauxii* were pulverised and hydro-distilled for three hours. All essential oils were stored by refrigeration until further analyses were carried out.

3.6 Extraction of plant materials from *Pterocarpus soyauxii*

The finely ground root bark (1.2 kilogram) of *Pterocarpus soyauxii* (PsRb) was extracted and fractionated using using hexane, ethyl acetate and methanol. The fractions were filtered and subsequently concentrated on a temperature controlled rotatory evaporator to about 50 mL and freeze dried. Partitioning with hexane yielded a deep green solid (20 g) coded PsRbH, ethyl acetate yielded a black solid (15 g) coded PsRbE while methanol gave a black coarse solid (35 g) coded PsRbM.

3.7 Phytochemical Screening

Qualitative phytochemical evaluation of extracts and fractions from *Calophyllum inophyllum* and *Pterocarpus soyauxii* were carried out using the following methods

3.7.1 Tests for saponins

Small quantity (5 mg) of extract were shaken into each of different graduated cylinder, after adding twenty (20) mL of deionized-water, the mixture was agitated for 20 minutes. Observation of persistent frothing indicated the presence of saponins (Evans *et al.*, 2002).

3.7.2 Test for alkaloids

3.7.2.1 Mayer's test for alkaloids

The extracts were heated with HCl (2%) in a water bath (100^{o}C), finely-filtered and treated with a small quantity of Mayer's reagent. Yellow precipitation confirmed that alkaloids were present (Evans *et al.,* 2002).

3.7.2.2 Dragendorff's test for alkaloids

To one (1) mL of extracts, one (1) mL of Dragendorff's solution was carefully added, formation of a precipitate revealed that alkaloids were present (Harborne, 1984; Evans, 1989).

3.7.2.3 Wagner's test for alkaloids

To two (2) mL of Wagner's reagent (iodine in KI) was introduced to one (1) mL of extract. There was no colour reaction or precipitate, indicating that the extracts were void of alkaloids. (Harborne, 1998).

3.7.3 Test for steroids

About five (5) milligram of sample was dissolved in two (2) mL of sulphuric acid and chloroform was added. The formation of a red-brown colour interphase was indicative of steroids. (Harborne, 1998).

3.7.4 Test for tannins and phenolic compounds

To about 1 mL of extracts a solution of ferric-chloride was added; subsequent formation of a dark-blue-greenish black precipitate indicative of the presence of tannins (Harborne, 1998).

3.7.5 Test for resins

To about five (5) mL of each extract, (5) mL copper (II) sulphate was added. The resulting mixtures were vigorously shaken. Green-coloured precipitate revealed the presence of resins (Harborne, 1998).

3.7.6 Test for carbohydrates

To five (5) mL of equi-volumes of Fehling's A and B; two (2) mL of each extract was added in a test tube. The resultant mixture was boiled for two (2) minutes. A brick-red precipitation of CuO indicated that carbohydrates were present (Soforowa, 1993).

3.7.7 Test for anthraquinones

Ten (10 g) of extract was shaken with five 5 mL of benzene, then filtered before adding 10 mL aqueous H_2SO_4 to the filtrate. Anthraquinone was detected when the mixture was vortexed and a pink or violet colour was observed.

3.7.8 Test for flavonoids

(a). One and half **(**1.5) mL of a 50 percent methanol was added to two (2) mL of the extract solution. The solution was warmed and magnesium metal was added; 5-6 drops of hydrochloric acid were added. The presence of flavonoids was suggested by the formation of a red colour.

(b). The presence of flavones was determined by treating 5 mg of the extract with sodium hydroxide; the formation of a yellow colour indicates the presence of flavones (Harborne, 1998).

3.7.9 Test for terpenoids

3.7.9.1 Libermann-Burchard test for terpenoids

One gram (1g) of sample was dissolved in 3 mL of acetic anhydride, with a few drops of chloroform added. Along the sides of the test tube, 3 mL glacial acetic acid was applied. The formation of bluish-green indicated the presence of terpenoids.

3.7.9.2 Salkowski test for terpenoids

Chloroform was used to dissolve the extracts and vortexed with a solution of concentrated tetraoxosulphate (VI) acid in an equal amount. The presence of steroidal components in the tested extracts was confirmed by blue-red colour in the chloroform layer (Harborne, 1998).

3.7.10 Test for glycosides

Two (2) mL of acetic-acid (glacial) containing one (1) drop of ferric chloride solution was used to treat 5 mL of extracts. When concentrated tetraoxosulphate (VI) acid was applied, the presence of glycosides was shown by a reddish-brown colouration at the middle of the two-layers. (Harborne, 1998).

3.8 Column chromatography of ethyl acetate fraction of *Calophyllum inophyllum*

Pod ethyl acetate fraction (20 g) from *Calophyllum inophyllum* (CiPdE) was pre-adsorbed on 30 g of silica-gel to form a homogenous solid and further subjected to column chromatographic purification (silica gel, 1400 g, 100-200 mesh size) using Hex (100%, 2000 mL); Hex : EtOAc (9:1, 1000 mL); (8:2, 1000 mL); (7:3, 1000 mL); (3:2, 1000 mL); (1:1, 1000 mL); (2:3, 1000 mL); (3:7, 1000 mL); (1:4, 1000 mL); (1:9, 1000 mL); (1:19, 1000 mL) EtOAc: MeOH (9:1, 1000 mL); (17:3, 1000 mL); (4:1, 1000 mL); (7:3, 1000 mL), (3:2, 1000 mL), and MeOH (100%, 1000 mL) separately. Seventy-two 72 chromatographic fractions (200 mL each) were collected and clustered to 6 sub-fractions (CiPdE 1 - CiPdE 6) based on the TLC analysis (Scheme 1). The micro-column chromatography (MCC) separation of CiPdE 2 (Rf = 0.6) and CiPdE 5 (Rf = 0.5) followed by recrystallization yielded four pure compounds each; a white crystalline solid CiPdE 1 (25 mg, R_f = 0.45 in 1:1 hexane:ethyl acetate), another white crystalline solid CiPdE 2 (16 mg, R_f = 0.4 in 1:1 hexane:ethyl acetate in 1:1 hexane:ethyl acetate), light yellow crystalline solid CiPdE 3 (25 mg, R_f = 0.5 in 1:1 hexane:ethyl acetate), and yellow solid CiPdE 4 (38 mg, R_f = 0.7 in 1:1 hexane:ethyl acetate).

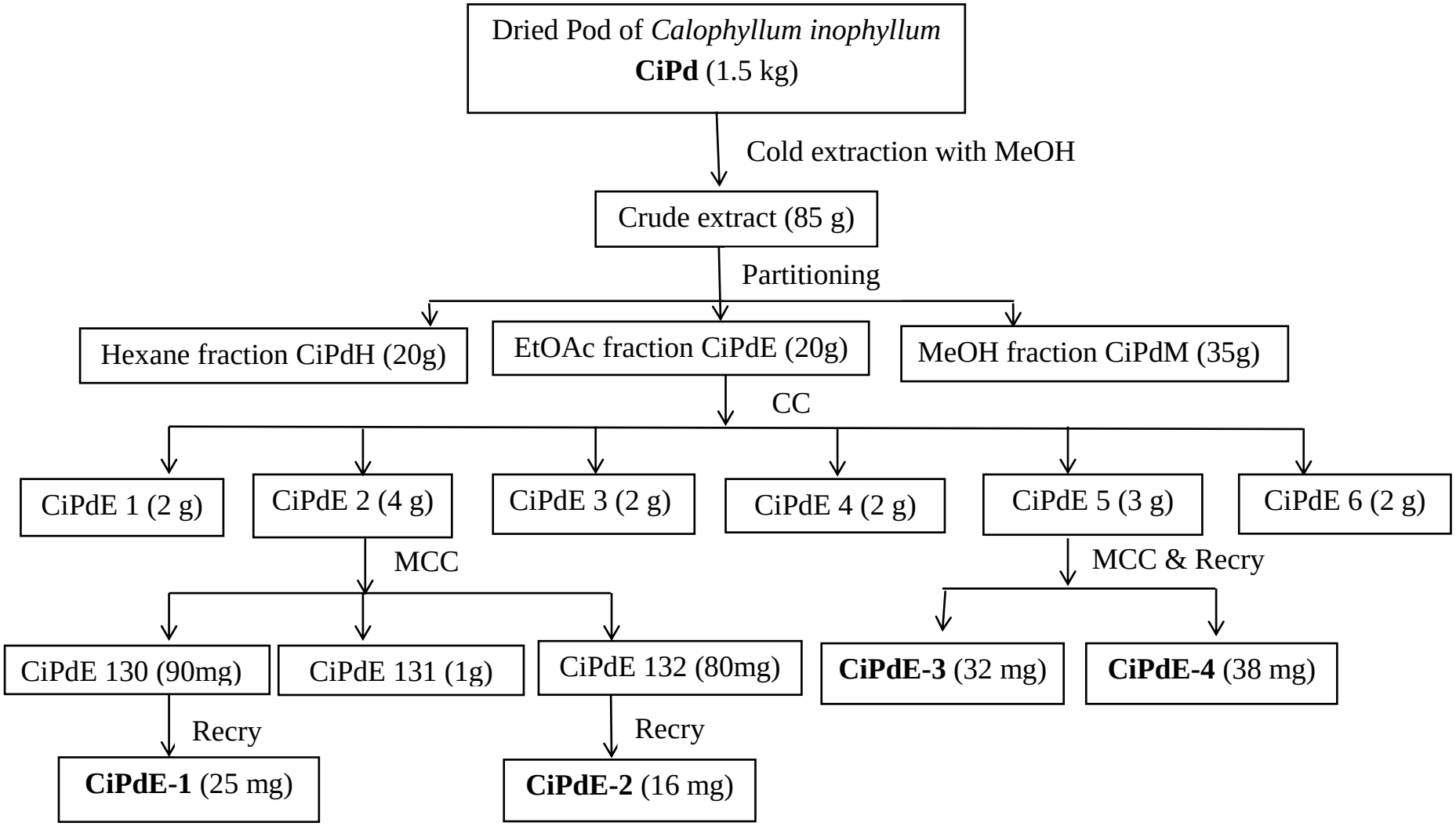
3.9 Column chromatography of ethyl acetate fraction of *Pterocarpus soyauxii*

Root bark ethyl acetate fraction (15 g) from *Pterocarpus soyauxii* (PsRbE) was pre-adsorbed on silica-gel (20g) to form a fine-solid which was then chromatographed by column chromatographic method (1000g, 100-200 mesh size-silical gel) using Hex (100%, 2000 mL); Hex : EtOAc (9:1, 1000 mL); (17:2, 1000 mL); (8:2, 1000 mL); (7:3, 1000 mL); (7:4, 1000 mL); (3:2, 1000 mL); (1:1, 1000 mL); (2:3, 1000 mL); (3:7, 1000 mL); (1:4, 1000 mL); (1:9, 1000 mL); (1:19, 1000 mL) EtOAc : MeOH (9:1, 1000 mL); (7:3, 1000 mL), (3:2, 1000 mL), and MeOH (100%, 1000 mL). A total of 142 eluents (200 mL each) were collected and pooled to 4 sub-fractions (PtRbE 1 to

PtRbE 4) on the basis of TLC analysis (Scheme 2). The sub-fractionation of PsRbE 1 and PsRbE 3 using micro-column chromatography (MCC) and subsequent recrystallization yielded four pure white crystalline solids; PsRbE-A (26 mg, Rf = 0.5 in 1:2 hexane:ethyl acetate), PsRbE-B (30 mg, R_f = 0.3 in 1:2 hexane:ethyl acetate), PsRbE-C (40 mg, R_f = 0.4 in 1:2 hexane:ethyl acetate), and PsRbE-D (40 mg, R_f = 0.6 1:2 hexane:ethyl acetate).

3.9.1 Characterisation of isolated compounds

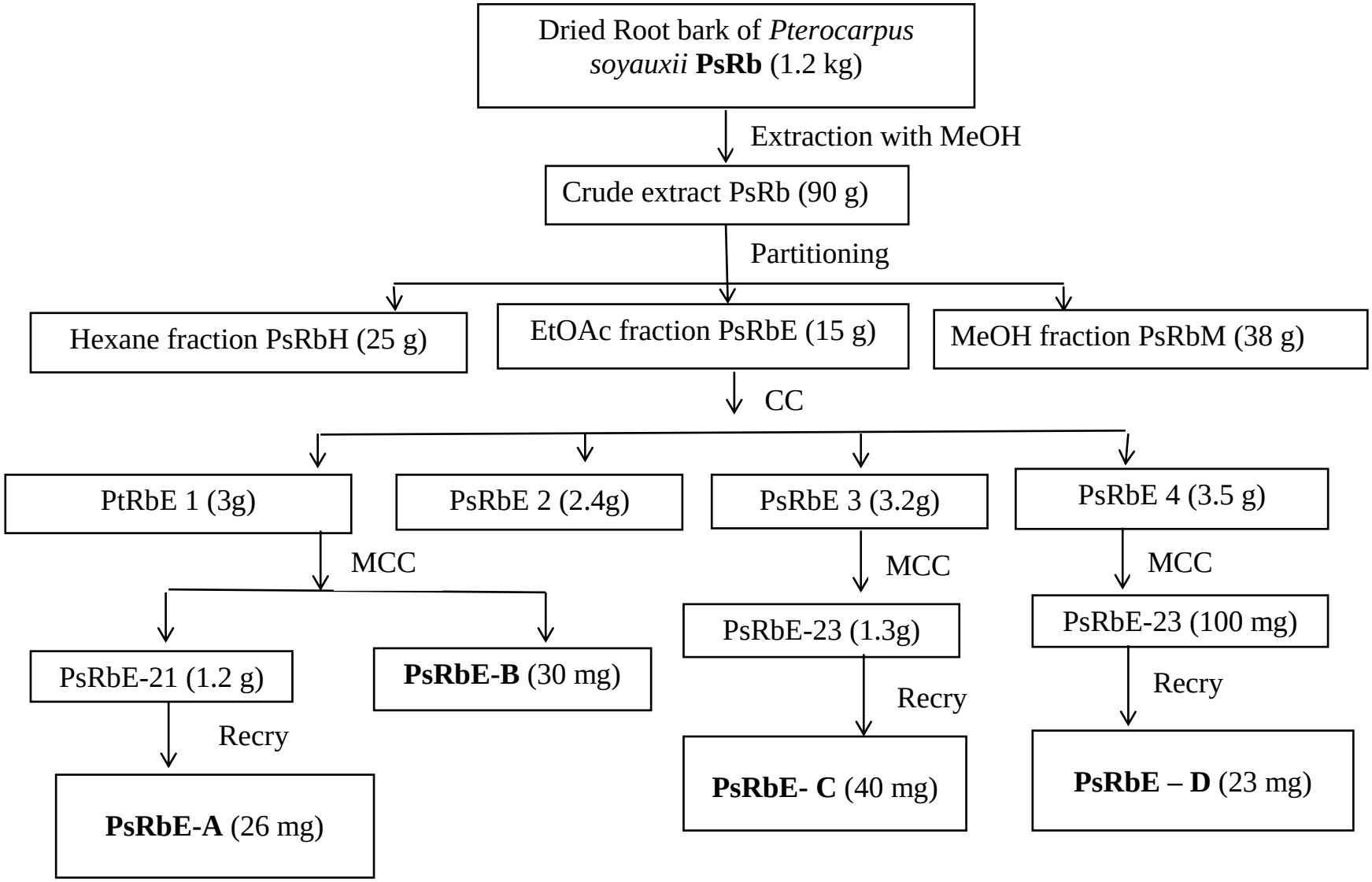
The pure compounds were characterized by subjecting them to full structural elucidation using several spectroscopic techniques in comparison with established literature data.



Key:

Column chromatography (CC), Micro-column chromatography (MCC), Recrystallization (Recry), Ethyl acetate (EtOAc), Methanol (MeOH)

Scheme 1: Isolation and extraction procedure from pod of *Calophyllum inophyllum*



Key:
Column chromatography (CC), Micro-column chromatography (MCC), Recrystallization (Recry), Ethyl acetate (EtOAc), Methanol (MeOH)

Scheme 2: Extraction and isolation procedure from root bark of *Pterocarpus soyauxii*

3.10 Biological Activities

3.10.1 Antidiabetic activity

3.10.1.1 Evaluation of inhibition activity (α-amylase)

Two hundred (200) µL of buffered sodium-phosphate (~0.03-Molar), 20.05 µL of enzyme-(~0.3 mg/mL), and the sample in concentrations ranging from 20 to 100 g/mL, was incubated for 15 minutes before being added to all test tubes with 200 µL of starch (5 mg/mL). By adding 400 mL DNS reagent and warming for 5 minutes, the reaction was stopped. After diluting with 15 mL of deionized water the UV was taken at 540 nm with spectrophotometer. The sample was not included in the control samples. The inhibition (percentage) was determined:

$$\text{Inhibition } (\%) = \frac{\textbf{Absorbance}_{\,(\text{control})} - \textbf{Absorbance}_{\,(\text{sample})}}{\textbf{Absorbance}_{(\text{control})}} \times 100$$

Where:

$\textbf{Absorbance}_{\,(Blank)}$ = Absorbance measurement of the control group.

$\textbf{Absorbance}_{\,(Sample)}$ = Absorbance measurement of sample.

The alpha amylase inhibitor assay was carried out three times in order to ensure reproducibility (Shaheen *et al.*, 2013; Davesh *et al.*, 2016).

3.10.1.2 Evaluation of yeast inhibitor activity (α-glucosidase)

The enzyme extract consisted of D-glucopyranoside-*p*-Nitrophenyl-acarbose and baker's-yeast dissolved in one hundred (100) mM of phosphate (PHS) buffer (pH 6.8). The substrate used was *p*-Nitrophenyl-D-glucopyranoside. At 30°C for 5 minutes, different concentrations of plant extracts were combined with three hundred and twenty (320) µL of one hundred (100) mM PHS buffer (pH = 6.8). Three (3) mL of fifty (50) mM NaOH was applied to the mixture and the absorbance measured (~410 nanometer) with a SHIMADZU ~UV-3101 PC spectrophotometer and 1 cm quartz cuvettes. There were no samples in the control group. The inhibition (%) was calculated thus:

$$\text{Inhibition } (\%) = \frac{\textbf{Absorbance}_{\,(\text{control})} - \textbf{Absorbance}_{(\text{sample})}}{\textbf{Absorbance}_{(\text{control})}} \times 100$$

Where:

$\mathbf{Absorbance}_{(Blank)}$ = Absorbance measurement of the control group.

$\mathbf{Absorbance}_{(Sample)}$ = Absorbance measurement of sample.

The IC_{50} were determined from the mean inhibitory values by non-linear regression analysis. (Davesh *et al.*, 2016).

3.10.2 Antioxidant activity of *Calophyllum inophyllum* and *Pterocarpus soyauxii*

The antioxidant potential of essential oils, crude extracts, fractions and isolates from *Calophyllum inophyllum* and *Pterocarpus soyauxii* were evaluated using standard procedures (Nabavi *et al.*, 2009).

3.10.2.1 Evaluation of DPPH antioxidant assay

Radical scavenging potency of essential oils/extracts/fractions/isolates from *Calophyllum inophyllum* and *Pterocarpus soyauxii* was determined using this method. As the radical is lowered to non-radicals by antioxidants, its dark purple colour fades, and the decrease in its absorbance is measured (517 nm). In order to obtain a concentration of 1 mg/mL, 2 mg of sample was dissolved in 2 mL of methanol. This stock solution was vortexed yielding sample solutions with concentrations varying from 1.0 mg/mL to 0.3125 mg/mL. The concentrations of standards and sample were prepared in triplicates. Usual positive controls included vitamin C (ascorbic acid) and butylated hydroxyl anisole (BHA). Three (3) mL of pure methanol solution containing DPPH was added to 0.5 mL of each triplicate's concentration (0.1M). The absorbance measurement was calculated at five hundred and seventeen nanometers (517 nm) against a blank (methanol DPPH) after 30 minutes of reaction time. In comparison to the blank the decrease in DPPH absorbance after adding test samples was used to measure the percentage inhibition (I %):

$$\mathbf{DPPH\ Inhibition}\ (\%) = \frac{\mathbf{Absorbance}_{(Blank)} - \mathbf{Absorbance}_{(sample)}}{\mathbf{Absorbance}_{(Blank)}} \times 100$$

Where:

$\mathbf{Absorbance}_{(Blank)}$ = Absorbance measurement of the blank

$\mathbf{Absorbance}_{(Sample)}$ = Absorbance measurement of sample.

(Davesh *et al.*, 2016).

3.10.2.2 Evaluation of Hydrogen peroxide (H_2O_2) scavenging activity

Samples from *Calophyllum inophyllum* and *Pterocarpus soyauxii* abilitiy to scavenge H_2O_2 radical was evaluated. In a solution of phosphate buffer at pH of 7.4, a 40 mM H_2O_2 solution was prepared. The concentration of hydrogen peroxide was determined using SHIMADZU ~UV-3101 PC spectrophotometer with quartz cuvettes. In a hydrogen peroxide solution, samples and standards dissolved in distilled water were added (0.6 mL, 40 mM). After thirty (30) minutes, the absorbance measurement of H_2O_2 was evaluated against a standard solution containing buffered phosphate. The absorbance (230 nm) of the reaction mixture was determined using Ascorbic acid and Butylated Hydroxyl Anisole as standards. The activity expressed in percentage of H_2O_2 scavenged was calculated as follows:

$$\% \, \mathbf{H_2O_2} = \frac{\mathbf{Abs}_{230\,(control)} - \mathbf{Abs}_{230\,(Sample)}}{\mathbf{Abs}_{230\,(control)}} \times 100$$

Where:

$\mathbf{Abs}_{230\,(Blank)}$ = Absorbance measurement of the blank at 230nm.

$\mathbf{Abs}_{230\,(sample)}$ = Absorbance measurement of samples at 230nm.

(Nabavi *et al.*, 2009).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Characterisation of extracted Volatile oil Constituents from *Calophyllum inophyllum* and *Pterocarpus soyauxii*

4.1.1 Characterisation of volatile oils constituents from *Calophyllum inophyllum*

Volatile oils from *Calophyllum inophyllum* gave characteristic odours (leafy, floral, nut-like, herbal and woody) and colours (pale yellow colour of leaf essential oil, white colour of seed essential oil, and pale-red colour of root parts). The variation in colour and odour is accrueable to the varieties of components in the essential oils (Table 4.1).

4.1.1.1 Yield of essential oils from *Calophyllum inophyllum*

Essential oils obtained from *C. inophyllum* gave 0.2 to 0.5% yields with fruit parts showing higher yield (0.6%), compared to root part with low yield (0.2%), which is traceable to its fiber composition (Table 4.1).

4.1.1.2 Chemical constitution of essential oils from *Calophyllum. Inophyllum*.

Essential oils extracted by hydrodistillation from leaf, leaf-stalk, flower, pod, pulp, stem and root parts of *C. inophyllum* were characterised by GC-MS resulting in 102 compounds (Appendix 1.1 to 1.10).

In the essential oil obtained from the leaf part, 71 compounds were characterised amounting to 54.9 percent of the essential oil; with prominent compounds being non-terpenes (27.9 percent), and sesquiterpenes (22.2 percent). The volatile oil in the leaf is mainly made up of hexadecanal (6.2 percent) and *cis*-cadina-1(6),4-diene (6.5 percent). Hexadecanal (6.2 percent) and *Cis*-cadina-1,4-diene (6.5 percent) are the most common compounds in leaf essential oil (5.4 percent) (Appendix 1.1).

Stalk of the leaf essential oil yielded twenty-two (22) constituents, accounting for 79.6 percent of the total, with monoterpenes dominating (75.6 percent). Monoterpenes like γ-terpinene (13.1 percent) and limonene (23.8 percent) are abundant in the essential

oil. The presence of phenyl-propanoids in the leaf can be responsible for the leaf's complex bioactivities. Twenty-five (25) compounds were identified in flower essential oil, accounting for 51.2 percent of the total. Sesquiterpenes make up more than half of all essential oils (32.9 percent). The existence of constituents such as β-alaskene (9.6 percent) and *cis*-cadina-1,4-diene (15.4 percent) may be responsible for the plant's effect notes (Appendix 1.2).

A total of twenty-five (25) constituents was found in the seed essential oil which makes up about 89.4%. γ-terpinene (14.0 percent) and limonene (25.4%) are the most abundant compounds in the seed essential oil. A total of 83.8 percent of identified essential oils are classified as monoterpenes (Appendix 1.3).

Pod essential oil is made up of sixty-nine (69) compounds which are responsible for 73.8 percent. The pod is very rich in anti-terpenes (~49 percent) and n-terpenes (25.0 percent). γ-Terpinene (9.8 percent) and limonene (16.9 percent) are predominant in the pod essential oil. Non-terpenes (48.8 percent) and monoterpenes (25.0 percent) are abundant in the pod. γ-Terpinene (9.8 percent) and Limonene (16.9 percent) are the most common constituents in pod essential oil. Sixty-nine (69) compounds were identified in the pod essential oil, accounting for 73.8 percent of the total. Non-terpenes (48.8 percent) and monoterpenes are abundant in the pod (25.0 percent). Limonene (16.9 percent), γ-terpinene (9.8 percent) and *p*-cymene (6.7 percent) are the most common constituents in pod essential oil (Appendix 1.4).

In pulp essential oil, fifteen (15) compounds were identified (46.1 percent). ‘As shown in Appx 4.5, it is entirely composed of sesquiterpenes (45.8 percent).

In stem wood essential oil, which accounts for 59.4 percent of the total, fifty-five compounds (55) were identified. Hexadecanal (6.9 percent) and *E*-nerolidol (5.9 percent) are the most common components in stem wood essential oil (5.6 %). As shown in Appx 4.7, the essential oil is high in monoterpenes (23.0 percent) and non-terpenes (28.1 percent). The colourless stem bark essential oil is made up of nine (9) compounds, accounting for 69.4% of the total. Hexadecanal (46.8 percent), *E*-anethole (6.1 percent) and limonene are all abundant in the essential oil (3.2 percent). Non-terpenes (60.9 percent) and monoterpenes (8.5 percent) dominated the essential oil, while sesquiterpenes were absent (Appendix 1.8).

There are fifty-one (51) compounds in root wood, accounting for 58.7% percent of the essential oil. Non-terpenes (45.8 percent) and sesquiterpenes (12.8 percent) predominate in this plant component, while monoterpenes are absent. Hexanedioc acid

(9.9 percent) and *E*-nerolidol (5.8 percent) (Appendix 1.9). The pale-yellow root bark essential oil contains twenty-four (24) compounds, which are strong sources of monoterpenes (44.0 percent), diterpenes (15.1 percent) and non-terpenes (14.5 percent). As shown in Appx 4.10, the essential oil contains high levels of 3Z-cembrene-A- (15.1 percent) and limonene (13.9 percent) (Appendix 1.10).

A unique compound in good amount eluted at 23.7 minutes showing a very similar fragmentation pattern to *cis*-thujopsene (Figure 4.1). This component is most likely the stereoisomer of *cis*-thujopsene. The mass spectrum of another notable compound, which was not named but was found in significant amounts in leaf essential oil (15.9 percent), at retention time 36.9 minutes.

The three (3) most common constituents in the twelve essential oils from the two plants are terpinene, cymene and ‘limonene. Cymene has been known as a powerful medicinal agent, and it was in a significant amount in eight of *Calophyllum inophyllum*'s essential oils. (Quintans-Junior *et al.,* 2013; DeOliveira *et al.,* 2015). A study revealed that the antimicrobial activity of *Carum copticum* essential oil was due to the appreciable dominance of cymene with terpinene. (Marino *et al.*, 1999). The large number of leaf stalk γ-terpinene (~13%), fruit pulp (~7%) and root (~8%) essential oils is credited to the plant's anti-inflammatory, anti-osteoarthritic, and antioxidant properties. Limonene has been shown to have medicinal effects in some plants and is present in large quantities in the essential oils of *Calophyllum inophyllum* stem (23.8%), stem-bark (3.2%), and root-bark (13.9%) (Table 4.2).

Table 4.1. Physicochemical properties of volatile oils from *C. inophyllum*

Parts	Percentage Yield	Odour	Colour
[a]Leaf	0.33	Leafy	Pale Yellow
[b]Leaf-stalk	0.31	Leafy	Colourless
[c]Flower	0.29	Pleasant	Colourless
[d]Seed	0.31	Pleasant	white
[e]Pod	0.51	Nut-like	Pale Red
[f]Pulp	0.56	Fruity	Pale Yellow
[g]Stem wood	0.34	Woody	Pale Yellow
[h]Stem Bark	0.31	Slightly choking	Colourless
[i]Root wood	0.22	Woody	Pale Red
[j]Root Bark	0.28	Nut-like	Pale Red

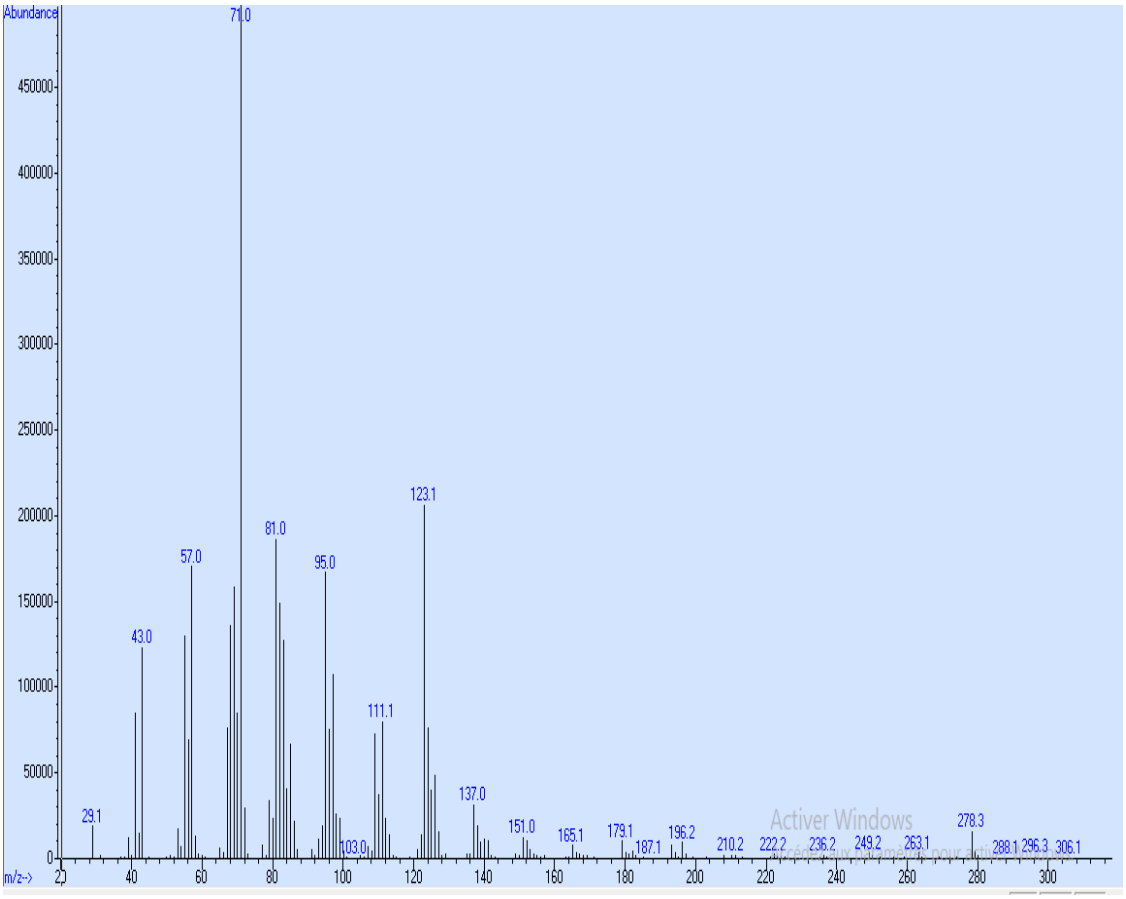


Fig. 4.1 Mass spectrum for non-identified compound with retention time 36.87 in leaf essential oil (15.85%) of *Calophyllum inophyllum*

Table 4.2 Chemical-content of essential oils from ten parts of *Calophyllum inophyllum*

S/N	RI	Compound	Class	[a]Leaf	[b]Leaf stalk	[c]Flower	[d]Seed	[e]Pod	[f]Pulp	[g]Stem wood	[h]Stem bark	[i]Root wood	[j]Root bark
1.	784	3-Hexanone[a]	Alkanone	0.13	0.05	-	-	4.76	-	0.14	1.72	0.17	0.07
2.	789	2-Hexanone[a]	Alkanone	0.50	0.07	-	-	3.21	-	1.00	1.85	0.82	0.12
3.	793	3-Hexanol[a]	Alkanol	0.09	-	-	-	3.16	-	0.12	-	0.11	-
4.	800	Hexanal[a]	Alkanal	0.92	0.42	0.06	0.67	3.82	-	0.84	-	1.24	0.16
5.	844	(*E*)-2-Hexenal[a]	Alkanal	1.05	-	-	-	-	-	0.06	-	0.10	-
6.	846	3-Hexen-1-ol[a]	Alkanol	0.23	-	-	-	-	-	-	-	-	-
7.	857	(*E*)-2-Hexen-1-ol[a]	Alkanol	0.99	-	-	-	-	-	-	-	-	-
8.	859	n-Hexanol[a]	Alkanol	3.33	-	-	-	-	-	0.14	-	0.09	
9.	923	Acetonyl acetone[a]	Alkanone	0.34	-	-	-	-	-	2.71		0.71	-
10.	926	α-Thujene[b]	MT	-	1.96	-	2.34	-	-	0.35	-	-	1.09
11.	932	**α-Pinene**[b]	MT	0.21	**7.88**	0.07	**9.39**	-	-	1.28	-	0.02	**4.44**
12.	945	Acetoxyhexane[a]	Ether	-	-	-	-	4.80	-	-	-	-	-
13.	947	Camphene[b]	MT	-	0.62	-	0.70	-	-	-	-	-	0.39
14.	958	Benzaldehyde[a]	Aldehyde	0.09	-	-	-	-			-	-	0.5
15.	969	Sabinene[b]	MT	-	0.72	-	0.93	-	-	-	-	-	-
16.	975	**β-Pinene**[b]	MT	0.11	**4.41**	-	**5.13**	-	-	0.73	-	-	2.54
17.	978	Octen-3-ol[a]	Alkanol	0.04	-	-	-	-	-	0.54	-	0.25	-
18.	987	6methyl-5-Hepten-2-one[a]	Alkanone	0.10	-	-	-	-	-	0.11	-	0.17	-
19.	991	Myrcene[b]	MT	0.30	2.81	-	3.21	-	-	1.19	-	-	1.67

S/N	RI	Compound	Class	[a]Leaf	[b]Leaf stalk	[c]Flower	[d]Seed	[e]Pod	[f]Pulp	[g]Stem wood	[h]Stem bark	[i]Root wood	[j]Root bark
20.	1002	*trans*-2-(2-Pentenyl) furan[a]	Aromatic	0.05	-	-	-	-	-	-	-	-	-
21.	1004	α-Phellandrene[b]	MT	-	0.27	-	0.32	-	-	0.26	-	-	-
22.	1010	δ-3-Carene[b]	MT	0.08	0.36	-	0.41	-	-	0.13	-	0.21	-
23.	1016	α-Terpinene[b]	MT	0.09	2.40	-	2.54	-	-	0.39	-	-	1.41
24.	1024	***p*-Cymene**[b]	MT	0.34	**9.28**	0.06	**10.03**	**8.50**	-	1.42	2.29	-	**5.39**
25.	1028	**Limonene**[b]	MT	0.75	**23.79**	0.18	**25.4**	**9.71**	-	3.47	3.24	-	**13.93**
26.	1030	**1,8-Cineole**[d]	Oxygenated MT	0.30	**5.33**	-	**5.69**	-	-	**5.63**	-	0.39	3.54
27.	1034	2,2,6-trimethyl Cyclohexanone,	Alkanone	0.05	-	-			-	-	-	-	-
28.	1039	β-Ocimene[b]	MT	-	0.56	-	0.65	-	-	0.11	-	-	0.36
29.	1043	Benzeneacetaldehyde	Aldehyde	0.08	-	-	-	-	-	-	-	-	-
30.	1049	(*E*)- β-Ocimene[b]	MT		0.84	-	0.90	-	-	0.15		-	0.51
31.	1058	**γ-Terpinene**[b]	MT	0.56	**13.06**	0.10	**14.00**	**6.77**	-	2.14	2.95	0.27	**7.75**
32.	1065	Acetophenone[a]	Alkanone	0.61	-	-	-	-		0.43		0.21	-
33.	1071	1-Octanol[a]	Alkanol	-	-	-	-	-	-	-		0.31	-
34.	1087	Terpinolene[b]	MT	-	0.70	-	0.84	-	-	-		-	0.53
35.	1100	Linalool[d]	Oxygenated MT	0.12	0.63	-	0.62	-	-	2.62		1.96	0.46
36.	1105	Nonanal[a]	Alkanal	0.31	-	-	-	-	-	-		1.79	-
37.	1116	α-Cyclo-citral	MT	0.04	-	-	-	-	-	-		-	-
38.	1134	2,2,6-trimethyl-Cyclohexanone[a]	Alkanone	-	-	-	-	-	-	0.03		0.02	-
39.	1143	Camphor[d]	Oxygenated MT	-	-	-	0.29	-	-	0.07		-	-

S/N	RI	Compound	Class	[a]Leaf	[b]Leaf stalk	[c]Flower	[d]Seed	[e]Pod	[f]Pulp	[g]Stem wood	[h]Stem bark	[i]Root wood	[j]Root bark
40.	1160	(*E*)-2-Nonenal[a]	Alkanal	0.08	-	-	-	-	-	0.33		0.69	-
41.	1176	Terpinen-4-ol[d]	Oxygenated MT	-	-	-	-	-	-	1.34		0.24	-
42.	1189	α-Terpineol[d]	Oxygenated MT	-	-	-	-	-	-	1.48		1.30	-
43.	1193	Methyl salicylate[a]	Aromatic	0.08	-	-	-	-	-	-		-	-
44.	1195	Myrtenol[a]	Alkanol	-	-	-	-	-	-	-		0.64	-
45.	1197	Methyl chavicol[a]	phenylpropanoid	-	0.59	-	0.73	-	-	0.09		-	-
46.	1198	Safranal[a]	Alkanal	0.10	-	-	-	-	-	-		-	0.06
47.	1206	Decanal[a]	Alkanal	0.09	-	-	-	-	-	0.46		0.73	-
48.	1220	β-Cyclo-citral[a]	MT	0.26	-	-	-	-	-	-		-	-
49.	1238	Ascaridole[d]	Oxygenated MT	-	-	-	-	-	-	0.08		-	-
50.	1258	Edulan II[a]	Ether	1.05	-	-	-	-	-	1.07		0.99	-
51.	1262	2-Decenal[a]	Alkanal	0.11	-	-	-	-	-	0.09		0.08	-
52.	1271	α-Citral[a]	Alkanal	0.04	-	-	-	-	-	0.12		0.14	-
53.	1285	***E* Anethole**[a]	Ether	0.13	2.80	-	3.66	**25.45**	-	0.87	**6.12**	-	2.04
54.	1293	(*E, Z*)-2,4-decadienal[a]	Alkanal	-	-	-	-	-	-	-		0.55	-
55.	1301	Carvacrol[d]	Oxygenated MT	-	-	-	0.42	-	-	0.12		-	-
56.	1314	Edulan I[a]	Ether	1.69	-	-	-	-	-	-		-	-
57.	1316	(*E, E*)-2,4-decadienal[a]	Alkanal	-	-	-	-	-	-	-		1.34	-
58.	1350	α-Cubebene[e]	ST		-	0.07	-	-	-	-		-	-
59.	1352	1,2'-di(hydro-1,1.6-trimethyl)naphthalene'[a]	Aromatic	0.14	-	-	-	-	-	-		-	-
60.	1363	2-Undecenal[a]	Alkanal	0.07	-	-	-	-	-	-		-	-

S/N	RI	Compound	Class	[a]Leaf	[b]Leaf stalk	[c]Flower	[d]Seed	[e]Pod	[f]Pulp	[g]Stem wood	[h]Stem bark	[i]Root wood	[j]Root bark
61.	1367	Cyclosativene[e]	ST	0.04	-	-	-	-	-	-		-	-
62.	1376	Copaene[e]	ST	0.17	-	0.31	-	-	0.40	0.10		0.15	-
63.	1382	(3*Z*)-3-Hexenyl hexanoate[a]	Ester	0.05	-	-	-	-	-	-		-	-
64.	1384	β-Bourbonene[e]	ST	0.06	-	0.27	-	-	-	-		-	-
65.	1387	n-Hexyl hexanoate[a]	Ester	0.77	-	-	-	-	-	-		-	-
66.	1391	7-*epi*-Sesquithujene[e]	ST	0.47	-	0.58			0.40	-		-	-
67.	1399	Cyperene[e]	ST		-	-	-	-	-	-		-	1.14
68.	1400	Tetradecane[a]	Alkane	0.10	-	-	-	-	-	-		4.19	-
69.	1405	Methyl eugenol[a]	phenylpropanoid	0.03	-	-	-	-	-	0.05		0.10	-
70.	1413	β-Cedrene[e]	ST	0.21	-	0.69	-	-	0.80	-		-	-
71.	1424	β-Copaene[e]	ST	-	-	-	-	-	0.20	-		-	-
72.	1428	α-Ionone[a]	Ester	0.44	-	-	-	-	-	-		-	-
73.	1454	6,10-dimethyl5,9-Undecadien-2-one[a]	Alkanone	0.89	-	0.09	-	-	-	0.39		0.97	-
74.	1458	β-Farnesene	ST	0.15	-	-	-	-	0.3	-		-	-
75.	1463	***cis*-Cadina 1,6 4 diene**[e]	ST	**6.50**	-	**15.42**	0.37	-	**15.60**	0.45		0.84	-
76.	1467	**β-Acoradiene**[e]	ST	2.54	-	**5.72**	0.15	-	**5.60**	0.25		-	-
77.	1477	γ-Muurolene[e]	ST	0.34	-	0.22	-	-	0.70	0.15		1.11	-
78.	1481	Germacrene[e]	ST	0.20	-	3.74	-	-	1.30	-		-	-
79.	1486	(*E*)-β-Ionone[a]	Nor-isoprenoid	1.94	-	-	-	-	-	0.07	-	1.31	
80.	1496	**β-Alaskene**[e]	ST	2.73	-	**9.63**	-	-	**8.40**	0.44	-	1.56	

S/N	RI	Compound	Class	[a]Leaf	[b]Leaf stalk	[c]Flower	[d]Seed	[e]Pod	[f]Pulp	[g]Stem wood	[h]Stem bark	[i]Root wood	[j]Root bark
81.	1510	β-Bisabolene[e]	ST	0.16	-	0.28	-	-	-	-	-	-	-
82.	1515	γ -Bisabolene[e]	ST	-	-	7.20	-	-	4.70	-	-	-	-
83.	1516	(*Z*)-γ-Bisabolene[e]	ST	2.01	-	-	-	-	-	-	-	1.22	-
84.	1523	δ-Cadinene[e]	ST	1.21	-	0.87			2.00	0.27	-	0.82	-
85.	1533	***cis*-Calamenene**[e]	ST	**5.41**	-	1.88	-	-	**5.70**	0.65	-	**5.83**	-
86.	1564	***E*-Nerolidol**[e]	ST	-	-	-	-	-	-	**5.87**	-	-	
87.	1579	(3*E'*,7*E'*)-4,8,12-Trimethyltrideca-1',3',7,11-tetraene[a]	Alkane	0.20	-	-	-	-	-	-	-	0.92	-
88.	1659	Neointermedeol[a]	Alcohol	-	-	-	-	-	-	-	-	1.48	-
89.	1681	(*Z*)-3-Heptadecene[a]	Alkane	0.77	-	-	-	-	-	-	-	-	-
90.	1688	α-Bisabolol[a]	Alcohol	0.28	-	0.50	-	-	-	-	-	4.36	-
91.	1817	**Hexadecanal**[a]	Alkanal	**6.16**		2.54	-	3.62	-	**6.87**	**46.80**		**10.61**
92.	1847	hexahydrofarnesyl acetone[a]	polyacetylene	0.78	-	-	-	-	-	0.19	-	0.73	-
93.	1972	Cembrene A 3Z[a]	Diterpene	-	-	-	-	-	-	-	-	-	15.05
94.	1880	1-Hexadecanol[a]	Alkanol	1.54	-	0.12	-	-	-	5.40	4.41	3.04	-
95.	1921	Farnesyl acetone[a]	Alkanone	0.63	-	-	-	-	-	-	-	1.12	-
96.	1973	n-Hexadecanoic acid[a]	Alkanoic acid	-	-	-	-	-	-	-	-	**9.86**	-
97.	1995	9-Octadecenal[a]	Alkanal	1.36	-	0.20	-	-	-	2.96	-	0.67	-
98.	2084	n-Octadecanol[a]	Alkanol										0.90
99.	2085	2-Octadecen-1-ol[a]	Alkanol	1.21	-	0.48	-	-	-	2.73	-	0.87	-
100.	2495	Pentacosane[a]	Alkane	-	-	-	-	-	-	-	-	1.08	-

S/N	RI	Compound	Class	[a]Leaf	[b]Leaf stalk	[c]Flower	[d]Seed	[e]Pod	[f]Pulp	[g]Stem wood	[h]Stem bark	[i]Root wood	[j]Root bark
101.	2599	Hexacosane[a]	Alkane	-	-	-	-	-	-	0.24	-	0.67	-
102.	2900	Nonacosane[a]	Alkane	-	-	-	-	-	-	0.23	-	0.32	-
		No. of Compounds		71	22	25	25	69	15	55	09	51	24
		Total		**54.94**	**79.55**	**51.24**	**89.39**	**73.80**	**46.10**	**59.40**	**69.38**	**58.73**	**74.66**

RI: retention index. **MT** = Monoterpene **SQ** = Sesquiterpene

Table 4.3. Classes of compounds in Essential oils from *Calophyllum. inophyllum*

S/N'	Class of compounds	[a]Leaf	[b]Leaf-stalk	[c]Flower	[d]Seed	[e]Pod	[f]Pulp	[g]Stem wood	[h]Stem bark	[i]Root wood	[j]Root bark
1	MT	3.16	75.62	0.41	83.81	24.98	-	22.96	8.48	-	44.01
2	ST	22.18	-	32.87	0.52	-	45.80	8.24	-	12.83	1.14
3	Diterpenes	-	-	-	-	-	-	-	-	-	15.05
4	Nor-isoprenoids	1.68	-	-	-	-	-	-	-	-	-
5	Phenylpropanoids	0.03	0.59	-	0.73	-	-	0.09	-	0.10	-
6	Non-terpenes	27.89	3.34	17.96	4.33	48.82	0.30	28.11	60.90	45.80	14.46
	Total	**54.94**	**79.55**	**51.24**	**89.39**	**73.80**	**46.10**	**59.40**	**69.38**	**58.73**	**74.66**

MT = Monoterpene **SQ** = Sesquiterpene

4.1.2 Characterisation of volatile oil constituents from *Pterocarpus soyauxii*

Volatile oils from root parts of *Pterocarpus soyauxii* gave characteristic woody and herbal odours. The essential oils were colourless in root wood and pale yellow for root bark essential oil. (Table 4.4).

4.1.2.1 Percentage yield of volatile oils from *Pterocarpus soyauxii*

Volatile oils from *Pterocarpus soyauxii* gave and yields of root wood (0.24 %) and root bark (0.21 %) essential oil respectively. The percentage yield of root wood' essential oil was higher than root bark essential oil, which may be attributed to the root wood's high fiber content, as shown in Table 4.4.

4.1.2.2 Chemical composition of volatile oils from *Pterocarpus soyauxii*

Essential oils from *Pterocarpus soyauxii* gave 95 characterised chemical constituents (Appendix 1.11 and 1.12). A total of sixty-five (65) chemical constituents were identified in root wood essential oil. This accounted for 81.72% of total identified compounds which was rich in monoterpenes (57.01%) and sesquiterpenes (15.6%). Predominant compounds in root essential oil are γ-terpinene (9.8%) and limonene (16.9%). The essential oils also contain *E*-anethole, methyl chavicol and methyl eugenol (phenyl-propanoids) while nor-isoprenoids were absent in the essential oil. Root bark oil contains 47 identified compounds which constitute about 67.5% which are more of non-terpenes (46.9%) and nor-isoprenoids (10.7%). The root bark is a rich source of phytol (6.6%), hexanol (6.3%), dihydroedulan (6.2%), methyl salicylate (4.5%) and α-ionone (4.5%). (Tables 4.5 and 4.6)

Table 4.4. Physicochemical properties of essential oils from *Pterocarpus soyauxii*

Plant parts	% Yield	Odour	Colour
Root	0.24	Woody	Colourless
Root Bark	0.21	Herbal	Pale Yellow

Table 4.5. Chemical constituents of volatile oils from root parts of *Pterocarpus soyauxii*

S/N.	RI	Compound	Class	% Composition	
				[a]Root wood	[b]Root bark
1.	784	3-Hexanone[a]	Alkanone	0.06	0.15
2.	789	2-Hexanone[a]	Alkanone	0.06	0.44
3.	793	3-Hexanol[a]	Alkanol	0.04	0.10
4.	800	Hexanal[a]	Alkanal	0.17	3.81
5.	844	(*E*)-2-Hexenal[a]	Alkanal	-	3.86
6.	854	Ethylbenzene[a]	Alkane	0.02	-
7.	857	(*E*)-2-Hexen-1-ol[a]	Alkanol	-	0.65
8.	859	n-Hexanol [a]	Alkanol	-	6.28
9.	863	*p*-Xylene	Alkane	-	1.06
10	923	Acetonyl acetone[a]	Alkanone	-	0.32
11	926	α-Thujene[b]	MT	1.43	-
12	932	α-Pinene[b]	MT	5.57	0.25
13	947	Camphene[b]	MT	0.49	-
14	958	Benzaldehyde	Alkanal	-	0.20
15	972	Sabinene[b]	MT	0.63	
16	975	β-Pinene[b]	MT	3.25	-
17	978	Octen-3-ol	Alkanal	-	0.39
18	987	6-methyl-5-Hepten-2one[a]	Alkanone	-	0.81
19	991	Myrcene[b]	MT	2.21	1.55
20	1002	*trans*-2-(2-Pentenyl)furan[a]	Alkane	-	0.11
21	1004	α-Phellandrene[b]	MT	0.24	-
22	1010	δ-3-Carene[b]	MT	0.28	0.09
23	1016	α-Terpinene[b]	MT	1.77	-
24	1024	*p*-Cymene[b]	MT	6.70	-
25	1028	**Limonene**[b]	MT	**16.85**	-
26	1030	1,8-Cineole[c]	Oxygenated MT	3.74	0.81
27	1034	2,2,6-trimethyl Cyclohexanone[a]	Alkanone	-	0.17
28	1039	β-Ocimene[b]	MT	0.47	-
29	1043	Benzeneacetaldehyde[a]	Alkanal	-	0.21
30	1049	(*E*)- β-Ocimene[b]	MT	0.71	-
31	1058	**γ-Terpinene**[b]	MT	**9.82**	0.24
32	1062	Artemisia ketone[a]	Alkanone	0.09	-
33	1065	Acetophenone[a]	Alkanone	-	-

S/N	RI	Compound		% Composition	
				[a]Root wood	[b]Root bark
34	1072	*cis*-Linalol oxide[a]	Alkanol	0.05	
35	1087	Terpinolene[B]	MT	0.57	-
36	1100	Linalool[a]	Oxygenated MT	0.54	0.55
37	1105	Nonanal[a]	Alkanal	0.11	0.18
38	1143	Camphor[c]	Oxygenated MT	0.22	-
39	1166	1,3-dimethoxy- Benzene	Alkane	-	1.27
40	1176	Terpinen-4-ol[c]	Oxygenated MT	0.28	-
41	1179	Naphthalene[a]	Alkane	-	2.69
42	1189	α-Terpineol[c]	Oxygenated MT	0.14	-
43	1193	Methyl salicylate[a]	Phenylpropanoid	-	4.48
44	1197	Methyl chavicol[a]	Phenylpropanoid	0.77	-
45	1206	Decanal[a]	Alkanal	-	-
46	1220	β-Cyclo-citral[a]	norisoprenoids	-	0.66
47	1235	Thymol methyl ether[a]	Ether	0.16	
48	1238	Ascaridole[a]	norisoprenoids	0.17	-
49	1244	Carvacrol-methyl ether[a]	Ether	0.10	
50	1257	Linalool acetate[a]	Oxygenated MT	0.14	-
51	1285	*E*-Anethole[a]	Phenylpropanoid	4.39	-
52	1287	Dihydroedulan	Oxygenated MT	-	6.19
53	1301	Carvacrol[c]	Oxygenated MT	0.48	-
54	1352	1,2'-dihydro-1,1,6'-trimethyl Naphthalene[a]	Alkane	-	0.19
55	1376	α-Copaene[d]	ST	0.06	-
56	1385	β-Bourbonene[d]	ST	0.04	
57	1391	7-*epi*-Sesquithujene[d]	ST	0.08	-
58	1400	Tetradecane[a]	Alkane	0.08	0.44
59	1405	Methyl eugenol[d]	Phenylpropanoid	0.09	-
60	1413	β-Funebrene[d]	ST	0.08	-
61	1428	αIonone[d]	Esther	-	4.48
62	1454	6,10'dimethyl.5,9'Undecadien-2-one[a]	Alkanone	0.05	2.02
63	1458	β-Farnesene[d]	ST	0.03	-
64	1463	*cis*-Cadina 1,6 4 diene[d]	ST	2.92	-
65	1467	β-Acoradiene[d]	ST	1.23	-
66	1477	γ-Muurolene[d]	ST	0.27	0.07
67	1481	Germacrene[d]	Sesquiterpene	0.15	-
68	1486	(*E*)-β-Ionone[d]	norisoprenoids	-	3.38

S/N	RI	Compound		% Composition	
				[a]Root wood	[b]Root bark
69	1496	*β*-Alaskene[d]	ST’	2.41	-
70	1501	*α*-Muurolene[d]	ST’	0.10	-
71	1509	*β*-Bisabolene[d]	ST’	0.06	0.19
72	1516	*γ*-Bisabolene[d]	ST’	1.31	-
73	1524	*δ*-Cadinene[d]	ST’	1.17	-
74	1534	*cis*-Calamenene[d]	ST’	3.58	
75	1544	*α*-Calacorene[d]	ST’	0.13	
76	1553	Cadala-1(10),3,8-triene[d]	ST’	0.15	
77	1616	*β*Copaen-4α-ol[d]	ST	0.82	
78	1668	Eudesma-4(15),7-dien-1β -ol[a]	Alkanol	1.14	
79	1682	(*Z*)-3-Heptadecene[a]	Alkane	-	1.27
80	1716	Pentadecanal[a]	Alkanal	-	2.43
81	1800	Octadecane[a]	Alkane	0.13	0.19
82	1818	Hexadecanal[a]	Alkane	1.03	0.24
83	1848	hexahydrofarnesyl acetone[a]	Alkanone	-	0.68
84	1881	1-Hexadecanol[a]	Alkanol	0.19	-
85	1922	Farnesyl acetone[a]	Alkanol	-	0.82
86	1927	Palmitic acid, methyl ester[a]	Ester	0.36	0.32
87	2086	Octadecanal[a]	Alkanal	0.55	0.20
88	2154	Phytol[a]	Diterpene	-	6.62
89	2190	Hexadecane[a]	Alkane	0.43	-
90	2200	Docosane[a]	Alkane	0.19	1.81
91	2324	Tricosane[a]	Alkane	-	1.08
92	2496	Pentacosane[a]	Alkane	-	1.64
93	2599	Hexacosane[a]	Alkane	0.17	1.31
94	2900	Nonacosane[a]	Alkane	-	0.57
		No. of Compounds		**65**	**47**
Total Yield				**81.72**	**67.47**

RI: retention index. **MT** = Monoterpene **SQ** = Sesquiterpene

Table 4.6 Classes of compounds in essential oils from *Pterocarpus soyauxii*

S/N	Class of Compound	% Composition	
		[a]Root wood	[b]Root bark
1.	MT	57.01	3.49
2.	ST	15.65	0.26
3.	Diterpenes	-	6.62
4.	Norisoprenoids	-	10.7
5.	Phenylpropanoids	5.25	-
6.	Non-terpenes	3.81	46.87
	Total (%)	**81.72**	**67.47**

MT = Monoterpene **SQ** = Sesquiterpene

4.2. Percentage yields and Qualitative phytochemical screening of extracts and fractions from *Calophyllum inophyllum* and *Pterocarpus soyauxii*

4.2.1 Percentage yields of pod extract and fractions from *Calophyllum inophyllum*

A total of 1.5 kg of pulverized pods of *Calophyllum inophyllum* was extracted and yielded 5.7% methanol extract. Fractionation of 85 grams of the pod methanol extract gave percentage yields (%) of 23.5, 23.5, and 41.2 for hexane, ethyl-acetate, and methanol fractions respectively as indicated on Table 4.7

4.2.2 Percentage yields of root bark fractionation of *Pterocarpus soyauxii*

A total of 1.2 kg of pulverized root bark of *Pterocarpus soyauxii* was extracted and yielded 7.5% methanol extract. Fractionation of 90 gram of the methanol extract gave percentage yields (%) of 27.7, 16.7, and 42.2 for hexane, ethyl acetate and methanol fractions respectively as indicated on Table 4.8.

4.2.3 Phytochemical screening of extracts and fractions from *Calophyllum inophyllum* and *Pterocarpus soyauxii*

4.2.3.1 Phytochemical screening of extracts and fractions from *Calophyllum inophyllum*

Phytochemical screening of crude methanol extracts from the leaf, leaf-stalk, flower, seed, pod, pulp, stem wood, stem-bark, root wood and root bark parts of *Calophyllum inophyllum* were carried out using conventional methods (Soforowa, 1993; Harborne, 1998; Edeoga *et al.*, 2005).

Saponins, glycosides, phenolics, anthraquinones, terpenoids, and flavonoids were present in all extracts. The phytochemicals identified corroborates earlier phytochemicals reported in literature (Pengsuparp *et al.*, 1996; Hang *et al.*, 2006; Li *et al.*, 2007). Studies reveal that plants rich in phenolics, terpenoids and flavonoids are good antioxidant and antidiabetic agents (Marie *et al.*, 2004; Janki *et al.*, 2012). The presence of these classes of phytochemicals in *Calophyllum inophyllum* could be responsible for the good antioxidant and antidiabetic activity of the plant.

All extracts had resins except flower and seed extracts while steroids were not detected in all extracts except seed methanol. Carbohydrates, reducing sugar and alkaloids were absent in all extracts (Table 4.9).

Phytochemical screening on the hexane fraction (CiPdH) revealed that tannins/phenolics, anthraquinones, resins, steroids, and terpenoids were present while carbohydrates, reducing sugar, and alkaloids were absent in all extracts. Ethyl acetate fraction (CiPdE) had saponins, tannins/phenolics, anthraquinones, steroids, terpenoids and flavonoids while resins, carbohydrates, reducing sugar, glycosides and alkaloids were absent. Methanol-fraction (CiPdM), revealed tannins/phenolics, saponins, glycosides, and anthraquinones were present while resins, carbohydrates, reducing sugar, steroids, terpenoids, and alkaloids were absent (Table 4.10). The selective presence of flavonoids in the ethyl acetate fraction compared to the hexane and methanol fraction could be responsible for the higher antioxidant and antidiabetic activity observed in this fraction. Some studies have shown that plants with high antioxidant and antidiabetic activity could be linked to the presence of flavonoids (Marie *et al.*, 2004; Janki *et al.*, 2012).

