

**PHYTOCHEMICAL ANALYSIS, BIOLOGICAL ACTIVITIES, AND
COMPUTATIONAL STUDY OF COMPOUNDS ISOLATED FROM
SELECTED MEDICINAL PLANTS OF ETHIOPIAN FLORA**



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A Dissertation Submitted to the Department of Applied Chemistry

School of Applied Natural Science

**In Partial Fulfillment of the Requirement for the Degree of Doctor of
Philosophy in Applied Chemistry (Natural Product Chemistry)**

Office of Graduate Studies

Adama Science and Technology University

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Declaration and Recommendation

Declaration:

I hereby declare that this dissertation entitled “**Phytochemical Analysis, Biological Activities, and Computational Study of Compounds Isolated from Selected Medicinal Plants of Ethiopian Flora**” is my original work and has not been submitted to any other university for the award of any academic degree. The references used in this dissertation are duly acknowledged by appropriate citations.

Name of student

Signature

Date

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We, the supervisors of this dissertation, hereby certify that we have read and revised the dissertation entitled “**Phytochemical Analysis, Biological Activities, and Computational Study of Compounds Isolated from Selected Medicinal Plants of Ethiopian Flora**” prepared under our guidance by **Teshome Degfie**, submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Natural Product chemistry. Therefore, we hereby recommend the submission of the dissertation to the department for further review and evaluation.

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Approvals

Approval of Supervisors

We hereby certify that the recommendations and suggestions given by the examiners committee are appropriately incorporated into the final version of the dissertation entitled “**Phytochemical Analysis, Biological Activities, and Computational Study of Compounds Isolated from Selected Medicinal Plants of Ethiopian Flora**” by Teshome Degfie.

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We, the undersigned, members of the examiners committee of the dissertation open defense by Teshome Degfie have read and evaluated the dissertation entitled “**Phytochemical Analysis, Biological Activities, and Computational Study of Compounds Isolated from Selected Medicinal Plants of Ethiopian Flora**” and examined the candidate during open defense. This is, therefore, to certify that the dissertation is accepted for partial fulfillment of the requirement of the degree of Doctor of Philosophy in applied chemistry (Natural Product Chemistry).

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Dedication

This PhD dissertation is dedicated to my family, especially to my wife, Marta Hailu Mulatu and my kids.

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List of Contents

Declaration and Recommendation	i
Approvals	iii
Acknowledgement.....	vi
Dedication	vii
List of Contents	viii
List of Tables.....	xi
List of Figures	xii
List of Appendixes	xiv
ABSTRACT	1
1. INTRODUCTION.....	3
1.1. Background of the study	3
1.2. Statement of the Problem	9
1.3. Justification of the Study.....	11
1.4. Objectives.....	11
1.4.1. General objective.....	11
1.4.2. Specific objectives.....	12
1.5. Significance of the Study	12
2. REVIEW OF LITERATURE.....	13
2.1. Ethnobotanical uses of medicinal plants selected for this study	13
2.1.1. <i>Cyphostemma cyphopetalum</i> (Fresen.) Wild & Drummond	13
2.1.2. <i>Hydnora johannis</i>	15
2.1.3. <i>Capparis tomentosa</i>	17
2.1.4. <i>Kalanchoe petitenia</i>	19
2.1.5. <i>Cadaba rotundifolia</i>	21
2.2. Phytochemistry of the selected medicinal plants.....	25
2.2.1. <i>Cyphostemma cyphopetalum</i>	25
2.2.2. <i>Hydnora johannis</i>	27
2.2.3. <i>Capparis tomentosa</i>	29
2.2.4. <i>Kalanchoe petitenia</i>	31
2.2.5. <i>Cadaba rotundifolia</i>	33
2.3. Biological activity	38
2.3.1. <i>Cyphostemma cyphopetalum</i> (Fresen.) Wild & Drummond	38

2.3.2. <i>Hydnora johannis</i>	39
2.3.3. <i>Capparis tomentosa</i>	40
2.3.4. <i>Kalanchoe petitiiana</i>	41
2.3.5. <i>Cadaba rotundifolia</i>	42
3. METHODOLOGY	45
3.1. General	45
3.2. Chemical and Reagents	45
3.3. Plant Material Collection and Identification	45
3.4. Extraction and Isolation	45
3.4.1. Extraction of plant parts	46
3.4.2. Isolation of compounds	46
3.4.3. Essential oil Extraction.....	48
3.5. GC-MS analysis of essential oils.....	49
3.6. Biological activity	49
3.6.1. <i>In vitro</i> Antibacterial Activity Assay	49
3.6.2. <i>In vitro</i> antifungal activity assay	50
3.6.3. <i>In vitro</i> cytotoxicity assay	51
3.6.4. Antioxidant activity	52
3.7. Computational Study	53
3.7.1. Molecular docking.....	53
3.7.2. <i>In silico</i> drug likeness, ADME and Toxicity prediction.....	54
3.7.3. Density Function Theory	55
3.8. Data analysis	55
4. RESULTS AND DISCUSSION	56
4.1. Phytochemical Analysis	56
4.1.1. Compounds isolated from roots of <i>C. cyphopetalum</i>	56
4.1.2. Compounds isolated from root extract of <i>H. johannis</i>	75
4.1.3. Compounds isolated from root extract of <i>C. tomentosa</i>	85
4.1.4. Compounds isolated from root extract of <i>K. petitiiana</i>	88
4.1.5. Compounds isolated from leaves extract of <i>C. rotundifolia</i>	90
4.1.6. GC-MS analysis of essential oils from leaves of <i>C. rotundifolia</i> , <i>C. tomentosa</i> and <i>K. petitiiana</i>	92
4.2. Biological activities.....	99
4.2.1. <i>In vitro</i> antibacterial activity	99

4.2.2. <i>In vitro</i> antifungal activity	106
4.2.3. Cytotoxicity tests	108
4.2.4. Antioxidant activity	110
4.3. Computational study of isolated compounds	112
4.3.1. Molecular docking analysis of isolated compounds.....	112
4.3.2. <i>In-Silico</i> Drug-likeness, ADME and Toxicity analysis	121
4.3.3. DFT analysis of compounds isolated from <i>C. cyphpetalum</i>	125
5. CONCLUSION AND RECCOMENDATION	127
5.1. Conclusion.....	127
5.2. Recommendations	128
Reference.....	130
Appendices	157

List of Tables

Table 1 Summary of the ethnobotanical information (habit, part used, disease treated) of the medicinal plant selected for this study.	23
Table 2: ¹ H (400 MHz, CD ₃ OD), and ¹³ C (100 MHz, CD ₃ OD) NMR of compound 28	57
Table 3: ¹ H and ¹³ C NMR spectra of compound 49 (400 MHz, CDCl ₃)	58
Table 4 1D and 2D spectra of isolated compound 100 (400 MHz, CDCl ₃)	61
Table 5: 1D and 2D NMR spectra of compound 101 (400 MHz, CD ₃ OD)	64
Table 6: 1D and 2D NMR spectra of compound 102 (400 MHz, CD ₃ OD)	67
Table 7: 1D and 2D NMR spectra of compound 103 (400 MHz, CD ₃ OD)	69
Table 8: ¹ H, and ¹³ C spectra of compound 104 (400 MHz, CD ₃ OD)	71
Table 9: 1D and 2D NMR spectra of compound 105 (400 MHz, CD ₃ OD)	73
Table 10: ¹ H (400 MHz, MeOD), ¹³ C (100 MHz) NMR spectra of compound 40	76
Table 11: ¹ H (400 MHz, CDCl ₃) and ¹³ C (100 MHz) NMR spectra of compound 41	78
Table 12: 1D and 2D NMR (400 CD ₃ OD) spectral data of compound 106	80
Table 13: ¹ H (400 MHz, MeOD), ¹³ C (100 MHz) NMR spectral data of compound 107	82
Table 14: ¹ H (400 MHz, CDCl ₃), ¹³ C NMR (100 MHz) spectral data of compound 108	84
Table 15: ¹ H (400 MHz, CDCl ₃), ¹³ C (101 MHz), and HMBC NMR data of compound 110	86
Table 16: 1D and 2D (400 MHz, CDCl ₃) spectra of compound 111	87
Table 17: 1D and 2D NMR (400 MHz, CDCl ₃) spectra of compound 112	90
Table 18: 1D and 2D (400 MHz, CDCl ₃) spectra of compound 113	91
Table 19: Percentage composition of essential oils from leaves of <i>C. tomentosa</i>	94
Table 20: Percentage composition of oils obtained from leaves of <i>K. petitiiana</i>	96
Table 21: Percentage composition of oils obtained from leaves of <i>C. rotundifolia</i>	99
Table 22: Bacterial growth inhibition zone (mean ± SD, in mm) of isolated compounds from roots of <i>C. cyphopetalum</i> against standard <i>E. coli</i> , <i>S. aureus</i> , and <i>P. aeruginosa</i> strains.	102
Table 23: Inhibition zone (mean ± SD, in mm) of roots extrcats of <i>H. johannis</i> , and isolated compounds 40 , 41 , 106-108	105
Table 24: Inhibition zone (in mm) of root extracts of <i>C. cyphopetalum</i> against <i>Candida albicans</i>	107
Table 25: Cytotoxicity of <i>C. cyphopetalum</i> , <i>H. johannis</i> , and <i>C. tomentosa</i> extracts against MCF-7 cell line	109
Table 26: Radical scavenging activity isolated compounds	111
Table 27: Molecular docking values of isolated compounds against DNA (PDB ID: 6F86) ..	115
Table 28: Molecular docking value of isolated compounds against PqsA (PDB ID: 5OE3) ..	116
Table 29: Molecular docking values against <i>S. aureus</i> PK (PDB ID: 3T07)	117
Table 30: <i>In silico</i> drug-likeness of isolated compounds computed by SwissADME	123
Table 31: ADME predictions of compounds 85-90, 93 and 94 computed by SwissADME ...	124
Table 32: Prediction of Toxicity of compounds computed by ProTox-II.	125
Table 33: Quantum chemical descriptors of the isolated compounds	126

List of Figures

Fig. 1: The structures of some natural products isolated from higher plants as clinical agents. .	7
Fig. 2: Aerial part of <i>C. cyphopetalum</i> (Photo by Teshome D, Sept 2019, Huruta)	14
Fig. 3: Photo of <i>H. johannis</i> (photo by Teshome D, Sept 2021, Huruta)	16
Fig. 4: Aerial part of <i>C. tomentosa</i> (Photo by Teshome D, Sept 2021, Adama)	18
Fig. 5: Aerial part of <i>K. petitiana</i> (Photo by Teshome D, Nov 2021, Huruta)	20
Fig. 6: Aerial part of <i>C. rotundifolia</i> (Photo by Teshome D, Sept 2021, Dire Dawa).	22
Fig. 7: Compounds reported from <i>C. digitatum</i>	25
Fig. 8: The structures of compounds reported from bark of <i>C. greveana</i>	26
Fig. 9: The structures of compounds reported from <i>C. crotalarioides</i>	27
Fig. 10: The structures of compounds reported from <i>H. johannis</i> roots.	28
Fig. 11: Chemical structure of some bioactive compounds from capper species (<i>C. spinosa</i> , <i>C. ovata</i> , <i>C. decidua</i> , and <i>C. diversifolia</i>)	30
Fig. 12: Compounds isolated from root and fruit of <i>C. tomentosa</i>	31
Fig. 13: Compounds reported from aerial part <i>K. petitiana</i> (61) and <i>K. murmorutu</i> (62)	32
Fig. 14: Compounds isolated from <i>K. pinnata</i> (63 & 64), <i>K. deigremontiana</i> (65), <i>K. gracilis</i> (66), and <i>K. tubiflor</i> (67)	33
Fig. 15: Compounds from ethanolic leaf extracts of <i>C. trifoliata</i>	34
Fig. 16: Structures of compounds reported from the aerial parts of <i>C. glandulosa</i>	35
Fig. 17: Compounds identified from methanol leaves of <i>C. farinosa</i>	36
Fig. 18: Structures of the compounds reported from the roots of <i>C. natalensis</i>	37
Fig. 19: Structure of compounds reported from areal part of <i>C. rotundifolia</i>	38
Fig. 20: Key ¹ H- ¹ H COSY and HMBC correlations of compound 100.	62
Fig. 21: Structure of compounds isolated from roots of <i>C. cyphopetalum</i>	74
Fig. 22: Structures of compounds isolated from the root of <i>H. johannis</i>	85
Fig. 23; Structure of compounds isolated from <i>C. tomentosa</i> (110 and 111), <i>K. petitiana</i> (112), and <i>C. rotundifolia</i> (113)	88
Fig. 24. GC profile of major compounds of oils from leaves of <i>C. tomentosa</i>	93
Fig. 25: Structures of major compounds (114- 117) from oil of leaves of <i>C. tomentosa</i>	93
Fig. 26: GC profile major compounds of oils from leaves of <i>K. petitiana</i>	95
Fig. 27: Structures of major compounds (118- 122) identified from oil of leaves of <i>K. petitiana</i>	95
Fig. 28: GC profile of major compounds of oil from leaves of <i>C. rotundifolia</i>	98
Fig. 29: Structure of major compounds identified from oil of leaves of <i>C. rotundifolia</i>	98
Fig. 30: <i>In-vitro</i> antifungal activity of <i>n</i> -hexane, DCM/MeOH (1:1), and MeOH (100%) extracts of roots of <i>C. cyphopetalum</i> against <i>C. albicans</i>	107
Fig. 31: <i>In-vitro</i> cytotoxic activity of DCM/MeOH, MeOH and/or <i>n</i> -hexane extracts of <i>C. cyphopetalum</i> , <i>H. johannis</i> , and <i>C. tomentosa</i>	109
Fig. 32: Percent of DPPH scavenging activity of the isolated compounds and the standard. .	111
Fig. 33: Binding affinity of the compounds toward DNA gyrase B, PqsA, and <i>S. aureus</i> PK.	118

Fig. 34: The 2D and 3D binding interactions of compounds 100 and standard (chloramphenicol) against DNA gyrase B (PDB ID: 6F86).	119
Fig. 35: The 2D and 3D binding interactions of compounds 102 and standard against PqsA (PDB ID: 5OE3).....	120
Fig. 36: The 2D and 3D binding interactions of compounds 106 and standard against <i>S. aureus</i> PK (PDB ID: 3T07).....	121

List of Appendices

Appendix 1A: ^1H NMR spectrum of compound 28	157
Appendix 1B: ^{13}C NMR spectrum of compound 28	157
Appendix 1C: DEPT-135 NMR spectrum of compound 28	158
Appendix 2A: IR spectrum of compound 49	158
Appendix 2B: ^1H NMR spectrum of compound 49	159
Appendix 2C: ^{13}C NMR spectrum of compound 49	159
Appendix 2D: DEPT-135 NMR spectrum of compound 49	160
Appendix 3A: IR spectrum of 3-hydroxyisoagatolacyone 100	160
Appendix 3B: ^1H NMR spectrum of compound 100	161
Appendix 3C: ^{13}C NMR spectrum of compound 100	161
Appendix 3D: DEPT-135 spectrum of compound 100	162
Appendix 3E: COSY spectrum of compound 100	162
Appendix 3F: HSQC NMR spectrum of compound 100	163
Appendix 3G: HMBC spectrum of compound 100	163
Appendix 4A: ^1H NMR spectrum of compound 101	164
Appendix 4B: ^{13}C NMR spectrum of compound 101	164
Appendix 4C: DEPT-135 spectrum of compound 101	165
Appendix 4D: COSY NMR spectrum of compound 101	165
Appendix 4E: HSQC NMR spectrum of compound 101	166
Appendix 4F : HMBC NMR spectrum of compound 101	166
Appendix 5A: ^1H NMR spectrum of compound 102	167
Appendix 5B: ^{13}C NMR spectrum of compound 102	167
Appendix 5C: DEPT-135 NMR spectrum of compound 102	168
Appendix 5D: COSY NMR spectrum of compound 102	168
Appendix 5E: HSQC NMR spectrum of compound 102	169
Appendix 5F: HMBC NMR spectrum of compound 102	169
Appendix 6A: ^1H NMR spectrum of compound 103	170
Appendix 6B: ^{13}C NMR spectrum of compound 103	170
Appendix 6C: DEPT-135 NMR spectrum of compound 103	171
Appendix 6D: COSY NMR spectrum of compound 103	171
Appendix 6E: HSQC NMR spectrum of compound 103	172
Appendix 6F: HMBC NMR spectrum of compound 103	172
Appendix 7A: ^1H NMR spectrum of compound 104	173
Appendix 7B: ^{13}C NMR spectrum of compound 104	173
Appendix 7C: DEPT-135 spectrum of compound 104	174
Appendix 7D: COSY NMR spectrum of compound 104	174
Appendix 7E: HSQC NMR spectrum of compound 104	175
Appendix 7F: HMBC NMR spectrum of compound 104	175
Appendix 8A: ^1H NMR spectrum of compound 105	176

Appendix 8B: ^{13}C NMR spectrum of compound 105	176
Appendix 8C: DEPT-135 NMR spectrum of compound 105	177
Appendix 8D: COSY NMR spectrum of compound 105	177
Appendix 8E: HSQC NMR spectrum of compound 105	178
Appendix 8F: HMBC NMR spectrum of compound 105	178
Appendix 9A: ^1H NMR spectrum of compound 40	179
Appendix 9B: ^{13}C NMR spectrum of compound 40	179
Appendix 9C: DEPT-135 NMR spectrum of compound 40	180
Appendix 10A: ^1H NMR spectrum of compound 41	180
Appendix 10B: ^{13}C NMR spectrum of compound 41	181
Appendix 10C: DEPT-135 NMR spectrum of compound 41	181
Appendix 11A: ^1H NMR spectrum of compound 106	182
Appendix 11B: ^{13}C NMR spectrum of compound 106	182
Appendix 11C: DEPT-135 spectrum of compound 106	183
Appendix 11D: COSY spectrum of Compound 106	183
Appendix 11E: HSQC spectrum of compound 106	184
Appendix 11F: HMBC spectrum of Compound 106	184
Appendix 12 A: ^1H NMR spectrum of Compound 107	185
Appendix 12 B: ^{13}C NMR spectrum of compound 107	185
Appendix 12C: DEPT-135 NMR spectrum of compound 107	186
Appendix 13C: Appendix 12D: DEPT NMR spectrum of compound 108	188
Appendix 13A: ^1H NMR spectrum of compound 108	186
Appendix 13 B: ^{13}C NMR spectrum of compound 108	187
Appendix 13C: Appendix 12D: DEPT NMR spectrum of compound 108	188
Appendix 14 A: GC of compound 109	189
Appendix 14B: Mass spectrum (MS) of compound 109	189
Appendix 15A: ^1H NMR spectrum of compound 110	190
Appendix 15B: ^{13}C NMR spectrum of compound 110	190
Appendix 15C: DEPT-135 NMR spectrum of compound 110	191
Appendix 16A: ^1H NMR spectrum of compound 111	191
Appendix 16B: ^{13}C NMR spectrum of compound 111	192
Appendix 16C: DEPT-135 NMR spectrum of compound 111	192
Appendix 16D: COSY NMR spectrum of compound 111	193
Appendix 16E HSQC NMR spectrum of compound 111	193
Appendix 16F: HMBC NMR spectrum of compound 111	194
Appendix 17A: ^1H NMR spectrum of compound 112	194
Appendix 17B: ^{13}C NMR spectrum of compound 112	195
Appendix 17C: DEPT-135 NMR spectrum of compound 112	195
Appendix 17D: COSY NMR spectrum of compound 112	196
Appendix 17E: HSQC NMR spectrum of compound 112	196
Appendix 17F: HMBC NMR spectrum of compound 112	197

Appendix 18A: ¹ H NMR spectrum of compound 113	197
Appendix 18B: ¹³ C NMR spectrum of compound 113	198
Appendix 18C: DEPT-135: ¹ H NMR spectrum of compound 113	198
Appendix 18D COSY NMR spectrum of compound 113	199
Appendix 18E: HSQC NMR spectrum of compound 113	199
Appendix 18F: HMBC NMR spectrum of compound 113	200
Appendix 19A: Chromatogram for essential oil from leaves of <i>C. tomentosa</i>	201
Appendix 19B: Chromatogram for essential oil from leaves of <i>K. petitiiana</i>	201
Appendix 19C: Chromatogram for essential oil from leaves of <i>C. rotundifolia</i>	202
Appendix 20A: Inhibition zone image of antibacterial activities	203
Appendix 21A: The 2D and 3D binding interactions of compounds 28, 49, 100, 101, 102, 103, 106 and 107 , and standard against DNA gyrase B (PDB ID: 6F86).....	204
Appendix 22A: The 2D and 3D binding interactions of compounds 28, 49, 100, 101, 102, 103, 106 and 107 , and standard against PqsA (PDB ID: 5OE3).....	206
Appendix 23A: The 2D and 3D binding interactions of compounds 28, 49, 100, 101, 102, 103, 106 and 107 , and standard against <i>S. aureus</i> PK (PDB ID: 3T07).	208

Abbreviations

1D	1-Dimensional
2D	2-Dimensional
ATCC	American Type Culture Collection
CLSI	Clinical and Laboratory Standards Institute
COSY	Correlation Spectroscopy
<i>d</i>	Doublet
<i>dd</i>	Doublet of a doublet
DEPT	Distortion-less Enhancement by Polarization Transfer
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
GC	Gas Chromatography
HMBC	Heteronuclear Multiple Bond Coherence
HSQC	Heteronuclear Single Quantum Coherence
IC ₅₀	50% Inhibitory Concentration
IR	Infra-Red
LD ₅₀	Lethal dose 50
MCF-7	Michigan Cancer Foundation-7
MHz	Megahertz
MIC	Minimum Inhibitory Concentration
MTT Diphenyltetrazolium Bromide	3-(4,5-Dimethylthiazol-2-Yl)-2,5-
ANCI	American National Cancer Institute
NMR	Nuclear Magnetic Resonance
PDA	Potato dextrose agar
<i>s</i>	Singlet
<i>t</i>	Triplet
TLC	Thin-Layer Chromatography
UV	Ultraviolet
WHO	World Health Organization

ABSTRACT

Drug resistance, the side effects and high cost associated with the existing antibiotics, reappearance of previously known diseases, and changes in genetic make-up are major problems in the treatment of human health. Thus, the search for alternatively safe and effective drugs with antimicrobial and anticancer agent has become a necessity. Plants have been known as potential source of therapeutic agents for the treatment of various diseases since the ancient days. Nearly 80% of the population in Ethiopia uses plant-based traditional medicine as their primary health care. In the traditional medicine of Ethiopia, *Cyphostema cyphopetalum*, *Hydnora johannis*, *Capparis tomentosa*, *Kalanchoe petitiata* and *Cadaba rotundifolia* are used as medicinal plants for the treatment of various ailments. The objective of this study was to analyse the phytochemical constituents, evaluate biological activities of extracts and isolated compounds, and predict computational study of compounds isolated from roots of *Cyphostemma cyphoptealum* and *Hydronea johannis*, stem bark of *Capparis tomentosa*, and leaves of *Kalanchoe petitiata* and *C.adaba rotundifolia*. Extraction by maceration was used to obtain crude extracts. Silica gel column chromatography was used to isolate compounds. Spectroscopic techniques (IR, 1D (^1H , ^{13}C , and DEPT -135) and 2D (COSY, HSQC, and HMBC NMR) were used to elucidate structures of isolated compounds. Hydrodistillation and GC-MS were used to extract and analyse the chemical composition of essential oils, respectively. Disc diffusion assay was used for in vitro antibacterial and antifungal activity evaluation of isolated compounds and extracts against bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes*, and fungal strain *Candida albicans*. The DPPH assay was used to evaluate antioxidant activity of isolated compounds. MTT assay was used to evaluate the cytotoxicity of extracts from roots of *C. cyphopetalum*, and *H. johannis*, and stem bark of *C. tomentosa* against MCF-7 cell lines. AutoDock Vina 4.2 (MGL tools 1.5.7) was used to perform the molecular docking analysis to predict the potential binding modes of the isolated compounds with the target bacterial proteins (DNA gyraseB, *S. aureus* PK, PqsA). SwissADME and PreADMET tools were used to predict the In silico drug likeness and ADME properties of isolated compounds. ProTox II tool to predict insilic toxicity. DFT analysis of isolated compounds was performed using Gaussian 09 and visualized through Gauss view 6.0. Silica gel column chromatographic separation of extracts of five selected medicinal plants afforded 20 compounds, including a new hydroxyl-spongiane diterpenoid lactone derivative, 3-hydroxyisoagatholactone(**100**) along with trans-resveratrol (**28**), β -sitosterol (**49**) and ϵ -viniferin (**101**) from dichloromethane/methanol (1:1), and gnetin H (**102**), tricuspidatol A (**103**), ϵ -viniferin-diol (**104**) and parthenostilbenin B (**105**) from methanol root extract of *C. cyphopetalum*. Silmilarily, catechin (**40**), oleic acid (**41**), β -sitosterol (**49**), catechin-7-O-glucoside (**106**), sitosterol-3-O-glucoside (**107**), caffeic acid derivative (**108**), and a pregn derivative (3,9-epoxypregn-16-ene-14,20-diol,7,11,18-triacetoxy-3-methox) (**109**) were isolated from roots of *H. johannis*. The dichloromethane/methaol extract of stem barck of *C. tomentosa* afforded tetradecanoic acid (**110**) and 4-hyroxybenzoate derivative (**111**), leaf extract of *K. petitiata* and *C. rotundifolia* afforded bis-(2-ethylhexyl) phthalate (**112**) and diethyl phthalate (**113**). The GC-MS analysis of essential oil obtained from leaves of *C.tomentosa*, *K. petitiata* and *C. rotundifolia* resulted in identification phenol, 2,4-bis(1,1-dimethylethyl)-(21.03 %), 1,5-Hexadiene, 2-methyl (23.07 %), Phenol, 2,4-bis(1,1-dimethylethyl)- (15.6%) as major compounds, respectively. Among the compounds isolated from *Cyphostema cyphopetalum*, at 50 $\mu\text{g/mL}$, the highest growth inhibition against *E. coli* was

exhibited by ϵ -viniferin (**101**) (8.55 ± 0.45 mm) and tricuspidatol A (**103**) (8.42 ± 0.20 mm). Whereas, against *P. aeruginosa* and *S. aureus* the highest inhibition zone was scored by gnetin H (**102**) (12.55 ± 0.65 mm) and ϵ -viniferin (**101**) (9.30 ± 1.39 mm) respectively, which is good compared to chloramphenicol (19.06 ± 2.28 , 11.76 ± 0.77 , and 15.74 ± 1.02 , at $30 \mu\text{g/mL}$, respectively). The DCM/MeOH extract of roots of *H. johannis* showed the highest antibacterial activity against *S. aureus* (10.75 ± 0.25 mm), *P. aeruginosa* (10.5 ± 0.5 mm), and *E. coli* (9.5 ± 0.5 mm), and n-hexane against *S. pyogenes* (9.5 ± 0.5 mm). Strong antibacterial activities were exhibited by compound **108** against *P. aeruginosa* (13.72 ± 0.05 mm, 0.25 mg/mL) and compound **107** against *S. aureus* (10.75 ± 0.25 mm) at 0.25 mg/mL , compared to amoxicillin (16.00 , 15.25 , respectively at 0.25 mg/mL). The DCM/MeOH (1:1) extract of roots of *C. cyphopetalum* showed moderate antifungal activity (8.5 ± 0.5 mm, at 12.5 mg/mL) compared to ketoconazole (17.3 ± 2.05 mm, at $10 \mu\text{g/disc}$). The methanol extracts of the roots of *C. cyphopetalum* and *H. johannis*, and dichloromethane/methanol (1:1) extract of stem bark extract of *C. tomentosa* showed strong anticancer activity against breast cancer cell line (MCF-7) at % cell viability $< 50\%$ each. At concentration of $12.5 \mu\text{g/mL}$, the highest percentage inhibition was recorded by compound **28** ($76.87 \pm 0.09\%$), followed by compound **101** ($74.30 \pm 0.12\%$), and **105** ($73.32 \pm 0.25\%$), with IC_{50} value of $0.052 \mu\text{g/mL}$, $0.017 \mu\text{g/mL}$, and $0.025 \mu\text{g/mL}$, respectively. The molecular docking analysis revealed that, highest binding affinity toward DNA gyrase was exhibited by compounds **101** and **107** (-7.6 kcal/mol). Compounds **102**, **107** and **40** (-8.8 , -8.7 , and -8.6 , respectively) displayed the next highest binding affinity towards protein PqsA whereas compound **107** (-7.4 kcal/mol) showed promising binding affinity towards *S. aureus* PK compared to chloramphenicol (-6.4 , -7.0 , and -4.6 kcal/mol , respectively). Compounds **28**, **100**, and **101** satisfy Lipinski's rule of five with zero violations. Compounds **40** and **107** exhibited negligible acute toxicity ($\text{LD}_{50} > 5000$, Toxicity class > 5). Compound **28** recorded the lowest HOMO-LUMO energy gap and good chemical activity. In this work, the computational study results are in agreement with the in vitro experimental studies obtained for compounds. Thus, compounds **28**, **100**, **101**, **102**, **106** and **107** may have potential to be used as lead molecules that could be developed into potent bacterial growth inhibitors. This supported the traditional uses of roots of *C. cyphopetalum* and *H. johannis* in the treatment of infectious diseases. The cytotoxicity results support the traditional uses of roots of *C. cyphopetalum*, *H. johannis*, and *C. tomentosa* in the treatment of breast cancer.

Key words: *C. cyphopetalum*, *H. johannis*, *C. tomentosa*, *K. petitiana*, *C. rotundifolia*

1. INTRODUCTION

1.1. Background of the study

Throughout human history, natural products have been utilized as medicine to prevent and treat diseases or as chemo-preventives, antioxidants, nutraceuticals, and diet supplements (Dia et al., 2012; Yuan et al., 2016). Natural product medicines obtained from various sources including terrestrial plants, microorganisms, marine organisms, terrestrial vertebrates and invertebrates (Rout et al., 2009). The major source of knowledge of using natural product in medication is the result of search of human beings for available foods and the treatment of diseases through palatable trails or untimely deaths by trial and error, for hundreds of centuries (Dias et al., 2012). The historic advantage claimed for the therapeutic uses of natural products is attributed to their huge phytochemical constituents with unique structural diversity, results in diversified biological activities (Brahmachari, 2012; Newman & Cragg, 2016). The chemical diversity of natural products substantiate the remarkable feature in relation to their long lasting advantage in traditional medicine practices, and continues to be a major source of lead compounds for drugs development (Newman & Cragg, 2016).

According to World Health Organization (WHO), traditional medicine is defined as the sum total of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve, or treat physical and mental illnesses (WHO, 2013). The application of traditional medicine is based on the rich experiences of innumerable healers over centuries, inherited from ancestors, healer-to-healer transfer, or developed through personal experiences over time. Formerly, traditional medication has been ignored by educated and modern societies. Recently, however, understanding the benefit of traditional medication in promoting the health care services, a number of ethno-medicinal as well as ethnobotanical studies are being carried out, which played significant roles in attitudinal changes (Wondimu, Asfaw, & Kelbessa, 2007). Most of the civilized nations have developed their own Materia-Medica, compiling details about various plants used for therapeutic purposes (H. Khan, 2014).

The practice of traditional medicine is well known throughout African, Asia, and Latin America. The traditional medicines such as the traditional Chinese medicine (TCM), Ayurveda (Indian

medicine), Kampo, traditional Korean medicine (TKM), and Unani (Arabian medicine) have been practiced all over the world for hundreds or even thousands of years, and grown into orderly-regulated systems of medicine and then distributed to other part of the world (Yuan et al., 2016). In Africa, although it is not organized as, for example, in India (Ayurveda) and China (TCM), traditional medicine is part of the people's culture (Kassaye, Amberbir, Getachew, & Mussema, 2006). Up to 80% of the population in Africa uses traditional medicine for their primary health care, which is mainly due to high cost of modern drugs, inadequacy and inaccessibility of modern health services, and cultural acceptability of traditional medicine (Alebie, Urga, & Worku, 2017; Vk, Tsegaye, Kebede, Elias, & Gemachu, 2015). Currently, many countries in Africa have a division, department, or task force on traditional medicine, usually attached to their Health Ministries (Mahomoodally, 2013).

In Ethiopia, the use of traditional medicine is widely spread. More than 70% of population of the country use traditional medicine, are more than 95% of the TMs are sourced from plants (Tuasha, Seifu, & Gadisa, 2019). The wide spread of traditional medicine in Ethiopia could be attributed to cultural acceptability of healers and local pharmacopeias, the relatively low cost of traditional medicine and difficult access to modern health facilities (Mahomoodally, 2013). In different localities of the country, it is customary to find traditional medicines in markets where food items and spices are sold (Enawgaw et al., 2013). The conditions that claimed to be treated include gastrointestinal disturbances, respiratory disorders, sexually transmitted infections, tuberculosis, impotency, hemorrhoids, rabies, intestinal parasites, skin problems, liver diseases, mental disorders, hypertension, diabetes, gynecological conditions rheumatism, malaria and others (Kassaye et al., 2006).

Along with consuming plants and their products for nutrition, human being relies on plant remedies to meet its health needs, either as their own 'traditional medicine' or as 'complementary and alternative medicine' (Dar, Shahnawaz, & Qazi, 2017). Out of the approximately 270,000 taxonomically classified terrestrial plants worldwide, about 15% (35,000-70,000 plants extracts) have been screened for their medicinal use (Mcchesney, Venkataraman, & Henri, 2015). In Africa, out of the 6,377 plant species in tropical region, more than 4,000 of them are used as medicinal plants (Rufaro, 2003). Many of the plants reported to show very promising medicinal properties warranting further investigations.

Historical experience of using plants, especially those with ethno-pharmacological uses, have been the primary sources of medicines for early drug discovery. In the early 19th century, the isolation of morphine (**1**) from opium poppy plant *Papaver somniferum*, by German pharmacist Friedrich Serturner, laid a basis for the use of medicinal plants in drug discovery (Joo, 2014; Manoharachary & Nagaraju, 2017; Njagi, 2013). This work paved the way for the discovery of many other active principles including quinine (1820) (**2**) from the Cinchona tree (*Cinchona officinalis*), strychnine (1819) (**3**) from seeds of *Strychnos nuxvomica* L., atropine (1819) (**4**) [racemic mixture of (+) and (-)-hyoscyamine] and hyoscine (**5**) from species of the *Atropa belladonna* L., *Hyoscyamus niger* L., and *Datura stramonium* L, cocaine (1860) (**6**) from *Erythroxylum coca* Lam. (coca leaves), digitoxin (**7**) from *Digitalis purpurea* leaves (Fig. 1), and others, of which some are still in use (Brahmachari, 2012; Halberstein, 2005; Khazir, Mir, & Cowan, 2013).

In the 20th century, additional important drugs were isolated from plants, including digoxin (**8**), artemisinin (**9**), paclitaxel (Taxol) (**10**), vinblastine (**11**), and vincristine (**12**) (Brahmachari, 2012; Halberstein, 2005; Khazir et al., 2013) (Fig. 1). In the same century, the advancements in pharmacology and understanding of human disease at molecular level shifted the physicians and pharmacists from the use of extractives in prescription to pure naturally occurring and synthetically modified natural products, or totally synthetic compounds (Butler, 2004; Kinghorn, 2001; Muller & Krauss, 2005). Around the middle of the 20th century, after World War II (1939–1945), the emphasis on seeking medicines from plants shifted to using microbes due to mass production of penicillin (1942) and many other lifesaving anti-infectives from microbial sources (Phillipson, 2001; Pina, Hussain, & Roque, 2010). However, in the post-World War-II years there were relatively few discoveries of new drugs from higher plants with the notable exception of reserpine (**13**) from the *Rauwolfia* species signaling the age of the tranquillisers and also vinblastine (**11**) and vincristine (**12**) (Fig. 1) from *Catharanthus roseus* which were effective in cancer chemotherapy (Phillipson, 2001).

Despite the successful isolation of new bioactive agents from natural sources, the arrival of successful synthetic clinical drugs, e.g. atenolol (**14**) (β -blocker) and captopril (**15**) (ACE-inhibitor) for treatment of hypertension, salbutamol (**16**) (Fig. 1) (adrenoceptor stimulant) for asthma (Phillipson, 2001) diminished the impact of phytochemistry on new drug development

and inevitably the innovative pharmaceutical industry turned to synthetic chemicals (Mcchesney et al., 2015; Phillipson, 2001). However, once more in the late 20th century, the isolation of three clinical drugs including anti-cancer drug taxol (**10**) (1993) (Newman & Cragg, 2016), from bark extract of Pacific yew tree (*Taxus brevifolia*), anti-breast, lung, and testicular cancers drug etoposide N (**17**) (1990) and antimalarial drug artemisinin (**9**) (Fig. 1) (1985) from *Artemisia annua* switched to the natural product world (Phillipson, 2001). The successful discoveries of bioactive principles denote not only the huge potential of medicinal plants for the production of many more novel drugs as well as the solid link between the folk medicine and drug development but also the necessity of natural products research. Fig. 1 displayed the structures of some natural products isolated from higher plants as clinical agents.

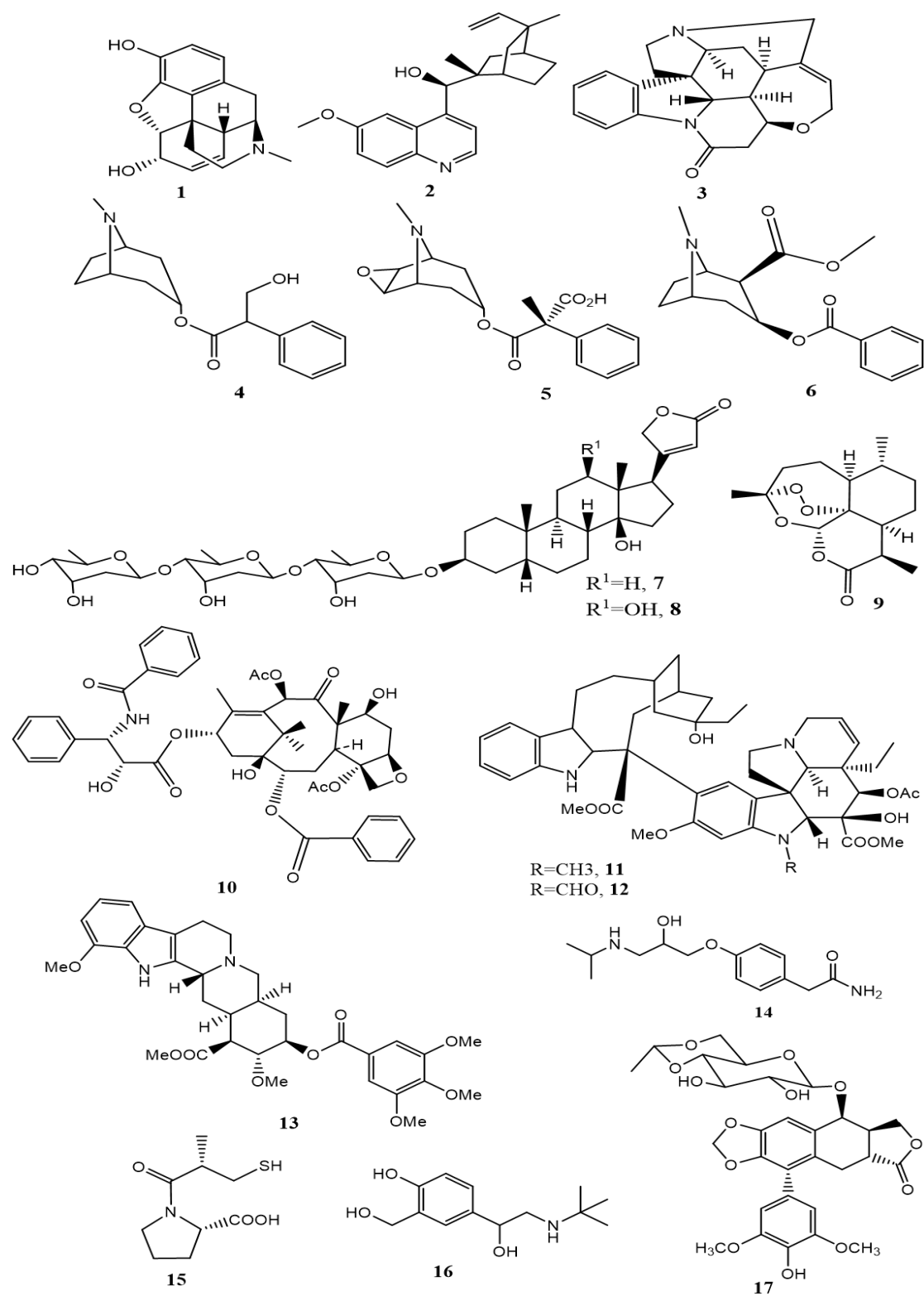


Fig. 1: The structures of some natural products isolated from higher plants as clinical agents.

Nowadays, the majority of significant drugs over the past 50 years have been isolated from plants or derivatives of isolated biochemical. Analysis of food and drug administration (FDA) new-drug approvals from 1981 to 2002 reveals that more than 90% of current therapeutic classes derive from a natural product prototype and interestingly, even today, roughly two-thirds to three-quarters of the world's population relies upon medicinal plants for its primary pharmaceutical (Newman et al., 2003). It is often noted that 25% of all drugs prescribed today come from higher plants (Dar et al., 2017).

Economically, medicinal plants are considerably essential. Due to their wide spread use, global trade in plant based drugs was estimated at US \$ 32-43 billion, which is expected to increase to US \$ 500 billion in the 21st century (Rao, 2016). China is a major source country with India and Chile playing a big role. The annual demand for plant material in China is around 700,000 tones (Bari, Ali, & Miah, 2017; Bukar, Dayom, & Uguru, 2016). The Indian Ayurveda medicine has a 70% share of the formal medicine market in India and it serves the need of more than 600 million people (Rao, 2016). In Africa, following the investments in new drug discovery program, the interest in plant derived allopathic drug increases. The South African medicinal plant market trade in raw material alone worth around R270 million annually (Street & Prinsloo, 2013).

Since decades, infectious diseases constitute a major public health problem all over worldwide (Assob et al., 2011). Antimicrobial resistance (AMR), emergence of new pathogens in addition to the resurgence of old ones, and the lack of effective new therapeutics exacerbate the problems, especially in the developing countries (Vikram et al., 2014; WHO, 2014). For several decades AMR has been a major threat to the effective treatment of an ever-increasing range of infections. According to the 2019 Centers for Disease Control and Prevention (CDC) antimicrobial resistant pathogens caused millions of deaths worldwide (Tamma et al., 2020).

Cancer is also one of the most common causes of death and its incidence is increasing worldwide (Khazaei et al., 2019). Breast cancer is the most frequently diagnosed cancer and the leading cause of death in women worldwide (Dandawate et al. 2016; Lukong et al., 2017). The burden of breast cancer mortality lies in the developing low-income and middle-income countries, where about 70% of such deaths occur (Lukong et al., 2017). In Africa, its burden has been rising steadily in the last three decades (Sharma, 2020). In Ethiopia the incidence of new cases

of breast cancer is currently increasing and has become one of the most common cancer types causing high rates of morbidity and mortality, constituting 33% of the cancers in women and 23% of all cancers in the country (Deressa et al., 2019; Memirie et al., 2018).

Adverse side effects of current treatment strategies and resistance of many microbial strains against existing antibiotics upsurge the need for alternative sources of drugs with effective and large spectra of biological activities. Traditional medicine through use of natural products plays a great role in the treatment of infectious diseases. Ethiopia is one of the top 25 biodiversity-rich countries in the world, and hosts two of the world's 34 biodiversity hotspots, namely the Eastern Afromontane and the Horn of Africa hotspots. It is also among the countries in the Horn of Africa regarded as major center of diversity and endemism for several plant species (Abdala, 2017). Its rich biodiversity makes the countries home for large number of plants with medicinal value to treat human and livestock ailments (Abera, 2014). It is reported that nearly 80% of the population in Ethiopia use plant-based traditional medicine as their primary health care system (Asfaw, 2014; Region & Meressa, 2018).

With respect to the great potential of medicinal plants for drug development, this project is planned to analyze phytochemical composition and examine the biological activities of selected medicinal plants collected from different parts of Ethiopia.

1.2. Statement of the Problem

Medicinal plants have been known as rich source of therapeutic agents for the treatment and prevention of various diseases including infectious and cancer related diseases since the ancient days of humankind (Dar et al., 2017; Halberstein, 2005; Sur et al., 2014). Infectious diseases are a significant cause of morbidity and mortality worldwide, accounting for approximately 50% of all deaths in tropical countries (Becker et al., 2006; Fonkwo, 2008). Although there has been an increase in the availability of novel antibiotics to combat human health problems in recent years (Tamma et al., 2020), the rapid development of AMR to the existing drugs is considered to be a challenging event in the treatment of several infectious diseases (Becker et al., 2006; Fonkwo, 2008; Tamma et al., 2020). AMR is estimated to account for more than 700,000 deaths per year worldwide, which will , if no appropriate measures are taken to cease its progress, will cost approximately 10 million lives per year by 2050 (Tadesse et al., 2017). In addition to the

challenges caused by AMR, the side effects associated with the existing antibiotics, the emergence of new pathogens in addition to the resurgence of old ones, and changes in genetic make-up of pathogens (Rani, Sharma, Chaturvedi, & Parkash Yadav, 2017), created immense clinical problems in the treatment of the spread of infectious diseases.

Cancer is also among the leading causes of morbidity and mortality in the globe. Breast cancer is the primary cause of cancer deaths in women in the globe, and is prevalent in developing nations, where about 70% of such deaths occur (Lukong et al., 2017b). Recent studies have shown that the incidence of breast cancer has significantly increased. According to the GLOBOCAN report, estimate 627,000 women died from breast cancer in 2018, accounting for 15% of all cancer-related deaths among women (GLOBOCAN, 2018). In Ethiopia, according to the Federal Ministry of health National Technical Advisory Group on Non-Communicable Diseases, breast cancer in women have been cited as important emerging public health problems (FDRE Health Minister, 2016). Despite the achievements in treating various diseases in recent decades, drug resistance to existing and/or novel drugs, adverse effects with existing antibiotics and their high cost, accessibility continue to pose a major problem in human health and contribute significantly to human mortality (Md.Shamsuzzaman & Hasan, 2019; X. Wang, Zhang, & Chen, 2019). This necessitates the search for new, safe, and low-cost drug candidates.

The use of plants and plant-based products for healing human diseases is an old experience and practice, and potentially plays a pivotal role in drug discovery (Khazir et al., 2013). The discovery of modern drugs such as (2), (7), (8), (9) and (12) (Fig. 1), from medicinal plants signifies the huge potential that still exists for the production of many more novel pharmaceuticals (Newman et al., 2003). In the areas of cancer and infectious disease, 60% and 75% of new drugs, respectively, originated from natural sources between 1981 and 2002 (Lam, 2007; Newman et al., 2003). In spite of numerous past successes in the development of plant-derived drug products, it has been estimated that only 5 to 15% of the existing species of higher plants have been systematically surveyed for the presence of biologically active compounds (Dias et al., 2012).

The Ethiopian flora is estimated to contain between 6,500 and 7,000 species of higher plants of which about 12% are endemic (Tuasha et al., 2018b). Many plant species are being used as medicine to treat various human and animal diseases (N. Mekonnen & Abebe, 2017). In contrast

to its broader utilization in the official health care delivery system, the medicinal flora of Ethiopia still remains virtually unexplored well from the point of view of its phytochemical constituent and biological/pharmacological activity.

Therefore, the medicinal plants that have been traditionally recommended to treat various infectious diseases and cancer in Ethiopia require specific evidence to support their claimed medical advantages. In this study the medicinal plants *C. cyphopetalum*, *H. johannis*, *C. tomentosa*, *K. petitiانا*, and *C. rotundifolia* are investigated for their phytochemical constituents and biological activities.

1.3. Justification of the Study

Africa is known for its large diversity of medicinal plants for the treatment of cancer and infectious diseases (Lukong et al., 2017b). Ethiopia is among the countries in the Horn of Africa regarded as major center of diversity, of which nearly 12% are endemic, for several plant species (Abdala, 2017). Its rich biodiversity makes the country home for large number of plants with medicinal value to treat human and livestock ailments (Abera, 2014). In the traditional medicinal system of Ethiopia, *C. cyphopetalum*, *H. johannis*, and *K. petitiانا* are among medicinal plant used to treat cancer and related diseases (Ayele, 2018; Belayneh & Bussa, 2014; Bussa & Belayneh, 2019; Kebede, Afework, Debella, Ergete, & Makonnen, 2016; Kefalew, Asfaw, & Kelbessa, 2015; Teklehaymanot & Giday, 2007). Likewise, *C. rotundifolia* and *C. tomentosa* are mentioned as among the dominant medicinal plants used to treat varies type of diseases in Ethiopia, and cancer in other in East African countries (Ayele, 2018; Kefalew et al., 2015; Teklehaymanot & Giday, 2007). Thus, there was a need to investigate these medicinal plants in order to understand better their chemical composition and biological activities.

1.4. Objectives

1.4.1. General objective

The overall objective is to study the phytochemical constituents, and evaluate *in vitro* biological activities of different parts of selected traditional medicinal plants and study their molecular docking against selected protein targets.

1.4.2. Specific objectives

- To successively extract the roots of *C. cyphoptealum*, roots of *H. johannis*, stem bark of *C. tomentosa*, leaves of *K. petitiانا*, and *C. rotundifolia* using *n*-hexane, dichloromethane/methanol (DCM/MeOH) (1:1), and methanol (MeOH).
- To extract essential oils from leaves extract of *C. rotundifolia*, *C. tomentosa* and *K. petitiانا* using hydrodistillation
- To analyze the composition of essential oils from leaves of *C. rotundifolia*, *C. tomentosa* and *K. petitiانا* using GC-MS.
- To isolate compounds from the roots of *C. cyphoptealum*, roots of *H. johannis*, stem bark of *C. tomentosa*, leaves of *K. petitiانا*, and *C. rotundifolia*
- To elucidate the structures of isolated compounds using spectroscopic methods (UV-Vis, IR, ¹H NMR, ¹³C NMR, DEPT-135, COSY, HMBC, HSQC, NOESY) and mass spectrometer.
- To evaluate the antibacterial activity of compounds isolated from root *C. cyphoptealum*, extracts and isolated compounds from root of *H. johannis* using agar disk diffusion against *E. coli*, *P.aeruginosa*, *S. aureus* and *S. pyogen*.
- To evaluate the antifungal activity of *n*-hexane, DCM/MeOH and MeOH root extracts of *C. cyphopetalum* using agar disk diffusion against *C. albicans*.
- To test cytotoxicity of DCM/MeOH and MeOH root extract of *C. cyphopetalum*, and *n*-hexane, DCM/MeOH and MeOH root extracts of *H. johannis*, and DCM/MeOH and MeOH of *C. tomentosa* using MTT assay.
- To evaluate the antioxidant activity of compounds isolated from *C. cyphopetalum* and *H. johannis* using DPPH radical scavenging assay.
- To predict the interaction of isolated compounds with the binding sites of DNA-ylrase, PqsA, *S. aurues* PK using the *in silico* molecular docking study.
- To predict the drug likeness and ADMET properties of isolated compounds.
- To describe the DFT of compounds isolated from roots of *C. cyphopetalum*.

1.5. Significance of the Study

In the effort to determine the biological activity and the search for active phytochemicals with medicinal values, there is a need for further research to be done on traditional medicinal plants.

The study outcome is anticipated to give secondary metabolite data from the studied medicinal plants, which will provide standard information about the chemical constituents and biological activity of various parts of the aforementioned plants. The computational analysis of the isolated compounds expected to provide scientific data to have a better understanding of the molecular mechanisms of interaction between the compounds with protein domains of selected bacterial strains. Provide a significant input in bridging the gap between modern medicine and traditional medicine.

2. REVIEW OF LITERATURE

2.1. Ethnobotanical uses of medicinal plants selected for this study

Ethnobotany is the scientific investigations involves an interdisciplinary approach encompassing the fields of botany, chemistry, pharmacology and anthropology (Limenih, Umer, & Wolde-mariam, 2015). Ethnobotanical studies are significant in revealing locally important plant species in relation to the discovery of crude drugs. Right from its beginning, the documentation of traditional knowledge, especially on the medicinal uses of plants, has provided many important up-date drugs (Teklehaymanot & Giday, 2007). In this section, the ethnobotanical uses of *C. cyphopetalum*, *H. johannis*, *C. tomentosa*, *C. rotundifolia*, and *K. petitenia* plants selected are described.

2.1.1. *Cyphostemma cyphopetalum* (Fresen.) Wild & Drummond

The genus *Cyphostemma* belongs to the family Vitaceae, with around 250 species which are widely distributed throughout Africa. The majority of species are erect, prostrate or climbing perennial herbs or shrubs, with or without tendrils (Sousa et al., 2011).

Cyphostemma cyphopetalum (Fresen.) Wild & Drummond (Syn: *Cissus cyphopetala*; *Cissus cyphopetala*; *Cyphostemma gallaense*; *Cissus javellensis*; *Cissus neghelliensis*; *Cissus digitata*), belongs to family Vitaceae, known by the vernacular name Milas Golgul (Am) (Demissew, 2019) Gindosh (Am) (Teklehaymanot, Giday, Medhin, & Mekonnen, 2007). Herbaceous climber with young stem, scrambling or trailing plant up to 4-6 m long. Grow at edges of evergreen forest, upland bush-land and scrub, riverine forest, Juniperus forest and scrub, *Acacia-Commiphora*, *Acacia-Combretum* and *Acacia-Balanites* woodland, wooded grassland and bush-land, persisting in cultivated areas, roadside; 250-2800 m. Occurs in most

Ethiopian regions, Cameroon, Sudan, Uganda, Kenya, Tanzania, Rwanda, E. Congo and Zambia (Dagne, 2016). In Ethiopia, *C. cyphopetalum* is distributed in different parts including Harerge, Afar, Ilubabor, Bale, Arsi, Welo, Shewa, Tigray, Gonder, Gojam, Welega, Sidamo, and Gamo gofa (Ayalew, Bekele, & Demissew, 2006). Fig. 2 shows the areal part of *C. cyphopetalum*.



Fig. 2: Aerial part of *C. cyphopetalum* (Photo by Teshome D, Sept 2019, Huruta)

Ethnobotanical studies indicated that most of the plant species which belongs to the genus *Cyphostemma* including: *C. cyphopetalum* (Yohannis et al., 2018), *C. adenocaulum* (Kefalew et al., 2015), *C. digitatum* (R. Khan et al., 2015), *C. junceum* (Teklehaymanot & Giday, 2007) and *C. vogelii* (Udegbumam & Anosa, 2013) are used in traditional medicine. According to reports, the root bark of *C. junceum* is crushed and is eaten with honey to treat a snake bite, the plant's whole part is applied for a toothache and spider bite through chewing and swallowing the juice, and the leaf is used for the evil eye by placing it on fire for fumigation (Teklay et al., 2013). Another study showed that the root of *C. junceum* is effective in the treatment of snake bite when the crushed fresh root is taken orally (Teklehaymanot & Giday, 2007).

According to the ethnobotanical report (Yohannis et al., 2018), the root of *C. cyphopetalum* is used to treat cancer by the local people in Menz Gera Midir District, North Shewa Zone of Ethiopia. In the study reported (Teklehaymanot et al., 2007), people around the monastery of Debre Libanos in Ethiopia, use the stem of *C. cyphopetalum* for the treatment of snake bite via chewing, the infusion of roots for rabies treatment through oral administration. The fresh root of *C. cyphopetalum* has been also reported to treat diarrhea, cough in horse and donkey, and inability to walk properly by crushing and drinking by the people in Southeastern Ethiopia (Mana Angetu District) (Lulekal et al., 2008). According to the study report (Chekole, 2017), the leaf of *C. cyphopetalum* is used to treat eczema (Atopic dermatitis) by the local community around Gubalafto (the plant is locally called Abawoldu).

In Kenya, the crushed and water added resultant concoction of tubers of *C. cyphopetalum* are used to eradicate all insects (Kipkore, Wanjohi, Rono, & Kigen, 2014). When used to kill household insects like mites, the concoction is left standing in the room for one day and after that, the room left for at least two days before occupation since the plant is very poisonous. It is also employed as a poison to kill unwanted animals. Decades ago studies in Kenya reported the used of leaves of *C. cyphopetalum* treat skin eruption, chicken-pox, dermatitis, enlarged glands and adenopathy by pounding the leaves at the site of local application (Kokwaro, 1976 & 1987).

2.1.2. *Hydnora johannis*

The Hydnoraceae are the only known angiosperm with no leaves or scales of any sort (Musselman & Visser, 1989; S. Yagi et al., 2012). *Hydnora* is a genus with eight species (Thorogood, 2019). The species of *Hydnora* are all chiefly hypogeous, root-parasitic herbs, with an extremely reduced vegetative body, which has no leaves or bracts and consists of a massive, fleshy horizontal rhizome like root covered with haustorial roots as wart like outgrowth (Al-Fatimi1 et al., 2016; Musselman & Visser, 1989). *Hydnora* is a genus of parasitic plants which occur in the semi-arid regions of Africa, Madagascar and southern Arabia (Thorogood, 2019). An extreme reduction in morphological features, including the complete lack of leaves, has led to *Hydnora*'s reputation as "the strangest plant in the world."

Hydnora johannis Becc. (Syn: *Hydnora abyssinica*; *Hydnora africana*). (Hydnoraceae) is identified by vernacular name Licce, Like, Likke, Lipti (af somali), DechMerech (Afan Oromo) (Bussa & Belayneh, 2019; Girma, Mulisa, & Amelo, 2018). The species is a subterranean

holoparasitic (non-photosynthetic) herb with often massive root systems spreading laterally from the host. It is an obligate root parasite found on *Acacia* and *Albizia* species as well as *Kigelia Africana*; growing between 1000– 1500 m altitudes (Thorogood, 2019). In Ethiopia, the species occurs in Shewa, Sidamo, Bale and Hararge. It has also observed occurring in Eritrea, Somalia, Kenya, Tanzania, Rwanda and Democratic republic of Congo. The species is widely distributed but seldom collected (Kitaba, 2006; Musselman & Visser, 1989). *H. johannis* protrudes a few cm through its apex from the ground surface, cylindrical in body shape and reddish brown in color. Fig. 3 shows the root and vegetative part of *H. johannis*.



Fig. 3: Photo of *H. johannis* (photo by Teshome D, Sept 2021, Huruta)

Traditionally, the plant is used as medicine for both human and livestock. The root is consumed to increase appetite. The root is also taken with honey for lung cancer, breast cancer and intestinal diseases. Swollen breast of cow will get relieved if it is washed with soil taken nearby the root of *H. johannis*. In Sudan, decoction of the roots of *H. johannis* is traditionally used for the treatment of dysentery, diarrhea, cholera and swelling tonsillitis (Sakina Yagi et al., 2013). Macerated root of *H. johannis* is used to treat cholera, diarrhea, dysentery and evil eye in the Blue Nile State, South-eastern Sudan (Musa et al., 2011). Likewise, in Ethiopia also the plant reported to possesses various ethno-medicinal uses. In Harari region, Eastern Ethiopia, the study

on ethno-medicine claims directed in *silico* prediction of anticancer activity disclosed that the crushed root of *H. johannis*, boiled with water containing exocarp/peel of coffee beans and taken orally for swollen breast and abdomen (Girma et al., 2018). Furthermore, *H. johannis* is reported to be one of the most valued traditional medicinal plants for the treatment of cancer, tumors, and inflammatory ailments by the traditional healers and local communities in Harari Region, Eastern Ethiopia (Bussa & Belayneh, 2019). The bark and root is grounded with *Echinops kebericho* tuber and put in the daily food as well as drink a solution for internal cancer. Decoction of the root is taken orally for external body swelling treatment. (Bussa & Belayneh, 2019). Root preparations from *H. johannis* were also prescribed as remedy for diarrhea, hemorrhage, wound and painful body swelling, locally called *GOFLA* (Oromo language) around Erer Valley of Babile Wereda, Eastern Ethiopia (Belayneh, Asfaw, Demissew, & Bussa, 2012).

2.1.3. *Capparis tomentosa*

Capparis tomentosa Lam. (Syn: *Capparis polymorpha*; *Capparis volkensii*; *Capparis corymbosa*), is a shrub which belongs to the family Capparidaceae (Akoto et al., 2008). In Ethiopia, this plant species is known by local name Gmero, Gimero (Amh). It is a small spiny tree or scrambling shrub found in tropical or other warm regions and sometimes can develop into a tree that can grow as high as 10 m tall. Leaves are elliptic, elliptic-oblong or ovate, 26–70 mm long and 13–80 mm wide, with tips usually round with a sharp point, light green to grayish green, soft and velvety. Hooked and sharp spines are found in the axils of the leaves. often grows in riverine forest or at temporary ponds at 500-2200 m altitudes (Orwa et al., 2009).

Flowers are fragrant with pale yellowish green petals, that surround a tuft of white or pink stamens, that grow up to 30 mm long. The flowers develop into red, globose, pendulous fruits that grow up to 50 mm in diameter. Seeds are dark brown, ovoid to kidney shaped and 1-15 mm long. The seeds are surrounded by a juicy, white to pinkish pulp. The plants flower in spring and early summer, from August to November. Flowers open in the morning and start to fade in the middle of the day. Fruits are produced in summer, from December to March. The leaves are stated to be poisonous or even lethal when eaten (Aweke, 2013; Bekele-Tesemma, 2007; Orwa et al., 2009). Fig.4. demonstrates the pictorial representation of the areal part of *C. tomentosa*.



Fig. 4: Aerial part of *C. tomentosa* (Photo by Teshome D, Sept 2021, Adama)

Capparis has been considered to comprise approximately 250 species and occur in all tropical and subtropical regions. *C. tomentosa*, which belongs to the genus *Capparis*, is well known in Botswana, Eritrea, Ethiopia, Lesotho, Namibia, South Africa, Swaziland (Orwa et al., 2009). In South Africa the plant is an important medicinal plant in many indigenous communities including for treatment of sexual and reproductive health problems. They are used to cure HIV opportunistic infections, such as tuberculosis, herpes zoster and herpes simplex and chronic diarrhea (Chinsebu et al., 2018). They are also used as an infusion to assist with epilepsy, sterility, threatened abortion, gonorrhea, syphilis and also to stop bleeding after childbirth (Steenkamp, 2003). Other plant parts also play an important role in traditional medicine. Decoctions of the roots, leaves and bark can be used to treat coughs, fever, and asthma. The decocted mixture of the root of *C. tomentosa* with roots of *Cyperus* species taken orally as aphrodisiac medicinal plant in sub-Saharan Africa (Ajao et al., 2018). Treatment should be taken in small quantities, as large doses can cause toxicity to the patient. Powdered roots can also be applied to wounds, abscesses and snakebites. The smoke inhaled from burning powdered bark, can be used for bronchitis, chest pains and headaches (Semenya & Maroyi, 2018). Decoction of the leaves is used for the treatment of asthma; a decoction prepared by scraping the bark and mixing it with goat soup is drunk for chest pains. Decoction of the root is a cough remedy, but it must be used with care, as it is highly poisonous when taken in large quantities (Orwa et al.,

2009). In Mozambique the entire plant part is used for treatment of cancer (Graham, Quinn, Fabricant, & Farnsworth, 2000). In Kitale, Kenya, the roots of *C. tomentosa* are boiled in water and taken as medicinal herb for management of various conditions such as diabetes mellitus, goiter, high blood pressure, boosting of CD4 count for HIV⁺ patients (Wangai et al., 2015).

Similarly, the ethnobotanical studies in Ethiopia explored that different part of the plant *C. tomentosa* played a crucial role in the folk medication to treat various ailments. The root of the plant is utilized in the medication of epilepsy, bleeding after delivery (Teklehaymanot & Giday, 2007), tooth ache, wound (Kefalew et al., 2015; Wondimu et al., 2007). The fruits are eaten in Ilubabor and Gambella (Bekele, 2007). Root of *C. tomentosa* is dried, powdered, and mixed with water to treat intestinal worm; three spoons are given per day for three days (AMENU, 2007). Around Dengego valleys, Eastern Ethiopia, the root and leaf concoction is taken orally to facilitate opening of nipple pores (Belayneh & Bussa, 2014). In different modes, the root and leaf of this plant are used to manage various mental illnesses in different part of Ethiopia (Wubetu, Sintayehu, Aeta, Reta, & Derebe, 2018). Inhaling of the smoke of mixed powder of root is utilized to treat malaria, Mich and evil eye by the community around Debre Libanos monastery in Ethiopia (Teklehaymanot et al., 2007). Generally, the ethnobotanical surveys conducted at different part of Ethiopia indicate *C. tomentosa* is an important medicinal plant in many indigenous communities in different parts of the country.

2.1.4. *Kalanchoe petitenae*

The genus *Kalanchoe* includes 139 species indigenous to Madagascar, southern and eastern Africa, and, to a lesser extent, tropical Africa, the Arabian Peninsula, and southeastern Asia. *Kalanchoe* spp. are generally amenable to cultivation and propagate readily from stem and leaf cuttings or plantlets produced along leaf margins (Currey & Erwin, 2011). *Kalanchoe* is a genus belonging to family Crassulaceae; closely related to Saxifragaceae from which it differs in the regular numerical plan, almost constantly separate ovaries and predominately fleshy habit (Kuma & Shibru, 2015). *Kalanchoe* spp. have ethnobotanical uses wherever they are found, sometimes being called “miracle leaf” for their use in treating various ailments (Richwagen, Lyles, Dale, & Quave, 2019).

Kalanchoe petitiana A. Rich, is a succulent species that belongs to family Crassulacea, is endemic to Ethiopia which is known by the local name Yeqolla Endahulla; Yeqola Endahula; Yekola Endahula (Amh) (Abebe, 2016). Amdohale, Bosoqe, Bosoke (Orm) (Kefalew et al., 2015). It is a drought resistant perennial herb, forming dense stands. This plant is disliked by domestic animals. There are two varieties of this species. *Var. petitiana*, habitat: Forest margins and open evergreen bush land, often in disturbed areas; 2000-3000 m altitudes. Found in most Ethiopian regions and western Eritrea is not known elsewhere (i.e. endemic to Ethiopia and Eritrea). *Var. neumannii*, habitat: Montane forest margins sometimes in rather wet sites; 2400-3000 m, occurs in most Ethiopian regions, not known elsewhere, endemic to Ethiopia (Dagne, 2016). Fig. 5 shows the aerial part of *K. petitiana*.



Fig. 5: Aerial part of *K. petitiana* (Photo by Teshome D, Nov 2021, Huruta)

In Ethiopian folk medication system, different parts of *K. petitiana* are used for curing a number of ailments such as breast tumor, skin cancer, epilepsy, trachoma, allergy, tonsils, intestinal parasites, gonorrhea, lymphadenopathy (Ebach), malignant wounds, rheumatic pain, bone fracture (Abebe, 2016; Ayele, 2018; Esubalew et al., 2017), at different part of the country. The leaves of *K. petitiana* are utilized to treat trachoma, syphilis and different swellings and to expel tapeworm around Entoto, Addis Ababa (Tadeg, Mohammed, Asres, & Gebre-Mariam, 2005), lymphadenopathy (Ebach) around Debre Libanos monastery in Ethiopia (Teklehaymanot et al., 2007). Around Ada'a District, East Shao of Ethiopia, the leaf is warmed up on a fire and paste on the swelling to treat both human and animal swelling (Kefalew et al., 2015). The boiled leaf

is administered orally to treat tonsil and dermally applied to treat wound by the traditional practitioners around Robe Town, Bale Zone, Oromia Region, Southeast Ethiopia (Birhanu & Ayalew, 2018). The fleshy leaves are used in baking local bread, Kocho (Qocho) or Bulla in dressing for wound sores. More uses of *Kalanchoe petitiiana* in Ethiopia include treating abdominal dropsy (Dagne, 2016). Around Dalle District, Sidama Zone, Ethiopia, oozed fluid from the leaf is spilled into the eyes to treat inflammation (Tuasha et al., 2018).

2.1.5. *Cadaba rotundifolia*

Cadaba rotundifolia Forssk. (Capparidaceae), known by the local name Goolf (amharic), Delensisa (afan oromo) (Belayneh & Bussa, 2014; Teklehaymanot & Giday, 2010). It is small shrub, up to 2-4 m, evergreen, grows in low-altitude bushland and semi-desert scrub, Riverine formations, stony ground or on sandy-loamy soil, sometimes growing in the shade of trees; 500-1200 m altitudes (Dagne, 2016). It is a much-branched shrub with young twigs densely covered with small glandular hairs, strongly scented. Grey-brown bark, smooth when young. Pale green leaves, wide oval to round, leathery, base rounded to heart shaped and a grooved stem. Yellow or white flowers (no petals) with deep yellow nectars, fading pink, in dense short heads. A cylindrical capsule fruit, green at first then becoming darker and covered with tiny rough hairs, splitting longitudinally from the tip to reveal a bright orange-red lining and dark brown seeds (PROTA4U, 2019). *C. rotundifolia* grows in most Ethiopian regions, and Sudan, Somalia, Socotra, Saudi Arabia and northern Kenya (Dagne, 2016). Fig. 6. Demonstrates the areal part of *Cadaba rotundifolia*.



Fig. 6: Areal part of *C. rotundifolia* (Photo by Teshome D, Sept 2021, Dire Dawa).

