

**ISOLATION AND CHARACTERIZATION OF
CYTOTOXIC CONSTITUENTS FROM SOME
VOLATILE OIL-CONTAINING MEDICINAL
PLANTS**

BY

**ROSE OSARIEME IMADE
PG\PHA1120161**

**DEPARTMENT OF PHARMACOGNOSY
FACULTY OF PHARMACY
UNIVERSITY OF BENIN
BENIN CITY**

AUGUST, 2021.

**ISOLATION AND CHARACTERIZATION OF
CYTOTOXIC CONSTITUENTS FROM SOME
VOLATILE OIL-CONTAINING MEDICINAL
PLANTS**

BY

**ROSE OSARIEME IMADE
PG\PHA1120161**

**A THESIS WRITTEN IN THE DEPARTMENT OF
PHARMACOGNOSY AND SUBMITTED TO THE
SCHOOL OF POSTGRADUATE STUDIES IN
PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE AWARD OF DOCTOR OF
PHILOSOPHY DEGREE OF THE UNIVERSITY OF
BENIN, BENIN CITY, NIGERIA**

AUGUST, 2021.

CERTIFICATION

This is to certify that this work was carried out by Mrs Rose Osarieme **IMADE** in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria.

Prof. B. A. Ayinde

(Supervisor)

Date

Dr (Mrs) J. O. Ofeimun

(Head of Department)

Date

CERTIFICATION OF THESIS ON PLAGIARISM

We certify that this thesis has undergone the plagiarism test and met the necessary requirement.

Prof. B. A. Ayinde

(Supervisor)

Date

Dr (Mrs) J. O. Ofeimun

(Head of Department)

Date

DEDICATION

I dedicate this work to God Almighty who causes me to triumph always. To Him be all the praise forever.

ACKNOWLEDGEMENT

I am grateful to God for His grace upon my life and for seeing me through this academic journey.

I thank my supervisor Prof. B. A. Ayinde for the privilege of being tutored by him. Your humility, concern and encouragement throughout the course of this research work was really amazing. You truly have mentored me, not only in my study but in other areas of life as well. May God bless you richly sir.

I also want to thank Prof. M. I. Choudhary, Director of the International Centre for Chemical and Biological Sciences (ICCBS), for his support during my three months stay at the centre and for providing me with state of the art facilities and a conducive environment for my research.

My sincere gratitude goes to all the laboratory staff of Pharmacognosy Department, especially Mr Kingsley Ugwu, for his kind assistance in the procurement and extraction of some of my plants.

I am forever grateful to my wonderful husband and children for their patience and sacrifice especially when I was away in Pakistan, I love you all dearly. My appreciation goes to my parents, Mr and Mrs Jesuorobo Idugboe for all their prayers as well as my siblings for their financial and moral support. God bless you all.

TABLE OF CONTENT

CONTENT	PAGE
TITLE PAGE-----	i
DEGREE PAGE-----	ii
CERTIFICATION-----	iii
CERTIFICATION OF THESIS ON PLAGIARISM -----	iv
DEDICATION-----	v
ACKNOWLEDGEMENT-----	vi
TABLE OF CONTENT-----	vii
LIST OF FIGURES-----	xiv
LIST OF TABLES-----	xvii
LIST OF ABBREVIATIONS-----	xix
ABSTRACT-----	xx
 CHAPTER ONE	 1
1.0 INTRODUCTION AND LITERATURE REVIEW-----	1
1.1.1 Pathogenesis of cancer-----	2
1.1.2 Genesis of a cancer cell-----	2
1.1.3 Special characteristics of cancer cells-----	3
1.2 Etiology of cancer-----	4
1.2.1 Environmental factors-----	4
1.2.2 Genetic factors-----	5
1.2.3 Oxidative stress-----	5

1.2.4	Hormonal and immune imbalance-----	6
1.3	Seven strategies for cancer inhibition-----	6
1.4	Some bench-top antitumor bioassay screening methods-----	8
1.5	Assays used <i>in vitro</i> to study cancer cell lines-----	10
1.6	Classes of secondary metabolites with antitumor activities-----	15
1.6.1	Alkaloids-----	15
1.6.2	Terpenes-----	19
1.6.3	Lignans-----	23
1.7	Essential oils as anticancer agents-----	24
1.8	The family Zingiberaceae-----	28
1.8.1	Genus <i>Zingiber</i> -----	29
1.8.1.1	<i>Zingiber officinale</i> Roscoe-----	29
1.9	The family Lamiaceae-----	35
1.9.1	Genus <i>Hyptis</i> -----	36
1.9.1.1	<i>Hyptis suaveolens</i> -----	36
1.9.2	Genus <i>Ocimum</i> -----	40
1.9.2.1	<i>Ocimum basilicum</i> -----	40
1.9.2.2	<i>Ocimum gratissimum</i> -----	45
1.10	The family Myrtaceae-----	49
1.10.1	Genus <i>Eugenia</i> -----	49
1.10.1.1	<i>Eugenia caryophyllus</i> Thunberg-----	50
1.10.2	Genus <i>Callistemon</i> -----	54
1.10.2.1	<i>Callistemon citrinus</i> -----	54
1.11	The family Mimosaceae-----	58
1.11.1	Genus <i>Tetrapleura</i> -----	58
1.11.1.1	<i>Tetrapleura tetraptera</i> Taubert-----	58
1.12	Rationale for the study-----	63

1.13	Aim and objectives-----	64
CHAPTER TWO		65
2.0	MATERIALS AND METHODS-----	65
2.1	Materials-----	65
2.1.1	Chemicals and reagents-----	65
2.1.2	Solvents-----	65
2.1.3	Consumables-----	65
2.1.4	Equipments-----	65
2.2	Collection and identification of plant material-----	66
2.3	Extraction of plant materials-----	66
2.4	Preliminary Biological Screening of Extracts-----	67
2.4.1	Determination of growth inhibitory effects on <i>Sorghum bicolor</i> radicle-----	67
2.4.2	Determination of cytotoxic effects using tadpoles (<i>Raniceps ranninus</i>) -----	67
2.4.3	Determination of cytotoxic effects using brine shrimps (<i>Artemia salina</i>) -----	68
2.5	Determination of Anticancer Activity using Cancer Cell Lines-----	68
2.5.1	Growth and maintenance of cells-----	69
2.5.2	Revival of cancer cell lines-----	69
2.5.3	Sub-culturing of cancer cells-----	69
2.5.4	Cell viability, concentration and culturing-----	69
2.5.5	Preparation of extracts, fractions and compounds for cytotoxicity assay-----	70
2.5.6	Anticancer activity using MTT-----	70
2.6	Column chromatography of <i>O. basilicum</i> volatile oil-----	71

2.6.1	Growth inhibitory and cytotoxic effects of the column chromatography fractions of <i>O. basilicum</i> volatile oil-----	73
2.6.2	Preparative TLC of fraction CF ₁ B and bioassay of its subfractions-----	73
2.7	Column chromatography of <i>O. gratissimum</i> volatile oil-----	75
2.7.1	Growth inhibitory and cytotoxic effects of the column chromatography fractions of <i>O. gratissimum</i> volatile oil-----	77
2.7.2	Preparative TLC of fraction CF ₁ G and bioassay of its subfractions-----	77
2.8	Column chromatography of <i>E. caryophyllus</i> volatile oil-----	79
2.8.1	Growth inhibitory and cytotoxic effects of the column chromatography fractions of <i>E. caryophyllus</i> volatile oil-----	81
2.8.2	Preparative TLC of fraction CF ₁ E and bioassay of its subfractions-----	81
2.9	Column chromatography of <i>C. citrinus</i> volatile oil-----	83
2.9.1	Growth inhibitory and cytotoxic effects of the column chromatography fractions of <i>C. citrinus</i> volatile oil-----	85
2.10	GC-MS analysis-----	85
2.11	Organic solvent partitioning of the methanol extract of <i>T. tetraptera</i> pod-----	85
2.11.1	Growth inhibitory and cytotoxic effects of the solvent fractions of <i>T. tetraptera</i> Pod-----	86
2.12	Vacuum liquid chromatography (VLC) of the chloroform fraction of <i>T. tetraptera</i> Pod-----	86
2.13	Column chromatography of bulked VLC fractions C and D and anticancer activity of the resulting sub-fractions-----	88
2.13.1	Preparative high pressure liquid chromatography of bulked subfraction C2-----	90

2.14	Column chromatography of bulked VLC fractions F and G-----	90
2.14.1	Preparative high pressure liquid chromatography of bulked subfraction F11-----	92
2.15	Statistical analysis-----	92
CHAPTER THREE		99
3.0	RESULTS-----	99
3.1	Yields of the plants essential oils/extract-----	99
3.2	Results of the growth inhibitory effects of the essential oils/extracts on radicle length of <i>S. bicolor</i> seeds-----	101
3.3	Result of the cytotoxic effects of the volatile oils/extract on tadpoles-----	108
3.4	Results of cytotoxicity assay on Brine Shrimp (<i>Artemia salina</i>) -----	110
3.5	Results of growth inhibitory effects of the volatile oils and extract on AU 565 cell line-----	112
3.6	Results of growth inhibitory effects of the volatile oils and extract on HeLa cell line-----	114
3.7	Result of GC-MS Analysis of <i>Z. officinale</i> essential oil-----	116
3.8	Result of GC-MS Analysis of <i>H. suaveolens</i> essential oil-----	118
3.9	Result of GC-MS Analysis of <i>O. basilicum</i> essential oil-----	120
3.10	Result of GC-MS analysis of <i>O. gratissimum</i> essential oil-----	122
3.11	Result of GC-MS analysis of <i>E. caryophyllus</i> essential oil-----	124
3.12	Result of GC-MS analysis of <i>C. citrinus</i> essential oil-----	126
3.13	Result of column chromatography of <i>O. basilicum</i> oil-----	128
3.14	Result of the growth inhibitory effects of the column fractions and subfractions of <i>O. basilicum</i> oil-----	130
3.15	Result of Inhibition effects of <i>O. basilicum</i> oil column fractions and preparative TLC sub-fractions on AU 565 and HeLa cell lines-----	132
3.16	Result of GC-MS analysis of preparative TLC sub-fraction SF ₂ B from <i>O.</i>	

	<i>basilicum</i> essential oil-----	134
3.17	Result of column chromatography of <i>O. gratissimum</i> oil-----	136
3.18	Result of the growth inhibitory effects of the column fractions and subfractions of <i>O. gratissimum</i> oil-----	138
3.19	Result of Inhibition effects of <i>O. gratissimum</i> oil column fractions and preparative TLC sub-fractions on AU 565 and HeLa cell lines-----	140
3.20	Result of GC-MS analysis of preparative TLC fraction SF ₂ G from <i>O.</i> <i>gratissimum</i> essential oil-----	142
3.21	Result of column chromatography of <i>E. caryophyllus</i> oil-----	144
3.22	Result of the growth inhibitory effects of the column fractions and subfractions of <i>E. caryophyllus</i> oil-----	146
3.23	Result of Inhibition effects of <i>E. caryophyllus</i> oil column fractions and preparative TLC sub-fractions on AU 565 and HeLa cell lines-----	148
3.24	Result of GC-MS analysis of preparative TLC fraction SF ₂ E from <i>E.</i> <i>caryophyllus</i> essential oil-----	150
3.25	Result of column chromatography of <i>C. citrinus</i> oil-----	152
3.26	Result of the growth inhibitory effects of the column fractions of <i>C. citrinus</i> oil----	154
3.27	Result of inhibition effects of <i>C. citrinus</i> oil column fractions on AU 565-----	156
3.28	Result of GC-MS analysis of CF ₁ C from <i>C. citrinus</i> essential oil-----	158
3.29	Result of the growth inhibitory effects of the solvent fractions of <i>T. tetraptera</i> pod extract-----	160
3.30	Result of inhibition effects of the solvent fractions on AU 565 cell line-----	162
3.31	Result of the growth inhibitory effects of the VLC fractions of <i>T. tetraptera</i> pod chloroform fraction-----	164
3.32	Result of inhibition effects of VLC fractions on AU 565 cell line-----	166

3.33	Result of inhibition effects of column subfractions from VLC fractions C and D on AU 565 cell line-----	168
3.34	Result of preparative HPLC of C2* subfractions (21-34) -----	170
3.35	Result of column chromatographic fractions of VLC fractions F and G-----	179
3.36	Result of reverse phase preparative HPLC of bulked fraction F11 obtained from column chromatography of F and G -----	181
3.37	Result of the inhibition/stimulation effects of TT2 and TRA on breast cancer cell lines-----	190
CHAPTER FOUR		192
4.1	DISCUSSION-----	192
CHAPTER FIVE		205
5.1	CONCLUSION-----	205
5.2	CONTRIBUTIONS TO KNOWLEDGE-----	206
REFERENCES-----		207
APPENDIX-----		228

LIST OF FIGURES

FIGURES	PAGE
Figure 1.1: Picture of the rhizome of <i>Zingiber officinale</i> Roscoe (Zingiberaceae) -----	31
Figure 1.2: Picture of <i>Hyptis suaveolens</i> Linn. (Lamiaceae) plant-----	37
Figure 1.3: Picture of <i>Ocimum basilicum</i> Linn. (Lamiaceae) plant-----	42
Figure 1.4: Picture of <i>Ocimum gratissimum</i> Linn. (Lamiaceae) plant-----	46
Figure 1.5: Picture of the flower buds of <i>Eugenia caryophyllus</i> (Myrtaceae) -----	51
Figure 1.6: Picture of <i>Callistemon citrinus</i> (Myrtaceae) shrub-----	55
Figure 1.7: Picture of the pod of <i>Tetrapleura tetraptera</i> (Mimosaceae) -----	60
Figure 3.1: Antiproliferative effect of the volatile oils/extract on the radicle length of <i>S. bicolor</i> seeds at 24 hours-----	102
Figure 3.2: Antiproliferative effect of the volatile oils/extract on the radicle length of <i>S. bicolor</i> seeds at 48 hours-----	103
Figure 3.3: Antiproliferative effect of the volatile oils/extract on the radicle length of <i>S. bicolor</i> seeds at 72 hours-----	104
Figure 3.4: Antiproliferative effect of the volatile oils/extract on the radical length of <i>S. bicolor</i> seeds at 96 hours-----	105
Figure 3.5: Effect of some of the volatile oils on the growth of <i>S. bicolor</i> seeds at 48 h---	106
Figure 3.6: Effect of some of the volatile oils on the growth of <i>S. bicolor</i> seeds at 48 h continued-----	107
Figure 3.7: Cytotoxic effects of the volatile oils and extract on tadpoles-----	109
Figure 3.8: Chromatogram showing the column fractions of <i>O. basilicum</i> oil before (1) and after (2) bulking-----	129

Figure 3.9: Chromatogram showing the preparative TLC fractions SF ₁ B and SF ₂ B of <i>O. basilicum</i> oil obtained from F1-----	129
Figure 3.10: Antiproliferative effect of <i>O. basilicum</i> oil column fractions and subfractions on the radical length of <i>S. bicolor</i> seeds-----	131
Figure 3.11: Chromatogram showing the column fractions of <i>O. gratissimum</i> oil before (1) and after (2) bulking-----	137
Figure 3.12: Chromatogram showing the preparative TLC fractions SF ₁ G and SF ₂ G of <i>O. gratissimum</i> oil obtained from F1-----	137
Figure 3.13: Antiproliferative effect of <i>O. gratissimum</i> oil column fractions and subfractions on the radical length of <i>S. bicolor</i> seeds-----	139
Figure 3.14: Chromatogram showing the column fractions of <i>E. caryophyllus</i> oil before (1) and after (2) bulking-----	145
Figure 3.15: Chromatogram showing preparative TLC fractions SF ₁ E and SF ₂ E of <i>E. caryophyllus</i> oil obtained from F1-----	145
Figure 3.16: Antiproliferative effect of <i>E. caryophyllus</i> oil column fractions and subfractions on the radicle length of <i>S. bicolor</i> seeds-----	147
Figure 3.17: Chromatogram showing the column fractions of <i>C. citrinus</i> oil before (1) and after (2) bulking-----	153
Figure 3.18: Antiproliferative effect of <i>C. citrinus</i> oil column fractions on the radicle length of <i>S. bicolor</i> seeds-----	155
Figure 3.19: Antiproliferative effect of the solvent fractions of <i>T. tetraptera</i> pod on the radical length of <i>S. bicolor</i> seeds at 5 mg/mL concentration-----	161
Figure 3.20: Antiproliferative effect of the VLC fractions of the chloroform fraction of <i>T. tetraptera</i> pod on the radical length of <i>S. bicolor</i> seeds-----	165
Figure 3.21: Chromatogram showing preparative HPLC fractions 12 and 21 obtained from column fraction C2*-----	171

Figure 3.22: Mass Spectral data of Compound TT2 (22-hydroxyisohopane) -----	172
Figure 3.23: ¹ HNMR Spectral data of Compound TT2 (22-hydroxyisohopane) -----	173
Figure 3.24: ¹³ C Spectral data (BB) of Compound TT2 (22-hydroxyisohopane) -----	174
Figure 3.25: ¹³ C Spectral data (DEPT 135) of Compound TT2 (22-hydroxyisohopane) ---	175
Figure 3.26: ¹³ C Spectral data (DEPT 90) of Compound TT2 (22-hydroxyisohopane) -----	176
Figure 3.27: Structure of 2-(5a,5b,8,8,11a,13b-Hexamethyl-icosahydro-cyclopenta[a] chrysen-3-yl)-propan-2-ol (22-hydroxyisohopane) -----	178
Figure 3.28: Chromatogram showing the column fractions of <i>T. tetraptera pod</i> obtained from VLC fraction F and G-----	180
Figure 3.29: Chromatogram showing preparative HPLC fractions 5 and 6 obtained from column fraction F11*-----	182
Figure 3.30: Mass Spectral data of Compound TRA (Aridanin) -----	183
Figure 3.31: ¹ HNMR Spectral data of Compound TRA (Aridanin) -----	184
Figure 3.32: ¹³ C Spectral data (BB) of Compound TRA (Aridanin) -----	185
Figure 3.33: ¹³ C Spectral data (DEPT 135) of Compound TRA (Aridanin) -----	186
Figure 3.34: ¹³ C Spectral data (DEPT 90) of Compound TRA (Aridanin) -----	187
Figure 3.35: Structure of Compound TRA (Aridanin) -----	189

LIST OF TABLES

TABLE	PAGE
Table 1.1: Chemical composition and concentrations of compounds present in ginger essential oil-----	34
Table 1.2: Chemical composition of the essential oil of <i>H. suaveolens</i> -----	39
Table 1.3: Chemical composition of <i>O. basilicum</i> L. oil-----	44
Table 1.4: Chemical composition of the essential oil of <i>O. gratissimum</i> -----	48
Table 1.5: Chemical composition of <i>Eugenia caryophyllus</i> essential oil-----	52
Table 1.6: Chemical composition of <i>C. citrinus</i> essential oil-----	57
Table 2.1: Column chromatography fractions of <i>O. basilicum</i> volatile oil-----	72
Table 2.2: Preparative TLC subfractions of <i>O. basilicum</i> volatile oil-----	74
Table 2.3: Column chromatography fractions of <i>O. gratissimum</i> volatile oil-----	76
Table 2.4: Preparative TLC subfractions of <i>O. gratissimum</i> volatile oil-----	78
Table 2.5: Column chromatography fractions of <i>E. caryophyllus</i> volatile oil-----	80
Table 2.6: Preparative TLC subfractions of <i>E. caryophyllus</i> volatile oil-----	82
Table 2.7: Column chromatography fractions of <i>C. citrinus</i> volatile oil-----	84
Table 2.8: The VLC subfractions of the chloroform fraction of <i>T. tetraptera</i> pod-----	87
Table 2.9: Column chromatography subfractions from VLC fractions C and D-----	89
Table 2.10: Column chromatography subfractions from bulked VLC fractions F and G---	91
Table 3.1: Percentage yield of the extracts-----	100
Table 3.2: Cytotoxic effects of the extracts against <i>Artemia salina</i> nauplii -----	111
Table 3.3: Inhibition/stimulation effects of the volatile oils and extract on AU 565 cell line-----	113
Table 3.4: Inhibition/stimulation effects of the volatile oils on HeLa cell line-----	115
Table 3.5: GC-MS Analysis of volatile composition of essential oil of <i>Z. officinale</i> -----	117
Table 3.6: GC-MS Analysis of volatile composition of essential oil of <i>H. suaveolens</i> -----	119

Table 3.7: GC-MS Analysis of volatile composition of essential oil of <i>O. basilicum</i> -----	121
Table 3.8: GC-MS Analysis of volatile composition of essential oil of <i>O. gratissimum</i> -----	123
Table 3.9: GC-MS Analysis of <i>E. caryophyllus</i> volatile oil-----	125
Table 3.10: GC-MS Analysis of <i>C. citrinus</i> volatile oil-----	127
Table 3.11 Inhibition/stimulation effects of <i>O. basilicum</i> oil column fractions and sub-fractions on AU 565 and HeLa cell lines at 50 µg/mL-----	133
Table 3.12: GC-MS Analysis of SF ₂ B-----	135
Table 3.13 Inhibition/stimulation effects of <i>O. gratissimum</i> oil column fractions and sub-fraction on AU 565 and HeLa cell lines at 50 µg/mL-----	141
Table 3.14: GC-MS Analysis of SF ₂ G-----	143
Table 3.15 Inhibition/stimulation effects of <i>E. caryophyllus</i> oil column fractions and sub-fraction on AU 565 and HeLa cell lines at 50 µg/mL-----	149
Table 3.16: GC-MS Analysis of SF ₂ E-----	151
Table 3.17 Inhibition/stimulation effects of <i>C. citrinus</i> oil column fractions on AU 565 cell line-----	157
Table 3.18: GC-MS Analysis of CF ₁ C-----	159
Table 3.19 Inhibition/stimulation effects of the solvent fractions on AU 565 cell line-----	163
Table 3.20 Inhibition/stimulation effects of the VLC fractions on AU 565 cell line-----	167
Table 3.21: Inhibition/stimulation effects of column chromatography subfractions from VLC fractions C and D on AU 565 cell line-----	169
Table 3.22: NMR Data of Compound TT2 in DMSO-----	177
Table 3.23: NMR Data of Compound TRA in DMSO-----	188
Table 3.24 Inhibition/stimulation effects of TT2 and TRA on breast cancer cell lines-----	191

LIST OF ABBREVIATIONS

ATCC	American type culture collection
ATP	Adenosine triphosphate
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
LDH	Lactate dehydrogenase
mRNA	messenger Ribonucleic acid
MTT	3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
qPCR	Quantitative Polymerase Chain Reaction
RFLP	Restricted fragment length polymorphism
ROS	Reactive oxygen species
RPMI	Roswell Park Memorial Institute
SDS PAGE	Sodium Do-decyl sulphate Poly Acrylamide Gel Electrophoresis
VEGF	Vascular endothelial growth factor

ABSTRACT

Plants have been recognized for their lifesaving and therapeutic properties. Various forms of cancer are prevalent in the society. *Zingiber officinale* (rhizome), *Eugenia caryophyllus* (flower), *Callistemon citrinus* (leaves), *Hyptis suaveolens* (leaves), *Ocimum basilicum* (leaves), *Ocimum gratissimum* (leaves) as well as *Tetrapleura tetraptera* pod are used in ethnomedicine in treating tumor-related ailments. This study was undertaken to evaluate the cytotoxic and growth inhibitory effects of these plants using predictive and confirmatory tests on cancer cell lines.

Preliminary screening of the essential oils of *Z. officinale* (rhizome), *E. caryophyllus* (flower), *C. citrinus* (leaves), *H. suaveolens* (leaves), *O. basilicum* (leaves), *O. gratissimum* (leaves) and *T. tetraptera* pod methanol extract was carried out using bench-top assay methods for cytotoxicity involving the use of tadpoles of *Raniceps ranninus* (10-40 µg/mL) and brine shrimp of *Artemia salina* (10-1000 µg/mL), and antiproliferation using radicle of *Sorghum bicolor* seeds (1-30 mg/mL). The essential oils were further tested on breast cancer (AU 565) and cervical cancer (HeLa) cell lines at 50 µg/mL. Essential oils of *E. caryophyllus*, *O. basilicum* and *O. gratissimum* were subjected to column chromatography and preparative TLC and the resulting fractions were also tested. *T. tetraptera* extract was partitioned into hexane, chloroform and aqueous fractions which were tested against AU 565. Vacuum liquid chromatography and other chromatographic techniques employed in the evaluation of the chloroform fraction led to the isolation of two compounds which were also tested for their effects on AU 565 and MDA-MB231 at 50 µM. The essential oils and their respective isolates were subjected to GCMS while NMR and Mass spectrometric analysis were carried out on the isolates obtained from the extract.

The results of the preliminary screening of the essential oils and extract showed a concentration dependent activity. Similar activities were also observed with the tadpole and brine shrimp mortality assays. *H. suaveolens* and *Z. officinale* oils showed the highest activity against AU 565 cells with 86.74 and 43% growth inhibitions respectively. The chromatographic procedures on the essential oils resulted in isolates with higher activities. GCMS revealed them to be thymol from *O. gratissimum* and eugenol from *E. caryophyllus* which produced +85.07 % and +18.82 % inhibitions against AU 565 cells respectively, as well as estragole from *O. basilicum* which gave +89.3 % inhibition against HeLa cells. The partitioning and isolation procedures carried out on *T. tetraptera* pod extract resulted in the isolation of two compounds. NMR and MS revealed the

compounds to be 22-hydroxyisohopane and (3-O-[β -D-glucopyranosyl-2'-acetamido-2'- deoxy]-oleanolic acid with 14.36 and 19 % inhibitions against breast cancer cell line respectively.

These results have shown the potency and justified the probable use of these plants in ethnomedicine in treating tumor-related ailments.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

The knowledge and use of plant-founded drugs all over the world is increasing. The African continent has an avalanche of food plants used for therapeutic purposes. There are reports of advances made in western medicine but there are still a large number of diseases for which standard drugs are yet to be derived. This has brought a pressing need to develop safer drugs for the cure of these diseases. Through researches on medicinal plants, there has been great development in the pharmacological assessment of various plants used in traditional systems of medicine. As a result, plants have been portrayed as a major source of medicines, not just as isolated active compounds which are formulated and dispensed in standardized dosage form but also as crude drugs (Adaikan *et al.*, 2001).

Presently, the goal of modern phytochemical research is to formulate preparations developed from medicinal plants that would meet present day standards of quality, safety, and efficacy. Not too long, plant essential oils have gained more recognition in phytomedicine (Buckle, 1999). Their widespread use has captured the attention and curiosity of scientists in basic research of essential oils; their potential anti-convulsant, anti-oxidant and anti-cancer activity have been evaluated recently (Mimica-Dukic, 2004).

Cancer is a disease of worldwide importance as it is one of the principal causes of death. It is a disease caused by division of cells not subject to control which leads to abnormal growth of tissue (Vogelstein and Kinzler, 2002). Currently, over a hundred types of cancers are known

differentiated by etiology and natural history. Among different cancers, breast and cervical cancers are the most prevalent malignant diseases in women (Jedi-Agba *et al.*, 2012).

Anticancer activity is the result of using biological and chemical or natural and synthetic agents to reverse, subdue or stop carcinogenic progression.

Several synthetic agents used to treat the disease kill normal cells along with the tumor cells and also have other severe side effects (Cragg *et al.*, 2009). There is therefore a pressing need for new treatment options with better effects.

Naturally occurring chemopreventive agents can induce apoptosis of cancer cells without side effects and drugs derived from them are very useful for the treatment of cancer (Musika and Indrapichate, 2014). Vinblastine and vincristine which are plant compounds, have been demonstrated to have therapeutic effects against Hodgkin's lymphoma and non-Hodgkin's lymphoma respectively while topotecan and irinotecan obtained from *Camptotheca acuminata* have been used for lung and ovarian cancers (Cragg and Newman, 2005a).