Table 4.7: Percentage yields obtained from Pod fractionation of *Calophyllum inophyllum*

Sample weight (g)	Methanol Extract (g)	% Yield	n-Hex fraction (g)	% Yield	EtOAc fraction (g)	% Yield	MeOH fraction (g)	% Yield
1500.0	85	5.7	20.0	23.5	20.0	23.5	35	41.2

Hex: Hexane, EtOAc: Ethyl Acetate, MeOH: Methanol

Table 4.8: Percentage yields obtained from root bark extraction and fractionation of *Pterocarpus soyauxii*

Sample weight (g)	Methanol Extract (g)	% Yield	n-Hex fraction (g)	% Yield	EtOAc fraction (g)	% Yield	MeOH fraction (g)	% Yield
1200.0	90	7.5	25.0	27.7	15.0	16.7	38	42.2

Hex: Hexane, EtOAc: Ethyl Acetate, MeOH: Methanol

Table 4.9 Phytochemical screening of ten (10) methanol extracts from *Calophyllum inophyllum*

Metabolites	**CiLf[a]**	**CiLs[b]**	**CiFl[c]**	**CiSd[d]**	**CiPd[e]**	**CiPp[f]**	**CiSw[g]**	**CiSb[h]**	**CiRw[i]**	**CiRb[j]**
Saponins	+	+	+	+	+	+	+	+	+	+
Tannins/Phenolics	+	+	+	+	+	+	+	+	+	+
Anthraquinones	+	+	+	+	+	+	+	+	+	+
Resins	+	+	-	-	+	+	+	+	+	+
Carbohydrates	-	-	-	-	-	-	-	-	-	-
Reducing sugar	-	-	-	-	-	-	-	-	-	-
Glycosides	+	+	+	+	+	+	+	+	+	+
steroids	+	+	+	-	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+	+	+	+

- = Not detected + = Detected
- **Key:** Ci = *Calophyllum inophyllum*, Lf = Leaf, Ls = Leaf stalk, Fl = Flower, Sd = seed, Pd = Pod, Pp = Pulp, Sw = Stem-wood, Sb = Stem-bark, Rw = Root-wood, Rb = Root-bark

Table 4.10 Phytochemical screening of pod methanol extract and fractions from *Calophyllum inophyllum*

Metabolites	**Crude Extract**	**CiPdH**	**CiPdE**	**CiPdM**
Saponins	+	-	+	+
Tannins/Phenolics	+	+	+	+
Anthraquinones	+	+	+	+
Resins	+	+	-	-
Carbohydrates	-	-	-	-
Reducing sugar	-	-	-	-
Glycosides	+	-	+	+
steroids	+	+	+	-
Terpenoids	+	+	+	-
Alkaloids	-	-	-	-
Flavonoids	+	-	+	-

- = Not detected + = Detected

Key: CiPdH: *Calophyllum inophyllum* Pod hexane fraction

CiPdE: *Calophyllum inophyllum* Pod ethyl acetate fraction

CiPdM: *Calophyllum inophyllum* Pod methanol fraction

4.2.3.2 Phytochemical screening of extracts and fractions from *Pterocarpus soyauxii*

Phytochemical screening of crude methanol extracts from leaf, stem and root parts from *Pterocarpus soyauxii* were determined using conventional methods. Saponins, phenolics, anthraquinones, carbohydrates, steroids, terpenoids and flavonoids were in all extracts. The classes of phytochemicals present in *Pterocarpus soyauxii* are similar to phytochemicals present in *Calophyllum inophyllum.* This result corroborates the good antioxidant and antidiabetic activities expressed by both plants. Resins were present in all extracts except the root bark part while glycosides were present in all extracts. Reducing sugar, and alkaloids were absent in all extracts (Table 4.11).

Phytochemical screening on the hexane fraction (PsRbH) showed the presence of tannins/phenolics, anthraquinones, carbohydrates, reducing sugar, steroids, terpenoids, and flavonoids while resins, saponins and alkaloids were absent. Ethyl acetate fraction (PsRbE) indicated the presence of saponins, tannins/phenolics, anthraquinones, carbohydrates, reducing sugar, terpenoids, glycosides, steroids, glycosides, and flavonoids while resins and alkaloids were absent. Tannins, phenolics, anthraquinones, and flavonoids were present in the methanol fraction (PsRbM) while saponins, resins, carbohydrates, reducing sugar, steroids, terpenoids and alkaloids were absent (Table 4.12).

The presence of flavonoids in all fractions could be responsible for the high antioxidant and antidiabetic activity observed in this plant. Studies have shown that plants with high antioxidant and antidiabetic activity could be linked to the presence of flavonoids (Marie *et al.,* 2004; Janki *et al.,* 2012).

Table 4.11 Phytochemical screening of methanol extract from four parts of *Pterocarpus soyauxii*

Metabolites	**PsLf**	**PsSt**	**PsRw**	**PsRb**
Saponins	+	+	+	+
Tannins/Phenolics	+	+	+	+
Anthraquinones	+	+	+	+
Resins	+	+	+	-
Carbohydrates	+	+	+	+
Reducing sugar	-	-	-	+
Glycosides	+	+	+	+
steroids	+	+	+	+
Terpenoids	+	+	+	+
Alkaloids	-	-	-	-
Flavonoids	+	+	+	+

- = Not detected + = Detected

Key: Ps = *Pterocarpus soyauxii* Lf = Leaf, St = Stem, Rw =Root~wood, Rb= Root~bark

Table 4.12. Phytochemical screening of root bark methanol extract and fractions from *Pterocarpus soyauxii*

Metabolites	**Crude Extract**	**PsRbH**	**PsRbE**	**PsRbM**
Saponins	+	+	+	+
Tannins/Phenolics	+	+	+	+
Anthraquinones	+	+	+	+
Resins	-	-	-	-
Carbohydrates	+	+	+	-
Reducing sugar	+	+	+	-
Glycosides	+	+	+	+
steroids	+	+	+	-
Terpenoids	+	+	+	-
Alkaloids	-	-	-	-
Flavonoids	+	+	+	+

- = Not detected + = Detected

Key: PsRbH: *Pterocarpus soyauxii* ‘Root bark hexane fraction

PsRbE: *Pterocarpus soyauxii* ‘Root.bark ethyl acetate fraction

PsRbM: *Pterocarpus soyauxii* ‘Root.bark methanol fraction

4.3 Bioassay results

4.3.1 Antidiabetic Activity of *Calophyllum inophyllum* and *Pterocarpus soyauxii*

4.3.1.1 Antidiabetic Activity of volatile oils from *Calophyllum inophyllum*

The percentage inhibitions of ten (10) volatile oils from *Calophyllum inophyllum* were obtained from 0.1 to 0.02 mg/mL. The IC_{50} (mg/mL) for the α-amylase inhibition was determined: [(^{a}Leaf, 0.046±0.3^{a}); (^{b}Leaf-stalk, 0.046±0.3^{b}); (^{c}Flower, 0.047±0.1^{c}); (^{d}Seed, 0.044±0.2^{d}); (^{e}Pod, 0.039±0.3^{e}); (^{f}Pulp, 0.049±0.1^{f}); (^{g}Stem-wood, 0.049±0.3^{g}); (^{h}Stem-bark, 0.051±0.3^{h}); (^{i}Root-wood, 0.050±0.2^{i}) and (^{j}Root-bark, 0.048±0.3^{j})] in reference-with acarbose the standard antidiabetic drug (*0.035±0.2). The pod essential oil exhibited the highest alpha amylase inhibition activity (^{e}0.039±0.3 mg/mL). This result highlights the pod as the most active part of *Calophyllum inophyllum* based on the volatile constituents identified in this plant. In the α-glucosidase assay, the essential oils gave IC_{50} (mg/mL): [(^{a}leaf, 0.049±0.2); (^{b}leaf-stalk, 0.048±0.1); (^{c}flower, 0.049±0.3); (^{d}seed, 0.046±0.2); (^{e}pod, 0.042±0.3); (^{f}pulp, 0.052±0.2); (^{g}stem-wood, 0.049±0.2); (^{h}stem-bark, 0.050±0.3); (^{i}root-wood, 0.051±0.1) and (^{j}root-bark, 0.049±0.3)] relative-to-acarbose (*0.037±0.3). The pod was most active in both studies with IC_{50} of 0.039±0.3 and 0.042±0.3 mg/mL respectively. The good antidiabetic activity of the pod essential oil must be due to the presence of some unique and biologically relevant volatile chemical contituents in the plant. The low antidabetic activity of the stem and root parts could be due to the high fibre content of these parts (Table 4.13).

4.3.1.2 Antidiabetic activity of volatile oils from *Pterocarpus soyauxii*

Alpha-Amylase and glucosidase inhibitory activities were carried out on the root parts' essential oils of *Pterocarpus soyauxii*. In the determination of α-amylase inhibition root wood and root bark essential oils gave IC_{50} (mg/mL) of 0.047±0.1 and 0.049±0.3 respectively compared to acarbose (0.033±0.2 mg/mL). Acarbose had the lowest IC_{50} value of 0.033±0.2 milligram/mL followed by the root bark (0.047±0.4) (Table 4.14). In the α-glucosidase model, IC_{50} (mg/mL) of 0.052±0.2 and 0.049±0.1 were obtained for root bark and root wood essential oils in relation to acarbose (0.035±0.3 mg/mL). The activities expressed must be due to the presence of some chemical contituents present in *Pterocarpus soyauxii* (Table 4.14).

Table 4.13 IC_{50} data of α-amylase and glucosidase-inhibition of volatile-oils from *C. inophyllum*

	Sample	α amylase inhibition (milligram/mL)	α glucosidase inhibition (milligram /mL)
Standard	Acarbose	0.035±0.2	0.037±0.3
	[a]Leaf	0.046±0.3	0.049±0.2
	[b]Leaf stalk	0.046±0.3	0.048±0.1
	[c]Flower	0.047±0.1	0.049±0.3
	[d]Seed	0.044±0.2	0.046±0.2
	[e]Pod	0.039±0.3	0.042±0.3
	[f]Pulp	0.049±0.1	0.052±0.2
	[g]Stem wood	0.049±0.5	0.049±0.2
	[h]Stem bark	0.051±0.3	0.050±0.2
	[i]Root wood	0.050±0.2	0.051±0.1
	[j]Root bark	0.048±0.3	0.049±0.3

Table 4.14: IC_{50} values of α-amylase and α-glucosidase inhibitory activity of *Pterocarpus soyauxii*

Plant sample	Plant part	α amylase inhibition (milligram/mL)	α glucosidase inhibition (milligram/mL)
Standard	Acarbose	0.033±0.2	0.035±0.3
	Root bark	0.047±0.1	0.049±0.1
	Root wood	0.049±0.3	0.052±0.2

4.3.1.3 Antidiabetic activity of Crude extracts from *Calophyllum inophyllum* and *Pterocarpus soyauxii*

4.3.1.3.1 Antidiabetic activity of Crude extracts from *Calophyllum inophyllum*

The antidiabetic potential of crude extracts from ten (10) parts of *Calophyllum inophyllum* was evaluated. The IC_{50} (mg/mL) were obtained in α'-amylased-inhibition: [(^{a}Leaf, 0.039±0.3); (^{b}Leaf-stalk, 0.042±0.1); (^{c}Flower, 0.039±0.3); (^{d}Seed, 0.035±0.2); (^{e}Pod, 0.028±0.3); (^{f}Pulp, 0.033±0.1); (^{g}Stem-wood, 0.039±0.2); (^{h}Stem bark, 0.040±0.5); (^{i}Root-wood, 0.036±0.3); and (^{j}Root bark, 0.039±0.1)] in relation to acarbose (*0.025±0.1). The Pod extract (^{e}0.028±0.3) was determined as the most active extract in the α-amylase antidiabetic assay. In the α-glucosidase antidiabetic assay the IC_{50} values (mg/mL) were obtained: [(^{a}Leaf, 0.043±0.3); (^{b}Leaf stalk, 0.048±0.2); (^{c}Flower, 0.042±0.2); (^{d}Seed, 0.039±0.1); (^{e}Pod, 0.032±0.2); (^{f}Pulp, 0.041±0.2); (^{g}Stem-wood, 0.046±0.1); (^{h}Stem-bark, 0.045±0.5); (^{i}Root-wood, 0.043±0.1); (^{j}bark, 0.042±0.2);] in comparison with acarbose the standard antidiabetic drug (*0.030±0.2). The Pod extract (^{e}0.032±0.2 mg/mL) was also determined as the most active extract in the α-glucosidase antidiabetic assay (Table 4.15).

4.3.1.3.2 Antidiabetic activity of Crude extracts from *Pterocarpus soyauxii*

In the α-amylase assay the following IC_{50} values were obtained: [(^{a}Leaf, 0.029±0.2); (^{b}Stem, 0.030±0.3); (^{c}Root wood, 0.028±0.2) and (^{d}Root bark, 0.024±0.2)] in comparison-tom acarbose (*0.025±0.1) meanwhile, the α-glucosidase gave [(^{a}Leaf, 0.034±0.4); (^{b}Stem, 0.035±0.2); (^{c}Root wood, 0.032±0.1); (^{d}Root bark, 0.027±0.3) in reference to acarbose (*0.030±0.2). In both assays the root bark extract exhibited a higher antidiabetic activity thereby making the root bark the most active fraction (Table 4.16). The crude extract being the most active extract was therefore, subjected to fractionation in order to obtain the hexane, ethyl acetate and methanol fractions respectively.

Table 4.15 IC_{50} values (mg/mL) of α-amylase and α-glucosidase inhibitory activity of crude extracts from *Calophyllum inophyllum*

Plant sample	Sample	α amylase inhibition (milligram/mL)	α glucosidase inhibition (milligram/mL)
Standard	Acarbose	0.025±0.1	0.030±0.2
	[a]Leaf	0.039±0.3	0.043±0.3
	[b]Leaf stalk	0.042±0.1	0.048±0.2
	[c]Flower	0.039±0.3	0.042±0.2
	[d]Seed	0.035±0.2	0.039±0.1
	[e]Pod	0.028±0.3	0.032±0.2
	[f]Pulp	0.033±0.1	0.041±0.2
	[g]Stem wood	0.039±0.2	0.046±0.1
	[h]Stem bark	0.040±0.2	0.045±0.5
	[i]Root wood	0.036±0.3	0.043±0.1
	[j]Root bark	0.039±0.1	0.042±0.2

Table 4.16 IC_{50} values (milligram/mL) of α-amylase and α-glucosidase inhibitory activity of crude extracts from *Pterocarpus soyauxii*

Plant sample	**Sample**	***α-amylase inhibition (milligram/mL)**	***α-glucosidase inhibition (milligram/mL)**
Standard	Acarbose	0.025±0.1	0.030±0.2
	[a]Leaf	0.029±0.2	0.034±0.2
	[b]Stem	0.030±0.3	0.035±0.2
	[c]Root wood	0.028±0.2	0.032±0.1
	[d]Root bark	0.024±0.4	0.027±0.3

4.3.1.3.3 Antidiabetic potential of fractions from Pod extract of *Calophyllum inophyllum*

The anti-diabetic potency of non-polar-hexane., ethyl-acetate and highly polar-methanol' fractions from the Pod of *Calophyllum inophyllum* was. The outlined IC_{50} (mg/mL) were obtained in the α-amylase-model: [(CiPdH, 0.038±0.3); (CiPdE, 0.032±0.2); (CiPdM, 0.042±0.3)] in comparison-to-acarbose (0.024±0.1) the α-glucosidase-model gave IC_{50} values of [(CiPdH, 0.046±0.3); (CiPdE, 0.036±0.3); (CiPdM, 0.046±0.2)] when compared-to-acarbose' (0.027±0.2). The ethyl acetate fraction with the highest inhibition efficiency was the most active fraction using the α-amylase and α-glucosidase (Table 4.17).

4.3.1.3.4 Antidiabetic activity of fractions from root bark extract of *Pterocarpus soyauxii*

The anti-diabetic activity of fractions from *Pterocarpus soyauxii* was evaluated. The outlined IC_{50} (mg/mL) were determined by the α-amylase-model: [(PsRbH, 0.036±0.3); (PsRbE, 0.031±0.2); (PsRbM, 0.043±0.1)] in reference-to-acarbose (0.029±0.1), the α-glucosidase assay gave IC_{50} values of [(PsRbH, 0.039±0.1); (PsRbE, 0.035±0.1); (PsRbM, 0.045±0.3)] in reference-to-acarbose (0.032±0.2) as indicated on Table 4.18. The ethyl acetate fraction with the highest inhibition efficiency was the most active fraction using the α-amylase and α-glucosidase models.

4.3.1.3.5 Antidiabetic activity of isolated compounds from *Calophyllum inophyllum*

The anti-diabetic activity of isolated compounds from *Calophyllum inophyllum* was evaluated. The IC_{50} values (mg/mL) in the α-amylase inhibition were: [(0.036±0.2 for compound CiPdE-1); (0.032±0.2 for compound CiPdE-2); (0.026±0.2 for compound CiPdE-3)] in relation to the standard drug acarbose (0.029±0.1). Compound CiPdE-3 had the highest α-amylase inhibition with an IC_{50} of 0.026±0.2 followed by CiPdE-2 (0.032±0.2). CiPdE-1 had the lowest activity with IC_{50} of 0.036±0.2. Interestingnly, CiPdE-3 (0.026±0.2) expressed a higher α-amylase activity compared to the standard drug acarbose (0.029±0.1).

In the α-glucosidase assay, the following IC_{50} values (mg/mL) were obtained: CiPdE-1 had 0.039±0.2; CiPdE-2 had 0.036±0.3; CiPdE-3 had 0.027±0.2 in comparison with the standard drug acarbose 0.030±0.2. Compound CiPdE-3 had the highest α-glucosidase inhibition with an IC_{50} of 0.027±0.2 followed by CiPdE-2 (0.036±0.3). CiPdE-1 had the lowest activity with IC_{50} of 0.039±0.2. CiPdE-3 (0.026±0.2) expressed α-amylase activity a higher than the standard drug acarbose (0.030±0.2) as indicated on Table 4.19.

4.3.1.3.6 Antidiabetic activity of isolated compounds from *Pterocarpus soyauxii*

The anti-diabetic potential of all isolated compounds from *Pterocarpus soyauxii* was evaluated. The IC_{50} data (mg/mL) were assessed in the α-amylase study: [(PsRbE-A, 0.027±0.3) (PsRbE-B, 0.032±0.2); (PsRbE-C, 0.042±0.1); (PsRbE-D, 0.038±0.3)] relative to acarbose (0.029±0.1). Compound PsRbE-A had the highest α-amylase inhibition with an IC_{50} of 0.027±0.3 followed by PsRbE-B (0.032±0.2). PsRbE-C had the lowest activity with IC_{50} of 0.042±0.1. PsRbE-A (0.027±0.3) expressed α-amylase activity higher than the standard drug acarbose (0.029±0.1) as indicated on Table 4.19.

In the α-glucosidase assay the IC_{50} data (mg/mL) were: (PsRbE-A, 0.029±0.1); (PsRbE-B, 0.037±0.1); (PsRbE-C, 0.044±0.3) and (PsRbE-D, 0.042±0.1)] relative to acarbose (0.030±0.2). PsRbE-A had the highest α-glucosidase assay with an IC_{50} of 0.029±0.3 followed by PsRbE-B (0.037±0.1). PsRbE-C had the lowest activity with IC_{50} of 0.044±0.3. PsRbE-A (0.029±0.3) expressed α-amylase activity higher than the standard drug acarbose (0.030±0.1) as indicated on Table 4.19.

Table 4.17 IC_{50} values (mg/mL) of α-amylase and α-glucosidase inhibitory activity of fractions from Pod extract of *Calophyllum inophyllum*

Plant sample	Fraction	*α amylase inhibition (milligram/mL)	*α glucosidase inhibition (milligram/mL)
Standard	Acarbose	0.024±0.1	0.027±0.2
	CiPdH	0.038±0.3	0.046±0.3
	CiPdE	0.032±0.2	0.036±0.3
	CiPdM	0.042±0.3	0.046±0.2

Key: CiPdH: Pod hexane fraction of *Calophyllum inophyllum*

CiPdE: Pod ethyl acetate fraction of *Calophyllum inophyllum*

CiPdM: Pod methanol fraction of *Calophyllum inophyllum*

Table 4.18 IC_{50} values (mg/mL) of α-amylase and glucosidase inhibitory activity of fractions from root bark fractions from *Pterocarpus soyauxii*

	Sample	***α amylase inhibition (milligram/mL)**	***α thrglucosidase inhibition (milligram/mL)**
Standard	Acarbose	0.029±0.1	0.032±0.2
	PsRbH	0.036±0.3	0.039±0.1
	PsRbE	0.031±0.2	0.035±0.1
	PsRbM	0.043±0.1	0.045±0.3

Key: PsRbH: *Pterocarpus soyauxii* Root bark hexane fraction

PsRbE: *Pterocarpus soyauxii* Root.bark ethyl acetate fraction

PsRbM: *Pterocarpus soyauxii* Root.bark methanol fraction

Table 4.19 IC_{50} values (milligram/milliLiter) of isolated compounds from *Calophyllum inophyllum*

	Isolated Compounds	***α amylase inhibition (milligram/mL)**	***α glucosidase inhibition (milligram/mL)**
Standard	Acarbose	0.029±0.1	0.030±0.2
	CiPdE-1	0.036±0.2	0.039±0.2
	CiPdE-2	0.032±0.2	0.036±0.3
	CiPdE-3	0.026±0.2	0.027±0.2

Table 4.20 IC_{50} values (mg/mL) of α-amylase and glucosidase inhibitory activity of isolated compounds from *Pterocarpus soyauxii*.

	Isolated Compounds	***α amylase inhibition (milligram/mL)**	***α glucosidase inhibition (milligram/mL)**
Standard	Acarbose	0.029±0.1	0.030±0.2
	PsRbE-A	0.027±0.3	0.029±0.1
	PsRbE-B	0.032±0.2	0.037±0.1
	PsRbE-C	0.042±0.1	0.044±0.3
	PsRbE-D	0.038±0.3	0.042±0.1

4.3.2 Antioxidant Activity of *Calophyllum inophyllum* and *Pterocarpus soyauxii*

4.3.2.1 Antioxidant activity of essential oils from *Calophyllum inophyllum*

The ten (10) essential oils had IC_{50} (mg/mL) values using DPPH model: [([a]leaf, 0.39±0.3); ([b]leaf-stalk, 0.42±0.1); ([c]flower, 0.39±0.3); ([d]seed, 0.35±0.2); ([e]pod, 0.30±0.3); ([f]fruit-pulp, 0.36±0.1); ([g]stem-wood, 0.39±0.1); ([h]stem-bark, 0.34±0.2); ([i]root-wood, 0.32±0.1) and ([j]root-bark, 0.39±0.1)] relative to standard antioxidants used: [Ascorbic acid (0.12±0.1) and BHA (0.091±0.2)] while the H_2O_2 model gave the IC_{50} values; [([a]leaf, 0.33±0.2); ([b]leaf-stalk, 0.38±0.2); ([c]flower, 0.32±0.2); ([d]seed, 0.37±0.1); ([e]pod, 0.30±0.2); ([f]fruit-pulp, 0.33±0.2); ([g]stem-wood, 0.36±0.1); ([h]stem-bark, 0.35±0.2); ([i]root-wood, 0.33±0.2) and ([j]root bark, 0.33±0.2)] relative to standard antioxidants used: [Ascorbic acid (0.12±0.2) and BHA (0.12±0.2)]. Pod had the highest antioxidant activity using both DPPH and H_2O_2 models. Result obtained from the antioxidant evaluations revealed that the essential oils contain bioactive chemical constituents with potent antioxidant properties (Table 4.21).

4.3.2.2 Antioxidant activity of essential oils from *Pterocarpus soyauxii*

As shown in Table 4.22, the antioxidant potential of root essential oils from *Pterocarpus soyauxii* were concentration dependent in relation to standards (ascorbic acid and BHA). The 50% inhibitory concentration (mg/mL) using the DPPH model showed that root bark essential oil expressed a higher radical scavenging activity (0.14±0.1) in relation to root wood essential oil (0.18±0.2) which are in close range with standards used (BHA, 0.091±0.1) and (Vitamin C, 0.12±0.1). In the H_2O_2 antioxidant assay, root bark essential oil gave a higher radical scavenging activity (0.16±0.3) relative to root wood essential oil (0.20±0.1) which are in close range to that of standards used [(BHA, 0.12±0.1) and (Vitamin C, 0.12±0.2)].

Table 4.21 IC_{50} values from DPPH and H_2O_2 scavenging activity of essential oils from *Calophyllum inophyllum*

Sample	Plant part	DPPH inhibition (mg/mL)	H_2O_2 inhibition (mg/mL)
Standard	Ascorbic acid	0.12±0.1	0.12±0.2
	BHA	0.09±0.1	0.12±0.2
	[a]Leaf	0.39±0.3	0.33±0.2
	[b]Leaf stalk	0.42±0.1	0.38±0.2
	[c]Flower	0.39±0.3	0.32±0.2
	[d]Seed	0.35±0.2	0.37±0.1
	[e]Pod	0.30±0.3	0.30±0.2
	[f]Pulp	0.36±0.1	0.33±0.2
	[g]Stem wood	0.39±0.1	0.36±0.1
	[h]Stem bark	0.34±0.2	0.35±0.2
	[i]Root wood	0.32±0.3	0.33±0.2
	[j]Root bark	0.39±0.1	0.33±0.2

Table 4.22 IC_{50} values from DPPH and H_2O_2 scavenging activity of essential oils from *Pterocarpus soyauxii*

Sample	**Plant part**	**DPPH inhibition (mg/mL)**	**H_2O_2 inhibition (mg/mL)**
Standard	Ascorbic acid	0.12±0.1	0.14±0.2
	BHA	0.09±0.1	0.12±0.1
	Root wood	0.18±0.2	0.20±0.1
	Root bark	0.14±0.1	0.16±0.3

4.3.2.3 Antioxidant activity of crude extracts from *Calophyllum inophyllum* and *Pterocarpus soyauxii*

4.3.2.3.1 Antioxidant activity of crude extracts from *Calophyllum inophyllum*

The antioxidant activity of ten (10) crude methanol extracts from *Calophyllum inophyllum* was evaluated (Table 4.23). The IC_{50} values (mg/mL) were determined using DPPH: [([a]Leaf, 0.38±0.2); ([b]Leaf stalk, 0.34±0.1); ([c]Flower, 0.36±0.3); ([d]Seed, 0.35±0.3); ([e]Pod, 0.26±0.1); ([f]Peel, 0.36±0.1); ([g]Stem-wood, 0.34±0.1); ([h]Stem-bark, 0.38±0.1); ([i]Root-wood, 0.36±0.3); and ([h]Root-bark, 0.34±0.2);] relative to standard antioxidants [(Ascorbic acid, 0.14±0.2); (BHA, 0.10±0.2)]. The Pod extract (0.26±0.1) was determined as the most active extract in the DPPH antioxidant assay.

In the H_2O_2 antioxidant assay the IC_{50} values (mg/mL) were obtained: [([a]Leaf, 0.41±0.2); ([b]Leaf stalk, 0.38±0.1); ([c]Flower, 0.40±0.2); ([d]Seed, 0.38±0.1); ([e]Pod, 0.32±0.2); ([f]Pulp, 0.33±0.2); ([g]Stem-wood, 0.36±0.1); ([h]Stem-bark., 0.41±0.1); ([i]Root-wood, 0.40±0.3); ([f]Root bark, 0.37±0.2);] in reference to standard antioxidants [(Ascorbic acid, 0.16±0.1); (BHA, 0.14±0.3);]. The Pod extract (0.32±0.2) was also determined as the most active extract in the H_2O_2 antioxidant assay (Table 4.23).

Due to the high antioxidant activity of the pod part using both DPPH and H_2O_2 models, it was selected for fractionation and subsequent column chromatographic purification.

4.3.2.3.2 Antioxidant activity of crude extracts from *Pterocarpus soyauxii*

In the DPPH assay, the IC_{50} values (mg/mL) were obtained: [([a]Leaf, 0.32±0.1); ([b]Stem, 0.31±0.3); ([c]Root-wood, 0.30±0.2); ([d]Root-bark, 0.27±0.2)] in reference to standard antioxidants [Ascorbic acid (0.14±0.2) and BHA (0.10±0.2)]. The H_2O_2 assay gave [([a]Leaf, 0.34±0.2); ([b]Stem, 0.34±0.1); ([c]Root-wood, 0.32±0.1); ([d]Root-bark, 0.30±0.3) in reference to standards [Ascorbic acid (0.16±0.1) and BHA (0.14±0.3)]. The antioxidant activity using the DPPH and H_2O_2 models revealed the root bark extract as the most potent (Table 4.24).

Table 4.23 IC_{50} values from DPPH and H_2O_2 scavenging activity of crude methanol extracts from *Calophyllum inophyllum*

	Plants parts	**DPPH inhibition (mg/mL)**	**H_2O_2 inhibition (mg/mL)**
Standards	Ascorbic acid	0.14±0.2	0.16±0.1
	BHA	0.10±0.2	0.14±0.3
	[a]Leaf	0.38±0.2	0.41±0.2
	[b]Leaf stalk	0.34±0.1	0.38±0.1
	[c]Flower	0.36±0.3	0.40±0.2
	[d]Seed	0.35±0.3	0.38±0.1
	[e]Pod	0.26±0.1	0.32±0.2
	[f]Pulp	0.36±0.1	0.33±0.2
	[g]Stem wood	0.34±0.1	0.36±0.1
	[h]Stem bark	0.38±0.1	0.41±0.1
	[i]Root wood	0.36±0.3	0.40±0.3
	[j]Root bark	0.34±0.2	0.37±0.2

Table 4.24 IC_{50} values from DPPH and H_2O_2 scavenging activity from crude methanol extracts from *Pterocarpus soyauxii*.

Plant sample	**Sample**	**DPPH inhibition (mg/mL)**	**H_2O_2 inhibition (mg/mL)**
Standard	Ascorbic acid	0.14±0.2	0.16±0.1
	BHA	0.10±0.2	0.14±0.3
	Leaf	0.32±0.1	0.34±0.2
	Stem	0.31±0.3	0.34±0.1
	Root wood	0.30±0.2	0.32±0.1
	Root bark	0.27±0.2	0.30±0.3

4.3.2.3.3 Antioxidant activity of fractions from pod extract of *Calophyllum inophyllum*

The antioxidant activity of all fractions from crude extract of *Calophyllum inophyllum* were evaluated based on the DPPH and H_2O_2 inhibitory models. The outlined IC_{50} values (mg/mL) were determined by DPPH inhibition: [(CiPdH, 0.40±0.3); (CiPdE, 0.34±0.1); (CiPdM, 0.37±0.3)] in reference to standards [Ascorbic acid (0.11±0.1) and BHA (0.10±0.1)] While the H_2O_2 assay gave IC_{50} values (mg/mL) of [(CiPdH, 0.42±0.1); (CiPdE, 0.36±0.2); (CiPdM, 0.40±0.1)] in reference to standards [Ascorbic acid (0.13±0.1) and BHA (0.14±0.3)]. The ethyl acetate fraction with the highest inhibition efficiency was the most active fraction using DPPH and H_2O_2 models (Table 4.25).

The results confirm the rich phytochemical constituents in the ethyl acetate fraction earlier identified during the phytochemical screening. The ethyl acetate being the most active fraction was selected for further purification by column chromatography

4.3.2.3.4 Antioxidant activity of fractions from root bark of *Pterocarpus soyauxii*

The antioxidant potential of three fractions from *Pterocarpus soyauxii* was evaluated based on DPPH and H_2O_2 inhibitory potential (Table 4.26). IC_{50} values (mg/mL) were obtained in this evaluation: [(PsRbH, 0.39±0.2); (PsRbE, 0.34±0.2); (PsRbM, 0.42±0.1)] in reference to standards [Ascorbic acid (0.11±0.1) and BHA (0.10±0.1)] While the H_2O_2 assay gave IC_{50} values (mg/mL) of [(PsRbH, 0.39±0.2); (PsRbE, 0.34±0.2); (PsRbM, 0.42±0.1)] in reference to standards [Ascorbic acid (0.13±0.1) and BHA (0.14±0.3)]. The ethyl acetate fraction with the highest inhibition efficiency was the most active fraction using both models.

The results from the antioxidant activity of the ethyl acetate fraction from the root bark of *Pterocarpus soyauxii* corroborates the earlier identified phytoconstituents during the phytochemical screening. The ethyl acetate being the most active fraction was selected for further purification by column chromatography.

Table 4.25 IC_{50} values from DPPH and H_2O_2 scavenging activity from Pod extract of *Calophyllum inophyllum*

	Sample	**DPPH inhibition (mg/mL)**	**H_2O_2 inhibition (mg/mL)**
Standard	Ascorbic acid	0.11±0.2	0.13±0.1
	BHA	0.10±0.1	0.14±0.3
	CiPdH	0.40±0.3	0.42±0.1
	CiPdE	0.34±0.1	0.36±0.2
	CiPdM	0.37±0.3	0.40±0.1

Key: CiPdH: Pod hexane fraction of *Calophyllum inophyllum*

CiPdE: Pod ethyl acetate fraction of *Calophyllum inophyllum*

CiPdM: Pod methanol fraction of *Calophyllum inophyllum*

Table 4.26 IC_{50} values from DPPH and H_2O_2 scavenging activity of fractions from Root bark extract of *Pterocarpus soyauxii*

	Sample	DPPH inhibition (mg/mL)	H_2O_2 inhibition (mg/mL)
Standard	Ascorbic acid	0.11±0.2	0.13±0.1
	BHA	0.10±0.1	0.14±0.3
	PsRbH	0.39±0.2	0.41±0.1
	PsRbE	0.34±0.2	0.37±0.1
	PsRbM	0.42±0.1	0.45±0.3

Key: PsRbH: *Pterocarpus soyauxii* Root bark hexane fraction

PsRbE: *Pterocarpus soyauxii* Root bark ethyl acetate fraction

PsRbM: *Pterocarpus soyauxii* Root bark methanol fraction

4.3.2.3.5 Antioxidant activity of isolated compounds from *Calophyllum inophyllum*

The antioxidant potential of isolated compounds from *Calophyllum inophyllum* was evaluated based on the DPPH and H_2O_2 inhibitory potential. The IC_{50} values (milligram/milliLiter) were obtained as follows: [(CiPdE-1, 0.30±0.1); (CiPdE-2, 0.26±0.2); (CiPdE-3, 0.16±0.1] relative to [Ascorbic acid (0.18±0.2) and BHA (0.15±0.4)]. In the H_2O_2 assay the IC_{50} (mg/mL) values were obtained: [(CiPdE-1, 0.33±0.6); (CiPdE-2, 0.29±0.3); (CiPdE-3, 0.20±0.2)] in comparison with [Ascorbic acid (0.22±0.2) and Butylated Hydroxyl Anisole (0.19±0.1)].

Compound CiPdE-3 had the highest antioxidant activity with an IC_{50} of 0.16±0.1 followed by CiPdE-2 (0.26±0.3). CiPdE-1 had the lowest activity with IC_{50} of 0.30±0.1. CiPdE-3 (0.16±0.2) expressed antioxidant activity higher than the standard drugs [Ascorbic acid (0.18±0.2) and BHA (0.15±0.4)] as indicated on Table 4.27.

4.3.2.3.6 Antioxidant activity of isolated compounds from *Pterocarpus soyauxii*

The antioxidant potential of all isolated compounds from *Pterocarpus soyauxii* was evaluated based on the DPPH and H_2O_2 inhibitory potential. The IC_{50} values (mg/mL) obtained from the DPPH inhibitions are: [(PsRbE-A, 0.17±0.3); (PsRbE-B, 0.36±0.2); (PsRbE-C, 0.38±0.1); (PsRbE-D, 0.40±0.3)] in reference to [Ascorbic acid (0.18±0.2) and BHA (0.15±0.1)]. In the H_2O_2 assay the IC_{50} values (mg/mL) were obtained: (PsRbE-A, 0.21±0.1); (PsRbE-B, 0.40±0.1); (PsRbE-C, 0.43±0.3); (PsRbE-D, 0.44±0.1)] relative to [Ascorbic acid (0.22±0.2) and BHA (0.19±0.4)].

Compound PsRbE-A had the highest antioxidant activity with an IC_{50} (mg/mL) of 0.17±0.3 followed by (PsRbE-B, 0.36±0.2). PsRbE-D, had the lowest activity with IC_{50} (mg/mL) of 0.40±0.3. PsRbE-A (0.17±0.3) expressed antioxidant activity two-fold higher than all other compounds isolated from this plant compared to standards used [Ascorbic acid (0.22±0.2 mg/mL) and BHA (0.19±0.4 mg/mL)] as indicated on Table 4.27. The synergy between the DPPH and H_2O_2 models corroborates the reliability and complementality of the two models. The high activity of PsRbE-A projects this compound as a suitable drug candidate for the production of new antioxidant agent.

Table 4.27 IC_{50} values from DPPH and H_2O_2 scavenging activity of isolated compounds from *Calophyllum inophyllum*

	Compounds	**DPPH inhibition (mg/mL)**	**H_2O_2 inhibition (mg/mL)**
Standard	Ascorbic acid	0.18±0.2	0.22±0.2
	BHA	0.15±0.1	0.19±0.1
	CiPdE-1	0.30±0.1	0.33±0.1
	CiPdE-2	0.26±0.2	0.29±0.3
	CiPdE-3	0.16±0.1	0.20±0.2

Table 4.28 IC_{50} values from DPPH and H_2O_2 scavenging activity of isolated compounds from *Pterocarpus soyauxii*

Plant sample	**Compound**	**DPPH inhibition (mg/mL)**	**H_2O_2 inhibition (mg/mL)**
Standard	Ascorbic acid	0.18±0.2	0.22±0.2
	BHA	0.15±0.1	0.19±0.1
	PsRbE-A	0.17±0.3	0.21±0.1
	PsRbE-B	0.36±0.2	0.40±0.1
	PsRbE-C	0.38±0.1	0.43±0.3
	PsRbE-D	0.40±0.3	0.44±0.4

4.4 Characterisation of non-volatile constituents from *Calophyllum inophyllum* and *Pterocarpus soyauxii*

4.4.1 Isolation of secondary metabolites from pod ethyl acetate fraction of *Calophyllum inophyllum*

Chromatographic separation of the EtOAc fraction (20 g) of *C. inophyllum* CiP (Scheme 1), gave four compounds. The compounds are: CiPdE-1 (25 mg), CiPdE-2 (16 mg), CiPdE-3 (32 mg), and CiPdE-4 (38 mg).

4.4.1.1 Characterisation of CiPdE-1

CiPdE-1 was separated from the pod (EtOAc fraction) as white-crystals (25 mg) with melting point of 250-258^{o}C (Sheme 1). Spraying of CiPdE-1 with vanillin sulphuric acid gave an oval single spot with an R_f value of 0.45.The Electrospray Ionisation positive (ESI+) mass spectrum of compound CiPdE-1 (Figure 4.2) gave *m/z* 449.4 [M + Na] which correspond to the molecular-formula $C_{30}H_{50}O$ (Figure 4.12). The IR $\bar{\upsilon}$ (cm^{-1}) spectrum of CiPdE-1 showed a band at 1714 cm^{-1} indicating the presence of carbonyl group (Figure 4.11). sp^3 hybridised C-H stretching formed absorption bands at 2926 cm^{-1} with a consequent deformation at 1462 cm^{-1}

^{1}H NMR spectrum of CiPdE-1 (Figure 4.3) revealed the presence of eight methyl groups, one 2^0 methyl appeared at δ 0.87 (d, *J=6.5* Hz, H-23) and seven quartenary methyl groups at δ 0.72 (s, H-24'), δ 0.82 (s, H-25'), δ 1.00 (s, H-26'), δ 1.05 (s, H-27'), δ 1.18 (s, H-28'), δ 0.99 (s, H-29') and δ 1.05 (s, H-30') suggesting the friedelane type triterpene. The signal at δ 2.27 was ascribed to H-4 while signals at δ 1.97 parts per million, δ 1.69 parts per million, δ 2.37 parts per million and δ 2.28 parts per million were matched to CH_2 hyrogens (H-1α', H-1β', H-2α' and H-2β' respectively). The signals visible on the proton spectrum were typical of common triterpenes. ^{13}C NMR spectrum (Figures 4.4) of CiPdE-1 showed 30 carbons ascribed to eight (8) CH_3 groups, eleven (11) sp^3 methylene carbons, four (4) sp^3 methines and seven (7) quaternary carbons from which one quaternary carbon at δ 213 ppm was typical for carbonyl carbon (C-3).

COSY experiment of CiPdE-1 (Figure 4.8) revealed cross-peak correlations between the proton at δ 2.37 (*d*, H-2) and one methylene (CH_2) proton δ 1.69 (H-1). COSY correlation was also detected between doublet protons H-21, δ 1.31 (H-2) and methyl proton H-29; methyl group at H-22 and methyl proton H-30. Similarly, methyl proton on H-25 correlated with one methylene proton (H-2). The HMBC experiment (Figure 4.9) showed significant correlations between δ 58.23 (C-4) and methyl group on H-24. Quartenary carbon δ 42.11 (C-5) and δ 37.44 (C-9) correlated with H-23 and H-25 respectively. Methine δ 59.32 (C-10) correlated with H-24 while methylene δ 35.20 (C-11) correlatd with both H-26 and H-27. Likewise, methylene δ 30.05 (C-12) correlated with carbons 26 and 27. Quartenary carbons δ 39.29 (C-13) and δ 38.31 (C-14') correlated with H-19α' and H-25 respectively. Methylene δ 30.05 (C-15) correlated with H-26 and H-7α. While methine δ 30.03 (C-17) correlated with H-16α and H-22α. Methine C-18 (δ 42.30) also correlated with H-28. Methylene C-19' (δ 35.34) and C-20' (δ 28.13) correlated with (30, 27) and (27, 28) respectively. Methylene C-21 (δ 32.76) and C-22 (δ 39.22) correlated with 30 and 28 respectively. Methyl C-27 (δ 18.63) correlated with H-19α and H-26 similarly methyl proton C-30 (δ 31.74) correlated with 29 and 19α. HSQC experiment was used as a diagnostic tool for assigning all protons bonded with individual carbons as indicated in Figure 4.7.

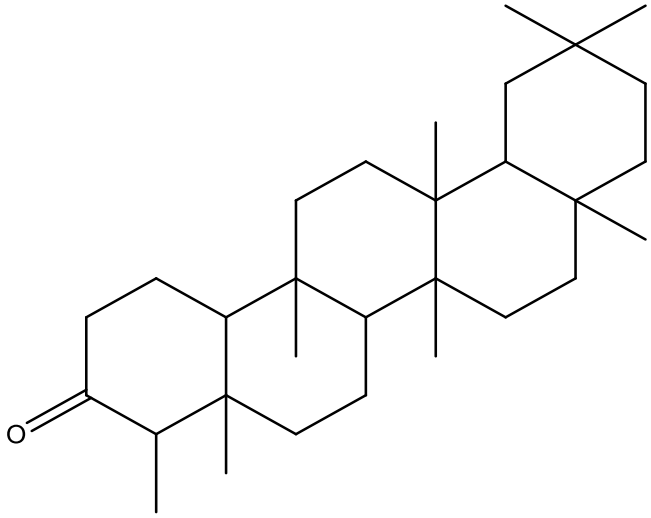


Fig. [4.2] Structure of **CiPdE-1** (Friedelin)

Table. 4.29 ^{1}Proton and ^{13}Carbon NMR data of **CiPdE-1** in $CDCl_3$

Position	**C.**	**δ ^{13}C.**	**δ ^{1}H.**	**M.**	**J_v**
1	$^{b}CH_2$	22.24	1.97, 1.69	*m.*	
2	$^{b}CH_2$	41.32	2.37 2.28	*dd.*	
3	^{d}C	213.31	-	-	
4	^{c}CH	58.23	2.26	*m.*	
5	^{d}C	42.11	-	-	
6	$^{b}CH_2$	41.24	1.73, 1.29	*d.*	
7	$^{b}CH_2$	18.24	1.49, 1.36	*m.*	
8	CH	52.93	1.40	*d.*	
9	^{d}C	37.44	-	-	
10	^{c}CH	59.32	1.53	*m.*	
11	$^{b}CH_2$	35.20	1.45, 1.24	*m.*	
12	$^{b}CH_2$	30.05	1.47, 1.27	*m.*	
13	^{d}C	39.29	-	-	
14	^{d}C	38.31	-	-	
15	$^{b}CH_2$	32.41	1.47, 1.27	*m.*	
16	$^{b}CH_2$	36.01	1.58, 1.35	*m.*	
17	^{c}CH	30.03	-	-	
18	^{c}CH	42.30	1.56	*m.*	
19	$^{b}CH_2$	35.34	1.37, 1.35	*m.*	
20	^{d}C	28.13	-	-	
21	$^{a}CH_2$	32.76	1.50, 1.31	*m.*	
22	$^{a}CH_2$	39.22	1.48, 0.95	*m.*	

23	$^{a}CH_3$	6.85	0.87	*d*	*J* = 6.45Hz
24	$^{a}CH_3$	14.56	0.72	*s*	
25	$^{a}CH_3$	17.89	0.82	*s*	
26	$^{a}CH_3$	20.22	1.00	*s*	
27	$^{a}CH_3$	18.63	1.05	*s*	
28	$^{a}CH_3$	32.06	1.18	*s*	
29	$^{a}CH_3$	35.0	1.05	*s*	
30	$^{a}CH_3$	31.74	0.95	*s*	