Ethnobotanical studies showed that various parts *C. rotundifolia* are traditionally utilized for medication of various ailments which include menstruation/Menometrorrhagia, external injury, wounds, skin infection, arthritis, eye sickness, tonsillitis, flue/Mitch, retained placenta, broken head, snake bite, livestock ailments, external parasite, brucellosis for livestock and malaria among others. The leaf and root extracts of *C. rotundifolia* traditionally prescribed for tumors and abscesses treatment in Sudan (Graham et al., 2000). Traditional healers in the region of Randa, Djibouti, reported to prescribe this plant as an antibiotic after soaking crushed fresh leaves in water for 12 h, and the water is taken orally (Hassan-Abdallah et al., 2013). Similarly, in the traditional medication practices of Ethiopian, *C. rotundifolia* are used to treat various ailments. In Easter Ethiopia, concoction and then filtration of the bark or leaf of the plant together with *Withania somnifera* is taken orally to treat extended flow of menstruation/Menometrorrhagia (Belayneh & Bussa, 2014). The leaf/young branch of the plant is used for treatment of external injury, wounds, skin infection (Teklehaymanot & Giday, 2010). Various parts of *C. rotundifolia* is utilized for the treatment of human ailments such as arthritis (stem), eye sickness (leaf), tonsillitis (leaf), flue/Mitch (leaf), retained placenta (root), broken head (leaf), snake bite (leaf); and livestock ailments retained placenta, external parasite, brucellosis for livestock, in Yalo Woreda of Afar region, Ethiopia (Teklehaymanot, 2017). The Cold water infusion of leaf of *C.*

rotundifolia is also orally administered for the treatment of malaria around Awash Fentale District of the Afar Region of Ethiopia (Alelign, Giday, Teklehaymanot, & Animut, 2018). Around Awash National Park, Ethiopia, the crushed, boiled and filtered fresh leaves of *C. rotundifolia* is utilized for Common cold treatment (Mengesha, 2016). Table 1 below shows the summarized ethnobotanical information of the studied medicinal plants

Table 1 Summary of the ethnobotanical information (habit, part used, disease treated) of the medicinal plant selected for this study.

S. No	Species of plant/Family	Hb	Disease treated	PU	Country	Reference
1	<i>Cyphostemma cyphopetalum</i> (Vitaceae)	Cl	• Cancer (Menz gera) • Snake bite, rabies (Debre libanos), Eczema, Diarrhea, cough in horse (south)	R S	Ethiopia	(Chekole, 2017; Lulekal et al., 2008; Yohannis et al., 2018)
			• Insecticide, kin eruption, chicken-pox, dermatitis, enlarged glands and adenopathy	R, L	Kenya	(Kipkore et al., 2014; Lewis, Elvin-Lewis, & Gnerre, 1987)
2	<i>Hydnora johannis</i> (Hydnoraceae)	P	• Swollen abdomen, lung cancer, breast cancer, intestinal diseases, diarrhea, hemorrhoid, external body swelling, internal cancer	R	Ethiopia	(Belayneh et al., 2012; Bussa & Belayneh, 2019; Girma et al., 2018).
			• Dysentery, diarrhea, cholera and swelling tonsillitis		Sudan	(Musa et al., 2011; Sakina Yagi et al., 2013)
			• Diabetics		Iran	(Sakina Yagi et al., 2013)
3	<i>Capparis tometosa</i> (Capparidaceae)	Sh	• HIV opportunistic infections (Tuberculosis, herpes zoster and herpes simplex and chronic diarrhea), asthma, fever,	R	S. Africa, India	(Ajao et al., 2018; Chinsebu et al., 2018; Semenya & Maroyi, 2018; Steenkamp, 2003)
			• Epilepsy, bleeding after delivery, tooth ache, wound, mental illness, facilitate opening of nipple pores	R L	Ethiopia	(AMENU, 2007; Belayneh &

						Bussa, 2014; Giday, Teklehaymanot, Animut, & Mekonnen, 2007; Wubetu et al., 2018)
			• Treatment of Cancer	Wp	Mozambique	(Graham et al., 2000).
			• Diabetes mellitus, goiter,	R	Kenya	(Wangai et al., 2015)
4	<i>Kalnchoe petitiانا</i> (Crassulaceae):	H	• Breast tumor, skin cancer, epilepsy, trachoma, allergy, tonsils, intestinal parasites, gonorrhea, lymphadenopathy (Ebach), malignant wounds, rheumatic pain, bone fracture	L	Ethiopia	(Ayele, 2018; Esubalew et al., 2017; Kefalew et al., 2015; Tuasha et al., 2018a, 2018b)
5	<i>Cadaba rotundifolia</i> (Capparidaceae)	Sh	• Extended flow of menstruation/ Menometrorrhagia, wounds, skin infection, arthritis, brucellosis for livestock	B L R St	Ethiopia	(Aleign et al., 2018; Teklehaymanot, 2017)
			• Tumors and abscesses,	L,	Sudan	(Graham et al., 2000)
			• Antibiotic (urinary diseases)	R,	Djibouti and Saud Arabia	(Hassan-Abdallah et al., 2013).
			• Stomach ulcer, cancer	L	Yemen	

Note: Hb=habit, PU=part used, Cl=climber, H=herb, P= parasitic, Sh=shrub, B= bark F= flower, L= leaf, St=stem, R=root, Wp=whole part.

2.2. Phytochemistry of the selected medicinal plants

The therapeutic effects of the medicinal plants can justifiably be attributed to, among others, their phytochemicals constituents (Nyamai, Ogola, Njagi, & Ngugi, 2016). In this part the phytochemical constituents of the selected medicinal plants are discussed.

2.2.1. *Cyphostemma cyphopetalum*

Phytochemical reports on the genus *cyphostemma* are limited. Only few compounds isolated from few species in this genus have been reported. According to the report by Chouna, et al., 2016, the chemical investigation of the methanol extract of bark and wood of *C. adenocaulis* led to the isolation of two ceanothane-type lupane derivatives triterpenoids. The chemical study on the acetone extracts of leaves of *C. digitatum*, which is obtained from Sana'a, isolated triterpenes which were identified as Lupeol (**18**), dioxo-urs-12-en-28-oic acid (**19**), and longistylin A (**20**), Piceid (**21**) as a stilbenoid glucoside (Mahweety, 2016). Fig. 7 depicts the structure of compounds identified from *C. digitatum*.

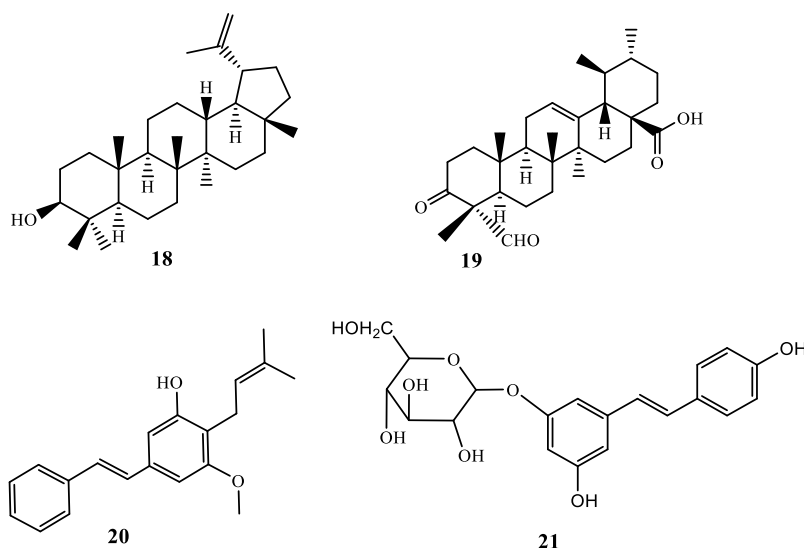


Fig. 7: Compounds reported from *C. digitatum*.

As reported in literature report (Cao et al., 2011), bioassay-guided fractionation of the crude ethanol extract from the bark of *Cyphostemma greveana* provided one macrolide, lasiodiplodin (**22**), three sesquiterpenoids, 12-hydroxy-15-oxoselina-4,11-diene (**23**), 1b,6a-dihydroxyeudesm-4(15)-ene (**24**), and (7R*)-opposit-4(15)-ene-1b,7-diol (**25**), and a

diterpenoid, 16,18-dihydroxykolavenic acid lactone (**26**). Fig 8 depicts the structure of macroline and terpene isolated from *C. greveana*.

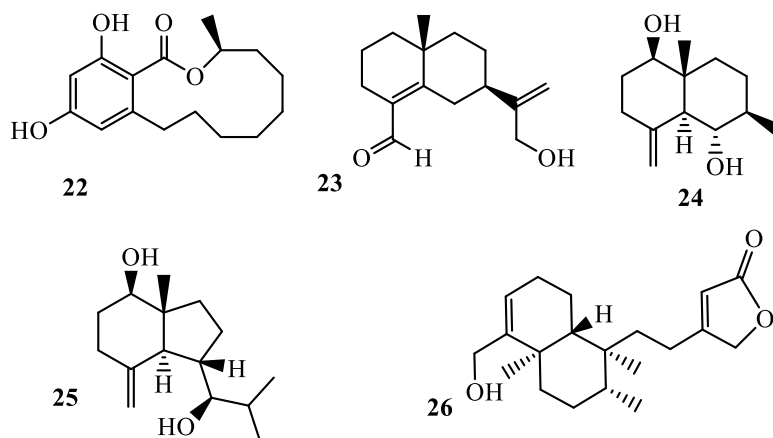


Fig. 8: The structures of compounds reported from bark of *C. greveana*.

Six antifungal constituents were isolated from the roots of *C. crotalarioides* (Vitaceae): a new product, *cis*- ϵ -viniferin (**27**) along with five known compounds: *trans*-resveratrol (**28**) and its oligomers: *trans*- ϵ -viniferin (**29**), gnetin C (**30**), gnetin E (**31**) and pallidol (**32**) (A. Bala et al., 2000). Together with the aforementioned compounds (**28-31**), three new dehydrodimers, cyphostemmins A (**33**) and B (**34**), have also been isolated from the roots of *C. crotalarioides* (A. E. Bala et al., 1999). Fig. 9 shows the structure of oligostelbines from root extract of *C. crotalarioids*.

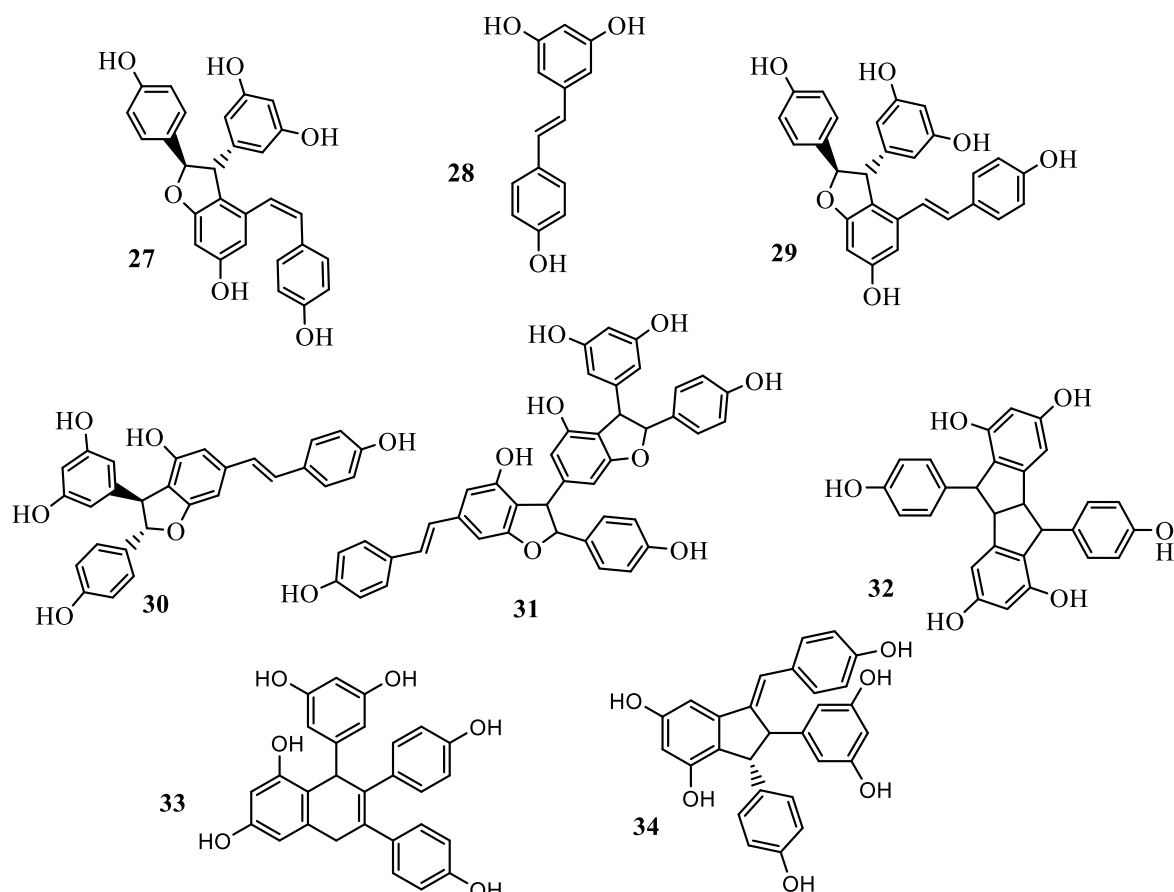


Fig. 9: The structures of compounds reported from *C. crotalarioides*.

2.2.2. *Hydnora johannis*

The species of *Hydnora* are all chiefly hypogaeous, root-parasitic herbs, with an extremely reduced vegetative body, which has no leaves or bracts and consists of a massive, fleshy horizontal rhizome like root covered with haustorial roots as wart like outgrowth (Thorogood, 2019). Phytochemical screening of the different extracts of *H. abyssinica* flowers revealed the presence of phenols like flavonoids and tannins in the polar extracts (ethyl acetate, ethanol and methanol), while the nonpolar *n*-hexane and dichlormethane extracts contained lipids, triglycerides, monoterpenes, essential oils and fixed oils (Al-Fatimi1 et al., 2016; Saadabi & Ayoub, 2009). Phytochemical analysis of the extracts of *H. africana* revealed the presence of alkaloids, saponins, tannins, flavonoids and steroids. These phytochemical compounds are known to be biologically active and thus aid the antimicrobial activities of plants (Nethathe & Ndip, 2011).

H. johannis, one of the species of *Hydnora*, is reported to be a phenolic-rich plant, where the water and ethanol (70%) extracts showed to contain high amount of total phenols (about 662 mg/g. in water and ethanol extract), proanthocyanidins (about 44.60 mg/g in water and ethanol extract) and flavonoids (25.75 mg/g in water extract and 31.82 mg/g in ethanol extract) (S. Yagi et al., 2012). By the same study, six compounds namely cirsiolol (3',4',5-trihydroxy-6-7-dimethoxy-flavone) (**35**), *trans* 3'5- dihydroxy-4'7-dimethoxydihydroflavonol (**36**), dl catechin (trans (±)2- (3,4-dihydroxyphenyl-3,4-dihydro-2H-1-benzopyran-3,5,7- triol)) (**37**), vanillin (4-hydroxy-3-methoxybenzaldehyde) (**38**), protocatechuic acid (3,4 dihydroxy benzoic acid) (**39**), catechin (**40**) and oleic acid (**41**) from the EtOAc fraction. Four compounds have also been identified by GC-MS as oleic acid (**41**), stigmasterol (**42**), myristic acid (**43**), and palmitic acid (**44**). The compounds (**37**) and (**39**), which exhibited *in vitro* anti-glycation and antioxidant properties, have also been reported from the ethanol extracts of the root of *H. johannis* (Asgharpour, Ranjkesh, & Taghi, 2019; Chinchansure, Korwar, Kulkarni, & Joshi, 2015). Fig. 10 shows the structure of the isolated compounds from root extracts of *H. johannis*.

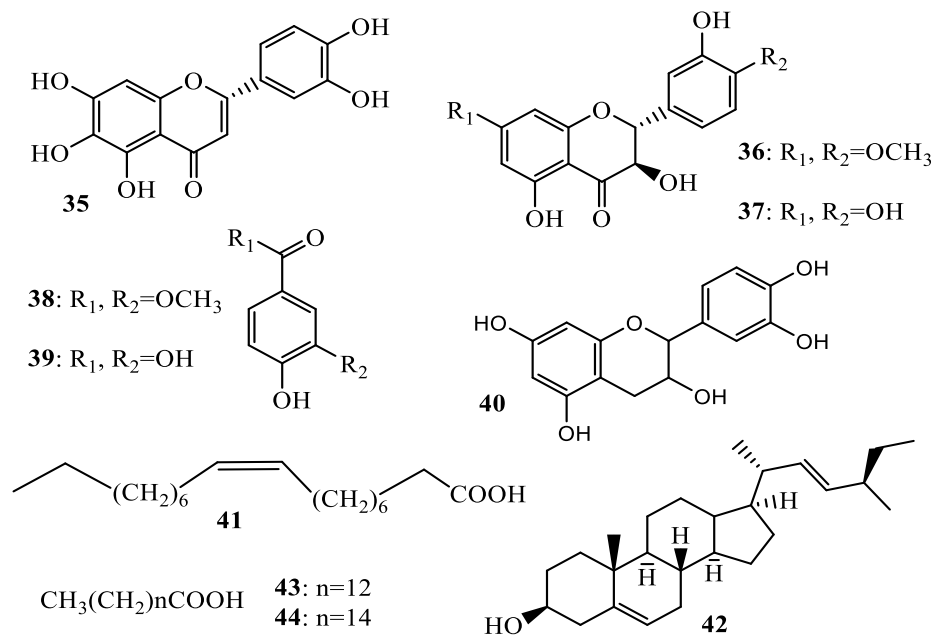


Fig. 10: The structures of compounds reported from *H. johannis* roots.

2.2.3. *Capparis tomentosa*

Capparis species, also known as Caper plants, are recognized as a potential source of valuable nutrients and bioactive compounds with physiological functions. Various chemical compounds have been isolated and determined from different parts of various *Capparis* species. GC-MS analysis of methanol extract of the leaf of one of *Capparis* species, *Capparis diversifolia* revealed the presence of thirty compounds among which the major phyto constituents include 6, 6, 7-trimethyl-9-oxo-3-oxabicyclo (3.3.1) nonane 2, 4-dinitrophenyl hydrazone (15.36%), 2,6-dimethyl-N-(2-methyl-phenylbenzyl) aniline (12.87%), 1, 12-octadecandiol (9.46%) and phytol (9.37%) (Jacintha Tamil Malar A.1*, Sarvana Gandhi A.2, 2016). On the other hand, some important classes of bioactive components such as spermidine, glucosinolate, alkaloids, phenols, glycosides, and flavonoids have been reported from different parts of caper species (*C. spinosa*, *C. ovata*, and *C. decidua*) (Gull et al., 2015; Rathee et al., 2010). This includes isocodonocarpine (**45**), 14-N-acetylcodonocarpine (**46**), capparisidine (**47**), capparidisine (**48**), β -sitosterole (**49**), capparine-A (**50**), guanosine (**51**), rutin (**52**), glucocapparine (**53**), and flazine (**54**) among others (Gull et al., 2015). Fig. 11 below demonstrates chemical structures of some bioactive of *C. diversifolia*.

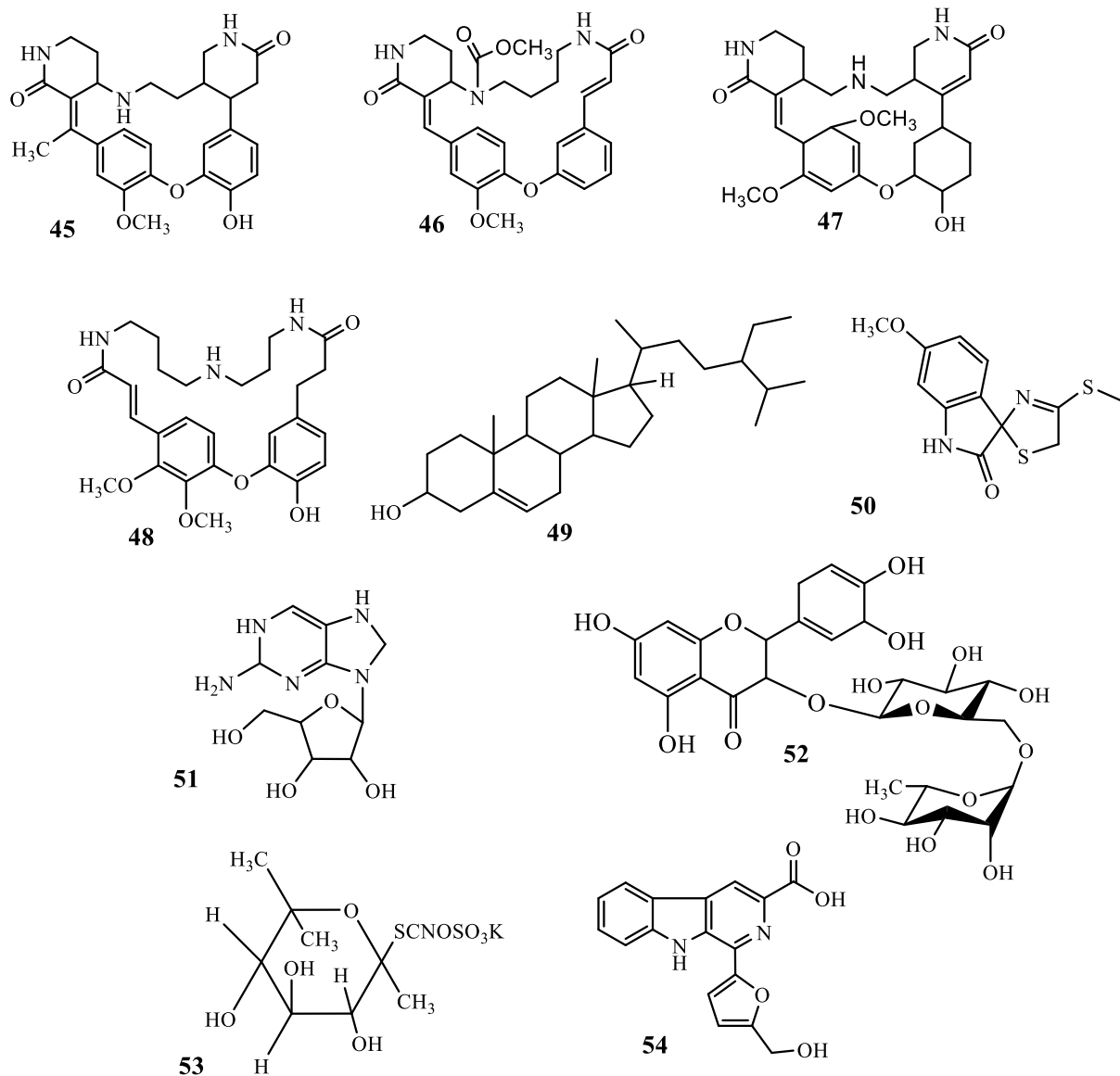


Fig. 11: Chemical structure of some bioactive compounds from capper species (*C. spinosa*, *C. ovata*, *C. decidua*, and *C. diversifolia*).

Different secondary metabolites, namely alkaloids, anthranoids, flavonoids, glycosides, polyphenols, saponins, steroids and tannins were reported from extracts of different parts of the *C. tomentosa* (Amusan et al., 2007). The alkaloids 3-hydroxy-4-methoxy-3-methyl-oxindole (55) and 4-methoxy-oxindole (56) have been isolated from the ethanol extract of roots of *C. tomentosa* (Dekker, Fourie, Mathee, & Snyckers, 1987). Separation of the petroleum ether (60–80°C) extract of the roots of this plant by chromatography on silica gel was reported the isolation

of two new compounds namely phytosterol 24-ethylcholestan-5-en-3-ol (**49**) and the dipeptide derivative N-benzoylphenylalanylaninol acetate (**57**) have been isolated. And, the fruits extracts are reported to possess L-stachydrine (**58**) (Akoto et al., 2008). Other research reports also indicated the seeds contain about 29% oil, consisting of 29% oleic acid (**41**), 26% linoleic acid (**59**), 23% palmitic acid (**44**) and 21% stearic acid (**60**) (Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Simons, 2009). Fig. 12 shows the structure of compounds isolated from root and fruit of *C. tomentosa*.

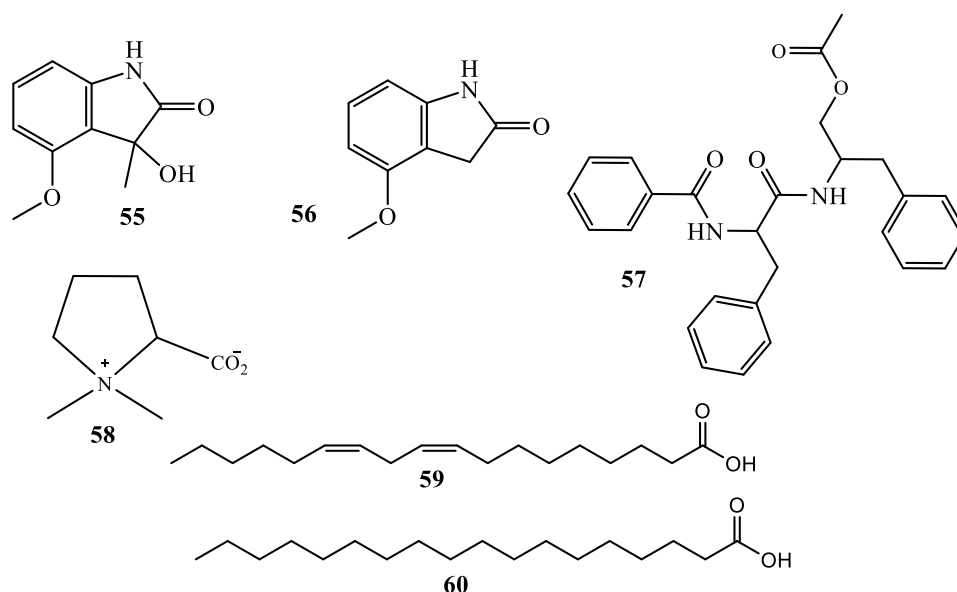


Fig. 12: Compounds isolated from root and fruit of *C. tomentosa*.

2.2.4. *Kalanchoe petiti*na

Phytochemical studies have demonstrated that different species from genus *Kalanchoe* synthesize phytochemical compounds with associated chemoprevention-related properties as a result of their secondary metabolism (Barreal, Dios, & Gallego, 2018). The *Kalanchoe* species are succulent plants. Phytochemical studies have determined that the crude extract of the different parts of various *Kalanchoe* species possesses polyphenols, alkaloids, steroids, saponins, tannins (Kolodziejczyk-czepas & Stochmal, 2017; A. Mekonnen, Sidamo, Asres, & Engidawork, 2013) glycosides, flavonoids, coumarins, anthocyanins, bufadienolides, triterpenoids, phenanthrenes and kalanchosine dimalate (Milad, El-ahmady, & Singab, 2014). Formerly, two decades ago, *K. petiti*ana has been shown to contain clionastanol and 24 α - and 24 β - alkylsterols. Alongside, the co-occurrence of both C-24 epimers (24 α , 24 β) of three 24-

ethylsterols lacking a Δ^{25} -bond, namely 24-ethyl-22E-dehydrocholesterol (**42**), 24-ethylcholesterol (**49**), 24-ethylcholestanol (**61**), from EtOH (95%) extract of the aerial parts of *K. petitiiana*, and as well the co-existence of both C-24 epimers of 24-ethyl-25-dehydrocholesterol (**62**) isolated from *K. murmorutu* have been reported (Akihisa, Gebreyesus, Hayashi, & Amura, 1992). Fig. 13 shows the clionastanol and 24 α - and 24 β - alkylsterols obtained from *K. prtitiiana*.

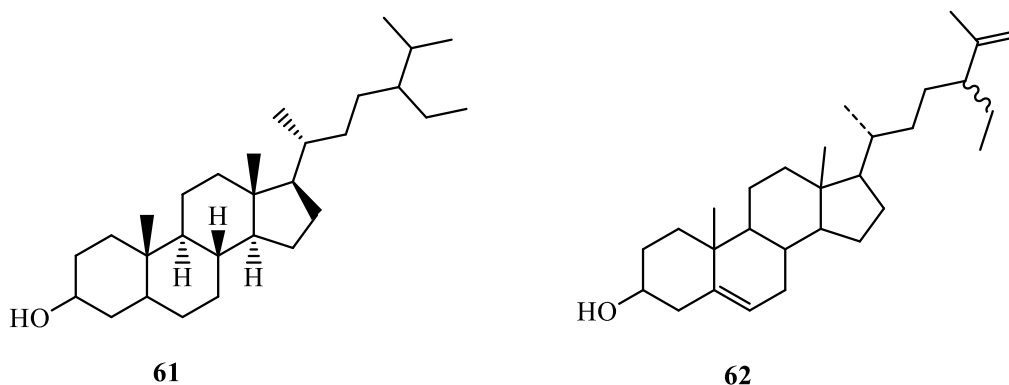


Fig. 13: Compounds reported from aerial part *K. petitiiana* (**61**) and *K. murmorutu* (**62**).

Study on quantitative determination of phenolic acid contents in leaves of *Kalanchoe* species (*K. pinnata*, *K. daigremontiana* ASE, *K. nyikae*, *K. milloti*) revealed presence of phenolic acids using the optimized LC–ESI-MS/MS method (Bogucka-Kocka, Zidorn, Kasprzycka, Szymczak, & Szewczyk, 2016). The result of this study indicated that, proportionally, larger amount of phenolic acids have been detected in the extracts of *K. daigremontiana* and *K. pinnata*, while *Kalanchoe nyikae* contain the lowest ones. Among the phenolic acids, ferulic, caffeic and protocatechuic acids have been reported to occur in larger amount, where the greatest amounts of ferulic and protocatechuic acids were detected in *K. daigremontiana* and *K. pinnata*. On top of this, chemical evaluation of bioassay guided fractions from methanol extracts of leaves of *K. pinnata* recognized two insecticidal bufadienolides named as bryophyllin A (**63**) and bryophylline C (**64**) (Supratman et al., 2000). It is also reported that water extracts of roots of *K. deigremontiana* are a rich source of various polyhydroxy C-24 structure of bufadienolides. From this plant, eight new bufadienolides that were named as kalandaigremosides (**65**) have been reported (Moniuszko-szajwaj, Pecio, Kowalczyk, & Stochmal, 2016). Similarly, bufadienolide glycosides (**66** & **67**) from *K. gracilis* and *K. tubilor* have also been reported

(Kolodziejczyk-czepas & Stochmal, 2017). Fig. 14 shows the structure of compounds isolated from *Kalanchoe* species.

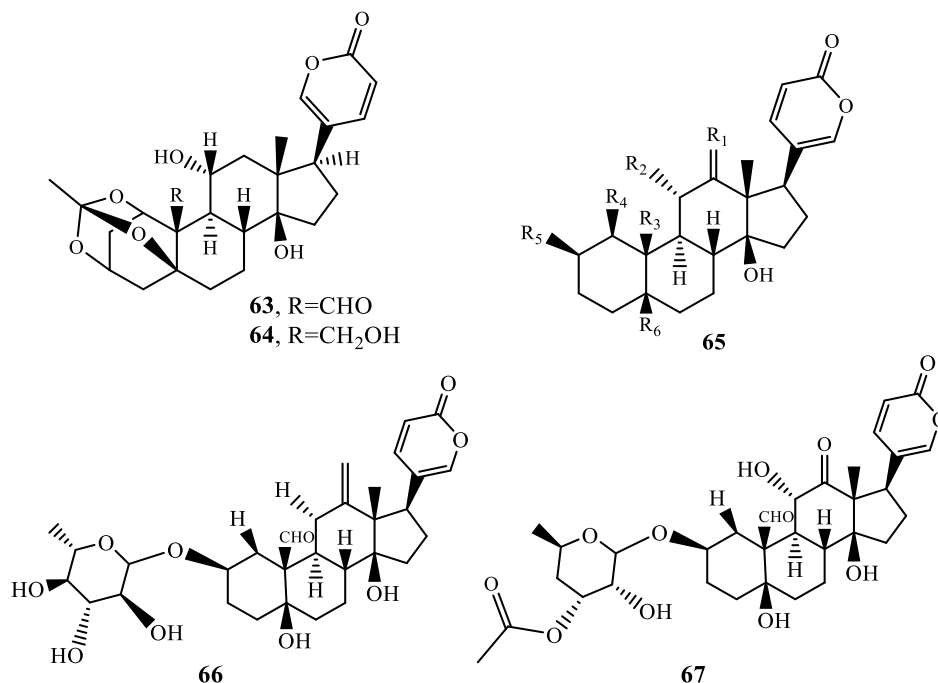


Fig. 14: Compounds isolated from *K. pinnata* (**63** & **64**), *K. deigremontiana* (**65**), *K. gracilis* (**66**), and *K. tubiflor* (**67**).

Until the completion of this report, to the best of our knowledge, we came across with limited phytochemical analysis report on *K. petitiiana* in Ethiopia. However, the other study reported that the fractions of hydroalcoholic (80% methanol) leaf extract of *K. petitiiana* possess saponins in all the fractions, whereas alkaloids and phenolic compounds in the aqueous and methanolic fractions (A. Mekonnen et al., 2013). Further, the detection of steroids in chloroform fraction, tannins and terpenoids in methanolic, and flavonoids in the aqueous fraction have been presented

2.2.5. *Cadaba rotundifolia*

Various chemical constituents, mainly compounds belonging to alkaloids, terpenes, tannins, steroids, glycosides, flavonoids, phenolic compounds, and sugar and proteins have been isolated from *Cadaba* species (Al-Musayeib, Mohamed, Ibrahim, & Ross, 2013; Mohamed, Ibrahim, Al-Musayeib, & Ross, 2014; Velmurugan & Kamaraj, 2011). The study on GC-MS analysis of

ethanol extract of leaf of *Cadaba trifoliata* reported 19 bioactive phytochemical compounds (Velmurugan & Kamaraj, 2011). Based on the peak area, the study identified phytochemical compounds with very high peak area: 1, 2-Benzenedicarboxylic acid, mono (2- ethylhexyl), ester (C₁₆H₂₂O₄) (**68**) with retention time (RT) 26.47 has peak area 43.52% and 1-methylpyrrolidine-2-carboxylic acid (C₆H₁₁NO₂) (**69**) with RT 8.18 has peak area 32.63%. The main important compounds phytol (C₂₀ H₄₀O) (**70**) with RT 19.91 ranks with peak area 1.15% was also reported (Velmurugan & Kamaraj, 2011). Fig. 15 shows the chemical structures of the phytochemical constituents from the leaf of *C. trifoliata*

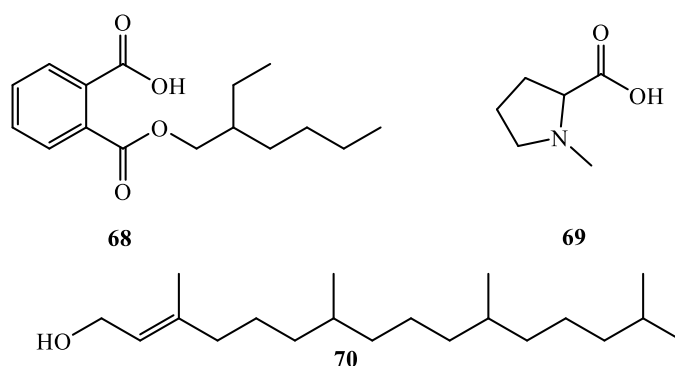


Fig. 15: Compounds from ethanolic leaf extracts of *C. trifoliata*.

Phytochemical study on the aerial part of EtOAc crude extract of *Cadaba glandulosa* revealed three new flavonoids namely, kaempferol-4-phenoxy-3,3,5-trimethylether (**71**), rhamnocitrin-4-(4-hydroxy-3-methoxy) phenoxy-3-methyl ether (**72**), and rhamnocitrin-3- O-neohesperoside-4'-O-rhamnoside (**73**), along with three known compounds; 4-methoxybenzaldehyde (**74**), kaempferol-3-methylether (**75**), and stachydrine (**57**) (Mohamed et al., 2014). Phenoxyflavonoids (**71** and **72**) found in the present study might be useful as chemotaxonomic markers for *cadaba* species. In Fig. 16 structure of compounds isolated from areal part of *C. glandulosa* are shown.

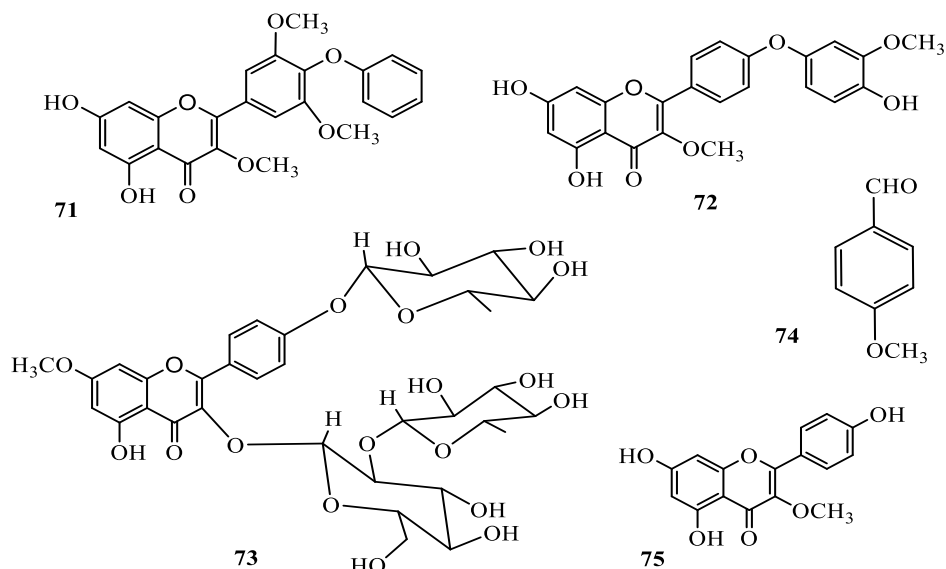


Fig. 16: Structures of compounds reported from the aerial parts of *C. glandulosa*.

From this genera, the methanol extract of leaves of *C. farinosa* Forssk (Al-Musayeib et al., 2013). Reported to contain lupeol-3-O-decanoate (76), lupeol (77), β -stigmasterol (42), ursolic acid (78), 12-aminododecanoic (79), stachydrine (80), 3-hydroxy-stachydrine (81), dillenetin-3-O- β -D-glucopyranoside (82), and quercetin-3-O- β -D-glucopyranoside (83) (Al-Musayeib et al., 2013; Velmurugan & Kamaraj, 2011). The study on the phyto-pharmacological perspective of *Cadaba farinosa* also reported the presence of chemical compounds including cadabicine (84), cadabicine methyl ether (85), cadabicine diacetate (86), α , β -dihydroferulic acid (aromatic acid) (87), 3(4-formyl Phenoxy)-4- Methoxy benzaldehyde (88), and cadibicilone (89) in large amount from the leafes extract of the plant (Telrandhe & Uplanchiwar, 2018). Fig 17 shows the compounds identified from leaves of *Cadaba farinose*

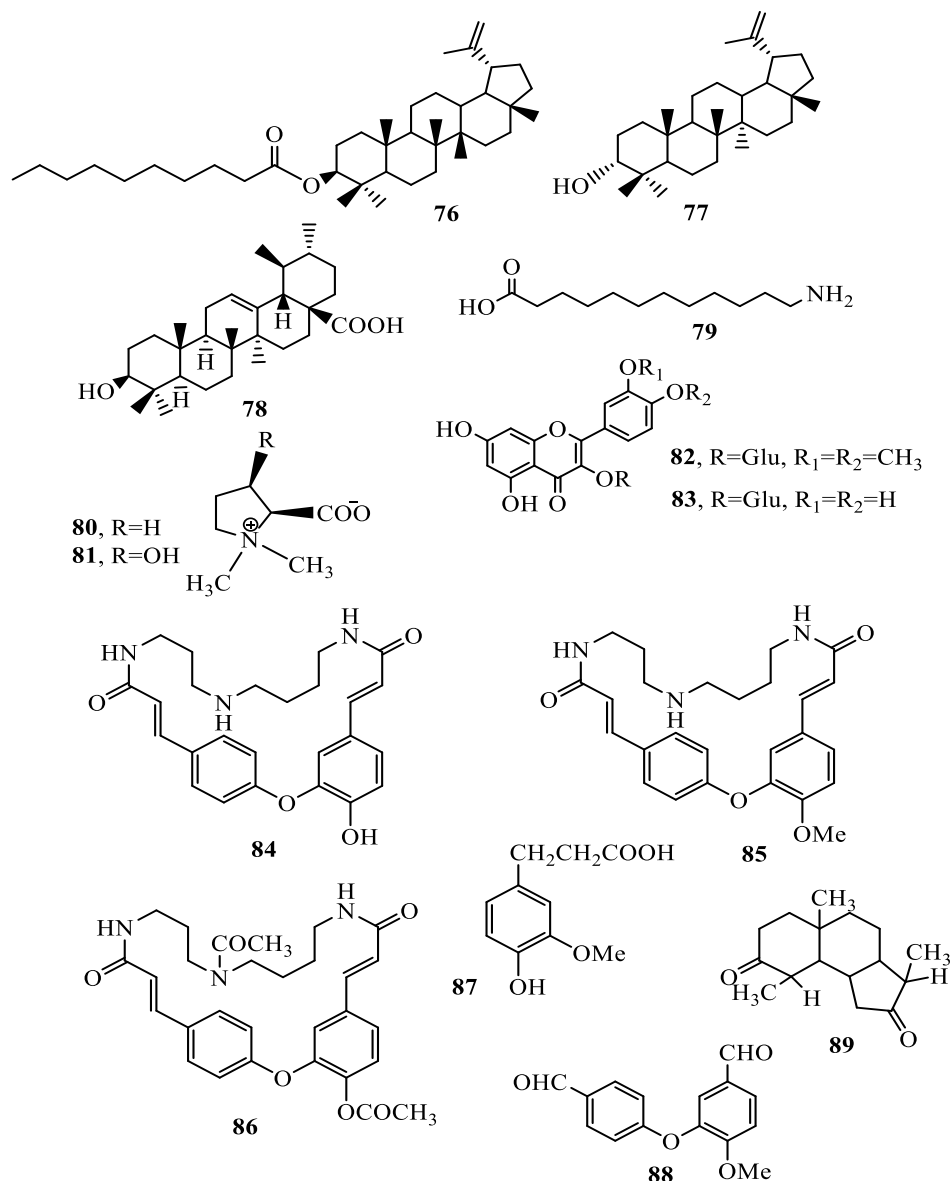


Fig. 17: Compounds identified from methanol leaves of *C. farinosa*

From the root extract of *C. natalensis* Sond., the novel macrocyclic dibenzo-diazacyclodecanedione (**90**) was isolated from ethyl acetate extract, while the (S)-2-ethyl-2-methyloxazolidin-5-one (**91**) was reported from heptane extract. In addition, five known compounds were obtained. Of these, (*R*)-5-ethyl-5-methyloxazolidin-2-one (**92**) is reported as a natural product for the first time. In addition, the isoquinoline derivatives thalifoline (**93**) and N-methylcorydaldine (**94**) have been reported (Monjane, Uamusse, & Sterner, 2016). Fig. 19 shows structures of the compounds isolated from the root of *C. natalensis*.

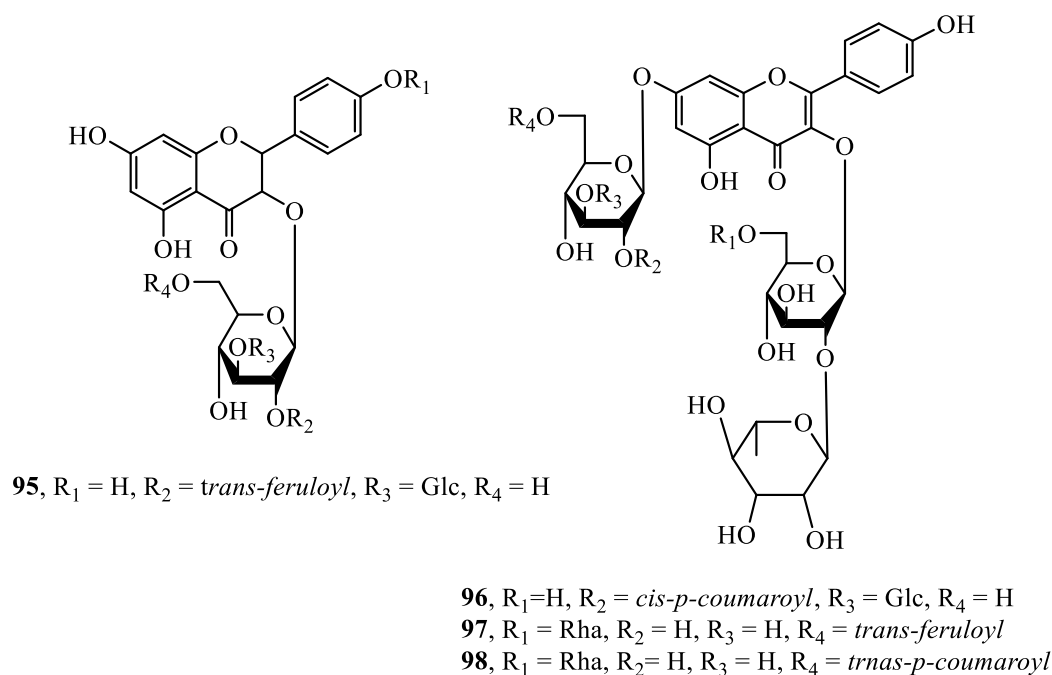


Fig. 19: Structure of compounds reported from areal part of *C. rotundifolia*

2.3. Biological activity

2.3.1. *Cyphostemma cyphopetalum* (Fresen.) Wild & Drummond

The study report by (R. Khan et al., 2015) revealed that stem and roots extracts of *C. digitatum* are good antioxidant and urease inhibitory activities, while the tested extract showed moderate antimicrobial effect against the selected microbes. According to the reported results, the methanolic extracts of the roots displayed highest antioxidant activity (93%) against DPPH while the crude methanolic extract of the stem showed highest antioxidant activity (66.163%) at 100 $\mu\text{g/mL}$ concentration. On the other hand, *C. crotalarioides* reported to have promising antifungal activity against *Fusarium nivale* (A. Bala et al., 2000). The same study reported the presence of resveratrol and some of its known natural oligomers from the roots of *C. crotalarioides*. These types of compounds were known to be produced by numerous plant species and proved to exhibit cancer chemopreventive, antifungal, and antibacterial activities. Compounds isolated from ethanol extract of *C. greveana* were reported to exhibit antiproliferative activities (Cao et al., 2011). Some other *Cyphostemma* species, *Cyphostemme glaucophilla* reported to possess hepatoprotective activity against carbon tetrachloride induced

hepatic damage in rats (Eleojo, And, & Chiletugo, 2012). Its aqueous leaves extract, among its numerous pharmacological properties, observed to serve as a cheap alternative in maintaining liver and kidney disorders.

The crude extracts and solvent fractions of the root and leaves of the *C. cyphopetalum* and *C. junceum* showed moderate antibacterial activity predominantly against *S. aureus* and the least towards *K. pneumonia*, methanol solvent fraction of the leaves revealed moderate antifungal activity against *C. albicans* (Tiki, 2018). Furthermore, the same study reported that the methanol solvent fraction of the leaves of *C. cyphopetalum* had moderate antifungal activity against *C. albicans*, and β -sitosterol (49), water fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum* and 80% methanol extracts of the leaves and roots of *C. cyphopetalum* had anti-inflammatory effect exceeding that of indomethacin.

2.3.2. *Hydnora johannis*

The ethanolic (70%) extract from roots of *H. johannis* and isolated compounds protocathechuic acid (39) and catechin (40) from the ethanolic extract were reported to have antiglycation effects, via inhibition of Advanced Glycation End Products (AGEs) formation (Asgharpour et al., 2019; Sakina Yagi et al., 2013). Thus, *H. johannis* roots extracts could be candidate for studies of herbal complement to diabetes treatment since it combines anti-oxidant and anti-AGE formation activities

The powder preparation as well as ethanolic extract of *H. johannis* roots has been reported to induced toxic effect on Wistar rats (S Yagi et al., 2011). The study on cytotoxic effect of the water and EtOH (70%) extracts against MRC5 (derived from non-cancer human fetal lung) and KB (derived from mouth epidermoid carcinoma) cells lines suggested that water and EtOH extracts exerted moderate cytotoxic effect against KB cells but not on normal MRC5 cells, while none of the tested phenolic components isolated from the 70% EtOH extract demonstrated any sign of toxicity at the highest concentration tested and no synergism was observed on different combination of pure compounds (S. Yagi et al., 2012).

The evaluation of the antibacterial activity of *H. johannis* root extracts established that the aqueous extract showed significant antibacterial activity against *E. faecalis* (MIC value of 16 μ g/mL) and *S. aureus* strains (MIC value of 64 μ g/mL) (S. Yagi et al., 2012). From ethno-

medicinal studies it is documented that the water extract is used in the treatment of diarrhea and dysentery in the folk medicine (Belayneh et al., 2012). However, the extract did not possess any activity against bacteria spp. that are mainly responsible for diarrhea (*Escherichia* spp., *Salmonella* spp., *Shigella* spp., or *Yersinia* spp) (S. Yagi et al., 2012), which suggests that the efficacy of this plant in the treatment of this disease was based on the physical and chemical properties of tannins.

The study on other *Hydnora* species, *Hydnora africana* Thumb indicated that essential oils isolated from this plant exhibited a moderate antioxidant activity that was dependent on the radical species. Similarly, the essential oils of *H. africana* showed activity against growth of some pathogenic bacteria that cause infections associated with diarrheal diseases (Wintola & Afolayan, 2017). The *in vitro* antimicrobial activity of this species against three bacterial species revealed that plant extractions with organic solvents (acetone, methanol, ethanol and ethyl acetate) provided stronger antibacterial activity than extraction with water (Nethathe & Ndip, 2011).

2.3.3. *Capparis tomentosa*

Capparis tomentosa is extensively used for the treatment of various both human and animal ailments. It is also known to be poisonous. *Capparis* species have been used in medicine since ancient times. The roots, flowers, and fruits of these plants with potential medicinal benefits, have been in use against infectious diseases without exerting side effects (Gull et al., 2015). Multiple biological activities have been reported from extracts of different part of *Capparis* species. The plant is reported to have significant pharmacological activities like hypercholesterolemic, anti-inflammatory and analgesic, antidiabeti, antimicrobial, antiplaque, antihypertensive, antihelmintic & purgativ activities (Rathee et al., 2010). Another member of *Capparis*, *C. ovata*, is reported to be effective against iron oxidative stresses in thalassemia patients (Gull et al., 2015). The isolates from the fruits of *C. spinose* were reported to process 1,1-diphenyl-2-picrylhydrazyl radical (DPPH*) scavenging components (Yang et al., 2010). These authors also indicated that ethyl acetate and aqueous fractions exhibited maximum DPPH* scavenging activities compared to the petroleum ether fractions.

The aqueous root extracts of *C. tomentosa* in alloxanised mice (at extracts dose of 50, 100 and 200 mg/kg body weight) showed dose dependent hypoglycemic activity (antidiabetic activity) that is significantly higher than that of the reference drug glibenclamide (Roy, Sedhain, & Ali, 2015; Wangai et al., 2015). The extract at a dose of 200 mg/kg body weight reduced blood glucose level from 28.4 mmol/l to 4.7 mmol/l whereas glibenclamide at a dose of 3 mg/kg body weight decreased glucose level to 4.9 mmol/l (Roy et al., 2015). The *in vitro* antioxidant activity of root extract of *C. tomentosa* using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay compared to ascorbic acid as a standards reported to show percent of inhibition of DPPH by $35.50 \pm 0.02\%$, in comparison to ascorbic acid equivalent and the reducing power observed to increase in a dose dependent manner (Wangai et al., 2015).

Extracts of the aerial parts of *C. tomentosa* exhibited significant *in-vitro* antimicrobial activity against the bacteria *S. aureus* and *B. cereus*, having stronger activity than the reference drug ampicillin, and against the fungi *A. flavus* and *C. albicans*, comparable to *griseofulvin* (Aweke, 2013). The methanol extract of areal part of *C. tomentosa* Lam. reported to be the most potent extract against breast cancer cell line (MCF7) with an IC_{50} value 9.05 $\mu\text{g/mL}$ (Mothana et al., 2011). In a related study on hepatocellular carcinoma cell line (HEPG2) and cervix cancer cell line (HELA) cell line revealed high sensitivity to *C. tomentosa* ($IC_{50} = 11.7 \mu\text{g/mL}$ for both) (Almehdar, Abdallah, Osman, & Abdel-Sattar, 2012). Sun-dried leaves of *C. tomentosa* reported to produce toxic effects in goats by causing structural and functional changes in the liver, kidneys and central nervous system (Ahmed & Adam, 1980).

2.3.4. *Kalanchoe petitiiana*

Reviews of pharmacological studies revealed that genus *Kalanchoe* had several pharmacological activities viz. antiviral, sedative, antiulcer, immunomodulatory, antileishmanial, CNS depressant, anti-inflammatory, thyroid peroxidase inhibitor, cytotoxic, hepatoprotective, antioxidant, analgesic, anticonvulsant, antimicrobial, B cell development inhibitor, cardiovascular activity, anti-hyperglycemic, antilarvicidal and insecticidal (Barreal et al., 2018; Milad et al., 2014; Stephenson, 2013; Zakharchenko et al., 2018).

The study on the phenolic acid composition, antioxidant, and cytotoxic activities in four *Kalanchoe* species (*K. daigremontiana*, *K. pinnata*, *K. milloti*, *K. nyikae*) demonstrated that *Kalanchoe* extracts have significant antioxidant and cytotoxic effects. It is reported that all tested

extracts possess significant and dose-dependent antioxidant capacities *in vitro*, as well as moderate cytotoxic activity (Bogucka-Kocka et al., 2016). The leaves extracts of another *Kalanchoe* species, *K. tubiflora*, also observed to inhibit cell proliferation by disrupting centrosome integrity and chromosome alignment in mitotic cells (Stephenson, 2013). This suggests that *Kalanchoe* species extracts can be used as a readily accessible source of natural antioxidants and potential anticancer compounds. In addition, investigation on the anti-hyperglycemic activity of the water-ethanol extract of *K. crenata* exhibited significant increase in the insulin sensitivity index compared with the initial time and to the untreated diabetic animals (Milad et al., 2014).

From the pharmacological point of view, *Kalanchoe* derived bufadienolides might be a promising group of steroid with biological actions including cardio-active, anticancer, antiviral properties (Kolodziejczyk-czepas & Stochmal, 2017). This suggests that therapeutic activities of *Kalanchoe*-derived medicines may be partly dependent on the presence of bufadienolides. Bufadienolides from the leaves of *K. pinnata* and *K. daigremontiana* showed potential cancer chemopreventive activity (Milad et al., 2014). The bufadienolides isolated from *Kalanchoe* species have also been reported to possess insecticidal activity against third instar larvae of silkworm (Supratman et al., 2000, 2001). So far, bufadienolides having an orthoacetate and α -pyrone moiety from *K. daigremontiana* (Crassulaceae), and bryophyllin A (**63**) and bryophyllin C (**64**) from *Kalanchoe pinnata* reported to show strong insecticidal activity (Milad et al., 2014).

Similar to other *Kalanchoe* species, the Ethiopian *K. petitiiana* reported to possess antimicrobial activity. The antimicrobial studies results of hydroalcoholic extracts of leaves of *K. petitiiana* hold up with the uses of the plant for treatment of various skin disorders, the majority of which are of infectious origin (Tadeg et al., 2005). It is reported that the *in vivo* wound healing activity of the methanolic and aqueous fractions from leaf extract of *K. petitiiana* facilitated wound healing activity (A. Mekonnen et al., 2013). However, the chloroform extract failed to show any wound healing activity. This result uphold with the traditional uses of the plant for the treatment of wounds and other dermatological disorders.

2.3.5. *Cadaba rotundifolia*

Several studies reported the pharmacological activity of the *Cadaba* species. The phyto-pharmacological perspective of *C. farinosa* Forsk indicated that the plant showed potent

hepatoprotective activity, antioxidant activity, antiprotozoal, schistosomicidal, antifungal activities, anticancer, wound healing, cytotoxic activity, antibacterial, and anti-diabetic activity (Telrandhe & Uplanchiwar, 2018). The study on the phytochemical analysis and anti-diabetic activity of *C. fruticose* reported that the oral administration of alcohol and aqueous leaf extracts of the plant at dose of 1000 mg/kg led to a significant blood glucose reduction (anti diabetic) (S. Arokiyaraj, R. Radha2, 2008). Ethanol and methanol extracts of wild and tissue cultured *C. fruticosa* were studied and compared for its antimicrobial activity against six human pathogenic organisms and anticancer activity against HeLa cancer cell lines, and it is reported to be a potential source of natural antimicrobial and anticancer activity (M. Bala, Pratap, & Verma, 2014; Verma, Devre, & Gangrade, 2014). The same study revealed that the highest zone of inhibition was obtained in *Escherichia coli* (14 ± 0.82 mm and 08 ± 1.05 mm) at 60 μ l concentration of wild and tissue cultured *C. fruticosa* ethanol extracts. Whereas in methanol extract the highest zone of inhibition (14 ± 0.14 mm and 09 ± 0.12 mm) in 60 μ l concentration against *S. pyogenes* and *S. aureus*. In both the cases the activity of the extract was less against fungal pathogens. The higher percentage of anticancer activity was observed in wild and tissue cultured *C. fruticosa* of ethanol extracts with 53.14 and 54.78 at 5 mg/ml concentration. Whereas in wild and tissue cultured *C. fruticosa* methanol extracts treatment were 52.58 and 51.39 respectively. The percentage of inhibition was increased with the increasing concentration. The IC_{50} value of wild and tissue cultured *C. fruticosa* ethanol extracts were 260.36 mg/mL and 268.05 mg/mL, methanol extracts were 273.71 mg/ml and 286.73 mg/ml respectively (S. Arokiyaraj, R. Radha2, 2008). The same study showed that the ethanol extract of *C. fruticose* also showed significant plasma glucose lowering effect when compared to aqueous extracts.

The isolated flavonoid compounds **72-75** (Fig. 16), from the methanol extracts of *C. glandulosa* Forssk exhibited potent anti-inflammatory activity comparable and moderate antioxidant activity (Mohamed et al., 2014). On the other hand, the evaluation of anti-inflammatory activity of other *Cadaba* speices, *C. trifoliata* (roxb) revealed that the aerial parts of the plant possess significantly acute and chronic anti-inflammatory property (Jesupillai, Nagarajan, Jegan, Sundarapandian, & Chidambaranathan, 2017). Other research work also reported that the leaf extracts of *C. trifoliata* exhibited antimicrobial activity, antipyretic activity, antibacterial activity and antihelmintic activity (Verma et al., 2014). The ethanol extract of the leaves demonstrated a high degree of activity against all the tested bacterial strains except *Erwinia* and

Acinetobacteria, whereas the aqueous extract of the leaves showed moderate activity against *E. coli*, *B. subtilis* and *S. aureus* and *Enterobacter aerogenes* (Verma et al., 2014). Three decades ago, El- Dirdiri and his coworkers reported the toxic effect of the dietary mixture of *A. bracteolate* and *C. rotundifolia* in the goats given the plant as dietary (Omer et al., 2012). Until the completion of this literature survey, no pharmacological activity of the plant *C. rotundifolia* has been reported.

3. METHODOLOGY

3.1. General

Melting points were determined using capillary tubes with Japan analytical melting point and Thiele tube apparatus. UV data were obtained on a Shimadzu UV-1201 spectrophotometer. IR spectra were recorded using KBr pellets on Perk-Elmer BX Infrared Spectrometer in the range 4000 to 400 cm^{-1} . The NMR spectra were recorded on Bruker Avance 400 MHz spectrometer with deuterated chloroform and methanol solvents using TMS as internal standard. GC-MS analysis was done by using a GC (7890 B, Agilent Technologies, USA) coupled with an MS (5977A Network, Agilent Technologies). Analytical TLC plates with silica gel 60 F254 TLC (Merck, Germany) were used to determine TLC profiles. Preparative thin layer chromatography (PTLC) separation using pre-coated glass plate 20×20cm (0.25 mm thick). UV lamp (254 and 365 nm), Iodine vapor, and vanillin spray to visualize spots on TLC plates. Silica gel column chromatography was performed at silica gel (60-120 mesh).

3.2. Chemical and Reagents

All chemicals, solvents and reagents used were analytical grade. The solvents were either Indian or France products.

3.3. Plant Material Collection and Identification

Plant samples were collected in time range of September to November 2019, and September to November 2021. Roots of *C. cyphoptealum* and *H. johannis*, and leaves of *K. petitiana* were collected from area of Huruta town, Lode Hetosa Woreda, Arsi Zone, Oromia, Ethiopia. Leaves and stem bark of *C. tomentosa* were collected from rural area of Adama town, and leaves of *C. rotundifolia* were collected from rural area of Dire Dawa city Administration, Eastern Ethiopia. The collected sample of plants *C. cyphoptealum*, *H. johannis*, *K. petitiana*, *C. tomentosa*, and *C. rotundifolia* were identified by Botanist Mr. Shambel Alemu and the specimens deposited at the National Herbarium, Addis Ababa University, Ethiopia (Voucher codes: TCY09/17, THJ10/17, TKP11/17, TCT11/17, and TCR10/17, respectively).

3.4. Extraction and Isolation

Briefly, the dried and powdered plant material (500 g) was defatted with *n*-hexane (1.5 L) three times for 72 hr each. The defatted marcs were further extracted successively with

dichloromethane/methanol (1:1), and methanol. The extracts were filtered using Whatman filter paper. The filtrates were dried using vacuum rotary evaporator at temperature of about 40 °C. Finally, after inspection of the TLC profile, each extract were kept under refrigerator at 4 °C for further experimental procedures.

3.4.1. Extraction of plant parts

The dried and powdered roots of *C. tomentosa* and *H. johannis*, stem bark of *C. tomentosa*, and leaves of *K. petitiannan* and *C. rotundifolia* (500 g each) were extracted sequentially with *n*-hexane, dichloromethane/methanol (DCM/MeOH) (1:1), and methanol (MeOH) (2 L each) by maceration, each for 72 h, thrice at room temperature while shaking over electrical shaker. In each case, the filtrates were concentrated *in vacuo* on a rotary evaporator at 40 °C. Dried extracts were collected in pre weighed vials separately to give 9 g *n*-hexane, 30.6 g DCM/MeOH and 24 g MeOH extracts of roots *C. cyphopetalum*, and 18.3 g, 30.1 g and 24 g extract roots of *H. johannis*, respectively. Similarly, 19, 27 and 22 g of *C. tomentosa*, 21, 19 and 16 g of *K. petitianna*, and 13, 11 and 17 g of *C. rotundifolia* were obtained, respectively, and store at 4°C for further experimental procedures.

3.4.2. Isolation of compounds

Isolation of compounds from the root of C. cyphopetalum

The dichloromethane/methanol (1:1) extract (10 g) from the root of *C. cyphopetalum* was adsorbed to 10 g of silica gel and applied to column chromatography (silica gel 180 g) and eluted with increasing gradient of *n*-hexane/EtOAc followed by dichloromethane/methanol mixtures, afforded four compounds (**28**, **49**, **100-105**). A total of 145 fractions were collected (each 50 mL). Fractions with similar *R_f* values and the same characteristic color on TLC were combined for further purification. Fractions 37-44, which collected with *n*-hexane/EtOAc (1:1), were mixed (20 mg) and subjected to isocratic elution with DCM/MeOH (95:5). A total of 17 sub-fractions were collected (each 15 mL). Sub-fraction 1-8 afforded compound **100** (53 mg), and sub-fraction 9-17 afforded compound **28** (69 mg). Fractions 17-36 (eluted by *n*-hexane/EtOAc (3:1)) were combined (110 mg) and purified to give compound **49** (45 mg). Fraction 45-52 which were collected from *n*-hexane/EtOAc (2:3) eluent, afforded compound **101** (41 mg).

Similarly, methanol extract (10 g) of the roots of *C. cyphopetalum* was subjected to silica gel column chromatography and eluted with increasing gradient of *n*-hexane/EtOAc followed by dichloromethane/methanol to give five compounds **28**, **102-105**. A total of 98 fractions were collected, and those with similar R_f values and the same characteristic color on TLC were combined for further purification. Fractions 27-29 which eluted with *n*-hexane/EtOAc (5:2) were mixed (44 mg) and purified further using isocratic elution by 5% MeOH in DCM. A total of 54 sub-fractions were collected. Sub-fraction 8-14 afforded compound **28** (11 mg, as pale brown), subfraction 22-30 afforded compound **102** (6 mg, brown) and subfraction 50-53 afforded **103** (20 mg, pale brown). Finally, fractions 53-57 that obtained with *n*-hexane/EtOAc (3:1) eluent were combined and further subjected to isocratic elution to collect 31 subfractions with 10% MeOH in DCM. Subfraction 20-27 afforded compound **104** (40 mg, pale brown) and subfraction 28-30 afforded compound **105** (7 mg, brown).

Isolation of compounds from the root extract of H. johannis

Dried dichloromethane/methanol (1:1) root extract (10 g) of *H. johannis* was adsorbed to 10 g of silica gel and subjected to silica gel column chromatography (silica gel 180 g) and eluted with increasing gradient of ethylacetate (EtOAc) in *n*-hexane followed by methanol in dichloromethane. A total of 119 fractions were collected (each 50 mL) and those with similar R_f values and the same characteristic color on TLC were combined for further purification. Compound **49** (58 mg) was obtained from fractions 27-31 which were eluted with *n*-hexane/EtOAc (7:3). Fractions 100-105 (eluted with DCM/MeOH (7:3)) were combined and purified in a small column with isocratic 5% MeOH in DCM. A total of 30 subfractions were collected, and sub-fraction 23-25 afforded compounds **106** (57 mg) and sub-fraction 12-19 afforded compound **107** (20 mg). Fractions 71-76 which eluted with *n*-hexane/EtOAc (1:4) were combined and further purified using small column with 0.5-2% MeOH in DCM and afforded compound **40** (55 mg). Fractions 34-37 were combined and purified in a small column with isocratic mode with 5% EtOAc in *n*-hexane to afford compounds **108** (43 mg), and **41** (55 mg). Fraction 19-26 which collected with 25-30 % EtOAc in *n*-hexane eluent afforded compound **109** (27 mg).

Isolation of compounds from the stem bark of C. tomentosa

The dichloromethane/methanol (1:1) extract (10 g) from the stem bark of *C. tomentosa* was adsorbed with 10 g of silica gel and applied on column chromatography (silica gel 180 g).

Elution was carried out with increasing gradient of EtOAc in *n*-hexane followed by methanol in dichloromethane. A total of 76 fractions were collected (each 50 mL). Fractions with similar R_f values and the same characteristic color on TLC were combined for further purification. Fractions 29-39 which eluted with 15% EtOAc in *n*-hexane afforded compound **110** (25 mg). Compound **111** (22 mg) was obtained from fraction 47-59 up on isocratic purification of with *n*-hexane/EtOAc (3:2).

Isolation of compounds from the leaves of K. petitiiana

The dichloromethane/methanol (1:1) extract (8 g) of the leaves of *K. petitiiana* was adsorbed to 8 g of silica gel and subjected to column chromatography (silica gel 180 g) and eluted with increasing gradient of *n*-hexane/EtOAc followed by DCM/MeOH. A total of 63 fractions were collected (each 50 mL). Fractions with similar R_f values and the same characteristic color on TLC were combined for further purification. Fractions 25-29 collected from 10-20 % EtOAc in *n*-hexane/EtOAc eluent, were combined and purified using isocratic elution with 5% EtOAc in *n*-hexane to afford compound **112** (17 mg).

Isolation of compounds from the leaves of C. rotundifolia

The dichloromethane/methanol crude extract (1:1) (8 g) was adsorbed to 8 g of silica gel and subjected to silica gel column chromatography (silica gel 180 g). Elution was carried out with increasing gradient of EtOAc in *n*-hexane followed by MeOH in DCM. A total of 69 fractions were collected (each 50 mL). Fractions with similar R_f values and the same characteristic color on TLC were combined for further purification. Re-purification of fractions 21-27 which were collected with *n*-hexane/EtOAc (7:3) afforded compound **113** (53 mg).

3.4.3. Essential oil Extraction

Air dried and powdered leaves of *C. tomentosa*, *K. petitiiana*, and *C. rotundifolia*, (each 500 g) were separately extracted using hydro-distillation with a Clevenger-type apparatus (Bousbia et al., 2009) and yielded essential oil. Then dried under anhydrous sodium sulphate, filtered and concentrated using rotary evaporator. The extracted oils were then stored at 4°C until analysis. Each extraction was performed at least three times.

3.5. GC-MS analysis of essential oils

GC-MS analysis was done by using a GC (7890B, Agilent Technologies, USA) coupled with an MS (5977A Network, Agilent Technologies). The GC had an HP 5MS column (non-polar column, Agilent Technologies), 30 m × 250 µm internal diameter (i.d.) and 0.25 µm film thickness. The carrier gas was helium flowing at a rate of 1 mL/ min. The injector temperature was 230 °C and the injection mode was split mode with split Ratio 10:1. The initial oven temperature was 40°C held for 5 min. It was raised to 250°C at 6°C/min held at this temperature for 20 min. The total run-time was 60 min. Mass spectra were recorded in EI mode at 70 eV, scanning the 50-500 m/z range. The volatile compounds in the oils were identified by comparing the mass spectra of the compounds in the oils with those in the database of NIST11 GC-MS libraries and by comparing with those reported in the literature.

3.6. Biological activity

Microorganism strain

In vitro antibacterial activities of extracts and isolated compounds were studied against *Escherichia coli* (*E. coli*, ATCC 25922), *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 27853), *Staphylococcus aureus* (*S. aureus*, ATCC 25923), and *Streptococcus pyogenes* (*S. pyogenes*, ATCC 19615) collected from Ethiopian Public Health Institute (EPHI). Antifungal activities were examined against *Candida albicans* (ATCC 26555) strains. These strains are among the drug resistant pathogenic strains to most of the first line drugs (Meshesha et al., 2017), and any substance toxic to these strains are also believed to be active on other strains (WHO, 2014).