1.1 Pathogenesis of cancer

Cancer can be described as an evolutionary process that comes from the accumulation of somatic mutation in the progeny of a normal cell, leading to a discriminatory growth advantage in the altered cells and ultimately to uncontrolled increase and spread of these cells (Merlo *et al.*, 2006; Greaves and Marley, 2012).

1.1.1 Genesis of a cancer cell

Epithelial tissues such as the skin, breast, colon, lungs and prostate are the most common human cancers spring up and collectively lead to several million deaths per year (Ferlay, 2010).

Normal healthy cells turn into cancer cells because of one or more alterations in their DNA, which can be hereditary or acquired. The development of cancer entails multiple steps, involving more than one genetic stage as well as other epigenetic factors such as hormonal action, co-carcinogen, and tumor promoter effects (Rang *et al.*, 2003).

The main types of genetic changes that can lead to development of cancer cells are:

1. The activation of proto-oncogenes to oncogenes: Proto-oncogenes are genes that normally control cell division, apoptosis and differentiation but which can be changed to oncogenes that induce malignancy by viral or carcinogen action.
2. Inactivation of tumour suppressor genes: Normal cells have genes with the ability to prohibit malignant change - termed tumour suppressor genes (anti-oncogenes). The loss of function of tumour suppressor genes can be the main cause of carcinogenesis (Rang *et al.*, 2003).

1.1.2 Special characteristics of cancer cells

These include:

a. Uncontrolled proliferation

The proliferation of cancer cells do not go through the normal processes that regulate cell division and tissue growth. This distinguishes them from normal cells. Nonfunctionality of tumor suppressor genes or change of proto-oncogenes into oncogenes removes restrictions which results in uncontrolled proliferation through changes in:

- I. Growth factors and their receptors
- II. The growth factor pathways e.g. the cytosolic and nuclear transducers
- III. The cell cycle transducers e.g. cyclins, cyclin-dependent kinases (cdks) or the cdk inhibitors

- IV. The apoptotic mechanisms that normally dispose of abnormal cells
- V. Telomerase expression
- VI. Local blood vessels, resulting from tumor-directed angiogenesis (Rang *et al.*, 2003).

b. Dedifferentiation and loss of function

The increase of normal cells involves division of the stem cells in a specific tissue leading to the formation of daughter cells. These daughter cells differentiate and become the mature cells of the relevant tissue and are able to carry out their programmed function. Cancer cells on the other hand dedifferentiate to varying degrees in different tumors (Rang *et al.*, 2003).

c. Invasiveness

Cancer cells through mutation, have lost the restraints that act on normal cells and also possess the ability to break down the extracellular matrix by secreting enzymes, thus enabling the cancer cells to pass through. A cancer of the rectal mucosa for example, can invade the tissues in the other layers of the rectum as well as other pelvic organs (Rang *et al.*, 2003).

d. Metastases

Cancer cells are able to metastasise due to some genetic changes which alter their responses to the regulatory factors that control the incumbent position of normal cells and tissues, thus enabling them to establish themselves in other parts of the body. Production of new blood vessels can be induced by these tumors thus making metastasis easier (Rang *et al.*, 2003).

1.2 Etiology of cancer

This refers to the factors that cause cancer and they are described as follows:

1.2.1 Environmental factors

The environmental factors include:

a. Radiations

The cumulative ultraviolet waves (100-400 nm) from solar system may lead to skin cancers (Berman and Cockerell, 2013). The ionizing and gamma radiations also act as carcinogen inducing DNA damages (Kadam *et al.*, 2013).

b. Chemical carcinogen

Tobacco smoking is strongly associated with cancer of the lungs (Baykara *et al.*, 2013) and is also involved in the etiology of other types of cancer like cancer of the pharynx, mouth, bladder and kidney (Sasco *et al.* 2014). Other chemical carcinogens are arsenic (Cooper *et al.*, 2013), asbestos, and aflatoxin B₁ (Palliyaguru and Wu, 2013).

c. Infections

The International Agency for Research on Cancer reported that there were about 2 million cancers globally in 2008 attributed to human papilloma virus, hepatitis virus and *Helicobacter pylori* bacteria (Khorasanizadeh *et al.*, 2013).

1.2.2 Genetic factors

There are several mutations required for development of a cancer (Burns *et al.*, 2013). Abnormal genetic manifestations in which DNA mutations accumulated were estimated to be about 300 of 30,000 human chromosomal genes. Mutations in as much as 1% of genes of total human genome may lead to the formation of different types of cancers (Futreal *et al.*, 1994). Hereditary mutations account for up to 5% of breast cancer in patients (Oluwagbemiga *et al.*, 2012).

1.2.3 Oxidative stress

During oxidative stress antioxidant cellular defense system is inefficient to inactivate reactive oxygen species (ROS) or there is excessive ROS production in cell or both (Manda *et al.*, 2009). Increased levels of ROS have been linked with cancer (Kobayashi and Suda, 2012). Cancer cells isolated from leukemia patients expressed high superoxide anion generation compared to normal blood lymphocytes (Zhou *et al.*, 2003). ROS promote metastasis and regulate positively the metastatic potential in cancer cells (Lim *et al.*, 2005). In cancer cells, ROS stimulates genetic instability and mutations associated with oncogene activation like RAS and c-MYC (Gibellini *et al.*, 2010).

1.2.4 Hormonal and immune imbalance

The most prevalent cancer in females is endometrial cancer associated with lower cumulative exposure to estrogen and/or higher exposure to progesterone (Dossus *et al.*, 2010). Although thyroid hormones are required for normal growth, the development and metabolism, especially during early childhood, with higher levels are associated with risk for colon, lung, prostate, and breast cancers (Moeller and Fuhrer, 2013). The human immunodeficiency virus (HIV) infection and patients with transplant are the examples of immune system dysfunction and are linked with cancer occurrence. Increased risk of epidemiology of HIV-infected population with lung cancer has been reported (Winston *et al.*, 2013).

1.3 Seven strategies for cancer inhibition

Strategies for cancer inhibition are dependent on the pathophysiology of cancer cells. The procancer events provide the targets for the anticancer strategies. The best results will be seen

when all are used together. Some natural compounds can be used to carry out each of these seven strategies. The seven strategies (Boik, 2001) are:

a. Reduce genetic instability

Genetic instability is worsened by oxidative stress. Cancer cells exist in an oxidative environment, and although some cells are killed in such an environment, many still continue to survive. As oxidative stress becomes elevated, the declining population of surviving cells become more unstable which leads to higher mutation rates. This eventually produces more aggressive and successful cancers. Thus one of the strategies of reducing genetic instability and in theory, cancer production is by reducing oxidative stress.

b. Prevent abnormal expression of genes

Genes that prevent cancer progression are usually under-expressed in cancer cells, and genes that promote cancer are commonly over-expressed. Therefore, cancer can be restrained by ensuring that the operation of those transcription factors that control the expression of these genes is normal.

c. Inhibit abnormal signal transduction

Signal transduction is a normal process required by healthy cells, but in cancer cells the amount is excessive, and the signals that flow increase proliferation and spread. The movement of a signal from outside the cell toward the nucleus depends on several proteins (including kinase enzymes and ras proteins), and so signal transduction can be prevented by blocking the actions of these proteins therefore bringing it down to the required levels.

d. Fostering normal cell-to-cell communication.

Normal cell-to-cell communication can be encouraged by improving gap junction communication and by normalizing cell adhesion molecules (CAM) activity.

e. Inhibit tumor angiogenesis

Angiogenesis is a normal process which is needed during wound healing but in tumors it is uncontrolled and further promoted if certain chemicals called angiogenic factors as well as certain environmental conditions e.g. hypoxic conditions are present. Cancer can be subdued by blocking the release or activity of angiogenic factors or by making the environmental condition unfavourable and so inhibit tumor angiogenesis.

f. Inhibit invasion and metastasis

In invasion, there is enzymatic digestion of healthy tissues around the tumor. It also involves the movement of tumor cells from the primary site. One can reduce invasion by preventing the digestion of local tissues through inhibition of the enzymes responsible. Normal tissues can also be protected from the enzymes as well as reducing the rate of migration of the infected cells. Metastasis involves the detachment of cells from the primary tumor. These cells enzymatically digest blood vessel walls to gain entry into and exit from the blood circulation, and while there, evade the immune system. Metastasis can therefore be prevented by keeping any one of these processes in check.

g. Increase the immune response

The body's immunity can be increased against cancer cells by stimulating the immune system and by reducing the evasion of cancer cells to immune attack. Healthy immune cells can kill cancer cells, as long as they can be detected as foreign to the body.

1.4 Some bench-top antitumor bioassay screening methods

Some of the bench-top assays used to screen for potential anticancer drugs are:

a. The use of *Artemia salina* (Leach) for lethality assay

Lethality testing using brine shrimp is a convenient monitor for screening and fractionation of plant extracts with the aim of discovering new bioactive products. The eggs of brine shrimp can remain viable for years when properly dried. Within 48 hours of being placed in water, the eggs hatch to provide large number of larvae. Brine shrimp lethality is indicative of cytotoxicity (Attur-Rahman, 2001), antibacterial activities and pesticidal effects (McLaughlin *et al.*, 1991). It has advantages of being fast, cost effective, and easy to carry out. Apart from Brine shrimp, mosquito larvae (*Culex quinquefasciatus*) (Moshen *et al.*, 1990) and tadpoles (*Raniceps ranninus*) (Ayinde and Agbakwuru, 2010) have also been used.

The lethality of materials towards these organisms is indicative of their ability to kill cancer cells in cell cultures. If the toxicity is transferred to tumor cells *in vivo*, the agent is said to have antitumor activity (Obuotor and Onajobi, 2000).

b. The use of crown gall tumors on potato discs

Crown gall is a neoplastic disease of plants caused by specific strains of the Gram negative bacterium, *Agrobacterium tumefaciens* (Braun and Stonier, 1958). The bacteria contain large tumor-inducing plasmids (T1) that transforms normal wounded plant cells into tumor cells. Galsky *et al.*, (1980) reported that compounds and plant extracts known to be active against 3PS (murine leukemia) actually inhibited the crown gall tumors on discs of potato tubers.

c. The use of *Lemna minor* (Duckweed): A simple Bioassay for Inhibition and Promotion of Plant Growth

Lemna minor is a very small monocotyledonous plant living in water. A general guideline for developing *Lemna* bioevaluation to screen plant extracts for determination of their effect on plant

growth was provided by Einhellig *et al.* (1985). It is a simple screening method for biodegradable herbicides. It has also been reported that natural antitumor compounds inhibit *Lemna* growth (Powell *et al.*, 1987).

1.5 Assays used *in vitro* to study cancer cell lines

Cancer research has benefitted immensely from cancer cell lines which provide a uniform sample and large number of single units. Essentially, cancer cell lines provide convenient representation of cancers and can be studied using several assays which can reflect a particular trait/aspect of cancer. Assays are performed for two major purposes (i) to ascertain the effect(s) of a physical or chemical agent on the cells and (ii) to understand the physiology or behaviour of cells under different conditions. Also, cells cultured in 6, 12, 24 and 96 well plates are utilized for the assays owing to the ease in which several concentrations of a variable being tested can be studied in either duplicates, triplicates or in more multiples so as to make the results meaningful and reliable (Ravi *et al.*, 2016).

Some of the assays used *invitro* to study cancer cell lines are:

a. Cytotoxicity

The ability of a drug to induce cell death is an integral part of chemotherapy. The assays which test for the cytotoxicity of a drug are classified as genomic or proteomic assays, functional assays, cytology assays and mitochondrial assays. Adenosine tri phosphate (ATP) assay is a functional assay which is the quickest cell viability/cytotoxicity assay. When the cell undergoes apoptosis or necrosis, it is seen that the ATP levels decline significantly. This

is used as a biomarker for cytotoxic effects. The levels of ATP are quantified using luciferase enzyme which results in luminescence which is recorded (Riss *et al*, 2011).

Lactate dehydrogenase leakage assay (LDH assay) is a commonly used cytotoxicity assay in the pharmaceutical industry. When there is a loss in membrane stability of a cell induced by cell death there is liberation of LDH into the culture medium which are thus directly proportional to the amount of cell death and can be detected by the addition of a tetrazolium salt (Francis *et al.*, 2013).

The resazurin reduction assay is similar to MTT and ATP assay. In this technique the resazurin when added to metabolically active cells gets converted to resorufin a pink fluorescent compound. The level of resorufin is indicative of cell viability. This assay is very sensitive and the quantification of resorufin is done by noting the fluorescence at the appropriate wavelength (Terry *et al.*, 2015).

In trypan blue assay, the dead cells whose membrane integrity has been disrupted will stain blue, whereas the live cells which maintain their membrane integrity exclude the dye. Thus the differential staining helps in characterisation of live and dead cells which gives the data for cell viability. This is a hemocytometer based assay (Altman *et al.*, 1993). The staining of cells with propidium iodide (PI) and acridine orange (AO) results in the live cells fluorescing green and dead cells fluoresce orange when viewed under a dark field fluorescence microscope. Here the percentage inhibition is derived from the cell viability value as in the case of trypan blue assay (Mascotti *et al.*, 2000).

b. Genotoxicity

Induction of damage to the genetic material of a cell either by a physical or chemical agent is termed genotoxicity. These agents can directly or indirectly affect the DNA of somatic or germ line cells causing mutations or other aberrations that can alter normal cellular functions. The propagation or effects of such damages are prevented by cells through complex processes such as the DNA repair and apoptosis. Various *in vitro* cell culture techniques are functional to examine the extent of damage caused to DNA *in vivo*. These cell culture models utilize assays such as comet assay, DNA fragmentation assay, cell viability assay and various high throughput technologies for the evaluation of DNA toxicity. Several cytogenetic techniques are used to test for genotoxicity. These include chromosomal numerical and structural aberrations, micronucleus assay, and sister chromatid exchange assay (Collins, 2004; Saud *et al.*, 2013).

c. Cell Migration

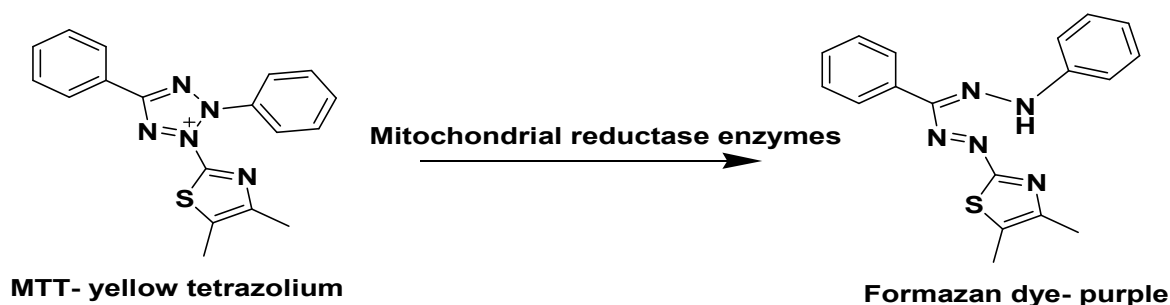
Drug development against cancer previously focused mainly on assays that screen cytotoxic agents which aid in inhibition of cell proliferation or stimulates apoptosis. The attention has now shifted to targeted therapies with development of inhibitors of invasion. Invasion and metastasis are accompanied by alterations in cell migration. Commonly used *in vitro* cell migration assays include the Boyden chamber assay and the scratch wound healing assays. (Chen, 2005; Chen and Nalbantoglu, 2014).

d. Cell Proliferation

Proliferation of cells in the presence of stimuli can be quantified using *in vitro* assays. These techniques help in monitoring cell division, the proliferative ability of cells, number of cells generated over time and DNA synthesis. Traditional proliferative assays are performed in

drug testing to evaluate the effect of drugs on cell multiplication. Conventionally, cell proliferation is being assessed by its DNA synthesizing capacity. The cell lines are incubated few hours to overnight with ^3H thymidine (a radioactive label). Proliferating cells integrate the radioactive label during the process of DNA synthesis which can be visualized and counted using a scintillation counter. A similar assay is performed to evaluate cell proliferation which utilizes 5bromo-2'-deoxyuridine (BrdU). BrdU also gets incorporated into the newly synthesized DNA which can be measured by a BrdU specific monoclonal antibody. A secondary antibody targeting the primary BrdU monoclonal antibody acts as a reporter generating a fluorescent signal on DNA synthesis indicating proliferation. This technique utilizes a calorimeter or a fluorescent microscope for the detection of reporter signals (Ravi *et al.*, 2016).

3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay is widely used for measuring cell proliferation with respect to cellular metabolism. This technique was the first high throughput assay designed for a 96 well plate format and involves a reduction of tetrazolium salts by dehydrogenase enzymes generating NADH and NADPH. The resulting metabolite which is formazan (purple colour) can be measured and quantified by spectrophotometry by measuring the absorbance at 570 nm. This chemical reaction yielding a purple product is displayed only in metabolically active cells thereby eliminating non-proliferating cells (Ravi *et al.*, 2016).



A high throughput quantification of cell proliferation can be achieved by measuring the amount of ATP generated in the cells. This technique is founded on the notion that dead cells generate slight amount of ATP. Enzyme luciferase generates light in the presence of ATP which can be efficiently detected by a luminometer. This assay is efficiently used to screen large number of samples and provides a reliable readout. Another high throughput analysis of cellular proliferation utilizes a chemical reaction involving the conversion of nonfluorescent dye resazurin (blue) to a fluorescent dye resorufin (pink) in a reducing growth medium due to cell proliferation. The fluorescent signal is then measured at particular wavelengths (570 and 600 nm) (Ravi *et al.*, 2016).

e. Gene expressions using mRNA

Gene expression studies on cell lines provide genetic information associated with cellular functions such as proliferation, apoptosis, cell division, adhesion and intercellular communication. Various cell lines are used for establishing gene expression profiles and genetic alterations in response to external and internal stimuli. *In vitro* cell culture models help estimating genetic expressions which are effectively used in drug testing and formulation of personalized medicine. mRNA as the study material and its quantification (rather than DNA) is widely being used to understand the alterations in gene expression. This is due to the possibility of post transcriptional changes in the sequence of DNA which will be

predominantly reflected in mRNA. Quantitative Polymerase Chain Reaction (qPCR) technique has been incorporated into the laboratory routine by various researchers working on cancer cell lines. Such studies are also useful for analyzing various metabolizing genes indicating drug effects (Shatha and Malek, 2013).

Gene polymorphism studies can also yield information about genetic alterations. Restricted fragment length polymorphism (RFLP) is a widely used technique to analyze nucleotide polymorphisms identified using primers. Specific primers are helpful in projecting the variations between normal and abnormal DNA sequences. Protein estimation and detection can also predict gene expressions changes at translational levels (Ravi *et al.*, 2016).

Western blot is a common technique used best for protein detection/identification which can be directly associated with genetic alterations. Alterations in protein levels are well established in various cell lines such as A549 and their associations with genes involved are well studied using western blot analysis and SDS PAGE (Sodium Do-decyl sulphate Poly Acrylamide Gel Electrophoresis) techniques. (Celline *et al.*, 2015).

f. Protein expression

Protein expression is a critical aspect to be studied on cancer cell lines as it directly reflects the genetic characteristics of a cell. Alterations in protein expressions are noted as vital biomarkers decisive of particular disorders, especially cancer. Several studies are being performed on various cancer cell lines for identification of biomarkers helpful in cancer diagnostic, therapeutic and prognostic applications. Western blot analysis is a well known tool for the identification of protein expression in cancer cell lines. For example, A549 cell line

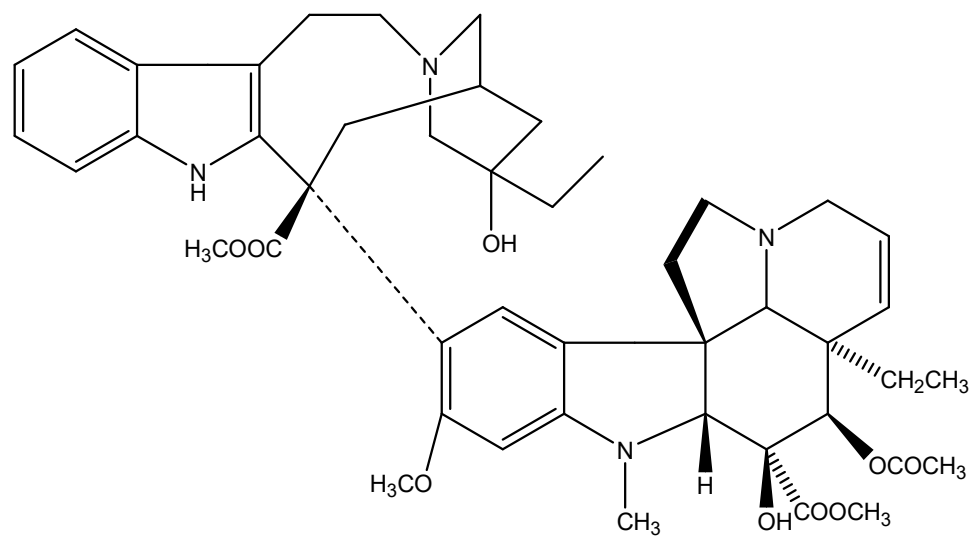
was efficiently utilized in a study which estimated the levels of and GAPDH proteins after drug exposures. (Celline *et al.*, 2015).

1.6 Classes of secondary metabolites with antitumor activities

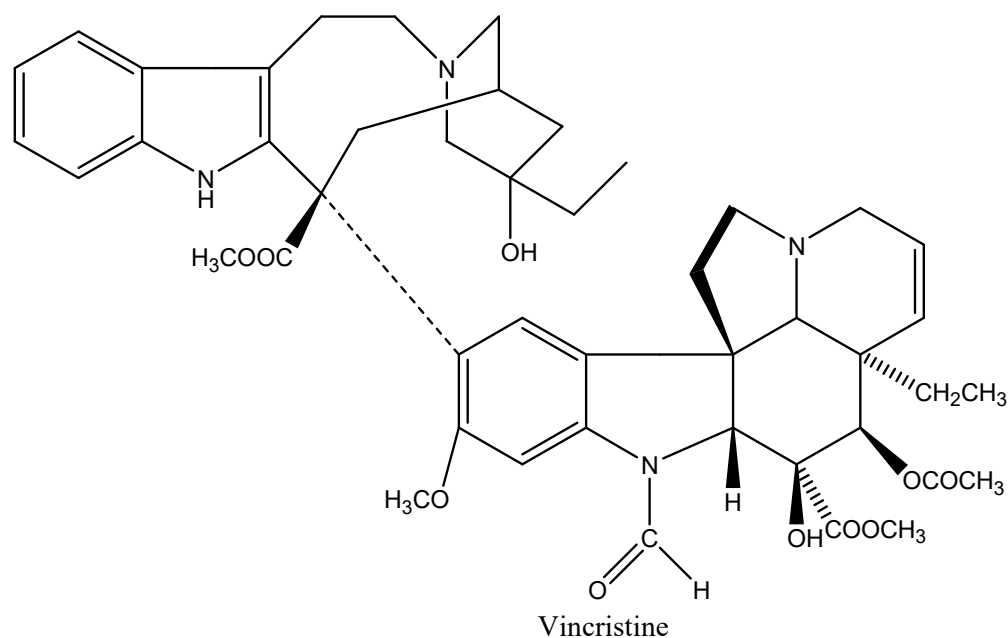
1.6.1 Alkaloids

a. Vinblastine and Vincristine (Bis-indole alkaloids)

Vinblastine and vincristine which are obtained from *Catharanthus roseus* G. Don. (Apocynaceae) were the first drugs used clinically for cancer treatment. They are mainly used together with other anticancer drugs for the treatment of various cancers such as leukemias, lung and breast cancers (Cragg and Newman, 2005b).

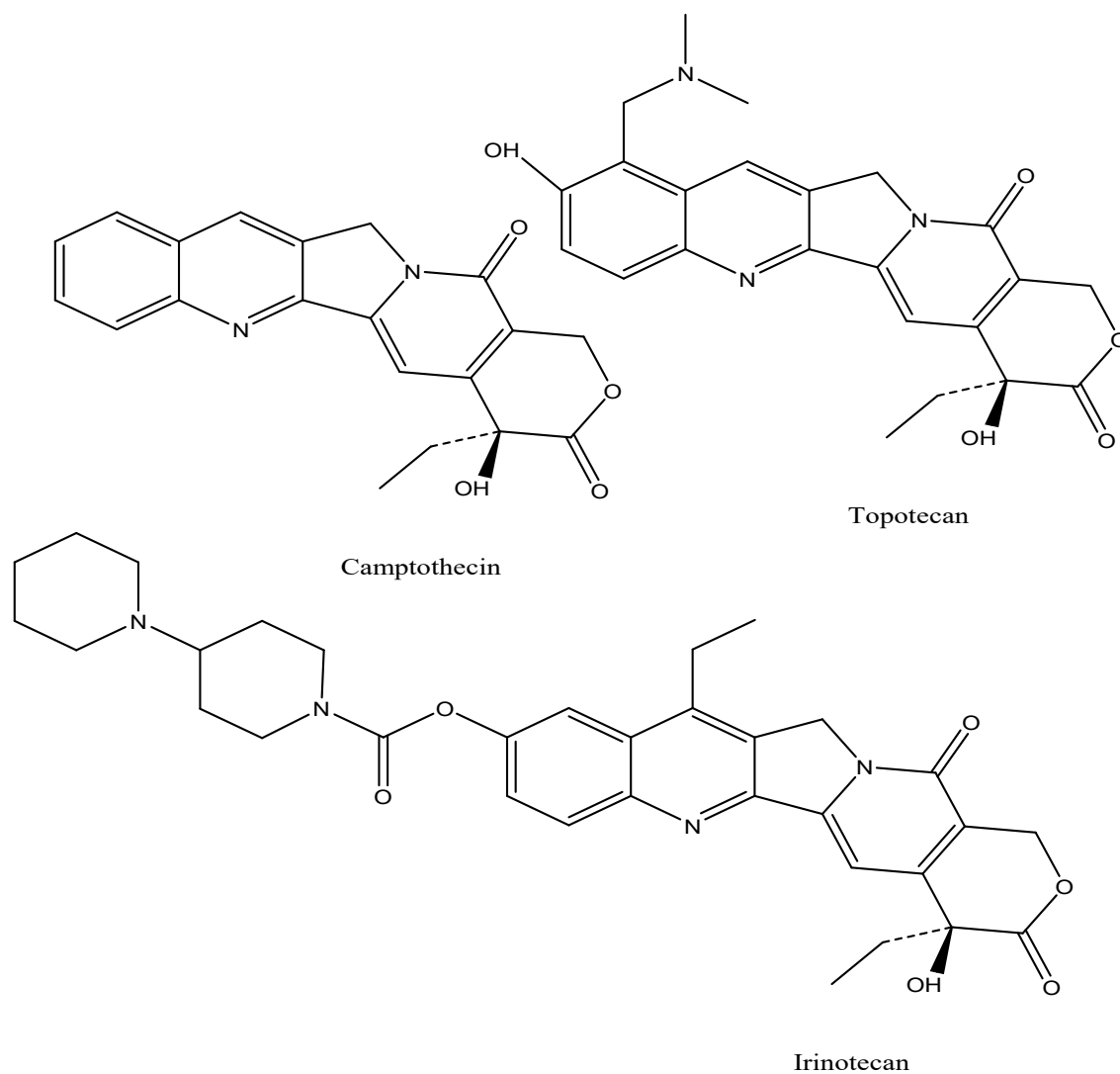


Vinblastine



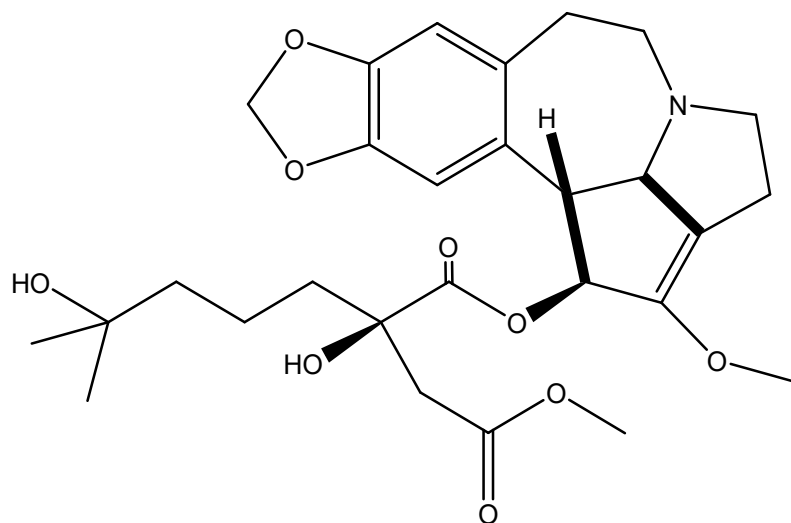
b. Camptothecin

Camptothecin (CPT) was isolated from *Camptotheca acuminata* Decne (Nyssaceae). It is a quinoline alkaloid which exhibits cytotoxicity by inhibiting DNA enzyme topoisomerase I but due to its toxicity on the bladder, it was dropped (Portmeisel and Pinedo, 1995). Topotecan and irinotecan are semi-synthetic derivatives of camptothecin. Topotecan is reportedly used for the treatment of ovarian and small cell lung cancers while irinotecan is used in cases of colo-rectal cancers. (Creemers *et al.*, 1995; Bertino, 1997).



c. Homoharringtonine

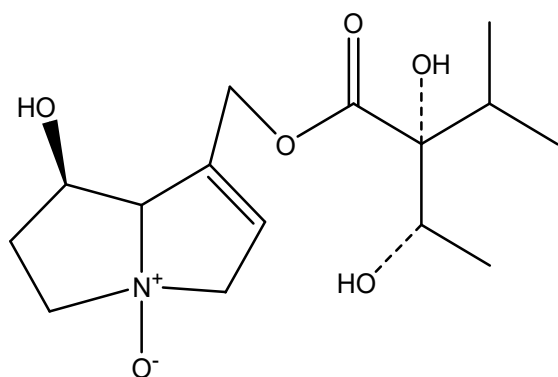
This was isolated from *Cephalotaxus harringtonia* var. Drupaceae (Sieb and Zucc.) (Cephalotaxaceae) and subjected to clinical application (Powell *et al.*, 1970). In China, a racemic mixture of harringtonine and homoharringtonine has been reported to be used for the treatment of both acute and chronic myelogenous leukemia (Cragg and Newman, 2005a).