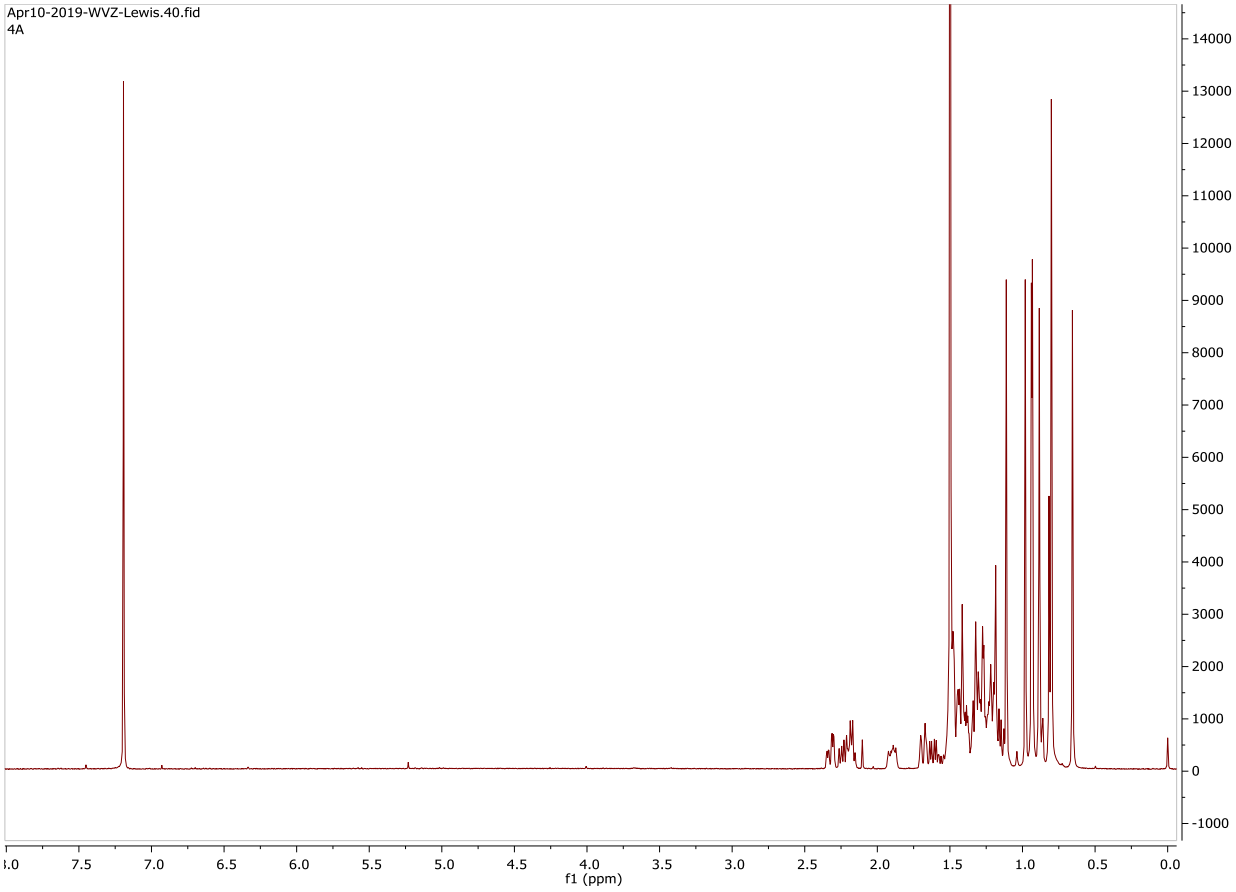


Fig. 4.3 ^{1}H NMR^ Spectrum (500MHz) of **CiPdE-1** in $CDCl_3$

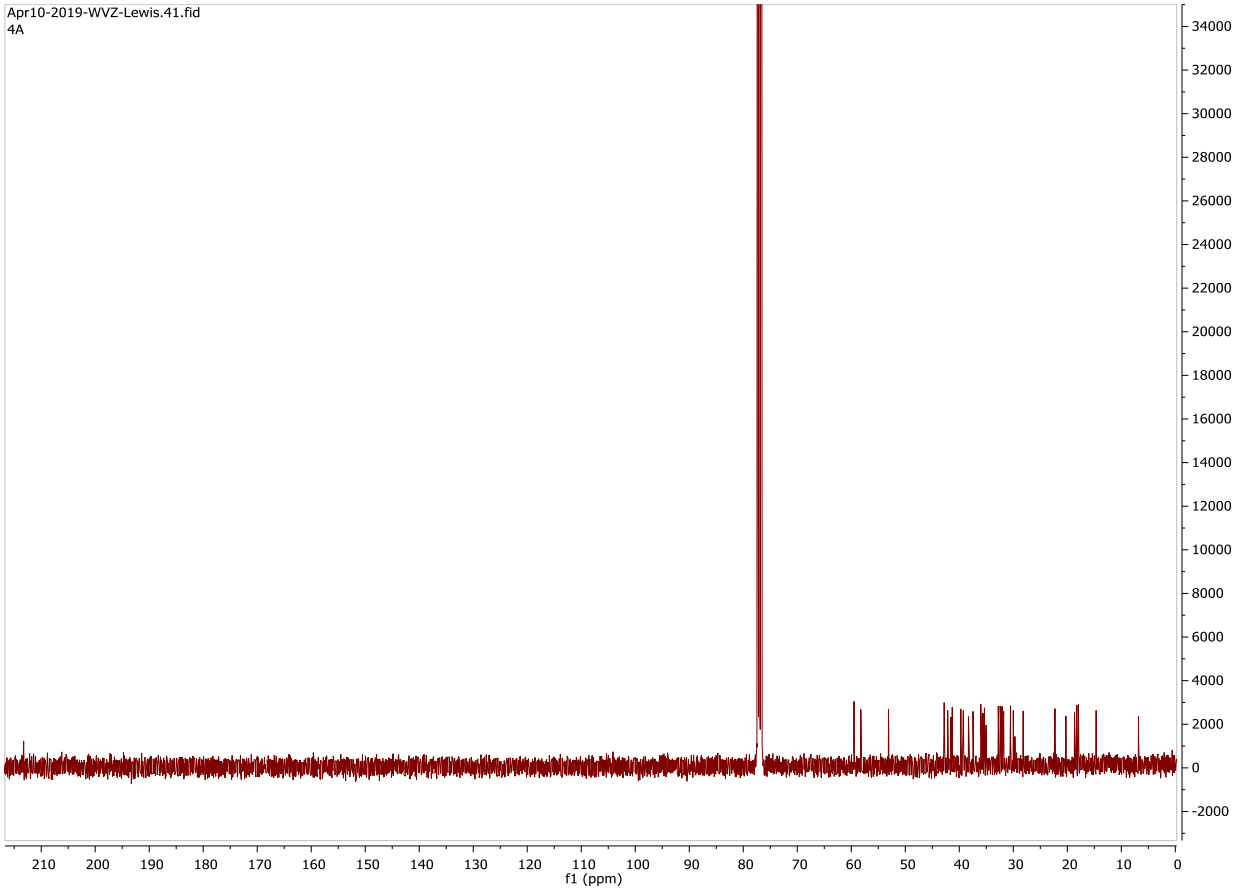


Fig. 4.4 ^{13}C NMR^ Spectrum (500MHz) of **CiPdE-1** in $CDCl_3$

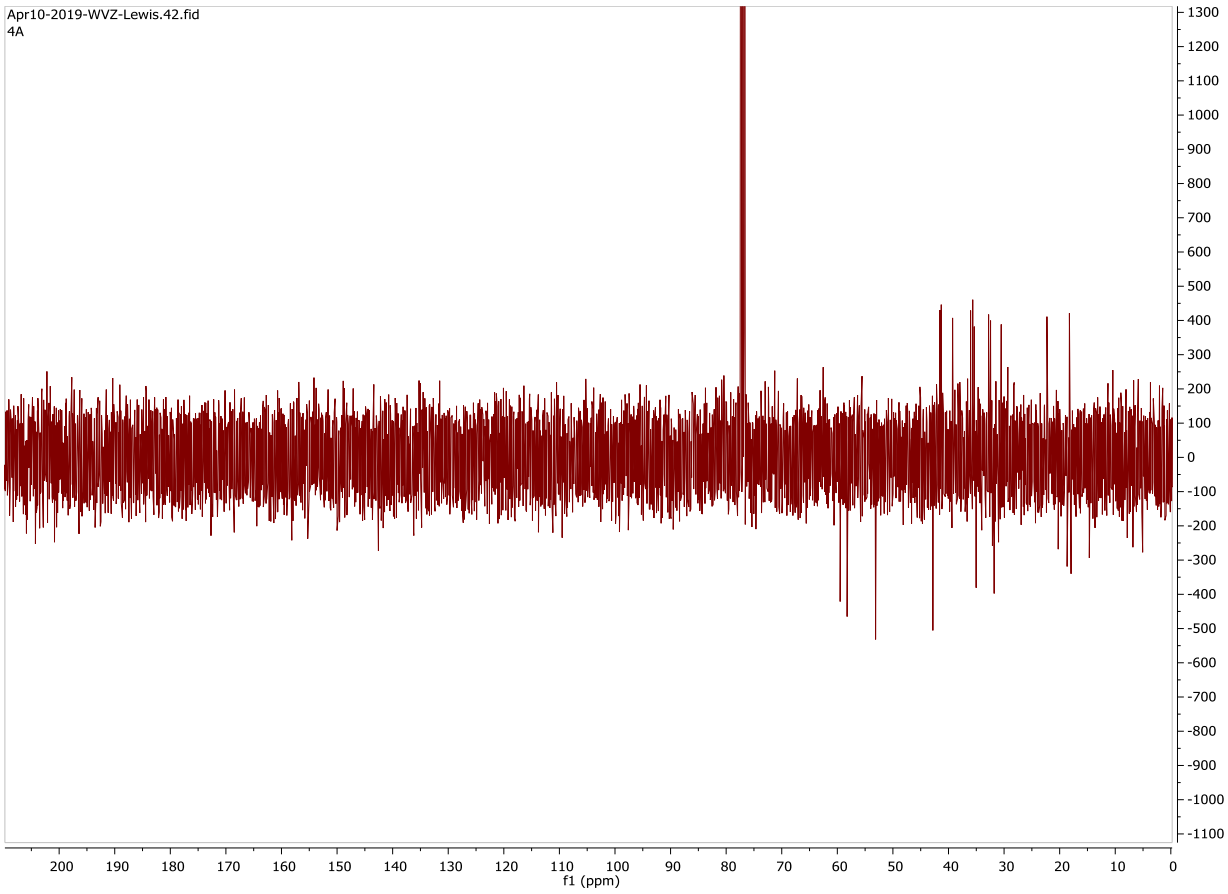


Fig. 4.5 DEPT* 135 NMR Spectrum of **CiPdE-1** in $CDCl_3$

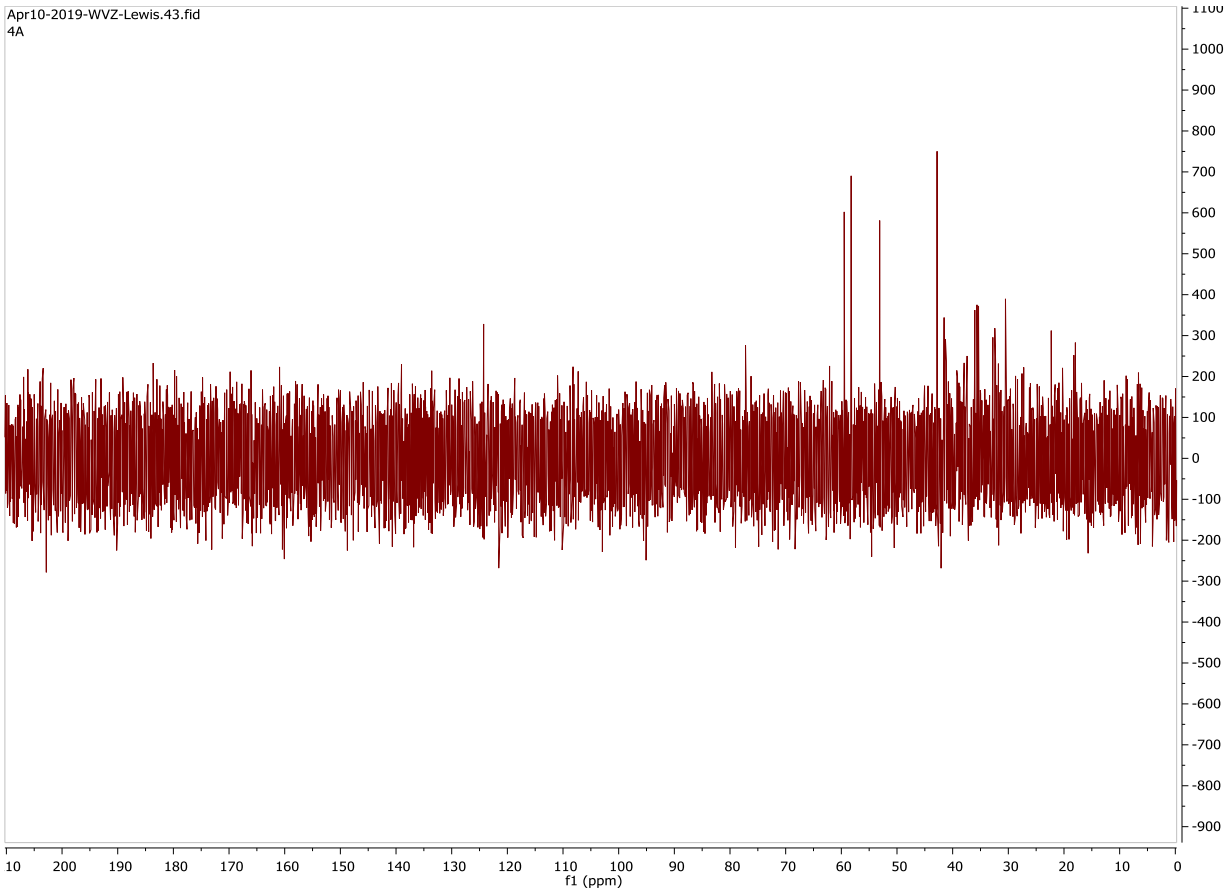


Fig. 4.6 DEPT* 90 NMR Spectrum of **CiPdE-1** in $CDCl_3$

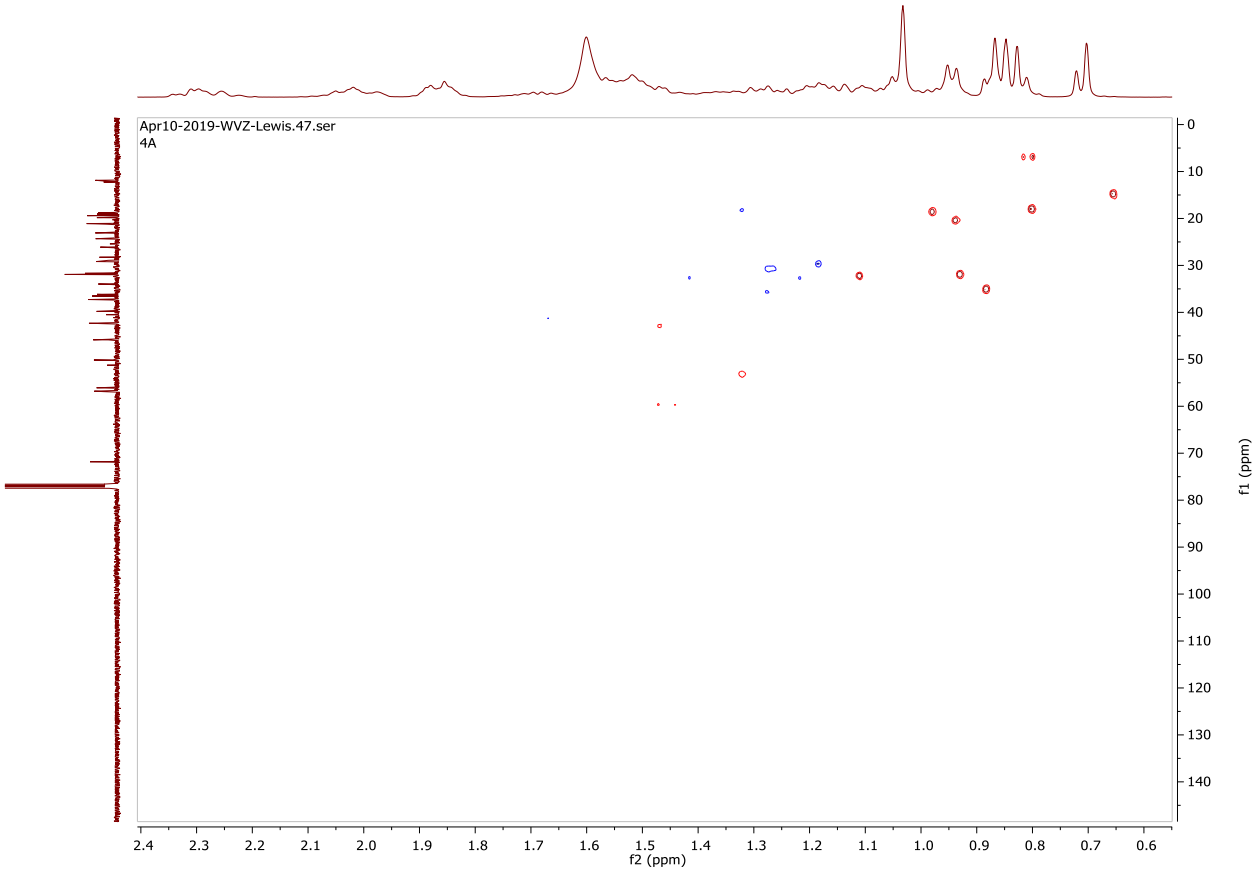


Fig. 4.7 HSQC* NMR' Spectrum of **CiPdE-1** in $CDCl_3$

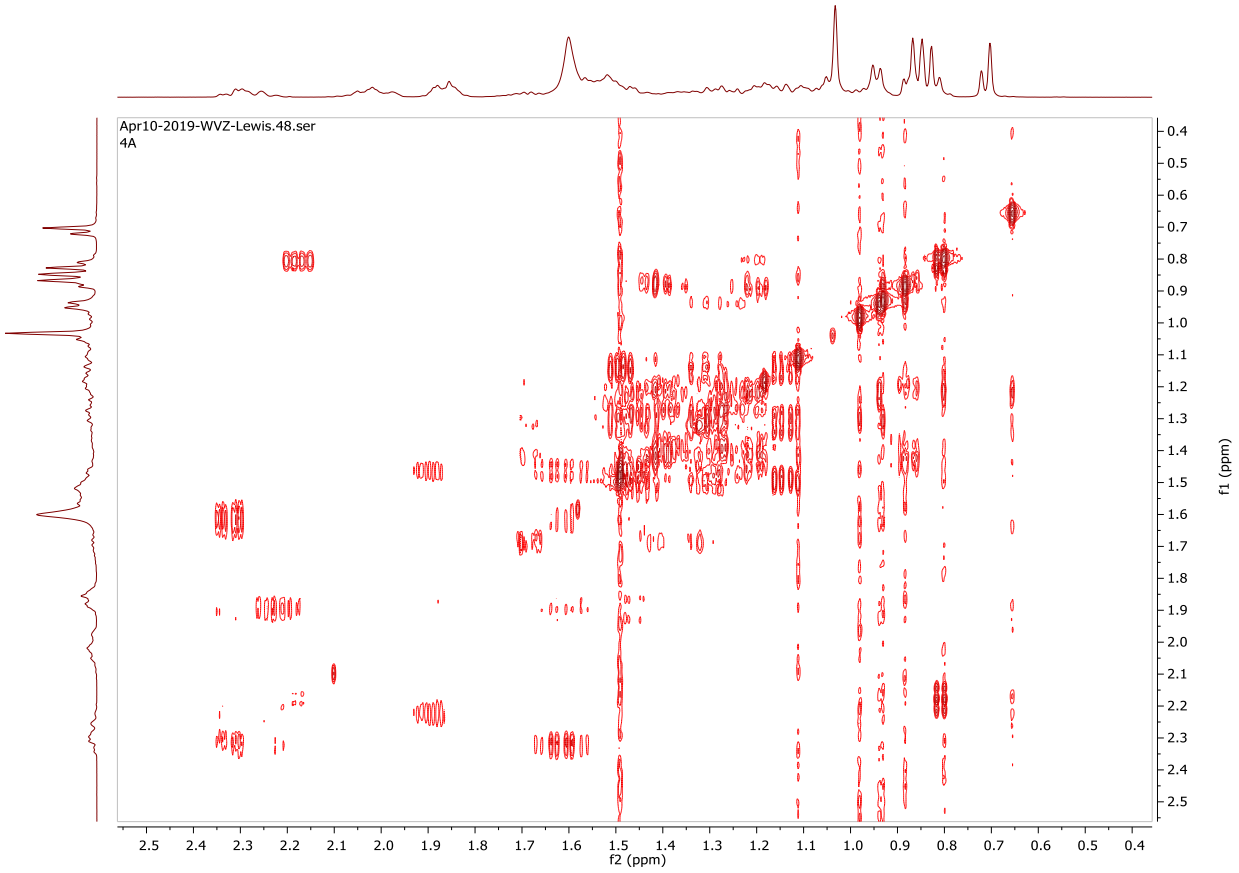


Fig. 4.8 COSY* Spectrum of **CiPdE-1** in $CDCl_3$

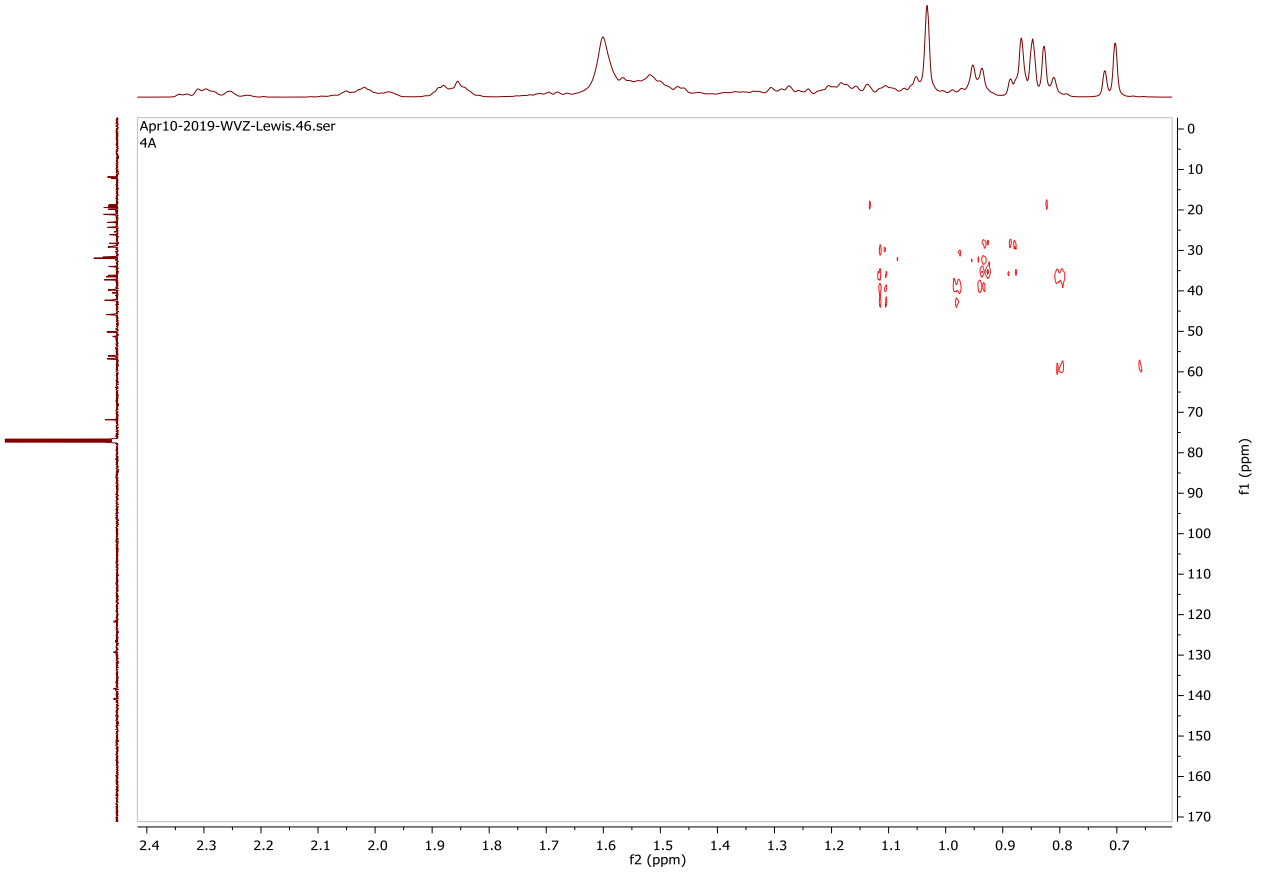


Fig. 4.9 HMBC* NMR' Spectrum of **CiPdE-1** in $CDCl_3$

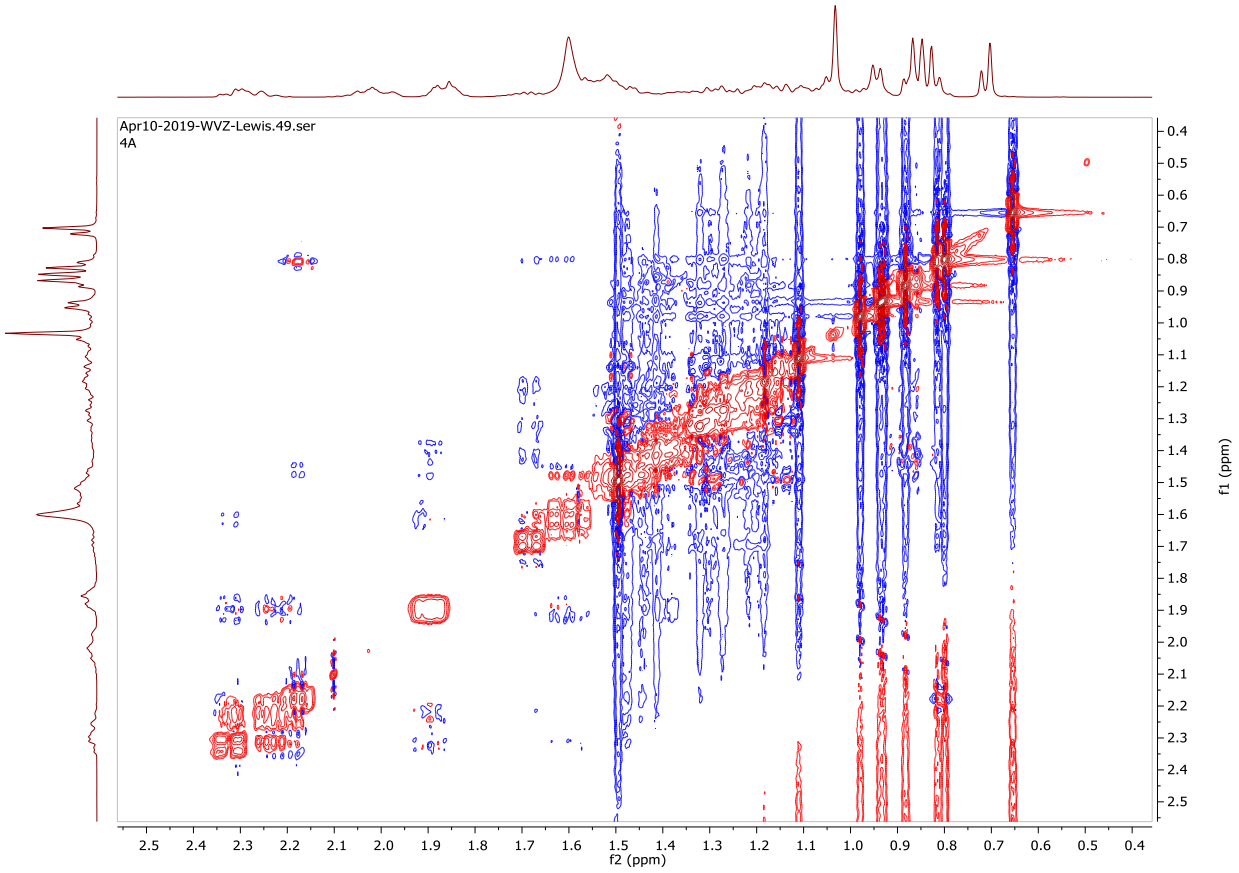


Fig. 4.10 NOESY* NMR' Spectrum of **CiPdE-1** in $CDCl_3$

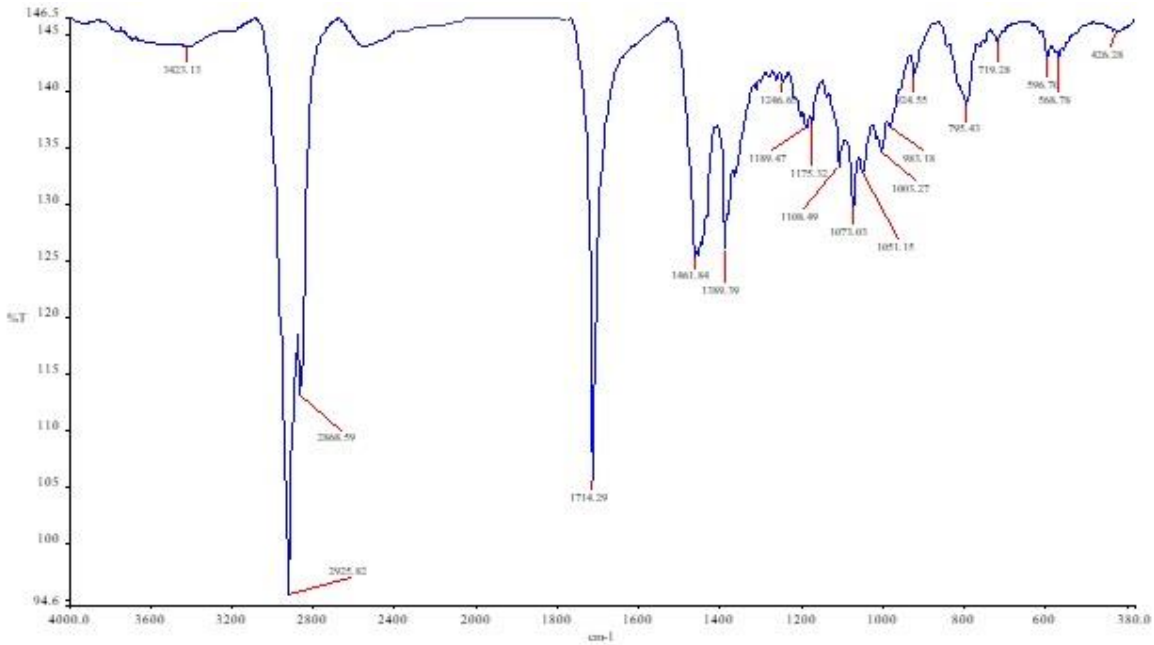


Fig. 4.11 FTIR* Spectrum of **CiPdE-1**

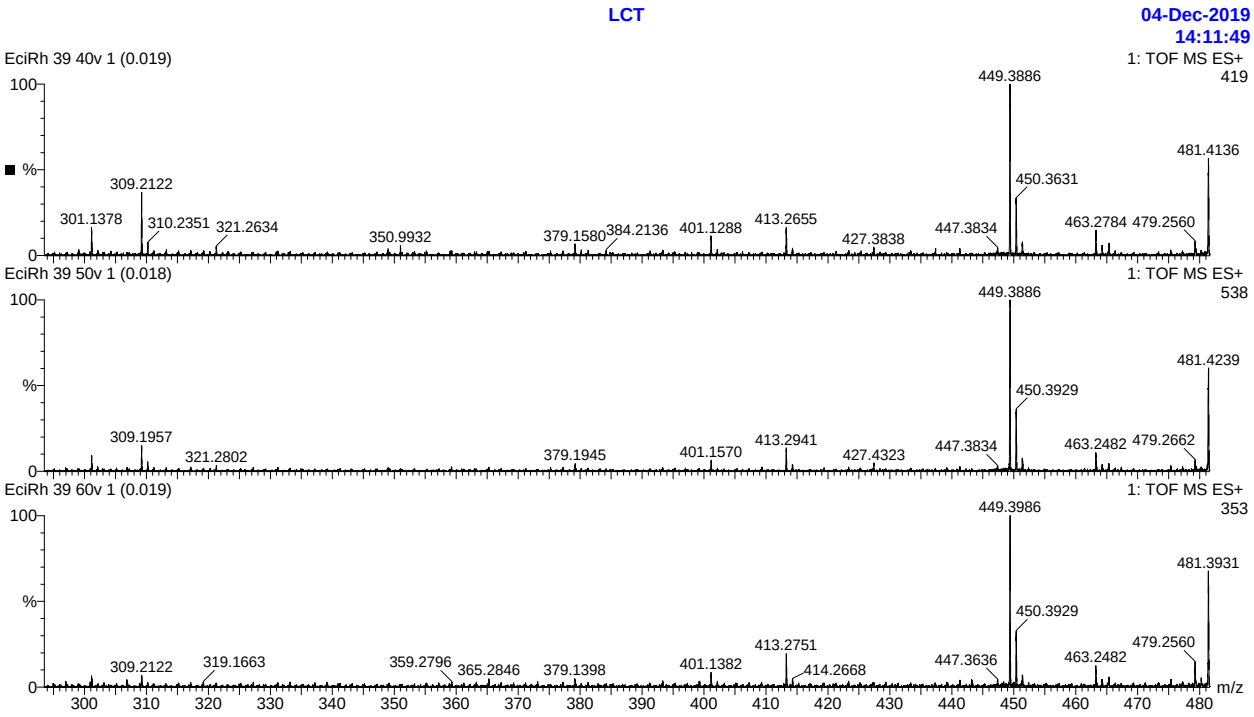


Fig. 4.12 High Resolution Mass Spectrum (TOF MS ES+) of **CiPdE-1**

4.4.1.2 Characterisation of CiPdE-2

CiPdE-2 was isolated as white-crystals (16 mg) from the pod ethyl acetate fraction of *Calophyllum inophyllum* (Scheme 1) with melting point 140-142^{o}C. This compound yielded a negative result in UV light, indicating the absence of conjugation in the structure. Spraying of CiPdE-2 with vanillin sulphuric acid solution gave an oval single spot with an R_f value of 0.5.

The mass spectrum of compound CiPdE-2 [**4.13**] gave molecular ion (M^+) with an *m/z* of 412 which supports the molecular formula $C_{29}H_{48}O$. The molecular ion (M^+) and fragmentation pattern of compound CiPdE-2 is consistent with that of stigmasterol. The IR $\bar{\upsilon}$ (cm^{-1}) spectrum of CiPdE-2, had an intense band (cm^{-1}) at 3422 (*br*) which indicates OH bond vibrations while the corresponding C=C vibrations had a weakly intense band (cm^{-1}) at 1645 (Figure 4.22). The methyl vibrations were observed as intense band (cm^{-1}) at 2933 cm^{-1}.

^{1}H NMR spectrum of CiPdE-2 (Figure 4.14), gave H-3 as triplet of triplet (tt) at δ 3.58 with *J=10.6, 4.6* Hz and H-6' showed a doublet at *δ'* 5.38 (olefine-type proton). Two olefine-type protons appeared downfield at *δ'* 5.15 and *δ'* 5.01 as doublet of doublet respectively which is diagnostic of H-22' and H-23' for stigmasterol. J values of *J=15.1, 8.5* Hz on both protons indicate the olefine-type hydrogens are coupled in an *ortho* fashion. Six (6) methyl protons were observed at (*δ'*) 1.54, 1.26, 1.04, 0.96, 0.84, 0.71 ppm. The compound had two methyl singlets at *δ'* 1.54, and *δ'* 1.26; three methyl doublets that were at (*δ'*) 1.04, 0.79, 0.71 and a methyl (CH_3) triplet at 1.04 ppm.

The ^{13}C NMR' of CiPdE-2 (Figure 4.15), gave recognizable signals at *δ'* 140.76 and *δ'* 121.72 ppm which were ascribed to 'C-5 and 'C-6 double bonds. Downfield Carbons *δ'* 138.31 and *δ'* 129.28 were correlated to 'C-22 and 'C-23 respectively. The 'C-5, 'C-6, 'C-22 and 'C-23 appeared as olefinic carbons. Carbon at *δ'* 71.78 was relative to C-3 β-hydroxyl group, *δ'* 19.87 and *δ'* 12.52 parts per million corresponds to angular carbon at 'C-19 and 'C-18. The ^{13}C-NMR and DEPT experiments differentiated twenty-nine (29) carbon signals including six (6) methyls, nine (9) methylenes, eleven (11) methine and three (3) quaternary carbons (Figure 4.16-4.17). The ^{1}H and ^{13}C values for the protons

and carbons were matched based on COSY, HMBC and HSQC correlation. COSY-experiment (Figure 4.19), gave cross-peak matches between the proton at *δ'* 5.35 (H-6) and CH_2 protons H-4 (*δ'* 2.3 and *δ'* 1.95); the doublet proton δ' 3.57 (H-3) and protons δ' 2.3 and *δ'* 1.86 on H-4. Also, the broad olefine-type proton at *δ'* 5.15 (H-23) correlated with the signal at *δ'* 1.29 (H-24). The exact positions of the double bond (between carbon 5 and 6) were detected using HMBC experiment with significant match between quartenary C-5 (140.76) and methylene protons at H-4α (2.3) and H-4β (1.9), methyl proton H-19 (0.84). Likewise, HMBC correlation exists between double bonded C-6 (121.72) and methylene protons at H-4α (2.3). Methine protons C-3 (71.78) and C-8 (29.17) correlated with methylene protons at H-4α (2.3) and methine proton at H-6 respectively. Other methine protons showed significant correlation as outlined C-9 (50.15) and methylene protons at 7α, methyl proton H-19 (0.84), methine C-14 (56.84) and H-18, H-21, C-17 (56.24) and H-18, C-20 (45.85) and H-18. Quartenry proton on C-10 (36.15) Correlated with H-6, C-13 (36.52) correlated with H-18, H-21. The positions of methyl protons were all confirmed using the HMBC experiment; upfield C-18 (12.52) correlated with H-21, C-19 (19.19) and H-11α, C-26 (20.29) and H-27, C-27(19.49) and H-26, C-29 and H-26, H-27. HSQC experiment was used as a diagnostic tool used to assign all protons attached to individual Carbons.

Comparison of the physical and spectroscopic data of stigmasterol (CiPdE-2) [**4.13**], correlated with literature data earlier reported for phytosterols (Mahato and Kundu, 1994; Li *et al*., 2006).

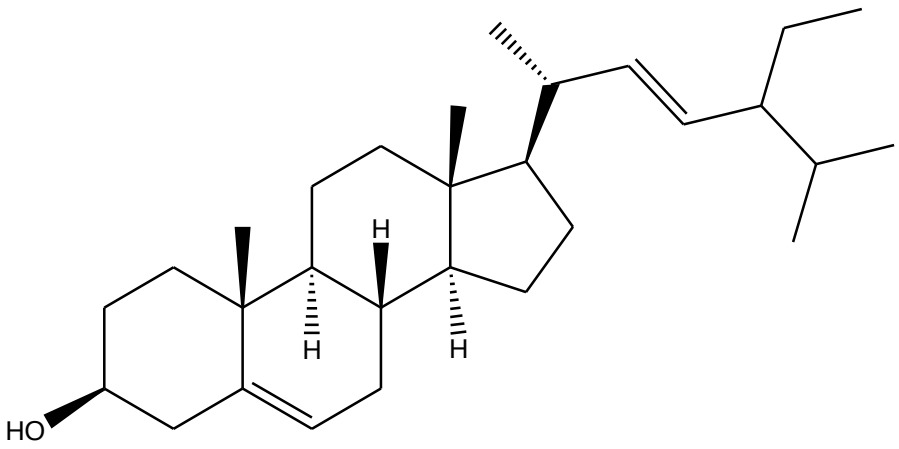


Fig. [4.13] Structure of CiPdE-2 (Stigmasterol)

Table 4.30. NMR spectral data of CiPdE-2 in $CDCl_3$

Position	**C**	**δ ^{13}C**	**δ ^{1}H**	**M"**	***J'* Values (*Hz*)**
1	$^{b}CH_2$	37.46	1.1, 1.84	2H, *m*	
2	$^{b}CH_2$	31.92	1.89, 1.43	2H, *m*	
3	^{c}CH	71.78	3.58	1H, *tt'*	*J=10.5, 4.6*
4	$^{b}CH_2$	42.45	2.31, 1.84	2H	
5	^{d}C	140.76	-	-	
6	^{c}CH	121.72	5.38	1H, *d'*	*J=5.0*
7	$^{b}CH_2$	34.06	1.42	2H	
8	^{c}CH	29.17	1.28	1H	
9	^{c}CH	50.15	0.99	1H	
10	^{d}C	36.15	-	-	
11	$^{b}CH_2$	22.99	1.29	2H, *m*	
12	$^{b}CH_2$	39.63	2.05	2H	
13	^{d}C	36.52	-	-	
14	^{c}CH	56.84	1.04	1H	
15	$^{b}CH_2$	24.21	1.12	2H	
16	$^{b}CH_2$	28.55	1.28	2H	
17	^{c}CH	56.24	1.15	1H	
18	$^{a}CH_3$	12.52	1.26	3H, *s*	
19	$^{a}CH_3$	19.19	0.84	3H, *d'*	*J=7.3*
20	^{c}CH	45.85	0.96	1H	
21	$^{a}CH_3$	21.21	1.54	3H, *s*	
22	^{c}CH	138.31	5.01	1H, *dd'*	*J=15.1, 8.4*

23	^{c}CH	129.28	5.15	1H, *dd'*	*J=15.1, 8.6*
24	^{c}CH	51.25	1.29	1H	
25	^{c}CH	31.90	1.89	1H	
26	$^{a}CH_3$	20.29	0.79	3H, *d'*	*J=6.8*
27	$^{a}CH_3$	19.49	1.04	3H, *d'*	*J=7.8*
28	$^{b}CH_2$	26.20	1.18	2H	
29	$^{a}CH_3$	11.87	0.71	3H, *d'*	*J=7.3*

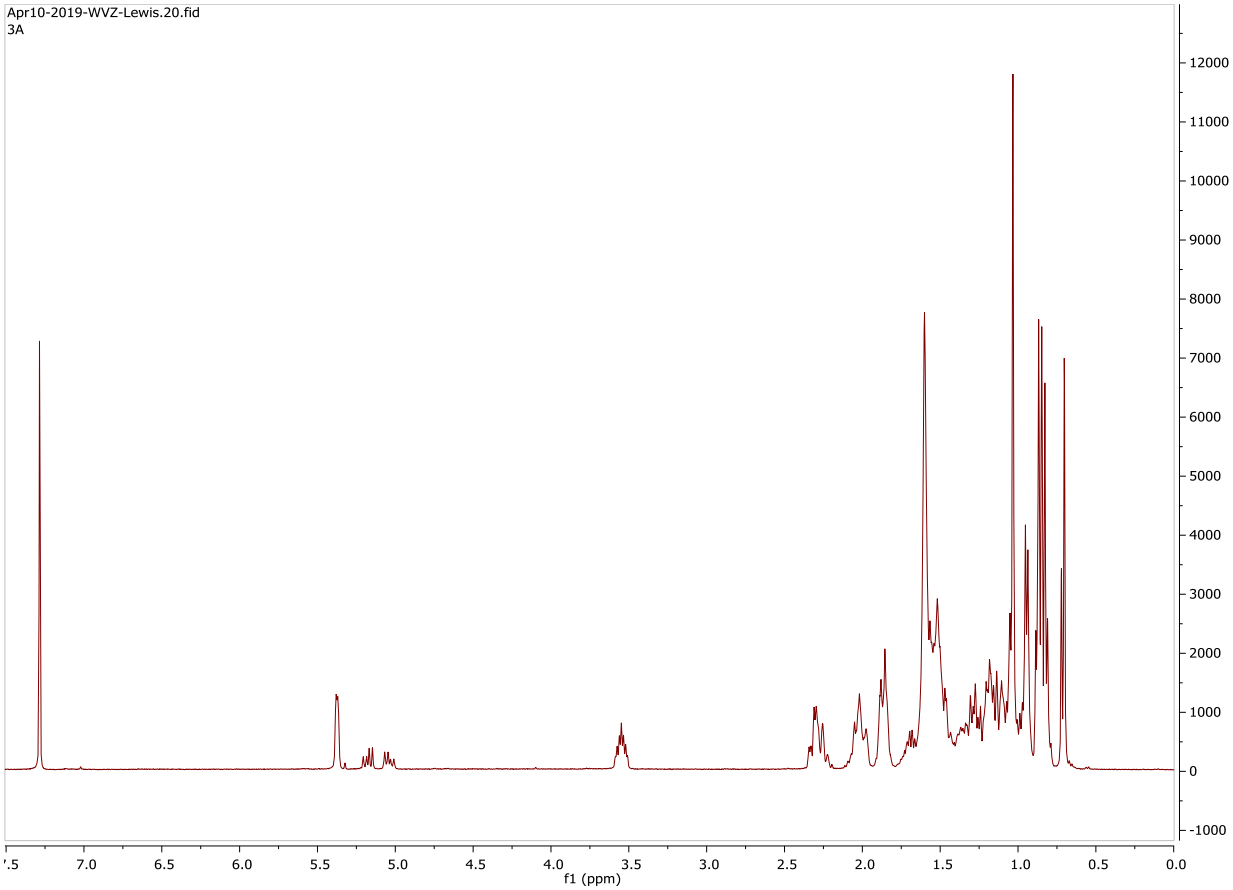


Fig. 4.14 ^{1}H' NMR^ Spectrum (500MHz) of **CiPdE-2** in $CDCl_3$

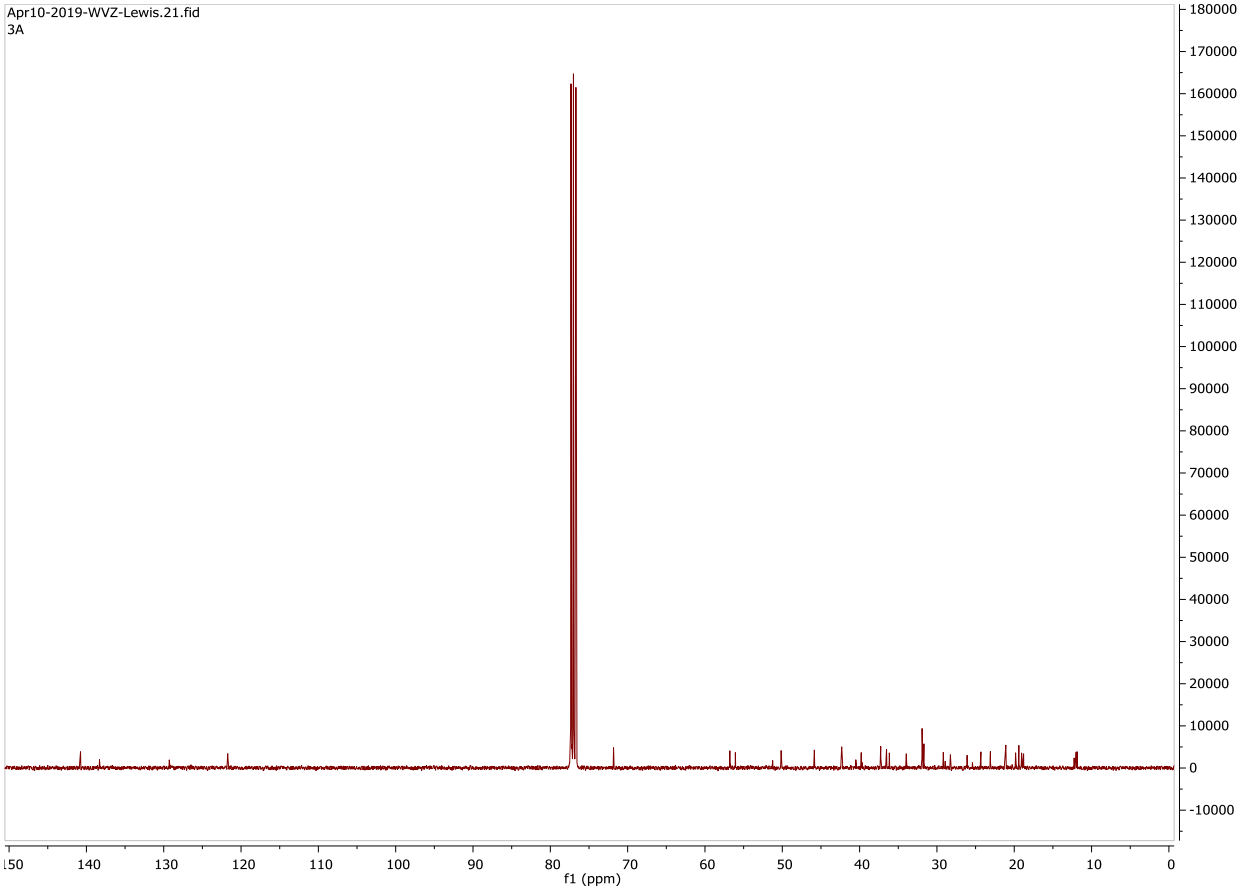


Fig. 4.15 ^{13}C' NMR^ Spectrum (500MHz) of **CiPdE-2**

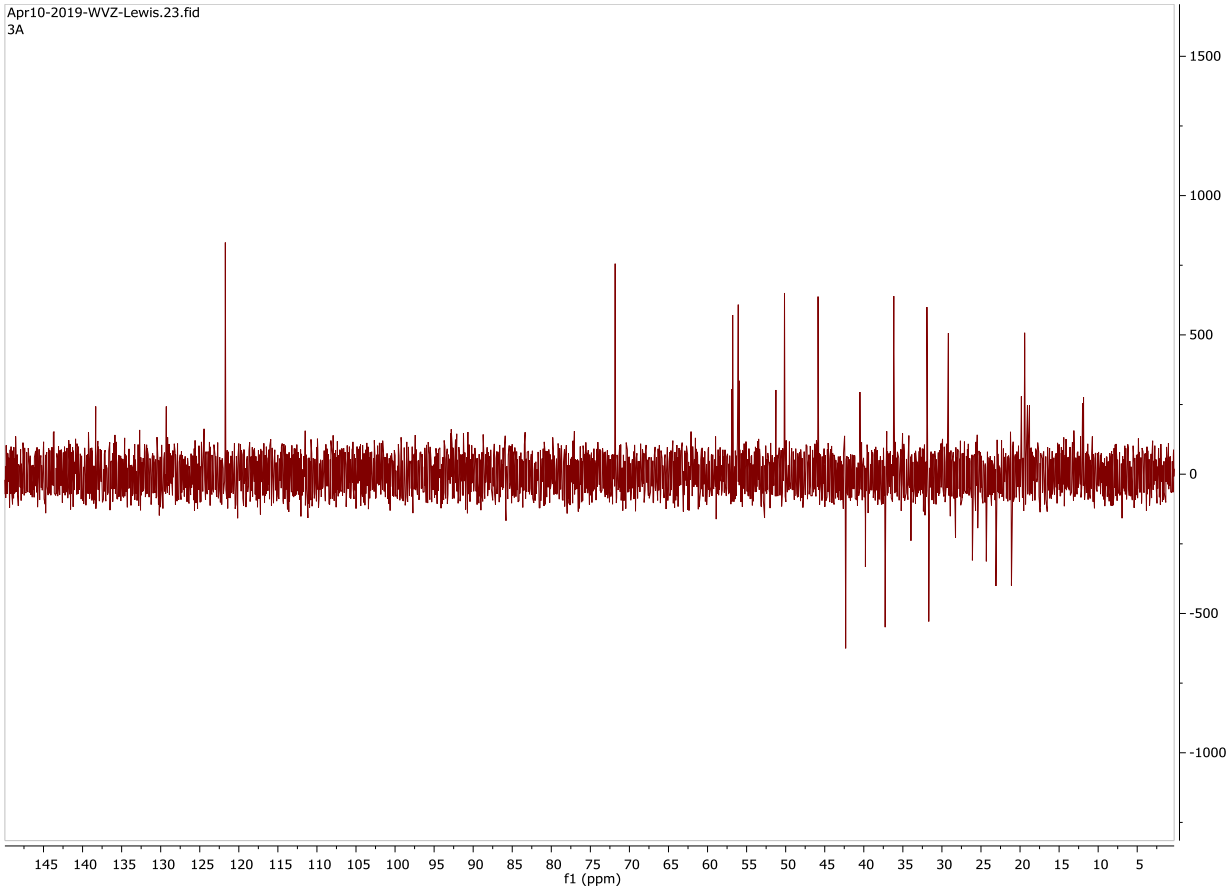


Fig. 4.16 DEPT$^{\wedge}$ 135 Spectrum of **CiPdE-2** in $CDCl_3$

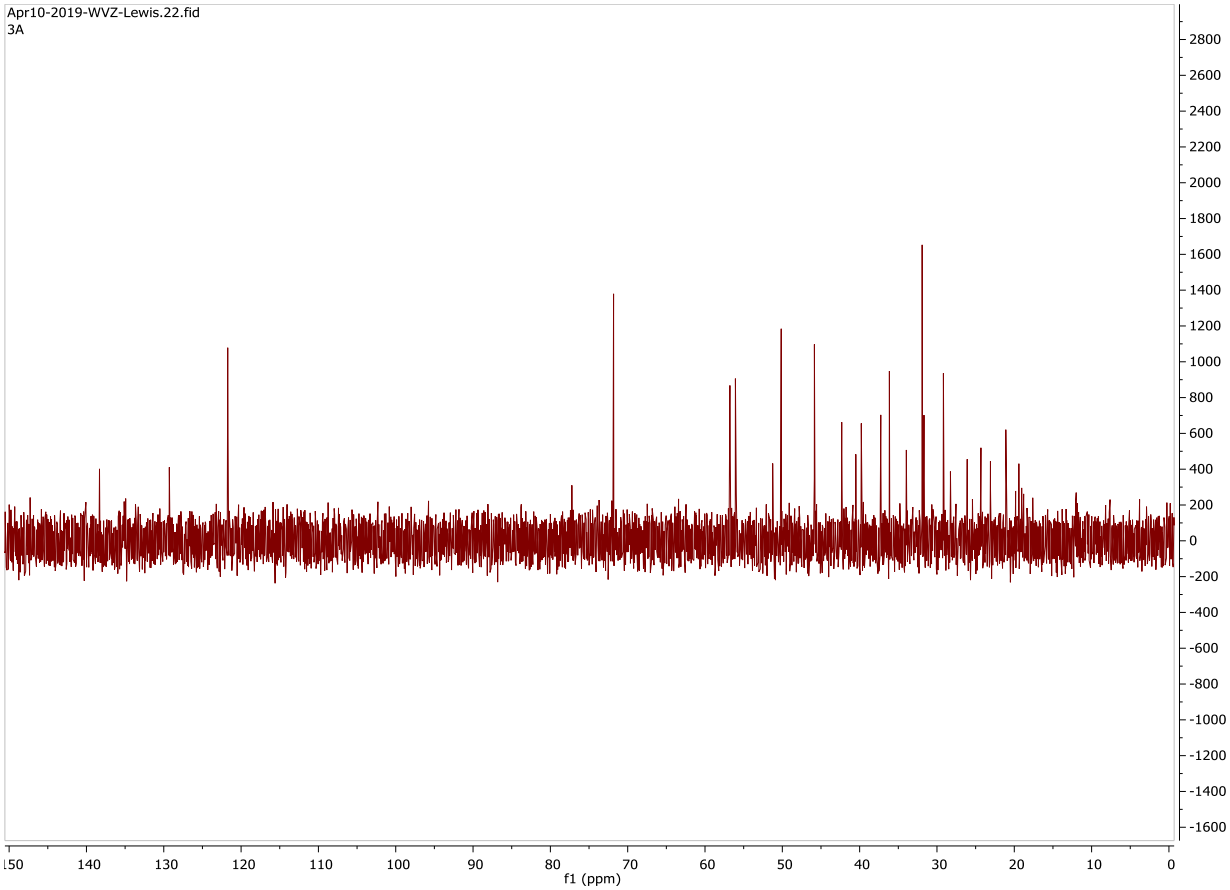


Fig. 4.17 DEPT. 90 Spectrum of **CiPdE-2** in $CDCl_3$

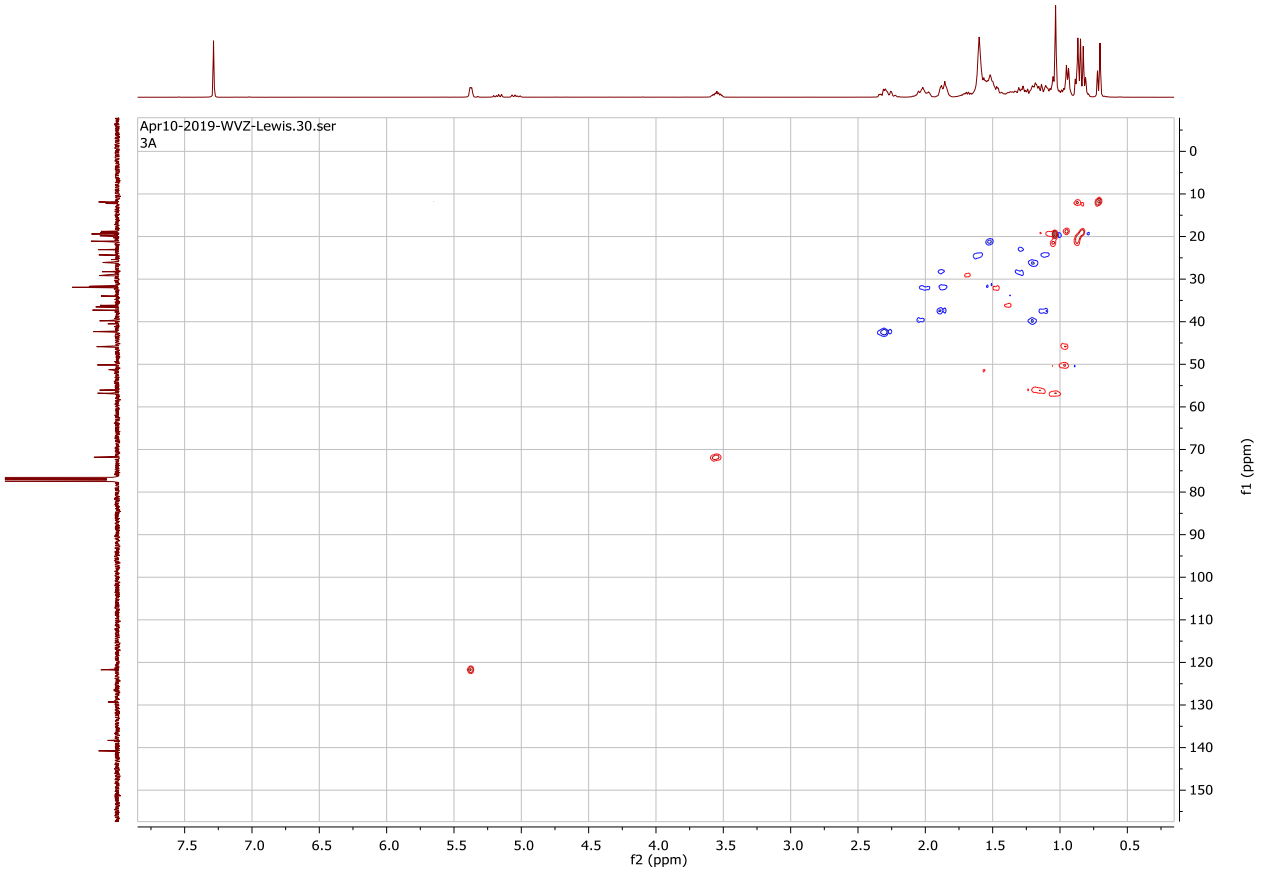


Fig. 4.18 HSQC* experiment of **CiPdE-2** in $CDCl_3$

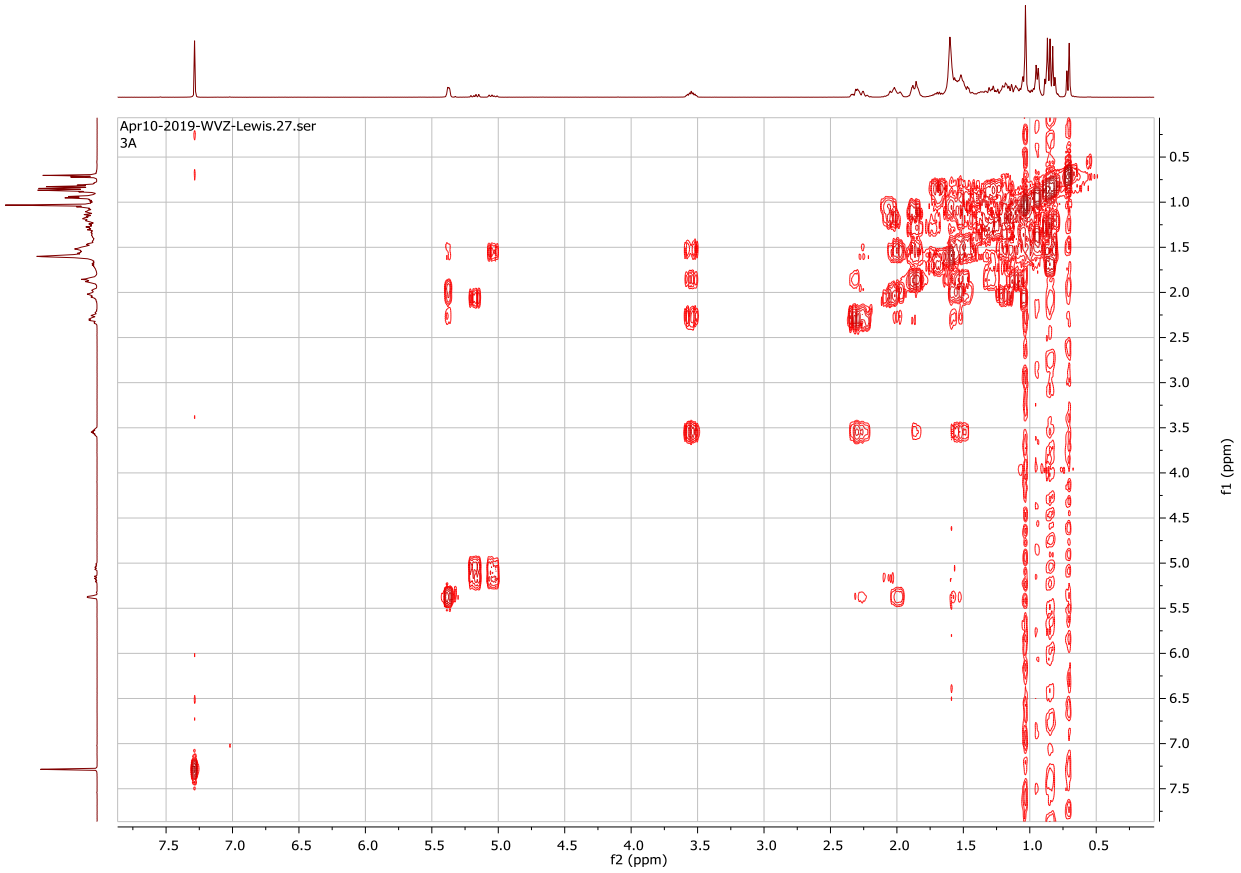


Fig. 4.19 COSY* experiment of CiPdE-2 in $CDCl_3$

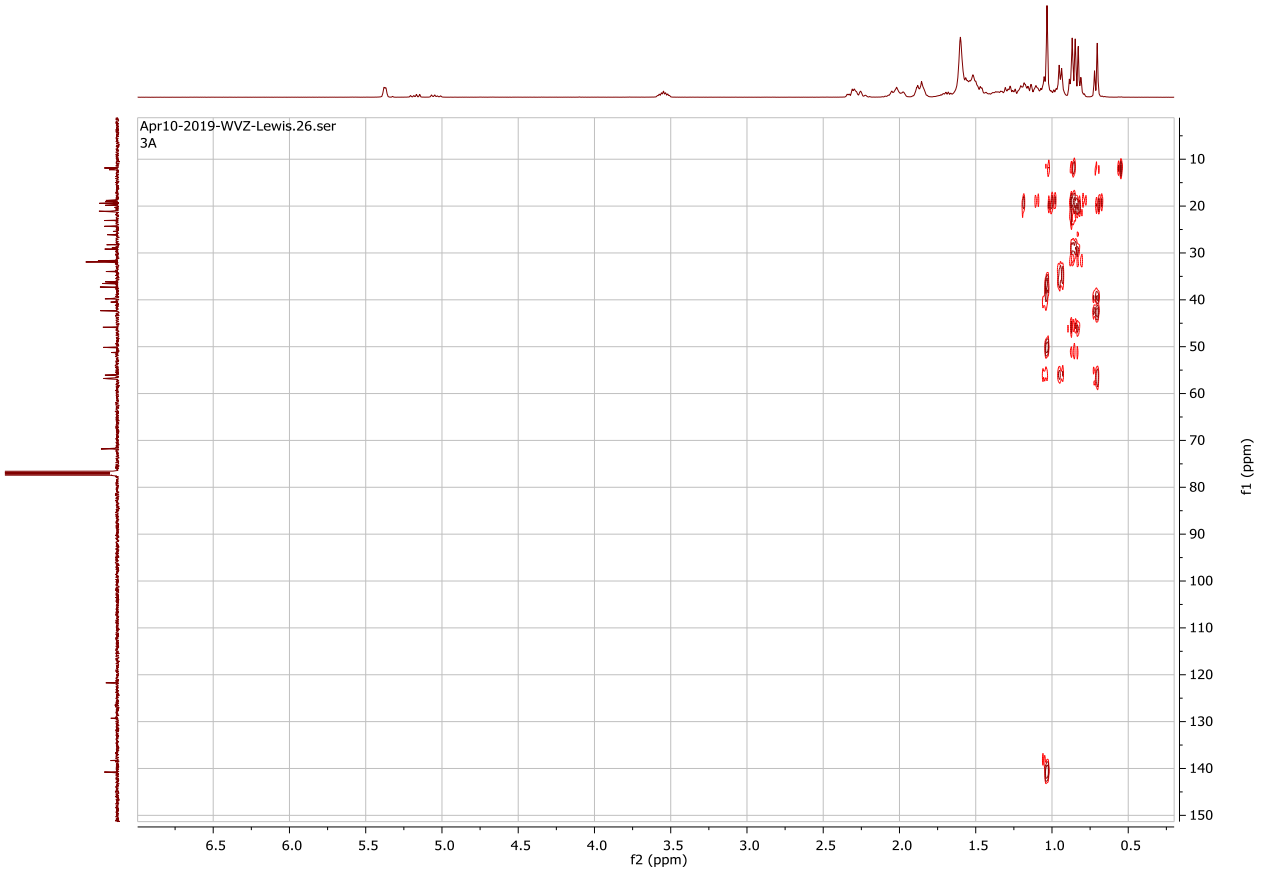


Fig. 4.20 HMBC* experiment of CiPdE-2 in $CDCl_3$

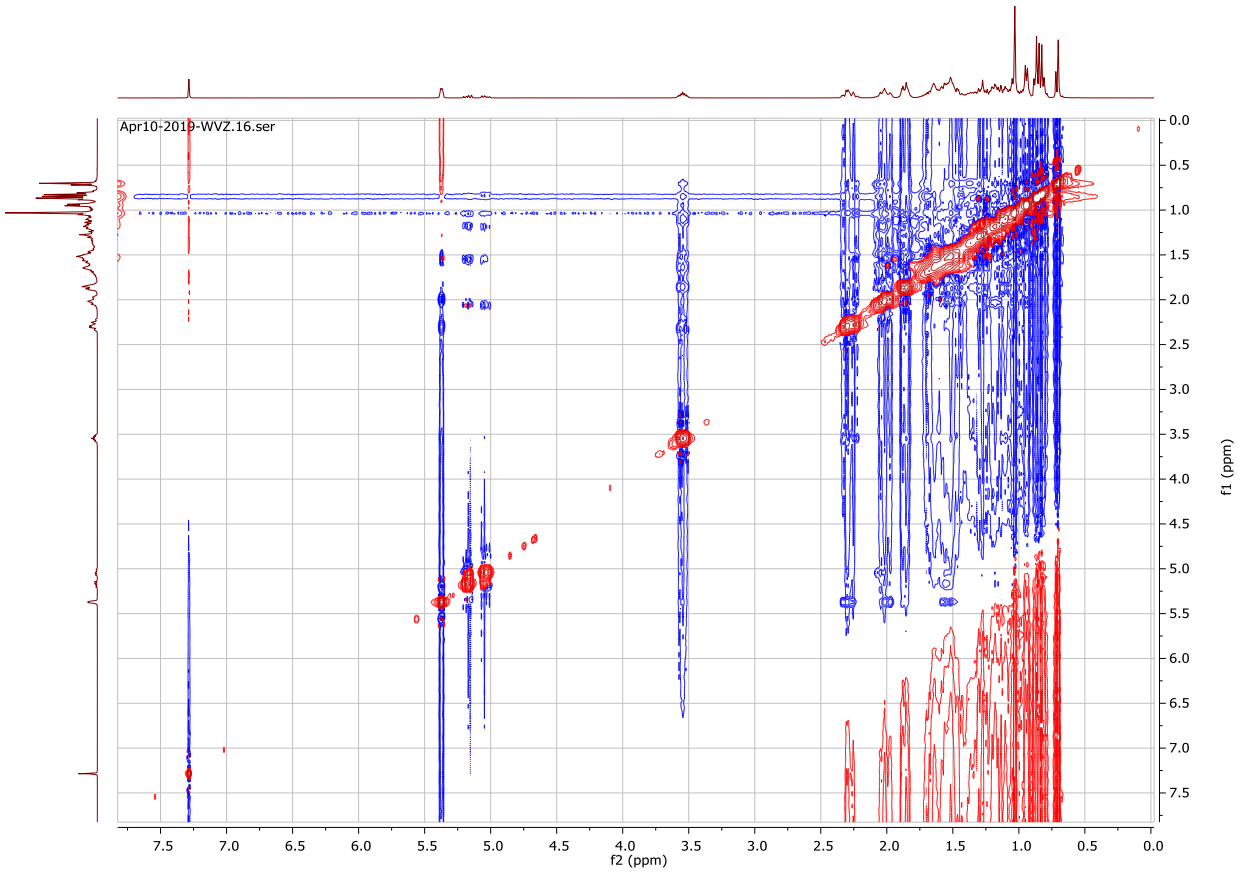


Fig. 4.21 NOESY experiment of CiPdE-2 in $CDCl_3$

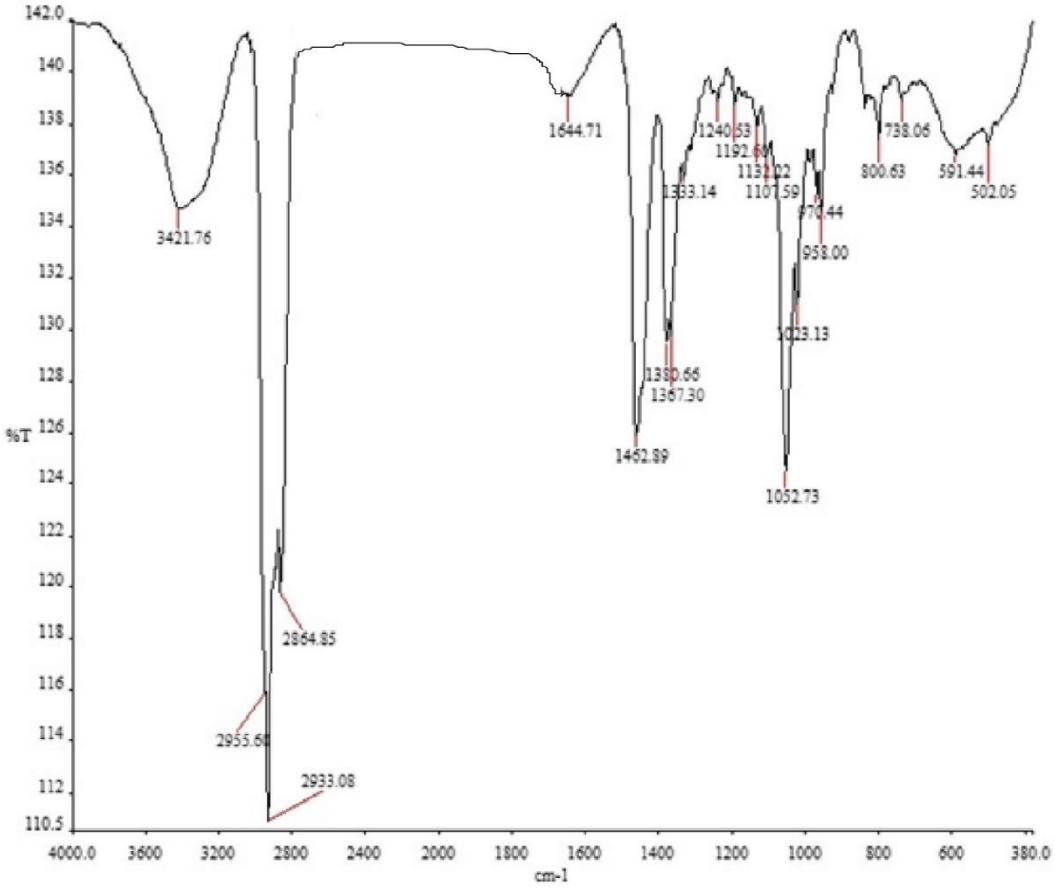


Fig. 4.22 FTIR Spectrum of CiPdE-2

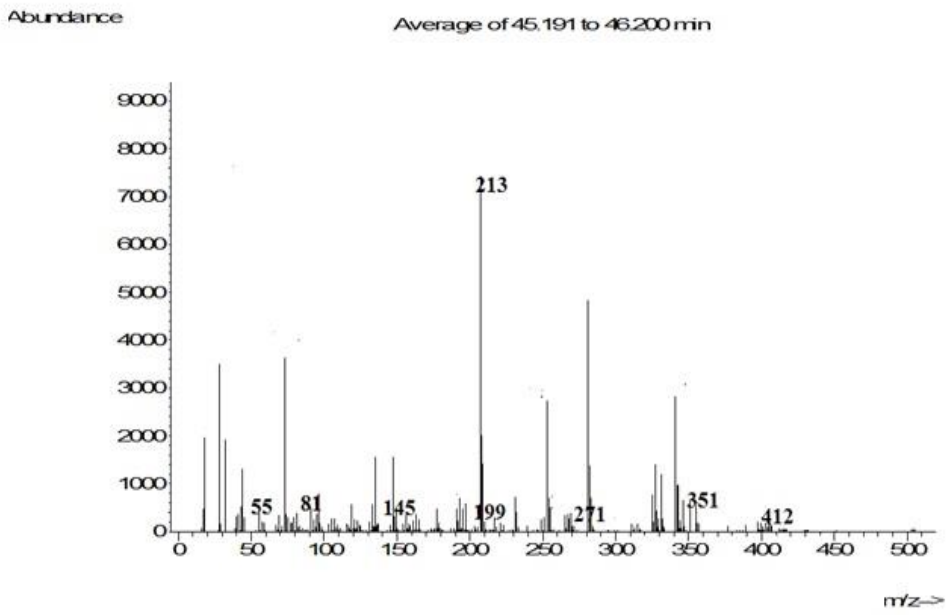


Fig. 4.23 Mass Spectrum of **CiPdE-2**

4.4.1.3. Characterization of CiPdE-3

CiPdE-3 **[4.24]** was isolated as yellow crystals from pod ethyl acetate fraction of *Calophyllum inophyllum* (Scheme 1) with melting point 182-184^{o}C. A colour change upon addition of $FeCl_3$ indicated the presence of phenolic groups in the compound with melting point 182-184^{o}C. Spraying of CiPdE-3 with vanillin sulphuric acid gave an oval single spot with an R_f value of 0.5.The **EI-MS** showed the base peak at *m/z* 274.2 [M^+], which corresponds to the ascribed molecular formula $C_{14}H_{10}O_6$ (Figure 4.36). The molecular mass of compound CiPdE-3 was confirmed by mass spectrometric studies, using Electrospray Ionisation positive (ESI+) technique (Figure 4.37). These experiments revealed base peak (*m/z*) 297.052 [M + Na], which corroborates the assigned molecular formula ($C_{14}H_{10}O_5$) of the compound.