Experiments were carried out at the microbiology laboratory of the medical laboratory Department, Haramaya University, in collaboration with microbiologists, and microbiology lab of Applied Biology department, Adama Science and Technology University. The degree of susceptibility of each bacterial strain to the isolated compounds was evaluated by using the agar medium disc-diffusion technique as per the standard protocols of Clinical and Laboratory Standards Institute (CLSI) (J. L. Rodriguez-Tudela et al., 2008).

3.6.1. *In vitro* Antibacterial Activity Assay

The *in vitro* antibacterial activities of plant extracts and isolated compounds were evaluated by using the agar medium disc-diffusion technique as per the standard protocols of Clinical and

Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Balouiri, Sadiki, & Ibnsouda, 2016; EUCAST, 2015).

Fresh colonies (about 3-5) with similar morphology of each bacterial species were aseptically transferred into a saline solution using a sterile inoculating loop, and bacterial turbidity was adjusted in reference to 0.5 McFarland standard solution (10^8 CFU/mL). The Mueller Hinton Agar (HiMedia) medium was prepared as per the manufacturer's instruction. The bacterial suspension was then streaked by swabbing with cotton swab onto Petri plates containing the medium. A sterilized Whatman No. 1 filter paper discs (6 mm in diameter) were prepared using a puncher to hold samples. A stock solutions of each extract and isolated compounds (5 mg in 5 mL) were prepared in 4% DMSO. Five different solutions, 1000, 500, 300, 100, and 50 μ g/mL, of each compound were prepared from their corresponding stock solutions. Standard antibiotic disc of chloramphenicol and amoxicillin, and DMSO were served as positive and negative controls, respectively. Each solution (100 μ L) was loaded onto separate paper discs (6 mm in diameter), placed discs onto the Petri plates with the bacterial culture inoculated MHA, and incubated at 37 °C for 18-24 hr. Inhibition zones were monitored by the paper disc's clear area and measured by caliper (in mm) (Balouiri et al., 2016; Zaidan et al., 2005). The experiment was done in duplicate aseptically, and the result was expressed as mean $\pm$ standard deviation using statistical analysis software of SPSS (version 20).

3.6.2. *In vitro* antifungal activity assay

The *in vitro* antifungal activities of *n*-hexane, DCM/MeOH (1:1), and MeOH extracts of roots of *C. cyphopetalum* were determined using the disc diffusion method (Balouiri et al., 2016). Potato dextrose agar (PDA) was prepared by dissolving the required amount of PDA powders with distilled water. Then the mixture was heated on hot plate and autoclaved to get boiled and uniform solution. The resulting autoclaved medium was transferred into separate Petri plates and allowed to solidify. Within 15 min of adjusting the turbidity of the suspension of inoculums, a sterile cotton swab was dipped into adjusted suspension and rotated several times by pressing firmly on the inside wall of the tube above the fluid level. This removes excess fluid from the swab. Then, the dried surface of PDA plates was inoculated by streaking using the swab three times over the entire surface by rotating the PDA plates approximately 60° each time to ensure an even distribution of the inoculums. Then, the PDA plates were left open in the hood for three

to five minutes to allow for any excess surface moisture to be absorbed (CLSI, 2012).

Concentrations 100, 50, 25, and 12.5 mg/mL of extracts were prepared from stock solutions. Discs of commercial Ketoconazole/Tilt (10 µg/disc) were used as positive controls while DMSO impregnated discs were used as negative controls. Sterilized filter paper discs of 6 mm diameter were prepared from sterile filter paper cut into small, circular pieces of equal size by a perforator and then they were impregnated each with 0.01 ml of the prepared test extracts. The impregnated discs were placed onto the surface of the inoculated agar plates using sterile forceps. Each disc was pressed down to ensure complete contact with the agar surface. The discs were distributed evenly so that they were no closer than 24 mm from center to center (CLSI, 2012). Then the PDA plates were sealed with Para film and incubated at 27°C for 3-5 days for fungal pathogens. After incubation, the diameters of the zone of inhibition around each disc were measured to the nearest millimeter along two axes (i.e. 90° to each other) using a transparent ruler and the means of the two readings were recorded. The experiment was done in duplicate aseptically, and the result was expressed as mean ± standard deviation using statistical analysis software of SPSS (version 20).

3.6.3. *In vitro* cytotoxicity assay

In vitro cytotoxicity testing is one of the major assays applied during toxin assessment, which focuses mainly on cell death or some measure of growth impairment. It is one of the commonly used tests in ensuring the safety of new products. Cytotoxicity studies are a useful step in determining the potential toxicity of a test substance, including plant extracts or biologically active compounds isolated from plants. In this study, *in vitro* cytotoxicity of *n*-hexane, DCM/MeOH (1:1), MeOH extracts of roots of *H. johannis*, DCM/MeOH (1:1), and MeOH extracts of roots of *C. cyphopetalum*, and DCM/MeOH (1:1), and MeOH extracts of stembark of *C. tomentosa* against breast cancer cell lines, Michigan Cancer Foundation-7 (MCF-7) were evaluated. The breast cancer cell lines MCF-7 were obtained from National Centre for Cell Science (NCCS), Pune, India.

Cell viability assay

Cell viability was measured using the MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Plumb, 2004). The assay detects the reduction of yellow

colored MTT by mitochondrial dehydrogenase to purple formazan product, which reflects the normal function of mitochondria and cell viability using MTT assay (Atta-ur-Rahman, M.Iqbal Choudhary, 2001; Bahuguna et al., 2017). The MFC-7 cells were cultured in T25 culture flask containing Dulbecco's Modified Eagle Medium (DMEM) containing DMEM supplemented with 10% FBS and 1% antibiotics (100U mL⁻¹ penicillin and 100µg mL⁻¹ streptomycin). The cells were incubated at 37 °C in 5% CO₂ atmosphere. Upon reaching confluence, the cells were detached using Trypsin–EDTA solution and were seeded at a density of 5000 cells per well. At 50% confluence, the culture medium was aspirated and the cells were treated with 20 µg of plant extract in DMSO (20 µL) for 24 h at 37 °C in the CO₂ incubator. Later, cells were incubated with MTT (4 mg mL⁻¹). The plates were incubated for 4 h at 37 °C in 5% CO₂ atmosphere. The purple formazan product was solubilized in DMSO. The absorbance was measured at 540 nm with a standard microplate reader. The percentage of viable cells is directly proportional to the level of formazan produced. Therefore, the higher the measured absorbance, the more viable the cells are present. The cell viability was expressed as the percentage of cytotoxicity (% cytotoxicity). The results are presented as the mean of triplicate experiments. The percentage cell viability is calculated as following equation:

$$\% \text{ of cell viability} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of negative control}} \times 100\%$$

3.6.4. Antioxidant activity

The antioxidant activities of isolated compounds **28**, **49**, **100-102**, **104**, and **105-107** were evaluated based on the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical (Braca, et al., 2010). Each sample was separately dissolved in methanol and serially diluted to give 200, 100, 50, and 25 µg/mL concentrations. Freshly prepared 0.04% DPPH solution in methanol was added to each concentration. The mixture was shaken and incubated in an oven at 37°C for 30 min. The absorbance of the resultant solution was measured at 517 nm using UV-Vis spectrophotometry. Ascorbic acid with a similar concentration of test sample was used as the positive control.

The DPPH radical scavenging activity of each of the tested compounds was reported by percentage inhibition using the following formula:

$$\%DPPH\ Inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of DPPH solution and A_{sample} is absorbance of the test sample (DPPH solution plus compound). (Galma et al., 2021; Narramore, Stevenson, Maxwell, Lawson, & Fishwick, 2019). The DPPH radical scavenging activity of the compounds was also expressed as IC_{50} , the concentration of the test compound to give a 50% decrease of the absorbance from that of the control solution.

3.7. Computational Study

3.7.1. Molecular docking

Molecular docking studies were performed to predict the interaction of isolated compounds with the binding sites of *E. coli* DNA gyrase B (PDB ID: 6F86), PqsA (PDB ID: 3T07), *S. aureus* pyruvate kinase (PK) (PDB ID: 5OE3) and human myeloperoxidase (PDB ID: 1DNU) were used as target proteins.

Ligand Preparation

The chemical structures of the isolated compounds (**28**, **49**, **100-102**, **106** and **107**) were drawn using the ChemOffice tool (Chem Draw 16.0) assigned with proper 2D orientation, and the energy of each molecule was minimized using ChemBio3D. Then the energy minimized ligand molecules were used as input for AutoDock Vina in order to carry out the docking simulation.

Protein Preparation

Crystal structures of the receptor protein molecules *E. coli* DNA gyrase B (PDB ID: 6F86), *P. aeruginosa* PqsA (PDB ID: 3T07), and *S. aureus* Pyruvate kinase (PK) (PDB ID: 5OE3) were obtained from the protein data bank (PDB). The proteins were prepared using protein preparation protocol (Narramore et al., 2019), applying default parameters (remove water molecules and cofactors). The target protein file was generated by leaving the associated residue with protein by using Auto Preparation of target protein file AutoDock 4.2 (MGL tools1.5.7).

Docking

The graphical user interface program was used to set the grid box size of 46×46×46 Å pointing in x, y, and z directions with a grid point spacing of 0.375 Å was considered. Lamarckian genetic algorithm (LGA) program with an adaptive whole method search in the Auto Dock was chosen

to calculate the different ligand conformers. The docking algorithm provided with AutoDock Vina was used to search for the best-docked conformation between ligand and protein. AutoDock Vina with the standard protocol was used to dock the ligands (isolated compounds) into the active site of proteins (Trott & Olson, 2009). Nine different conformations were generated for each ligand scored using AutoDockVina scoring functions. The conformations with the most favorable (least) free binding energy were selected for analyzing the interactions between the target receptor and ligands by Discovery studio visualizer and PyMOL. The ligands are represented in different colors, H-bonds and the interacting residues are represented in stick model representation.

3.7.2. *In silico* drug likeness, ADME and Toxicity prediction

Attempting to develop new drugs in a shorter time and at lower cost remains challenges in drug development (Jia, Li, Hao, & Yang, 2020). Undesirable pharmacokinetic properties and intolerable toxicity are the main causes of the failure of drug candidates at the clinical trial stage (Protti, Rodrigues, Fonseca, & Alves, 2021). The concept of drug-likeness and ADMET were proposed to provide useful guidelines during the early stages of drug discovery to improve the chance of a chemical entering and passing clinical (Protti et al., 2021; Tian et al., 2015)

In this study, drug likeness and ADMET properties of isolated compounds **28, 49, 100-102, 106 and 107** were predicted. Structure of the isolated compounds were converted to their canonical simplified molecular-input line-entry system (SMILE) and submitted to SwissADME and PreADMET tool to estimate the drug-likeness and pharmacokinetic parameters (Banerjee, Eckert, Schrey, & Preissner, 2018; Daina, Michielin, & Zoete, 2017). The drug-likeness of isolated compounds was predicted based on the concept of Lipinski's rule of five (Lipinski, Lombardo, Dominy, & Feeney, 1997). Veber's rule was employed to predict the oral bioavailability of the isolated compounds (Veber et al., 2002). Lipinski's rule of five established that a compound with molecular weight (MW) <500 Daltons, H-bond donors (NHD) <5, H-bond, acceptors (NHA) <10, and logarithm of the *n*-octanol/ water partition coefficient (logP)<5 has a drug like activity (Lipinski et al., 1997). Veber's rule states a compound with rotatable bonds (RTB)<10 and a polar surface area (TPSA)<140 Å² should present good oral bioavailability (Veber et al., 2002). To estimate the pharmacokinetic properties, the ADMET Gastro Intestinal Absorption (GIA), Blood Brain Barrier (BBB) permeability, permeability

glycoprotein (P-gp), and inhibitory interaction of cytochromes (CYP), Lethal dose 50 (LD₅₀), and organ toxicities were predicted. Organ toxicities and toxicological endpoints were predicted using Pro Tox II tool (Banerjee et al., 2018).

3.7.3. Density Function Theory

Density functional theory (DFT) is emerging as a very powerful technique to study biomolecular systems to know the reaction coordinates and the transition state of a reaction is fundamental for developing mechanism-based inhibitors that usually mimic the transition state (Seeliger & Groot, 2010). In the present study, the DFT analysis of isolated compounds **28**, **49**, **100-103** was performed using Gaussian 09 and visualized through Gauss view 6.0. The structural coordinates were optimized using B3LYP/6-31 G (d, p) level basis set without any symmetrical constraints. The molecular electrostatic potential map and energies of the compounds were obtained from the optimized geometry. Koopman's approximation was used to estimate the highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), energy gap, and related reactive parameters (electronegativity, chemical potential, hardness, softness, electrophilicity) (Mumit et al., 2020).

3.8. Data analysis

Each experiment was performed in triplicates for statistical analysis (mean $\pm$ standard deviation). The IC₅₀ values of the compound scavenging the DPPH radical by 50% were determined from each dose-response curve by GraphPad Prism.

4. RESULTS AND DISCUSSION

4.1. Phytochemical Analysis

A total of 20 compounds were isolated and fully characterized using IR, ^1H NMR, ^{13}C NMR, DEPT-135, 2D NMR (HSQC, COSY, and HMBC) techniques and in comparison with the literature data. The chemical composition of essential oils of leaves of *C. tomentosa*, *K. petitiiana* and *C. rotundifolia* were analyzed by GC-MS.

4.1.1. Compounds isolated from roots of *C. cyphopetalum*

The DCM/MeOH (1:1) and MeOH (100%) extracts of the roots of *C. cyphopetalum* each separately subjected to silica gel column chromatography to afford compounds **28**, **49**, **100**, **101** and from DCM/MeOH (1:1) and **28**, **102-105** from MeOH extracts, including a new hydroxyl-spongiane diterpenoid lactone, 3-hydroxyisoagatholactone (**100**). In this study compound **28** is isolated from both DCM/MeOH and MeOH extracts. All the compounds are reported from this plant species for the first time.

Compound **28**

Compound **28** (29 mg) was obtained as a solid with melting point of 258-260 °C. The TLC profile R_f value of 0.32 with DCM/MeOH (9.5/0.5) as eluent. The ^1H NMR displayed two doublet ($J = 8.5$ Hz) aromatic proton signals at δ 7.35 (H-2/6) and 6.73 (H-3/5) that are assignable to *p*-substituted phenyl group (Table 2, Appendix 1A). The aromatic proton signals at δ 6.42 (2H, C-2'/6'), 6.13 (1H, C-4') suggested the presence of 1, 3, 5 tri-substituted benzene ring. The two doublets ($J = 16.3$ Hz) at δ 6.78 and 6.92 correspond to the *trans* olefinic protons. The ^{13}C NMR (101 MHz, MeOD) spectral data together with its DEPT-135 showed the presence of ten well resolved carbons (Table 2, Appendix 1B and 1C). The carbon signals at δ 159.5 (C-3'/5'), 158.2 (C-4), 141.2 (C-1'), and 130.3 (C-1) ascribed to quaternary aromatic carbons. The signals at δ 128.7 (C-2/6), 116.4 (C-3/5), 105.7 (C-2'/6'), and 102.6 (C-4') suggested the aromatic methine carbon. Olefinic carbon signals were recognized at 129.3 (C-1''), and 126.9 (C-2''). The observed spectral data is found to be in good agreement with previously reported data of stilbene derivative (a *trans*-resveratrol) (Commodari, Khiat, Ibrahim, Brizius, & Kalkstein, 2005; Mattivi, Reniero, & Korhammer, 1995) (Fig 21).

Table 2: ^1H (400 MHz, CD_3OD), and ^{13}C (100 MHz, CD_3OD) NMR of compound **28**
 compared to literature (Commodari et al., 2005; Mattivi et al., 1995), 400 MHz,
 $\text{DMSO}-d_6$, δ (ppm) and J (Hz)

.Compound 28			(Commodari et al., 2005; Mattivi et al., 1995)	
No	^1H (J)	^{13}C	^1H NMR	^{13}C NMR
1	-	130.4	-	128.07
2, 6	7.35 (d, 8.5 Hz, 2H)	128.7	7.35 (d)	127.75
3, 5	6.77 (d, 8.5 Hz, 2H)	116.4	6.71 (d)	115.43
4		158.2		157.16
1''	6.96 (d, 16.3 Hz, 1H)	129.3	6.87 (d)	128.02
2''	6.80 (d, 16.3 Hz, 1H)	126.9	6.76 (d)	125.60
1'	-	141.2	-	139.19
2', 6'	6.45 (d, 2.1 Hz, 2H)	105.7	6.34 (brs)	104.23
3', 5'		159.6		158.28
4'	6.16 (bs, 1H)	102.6	6.07 (brs)	101.67
4-OH	9.29 (OH)	OH	9.48 (OH)	
3', 5'- OH	9.11 (OH)	OH	9.12 (OH)	

Compound 49

Compound **49** (45 mg) was obtained as a solid with melting point in the rage of 134 to 136°C. The TLC profile showed R_f value of 0.65 with DCM/MeOH (9:1) mobile phase. The IR (KBr) spectrum showed absorptions at 3434 cm^{-1} and $2919/2850\text{ cm}^{-1}$, that indicated the hydroxyl group and sp^3 C-H stretching, respectively. The absorptions at 1464 cm^{-1} indicate the presence of methylene C-H stretching (Appendix 2A). The ^1H NMR (400 MHz, CDCl_3) displayed signal assignable to olefinic proton at δ 5.33 (bs, 1H, H-6), oxygenated methine proton at δ 3.50 (m, 1H, H-3), methylene protons at δ 2.04 (m, 2H, H-7) and diastereotopic methylene protons at δ 2.31 (m, 2H, H-4) (Table 3, Appendix 2B). The signals for six methyl protons were observed at δ 0.66 (H-18), 0.79 (H-27), 0.81 (H-29), 0.83 (H-26), 0.86 (H-19), and 0.99 (H-21). The remaining proton signals were overlapping in the range of δ 1.26 to 2.00. The ^{13}C and DEPT spectrum indicates the presence of olefinic carbon at δ 140.7 (C-5) and 121.7 (C-6) of which the former suggests sp^2 quaternary carbon, hydroxylated sp^3 carbon at δ 71.8 (C3), two sp^3

quaternary carbons at δ 36.5 (C-10) and 42.3 (C-13), and methine carbons at δ 32.0 (C-8), 50.2 (C-9), 56.8 (C-14), 56.1 (C-17), 36.2 (C-20), 45.9 (C-24) and 29.2 (C-25) (Appendix 2C). Its DEPT-135 clearly displayed the presence of 11 methylene signals (Appendix 2D). The observed spectral data is found to be in good agreement with previously reported data of β -sitosterol (Pierre & Moses, 2015) (Fig 21).

Table 3: ^1H and ^{13}C NMR spectra of compound **49** (400 MHz, CDCl_3) compared to literature (Pierre & Moses, 2015), 400 MHz, CDCl_3 , δ (ppm) and J (Hz)

Compound 49		(Pierre & Moses, 2015)	
No	^1H (J)	^{13}C	^{13}C
1		37.2	37.2
2		31.6	31.6
3	3.50 (m, 1H)	71.9	71.8
4	2.31 (m, 2H)	42.2	42.2
5		140.8	140.7
6	5.33 (bs, 1H)	121.8	121.7
7	2.04 (m, 2H)	32.0	31.9
8		32.0	31.9
9		50.2	50.1
10		36.6	36.5
11		21.1	21.0
12		39.8	39.7
13		42.4	42.3
14		56.8	56.7
15		24.8	24.3
16		28.2	29.7
17		56.1	56.0
18	0.66 (s, 3H)	11.9	12.2
19	0.86 (s, 3H)	19.1	19.4
20		36.2	36.5
21	0.99 (d, 3H)	18.8	18.9
22		34.0	33.9
23		26.1	26.2
24		45.9	45.8
25		29.2	29.1
26	0.83 (d, 3H)	19.9	19.8
27	0.79 (d, 3H)	19.5	19.4
28		23.1	23.0
29	0.81 (t, 3H)	12.0	12.2

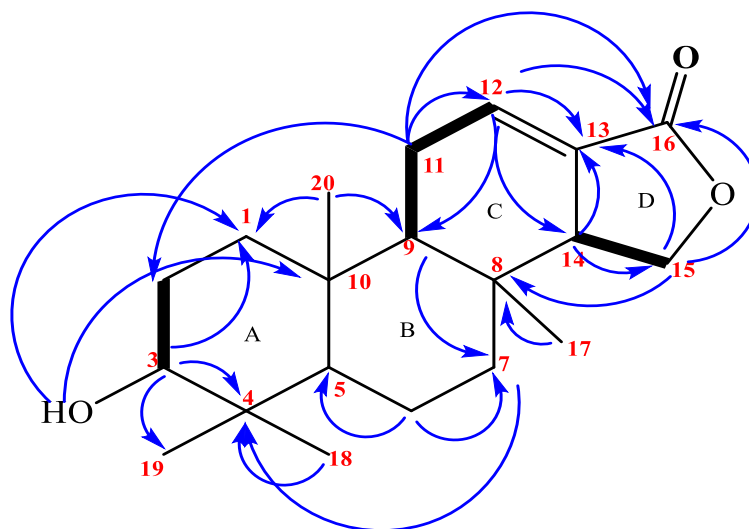
Compound 100

Compound **100** (53 mg) was isolated as a yellowish solid with melting point of 152-155 °C. The characteristic UV absorption at $\lambda_{\text{max}} = 220$ nm suggesting π to π^* transition of electrons. Its IR spectrum displayed absorption bands at $\nu_{\text{max}} = 1761$ and 1687 cm^{-1} attributed to the presence of an α , β -unsaturated γ -lactone ring (Cimino, De Rosa, De Stefano, & Minale, 1974) (Appendix 3A). In addition, the IR spectrum showed bands for hydroxyl (3506 cm^{-1}) and sp^3 C-H stretches (2850 cm^{-1}). The ^1H NMR spectra displayed signals for an olefinic proton at δ_{H} 6.88 (dd, $J = 3.5, 3.5$ Hz, H-12), while the diastereotopic oxygenated methylene protons in the lactone ring resonated at δ_{H} 4.36 and 4.03 (t, 1H each, $J = 9.1$, H-15a/b) (Table 4, Appendix 3B). The signals at δ_{H} 3.45 (m, 1H, H-3) suggests an sp^3 oxygenated methine proton. The signals at δ_{H} 2.27 and 2.78 were assignable to the methylene and methine protons at H-11 and H-14, respectively. The ^{13}C NMR spectra of compound **100** displayed 20 well-resolved carbon peaks (Table 4, Appendix 3C). The carbon signal at δ_{C} 29.7 is attributed to hexane impurity. The DEPT-135 spectrum revealed five quaternary, six methylene, five methine, and four methyl carbones (Table 4, Appendix 3D). The signal at δ_{C} 170.3 in the ^{13}C NMR spectra was assignable to the lactone carbonyl at C-16, those at δ_{C} 136.4 and 126.9 to the olefinic carbons at C-12 and C-13, respectively. In addition, signals at δ_{C} 53.9 and 67.2 were assignable to C-14 and C-15, respectively, on γ -lactone ring. The $^1\text{H}/^{13}\text{C}$ NMR data were consistent with published data reported for a spongiane diterpene lactone skeleton (**99**) which lacks a C-3 hydroxyl group (Cimino et al., 1974; González et al., 1984). The COSY spectra showed correlations between H-2 (δ_{H} 1.52) and H-3 (δ_{H} 3.42), H-11 (δ_{H} 2.32) with H-9 (δ_{H} 1.36) and H-12 (δ_{H} 6.88), H-14 (δ_{H} 2.79) and H-15a (δ_{H} 4.36) and H-15b (δ_{H} 4.03) (Table 4, Fig. 20, Appendix 3E). All protonated carbons were assigned by using the result from HSQC spectrum (Appendix 3F). The HMBC correlations observed between proton at δ_{H} 6.88 (H-12) to C-13 (δ_{C} 126.9) and C-16 (δ_{C} 170.3) were in agreement with α , β -unsaturated ester carbonyl moiety (Table 4, Fig. 20, Appendix 3G). In addition, the same olefinic proton at δ_{H} 6.88 (H-12) showed correlations with C-9 (δ_{C} 53.9), C-11 (δ_{C} 24.1), and C-14 (δ_{C} 51.0). Furthermore, the HMBC spectral data also displayed correlations of δ_{H} 1.60 (H-1) to C-5 (δ_{C} 49.3), C-8 (δ_{C} 34.4), and C-9 (δ_{C} 51.1), δ_{H} 1.52 (H-2) to C-1 (δ_{C} 32.7) and C-3 (δ_{C} 75.7), δ_{H} 3.42 (H-3) to C-1 (δ_{C} 32.7), C-4 (δ_{C} 37.4), C-5 (δ_{C} 49.3), and C-19 (δ_{C} 22.1), δ_{H} 0.76 (H-17) to C-7 (δ_{C} 40.6), C-8 (δ_{C} 34.4), and C-14 (δ_{C} 53.9), δ_{H} 0.87 (H-20) to C-1 (δ_{C} 32.7), C-9 (δ_{C} 51.1), and C-10 (δ_{C} 37.0), and δ_{H} 0.89 (H-19) to C-4 (δ_{C}

37.4), C-5 (δ_C 49.3), C-10 (δ_C 37.0), and C-20 (δ_C 15.1). Based on the above spectral data analysis and comparison with literature data, compound **100** was elucidated as a spongiane diterpenoid lactone, 3-hydroxyisoagatholactone, reported herein as a novel compound. The structure of the compounds isolated and fully characterized are listed in Fig 21.

Table 4 1D and 2D spectral data of isolated compound **100** (400 MHz, CDCl₃) compared to ¹³C NMR of spongian diterpene lactone skeleton (**99**) (González et al., 1984), (360 MHz, CDCl₃), δ (ppm) and J (Hz)

Compound 100					¹³ C, (Cimino et al., 1974; González et al., 1984).
No	¹ H (J)	¹³ C	COSY	HMBC	
1	1.60 (m, 2H)	32.7		C-2, 3, 10	39.1
2	1.52 (m, 2H)	25.0	H-3	C-1, 3	18.3
3	3.42 (t, 2.9, 1H)	75.7	H-2	C-1, 4, 5, 19	41.7
4		37.4			33.1
5	1.36 (m, 1H)	49.3		C-10	56.6
6	1.39 (m, 2H)	17.9		C-5, 7	18.7
7	1.45 (m, 2H)	40.6		C-6, 8	40.7
8		34.4			34.4
9	1.36 (m, 1H)	51.1		C-7, 10	51.1
10		37.0			37.2
11	2.31 (m, 2H)	24.1	H-9, H- 12	C-9, 12, 13	24.1
12	6.85 (dd, 3.5, 3.5, 1H)	136.4	H-11	C-11, 13, 14, 16	136.2
13		126.9			127.0
14	2.79 (m, 1H)	53.9	H-15	C-13, 15	54.4
15	4.03, 4.36 (each t, 9.1, 1H)	67.2	H-14	C-8, 13, 14	67.70
16		170.3			170.0
17	0.78 (s, 3H)	14.1		C-8	14.1
18	0.94 (s, 3H)	28.3		C-4	33.3
19	0.97 (s, 3H)	22.1		C-3, 4	21.56
20	0.87 (s, 3H)	15.1		C-1, 9, 10,	15.8



— ^1H - ^1H COSY $\rightarrow$ HMBC

Fig. 20: Key ^1H - ^1H COSY and HMBC correlations of compound 100.

Compound 101

Compound **101** (41 mg) was obtained as a brown solid with melting point of 157-159 °C. The TLC profile showed R_f value of 0.47 with DCM/MeH (9:1) as mobile phase. The ^1H NMR (400 MHz, Methanol- D_4) displayed two group of AA'BB' coupling aromatic protons signals at δ 7.16 (d, J = 8.6 Hz, H-2/6)/6.79 (d, J = 8.6 Hz, H-3/5), and at δ 7.06 (d, J = 8.7 Hz, H-2'/6')/6.67 (d, J = 8.8 Hz, H-3'/5') that showed the presence of two *p*-substituted phenyl structures (Table 5, Appendix 4A). The proton signals at δ 6.19 (d, J = 3.0 Hz, H-10, 14) and 6.18 (d, J = 2.0 Hz, H-12) are assignable to 1, 3, 5-trisubstituted type benzene ring. The ^1H NMR spectrum also indicated the presence of *trans* olefinic protons at 6.84 (d, J = 16.4 Hz, H-7') and 6.59 (d, J = 16.4 Hz, H-8'). The proton signals at δ 5.39 (d, J = 6.6 Hz, H-7) and δ 4.37 (d, J = 6.6 Hz, H-8) suggested the presence of benzofuran ring. The ^{13}C spectrum of compound **101** displayed 22 well resolved peaks assignable to 28 carbons (Table 5, Appendix 4B). The DETP-135 (Appendix 4C) along with the ^{13}C NMR spectrum identify the presence of signals of eleven aromatic quaternary, thirteen aromatic methine, two olefinic, and two aliphatic methine carbons. The signals at δ 162.6 (C-11'), 159.9 (C-11, 13), 159.7 (C-13'), 159.4 (C-4), 158.3 (C-4'), 147.3 (C-9), 136.8 (C-9'), 133.8 (C-1), 131.1 (C-1'), and 120.0 (C-10') ascribed for the quaternary aromatic carbons. The signals at δ 128.7 (C-2'/6'), 128.1 (C-2/6), 120.0 (C-10'), 116.3 (C-3/5), 116.2 (C-3'/5'), 107.4 (C-10/14), 102.1 (C-12), and 96.8 (C-12') represent the aromatic methine

carbon. The olefinic carbon signals appeared at 130.3 (C-7') and 123.6 (C-8'). The two aliphatic methine signals appeared at 94.7 (C-7) and 58.2 (C-8) correspond to the bezofurane aliphatic methines. All proton multiplicities and protonated carbons were assigned by the result from COSY and HSQC spectrum (Appendix 4D and 4E, respectively). In the HMBC spectrum, the correlations H2/6, H3/5, H2'/6', and H3'/5' with C3/5, 4, C1, 4, C4', and C2'/6', respectively verified the presence of two *p*-substituted phenyl structures (Appendix 4F). Correlations of aliphatic proton H7 with C1, 2, 4 indicate attachment of the *p*-substituted phenyl ring to the bezofurane ring at C-7 (Fig. 21). Similarly, HMBC correlation of H8 with C9, C10/14 showed the connectivity of 1, 3, 5-trisubstituted benzene ring with the benzofurane ring at C-8. Correlation of the olefinic proton H7' with C2'/6', 8', 9', and H8' with C1', C14' represented the structure of *trans* resveratrol. Thus, based on the above spectral data and comparison with literature (Kurihara, Kawabata, Ichikawa, & Mizutani, 1990; Liu et al., 2021), the structure of compound **101** was found to be similar with previously reported structure of ϵ -viniferin, a stilbene dimer (Fig. 21), isolated for the first time from the roots of *C. cyphopetalum*.

Table 5: 1D and 2D NMR spectra of compound **101** (400 MHz, CD₃OD) compared to literature [195], (500 MHz, acetone-*d*₆), δ (ppm) and *J* (Hz)

.Compound 101					[195]	
No	¹ H (<i>J</i>)	¹³ C	COSY	HMBC	¹ H (<i>J</i>)	¹³ C
1		133.8				134.4
2, 6	7.16 (d, 8.6Hz, 2H)	128.2	H-3,5	C-4, 3/5	7.14 (d, 9.0 Hz)	128.7
3, 5	6.79 (d, 8.6Hz, 2H)	116.3	H-2,6	C-1, 4,	6.76 (d, 9.0 Hz)	116.8
4		159.4				158.9
7	5.39 (d, 6.6 Hz, 1H)	94.8		C-1, 8,	5.36 (d, 6.5 Hz)	95.3
8	4.37 (d, 6.6 Hz, 1H)	58.2	H-10/14	C-1, 7, 9, 11'	4.35 (d, 6.5 Hz)	58.8
9		147.3				147.9
10, 14	6.19 (d, 3.0 Hz, 2H)	107.6		C-8	6.16 (d, 2.0 Hz)	108.0
11, 13		159.9				160.5
12	6.18 (d, 2.0 Hz, 1H)	102.2			6.18 (t, 2.0 Hz)	102.7
1'		131.1				130.8
2', 6'	7.06 (d, 8.7 Hz, 2H)	128.8		C-4, 3'/5'	7.03 (d, 9.0 Hz)	129.3
3', 5'	6.67 (d, 8.8 Hz, 2H)	116.3		C-4',C2'/6'	6.65 (d, 9.0 Hz)	116.9
4'		158.3				159.0
7'	6.84 (d, 16.4 Hz, 1H)	130.3	H-8'	C-2'/6', 8'	6.81(d, 16.0 Hz)	130.9
8'	6.59 (d, 16.5 Hz, 1H)	123.6	H-7'	C-1',7', 9', 14'	6.56(d, 16.0 Hz)	124.2
9'		136.8				137.4
10'		120.0				120.6
11'		162.7				163.2
12'	6.25 (s, 1H)	96.8		C-9',	6.25 (d, 2.0 Hz)	97.4
13'		159.7				160.2
14'	6.63 (s, 1H)	104.3		C-7', 9',	6.63 (d, 2.0 Hz)	104.9

Compound 102

Compound **102** (6 mg) was obtained as brown solid with melting point of 183-186 °C. The TLC profile showed R_f value of 0.30 in DCM/MeOH (9/1) eluent. The ^1H NMR (400 MHz, MeOD) spectra displayed three sets of aromatic signals at 7.15 (2H, H-2/ 6)/6.72 (2H, H-3/5), 7.04 (2H, H-2''/6'')/6.66 (2H, H-3''/5''), and 6.95 (2H, H-2'/6')/6.77 (2H, H-3'/5'), that are assigned to three *p*-substituted phenyl moieties (A1, B2, and C1 rings) (Table 6, Appendix 5A). The two AX_2 type coupling proton signals at 5.93 (d, $J = 2.2$ Hz, 2H)/6.10 (d, $J = 2.2$ Hz, 1H), and 6.17 (d, $J = 2.3$ Hz, 2H)/ 6.22 (d, $J = 2.3$ Hz, 1H) attributed to two 1, 3, 5-trisubstituted aromatic rings (A2 and C2 rings). The proton signals at δ 6.82 ($J=16.4$ Hz) and 6.58 ($J=16.4$ Hz) suggests the presence of *trans*-coupled olefinic protons. The two sets of doublet ($J=6.6$ Hz) aliphatic proton signals at 5.37, H-7)/4.35 (H-8) and 5.19 (H-7'')/3.79 (H-8'') are in good agreement with methine proton signals of two 1, 2-diarylbenzofuranse. The ^{13}C NMR (101 MHz, MeOD) spectra displayed 32 peaks assignable 42 carbones (Table 6 Appendix 5B). The DEPT-135 (Table 6 Appendix 5C) along with its ^{13}C nar displayed the presence aromatic quaternary carbons at δ 162.8 (C-13'), 160.1 (C-11'), 159.7 (C-11/13), 158.5 (C-11''/13''), 158.4 (C-4), 157.8 (C-4''), 147.3 (C-9), 147.4 (C-9''), 136.9 (C-1), 137.8 (C-1''), 133.9 (C-9'), 133.8 (C-1'), 120.3 (C-14'), and 120.0 (C-10'). The carbon signals at 128.7 (C-2'/6'), 128.5 (C-2''/6''), 128.2 (C-2/6), 116.4 (C-2/6), 116.2 (C-3''/5''), 115.8 (C-3'/5'), and 96.6 (C-12') are in agreement with aromatic methine carbon signals of the three *p*-substituted phenyl group. The aromatic methine signals at 107.1 (C-10/14), 106.7 (C-10'', 14''), 101.8 (C-12''), and 101.5 (C-12) ascribed to the carbons of the 1, 3, 5-trisubstituted aromatic rings. The carbon signals at 128.7 (C-7'), and 123.7 (C-8') correspond to olefinic carbon signals. The carbon signals appeared at 94.9, 94.8, 58.3, and 57.7 are respectively assignable to the C-7, 7'', 8, and 8'' of aliphatic methine carbons of the two 1, 2-diarylbenzofurane rings.

The COSY spectrum showed ^1H - ^1H correlation of aromatic protons H2/6 with H3/5, H2''/6'' with H3''/5'', olefinic protons at H7' with H8', and aliphatic protons H7 with H8, and H7''with H8'', among others (Table 6, Appendix 5D). The HSQC spectral data supported each of the carbon-hydrogen connectivity (Appendix 5E). The HMBC spectrum revealed correlations at H7' and H8' with C8', 9' and C1', 14', respectively, demonstrating that aromatic ring A1 and ring A2 are connected across the C7'–C8' pattern (Table 6, Appendix 5F). The HMBC correlations

H7 with C2/6, and H7" with C1", 2"/6", respectively suggested the attachment of ring B1 and ring C1 (*p*-substituted groups) with each benzofurane rings across the C1–C7, and C1"–C7" patterns. Similarly, the correlations H8 with C9, 10/14, and H8" with C9", 10"/14" support the connection of ring B2, and ring C2 (1, 3, 5-trisubstituted benzene rings), with the benzofurane rings across the C8–C9 and C8"–C9" patterns. The reported spectral data is found to be in good agreement with previously reported data of Gnetin H, stilbene trimer (He et al., 2010; Ito et al., 2012). Thus, based on the above spectral data and the data from literature, the chemical structure of compound **102** was proposed to be identical with that of Gnetin H, stilbene trimer, which is reported from the root of *C. cyphopetalum* for the first time (Fig. 21).

Table 6: 1D and 2D NMR spectra of compound **102** (400 MHz, CD₃OD) compared to literature (He et al., 2010; Ito et al., 2012), 400 MHz, acetone *d*₆, δ (ppm) and *J* (Hz)

Compound 102					(He et al., 2010; Ito et al., 2012)	
No	¹ H (J)	¹³ C	COSY	HMBC	¹ H (J)	¹³ C
1		136.9				134.7
2, 6	7.15 (d, 8.6 Hz, 2H)	128.2	H-3/5	C-3/5, 7	7.18 (d, 8.5)	128.6
3, 5	6.77 (d, 8.6 Hz, 2H)	116.4			6.79 (d, 8.5)	116.9
4		158.4				159.0
7	5.37 (d, 6.6 Hz, 1H)	94.9	H-8	C-2/6, 8, 9	5.40 (d, 5.5)	95.3
8	4.35 (d, 6.6 Hz, 1H)	58.3		C-9,14/10'	4.40 (d, 5.5)	59.4
9		147.3				148.0
10, 14	6.17 (d, 2.3 Hz, 2H)	107.0	H-14	C-9,	6.14 (s)	107.8
11, 13		159.7				160.6
12	6.24 (s, 1H)	101.5	H-10/14		6.14 (s)	102.7
1'		133.8				131.1
2', 6'	6.92 (d, 8.4 Hz, 2H)	128.2	H-3'/5'		6.69 (d, 8.5)	129.2
3' 5'	6.72 (d, 8.6 Hz, 2H)	115.7		C-2'/3',7'	6.51 (d, 8.5)	116.7
4'		158.4				158.8
7'	6.82 (d, 16.4 Hz, 1H)	128.7	H-8'	C-8',9'	6.38 (s)	129.2
8'	6.58 (d, 16.9 Hz, 1H)	123.7		C-1',14'		123.0
9'		133.9				135.0
10'		120.0				120.8
11'		160.0				163.5
12'	6.18 (s, 1H)	96.6			6.42 (s)	92.0
13'		162.8				163.5
14'		120.3				120.8
1''		137.8				134.2
2'', 6''	7.04 (d, 8.8 Hz, 2H)	128.5	H-3''/5''	C-3''/5''	7.18(dd, 2.0, 8.5)	128.6
3'', 5''	6.66 (d, 8.6 Hz, 2H).	116.2			6.78 (dd, 2.0, 8.5)	116.9
4''		157.8				159.0
7''	5.19 (d, 6.2 Hz, 1H)	94.8	H-8''	C-1'',8'',9	5.40 (d, 5.5)	95.3
8''	3.79 (d, 6.4 Hz, 1H)	57.7		C-7'',9''	4.40 (d, 5.5)	59.4
9''		147.4				148.0
10'', 14''	5.93 (d, 2.2 Hz, 2H)	106.7	H-10,14''	C-9'',8''	6.14 (s)	107.8
11'', 13''		158.5				160.6
12''	6.18(dd, 6.8, 2.3Hz, 1H)	101.8	H-10''/14''		6.14 (s)	102.7

Compound 103

Compound **103** (20 mg) was obtained as a pale brown solid with melting point 164-167°C, and TLC *R_f* value of 0.27 in DCM/MeOH (9:1) as eluent. The ¹H NMR (400 MHz, MeOD) displayed

two AA'BB' type spin system aromatic signals at 7.27 (d, $J = 8.5$ Hz, 4H, H-2/6, 2'/6') and 6.76 (d, $J = 8.5$ Hz, 4H, H-3/5, 3'/5') that suggested the presence *p*-substituted phenyl moieties (ring A and D) (Table 7, Appendix 6A). The high intensity signal at 6.02 (s, 6H) attributed to six protons of two 1,3,5-trisubstituted resorcinol moieties (B and D rings). The two vicinal coupling methine protons signals at δ 5.40 (d, $J=4.4$ Hz, 2H), and 3.58 (d, $J = 4.4$ Hz, 2H,) are respectively assignable to the H-7/7', and H-8/8' protons of the tetrahydrofuran ring (ring C). The ^{13}C NMR (101 MHz, MeOD) displayed 10 well resolved signals (Table 7, Appendix 6B). The DEPT-135 (Appendix 6C) was used to analyze the types of the alkyl groups, and the signals at δ 158.8 (C-11/13, 11'/13'), 158.0 (C-4/4'), 142.1 (C-9/9'), and 133.9 (C1/1') assigned to ten sp^2 aromatic quaternary carbons. The aromatic carbon signals at 128.8 (C-2/6, 2'/6') and 116.1 (C-3/5, 3'/5') ascribed for the *p*-substituted phenyl moieties. Similarly, the carbon signals at 109.0 (C-10/14, 10'/14') and 101.8 (C-12/12') are consistent with aromatic methine carbons of the of two 1,3,5-trisubstituted groups. The two sp^3 methine signals at δ 85.9 (C-7/7') and 59.1 (C-8/8') correspond to the carbons of the tetrahydrofuran moiety.

The 2D spectral data was also used to analyze the structure of compound **103**. The COSY correlations at H2/6 with H3/5, H2'/6' with H3'/5', and H7/7' with H8/8' supported AA'BB' type proton couplings of the two *p*-substituted phenols (Appendix 6D). The HSQC displayed the correlation of the carbon atoms with their respective carbons (Appendix 6E). The HMBC showed correlations H-7/7' with C-2/6, 2'/6', and H-2'/6', 2/6 with C-7/7' which suggested the connection of ring A and ring D (*p*-substituted phenyl groups) to the tetrahydrofuran (ring C) across C-1–C-7 and C-1'–C-7' connectivity pattern, respectively (Appendix 6F). The correlations H8/8' with C9/9', 10/14, 10'/14' signified the linkage of ring B and E to the tetrahydrofuran ring (ring C) across C-8–C-9 and C-8'–C-9' connectivity pattern, respectively. The reported spectral data is found to be in good agreement with previously reported data of Tricuspidatol A, stilbene dimer (Cichewicz, Kouzi, & Hamann, 2000; Lins, Felicio, Braggio, & Roque, 1991). Thus, based on the above spectral data and the data from literature, the chemical structure of compound **103** was suggested to be identical with that of Tricuspidatol A (Fig. 21), which was obtained for the first time from the root of *C. cyphopetalum*.

Table 7: 1D and 2D NMR spectra of compound **103** (400 MHz, CD₃OD) compared to literature (Cichewicz et al., 2000; Lins et al., 1991), 400 MHz, acetone-*d*₆, δ (ppm) and *J* (Hz)

Compound 103					(Cichewicz et al., 2000; Lins et al., 1991)	
No	¹ H (<i>J</i>)	¹³ C,	COSY	HMBC	¹ H	¹³ C
1, 1'		133.9				131.7
2/6,	7.27 (d, 8.4	128.8	H-3/5;	C-4/7; C-4'/7'	7.17 d	128.1
2'/6'	Hz, 4H)		3'/5'		(8.4)	
3/5,	6.76 (d, 8.4	116.1		C-1; C-1'	6.64 d	114.2
3'/5'	Hz, 4H)				(8.4)	
4, 4'		158.0				155.9
7, 7'	5.40 (d, 4.4	85.9	H-8, 8'	C-2/6; C-2'/6'	5.82 d	83.6
	Hz, 2H)				(4.4)	
8, 8'	3.58 (d, 4.4	59.0		C-9, 10/14;	3.84 dd	59.2
	Hz, 2H)			C-9', 10'/14'	(4.4, 6.2	
9, 9'		142.1				142.6
10/14;	6.02 (s, 4H)	109.0		C-8, 12; C-8',	6.08 d	108.0
10'/14'				12'	(2.2)	
11/13;		158.8				157.7
11'/13'						
12, 12'	6.02 (bs, 2H)	101.8			6.05 t (2.2	100.5

Compound 104

Compound **104** (40 mg) was obtained as an amorphous powder with TLC R_f value of 0.33 in DCM/MeOH (9:1) eluent. The ¹H NMR (400 MHz, MeOD) spectral data revealed the presence of two *ortho*-coupled aromatic proton signals [δ 7.14 (d, *J* = 8.6 Hz, H-2'/6')/ 6.71 (d, *J* = 8.6 Hz, H-3'/5'), and 6.93 (d, *J* = 8.5 Hz, H-2/6)/ 6.58 (d, *J* = 8.5 Hz, H-3/5)] assignable to two *p*-substituted phenyl groups (ring A1 and B1) (Table 8, Appendix 7A). The aromatic proton signals at 6.09 (brs, 1H, H-12), and 5.93 (d, *J* = 2.2 Hz, 2H, H-10/14) are in agreement with the protons signals of 1,3,5-dtrisubstituted benzene ring (ring A2). The aliphatic methine proton signals at 4.07 (d, *J* = 6.6 Hz, H-7'/8'), 5.18 (d, *J* = 6.2 Hz, H-7), and 3.78 (d, *J* = 6.2 Hz, H-8)

attributed to the bezofurane ring (ring B2). The ^{13}C NMR spectrum of compound **104** displayed 22 well resolved carbon signals assignable to 28 carbons of the compound (Table 8, Appendix 7B). The DEPT-135 spectrum of compound **104** revealed the presence of eleven aromatic quaternary [δ 162.7 (C-11'), 158.4 (C-4), 160.0 (C-11/13), 157.8 (C-4'), 159.5 (C-13'), 147.3 (C-9), 146.8 (C-9'), 131.6.8 (C-1'), 133.8 (C-1), 120.3 (C-10')], thirteen aromatic methine [131.0 (C-2/6), 128.5 (C-2'/6'), 116.3 (C-3/5), 115.9 (C-3'/5'), 107.2 (C-10/14), 108.0 (C14'), 101.8 (C-12), 96.6 (C-12'), four aliphatic methine [94.9 (C-7), 72.9 (C-7'), 73.2 (C-8'), 57.5 (C-8)] carbon signals (Table 8, Appendix 7C). The aromatic methine signals at 128.5 (C-2/6), 116.3 (C-3/5), 131.6 (C-2'/6'), and 116.3 (C-3'/5') are in agreement with the *p*-substituted phenyl groups (ring A1 and B1). Each H-C connectivity in the structure was determined by the COSY (Appendix 7D) and HSQC (Appendix 7E) correlations. The COSY correlation of δ_{H} 5.18 (H7) with H3.78 (H8) shows a connectivity at C7-C8. The HMBC correlations H8'with C9', and H7 with C2'/6', 14' signified that ring B1and ring B2 attached to each other across the C-7–C-8 pattern (Appendix 7F). The correlations of H8 with C10/14, and H7 with C9 indicated the attachment of the resorcinol moiety (ring A2) with the furan ring (ring C) across the C-8–C-9 pattern. The spectral data of compound **104** was found to be in agreement with previously reported data of ϵ -viniferin diol, stilbene dimer (Oshima et al., 1995). Thus, based on the above spectral data and the data from literature, the chemical structure of compound **104** was proposed to be identical with that of ϵ -viniferin diol, stilbene trimer (Fig. 21), which is reported from the root of *C. cyphoptealum* for the first time.

Table 8: ^1H , and ^{13}C spectra of compound **104** (400 MHz, CD_3OD) compared to literature (Oshima et al., 1995), (500, acetone- d_6), δ (ppm) and J (Hz)

No	Compound 104		(Oshima et al., 1995)	
	$^1\text{H}(J)$	^{13}C	$^1\text{H}(J)$	^{13}C
1		133.8		135.0
2, 6	6.93 (d, 8.5 Hz, 2H)	131.1	7.15 (d, 8.4)	129.2
3, 5	6.58 (d, 8.5 Hz, 2H)	116.2	6.83 (d, 8.4)	116.5
4		158.4		159.1
7	5.18 (d, 6.2 Hz, 1H)	94.9	5.35 (d, 5.1)	93.7
8	3.78 (d, 6.2 Hz, 1H)	57.5	4.4 (d, 5.1)	56.9
9		147.4		148.0
10, 14	5.93 (d, 2.2 Hz, 2H)	107.3	6.04 (d, 2.2)	106.9
11, 13		160.1		160.0
12	6.09 (brs, 1H)	101.8	6.04 (, 2.2)	102.0
1'		131.6		131.2
2', 6'	7.14 (d, 8.6 Hz, 2H)	128.5	6.93 (d, 8.4)	127.5
3', 5'	6.71 (d, 8.6 Hz, 2H)	115.9	6.65 (d, 8.4)	115.4
4'		157.8		158.0
7'	4.07 (d, 6.6 Hz, 1H)	72.9	4.93 (d, 8.7)	75.1
8'	4.07 (d, 6.6 Hz, 1H)	73.2	4.58 (d, 8.7)	75.9
9'		146.8		148.5
10'		120.3		119.2
11'		162.8		161.9
12'	6.23 (d, 2.2 Hz, 1H)	96.6	6.37 (d, 2.2)	96.7
13'		159.5		159.1
14'	6.53 (brs, 1H)	107.7	6.67 (d, 2.2)	108.2

Compound 105

Compound **105** (7 mg) was obtained as a brown amorphous. The TLC R_f value was computed to be 0.58 with DCM/MeOH (9:1) as mobile phase. The ^1H NMR (400 MHz, MeOD) spectrum showed two sets of AA'BB' type signals at δ 7.30 (d, J = 8.6 Hz, 2H, H-2/6)/ 6.79 (d, J = 8.6 Hz, H-3/5), and 6.97 (d, J = 8.6 Hz, H-2'/6')/ 6.71 (d, J = 8.6 Hz, H-3'/5') that are assignable to two *p*-substituted phenyl groups (ring A1 and B1) (Table 9, Appendix 8A). The signal at 6.05 (s, H-10'/14'), and 6.17 (d, J = 2.0 Hz, H-12') are attributed to the aromatic methine protons of 1, 3, 5-trisubstituted benzene ring (ring B2). The signals at 6.25 (d, J = 2.4 Hz, H-14) and 6.19 (d, J = 2.4 Hz, H-12) suggested the presence of 3,5-dioxybenzene ring (ring A2). The group of aliphatic methine signals at 5.43 (d, J = 6.6 Hz, H-7), 3.59 (dd, J = 6.6, 6.3 Hz, H-8), 4.10 (d, J = 6.5 Hz, H-7'), and 4.20 (dd, J = 6.6, 6.3 Hz, H-8') ascribed to suggested a resveratrol dimer with an indane skeleton. The ^{13}C NMR (101 MHz, MeOD) spectrum displayed 23 well resolved carbon signals (Appendix 8B), and the DEPT-135 spectrum (Appendix 8C) shows the carbon signals to be aromatic quaternary [δ 158.0 (C-13), 158.8 (C-11'/13'), 156.7 (C-11), 157.4 (C-4), 156.4 (C-4')], 150.2 (C-9'), 146.2 (C-9), 138.1 (C-1'), 131.2 (C-1), 125.6 (C-10)], aromatic methine signals [130.8 (C-2'/6'), 128.8 (C-2/6), 116.1 (C-3/5), 116.0 (C-3'/5'), 106.7 (C-10'/14'), 106.9 (C-14), 103.6 (C-12), 101.84 (C-12)], and aliphatic methine carbon signals [85.9 (C-7), 60.9 (C-8), 59.1 (C-8'), 55.6 (C-7')] (Table 9). The sp^2 methine signals at 130.8 (C-2'/6'), 128.8 (C-2/6), 116.1 (C-3/5), 116.0 (C-3'/5') supported the presence of two AA'BB' type aromatic rings (A1 and B1). The aliphatic carbon at δ 85.9 attributed to the oxy-methine carbon (C-7).

The COSY spectrum showed correlations of H-2/6 with H-3/5, and H-2'/6' with H-3'/5'. H-7 with H-8 and H-7' with H-8' that is in agreement with the AA'BB' coupling system of the *p*-substituted phenyl group (Appendix 8D). The HSQC displayed the each H-C connectivity (Appendix 8E). The HMBC spectrum showed correlation of H-2/6 with C-7, and H-2'/6' with C-7' that signify the *p*-substituted phenyl rings (ring A1 and B1) attached across the C-1–C-7 and C-1'–C-7' pattern (Appendix 8F). The correlation of H-8' with C-10'/14' suggested the connectivity of the 1,3,5-trisubstituted benzene ring (B2) across C-8'–C-9' pattern. The H-7 with C-10'/14' suggested that the methoxy group is oriented towards the resorcinol ring (B2). The reported spectral data was found to be in good agreement with previously reported data of parthenostilbenin B, stilbene dimer (Kim, Saleem, Seo, Jin, & Lee, 2005). Thus, the chemical structure of compound **105** was

found to be identical with that of parthenostilbenin B, (Fig. 21), which was reported from the root of *C. cyphopetalum* for the first time.

Table 9: 1D and 2D NMR spectra of compound **105** (400 MHz, CD₃OD) compared to literature (Kim et al., 2005), 300 MHz, acetone-*d*₆, δ (ppm) and *J* (Hz).

Compound 105					(Kim et al., 2005)	
No	¹ H (<i>J</i>)	¹³ C	COSY	HMBC	¹ H	¹³ C
1		131.2				131.3
2, 6	7.30 (d, <i>J</i> = 8.6 Hz, 2H)	128.8	H-3, 5	C-7	7.05 (d, 8.5)	129.9
3, 5	6.79 (d, <i>J</i> = 8.6 Hz, 2H)	116.1	H-2, 6		6.82 (d, 8.5)	115.1
4		157.4				157.4
7	5.43 (d, <i>J</i> = 6.6 Hz, 1H)	85.9	H-8	C-1, 2,6	4.30 (d, 8.0)	86.9
8	3.59 (dd, <i>J</i> = 6.6, 6.3 Hz, 1H)	60.9	H-7	C-14	3.42 (dd, 8.4, 3.5)	60.7
9		146.2				146.3
10		125.6				122.7
11		156.7				154.5
12	6.19 (d, <i>J</i> = 2.4 Hz, 1H)	103.6	H-10/14		6.27 (d, 2.0)	102.0
13		158.0				158.1
14	6.25 (d, <i>J</i> = 2.4 Hz, 1H)	106.9			5.74 (d, 1.8)	105.3
1'		138.1				137.4
2', 6'	6.97 (d, <i>J</i> = 8.6 Hz, 2H)	128.8	H-3', 5'		6.89 (d, 8.4)	128.8
3', 5'	6.71 (d, <i>J</i> = 8.6 Hz, 2H)	116.1		C-1', 7'	6.77 (d, 8.6)	115.1
4'		156.4				155.9
7'	4.10 (d, <i>J</i> = 6.5 Hz, 1H)	55.6		C-1', 8'	3.93 (d, 3.0)	55.2
8'	4.20 (dd, <i>J</i> = 6.6, 6.3 Hz, 1H)	59.1		C-1', 9'	3.46 (t, 3.5)	58.8
9'		150.3				150.5
10', 14'	6.05 (s, 2H)	106.9		C-8'	6.09 (d, 1.8)	105.8
11', 13'		158.8				158.9
12'	6.17 (d, <i>J</i> = 2.0 Hz, 1H)	101.8		C-10', 14', 8'	6.20 (d, 1.8)	100.7
OCH ₃	3.58 (d, <i>J</i> = 4.8 Hz, 3H)	55.6		C-10', 14'	2.96 (3H, s)	55.8

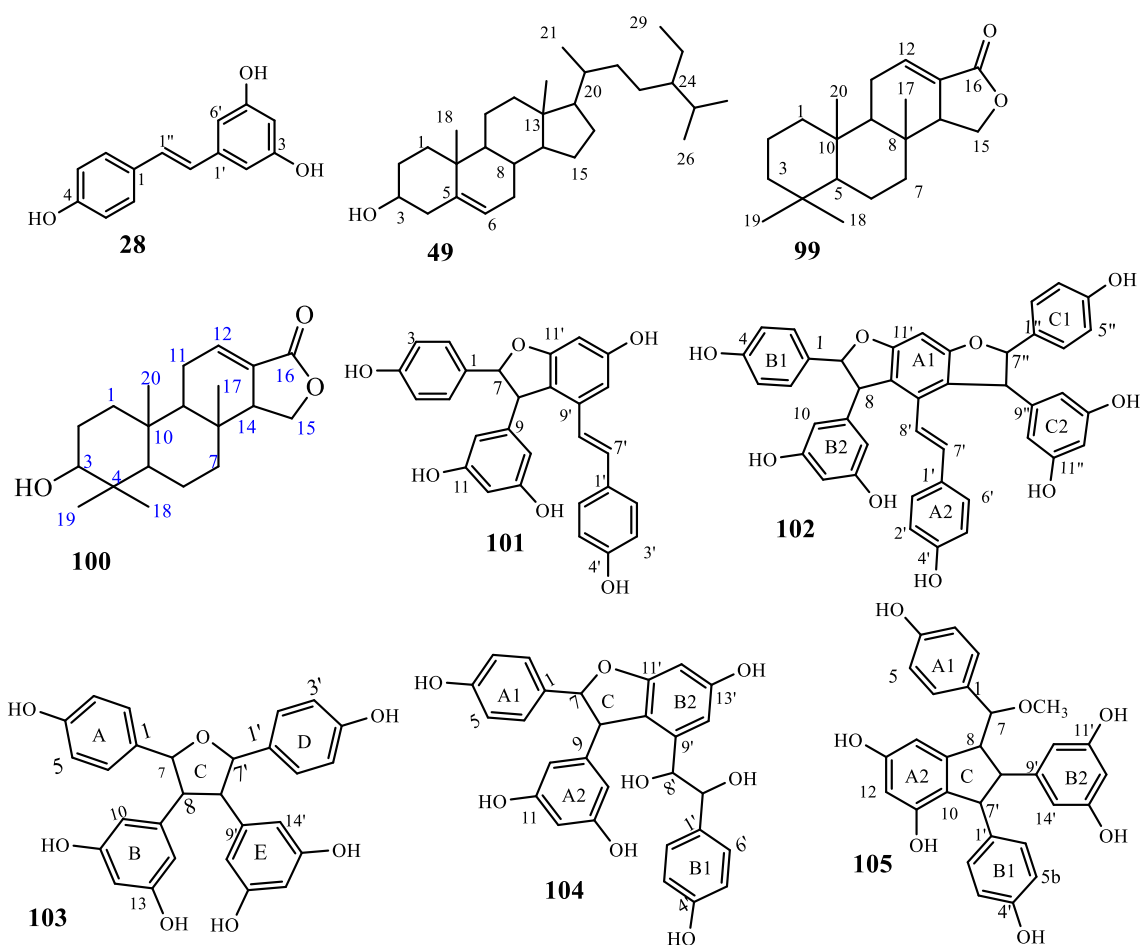


Fig. 21: Structure of compounds isolated from roots of *C. cyphopetalum* and previously reported spongiane diterpen lactone (**99**) (González et al., 1984)

4.1.2. Compounds isolated from root extract of *H. johannis*

The dichloromethane/methanol (1:1) root extracts of *H. johannis* was subjected to gravity column chromatography over silica gel to afford six compounds (**40**), oleic acid (**41**) **49**, **106-109**) (Fig. 22), reported from this plant for the first time. The structures of each of the isolated compounds were elucidated by different spectroscopic techniques and comparison of their spectroscopic data to those previously reported in the literature.

Compound **40**

Compound **40** was obtained as brown solid with TLC profile R_f value of 0.32 with DCM/MeOH (9/1) as eluent. The ^1H NMR (400 MHz, MeOD) displayed aromatic proton signals at δ 6.74 (d, $J = 1.9$ Hz, 1H), 6.68 (d, $J = 8.1$ Hz, 1H), 6.65 (dd, $J = 8.1, 1.9$ Hz, 1H), 5.84 (d, $J = 1.2$ Hz, 1H), 5.81 (d, $J = 1.2$ Hz, 1H), and aliphatic proton signals at δ 4.46 (d, $J = 7.9$ Hz, 1H), 3.88 (m, 1H), 2.81 (dd, $J = 16.0, 5.5$ Hz, 1H), 2.41 (dd, $J = 16.0, 5.5$ Hz, 1H) (Table 10, Appendix 9A). The aromatic methine proton signals at δ 6.74 (d), 6.6 (d), 6.65 (dd), δ 5.84 (d) and 5.81 (d) were assigned to H-2', H-5' H-6', H-6, and H-8 of a flavan-3-ol moiety, respectively (Fig 22). The aliphatic methine proton signals at δ 4.58 (d), 3.94 (m), and methine protons at 2.87 (dd)/2.52 (dd) were assigned to H-2, H-3, and methylene proton H-4, respectively. The ^{13}C NMR (100 MHz) (Appendix 9B), together with its DEPT-135 spectra (Appendix 9C) of compound **40** displayed 15 carbon signals corresponding to seven quaternary carbons at δ 155.9 (C-7), 155.7 (C-5), 155.3 (C-9), 144.6 (C-3'), 144.4 (C-4'), 130.2 (C-1'), 99.8 (C-10), seven methines carbons at δ 119.2 (C-6'), 115.1 (C-5'), 114.1 (C-2'), 95.3 (C-6), 94.6 (C-8), 81.3 (C-2) and 67.4 (C-3), and one methylene at δ 27.3 (C-4) (Table 10). The obtained spectral data is found to be in good agreement with previously reported data of catechin (Cho et al., 2020; Ismail et al., 2021). Thus, based on the mentioned spectral data and the data from literature, the chemical structure of compound **40** was drawn as it is shown in Fig. 22.

Table 10: ^1H (400 MHz, MeOD), ^{13}C (100 MHz) NMR spectra of compound **40**
compared to literature (Cho et al., 2020), 400 MHz, CD_3OD , δ (ppm) and J (Hz).

No	Compound 40		^{13}C , (Cho et al., 2020)
	^1H	^{13}C	
2	4.46 (d, $J = 7.9$ Hz, 1H)	81.3	81.4
3	3.88 (m, 1H)	67.4	66.8
4	2.81 (dd, $J = 16.0, 5.5$ Hz, 1H), 2.41 (dd, $J = 16.0, 5.5$ Hz, 1H),	27.3	28.2
5		155.7	156.6
6	5.81 (d, $J = 1.2$ Hz, 1H)	95.3	95.6
7		155.9	156.9
8	5.84 (d, $J = 1.2$ Hz, 1H)	94.6	94.4
9		155.3	155.8
10		99.3	99.6
1'		130.2	131.1
2'	6.74 (d, $J = 1.9$ Hz, 1H)	114.1	114.9
3'		144.6	145.3
4'		144.4	145.3
5'	6.68 (d, $J = 8.1$ Hz, 1H)	115.1	115.6
6'	6.65 (dd, $J = 8.1, 1.9$ Hz, 1H)	119.2	118.9

Compound 41

Compound **41** was obtained as yellow amorphous solid with melting point of 13.3-14 °C. The TLC profile displayed R_f value determined as 0.47 (10% EtOAc in *n*-hexane). The ^1H -NMR (400 MHz, CDCl_3) spectrum showed multiplet signal at δ 0.99 which assigned to methyl protons (H18) (Table 11, Appendix 10A). The multiplicities at δ 1.83 (m) are assignable to allylic protons and was assigned to the two methylene protons at the position adjacent to the double bond (H8 and H11). The signal at δ H 2.31 assigned to methylene protons adjacent to carbonyl carbon. The multiplet signals at δ H 5.33 is attributed to olefinic methylene protons (H9 and H10). The ^{13}C -NMR (101 MHz, CDCl_3) spectrum (Appendix 10B), together with its DEPT-135

(Appendix 10C) displayed methylene signals at δ 34.0 (C2), 31.6 (C8, C11), 29.5 (C14, C15), 29.4 (C13), 29.3 (C12), 29.2 (C6, C7), 27.3 (C3), 25.0 (C4), 24.8 (C3), 22.8 (C16), and 21.2 (C17), olefinic carbon signals at δ 130.1 (9), 129.8 (C10), signals of methyl group at δ 14.2 (C18), and signals of carbonyl carbon at δ 179.6 (CO) (Table 11). The methylene carbon signals resonated in the range of 34.0 to 21.2 indicate the presence of open aliphatic chain. The signals at 130.1 (9), 129.8 (C10) are characteristics of olefinic carbons of oleic acid chain positioned at a lower frequency separation (Akintayo, Adeyeye, Akintayo, & Oyewusi, 2004). The signal at δ 14.2 (C18) represent a terminal methyl group. The carbonyl signal at δ 179.6 is assignable to carboxylic carbonyl carbon. Based on the above spectral data and comparison of this data with the literature values (Akintayo et al., 2004; Dessalegn Wako Kumsa, 2018), compound **41** is proposed to be same as oleic acid, Fig. 22.

Table 11: ^1H (400 MHz, CDCl_3) and ^{13}C (100 MHz) NMR spectra of compound **41** compared to literature (Akintayo et al., 2004; Dessalegn Wako Kumsa, 2018), 400 MHz, CDCl_3 , δ (ppm) and J (Hz).

Compound 41			(Akintayo et al., 2004; Dessalegn 2018)	
No	^1H	^{13}C	^1H	^{13}C
2	2.31 (m, 2H)	34.0	2.51(2H,t,H-2)	34.1
3	1.63 (m, 2H)	24.8	1.49(2H,m,H-3)	24.8
4	2.01 (m, 2H)	25.0	2.04(2H,m,H-4)	25.6
5	2.01 (m, 2H)	27.3	2.04(2H,m,H-5)	27.0
6	2.01(m, 2H)	29.2	2.04(2H,m,H-6)	29.0
7	2.01 (m, 2H)	29.2	2.04(2H,m,H-7)	29.1
8	1.83 (m, 2H)	31.6	1.97(2H,m,H-8)	31.3
9	5.33 (m,1H)	130.1	5.33(1H,d)	130.0
10	5.33 (m, 1H)	129.8	5.33	128.70
11	1.83 (m, 2H)	31.6	2.19(2H,m,H-11)	31.7
12	2.01 (m, 2H)	29.3	2,04(2H,m,H-12)	29.3
13	2.01 (m, 2H)	29.4	2,04(2H,m,H-13)	29.4
14	2.01 (m, 2H)	29.5	2.04(2H,m,H-14)	29.5
15	2.01 (m, 2H)	29.5	2.04(2H,m,H-15)	29.5
16	1.83 (m, 2H)	22.8	2.19(2H,m,H-16)	22.8
17	1.83 (m, 2H)	21.2	2.19(2H,m,H-17)	18
18	0.99 (m, 3H)	14.2	0.96(3H,t,H-18)	14.3
C=O		179.6		176.8

Compound **49**

Compound **49** (20 mg) was obtained as a solid with melting point in the rage of 134 to 136°C. The TLC profile showed R_f value of 0.62 with DCM/MeOH (9:1) mobile phase. The NMR spectral data of Compound 49 presented in Table 3 and Appendix 1A-3B (Pierre & Moses, 2015) (Fig 21).

Compound **106**

Compound **106** (57 mg) was obtained as a solid. The TLC profile showed R_f value of 0.47 with DCM/MeOH (9/1) mobile phase. The ^1H NMR (400 MHz, CD_3OD) spectrum revealed the presence aromatic protons at δ 6.86 (d, J = 1.9 Hz, 1H, H-2'), δ 6.78 (d, J = 8.1 Hz, 1H, H-5')

and δ 6.73 (dd, J = 8.1, 1.9 Hz, 1H, H-6') with AX2 spin system (ring B), and at δ 5.95 (d, J = 2.3 Hz, 1H, H6) and δ 5.87 (d, J = 2.3 Hz 1H, H8) assignable to AX type system (ring A) (Table 12 Appendix 11A). The protons signals at δ 4.58 (d, J = 7.5 Hz, 1H, H2)/3.94 (m, 1H, H3), and at 2.87 (dd, J = 16.1, 5.5 Hz, 1H, H4a)/2.52 (dd, J = 16.1, 5.5 Hz, 1H, H4b) were assignable to methine and methylene protons of ring C, respectively. The ^1H NMR also displayed proton signals assignable to sugar moiety at δ 4.31 (d, J = 7.7 Hz, 1H, H-1''), and ranging δ 3.21 to 3.71 (6H, H-2'' to H-6''). The ^{13}C NMR (100 MHz, CD_3OD) (Appendix 11B) and DEPT-135 (Appendix 11C) spectra displayed seven aromatic quaternary signals at δ 157.66 (C-5), 157.44 (C-7), 156.79 (C-9), 146.10 (C-3'), and 146.07 (C-4') assignable to oxygenated sp^2 aromatic quaternary carbons, δ 132.10 (C-1') and 100.79 (C-10), and five sp^2 methine signals at δ 96.2 (C-6), 94.5 (C-8), 115.2 (C-2'), 116.1 (C-5') and 120.0 (C-6'), assigned to aromatic carbons of ring A and B (Table 12). The ^{13}C spectrum also displayed two sp^3 oxy-methine signals δ 82.6 (C-2) and 68.6 (C-3), and one methylene signals at δ 28.3 (C-4), assignable to methine and methylene carbons of ring C. Furthermore, the ^{13}C spectrum revealed characteristic sugar peaks at δ 104 (C-1''), 75.0 (C2''), 77.9 (C-3''), 72.0 (C-4''), 77.7 (C-5''), and 61.6 (C-1''). The methylene absorption at δ 61.6 suggests the sugar to be glucopyranose group. The linkages and substitution patterns were established from the COSY (Appendix 11D), HSQC (Appendix 11E) and HMBC analyses (Table 12, Appendix 11F). The HMBC spectrum displayed 3J correlations between proton at δ 4.31 (H-1'') with 96.2 (C-6) and proton 5.87 (H-8) with 104.1 (C1'') which suggest the attachment of the glucopyranose moiety to C-7 of ring A. Based on the reported spectral data and the data from literature (Friedrich & Galensa, 2002), the structure of compound **106** is identical with the falvan-3-ol-7-O-glycoside (catechin-7-O-glycoside, (Fig. 22), reported herein for the first time from roots of *H. johannis*.