Homoharringtonine

d. Indicine-N-oxide

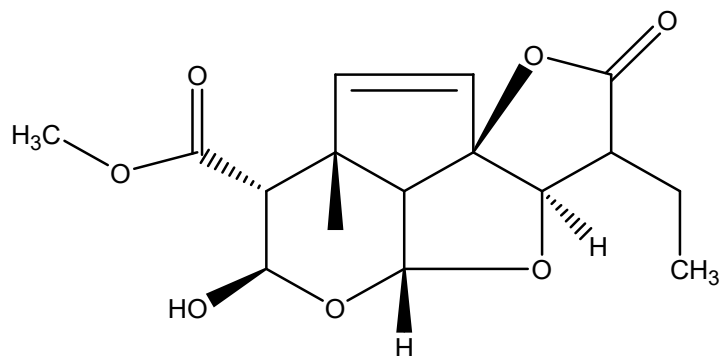
Indicine-N-oxide is obtained from *Heliotropium indicum* and has been shown to exhibit antitumor activity at low dose level against P-388 leukemia test system, B16 melanoma and L1210 leukemia (Kugelman *et al.*, 1976).



Indicine-N-oxide

1.6.2 Terpenes

a. Oxygenated terpenes (Iridoids)



Allamandine

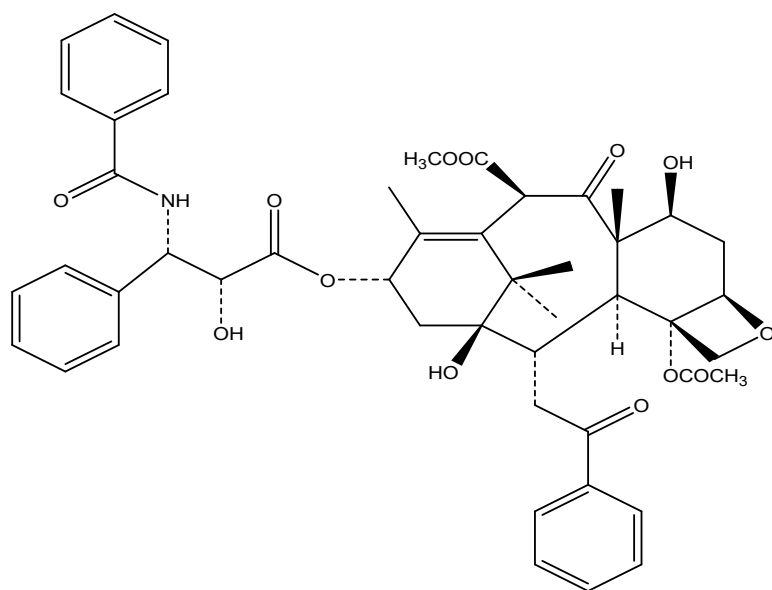
b. Sesquiterpenes

Eupatoriopicrin and eupatopicrinacetone are sesquiterpenes isolated from *Eupatorium cannabinum* L. (Asteraceae). They were found to exhibit cytotoxicity against Groningen lung carcinoma (Woerdenbag *et al.*, 1987). Vernoleptin obtained from *Vernonia amygdalina* Del has also been shown to be active in the KB, P-388, and Walker muscular (WA) test systems (Erasto *et al.*, 2006).

c. Diterpenes

i. Taxol

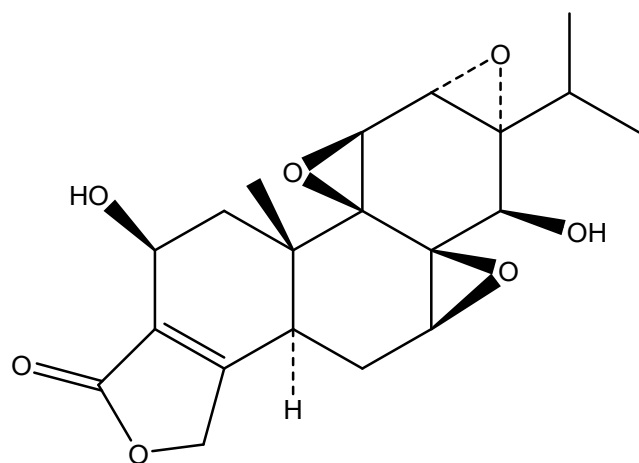
Paclitaxel (Taxol) was discovered from the bark of *Taxus brevifolia* Nutt. (Taxaceae). It possesses activity against ovarian cancer, small and non-small cell lung cancer as well as breast cancer (Rowinsky *et al.*, 1992).



Paclitaxel

ii. Triptiolide

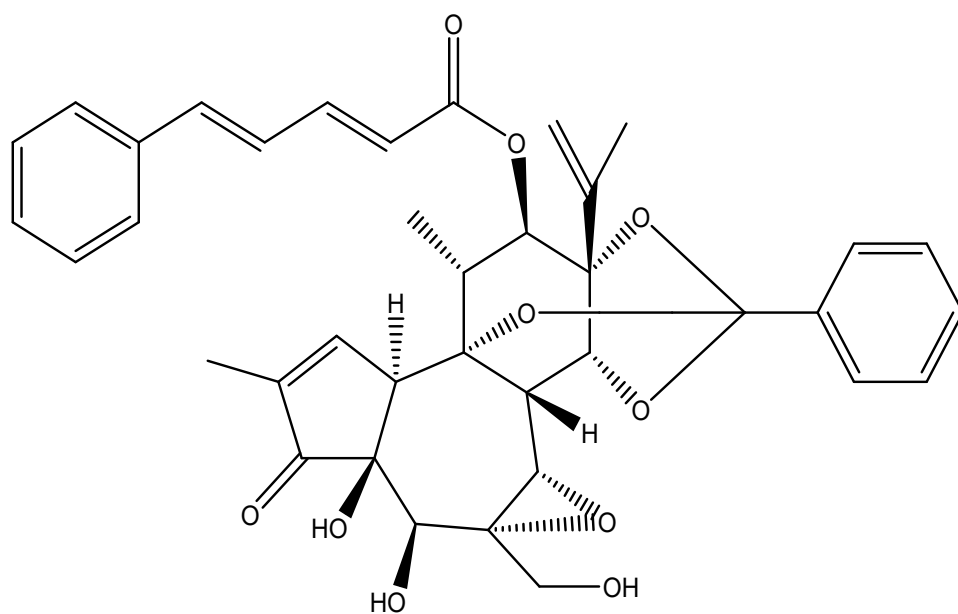
Triptiolide (TPL) is a diterpenoid triepoxide obtained from the Chinese herb *Tripterygium wilfordii* Hook F. *Invitro* experiments showed that TPL inhibited the multiplication and colony formation of tumour cells at extremely low concentrations and was more potent than Taxol. Its antitumor effect was reported to be at the same level or greater than that of typical antitumor drugs such as mitomycin, and cisplatin (Shamon *et al.*, 1997).



Tripdiolide

iii. Mezerein

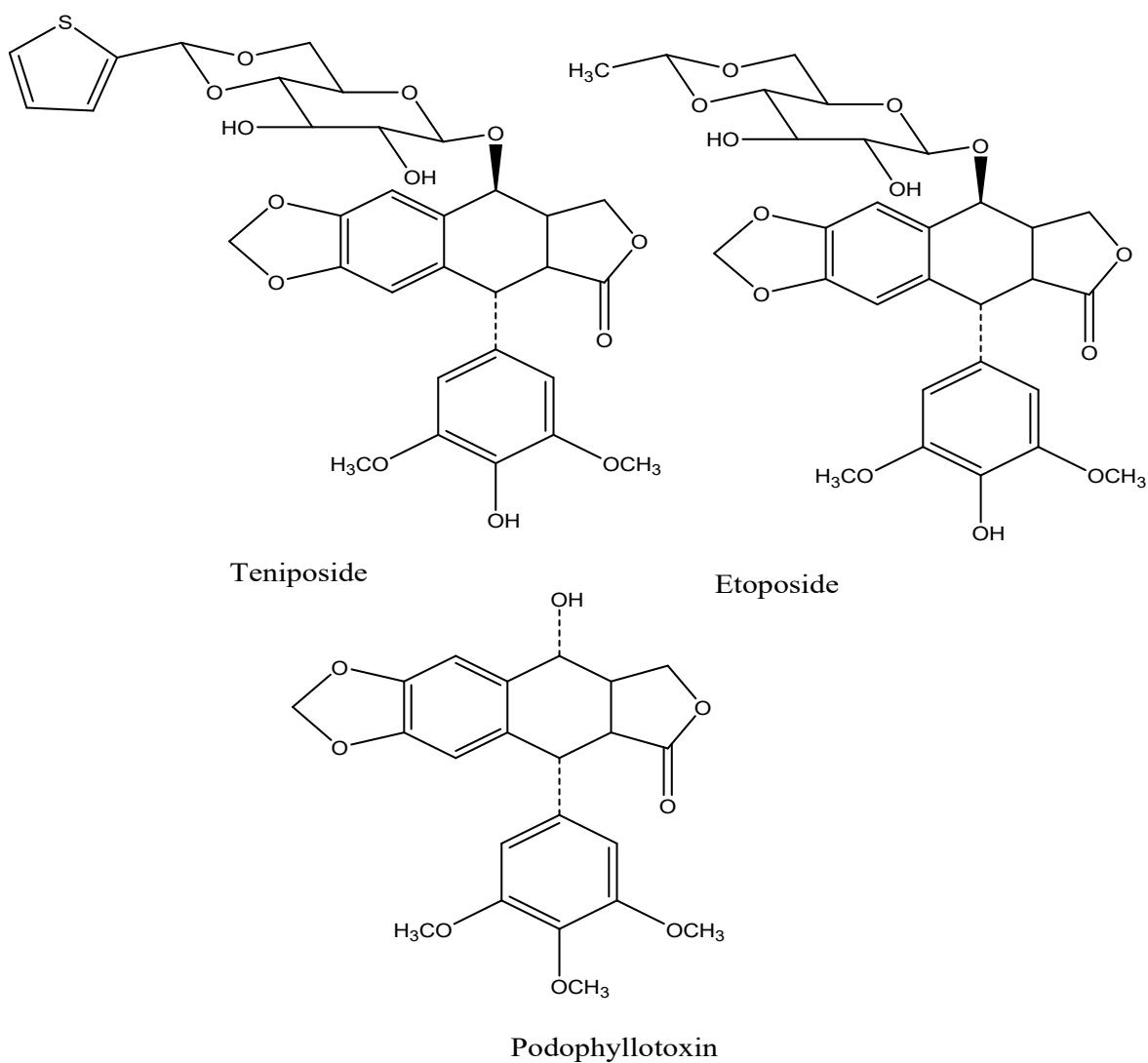
This is an antitumor compound purified from *Daphne mezereum* L. (Thymelaceae). The plant has been used for centuries in many countries for the treatment of cancer (Barton *et al.*, 1989).



Mezerein

1.6.3 Lignans

Podophyllotoxin was isolated from the roots of *Podophyllum peltatum* Linnaeus and *P. emodi* Wallich (Berberidaceae) (Stahelin, 1973). It possesses active antitumor properties along with its isomer, epipodophyllotoxin. Semi-synthetic derivatives of epipodophyllotoxin such as etoposide and teniposide are also used in the treatment of lymphomas, testicular and bronchial cancers (Cragg and Newman, 2005a).



1.7 Essential oils as anticancer agents

Essential oils (EOs) are natural, complex, volatile, and odourific oils synthesized by the secretory cells of aromatic plants. Essential oils and their constituents have been widely used in ethnomedicine. They have found applications in the pharmaceutical, food, cosmetic, and beverage industries (Nogueira de Milo *et al.*, 2011). Their biological activity depends on their constituents. These oils contain majorly terpenes obtained from aromatic plants; mainly monoterpenes and sesquiterpenes (Daferera *et al.*, 2002).

Thus in conducting studies on essential oils, the chemical characterization as well as the possibility of linking the major components with particular biological properties comes into play.

Several studies have cited the activities of EOs and their components against various cancer cells.

Examples include:

a. Prostate cancer

EOs of *Hypericum hircinum*, *Thymus vulgaris* and *Solanum erianthum* leaf demonstrated antiproliferative activity against human prostatic adenocarcinoma (PC3) (Zu *et al.*, 2010; Essien *et al.*, 2012; Quassinti *et al.*, 2013). *Pinus wallichiana* EO showed significant inhibitory activity on prostate cancer cells (Yousuf *et al.*, 2012)

b. Glioblastoma

Quassinti *et al.* in 2013, stated that *Hypericum hircinum* EO showed antiproliferative activity against human glioblastoma tumor cells (T98G). Other studies also demonstrated that *Lippia multiflora* Moldenk and *Ageratum conyzoides* Linn. inhibited the growth of SF-767 glioblastoma and SF-763 cell lines respectively (Bayala *et al.*, 2014).

c. Melanoma

EOs of *Afrostryax lepidophyllus* and *Scorodophloeus zenkeri* were found to exhibit significant anticancer effects on human malignant melanoma (A375) cell line (Fogang *et al.*, 2014). EOs from the leaves of *Neolitsea variabilissima* (Su *et al.*, 2013) and *Casearia sylvestris* (Bou *et al.*, 2013) also showed strong growth-inhibitory activity against human melanoma cancer.

d. Breast cancer

EOs extracted from leaves of *Satureja khuzistanica* Jamzad (Yousefzadi *et al.*, 2014), *Casearia sylvestris* (Bou *et al.*, 2013), *Cedrelopsis grevei* (Afoulous *et al.*, 2013) and *Solanum spirale* Roxb (Keawsa-ard *et al.*, 2012) were found to have cytotoxic effects against MCF7 cells in a dose-dependent manner. Likewise, human breast cancer cell lines such as MDA-MB-231 and MCF7 were shown to be sensitive to *Boswellia sacra* EO as demonstrated by increased cell death (Suhail *et al.*, 2011).

e. Colon cancer

Geraniol, a component of EOs has been found to have chemopreventive activities as it has inhibitory effects on Caco-2 colon cancer cells (Carnesecchi *et al.*, 2001). EO from *Satureja khuzistanica* to a large extent, reduced the cell viability of SW480 cell line (Yousefzadi *et al.*, 2014). Volatile oil extracted from blood oranges were revealed to have pro-apoptotic and anti-angiogenesis effect on colon cancer cells (Chidambara *et al.*, 2012).

f. Ovary cancer

Do N Fontes *et al.* (2013) demonstrated that EO obtained from *Guatteria pogonopus* leaves possessed high *in vitro* and *in vivo* antitumor activity against ovarian adenocarcinoma OVCAR-8.

Malus domestica leaves EO at 1000 µg/ml produced 68.3% inhibition against CHOK1 cells (Walia *et al.*, 2012).

g. Liver cancer

EOs from *Thymus citriodorus* (Wu *et al.*, 2013a), *Artemisia indica* (Rashid *et al.*, 2013), and *Pituranthos tortuosus* (Abdallah and Ezzat, 2011) leaves were proven to have highly significant activity against liver cancer cells HepG2 while *Zanthoxylum schinifolium* essential oils induced death of human hepatoma cell line (Paik *et al.*, 2005).

h. Uterus and cervix cancers

Cytotoxic activity was observed on uterus carcinoma (Siha) cell line with EO of *Casearia sylvestris* leaves (Bou *et al.*, 2013). A similar result was seen with *Schinus terebinthifolius* Raddi according to Santana *et al.* (2012). From the EO of *Curcuma wenyujin* rhizome, furanodiene, a sesquiterpene was extracted and discovered to also inhibit the growth of uterine cervical (U14) tumors in mice (Sun *et al.*, 2009).

i. Lung cancer

Xylopiia frutescens leaf EO, according to Ferraz *et al.* (2013), demonstrated *in vitro* and *in vivo* anticancer effects on bronchoalveolar lung carcinoma cell line (NCI-H358M). Investigations also proved that *Tridax procumbens* L. prevents lung metastasis of B16F-10 cell line in mice (Manjamalai *et al.*, 2012). Vapor of volatile oil from *Litsea cubeba* seeds were said to cause cell death of human NSCLC cells, A549 (Seal *et al.*, 2012).

j. Oral cancer

EO reportedly obtained from the leaf of *Neolitsea variabilissima* showed cytotoxic activity against human oral cancer (Su *et al.*, 2013). *Solanum spirale* Roxb. Leaves EO demonstrated strong cytotoxic effects against KB cell line (Keawsa-ard *et al.*, 2012). *Pinus densiflora* leaf EO also retarded the proliferation of YD-8 oral squamous cell carcinoma (Jo *et al.*, 2012).

k. Leukemia

Leaves of *N. variabilissima* (Su *et al.*, 2013) and *Casearia sylvestris* (Bou *et al.*, 2013) showed anticancer activity against leukemia HL-60 cell line while EO from *Artemisia indica* showed dose dependent antiproliferation of THP-1 cell line (Rashid *et al.*, 2013). *Malus domestica* leaves EO at 1000 µg/ml also produced 65.7% inhibition of human acute monocytic leukemia cell (THP-1) (Walia *et al.*, 2012).

l. Kidney cancer

Yousefzadi *et al.* in 2014, reported that *Satureja khuzistanica* EO, in a dose-dependent manner, significantly reduced proliferation of Vero cell line. EO of *Platycladus orientalis*, *Prangos asperula* (Loizzo *et al.*, 2008) and *Sideritis perfoliata* (Loizzo *et al.*, 2007) were also found to exert anticancer effects on renal adenocarcinoma cell line (ACHN).

m. Bone cancer

Pyrolae herba EO reportedly demonstrated significant antitumor effects on SW1353 cells in a concentration dependent manner. (Cai *et al.*, 2013).

n. Pancreas cancer

Human pancreas cancer cells showed sensitivity to EO obtained from *Boswellia* species demonstrated by reduced cell viability and apoptosis. (Ni *et al.*, 2012). Also, EO from of *Kadsura longipedunculata* stem bark showed cytotoxic effect against human pancreas carcinoma (MIA PaCa-2) (Mulyaningsih *et al.*, 2010).

o. Skin cancer

Schefflera heptaphylla (L.) Frodin EO and its major compound pinene, demonstrated high cytotoxic activity against A375 cell line according to Li *et al.* (2009).

1.8 The family Zingiberaceae

Zingiberaceae family contains flowering plants made up of 50 genera and about 1600 species (Christenhusz and Byng, 2016) of aromatic and perennial herbs. They have creeping horizontal or tuberous rhizomes and are found all over tropical Asia, Africa, and America. The family is characterized by small to large sized plants. They are herbs with distichous leaves that have basal sheaths which overlap to form a pseudostem. The plants are either epiphytic or self supporting. Flowers are hermaphroditic, zygomorphic, with indeterminate cymose inflorescences. The bracts are spirally arranged and conspicuous. The perianth is made up of two whorls, a fused tubular calyx, and a tubular corolla in which one lobe is larger than the other two. Their flowers usually have two of their stamenoids joined to form a petaloid lip, and have only one fertile stamen. The ovary is inferior and topped by two nectarines while the stigma is funnel-shaped. Some genera in the family produce volatile oils useful in the perfume industry (*Alpinia*, *Hedychium*). Many of the family's species are important for ornamental, culinary or medicinal purposes. Examples of genera with plants used for ornamental purposes include *Alpinia*, *Globba*, *Hedychium*, and

Kaempferia.. Spices include *Zingiber*, *Aframomum*, and *Curcuma*. Generally, plants that are in the Zingiberaceae family contain polyphenols. Ethnomedicinally, *Aframomum citratum* Schum is used to treat bacteria, malaria, and infections (Titanji *et al.*, (2008). *Curcuma longa* was reported to have neuroprotective properties and was found to also ameliorate attention and working memory (Ahmad, 2020).

1.8.1 Genus *Zingiber*

The genus *Zingiber* is commonly found in Southeast Asia especially Thailand, China, the Indian Sub-continent, and New Guinea (Govaerts, 2004). The genus has about 150 species; the main ones include *Z. barbatum*, *Z. mioga*, *Z. officinale*, and *Z. zerumbet*.

1.8.1.1 *Zingiber officinale* Roscoe

Z. officinale is a herbaceous perennial plant with tubers. Its widespread cultivation is seen in most tropical and subtropical countries, like Africa, India, Australia and China. China and India are the world's top producers (Langner *et al.*, 1998).

i. Morphology of *Zingiber officinale*

Its leaves are elongated, oblong-lanceolate and glabrous, and are attached to the stem by their sheaths. It possesses spikes that are usually radical on the leafy stem and its peduncle can be short or long. Bracts are persistent and usually single. The calyx is cylindrical, and shortly three-lobed while the corolla tube is cylindrical, and its segments lanceolate. It has a three celled ovary with many ovules which are superposed and a filiform style. Its stigma is small and subglobose. The capsule is oblong and dehiscent with large, globose, arillate seeds. Rhizomes are present which are stout and tuberous with stems approximately 0.6 to 1.2 m high. Its flowers are greenish with a small dark purple or purplish black lip, in radial spikes 3.8 to 7.5 cm long and 2.5

cm diameter on peduncles 15-30 cm long. Stamens are dark purple, as long as the lip, but shorter than the corolla (Evans, 2002).

ii. Morphology of *Zingiber officinale* rhizome

The rhizomes appear horizontal with sympodial branching. It has a length of 5 to 15 cm, width 3 to 6 cm and thickness 0.5 to 1.5 cm. It is laterally flattened on the upper side with short flattened obovate, oblique fingers. Each branch is 1 to 3 cm long with a depressed scar of the stem at its apex. The surface has longitudinal striations and sometimes projecting fibers. Its fracture is short, starchy and fibrous. Fractured surface shows a narrow bark, a wide stele, a marked endodermis and many scattered gray-coloured fibro-vascular bundles as well as smaller yellowish secretion cells. It has a buff colour, an aromatic odour and a pungent taste (Evans, 2002).

Z. officinale rhizome is locally known as *jinja* in Igbo, *cithar* in Hausa and *ata-ile* in Yoruba.



Plate 1.1: Picture of the rhizome of *Zingiber officinale* Roscoe (Zingiberaceae)

iii. Medicinal uses of *Zingiber officinale* rhizome

It is used to treat common cold disorders, asthma, stroke, nervous disease, gastrointestinal constipation, toothache, inflammation, oxidant stress, hypercholesterolaemia, migraine, helminthiasis and schistosomiasis (Iqbal *et al.*, 2006; Lin *et al.*, 2010; Iwami *et al.*, 2011).

iv. Reported biological/pharmacological activities of *Zingiber officinale* rhizome

The anti-inflammatory properties of *Z. officinale* rhizome have been reported, it retards prostaglandin synthesis by inhibiting cyclooxygenase and cyclooxygenase-1 (Vanden and Letsky, 2000). Ginger essential oil was also found to exhibit strong cytotoxicity towards Bel-7402 and HO-8910 tumour cell lines (Wang *et al.*, 2012). Its component gingerol, possesses antiangiogenic activity and may inhibit tumor growth and metastasis (Kim *et al.*, 2005).

Z. officinale rhizome has also been reported to show anticoagulant effects by inhibiting platelet aggregation (Wang *et al.*, 2003) and decreasing platelet thromboxane production *in vitro* (Nurtjahja-Tjendraputra *et al.*, 2003). Fuhrman *et al.* (2000) demonstrated the antioxidant effects while Srivastava (1984) reported that it retards the growth of *Helicobacter pylori* *in vitro*.

Based on the results published by Fuhrman *et al.*, (2000) its hypocholesterolemic, hypolipidemic, and antiatherosclerotic activities were demonstrated. Other pharmacological activities such as antiarthritic (Funk *et al.*, 2009), antimicrobial (Miri *et al.*, 2008), radio protective (Kim *et al.*, 2007) and antigenotoxic activities (Beg *et al.*, 2008) have also been reported.