IR $\bar{\upsilon}$ (cm^{-1}) spectrum, gave an intensely broad band (cm^{-1}) at 3222 and moderately intense band cm^{-1} at 988 which confirmed the presence of OH vibrations with corresponding bending absorption as a weak peak (cm^{-1}) at 1440 (Figure 4.35). The peak at 3458 cm^{-1} reflects hydrogen bonded OH bond vibrations. The vibrations as a consequence of methyl C-H absorption was observed by the moderately intense band (cm^{-1}) at 3096 and 2934. The methyl of the methoxyl group attached at position 2 showed characteristic bending absorption at 1372 cm^{-1}. C-H stretch for sp^2 C-H occurred at 3096 cm^{-1} and it's out of plane bending was observed in the range of 1000 to 650 cm^{-1}. C=C aromatic stretch was observed at 1611 cm^{-1} and 1460 cm^{-1} due to extension of the conjugation of benzene rings by the carbonyl group. C-O stretch vibrations occurred at 1264-1074 cm^{-1} which is characteristic of phenyl alkyl ethers. C=O absorption appeared at 1653 cm^{-1} characteristic of a conjugated phenyl keto-carbonyl group. Strong absorption was observed at about 1247 cm^{-1} and 1074 cm^{-1}.

^{1}H-NMR of CiPdE-3 (Figure 4.25), gave a hydroxyl proton as singlet at δ 12.52 and H'-4 an aromatic proton showed a singlet at δ' 6.52. 3H methoxyl proton showed sharp recognizable peak overlapping with solvent peak at δ' 3.45. H-7 appeared as a singlet with chemical shift of δ 6.95. H-7 and H-8 (aromatic hydrogens) appeared as doublets with chemical shifts δ 6.98 (*d*, 1H, *J' = 7.6 Hz*, H-5) and δ 7.25 (*d*, 1H, *J' = 7.6 Hz*, H-8) respectively. J value of *7.6* Hz is indicative of *ortho* coupling. The compound showed the

presence of two methyl singlet at δ 1.53 and δ 1.26; three methyl (CH_3) doublets that appeared at δ' 0.92, δ' 0.79, δ' 0.69 ppm; and a methyl triplet at δ' 1.04. ^{13}C NMR and DEPT experiment afforded 8 quaternary carbons, 5 methine carbons and one methyl carbon atom. ^{13}C NMR showed recognizable signals at 60.4 ppm for CH_3 of the methoxyl group (OCH_3) attached to C-2. Downfield Carbon at δ 181.0 ppm was ascribed to the carbonyl carbon at C'-9 while C'-1, C'-3, and C'-6 are downfield protons attached to OH groups with chemical shifts δ 154.4, δ 159.7 and δ 120.83 respectively. C-4, C-5, C-7, and C-8 are aromatic protons with chemical shifts δ 94.7 (C'-4), δ 146.9 (C'-5), δ 124.53 (C'-7), and δ 120.93 (C'-8) respectively. Two α carbons to the carbonyl carbons are δ 114.96 (C-8a) and δ 102.74 (C-9a). δ 152.9 (C-4a) and δ 145.3 (C-10a) are carbons linking the oxygen atom to the benzene rings.

COSY experiment (Figure 4.32), showed cross-peak correlation at δ 3.75 (Me). The exact position for the methoxy group (OCH_3) at C-2 was dediphered by the HMBC experiment where significant 2J and 3J correlations were observed from the methoxyl proton at δ' 3.75 (H-2) and the acetoxyl carbonyl carbon at δ 131.37 ppm. HSQC was used to confirm that methyl methoxyl proton (3H) at δ 3.75 ppm (H-2) is bonded with carbon δ 60.43 ppm (Figure 4.31).

Comparison of the physical and spectroscopic data of 1,3,5-trihydroxy-2-methoxyxanthone (CiPdE-3) [**4.24**], correlated with literature data earlier reported for xanthones (Mahato and Kundu, 1994; Li *et al*., 2006).

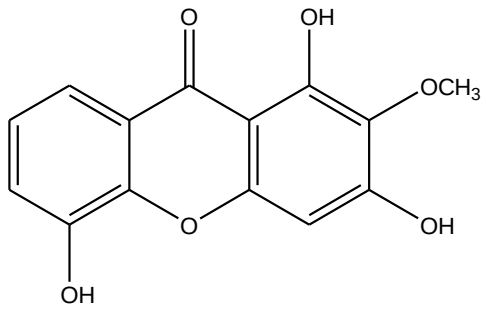


Fig. [4.24] Structure of CiPdE-3 (1,3,5-trihydroxy-2-methoxyxanthone)

Table. 4.31 ^{1}Proton and ^{13}Carbon NMR data of CiPdE-3 in DMSO

Position	**C.**	**Δ. ^{13}C.**	**δ ^{1}H.**
1	^{c}C-OH	154.4	δ 12.52 (*s*, 1H., 1-OH)
2	^{d}C	131.2	-
3	^{c}C-OH	159.7	-
4	^{b}CH	94.7	*δ'* 6.52 (*s*, 1H., H-4)
4a	C	152.9	-
5	^{c}C-OH	146.5	-
6	^{b}CH	121.0	-
7	^{b}CH	125.0	*δ* 6.98 (*dd.*, 2H., *J'* = 7.6 Hz, H-5)
8	^{b}CH	115.0	*δ* 7.25 (*d.*, 1H., *J'* = 7.6 Hz, H-8)
8a	^{d}C	121.0	-
9	^{d}C	181.0	-
9a	^{d}C	102.7	-
10	^{d}C	145.3	-
OMe	$^{a}CH_3$	60.5	*δ'* 3.75 (*s*, 3H., 2-OMe)

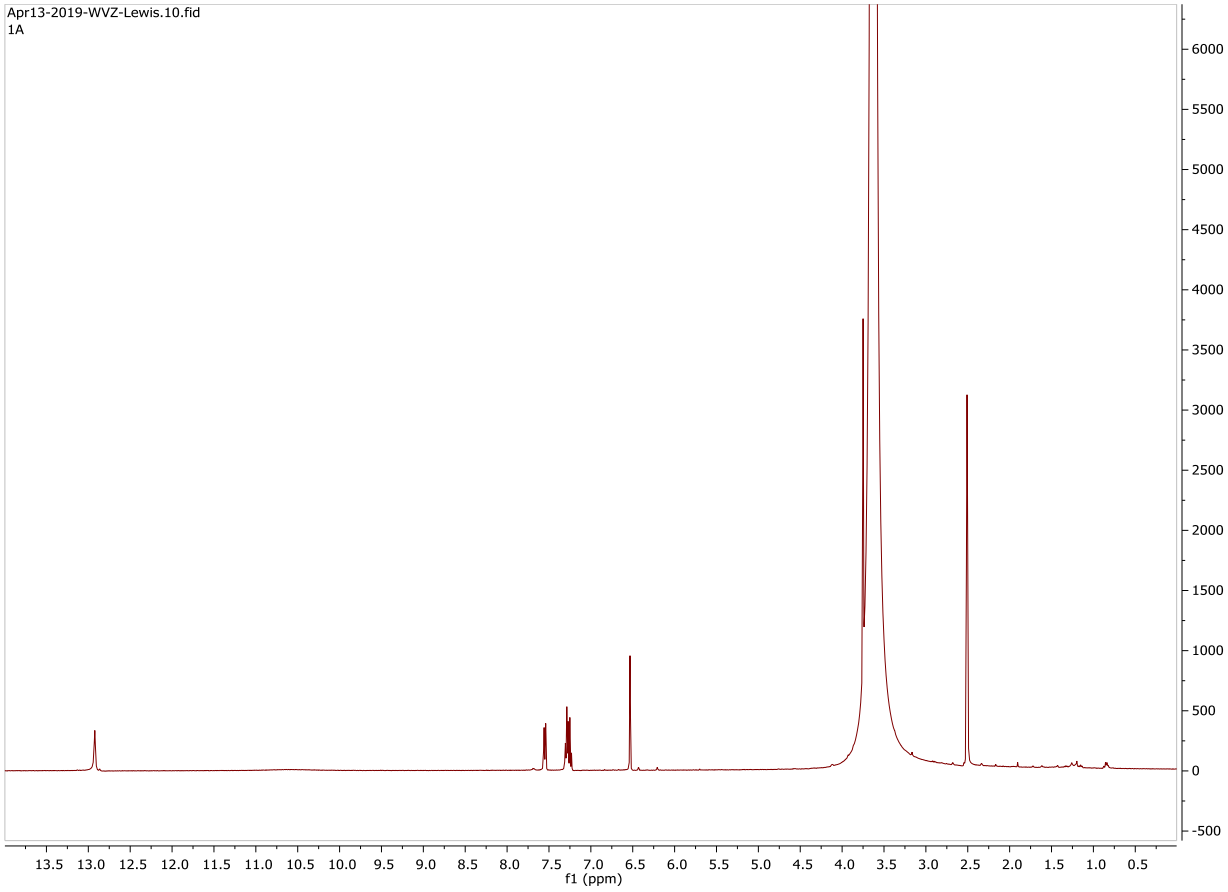


Fig. 4.25 Proton (^{1}H NMR) * Spectrum (500MHz) of **CiPdE-3**

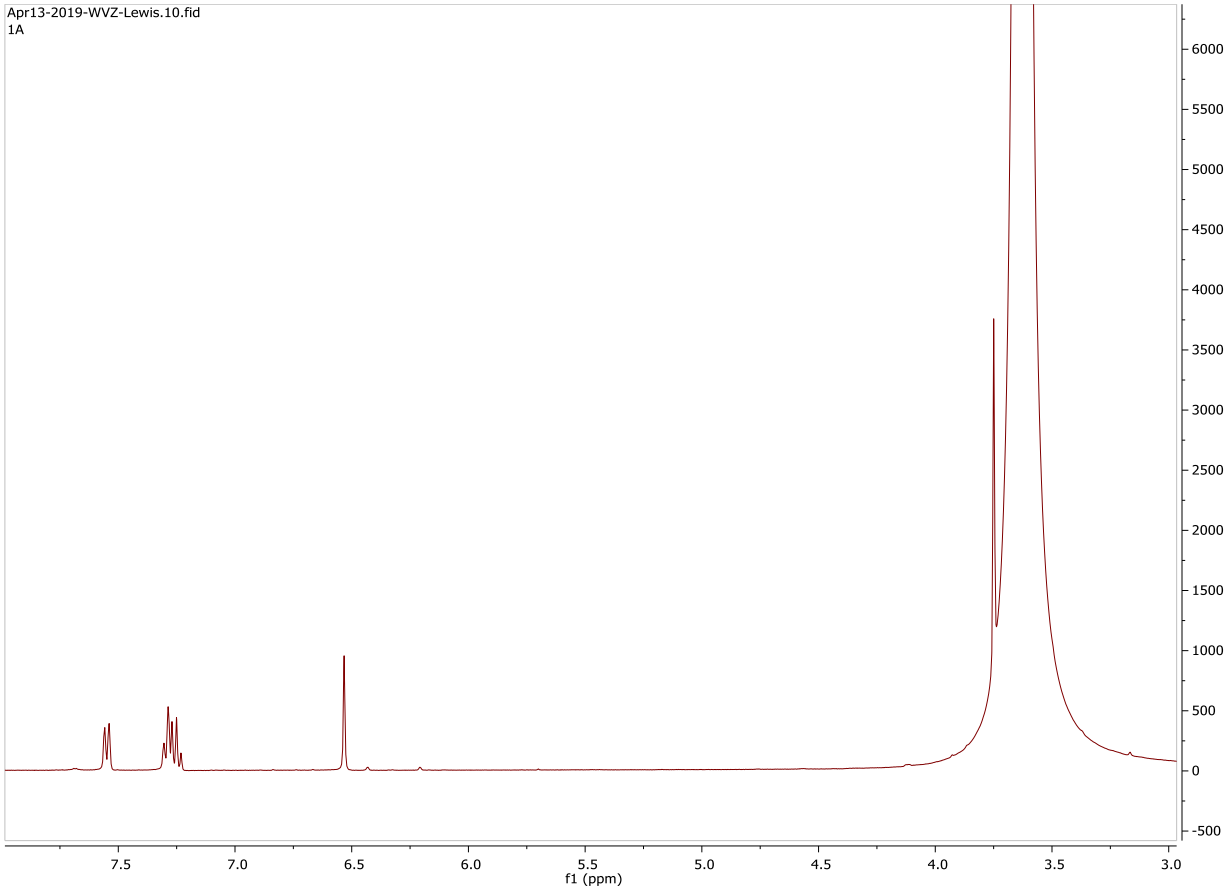


Fig. 4.26 ^{1}H NMR* Spectrum (500MHz) of **CiPdE-3** (Expanded copy 3-8ppm)

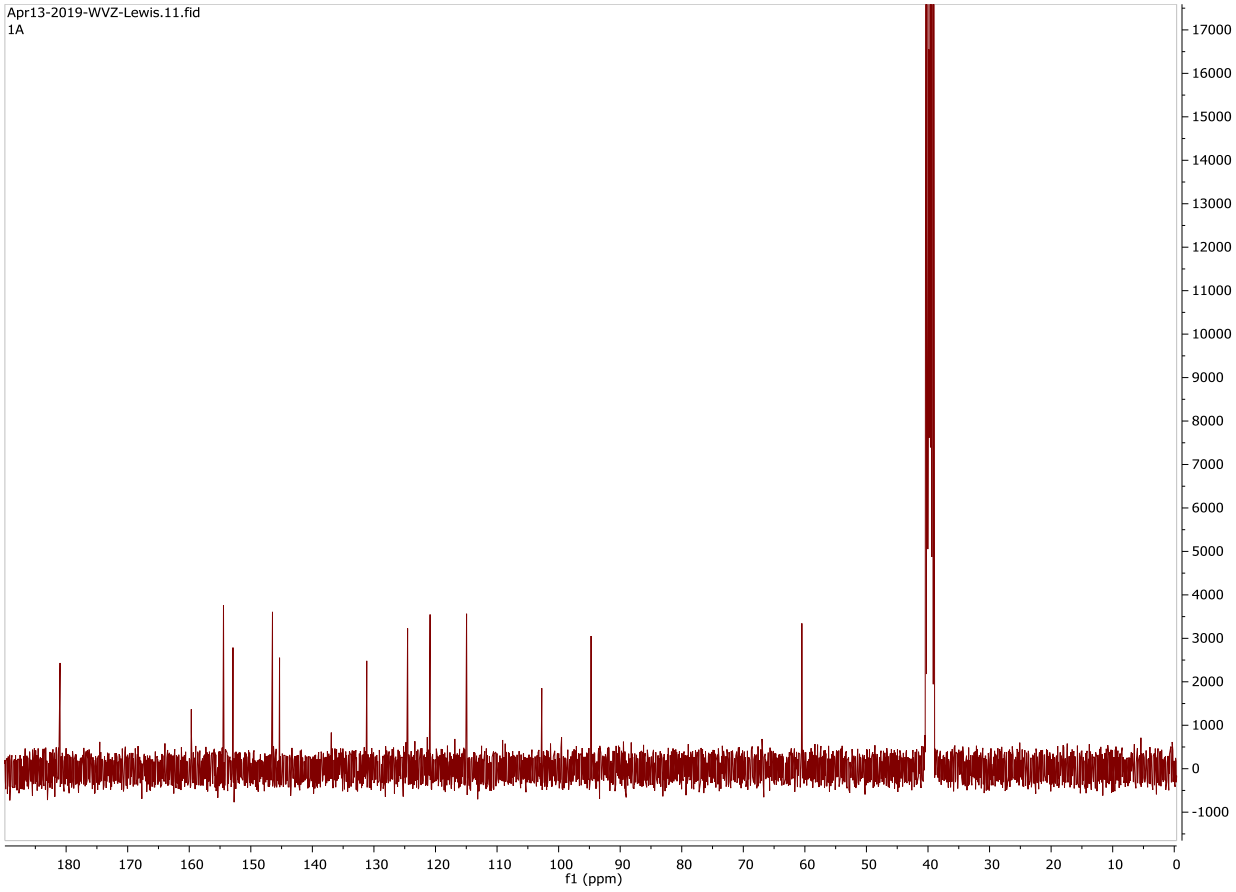


Fig. 4.27 ^{13}C NMR* Spectrum (500MHz) of **CiPdE-3** in DMSO

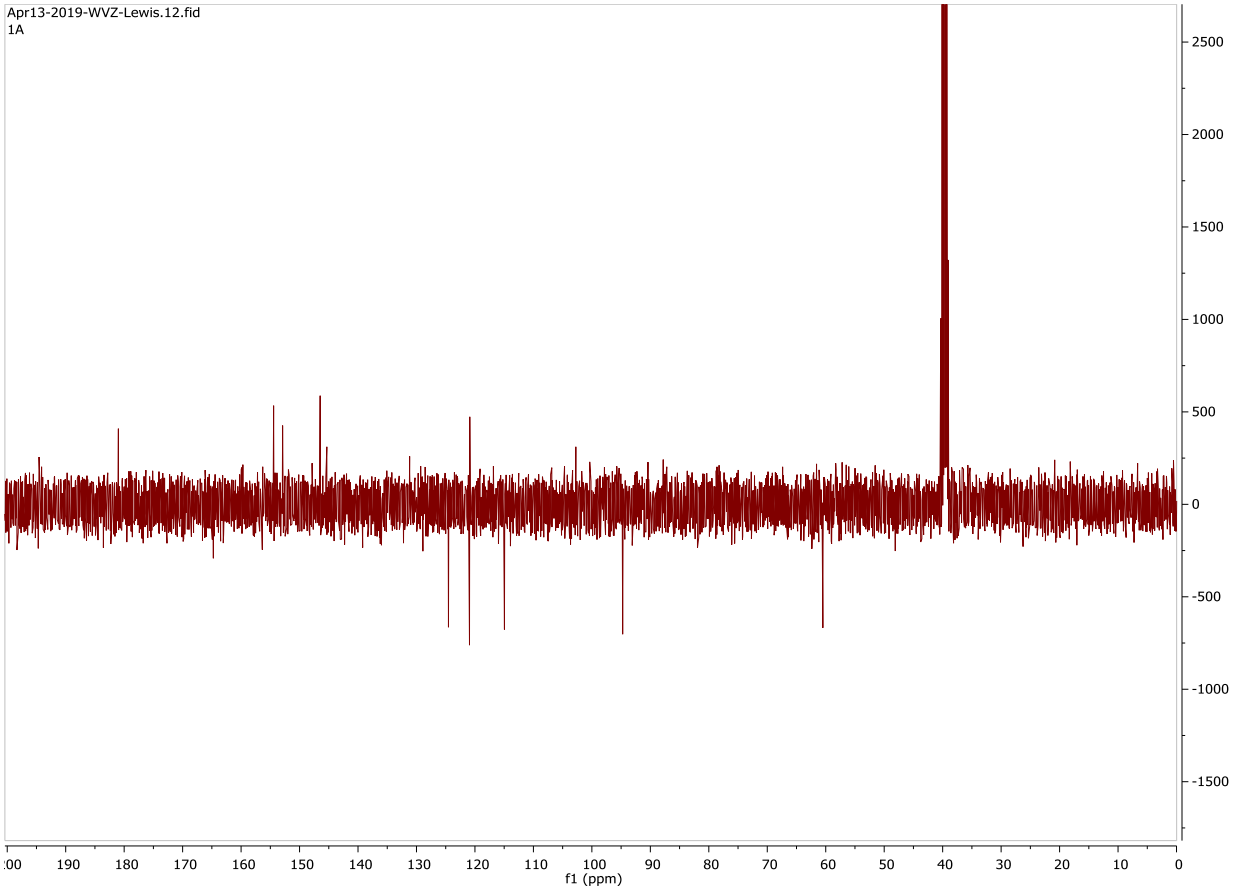


Fig. 4.28 DEPT. 135 Spectrum. of **CiPdE-3** in DMSO

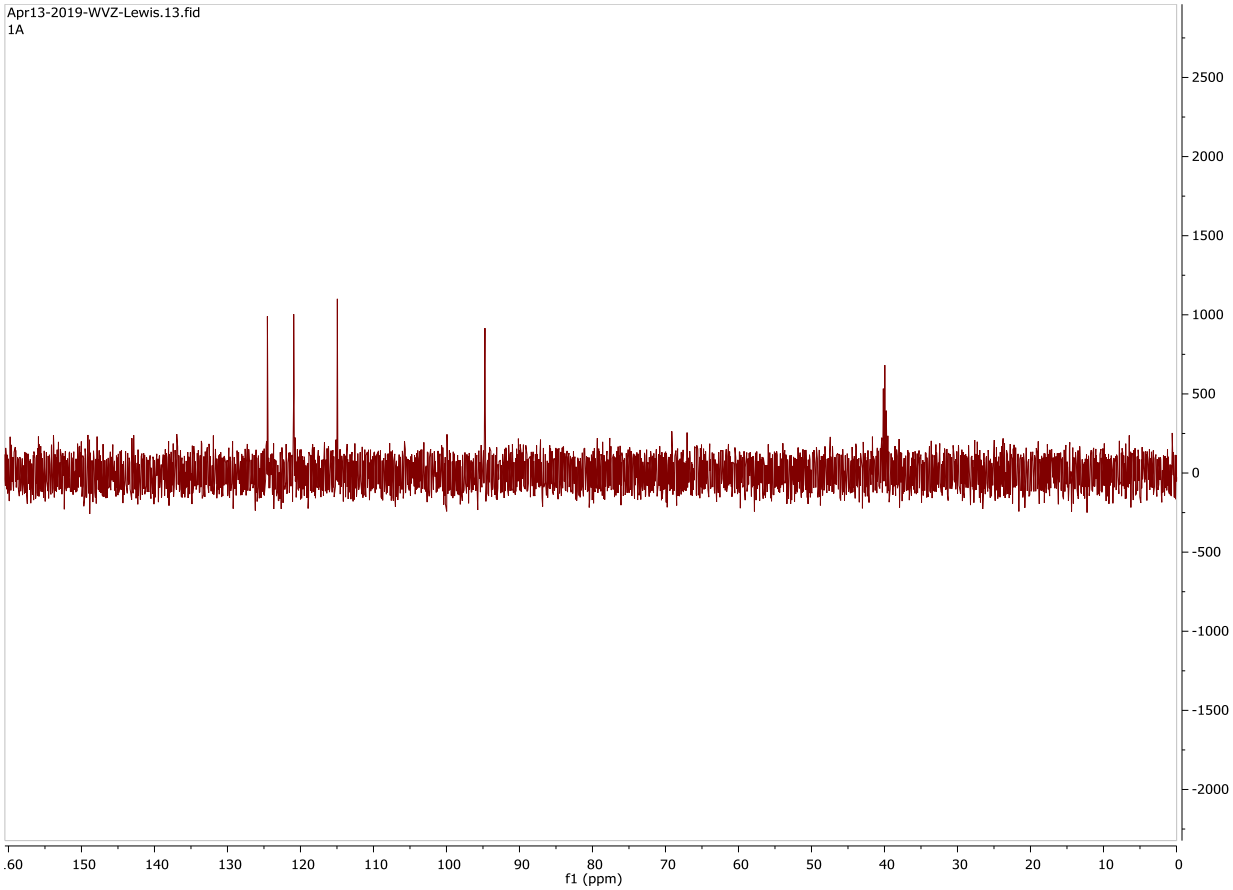


Fig. 4.29 DEPT 90 Spectrum of **CiPdE-3** in DMSO

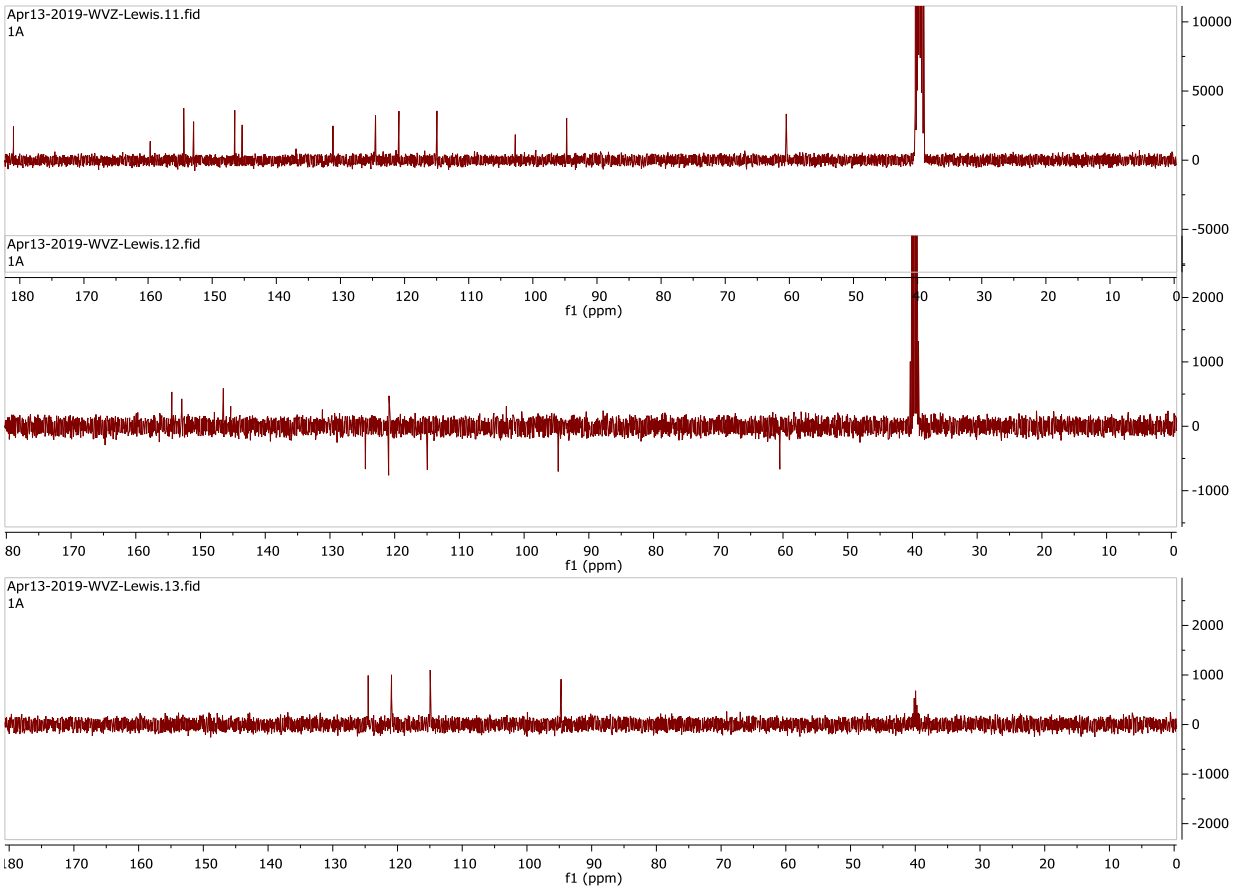


Fig. 4.30 Stacked spectra of DEPT. experiments of **CiPdE-3** in DMSO

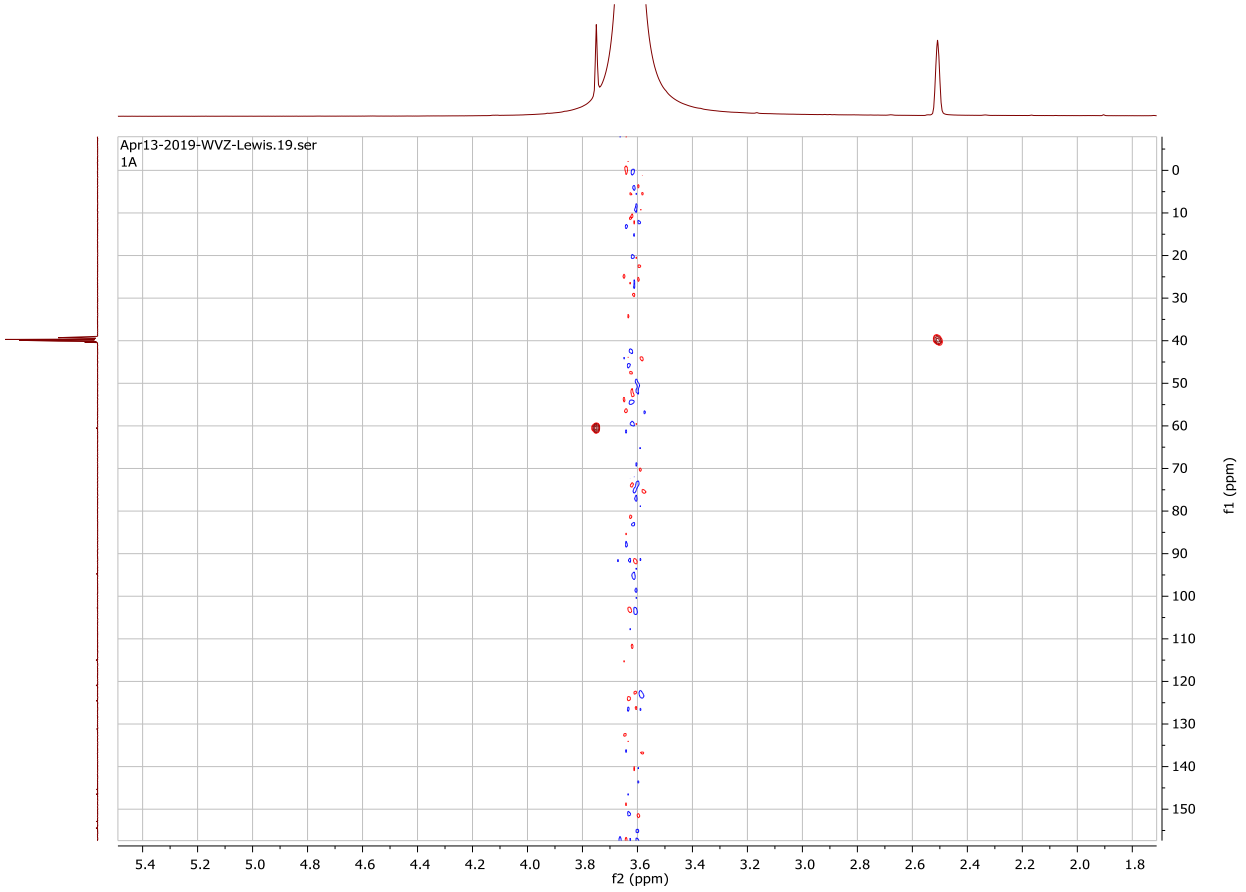


Fig. 4.31 HSQC. Spectrum of **CiPdE-3** in DMSO

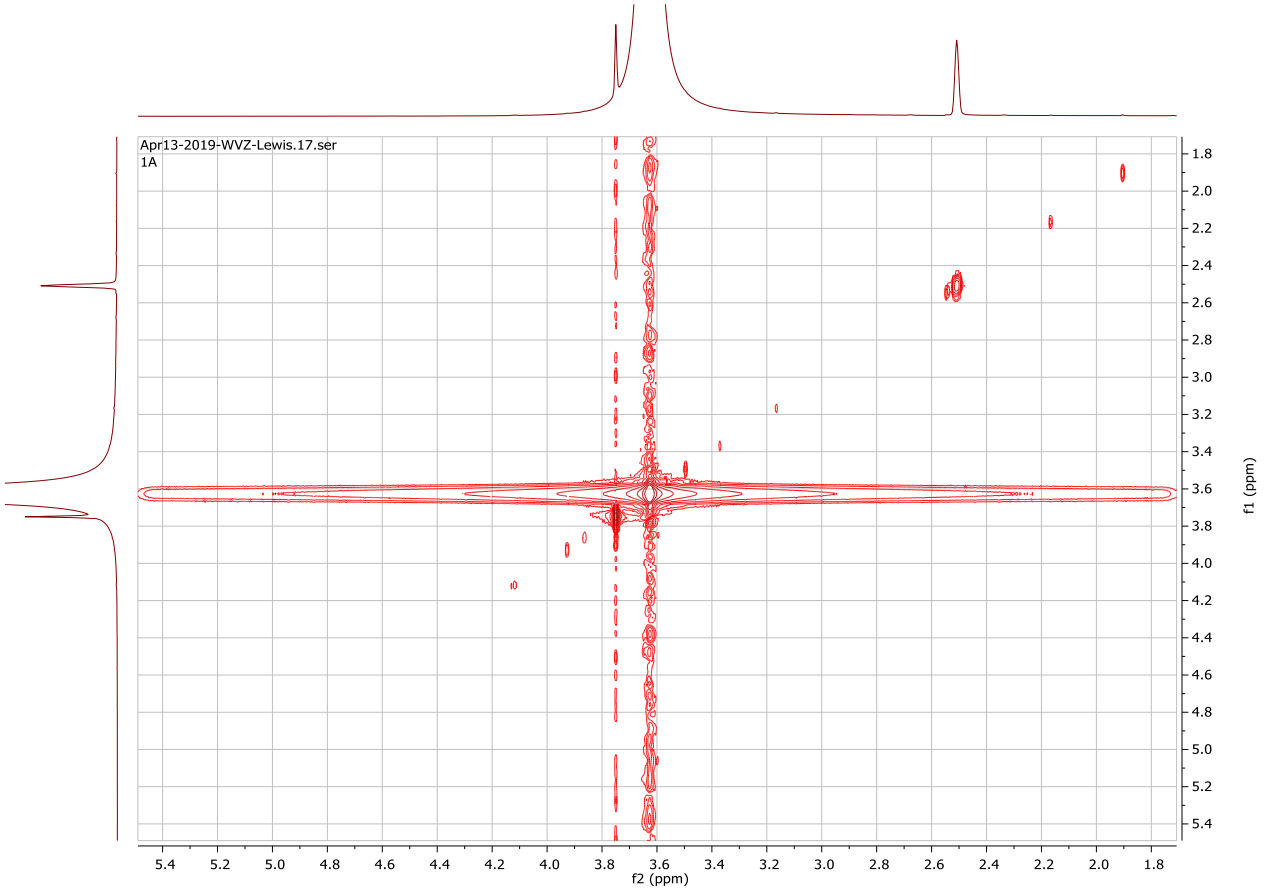


Fig. 4.32 COSY Spectrum of **CiPdE-3** in DMSO

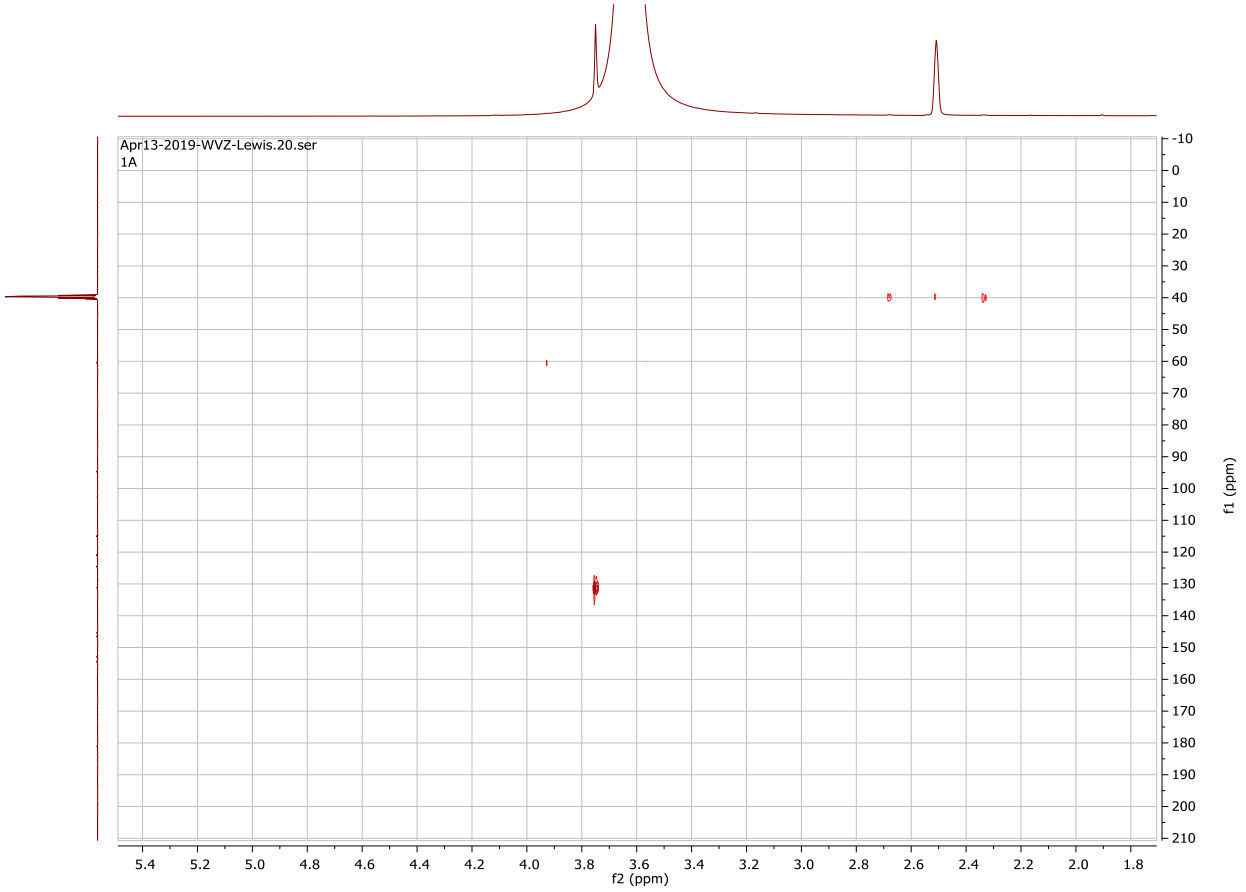


Fig. 4.33 HMBC Spectrum of **CiPdE-3** in DMSO

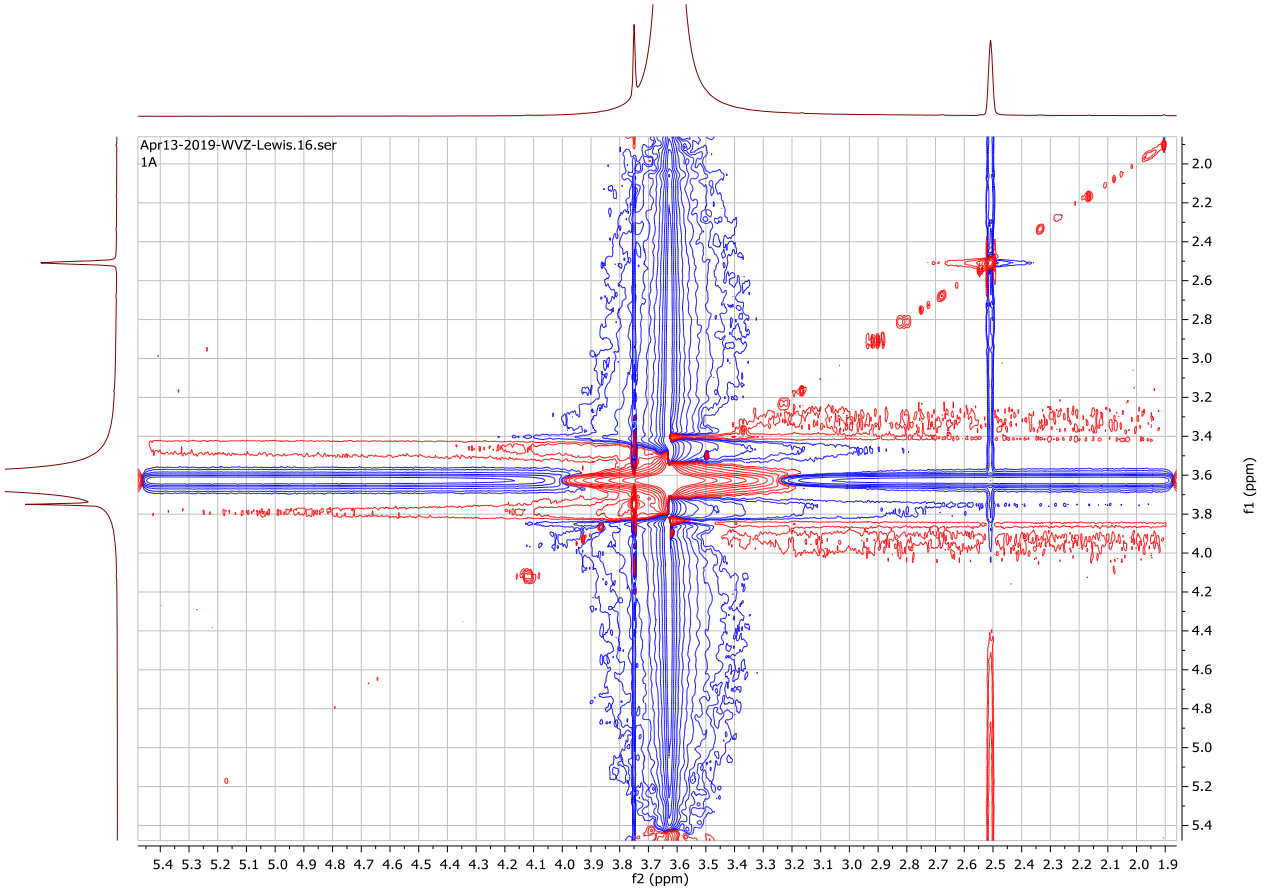


Fig. 4.34 NOESY Spectrum of **CiPdE-3** in DMSO

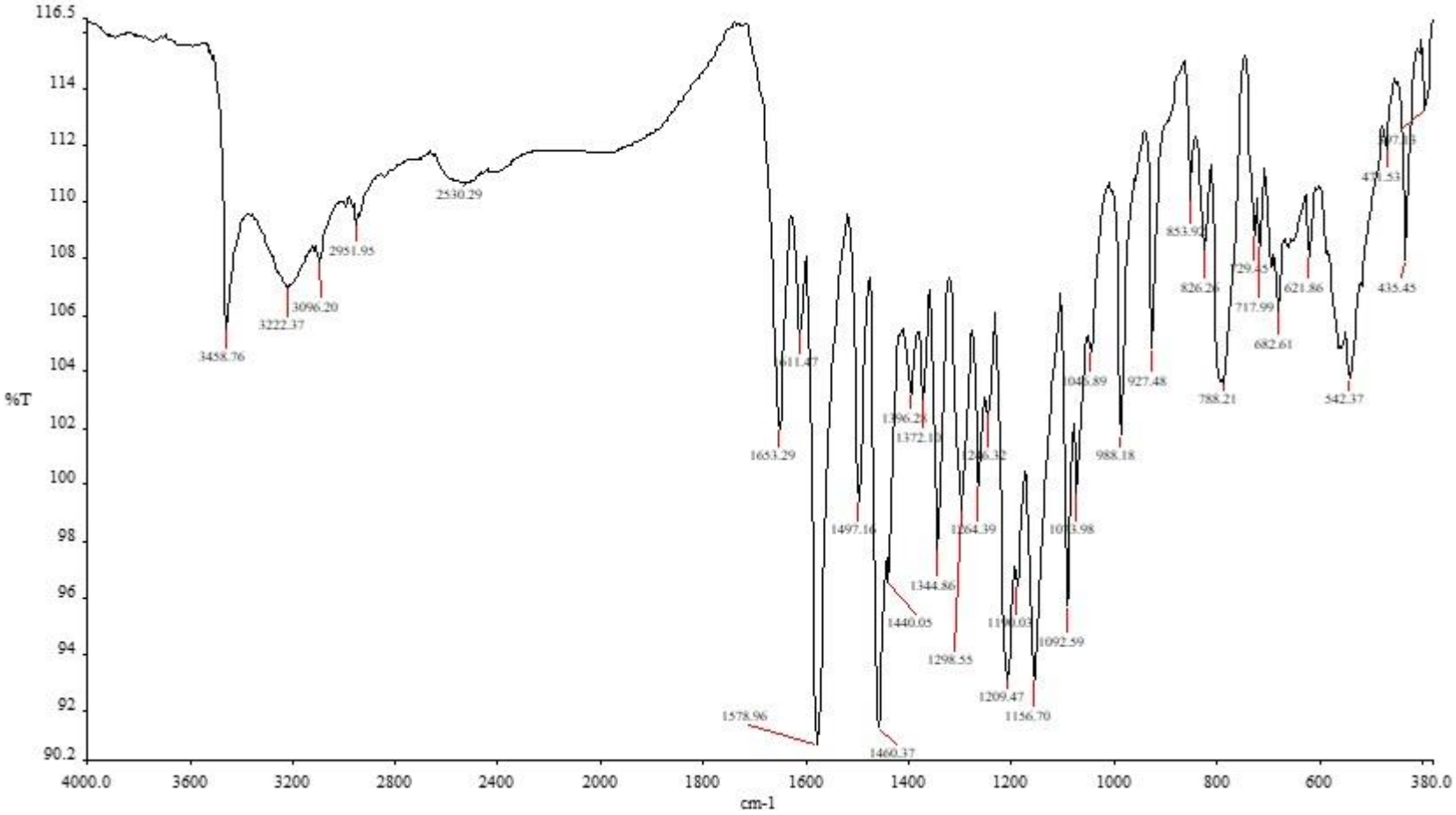


Fig. 4.35 FTIR Spectrum of **CiPdE-3**

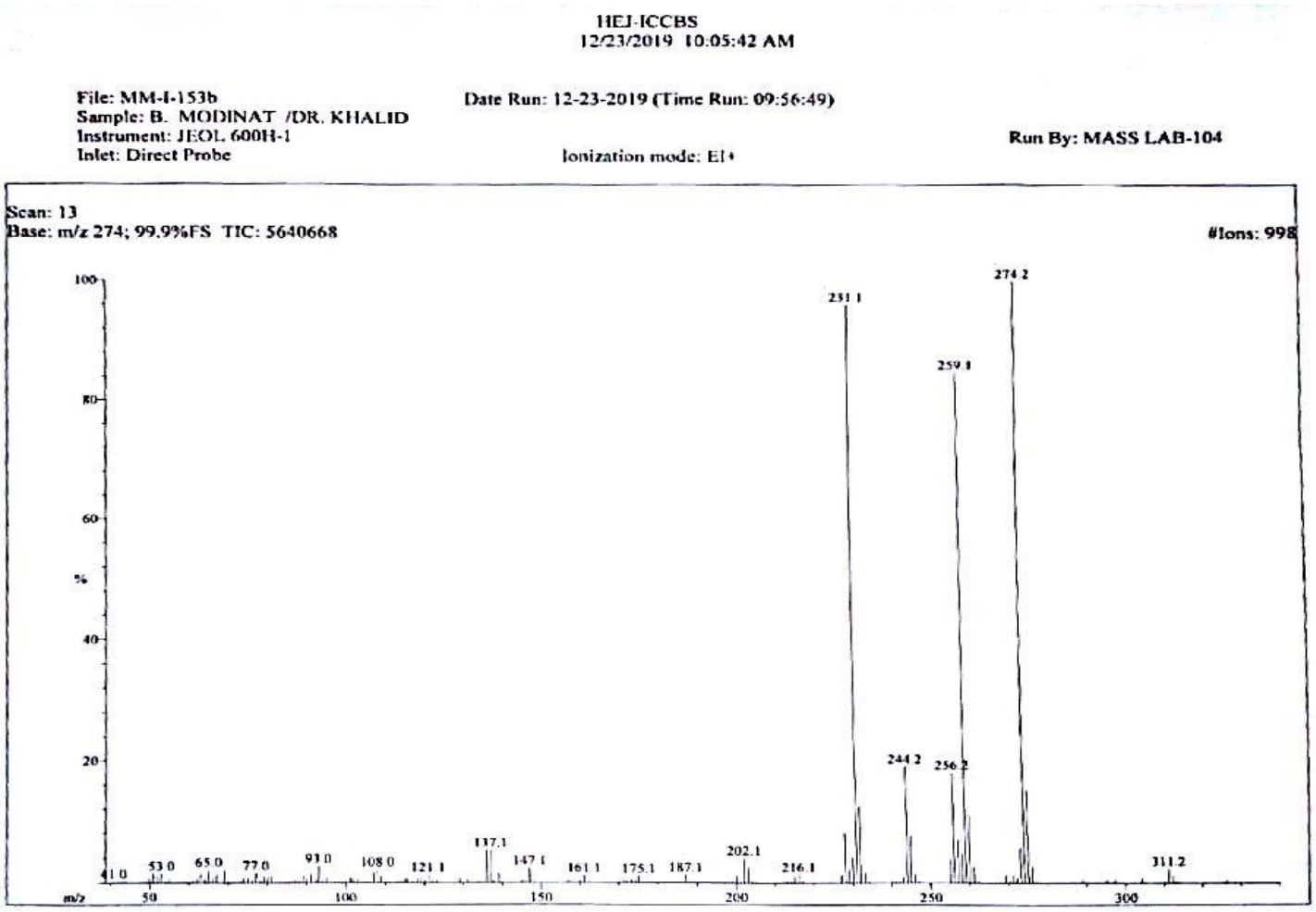


Fig. 4.36 Electron Ionization (EI) Mass spectrum of **CiPdE**-3

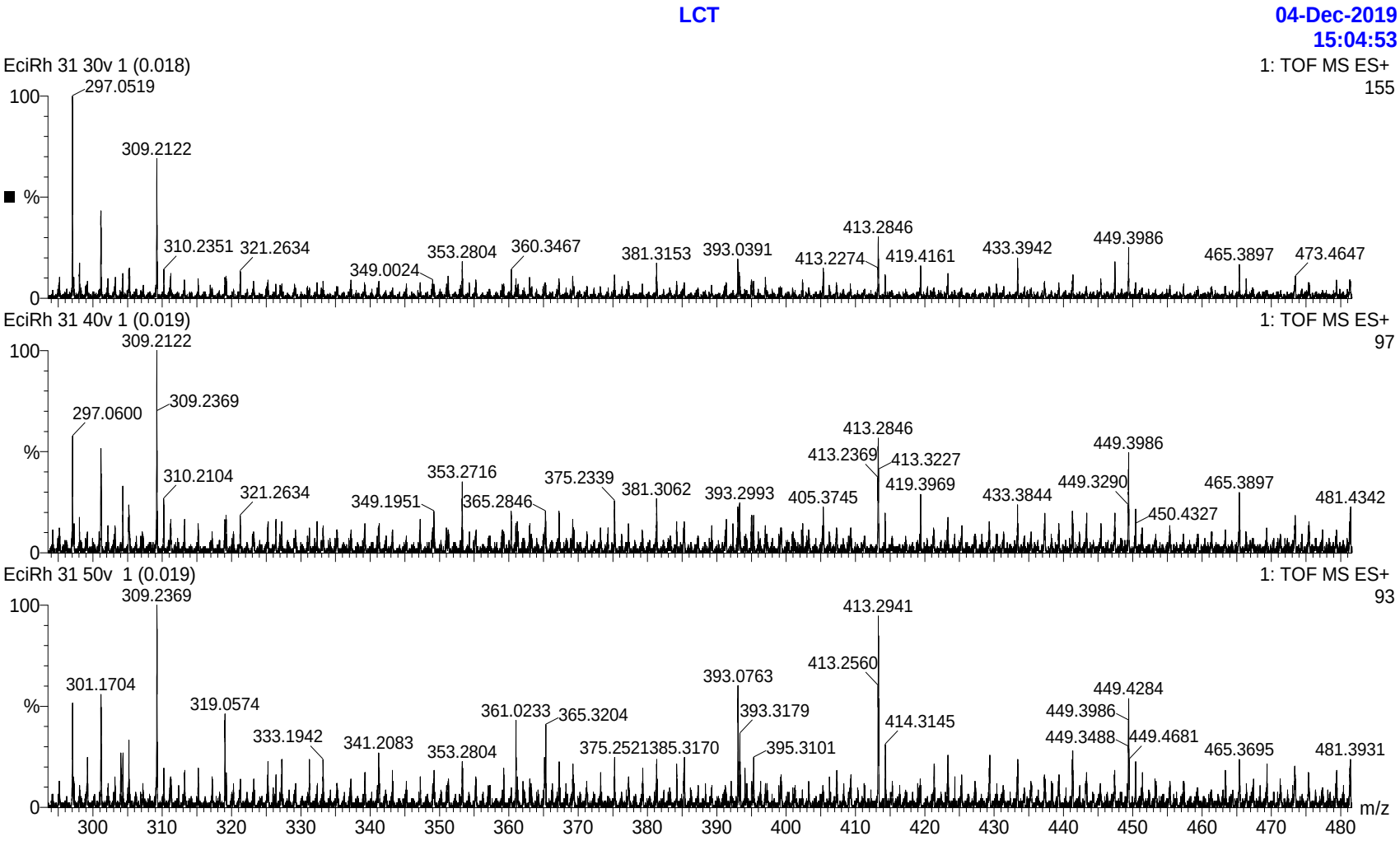


Fig. 4.37 High Resolution Mass spectrum (TOF ES+) of **CiPdE**-3

4.4.2 Isolation from root bark ethyl acetate fraction of *Pterocarpus soyauxii*

The chromatographic separation of ethyl acetate fraction from the root bark of *Pterocarpus soyauxii* PsRb, (15 g) gave four-compounds as described in Sheme 2. The compounds are: PsRbE-A (26 mg), PsRbE-B (30 mg), PsRbE-C (40 mg), and PsRbE-D (23 mg).

4.4.2.1 Characterisation of PsRbE-A

PsRbE-A was isolated as white-crystals from root bark ethyl acetate fraction of *Pterocarpus soyauxii* (Scheme 2) with melting point 282-294^{o}C. Under UV light and treatment with $FeCl_3$ produced no spot. The compound was found to be non-phenolic due to a negative $FeCl_3$ test result. Vannillin Sulphuric acid spray revealed the compound as a single spot with R_f of 0.5. In addition, PsRbE-A gave a positive Liebermann-Burchard test, suggesting that it is a pentacyclic triterpene.

The Electron ionization mass spectron (EIMS) of PsRbE-A (Figure 4.48) showed a molecular ion peak $[M]^+$ at mass to charge (*m/z*) 444 which is consistent with the molecular formula $C_{30}H_{52}O_2$. IR $\bar{\upsilon}$ (cm^{-1}) spectrum of PsRbE-A (Figure 4.47) showed an intensely broad band (cm^{-1}) at 3399.85 and 3623.03 indicating the presence of free and hydrogen bonded OH groups respectively. The stretching/bending-type absorption of CH_3 were intense (cm^{-1}) at 2931 with corresponding CH_2 peak at 2868 cm^{-1}.

^{1}H NMR spectrum (Figure 4.39) of PsRbE-A showed signals for seven tertiary methyl groups at δ' 0.85, δ' 0.93, δ' 0.94, δ' 0.94, δ' 0.95 δ' 1.00 and δ' 1.17. A secondary methyl group was observed at δ' 0.94 indicating a triterpene skeleton (Mahato and Kundu, 1994). The prominent signals of ^{1}H NMR at δ 3.55 and δ 3.99 corresponding to methine protons on H-2 and H-3 with *J* values of *3.0* and *2.9* Hz which is diagnostic of pentacyclic triterpenes skeleton confirming that PsRbE-A was non-aromatic (**4.38**).

^{13}C NMR spectrum of PsRbE-A showed a total of 30 carbon signals differentiated as eight (8) methyls, ten (10) sp^3 methylenes, six (6) methine and six (6) quaternary carbons classified based on the HSQC data (Figures 4.43). Deshielded signals at δ' 71.39 and δ' 76.51 ppm were matched to carbons C'-2 and C'-3. The COSY spectrum (Figure 4.30) was also analyzed and the data obtained helped confirm the chemical shift correlation between δ' 3.99 (H-2) and δ' 3.55 (H-3). In the HMBC spectrum

correlations were observed between signal at δ' 76.51 (C-3) with δ' 0.96 (H-23) and δ' 0.94 (H-24). δ' 53.23(C-8) with signals at δ' 1.01 (H-25), δ' 0.94 (H-24) and δ' 0.86 (H-26) (Figure 4.45); signal at δ 43.67 (C-18) with signals at δ' 1.17 (H-29), δ 1.01 (H-25) and δ' 0.94 (H-24) which is diagnostic of pentacyclic triterpenes. Comparison of the physical and spectroscopic data of 2,3-dihydroxyfriedelin (PsRbE-A) (**4.38**), established a correlation with literature data earlier reported for pentacyclic triterpenes (Mahato and Kundu, 1994). This compound is isolated and characterized for the first time from *P. soyauxii* Taub.