Table 12: 1D and 2D NMR (400 CD₃OD) spectral data of compound **106** compared to literature (Friedrich & Galensa, 2002), 600 MHz, MeOH-*d*₄, δ (ppm) and *J* (Hz)

Compound 106				¹³ C, (Friedrich & Galensa, 2002)
No	¹ H	¹³ C	HMBC	
2	4.58 (d, <i>J</i> = 7.5 Hz, 1H)	82.6	C-3,4,9,1',2',6'	83.0
3	3.94 (m, 1H)	68.6		68.6
4	2.87 (dd, <i>J</i> = 16.2, 5.5 Hz, 1H) 2.52 (dd, <i>J</i> = 16.2, 8.2 Hz, 1H)	28.3	C-9, 10	28.6
5		157.4		157.6
6	5.95 (d, <i>J</i> = 2.3 Hz, 1H)	96.2	C-9	97.5
7		157.6		158.7
8	5.87 (d, <i>J</i> = 2.3 Hz, 1H)	95.4	C-1''	97.0
9		156.7		156.9
10		100.7		103.7
1'		132.1		132.1
2'	6.86 (d, <i>J</i> = 1.9 Hz, 1H)	115.2	C-2, 3'	115.3
3'		146.1		146.3
4'		146.0		146.3
5'	6.78 (d, <i>J</i> = 8.1 Hz, 1H)	116.1	C-1'	116.1
6'	6.73 (dd, <i>J</i> = 8.1, 1.9 Hz, 1H)	120.0		120.0
1''	4.31 (d, <i>J</i> = 7.7 Hz, 1H)	104.1	C-6	102.3
2''	3.20	75.0		74.9
3''	3.31	77.9		78.1
4''	3.30	72.0		71.4
5''	3.46	77.7		78.1
6''	3.71	61.6		62.6

Compound 107

Compound **107** (20 mg) was obtained as a light yellow amorphous solid. The TLC profile showed R_f value of 0.56 with DCM/MeOH (9/1) mobile phase. The ¹H NMR (400 MHz,

CD₃OD) spectrum displayed signals assignable to olefinic protons at δ 5.25 (H-6), oxygenated methine proton at δ 4.29 (H-3), methylene protons at δ 2.31 (dd, 2H, H-4), and six methyl protons at δ 1.14 (H-19), 0.89 (H-29), 0.81 (H-21), 0.80 (H-18), 0.73 (H-26), and 0.71 (H-27) (Table 13, Appendix 12A). The proton signals observed in the range of 3.12 to 4.29 were assignable to sugar moiety. The remaining proton signals overlapped in the range of δ 1.26 to 2.00. The ¹³C NMR (101 MHz, CDCl₃) spectrum displayed 35 carbon signals including olefinic carbon at δ 140.7 (C-5) and 121.7 (C-6), of which the former suggest sp² quaternary carbon, sp³oxy-carbon at δ 78.9 (C-3), two sp³ quaternary carbons at δ 36.5 (C-10) and 42.3 (C-13), and methine carbons at δ 31.6 (C-8), 49.9 (C-9), 56.5 (C-14), 55.8 (C-17), 35.9 (C-20), 45.6 (C-24) and 28.8 (C-25), six methyl carbons at δ 19.5 (C-19), 19.0 (C-26), 18.7 (C-27), 18.5 (C-21), 11.6 (C-29) and 11.5 (C-18)), and six carbons assignable to sugar moiety at 100.8 (C-1'), 76.1 (C-5'), 75.5 (C-3'), 73.3 (C-2'), 69.7 (C-4') and 61.4 (C-6') (Table 13, Appendix 12B). The DEPT-135 spectrum displayed the presence of 12 methylene signals including one belong to the glucopyranose moiety (Appendix 12C). The observed spectral data is found to be in good agreement with previously reported data of β -Sitosterol-3-O- β -D-glucoside (Peshin & Kar, 2017), Fig 22, reported herein for the first time from the roots of *H. johannis*.

Table 13: ^1H (400 MHz, MeOD), ^{13}C (100 MHz) NMR spectral data of compound **107**
compared to literature (Peshin & Kar, 2017), (400 MHz, CD_3Cl , δ (ppm) and J (Hz))

No	Compound 107		^{13}C (Peshin & Kar, 2017)	No	Compound 107		^{13}C (Peshin & Kar, 2017)
	^1H	^{13}C			^1H	^{13}C	
1	1.89 (m, 2H)	37.0	36.7	19	1.14 (s, 3H)	19.5	19.4
2	1.37 (m, 2H)	29.4	29.2	20		35.9	35.4
3	3.59 (m, 1H)	78.9	76.8	21	0.81 (d, 3H)	18.5	18.5
4	2.31 (dd, 2H)	39.5	39.2	22	1.18 (m, 2H)	33.7	33.2
5		140.7	140.4	23	1.16 (m, 2H)	25.7	25.3
6	5.25 (bs, 1H)	121.7	121.2	24	(m, 1H)	45.6	45.1
7	1.94 (s, 2H)	31.7	31.3	25	1.50 (m, 1H))	28.8	28.6
8	1.48 (m, 1H)	31.6	31.2	26	0.73 (d, 3H)	19.0	19.6
9	0.89 (m, 1H)	49.9	49.5	27	0.71 (d, 3H)	18.7	18.8
10		36.5	36.1	28	1.37 (m, 2H)	22.8	22.1
11	1.48 (m, 2H)	20.8	20.5	29	0.89 (d, 3H)	11.6	11.7
12	1.15 (m, 2H)	38.4	38.2	1'	4.42 (d)	100.8	100.7
13		42.3	41.8	2'	3.20 (m)	73.3	73.4
14	1.04 (m, 1H)	56.5	56.1	3'	3.31 (m)	75.5	76.8
15		24.0	23.8	4'	3.30 (m)	69.8	70.0
16	1.75 (m, 2H)	28.0	27.7	5'	3.46 (m)	76.1	76.7
17	1.13 (m, 1H)	55.8	55.3	6'	3.71 (m)	61.4	62.8
18	0.80 (s, 3H)	11.5	11.6				

Compound 108

Compound **108** (43 mg) was isolated as a yellowish amorphous powder. The TLC profile showed R_f value of 0.48 with n-hexane/EtOAc(1/1) mobile phase. The ^1H -NMR (400 MHz, CDCl_3) spectral displayed signals for aromatic proton with ABX spin system at 7.08 (d, $J = 8.1$ Hz, 1H), 7.05 (d, $J = 2.0$ Hz, 1H) and 6.92 (d, $J = 8.1$ Hz, 1H), and *trans*-olefinic protons at 7.62 (d, $J = 15.9$ Hz, 1H) and 6.30 (d, $J = 15.9$ Hz, 1H) (Table 14, Appendix 13A). The ABX coupled aromatic proton signals [7.08, 7.05 and 6.92] and *trans*-olefinic signals [7.62 and 6.30] were, respectively, assigned to H-6, H-2 and H-5, and H-7 and H-8 of a *trans*-caffeoy moiety. The ^1H NMR also displayed signals at δ 4.34/4.28 (dd, $J = 12.0, 4.4$ Hz, 2H) and 4.15 (m, 1H), methyl 0.89 (t, $J = 6.8$ Hz, 3H), and signals overlapping in the range of δ 2.37 to 1.62. The signals at δ

4.34/4.28 (dd, $J = 12.0, 4.4$ Hz, 2H) and 4.15 (m, 1H) are attributed to sp^3 oxy-methylene protons (H-1') and oxy-methine (H-2') groups. The signals overlapping in the range of δ 2.37 to 1.62 were assigned to the methylene protons H-3'-8', and the signal at 0.89 represents the terminal methyl group (H-9'). The obtained 1H NMR spectral data is in good agreement with *trans*-caffeic acid hydroxyalkyl ester.

The ^{13}C NMR (101 MHz, $CDCl_3$) spectrum (Appendix 13B) together with its DEPT-135 (Appendix 13C) displayed three aromatic quaternary carbons signals at δ 147.9 (C4), 146.7 (C3), and 130.0 (C1), three aromatic methine carbons at δ 114.7 (C2), 115.8 (C5), and 123.0 (C6), two olefinic carbons at δ 144.7 (C7) and 115.6 (C8), one carbonyl carbon at δ 167.5 (C9) (Table 14). The ^{13}C NMR also displayed oxygenated aliphatic carbons at δ 68.3 and 65.0 which were assigned to methylene and methine carbons at C1' and C2' of the ester side chain, respectively. The carbon signals at δ 34.1, 31.9, 29.1, 27.1, 24.8, and 22.7 were assigned to the methylene (C3'-8'), and methyl carbons at δ 14.1 and methyl carbon (C9') of the hydroxy alkyl ester side chain. Based on the obtained spectral data and the data from literature survey (Chowdhury, Al Hasan, Shahidullah Tareq, Ahsan, & Zafrul Azam, 2013; Nguyen et al., 2015; Saleem, Hyoungh, Jin, & Yong, 2004), compound **108** was proposed to be 2-hydroxynonyl (*E*)-3-(3,4-dihydroxyphenyl)acrylate (caffeic acid-2-hydroxynonyl ester), Fig. 22.

Table 14: ^1H (400 MHz, CDCl_3), ^{13}C NMR (100 MHz) spectral data of compound **108** compared to literature (Chowdhury et al., 2013; Nguyen et al., 2015), (400 MHz, methanol- d_4), δ (ppm) and J (Hz)

No	Compound 108		(Chowdhury et al., 2013; Nguyen et al., 2015)	
	^1H	^{13}C	^1H	^{13}C
1		128.9		127.9
2	7.05 (d, $J = 2.0$ Hz, 1H)	114.7	7.05, br, s	115.3.
3		146.7		146.9
4		147.9		149.5
5	6.92 (d, $J = 8.1$ Hz, 1H)	115.6	6.78, d, 8.0	115.0
6	7.08 (d, $J = 8.1$ Hz, 1H)	123.05	6.95, br, d, 8.0	123.1
7	7.62 (d, $J = 15.9$ Hz, 1H)	144.7	7.58, d, 16.0	147.2
8	6.30 (d, $J = 15.9$ Hz, 1H)	115.8	6.29, d, 16.0	116.3
9		167.5		169.4
1'	4.34/4.28 (dd, $J = 12.0, 4.4$ Hz, 2H)	65.1		66.7
2'	4.15 (m, 1H)	68.9		71.4
3'		34.1		
4'		29.1		
5'		27.1		
6'		24.8		
7'		31.9		
8'		22.7		
9'	0.89 (t, $J = 6.8$ Hz, 3H)	14.1		

Compound 109

Compound **109** (3,9-epoxypregn-16-ene-14,20-diol,7,11,18-triacetoxy-3-methox) was obtained from the GC/MS analysis of fraction 19-26, which were eluted with 25-30% EtOAc in *n*-hexane from DCM/MeOH roots of *H. johannis*. According to the GC/MS analysis result, compound **109** was found to be the predominant compound with retention time of 4.00 min, with molecular ion signal at m/z 518 indicating an elemental composition of $\text{C}_{28}\text{H}_{40}\text{O}_{10}$. The mass spectrum data was compared with National Institute of Standards and Technology (NIST) library databases. The structure of compound **109** reported in this study is found to be similar with the previously reported structure of pregn derivative from methanolic extract of *Cyclamen persicum* (Ibraheam et al., 2017), Fig. 22.

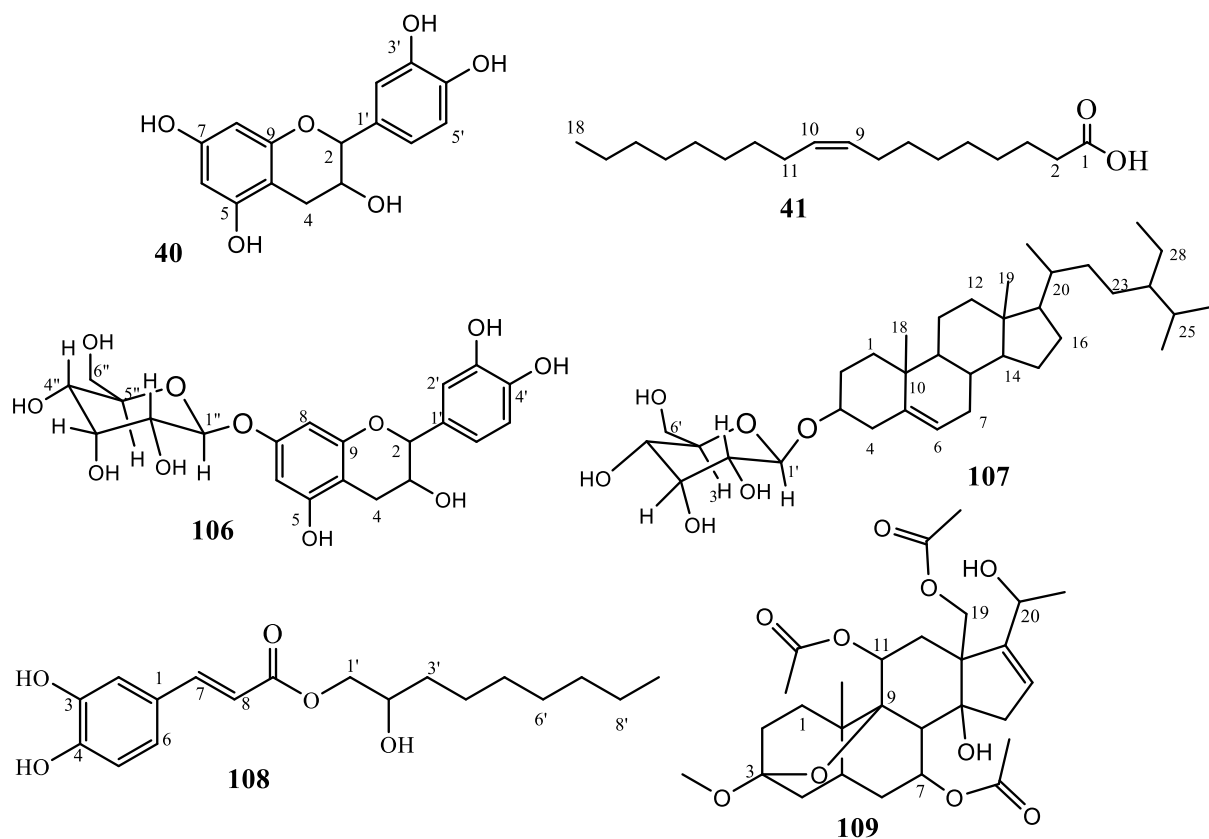


Fig. 22: Structures of compounds isolated from the root of *H. johannis*.

4.1.3. Compounds isolated from root extract of *C. tomentosa*

The dichloromethane/methanol (1:1) stem bark extract of *C. tomentosa* was subjected to gravity column chromatography over silica gel to afford two compounds (**110** and **111**) (Fig. 23). The structures of each of the isolated compounds were elucidated by 1D and 2D NMR spectroscopic techniques and comparison of their spectroscopic data to those previously reported in the literature.

Compound 110

Compound **110** (25 mg) was obtained as a white amorphous from 20% EtOAc in *n*-hexane fraction with R_f value of 0.57. The ^1H -NMR (400 MHz, CDCl_3) spectra displayed proton signals at δ 2.27 (t, J = 7.5 Hz, 2H) assignable to methylene group adjacent to carbonyl carbon, signal overlapping in the range of 1.23 to 1.54 attributed to methylene groups in long aliphatic carbon chain, and proton signal at δ 0.80 (t, J = 6.1 Hz, 3H) which represent terminal methyl proton on aliphatic chain (Table 15, Appendix 15A). The ^{13}C NMR (101 MHz, CDCl_3) spectrum revealed the presence of 14 carbon (Appendix 15B), and the corresponding DEPT-135 (Appendix 15C)

indicate multiplicities including one quaternary (δ 179.0), one methyl (δ 14.1), and twelve methylene (33.9, 31.9, 29.7, 29.6, 29.6, 29.4, 29.3, 29.2, 29.0, 24.7, and 22.6) signals (Table 15). The quaternary carbon signal at δ 179.0 (C1) is ascribed to carbonyl carbon of carboxylic moiety. The signal at δ 33.9 (C2) is attributed to the methylene group attached to the carbonyl carbon, and the signals at δ 24.7, 29.7, 29.6, 29.6, 29.4, 29.3, 29.2, 29.0, 31.9, and 22.6 are due to long aliphatic methylene carbon chain, and the signal at δ 14.1 correspond to the terminal methyl group. Based on the above spectral data and comparison of this data with the literature values compound **110** (Fig. 23) is proposed to be tetradecanoic acid (Ejeta, 2017; Njagi, 2013).

Table 15: ^1H (400 MHz, CDCl_3), ^{13}C (101 MHz), and HMBC NMR data of compound **110** compared to literature (Ejeta, 2017; Njagi, 2013), 400 MHz, CDCl_3 , δ (ppm) and J (Hz)

Compound 110			^{13}C , (Ejeta, 2017; Njagi, 2013)
No	^1H	^{13}C	
1		179.0	179.2
2	2.27 (t, J = 7.5 Hz, 2H)	33.9	33.9
3	1.54 (m, 2H)	24.7	24.6
4-11	1.23 (bm, 16H)	29.0-29.7	29.0-29.6
12	1.24(m, 2H)	31.9	31.9
13	1.26 (m, 2H)	22.6	22.6
14	δ 0.80 (t, J = 6.1 Hz, 3H)	14.1	14.1
OH	9.68 (s)		

Compound 111

Compound **111** (22 mg) was obtained as a white amorphous isolated from 30% EtOAc in *n*-hexane fraction with R_f value of 0.45. The ^1H NMR (400 MHz, CDCl_3) spectral data showed two doublets at 8.01 (d, J = 8.2 Hz, 2H), 6.90 (d, J = 8.2 Hz, 2H) which are evident for the presence of 1, 4-disubstituted aromatic group. Proton signals observed at 4.20 (m, 2H, H1'), 1.72 – 1.59 (m, 2H, H2'), 1.59(m, 2H, H3'), 1.65 (m, 2H, H4'), 0.90 (t, J = 6.0 Hz, 3H, H5') are attributed to the aliphatic chain (Table 16, Appendix 16A). The ^{13}C NMR (101 MHz, CDCl_3) spectra displayed 11 well resolved signals assignable to 13 carbon (Table 16, Appendix 16B). The DEPT-135 of compound 100 indicated the presence of three set of quaternary and three

methylene groups (Appendix 16C). The carbon signals at δ 167.5(C7), 160.7 (C4), 132.5 (C2/6), 127.9 (C1), 115.3 (C3/5) are assignable to a *p*-substituted benzoate moiety. The methylene carbon signals at 65.1 (C1'), 24.9 (C2'), 31.9 (C3') and 22.7 (C4'), and methyl signals at 14.1 (C5') belongs to the alkyl side chain. The COSY correlation of δ 8.01 (H-2/6) with 6.90 (H-3/5) evident for the 1'4-substituted aromatic group (Appendix 16D). The COSY correlation of δ 4.20 (H-1') to 1.6 (H-2'), 1.65 (H-4') to 1.6 (H-2') supports the benzoate linkage. The C-H linkages were established HSQC analyses (Table 16, Appendix 16E). The HMBC correlation at 4.20 (H1')/132.5 (C2/6) verified the presence of C7-C1' ester linkage (Appendix 16F). Based on the above spectral data and comparison of this data with the literature values (Ren, Chen, & Zhao, 2012; Shah et al., 2017), compound **111** is proposed to penthyl-4-hydroxybenzoate (Fig. 23).

Table 16: 1D and 2D (400 MHz, CDCl₃) spectra of compound **111** compared to literature

(Ren et al., 2012; Shah et al., 2017), 400 MHz, DMSO-d₆ (δ (ppm) and *J* (Hz))

No	Compound 111				¹³ C (Ren et al., 2012; Shah et al., 2017)
	¹ H	¹³ C	COSY	HMBC	
1		127.9			127.3
2/6	8.01 (d, <i>J</i> = 8.2 Hz, 2H)	132.5	H3/5	H1'	131.6
3/5	6.90 (d, <i>J</i> = 8.2 Hz, 2H)	115.3			115.1
4		160.7			161.4
7		167.5			167.8
1'	4.20 (m, 2H)	65.1	H2'	C2/6	64.0
2'	1.72– 1.59 (m, 2H)	24.9			23.5
3'	1.59 (m, 2H)	31.9			32.1
4'	1.65 (m, 2H)	22.7			21.2
5'	0.90 (t, <i>J</i> = 6.0 Hz, 3H).	14.1			14

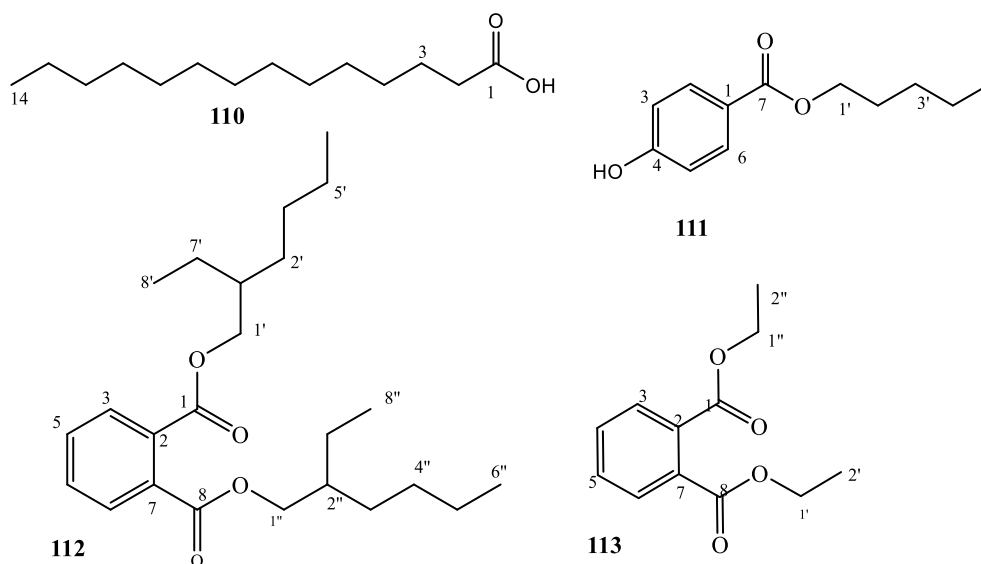


Fig. 23; Structure of compounds isolated from *C. tomentosa* (**110** and **111**), *K. petitiana* (**112**), and *C. rotundifolia* (**113**)

4.1.4. Compounds isolated from root extract of *K. petitiana*

The dichloromethane/methanol (1:1) leaves extract of *K. petitiana* was subjected to gravity column chromatography over silica gel to afford one compound (**112**). The structure of the isolated compound was elucidated by 1D and 2D spectroscopic techniques and comparison of its spectroscopic data to those previously reported in the literature.

Compound 112

Compound **112** (17 mg) was isolated as a amorphous with TLC R_f value of 0.42 with *n*-hexane/EtOAc (7/3) mobile phase. The ^1H NMR (400 MHz, CDCl_3) spectra displayed two AB coupled proton signals [δ 7.73 (dd, $J = 5.7, 3.3$ Hz, 2H, H3/6) and 7.55 (dd, $J = 5.7, 3.3$ Hz, 2H, H4/5)] assignable to 1, 2-sunbstituted aromatic moiety, aliphatic proton signals at δ 4.23 (m, 4H) corresponding to oxy-methylene protons (H1'/1''), and two terminal methyl proton signals at δ 0.94 (m, 3H, H8'/8'') and 0.92 (m, 3H, H6'/6'') (Table 17, Appendix 17A). The methine protons at H2'/2'' are represented by the signal at δ 1.70. The proton signal overlapped in the range δ 1.34 – 1.44 were assigned for five methylene groups (H3'/3'', 4'/4'', 5'/5'', and 7'/7''). The ^{13}C NMR (101 MHz, CDCl_3) spectrum (Appendix 17B) displayed 12 well resolved carbon signals, and were assigned by DEPT experiment (Appendix 17C) to be two quaternary (δ 167.7

(C1/8) and 132.4 (C2/7)), three methine (130.9 (C4/5), 128.8 (C3/6) and 38.7 (C2'/2'')) and five methylene carbons (68.1 (C1'/1''), 30.3 (C3'/3''), 28.9 (4'/4''), 23.7 (C7'/7''), and 23.0 (C5'/5''), with two methyl groups (14.0 (C6'/6''), and 10.9 (8'/8'')) (Table 17). The quaternary signal at 167.7 (C1/8) is a characteristic of a carbonyl carbon of a benzoate moiety. The carbon signals at δ 132.4 (C2/7), 130.9 (C4/5) and 128.82 (C3/6) are assignable to 1, 2-substituted aromatic ring. The five methylene and one methine carbons (68.1 (C1'/1''), 30.3 (C3'/3''), 28.9 (4'/4''), 23.7 (C7'/7''), 23.0 (C5'/5''), and 38.7 (C2'/2''), respectively) exhibited chemical shifts values that coincided with those for a 2-ethylhexyl moiety.

The COSY correlations of H1'/1'' (δ 4.23) with H2'/2'' (δ 1.70), H2'2'' (1.70) with H3'/3'' (1.38) and H7'/7'' (1.44), H5'/5'' (δ 1.34) with H6'/6'' (δ 0.92), H7/7'' (δ 1.44) with H8'/8'' (0.94) supported the multiplicities in the 2-ethylhexyl moiety (Appendix 17D). The C-H linkages in the structure were obtained from the HSQC spectrum (Appendix 17E). The proton correlation at H3/6 (δ 7.73)/H4/6 (δ 7.55) is evidence for the multiplicities in the 1, 2-substituted aromatic moiety. The HMBC correlation at δ 4.23 (H1'/1'') to C1/8 (δ 167.7) shows linkage of oxygenated methylene group (δ 68.1, C1'/1'') belonging to two 2-ethylhexyl moieties to the carbonyl (δ 167.7, C1/8) of the benzoate moiety (Appendix 17F). Based on the above spectral data and comparison with the literature values, compound **112** is deduced to bis (2-ethylhexyl) phthalate (Habib & Karim, 2009; Lotfy, Hassan, Hetta, El-Gendy, & Mohammed, 2018), Fig 23. The isolation di-(2-ethylhexyl) phthalate including other phthalates from natural sources (Dissanayake, Wagner, & Nair, 2016; Ga & De, 2019; Habib & Karim, 2009; Lotfy et al., 2018) has changed the notion considering phthalates as totally synthetic.

Table 17: 1D and 2D NMR (400 MHz, CDCl₃) spectra of compound **112** compared to literature (Habib & Karim, 2009; Lotfy et al., 2018), 400 MHz CDCl₃, δ (ppm) and J (Hz)

No	Compound 112				¹³ C (Habib & Karim, 2009; Lotfy et al., 2018)
	¹ H	¹³ C	COSY	HMBC	
1, 8		167.7			166.5
2, 7		132.4			131.5
3, 6	7.73 (dd, J = 5.7, 3.3 Hz, 2H)	128.8	H-4/5	C1/8	127.8
4, 5	7.55 (dd, J = 5.7, 3.3 Hz, 2H)	130.9		C3/6	129.8
1', 1''	4.26 (d, J = 6.2 Hz, 1H) 4.23 (d, J = 6.2 Hz, 1H)	68.1	H-2'/2''	C1/8, C2'/2'', C7'/7''	68.1
2', 2''	1.70 (d, J = 6.1 Hz, 1H)	38.7	H-7'/7'', 3'/3'', 4'/4''	C3'/3'', C4'/4''	38.7
3', 3''	1.38 (m, 2H)	30.3			30.4
4', 4''	1.34 (m, 2H)	28.9			28.9
5', 5''	1.34 (m, 2H)	23.0	H-6'/6''		23.0
6', 6''	0.92 (m, 3H)	14.0		C5'/5''	14.0
7', 7''	1.44 (m, 2H)	23.7	H-8'/8''		23.7
8', 8''	0.94 (m, 3H)	10.9		C5'/5'', C2'/2''	10.9

4.1.5. Compounds isolated from leaves extract of *C. rotundifolia*

The dichloromethane/methanol (1:1) leaves extract of *C. rotundifolia* (8 g) was subjected to gravity column chromatography over silica gel and afforded one compound (**113**). The structure of the isolated compound was elucidated by 1D and 2D spectroscopic techniques and comparison of its spectroscopic data to those previously reported in the literature

Compound 113

Compound **113** (13 mg) was isolated as a color less amorphous with TLC profile R_f value of 0.53 with *n*-hexane/EtOAc (7/3) as mobile phase. The ¹H-NMR spectrum provided two AB coupled proton signals at δ 7.74 (d, J = 5.7, Hz, 2H) and 7.55 (dd, J = 5.7, 3.3 Hz, 2H) evidence for 1, 2-substituted aromatic moiety (Table 18, Appendix 18A). The ¹H NMR also displayed

proton signals δ 4.39 (q, $J = 7.2$ Hz, 4H) and 1.39 (t, $J = 7.2$ Hz, 6H) assignable to oxy-methylene (-OCH₂) and methyl protons, respectively. The ¹³C NMR (101 MHz, CDCl₃) spectrum provided six well resolved carbon signals including δ 167.6, 132.2, 130.9, 128.8, 61.6, and 14.1 allocated to 12 carbons (Table 18, Appendix 18B). The multiplicity of each carbon atom was determined by the DEPT-135 experiment (Appendix 18C). The signal at δ 167.6 (C1, 8) identified to be represent a carbonyl group in the isolate molecule. The carbon signals at δ 132.2 (C2, 7), 130.9 (C4, 5), 128.8 (C3, 6) are quaternary and methine aromatic carbons, respectively. The signal at δ 61.6 coincide with an oxygenated methylene group. The proton signals multiplicities and carbon-hydrogen linkages were obtained from the COSY and HSQC spectrum (Appendix 18D and 18E, respectively). The COSY correlation at 7.75 (H4, 5)/7.55(H3, 6), 4.39 (H1'/1'')/1.41 (H2', 2'') are evidence for the multiplicities in the 1, 2-substituted aromatic moiety and oxy-ethyl groups, respectively. The HMBC correlations of δ 4.239 (H1', 1'') with δ 167.6 (C1, 8) and δ 4.39 (H1', 1'') with 14.1 (C2', 2'') (Appendix 18F) indicate that the oxygenated methylene (δ 61.6) belonging to two ethylene moieties were linked to the carbonyl (δ 167.6) of the benzoate. Thus, based on the spectral data and comparison of this data with the literature, compound **113** was identified as diethyl phthalate (Dissanayake et al., 2016; Koko, Mesaik, Ranjitt, Galal, & Choudhary, 2015), Fig 23.

Table 18: 1D and 2D (400 MHz, CDCl₃) spectra of compound **113** compared to literature (Koko et al., 2015) (400 MHz, CDCl₃), δ (ppm) and J (Hz).

No	Compound 113				¹³ C, (Koko et al., 2015)
	¹ H	¹³ C	COSY	HMBC	
1/8	-	167.6			165.8
2/7	-	132.2			132.6
3/6	7.74 (d, $J = 5.7$, Hz, 2H)	128.8	H4/5	C4/5	128.3
4/5	7.55 (dd, $J = 5.7$, 3.3 Hz, 2H))	130.9			129.5
1'/1''	4.39 (q, $J = 7.2$ Hz, 4H)	61.6	H2'/2''	C1'/1''	64.1
2'/2''	1.39 (t, $J = 7.1$ Hz, 3H)	14.1		C1'/1''	10.3

4.1.6. GC-MS analysis of essential oils from leaves of *C. rotundifolia*, *C. tomentosa* and *K. petitiiana*

The leaves of *C. tomentosa*, *K. petitiiana* and *C. rotundifolia* were subjected to hydrodistillation to collect essential oil. The chemical profile of the volatile oil collected from each plant was analysed by means of GC-MS. The GC chromatograms are depicted in Appendix 19A-19C, respectively.

Analysis of the oil of *C. tomentosa* resulted in identification of 25 volatile compounds comprising 89.7 % of the total. The chemical profile of the oil, the percentage composition, and the retention times are presented in Table 19. The major constituents in the oil from leaves of *C. tomentosa* were Phenol, 2, 4-bis(1,1-dimethylethyl)- (21.03 %) (**114**), cetene (10.11 %) (**115**), 1-octadecene (12.07 %) (**116**), and cycloeicosane (10.15 %) (**117**) (Fig. 24). The structure of major compounds (**114-117**) are shown in Fig. 25

The essential oil reported in previous work (J, Ndung, & Gitu, 2013), from leaves and fruits of *C. tomentosa*, which were obtained by steam distillation using a Clevenger type apparatus, reported to have β -Phellandrene and β pinene as a major component, which is different from the major component phenol (2,4-bis(1,1-dimethylethyl)- (21.03 %) reported in this work. This can be explained based on the fact that, the same plant species in different conditions such as type of soil, climatic conditions, stage of growth and other factors, can give different chemical composition of volatile compounds (Kulisic-Bilusic, Blažević, Dejanović, Miloš, & Pifat, 2009).

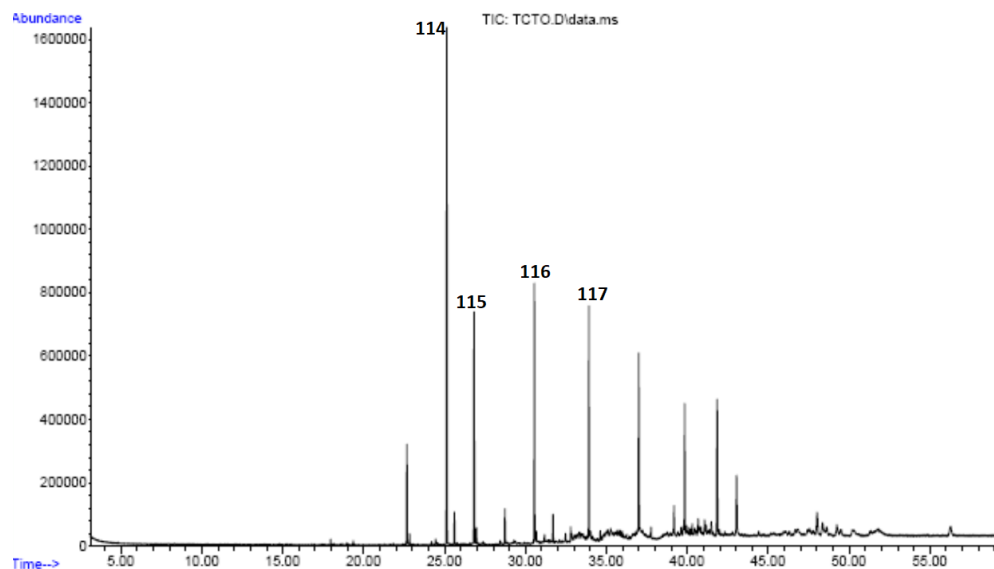


Fig. 24. GC profile of major compounds of oils from leaves of *C. tomentosa*.

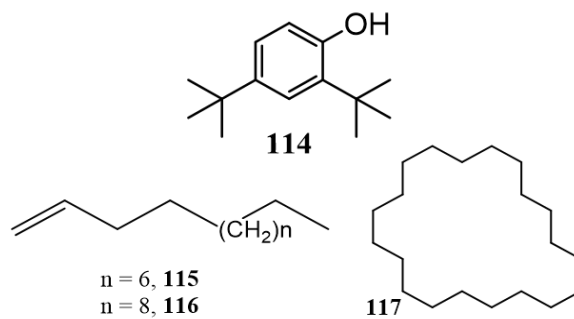


Fig. 25: Structures of major compounds (**114- 117**) from oil of leaves of *C. tomentosa*

Table 19: Percentage composition of essential oils from leaves of *C. tomentosa*

Name of Compound	RT	MW	% total
2-Tetradecene, (E)-	22.66	196.219	3.89
Tetradecane	22.83	198.235	0.42
Phenol, 2,4-bis(1,1-dimethylethyl)-	25.13	206.167	21.03
Xyloidone	25.60	240.079	1.41
Cetene	26.81	224.25	10.11
Hexadecane	26.96	226.266	0.66
2-Propenoic acid, pentadecyl ester	28.72	282.256	1.45
1-Octadecene	30.54	252.282	12.07
Octadecane	30.66	254.297	0.51
2H-Pyran, tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-	31.17	154.136	0.65
Phthalic acid, butyl isohexyl ester	31.69	306.183	1.42
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	32.46	276.173	0.81
Hexadecanoic acid, methyl ester	32.79	270.256	0.24
Cycloeicosane	33.91	280.313	10.15
1-Decanol, 2-octyl-	34.61	270.292	0.44
1-Dodecanol, 2-hexyl-	37.75	270.292	0.53
[1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1-dimethylethyl)-	39.17	410.318	1.64
Phthalic acid, isobutyl 2-methylpent-3-yl ester	39.76	306.183	0.49
Cyclotetracosane	39.83	336.376	6.55
Phthalic acid, 2-ethylbutyl nonyl ester	40.30	376.261	0.47
Phthalic acid, 4-methylpent-2-yl pentyl ester	40.66	320.199	3.55
Phthalic acid, 5-methylhex-2-yl pentyl ester	41.06	334.214	0.84
Phthalic acid, hexadecyl pentyl ester	41.48	460.355	0.73
Bis(2-ethylhexyl) phthalate	41.84	390.277	8.37
Phthalic acid, 4-methylhept-3-yl nonyl ester	49.25	404.293	1.18
Total			89.7 %

MW: Molecular weight; RT: Retention time in min

The GC-MS analysis of essential oil of leaves of *K. petitiiana* revealed the presence of 54 volatile compounds accounting for 97.2 % of the the total. Table 20 shows the chemical profile of the oil, the percentage composition, and the retention times. The identified major volatile compounds were 1,5-Hexadiene, 2-methyl (23.07%) (**118**), 2-Butenoic acid, 2-methyl-, 2-methylpropyl ester (, (E) (4.77 %) (**119**), Cyclobutylcarboxamide, N-allyl- (3.27 %) (**120**), Phthalic acid, isobutyl trans-hex-3-enyl ester (9.82 %) (**121**), and Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-[1S-(1.α.,2.β.,4.β.)]-(6.12%) (**122**) (Fig. 26). The remaining

volatile compounds constitutes 0.12-1.27 %. The structure of major compounds (**118-122**) from the essential oil of leaves of *K. petitiiana* are shown in Fig. 27.

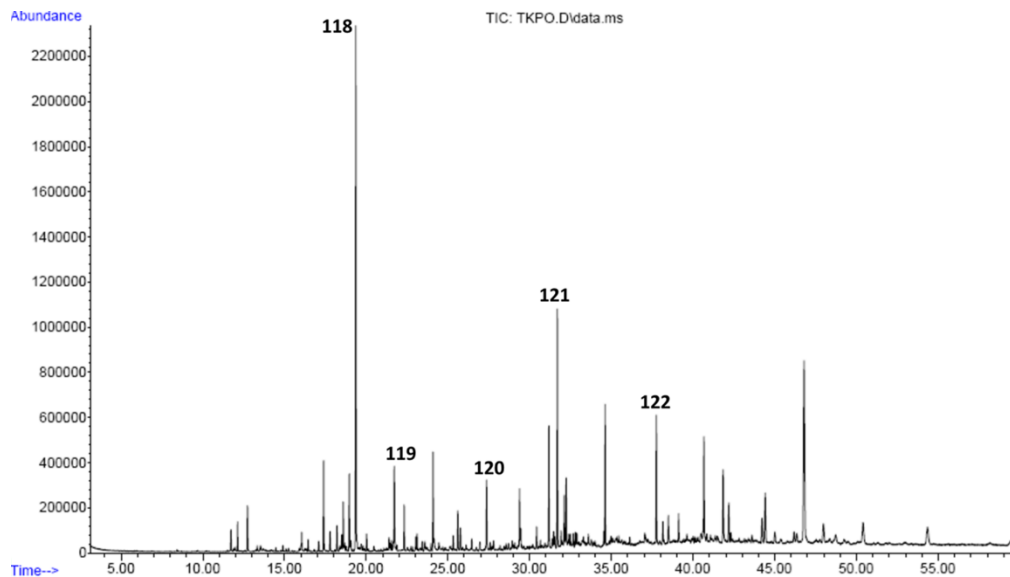


Fig. 26: GC profile major compounds of oils from leaves of *K. petitiiana*.

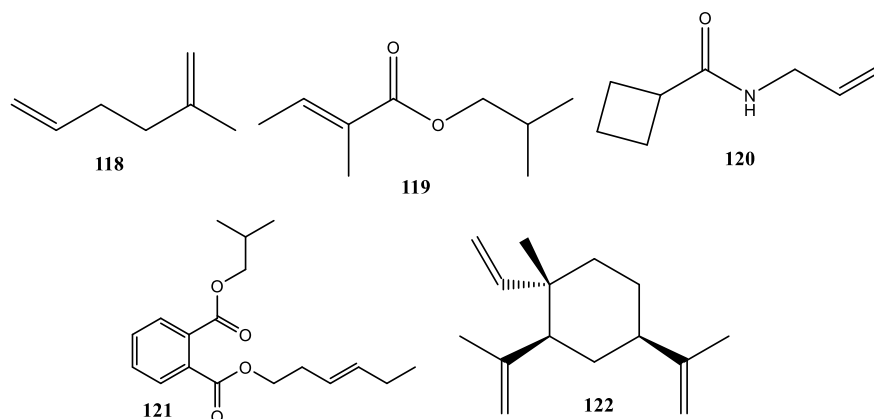


Fig. 27: Structures of major compounds (**118- 122**) identified from oil of leaves of *K. petitiiana*

Table 20: Percentage composition of oils obtained from leaves of *K. petitiiana*

Name of compound	RT	MW	% total
3-Isopropyl-4-methyl-1-pentyn-3-ol	11.721	140.12	1.05
Cyclotetrasiloxane, octamethyl-	12.131	296.08	1.08
Spiro[4.4]nonan-1-one	12.738	138.10	1.89
Bicyclo[4.1.0]hepta-1,3,5-triene	16.053	90.05	1.26
Cyclopentasiloxane, decamethyl-	16.442	370.09	0.43
Succinic acid, butyl 4-heptyl ester	17.096	272.20	0.53
Dodecane, 1,1-dimethoxy-	18.497	230.23	0.94
Butane, 2-methoxy-3-methyl-	18.596	102.10	2.28
8-Tetradecyn-1-ol	18.959	210.20	2.31
1,5-Hexadiene, 2-methyl-	19.359	96.09	23.07
Cyclohexa-2,4-dienylmethanol	21.398	110.07	0.52
p-Mentha-2,5-dien-7-ol, trans-	21.553	152.12	0.43
2-Butenoic acid, 2-methyl-, 2-methylpropyl ester, (E)-	21.714	156.12	4.77
6-Undecanol	22.326	172.18	2.16
2,2-Dimethylhex-4-enylamine	23.032	127.14	0.73
2-Pyrimidinamine, 4-methoxy-6-methyl-	23.11	139.08	0.82
5-(Diethylamino)-2-nitrosophenol	23.587	194.11	0.55
Furan, 2,5-diethyltetrahydro-	23.961	128.12	0.29
Cyclododecane	24.443	168.19	0.49
Cyclohexanol, 4-(1-methylethyl)-	26.457	142.14	0.74
Hexadecane	26.96	226.27	0.53
Cyclobutylcarboxamide, N-allyl-	27.365	139.10	3.27
Thiophen-2-methylamine, N-ethyl-N-(2-methylphenyl)-	27.588	231.11	0.45
3-Oxatricyclo[3.2.1.0(2,4)]octane, (1.alpha.,2.beta.,4.beta.,5.alpha.)-	28.553	110.07	0.42
2-Hexenoic acid, 2-hexenyl ester, (E,E)-	30.431	196.15	1.06
Hexacosane	30.66	366.42	0.36
Cyclopentane, decyl-	31.381	210.24	0.50
5-Nitrobenzimidazole	31.469	163.04	0.90
1,4-Bis-furan-2-ylmethyl-piperazine	31.542	246.14	0.67
Phthalic acid, isobutyl trans-hex-3-enyl ester	31.697	304.17	9.82
1H-Benzimidazole, 5-nitro-	32.128	163.04	2.91
1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	32.491	304.17	0.75
Pentadecanoic acid, 14-methyl-, methyl ester	32.797	270.26	0.77
2,4-Nonadienal	32.885	138.10	0.95
Dibutyl phthalate	33.285	278.15	1.07
1,2-Hydrazinedicarboxylic acid, dimethyl ester	33.596	148.05	0.71
Thymidine, 5'-amino-5'-deoxy-	34.541	241.11	0.70

Butanoic acid, 4-(1,1-dimethylethoxy)-3-hydroxy-2-methyl-, ethyl ester, [r-(R*,S*)]-	35.454	218.15	0.33
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	37.052	204.19	6.12
Indane	39.112	118.08	1.52
Prazepam - 3-hydroxy	39.62	340.10	0.51
Phthalic acid, isobutyl 1-methoxydec-4-yl ester	40.113	392.26	0.37
Dimethyl 2-hydroxy-2-methylbutane-1,4-dioate	40.487	176.07	0.96
Adamantane, 1-isothiocyanato-3-methyl-	40.845	207.11	0.58
Tricyclo[4.3.1.1(3,8)]undecane-1-carboxylic acid, methyl ester	41.073	208.15	0.21
Diisooctyl phthalate	41.841	390.28	2.18
1,3-Dicyclohexyl-2-(4-oxo-pentanoyl)-isourea	42.313	322.23	0.52
Benzonitrile, 4-methyl-	44.223	117.06	1.86
2-Pyrrolidinone, 1-octadecyl-	46.179	337.33	1.00
5,6-Tetramethylenetetrahydro-1,3-oxazine-2-thione-4-spirocyclohexane	46.35	239.13	0.89
Decanedioic acid, bis(2-ethylhexyl) ester	47.979	426.37	2.04
1-[2-Deoxy-.beta.-d-erythro-pentofuranosyl]pyrrole-2-carbonitrile	48.737	208.09	1.24
Eicosane	50.403	282.33	1.91
2,4,6-Cycloheptatriene-1-carbonitrile	54.346	117.06	2.86
Total			97.2 %

MW: Molecular weight; RT: Retention time in min

Similarly, 23 compounds which account for 95.47 % of the total were identified in the oil from leaves of *C. rotundifolia* and presented in Table 21. Based on the obtained GC and GC-MS result, the major compounds were identified to be Phenol, 2,4-bis(1,1-dimethylethyl)- (15.6%) (**123**), 3-Hexen-1-ol, benzoate, (Z)- (8.01%) (**124**), Cyclohexadecane (8.34%) (**125**), 1-Nonadecene (7.47%) (**126**), 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (8.24%) (**127**), Cycloeicosane (6.5%) (**117**), and Decanedioic acid, bis(2-ethylhexyl) ester (9.34%) (**128**) (Fig. 28). The structure of the major compounds (**117**, **123-128**) from the essential oil of leaves of *C. rotundifolia* are shown in Fig. 29

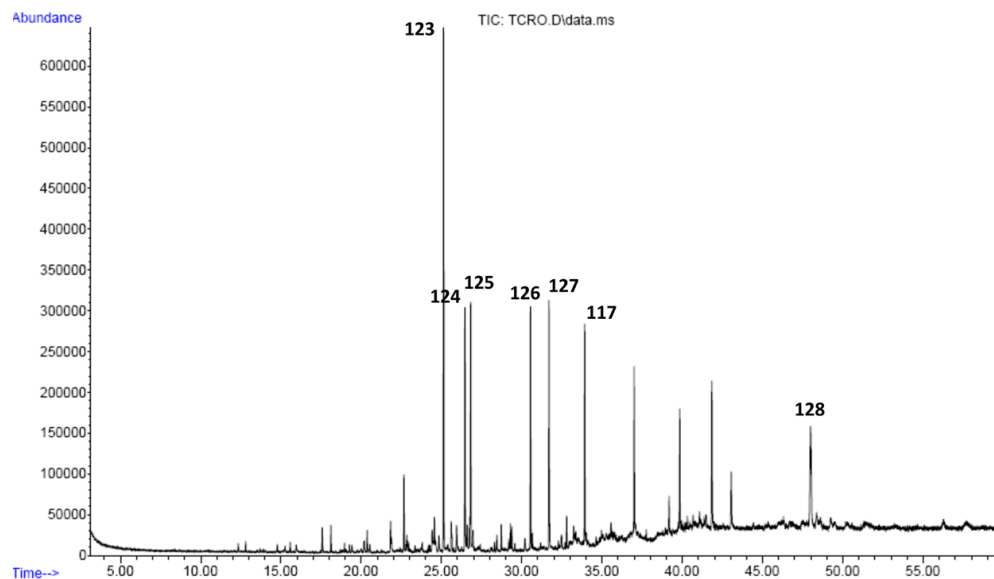


Fig. 28: GC profile of major compounds of oil from leaves of *C. rotundifolia*.

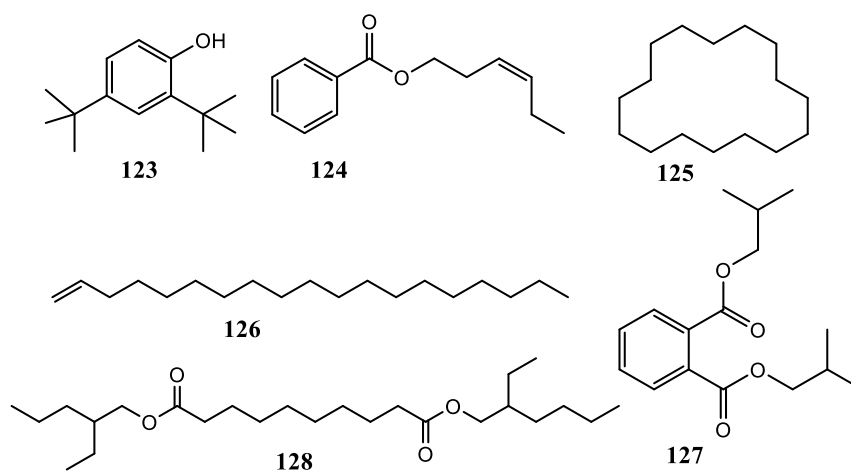


Fig. 29: Structure of major compounds identified from oil of leaves of *C. rotundifolia*.

Table 21: Percentage composition of oils obtained from leaves of *C. rotundifolia*

Name of Compound	RT	MW	% total
Benzeneacetic acid, methyl ester	17.57	150.07	1.09
L-. α .-Terpineol	18.11	154.14	0.91
Acetophenone, 2',4'-dimethoxy-3'-methyl-	20.37	194.09	0.78
Eugenol	21.82	164.08	1.86
2-Tetradecene, (<i>E</i>)-	22.66	196.22	2.55
<i>trans</i> -.B.-Ionone	24.55	192.15	1.31
Phenol, 2,4-bis(1,1-dimethylethyl)-	25.12	206.17	15.62
1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-3-methyl-	25.93	178.06	1.54
3-Hexen-1-ol, benzoate, (<i>Z</i>)-	26.45	204.12	8.01
Benzoic acid, hexyl ester	26.60	206.13	0.87
Cyclohexadecane	26.81	224.25	8.34
2-Propenoic acid, pentadecyl ester	28.73	282.26	0.88
1,7-di-iso-propylnaphthalene	29.29	212.16	0.77
1-Nonadecene	30.54	266.30	7.47
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	31.69	278.15	8.24
Hexadecanoic acid, methyl ester	32.79	270.26	1.16
Isoxazole, 5-methoxy-3-phenyl-	33.23	175.06	0.90
Cycloeicosane	33.91	280.31	6.50
Behenic alcohol	37	326.36	5.33
[1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1-dimethylethyl)-	39.1	410.31	1.85
Cyclotetracosane	39.83	336.38	4.11
Diisooctyl phthalate	41.84	390.28	6.02
Decanedioic acid, bis(2-ethylhexyl) ester	47.98	426.37	9.34
Total			95.47 %

MW: Molecular weight; RT: Retention time in min

4.2. Biological activity

4.2.1. *In vitro* antibacterial activity

In vitro antibacterial activities of compounds **28**, **49**, **100-105** isolated from the root extract of *C. cyphopetalum* were tested against *E. coli*, *S. aureus*, and *P. aeruginosa* pathogens at

concentrations of 50, 100, 300, 500, and 1000 $\mu\text{g/mL}$. The obtained mean inhibition zone diameters (in mm) were presented in Table 22. Appendix 20A displayed the inhibition diameter of some of the tested compounds. Based on the obtained result, compound **28**, **101-105** exhibited growth inhibition effect (>7 mm inhibition zone diameter) against *E. coli* at all tested concentrations.

At the lowest concentration of 50 $\mu\text{g/mL}$, ϵ -viniferin (**101**) recorded the highest mean inhibition zone against *E. coli* (8.55 ± 0.45 mm) and *S. aureus* (9.30 ± 1.39 mm), followed by tricuspidatol (**103**) (8.42 ± 0.20 and 7.36 ± 0.20 mm, respectively), which is moderate in comparison to the standard chloramphenicol (30 $\mu\text{g/mL}$). Against the gram-negative bacterium *P. aeruginosa*, gnetin (**102**) scored better mean inhibition zone (12.55 ± 0.65 mm) than chloramphenicol (11.76 ± 0.77 mm at 30 $\mu\text{g/mL}$), followed by ϵ -viniferin diol (**104**, 9.42 ± 0.42 mm) at 50 $\mu\text{g/mL}$. The highest mean inhibitory value (10.45 ± 0.75) at the highest concentration (1000 $\mu\text{g/mL}$) was also exhibited by ϵ -viniferin (**101**), followed by gnetin H (**102**) (10.02 ± 0.13 mm) and parthenostilbenin B (**105**) (9.52 ± 0.19 mm) against *E. coli*. Compounds **100**, **28**, **103** and **104** exhibited moderate inhibitory effects against *E. coli* (8.92 ± 0.04 , 8.83 ± 0.02 , 8.72 ± 0.0 , and 7.32 ± 1.9 mm, respectively). Against the bacterium *P. aeruginosa*, gnetin H (**102**) exhibited better activity (14.60 ± 0.1 mm) than chloramphenicol (11.76 ± 0.77 mm at 30 $\mu\text{g/mL}$). Against the gram positive bacterium, *S. aureus*, the highest activity was recorded by gnetin H (**102**), followed by ϵ -viniferin (**101**), β -sitosterol (**49**), tricuspidatol A (**103**), and *trans*-resveratrol (**28**) with inhibition zone diameter of 12.45 ± 0.37 mm, 12.22 ± 0.74 mm, 10.93 ± 1.54 mm, 10.79 ± 0.24 mm, 8.62 ± 1.5 mm, respectively, at the concentration of 1000 $\mu\text{g/mL}$. Compounds **100**, **104**, and **105** exhibited no activity against *S. aureus* at all concentration.

Over all, compounds **101**, **28**, **102-105** exhibited antibacterial activities against *E. coli* at all tested concentrations, which is moderate compared to chloramphenicol. Similarly, all the compounds displayed dose-dependent pattern inhibitory activity at all concentration against the bacterium *P. aeruginosa*, except β -sitosterol (**49**) at 50 and 100 $\mu\text{g/mL}$. Gnetin H (**89**) displayed inhibitory activity higher than that of the standard, chloramphenicol (11.76 ± 0.77 mm at 30 $\mu\text{g/mL}$) against *P. aeruginosa*, at all tested concentrations. Against the Gram-positive bacterium, *S. aureus*, gnetin H (**102**), and ϵ -viniferin (**101**) showed growth inhibitory activity in good comparison with that of the standard, chloramphenicol (15.74 ± 1.02 mm). The result of the present finding generally indicates that, the isolated compounds displayed an ability to inhibit

the growth of the Gram-negative bacterial strains at all tested concentration. Compounds **101** and **104** displayed growth inhibition against Gram-positive bacterium at all concentrations whereas compounds **49**, **28** and **102** displayed promising activity at higher concentrations.

Table 22: Bacterial growth inhibition zone (mean $\pm$ SD, in mm) of isolated compounds from roots of *C. cyphopetalum* against standard *E. coli*, *S. aureus*, and *P. aeruginosa* strains.

B. strains	compounds	Concentrations ($\mu\text{g/mL}$)				
		50	100	300	500	1000
<i>E. coli</i>	28	8.16 $\pm$ 0.15	8.28 $\pm$ 0.13	8.41 $\pm$ 0.11	8.42 $\pm$ 0.02	8.72 $\pm$ 0.02
	49	NA	NA	NA	NA	7.54 $\pm$ 0.15
	100	NA	NA	NA	NA	7.32 $\pm$ 1.9
	101	8.55 $\pm$ 0.45	8.70 $\pm$ 0.20	9.35 $\pm$ 1.05	9.90 $\pm$ 0.30	10.45 $\pm$ 0.75
	102	7.43 $\pm$ 0.02	8.54 $\pm$ 0.16	9.86 $\pm$ 0.18	9.98 $\pm$ 0.13	10.02 $\pm$ 0.13
	103	8.42 $\pm$ 0.20	8.67 $\pm$ 0.05	8.74 $\pm$ 0.04	8.75 $\pm$ 0.03	8.92 $\pm$ 0.04
	104	8.06 $\pm$ 0.04	8.39 $\pm$ 0.16	8.55 $\pm$ 0.05	8.74 $\pm$ 0.04	8.83 $\pm$ 0.02
	105	8.19 $\pm$ 0.04	8.67 $\pm$ 0.07	8.76 $\pm$ 0.06	9.16 $\pm$ 0.28	9.52 $\pm$ 0.19
	CA	19.06 $\pm$ 2.28				
<i>P. aeruginosa</i>	28	7.43 $\pm$ 0.02	7.60 $\pm$ 0.48	9.28 $\pm$ 0.33	9.24 $\pm$ 0.29	10.46 $\pm$ 0.11
	49	NA	NA	7.05 $\pm$ 0.65	7.8 $\pm$ 0.2	7.85 $\pm$ 0.15
	100	NA	6.55 $\pm$ 0.15	6.65 $\pm$ 0.45	6.92 $\pm$ 0.55	7.01 $\pm$ 0.60
	101	7.76 $\pm$ 0.61	8.26 $\pm$ 0.19	8.78 $\pm$ 0.01	9.07 $\pm$ 0.27	10.36 $\pm$ 0.01
	102	12.55 $\pm$ 0.65	13.35 $\pm$ 0.05	13.25 $\pm$ 0.95	13.75 $\pm$ 0.05	14.60 $\pm$ 0.1
	103	7.67 $\pm$ 0.46	8.62 $\pm$ 0.02	8.21 $\pm$ 0.42	8.34 $\pm$ 0.34	10.32 $\pm$ 0.29
	104	9.42 $\pm$ 0.42	8.55 $\pm$ 0.35	8.20 $\pm$ 0.12	9.13 $\pm$ 0.14	9.51 $\pm$ 0.39
	105	7.82 $\pm$ 0.44	8.02 $\pm$ 0.32	7.71 $\pm$ 0.25	8.00 $\pm$ 0.12	9.44 $\pm$ 0.32
	CA	11.76 $\pm$ 0.77				
<i>S. aureus</i>	28	NA	NA	NA	8.60 $\pm$ 0.7	8.62 $\pm$ 1.5
	49	NA	7.20 $\pm$ 0.56	7.21 $\pm$ 0.19	7.69 $\pm$ 0.58	10.9 $\pm$ 1.54
	100	NA	NA	NA	NA	NA
	101	9.30 $\pm$ 1.39	10.00 $\pm$ 1.3	10.66 $\pm$ 0.73	10.75 $\pm$ 0.2	12.22 $\pm$ 0.74
	102	NA	NA	7.72 $\pm$ 0.39	8.82 $\pm$ 0.09	12.45 $\pm$ 0.37
	103	7.36 $\pm$ 0.20	7.87 $\pm$ 0.01	8.06 $\pm$ 0.14	8.84 $\pm$ 0.11	10.79 $\pm$ 0.24
	104	NA	NA	NA	NA	NA
	105	NA	NA	NA	NA	NA
	CA	15.74 $\pm$ 1.02				

Note: 1. CA-Chloramphenicol, impregnated purchased disc (30 $\mu\text{g/mL}$), NA = Not active

Similarly, *in vitro* antibacterial effects of *n*-hexane, DCM/MeOH (1:1), and MeOH (100%) extracts of the root of *H. johannis*, as well as compounds **40**, **41**, **106** – **108** isolated from DCM/MeOH (1:1) extract were evaluated against the pathogens *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. pyogen*. Extract concentrations of 0.25, 0.50, and 1.00 mg/mL and compounds concentrations of 0.25 and 0.50 mg/mL were prepared from the stock solution. The obtained mean inhibition zone diameters were compared with the standard drug amoxicillin. Table 23 displayed the average inhibitory zone diameters (in mm) of the extracts and identified compounds **40**, **41**, **106** – **108** against the growth of each bacterial strain.

Based on the obtained result, the *n*-hexane, DCM/MeOH (1:1) and MeOH (100%) extracts caused bacterial growth inhibition in a dose dependent manner. At the lowest test concentration of 0.25 mg/mL, the highest activity was exhibited by DCM/MeOH against *S. aureus*, *P. aeruginosa*, and *E. coli* with mean inhibition diameter of 10.75 ± 0.25 , 10.5 ± 0.5 , and 9.5 ± 0.5 mm, respectively. This could be because there is a possibility that the DCM/MeOH extract is meant to have variety of functional groups. Against *S. pyogen*, the *n*-hexane extract showed the highest activity with inhibition diameter of 9.5 ± 0.5 mm. The MeOH extract displayed highest inhibitory effect against *S. aureus*, with inhibition zone of 8.5 ± 0.0 mm, and then against *S. pyogen* (8.25 ± 0.25 mm), *P. aeruginosa* (8.00 ± 1.0 mm), and *E. coli* (7.75 ± 0.75 mm).

With regard to the isolated compounds from the roots of *H. johannis*, at 0.25 mg/mL test concentration, compound **40** showed the greatest activity against *P. aeruginosa*, *E. coli*, and *S. pyogen*, with mean inhibition zones of 13.72 ± 0.05 , 12.25 ± 0.1 , and 11.62 ± 0.12 mm, respectively. These results are promising when compared to amoxicillin (16.00 ± 0.0 mm, at 30 μ g/mL). Compound **106** exhibited the next highest activity against *P. aeruginosa*, *E. coli*, and *S. pyogen* with zone of inhibition diameter of 12.0 ± 0.0 , 11.5 ± 0.5 , and 11.0 ± 0.0 mm at 0.25 mg/mL compared to chloramphenicol (11.76 ± 0.77 , 19.06 ± 2.28 , 15.74 ± 1.02 mm, respectively, at 30 μ g/mL). Against the gram positive *S. aureus* bacteria at 0.25 mg/mL, compound **106** showed the highest growth inhibition with a mean inhibition zone of $10.75 \text{ mm} \pm 0.25$ mm. Compared amoxicillin (16.0 ± 0.0 , 0.25 mg/mL), compounds **107**, **108**, and **41** scored moderated mean inhibition zone (10.75 ± 0.25 , 10.32 ± 0.12 , and 10.41 ± 0.48 mm, respectively) against *P. aeruginosa*. At concentration of 0.50 mg/mL, the largest mean inhibition zone of 14.8 ± 0.2 mm was measured for compound **40** against *P. aeruginosa*, which is promising compared to the standard (16.0 ± 0.0 mm). Against *P. aeruginosa*, the next growth inhibition is scored by

compound **106** (13.5 ± 0.5 mm) followed by compound **108** (11.87 ± 0.97 mm), compound **107** (11.75 ± 0.75 mm), and compound **41** (10.41 ± 0.48 mm). At the same test concentration of 0.5 mg/mL, the highest growth inhibition activity against *E. coli* was shown by compound **40** with mean inhibition zone of 13.89 ± 0.09 mm, followed by compound **106** (12.75 ± 0.75 mm). Overall, the experimental result shows that, despite their variation in antibacterial activities relative to the standard compounds the extracts (*n*- hexane, DCM/MeOH, and MeOH) and evaluated compounds (**100, 49, 101, 28, 102, 103, 104, 105, 106, 107, 40, 108, 41**) exhibited promising growth inhibitory effect against the tested bacterial strains, which support the use of *C. cyphopetalum* and *H. johannis* in tradition medication system for the treatment of bacterial infectious diseases.

Table 23: Inhibition zone (mean $\pm$ SD, in mm) of roots extrcats of *H. johannis*, and isolated compounds **40**, **41**, **106-108**

Samples	Conc. (mg/mL)	Bacterial strains			
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
n-Hexane	0.25	8.25 $\pm$ 0.25	8.50 $\pm$ 0.5	9.25 $\pm$ 0.25	9.5 $\pm$ 0.5
	0.5	10.25 $\pm$ 0.25	9.50 $\pm$ 0.5	10.50 $\pm$ 0.5	11.25 $\pm$ 0.25
	1	11.75 $\pm$ 0.25	11.50 $\pm$ 0.5	12.50 $\pm$ 0.5	13.75 $\pm$ 0.75
DCM/MeOH	0.25	9.5 $\pm$ 0.5	10.5 $\pm$ 0.5	10.75 $\pm$ 0.25	8.5 $\pm$ 0.5
	0.5	11 $\pm$ 0.0	12.25 $\pm$ 0.25	11.5 $\pm$ 0.5	9.5 $\pm$ 0.5
	1.0	13.75 $\pm$ 0.25	14.25 $\pm$ 0.25	13 $\pm$ 0.0	11.5 $\pm$ 0.5
MeOH	0.25	7.75 $\pm$ 0.75	8.00 $\pm$ 1.0	8.5 $\pm$ 0.0	8.25 $\pm$ 0.25
	0.5	9 $\pm$ 1.0	9.25 $\pm$ 1.25	10 $\pm$ 1.0	9.75 $\pm$ 0.75
	1.0	10.25 $\pm$ 0.5	11.5 $\pm$ 1.0	12.25 $\pm$ 0.75	11.5 $\pm$ 0.5
40	0.25	12.25 $\pm$ 0.1	13.72 $\pm$ 0.05	10.20 $\pm$ 0.21	11.62 $\pm$ 0.12
	0.5	13.89 $\pm$ 0.09	14.8 $\pm$ 0.2	10.82 $\pm$ 0.05	12.66 $\pm$ 0.05
41	0.25	7.35 $\pm$ 0.15	7.89 $\pm$ 0.05	7.10 $\pm$ 0.49	8.13 $\pm$ 0.19
	0.5	9.71 $\pm$ 0.06	10.41 $\pm$ 0.48	8.95 $\pm$ 0.05	9.62 $\pm$ 0.12
106	0.25	11.5 $\pm$ 0.5	12.00 $\pm$ 0.0	10.75 $\pm$ 0.25	11.00 $\pm$ 0.0
	0.5	12.75 $\pm$ 0.75	13.50 $\pm$ 0.5	11.75 $\pm$ 0.25	12.00 $\pm$ 0.0
107	0.25	9.0 $\pm$ 0.0	10.75 $\pm$ 0.25	10.00 $\pm$ 0.5	9.50 $\pm$ 1.5
	0.5	11.25 $\pm$ 0.25	11.75 $\pm$ 0.75	11.50 $\pm$ 0.5	11.0 $\pm$ 0.5
108	0.25	9.19 $\pm$ 0.06	10.32 $\pm$ 0.12	8.97 $\pm$ 0.22	8.2 $\pm$ 0.3
	0.5	11.04 $\pm$ 0.05	11.87 $\pm$ 0.97	10.00 $\pm$ 0.89	10.15 $\pm$ 0.05
Amoxicillin (0.25 mg/mL)		15.25 $\pm$ 0.25	16.0 $\pm$ 0.0	15.25 $\pm$ 0.25	16.25 $\pm$ 0.25

4.2.2. *In vitro* antifungal activity

In this study, *in vitro* antifungal activity of *n*-hexane, DCM/MeOH (1:1), and MeOH (100%) extracts from the roots of *C. cyphopetalum* was evaluated against *C. albicans* at extracts concentrations of 12.5, 25, 50, and 100 mg/mL. Table 24 displayed the mean inhibition zone diameters (in mm) of the extracts against the growth of *C. albicans* fungal strain.

Based on the study result, the tested extracts showed antifungal activity in a dose dependent manner (Table 24, Fig. 30). The DCM/MeOH (1:1) and *n*-hexane extracts showed inhibition activity against *C. albicans* at test concentration of 12.5 mg/mL (Table 24). The highest activity was exhibited by DCM/MeOH (1:1) extract with mean inhibition zone of 8.5 ± 0.5 mm, followed by *n*-hexane (7.75 ± 0.25 mm), which is moderate in comparison to the standard Ketoconazole (10 µg/disc) (17.3 ± 2.05 mm). The MeOH (100%) extract displayed no growth inhibition effect against *C. albicans* at concentration of 12.5 mg/mL. At higher concentrations (50 and 100 mg/mL), the *n*-hexane and DCM/MeOH extracts showed similar activity (Fig. 30). At concentration of 50 mg/mL, the DCM/MeOH (1:1) and *n*-hexane extracts scored good activity (13.5 ± 0.5 and 13.25 ± 0.25 mm, respectively) in comparison to the standard. At the largest test concentration of 100 mg/mL, DCM/MeOH (15.5 ± 0.5 mm) and *n*-hexane extracts (15.0 ± 0.0 mm) showed strong fungal growth inhibition comparison to the standard Ketoconazole (10 µg/disc) (17.3 ± 2.05 mm). Methanol extract of roots of *C. cyphopetalum* showed considerable antifungal activity (11.0 ± 0.5 mm) at 100 mg/mL; however, it displayed low antifungal activity relative to the DCM/MeOH and *n*-hexane extracts at all concentrations, but The *in vitro* antifungal activity result reported in this work shows that, *n*-hexane, and DCM/MeOH and MeOH extracts exhibited promising growth inhibitory effect against *C. albicans* strains, which support the use of *C. cyphopetalum* in traditional medication system.