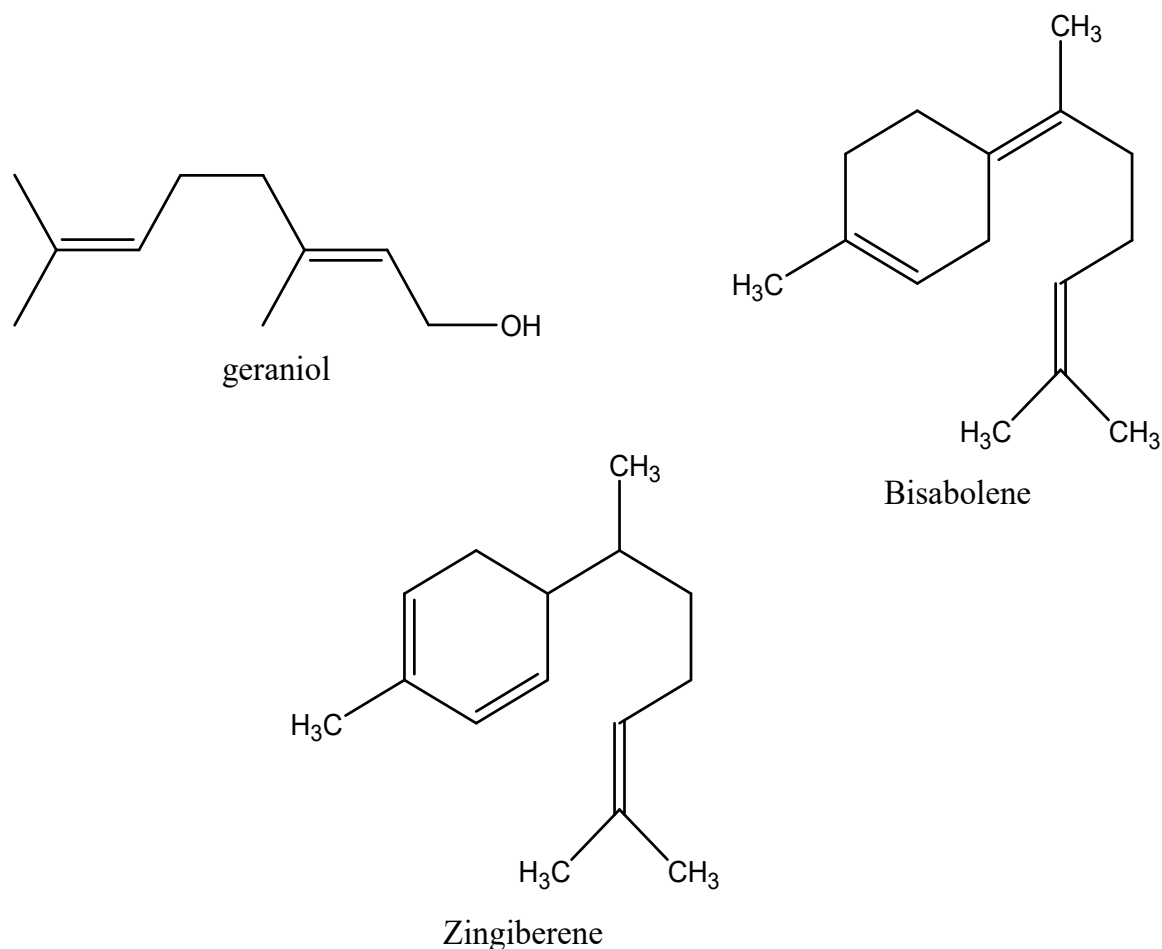
v. Reported phytochemical constituents of *Zingiber officinale* rhizome

The constituents are many and vary which is due to differences in the place of origin and the condition of the rhizome; whether its fresh or dry. The main pungent constituents are gingerol,

paradol, shogol, and zingerone (Kamaliroosta *et al.*, 2013). Its essential oil components are listed in Table 1.1.

Table 1.1: Chemical composition and concentrations of compounds present in ginger essential oil (Kamalirroosta *et al.*, 2013)

Compounds	Concentrations (%)
Camphene	0 .73
Beta-Phellandrene	0 .93
Endo- Borneol	0 .97
Geraniol	0 .97
Geraly Acetate	1 .37
R-Curcumene	15.88
Zingiberene	31.79
Alpha Farnesene	5 .71
Beta-Bisabolene	9 .29
4, 5- Dimethyl-11-Methylene Tricycle	2 .35
Gamma-Cadinene	3 .56
Beta-Sesquiphellandrene	15.57
Delta-Cadinene	0 .64
Nerolidol	2 .01
7Alpha-(1-Hydroxyl-1-Methylethyl)	2 .00
Germacrene B	1 .10
Alpha-Eudesmol	3 .23



1.9 The family Lamiaceae

Lamiaceae family is made up of flowering plants which can be found in almost all parts of the world (Heywood *et al.*, 2007). It contains about 240 genera and 7200 species (Harley *et al.*, 2010). The largest genera in this family are *Salvia* (900), *Scutellaria* (360), *Stachys* (300), *Plectanthrus* (300), *Hyptis* (280), *Teucrium* (250), *Vitex* (250), *Thymus* (220), and *Nepeta* (200). Their flowers typically have petals fused into an upper lip and a lower lip and are bilaterally symmetrical with five united petals and five united sepals. They are usually bisexual and verticillastrate. The leaves emerge oppositely and are decussate or whorled. Cross section of the

stem shows that they are frequently square (Parnell and Curtis, 2012), but this is not found in all members of the family. Many members of the family are used as spice or in medicinal preparations and as sources of essential oils. Chemical constituents commonly found in the family include diterpenes, triterpenes, saponins, pyridine and pyrrolidine alkaloids, and tannins (Evans, 2002).

1.9.1 Genus *Hyptis*

The *Hyptis* genus comprises of 280 species that are mostly found in tropical America. Majority of plants in this genus are highly aromatic and are seen as weed in countries like Australia. They are generally used in traditional medicine for the treatment of various illnesses and have been found to have high pharmacological activities which include mycotoxic, antifertility, tumorigenic, and phytotoxic activities (Kumar *et al.*, 2005; Chukwujekwu *et al.*, 2005).

1.9.1.1 *Hyptis suaveolens*

Hyptis suaveolens L. Poit popularly called Mintweed, is native to tropical America and now it is seen as a worldwide weed (Chukwujekwu *et al.*, 2005). It is a fast-growing, herbaceous, perennial and aromatic plant. It is known locally as *jogbo* in Yoruba and *daddooyar goonaa* in Hausa.

i. Description of *Hyptis suaveolens*

The plant has a sweet odour with tetragonal hispid stem. Its leaves are long, petioled, pubescent, and broadly elliptic-ovate. Its base is oblique, truncate or acute with a coarsely serrulate margin and an acute and chartaceous apex (Kumar *et al.*, 2005). Flowers are blue and arranged in short stalked axillary cymes with an ovoid calyx which is companulate and subequally 5 lobed and often accrescent. Corolla is bilabiate with lower deflexed and seccate lip. Its stamens are

didynamous while the anther cells are confluent and glandular. It has gynobasic style with a shortly bifid stigma and a 4 partite ovary (Kumar *et al.*, 2005).



Plate 1.2: Picture of *Hyptis suaveolens* L. Poit (Lamiaceae) growing in its natural habitat

ii. Ethnomedicinal uses of *Hyptis suaveolens*

Most parts of mintweed are useful in traditional medicine for treatment of various symptoms such as gastrointestinal disorders, colds, respiratory tract infections, pain, cramps, fever, and skin diseases (Grassi *et al*, 2006). Its leaves have been used as a galactagogue, carminative, stimulant, sudorific, and as a cure for parasitic cutaneous disease (Mandal *et al*, 2007).

iii. Reported biological/pharmacological activities of *Hyptis suaveolens*

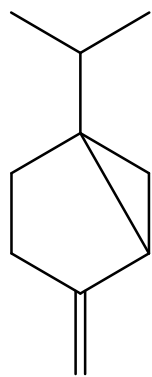
The extract was reported to exhibit insecticidal activity against rice weevils, *Sitophilus oryzae* (Buatone and Indrapichate, 2011) and mosquitoes (*Aedes aegypti*) (Yongkhamcha and Indrapichate, 2012). It was also reported to possess anti-HIV (Kunkongkapan *et al*, 2007) and antiparasitic activity (Nayak *et al*, 2010). Reports of its antimicrobial (Nantitanon *et al*, 2007), antifungal (Zollo *et al*, 1998), antiplasmodial (Chukwujekwu *et al*, 2005; Ziegler *et al*, 2002), and antinociceptive (Santos *et al*, 2007) have been documented. Its antidiarrhoeal (Shaikat *et al*, 2012), anti-hyperglycemic (Mishra *et al.*, 2011), anti-inflammatory (Mahesh, 2001), and anticancer activities (Gurunagarajan and Pemaiah, 2011) have also been demonstrated.

iv. Reported phytochemical constituents of *Hyptis suaveolens*

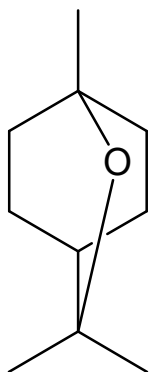
Phytochemical evaluation showed that the extract comprises of alkaloids, glycosides, saponins, tannins and flavonoids as major active constituents (Shaikat *et al.*, 2012). The essential oil has been reported to contain sabinene and trans α -caryophyllene as major constituents (Table 1.2).

Table 1.2: Chemical composition of the essential oil of *H. suaveolens* (Poonkodi *et al.*, 2017)

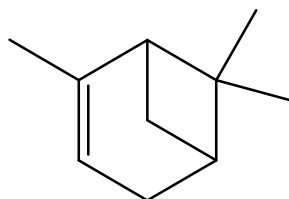
Compound	Concentration (%)
α - Pinene	0.7
3-Hexene	1.14
Sabinene	29.42
1,8-Cineole	4.61
Eucaliptol	3
Germacrene B	0.52
α -Copaene	0.86
α – Elemene	1.04
Transα-Caryophyllene	13.66
α –Selinene	2.32
α –Humulene	1.37
δ -Cadinene	0.65
Caryophyllene oxide	2.08
Z- α -trans-Bergamotol	2.47
Beta-Elemene	4.25
Rimuene	5.73
Germacrene	0.85
Gama-Ellemene	1.97
Espatulenol	7.1
4-Epidehydroabietol	2.12



Sabinene



Eucalyptol



Alpha-pinene

1.9.2 Genus *Ocimum*

The genus *Ocimum* is composed of aromatic plants which are both annual and perennial shrubs in Lamiaceae family. The greatest number of species is found in Africa with distributions in other tropical and warm temperate regions of all 6 continents (Jirovetz and Buchbauer, 2001). The genus consists of more than 150 species (Mondal *et al.*, 2007) which include *O. sanctum* L., *O. canum* Sims, *O. basilicum* L., *O. americanum* L., *O. kilimandscharicum* Guerke, *O. gratissimum* L., and *O. micranthum* Willd (Silva *et al.*, 2008; Zheljazkov *et al.*, 2007)

1.9.2.1 *Ocimum basilicum*

O. basilicum Linn. plant is locally called *efirin* in Yoruba, *daddooya* in Hausa, and *nchuanwu* in Igbo. It is native to tropical regions from central Africa to Southeast Asia (Buchbauer, 2012).

i. Description of *Ocimum basilicum*

It is an erect herb that grows between 0.3 and 1.3 m high, with light green leaves. Its leaves are simple, opposite, between 3 to 11 cm long and 1 to 6 cm wide. They are also acute, ovate and contain numerous oil glands (Poonkodi, 2016). Sweet basil flowers are white to purple and grow

from a central inflorescence arranged in a terminal spike. It has a thick central tap root (Evans, 2002)



Plate 1.3: Picture of the leaves and flowering tops of *Ocimum basilicum* Linn (Lamiaceae)

ii. Ethnomedicinal uses of *Ocimum basilicum*

It has long been utilized traditionally for curing various ailments. Examples include coughs, stings, pyrexia, strong aching, anxiousness, gripe, platonic transmissions, worms, infective diseases, acne, diarrhea, headaches, constipation, warts, and kidney malfunction (Akgül, 1989; Simon *et al.*, 1990; Lachowicz *et al.*, 1996).

iii. Reported biological/pharmacological activities of *Ocimum basilicum*

Its antibacterial activity (Akgül, 1989; Simon *et al.*, 1990), antioxidant effects (Javanmardi *et al.*, 2002; Srivastava *et al.*, 2014), and anti-inflammatory activity (Okoye *et al.*, 2014) have been cited. The insecticidal activity of its essential oil constituents against stored grain insects (*Sitophilus oryzae*, *Tribolium castaneum*, *Bruchus chinensis* and *Stagobiom paniceum*) was analyzed and reported by Deshpande and Tipnis (1997).

Oliveira *et al.*, (2009) and Baba *et al.*, (2012), documented its larvicidal activity while Sethi *et al.*, (2013) cited its anticonvulsant activity. It has also been reported to have fungicidal effects against *Aspergillus niger*, *A. flavus* Link, *Fusarium oxysporum* Schl. ex Fries and *Penicillium* species (Manosroi *et al.*, 2006).

Marotti *et al.*, 1996 evaluated and published the cytotoxic and apoptosis induction activity of the essential oil on P388 (murine leukemia) and HeLa (human cervical adenocarcinoma) cell lines.

iv. Reported phytochemical constituents of *Ocimum basilicum*

Basil essential oil contains monoterpenes and phenyl propanoid derivatives (Vina and Murillo, 2003; Lawrence and Reynolds, 1993). However, methyl eugenol was reported to be the major constituent of the volatile oil (Table 1.3).

Table 1.3: Chemical composition of *O. basilicum* L. oil (Ozcan and Chalchat, 2002)

Compound	Concentration (%)
Limonene	0.009
3,7-dimethyloct-1,5-dien-3,7-diol	0.327
α -terpineol	0.003
n-octyl acetate	0.021
endo-fenchyl acetate	0.036
Nerol	0.825
Neral	0.009
geraniol	0.259
carvacrol	0.028
bicycloelemene	0.012
exo-2-hydroxycineole acetate	0.048
α -cubebene	6.170
3,7-dimethylocta-1,7-dien-3,6-diol	0.025
geranyl acetate	0.088
α -ylangene	0.016
β -bourbonene	0.042
β -elemene	0.241
β -cubebene	0.297
methyl eugenol	78.016
β -copaene	0.087
α -guaiene	0.198
cadina-3,5-diene	0.147
epsilon-muurolene	0.736
α -humulene	0.117
cis-muurola-4(14),5-diene	0.101
β -acoradiene	0.071
α -acoradiene	0.052

1.9.2.2 *Ocimum gratissimum*

The species *O. gratissimum* L. is locally called *efirin-nla* in Yoruba, *daddooya ta gidaa* in Hausa and *nchanwa* in Igbo. It is native to South Africa, Asia and Brazil (Sartoratto *et al.*, 2004).

i. Description of *Ocimum gratissimum*

O. gratissimum L. (Lamiaceae) is an aromatic perennial erect herb with many branches which usually grows not more than 1 m in height with a woody base. Its leaves are opposite, pubescent with blades elliptical to ovate. Its base is cuneate, and its apex, acute. Inflorescence is arranged in a terminal simple or branched raceme. Its bracts are sessile, ovate, acuminate and caducous.

Flowers are small, hermaphrodite and arranged in 6-10-flowered verticillasters. Its corolla is campanulate and 3.5-5 mm long; it is 2-lipped and greenish-white in colour. It has 4 stamens which are declinate, in 2 pairs, and inserted on the corolla tube. It has a superior ovary, consisting of 2 carpels, each 2-celled.

Fruit consists of 4, dry, 1-seeded nutlets enclosed in the persistent calyx (Orwa *et al.*, 2009).



Plate 1.4: Picture of *Ocimum gratissimum* Linn. (Lamiaceae) growing in its natural habitat

ii. Ethnomedicinal uses of *Ocimum gratissimum*

It is widely used in traditional medicine in several countries, mainly in the form of teas and infusions of its leaves (Rabelo *et al.*, 2003). It is used to treat abdominal pain and cough (Matasyoh *et al.*, 2007) and colds, pruritus, stress and headache (Albuquerque *et al.*, 2007). Also, there are reports of the use of *O. gratissimum* to treat upper respiratory tract infections, pneumonia, conjunctivitis and skin diseases (Adebolu and Oladimeji, 2005).

iii. Reported biological/pharmacological activities of *Ocimum gratissimum*

Various scientific studies have identified some of the plant's activities, such as antidiabetic (Akpan *et al.*, 2014), antioxidant (Ojo *et al.*, 2014), anxiolytic (Venuprasad *et al.*, 2014), sedative (Tankam and Ito, 2014) anti-inflammatory (Okoye *et al.*, 2014), hepatoprotective (Awogbindin *et al.*, 2014), antitumor (Lin *et al.*, 2014), gastroprotective (Gege-Adebayo *et al.*, 2013), and hypolipidemic (Ekoh *et al.*, 2014). The antibacterial activity of this plant species has been described in many studies that tested various parts of the plant (Matias *et al.*, 2010; Matasyoh *et al.*, 2008)

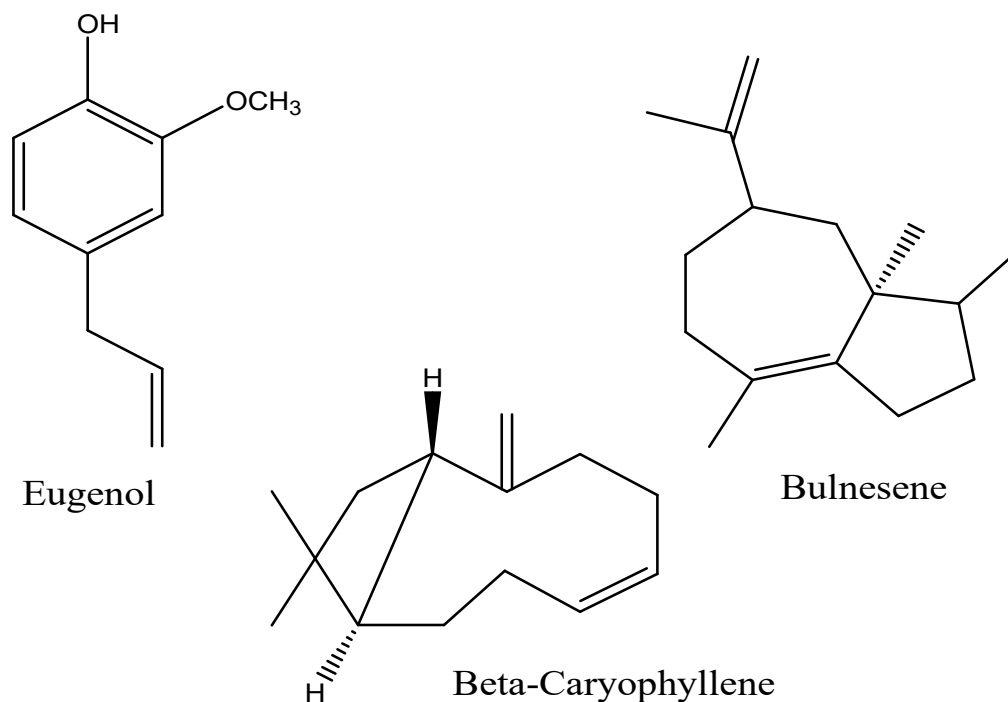
iv. Reported phytochemical constituents of *Ocimum gratissimum*

Several compounds have been reportedly found in the essential oil of *O. gratissimum* with eugenol as the major constituent (57%) alongside β -Caryophyllene, bulnesene and others (Table 1.4).

Table 1.4: Chemical composition of the essential oil of *O. gratissimum* (Joshi, 2017)

Compound	Concentration (%)
α -Pinene	0.2
β -Pinene	0.1
Myrcene	0.1
E- β -Ocimene	0.1
γ -Terpinene	0.2
Terpinolene	0.3
1,8-Cineole	0.7
Linalool	0.2
Camphor	0.3
Borneol	0.1
Terpin-4-ol	0.7
Isobornyl formate	0.9
α -Copaene	0.6
β -Bourbonene	0.5
β -Elemene	0.1
β -Caryophyllene	14.2
α -Bulnesene	15.6
δ -Cadinene	0.4
Elemol	1.2
Caryophyllene oxide	2.2
1,10-di-epi-Cubenol	0.5
1-epi-Cubenol	0.8
γ -Eudesmol	0.6
β -Eudesmol	0.9
epi- α -Cadinol	0.3

Eugenol	57.1
Methyl eugenol	0.2



1.10 The family Myrtaceae

It is a family of shrubs and trees in the order Myrtales, containing about 132 genera and 5,950 species that are widely distributed in the tropical and warm temperate regions of the world such as Australia, Chile, Argentina, and Philippines (Christenhusz and Byng, 2016). Their leaves are leathery and evergreen containing oil glands, and are alternate to mostly opposite, simple, and usually entire. The flowers have a base number of five petals, though in several genera the petals are minute or absent. The stamens are usually very conspicuous, brightly colored and numerous. Important genera in this family include *Callistemon*, *Eugenia*, *Melaleuca* and *Eucalyptus* (Angiosperm Phylogeny Group, 2009).

1.10.1 Genus *Eugenia*

Eugenia is a genus of flowering plants that comprises of about 1200-1800 species (Jacqueline, 2017). Its native range extends from Africa and Madagascar to Southern Asia. Most species are evergreen trees and shrubs. Several species are grown as ornamental plants and a few produce edible fruits.

1.10.1.1 *Eugenia caryophyllus* Thunberg

E. caryophyllus, popularly called clove, is known as *kanafuru* in Yoruba, *kloovu* in Igbo, and *kanumfarii* in Hausa. It is one of the most economically important herbs in traditional medicine (Amla *et al.*, 2007). It is native to Indonesia and used as a spice in virtually all of the world's cuisine. Since ancient times, clove has been cultivated in India, Madagascar, Sri Lanka, Indonesia and Southern China. Recently, other countries like Bangladesh, Burma, Thailand and Malaysia cultivate clove on a small scale (Hossain *et al.*, 2012).

i. Description of *Eugenia caryophyllus*

The clove tree is an evergreen that grows to a height ranging from 8 to 12 m, having large leaves and sanguine flowers in numerous groups of terminal clusters. The flower buds are at first a pale colour and gradually become green, after which they develop into a bright red, when they are ready for collection. Cloves are harvested when they reach 1.5 to 2 cm long. They consist of a long calyx, terminating in four spreading sepals and four unopened petals which form a small ball in the center (Goni *et al.*, 2016).



Plate 1.5: Picture of the flower buds of *Eugenia caryophyllus* Thunberg (Myrtaceae)

ii. Ethnomedicinal uses of *Eugenia caryophyllus*

The dried buds of *E. caryophyllus* L have been widely used as a spice. Its essential oil is used in aromatherapy and to treat toothache in dental emergencies (Kim *et al.*, 1998). It is also used in the treatment of indigestion, loose stools, flatulence and nausea.

iii. Reported biological/pharmacological activities of *Eugenia caryophyllus*

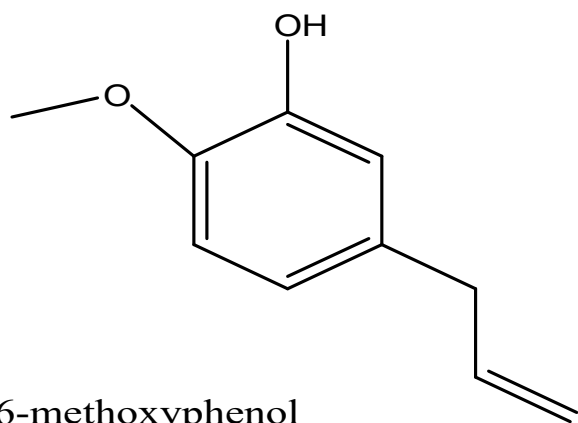
It has been reported to possess antibacterial, antifungal, insecticidal and antioxidant activities (Niwano *et al.*, 2011). Clove essential oil has been reported to show anticarcinogenic (Zheng *et al.*, 1992) and antimutagenic potential because of its strong free radical scavenging activity (Miyazawa and Hisama, 2001). Several preliminary studies suggested chemo preventive role of clove oil, particularly in cases of lung, skin and digestive cancers (Schonfelder and Schonfelder, 2004). In an another study, eugenol obtained from the essential oil was reported to suppress growth of malignant melanoma WM1205Lu of both anchorage-dependent and anchorage independent growth, decreased size of tumors and inhibited melanoma invasion and metastasis by the inhibition of two transition factors of the E2F family (Ghosh *et al.*, 2005).

iv. Reported phytochemical constituents of *Eugenia caryophyllus*

E. caryophyllus contains a wide range of bioactive compounds which includes flavonoids (eugenin, rhamnetin, kaempferol, and eugenitin), triterpenoids (oleanolic acid, stigmasterol, and campesterol), and several sesquiterpenes (Park and Shin, 2005). Its essential oil components are listed in Table 1.5.

Table 1.5: Chemical composition of *Eugenia caryophyllus* essential oil (Mohammed *et al.*, 2015)

Compound	Concentration (%)
2-Cyclohexen-1-one,2-methyl-5-(1-methylethenyl)	0.20
Phenol, 4-(2-propenyl)	0.17
3-Allyl-6-methoxyphenol	87.98
Caryophyllene	3.58
Alpha-Caryophyllene	0.55
Eugenol	7.38
1,2-Benzenedicarboxylic acid, bis(2methylpropyl) ester	0.14



1.10.2 Genus *Callistemon*

The genus *Callistemon* consists of over 30 species spread around the world (Spencer and Lumley, 1991) out of which 10 species are found in India.

1.10.2.1 *Callistemon citrinus*

Callistemon citrinus Curtis, Skeels is widely distributed across the world but it is more prevalent in tropical Asia, Queensland, New South Wales, South America and Australia, (Oyedeki *et al.*, 2009).

i. Description of *Callistemon citrinus*

C. citrinus leaves are evergreen, aromatic, alternate and lanceolate with entire margin and anomocytic stomata. Stems are grey in colour and it grows to about 5-8 m in height. Its leaves are lanceolate, 3 - 6 mm wide and 40-70 mm long. The flowers are borne in spikes 140-150 mm long with prominent red stamens 15-25 mm long. Petals are greenish or pale colored, tiny and inconspicuous (Spencer and Lumley, 1991). Flowers mature into woody capsules (Brophy *et al.*, 1997).



Figure 1.6: Picture of *Callistemon citrinus* Curtis, Skeels (Myrtaceae) growing within University of Benin, Ugbowo campus

ii. Ethnomedicinal uses of *C. citrinus*

C. citrinus hot water infusion is used for the treatment of cough, bronchitis, rheumatism, tuberculosis, antibacterial, and antifungal purposes. It's also used as an insecticide, for urinary incontinence and cleansing genitourinary tract (Tabuti *et al.*, 2010; Sutar *et al.*, 2014).

iii. Reported biological/pharmacological activities of *C. citrinus*

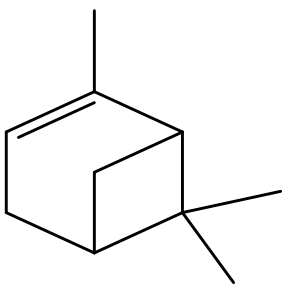
Antimicrobial, (Oyedeji *et al.*, 2009) antithrombin (Chistokhodova *et al.*, 2002)., repellent, and *in-vitro* antihelmintic activity against earthworms, tapeworms and hookworms (Srivastava *et al.*, 2001) as well as larvicidal and pupicidal values have been reported for the genus. They have been reported to provide effective control of *Ephestia kuehniella* by exerting negative effects on its immune cells (Ghasemi *et al.*, 2014).

iv. Reported phytochemical constituents of *C. citrinus*

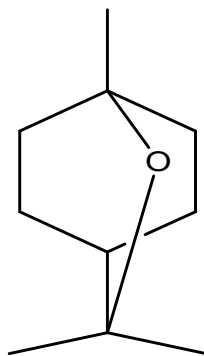
The essential oil components of *C. citrinus* are many with 1, 8-cineole as the major constituent (Table 1.6).

Table 1.6: Chemical composition of *C. citrinus* essential oil (Gupta *et al.*, 2008)

Compound	Concentration (%)
α-Pinene	24.1
β -Pinene	0.9
Myrcene	0.4
α -Phellandrene	0.6
1,8-Cineol	58.0
Terpinolene	0.5
Linalool	0.1
(E) - β - Terpineol	1.8
Eugenol	0.2
β -Caryophyllene	0.2
Allo-aromadendrene	0.2
Spathulenol	0.2
β -Copaen-4- α -ol	0.4
Cubenol	0.4
T-Cadinol	0.1
Tridecanoic acid	0.8



Pinene



1,8 Cineole

1.11 The family Mimosaceae

This family includes 40 genera and 2000 species. It composes of trees or shrubs with leaves that are mostly bipinnate, often with large glands on the rachis. Their flowers are hermaphrodite, small, spicate, racemose or capitates. They are actinomorphic and usually 5-merous. Calyx is tubular, valvate or very rarely imbricate, 5-lobed or toothed. Petals are valvate, free or connate into a short tube. Stamens equal in number to the sepals or more numerous, free or monadelphous; anthers small, 2-celled, opening lengthwise, often with a deciduous gland at the apex. Ovary is superior and consist of one carpel. Its fruit is mostly dehiscent with seeds that have scanty or no endosperm. They are found mainly in the tropics and subtropics, and are easily recognized by their usually bipinnate leaves and small flowers with valvate petals (Adesina *et al.*, 2016).