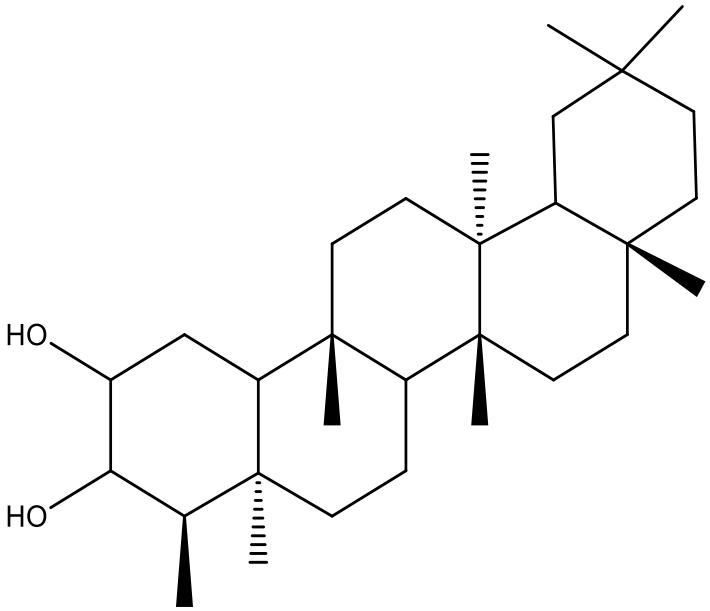


Fig. [4.38] Structure of PsRbE-A (2,3-dihydroxyfriedelin)

Table 4.32 ^{1}H NMR and ^{13}C NMR' spectral data of PsRbE-A in $CDCl_3$

Position	**C**	**δ ^{13}C**	**δ ^{1}H'**	**M**	***J'***
1	CH_2	32.32	1.28		
2	CH	71.39	3.99	q	2.9 Hz
3	CH	76.51	3.55	d	3.0 Hz
4	CH	42.82	1.55	tdd	
5	C	38.39	-	-	
6	CH_2	35.49	1.41	m	
7	CH_2	30.61	1.31	m	
8	CH	53.23	1.36	m	
9	C	37.79	-	-	
10	CH	52.27	1.39	m	
11	CH_2	17.51	1.4	m	
12	CH_2	35.34	1.4	m	
13	C	36.51	-	-	
14	C	39.7	-	-	
15	CH_2	23.93	1.83	tdd	
16	CH_2	39.28	1.47	m	
17	C	30.02	-	-	
18	CH	43.67	1.6	m	
19	CH_2	36.05	-		
20	C	28.18	-		
21	CH_2	32.8	1.48	m	
22	CH_2	41.36	1.75	t	
23	CH_3	10.9	0.94	d	2.4 Hz
24	CH_3	15.91	0.93	s	
25	CH_3	18.74	0.85	s	
26	CH_3	18.16	0.94	s	

27	CH_3	20.14	0.95	s
28	CH_3	35.04	1.17	s
29	CH_3	31.78	1.01	s
30	CH_3	32.12	0.99	s

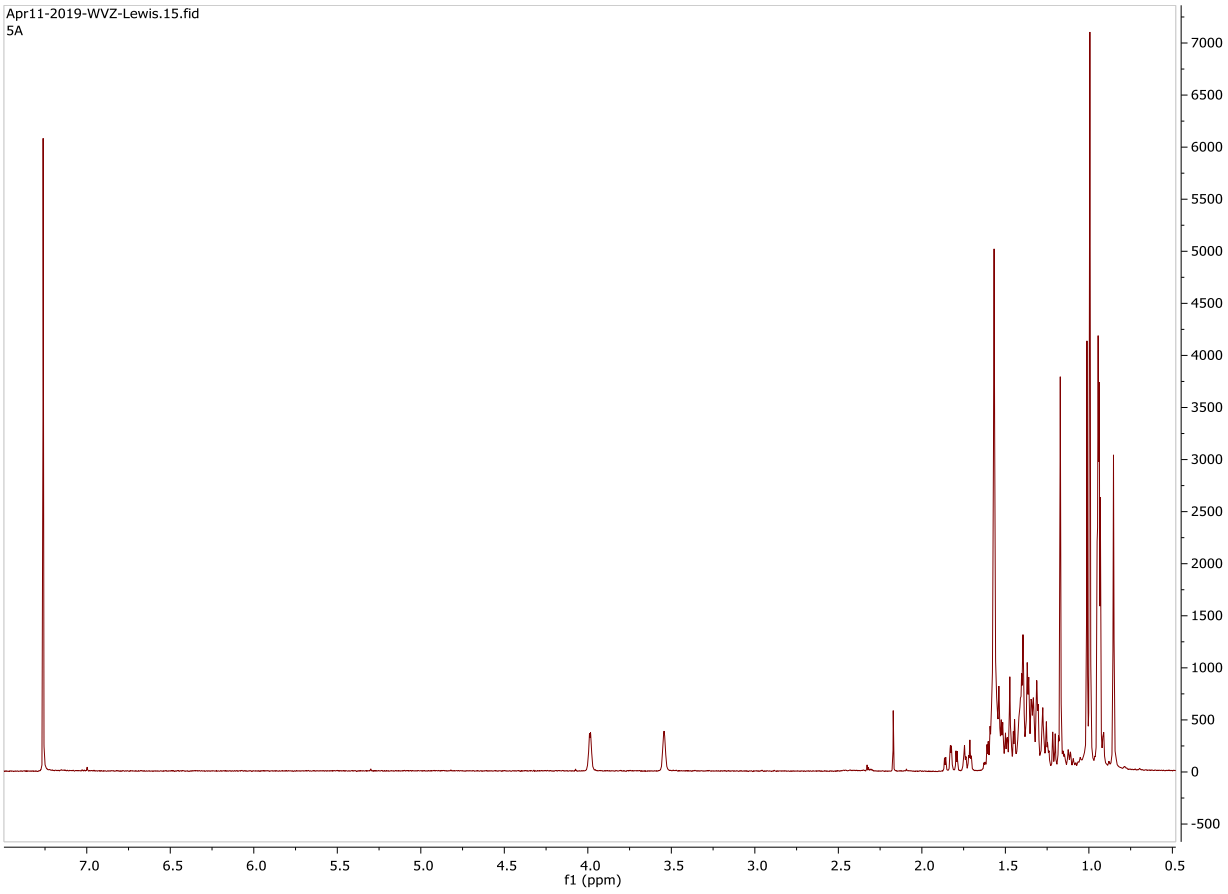


Fig. 4.39 ^{1}H NMR' Spectrum (500 MHz) of PsRbE-A in $CDCl_3$

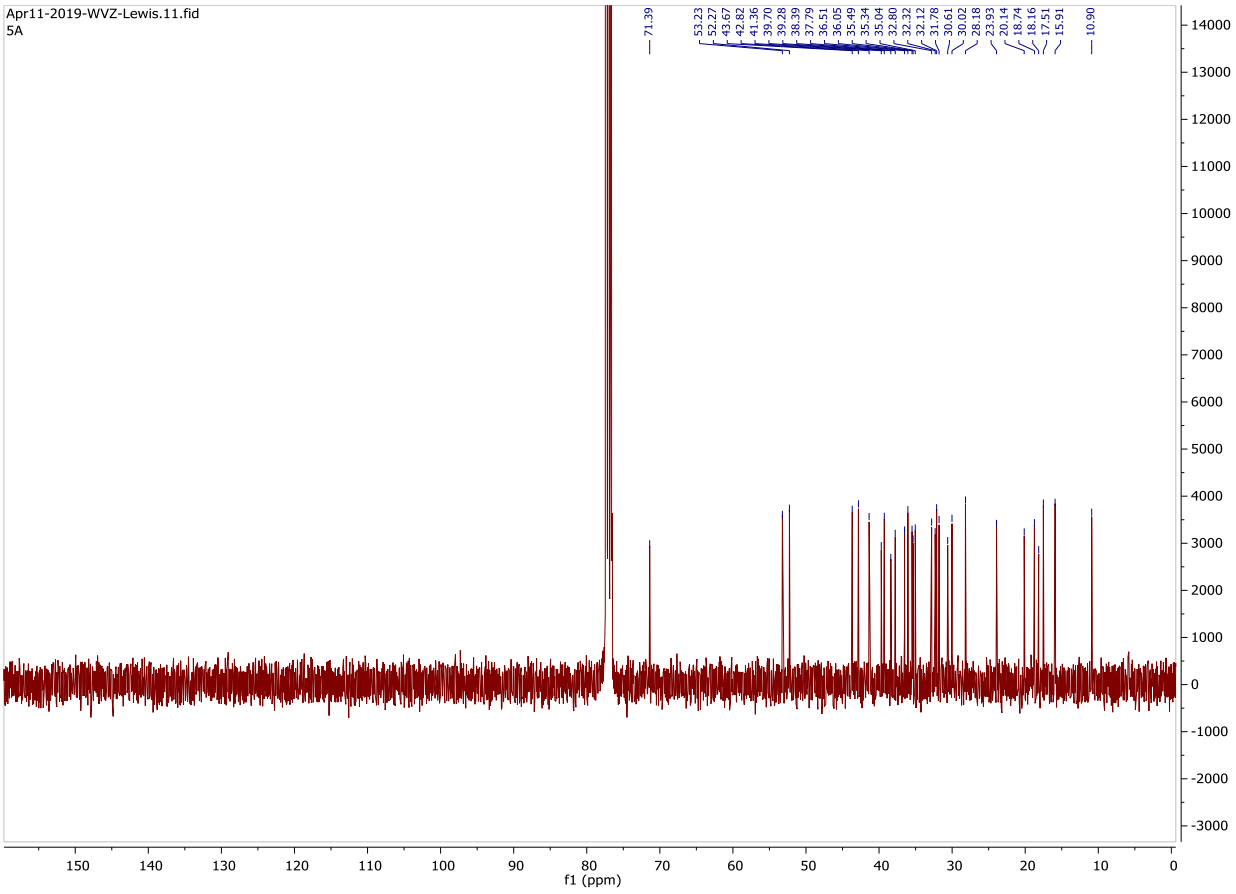


Fig. 4.40 ^{13}C' NMR^ Spectrum (500 MHz) of PsRbE-A in $CDCl_3$

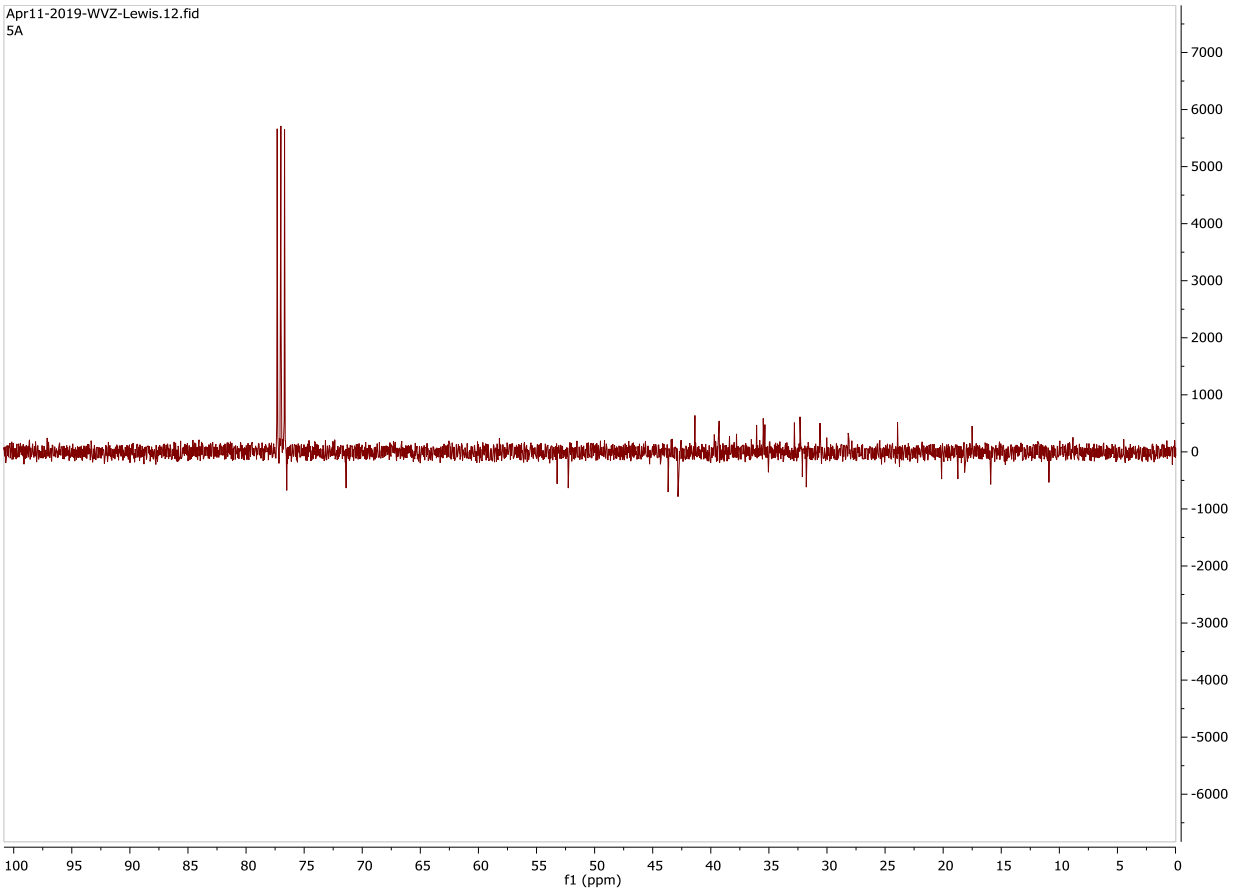


Fig. 4.41 DEPT$^{\wedge}$ 135 Spectrum of PsRbE-A in $CDCl_3$

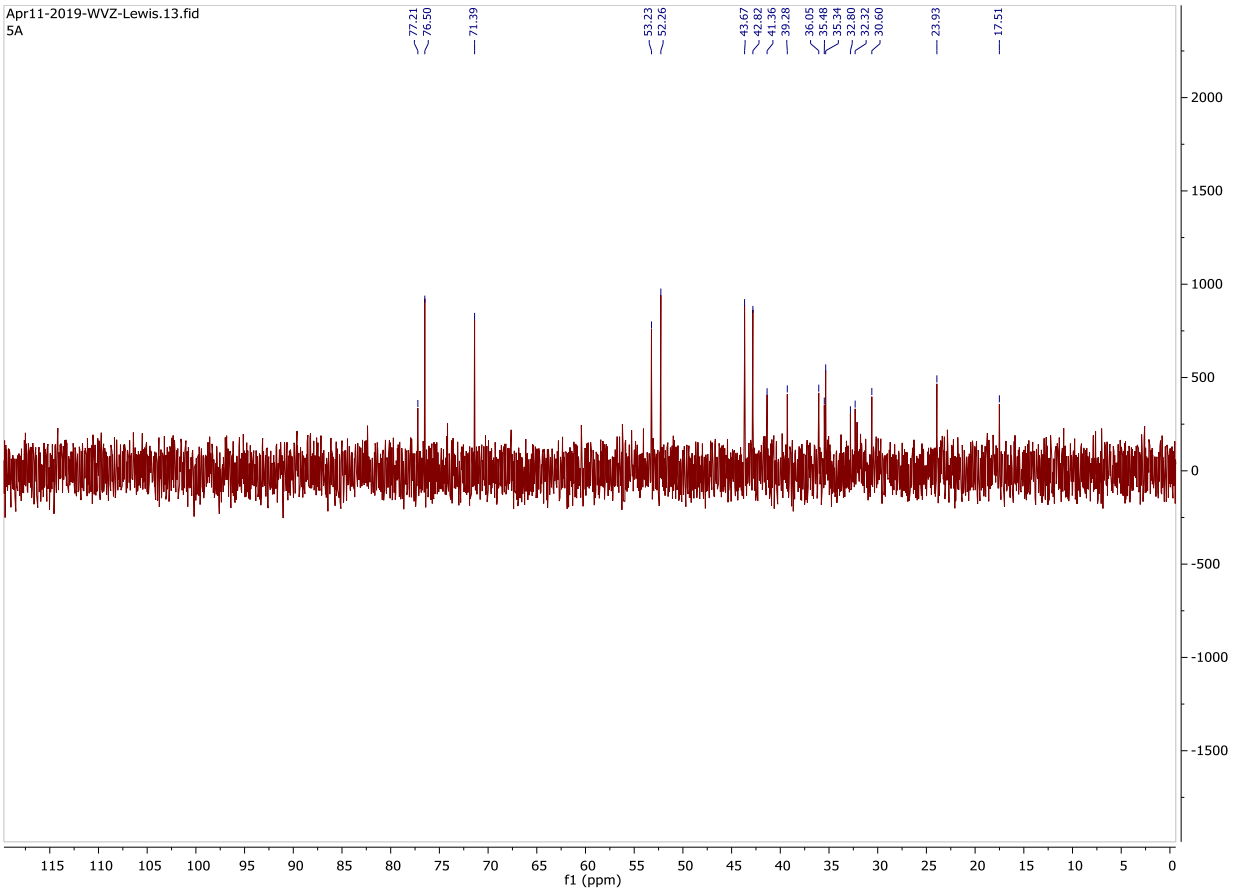


Fig. 4.42 DEPT 90 Spectrum of PsRbE-A

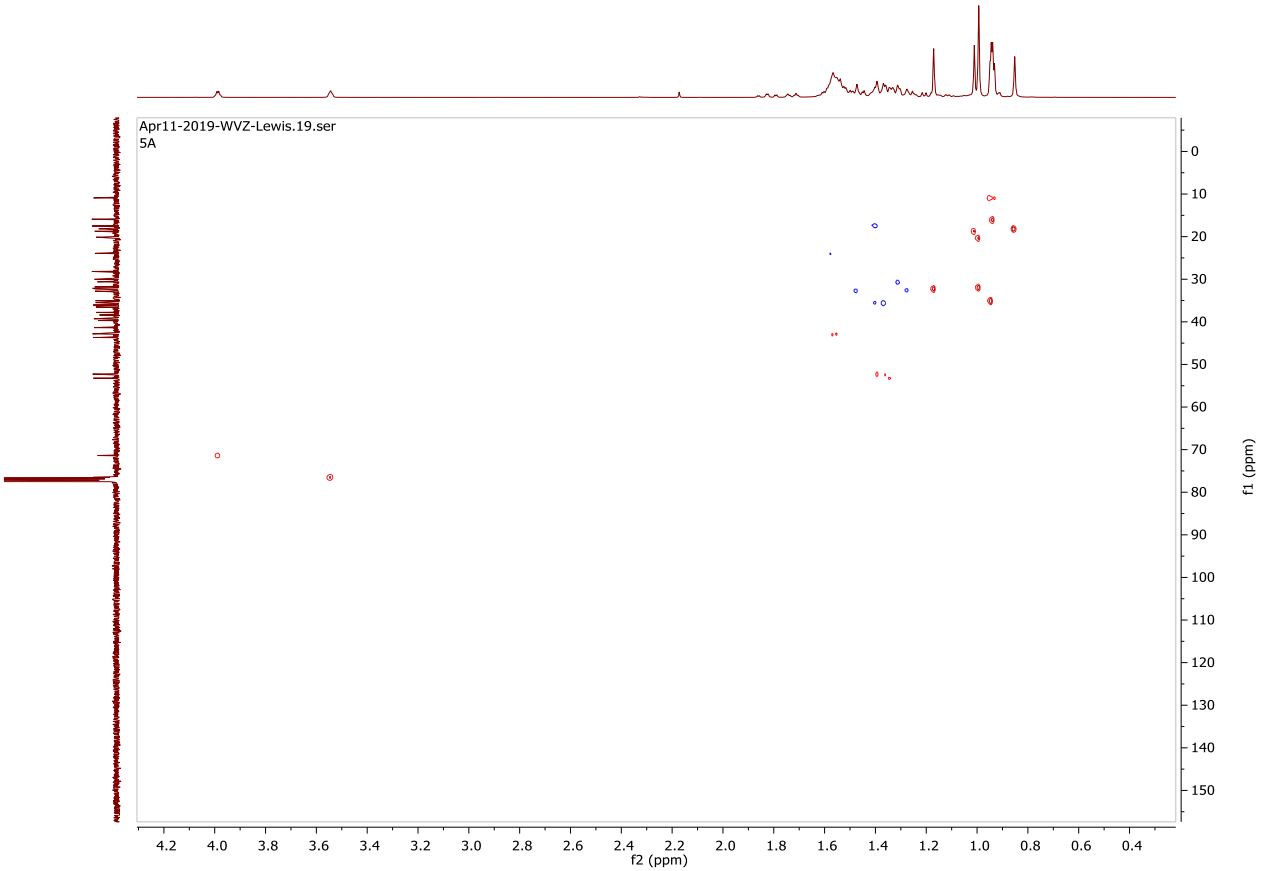


Fig. 4.43 HSQC Spectrum of PsRbE-A in $CDCl_3$

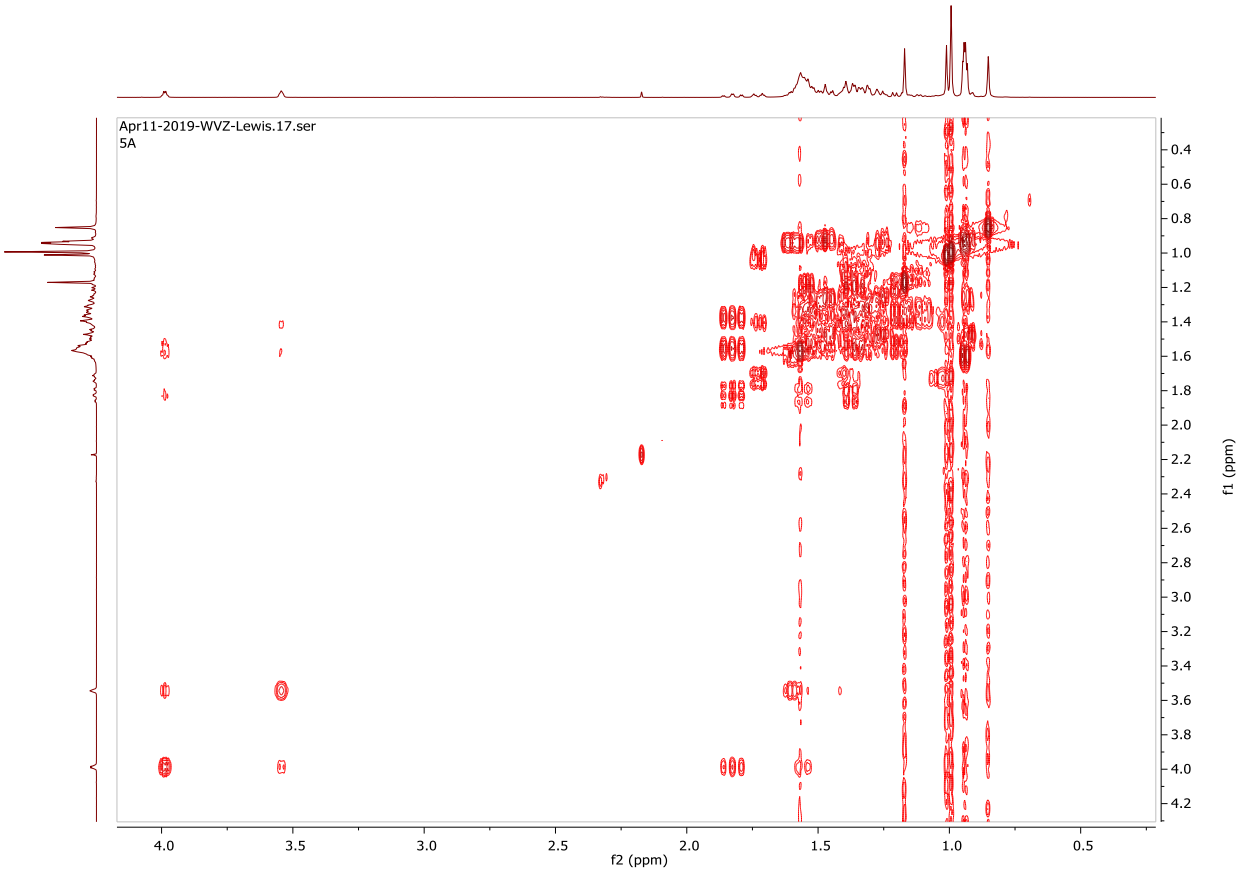


Fig. 4.44 COSY Spectrum of PsRbE-A in $CDCl_3$

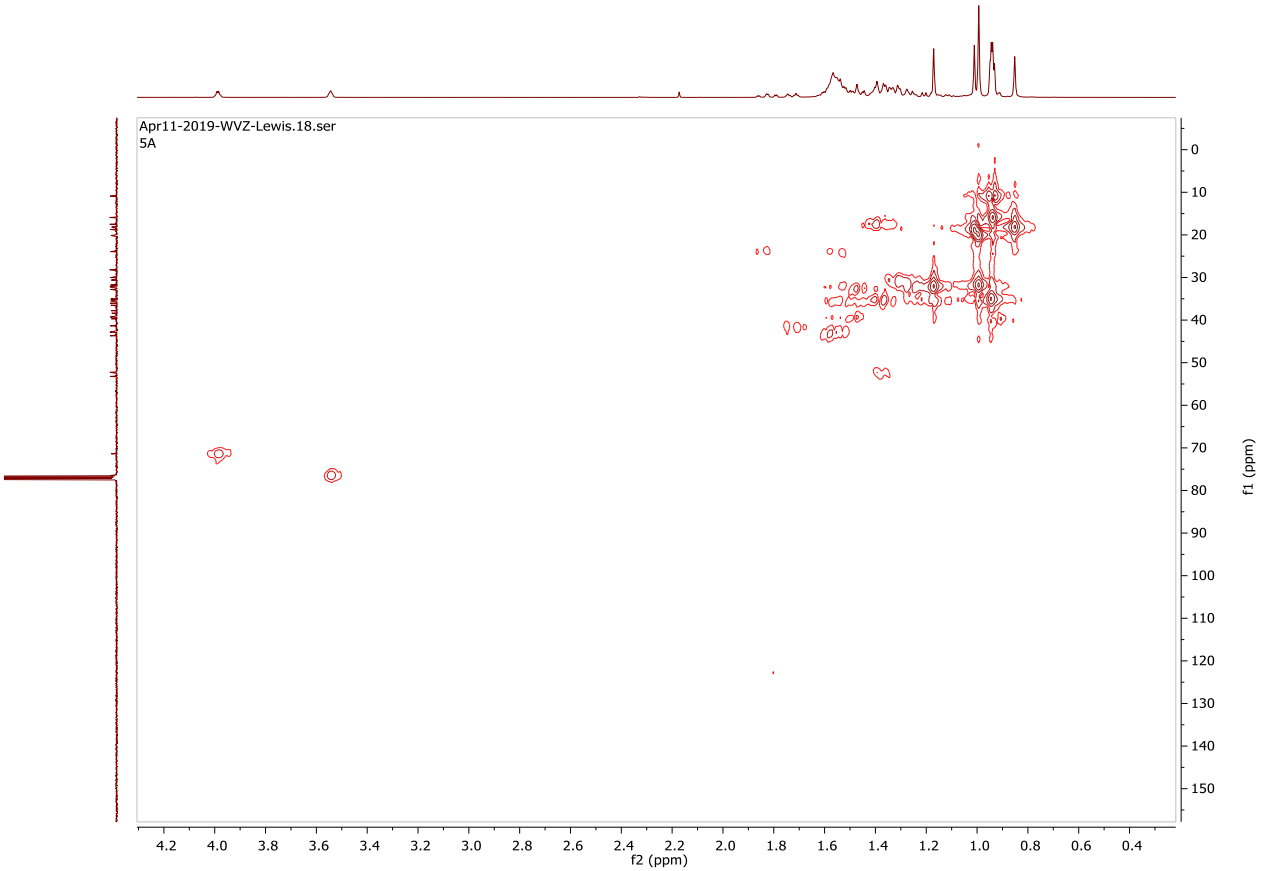


Fig. 4.45 HMBC Spectrum of PsRbE-A

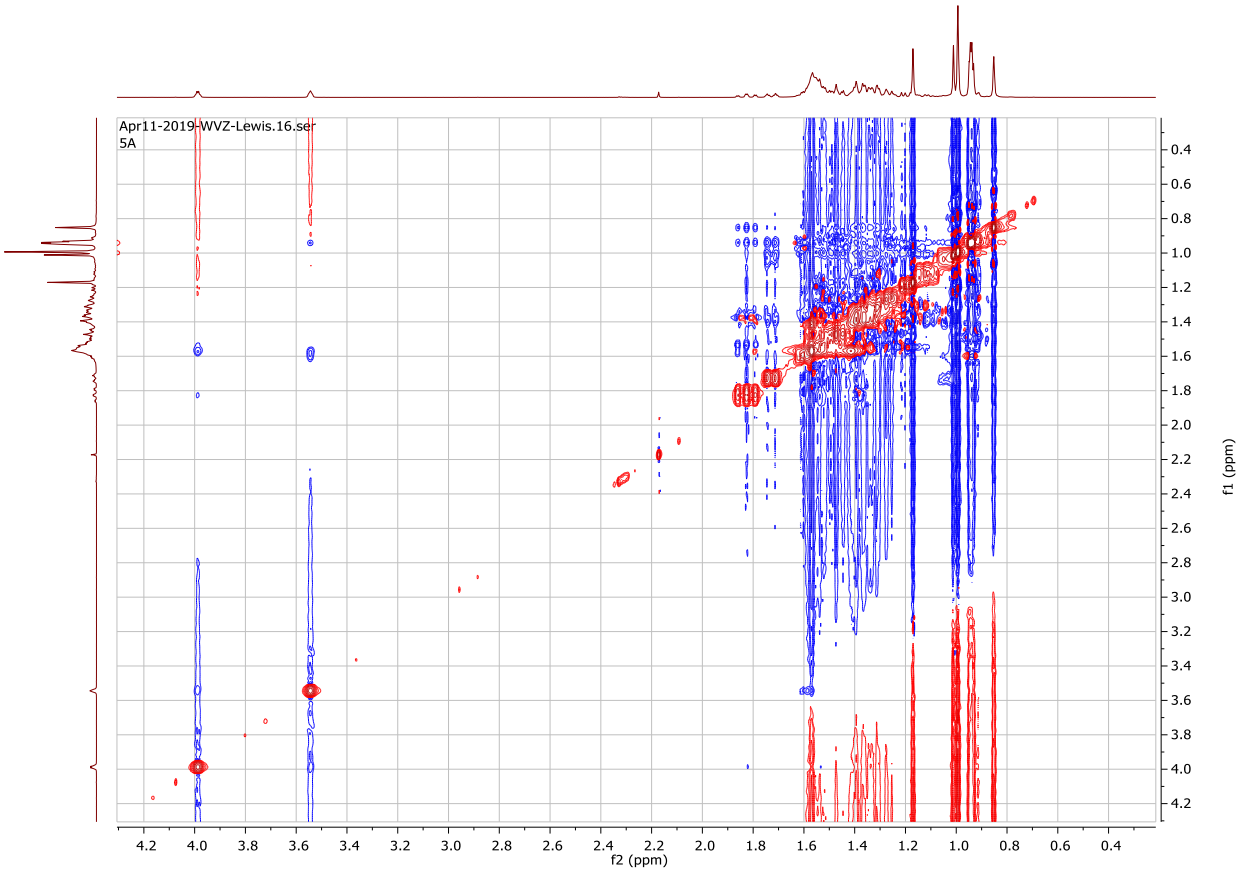


Fig. 4.46 NOESY Spectrum of PsRbE-A

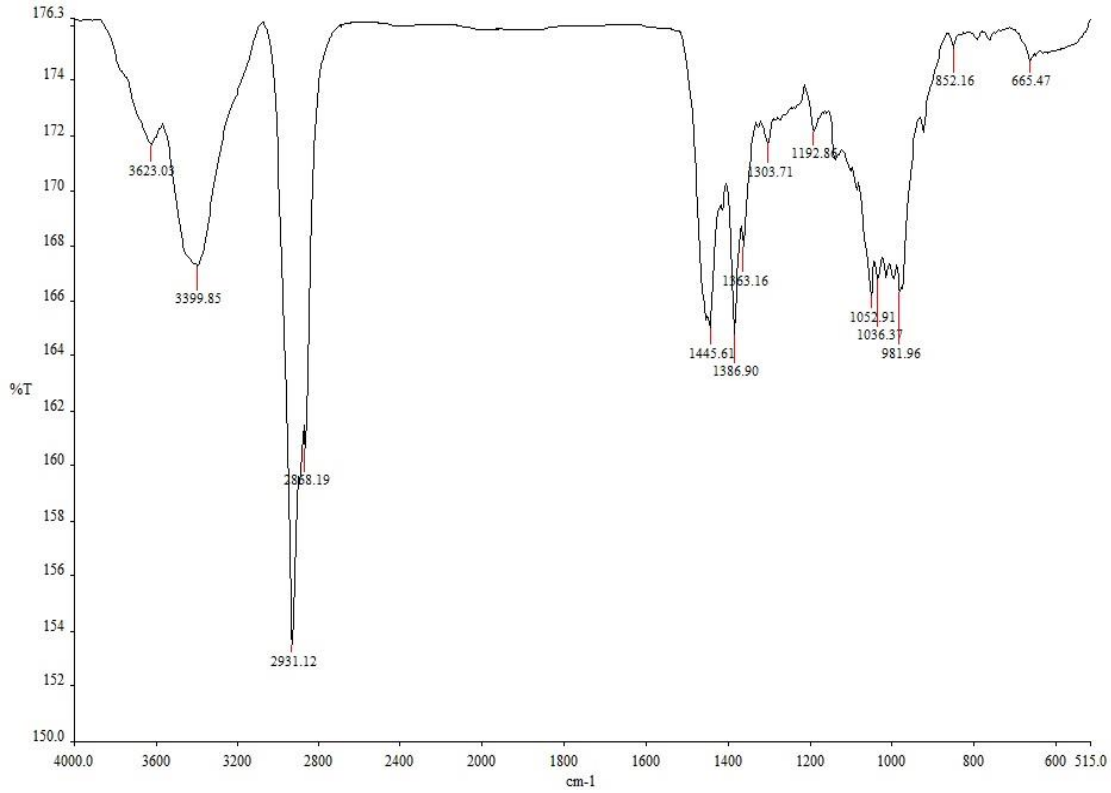


Fig. 4.47 IR Spectrum of PsRbE-A

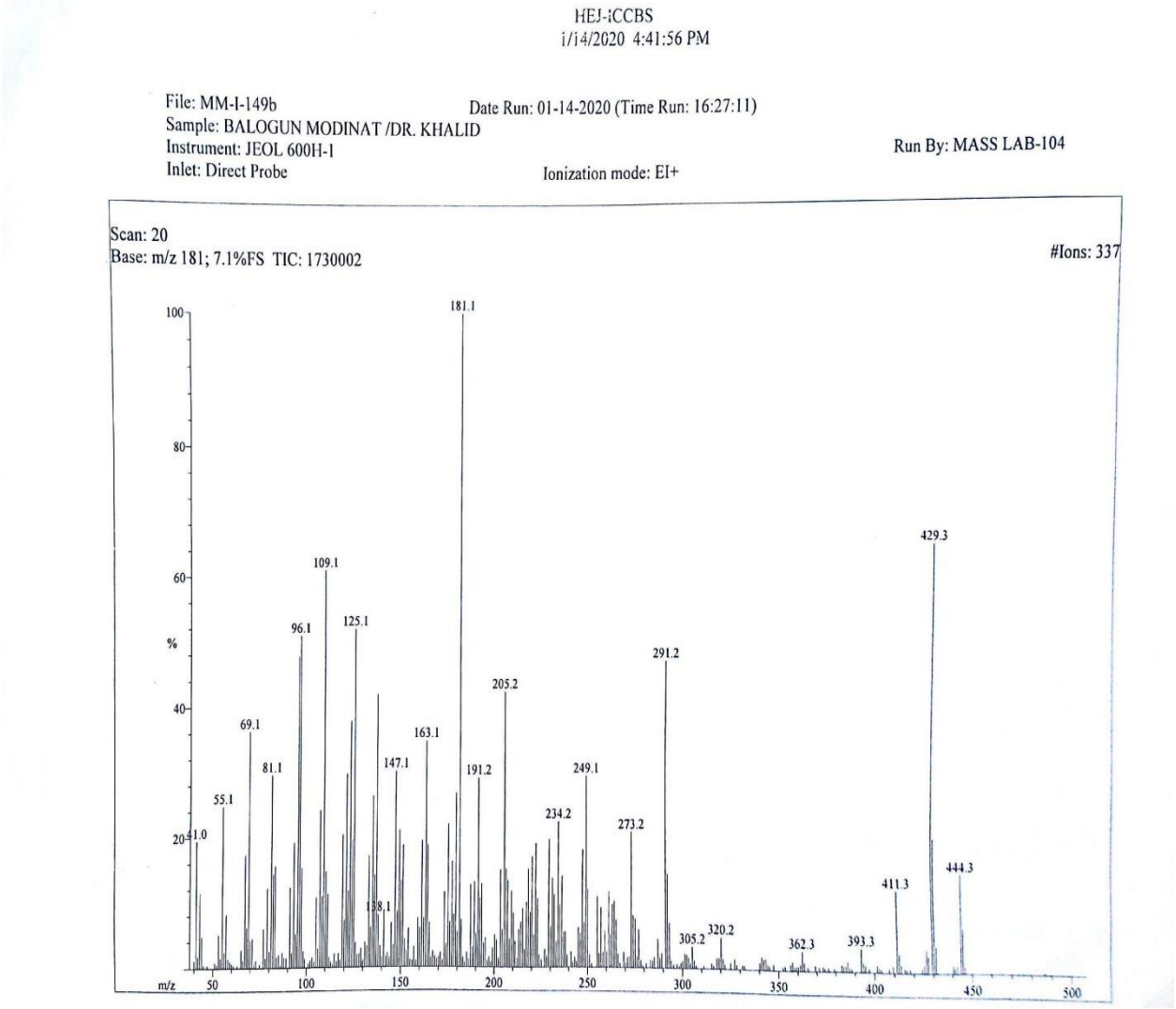


Fig. 4.48 Mass Spectrum (**EIMS**) of PsRbE-A

4.4.2.2 Characterisation of compound PsRbE-B

PsRbE-B was isolated from the root bark ethyl acetate fraction of *Pterocarpus soyauxii* (Scheme 2) as amorphous white powder (30 mg) with melting point 216-218^{o}C. The compound gave a single spot on the chromatographic plate using hexane: ethyl acetate solvent system (2:1) with R_f value of 0.3. Spraying of PsRbE-B with vanillin sulphuric acid gave a single spot confirming the purity of the isolated compound. PsRbE-B gave a positive Liebermann-Burchard test indicating that it is a pentacyclic triterpenes. IR ῡ (cm^{-1}) spectrum of PsRbE-B (Figure 4.57) showed a peak at 3393 cm^{-1} attributed to OH bond vibration. The C=C peaks were observed around 1650 cm^{-1} as weakly intense band. (**4.49**). The molecular mass of PsRbE-B was confirmed by mass spectrometric analysis, using Electrospray Ionisation positive (ESI+) technique (Figure 4.48). This experiment gave base peak (*m/z*) 449.3886 [M + Na], which corroborates the assigned molecular formula ($C_{30}H_{50}O$) of the compound.

^{1}H NMR spectrum (Figure 4.58) of PsRbE-B showed signals for six tertiary groups at δ' 0.78, δ' 0.79, δ' 0.83, δ' 0.97 δ' 0.98 and δ' 1.02 and one isopropenyl moiety at δ' 1.68 indicating a lupane skeleton (Mahato and Kundu, 1994). The most common signals of ^{1}H NMR at δ' 4.56 and δ' 4.68 corresponding to exocyclic vinyl hydrogens present at C-29 supports the skeleton of 3β-hydroxylup-20(29)-ene (**4.49**) commonly known as lupeol. Additional confirmation includes the signals at δ' 2.38 and δ' 3.18 for hydrogens at C-19 and C-3 respectively. The ^{13}C NMR spectrum (Figure 4.51) revealed 30 carbon signals differentiated as seven (7) methyls, ten (11) methylenes, six (6) methine and six (6) quaternary carbons which were differentiatred based on the HSQC spectra is typical of lupeol (Figure 4.52). ^{13}C NMR peak at δ 109.5, corresponds to the exocyclic carbon on C-29. The deshielded signal at δ 79.2 was assigned to hydroxyl group at C-3. The COSY spectrum (Figure 4.55) was also analyzed and the data obtained helped confirm the chemical shift attribution of H-29α and H-29β, δ' 2.38 (H-19) and δ' 3.18 (H-3). In the HMBC. spectrum, (Figure 4.54) correlations was seen clearly between signals at δ^{C} 151.2 (C-20) with δ 4.56 (H-29α) and δ 4.68 (H-29β), δ 2.38 (H-19) and δ 3.18 (H-3), δ 1.68 (H-30) and δ 1.57 (H-2α). Also, signal at δC 109.5 (C-29) correlated with signals at δ 2.38 (H-19) and δ 1.68 (H-30) which is diagnostic of 3β-hydroxylup-20(29)-ene (**4.49**). Correlation between the downfield methyl group resonance (CH_3) at δ 1.68 and the two non-equivalent

methylene proton resonances at δ 4.68 and δ 4.56 in the NOESY spectrum were ascribed to H-29α and H-29β as indicated in figure 4.56.

Comparison of the physical and spectroscopic data of (PsRbE-B**)** 3β-hydroxylup-20(29)-ene (**4.49**), correlated with literature data earlier reported (Gongalez, 1987; Tolstiva *et al*., 2006). This is the first time lupeol is characterized and reported to be a major chemical compound in *Pterocarpus soyauxii*.

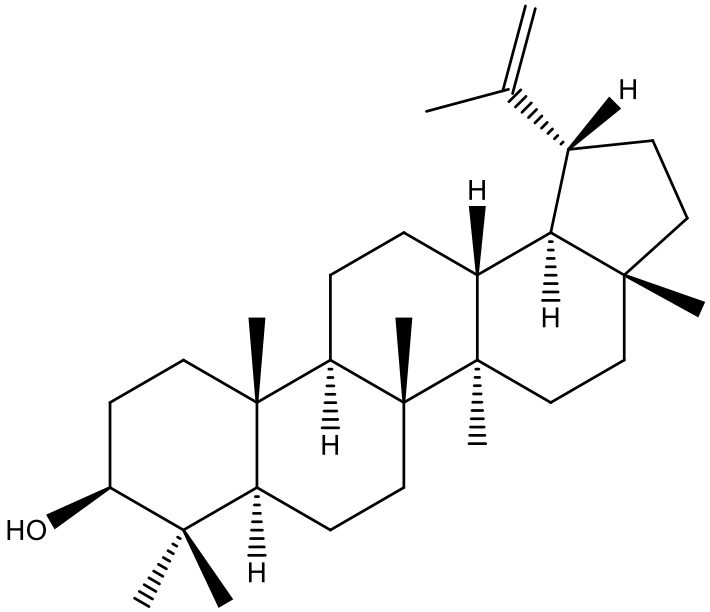


Fig. [4.49] Structure of PsRbE-B (3β-hydroxylup-20(29)-ene)

Table. 4.33. ^{1}Proton and ^{13}Carbon NMR*-data of PsRbE-B in $CDCl_3$

Position	**C.**	**δ ^{13}C.**	**δ ^{1}H.**	**M*v***
1	$^{b}CH_2$	38.9	0.94, 1.65	*m*
2	$^{b}CH_2$	27.6	1.57, 1.65	*m*
3	^{c}CH	79.2	3.18	*dd*
4	^{d}C	38.9	-	
5	^{c}CH	55.5	0.67	*d*
6	$^{b}CH_2$	18.5	1.39, 1.57	*m*
7	$^{b}CH_2$	34.5	1.38, 1.38	
8	^{d}C	40.2	-	
9	^{c}CH	50.7	1.28	*m*
10	^{d}C	37.4	-	
11	$^{b}CH_2$	21.2	1.22 ,1.41	*m*
12	$^{b}CH_2$	25.4	1.06, 1.67	*m*
13	^{c}CH	38.3	1.65	*t*
14	^{d}C	43.1	-	
15	$^{b}CH_2$	27.6	1.01, 1.67	*m*
16	$^{b}CH_2$	35.8	1.38, 1.41	*m*
17	^{d}C	43.2	-	
18	^{c}CH	48.5	1.36	*t*
19	^{c}CH	48.2	2.38	*m*
20	^{d}C	151.2	-	
21	$^{b}CH_2$	30.0	1.33, 1.92	*m*
22	$^{b}CH_2$	40.2	1.19, 1.38	*m*
23	$^{a}CH_3$	28.2	0.97	*s*
24	$^{a}CH_3$	15.6	0.78	*s*
25	$^{a}CH_3$	16.3	0.83	*s*
26	$^{a}CH_3$	16.2	1.02	*s*
27	$^{a}CH_3$	14.7	0.98	*s*
28	$^{a}CH_3$	18.2	0.79	*s*
29	^{c}CH	109.5	4.56, 4.68	*brs*
30	$^{a}CH_3$	19.5	1.68	*s*

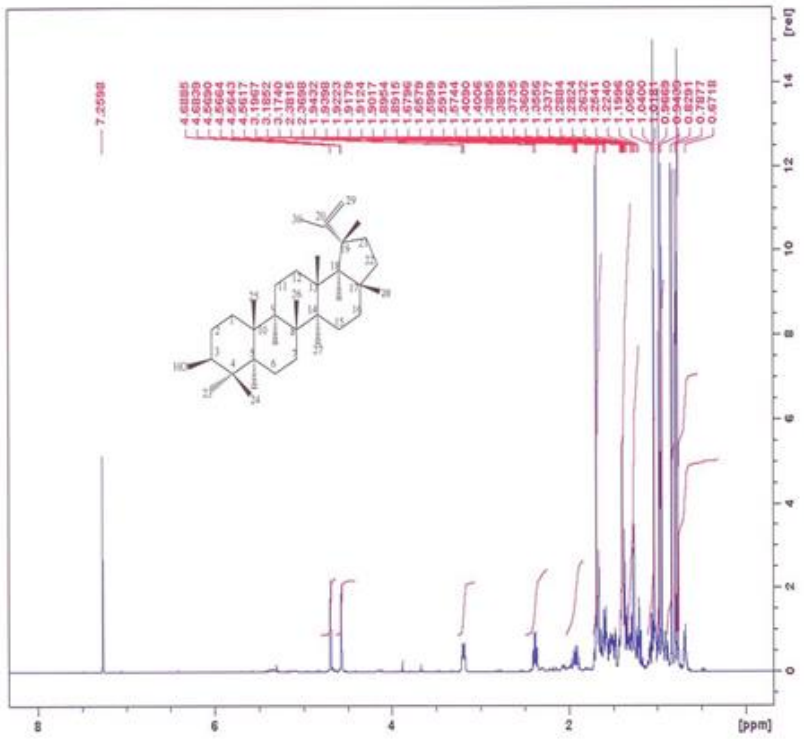


Fig. 4.50 Proton (^{1}H) NMR (500MHz) spectrum of **PsRbE-B** in $CDCl_3$

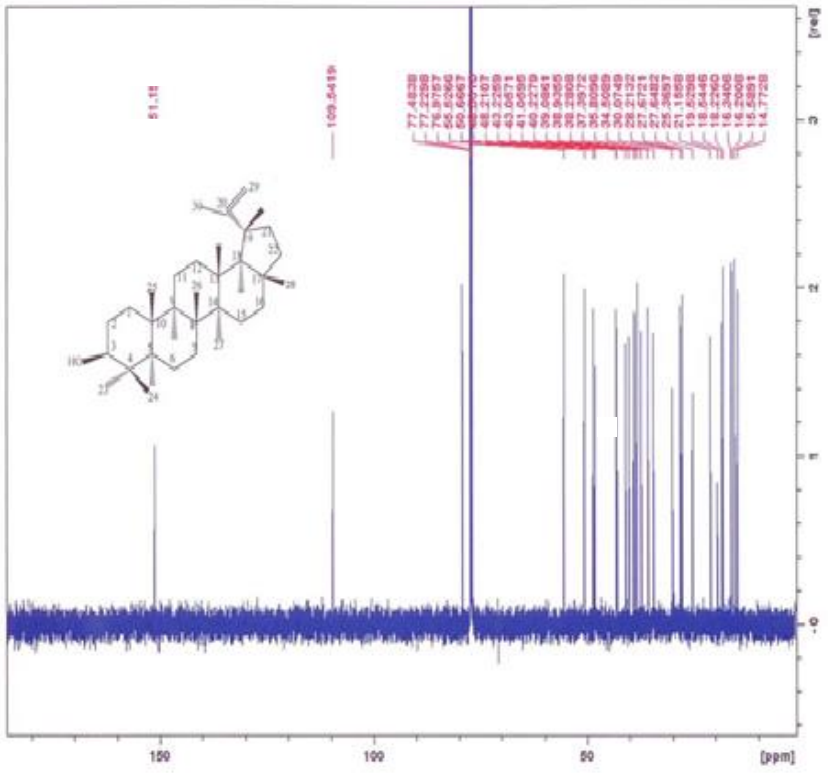


Fig. 4.51 ^{13}C NMR spectrum of **PsRbE-B** in $CDCl_3$

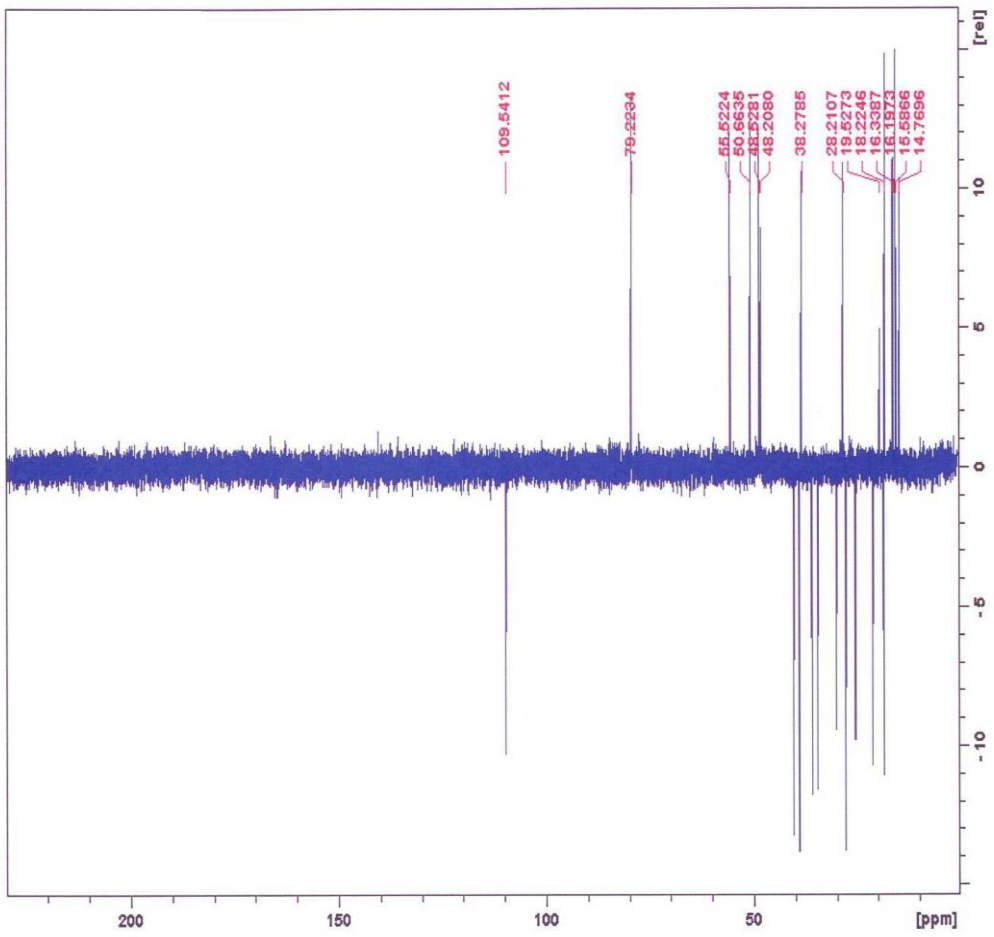


Fig. 4.52 DEPT 135 spectrum of **PsRbE-B** in $CDCl_3$

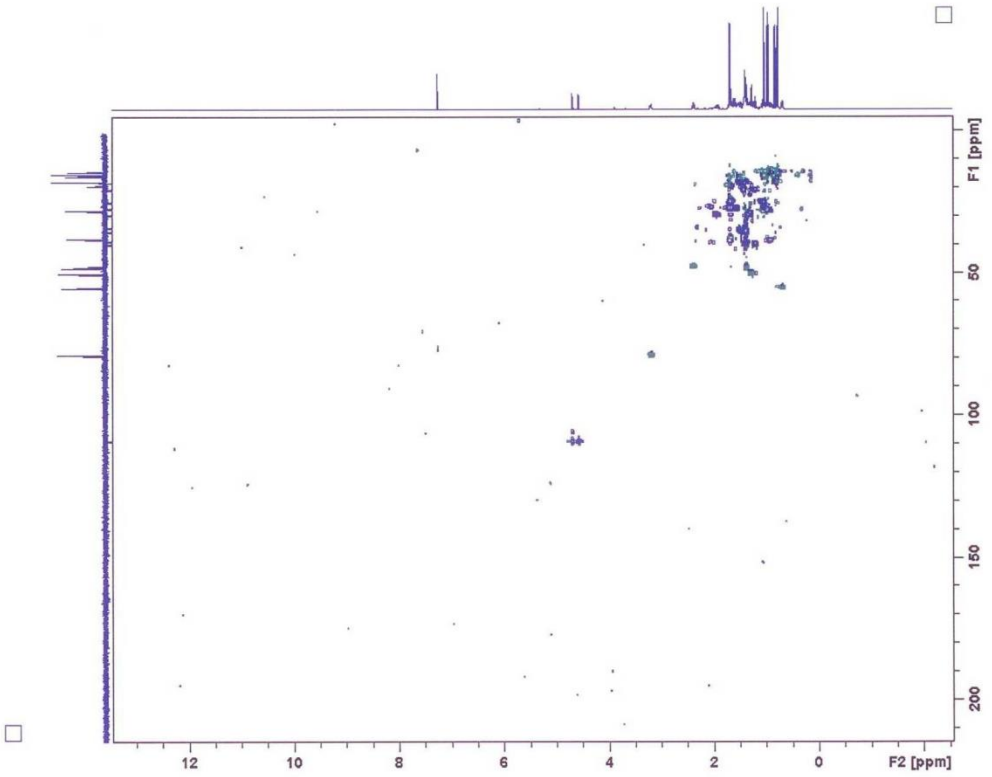


Fig. 4.53 HSQC DEPT spectrum of **PsRbE-B** in $CDCl_3$ (expanded)