Table 24: Inhibition zone (in mm) of root extracts of *C. cyphopetalum* against *Candida albicans*.

Samples	Concentrations (mg/mL) and inhibition zone (mm)			
	12.5	25	50	100
<i>n</i> -hexane	7.75±0.25	9.75±0.25	13.25±0.25	15.0±0.00
DCM/MeOH	8.5±0.5	11.75±0.25	13.5±0.5	15.5±0.5
MeOH	NA	7.75±0.25	8.75±0.25	11.0±0.5
Ketoconazole/Tilt (10 µg/disc)	17.3±2.05			

NA = not active

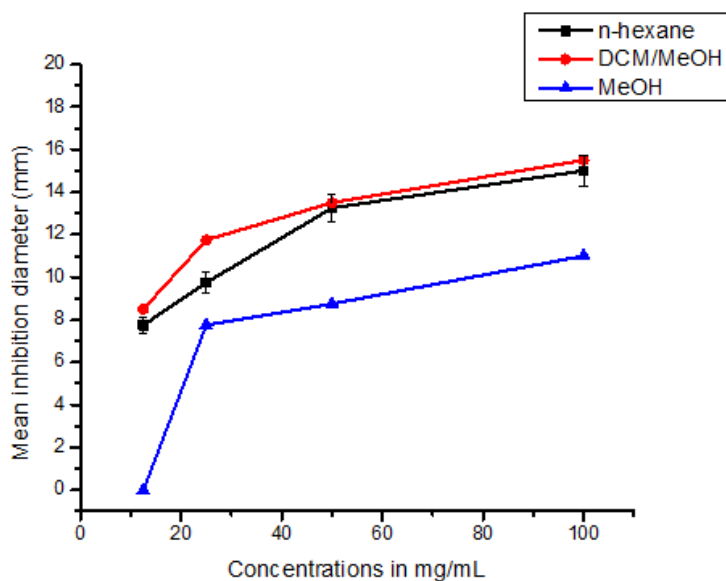


Fig. 30: *In-vitro* antifungal activity of *n*-hexane, DCM/MeOH (1:1), and MeOH (100%) extracts of roots of *C. cyphopetalum* against *C. albicans*.

4.2.3. Cytotoxicity tests

Breast cancer is the leading deadly disease for women worldwide (R. Wang et al., 2018). Phytochemicals isolated from medicinal plants are considered as promising agents for the clinical treatment of breast cancer. The present study reported the anticancer activity of *n*-hexane, DCM/MeOH (1:1), MeOH extracts of roots of *H. johannis*, DCM/MeOH (1:1) and MeOH extracts from roots of *C. cyphostemma* and stem bark of *C. tomentosa* against human breast cancer MCF-7 cell line by MTT assay, at extracts concentration of 20 µg /mL. MCF-7 cell line is a commonly used suitable model breast cancer cell for investigation of anticancer drugs (Comşa, Cîmpean, & Raica, 2015). Table 25 displayed the % cell viability tested extracts against MCF-7 cell lines.

The studied extracts recorded potential anticancer property. The MeOH extract of roots of *C. cyphopetalum*(CCME), *n*-hexane extract of roots *H. johannis* (HJHE), and DCM/MeOH extract of stem bark of *C. tomentosa* (CTDME) exhibited strong anticancer activity against breast cancer cell line (MCF-7) with % cell viability of 27.8 %, 28.9 % and 44.9 %, respectively (Table 25, Fig. 31). The MeOH extract of root of *H. johannis* (HJME), DCM/MeOH extract of *C.cyphopetalum* (CCDME), MeOH extract of *C.tomentosa* (CTME) and DCM/MEOH extract of *H.johannis* (HJDME) extracts have shown moderate cytotoxicity with percentage viability of 51.6%, 53.9%, 58.9%, and 59.5%, respectively. The obtained result indicates that the CCME HJHE and CTDME extracts (20 µg/mL) exhibited % cell viability <50% (estimated IC₅₀ < 30 µg/mL), which is acceptable according to the American National Cancer Institute (NCI) criteria of cytotoxic activity for the crude extracts (Talib & Mahasneh, 2010). The experimental result shows that the evaluated extracts exhibited promising cytotoxicity effect against MCF-7 cell line, which support the use of *C. cyphopetalum*, *H. johannis*, and *C. tomentosa* in tradition medication system for breast cancer treatment.

Table 25: Cytotoxicity of *C. cyphopetalum*, *H. johannis*, and *C. tomentosa* extracts against MCF-7 cell line

Samples	% of cell viability	SD	Estimated IC ₅₀ (µg/mL)
DMSO	100.0	0.14	
CCDME	53.9	0.03	
CCME	27.8	0.13	< 20
HJHE	28.9	0.19	< 20
HJDME	59.5	0.15	
HJME	51.6	0.28	20
CTDME	44.9	0.32	< 20
CTME	58.9	0.06	

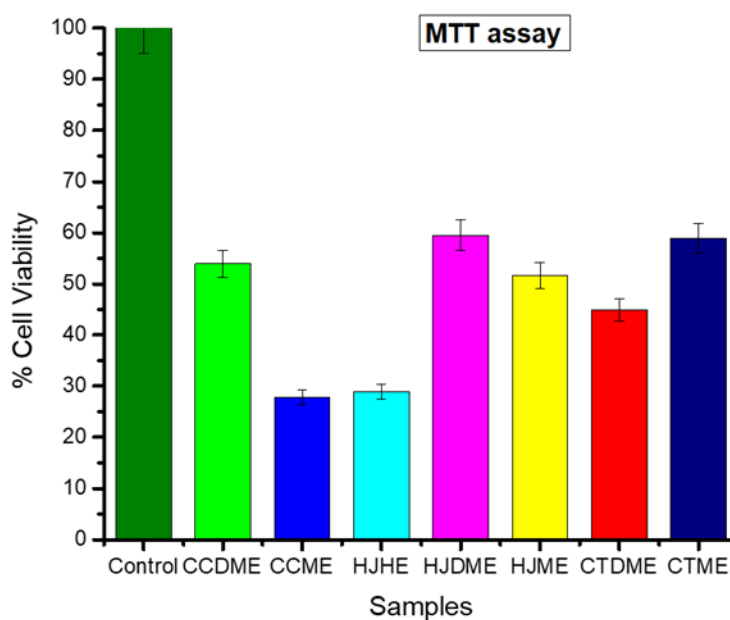


Fig. 31: *In-vitro* cytotoxic activity of DCM/MeOH, MeOH and/or n-hexane extracts of *C. cyphopetalum*, *H. johannis*, and *C. tomentosa*.

Note = CCMDE= DCM/MeOH and CCME=MeOH extracts of *C. cyphopetalum*; HJHE=n-hexane, HJDME=DCM/MeOH, and HJME=MeOH extracts of *H. johannis*; CTDME=DCM/MeOH and CTME=MeOH extracts of *C. tomentosa*.

4.2.4. Antioxidant activity

The radical scavenging activity of isolated compounds **28**, **49**, **100**, **101**, **102**, **104**, and **105**, isolated from roots of *C. cyphopetalum*, **106**, and **107** which isolated from the root of *H. Johannis* were evaluated using the DPPH assay, a simple method to evaluate antioxidant activities by measuring absorbance at 517 nm due to the formation of stable DPPH radical (Gülçin, 2010). At all the tested concentrations of each compound, the purple color of the DPPH solution was changed to yellow color indicating potential as radical scavenger of the compounds. The % of DPPH scavenging activity exhibited by each compound is presented in Table 26. The compounds have shown radical scavenging activity in dose-dependent manner (Fig 32). At concentration of 12.5 µg/mL, compounds **28**, **101**, and **105** displayed good DPPH radical scavenging activity (76.87, 74.30, and 73.32 %, respectively) compared to ascorbic acid (80.91 %). The highest percentage inhibition was recorded by compound **28** (76.87 ± 0.09 %) at 12.5 µg/mL, followed by compound **101** (74.30 ± 0.12 %), and **105** (73.32 ± 0.25 %), with IC₅₀ value of 0.052 µg/mL, 0.017 µg/mL, and 0.025 µg/mL, respectively. The highest DPPH scavenging activity of compound **28** is justifiably due to the stability of catechin radical. At the same concentration, compound **104** (69.40 ± 0.11 %) and **106** (67.87 ± 0.02 %) has shown moderate radical scavenging activity. Compounds **107** (52.75 ± 0.01 %), **100** (55.69 ± 0.20 %) **49** (59.61 ± 0.22 %) recorded low radical scavenging activity compared to other compounds in this report. At concentration of 200 µg/mL, compound **28** exhibited better antioxidant activity (88.86 %) compared to that of ascorbic acid (87.64 %). At the same concentration, compounds **101**, **102**, **104** and **105** (85.07, 83.23, 82.99, 83.72 %, respectively) also displayed good antioxidant activity compared to ascorbic, followed by compounds **49**, **100**, **106** and **107** (76.74, 74.05, 79.74 %, respectively). Compound **107** showed the lowest activity (67.07 %).

Table 26: Radical scavenging activity isolated compounds

Compounds	% DPPH inhibition at					IC ₅₀ ($\mu\text{g/mL}$)
	200 $\mu\text{g/mL}$	100 $\mu\text{g/mL}$	50 $\mu\text{g/mL}$	25 $\mu\text{g/mL}$	12.5 $\mu\text{g/mL}$	
28	88.86 $\pm$ 0.02	86.41 $\pm$ 0.11	82.86 $\pm$ 0.27	79.44 $\pm$ 2.64	76.87 $\pm$ 0.09	0.052
49	76.74 $\pm$ 0.11	69.40 $\pm$ 0.21	65.73 $\pm$ 0.37	63.28 $\pm$ 0.33	59.61 $\pm$ 0.22	2.72
100	74.05 $\pm$ 0.30	66.22 $\pm$ 0.41	62.79 $\pm$ 0.49	58.63 $\pm$ 0.01	55.69 $\pm$ 0.20	6.05
101	85.07 $\pm$ 0.05	80.17 $\pm$ 0.10	77.85 $\pm$ 0.06	76.74 $\pm$ 0.18	74.30 $\pm$ 0.12	0.017
102	83.23 $\pm$ 0.23	79.19 $\pm$ 0.29	75.28 $\pm$ 0.71	74.17 $\pm$ 0.31	71.85 $\pm$ 0.63	0.063
104	82.99 $\pm$ 0.04	79.07 $\pm$ 0.63	76.38 $\pm$ 0.01	74.42 $\pm$ 0.23	69.40 $\pm$ 0.11	0.157
105	83.72 $\pm$ 0.31	79.68 $\pm$ 0.17	77.85 $\pm$ 1.13	76.87 $\pm$ 0.98	73.32 $\pm$ 0.25	0.025
106	79.74 $\pm$ 0.04	77.36 $\pm$ 0.02	75.03 $\pm$ 0.01	70.99 $\pm$ 0.1	67.87 $\pm$ 0.02	0.190
107	67.07 $\pm$ 0.01	65.48 $\pm$ 0.01	61.44 $\pm$ 0.03	57.77 $\pm$ 0.02	52.75 $\pm$ 0.01	6.250
Asc Acid	87.64 $\pm$ 0.13	81.64 $\pm$ 0.11	82.62 $\pm$ 0.13	82.25 $\pm$ 0.05	80.91 $\pm$ 0.21	0.0012

Ascorbic acid was used as positive control with DPPH inhibition of 80.91% at 12.5 $\mu\text{g/mL}$

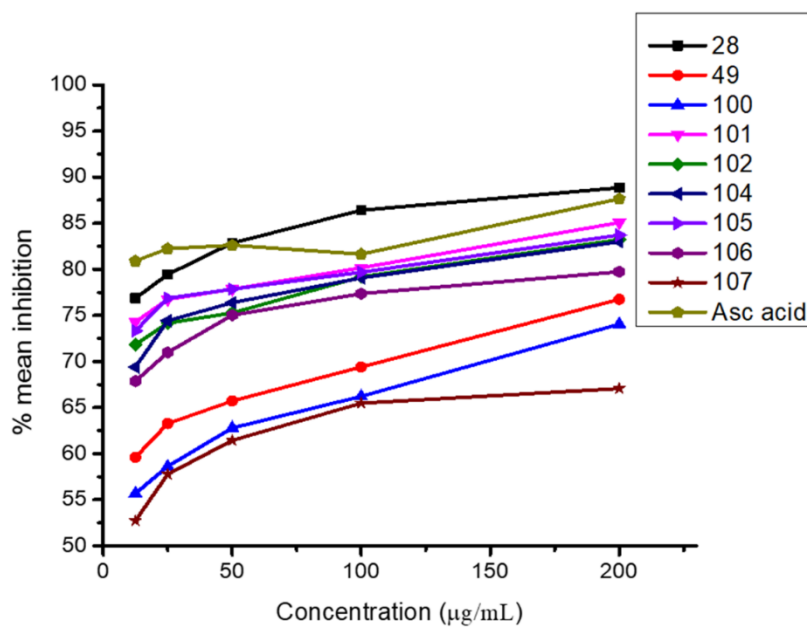


Fig. 32: Percent of DPPH scavenging activity of the isolated compounds and the standard.

4.3. Computational study of isolated compounds

4.3.1. Molecular docking analysis of isolated compounds

Molecular docking has become a crucial technique in structure-based rational drug discover to facilitate and speed up the drug development process due to its capability to predict the orientation of the ligand when it is coupled to a protein receptor or enzyme (L. G. Ferreira, Santos, Oliva, & Andricopulo, 2015; Pagadala, Syed, & Tuszynski, 2017). By using this tool, researchers can virtually screen the relationship between the ligand and target protein, while also being able to predict how a specific species will bind to a specific target protein (Mumit et al., 2020). In this study, molecular docking evaluation of isolated compounds **28**, **49**, **100**, **101**, **102**, **103**, **106**, and **107** was carried out to study their binding pattern with the binding site of proteins *E.coli* DNA gyrase B (PDB ID 6F86), PqsA (PDB ID: 5OE3), *S. aureus* Pyruvate Kinase (PK) (PDB ID: 3T07) and compared with the standard chloramphenicol.

E. coli DNA gyrase, PqsA *P. aeruginosa* and *S aureus* PK were chosen as target enzymes based on their significance in the corresponding organism. *E.coli* DNA gyrase B, an essential enzyme involved in DNA supercoiling, is a successful target in antibacterial compound screening (Alt, Mitchenall, Maxwell, & Heide, 2011). Due to the importance of pyruvate level in organisms for biofilm formation and the maintenance of reductive conditions, pyruvate kinase may be a potential target for drugs against *S aureus* infections (K et al., 2012; Zoraghi et al., 2011). The enzyme PqsA is useful as a means to screen inhibitors of *Pseudomonas* quinolone signal (PQS) biosynthesis, which is predicted to control the expression of multiple poisonousness factors in *P. aeruginosa*. (Chen et al., 2022; Duraipandiyar, Balachandran, Raj, Arasu, & Ignacimuthu, 2014).

The molecular values including docking scores and binding interactions of the best poses for each of the docking studies of the ligands with the target proteins DNA gyrase, PqsA, and *S. aureus* PK are presented in Table 27-29. Fig. 33 is presented to compare the binding affinity of each compounds docked to DNA gyrase B, PqsA, and *S. aureus* PK. The binding interactions of the best poses for each of the docking studies of the ligands with each target proteins are presented in Fig 34-36. A greater negative value of binding energy of any isolated compound corresponds to a stronger binding target protein. The docking of isolated compounds to the target protein DNA gyrase B, PqsA, and *S. aureus* PK exhibited binding affinities in the range of -6.5

to -7.6 kcal/mol, -6.4 to -8.8 kcal/mol and -4.4 to -7.4 kcal/mol, respectively (Table 27-29). Each of the compounds docked to the target proteins have shown hydrogen bond (except compound **100** to DNA gyrase and *S. aureus* PK, compound **49** to PqsA and *S. aureus* PK), hydrophobic/Pi-Cation and Van der Waals interaction with residual amino acids in the binding pocket. When docked to protein DNA gyrase B, all the isolated compounds displayed higher binding energy (-6.5 to -7.6 kcal/mol) than the standard chloramphenicol (-6.4 kcal/mol). Compounds **101** and **106** scored the highest binding energy (-7.6 kcal/mol) followed by compounds **49** and **102** (-7.4 kcal/mol) (Table 27). The highest binding energy of compound **101** and **106** (-7.6 kcal/mol each) in DNA gyrase complex arise from hydrogen bond with amino acids Asp-73, and Glu-50, Gly-77 and Ser-12, respectively, along with hydrophobic and van der Waals interactions (Fig. 34, Appendix 21). Compound **107**, relative to the other compounds, scored the lowest binding energy (-6.5 kcal/mol) but sufficient in comparison to chloramphenicol. The hydrophobic and van der Waals interactions observed to be dominant in compound **100**-DNA gyrase complex. Compound **49** and **102** (-7.4 kcal/mol each) showed hydrogen bond interaction with amino acids Glu-50 in binding pocket of DNA gyrase.

Towards the target enzyme PqsA of *P. aeruginosa*, compounds **28**, **101**, **102**, **103**, **106**, **107** scored better binding affinities (with binding energies in the range of -7.1 to -8.8 kcal/mol) than the standard chloramphenicol (-7.0 kcal/mol) (Table 28). All compounds have shown hydrogen bond (except compound **49**), hydrophobic and van der Waals interactions (Fig 35). The highest binding affinity was measured for compound **102** (-8.8 kcal/mol) followed by compound **106** and **107** (-8.7 and -8.6 kcal/mol, respectively). The comparably better binding energy of compound **102** (-8.8 kcal/mol) than chloramphenicol (-7.0 kcal/mol) is attributed to six hydrogen bond interactions (Thr-380, Tyr-378, Asp-382, Gly-300, and Gly-279 of PqsA) along with hydrophobic interactions (Ser-280, Arg-397, Ala-303, Phe-209, Gly-302 and Ile-301) and van der Waals interaction (Asp-299, His-394, Gly-396, Pro-281, Ala-278, Arg-372, Phe-310, Tyr-211 and Thr-304), respectively (Appendix 22). Despite its interaction without hydrogen bond, compound **49** has shown binding energy of -7.0 kcal/mol comparably equivalent to the standard chloramphenicol (-7.0 kcal/mol). Compound **100** score the lowest binding affinity with binding energy of -6.4 kcal/mol relative to other compounds, but moderate in comparison to the standard (-7.0 kcal/mol).

With regard to docking poses with target protein Pyruvate kinase (PK) of the Gram positive bacterial strain *S. aureus*, compounds **106**, **107**, **102**, **101**, and **100** scored better binding affinities (-5.4 to -7.4 kcal/mol) than chloramphenicol (-4.6 kcal/mol) (Table 29). Among these, compound **106** scored the highest binding affinity (-7.4 kcal/mol). A total of seven hydrogen bonds via amino acids Glu-234, Leu-233, Ser-236, Asp-237, Lys-271, Asn-357 and Leu-269, Leu-269, and residual hydrophobic and van der Waals interactions were observed in the compound **106**-*S. aureus* PK complex (Fig. 36). Compound **107** has shown hydrogen bond with amino acid Ser-411, hydrophobic with Val-419, and van der Waals with Glu-440, Val-420, Asp-58, Ile-60, Val-416, B: Lys-5, Gln-417 Thr-441 of the *S. aureus* PK binding pocket (Appendix 23). Compounds **49** and **103** (-4.6 kcal/mol, each), and compound **28** (-4.4 kcal/mol) scored binding energies comparable to the standard.

Over all, the binding affinities of the docked compounds are in agreement with the *in vitro* antibacterial and antibacterial activity results. In DNA gyrase B docking poses, all the tested compounds scored binding affinities better than the standard chloramphenicol. Except compound **85**, **100** with PqsA and **28** with *S. aureus* PK, all the docked compounds has shown binding affinities better than chloramphenicol. The binding affinity, H-bond and residual amino acid interactions of the compounds and chloramphenicol are summarized in Table 27-29. The mapped binding interactions of isolated compounds **28**, **49**, **100**, **101**, **102**, **103**, **106**, and **107** against bacterial proteins are displayed in Fig. 34-36, Appendices 21-23. Ribbon model shows the binding pocket structure of target proteins with the compounds. Hydrogen bond between compounds and amino acids are shown as green dash lines, hydrophobic interaction are shown as pink lines.

Table 27: Molecular docking values of isolated compounds against DNA (PDB ID: 6F86)

Compounds	Affinity (kcal/mol)	H-bond	Residual interactions	
			Hydrophobic/Pi-Cation	Van der Waals
28	-6.6	Asn-46	Thr-165, Ala-47, Val-167	Met-95, Val-120, Val-43, Val-71, Asp-73
49	-7.4	Glu-50	Ile-78, Val-120, Ile-94, Val-93, Pro-79	Ser-121, Val-97, Gly-119
100	-6.9	-	Ile-78, Pro-79,	Gly-77, Glu-50, Thr-165, Asp-73, Ala-47, Ile-94
101	-7.6	Asp-73	Ile-94, Asn-46, Ile-78, Arg-76, Pro-79	Ala-47, Gly-77
102	-7.4	Glu-50	Ile-78, Ile-94, Pro-79, Arg-136	Asp-49, Ala-53, Arg-136
103	-6.8	Asn-46, Thr-165	HOH-616, Ile-94, Pro-79, Ile-78, Glu-50, Arg-76	Asp-49, Gly-77, Arg-136, Hoh-685
106	-7.6	Glu-50, Gly-77, Ser-121	Ile-94, Ile-78	Arg-76, Pro-79, Gly-75, Asp-73, Thr-165, Gly-164, Asn-46, Gly-117, Val-118, Leu-98, Val-97, Gly-119, Val-120
107	-6.5	Asn-46, Gly-77, Arg-76	Leu-52, Ala-53, Pro-79	Sp-49, Ile-78, Ile-94, Ala-47, Gly-75
Chloramphenicol	-6.4	Glu-50, Gly-77, Asn-46	Ile-78	Val-43, Asp-49, Arg-76, Asp-73, Ala-47, Thr-165

Table 28: Molecular docking value of isolated compounds against PqsA (PDB ID: 5OE3)
in complex with anthraniloyl-AMP (PDB ID: 5OE3)

Compounds	Affinity (kcal/mol)	H-bond	Residual interactions	
			Hydrophobic/Pi-Cation	Van dar Waals
28	-7.5	Thr-304, Val-309	Gly-302, His-308, Ala-303	Gly-210, Tyr-211, Gly-279, Thr-164, Glu-305
49	-7.0	--	Ile-301, Phe-209, Val-309, Tyr-211, Ala-278	Gly-210, Gly-279, Gly-302, Thr-304, His-308, Thr-164, Glu-305, Lys-172, Arg-397, Thr-380, Tyr-378, Arg-372
100	-6.4	Arg-372, Lys-172	Ala-303, Thr-380	Arg-397, Gly-279, Phe-209, Gly-302, Thr-304, Thr-164, Tyr-362, Tyr-378
101	-7.1	Asp-382, Gly-279	Ser-280, Ile-301, Thr-164	Pro-281, Gly-210, Tyr-211, Phe-209, Thr-304, Glu-305, Tyr-378, Lys-172
102	-8.8	Thr-380, Tyr-378, Asp-382, Gly-300, Gly-279	Ser-280, Arg-397, Ala-303, Phe-209, Gly-302, Ile-301	Asp-299, His-394, Gly-396, Pro-281, Ala-278, Arg-372, Phe-310, Tyr-211, Thr-304
103	-7.2	Thr-380	Ala-303, Glu-305, Thr-164, Phe-209, Gly-302, Gly-279	Tyr-378, Tyr-211, Gly-210
106	-8.7	Pro-85, Lys-86, Ala-108	Ala-124, Leu-60, Arg-128, Ala-108	Arg-106, A: Pro-111, Ala-110, Asp-109, Asn-61, Phe-134, Leu-66, Ser-65, Ala-125, A: Gly-127, Ala-126
107	-8.6	Gly-279, Asp-282	Val-254, A: Pro-205 A: Ile-204, A: Ile-257, Phe-209	Pro-234, Tyr-211, Phe-208, Trp-233, Gly-210, Ala-303, Gly-302, Arg-397, His-394, Ile-301, Thr-304
Chloramphenicol	-7.0	Gly-279	Phe-209, Ala-278, His-308, Gly-302	Ser-280, Ile-307, His-394, Asp-382, Ala-303, Val-309, Gly-210, Gly-307, Tyr-211

Table 29: Molecular docking values against *S. aureus* PK (PDB ID: 3T07)

in complex with a naturally occurring bis-indole alkaloid

Compounds	Affinity (kcal/mol)	H-bond	Residual interactions	
			Hydrophobic/Pi-Cation	Van der Waals
28	-4.4	Asn-583, Asp-571	Leu-370	Leu-499, Leu-471, Gln-574
49	-4.6	--	His-365, Ile-361	Thr-366, Asn-369, Ser-362, Ala-358, Thr-353
100	-4.8	--	Leu-370, Leu-449, Ile-469	Asn-583
101	-5.4	Thr-353	Ala-358, Ile-361, Ser-362, His-365	Thr-348, Ser-354, Leu-355
102	-5.8	Asn-369	His-365, Ile-361, Leu-370	Thr-366, Ser-362, Thr-348
103	-4.6	--	Ile-361, Thr-353, Ala-358	Thr-348, Ser-354, Asn-357
106	-7.4	Glu-234, Leu-233, Ser-236, Asp-237, Lys-271, Asn-357, Leu-269	Val-356	Arg-204, Val-235, Glu-352, Lys-390, Gly-270, Arg-386
107	-7.0	Ser-411	Val-419	Glu-440, Val-420, Asp-58, Ile-60, Val-416, B: Lys-5, Gln-417 Thr-441
Chloramphenicol	-4.6	Ala-358, Ser-362	--	Lu-355, Thr-353, Thr-348, His-365

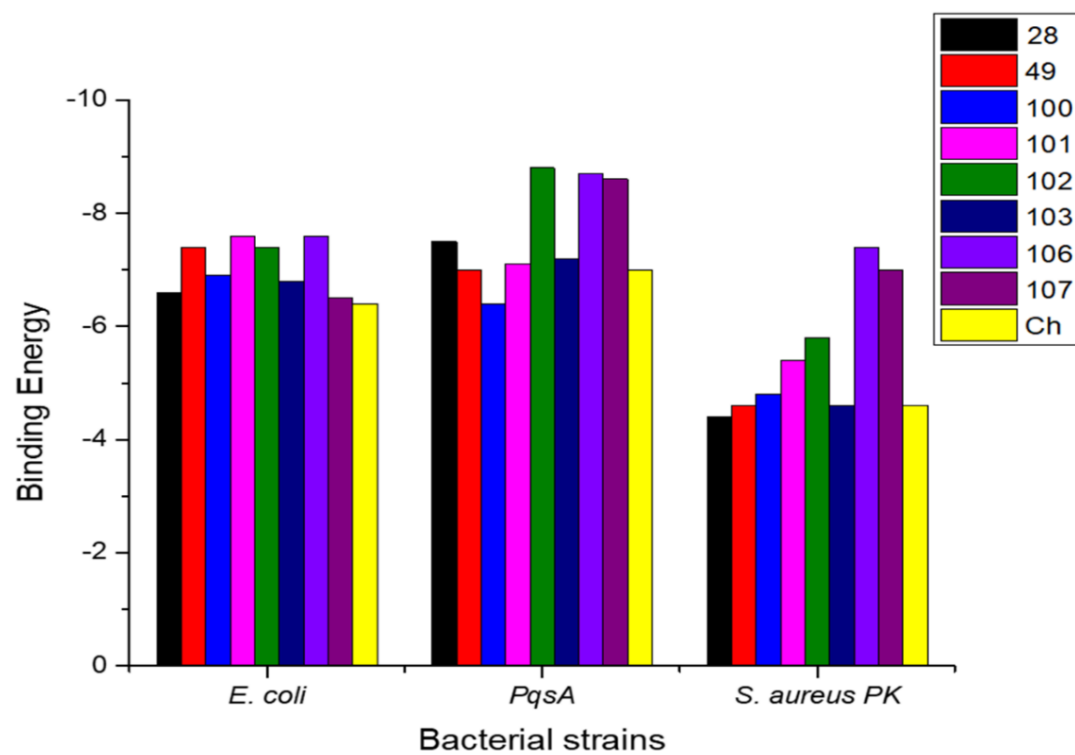


Fig. 33: Binding affinity of the compounds toward DNA gyrase B, PqsA, and *S. aureus* PK.

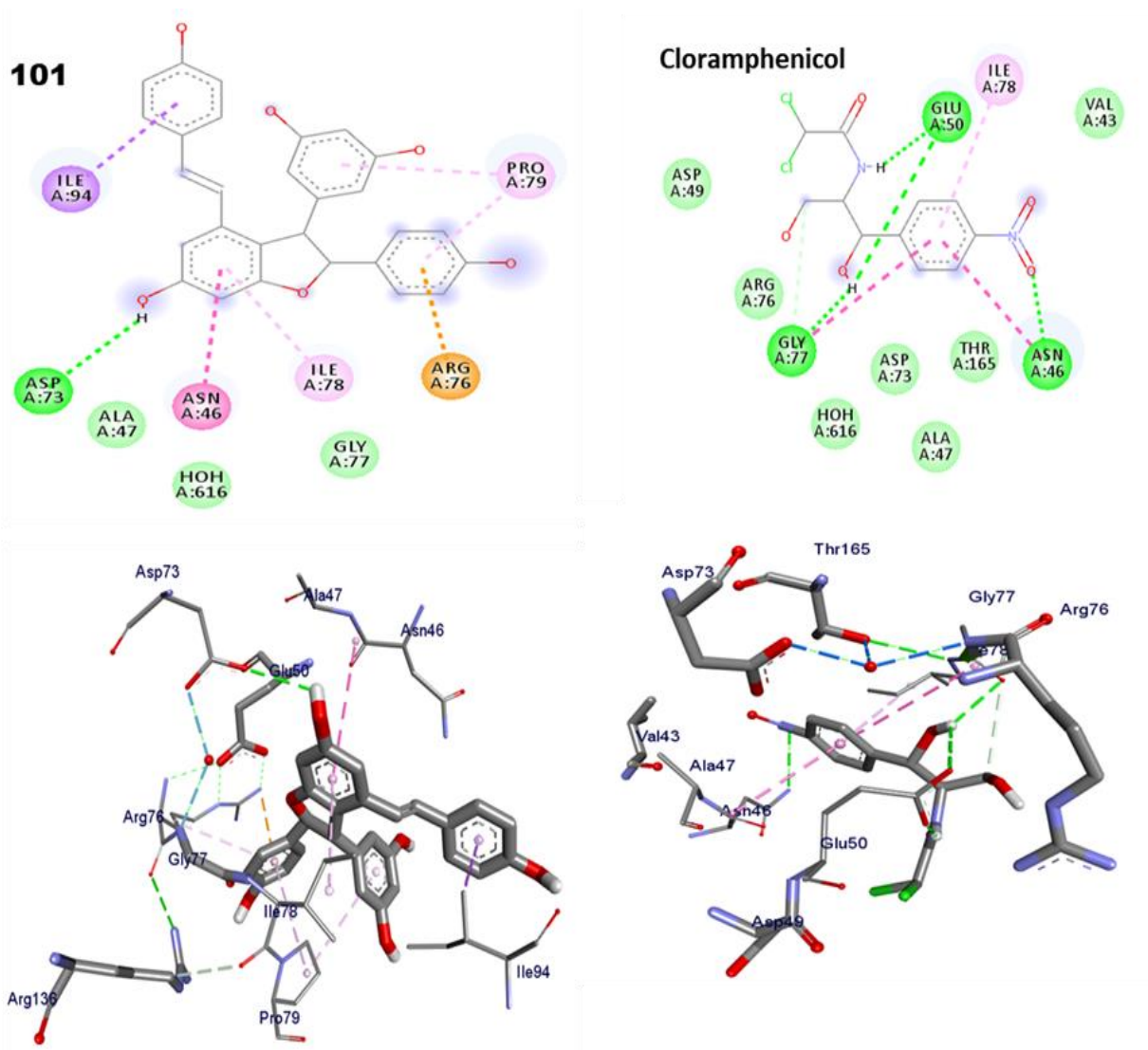


Fig. 34: The 2D and 3D binding interactions of compounds **100** and standard (chloramphenicol) against DNA gyrase B (PDB ID: 6F86).

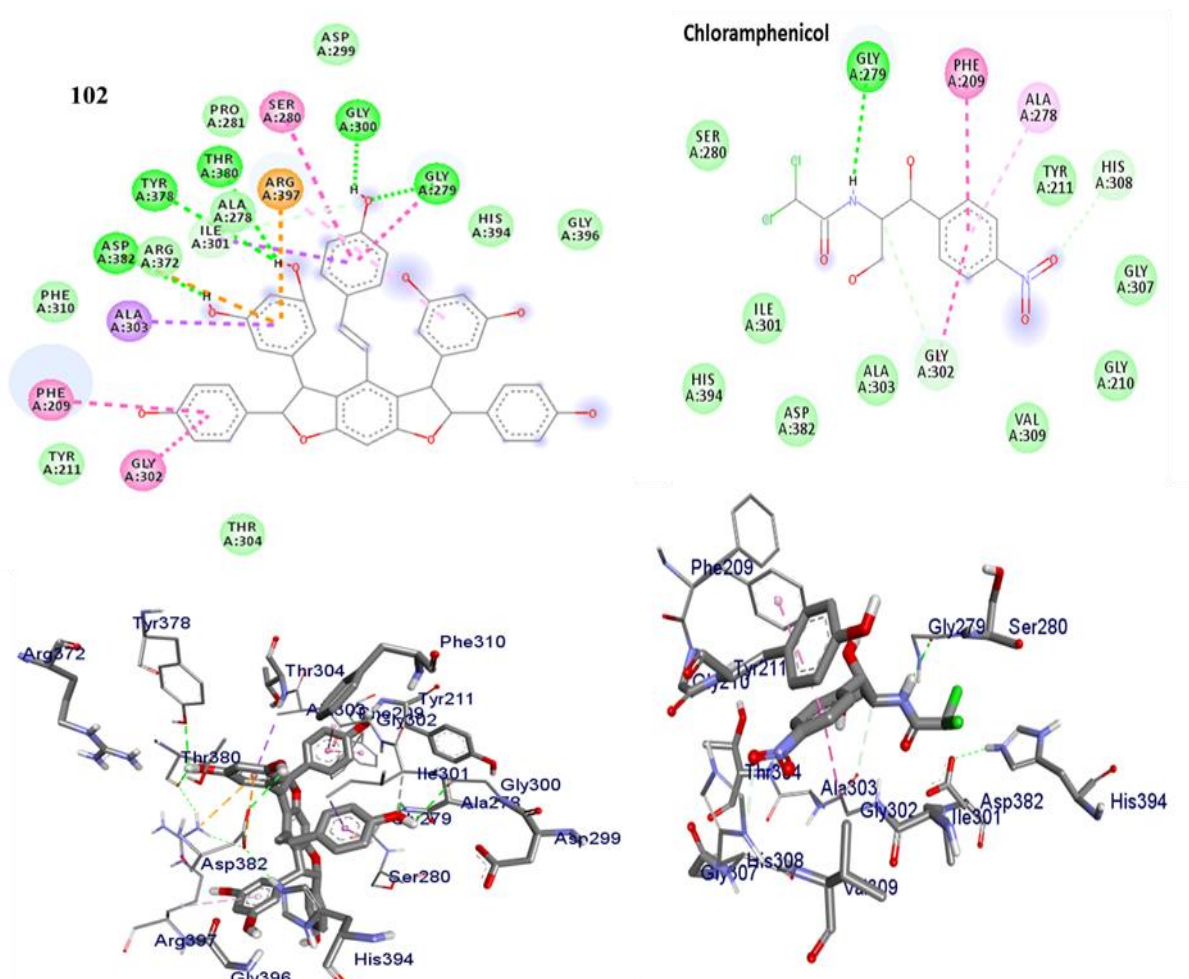


Fig. 35: The 2D and 3D binding interactions of compounds **102** and standard against PqsA (PDB ID: 5OE3).

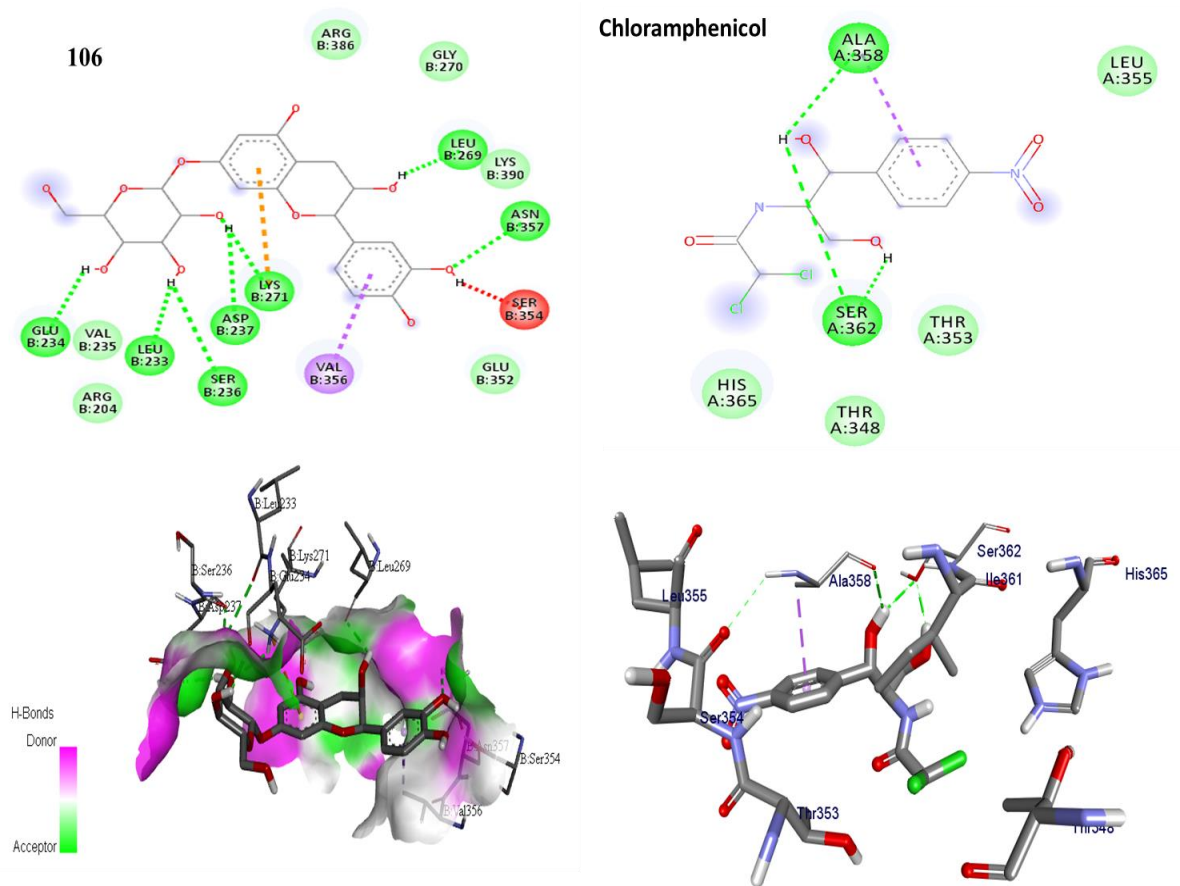


Fig. 36: The 2D and 3D binding interactions of compounds **106** and standard against *S. aureus* PK (PDB ID: 3T07).

4.3.2. *In-Silico* Drug-likeness, ADME and Toxicity analysis

In silico pharmacokinetic (drug likeness and ADME) predictions have been playing an important part in drug development for the selection of compounds with desirable bioavailability during the early phases of drug discovery in hit-to-lead and lead-optimization campaigns (Jia et al., 2020; Odoemelam et al., 2022). Based on the fact that natural products are excellent sources of drug candidates and that it is valuable to obtain their *in silico* pharmacokinetic profile (Durán-Iturbide, Díaz-Eufracio, & Medina-Franco, 2020). The *in silico* drug likeness and ADMET predictions of the isolated compounds **28**, **49**, **100 - 103**, **106**, and **107** were determined based the concept of the Lipinski's rule of five (Lipinski et al., 1997) and Veber's rule (Wong, Hag Ali, & Boey, 2012). The Lipinski's rule of five established that compounds with molecular weight (MW) <500 Daltons, H-bond donors (NHD) <5, H-bond, acceptors (NHA) <10, and

logarithm of the *n*-octanol/ water partition coefficient ($\log P$) < 5 are likely to be considered as a potential drug candidate (Lipinski et al., 1997). Veber's rule says that a compound with rotatable bonds (RTB) < 10 and a polar surface area (TPSA) < 140 Å² should present good oral bioavailability (Veber et al., 2002).

Drug likeness

The chemical structures of isolated compounds were converted to their corresponding canonical simplified molecular-input line-entry system (SMILE) and submitted to the SwissADME tool to generate the physicochemical and pharmacokinetic properties of the compounds. The drug likeness prediction results of the studied compounds are presented in Table 30. Based on the SwissADME prediction outcome, compounds **100**, **49**, **101** and **28** satisfy both Lipinski's rule of five and Veber's rule with zero violations (Table 30). This implies that compounds **100**, **49**, **101** and **28** are being likely drug candidates according to the Lipinski's rule of five. Compounds **102**, **103** and **106** violated the rule because of high HBD (>5) each. Compounds **100**, **49**, **101**, **28**, **102**, **103** and **106** recorded lipophilicity (iLogP) value of less than five (range from 1.79 to 4.79), implying their optimal lipophilicity, except compound **107** (5.29). The number of rotatable bond (NRB) values for all the studied compounds are less than ten, indicating the conformation stability of the compounds (Anza, Endale, Science, Eswaramoorthy, & Science, 2021). The total polar surface (TPSA) influences permeability and bioavailability of a molecule. The predicted TPSA shows compounds **28**, **49**, **100**, **101**, **102**, and **107** have TPSA less than 140 Å², which indicate their excellent absorption in the intestine. Compound **102** and **106** violate the Veber's rule because of high TPSA (>140 Å²).

Table 30: *In silico* drug-likeness of isolated compounds computed by SwissADME

Compounds	Formula	MW (g/mol)	NHD	NHA	LogP (iLogP)	LRO5 Violation	NRB	TPSA (Å ²)	Veber's rule violation
28	C ₁₄ H ₁₂ O ₃	228.24	3	3	1.71	0	2	60.69	0
49	C ₂₉ H ₅₀ O	414.71	1	1	4.79	0	6	20.23	0
100	C ₂₀ H ₃₀ O ₃	318.45	1	3	2.92	0	0	46.53	0
101	C ₂₈ H ₂₂ O ₆	454.47	5	6	2.41	0	4	110.38	0
102	C ₄₂ H ₃₂ O ₉	680.7	7	9	3.39	1	6	160.07	1
103	C ₂₈ H ₂₄ O ₇	472.49	6	7	2.04	1	4	130.61	0
106	C ₂₁ H ₂₄ O ₁₁	452.41	8	4	1.55	1	4	189.53	1
107	C ₃₅ H ₆₀ O ₆	576.85	11	6	5.29	2	9	99.38	0

NHD: Number of Hydrogen donor; NHA: Number of Hydrogen acceptor; NRB: Number of rotatable bonds; TPSA: total polar surface area, MW: Molecular weight

ADME properties

Prediction of ADME properties are very important in the drug discovery stages (Joung et al., 2012). In this study, *in silico* prediction of ADME properties of isolated compound **28, 49, 100, 101, 102, 103, 106, and 107** were performed with the SwissADME tool (Daina et al., 2017). The main ADME parameters including gastrointestinal absorption, BBB permeability, transporter P-glycoprotein (P-gp) binding, skin permeation (logKp) and drug-mobilizing CYP450 isoforms (CYP1A2, 2C9, 2C19, 2D6 and 3A4) inhibition were considered to assess the pharmacokinetic behavior of the tested compounds (L. L. G. Ferreira & Andricopulo, 2019). These pharmacokinetic parameters stated to comprises an efficient ADME barrier for many orally administered drugs (Yim et al., 2020). Table 31 displayed the ADME prediction outcomes of the tested compounds.

Based on the obtained prediction result, the logKp value of the studied compounds predicted to be within the range of -2.2 to -10.08 cm/s. The more negative the log Kp (with Kp in cm/s), the

less skin permeant is the molecule (Daina et al., 2017). In the SwissADME prediction, compounds **100**, **101** and **28** have high gastro intestinal absorption. Compound **85** and **28** exhibited blood brain barrier (BBB) permeation, which is fundamental for the distribution of central-acting molecules. High gastrointestinal (GI) and blood-brain barrier (BBB) permeation can be considered as favorable absorption and distribution properties of drug molecules (Anza et al., 2021). The ADME prediction outcome also displayed that all compounds under investigation are found to be non-substrate of permeability glycoprotein (P-gp), and hence non-inhibitors of most of the selected cytochromes (CYP). However, compound **28** predicted to show inhibitory effect towards CYP1A2, CYP2C9 and CYP3A4, compound **100** and **101** each exhibited inhibition of CYP2C9 and CYP3A4, and compound **90** inhibited CYP3A4.

Table 31: ADME predictions of compounds 85-90, 93 and 94 computed by SwissADME

Compo unds	logKp cm/s	GIA	BBB	Inhibitor Interaction (SwissADME/PreADMET)					
				P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
28	-5.47	High	Yes	No	Yes	No	Yes	No	Yes
49	-2.2	Low	No	No	No	No	No	No	No
100	-5.23	High	Yes	No	No	No	Yes	No	No
101	-5.24	High	No	No	No	No	Yes	No	No
102	-5	Low	No	No	No	No	No	No	No
103	-6.14	Low	No	No	No	No	No	No	Yes
106	-10.08	Low	No	No	No	No	No	No	No
107	-4.32	Low	No	No	No	No	No	No	No

LogKp = Skin permeation value, GI = Gastro-Intestinal, BBB = Blood Brain Barrier, P-gp = P-glycoprotein, CYP = Cytochrome-P.

Toxicity prediction

Toxicity is the degree to which a substance can damage an organism or organs of the organism, such as cells and tissues, and is one of the most significant reasons for failure in late-stage drug

development (Durán-Iturbide et al., 2020). Determining the toxicity of bioactive compounds would thus be necessary to identify their harmful effects on humans, animals, plants, or the environment. The toxicity profiles of the isolated studied compounds **28**, **49**, **100**, **101**, **102**, **103**, **106**, and **107** were carried out using ProTox II. The predicted toxicity results of the studied compounds were presented in Table 32. Compound **106** and **107** exhibited negligible acute toxicity ($LD_{50} > 5000$, Toxicity class > 5). The ProTox-II prediction also gives toxicological endpoints such as hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity results. Organ toxicity prediction results indicated that none of the studied compounds have shown hepatotoxicity, mutagenicity, and cytotoxicity. Compounds **49**, **100**, **101**, and **106** have shown immunotoxicity. Compound **102** showed carcinogenicity and immunotoxicity. Based on the ADMET prediction analysis and the presented toxicity data, the studied compounds can be considered as good candidates for drug development process.

Table 32: Prediction of Toxicity of compounds computed by ProTox-II.

Compounds	LD ₅₀ (mg/Kg)	Toxicity class	Toxicity				
			Hepato	Carcino	Immuno	Mutagen	Cyto
28	1560	4	No	No	No	No	No
49	890	4	No	No	Yes	No	No
100	5000	5	No	No	Yes	No	No
101	500	4	No	No	Yes	No	No
102	500	4	No	Yes	Yes	No	No
103	500	4	No	No	No	No	No
106	10,000	6	No	No	No	No	No
107	8000	6	No	No	Yes	No	No

4.3.3. DFT analysis of compounds isolated from *C. cyphpetalum*

Fortier molecular orbitals play a crucial role in determining the electron transition, optical properties, and chemical reactivity of compound (Pandey et al., 2017). The energies of frontier molecular orbitals are listed in Table 33. The HOMO–LUMO energy gap is related to the occurrence of intramolecular charge transfer within the system and can be used as an indicator of the biological activity of the system. A large HOMO–LUMO energy gap indicates that greater excitation energies are required for reaction, thus the harder and most stable (less reactive) the

molecules are. The calculated energy gaps (eV) of compound **28**, **49**, and **100 - 103** were found to be 3.939, 6.181, 5.760, 4.034, 4.125 and 5.502, respectively (Table 33). Compound **28** recorded the lowest energy gap (3.939 eV) indicating its good chemical reactivity, in agreement with its strong scavenging activity. The lowest chemical hardness (1.970 eV) or highest chemical softness (0.254 eV) of compound **28** also indicate that it's better reactivity than all the studied compounds.

Table 33: Quantum chemical descriptors of the isolated compounds

Compounds	HOMO	LUMO	Eg (eV)	χ (eV)	μ (eV)	η (eV)	σ (eV ⁻¹)	ω (eV)	Nu
28	-5.660	-1.721	3.939	3.691	-3.691	1.970	0.254	3.458	0.289
49	-6.410	-0.229	6.181	3.319	-3.319	3.091	0.162	1.782	0.561
100	-7.215	-1.455	5.760	4.335	-4.335	2.880	0.174	3.262	0.307
101	-5.682	-1.647	4.034	3.665	-3.665	2.017	0.248	3.329	0.300
102	-5.721	-1.596	4.125	3.658	-3.658	2.063	0.242	3.244	0.308
103	-6.200	-0.698	5.502	3.449	-3.449	2.751	0.182	2.162	0.463

Eg=Energy Gap, χ =Electronegativity, σ =Global softness, ω = Electrophilicity (eV) μ = chemical potential, η = global hardness, Nu = nucleophilicity, $\sigma=1/\eta$

5. CONCLUSION AND RECCOMENDATION

5.1. Conclusion

From the roots extracts of *C. cyphopetalum*, eight compounds including a new hydroxyl-spongiane diterpenoid lactone, 3-hydroxyisoagatholactone (**100**), along with *trans*-resveratrol (**28**), β -sitosterol (**49**), and ϵ -viniferin (**101**) from dichloromethane/methanol (1:1) roots extract, *trans*-resveratrol (**28**), gnetin H (**102**), tricuspidatol A (**103**), ϵ -viniferin-diol (**104**) and parthenostilbenin B (**105**) from methanol extract, reported herein for the first time from this plant. When compared to ketoconazole (17.32 ± 0.5 mm, at 10 g), extracts of DCM/MeOH (1:1) demonstrated moderate antifungal efficacy (8.50 ± 0.5 mm, at 12.5 mg/mL). At concentration of 50 μ g/mL, compared to chloramphenicol (30 μ g/mL), ϵ -viniferin (**101**) and tricuspidatol A (**103**) showed the highest activity against *E. coli* (8.55 ± 0.45 and 8.42 ± 0.20 mm) and *S. aureus* (9.30 ± 1.39 and 7.36 ± 0.20 mm), and gnetin H (**102**) and ϵ -viniferin-diol (**104**) against *P. aeruginosa* (12.55 ± 0.65 and 9.42 ± 0.42 mm), respectively.

From the DCM/MeOH (1:1) extract of roots of *H. johannis*, six compounds including β -sitosterol (**49**), catechin-7-O-glucoside (**106**), β -sitosterol-3-O-glucoside (**107**), catechin (**40**), caffeic acid-2-hydroxynonyl ester (**108**), oleic acid (**41**), and 3,9-epoxypregn-16-ene-14,20-diol,7,11,18-triacetoxy-3-methoxy (**109**) were isolated. Similarly, the DCM/MeOH extract of stem bark of *C. tomentosa* afforded tetradecanoic acid (**110**) and 4-hydroxybenzoate derivative (**111**), leaf extract of *K. petitiiana* and *C. rotundifolia* afforded bis-(2-ethylhexyl) phthalate (**112**) and diethyl phthalate (**113**). Based on the invitro antibacterial activity, the DCM/MeOH extract of roots of *H. johannis* showed the highest activity against *S. aureus*, *P. aeruginosa*, and *E.coli*, followed by *n*-hexane. Against *S. pyogenes*, *n*-hexane extract showed the highest activity. Catechin-7-O-glucoside (**106**) was found to be strong chemical agent (11.5 ± 0.5 , 12.0 ± 0.0 , 10.75 ± 0.25 , 11.0 ± 0.0 mm) against all tested bacterial stains (*E.coli*, *P. aeruginosa*, *S.aureus*, and *S. pyogen*, respectively), in comparison to the standard rug amoxacillin, indicating its strong antibacterial ctivity

The GC-MS chemical profiling of essential oil from leaves of *C. tomentosa*, *K. petitiiana* and *C. rotundifolia* resulted in identification 25 (89.7 %), 54 (97.2 %), and 23 (95.47 %) volatile compounds, respectively.

The MCF-7 cells were found to have low viability when exposed to MeOH extracts of roots from *C cyphopetalum* (CCME) and *H johannis* (HJME), as well as DCM/MeOH extract of stem bark from *C tomentosa* (CTDME) (27.1%, 28.9 % and 44.9%, respectively). This suggests that the samples might be suitable for breast cancer therapy and supports the traditional treatment system of *C cyphopetalum*, *H johannis*, and *C tomentosa*.

The molecular docking analysis revealed that, highest binding affinity toward DNA gyrase was scored by compounds **101** and **106** (-7.6 kcal/mol), compounds **102**, **106** and **40** (-8.8, -8.7, and -8.6, respectively) scored towards protein PqsA, whereas compound **106** (-7.4 kcal/mol) scored the highest towards *S. aureus* PK compared to chloramphenicol (-6.4, -7.0, and -4.6 kcal/mol, respectively). The binding affinities scored by compound **101** and **102** support the *in vitro* antibacterial activities. This suggests that the compounds have potential to be used as antibacterial agents, which supports the traditional uses of the plants. The drug likeness prediction result shows that compound **28**, **49**, **100**, **101** satisfy Lipinski's rule of five and Veber's rule with zero violations. The ADME prediction shows that compounds **100**, **101** and **28** exhibit favorable absorption and distribution properties. Toxicity prediction shows, none of the studied compounds have shown hepatotoxicity, mutagenicity, and cytotoxicity. The DFT computation indicates that compound **28** recorded the lowest energy gap and better chemical reactivity. The results obtained from molecular docking, drug-likeness properties, ADMET analysis and the DFT calculation are in a good agreement with those obtained from experimental studies of *C. cyphopetalum* and *H. johannis* suggesting the potential use of the isolated compounds as medicine which corroborate the traditional use of selected medicinal plants.

5.2. Recommendations

- *In vivo* antibacterial studies against animal models for compounds which exhibited sizable *in vitro* antibacterial activity ϵ -viniferin (**101**), tricuspidatol A (**103**), gnetin H (**102**), ϵ -viniferin-diol (**104**), catechin-7-O-glucoside (**106**) needs to be carried out to determine their bioactivity.
- Considering the promising antifungal activity displayed by *n*-hexane and DCM/MeOH (1:1) extracts of *C. cyphopetalum* against *C. albicans*, *in vitro* and *in vivo* antifungal activities of isolated compounds needs to be done to identify the possible antifungal principle.

- Cytotoxicity of compounds isolated from roots of *C. cyphopetalum* and *H. johannis* and their structure-activity relationship needs to be carried out to determine potential activity against breast cancer.
- Cytotoxicity of leave extracts of *K. petitiiana* and *C. rotundifolia* against breast cancer cell lines (MCF-7) needs to be done to support the traditional use of the plants for breast cancer treatment.
- *In vitro* antibacterial, antifungal and antioxidant activities, and cytotoxicity of essential oils obtained from leaves of *C. toemntosa*, *K. petitiiana* and *C. rotundifolia* needs to be carried out.
- More phytochemical anlysis and biological activities of extrcats and isolated compounds of leaves *K. petitiiana* and *C. rotundifolia* needs to be carried out support the uses of these plants in traditional medicin.

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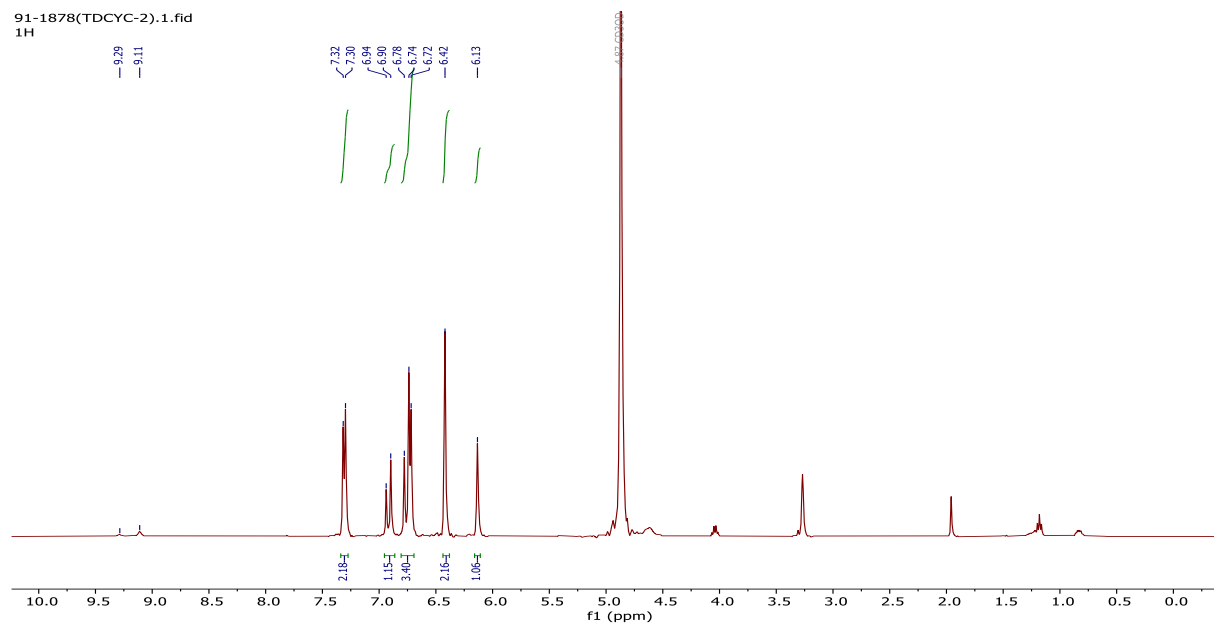
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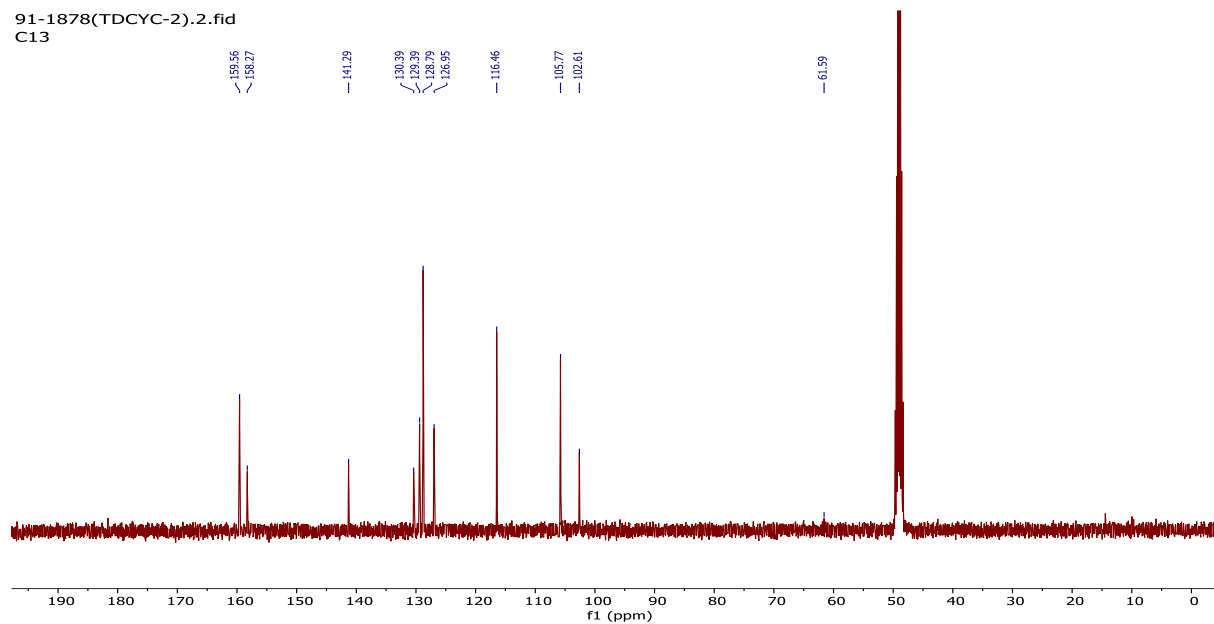
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Appendices

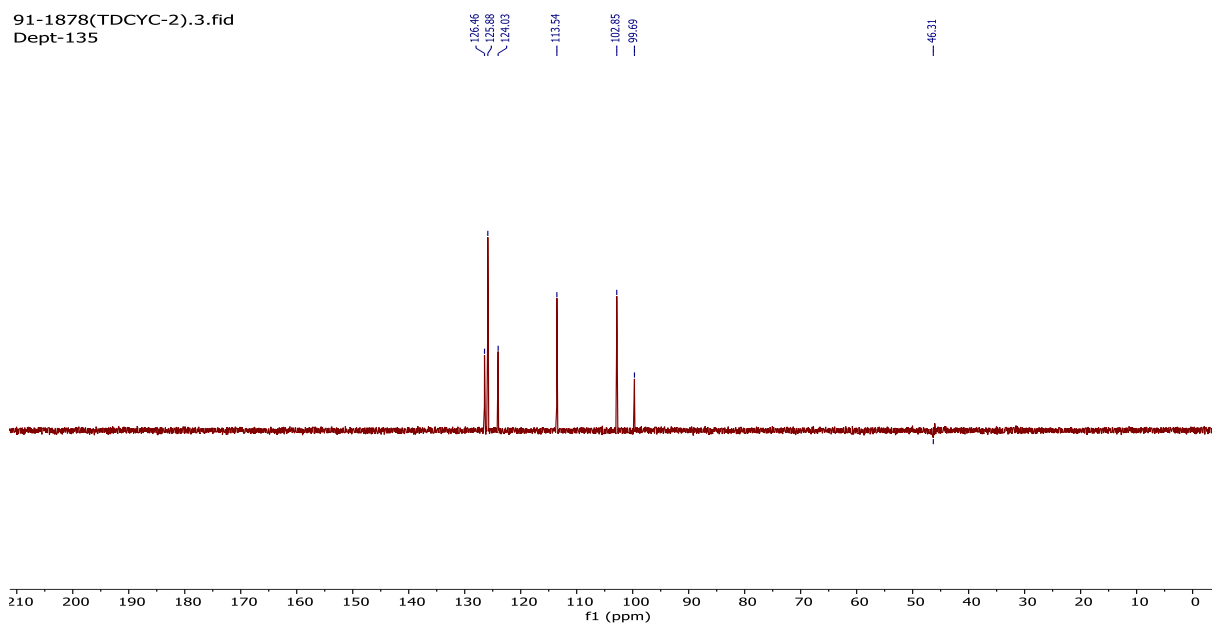
Appendix 1A: ^1H NMR spectrum of compound **28**



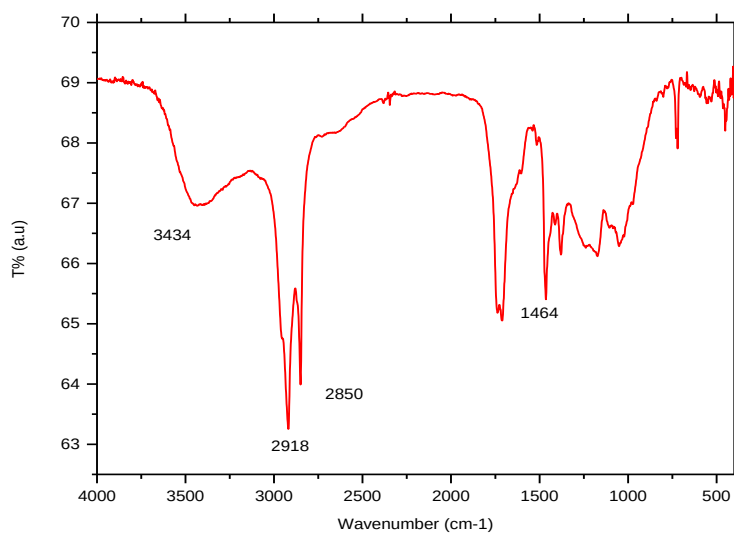
Appendix 1B: ^{13}C NMR spectrum of compound **28**



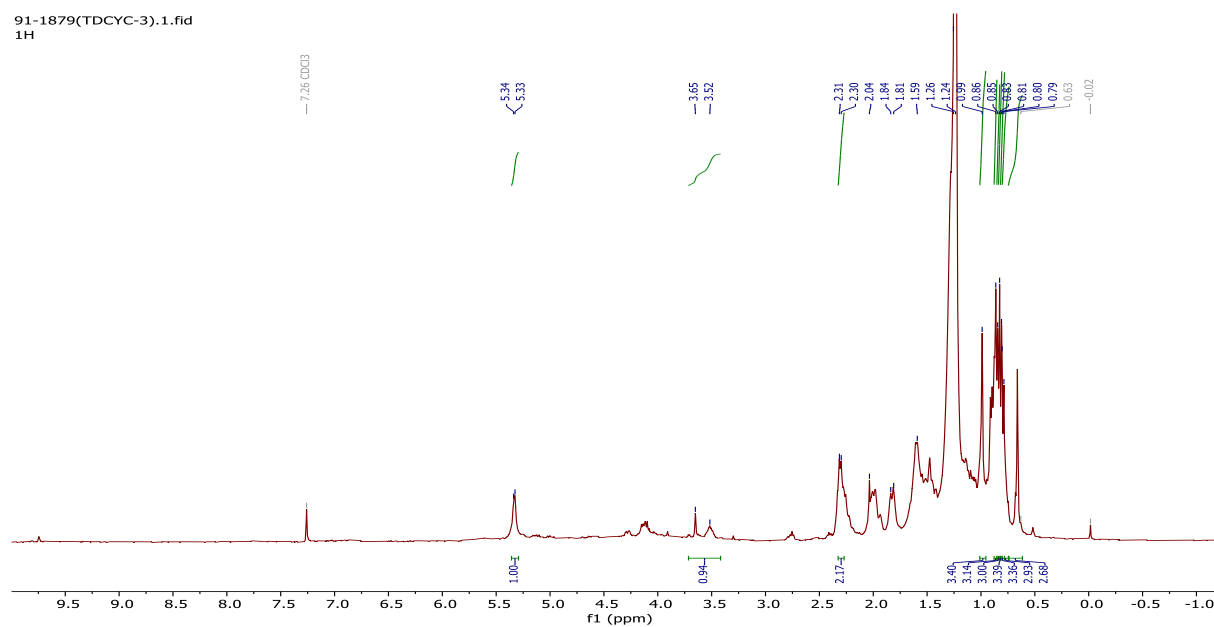
Appendix 1C: DEPT-135 NMR spectrum of compound **28**



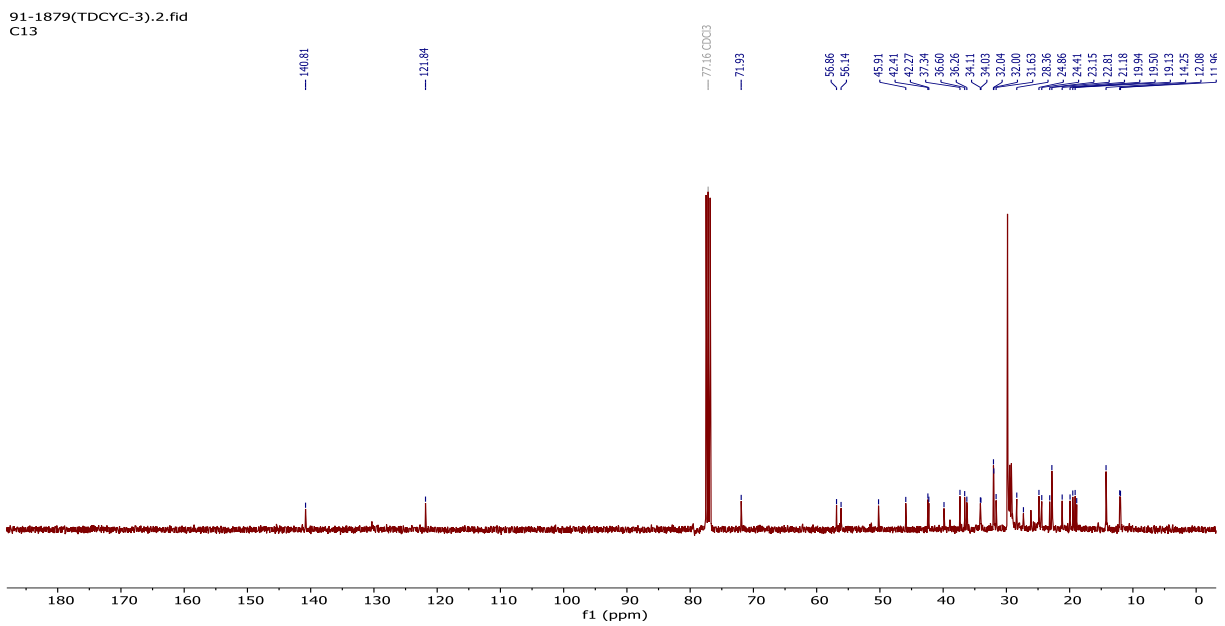
Appendix 2A: IR spectrum of compound **49**