1.11.1 Genus *Tetrapleura*

This genus is native to west tropical Africa to Kenya and Angola. Species in this genus include *Tetrapleura chevalieri* (Harms) Baker f. and *Tetrapleura tetraptera* (Schumach. and Thonn) Taub.

1.11.1.1 *Tetrapleura tetraptera* Taubert

T. tetraptera is deciduous and grows on the fringe of the West and Central African rainforest zone. It is found in Uganda, Mali, Burkina Faso, Mauritania and countries from Gambia to Nigeria. It grows most luxuriantly in the rain forest, reaching 20-25 m in height, with a girth of about 1.2-3 m (Adesina *et al.*, 2016).

T. tetraptera is locally known as *Uyayak* in Ibibio, *Edeminang* in Efik, *Osakirisa* or *Oshosho* in Igbo, *Dawo* in Hausa, *Ighimiakia* in Bini and *Aridan* in Yoruba (all in Nigeria) (Ojewole and Adewunmi, 2004)

i. Description of *T. tetraptera* Taubert

The stem bark is fairly smooth, greyish-brown and thin. Leaves are sessile, glabrous or minutely hairy with a common stalk 15-30 cm long and slightly channeled on the upper phase. The fruit is persistent, hanging at the ends of branches on stout stalks 25 cm long. The fruit appears green when still tender but on maturity and ripening, it is shiny, glabrous and dark-purple-brown. It is 15-25 cm long by 4-5 cm broad (depending on size), with four longitudinal, winglike ridges nearly 2.5-3.0 cm broad. Two of these wings are woody while the other two are soft and are used for food, drinks and medicine, It has small, black, hard and flat seeds which are hidden in the pods (Adesina *et al.*, 2016).



Plate 1.7: Picture of the pod of *Tetrapleura tetraptera* Taubert (Mimosaceae)

ii. Ethnomedicinal uses of *Tetrapleura tetraptera*

The fruits are used in Nigeria for manufacturing seasoning spices, pomades and soaps due to its pleasant aroma (Essien *et al*, 1994 and Okwu, 2001) while it is used in Ghana as vitamin. It is also commonly used in soups of nursing mothers to prevent post partum contractions (Nwaiwu and Akali, 1986). An infusion of the whole fruit is usually taken for relief from feverish conditions, constipation and as an emetic. Adesina *et al.* (2016) also reported its use in the management of convulsion, leprosy, inflammation, rheumatic pains, arthritis, diabetes mellitus, epilepsy, jaundice, hypertension, schistosomiasis, asthma, malaria, and microbial infections.

iii. Reported biological/pharmacological activities of *T. tetraptera*

It is reported to have antitumor activity, anti-inflammatory properties and also inhibit HIV replication due to the presence of caffeinic acid in the fruits (Adesina, *et al*, 1980). Activity-guided fractionation of the methanol extract of the fruits afforded four saponins, which exhibited strong molluscicidal properties against the schistosomiasis-transmitting snails *Biomphalaria Glabrata* (Maillard *et al.* 1989).

Ojewole and Adesina in 1983 demonstrated the cardiovascular and neuromuscular actions of the fruit of *T. tetraptera*. Pharmacological examination through *in vitro* and *in vivo* experiments showed that it possesses hypotensive effects in anaethetized rats (Ojewole and Adesina, 1983).

Nwaiwu and Alkali (1986) also reported the anticonvulsant activity of the volatile oil from the fresh fruits of *T. tetraptera* in mice and the results obtained showed that the oil given intraperitonally offered some protection against leptazol-induced convulsions.

Noamesi *et al.* (1994) demonstrated the antiulcer effects of the stem bark of *Tetrapleura tetraptera* using HCl/EtOH- induced ulceration in fasted rat stomachs.

Okochi *et al.* (1999) also reported the trypanocidal effect of the water extract of *T. tetraptera* against *Trypanosoma brucei* in laboratory rats. The anti-inflammatory and hypoglycaemic effect of *Tetrapleura tetraptera* fruit aqueous extract was demonstrated in rats using fresh egg albumin-induced pedal oedema and streptozotocin-induced diabetes mellitus (Ojewole and Adewunmi, 2004).

Aderibigbe *et al.* (2007a) documented its anticonvulsant, analgesic and hypothermic activities in mice.

iv. Phytochemical constituents of *Tetrapleura tetraptera*

The soft parts of the fruit and the bark are known to contain sugars, tannins, traces of saponin and amino acids (Adesina *et.al.*, 1980).

L- γ - Ethylideneglutamic acid and L- γ Methyleneglutamic acid were isolated from the seeds of *T. tetraptera* (Gmelin and Larson, 1967). Activity-directed fractionation of *T. tetraptera* fruit extract yielded 7-Hydroxy-6-methoxy-2H-1-benzopyran-2-one (Scopoletin) (Adesina, 1978; Adesina *et al.*, 1980; Essien *et al.*, 1983). Further activity-directed analysis of the fruit by Adesina and Reisch, (1985) gave 3-O-[β -Dglucopyranosyl]-2'-acetamido-2'- deoxy]-oleanolic acid (Aridanin). Hentriacontane, carbohydrate residues, phenylpropanoids- ferulic acid, caffeic acid and p-coumaric acid have also been isolated (Essien *et al.*, 1983).

Activity-guided investigation of the fruit alcoholic extracts afforded 3[(2-acetamido-2-deoxy- β -D-glucopyranosyl) oxy]16 α -hydroxyolean-12-en-28-oic acid, 3-{[O- β D-galactopyranosyl]-

(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)oxy}]olean-12-en-28oic, 3-{(O-β-D-glucopyranosyl-(1→6)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)oxy}olean-12-en-28-oic, and 3-{[O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-oxy}27-hydroxyolean-12-en-28-oic acid (Maillard *et al.*, 1989; Maillard *et al.*, 1992).

Three flavonoids have also been reported isolated from the ethanolic extract of the mature fruit (Fleischer *et al.*, 2006); these were identified as 2', 4, 4'- trihydroxychalcone-Isoliquiritigenin, 2', 3, 4, 4'-tetrahydroxychalcone-Butein and 4', 5, 7trihydroxyflavanone-Naringenin.

Noté *et al.* in 2009 documented the presence of tetrapteroside A, 3-O-{6-O-[(2E,6S)-2,6-dimethyl-6-hydroxyocta2,7-dienoyl]-β-D-glucopyranosyl-(1→2)-β-glucopyranosyl-(1→3)-β-D-glucopyranosyl(1→4)-[β-D-glucopyranosyl-(1→2)]-β-lucopyranosyl}-3,27-dihydroxyoleanolic acid and tetrapteroside B, 3-O-{β-D-glucopyranosyl-(1→2)-6-O-(E)-feruloyl]-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl(1→4)-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl}-3,27-dihydroxyoleanolic acid).

1.12 Rationale for the study

Cancer is now a major cause of death in the world. Currently, over a hundred types of cancers are known. Among different cancers, breast and cervical cancers are the most prevalent malignant diseases in women. Different forms of cancer are treated with synthetic drugs and chemotherapy which are accompanied with scarcity, prohibitively high costs and life threatening side effects. There is therefore an urgent need to evaluate medicinal plants used to treat tumor-related ailments in ethnomedicine with a view to explore their abundance, claimed potency with low or no side effects and probable presence of novel anticancer constituents. This step becomes

imperative considering the fact that most of the plants are used naturally as local aromatic spices due to the presence of essential oils.

1.13 Aim and objectives

The aim of this study was to investigate the cytotoxic and antiproliferative activities of the essential oils of *Zingiber officinale* (rhizome), *Eugenia caryophyllus* (flower), *Callistemon citrinus* (leaves), *Hyptis suaveolens* (leaves), *Ocimum basilicum* (leaves), *Ocimum gratissimum* (leaves) as well as *Tetrapleura tetraptera* pod extract and to isolate and characterize active constituents from the essential oils/extract.

The objectives were to:

1. evaluate the cytotoxic and antiproliferative activities of the selected plants using bench-top experiments with tadpoles and guinea corn radicle
2. isolate and characterize active constituents from the volatile oils/extract using bio-activity guided methods
3. evaluate the anticancer potential of the isolated compounds
4. analyze/characterize the essential oils and compounds using GCMS and other spectroscopic methods (where applicable).

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals and reagents

The chemicals used include dimethyl sulfoxide (DMSO), fetal bovine serum (FBS), gentamycin sulphate, streptomycin, penicillin, trypan blue and trypsin-EDTA (Sigma Co ,St. Louis, Mo, USA), Doxorubicin (ICN, USA), Roswell Park Memorial Institute- 1640 medium (RPMI-1640), 3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) (Sigma-Aldrich).

2.1.2 Solvents

Solvents of analytical grade were purchased and re-distilled before use. These include methanol, n-hexane, dichloromethane, Acetone, ethyl acetate, chloroform (BDH Chemicals, Poole, England), dimethylsulphoxide (DMSO) (Riedel-de Hean, Germany), and distilled water.

2.1.3 Consumables

Culture plates (96-well, transparent with flat-bottom), flasks (25cm² and 75 cm²), Vials, centrifuge tubes (15 and 50 mL), serological pipette (1, 5, and 10 mL), were purchased from Falcon BD (USA), Micro centrifuge tubes (2 mL) were purchased from Kartel (Italy).

2.1.4 Equipment

The equipment used include analytical balance, lamp, milligram balance (Precisia, Switzerland), centrifuge and incubator, CO₂ (Kendro Lab Products, Germany), Spectrophotometer, Safety cabinet class 2 (Heraeus, Germany), Microscope: TS-100 (Nikon, Japan), multiwell micro plate

reader (Stat fax 2100, Awareness technology, USA), multiwell plate shaker (PMS 1000, Grant Instruments, England), Ultrasonic bath (MXB6, Grant, England) and Neubauer's chamber (0.1 mm and 0.0025 mm², HBG, Germany), recycling HPLC (Japan analytical company limited, model no LC 908).

2.2 Collection and identification of plant material

The plants *Hyptis suaveolens* (leaves), *Ocimum basilicum* (leaves), *Ocimum gratissimum* (leaves), *Eugenia caryophyllus* (flower buds), *Callistemon citrinus* (leaves), *Zingiber officinale* (rhizome) and *Tetrapleura tetraptera* pod were collected between September and November 2016 in Benin City, Edo State, Nigeria. The identity of the plants were confirmed by Dr Henry Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria where voucher numbers UBH C384 (*E. caryophyllus*), UBH C383 (*C. citrinus*), UBH Z384 (*Zingiber officinale*), UBH O341 (*Ocimum gratissimum*), UBH O461 (*Ocimum basilicum*), UBH H462 (*Hyptis suaveolens*) were obtained. *T. tetraptera* was authenticated at Forest Research Institute of Nigeria (FRIN), Ibadan and herbarium specimen number FHI 110614 was assigned to it.

2.3 Extraction of plant materials

About 2 kg each of *Z. officinale* (rhizome), *H. suaveolens* (leaves), *O. basilicum* (leaves), *O. gratissimum* (leaves), *E. caryophyllus* (flower buds), and *C. citrinus* (leaves) were extracted differently in batches by hydro-distillation method with the aid of a Clavenger-type apparatus to obtain their volatile oils.

T. tetraptera pod was air-dried for 3 days after which it was powdered using an electric milling machine. About 2.2 kg of the powdered material was exhaustively extracted with methanol using

a Soxhlet apparatus. The extract was concentrated over a water bath (maintained at 50⁰C) and kept in the refrigerator until required.

2.4 Preliminary Biological Screening of Extracts

2.4.1 Determination of growth inhibitory effects on *Sorghum bicolor* radicle

Guinea corn (*S. bicolor*) seeds obtained from Uselu market in Benin City were cleansed with absolute alcohol (95% ethanol), and a simple viability test of the seeds was determined by adding water and immediately decanting thereby removing the floating compromised seeds. Those that remained submerged in water were selected and dried before use.

The extract and volatile oils were separately constituted into concentrations of 1, 2, 5, 10, 20 and 30 mg/mL using 2% Tween 80 in distilled water. Ten (10) mL of the different concentrations were poured into Petri dishes covered with filter paper (Whatman No 1) underlaid with cotton wool after which twenty (20) viable seeds were spread on each filter paper and incubated in a dark environment. Lower concentrations of 0.1, 0.25 and 0.5 were also included for *S. aromaticum* oil. The lengths (mm) of the radicles emerging from the seeds were taken at 24, 48, 72 and 96 h. The control seeds were treated with 10 ml of distilled water containing 2% Tween 80. The experiments were carried out in triplicates for all concentrations and controls (Ayinde and Agbakwuru, 2010).

2.4.2 Determination of cytotoxic effects using tadpoles (*Raniceps ranninus*)

Tadpoles of *R. ranninus* were obtained from stagnant water in the Faculty of Pharmacy, University of Benin and identified by Professor M. I. Aisien, Animal Parasitologist, Department of Animal and Environmental Biology, Faculty of Life Science, University of Benin.

Ten (10) tadpoles of same size were selected with the aid of a Pasteur pipette into 50-ml capacity beakers containing 15 mL of the water from the habitat of the tadpoles, which was made up to 49 mL with distilled water. The volume was separately made up to 50 mL with 0.25, 0.5, 1, 2, and 5 mg/mL of each of the volatile oils and extract thereby making final concentrations of 5, 10, 20, 40, and 100 µg/mL, respectively. The controls for each of the experiments contained 2% Tween 80 in distilled water. The tadpoles were observed for 24 h and their mortality was indicated by lack of motion and complete submergence in water. The experiment was carried out in triplicates for each concentration. (Ayinde and Agbakwuru, 2010).

2.4.3 Determination of cytotoxic effects using brine shrimps (*Artemia salina*)

About 20 mg of each volatile oil and the extract was dissolved separately in 2 mL distilled water and from this solution, concentrations of 10, 100 and 1000 µg/mL were obtained. The solvents were allowed to evaporate overnight. After 48 h of hatching and maturation of nauplii, 10 larvae were transferred to each vial using a Pasteur pipette. The volume was made up to 5 mL with seawater (38 g/L, pH 7.4) and the vials were incubated at 25-27°C for 24 h under illumination. Other vials containing the solvent and reference cytotoxic drug (etoposide) served as the negative and positive controls respectively (McLaughlin, 1991).

2.5 Determination of Anticancer Activity using Cancer Cell Lines

The anticancer activity was performed using MTT assay on human breast cancer (AU 565 and MDA-MB231) and cervical cancer (HeLa) cell lines in the presence of various concentrations of plant extracts and fractions (Puig *et al.*, 2011).

2.5.1 Growth and maintenance of cells

Au565 and HeLa were grown in ATCC modified complete RPMI medium, supplemented with 90% FBS, 1% penicillin, 1% streptomycin and then filtered by 0.22 micrometer steric cup filter assembly (Puig *et al.*, 2011).

2.5.2 Revival of cancer cell lines

Cryovials of the cells were thawed by hand thumbing for a few minutes, and immediately transferred into a falcon tube containing 9 ml of complete ATCC RPMI. Cells were centrifuged at 1000 rpm for 5 minutes at 4°C then supernatant was discarded gently, and pellet was resuspended in 1 ml of complete growth media. Cell suspension was then transferred into a 25 cc flask containing 5 ml of complete media after which the cells were incubated at 37 °C under 5% humidified atmosphere of CO₂ until confluent to 60% (Kritsanawong *et al.*, 2016).

2.5.3 Sub-culturing of cancer cells

When the cells were grown up to 60-70% visible confluency in the flask, the media was removed after which the cells were further washed with PBS (Phosphate Buffer Saline) in order to adjust the pH, remove dead or detached cells as well as traces of media left behind. After removal of PBS with 10 mL pipette, the cells were trypsinized using trypsin EDTA (1 ml for 25 cc and 2 ml for 75 cc flask) to allow cell detachment. The enzyme was de-activated after 5 – 7 min by adding a volume of complete media which was double the volume of trypsin. The cells were then centrifuged at 1000 rpm for 5 min at 23 °C, pellet was resuspended in 1 ml complete media, and cells were sub-cultured (Puig *et al.*, 2011; Kritsanawong *et al.*, 2016).

2.5.4 Cell viability, concentration and culturing

Cell viability and concentration were determined using trypan blue dye exclusion method with hemocytometer. Trypan blue solution (10 μ L, 0.4% in 0.9% saline) was mixed with the cell suspension (10 μ L) and a sample (10 μ L) was placed on the hemocytometer. The cells were counted, blue cells were considered as non-viable while the bright and translucent (unstained) cells as viable. After cell viability and cell concentration determination, the cancer cells were placed in wells of the 96-well plate at a density of 10,000 cells/well/100 μ L and allowed to incubate for 24 h and after which it was treated with the extracts and compounds (Puig *et al.*, 2011; Kritsanawong *et al.*, 2016).

2.5.5 Preparation of extracts, fractions and compounds for cytotoxicity assay

The stock solution of plant extracts and fractions (20 mg/mL) were prepared in sterile DMSO (100%) and later diluted to 1 - 50 μ g/mL. The stock solutions for doxorubicin (1 mM) were prepared in sterile distilled water and kept at -80°C until required. For the compounds, a working concentration of 50 μ M was used. The dilutions were prepared in RPMI-1640 containing gentamycin (50 μ g/mL) on the day of the experiment (Puig *et al.*, 2011; Kritsanawong *et al.*, 2016).

2.5.6 Anticancer activity using MTT

The cancer cells were cultured in microplates (tissue culture treated, 96 well, flat bottoms) to the final volume of 100 μ L per well culture medium in a specific environmental condition such as, 37 °C temperature and, 5% CO₂ for the healthy growth of the cells. The next day, when the cells were adhered, the culture media was removed from each well. The cells were treated in

triplicates with decreasing concentrations of the extract and the volatile oils. The cells culture was allowed to incubate for 48 hours at 37 °C and in humidified atmosphere of 5% CO₂. After the incubation of 48 h, 200 µL of MTT (0.5mM) dye was added in each well and then incubated for another 4 h at 37°C in 5% of CO₂. The resulting formazan crystals were dissolved in 100 µL of DMSO and shaken thoroughly for a minute on the shaker (Kritsanawong *et al.*, 2016).

The absorbance of the resulting solution was measured at 570 nm against a background control which is blank using a microplate reader. The measured absorbance directly correlates to the number of viable cells (Puig *et al.*, 2011; Kritsanawong *et al.*, 2016).

The extract/volatile oils which possessed more than 50% inhibition were considered active. IC₅₀ (concentration at which 50% inhibition of the cells occurred) was calculated by EZ-Fit software. The experiments were performed in triplicates. The major components of some of the volatile oils were isolated. Bioactivity directed fractionation was carried out on *T. tetraptera pod.* These assays were repeated for the fractions and subsequent isolates at various concentrations.

2.6 Column chromatography of *O. basilicum* volatile oil

O. basilicum oil (13 mL) was triturated with silica (200-400 mesh) and subjected to column chromatography. Isocratic elution was carried out using 1200 mL of C₆H₁₄ (100%) with 20 mL collected in each test tube. The resulting fractions (67) were collected and bulked based on their TLC profile as shown in table 2.1.

Table 2.1: Column chromatography fractions of *O. basilicum* volatile oil

Fractions	Codes	Weight (mg)
4-8	CF ₁ B	7800
9	-	-
10-14	CF ₂ B	20
15-20	CF ₃ B	30
21-47	CF ₄ B	220
48-67	CF ₅ B	200

2.6.1 Growth inhibitory and cytotoxic effects of the column chromatography fractions of *O. basilicum* volatile oil

The growth inhibitory activities of the fractions were determined at 2 mg/mL following the method stated in section 2.4.1 and the anticancer activity determined at concentrations between 1 - 50 µg/mL using the MTT assay (2.5.6).

2.6.2 Preparative TLC of fraction CF₁B and bioassay of its subfractions

Due to its high yield and conspicuous spots as well as its high growth inhibitory activity, fraction CF₁B (5 g) was subjected to preparative TLC analysis on 0.5 mm thick Silica gel G (10-40 mesh) using C₆H₁₄ (100%). Multiple developments were carried out. Two conspicuous bands were scrapped, eluted, concentrated and coded as SF₁B and SF₂B. TLC analyses of these products were carried out using C₆H₁₄ (100%). Their weights were noted (table 2.2) after which they were subjected to growth inhibitory tests at 2 mg/mL concentration and cytotoxic analyses on AU 565 and HeLa at 50 µg/mL.

Table 2.2: Preparative TLC subfractions of *O. basilicum* volatile oil

Bands	Codes	Weight (mg)
1	SF ₁ B	40
2	SF ₂ B	1130

2.7 Column chromatography of *O. gratissimum* volatile oil

O. gratissimum oil (15 mL) was triturated with silica (200-400 mesh) and subjected to column chromatography. Gradient elution was carried out using 200 mL each of C₆H₁₄ (100%) and C₆H₁₄- CH₃COOC₂H₅ (99:1, 98:2, 97:3, 96:4, 95:5, 94:6, 93:7, 92:8, 91:9, 90:10) with 20 mL collected in each test tube. One hundred and eighteen (118) fractions were collected and bulked according to their TLC profile as shown in Table 2.3.

Table 2.3: Column chromatography fractions of *O. gratissimum* volatile oil

Fractions	Codes	Weight (mg)
5-12	CF ₁ G	4300
13-28	CF ₂ G	1680
29-40	CF ₃ G	190
41-61	CF ₄ G	120
62-90	CF ₅ G	50
91-118	CF ₆ G	10

2.7.1 Growth inhibitory and cytotoxic effects of the column chromatography fractions of *O. gratissimum* volatile oil

The growth inhibitory activities of the fractions were determined at 1 mg/mL following the method stated in section 2.4.1 and the anticancer activity determined at concentrations between 1 - 50 µg/mL following methods stated in section 2.5.6.

2.7.2 Preparative TLC of fraction CF₁G and bioassay of its subfractions

As a result of the high yield, conspicuous nature and resolutions of its spots on TLC plate, coupled with high growth inhibitory activity, fraction CF₁G (5 mL) was subjected to preparative TLC analysis on 0.5 mm thick Silica gel (10-40 mesh) using C₆H₁₄- CH₃COOC₂H₅ (9.7:0.3). Multiple developments were carried out. The conspicuous bands were scrapped, eluted, concentrated and coded as SF₁G and SF₂G. TLC analyses of these products were carried out using C₆H₁₄- CH₃COOC₂H₅ (9.7:0.3). Their weights were noted (table 2.4) then they were subjected to growth inhibitory tests at 1 mg/mL concentration and cytotoxic analyses on AU 565 and HeLa at 50 µg/mL.

Table 2.4: Preparative TLC subfractions of *O. gratissimum* volatile oil

Bands	Codes	Weight (mg)
1	SF ₁ G	390
2	SF ₂ G	680

2.8 Column chromatography of *E. caryophyllus* volatile oil

E. caryophyllus oil (15 mL) was subjected to column chromatography using silica gel (200-400 mesh). Gradient elution was done using 200 mL each of C₆H₁₄ (100%) and C₆H₁₄-CH₃COOC₂H₅ (99.5:0.5, 99:1, 98.5:1.5, 98:2, 97.5:2.5, 97:3, 96:4, 95:5, 94:6, 93:7, 92:8, 91:9, 90:10) with 20 mL collected in each test tube. The fractions (58) were collected and bulked according to their TLC profile as shown in table 2.5.

Table 2.5: Column chromatography fractions of *E. caryophyllus* volatile oil

Fractions	Codes	Weight (mg)
4-6	CF ₁ E	12,160
7-11	CF ₂ E	3210
12-20	CF ₃ E	1400
21-32	CF ₄ E	2200
33-58	CF ₅ E	500

2.8.1 Growth inhibitory and cytotoxic effects of the column chromatography fractions of *E. caryophyllus* volatile oil

The growth inhibitory activities of the fractions were determined at 0.25 mg/mL and the anticancer activity determined at concentrations between 1 - 50 µg/mL.

2.8.2 Preparative TLC of fraction CF₁E and bioassay of its subfractions

Fraction CF₁E (5 mL) was subjected to preparative TLC analysis on 0.5 mm thick Silica gel (10-40 mesh) using C₆H₁₄-CH₂Cl₂ (9:1). This was as a result of its high yield and conspicuous nature, coupled with its high growth inhibitory activity. Multiple developments were carried out. The conspicuous bands were scrapped, eluted, concentrated and coded as SF₁E and SF₂E. TLC analyses of these products were carried out using C₆H₁₄-CH₂Cl₂ (9:1). Their weights were noted (table 2.6) after which they were subjected to growth inhibitory tests at 0.25 mg/mL concentration and cytotoxic analyses using AU 565 and HeLa at 50 µg/mL.

Table 2.6: Preparative TLC subfractions of *E. caryophyllus* volatile oil

Bands	Codes	Weight (mg)
1	SF ₁ E	30
2	SF ₂ E	700

2.9 Column chromatography of *C. citrinus* volatile oil

C. citrinus oil (20 mL) was triturated with silica and subjected to column chromatography. Gradient elution was carried out with 200 mL each of C_6H_{14} (100%), and C_6H_{14} - $CH_3COOC_2H_5$ (99:1, 98:2, 97:3, 96:4, 95:5, 94:6, 93:7, 92:8) with 20 mL collected in each test tube. Based on the TLC profiles obtained, similar fractions were bulked together as shown in Table 2.7.

Table 2.7: Column chromatography fractions of *C. citrinus* volatile oil

Fractions	Codes	Weight (mg)
3-5	CF ₁ C	1930
6-14	CF ₂ C	1550
15-21	CF ₃ C	70
22-38	CF ₄ C	770
39-66	CF ₅ C	400
67-75	CF ₆ C	90

2.9.1 Growth inhibitory and cytotoxic effects of the column chromatography fractions of *C. citrinus* volatile oil

The growth inhibitory activities of the fractions were determined at 1 mg/mL following the method stated in section 2.4.1 and the anticancer activity on AU 565 and HeLa determined at concentrations between 1-50 µg/mL following methods stated in sections 2.5.6.

2.10 GC-MS analysis

Gas chromatography-mass spectroscopy was carried out on the volatile oils and sub-fractions. An Agilent technologies 7000 GC/MS triple quadrupole mass spectrometer with OPTIMA-5-ZB-5 column having the following dimensions: length – 30 m, internal diameter - 250 µm and thickness - 0.25 µm. Electron ionization (EI) with ionization energy of 70eV was used. Helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1.129 mL/min and injection volume was 2 µL (split ratio 15:1). Ion-source temperature was 250°C. The oven temperature was programmed from 50°C (isothermal for 15 min.), with a reduction to 8°C/min, to a further increase to 180°C for 15 min, then 15°C/min and finally to 290°C for 5 min. Total run time was 58.583 min. Software adopted to handle mass spectra and chromatogram was a Chemstation and compounds were identified from NIST library match.

2.11 Organic solvent partitioning of the methanol extract of *T. tetraptera* pod

Purification was carried out on the methanol extract of *T. tetraptera* pod. 150g of the methanol pod extract was re-dissolved in methanol-water (1:1) and partitioned exhaustively in three aliquots with hexane (900 mL x 3) and chloroform (900 mL x 4) in a separating funnel. This was

repeated until a clear lower layer was obtained. The resulting hexane, chloroform and aqueous fractions were concentrated to dryness over a water bath (at 50°C) and their respective yields noted.

2.11.1 Growth inhibitory and cytotoxic effects of the solvent fractions of *T. tetraptera* pod

The growth inhibitory activities of the solvent fractions on *S. bicolor* seeds were determined at 5 mg/mL following the method stated in section 2.4.1. The anticancer activity was determined at concentrations between 1-50 µg/mL following the MTT assay procedure stated in section 2.5.6.