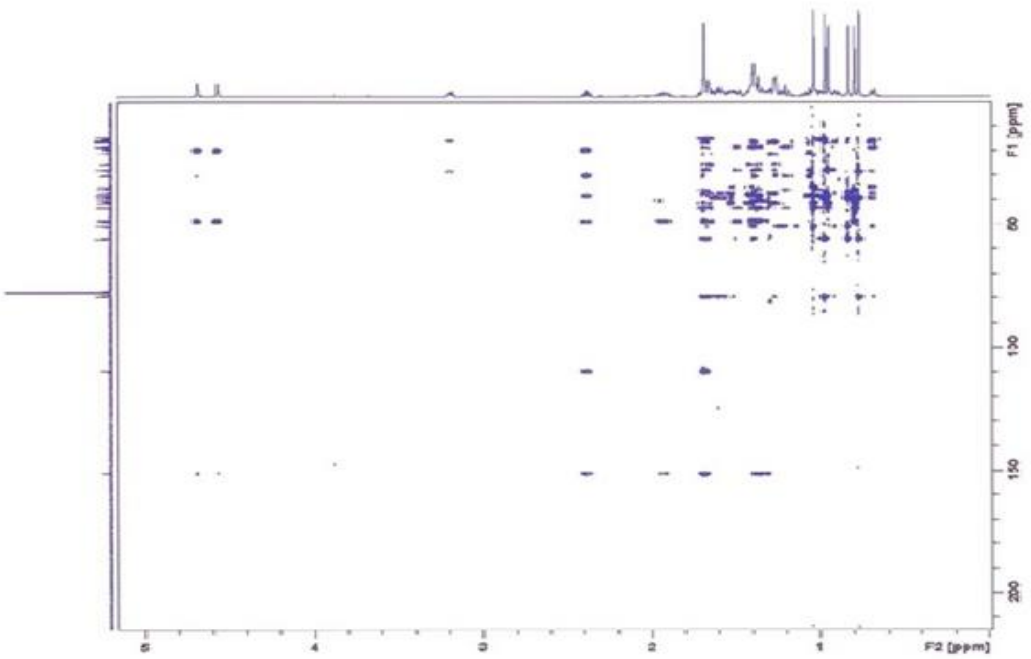


Fig. 4.54 HMBC spectrum of **PsRbE-B** in $CDCl_3$

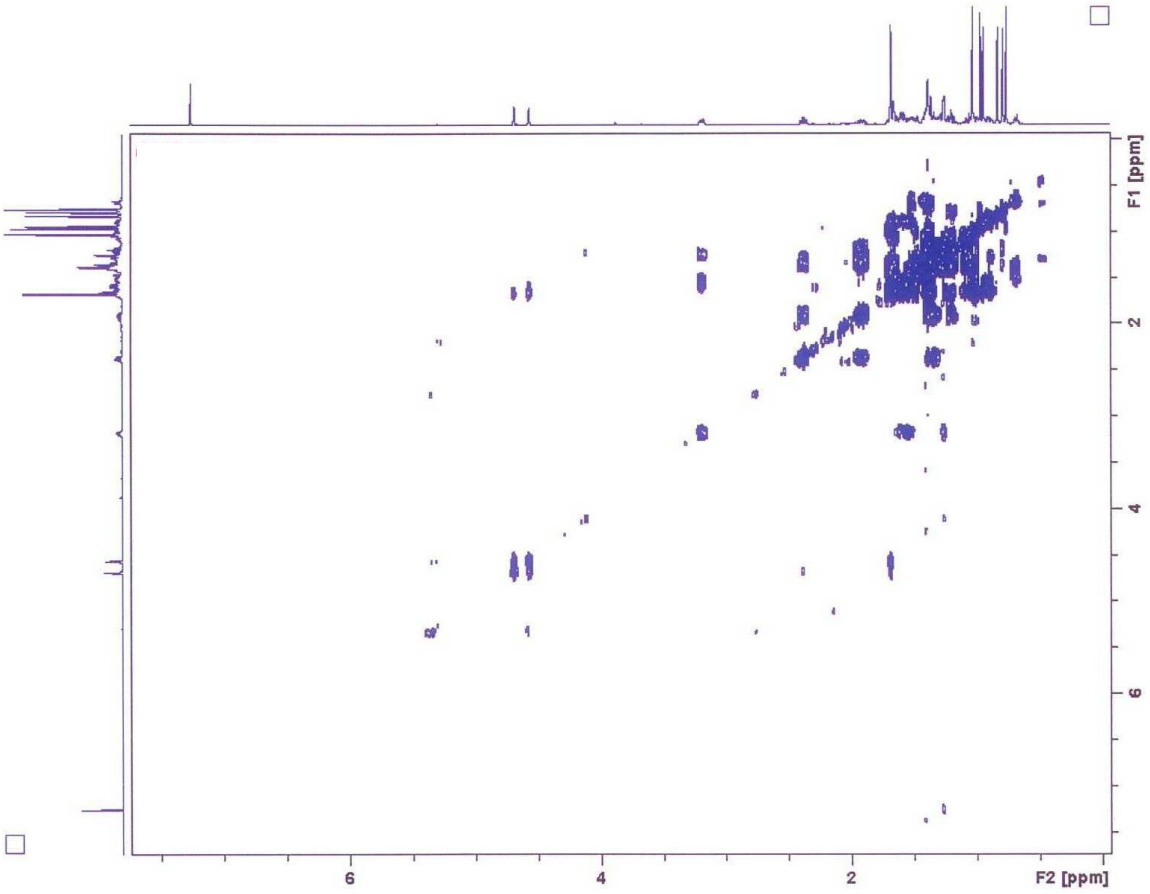


Fig. 4.55 COSY spectrum of **PsRbE-B** in $CDCl_3$

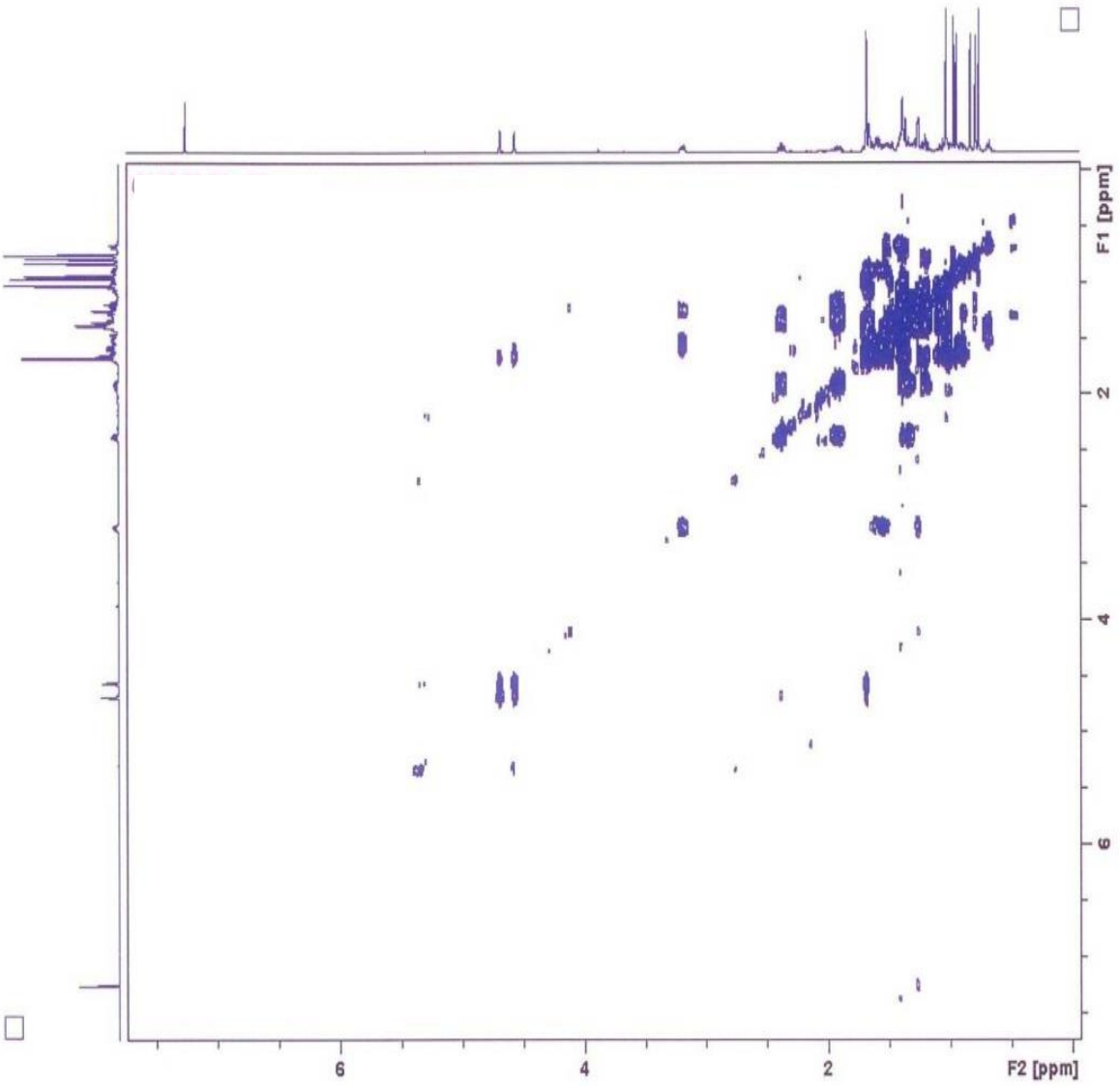


Fig. 4.56 NOESY spectrum of **PsRbE-B** in $CDCl_3$

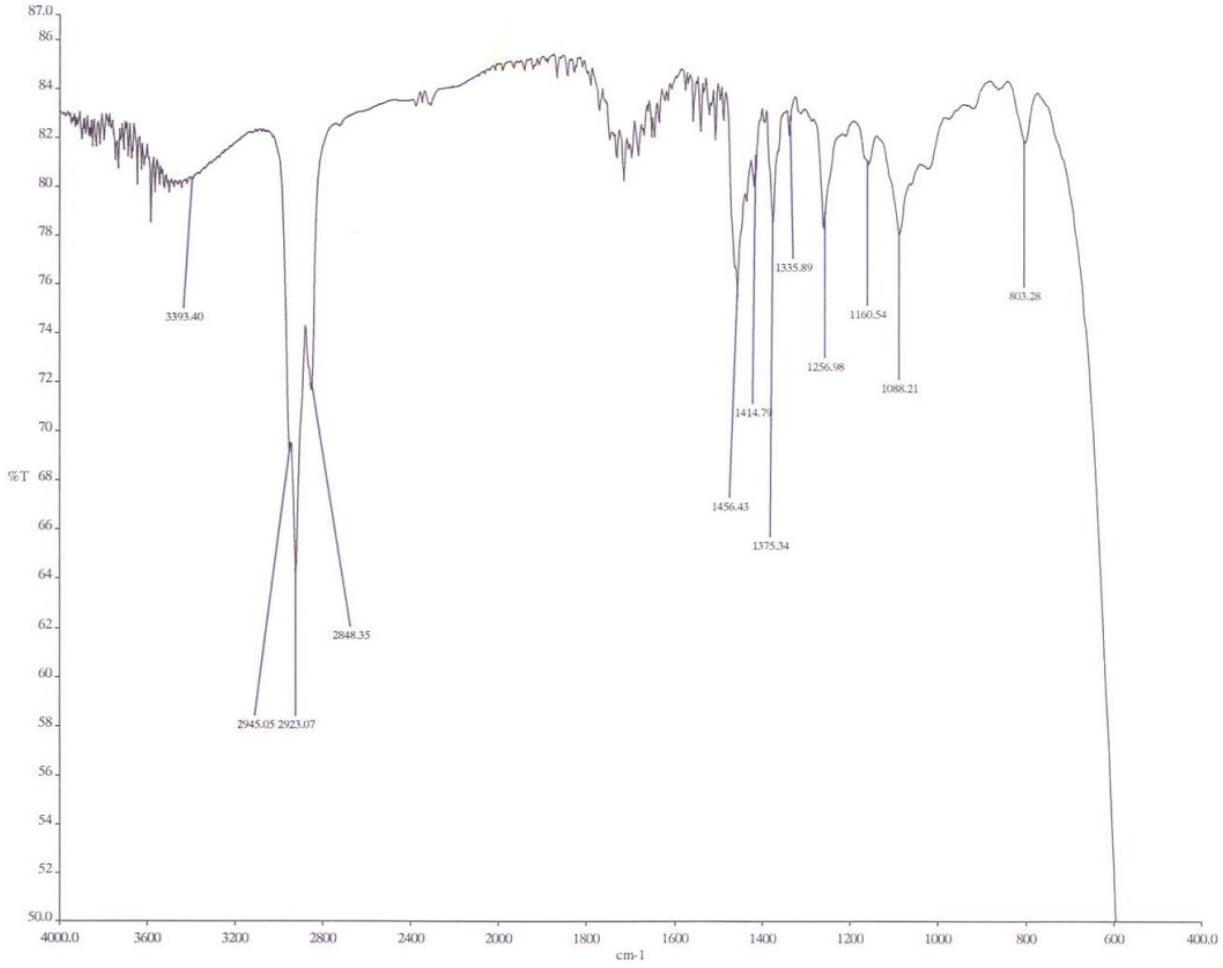


Fig. 4.57 FTIR spectrum of **PsRbE-B**

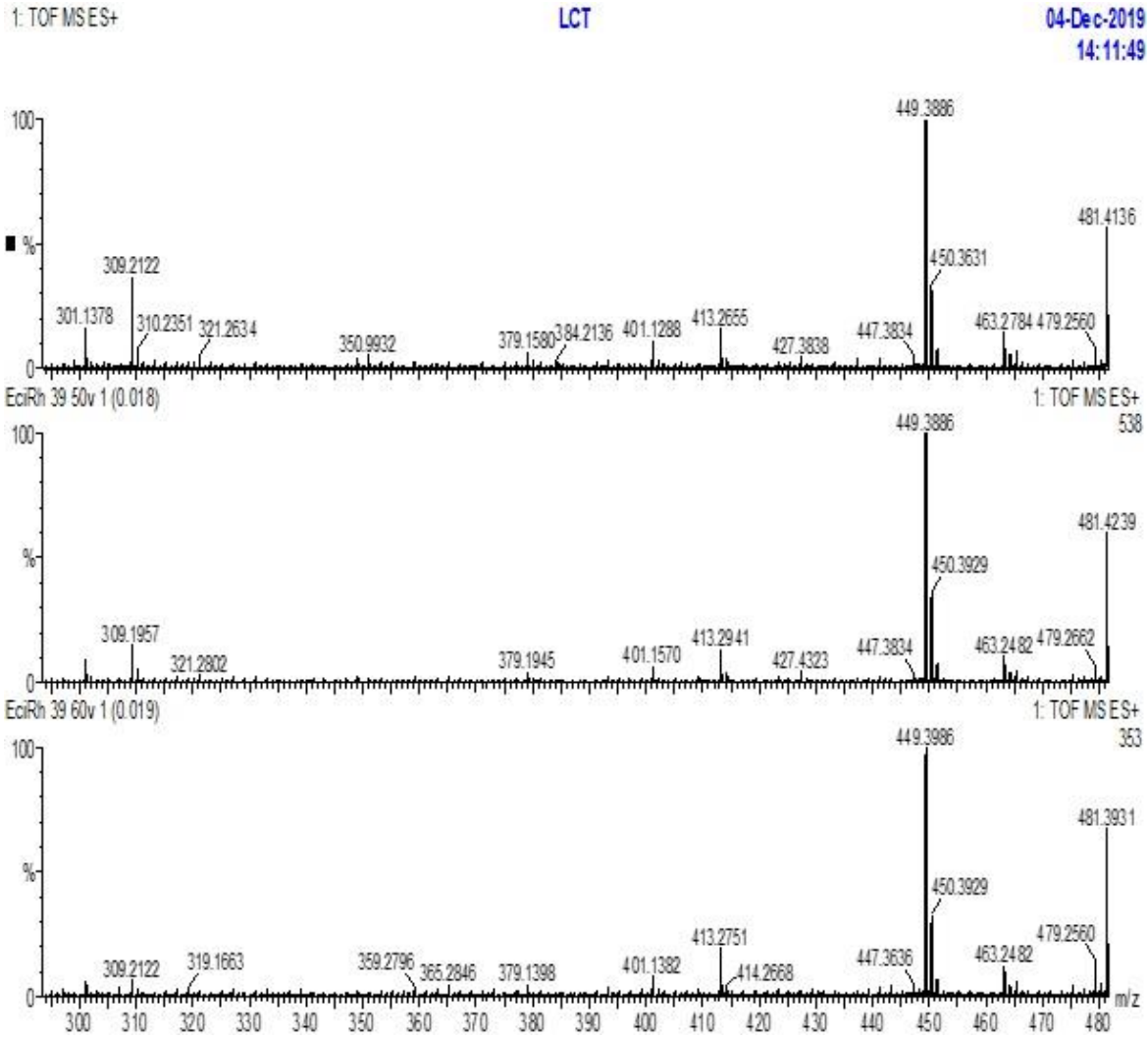


Fig. 4.58 High Resolution Time of Flight (HR TOF) Mass Spectrum of **PsRbE-B**

4.4.2.3 Characterisation of PsRbE-C

PsRbE-C was also isolated as white-crystals from the root bark of *Pterocarpus soyauxii* (Scheme 2) with melting point 142^{o}C-144^{o}C. Spraying of PsRbE-C with vanillin sulphuric acid gave a single spot with an R_f of 0.4 confirming the purity of the isolated compound. The isolated compound was elucidated using various techniques. The mass spectrum of PsRbE-C (Figure 4.69) showed molecular ion (M^+) at 412 which is in consonance with the molecular formula $C_{29}H_{48}O$. The IR $\bar{\upsilon}$ (cm^{-1}) spectrum showed an intensely broad band (cm^{-1}) at 3422 which indicates the presence of OH vibrations. The C=C vibrations was observed at 1659 cm^{-1} as weakly intense-band. The stretching/bending-type bond vibrations (cm^{-1}) of CH_3 were noticed at 2934. CH_2 vibration showed absorptions (cm^{-1}) at 2868 and 1376 (Figure 4.68).

^{1}H NMR spectrum of PsRbE-C (Figure 4.60) showed H-3 proton as a triplet of triplet at *δ'* 3.52 (*J=10.6* Hz) and H-6 olefinic proton showed a doublet at *δ'* 5.35. Two olefinic protons appeared downfield at *δ'* 5.15 and *δ'* 5.01 as doublet of doublet respectively which was identical with the chemical shift of H-22 and H-23 which is diagnostic of stigmasterol. *J* values of *15.1* and *8.5* Hz on both protons indicate the olefinic protons are coupled in an *ortho* fashion. PsRbE-C showed six methyl groups at *δ'* 1.53, *δ'* 1.26, *δ'* 1.04, *δ'* 0.92, *δ'* 0.79, *δ'* 0.69 ppm. Two secondary methyl groups were observed at showed the presence of two CH_3 singlets at *δ* 1.53, and *δ* 1.26; three CH_3 doublets that appeared at *δ* 0.92, δ_H 0.79, δ_H 0.69 ppm; and a CH_3 triplet at δ_H 1.04. ^{13}C NMR spectrum (Figure 4.61) showed recognizable down-field carbons at *δ* 141.22 and *δ'* 122.18 ppm which were ascribed to C-5 and C-6 double bonds respectively. Also, downfield carbons *δ'* 138.31 and *δ'* 129.28 ppm were ascribed to olefinic C-22 and C-23 respectively. This spectrum showed C-5, C-6, C-22 and C-23 as olefinic carbons. *δ'* 71.83 was matched to C-3 β-hydroxyl group, *δ'* 19.87 ppm and *δ'* 12.52 corresponds to angular carbon at C-19 and C-18. DEPT experiments diffentiated twenty-nine (29) carbon signals including six (6) methyls, nine (9) methylenes, eleven (11) methine and three (3) quaternary carbons (figure 4.62 and 4.63). COSY experiment showed cross-peak correlations between the downfield proton *δ* 5.35 (H-6) and methylene protons *δ'* 2.3 (H-4α) and *δ'* 1.95 (H-4β); the doublet proton δ' 3.52 (H-3) and proton δ' 2.3 (H-4), *δ'* 1.84 (H-4) and *δ'* 1.43 (H-2).

Similarly, the olefinic proton at *δ'* 5.01 (H-22) correlated with *δ'* 1.55 (H-20) as indicated in Figure 4.65. The exact positions for the double bond between carbon 5 and 6 were detected by HMBC experiment (Figure 4.66) where significant correlations were observed between quartenary C-5 (δ 140.76) and methylene protons at δ' 2.3 (H-4α) and δ' 1.84 (H-4β), methyl proton δ' 0.7 (H-19). Likewise, HMBC correlation exists between double bonded *δ'* 122.18 (C-6) and CH_2 protons at *δ'* 2.3 (H-4α). Methine protons *δ'* 71.83 (C-3) and *δ'* 29.17 (C-8) correlated with methylene protons at *δ'* 2.3 (H-4α) and methine proton at H-6 respectively. Other methine protons showed significant correlation as outlined C-9 (50.61) and CH_2 protons at H-7α, methyl proton H-19 (δ 0.7), methine C-14 (δ 50.61) and H-18, H-21, C-17 (δ 56.54) and H-18, C-20 (δ 40.96) and H-18. Quartenry proton on C-10 (δ 36.62) Correlated with H-6, while C-13(36.98) correlated with H-18 and H-21. The positions of methyl protons were all confirmed using the HMBC experiment; upfield C-18 (12.52) correlated with H-21, C-19(19.87) and H-11α, C-26(20.29) and H-27, C-27 (19.83) and H-26, C-29 and H-26, H-27. HSQC experiment was used as a diagnostic tool used to assign all protons attached to individual Carbons (Figure 4.64). Comparison of the physical and spectroscopic data of compound PsRbE-C (**4.59**), correlated with literature data earlier reported for stigmasterol (Mahato and Kundu, 1994; Li *et al*., 2006; Habib *et al.*, 2007).

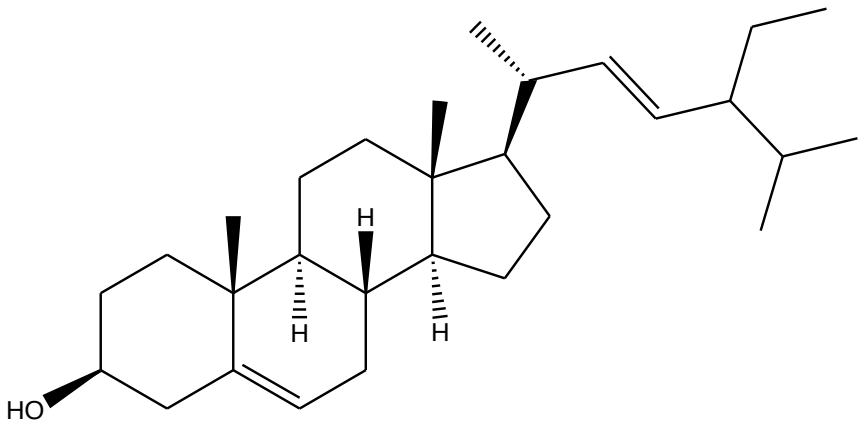


Fig. [4.59] Structure of PsRbE-C (Stigmasterol)

Table. 4.34 ^{1}Proton and ^{13}Carbon NMR*-data of PsRbE-C in $CDCl_3$

Position	**C.**	**δ ^{13}C.**	**δ ^{1}H.**	**M"**	***Jv.* (*Hz.*)**
1	$^{b}CH_2$	37.73	1.1, 1.84	2H, *m*	
2	$^{b}CH_2$	31.92	1.89, 1.43	2H, *m*	
3	^{c}CH	71.83	3.52	1H, *tt'*	*J=10.5, 4.6*
4	$^{b}CH_2$	42.34	2.31, 1.84	2H	
5	^{d}C	140.76	-	-	
6	^{c}CH	121.72	5.35	1H, *d'*	*J=5.0*
7	$^{b}CH_2$	34.06	1.42	2H	
8	^{c}CH	29.17	1.28	1H	
9	^{c}CH	50.15	0.99	1H	
10	^{d}C	36.15	-	-	
11	$^{b}CH_2$	23.08	1.29	2H, *m*	
12	$^{b}CH_2$	39.79	2.05	2H	
13	^{d}C	36.52	-	-	
14	^{c}CH	56.78	1.04	1H	
15	$^{b}CH_2$	24.31	1.12	2H	
16	$^{b}CH_2$	28.26	1.28	2H	
17	^{c}CH	56.07	1.1	1H	
18	$^{a}CH_3$	12.52	1.26	3H, *s*	
19	$^{a}CH_3$	19.87	0.69	3H, *d'*	*J=7.3*
20	^{c}CH	45.85	1.55	1H	
21	$^{a}CH_3$	21.09	1.53	3H, *s*	
22	^{c}CH	138.31	5.01	1H, *dd'*	*J=15.1, 8.4*

23	^{c}CH	129.28	5.15	1H, *dd'*	*J=15.1, 8.6*
24	^{c}CH	51.25	1.29	1H	
25	^{c}CH	32.38	1.97	1H	
26	$^{a}CH_3$	20.29	0.79	3H, *d'*	*J=6.8*
27	$^{a}CH_3$	19.83	1.02	3H, *d'*	*J=7.8*
28	$^{b}CH_2$	26.09	2.21	2H	
29	$^{a}CH_3$	11.86	1.04	3H, *t'*	*J=3.2, 2.3*

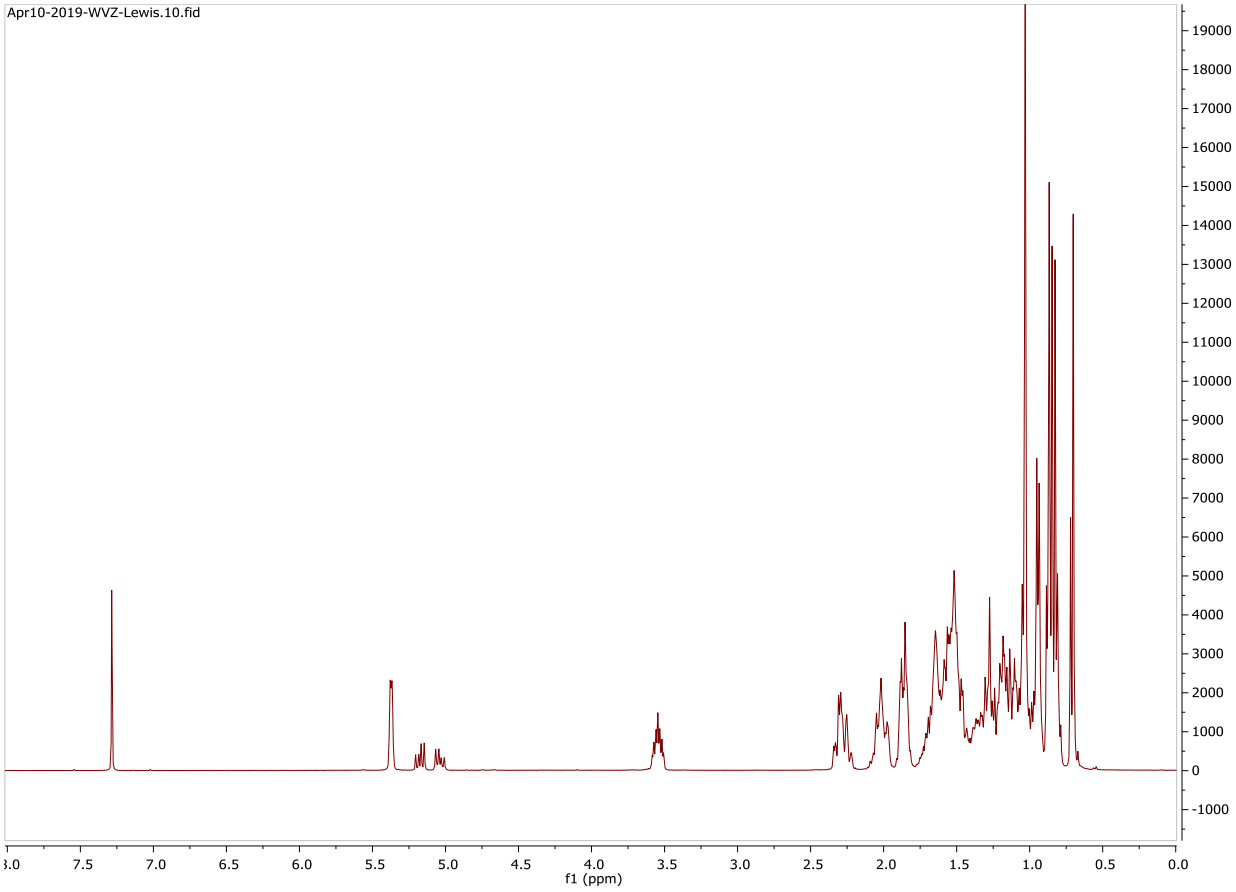


Fig. 4.60 ^{1}H NMR (500 MHz) Spectrum of PsRbE-C in $CDCl_3$

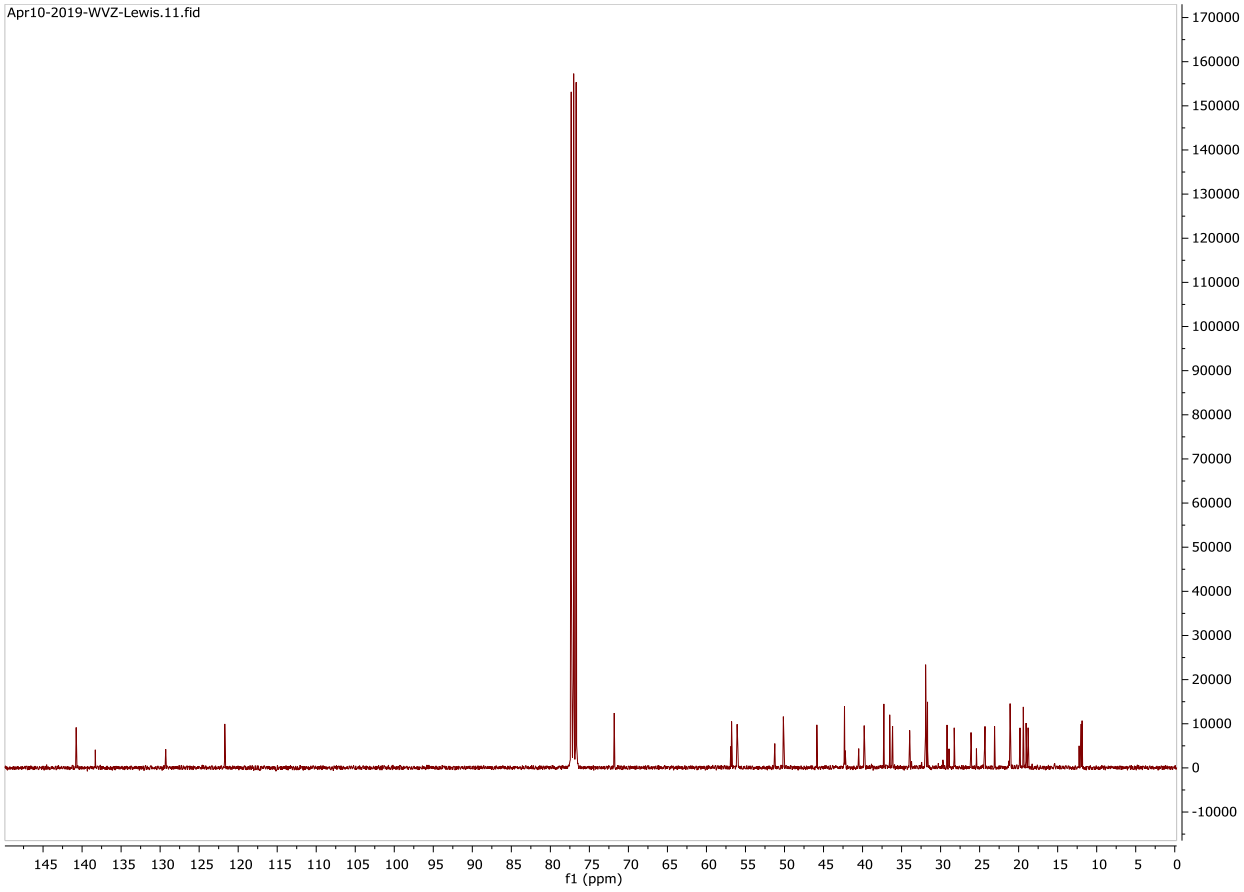


Fig. 4.61 ^{13}C NMR* Spectrum (500 MHz) of PsRbE-C in $CDCl_3$

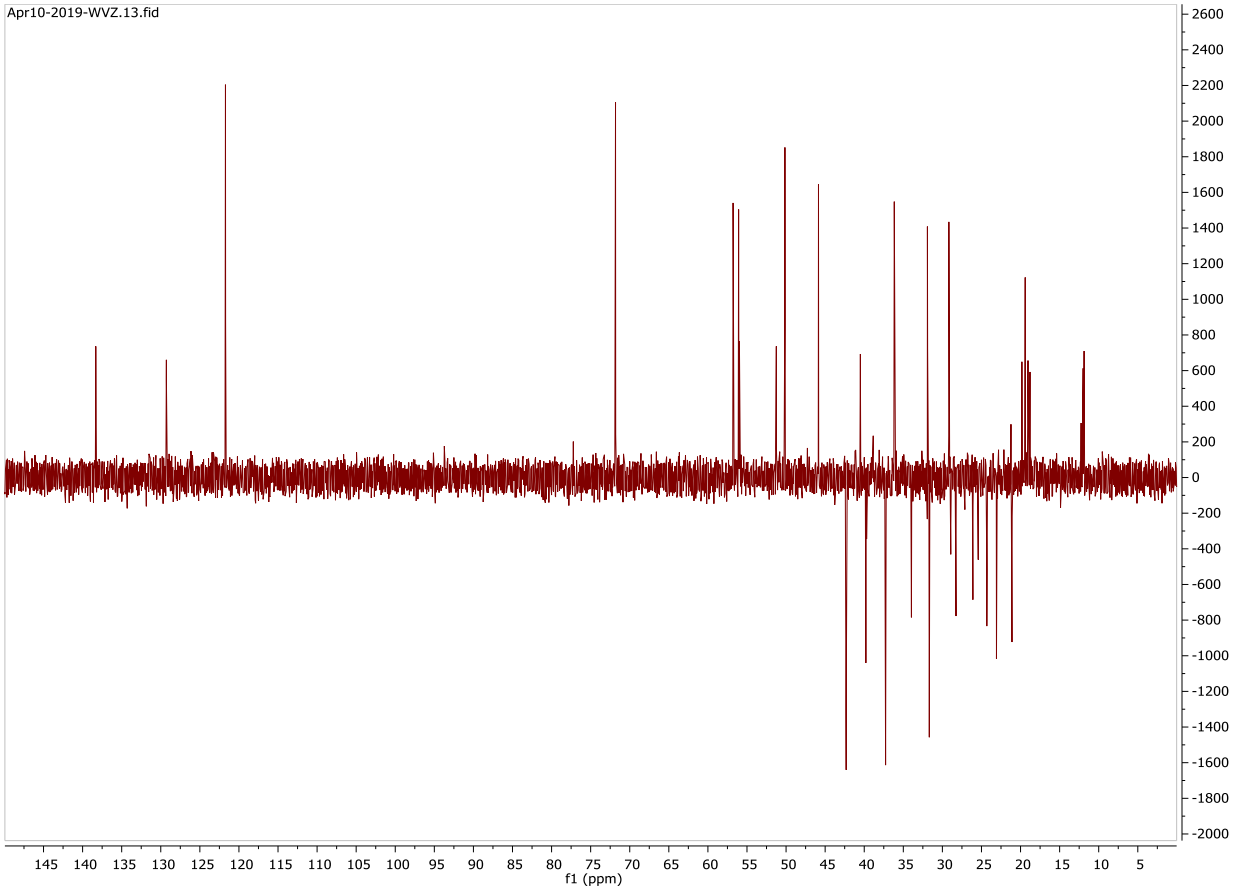


Fig. 4.62 DEPT 135 Spectrum of PsRbE-C in $CDCl_3$

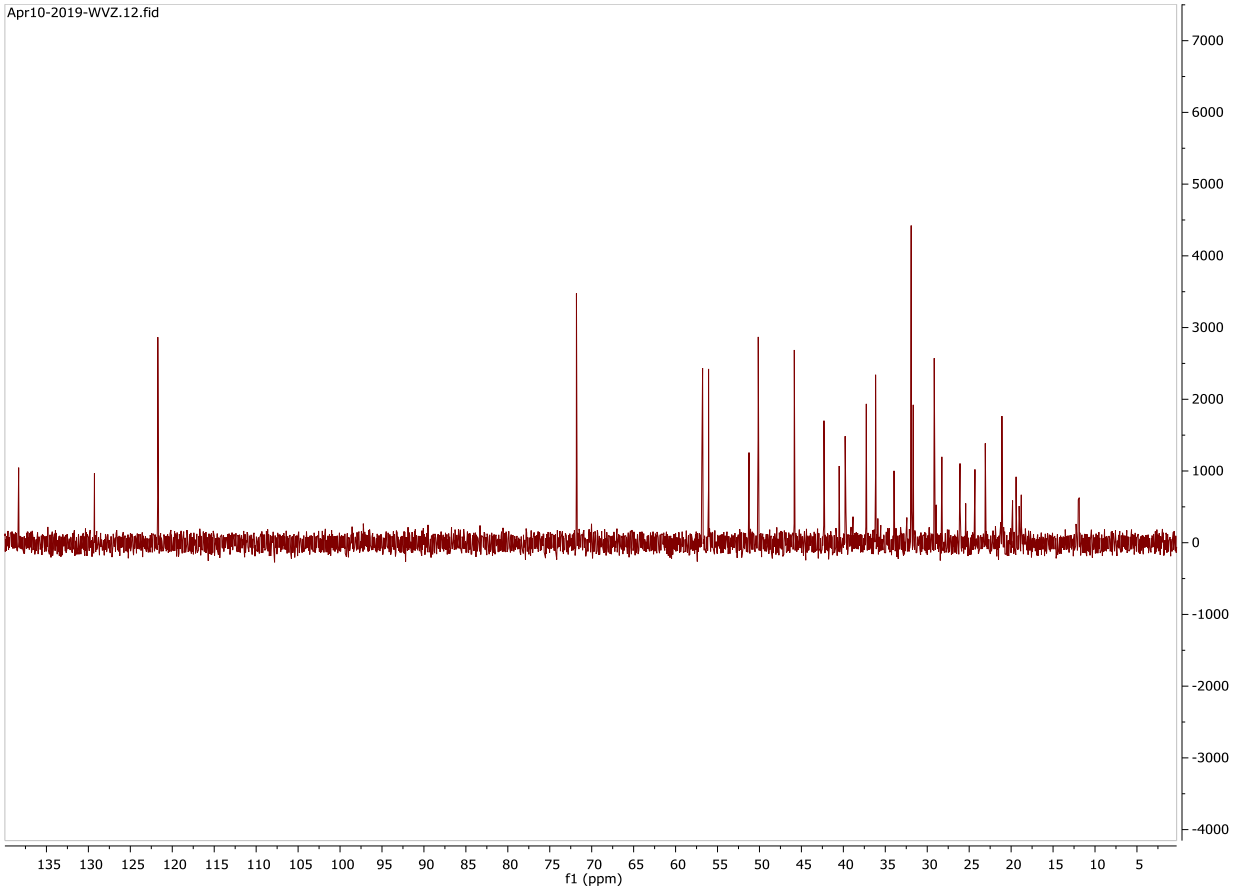


Fig. 4.63 DEPT 90 Spectrum of PsRbE-C

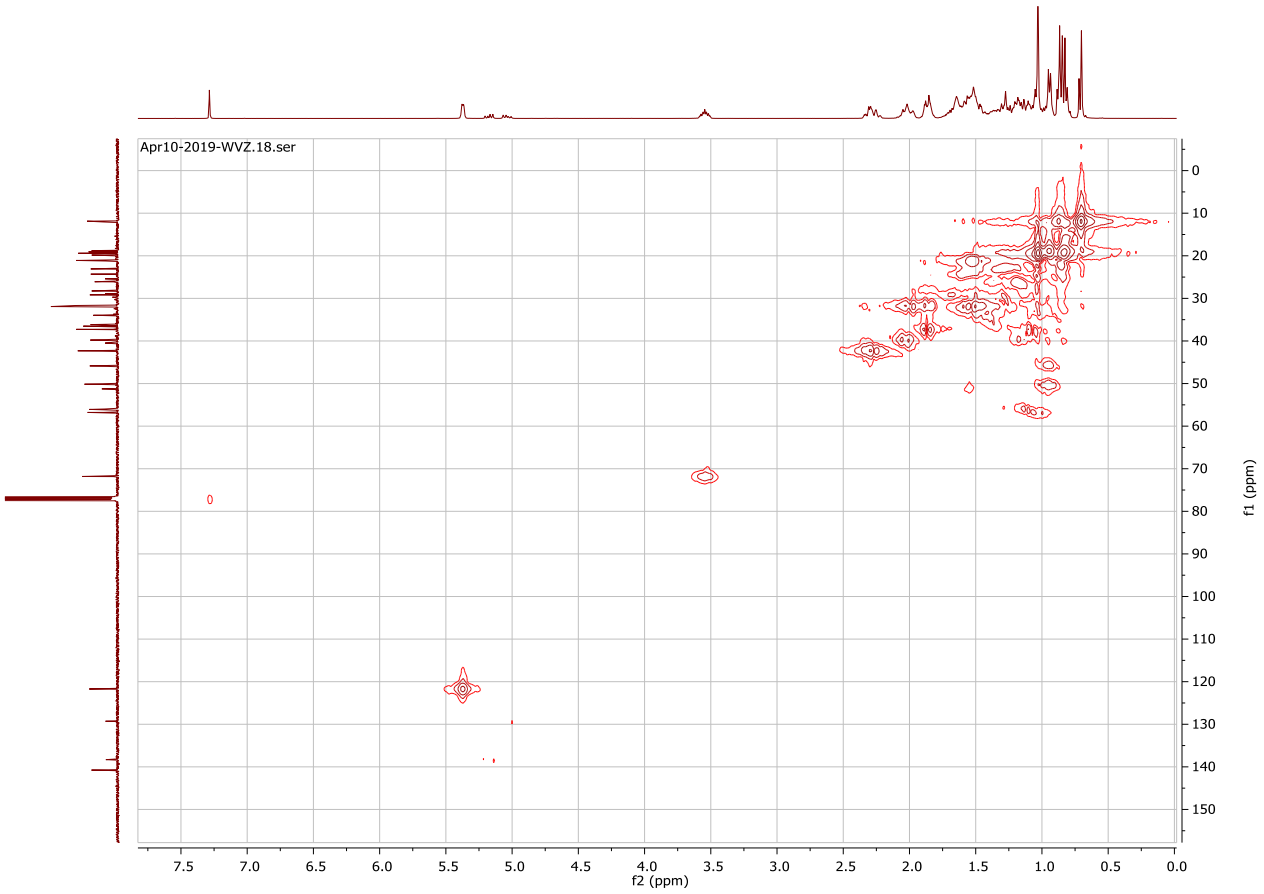


Fig. 4.64 HSQC Spectrum of PsRbE-C in $CDCl_3$

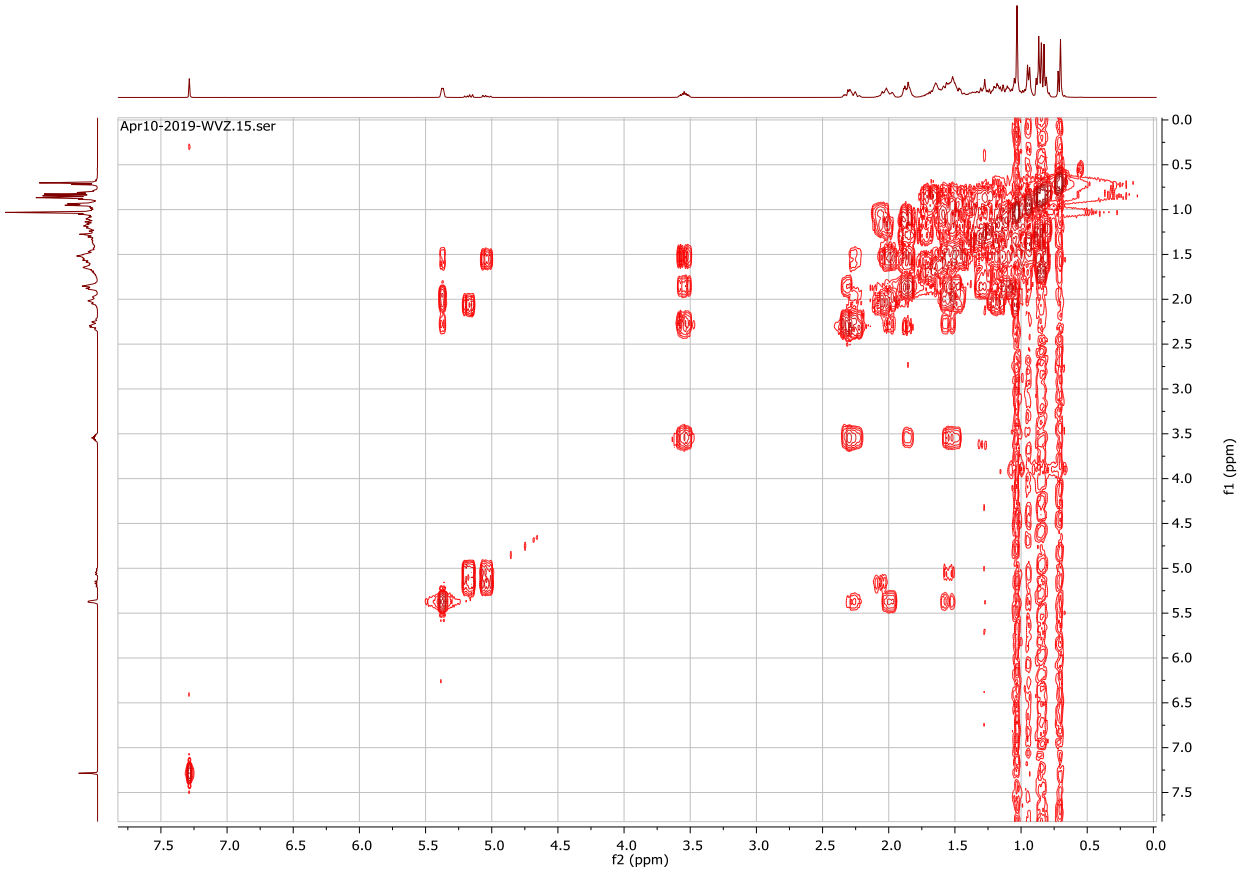


Fig. 4.65 COSY Spectrum of PsRbE-C

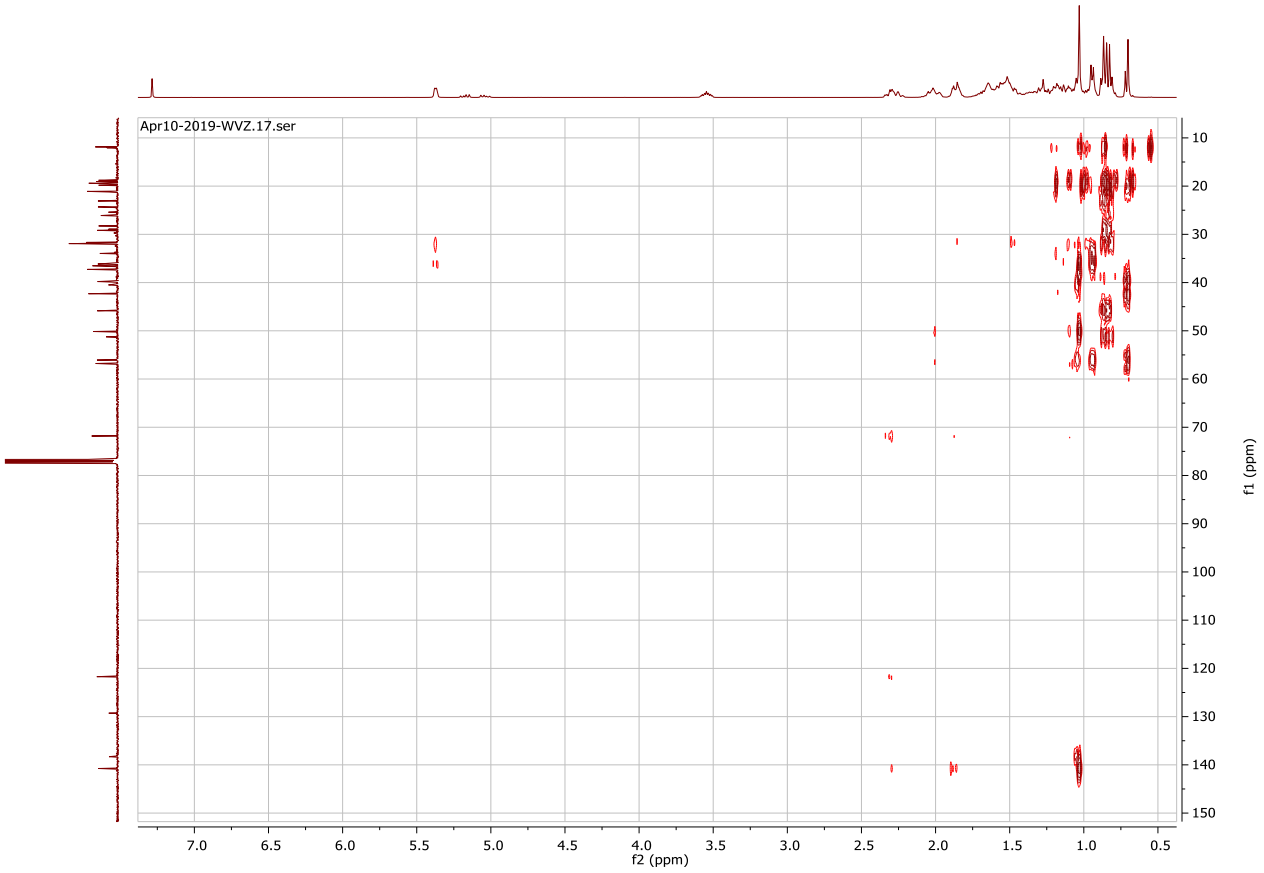


Fig. 4.66 HMBC Spectrum of PsRbE-C in $CDCl_3$

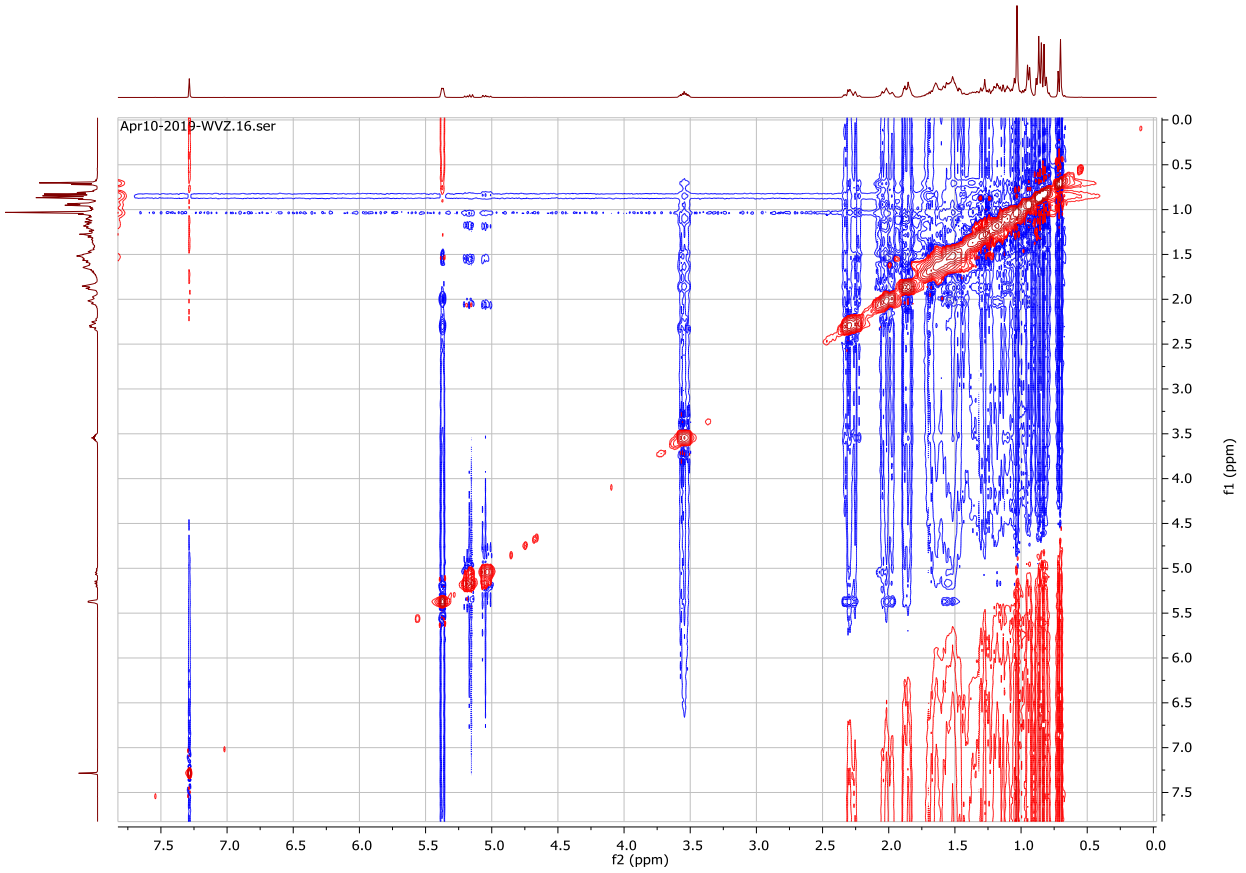


Fig. 4.67 NOESY Spectrum of PsRbE-C

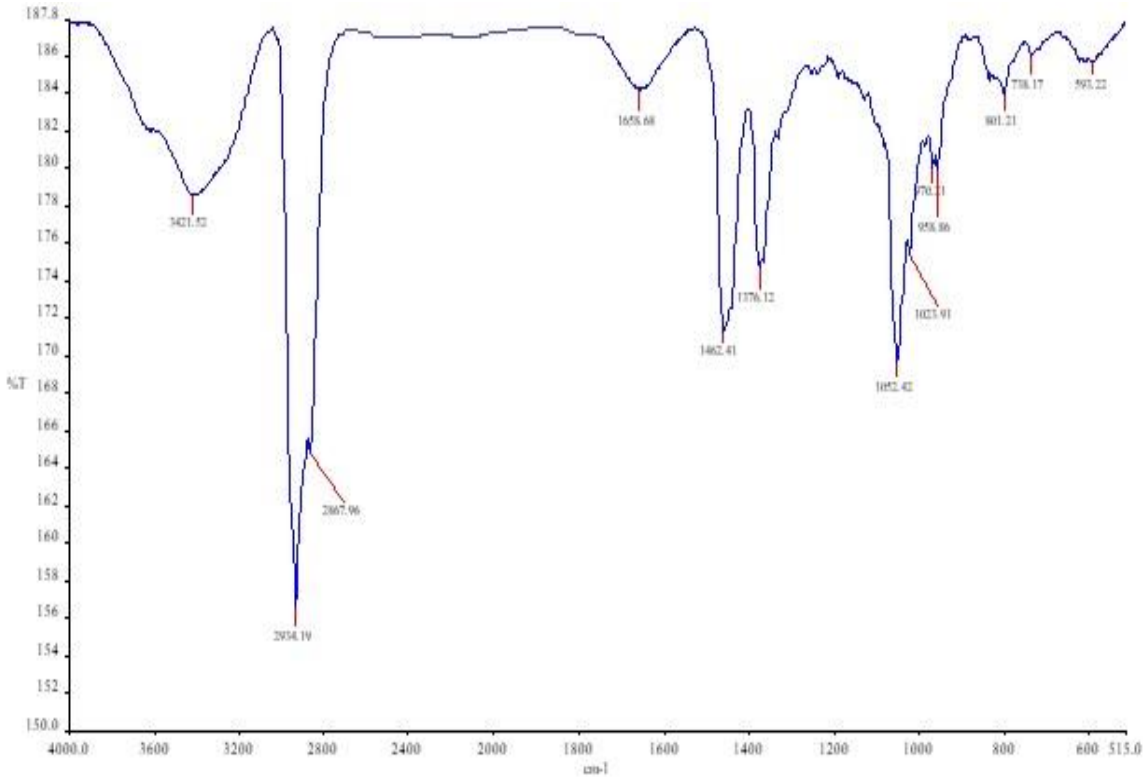


Fig. 4.68 FTIR Spectrum of PsRbE-C

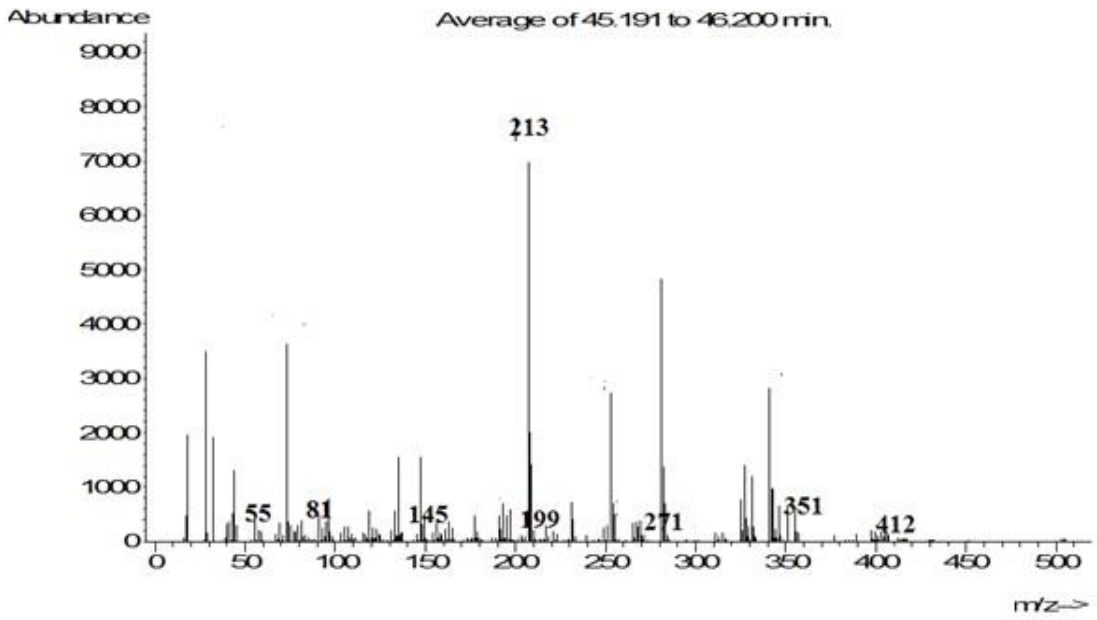


Fig. 4.69 Mass Spectrum of PsRbE-C

4.4.2.4. Characterisation of PsRbE-D

PsRbE-D was separated as pure-white crystals (melting point 136-138^{o}C) from the root bark part of *Pterocarpus soyauxii* (Scheme 2). Spraying of PsRbE- D with vanillin sulphuric acid an oval spot using hexane: ethyl acetate solvent system (1:2) showing R_f value of 0.6. Various spectroscopic techniques were used to classify the isolated pure compound and elucidate its structure. The mass spectrum (Figure 4.86) of PsRbE-D showed base beak with *m/z* 213 and molecular ion of 414 which is in consonance with the molecular formula $C_{29}H_{50}O$. This fragmentation pattern is consistent with that of β-sitosterol [**4.7**]. The IR $\bar{\upsilon}$ (cm^{-1}) spectrum of PsRbE-D showed broad band (cm^{-1}) at 3424 and moderately intense band at 1053 and 593 for OH group vibrations. The corresponding C=C vibrations (cm^{-1}) were detected as a weak band at 1657. The intense band (cm^{-1}) at 2956 revealed the stretching and bending vibrations of the methyl element. The band at 2935 cm^{-1} and the medium band at 1376 cm^{-1} showed the vibration of the methylene component (Figure 4.79).

^{1}H NMR spectra of PsRbE-D (Figure 4.71) showed one olefinic methine proton at δ' 5.33. It showed a proton corresponding to C-3 hydroxy group which appeared as a triplet of doublet of doublets at δ' 3.52. ^{13}C NMR of PsRbE-D gave recognizable signals at δ' 140.75 and δ' 121.72 ppm which were ascribed to C-5 and C-6 respectively. δ' 71.83 was ascribed to the β-hydroxyl group C-3, δ' 19.82 ppm and δ' 11.99 corresponds to angular carbon at C-19 and C-18. PsRbE-D had twenty-nine (29) carbon signals including six (6) CH_3, nine (11) CH_2 and three (3) carbons differentiated using ^{90}DEPT and ^{135}DEPT experiments (Figure 4.73 and Figure 4.74). The COSY experiment showed cross-peak match between protons at *δ'* 5.33 (H-6), *δ'* 2.23 (H-4), and *δ'* 1.87 (H-7). COSY correlation was observed between the triplet proton δ' 3.52 (H-3) and protons δ' 2.3 (H-4). Also, *δ'* 1.98 (H-25) and *δ'* 0.94 (H-26, H-27) correlated. Similarly, the terminal proton at *δ'* 1.56 (H-28) correlates with *δ'* 0.90 (H-29).

The HMBC experiment (Figure 4.77) showed significant match between quartenary *δ* 140.75 (C-5) and methyl protons at *δ* 2.3 (H-19), likewise methine proton *δ* 0.95 (H-9) matches methyl proton *δ* 0.94 (H-19). HMBC correlation exists between *δ* 56.78 (C-14) and singlet methyl protons at *δ* 1.01 (H-18). *δ* 29.17 (C-23) and *δ* 19.4 (C-26) correlated

with H-27. HSQC experiment was used as a diagnostic tool used to assign all protons attached to individual carbons. The isolated compound was identified as β-sitosterol (**4.70**). Spectroscopic data of PsRbE-D is in consonance with earlier reports in literature (Li *et al*., 2006; Habib *et al.*, 2007).