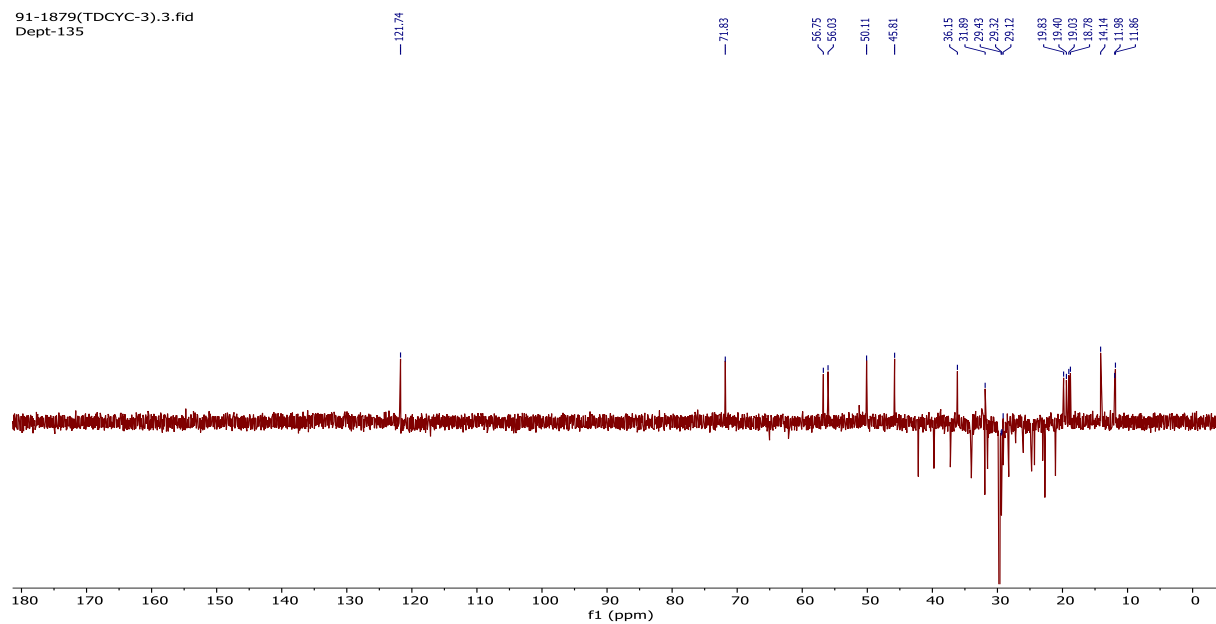
Appendix 2B: ^1H NMR spectrum of compound **49**



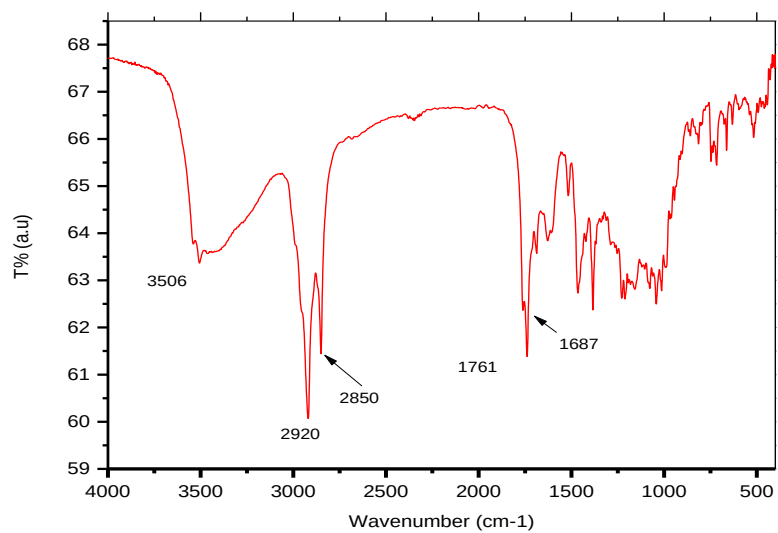
Appendix 2C: ^{13}C NMR spectrum of compound **49**



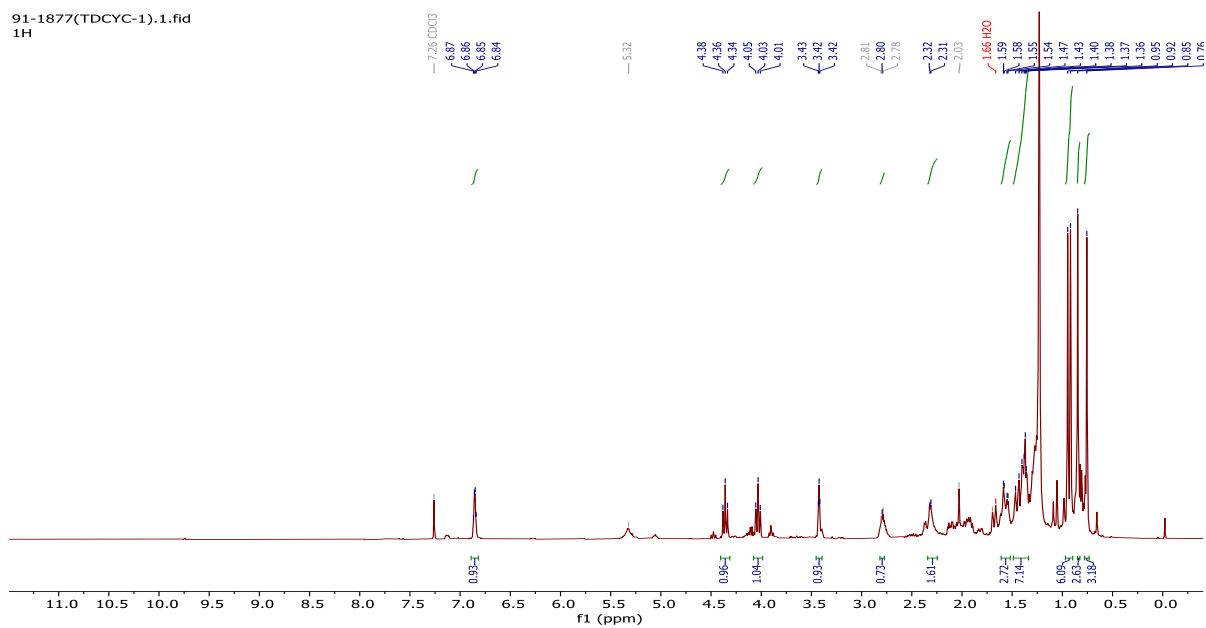
Appendix 2D: DEPT-135 NMR spectrum of compound **49**



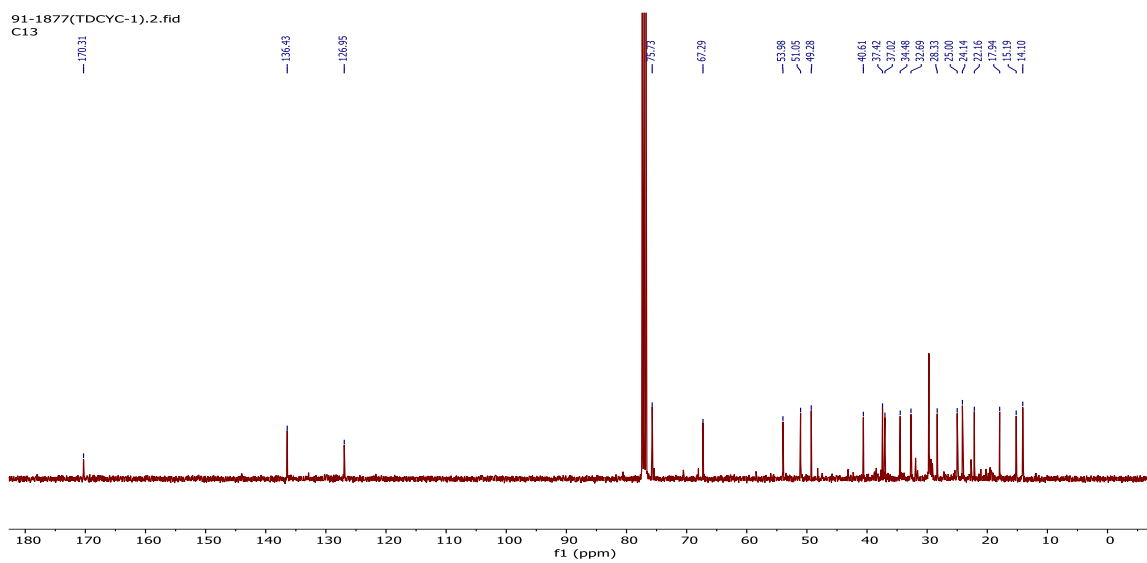
Appendix 3A: IR spectrum of 3-hydroxyisoagatolacyone **100**



Appendix 3B: ^1H NMR spectrum of compound **100**

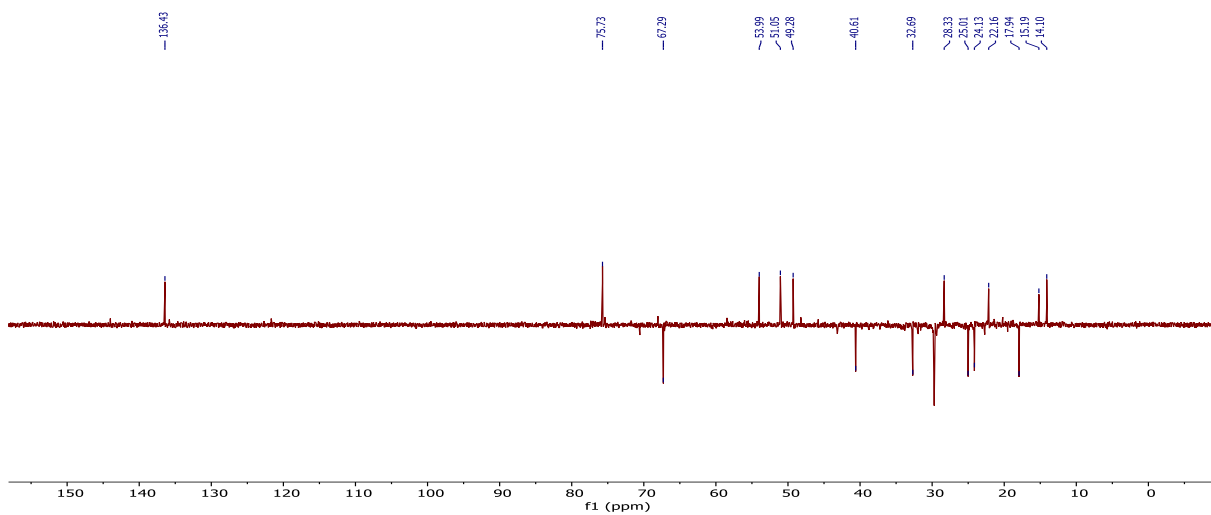


Appendix 3C: ^{13}C NMR spectrum of compound **100**

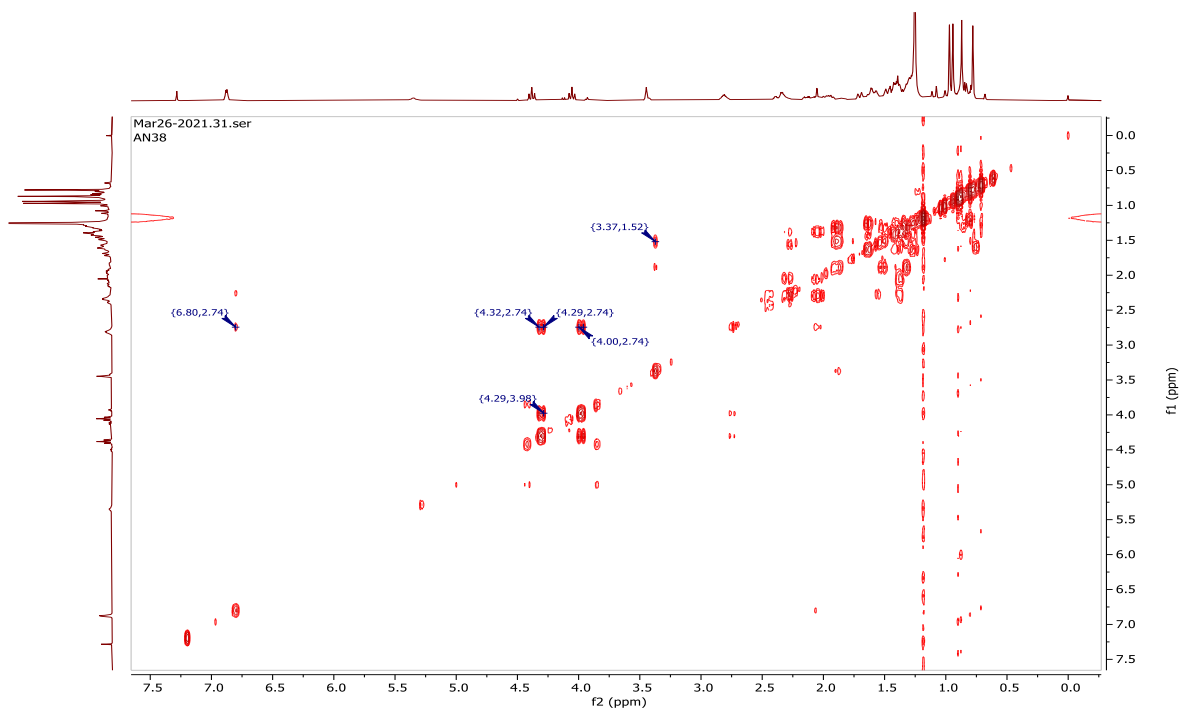


Appendix 3D: DEPT-135 spectrum of compound **100**

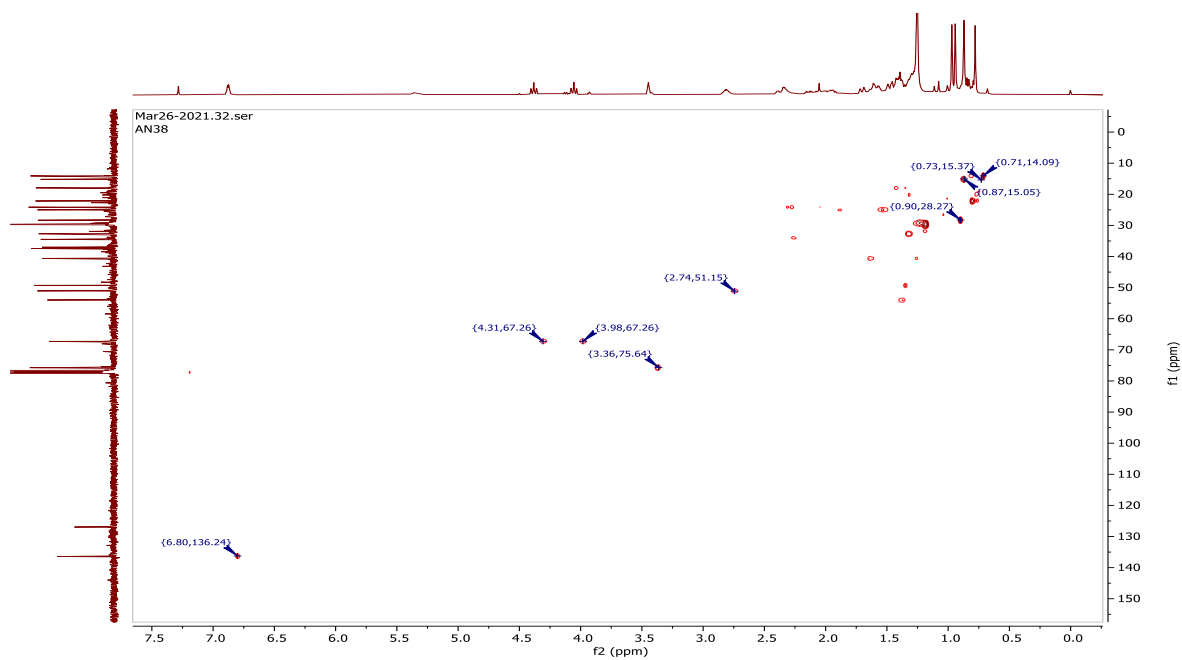
91-1877(TDCYC-1).3.fid
Dept-135



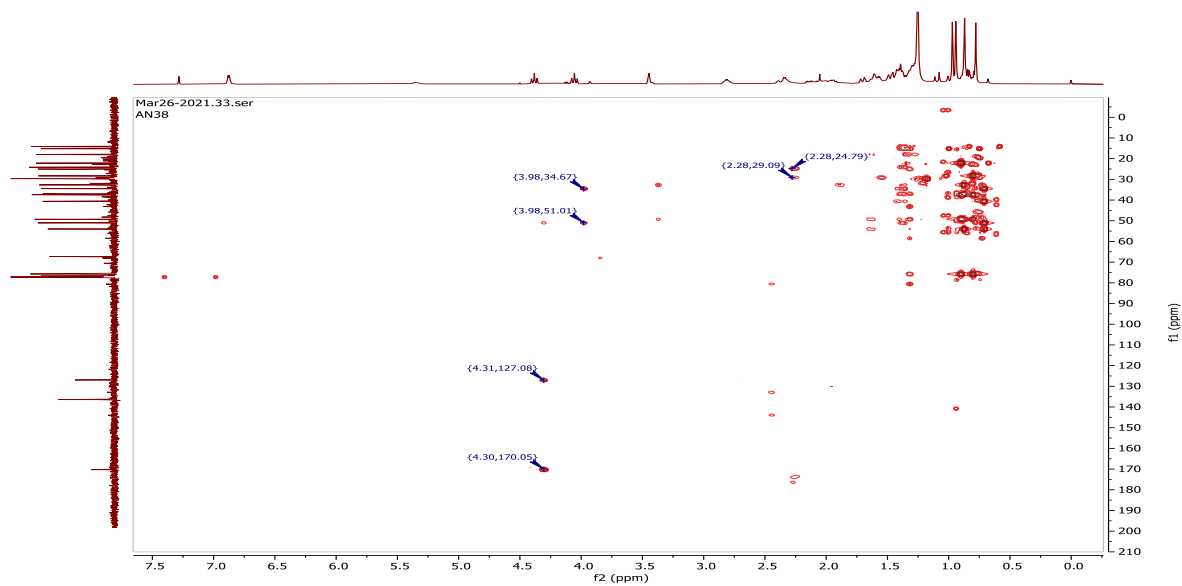
Appendix 3E: COSY spectrum of compound **100**



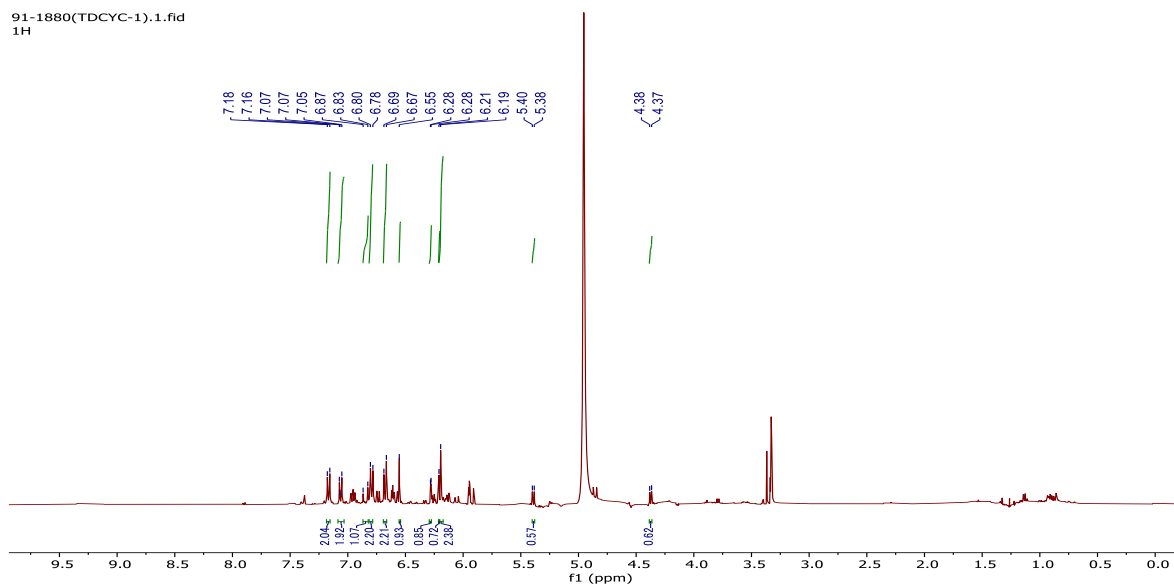
Appendix 3F: HSQC NMR spectrum of compound **100**



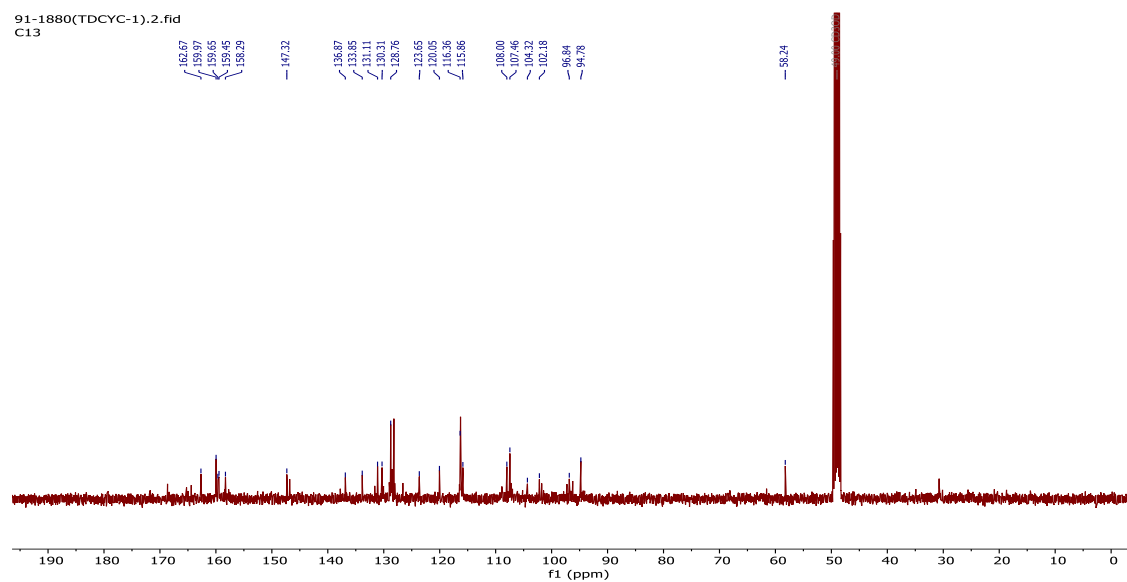
Appendix 3G: HMBC spectrum of compound **100**



Appendix 4A: ^1H NMR spectrum of compound **101**

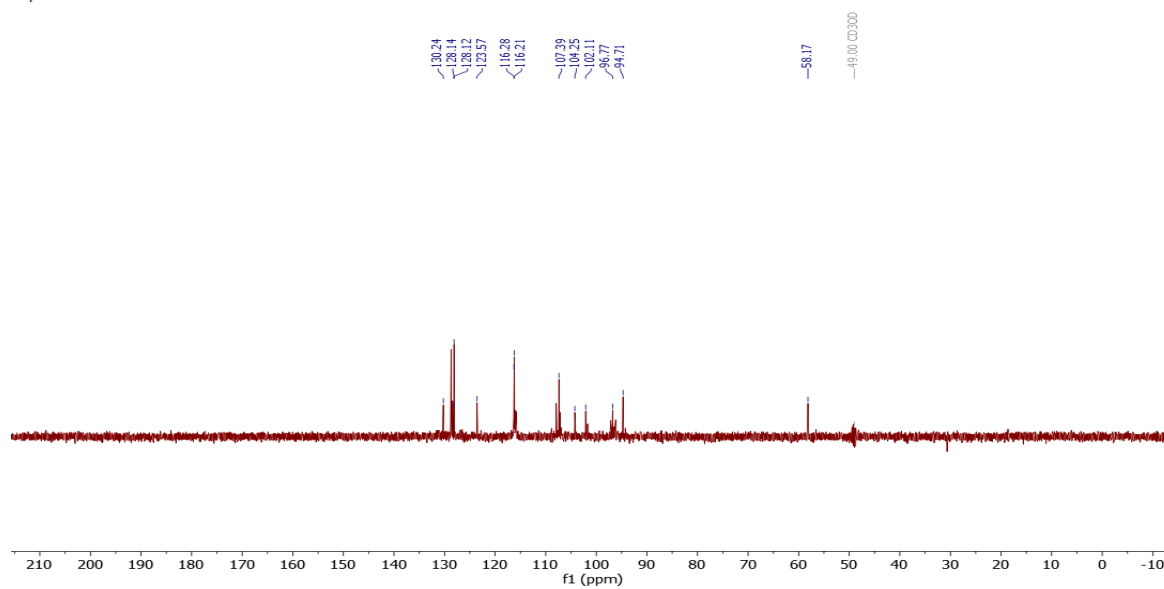


Appendix 4B: ^{13}C NMR spectrum of compound **101**

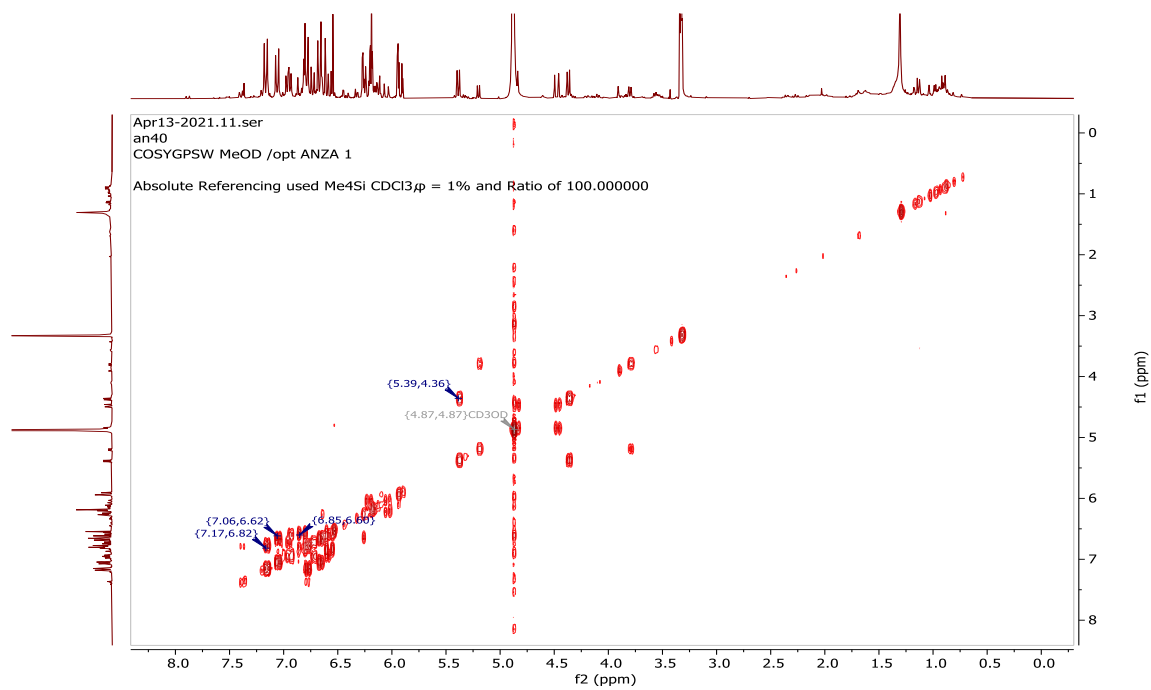


Appendix 4C: DEPT-135 spectrum of compound **101**

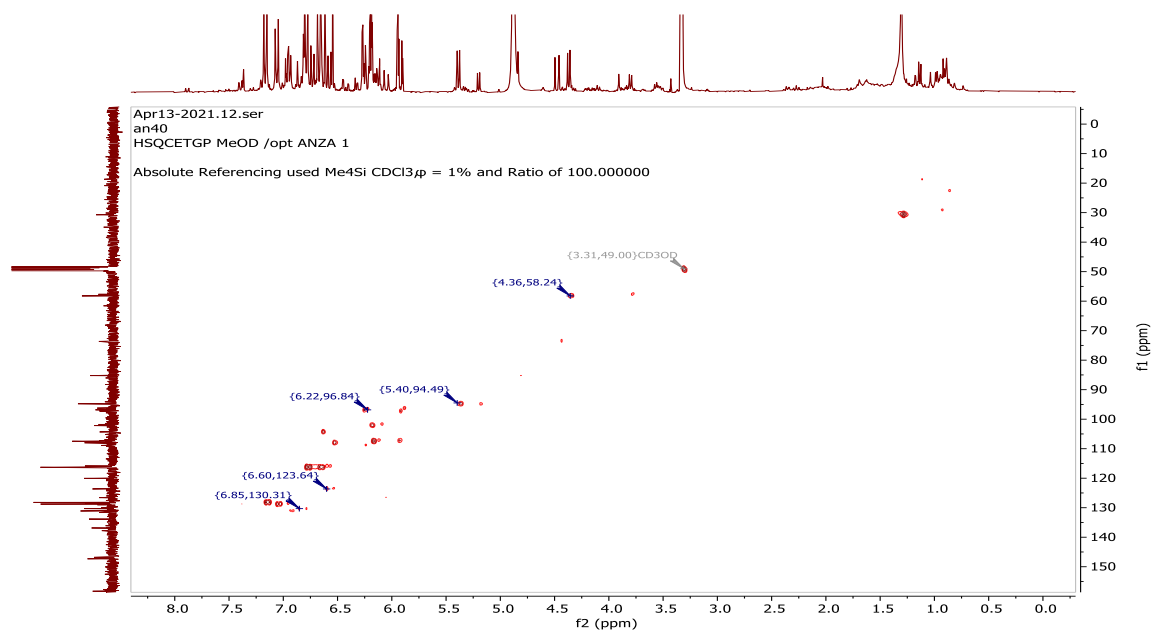
91-1880(TDCYC-1).3.fid
Dept-135



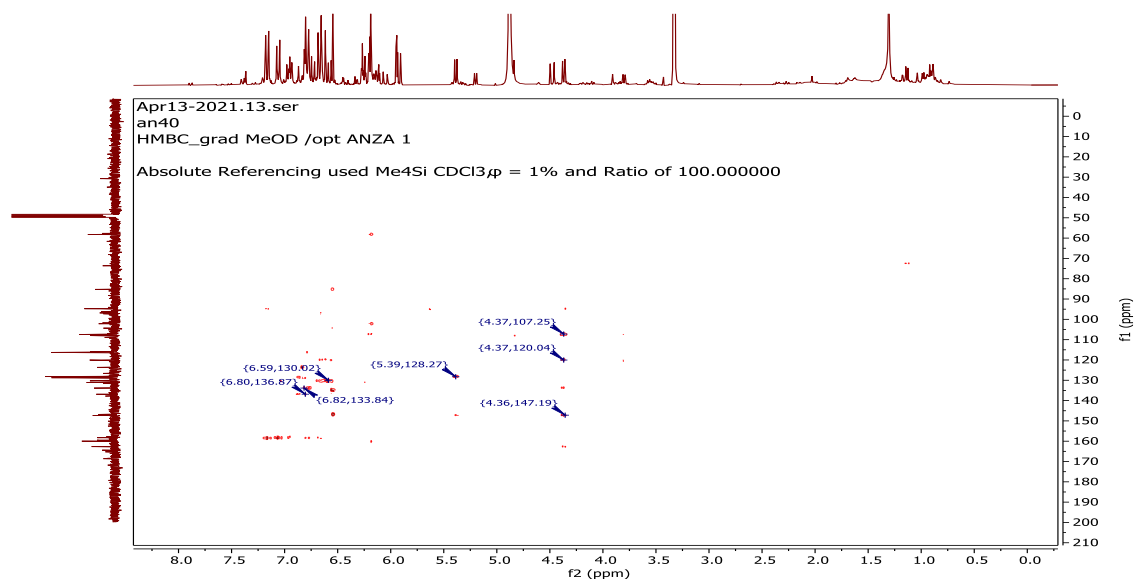
Appendix 4D: COSY NMR spectrum of compound **101**



Appendix 4E: HSQC NMR spectrum of compound **101**



Appendix 4F : HMBC NMR spectrum of compound **101**



TDCYC6.10.fid
TDCYC6

7.16, 7.13, 7.05, 7.03, 6.96, 6.94, 6.92, 6.86, 6.72, 6.66, 6.64, 6.59, 6.57, 6.22, 6.17, 5.93, 5.38, 5.36, 5.19 H₂O, 5.16, 4.35, 4.34, 3.79, 3.78, 3.3, 2.1, 2.0, 2.00, 2.10, 2.44, 4.27, 2.03, 2.44, 1.82, 1.97, 2.15, 1.61, 0.94, 0.80, 1.14, 0.97

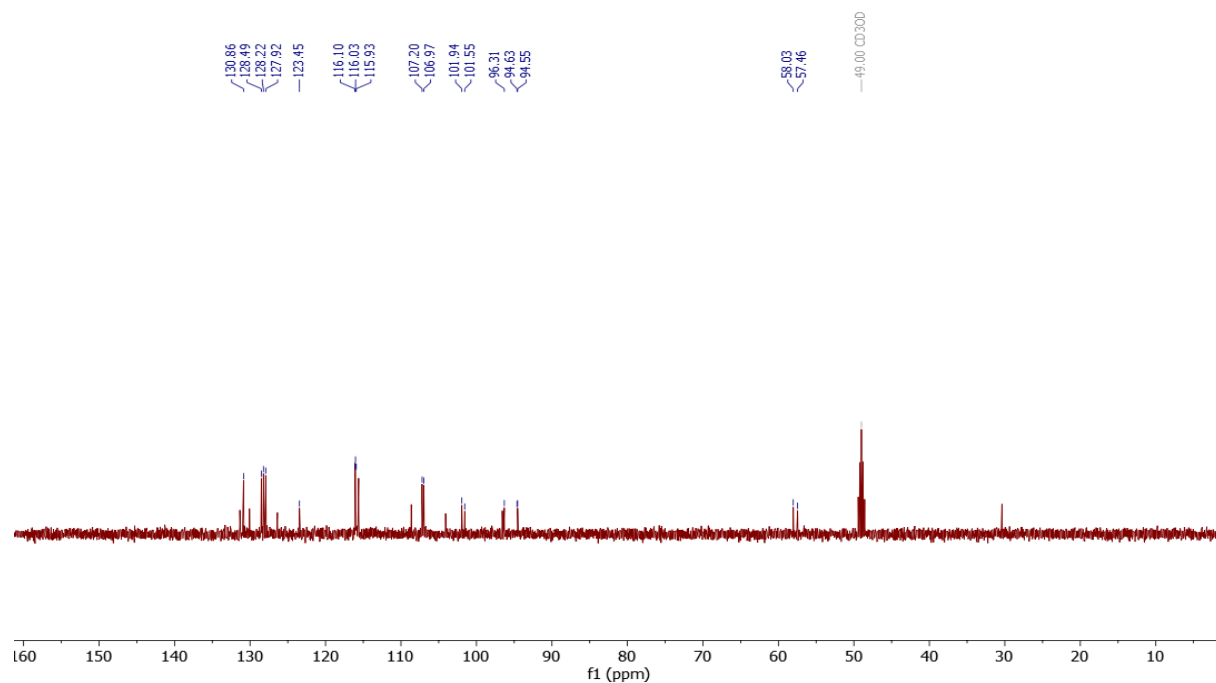
TDCYC6.12.fid
TDCYC6

162.76
160.06
159.55
158.55
158.41
157.84
147.37
147.35
137.82
136.94
133.92
133.87
131.14
128.78
128.51
128.21
122.74
120.39
120.06
116.39
116.31
116.21
107.49
107.35
102.23
101.84
96.60
94.92
94.84
58.31
57.74

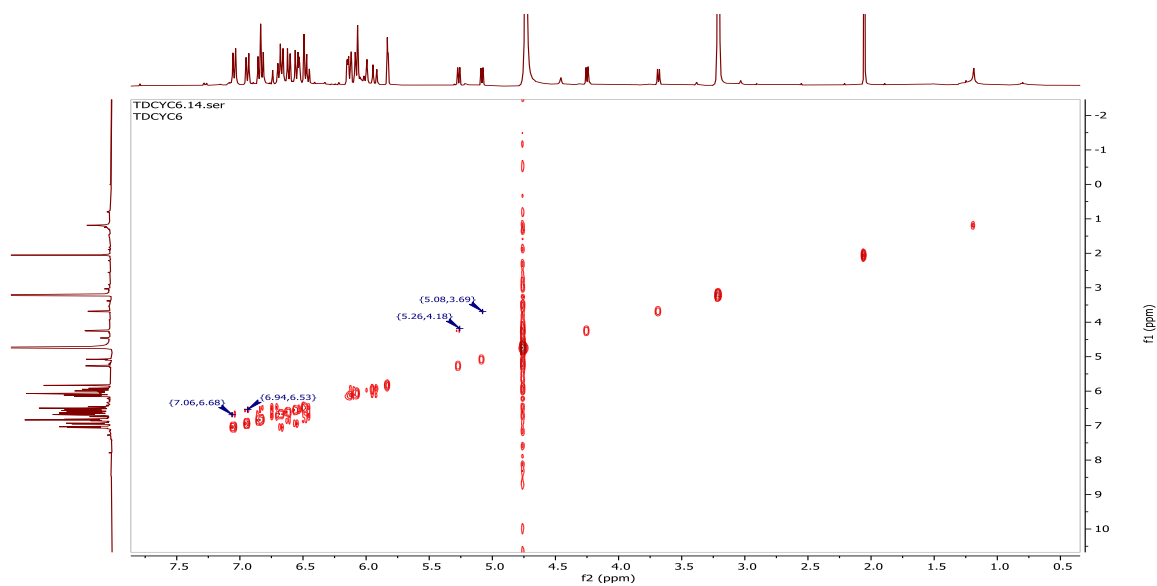
f1 (ppm)

Appendix 5C: DEPT-135 NMR spectrum of compound **102**

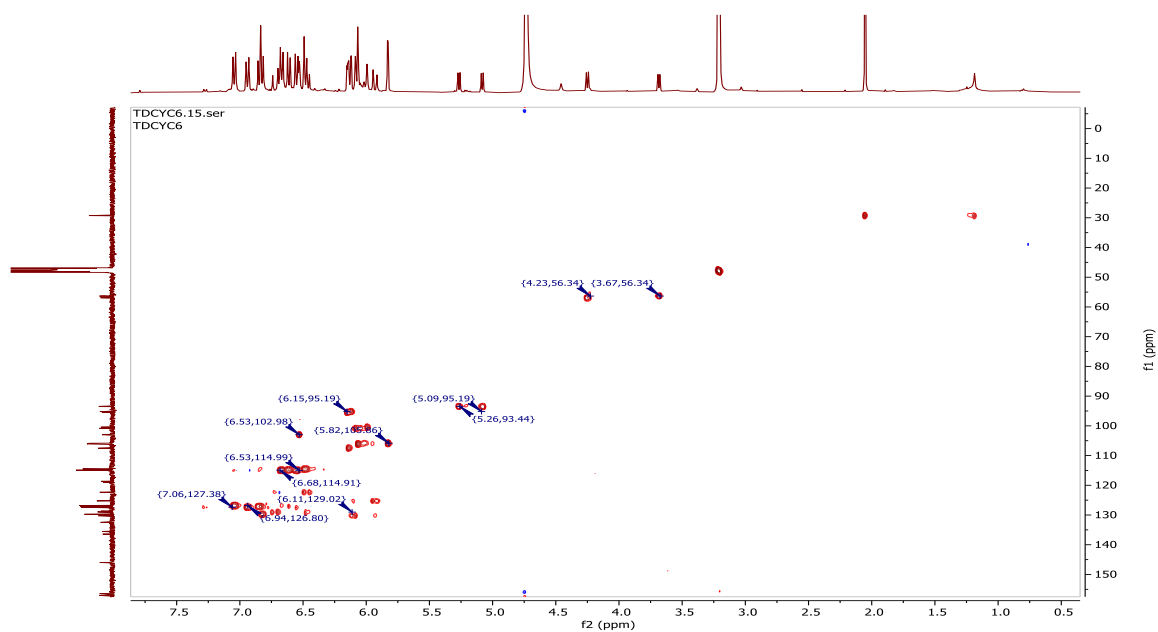
TDCYC6.13.fid
TDCYC6



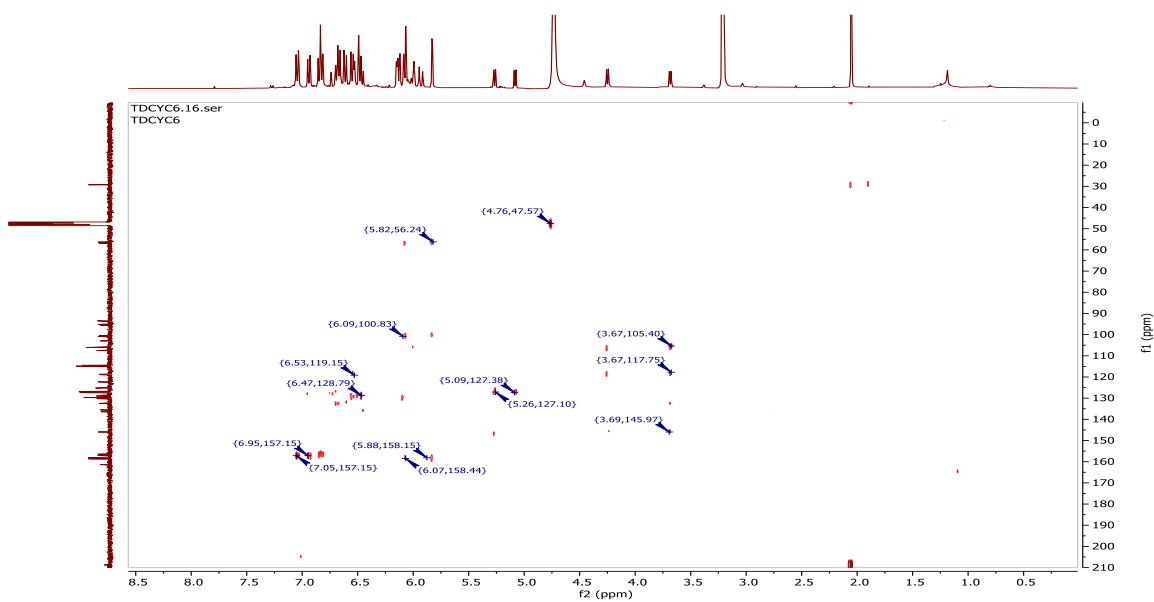
Appendix 5D: COSY NMR spectrum of compound **102**



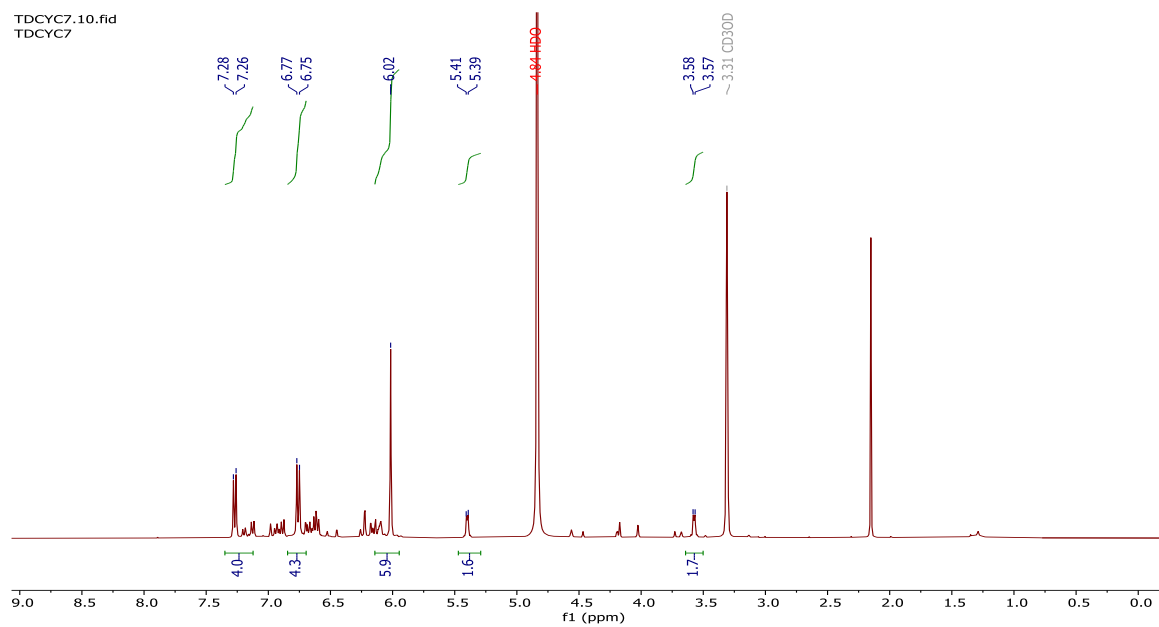
Appendix 5E: HSQC NMR spectrum of compound **102**



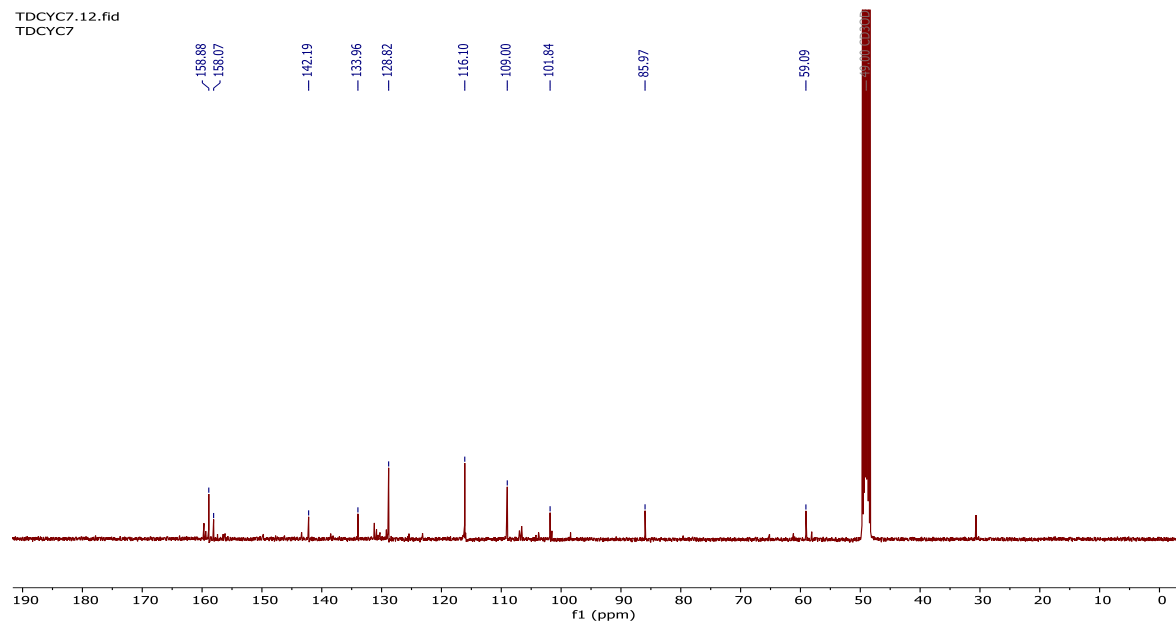
Appendix 5F: HMBC NMR spectrum of compound **102**



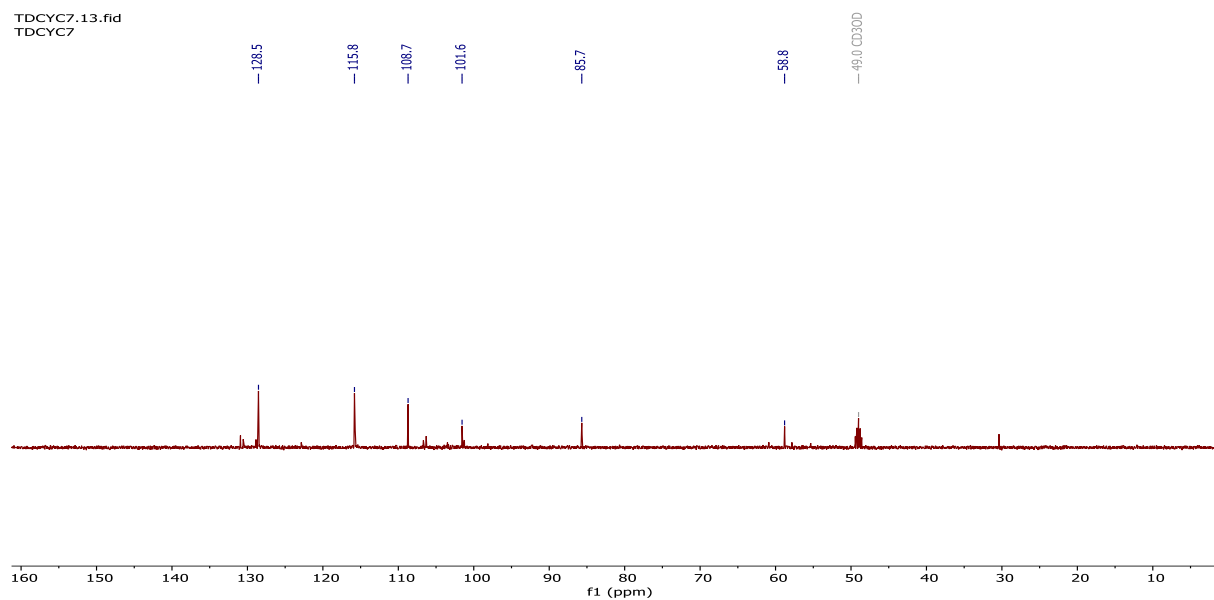
Appendix 6A: ^1H NMR spectrum of compound **103**



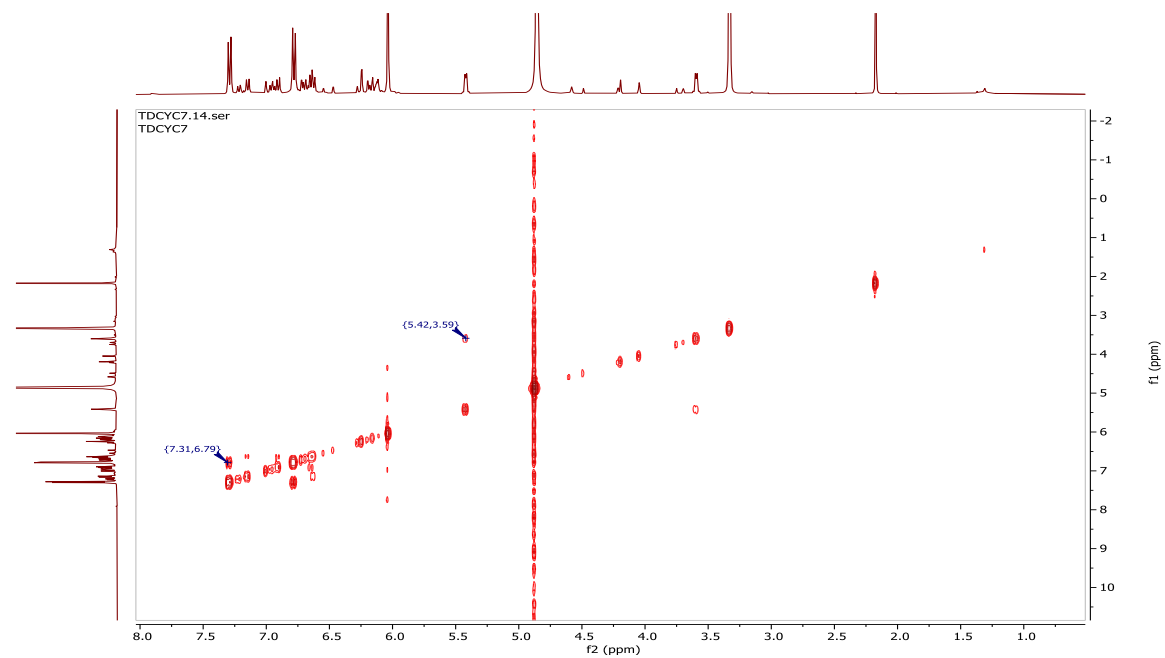
Appendix 6B: ^{13}C NMR spectrum of compound **103**



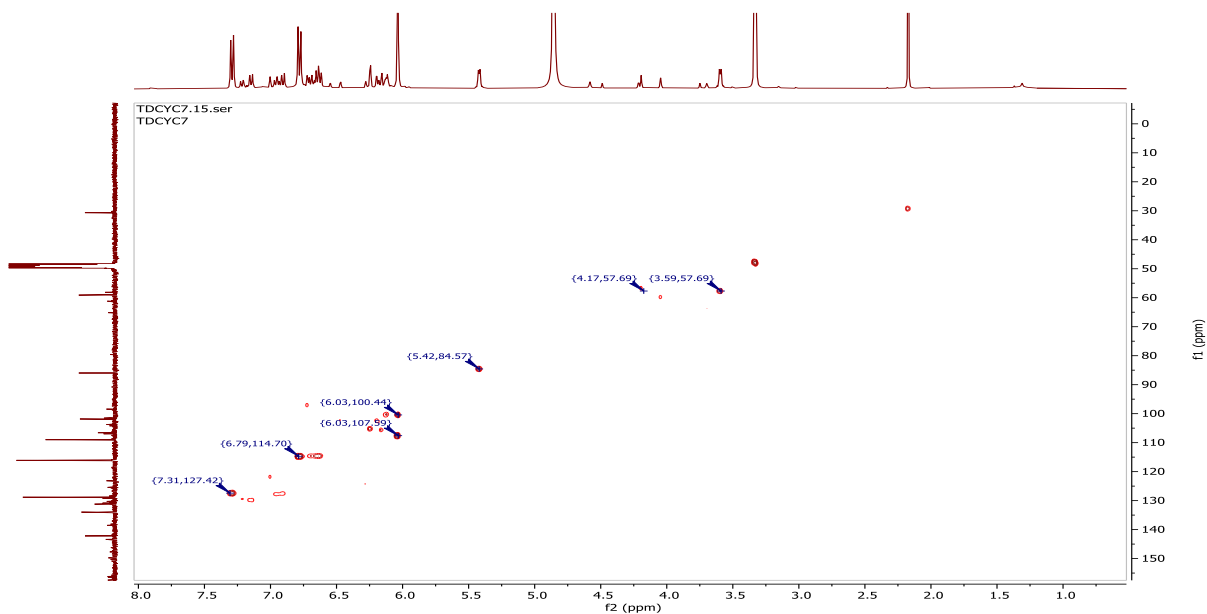
Appendix 6C: DEPT-135 NMR spectrum of compound **103**



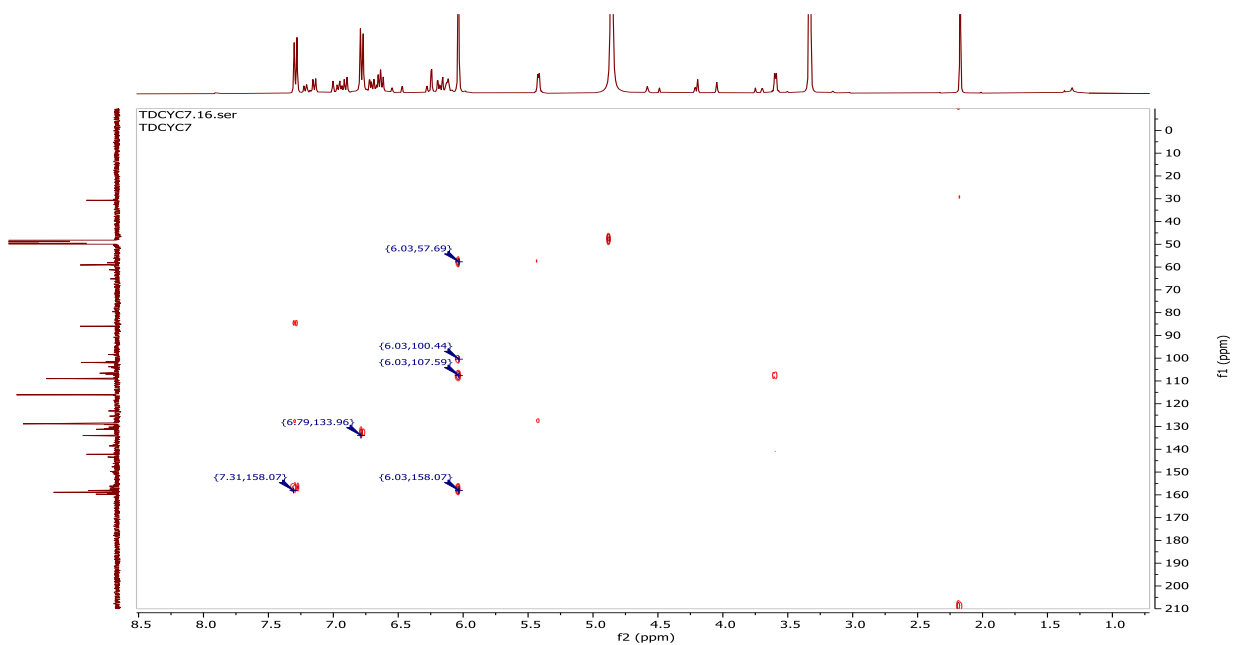
Appendix 6D: COSY NMR spectrum of compound **103**



Appendix 6E: HSQC NMR spectrum of compound **103**



Appendix 6F: HMBC NMR spectrum of compound **103**

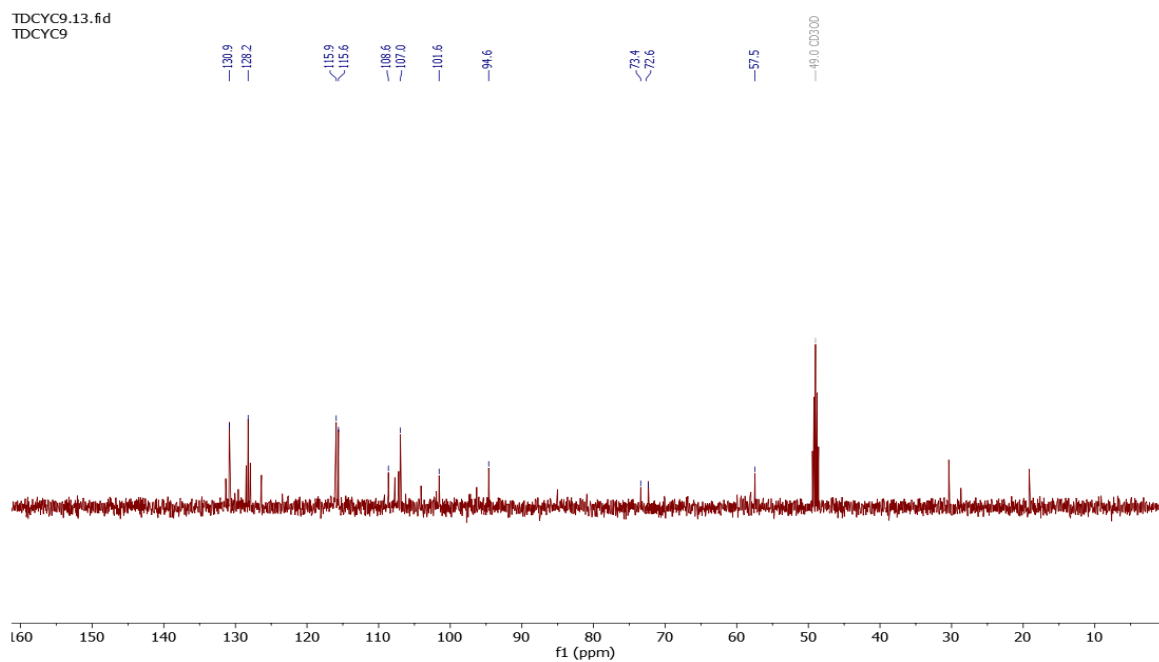


[illegible]

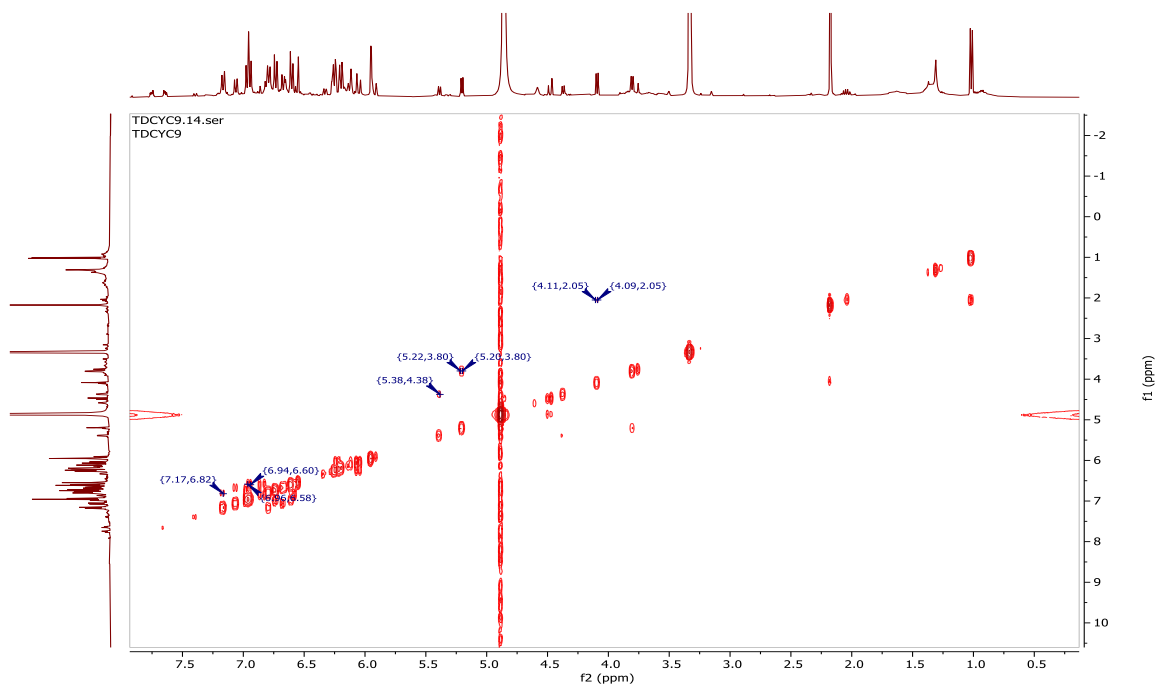
163.79
160.06
159.56
158.41
157.83
147.35
146.89
133.86
131.14
130.68
128.51
120.38
116.21
115.90
108.02
107.26
101.84
96.60
94.92
73.70
72.92
57.74

f1 (ppm)

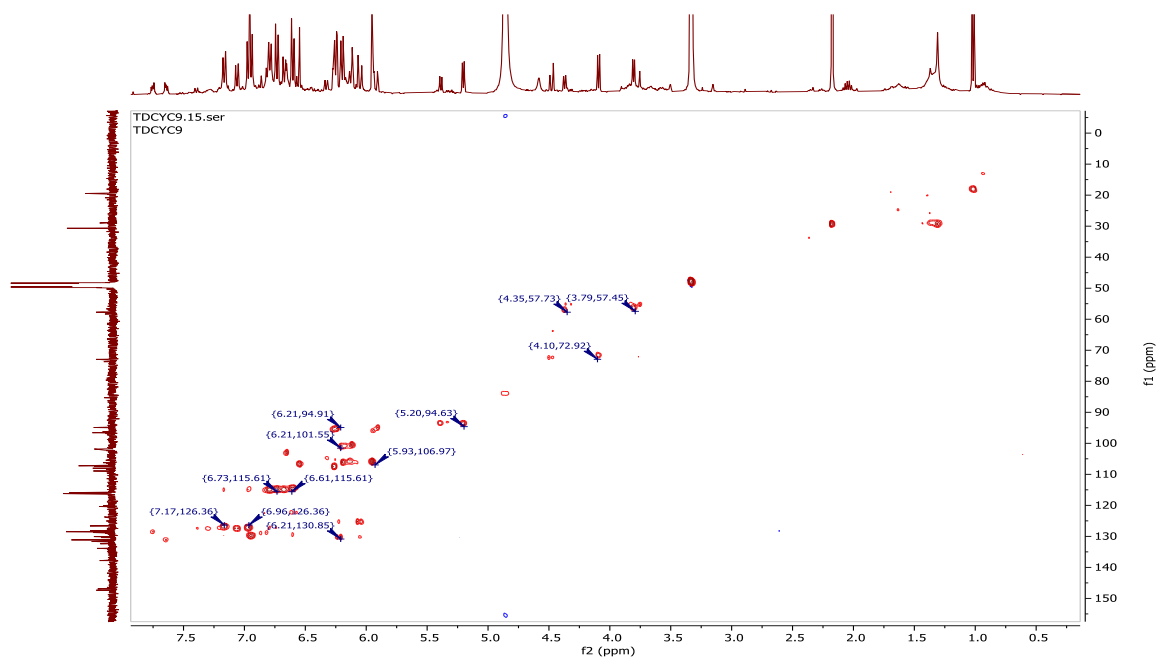
Appendix 7C: DEPT-135 spectrum of compound **104**



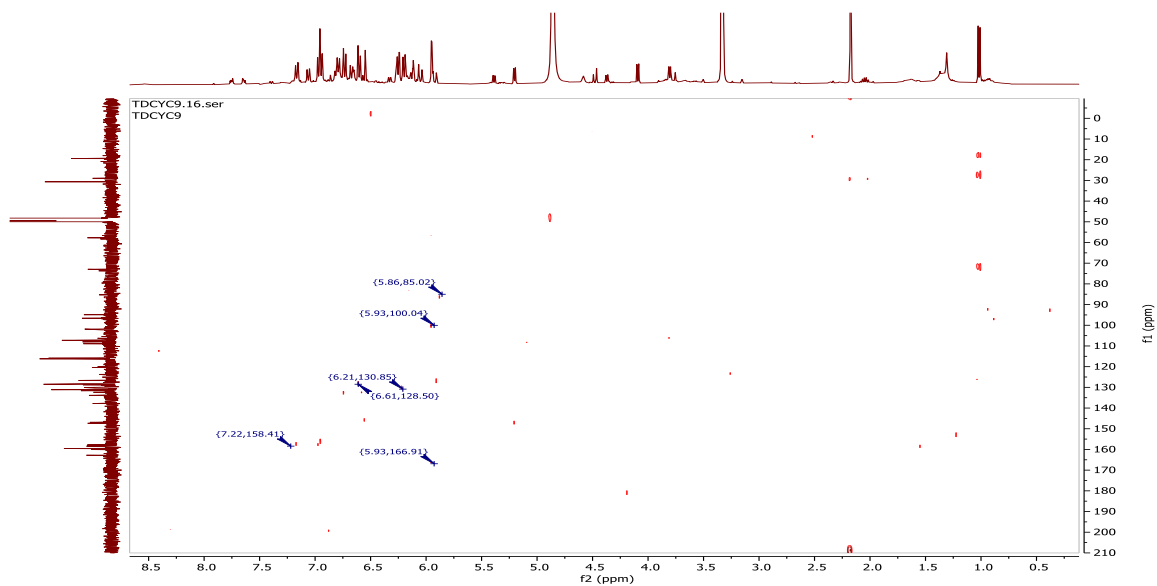
Appendix 7D: COSY NMR spectrum of compound **104**



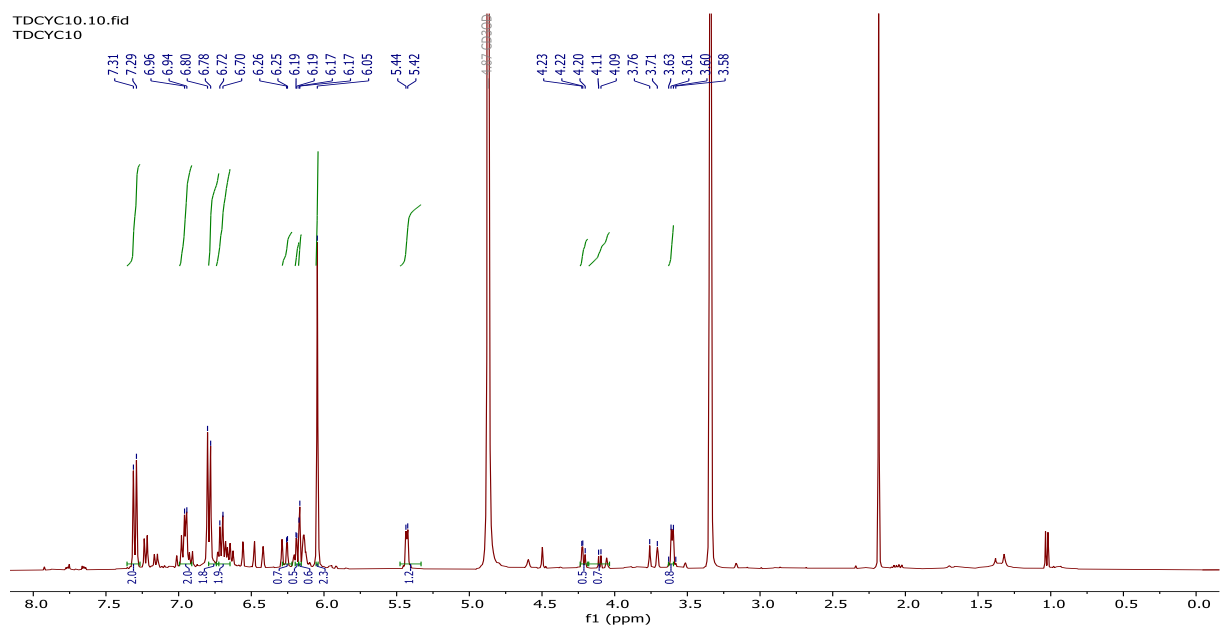
Appendix 7E: HSQC NMR spectrum of compound **104**