2.12 Vacuum liquid chromatography (VLC) of the chloroform fraction of *T. tetraptera* pod

About 31 g of the chloroform fraction was loaded on silica gel G (60-120 µm) in a Sinta glass (No. 5) attached to a Buchner flask connected to a vacuum pump. Gradient elution was performed using 400 mL of the following solvents: C₆H₁₄ (100%), C₆H₁₄-CHCl₃ (1:1), CHCl₃ (100%), CHCl₃-CH₃COOC₂H₅ (1:1), CH₃COOC₂H₅ (100%), CH₃COOC₂H₅-CH₃OH (1:1), CH₃OH (100%), CH₃OH-H₂O (1:1). The various fractions were reduced to dryness over a water bath and their respective yields noted (Table 2.8).

The fractions were further subjected to antiproliferative activity on *S. bicolor* seeds at 2 mg/mL as stated in section 2.4.1 and anticancer activity using MTT assay on AU 565 at concentrations between 1-50 µg/mL.

Table 2.8: The VLC subfractions of the chloroform fraction of *T. tetraptera* pod

Fractions	Weight (mg)	% Yield
A - Hexane (100%)	40	0.13
B - Hexane:Chloroform (1:1)	570	1.84
C - Chloroform (100%)	420	1.35
D - Chloroform:Ethylacetate (1:1)	610	1.97
E - Ethylacetate (100%)	200	0.65
F - Ethylacetate:Methanol (1:1)	3170	10.23
G - Methanol (100%)	4380	14.13
H - Methanol:Water (1:1)	8290	26.74

2.13 Column chromatography of bulked VLC fractions C and D and anticancer activity of the resulting sub-fractions

VLC fractions C and D (1 g) were bulked together based on similarity in their TLC profile and biological activities. The combination was subjected to column chromatography using silica gel (200-400 mesh). Gradient elution was done using 200 mL of C_6H_{14} (100%), C_6H_{14} - $CH_3COOC_2H_5$ (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95) and CH_3OH (100%). Ninety-six (96) fractions were collected and bulked based on their TLC profile as shown below (table 2.9). They were later subjected to anticancer activity on AU565 at 1-50 $\mu g/mL$ concentrations.

Table 2.9: Column chromatography subfractions from VLC fractions C and D

Codes	Fractions	Weight (mg)
C1	3-20	40
C2	21-34	80
C3	35-37	10
C4	38-50	170
C5	51-68	160
C6	69-96	70

2.13.1 Preparative high pressure liquid chromatography of bulked subfraction C2

Having shown the highest anticancer activity, and also based on the conspicuous nature and resolution of its spots, subfraction C2 was subjected to normal phase Prep-HPLC. The column specifications are internal diameter (10 mm), length (250 mm), containing silica with particle size of 4 μm , UV detector (UV – 50), RI detector (RI – 5), flow rate (4 mm/min). Isocratic elution was carried out using C_6H_{14} - $\text{CH}_3\text{COOC}_2\text{H}_5$ (7:3). Twenty five (25) fractions were obtained with fractions 11-13 and 19-22 showing single peaks and producing white precipitates. Having shown similar spots on commercial TLC plates, they were bulked separately and coded as TT1 and TT2 respectively. Spectroscopic analyses of the two compounds were carried out using NMR and mass spectroscopy after which they were subjected to anticancer activity on AU 565 cell line at 50 μM .

2.14 Column chromatography of bulked VLC fractions F and G (from 2.12)

Based on the similar TLC profile and biological activities obtained, VLC fractions F and G were bulked together and 4 g was subjected to column chromatography. Gradient elution was done using 200 mL of C_6H_{14} (100%), C_6H_{14} - CHCl_3 (80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90), CHCl_3 (100%), CHCl_3 - $\text{CH}_3\text{COOC}_2\text{H}_5$ (80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90), $\text{CH}_3\text{COOC}_2\text{H}_5$ (100%) and $\text{CH}_3\text{COOC}_2\text{H}_5$ - CH_3OH (80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90). Seventy-eight (78) fractions were collected and bulked based on their TLC profile as shown below (table 2.10).

Table 2.10: Column chromatography subfractions from bulked VLC fractions F and G

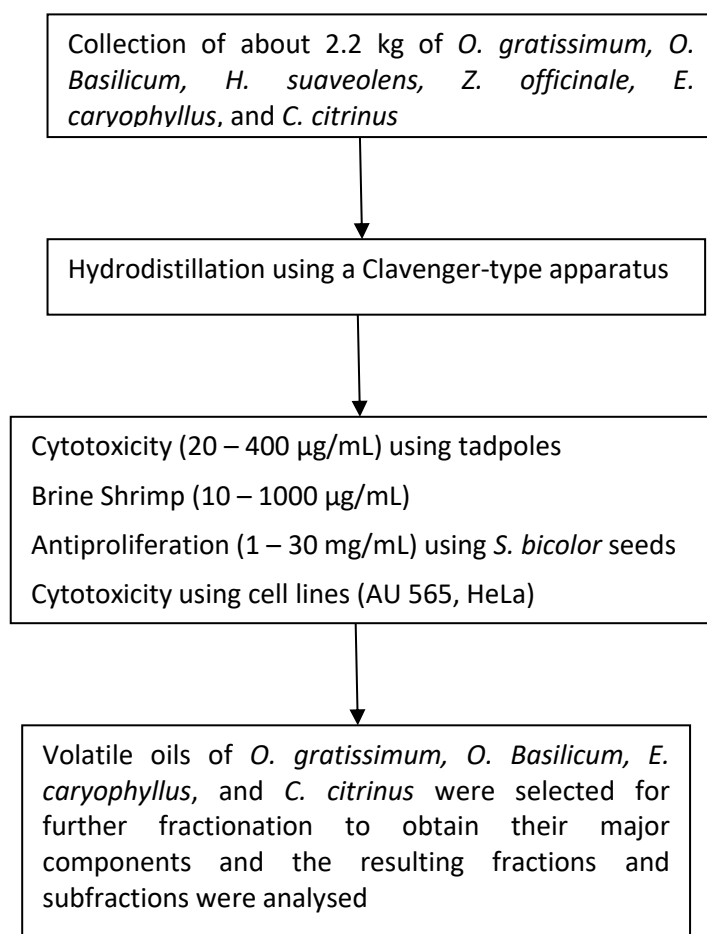
Codes	Fractions	Weight (mg)
F1	1-13	5
F2	14-15	3
F3	16-20	13
F4	21-24	14
F5	25-28	429
F6	29-34	28
F7	35-39	58
F8	40-45	128
F9	46-53	136
F10	54-57	300
F11	58-62	260
F12	63-66	713
F13	67-69	180
F14	70-74	420
F15	75-78	470

2.14.1 Preparative high pressure liquid chromatography of bulked subfraction F11

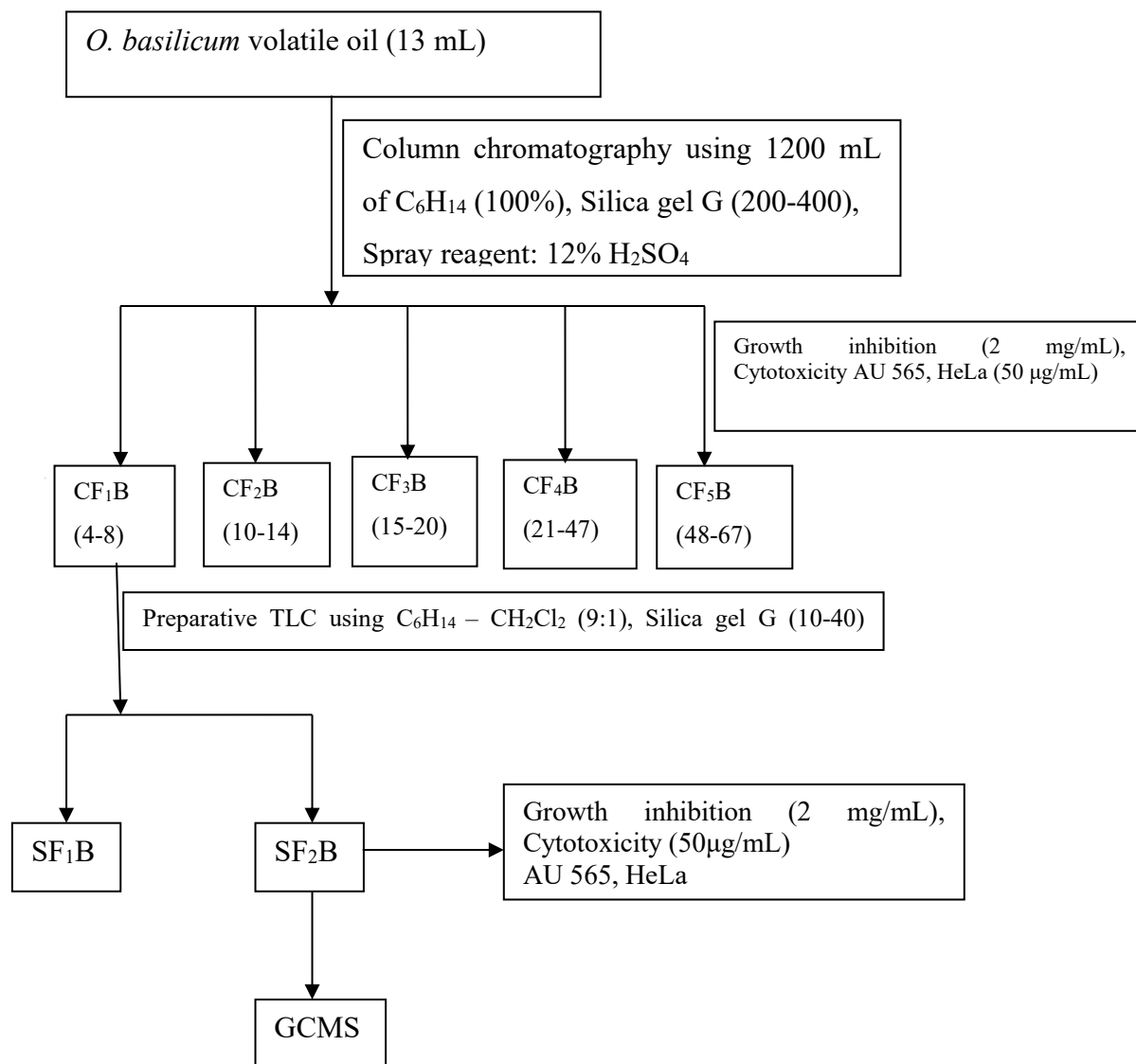
Subfraction F11 produced white precipitates which settled at the bottom of the test tube. The upper yellow solution was decanted and the precipitate rinsed repeatedly with ethylacetate. The washed precipitate (170 mg) was subjected to reversed phase Prep-HPLC with column specifications of internal diameter (10 mm), length (250 mm), particle size (4 μ m), octadecyl silane H80, UV detector (UV – 50), RI detector (RI – 5), flow rate (4 mm/min). Isocratic elution was carried out using CH₃OH-H₂O (9:1). Seven (7) fractions were obtained with fraction 6 showing one spot on commercial TLC plate. It was reduced to dryness, coded as TRA and subjected to spectroscopic analyses using ¹NMR and mass spectroscopy. It was also subjected to anticancer activity on MDA-MB231 at 50 μ M concentration.

2.15 Statistical analysis

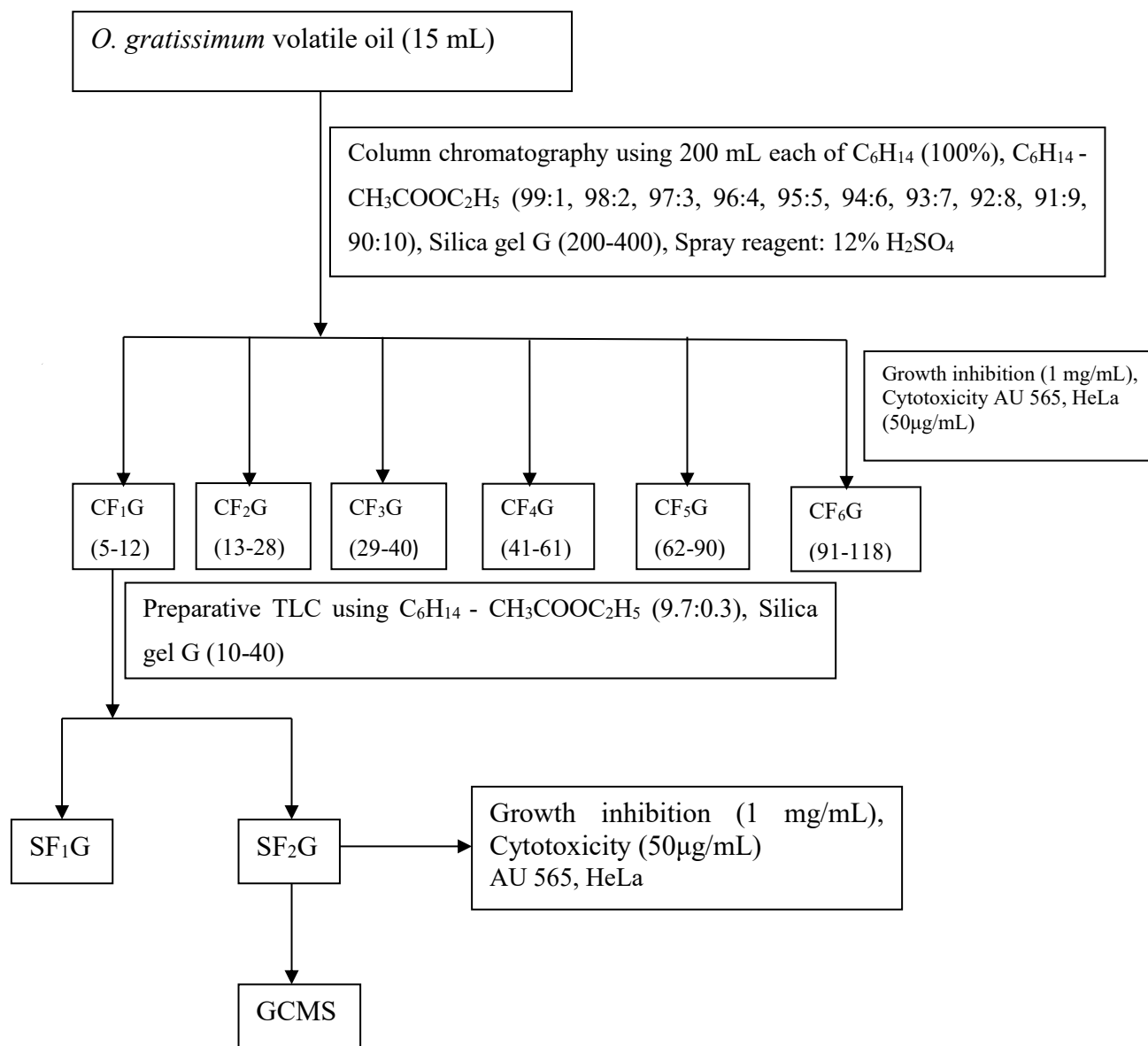
All data were expressed as mean $\pm$ SD and One-way Analysis of Variance (ANOVA) statistical test using SPSS version 21 was used to test for significance. $P < 0.05$ was considered significant. The entire methodology is summarized in the flow charts below.



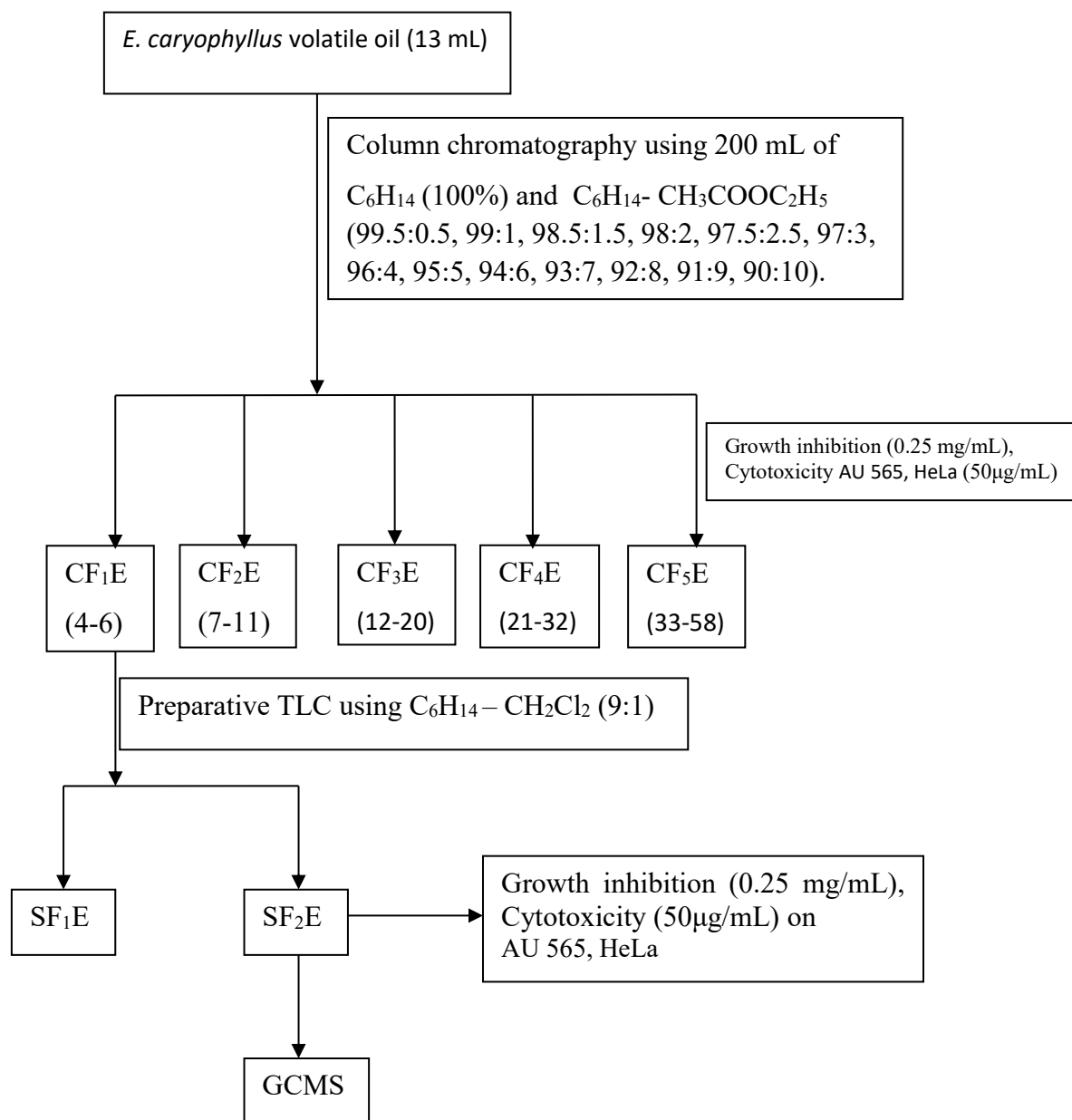
Flow chart 2.1: Flow chart showing extraction of six plant materials and preliminary screening of their volatile oils



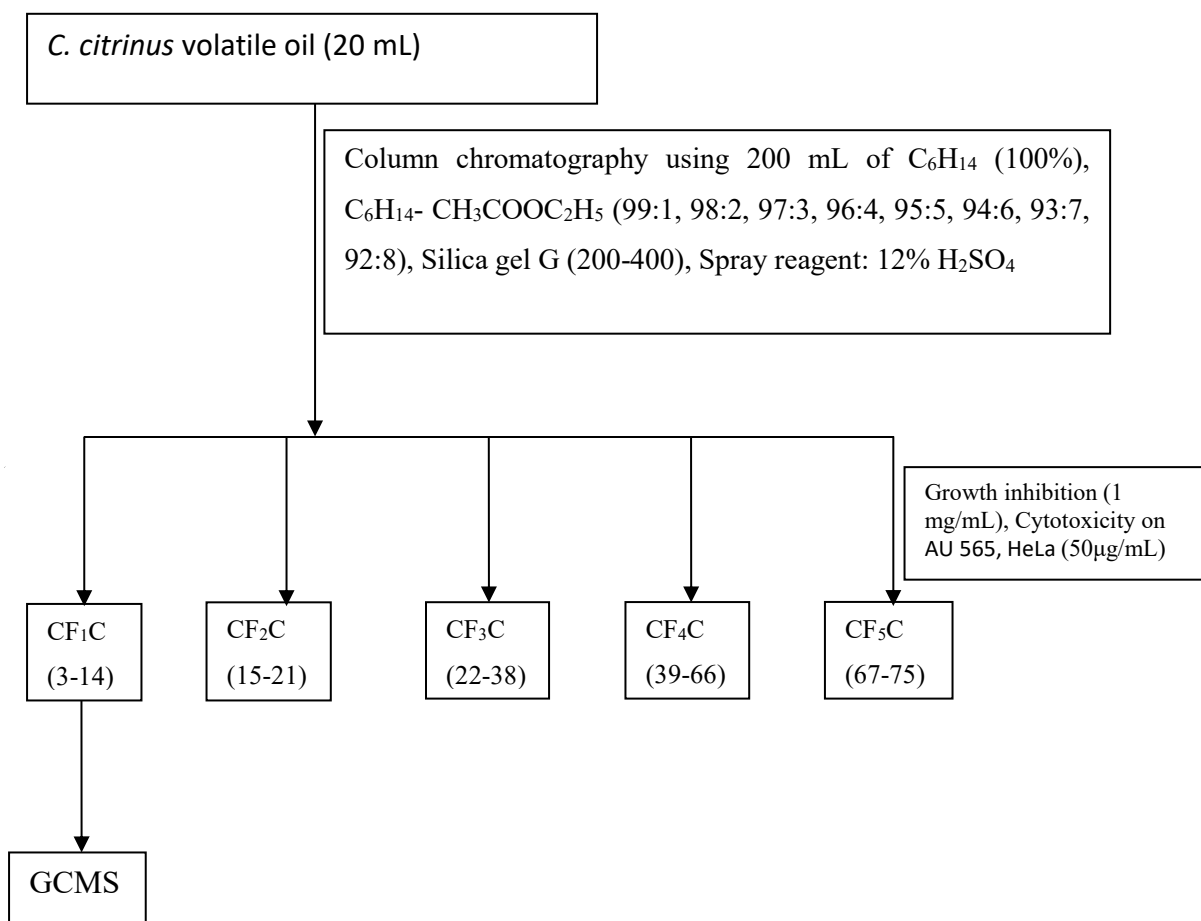
Flow chart 2.2: Flow chart showing bioactivity guided fractionation of *O. basilicum* volatile oil



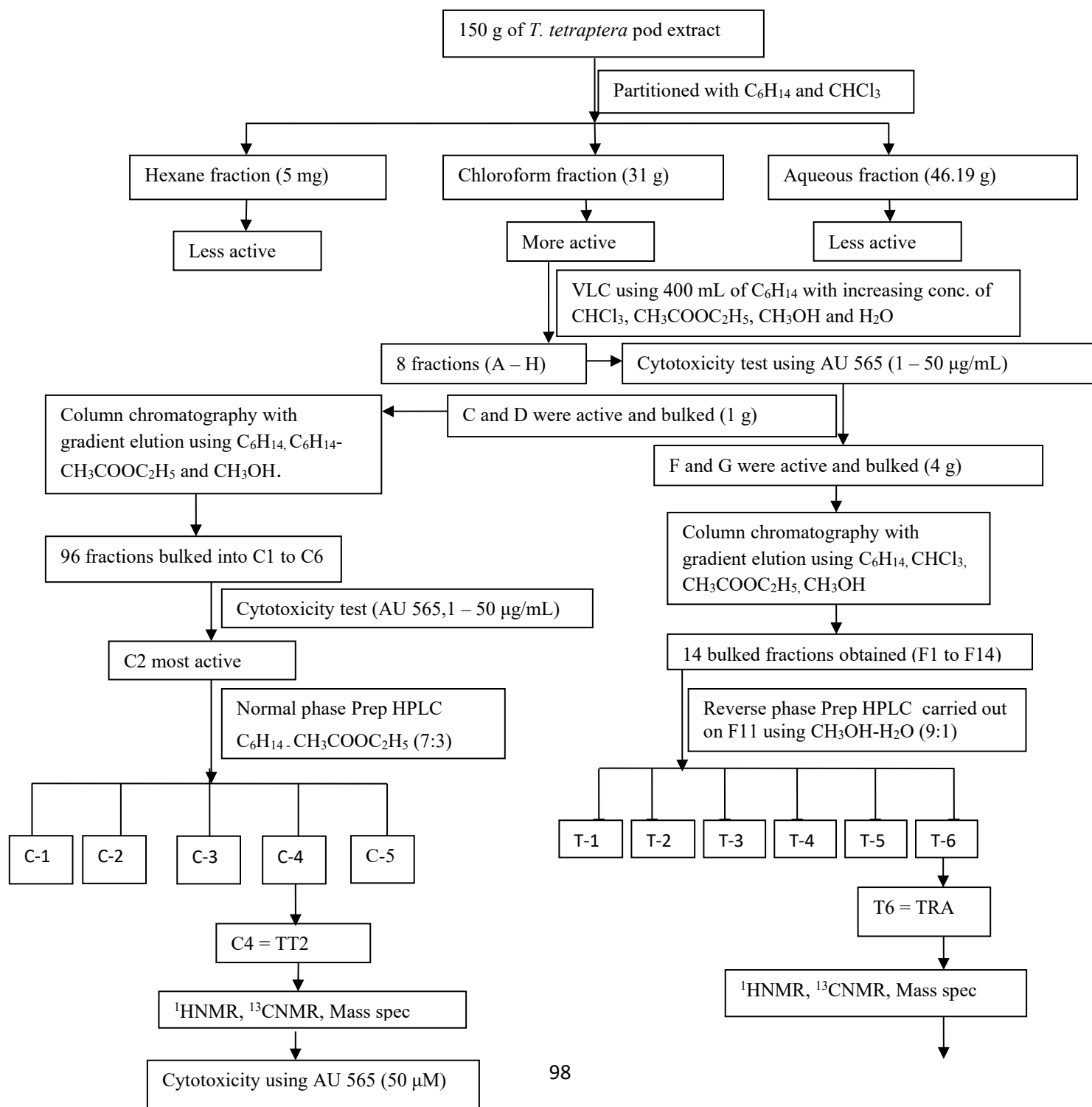
Flow chart 2.3: Flow chart showing bioactivity guided fractionation of *O. gratissimum* volatile oil



Flow chart 2.4: Flow chart showing bioactivity guided fractionation of *E. caryophyllus* volatile oil



Flow chart 2.5: Flow chart showing bioactivity guided fractionation of *C. citrinus* volatile oil



Flow chart 2.6: Flow chart showing bioactivity guided fractionation of *T. tetraptera* pod

CHAPTER THREE

3.0 RESULTS

3.1 Yields of the plants essential oils/extract

The yield varied from plant to plant. *E. caryophyllus* bud gave the highest yield of 15.13% while the rhizome of *Z. officinale* produced the least yield of 0.16%. However, the extraction of *T. tetraptera* pod produced a yield of 15% (Table 3.1).

Table 3.1: Percentage yield of the extracts

Plant	Part	Yield (%)
<i>Zingiber officinale</i>	Rhizome	0.16
<i>Hyptis suaveolens</i>	Leaf	0.2
<i>Ocimum basilicum</i>	Leaf	0.33
<i>Ocimum gratissimum</i>	Leaf	1.6
<i>Eugenia caryophyllus</i>	Flower bud	15.13
<i>Callistemom citrinus</i>	Leaf	0.6
<i>Tetrapleura tetraptera</i>	Pod	15

3.2 Results of the growth inhibitory effects of the essential oils/extracts on radicle length of *S. bicolor* seeds

The volatile oils of all the plants were observed to remarkably inhibit the growth of the radicles in a concentration dependent manner. These inhibitory effects were sustained throughout the period of the assay. *E. caryophyllus*, *Z. officinale*, and *O. gratissimum* produced the highest activities.