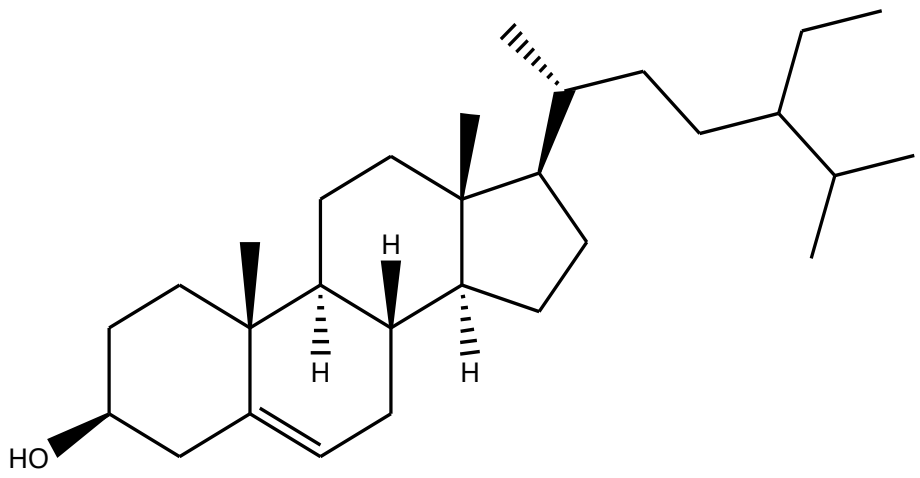


Fig. [4.70] Structure of PsRbE-D **(**β-sitosterol**)**

Table, 4.35 ^{1}Proton and ^{13}Carbon NMR- data of PsRbE-D in $CDCl_3$

Position	C.	**δ $^{13}C_a$**	**δ $^{1}H_a$**	**M"**	***Jv* (*Hz.*)**
1	$^{b}CH_2$	37.26	1.04, 1.8	2H, *m*	
2	$^{b}CH_2$	31.67	1.89, 1.44	2H, *m*	
3a	^{c}CH	71.83	3.52	1H, *tt'*	*J=10.6, 5.4, 4.7*
3b	OH	-	4.76	1H	
4	$^{b}CH_2$	42.30	2.3, 1.84	2H	
5	^{d}C	140.75	-	-	
6	^{c}CH	121.72	5.33	1H, *m*	
7	$^{b}CH_2$	28.25		2H, *m*	
8	^{c}CH	31.91	1.4	1H	
9	^{c}CH	50.15	0.95	1H	
10	^{d}C	36.15	-		
11	$^{b}CH_2$	24.31	1.29	2H	
12	$^{b}CH_2$	36.52	2.01	2H	
13	^{d}C	39.79	-	-	
14	^{c}CH	56.78	1.0	1H	
15	$^{b}CH_2$	21.08	1.4	2H	
16	$^{b}CH_2$	29.17		2H	
17	^{c}CH	55.97	1.1	1H	
18	$^{a}CH_3$	11.99	1.01	3H', *s*	
19	$^{a}CH_3$	19.82	0.94	3H', *s*	
20	^{c}CH	36.15	0.95	1H	
21	$^{a}CH_3$	23.08	1.53	3H'	

22	$^{b}CH_2$	33.96		2H	
23	$^{b}CH_2$	29.17		2H	
24	^{c}CH	51.71		1H	
25	^{c}CH	31.67	1.97	1H	
26	$^{a}CH_3$	19.4	0.7	3H', *d'*	*J=6.7*
27	$^{a}CH_3$	19.04	0.82	3H', *d'*	*J=6.7*
28	$^{b}CH_2$	26.10	1.5		
29	$^{a}CH_3$	11.86	0.84 (t, H)	3H', *t'*	*J=3.2, 2.3*

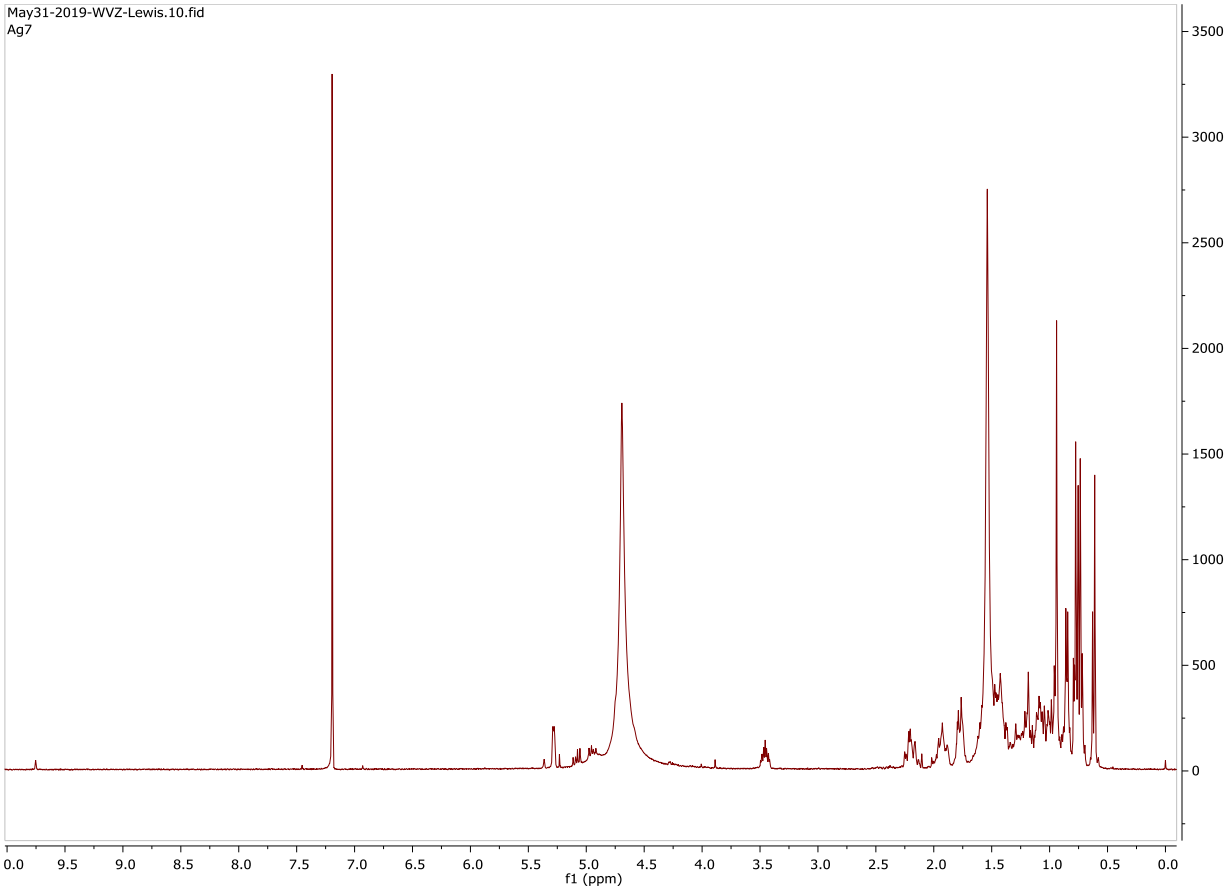


Fig. 4.71 Proton (^{1}H) NMR Spectrum (500MHz) of **PsRbE-D** in $CDCl_3$

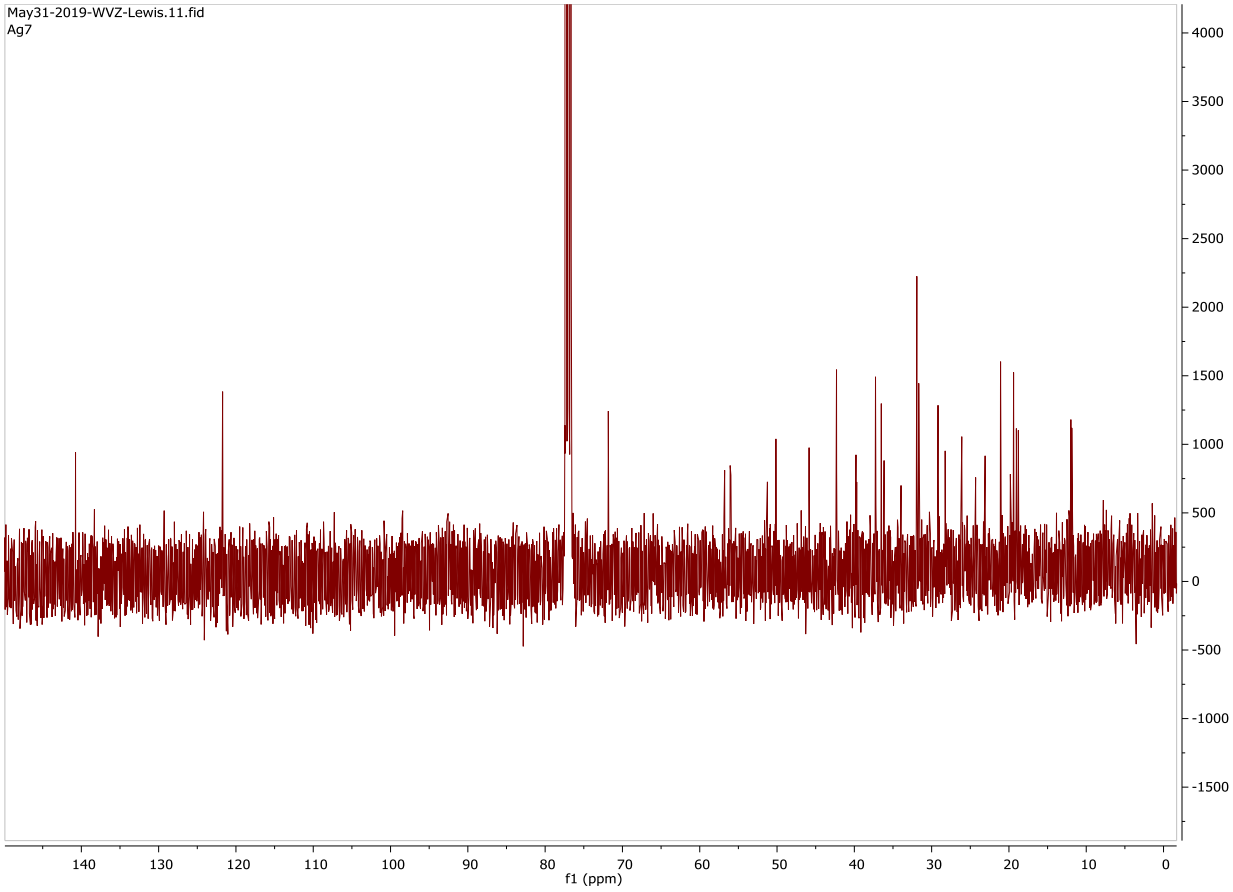


Fig. 4.72 ^{13}C NMR Spectrum (500MHz) of **PsRbE**-D in $CDCl_3$

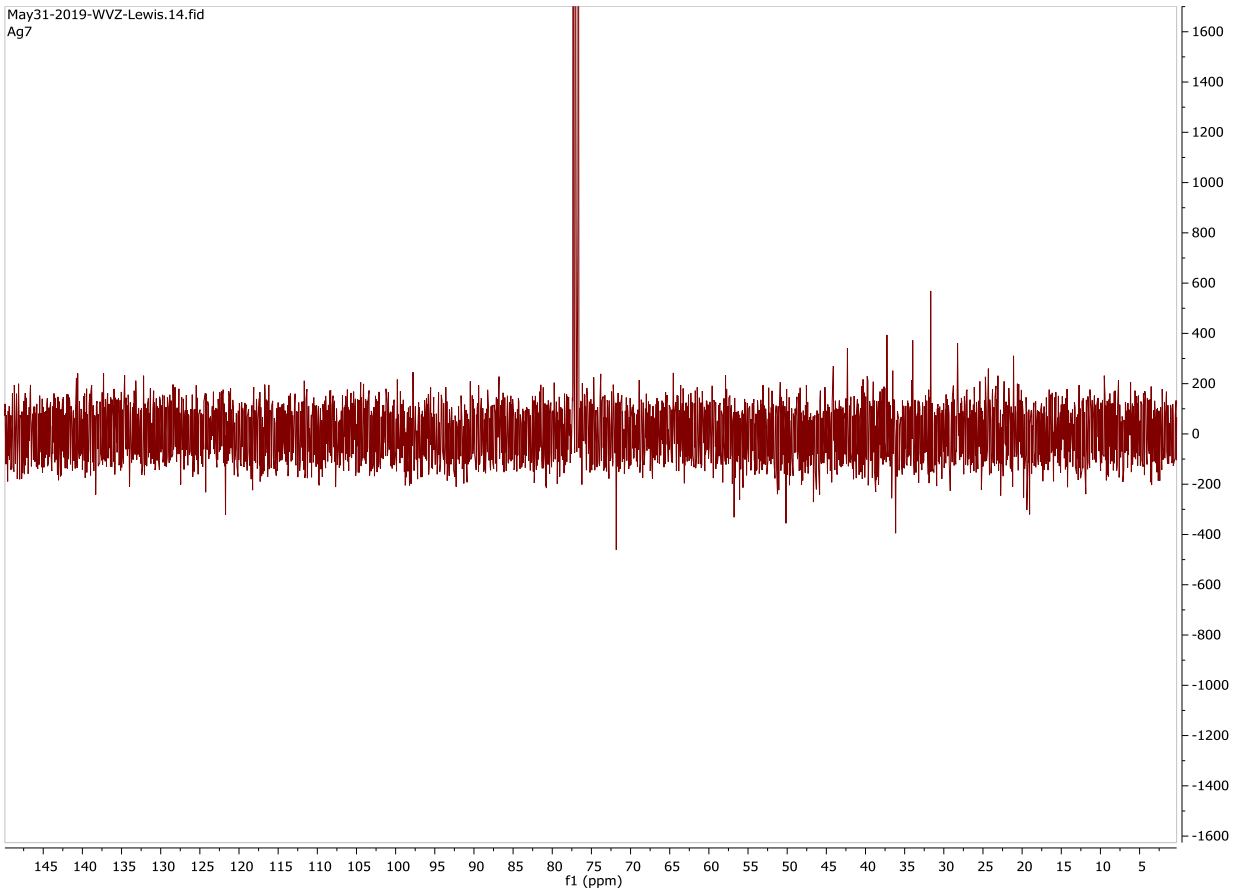


Fig. 4.73 DEPT 135 Spectrum of **PsRbE-D** in $CDCl_3$

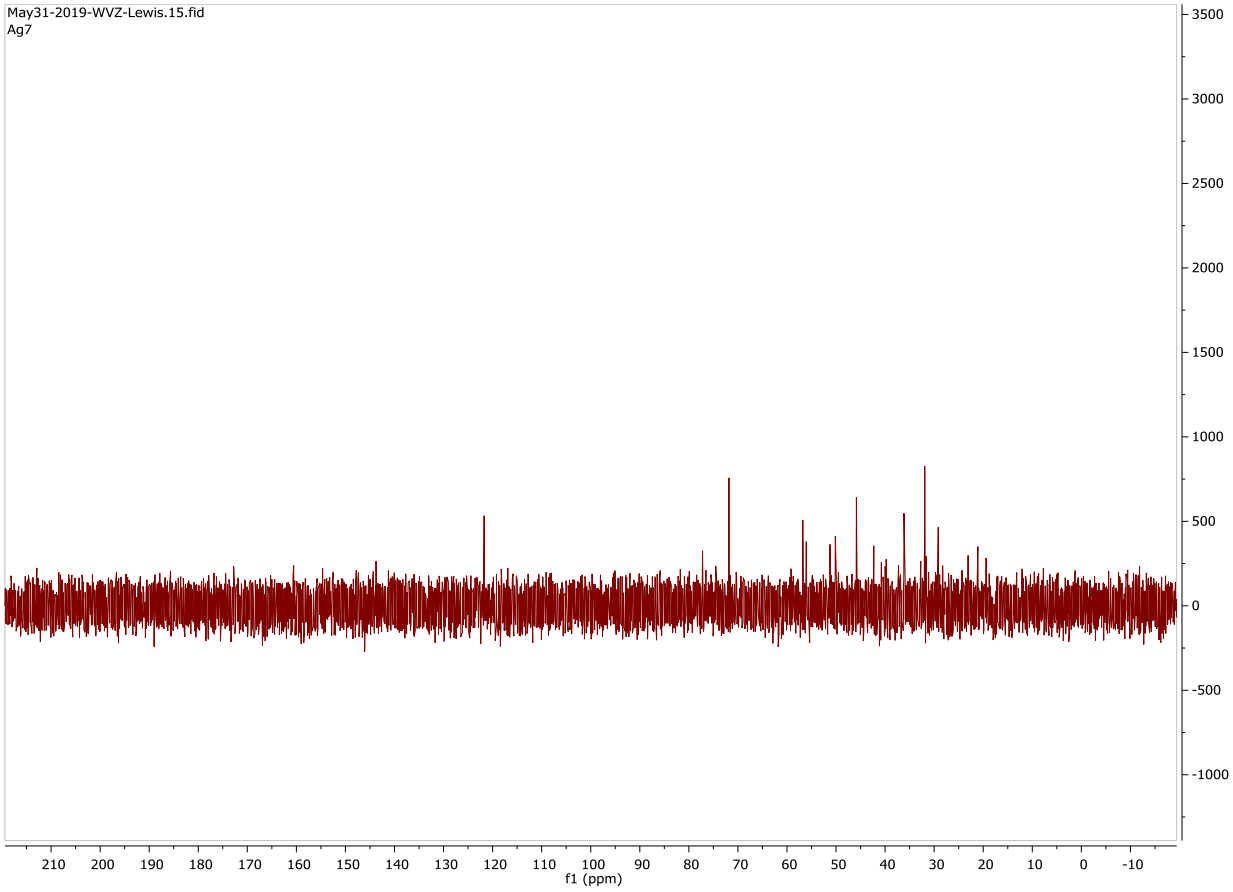


Fig. 4.74 DEPT 90 Spectrum of **PsRbE-D** in $CDCl_3$

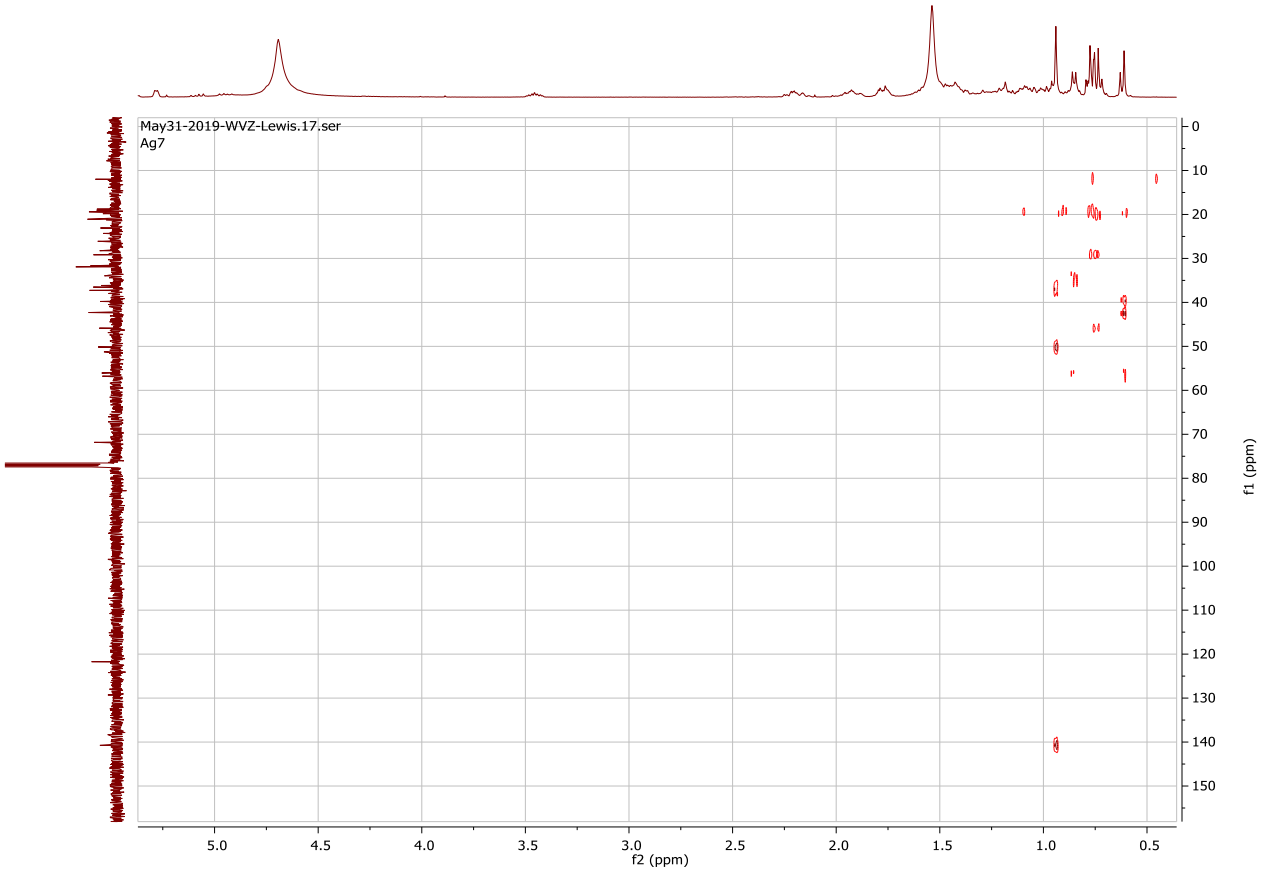


Fig. 4.75 HSQC Spectrum of **PsRbE-D** in $CDCl_3$

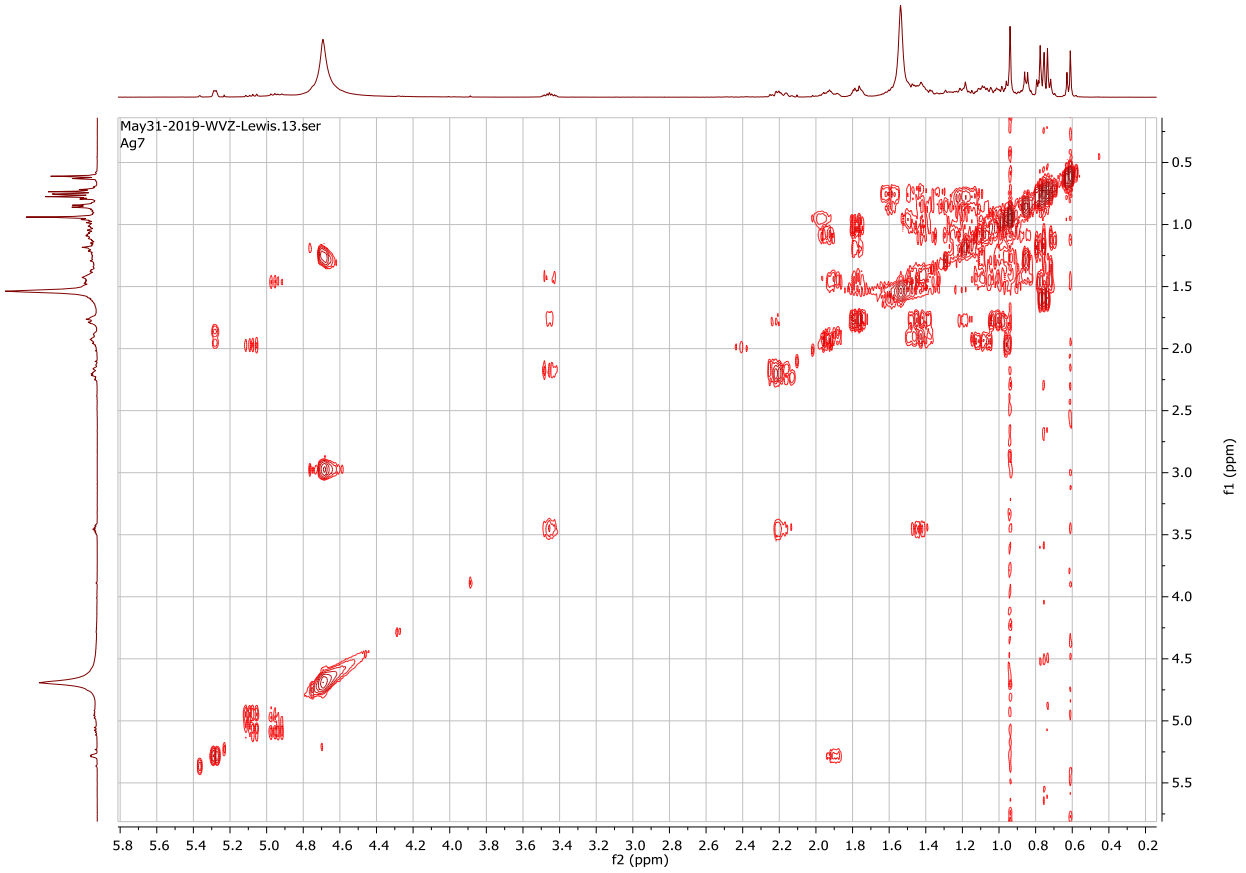


Fig. 4.76 COSY Spectrum of **PsRbE-D** in $CDCl_3$

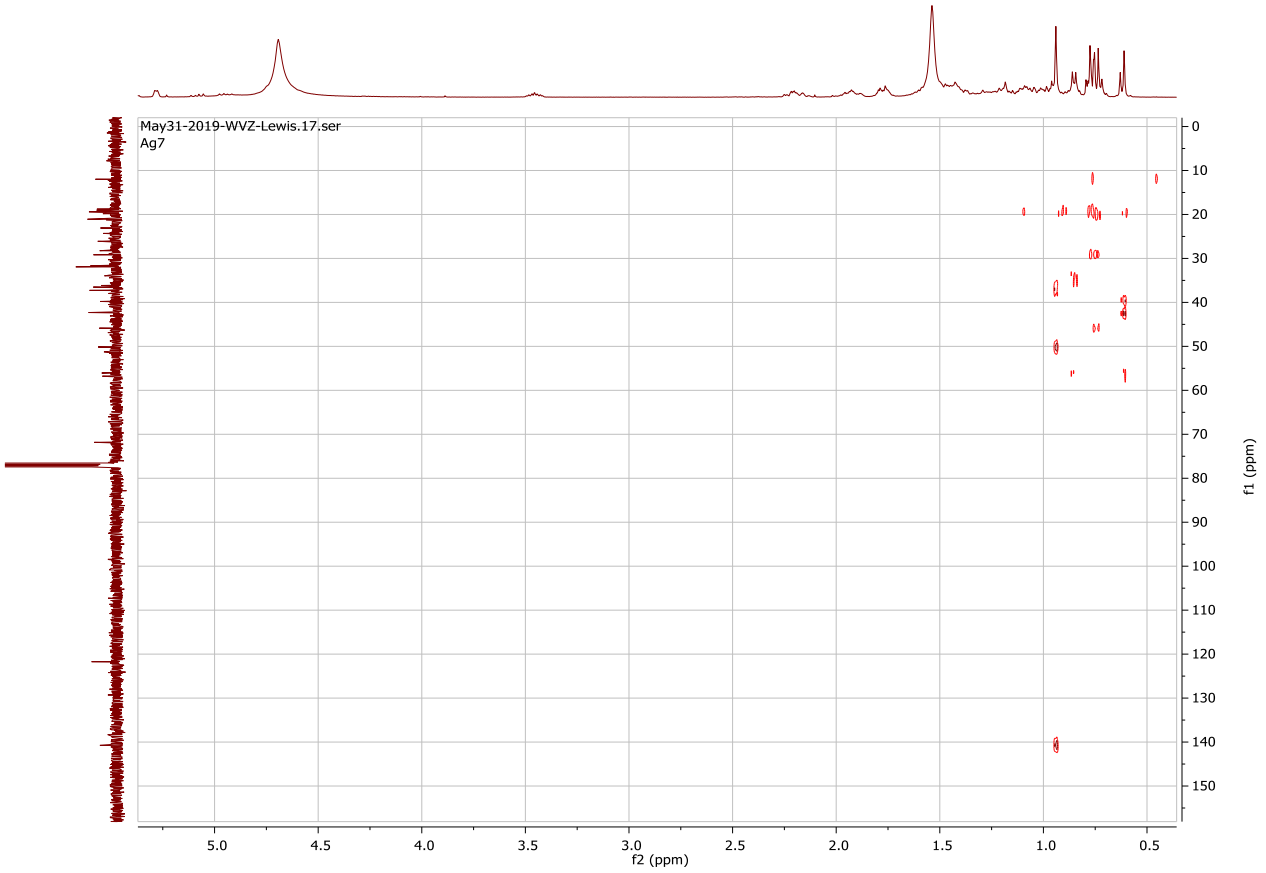


Fig. 4.77 HMBC Spectrum of **PsRbE-D** in $CDCl_3$

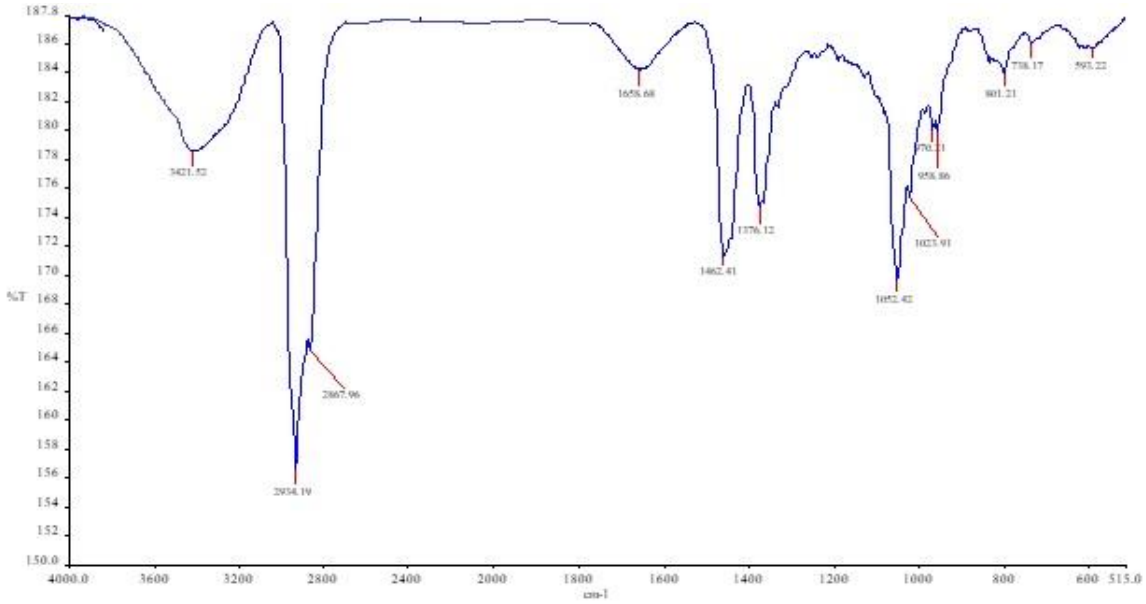


Fig. 4.78 FTIR Spectrum of **PsRbE-D**

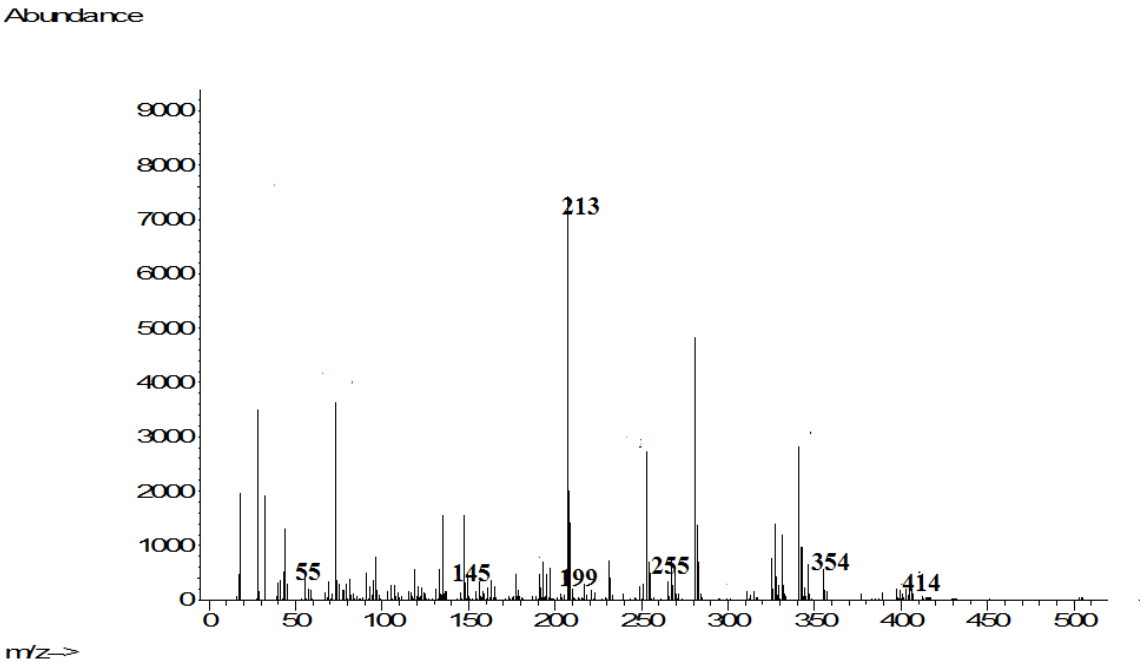


Fig. 4.79 Mass Spectrum of **PsRbE-D**

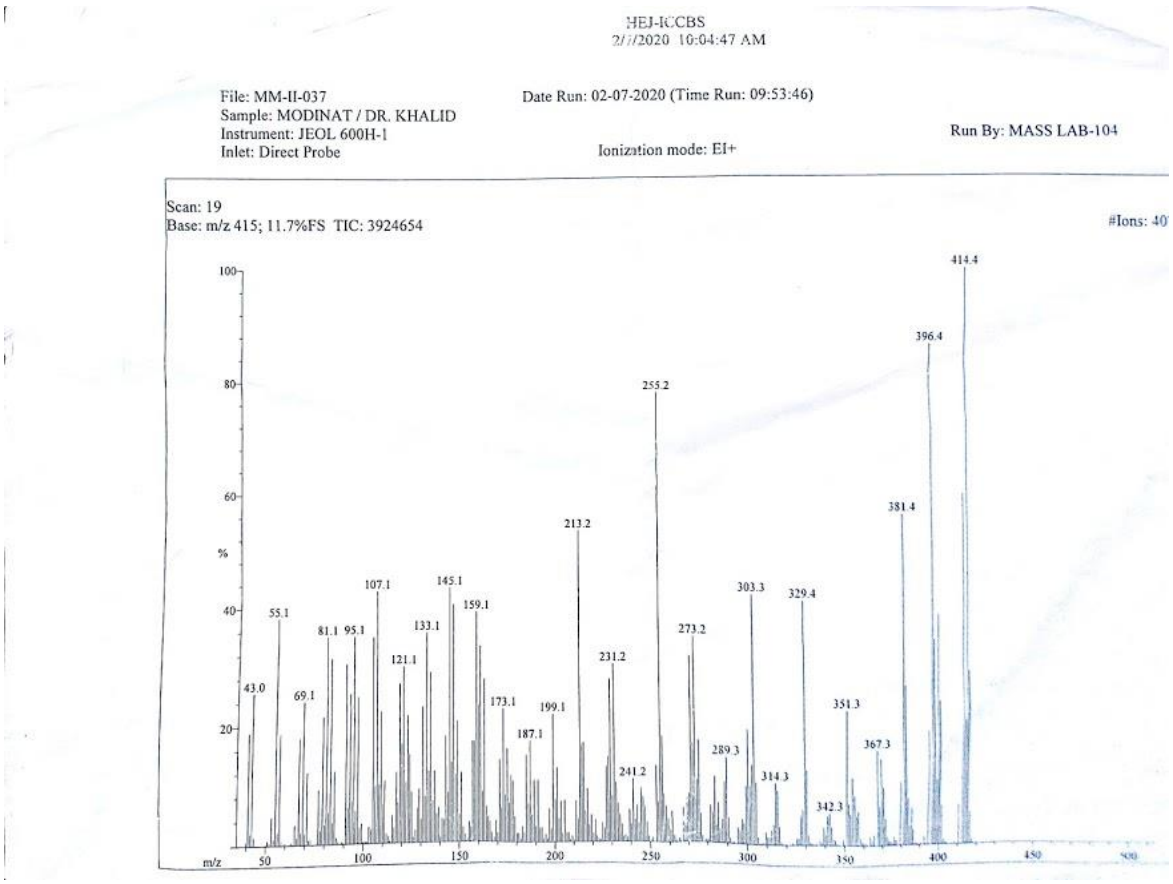


Fig. 4.80 EI-MS Mass Spectrum of **PsRbE-D**

4.5 Antidiabetic and Antioxidant activities of isolated compounds

Chromatographic purification of ethyl acetate (EtOAc) fractions from *Calophyllum inophyllum* and *Pterocarpus soyauxii* gave seven (7) compounds. Friedelan-3-one, 1,3,5-trihydroxy,2-methoxyxanthone and Stigmasterol were isolated from *Calophyllum inophyllum* while 3β-hydroxylup-20(29)-ene, 2,3 dihydroxyfriedelinol, stigmast-5-en-3-ol and stigmasterol were isolated from *Pterocarpus soyauxii*. 1,3,5-trihydroxyl-2-methoxylxanthone, 3β-hydroxylup-20(29)-ene and 2,3-dihydroxyfriedelinol are reported for the first time in *Calophyllum inophyllum*. The anti-diabetic and antioxidant potential of all isolated compounds from *Calophyllum inophyllum* and *Pterocarpus soyauxii* were evaluated based on their minimum inhibitory concentration. 1,3,5-trihydroxy-2-methoxyxanthone had the highest antidiabetic and antioxidant activity due to the polyhroxylated nature of the compound followed by 2,3 dihydroxyfriedelinol. These activities must be due to the presence of the hydroxylated and polyhydroxylated nature of these compounds.

The biological activities of some chemical consituents from *Calophyllum* and *Pterocarpus* species have been reported earlier in literature. Chemical constituents from *Calophyllum inophyllum* has been reported to exhibit activity against dermatitis (Burkhil, 1994; Uma *et al.,* 2012). Xanthones isolated from this plant has been responsible for their anti-microbial and cytotoxic properties (Kashman *et al*., 1992; Hang *et al.,* 2006). A xanthone derivative from *Calophyllum inophyllum* root bark has been discovered to be a potential anti-inflammatory, antimicrobial and cytotoxic agent (Marie *et al.,* 2004). Triterpenes in *Calophyllum inophyllum* leaves showed good lipid inhibition *in-vivo* (Janki *et al.,* 2012). Calophyllolide also, has been found to be an anticoagulant (Bhalla *et al*., 1980). The plant has also been discovered to be an effective anticancer agent (Itoigawa *et al.,* 2001). Chemical constituents from *Pterocarpus soyauxii* has been reported to possess anti-inflammatory properties and can be used to treat skin ailments. (Oteng-Gang and Mbachu, 1990; Bremaud *et al*., 2011). Triterpenes isolated from the root parts exhibited activities against Dysmenorrhoea, uterine haemorrhage and dysentery (Lainé *et al.,* 1985). The plant exhibited anti-inflammatory activity and ameliorates Broncho-pulmonary infections (Nzokou and Kamdem, 2003). The presence of saponins in *Pterocarpus soyauxii* leaf

extracts finds application in the mitigation of hormonal disorders (Francis *et al.*, 2002). The presence of tannins in *Pterocarpus soyauxii* supports its role in mitigating haemorrhoids and ulcers. (Villegas *et al.,* 1997) Pterocarpin, formononetin, and prunetin are some of the isoflavonoids in the plant with antimicrobial potential (Arnone *et al*., 1977).

This work reveals the antioxidant and antidiabetic activities of 1,3,5-trihydroxy-2-methoxyxanthone, 3β-hydroxylup-20(29)-ene and 2,3-dihydroxyfriedelinol isolated for the first time from *Calophyllum inophyllum* pod and *Pterocarpus soyauxii*. In view of the activities reported, the presence of 1,3,5-trihydroxy-2-methoxylxanthone and 2,3 dihydroxyfriedelinol in both plants could further explain the ethnomedicinal applications of *Calophyllum inophyllum* pod and *Pterocarpus soyauxii* as antioxidant and antidiabetic agents.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Summary

Gas Chromatography-Mass Spectrometry analysis of leaf[a], leaf-stalk[b], flower[c], pod[d], fruit-pulp[e], stem wood[f], stem bark[g], root wood[h] and root bark[i] essential oils from *Calophyllum inophyllum* gave one hundred and two (102) constituents. GC-MS of *Pterocarpus soyauxii* had ninety-five (95) identified compounds in root essential oils. The essential oils are rich sources of monoterpenes, sesquiterpenes, and their oxygenated derivatives.

The antidiabetic and antioxidant bioassay study guided the choice of extracts and fractions used for this study. Chromatographic purification of ethyl acetate (EtOAc) fraction from *Calophyllum inophyllum* pod and *Pterocarpus soyauxii* roots gave seven (7) compounds. These compounds include: CiPdE-1 (Friedelan-3-one), CiPdE-2 (Stigmasterol), and CiPdE-3 (1,3,5-trihydroxy-2-methoxylxanthone). Four compounds were isolated from *Pterocarpus soyauxii,* the compounds include: PsRbE-A (3β-hydroxylup-20(29)-ene), PsRbE-B (2,3-dihydroxyfriedelinol). PsRbE-C (Stigmasterol), PsRbE-D (Stigmast-5-en-3-ol), and 1,3,5-trihydroxy-2-methoxylxanthone, 3β-hydroxylup-20(29)-ene, and 2,3-dihydroxyfriedelinol are reported for the first time in the respective parts of the plants.

Essential oils from *Calophyllum inophyllum* showed a good α-amylase and α-glucosidase antidiabetic activity with IC_{50} (mg/mL); (0.037 to 0.049) and (0.042 to 0.052) respectively. *Pterocarpus soyauxii* showed significant α-amylase and α-glucosidase inhibitory activity with IC_{50} values (mg/mL); (0.047 to 0.049) and (0.049 to 0.052) respectively. DPPH and H_2O_2 antioxidant activities of essential oils from *Calophyllum inophyllum* showed significant inhibitory activity at concentration of 1-0.03125 mg/mL with IC_{50} values of (0.30 to 0.42) and (0.30 to 0.38) respectively. *Pterocarpus soyauxii* showed significant inhibitory activity with IC_{50} (mg/mL) from (0.14 to 0.18) and (0.16 to 0.20±0.1) respectively. Isolated compounds showed dose dependent anti-diabetic and antioxidant activity with mean IC_{50} (mg/mL) of 0.035 and 0.30 respectively.

5.2 Conclusion

The antidiabetic and antioxidant activities of *Calophyllum inophyllum* and *Pterocarpus soyauxii* could be attributed to the presence of the characterized volatile and non-volatile chemical constituents which is a validation of their ethno-medicinal uses.

5.3 Contribution to Knowledge

This study has identified the pod part of *Calophyllum inophyllum* and root bark of *Pterocarpus soyauxii* as the most active parts due to their selectively high antidiabetic and antioxidant activities compared with standard drugs used.

Extraction and fractionation in different solvent systems has identified the mid-polarity solvent, ethyl acetate as the most active solvent for optimal antidiabetic and antioxidant activity of *Calophyllum inophyllum* and *Pterocarpus soyauxii*.

The essential oils from *Calophyllum inophyllum* and *Pterocarpus soyauxii* were found to be dominated by monoterpenes and sesquiterpenes. Friedelan-3-one and 1,3,5-trihydroxy-2-methoxyxanthone from *Calophyllum inophyllum* pod as well as 3β-hydroxylup-20(29)-ene and 2,3-dihydroxyfriedelinol from *Pterocarpus soyauxii* root bark exhibited antidiabetic and antioxidant activities which is being reported for the first time.

Studies on the antidiabetic and antioxidant activities of volatile and non-volatile chemical constituents from these plants were carried out for the first time.

5.4 Recommendations

The antidiabetic and antioxidant activities of volatile and non volatile compounds from *C. inophyllum* and *Pterocarpus soyauxii* were evaluated in-vitro, an in-vivo study would further help in translating this research to prescription drugs for orthodox therapies.

References

Adams, R.P. 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. 4th Edition *Allured Publishing Corporation*, Carol Stream

Aidi-Wannes, W., Mhamdi, B., Saidani-Tounsi, M. and Marzouk, B. 2017. Lipid and volatile composition of borage (*Borago officinalis* L.) leaf. *Trends in Phytochemical Research* 1.3: 143-148.

Aksenov, A., da Silva, R., Knight, R., Lopes, N. P., and Dorrestein, P. 2017. Global chemical analysis of biology by mass spectrometry. *Nature Chemistry Review* 1(4): 54

Ali, J. Haider, S.M.S. Ali, S.M. Haider, T. Anwar, A. Hashmi, A.A. 2023. Overall Clinical Features of Type 2 Diabetes Mellitus With Respect to Gender. *Clinical Medicine* 4.15(3):35771.

Akerele, O. 1993. Nature's Medicinal bounty: Don't Throw it away. *World Health Forum.* 14.4: 390-395

Ames, B. N., Shigenag, M. K. and Hagen, T. M. 1993. Oxidants, antioxidant and the degenerative diseases of aging. *Proceeding of the National Academic Science*, 90: 7915-7922.

Arnone, A., Camarda, L., Merlini, L., Nasini, G. and Taylor, D. A. H. 1977. Colouring matters of the West African red woods *Pterocarpus osun* and *Pterocarpus soyauxii*. Structures of santarubins A and B. *Journal of Chemistry Society Perkin Trans* 1.19: 2116-2118.

Assanta, M. A. and Robert, C. 2011. *Gnetum africanum*; a wild food plant from the African forest with many nutritional and medicinal purposes. *African Journal Herbal Medicine* 14: 1289-1297.

Ayat, A., Mohamad, E. A., Mohamad, J. K. and Foroogh, N. 2015. *In vitro* α-amylase and α- glucosidases inhibitory effects of some plant extracts. *International Journal of Pharmacognosy and Phytochemical Research* 131.4: 281-303.

Ayu, M. Reka, M. Sari, A. Budiman, Y. Nur, R. Talha, B. Firzan, N. Jesus, S. 2022. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. *Food Chemistry.* 13.2: 100217

Balick, M. J. and Cox, P. A. R. 1996. Plants people and culture. The Science of Ethnobotany. *Scientific American Library, New York, USA*. 219.

Barend, C. B. and Brandt, B. E. V. 1987. Flavonoid analogs from *Pterocarpus* species. *Phytochemistry* 26, 531-535.

Bhalla, T. N., Saxena, R. C., Nigam, S. K., Misra, G. and Bhargava, K. P. 1980. Calophyllolide-A new nonsteroidal anti-inflammatory agent. *Industrial Journal of Medicinal Research* 72: 762-765.

Bohm, B. A. 1994. Flavonoid and Condensed tannins from Leaves of *Vaccinium vaticulatum* and *V. calycinium*. *Pacific Science* 48: 458-463.

Boutati, E. I. and Raptis, S. A. 2004. Post prandial hyperglycemia in type 2 diabetes: Pathophysiological aspect, teleogical notions and flags for clinical practice. *Diabetes Research Review* 20.2: 13-23.

Bremaud, I., Amusant, N., Minato, K., Gril, J. and Thibaut, B. 2011. Effect of extractives on vibrational properties of African Padauk (*Pterocarpus soyauxii* Taub). *Wood Science and Technology* 45: 461-472.

Bruhn, J. G. and Bohlin, L. 1997. Molecular pharmacognosy: an explanatory model. *Drug Discovery Today* 2.6: 243–246.

Buchbauer, G. 2000. The detailed analysis of essential oils leads to the understanding of their properties. *Perfumer and Flavourist* 25: 64-67.

Burkhil, H. M. 1994. The useful plants of west tropical Africa. Edn 2, Families E-I. XX *Royal Botanic Gardens Review* 2: 522.

Burnham, R. J. and Johnson, K. R. 2004. South American palaeobotany and the origins of neotropical rainforests. Philosophical Transactions of the Royal Society B: *Biological Sciences.* 359.44: 1595–1610

Cardoso, D., Pennington, R. T., de Queiroz, L. P., Boatwright, J. S., Van-Wyk, B. E., Wojciechowskie, M. F. and Lavin, M. 2013. Reconstructing the deep-branching relationships of the papilionoid legumes. *South African Journal of Botany* 89: 58-75

Cardellina, J. H. 2002. Challenges and opportunities confronting the botanical dietary supplement industry. *Journal of Natural Products* 65.7: 1073-1084.

Chapman, V. 1997. Natural Product. Dictionary of Natural product. *Chapman and Hall*. 866-1042.

Christenhusz, M. J. M. and Byng, J. W. 2016. The number of known plants species in the world and its annual increase. *Phytotaxa*. 261.3: 201–217.

Christophe, B., Manteghetti, M., Gross, R., Baissac, Y., Jacob, M., Petit, P., Sauvaire, Y., and Ribes, G. 2000. 4-Hydroxyisoleucine: effects of synthetic and natural analogues on insulin secretion. *European Journal of Pharmacy* 390: 339-345.

Cooper, R. and George, N. 2015. Natural Products Chemistry. Sources, Separations and Structures. *CRC press*. 203-210.

Cuatrecasas, P., Wilchek, M., and Anfinsen.C. B. 1968. Selective enzyme purification by affinity chromatography. *Proceedings of the National Academy of Science USA*, 61: 636–43.

Das, M., and Dasgupta, D. 1998. Pseudo-affinity column chromatography based rapid purification procedure for T7 RNA polymerase. *Preparatory Biochemistry and Biotechnol*ogy 283: 39-48.

Da Silva, R.F. Carneiro, C.N.; de Sousa, C.B.d.C.; Gomez, F.J.V.; Espino, M.; Boiteux, J.; Fernández, M.d.; Silva, M.F.; Dias, F.d. 2022. Sustainable Extraction Bioactive Compounds Procedures in Medicinal Plants Based on the Principles of Green Analytical Chemistry: A Review. *Microchemistry Journal* 175, 107184

Davesh, K. K., Manjulika, Y., Sanjukta, C., Amrita, K. S. and Geeta, W. 2016. α-Amylase and α-Glucosidase inhibitory activity assessment of *Cucurbita maxima* seeds. *International Journal of Phytomedicine.* 8: 312-318.

de Sales, P. M., Simeoni, L. A., Magalhães, P. O. and Silveira, D. 2012. α-Amylase inhibitors: A review of raw material and isolated compounds from plant source. *Journal of Pharmacy and Pharmaceutical Sciences* 15: 141-183.

Denise, F. Samary, A. Griselda, R. Natalia, V. Marcela, H. and Gabriela, G. 2023. Anthocyanins: Potential Therapeutic Approaches towards Obesity and Diabetes Mellitus Type 2. *Antidiabetics* 28(3), 1237-1248

DeFronzo, R. A. 1999. Pharmacologic therapy for type 2 diabetes mellitus. Ann. Intern. Med. *descriptions, illustrations, identification, and information retrieval*. [online] Accessed 15 April 2020

DeOliveira, T. M., deCarvalho, R. B. F., daCosta, I. H. F., de Oliveira, G. A. L., deSouza, A. A., deLima, S. G. and deFreitas, R. M. 2015. Evaluation of *p*-cymene, a natural antioxidant. *Pharmaceutical Biol*iogy 53: 423-428

Djafarou, S., Mermer, A. Barut, B. Yılmaz, G.T. Amine, K. I. Boulebd, H. 2023. Synthesis and evaluation of the antioxidant and anti-tyrosinase activities of

thiazolyl hydrazone derivatives and their application in the anti-browning of fresh-cut potato. *Food Chemistry* 15(2):414-745

Donald, P. L., Lampman, G. M., Kritz, G. S. and Randall, G. 2006. Engel Introduction to Organic Laboratory Techniques 4: 797-817.

Dudley H. W. 1980. Spectroscopy method in organic chemistry. *McGraw Hill.* 252-257

Dweck, A. C., and Meadowsy, T. 2002. Tamanu (*Calophyllum inophyllum*) – the African, Asian, Polynesian and Pacific Panacea. *International Journal of Cosmetic Science,* 24, 1–8.

Edeoga, H. O., Okwu D. E. and Mbaebia G. A. 2005. Phytochemical Constituents of Some Nigerian medicinal plants. *African journal of Bio-technology*. 47: 685-688

Ee, G. C. L., Jong, V. Y. M., Sukari, M. A., Rahmani, M. and Kua, A. S. M. 2009. Xanthones from *Calophyllum inophyllum. Journal of Science and Technology.* 17.2: 307-312.

Eiceman, G. A. 2000. Gas Chromatography: In: *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation.* 10627.

El-Asbahani, A., Miladi, K., Badri, W., Sala, M., Addi, E. H. A., Casabianca, H., El Mousadik, A., Hartmann, D., Jilale, A., Renaud, F. N. R. and Elaissari, A. 2009. Essential oils: From extraction to encapsulation. *International Journal of Pharmacy* 483: 220–243.

Elnaz, S. Vinuthaa, M. Hao, W. Roshanak, K. Johanna, W. Richard, W. 2023. Original Research Formulation and Biomedical Activity of Oil-in-Water Nanoemulsion Combining *Tinospora smilacina* Water Extract and *Calophyllum inophyllum* Seeds Oil 16;1159–1174

Ernst, R. R. and Anderson, W. A. 1966. Application of Fourier Transform Spectroscopy to Magnetic Resonance. *Review of Science Instrumentation* 37, 93.

Evans, W. C., Trease, G. E. and Evans, D. 2002. Trease and Evans Pharmacognosy. *Edinburgh, Saunders*: 15: 249-454.

Evans, W. C., and Trease, G. E. 1989. Pharmacognosy. *Balliere Tindall*. 13: 97-105.

Farnsworth, N. R., Akerele, O., Bingel, A. S. and Soejarto, D. D. 1985. Medicinal Plants in Therapy. *Bulletin of the World Health Organisation* 63.6: 965-81

Finar, L. 1973. Organic Chemistry "Natural Product". *Academic Press* 5: 458-470.

Finar, I. L. 1975. Stereochemistry and the chemistry of natural products, *Longman Publishing Group* 2: 5

Francis, G., Kerem, Z., Makkar, H. and Becker, K. 2002. The biological action of saponins in animal systems: A review. *British Journal of Nutrition* 88.6: 587-605.

Gelosa, D. and Sliepcevich, A. 2009. Fundamentals of chemistry, Chromatography techniques, Politecnio di Milano, *Encclopeadia of life support systems*, 1: 16-27.

Ghasemzadeh, A., Jaafar, H. Z. E. and Rahmat, A.2010. Antioxidant activities, total Phenolics and flavonoids content in two varieties of Malaysia Young Ginger (*Zingiber officinale* Roscoe) 15: 4324-4333.

Gongalez, A. G., Ferro, E. A. and Ravelo, A. G. 1987. Triterpenes from *Mayterus horrid Phytochemistry* 26: 2785-2788.

Gryglewski, R. J., Korbut, R. and Robak, J. 1987. On the mechanism of antithrombotic action of flavonoids. *Biochemical Pharmacology* 36: 317-321.

Gurib-Fakim, A. 2006. Medicinal plants: Drugs of tomorrow and traditions of yesterday, *Molecular Aspects of Medicine*, 27.1: 1-93.

Gyamfi, M. A. and Aniya, Y. 1983. Antioxidant properties of Thonningianin A, isolated from the African medicinal herb, *Thonningia sanguine. Biochemical Pharmacology* 63.1: 725-37.

Habib, M. R., Nikkon, F., Rahman, M., Haque, M. E. and 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.

Hanefeld, M., Schmechel, H., Julius, U. and Schwanebeck, U. 1996. Determinants for coronary heart disease in non-insulin dependent diabetes mellitus: lessons from the diabetes intervention study. *Diabetes Research and Clinical Practice* 30: 67-70.

Hang, N. T. M., Chien, N. Q. and Hung, N. V. 2006. Triterpenes from the leaves of Vietnamese plant *C. inophyllum* L. *Journal of Chemistry* 44: 115-118.

Harbone, J. B. 1984. Phytochemical methods. *Chapman and Hall*. 245-248.

Harbone, J. B. 1993. A Guide to Modern Techniques of Plants Analysis. *Phytochemical* 3: 46-60.

Harborne, J. 1998. Phytochemical Methods, A guide to modern Techniques of plant analysis.

Harborne, J. B. 1999. An overview of antinutritional factors in higher plants. In: Secondary plants products. Antinutritional and beneficial actions in animal feeding Caygill JC and Mueller-Harvey I, eds. *Nottingham University Press, UK*,7-16.

Harris, D. C. 2004. Exploring chemical analysis. 3rd ed, WH. Freeman and Co. 3:2

Harvey A. L. 2008. Natural products in drug discovery. *Drug Discovery Today* 13:894-901.

Hathurusingha, H. M. S. D. and Ashwath, N. 2007. Beauty leaf (*Calophyllum inophyllum* L.) tree: A tree with great economic potential. In: Proceedings of 12th *International Forestry and Environment Symposium, Kalutara, SriLanka*, 20.

He, J. Tang, P. Liu, M. Liao, G. Lu, R. Yang, X. 2023. Triterpenoid saponins and C_{21} steroidal glycosides from *Gymnema tingens* and their glucose uptake activities. *Royal Soceity of Chemistry Advances*. 8;13(11):7503-7513

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 of Ethnopharmacology* 92.2-3: 147-162.

Hind, L., Nabila, B., Sara, A. and Rabah, D. 2007. *In vitro* α-amylase and α-glucosidase inhibitory activity of *Ononis angustissima* extracts. *Journal of Applied Pharmaceutical Sci*ences 7.2, 191-198.

Holzgrabe, U., Waver, I. and Diehl, B. 1999. NMR Spectroscopy in Drug Development and Analysis. *Wiley-VCH*. 1: 16-61.

Hussain, A. I., Anwar, F., Nigam, P. S., Ashraf, M., and Gilani, A. H. 2010. Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four Mentha species. *Journal of Science and Food Agric*ulture 90: 1827-1836.

Irene, M., Estrella S., Manuel, V., Casilda, N., and Esther, S. 2016. Chemical Composition, Antioxidant and Antimicrobial Activity of Essential Oils from OrganicFennel, Parsley, and Lavender from Spain *Agricultural Journal of foods* 5: 18.

Itoigawa, M., Ito, C., Tan, H. T. W., Kuchide, M., and Tokuda, H. 2001. Cancer chemo-preventive agents, 4-phenylcoumarins from *C. inophyllum. Cancer Lett*: 169: 15-19.

Janki, P., Atul, S., Khanna, A. K., Bhatia, G., Awasthi, S. K. and Narender, T. 2012. Antidyslipidemic and antioxidant activity of the constituents isolated from the leaves of *Calophyllum inophyllum. Phytomedicine* 19.14: 1245-1249.

Jeener, M. 1971. Unpublished lecture at the Ampere International Summer School II InBasko polje, Yugoslavia.

Jelena, S., Stanojević, L. P., Stanojević, D. J., Cvetković, B. R. and Danilović. I. 2015. Chemical composition, antioxidant and antimicrobial activity of the turmeric essential oil (*Curcuma longa* L.). *Journal of Advance Technologies*, 4.2: 19-25.

Judd, W. S., Campbell, C. S. Kellogg, E. A. Stevens, P.F. Donoghue, M. J. 2002. Plant systematics: a phylogenetic approach, Sinauer Axxoc, 287-292.

Kahn, S. E., Cooper, M. E. and Del-Prato, S. 2014. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. *Lancet* 383:1068–83.

Kashman, Y.K., Gustafson, R., Fuller, R.W., Cardellina, J.H., McMahon, J.B., Currens, M.J., Buckheit, R.W., Hughes, S.H., Cragg, G.M., and Boyd, M.R. 1992. The Calanolide, a novel HIV-Inhibitory class of coumarin derivatives from the tropical rainforest tree. *Calophyllum lanigerum*. *Journal of Medicinal Chemistry* 35, 2735-2743.

Kemp, W. 1993. Organic Spectroscopy. 3rd Edition, ELBS, Macmillan

Kijjoa, A., Gonzalez, M. J., Pinto, M. M., Silva, A. M., Anantachoke, C. and Herz, W., 2000. Xanthones from *Calophyllum teysmannii* var. inophylloide. *Phytochemistry*, 55: 833-836.

Kinghorn, A. D. (2001). Pharmacognosy in the 21st century. *Journal of Pharmacy and Pharmacology* 53.2: 135-148

Kwon, Y. I., Apostolidis, E., Kim, Y. C. and Shetty, K. 2007. Health benefits of traditional corn, beans and pumpkin: *In vitro* studies for hyperglycemia and hypertension management. *Journal of Medicinal Food.* 10: 266-275.

Lainé, C., Baniakina, J., Vaquette, J., Chaumont, J. P. and Simeray, J. 1985. Antifungal activity of the barks of trunks of seven phanerogams from the Congo. *Plantes Médicinales et Phytotherapie* 19.2: 75-83.

Lasztity, R., Hidvegi, M. and Bata, A. 1998. Saponins in food. *Food Review International*: 14: 371-390.

Lavin, M., Pennington, R. T., Klitgaard, B. B., Sprent, J. I., de Lima, H. C., Gasson, P. E. 2001. The dalbergioid legumes (Fabaceae): delimitation of a pantropical monophyletic clade *American Journal of Botany* 88.3: 503-533

Lavin, M. and Pennigton, R. T. 2001. Delimitation of a pantropic legumes. *Journal of Botanical Science* 8, 503-511.

Li, Y. Z., Li L. Z., Hua, H. M., Li, Z. G. and Liu, M. S. 2007. Studies on flavonoids from stems and leaves of *C. inophyllum. Journal of Chinese Materia Medica* 32: 692-694.

Li, C., Bu, P. B., Yue, D. K. and Sun, Y. F.2006. Chemical constituents from roots of *Ficus hirta* Zhongguo Zhong, *Yao, Za, Zhi.*, 31:131-133.

Li,, X. W., Li, J., Robinson, N. K. B. and Steven, P. (1990). Clusiaceae (Guttiferae). *Flora of China*, [e-journal] 13, 1. Available at: <http://www.efloras.org/florataxon.aspx?flora_id=2andtaxon_id=10203> [Accessed 19 April 2019].

Li, Y. Z., Li, Z. L., Yin, S. L., Shi, G., Liu, M. S., Jing, Y. K. and Hua, H. M. 2010. Triterpenoids from *Calophyllum inophyllum* and their growth inhibitory effects on human leukemia HL-60 cells. *Fitoterapia* 81: 586–589.

Lombardino, J. G. and Lowe, J. A. 2004. The role of the medicinal chemist in drug discovery-then and now. *Nature Reviews Drug Discovery* 3.10: 853-862.

Luksamee, V. Chakhriya, C. Waraporn, R. Nararak, L. 2023. Phytochemical characterization of bioactive compounds extracted with different solvents from *Calophyllum inophyllum* flowers and activity against pathogenic bacteria. South *African Journal of Botany* 154, 346-355

Maduka, H. C., Ugwu, C. E., Okpogba, A. N., Ogueche, P. N., Dike, C. C., Okonkwo, C. O. and Nwanyanwu, A. C. 2018. Phytochemical Analysis and Antioxidant Properties of the Ethanolic Extract from *Tetracarpidium conophorum* (African Walnut) and *Pterocarpus soyauxii* (oha) Leaf. 8.1: 15.

Magallón, S. A., and Sanderson, M. J. 2001. Absolute diversification rates in angiosperm clades. *Evolution.* 55 (9): 1762–1780.

Mahato, S. B. and Kundu, A. P. 1994. ^{13}C NMR Spectra of Pentacyclic Triterpenoids-A Compilation and some salient features. *Phytochemistry.* 37.6: 1517-1575.

Mamta, S., Jyoti S., Rajeev, N., Dharmendra, S. and Abhishek, G. 2013. Phytochemistry of Medicinal Plants, *Journal of Pharmacognosy and Phytochemistry*, 8192.1: 168-182.

Mangan, J. L. 1998. Nutritional effect of tannins in animal feeds. *Natural Research and Reviews* 1: 209-231.

Manuchair. E. 2002. Pharm. dynamic basis of herbal medicine.*CRC press*. 14.2: 356-360.

Marie, C., Yimdjo, A. G., Azebaze, A. E., Nkengfack A., Michele M., Bernard B. and Zacharias T. 2004. Fomum a Antimicrobial and cytotoxic agents from *Calophyllum inophyllum Phtochemisty* 65: 2789-2795

Marino, M., Bersani, C. and Comi, G. 1999. Antimocrobial activity of the essential oils of *Thymus Vulgaris* L. measured using a biompedometric method. *Journal of Food protection* 62: 1017-1023

Masahiro N., Masahiro K., Minemitsu N., Akio K. and Yoshimi N. 2005. Non-reductive Scavenging of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) by Peroxyradical: A

Useful Method for Quantitative Analysis of Peroxyradical. Chem Pharm Bull 53.6: 714-716

Matsuda, H., Nishida, N. and Yoshikawa, M. 2002. Antidiabetic principles of natural medicines. V. Aldose reductase inhibitors from *Myrcia multiflora* DC. (2): Structures of myrciacitrins III, IV, and V. *Chemcal Pharmacy Bulletin* 50.14: 429-431.

Mc-Leod, M. N. 1974. Plant tannins: Their role in forage quality. *Nutrition Abstract Review* 44: 803-812.

Meyer, V. R. 1988. Practical High Performance Liquid Chromatography, 342.