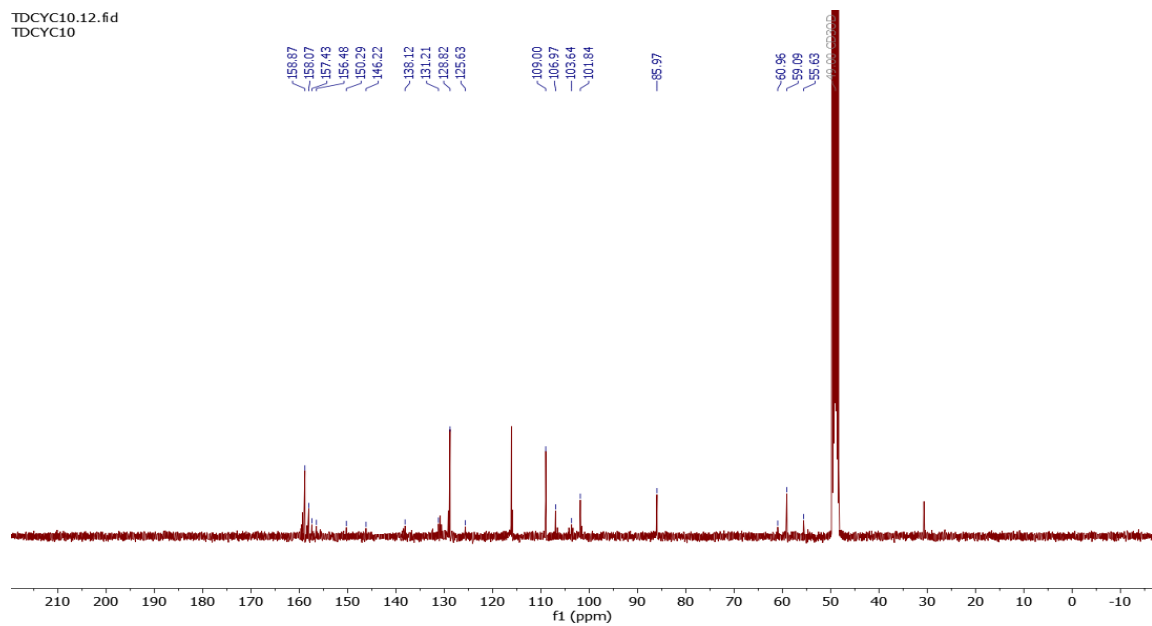
Appendix 7F: HMBC NMR spectrum of compound **104**



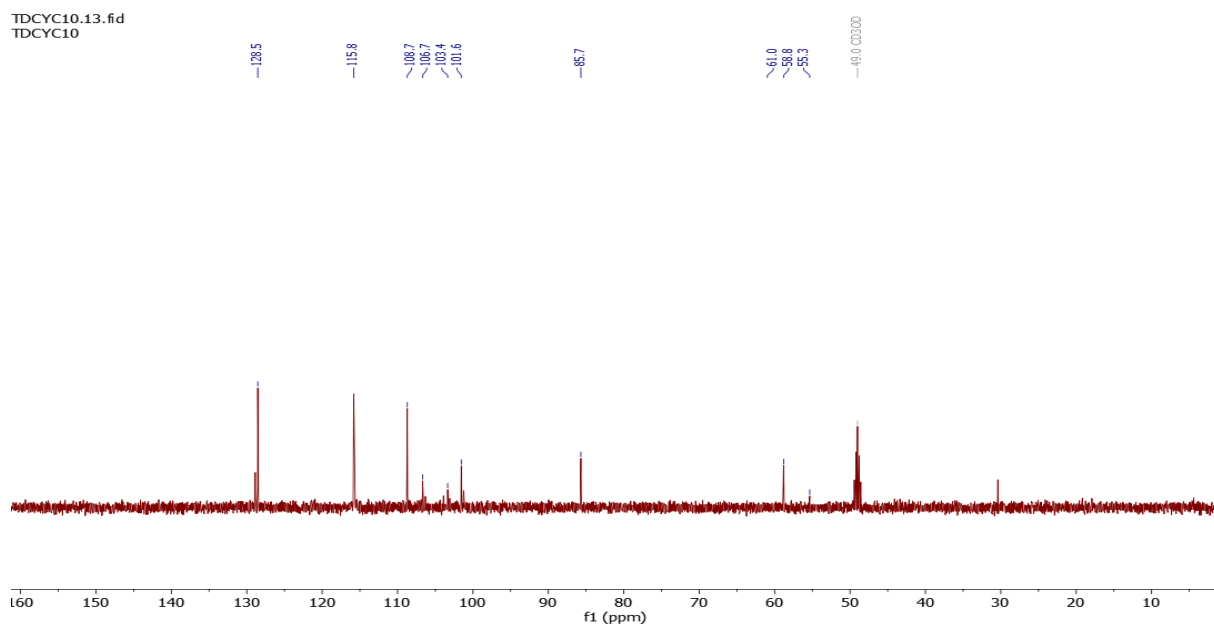
Appendix 8A: ^1H NMR spectrum of compound **105**



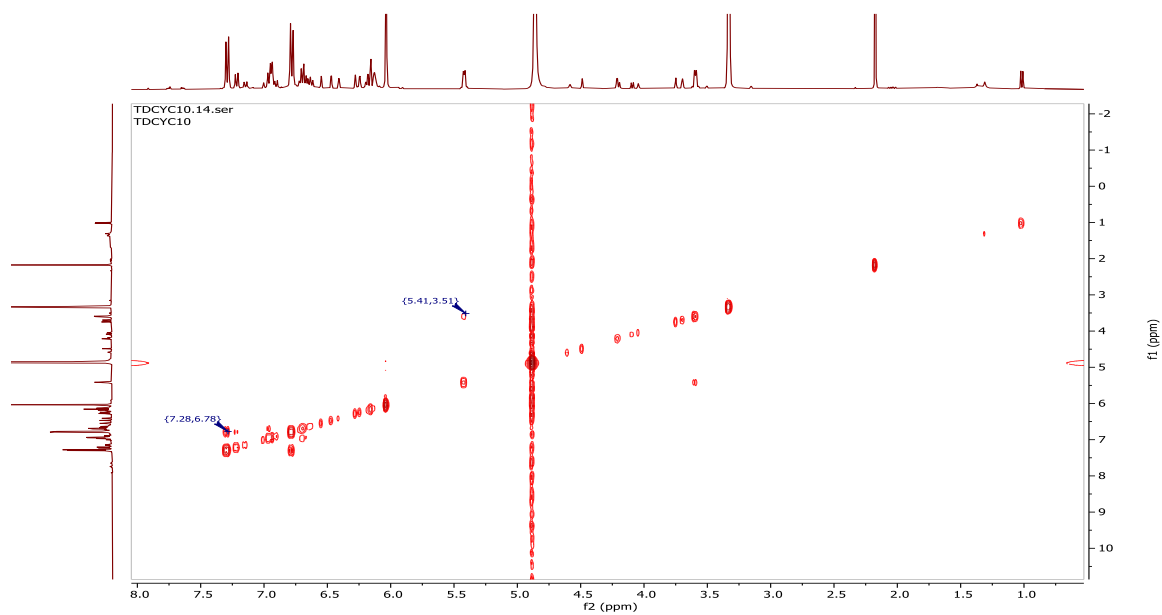
Appendix 8B: ^{13}C NMR spectrum of compound **105**



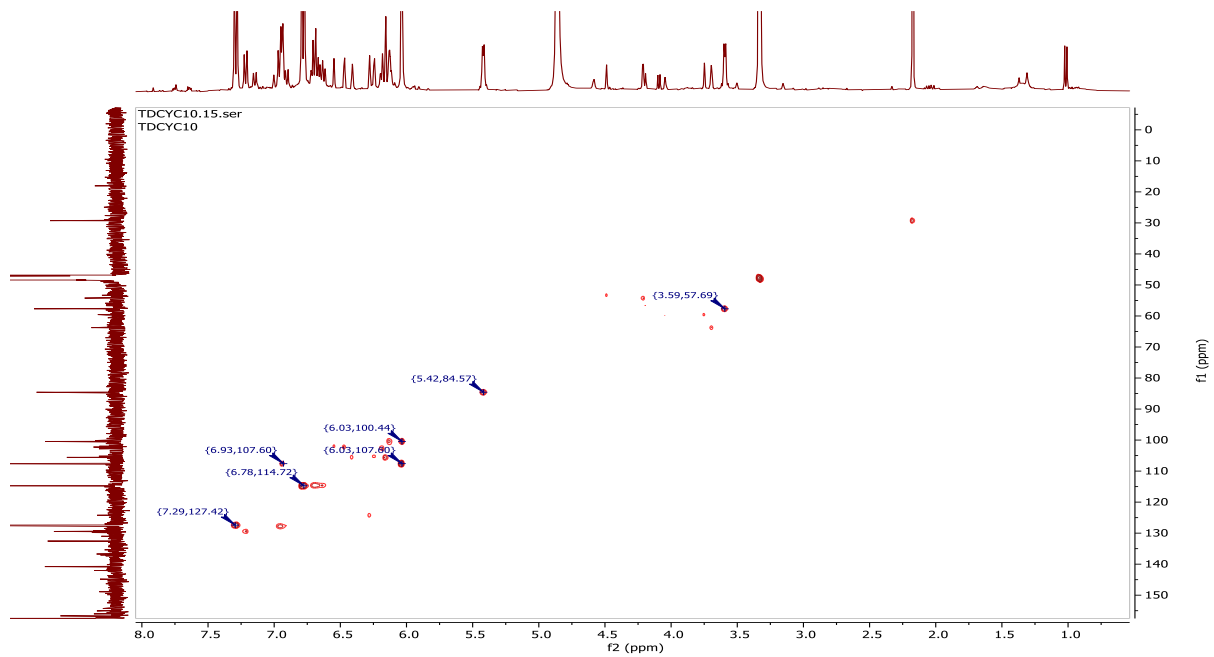
Appendix 8C: DEPT-135 NMR spectrum of compound **105**



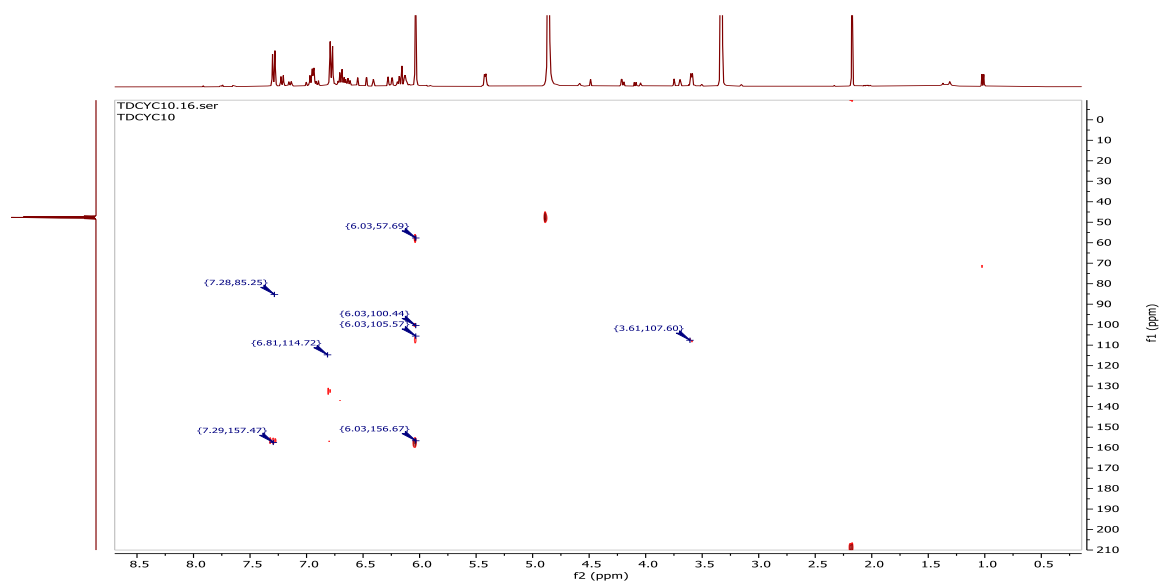
Appendix 8D: COSY NMR spectrum of compound **105**



Appendix 8E: HSQC NMR spectrum of compound **105**

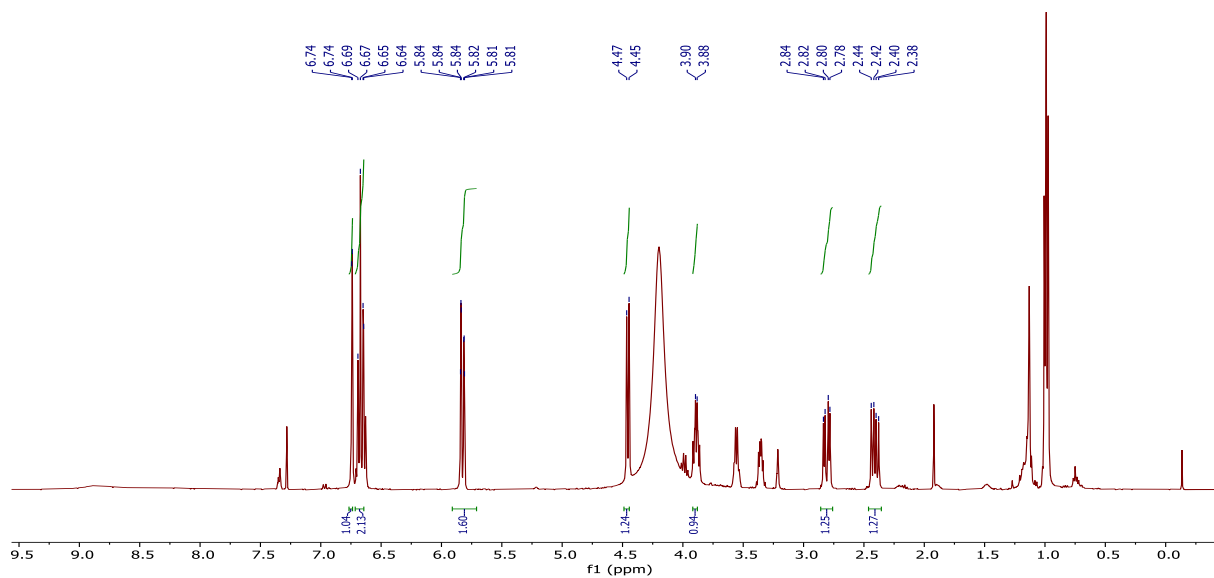


Appendix 8F: HMBC NMR spectrum of compound **105**



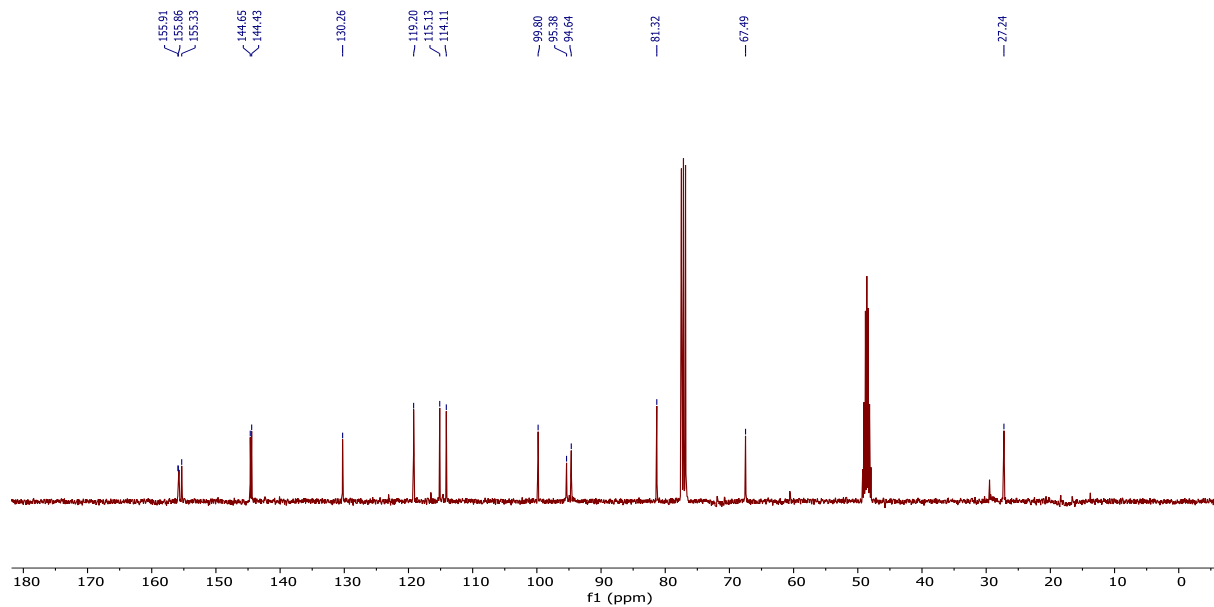
Appendix 9A: ^1H NMR spectrum of compound **40**

91-1884(TDHC-4).1.fid
 ^1H



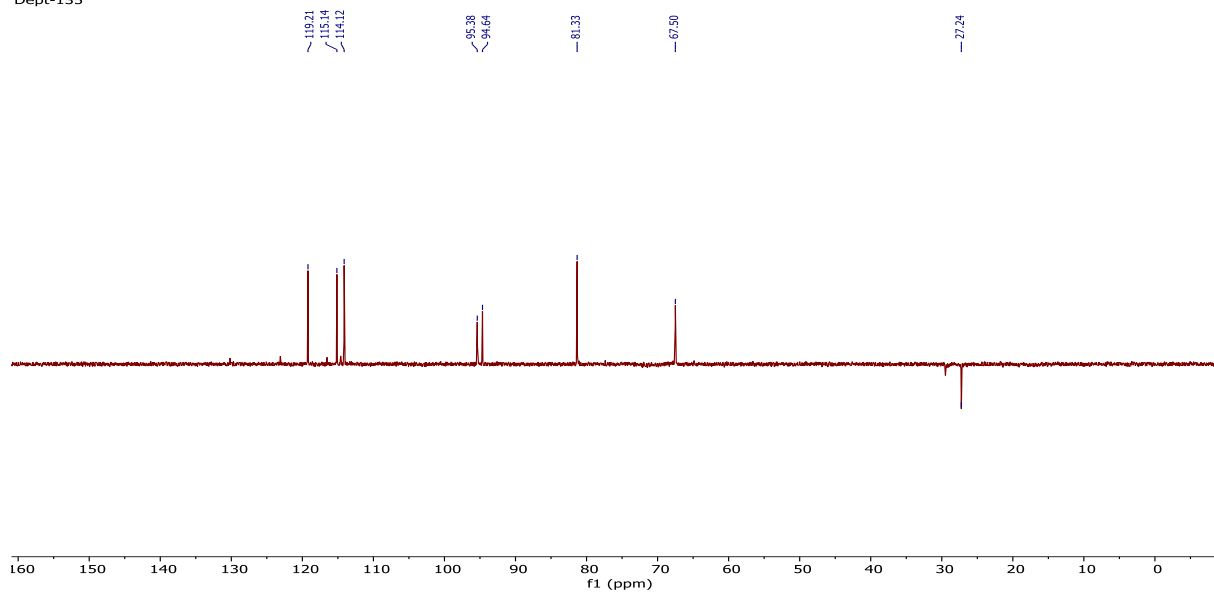
Appendix 9B: ^{13}C NMR spectrum of compound **40**

91-1884(TDHC-4).2.fid
 ^{13}C



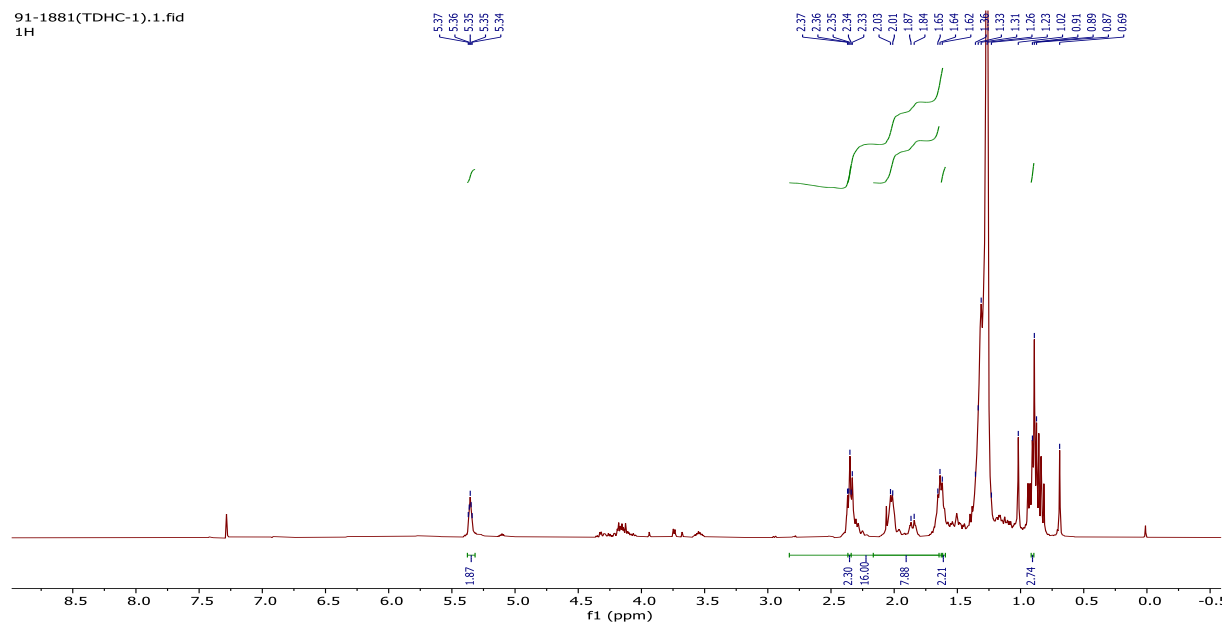
Appendix 9C: DEPT-135 NMR spectrum of compound **40**

91-1884(TDHC-4).3.fid
Dept-135

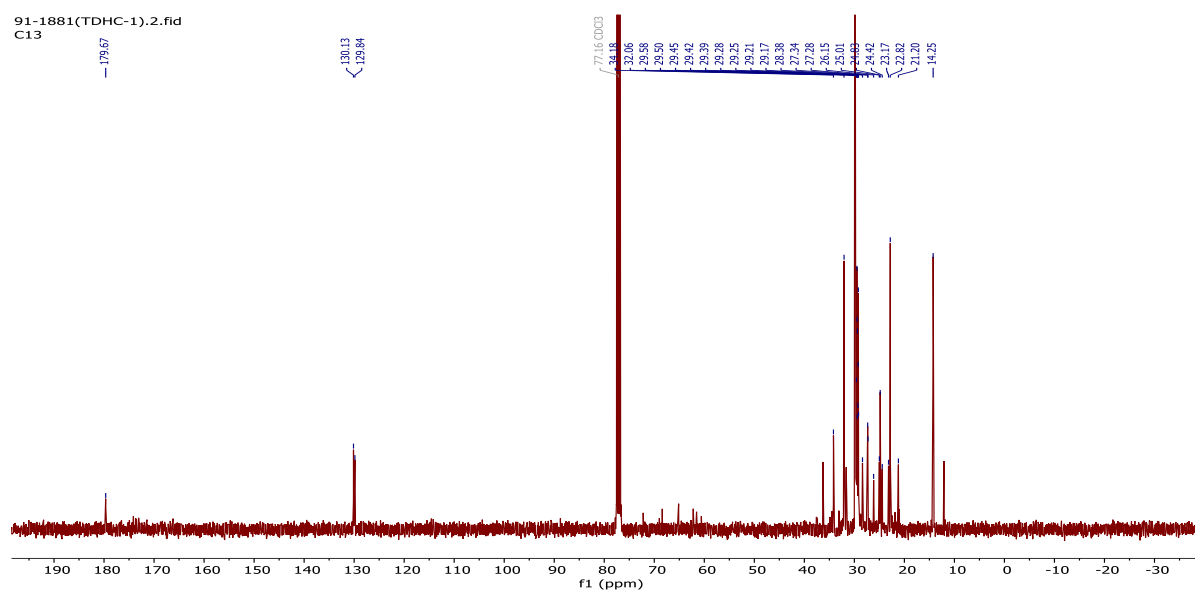


Appendix 10A: ^1H NMR spectrum of compound **41**

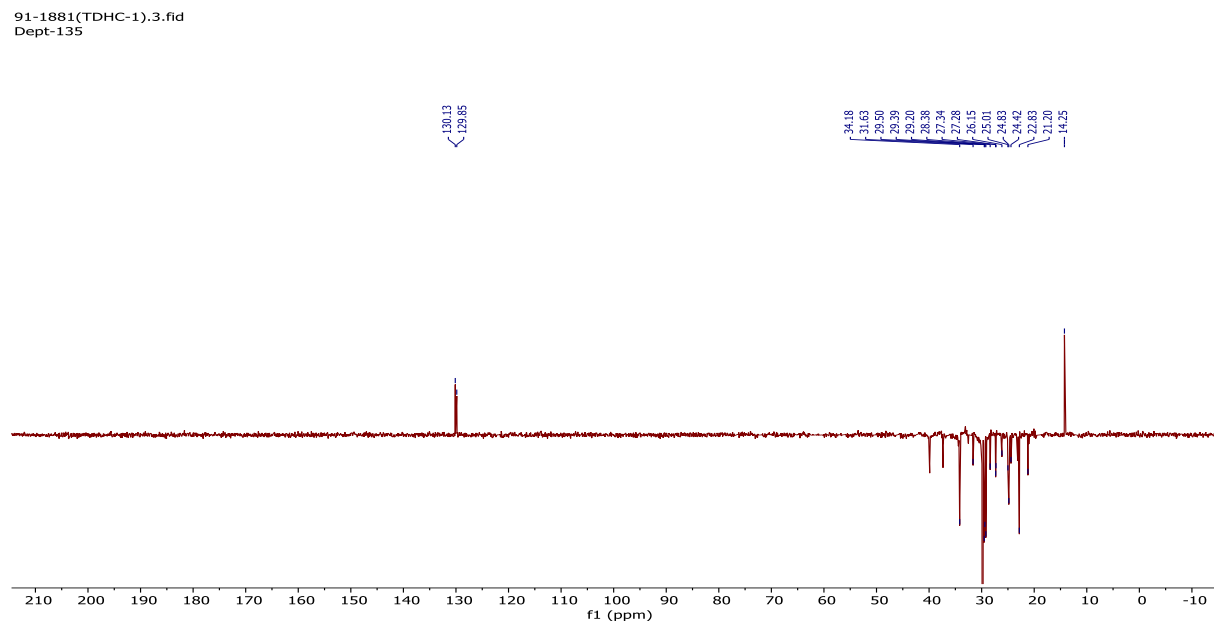
91-1881(TDHC-1).1.fid
 ^1H



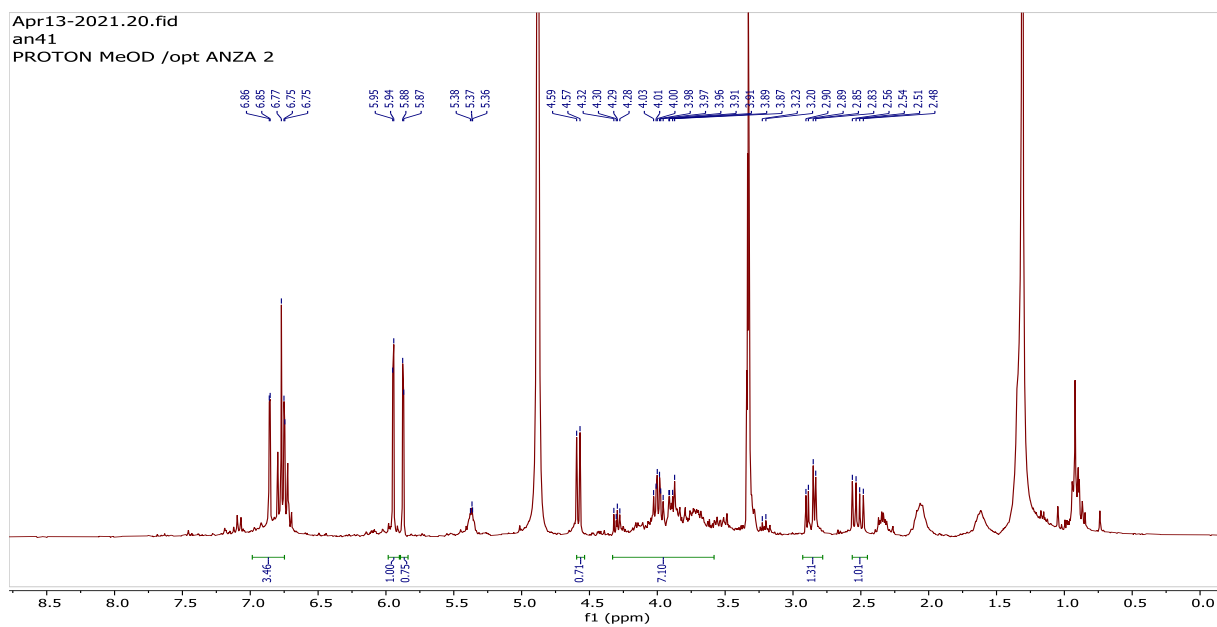
Appendix 10B: ^{13}C NMR spectrum of compound **41**



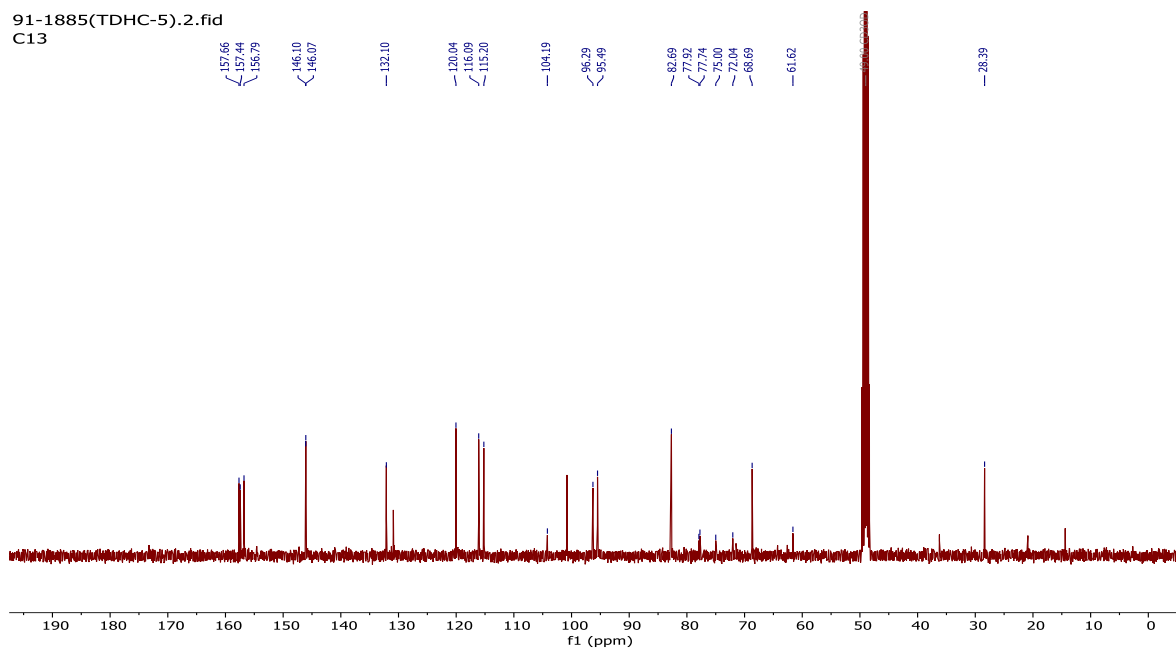
Appendix 10C: DEPT-135 NMR spectrum of compound **41**



Appendix 11A: ^1H NMR spectrum of compound **106**

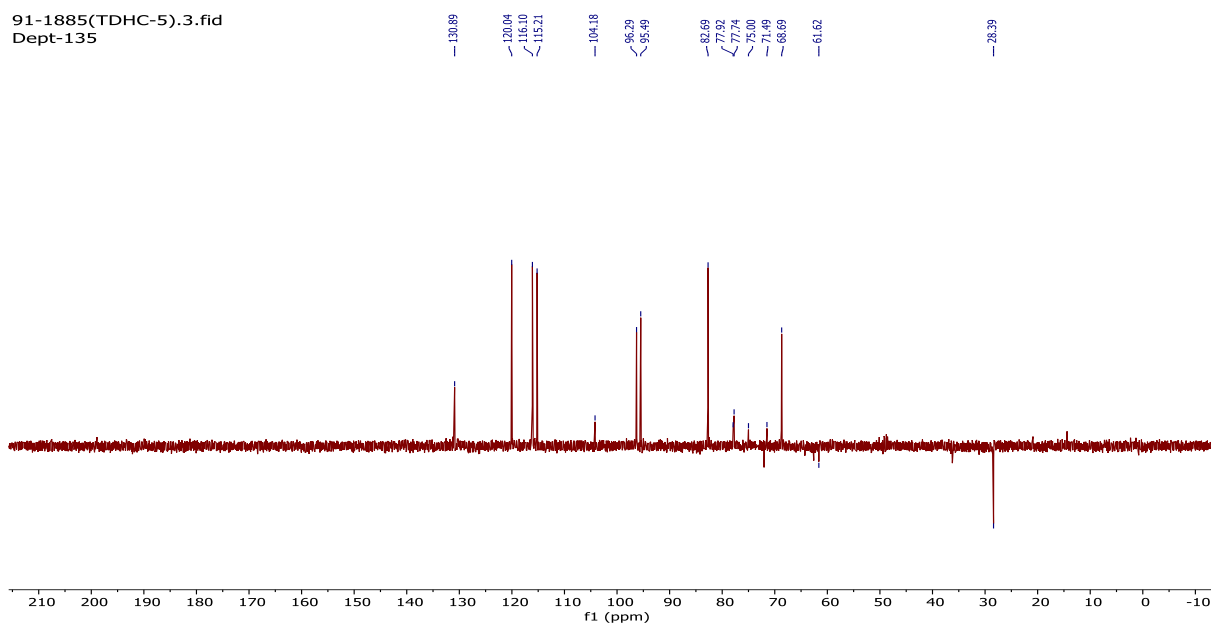


Appendix 11B: ^{13}C NMR spectrum of compound **106**

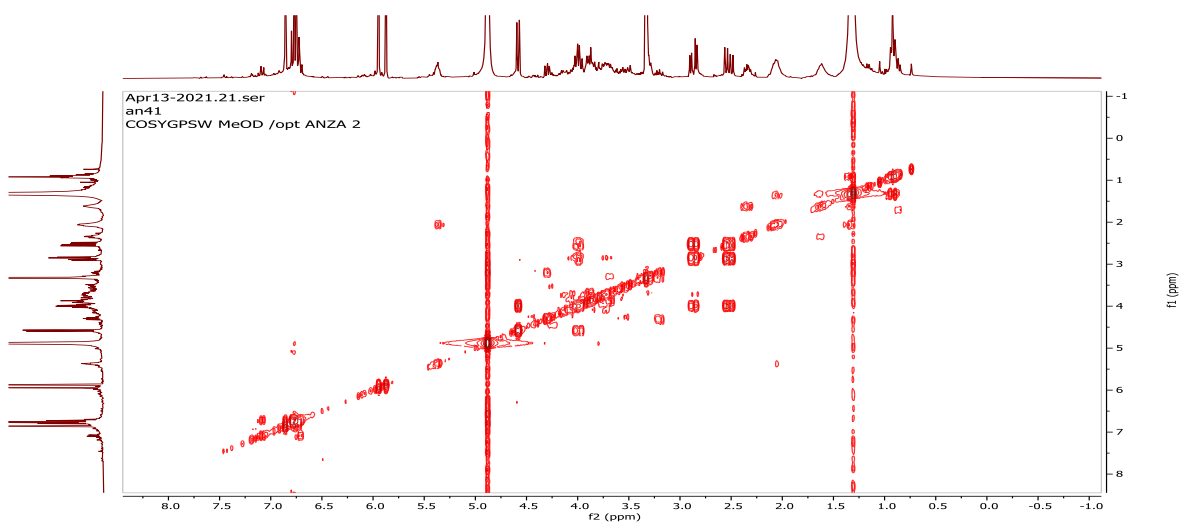


Appendix 11C: DEPT-135 spectrum of compound **106**

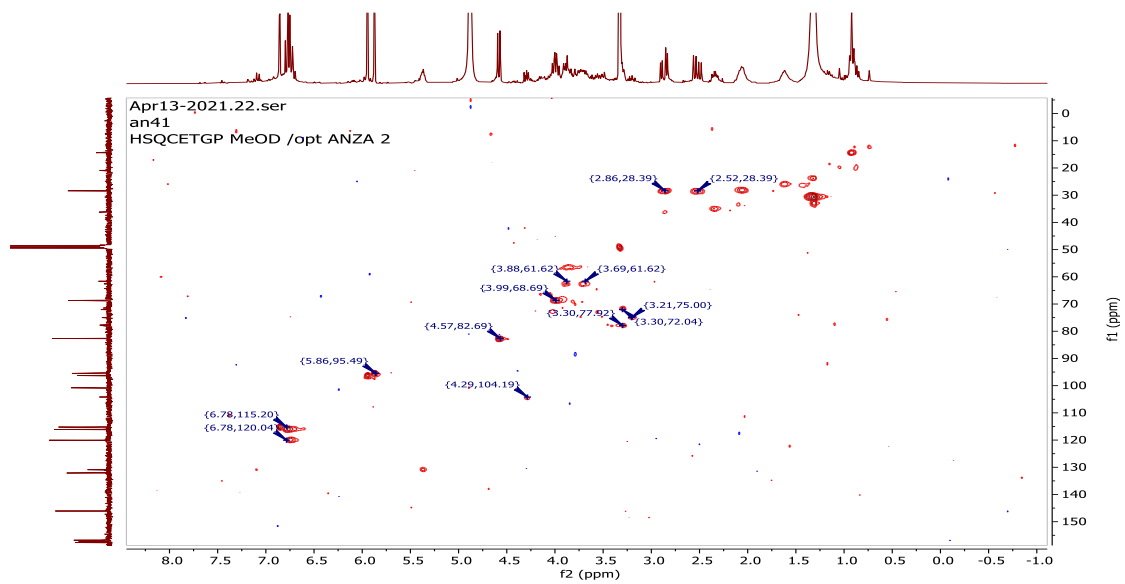
91-1885(TDHC-5).3.fid
Dept-135



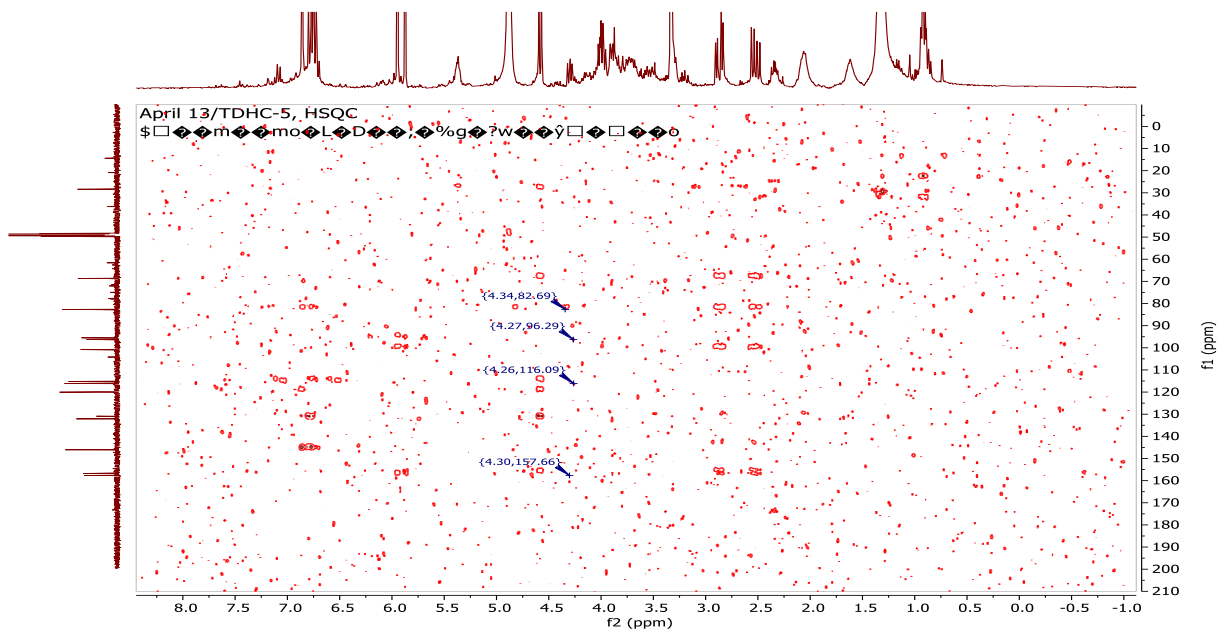
Appendix 11D: COSY spectrum of Compound **106**



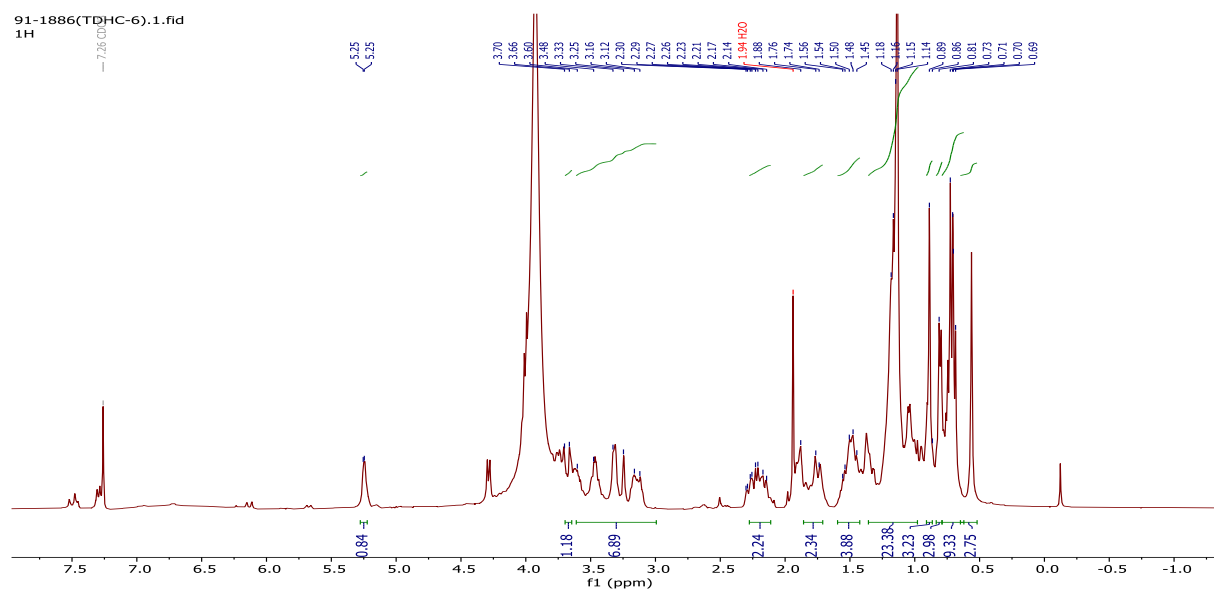
Appendix 11E: HSQC spectrum of compound **106**



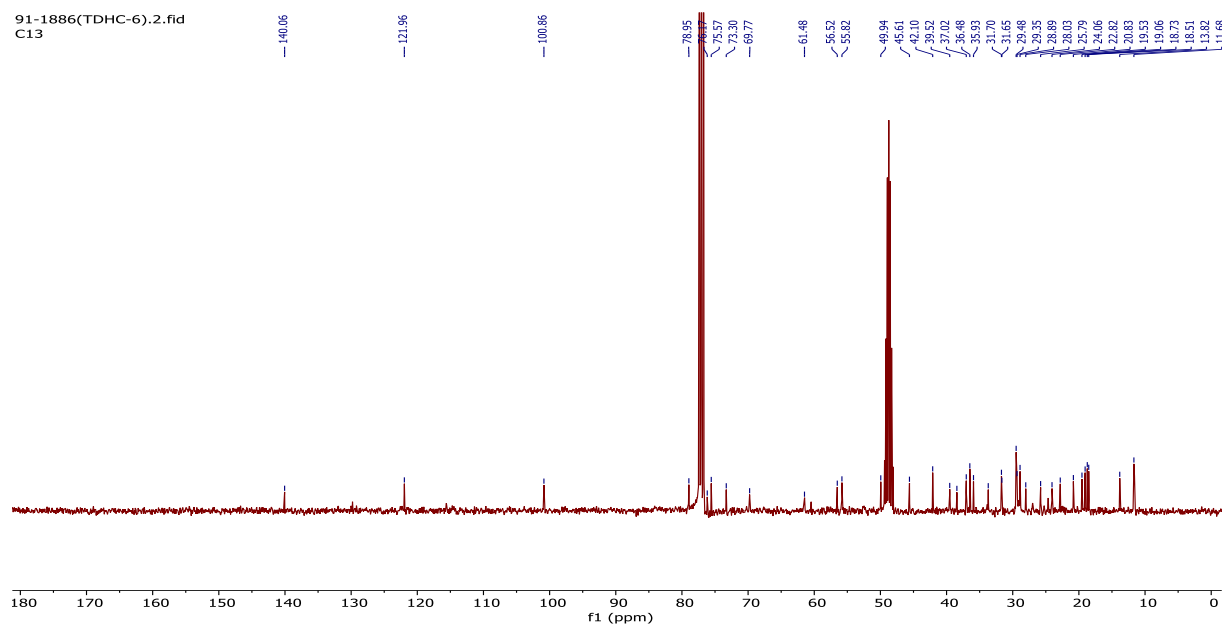
Appendix 11F: HMBC spectrum of Compound **106**



Appendix 12 A: ^1H NMR spectrum of Compound **107**

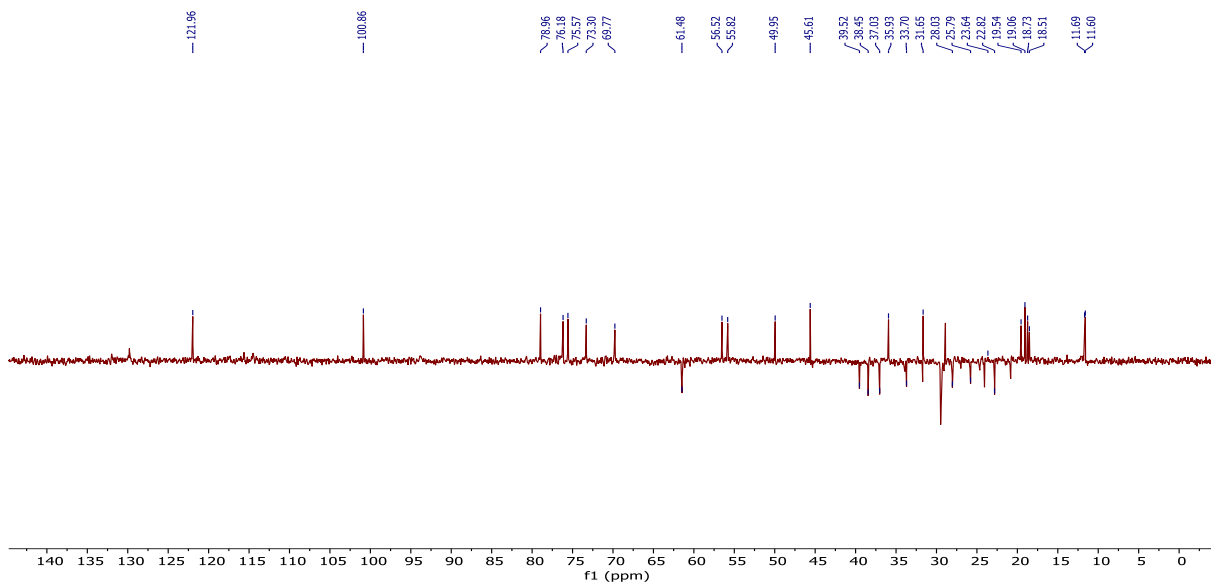


Appendix 12 B: ^{13}C NMR spectrum of compound **107**



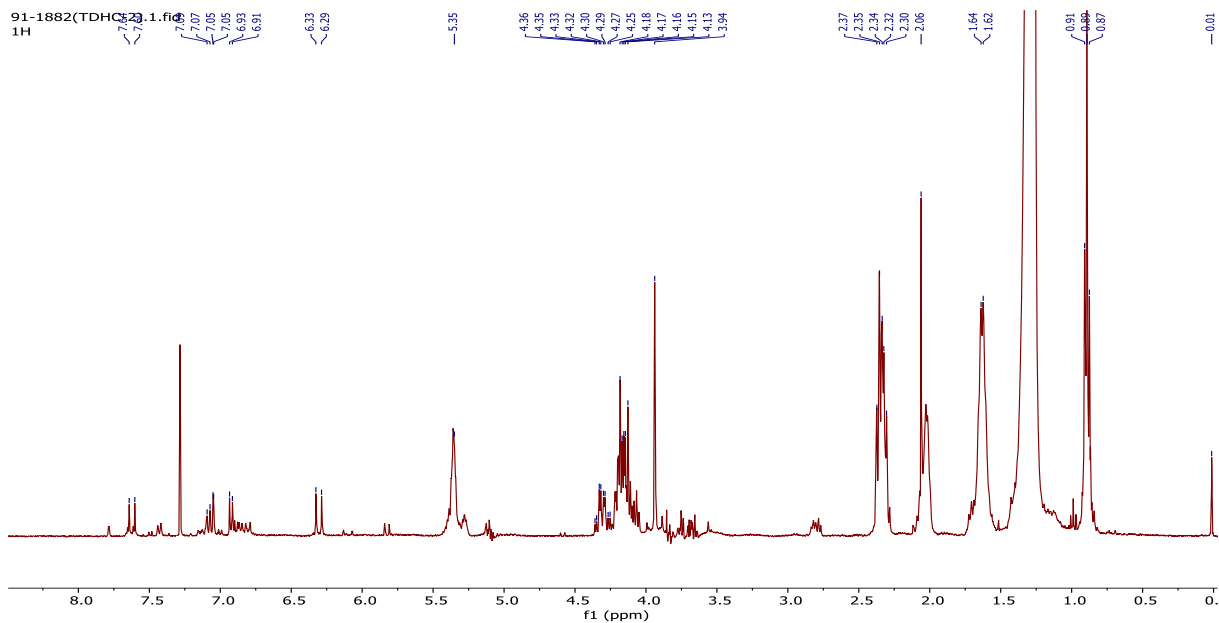
Appendix 12C: DEPT-135 NMR spectrum of compound **107**

91-1886(TDHC-6).3.fid
Dept-135

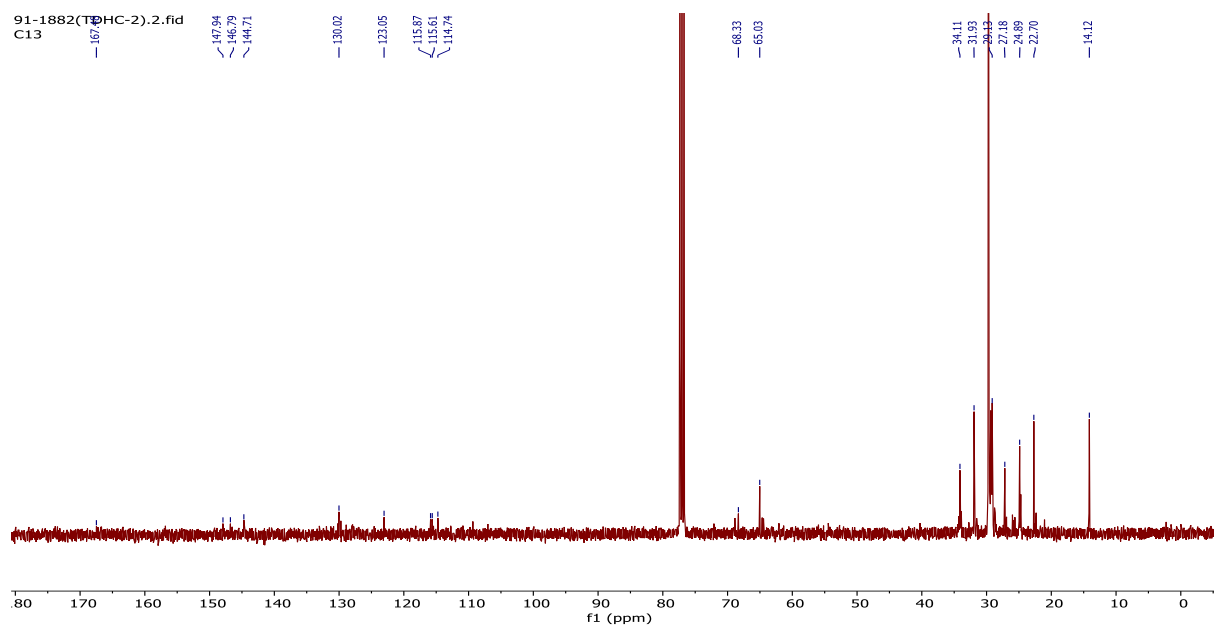


Appendix 13A: ^1H NMR spectrum of compound **108**

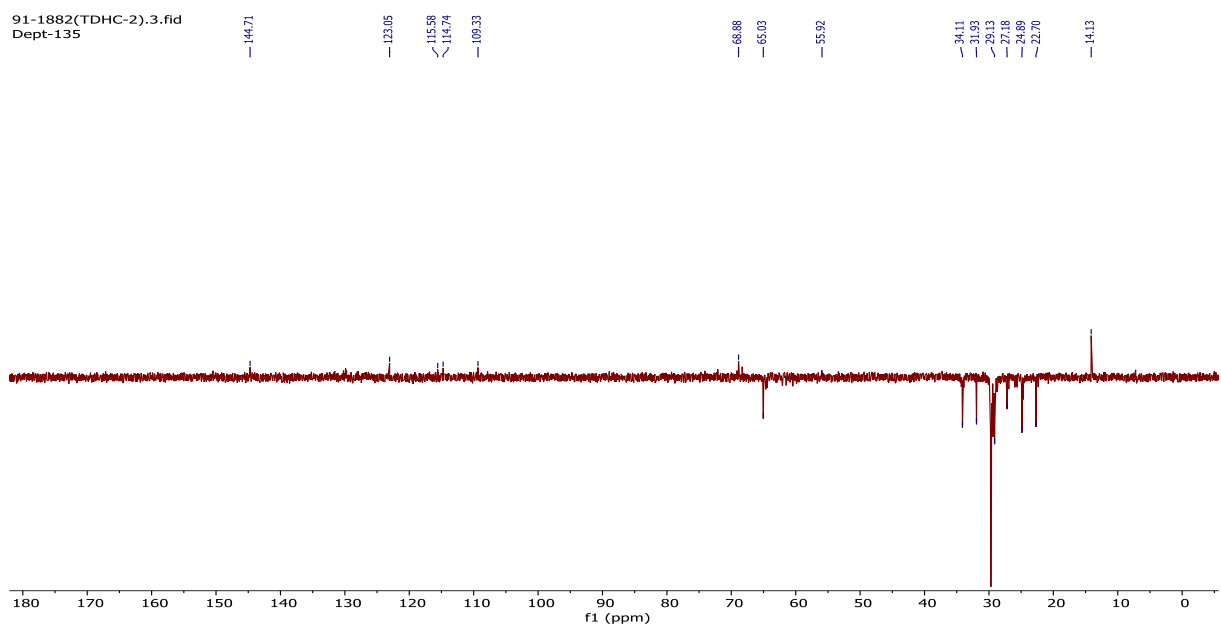
91-1882(TDHC-2).1.fid
 ^1H



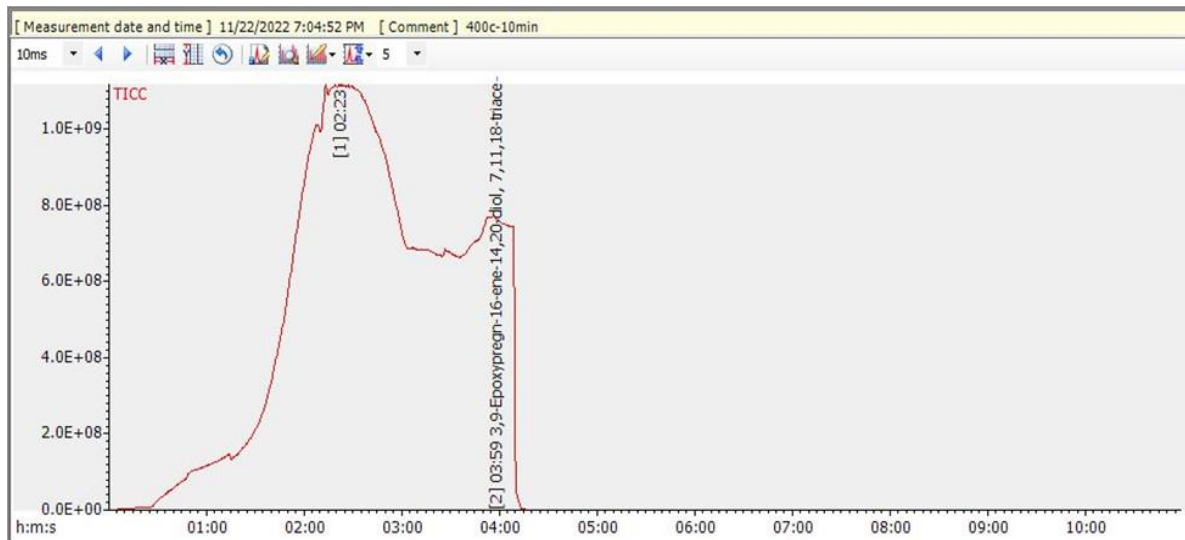
Appendix 13 B: ^{13}C NMR spectrum of compound **108**



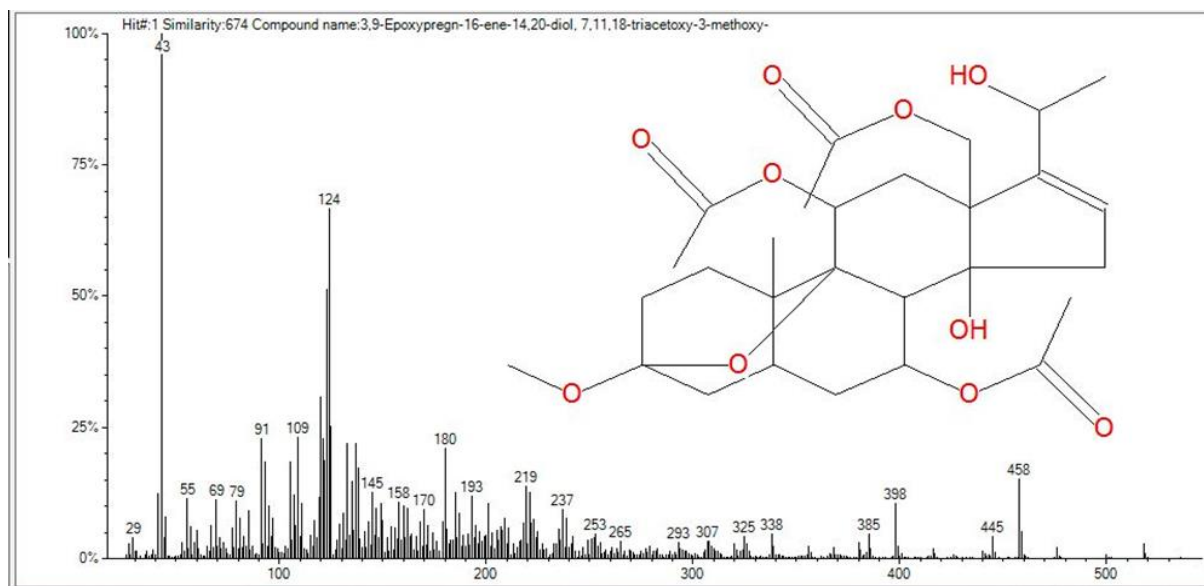
Appendix 13C: Appendix 12D: DEPT NMR spectrum of compound **108**



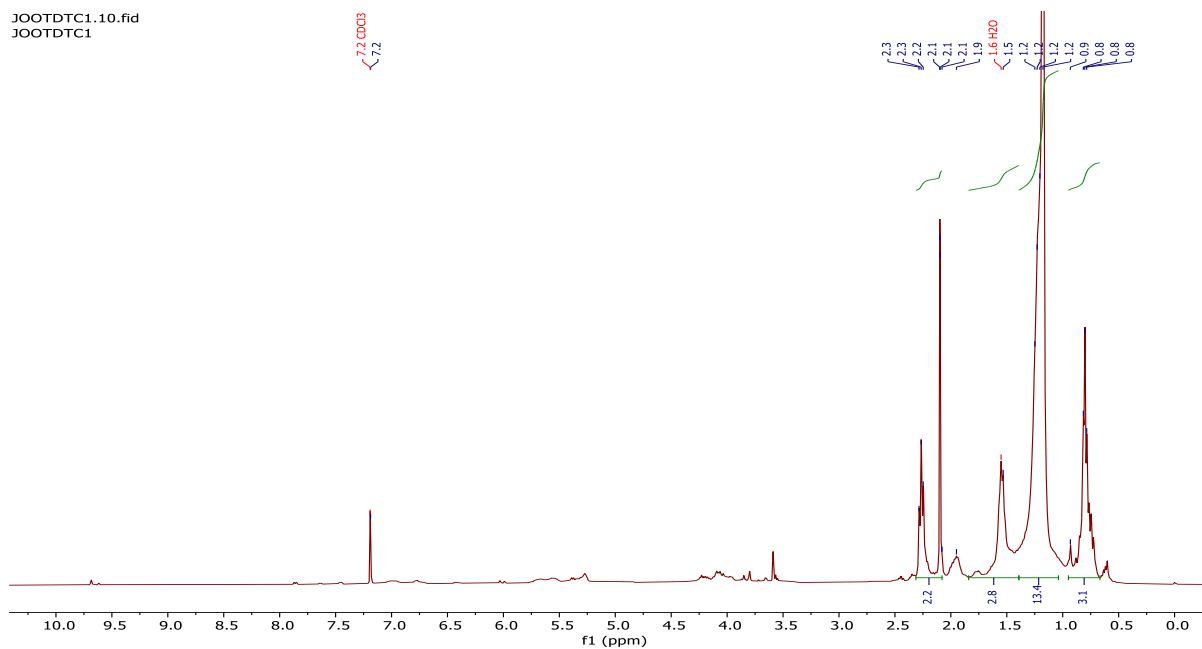
Appendix 14 A: GC of compound **109**



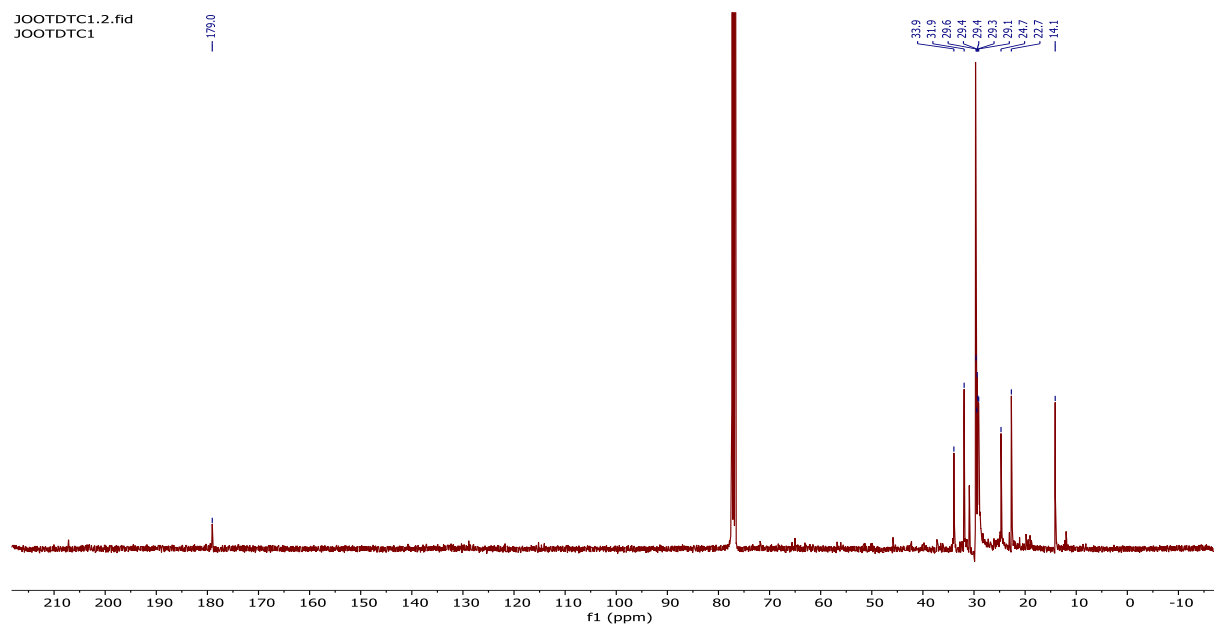
Appendix 14B: Mass spectrum (MS) of compound **109** with RT of 4.00 min



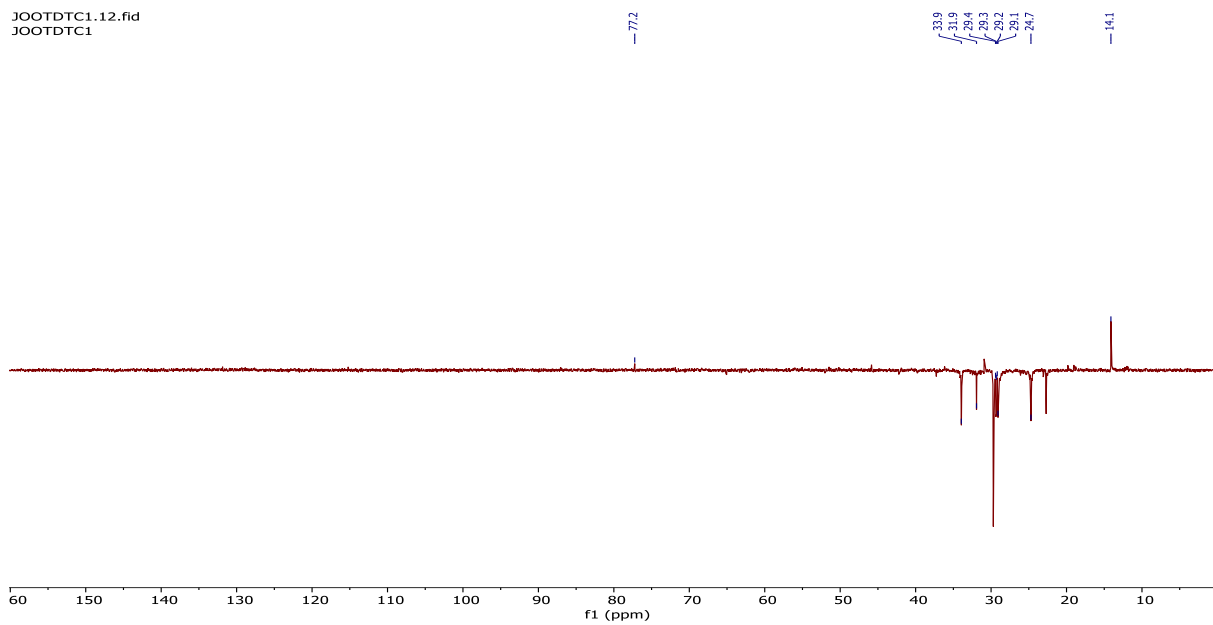
Appendix 15A: ^1H NMR spectrum of compound **110**



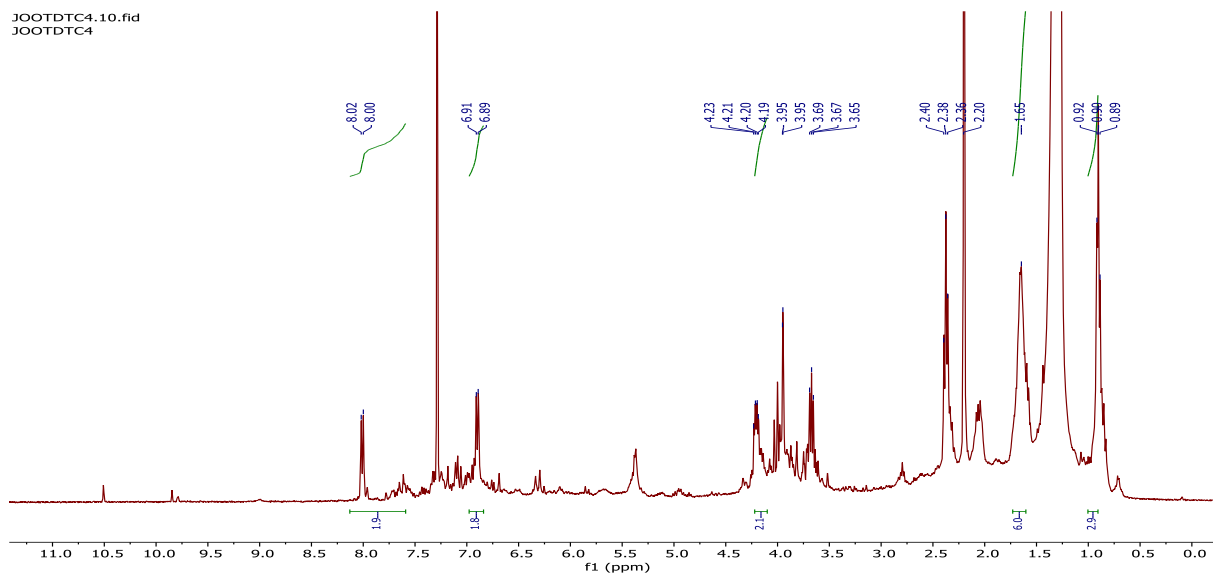
Appendix 15B: ^{13}C NMR spectrum of compound **110**