5 mg/mL of *E. caryophyllus* oil completely inhibited the growth of the radicles (Figure 3.1 – 3.4). The different concentrations of *C. citrinus* and *Z. officinale* essential oils were observed to also retard the growth of the seed radicles. An average length of 14.90 ± 0.57 mm in the controls at 96 h was reduced to 4.68 ± 0.54 mm in seeds treated with 5 mg/ml concentration of *Z. officinale* oil, showing 68.59% reduction (Figure 3.4).

At the same concentration, *C. citrinus* oil reduced the control length of 13.68 ± 0.20 to 7.65 ± 1.05 , indicating 44.08% reduction.

T. tetraptera pod extract gave radicle length of 8.98 ± 0.15 mm at 96 h as against the control which produced 31.32 ± 0.31 mm, indicating 71.33% reduction in length (Figure 3.1-3.4).

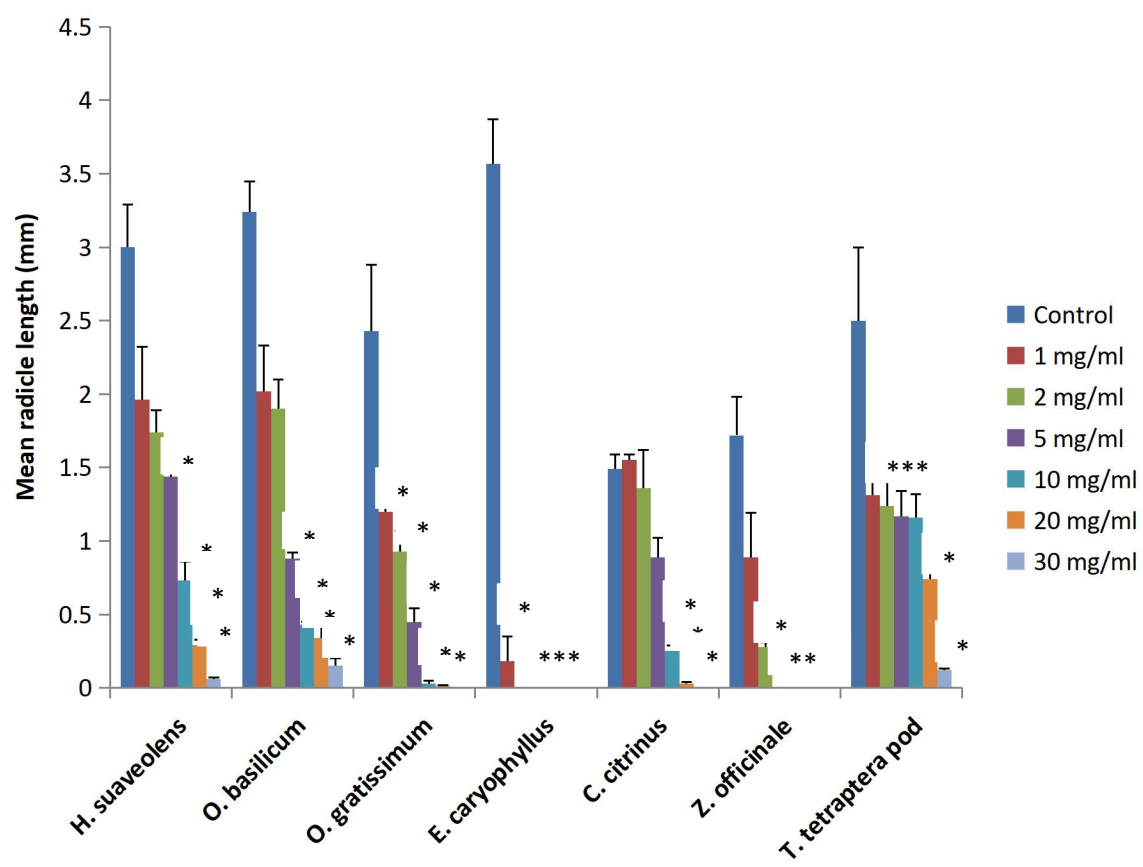


Figure 3.1: Antiproliferative effect of the volatile oils/extract on the radicle length of *S. bicolor* seeds at 24 hours. Values are Mean $\pm$ SEM, n = 20. *P<0.05 compared to control. The most active were *E. caryophyllus*, *Z. officinale*, and *O. gratissimum*

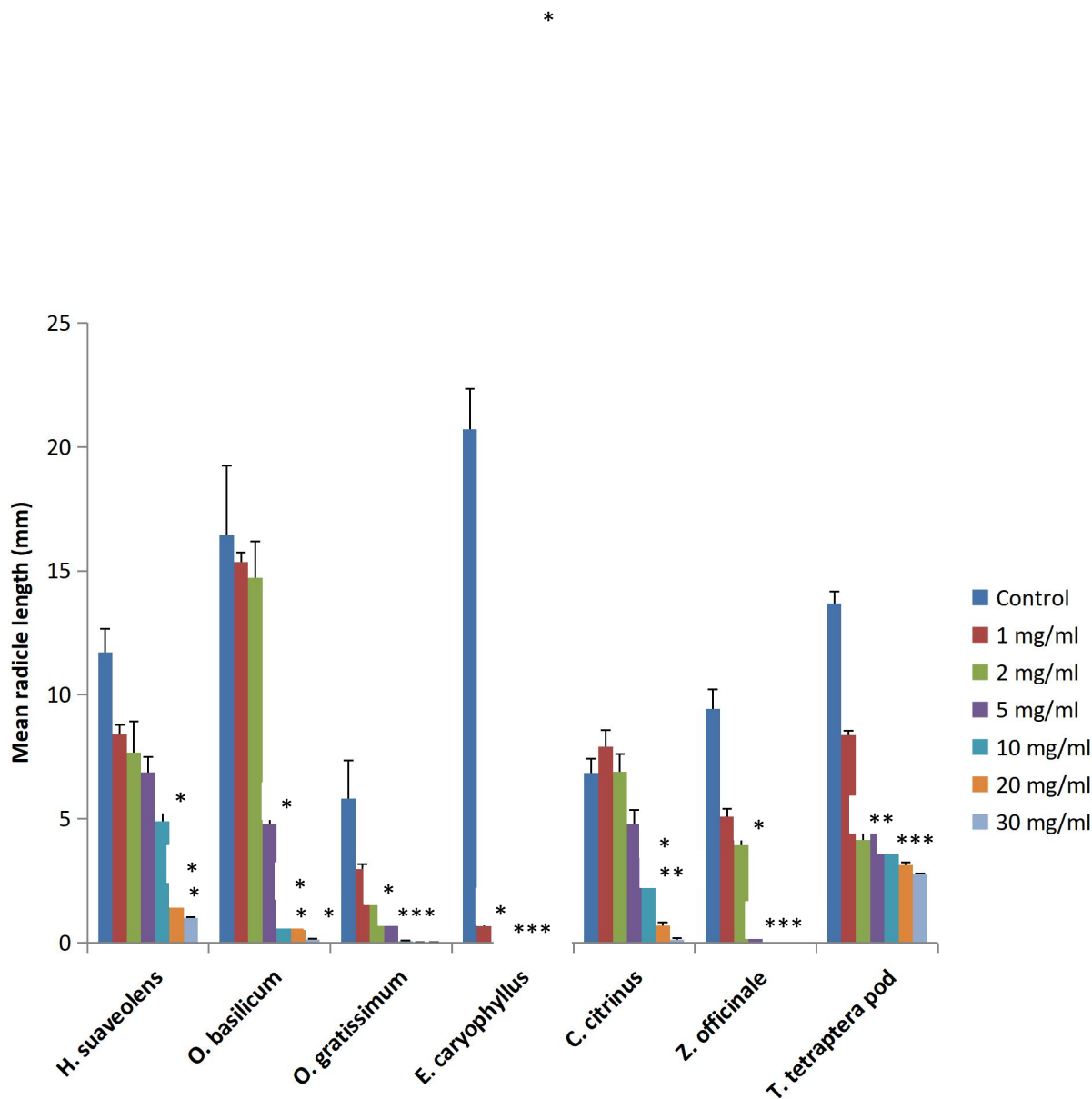


Figure 3.2: Antiproliferative effect of the volatile oils/extract on the radicle length of *S. bicolor* seeds at 48 hours. Values are Mean $\pm$ SEM, n = 20. *P<0.05 compared to control. The most active were *E. caryophyllus*, *Z. officinale*, and *O. gratissimum*

*

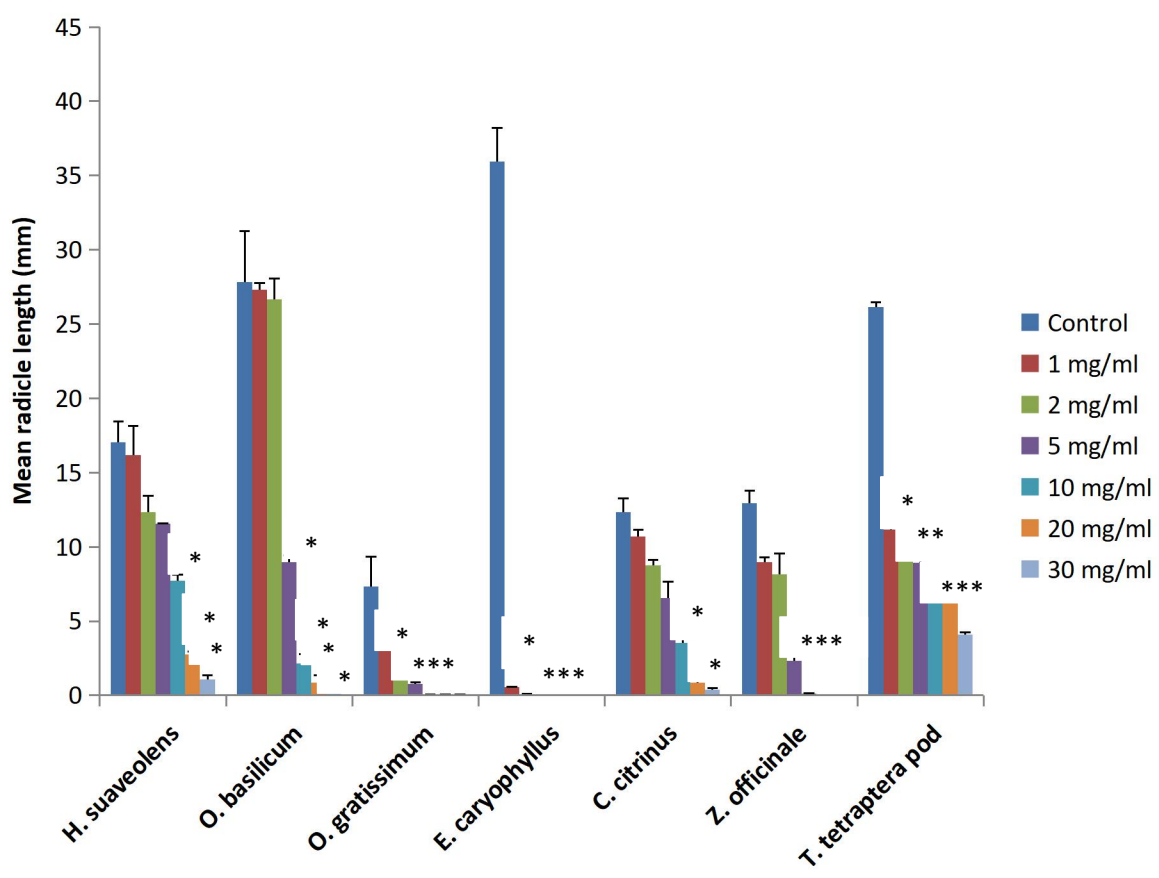


Figure 3.3: Antiproliferative effect of the volatile oils/extract on the radicle length of *S. bicolor* seeds at 72 hours. Values are Mean $\pm$ SEM, n = 20. *P<0.05 compared to control. The most active were *E. caryophyllus*, *Z. officinale*, and *O. gratissimum*.

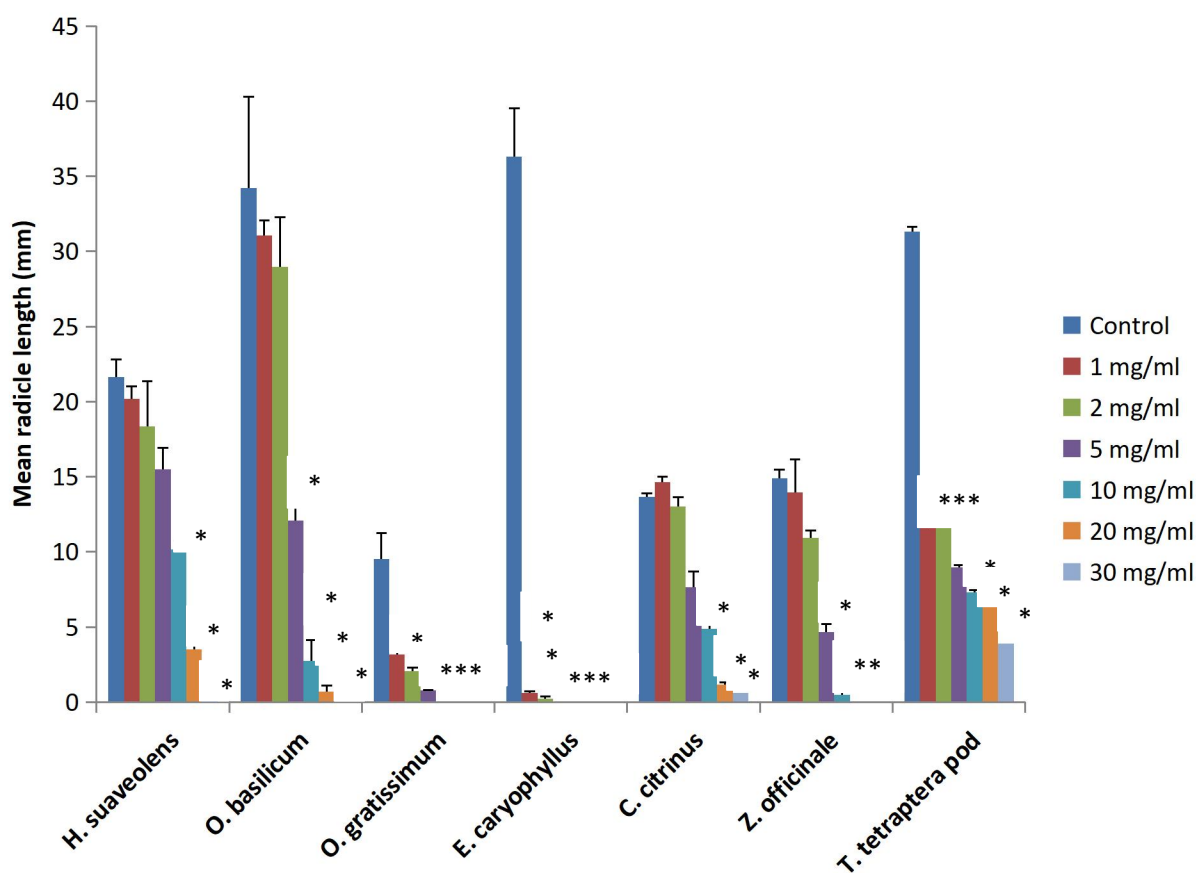


Figure 3.4: Antiproliferative effect of the volatile oils/extract on the radical length of *S. bicolor* seeds at 96 hours. Values are Mean $\pm$ SEM, n = 20. *P<0.05 compared to control. The most active were *E. caryophyllus*, *Z. officinale*, and *O. gratissimum*.

A



B



C



D



Figure 3.5: Effect of some of the volatile oils on the growth of *S. bicolor* seeds at 48 h
 A - Control, B - Seeds treated with 5 mg/mL of *O. gratissimum* volatile oil
 C - Seeds treated with 5 mg/mL of *E. caryophyllus* volatile oil
 D - Seeds treated with 5 mg/mL of *Z. officinale* volatile oil

E



F



G



Figure 3.6: Effect of some of the volatile oils on the growth of *S. bicolor* seeds at 48 h continued
 E - Seeds treated with 5 mg/mL of *H. suaveolens* volatile oil
 F - Seeds treated with 5 mg/mL of *C. citrinus* volatile oil
 G - Seeds treated with 5 mg/mL of *O. basilicum* volatile oil

3.3 Result of the cytotoxic effects of the volatile oils/extract on tadpoles

A concentration dependent activity was observed in the tadpole mortality test. While 100% mortalities were achieved at 20 $\mu\text{g/mL}$ for some volatile oils, others showed no mortality at 40 $\mu\text{g/mL}$. *T. tetraptera* pod produced $93.33 \pm 6.67\%$ mortality at 10 $\mu\text{g/mL}$ while *H. suaveolens* volatile oil gave 50% mortality at the same concentration. *O. gratissimum*, *H. suaveolens*, *E. caryophyllus*, *Z. officinale*, and *T. tetraptera* pod produced 100% mortality at 20 $\mu\text{g/mL}$, but no deaths were observed with *O. basilicum* and *C. citrinus* volatile oils at the same concentration (Figure 3.7).

An LC_{50} of 12.8, 15.8, 25.2, 8.0, 15.8, 8.4, and 25.2 $\mu\text{g/mL}$ were obtained for *E. caryophyllus*, *Z. officinale*, *C. citrinus*, *H. suaveolens*, *O. gratissimum*, *T. tetraptera* pod and *O. basilicum* respectively.

Generally, *H. suaveolens*, *T. tetraptera* pod and *E. caryophyllus* demonstrated the highest activity.

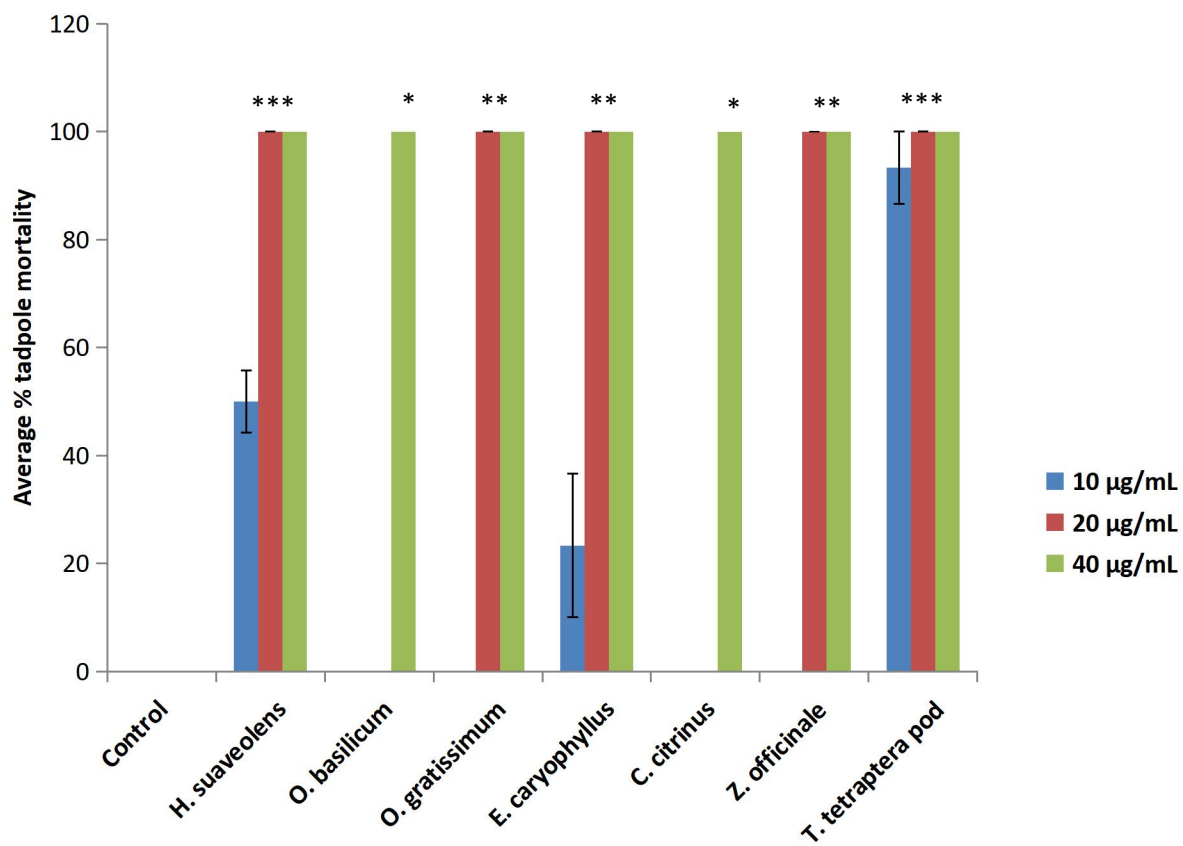


Figure 3.7: Cytotoxic effects of the volatile oils and extract on tadpoles. Values are Mean $\pm$ SEM, n = 10. The most active were *T. tetraptera pod*, *H. suaveolens* and *E. caryophyllus*. The activities of all the materials were significantly different from the control (*P < 0.05)

3.4 Results of cytotoxicity assay on Brine Shrimp (*Artemia salina*)

A dose-dependent response was observed when the shrimps came in contact with different concentrations of the volatile oils and extract. The activities of *Z. officinale*, *H. suaveolens*, and *E. caryophyllus* were comparable; at 1000 µg/mL, they all gave 100% mortality. Activities of *O. gratissimum* and *C. citrinus* were also comparable producing 80 and 60% mortality respectively at the same concentration.

O. basilicum and *T. tetraptera* pod showed the lowest activities with 20 and 26.66% mortalities (Table 3.2).

Table 3.2: Cytotoxic effects of the extracts against *Artemia salina* nauplii

Extracts	Concentration (µg/mL) / % Mortality			
	10	100	1000	LC ₅₀ (µg/mL)
<i>Z. officinale</i>	10.00 ± 0.00	13.33 ± 1.18	***100 ± 0.00	157.75
<i>H. suaveolens</i>	3.33 ± 0.03	6.66 ± 1.00	***100 ± 0.00	188.67
<i>O. basilicum</i>	6.66 ± 0.00	13.33 ± 0.30	20 ± 1.40	> 1000
<i>O. gratissimum</i>	13.33 ± 0.40	26.66 ± 0.00	***80 ± 0.03	200.34
<i>E. caryophyllus</i>	0.00 ± 0.00	26.66 ± 1.00	***100 ± 0.00	143.17
<i>C. citrinus</i>	20 ± 0.50	26.66 ± 0.00	**60 ± 3.30	528.48
<i>T. tetraptera</i> pod	6.66 ± 0.00	23.33 ± 0.00	26.66 ± 0.70	> 1000
Ectoposide (+ve control)	*50.30 ± 0.5	***89.10 ± 1.98	***100 ± 0.00	10.00

Distilled water (-ve control)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-
----------------------------------	-------------	-------------	-------------	---

Values are expressed as the mean ± SEM of three independent observations. Standard drug; Ectoposide LC₅₀ = 10 µg/mL. * indicates significance compared to negative control. *P < 0.05, **P < 0.01, ***P < 0.001. The order of activity: *E. caryophyllus* > *Z. officinale* > *H. suaveolens* > *O. gratissimum* > *C. citrinus* > *T. tetraptera* pod > *O. basilicum*

3.5 Results of growth inhibitory effects of the volatile oils and extract on AU 565 cell line

H. suaveolens and *Z. officinale* showed the highest cytotoxicity on AU 565 cells at 50 µg/mL with 86.74 and 43% inhibitions respectively. *T. tetraptera* pod produced the least activity of - 1.50%. The other plant products showed low inhibitions at this concentration (Table 3.3).

Table 3.3 Inhibition/stimulation effects of the volatile oils and extract on AU 565 cell line

Volatile oil/Extract	Conc (µg/mL)	% Inhibition/Stimulation	IC ₅₀
<i>Z. officinale</i>	50	+43.01	-
<i>H. suaveolens</i>	50	+86.74	34.75 ± 1.11
<i>O. basilicum</i>	50	-1.02	-
<i>O. gratissimum</i>	50	+17.82	-
<i>E. caryophyllus</i>	50	+16.52	-
<i>C. citrinus</i>	50	+11.80	-
<i>T. tetraptera</i> pod	50	-1.50	-
Doxorubicin	50 µM	+97.89	0.085 ± 0.03

Each value represents % mean $\pm$ SEM of three independent experiments. IC₅₀ = Concentration of volatile oil/extract causing 50% inhibition of cells. The order of activity is *H. suaveolens* > *Z. officinale* > *O. gratissimum* > *E. caryophyllus* > *C. citrinus* > *O. basilicum* > *T. tetraptera* pod

3.6 Results of growth inhibitory effects of the volatile oils and extract on HeLa cell line

The volatile oils were evaluated for cytotoxicity against HeLa at 50 μ g/mL. *Z. officinale* oil produced the highest effect with 50% inhibition. Activities of *O. basilicum* and *H. suaveolens* gave comparable inhibitions of 23 and 21.80% respectively. *C. citrinus*, *E. caryophyllus* and *O. gratissimum* showed the least activities with -7.60, -11.07, and -14.30% inhibitions (Table 3.4).

Table 3.4 **Inhibition/stimulation effects of the volatile oils on HeLa cell line**

Volatile oil	Concentration (µg/mL)	% Inhibition/ Stimulation	IC ₅₀
<i>Z. officinale</i>	50	+50.00	29.90 ± 0.1
<i>H. suaveolens</i>	50	+21.80	-
<i>O. basilicum</i>	50	+23.02	-
<i>O. gratissimum</i>	50	-14.30	-
<i>E. caryophyllus</i>	50	-11.07	-
<i>C. citrinus</i>	50	-7.60	-

The order of activity is *Z. officinale* > *O. basilicum* > *H. suaveolens* > *C. citrinus* > *E. caryophyllus* > *O. gratissimum*

3.7 Result of GC-MS Analysis of *Z. officinale* essential oil

Analysis of the volatile oil of *Z. officinale* revealed the presence of different forms of terpenes. α and β -citral were present in the highest quantities with relative abundance of 11.68 and 10.18% respectively. Camphene, borneol, geraniol, and zingiberene were 8.87, 5.01, 6.25, and 7.35% respectively. A total of 33 components were obtained (Table 3.5 and Appendix 1).

Table 3.5: GC-MS Analysis of volatile composition of essential oil of *Z. officinale*

S/No	Retention time (minutes)	Compound	% Concentration
1	19.1	1R- α -Pinene	3.56
2	19.86	Camphene	8.87
3	20.87	Sabinene	0.22
4	20.99	L- β -pinene	0.55
5	21.54	β -Myrcene	1.63
6	22.70	o-Cymene	0.17
7	22.87	β -Phellandrene	1.5
8	22.99	Cineole	3.18
9	23.71	γ -Terpinene	0.1
10	24.79	β -Linalool	3.40
11	25.23	Fenchol	0.11
12	26.01	L-camphor	0.17
13	26.11	β -Citronellal	0.33
14	26.42	β -Terpineol	0.09
15	26.55	Borneol	5.01
16	27.03	α -Terpineol	3.3
17	27.73	(R)-(+)- β -Citronellol	2.89
18	28.08	β-Citral	10.18
19	28.32	Geraniol	6.25
20	28.66	α-Citral	11.68
21	30.69	Copaene	0.2
22	30.91	β -Elemene	0.29
23	31.82	(Z)- β -Farnesene	0.24
24	32.31	Aromadendrene	0.18

25	32.46	Curcumene	2.21
26	32.75	Zingiberene	7.35
27	32.84	Farnesene	2.86
28	33.00	β -Bisabolene	2.14
29	33.07	γ -Cadinene	0.68
30	33.37	β -Sesquiphellandrene	3.99
31	34.47	γ -Elemene	0.12
32	34.97	Cubenol	0.47
33	37.14	β -Eudesmol	0.96

3.8 Result of GC-MS Analysis of *H. suaveolens* essential oil

GC-MS of *H. suaveolens* volatile oil revealed the major components as sabinene (10.64%) and (-)-4-terpineol (7.27%). Cineole, 1-Octen-3-ol, D-Limonene, and α -Bergamotene were also present with relative abundance of 0.86, 3.28, 3.54, and 4.45% respectively. A total of 31 constituents were obtained (Table 3.6 and Appendix 2).