Mohamed, A.A., Alotaibi, B.M. 2023. Essential oils of some medicinal plants and their biological activities: a mini review. *Journal of Umm Al-Qura Univiversity Appllied Science* 9, 40–49

Mohammadhosseini, M., Akbarzadeh, A., Hashemi-Moghaddam, H., Mohammadi Nafchi, A., Mashayekhi, H.A. and Aryanpour, A. 2016. Chemical composition of the essential oils from the aerial parts of *Artemisia sieberi* by using conventional hydrodistillation and microwave assisted hydrodistillation: A comparative study. *Journal of Essential Oil-Bearing Plants* 19.1: 32-45.

Mohammadhosseini, M. 2017. The ethnobotanical, phytochemical and pharmacological properties and medicinal applications of essential oils and extracts of different *Ziziphora* species. *Indian Crops Production* 105: 164-192.

Mole, S. and Waterman, P. G. 1987. Tannic acid and proteolytic enzymes: enzyme inhibition or substrate deprivation. *Phytochemistry.* 26: 99-102.

Molyneux, R. J., Nash, R. J. and Asano, N. 1996. Alkaloids: Chemical and Biological Perspectives, Vol. 11, Pelletier SW, ed. *Pergamon, Oxford*, 303.

Morrison, R. T. and Bough R. N. 2004. Spectroscopy and structure. Organic Chemistry. *Prentice Hall*, 6: 586-600.

Morrissey, J. P. and Osbourn, A. E. 1999. Fungal resistance to plant antibiotics as a mechanism of pathogenesis. *Microbiological and Molecular Biological Reviews,* 63: 708-724.

Nabavi, S. M., Ebrahimzadeh, M. A., Nabavi, S. F., Fazelian, M. and Eslami, B. 2009. In vitro antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. *Pharmacognosy Magazine*, *4*.18: 123-127.

Nickavar, B. and Yousefian, N. 2009. An historical perspective of natural products chemistry. *Iranian Journal of Pharmaceutical Research* 8.1: 53-57

National Institute of Standards and Technology 2017. Mass spectroscopic library for identification of Essential oils 6th Edition. Bureau Press, Gaithersburg

Nzokou, P. and Kamdem, D. P. 2003. Fungal decay resistance of non-durable aspen wood treated with extractives from African padauk *(Pterocarpus soyauxii). Journal of Tropical Forest Products.* 9.1-2: 125-133.

Obute, G. C. and Osuji, I. C. 2002. Environmental awareness and dividends: A scientific discourse. *African Journal of Interdisciplinary Studies.* 3.1: 90-94.

Ong, H. C., Mah'liaa, T. M. I., Masjukia', H. H. and Norhasyima, R. S. 2011. Comparison of palm oil, *Jatropha curcas* and *Calophyllum inophyllum* for biodiesel. *Journal of Renewable and Sustainable Energy Reviews*, 15: 3500–3519.

Onocha, P. A., Oloyede, G. K, and Afolabi, Q. O. 2011. Chemical Composition, Cytotoxicity and Antioxidant Activity of Essential Oils of Acalyphahispida Flowers. *International Journal of Pharmacology*, 7.1: 144-148.

Ortiz-Andrade., R. R., Garcia-Jimenez, S., Castillo-Espana, P., Ramirez-Avila, G., Villalobos-Molina, R. and EstradaSoto, S. 2007. Alpha-glucosidase inhibitory activity of the methanolic extract from *Tournefortia hartwegiana*: an antihyperglycemic agent. *Journal of Ethnopharmacology* 109, 48-53.

Oteng-Gang, K. and Mbachu, J. I. 1990. Changes in the ascorbic acid content of some tropical leafy vegetables during traditional cooking and local processing. *Food Chemistry* 23, 9-17.

Padhee, D. K. 2001. Medicinal plants of Orissa. Conservation and utilization of medicinal and aromatic plants. *Allied Publisher Limited*. 22.

Pengsuparp, T., Serit, M., Hughes, S. H., Soejarto, D. D. and Pezzuto, J. M. 1996. Specific Inhibition of Human Immunodeficiency Virus Type 1 Reverse Transcriptase Mediated by Soulattrolide, a Coumarin Isolated from the Latex of *Calophyllum teysmannii*. *Journal of Natural Products* 59: 839-842.

Porath, J. 1997. From gel filtration to adsorptive size exclusion. *Journal of Protein Chemistry* 16, 463-8.

Pridham, J. B. 1960. In: Phenolics in Plants in Health and Disease, *Pergamon Press, New York*, 34-35.

Quintans-Junior, L., Moreira, J. C., Pasquali, M. A., Rabie, S. M., Pires, A. S., Schroder, R., Rabelo, T. K., Santos, J. P., Lima, P. S. and Cavalcanti, S. C. 2013. Antinociceptive Activity and Redox profile of the Monoterpenes (+-Camphene, *p*-Cymene, and Geranyl Acetate in Experimental Models *ISRN* 459530

Rao, R. V. K., Ali, N. and Reddy, M. N. 1978. Occurrence of both sapogenins and alkaloid lycorine in Curculigoorchioides. *Indian Journal of Pharmaceutical Science*, 40: 104-105.

Rojas, R., Bustamante, B., Bauer, J., Ferrandez, I., Alban, J. and Lock, O. 2003. Antimicrobial activity of selected *Peruvian* medicinal plants, *Journal of Ethnopharmacology* 88: 199-204.

Rojo, J. P. 1972. *Pterocarpus soyauxii* (Leguminosae-Papilionaceae) revised for the world. *Phanerogamarum Monographiae*. *J. Cramer, Lehre, Germany*. 5: 119

Sagar B., Kedare R., and Singh P. 2011. Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology* 48.4: 412–422

Saha, J. B. T, Abia, D., Dumarcay, S., Ndikontar, M. K., Gerardin, P., Ngamveng-Noah, J. and Perrin D. 2013. Antioxidant activities, total phenolic contents and chemical compositions of extracts from four Cameroonian woods: Padouk (*Pterocarpus soyauxii* Taubb), tali (*Erythrophleum suaveolens*), moabi (*Baillonella toxisperma*), andmovingui (*Distemonanthus benthamianus*). *Indian Crops Production* 41: 71-7

Samuelsson, G. 2004. Drugs of Natural Origin: a Textbook of Pharmacognosy, 5th Swedish Pharmaceutical Press, Stockholm 4.68: 612-662

Santhakumari, P., Prakasam, A. and Pugalendi, K. V. 2006. Pugalendi antihyperglycemic activity of piper betle leaf on Streptozotocin-induced diabetic rats. *Journal of Medical Food.* 9(1), 108-112.

Sarker, S. D., Latif, Z., and Gray, A. I. 2006. Natural Products Isolation. New Jersey: *Humana Press*. 20: 391-414

Schofield, P., Mbugua, D. M., and Pell, A. N. 2009. Analysis of condensed tannins: a review. *Animal Feed Science Technology*, 91: 21-40.

Schmidt-Rohr, K. Mao, J.D. 2000. Efficient CH-group selection and identification in ^{13}C solid-state NMR by dipolar DEPT and ^{1}H chemical-shift filtering. *Journal of the American Chemical Soceity* 124(46):13938-14846

Seidel, V. 2006. Initial and Bulk Extraction. *In:* Sarker, S. D., Latif, Z., Gray, A. I. (eds.) *Natural Products Isolation. New Jersey: Humana Press.* 20:2, 27-35

Shaw, J. E., Sicree, R. A., Zimmet, P. Z. 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice* 87.1: 4-14.

Shaheen, S. J., Kaskoos, R. A., Hamad, K. J., and Ahamad, J. 2013. *In-vitro* antioxidant and α-amylase inhibition activity of *Cucurbita maxima*. *Journal of Pharmacognosy Phytochemistry* 2: 121-124.

Shihabudeen, M. S., Hansi, P., Kavitha, T. 2011. Cinnamon extract inhibits a-glucosidase activity and dampens postprandial glucose excursion in diabetic rats. *Nutritional Metabolism.* 8: 46-51.

Shaw, J. E., Sicree, R. A., Zimmet, P. Z. 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice* 87.1: 4-14.

Shenoy, K. and Shirwaiker, A. 2002. Anti-inflammatory and free radical scavenging studies of Hyptis suavolens. *Indian drug* 39: 574-577

Sies, H. 1997. Oxidative stress, oxidants and antioxidants. *Experimental physiology*, 82, 291-295.

Singh, G., Marimuthu, P., De Heluani, C. S. and Catalan, C. 2005a. Antimicrobial and antioxidant potentials of essential oil and acetone extract of M*yristica fragranshoutt*. (Aril part). *Journal of Food Science.* 70: 141-148.

Singh, G., Marimuthu, P., Murali, H. S. and Bawa, A. S. 2005b. Antioxidative and antibacterial potentials of essential oils and extracts isolated from various spice materials. *Journal of Food Safety* 25: 130-145.

Skoog, D. A., Holler, F. J., Crouch, S. R 2007. Principles of Instrumental Analysis.6th Edition.Brooks/Cole Cengage Learning, *Chapters* 11, 20-27.

Sofowora, A. 1993. Medicinal plants and Traditional Medicine in Africa. *Spectrum Books. Ibadan, Nigeria* 34-188.

Song, J. Zhang, B., Li, M., Zhang, J. 2023. The current scenario of naturally occurring indole alkaloids with anticancer potential. *Fitoterapia.* 165:105430.

Spinor, C., Marco', D. and Subramaniam, S. 1998. Anti-HIV coumarins from *Calophyllum* seed oil. *Bioorganic Medicinal Chemistry Letters* 8: 3475-3478.

Sutar, N., Garai, R., Sharma, US. and Sharma. 1999. Anthelmintic activity of Platycladusorientalis leaves extract. *International Journal of Parasitology Research,* 2.2: 1-3.

Tabuti, J. R., Lye, K. A. and Dhyllon, S. S. 2003. Traditional herbal drugs of Budamogii, Uganda. Plants use and administration. *Journal of Ethnopharmacology* 88.1: 19-44.

Taher, M., Attoumani, N., Susanti, D., Ichwan, S. J. A. and Ahmad, F. 2010. Antioxidant Activity of Leaves of *Calophyllum rubiginosum. American Journal of Applied Sciences*, 7.10: 1305-1309.

Tchamadeu, M. C., Dzeufiet, P. D., Nana, P., Kouambou, N. C., Ngueguim, T. F., Allard, J., Blaes, N., Siagat, R., Zapfack, L., Girolami, J. P., Tack, I., Kamtchouing, P. and Dimo, T. 2011. Acute and sub-chronic oral toxicity studies of an aqueous stem bark extract of *Pterocarpus soyauxii* Taub (Papilionaceae) in rodents. *Journal of Ethnopharmacology* 133: 329-335.

Tolstiva, T. G, Sorokina, I. V, Tolstikov, A. G. and Flekhter, O. B. 2006. Biological activity and Pharmacological prospects of lupine terpenoids: Natural lupane derivatives. *Russian Journal of Bioorganic Chemistry* 32: 37-49.

Tong, Z. He, W. Fan, X. and Guo, A. 2022. Biological Function of Plant Tannin and Its Application in Animal Health. *Frontier of Veterinary Science.* 1.4:803657

Tongnuanchan, P. and Benjakul, S. 2014. Essential oils: extraction, bioactivities, and their uses for food preservation. *Journal of Food Science*, 79: 1231-1249.

Traore, F., Faure, R., Ollivier, E., Gasquet, M., Azas, N., Debrauwe, L., Keita, A., Timon-David, P. and Balansard, G. 2000. Structure and antiprotozoal activity of triterpenoid saponins from *Glinus oppositifolius*. *Planta Medica*. 66: 368-371.

Tyler, V. E. 1999. Phytomedicines: back to the future. *Journal of Natural Products* 62 58:9-1592.

Uddin, M. S. Chowdhury, V. Uddin, S. B. 2023. Ethnomedicinal study of the khumi indigenous community in Bandarban District, Bangladesh. *Drug Discovery* 17: 1004

Uma, S. M. Murthy, P. N. Sudhir, K. S. and Kanhu, C. S. 2012. Formulation and evaluation of herbal tablet containing methanolic extract of *Calophyllum inophyllum*. *International Journal of Pharmacy* 2.1: 181-186.

Usama, R. A. Ahmed, M. S. Abeer, H. E. 2022. Natural Products' Extraction and Isolation-Between Conventional and Modern Techniques. *Frontier of Natural Products*. 1.4: 202

vanAgtmael, M. A., Eggelte, T. A. and vanBoxtel, C. J. (1999). Artemisinin drugs in the treatment of malaria: from medicinal herb to registered medication. *Trends in Pharmacological Sciences* 20.5: 199-205.

Vansoest, P. J. (1994). Nutritional ecology of the ruminant, *Cornell University Press. Ithaca, New York,* 2: 476.

Villegas, L. F., Fernandez, I. D, Maldonads, H., Torres, R., Zavaleta, A. and Vaisberg, A. J. (1997). Evaluation of the wound-healing activity of selected traditional medicinal plants from Peru. *Journal of Ethnopharmacology*. 55.3: 193-200.

Walton, N. J., Mayer, M.J. and Narbad, A. 2003. Molecules of Interest: Vanillin. *Phytochemistry,* 63: 505-515.

Wink, M., Schmeller, T. and Latz-Briining, B. (1998). Modes of action of allelochemical alkaloids: Intraction with neuroreceptors, DNA and other molecular targets. *Journal of chemical Ecology*, 24: 1888-1937.

Xi, X.; Wang, J.; Qin, Y.; You, Y.; Huang, W.; Zhan, J. 2022. The Biphasic Effect of Flavonoids on Oxidative Stress and Cell Proliferation in Breast Cancer Cells. *Antioxidants* 11, 622

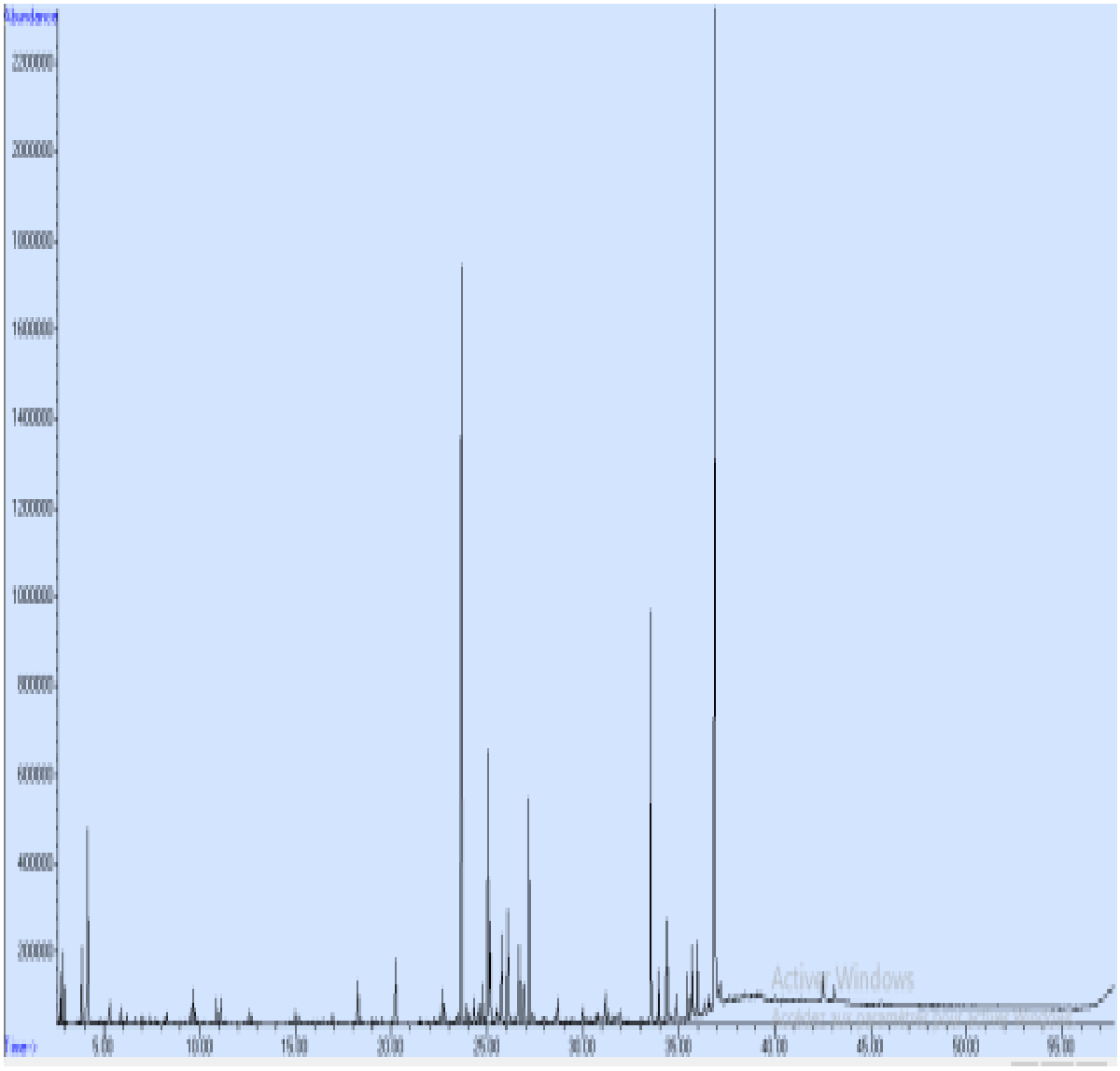
Yimdjo, M. C., Azebaze, A. G., Nkengfack, A. E., Meyer, A. M., Bodo, B., Yu, L. U. and Young W.J. 2016. Inhibitory effects of *Calophyllum inophyllum* extract on atopic dermatitis induced by DNCB in mouse. *African Journal of Pharmacognosy and chemical Technology* 4.6: 165-173.

Zarith, A. A. A., Akil, A., Siti, H. M. S., Alptug, K., Muhammad, M. A., David, L., Mohd, R., Magdah, G., Mohammad, A. K. and Ghulam, M. A. 2018. Essential Oils: Extraction Techniques, Pharmaceutical and Therapeutic Potential-A Review. *Current Drug Metabolism,* 19: 1-11

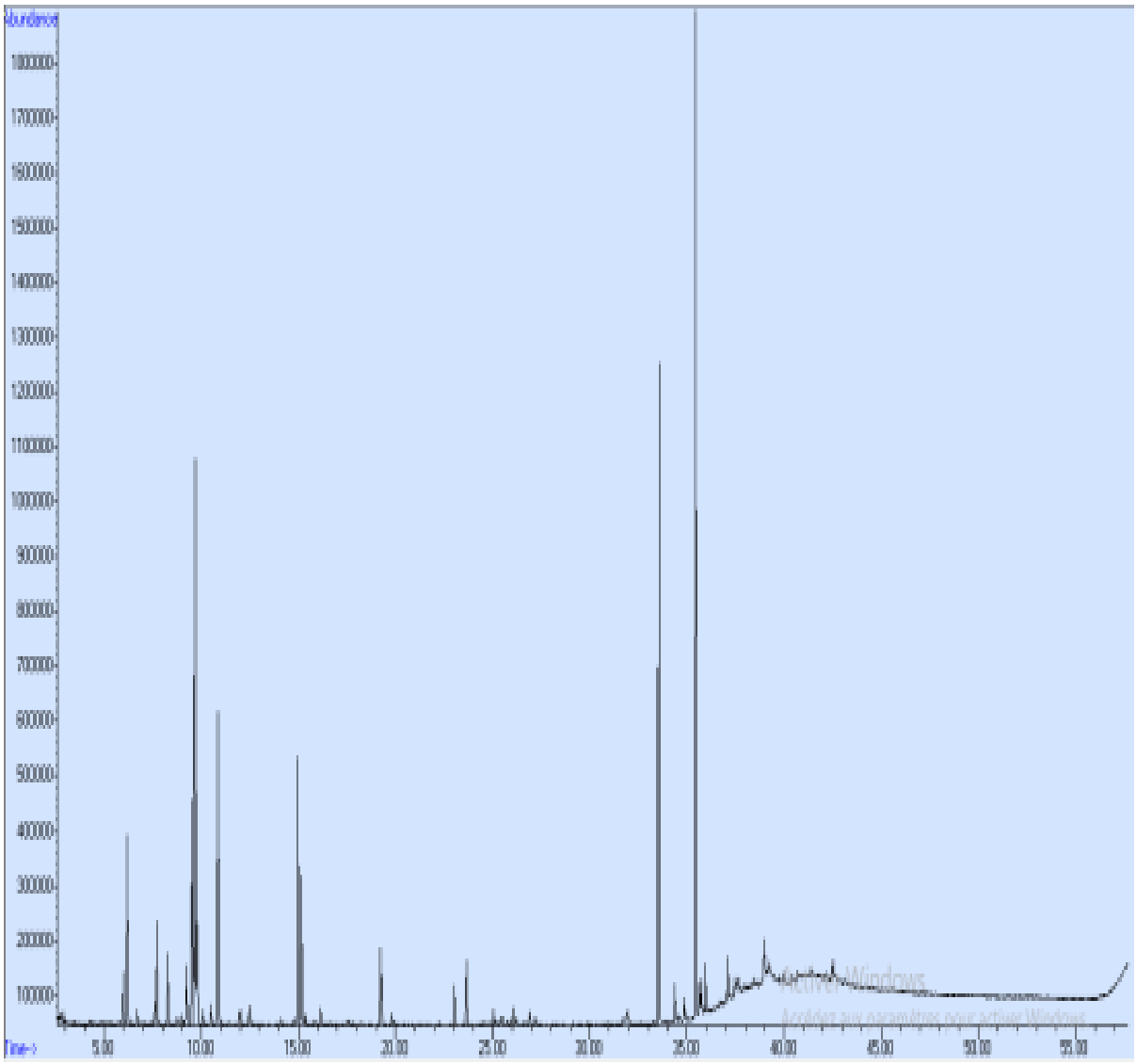
Zushang, S., Ping, W., Wei, Y. and Shiyou, L. 2013. Flavonoids and 3-Arylcoumarin from *Pterocarpus soyauxii*. *Planta Medica* 79: 487-491

Zygler, A. Słomińska, M. Namieśnik, J. 2012. Soxhlet Extraction and New Developments. *Academic Press* 2; 65-82

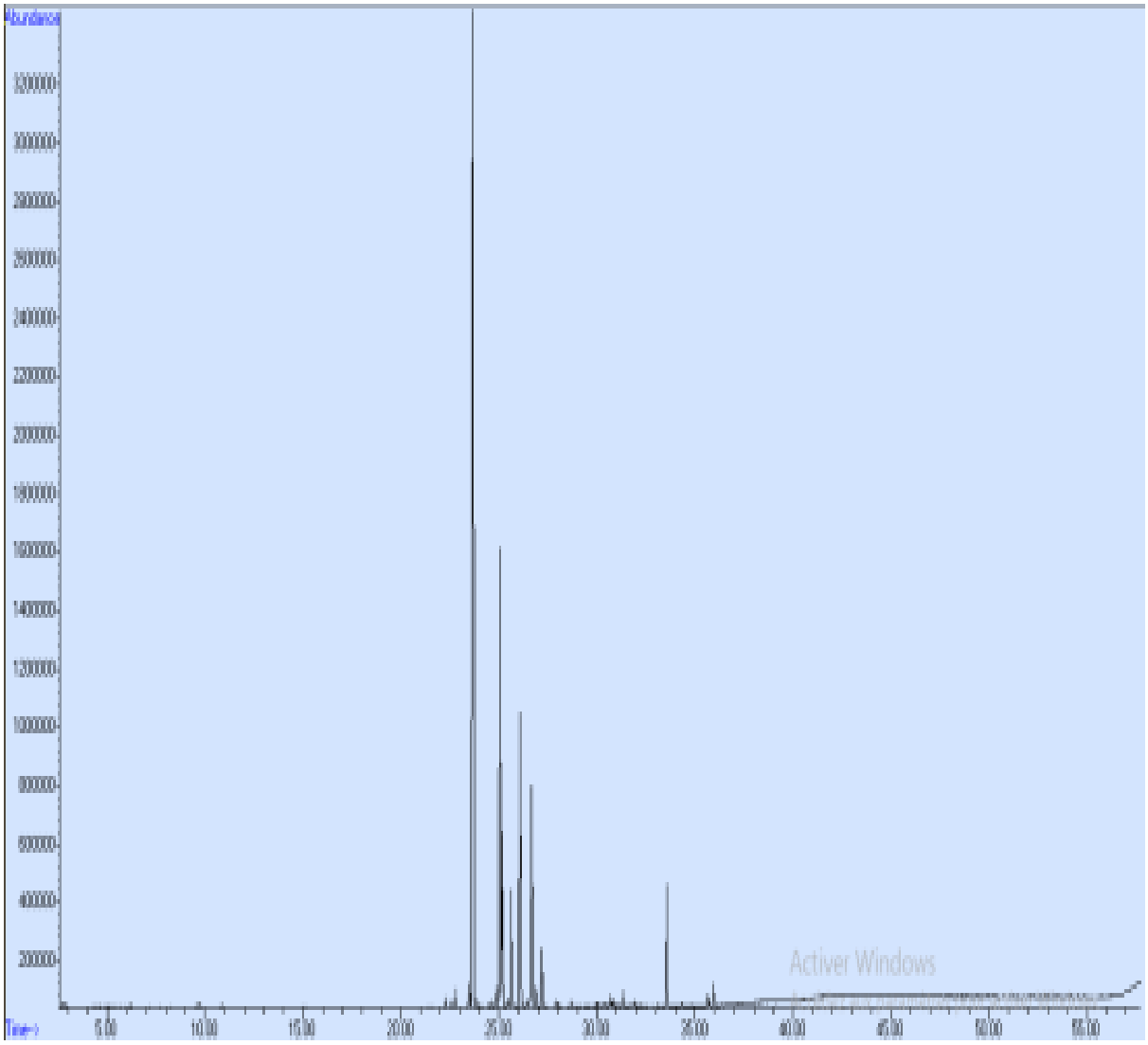
Appendices



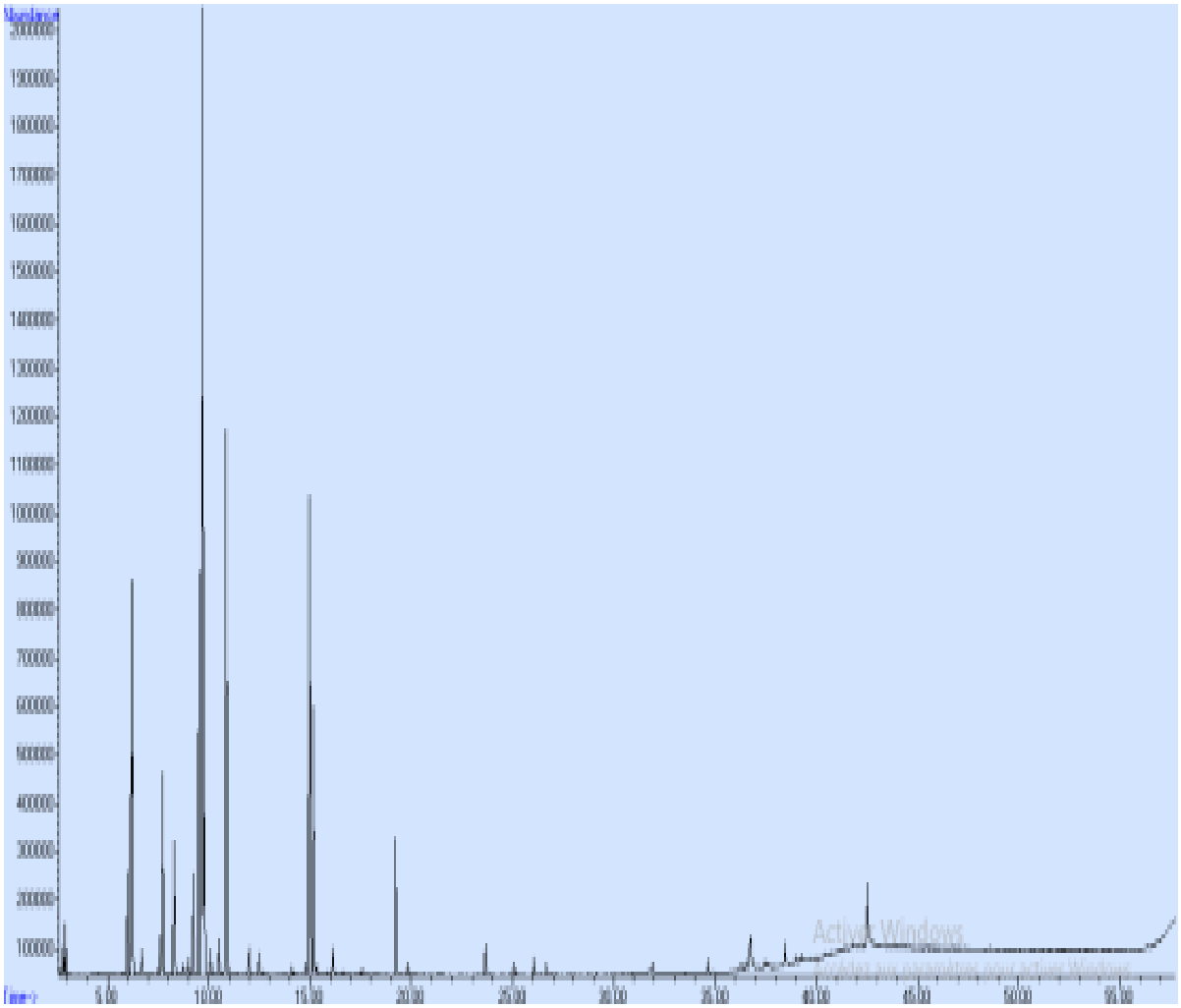
Appx. 1.1 Chromatogram of volatile oil from leaves of *Calophyllum inophyllum*



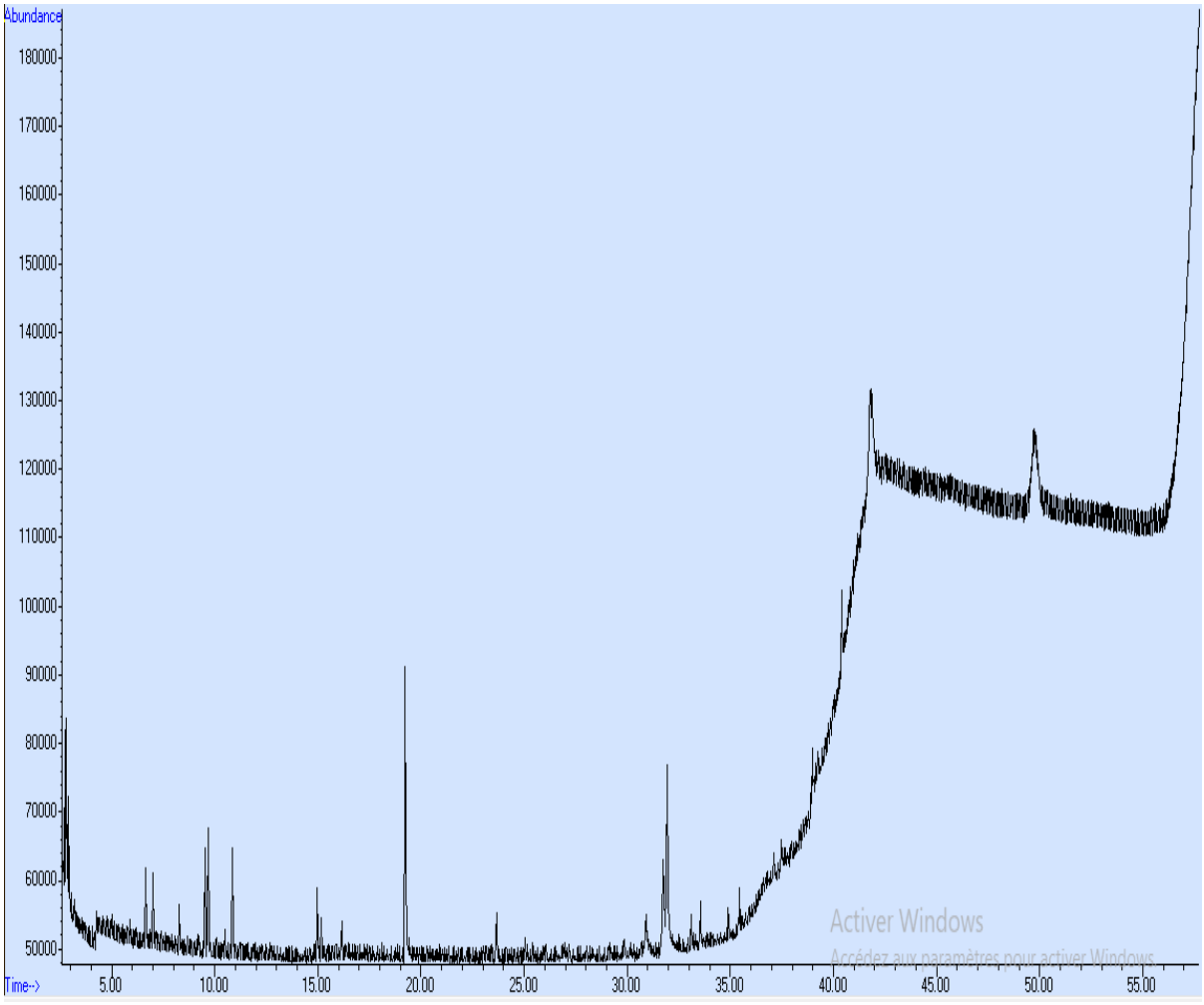
Appx. 1.2 Chromatogram of volatile oil from leaf-stalk of *Calophyllum inophyllum*



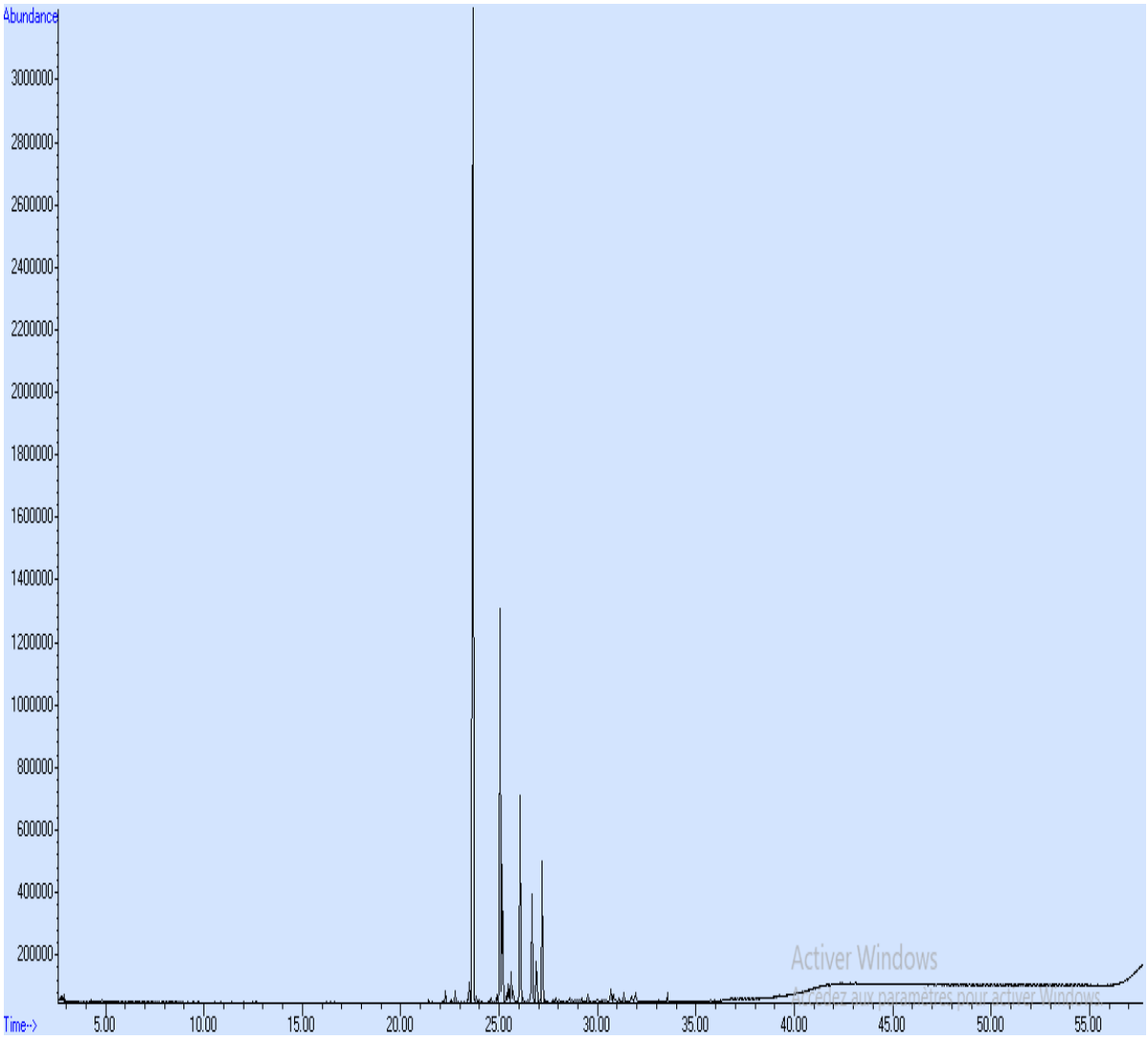
Appx. 1.3 Chromatogram of volatile oil from flower of *Calophyllum inophyllum*



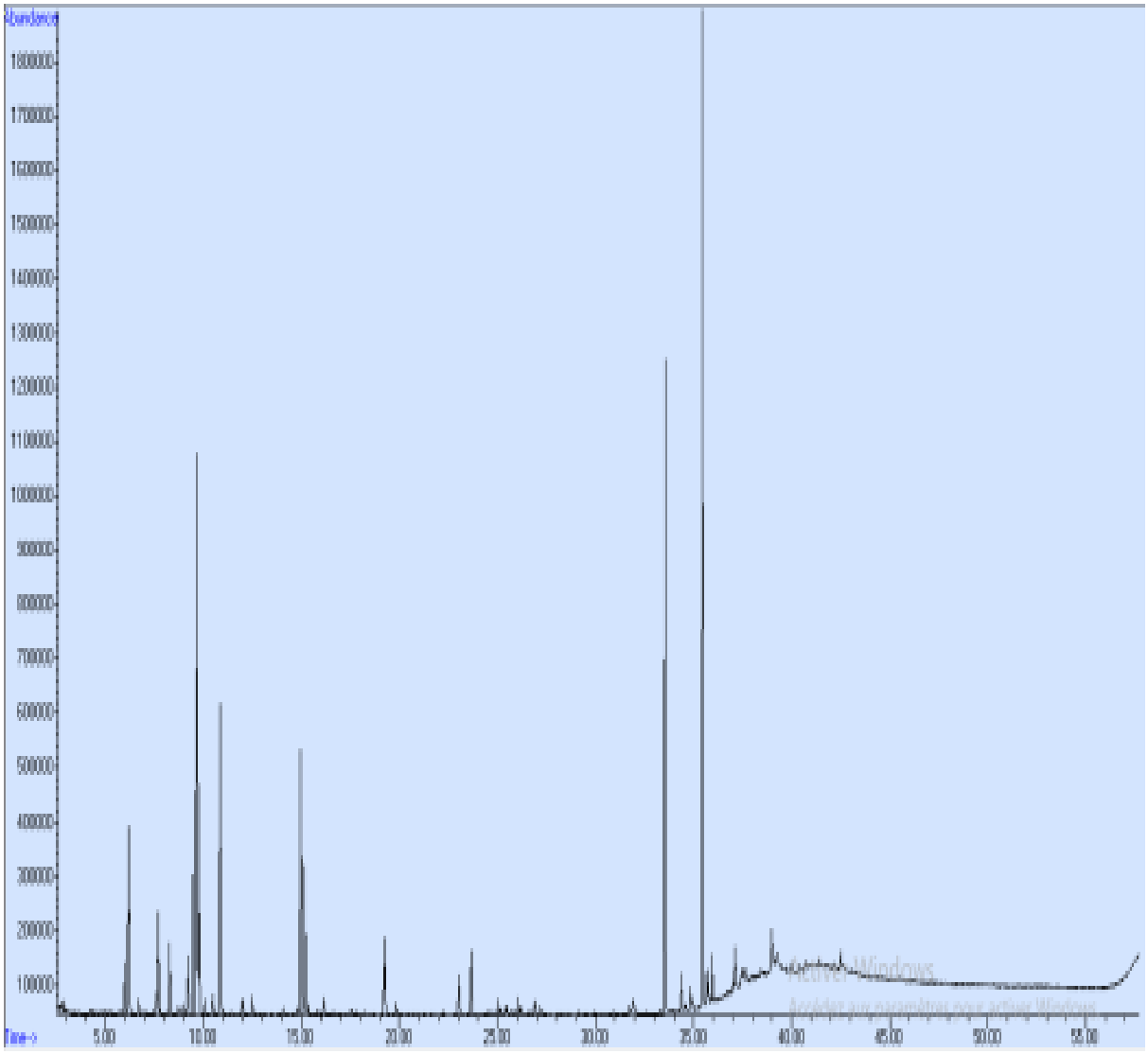
Appx. 1.4 Chromatogram of seed volatile oil from *Calophyllum inophyllum*



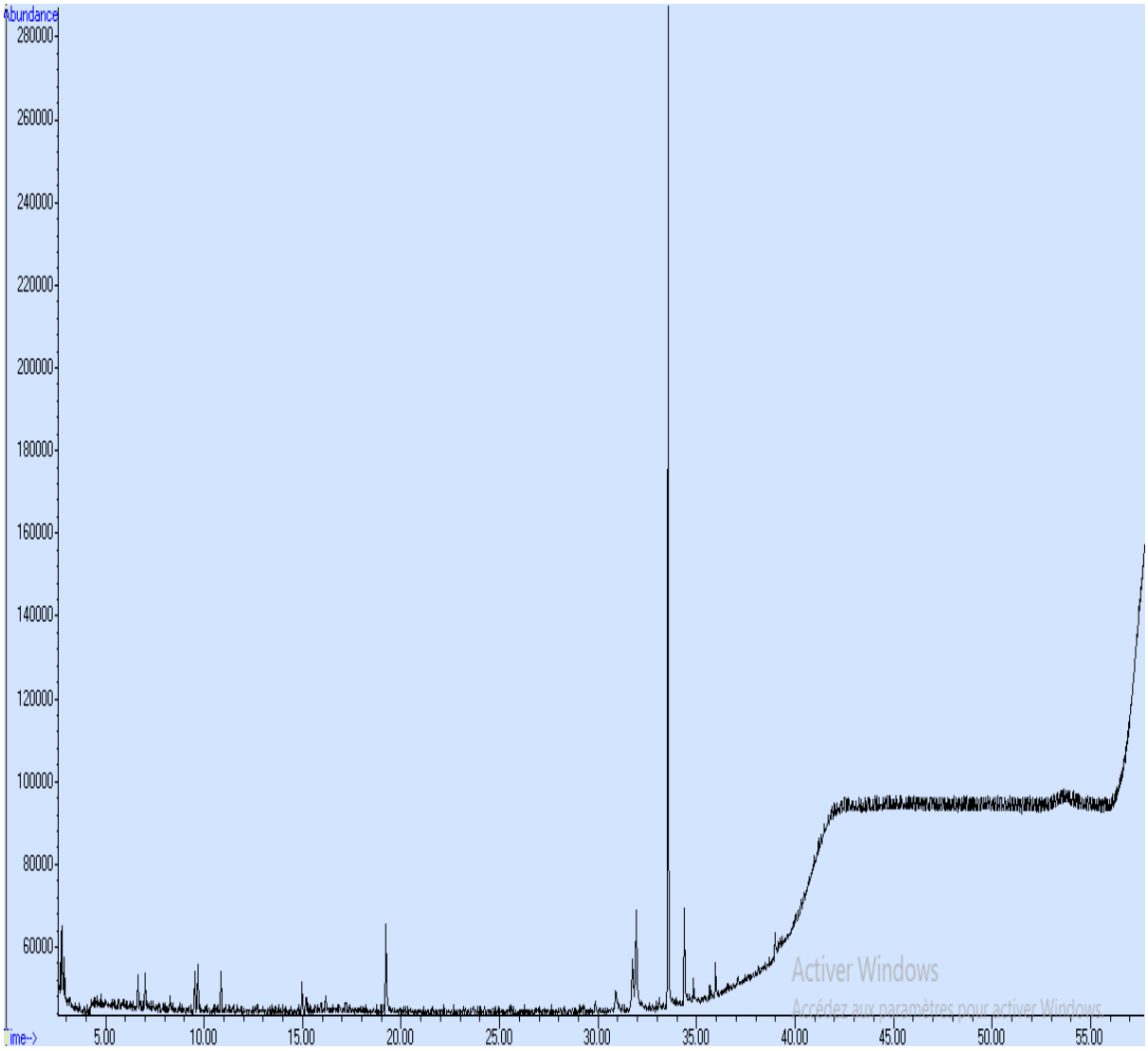
Appx. 1.5 Chromatogram of pod volatile oil from *Calophyllum inophyllum*



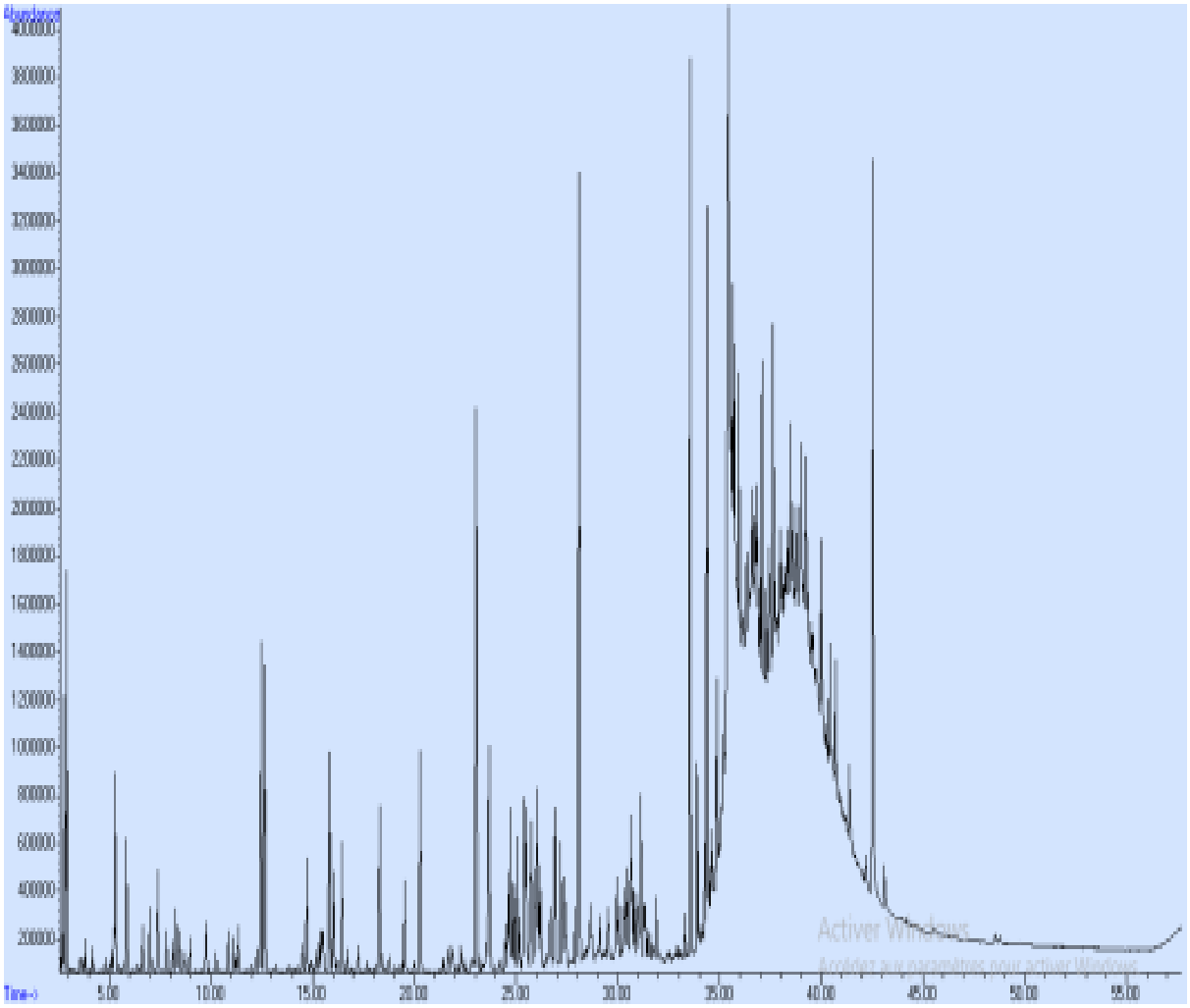
Appx. 1.6 Chromatogram of fruit-pulp volatile oil from *Calophyllum inophyllum*



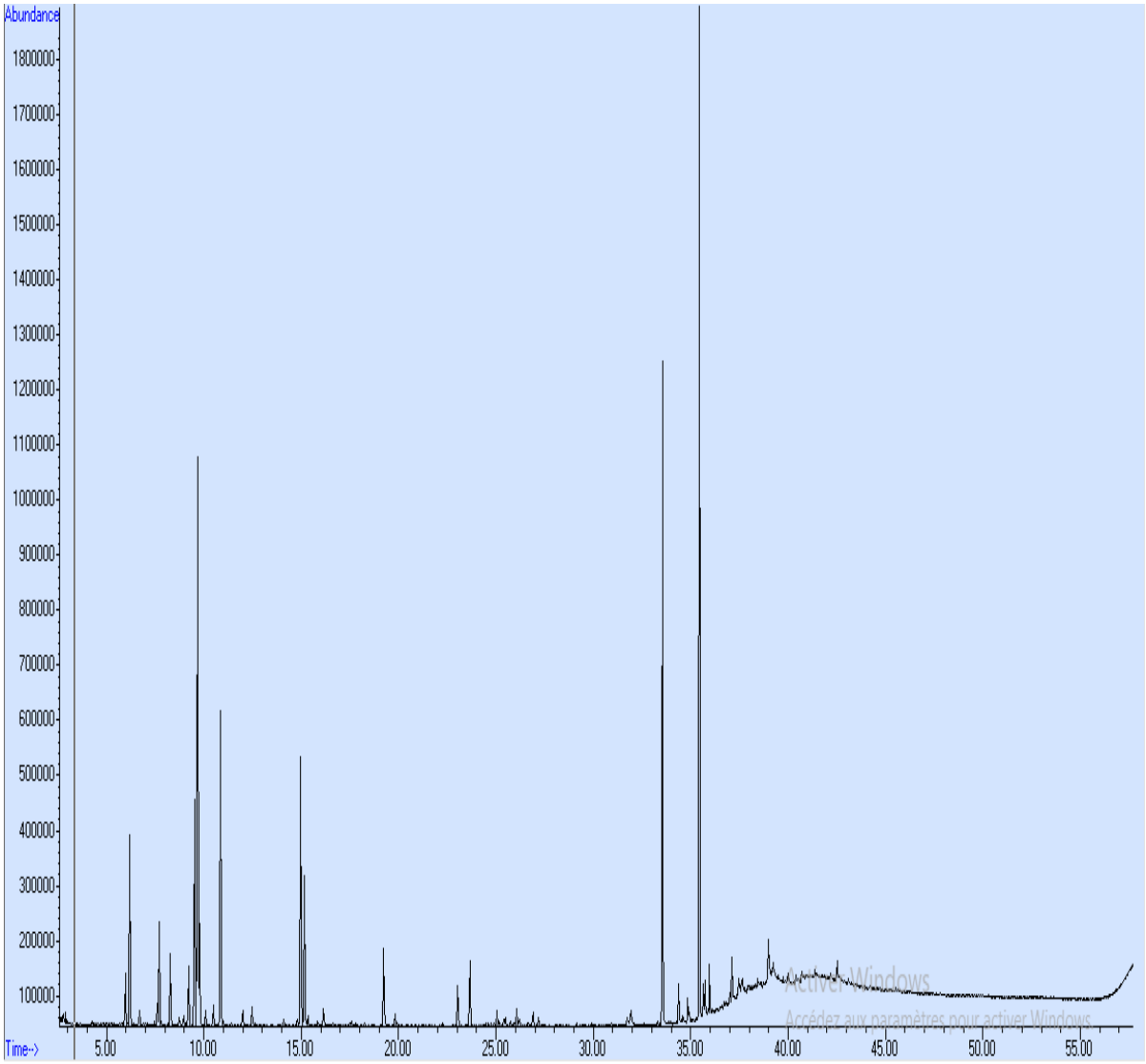
Appx. 1.7 Chromatogram of stem wood volatile oil from *Calophyllum inophyllum*



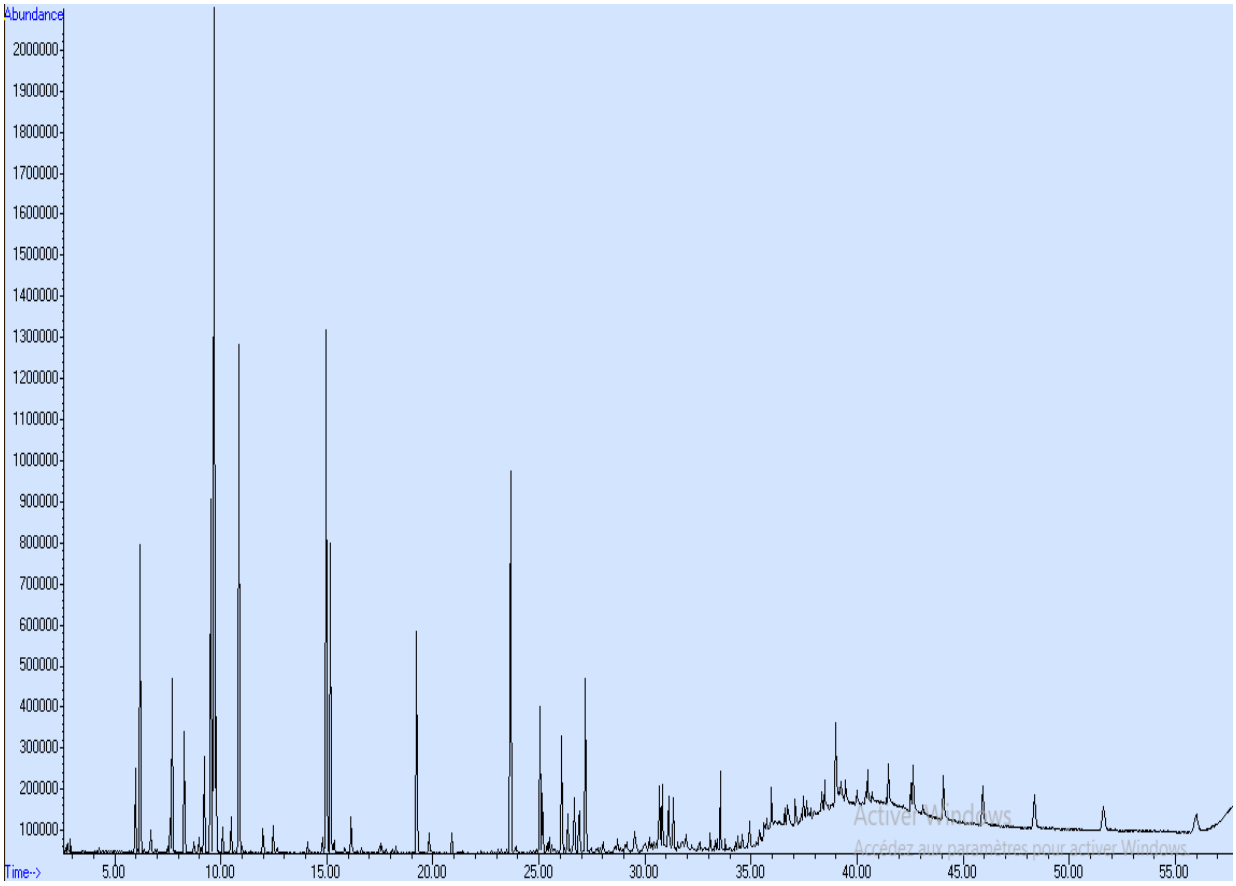
Appx. 1.8 Chromatogram of stem bark volatile oil from *Calophyllum inophyllum*



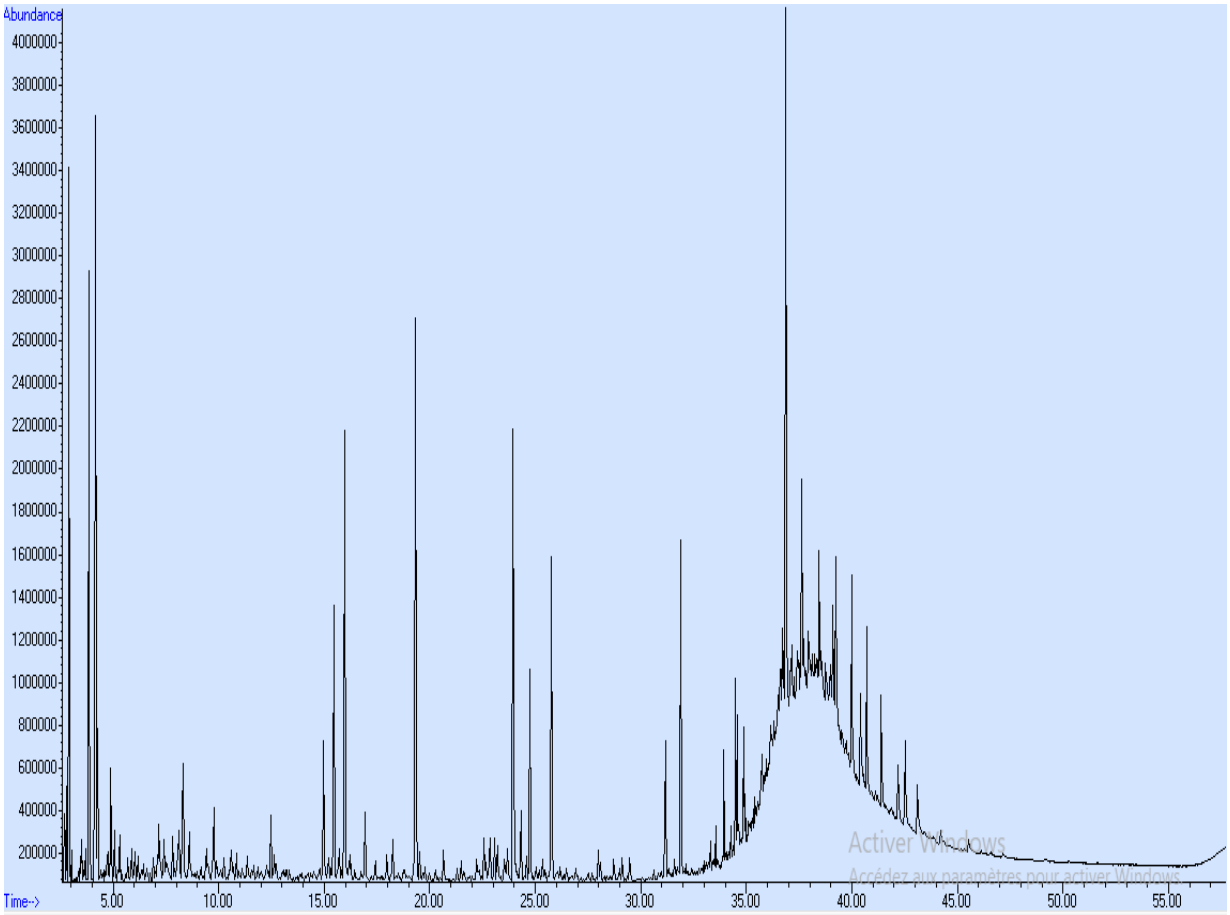
Appx. 1.9 Chromatogram of root wood volatile oil from *Calophyllum inophyllum*



Appx. 1.10 Chromatogram of root bark volatile oil from *Calophyllum inophyllum*



Appx. 1.11 Chromatogram of root wood essential oil from *Pterocarpus soyauxii*



Appx. 1.12 Chromatogram of root bark essential oil from *Pterocarpus soyauxii*