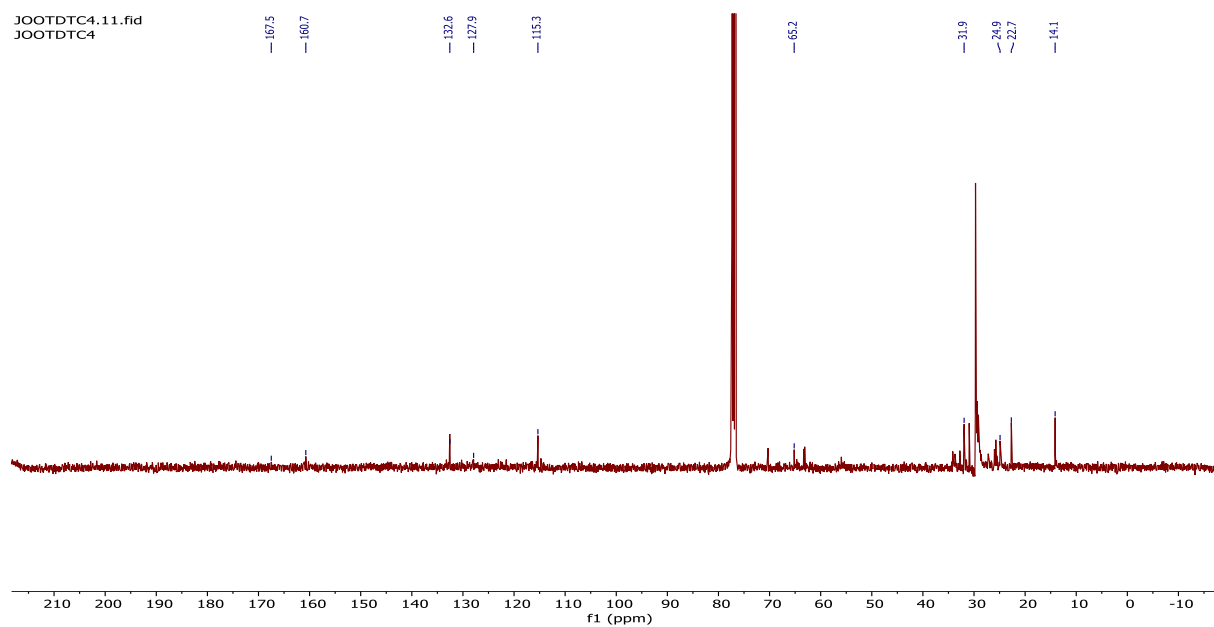
Appendix 15C: DEPT-135 NMR spectrum of compound **110**



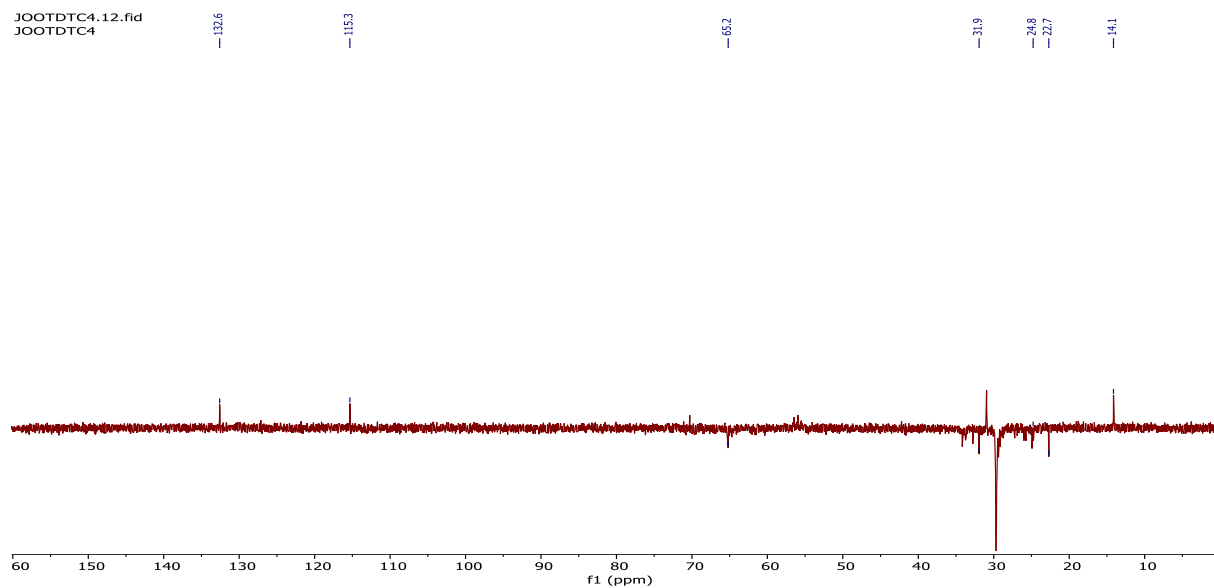
Appendix 16A: ^1H NMR spectrum of compound **111**



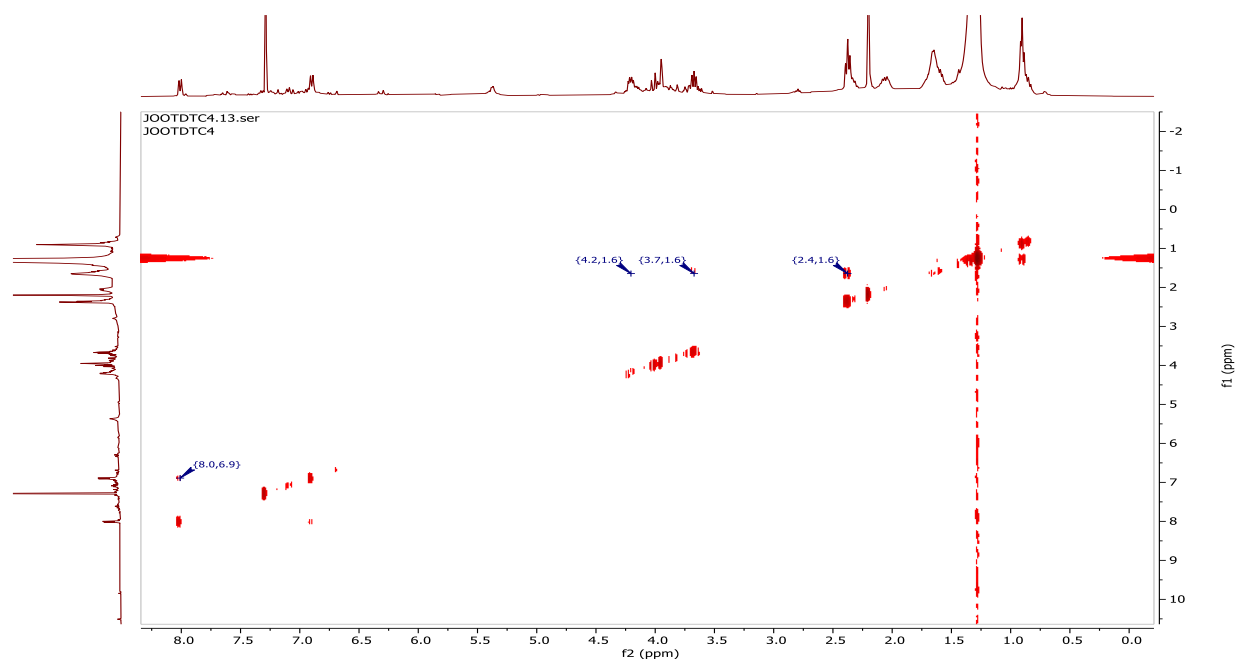
Appendix 16B: ^{13}C NMR spectrum of compound **111**



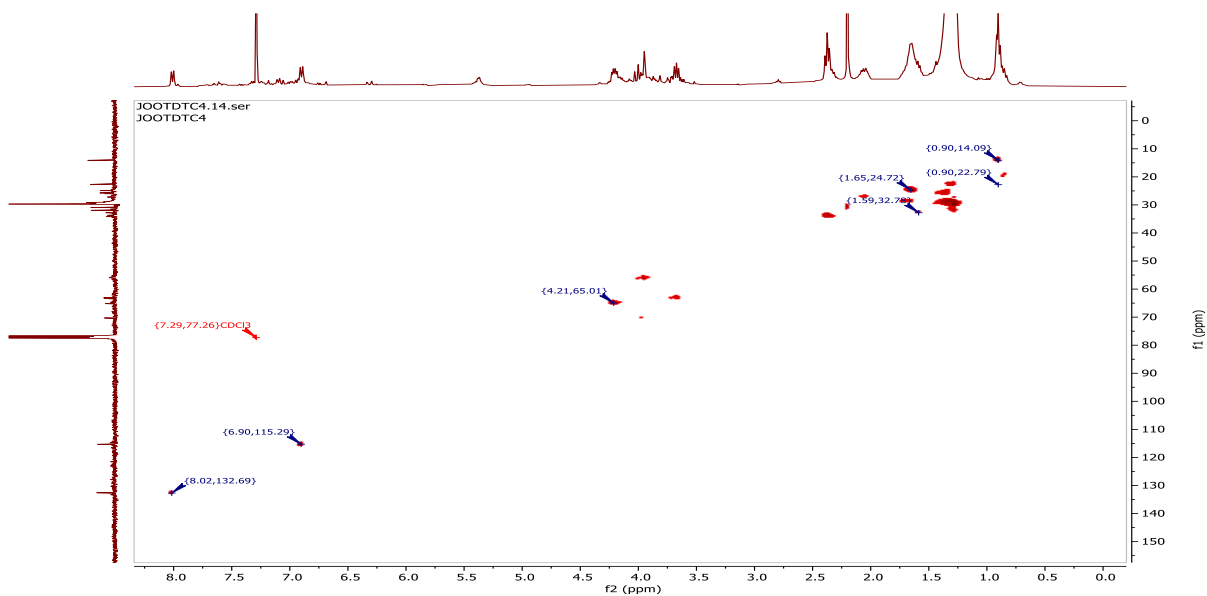
Appendix 16C: DEPT-135 NMR spectrum of compound **111**



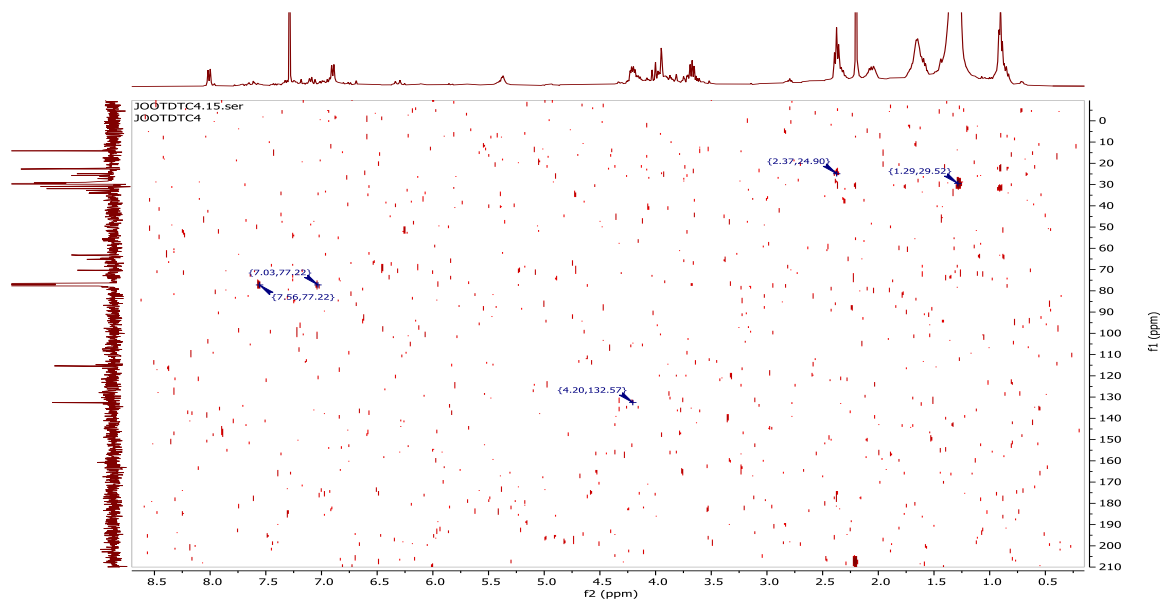
Appendix 16D: COSY NMR spectrum of compound **111**



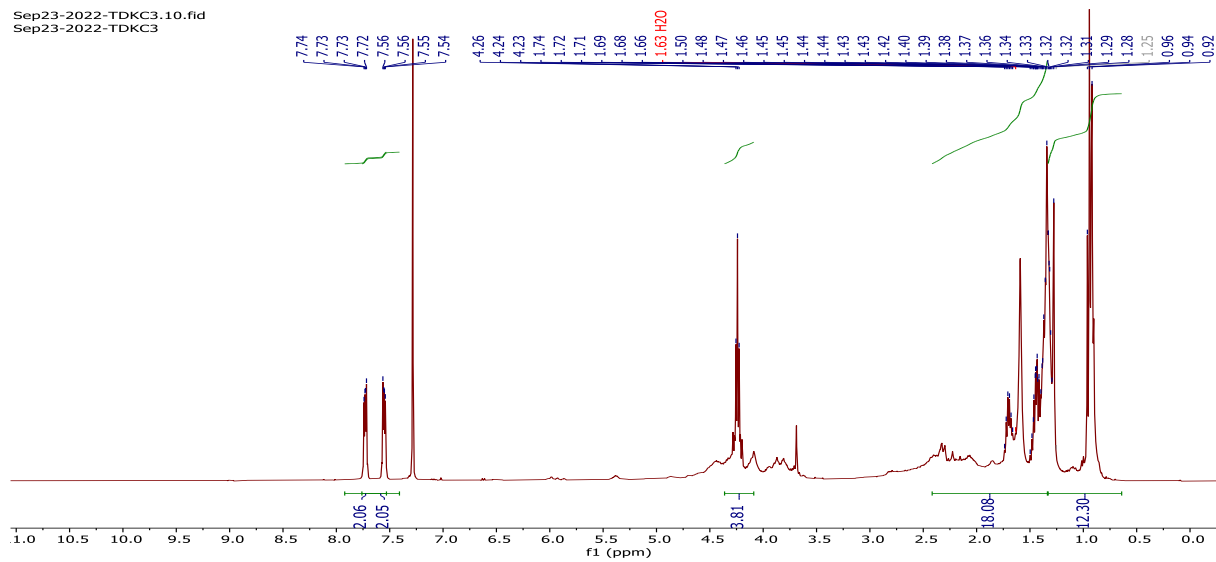
Appendix 16E HSQC NMR spectrum of compound **111**



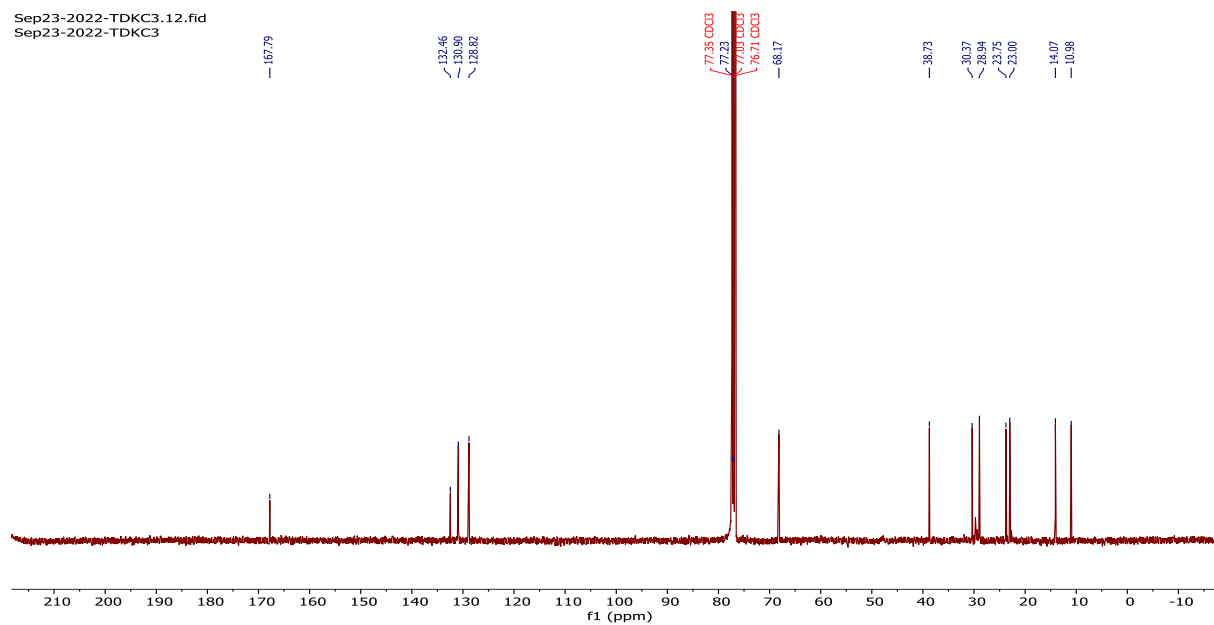
Appendix 16F: HMBC NMR spectrum of compound **111**



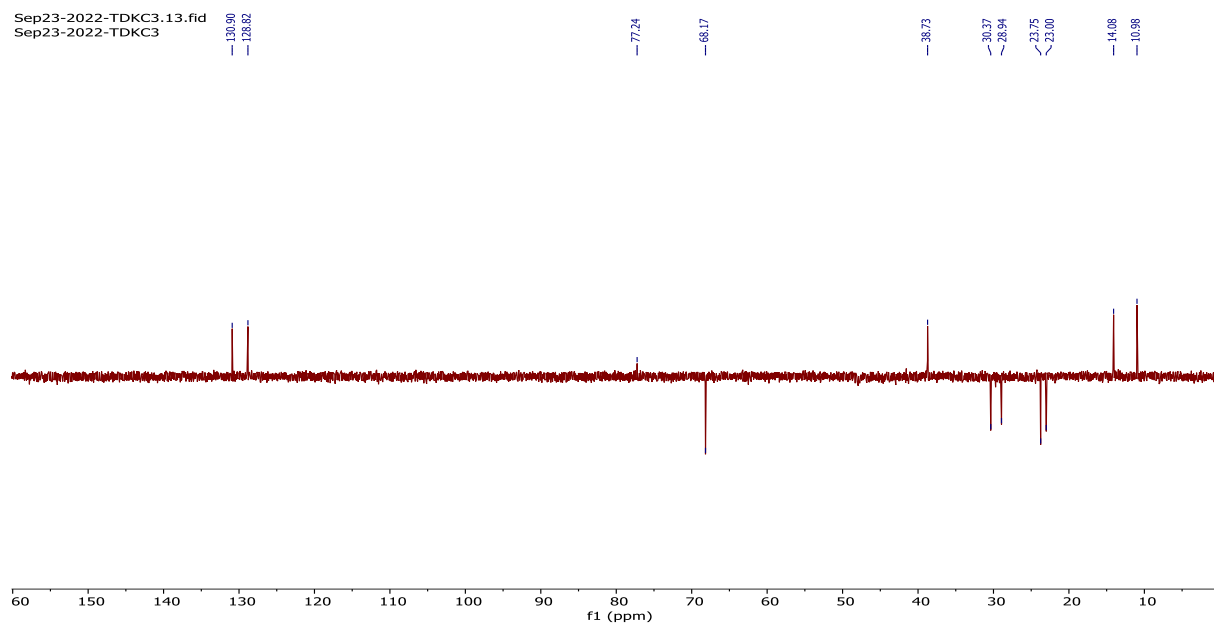
Appendix 17A: ^1H NMR spectrum of compound **112**



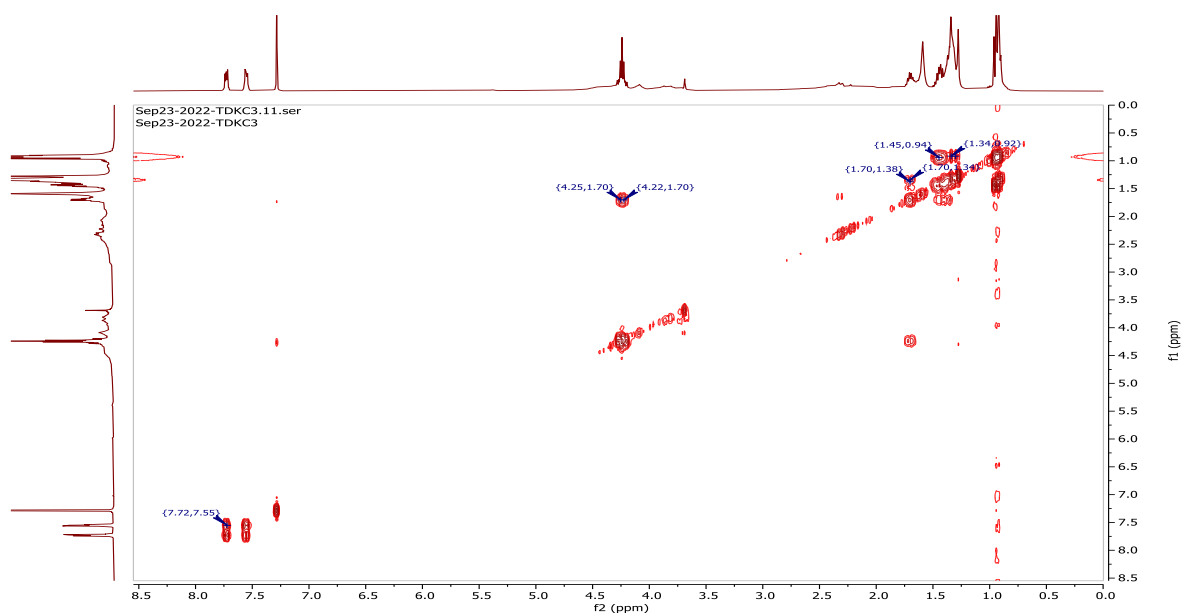
Appendix 17B: ^{13}C NMR spectrum of compound **112**



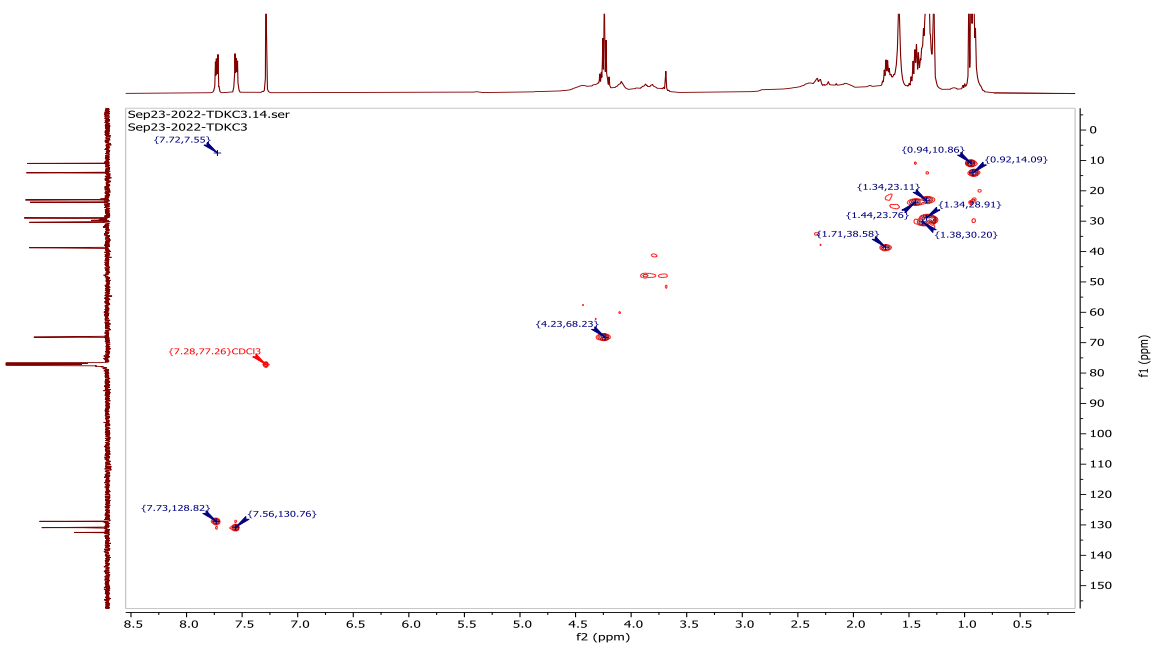
Appendix 17C: DEPT-135 NMR spectrum of compound **112**



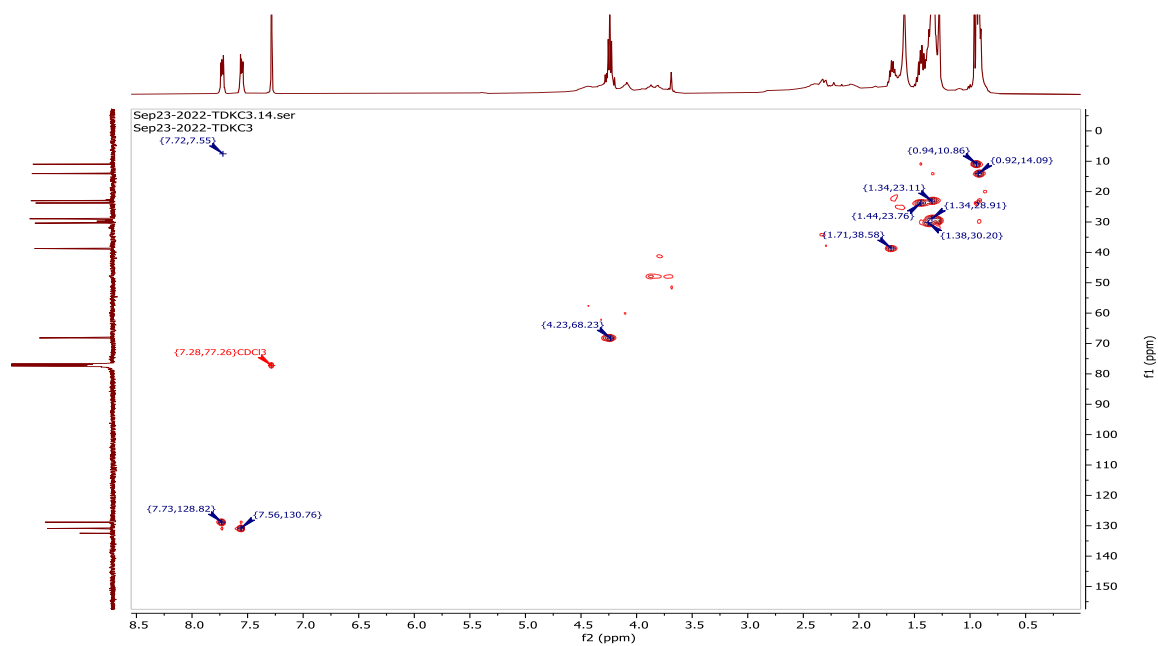
Appendix 17D: COSY NMR spectrum of compound **112**



Appendix 17E: HSQC NMR spectrum of compound **112**

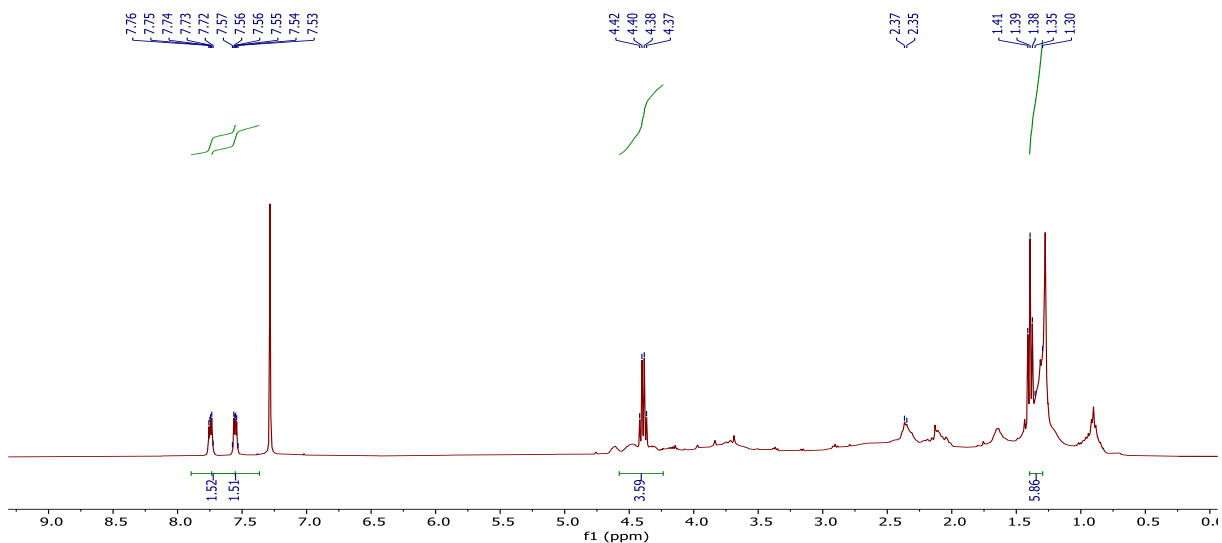


Appendix 17F: HMBC NMR spectrum of compound **112**



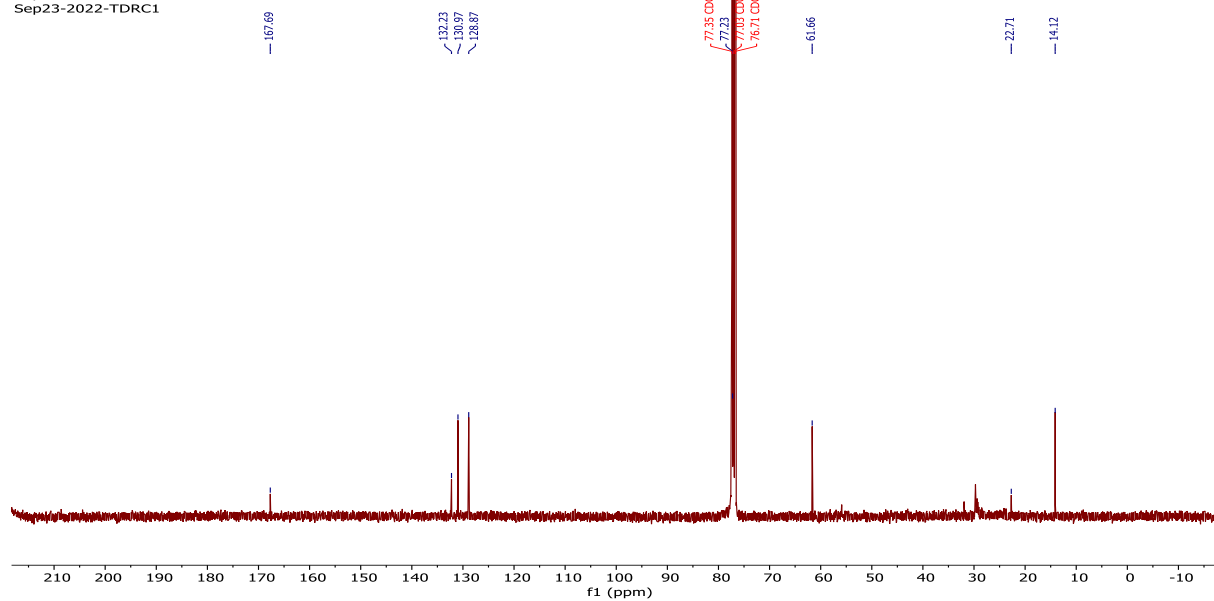
Appendix 18A: ^1H NMR spectrum of compound **113**

Sep23-2022-TDRC1.10.fid
Sep23-2022-TDRC1



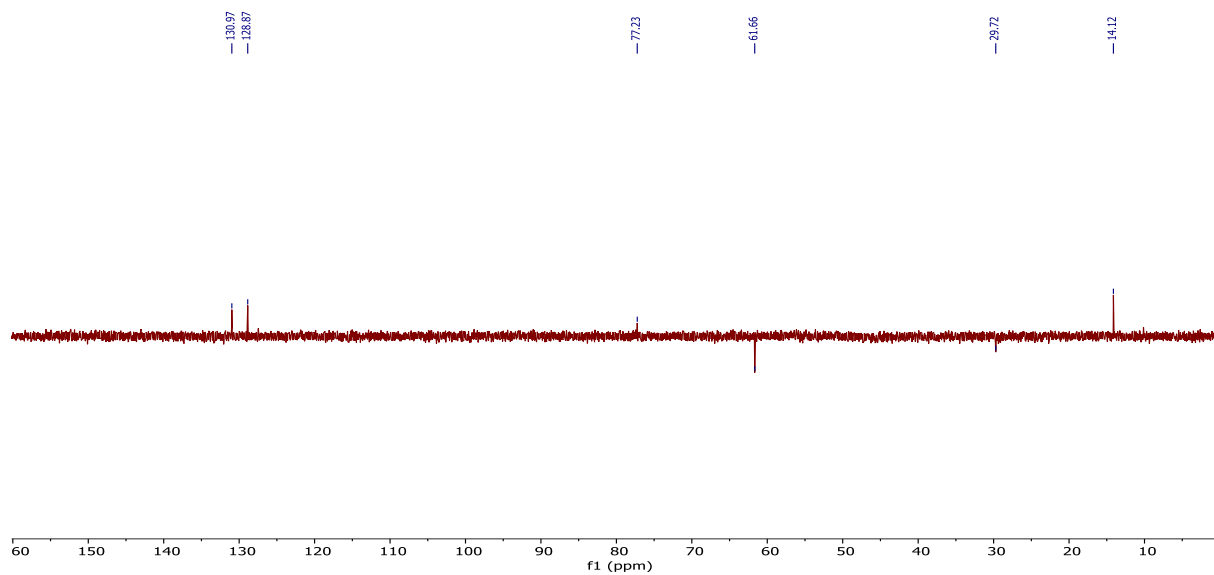
Appendix 18B: ^{13}C NMR spectrum of compound **113**

Sep23-2022-TDRC1.13.fid
Sep23-2022-TDRC1

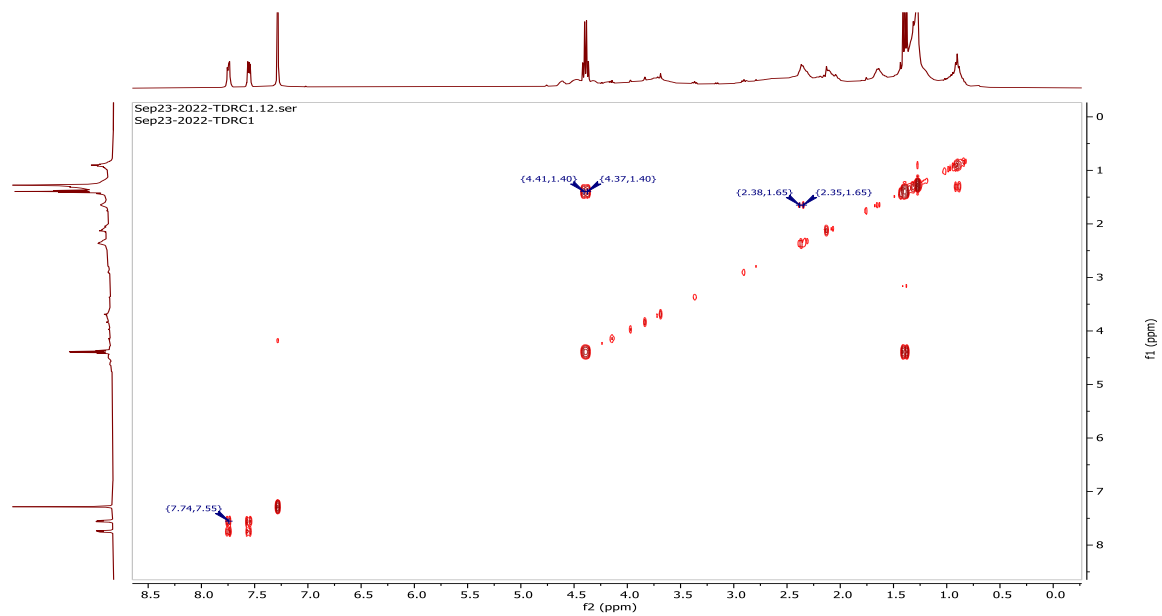


Appendix 18C: DEPT-135: ^1H NMR spectrum of compound **113**

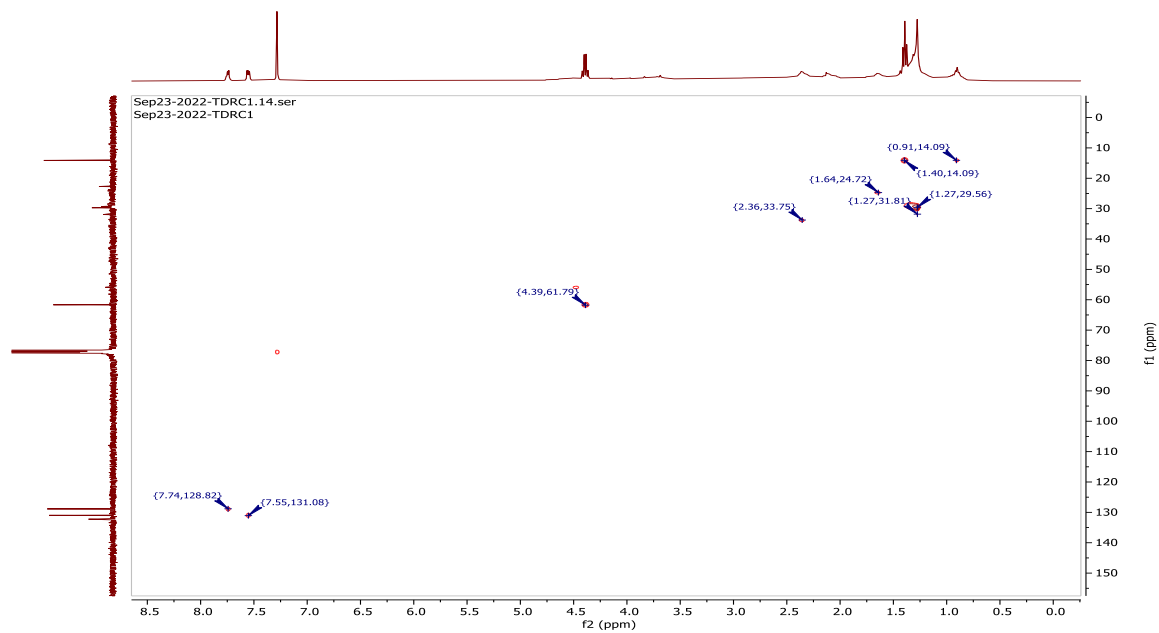
Sep23-2022-TDRC1.16.fid
Sep23-2022-TDRC1



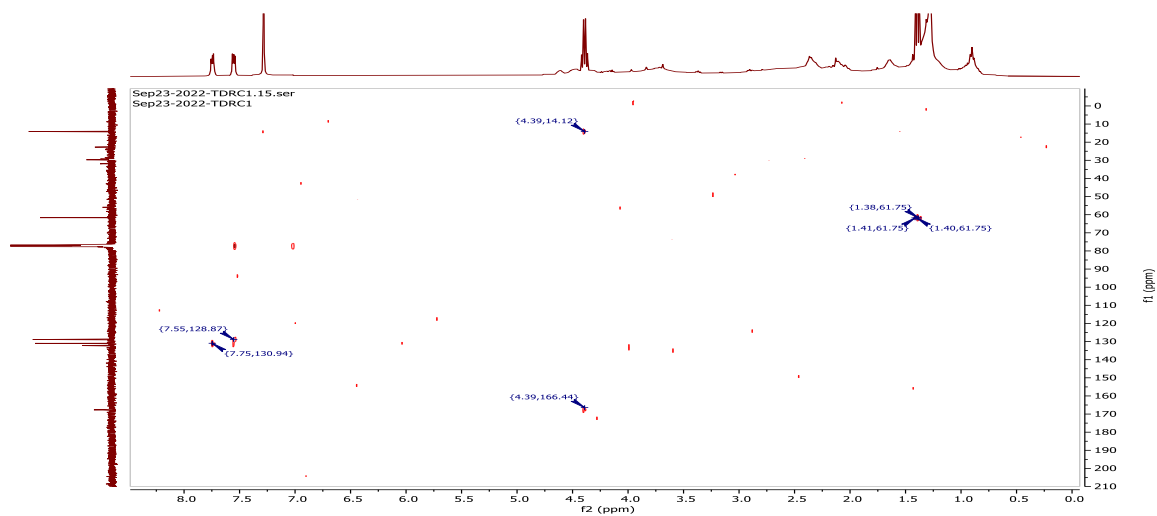
Appendix 18D COSY NMR spectrum of compound **113**



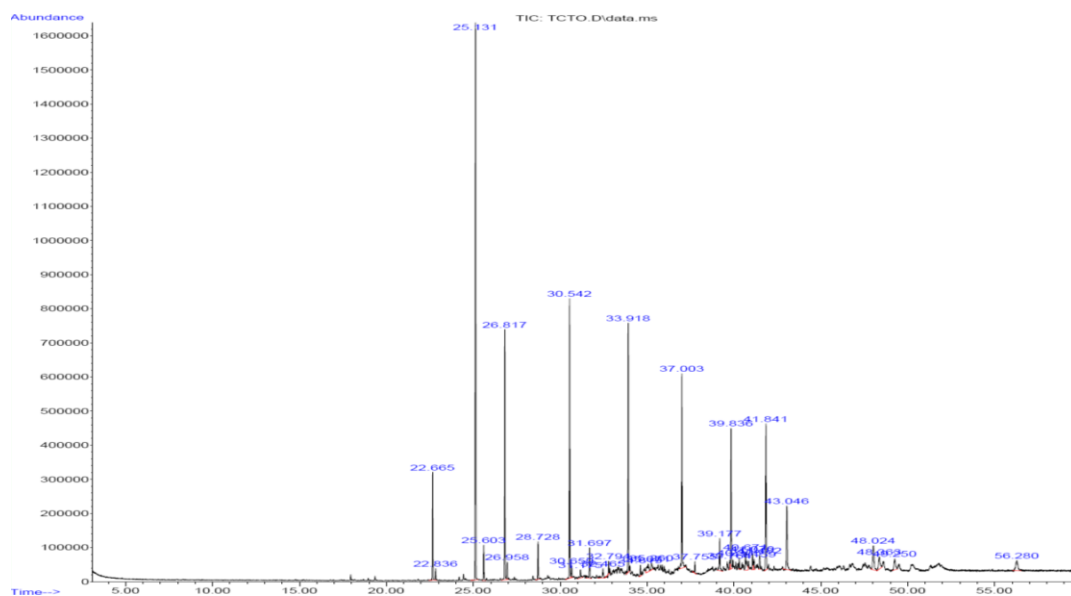
Appendix 18E: HSQC NMR spectrum of compound **113**



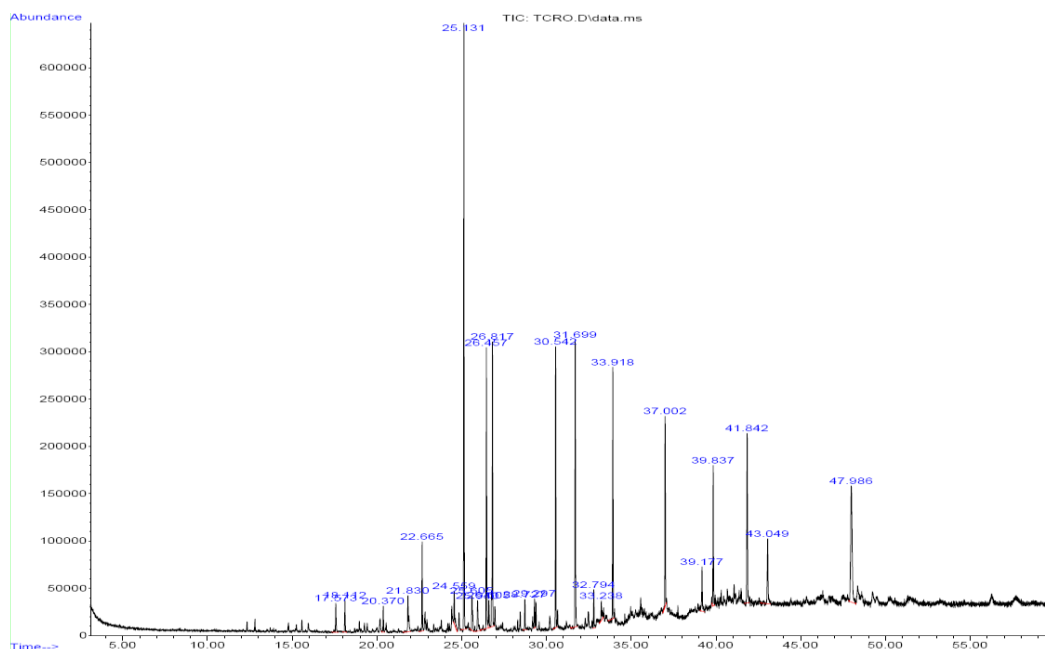
Appendix 18F: HMBC NMR spectrum of compound **113**



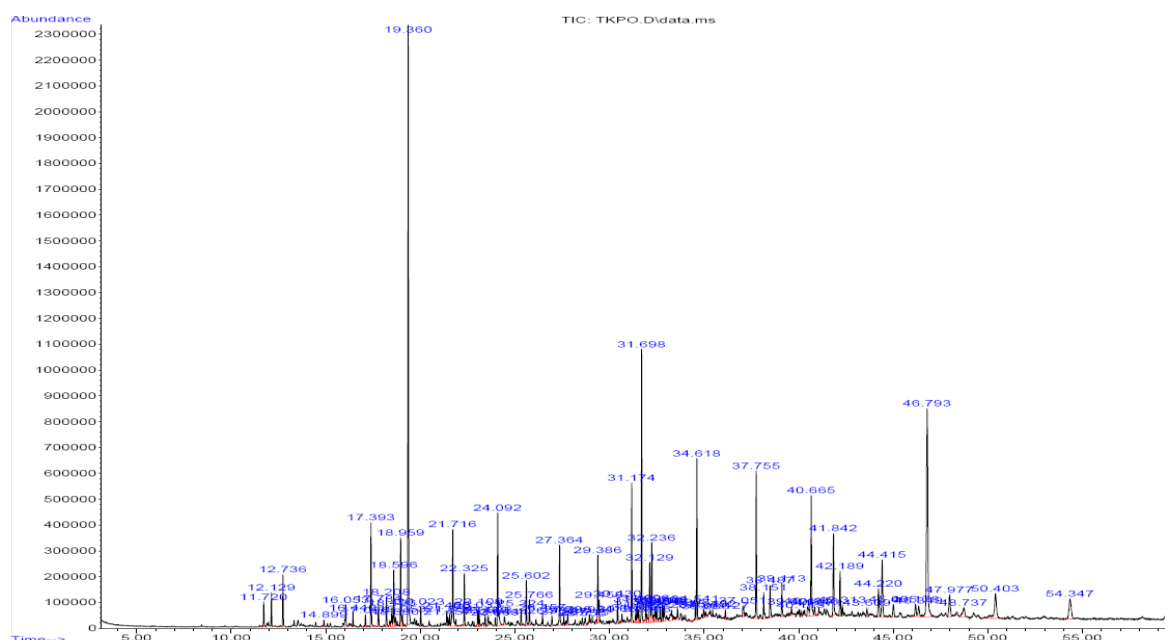
Appendix 19A: Chromatogram for essential oil from leaves of *C. tomentosa*



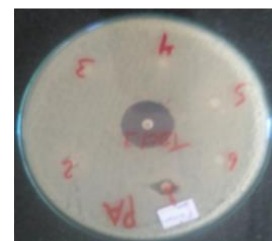
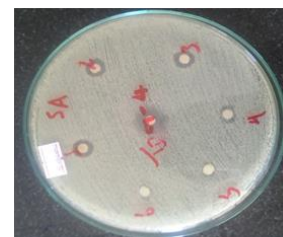
Appendix 19B: Chromatogram for essential oil from leaves of *K. petitiiana*



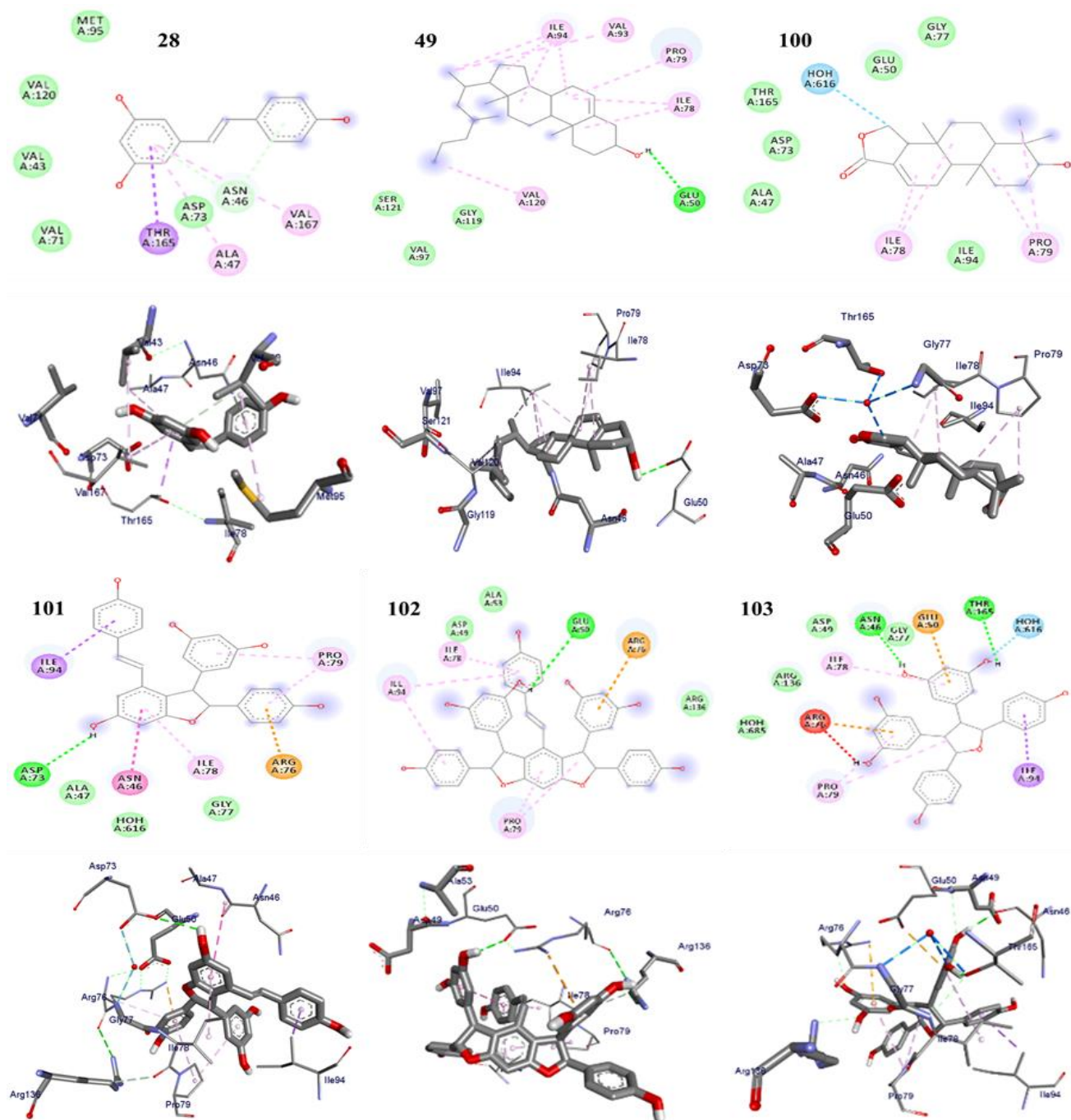
Appendix 19C: Chromatogram for essential oil from leaves of *C. rotundifolia*



Appendix 20A: Inhibition zone image of antibacterial activity of some of the tested compounds.

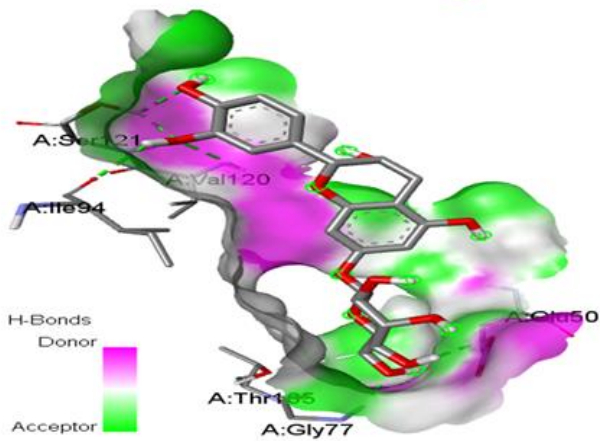
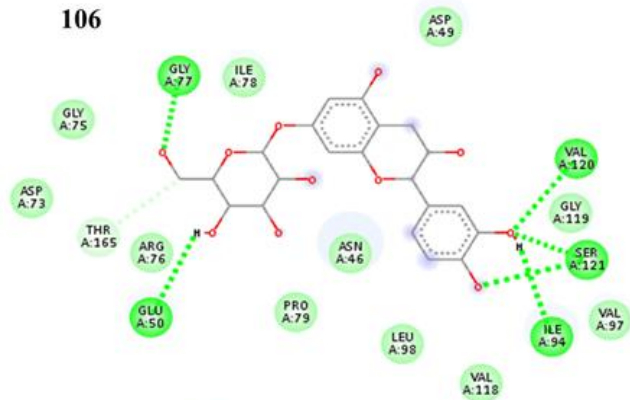


Appendix 21A: The 2D and 3D binding interactions of compounds **28**, **49**, **100**, **101**, **102**, **103**, **106** and **107**, and standard against DNA gyrase B (PDB ID: 6F86).

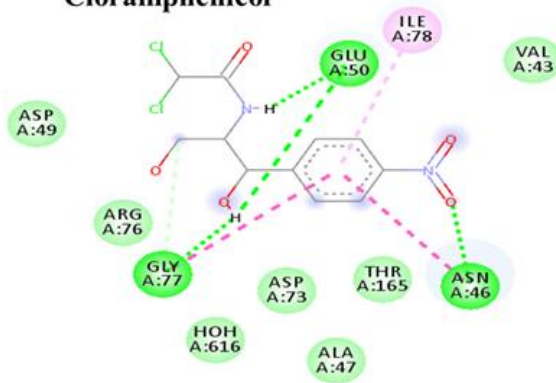


Appendix 21A: continuous . . .

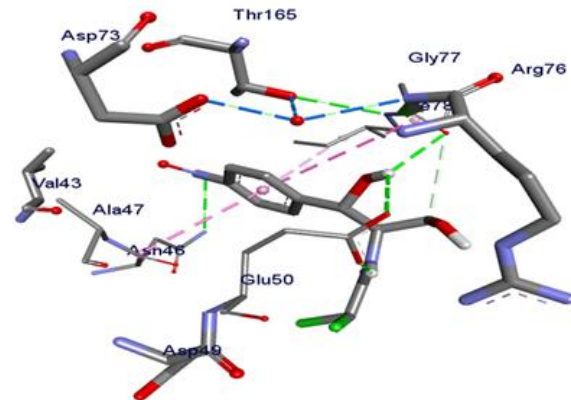
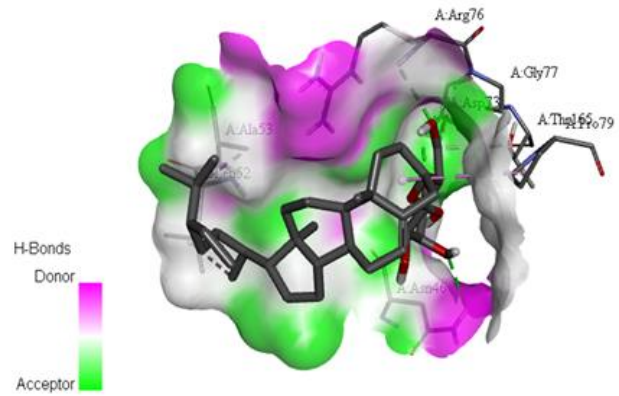
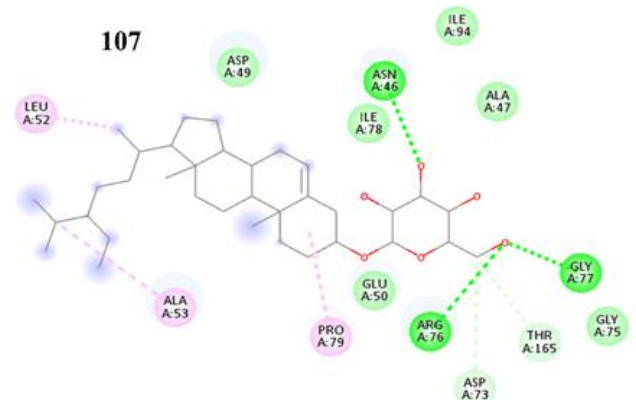
106



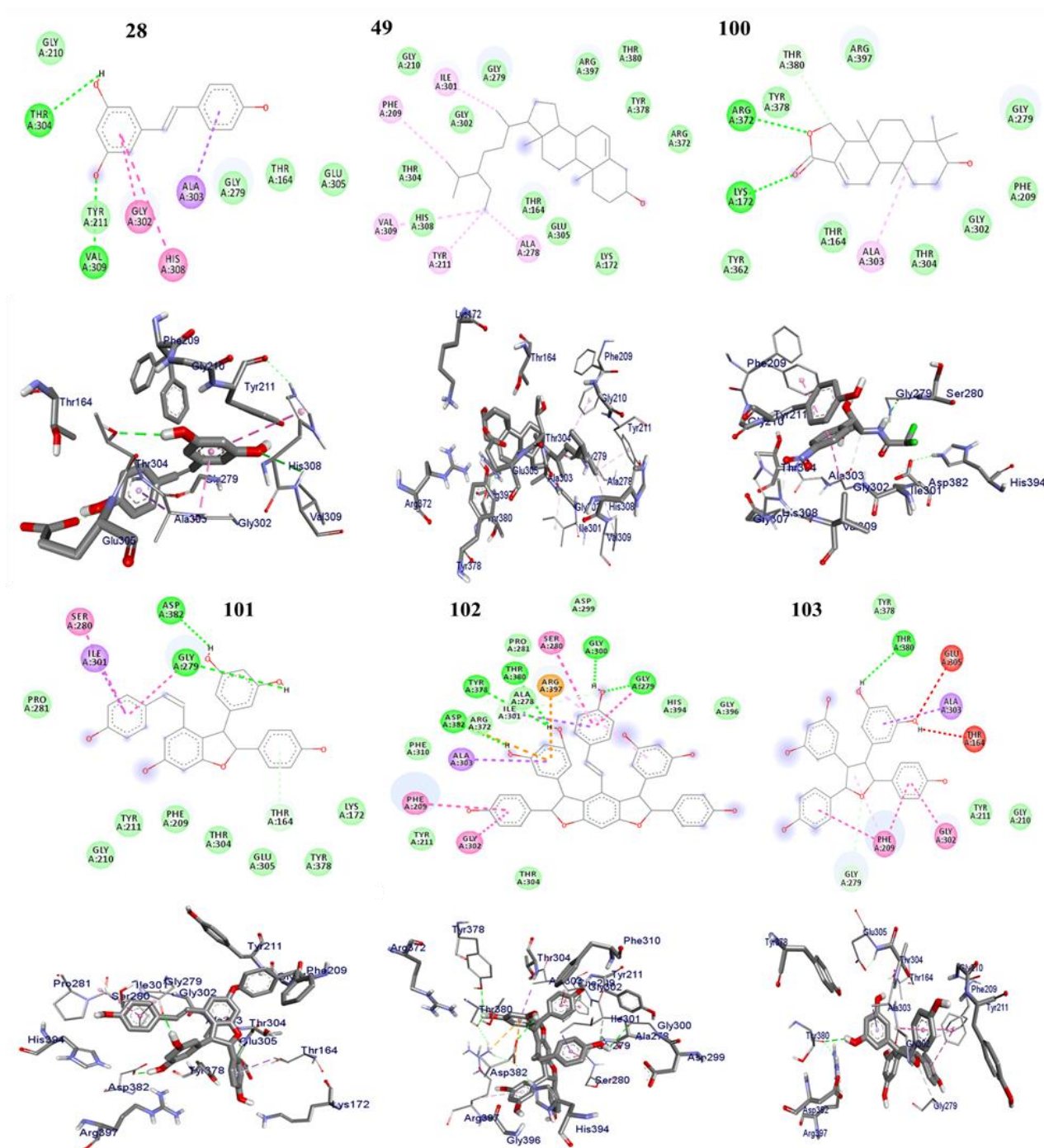
Cloramphenicol



107

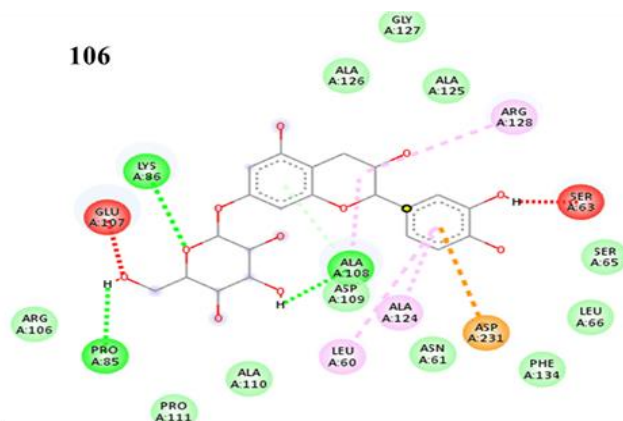


Appendix 22A: The 2D and 3D binding interactions of compounds **28**, **49**, **100**, **101**, **102**, **103**, **106** and **107**, and standard against PqsA (PDB ID: 5OE3).

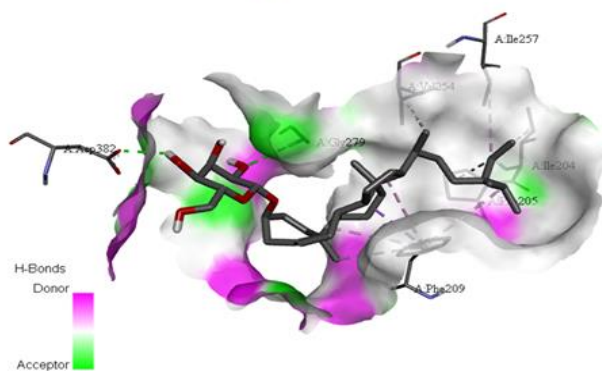
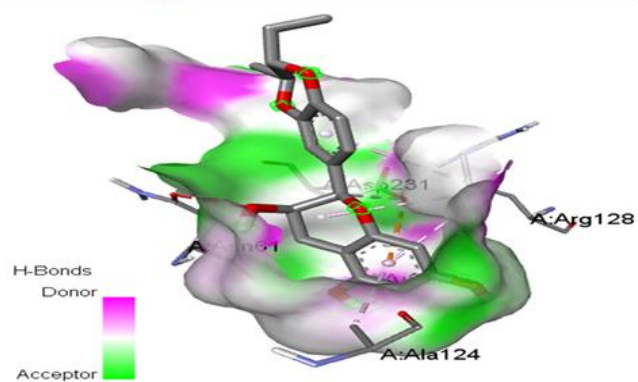
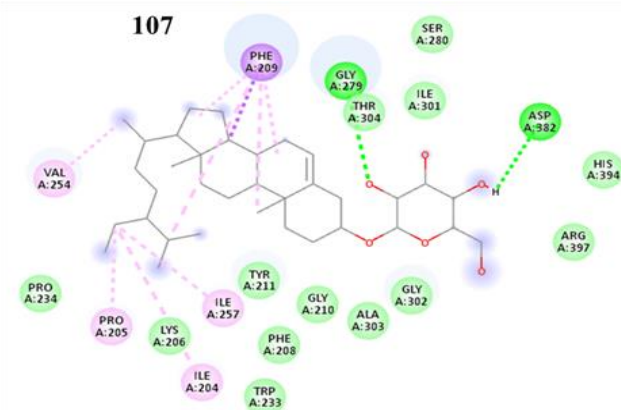


Appendix 22: continuous

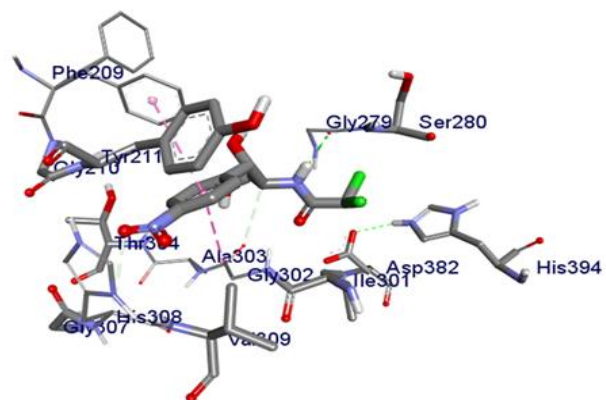
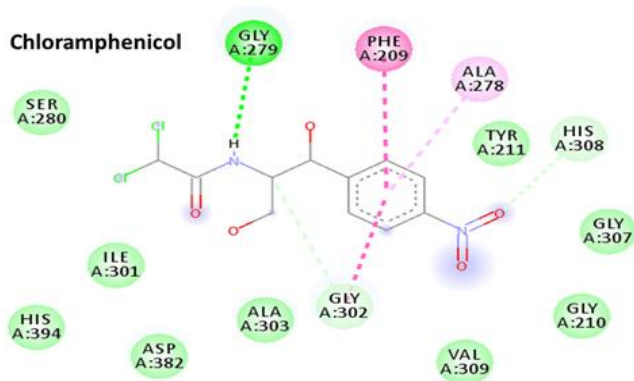
106



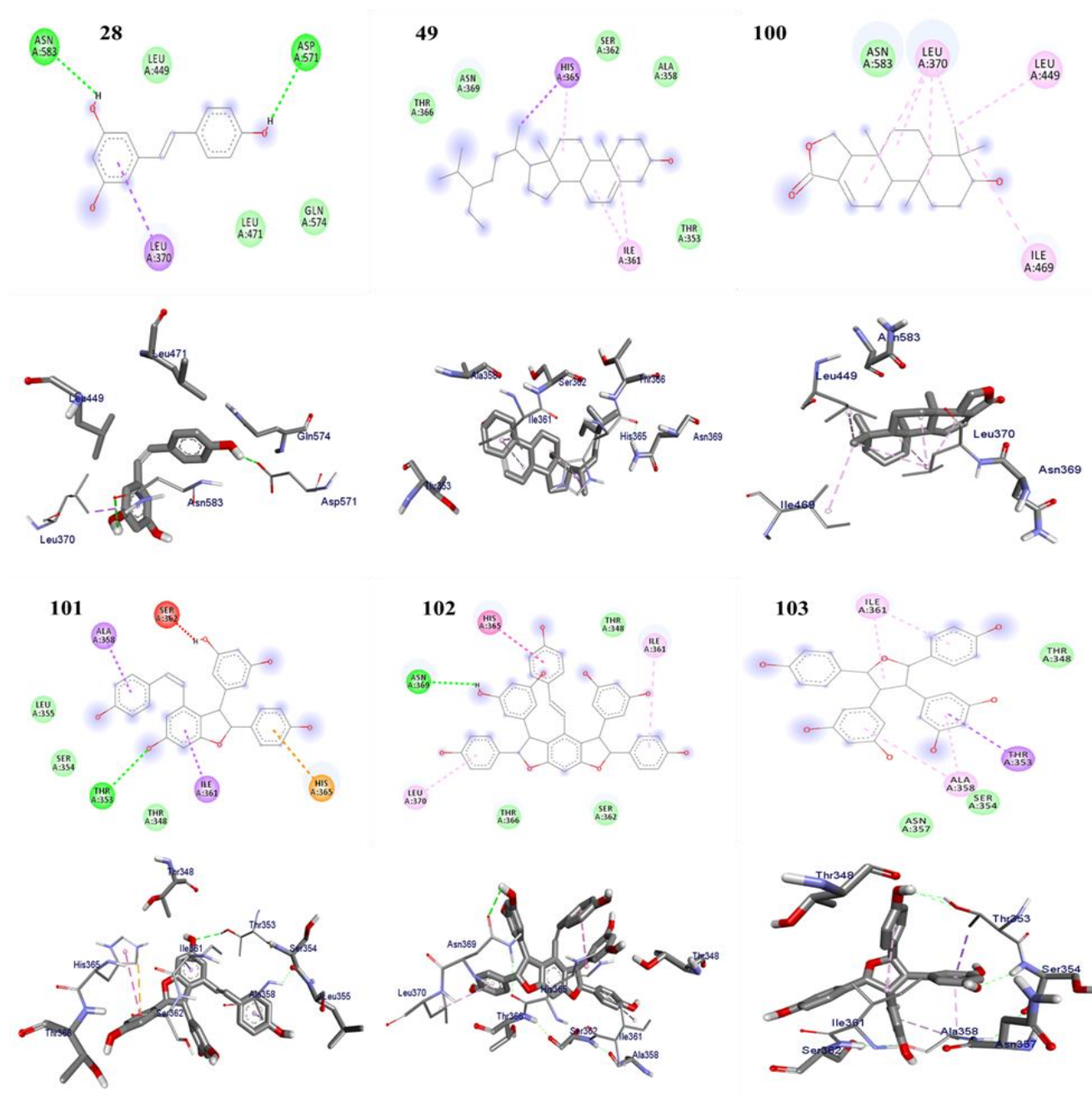
107



Chloramphenicol



Appendix 23A: The 2D and 3D binding interactions of compounds **28**, **49**, **100**, **101**, **102**, **103**, **106** and **107**, and standard against *S. aureus* PK (PDB ID: 3T07).



Appendix 23: continuous