Table 3.6: GC-MS Analysis of volatile composition of essential oil of *H. suaveolens*

S/No	Retention time (minutes)	Compound	% Concentration
1	19.07	1R- α -Pinene	0.9
2	20.95	Sabinene	10.64
3	21.06	L- β -pinene	1.87
4	21.18	1-Octen-3-ol	3.28
5	22.71	o-Cymene	4.45
6	22.85	D-Limonene	3.54
7	22.93	Cineole	0.86
8	23.98	Terpineol, cis- β -	2.45
9	24.82	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1 α ,2 β ,5 α)-	2.45
10	25.23	Fenchol	1.01
11	25.41	Terpineol, cis- β -	0.57
12	25.46	Cyclohexanone, 2-ethyl-	0.14
13	26.78	(-)-4-Terpineol	7.27
14	26.88	p-Cymen-8-ol	3.32
15	27.02	α -Terpineol	0.46
16	28.11	D-Carvone	0.28
17	30.00	p-Cymen-8-ol	0.33
20	31.54	Caryophyllene	1.7
21	31.64	α -Bergamotene	4.45
22	32.82	β -Selinene	2.13

23	32.97	α -Selinene	0.68
24	34.33	Caryophyllene oxide	2.28
25	35.33	Caryophyllene oxide	5.35
26	35.40	Caryophyllene oxide	2.82
27	35.97	Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl	3.22
28	37.30	Ledol	1.14
29	38.37	Bergamotol, Z-α-trans-	5.79
30	51.46	7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	4.71
31	53.39	Retinol	0.77

3.9 Result of GC-MS Analysis of *O. basilicum* essential oil

The major components in *O. basilicum* oil were estragole (52.8%) and β -linalool (16.1%). Sabinene and β -Farnesene were present in the lowest quantities with relative abundance of 0.16 and 0.18% respectively. A total of 22 components were revealed (Table 3.7 and Appendix 3).

Table 3.7: GC-MS Analysis of volatile composition of essential oil of *O. basilicum*

S/No	Retention time (minutes)	Compound	% Concentration
1	20.87	Sabinene	0.16
2	22.67	o-Cymene	0.37
3	22.81	D-Limonene	0.92
4	22.9	Cineole	5.00
5	23.36	β -Ocimene	0.40
6	23.70	γ -Terpinene	0.31
7	24.55	Fenchone	0.68
8	24.79	β -Linalool	16.1
9	26.00	d-Camphor	1.08
10	26.47	α -Terpineol	0.31
11	26.72	4-Terpineol	3.75
12	27.00	Terpineol	1.09
13	27.21	Estragole	52.8
14	28.15	Chavicol	0.23
15	30.19	Eugenol	3.47
16	31.52	Caryophyllene	1.85
17	31.62	α -Bergamotene	2.2
18	31.81	β -Farnesene	0.18
19	32.14	Humulene	0.68
20	32.65	Germacrene D	0.4
21	32.96	β -Bisabolene	4.48

22	33.29	γ -Cadinene	0.47
----	-------	--------------------	------

3.10 Result of GC-MS analysis of *O. gratissimum* essential oil

Thymol (22.49%), p-cymene (13.47%), and γ -terpinene (11.59%) were present in the highest quantities in *O. gratissimum* oil. β -myrcene, carvacrol, eugenol, and β -selinene were also present with relative abundance of 4.58, 2.6, 0.28, and 4.15% respectively. A total of 25 components were obtained (Table 3.8 and Appendix 4).

Table 3.8: GC-MS Analysis of volatile composition of essential oil of *O. gratissimum*

S/No	Retention time (minutes)	Compound	% Concentration
1	19.08	1R- α -Pinene	1.69
2	20.87	Sabinene	0.57
3	20.99	L- β -pinene	0.64
4	21.55	β -Myrcene	4.58
5	22.21	1R- α -Pinene	0.32
6	22.43	α -Terpinene	1.15
7	22.77	p-Cymene	13.47
8	22.93	Cineole	0.19
9	23.77	γ -Terpinene	11.59
10	24.76	β -Linalool	0.3
11	25.28	Thujone	0.36
12	26.49	Isoborneol	0.18
13	29.14	Thymol	22.49
14	29.23	Carvacrol	2.6
15	30.18	Copaene	0.17
16	30.23	Eugenol	0.28
17	30.71	Copaene	1.42
18	30.92	β -Elemene	0.14
19	31.54	Caryophyllene	1.07
20	31.62	α -Bergamotene	0.36
21	32.16	α -Caryophyllene	0.31

22	32.83	β -Selinene	4.15
23	32.98	α -Selinene	1.35
24	33.41	δ -Cadinene	0.51
25	35.09	Caryophyllene oxide	0.37

3.11 Result of GC-MS analysis of *E. caryophyllus* essential oil

Eugenol (64.84%) and acetyeugenol (22%) were present in the highest quantities in *E. caryophyllus* oil. α and β -Caryophyllene were also present with relative abundance of 1.66 and 10.56% respectively. A total of 5 constituents were present in the oil (Table 3.9 and Appendix 5).

Table 3.9: GC-MS Analysis of *E. caryophyllus* volatile oil

S/No	Retention time (minutes)	Compound	% Concentration
1	30.31	Eugenol	64.84
2	31.54	β -Caryophyllene	10.56
3	32.15	α -Caryophyllene	1.66
4	33.28	Acetyeugenol	22
5	35.16	Caryophyllene oxide	0.43

3.12 Result of GC-MS analysis of *C. citrinus* essential oil

C. citrinus oil revealed the presence of cineole (36.06%) and α -Pinene (21.41%) as the major components. O-Cymene, α -Terpineol, and 1,1'-(5-hydroxy-2,2-dimethylbicyclo[4.1.0]heptane-1,7-diyl)bis-, (1 α ,5 β ,6 α ,7 α)-ethanone were also present with relative abundance of 7.54, 9.94, and 1.13% respectively. A total of 20 constituents were present in the oil (Table 3.10 and Appendix 6).

Table 3.10: GC-MS Analysis of *C. citrinus* volatile oil

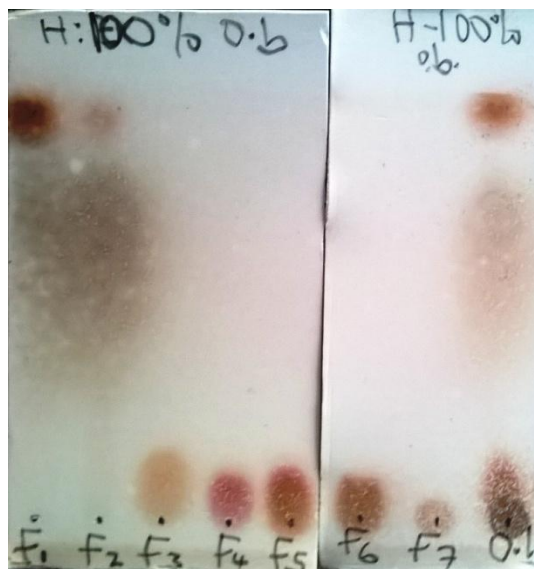
S/No	Retention time	Compound	% Concentration
1	7.99	3-Pentanone, 2,4-dimethyl-	0.55
2	18.76	Bicyclo[3.1.0]hexane, 4-methyl-1-(1-methylethyl)-, didehydro deriv.	1.51
3	19.15	-α-Pinene	21.41
4	20.99	.L- β -pinene	1.16
5	22.01	α -Phellandrene	4.47
6	22.72	o-Cymene	7.54
7	22.99	Cineole	36.06
8	23.71	γ -Terpinene	1.06
9	24.76	β -Linalool	1.53
10	26.72	4-Terpineol	2.8
11	27.02	α -Terpineol	9.94
12	30.49	Geraniol acetate	0.11
13	31.52	β -Caryophyllene	2.09
14	32.14	α -Caryophyllene	0.12
15	33.27	7-Isopropyl-7-methyl-nona-3,5-diene-2,8-dione	0.97
16	33.75	Ethanone, 1,1'-(5-hydroxy-2,2-dimethylbicyclo[4.1.0]heptane-1,7-diyl)bis-, (1 α ,5 β ,6 α ,7 α)-	1.13
17	34.93	(-)-Spathulenol	0.73
18	35.92	β -Caryophyllene oxide	1.06
19	38.52	Flavanone	3.92
20	29.09	.Carvacrol	0.35

3.13 Result of column chromatography of *O. basilicum* oil

The exercise produced 67 test tube fractions which were pooled together based on their spot similarities on the plates as shown below (Figure 3.8). Fractions 4-8 and 10-14 were separately bulked and coded CF₁B and CF₂B. Preparative TLC of CF₁B yielded SF₁B and SF₂B (Figure 3.9).



1



2

Figure 3.8: Chromatogram showing the column fractions of *O. basilicum* oil before (1) and after (2) bulking. Adsorbent- Silica gel GF₂₅₄, Solvent system: C₆H₁₄ (100%). Spray reagent: 12% H₂SO₄, heated at 110°C. Fractions 4-8, 10-14 were bulked



Figure 3.9: Chromatogram showing the preparative TLC fractions SF₁B and SF₂B of *O. basilicum* oil obtained from F1 (CF₁B). Adsorbent- Silica gel GF₂₅₄, Solvent system: C₆H₁₄ (100%). Spray reagent: 12% H₂SO₄

3.14 Result of the growth inhibitory effects of the column fractions and subfractions of *O. basilicum* oil

Proliferation of the radicles at 2 mg/mL was greatly retarded by the column fractions. At 96 h the radicle length of the control was 6.18 mm while CF₁B gave 4.07 mm indicating 34.14% growth inhibition. Subfraction 2 (SF₂B) obtained by preparative TLC of CF₁B gave a radicle length of 1.28 mm indicating a growth inhibition of 79.29% (Figure 3.10).

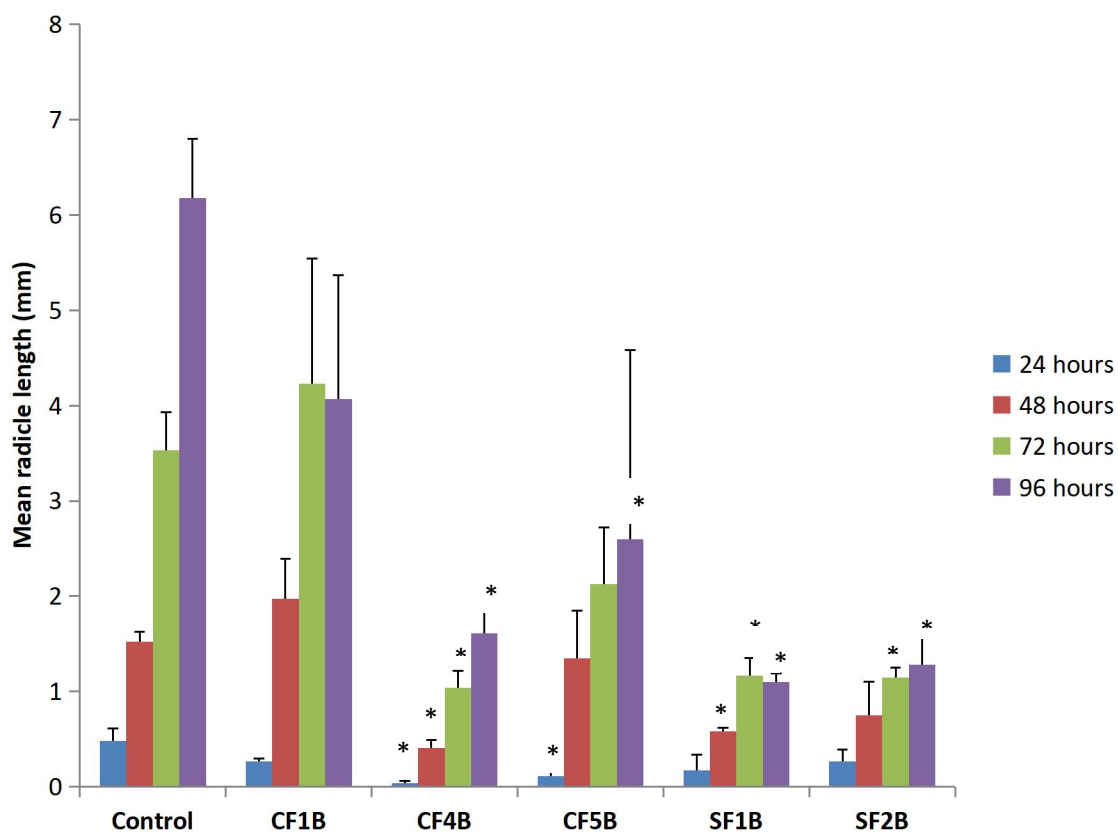


Figure 3.10: Antiproliferative effect of *O. basilicum* oil column fractions and subfractions on the radical length of *S. bicolor* seeds. Values are Mean $\pm$ SEM, n = 20. *P < 0.05 compared to control

3.15 Result of Inhibition effects of *O. basilicum* oil column fractions and preparative TLC sub-fractions on AU 565 and HeLa cell lines

The inhibitory activities of the column fractions against AU 565 were varied with CF₅B producing the highest activity of +48.41%. The least activity was shown by CF₄B (-7.78%).

Preparative TLC fraction SF₂B was active against HeLa with +89.3% inhibition and IC₅₀ of 18.70 ± 1 was obtained. It also produced 33% inhibition against AU 565 cell line (Table 3.11).

Table 3.11 Inhibition/stimulation effects of *O. basilicum* oil column fractions and sub-fractions on AU 565 and HeLa cell lines at 50 µg/mL

Fractions	Cell line	% Inhibition/Stimulation	IC ₅₀
Column fractions			
CF ₁ B	AU 565	-1.17	-
CF ₂ B	AU 565	+14.90	-
CF ₄ B	AU 565	-7.78	-
CF ₅ B	AU 565	+48.41	-
Preparative TLC fraction			
SF ₂ B	AU 565	+33.19	-
SF ₂ B	HeLa	+89.3	18.70 ± 1

Each value represents % mean ± SEM of three independent experiments as compared to control

3.16 Result of GC-MS analysis of preparative TLC sub-fraction SF₂B from *O. basilicum* essential oil

Preparative TLC fraction SF₂B from *O. basilicum* oil showed an increase in the quantity of estragole from 52.8 to 88.61%. 4-Methoxycinnamaldehyde was also present with relative abundance of 6.47% (Table 3.12 and Appendix 7).

Table 3.12: GC-MS Analysis of SF₂B

S/No	Retention time	Compound	% Concentration
1	27.38	Estragole	78.61
2	34.43	4-Methoxycinnamaldehyde	6.47

3.17 Result of column chromatography of *O. gratissimum* oil

The procedure produced 118 test tube fractions which were bulked together based on their spot similarities on the plates as shown below (Figure 3.11). Fractions 5-12 and 13-28 were separately bulked and coded CF₁G and CF₂G. Preparative TLC of CF₁G yielded SF₁G and SF₂G (Figure 3.12).

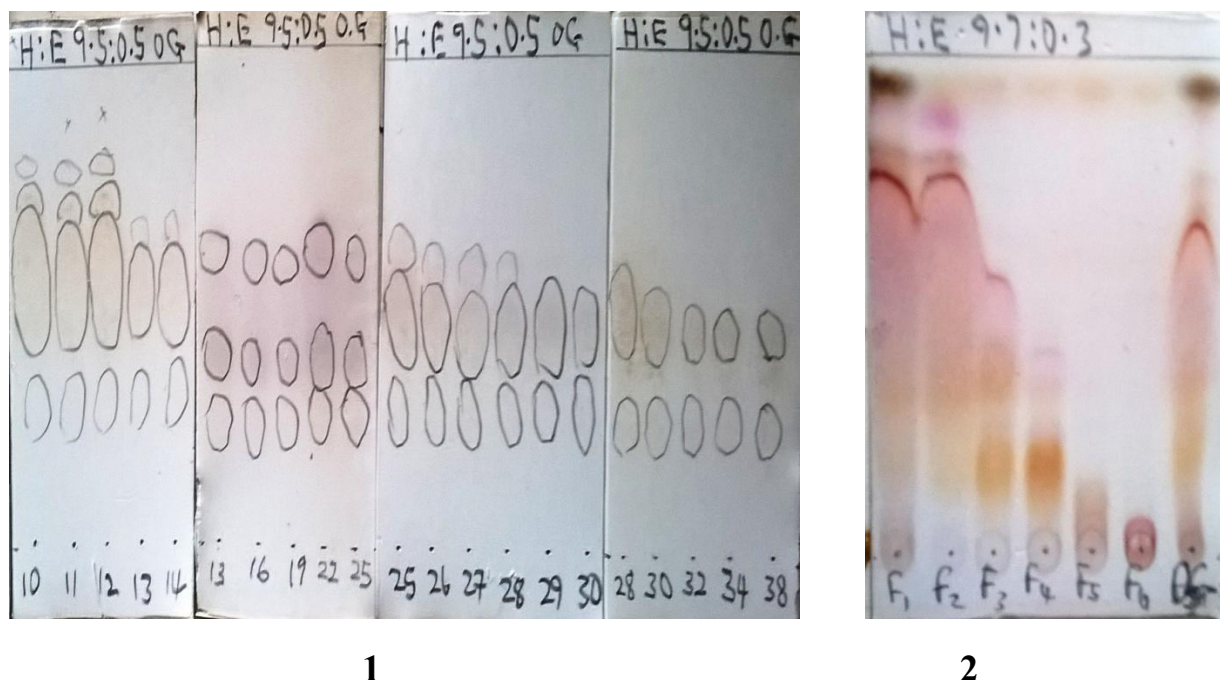


Figure 3.11: Chromatogram showing the column fractions of *O. gratissimum* oil before (1) and after (2) bulking. Adsorbent- Silica gel GF₂₅₄, Solvent system: C₆H₁₄-CH₃COOC₂H₅ (9.5:0.5, 9.7:0.3). Spray reagent: 12% H₂SO₄, heated at 110⁰C. Fractions 5-12, 13-28, 29-40 were bulked



Figure 3.12: Chromatogram showing the preparative TLC fractions SF₁G and SF₂G of *O. gratissimum* oil obtained from F1 (CF₁G). Adsorbent- Silica gel GF₂₅₄, Solvent system: C₆H₁₄-CH₃COOC₂H₅ (9.7:0.3). Spray reagent: 12% H₂SO₄

3.18 Result of the growth inhibitory effects of the column fractions and subfractions of *O. gratissimum* oil

At 1 mg/mL concentration, the growth of the radicles was greatly retarded by the fractions. At 24 and 96 h the radicle length of the control was 0.92 and 7.9 mm respectively while CF₁G gave 0.12 and 1.48 mm indicating 81.27 and 86.96% growth inhibitions respectively. Subfraction 2 (SF₂G) obtained by preparative TLC of CF₁G gave a radicle length of 0.06 and 0.63 mm at these times indicating 93.48 and 92.03% growth inhibitions respectively (Figure 3.13).

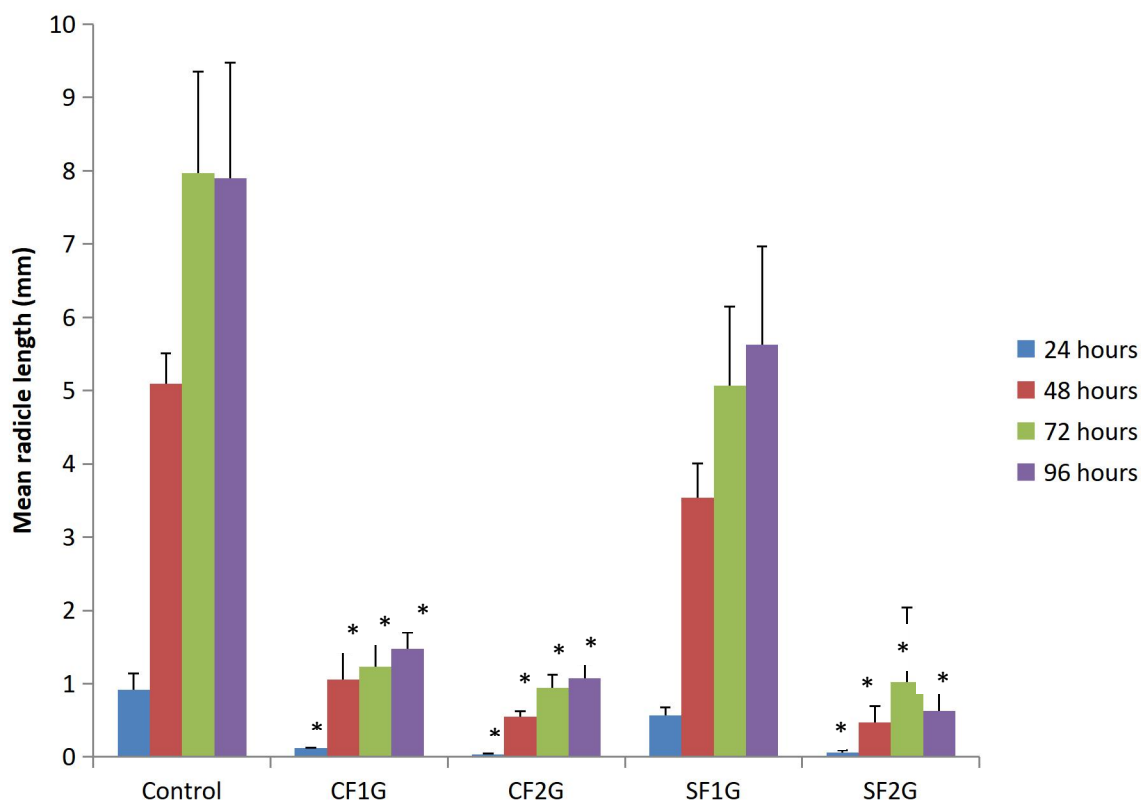


Figure 3.13: Growth inhibitory effect of *O. gratissimum* oil column fractions and subfractions on the radical length of *S. bicolor* seeds. Values are Mean $\pm$ SEM, n = 20. *P < 0.05 compared to control. The fractions and subfractions remarkably inhibited the growth of the radicles with SF₂G showing the highest inhibitory effects.

3.19 Result of Inhibition effects of *O. gratissimum* oil column fractions and preparative TLC sub-fractions on AU 565 and HeLa cell lines

Fraction CF₄G- CF₆G gave the highest inhibitory activity of +34.42 against AU 565. The least activity was shown by CF₁G (12.98%).

Preparative TLC fraction SF₂G was active against AU 565 with 85.07% inhibition. An IC₅₀ of 15.96 ± 1.61 was obtained for it. No inhibitory activity was observed against HeLa cell line (Table 3.13).

Table 3.13 Inhibition/stimulation effects of *O. gratissimum* oil column fractions and sub-fraction on AU 565 and HeLa cell lines at 50 µg/mL

Fractions	Cell line	% Inhibition/Stimulation	IC ₅₀
Column fractions			
CF ₁ G	AU 565	+12.98	-
CF ₃ G	AU 565	+25.30	-
CF ₄ G-CF ₆ G	AU 565	+34.42	-
Preparative TLC fraction			
SF ₂ G	AU 565	+85.07	15.96 ± 1.61
SF ₂ G	HeLa	-29.20	-

Each value represents % mean ± SEM of three independent experiments as compared to control

3.20 Result of GC-MS analysis of preparative TLC fraction SF₂G from *O. gratissimum* essential oil

Thymol was revealed to be the major component in preparative TLC fraction SF₂G from *O. gratissimum* essential oil with a relative abundance of 94.31%.

L-4-terpineol was also present with relative abundance of 2.67% (Table 3.14).

Table 3.14: GC-MS Analysis of SF₂G

S/No	Retention time (minutes)	Compound	% Concentration
1	26.71	L-4-terpineol	2.67
2	28.96	Thymol	94.31

3.21 Result of column chromatography of *E. caryophyllus* oil

The procedure produced 58 test tube fractions which were bulked together based on their spot similarities on the plates as shown below (Figure 3.14). Fractions 4-6 and 7-11 were separately bulked and coded CF₁E and CF₂E. Preparative TLC of CF₁E yielded SF₁E and SF₂E (Figure 3.15).

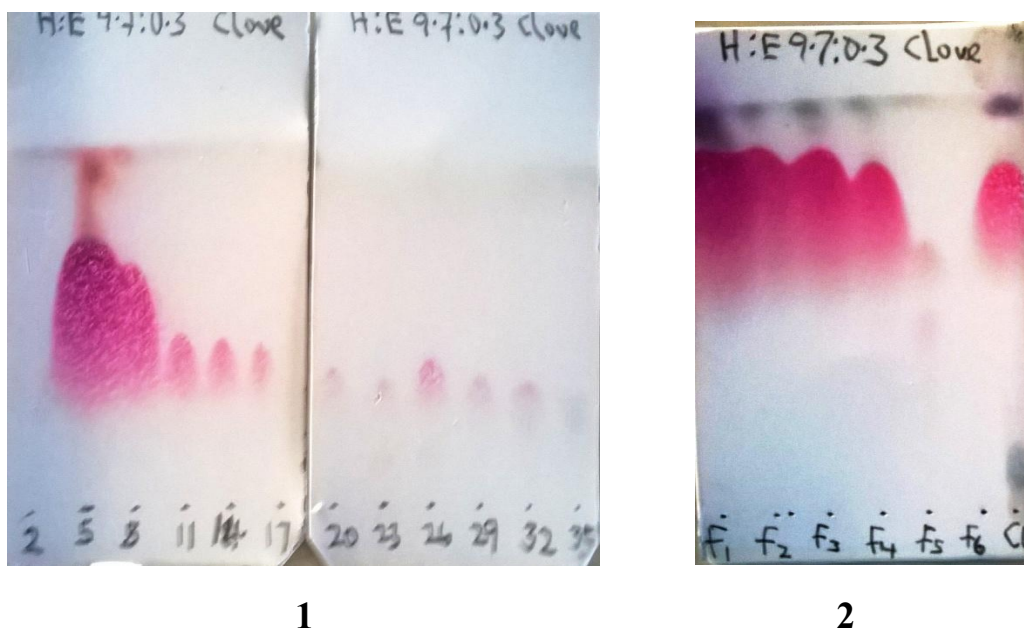


Figure 3.14: Chromatogram showing the column fractions of *E. caryophyllus* oil before (1) and after (2) bulking. Adsorbent- Silica gel GF₂₅₄, Solvent system: C₆H₁₄-CH₃COOC₂H₅ (9.7:0.3). Spray reagent: 12% H₂SO₄, heated at 110⁰C. Fractions 4-6, 7-11 were bulked.



Figure 3.15: Chromatogram showing preparative TLC fractions SF₁E and SF₂E of *E. caryophyllus* oil obtained from F1 (CF₁E). Adsorbent- Silica gel GF₂₅₄, Solvent system: C₆H₁₄-CH₂Cl₂ (9:1). Spray reagent: 12% H₂SO₄

3.22 Result of the growth inhibitory effects of the column fractions and subfractions of *E. caryophyllus* oil

A concentration of 0.25 mg/mL was used due to its high growth inhibitory activity. At 24 h, CF₁E produced 94.05% inhibition while CF₃E showed 100% inhibition. At 96 h, CF₁E and CF₃E gave 97.32 and 98.44% growth inhibitions respectively. SF₂E obtained by preparative TLC of CF₁E produced growth inhibitions of 97.62 and 97.99% respectively at 24 and 96 h (Figure 3.16).

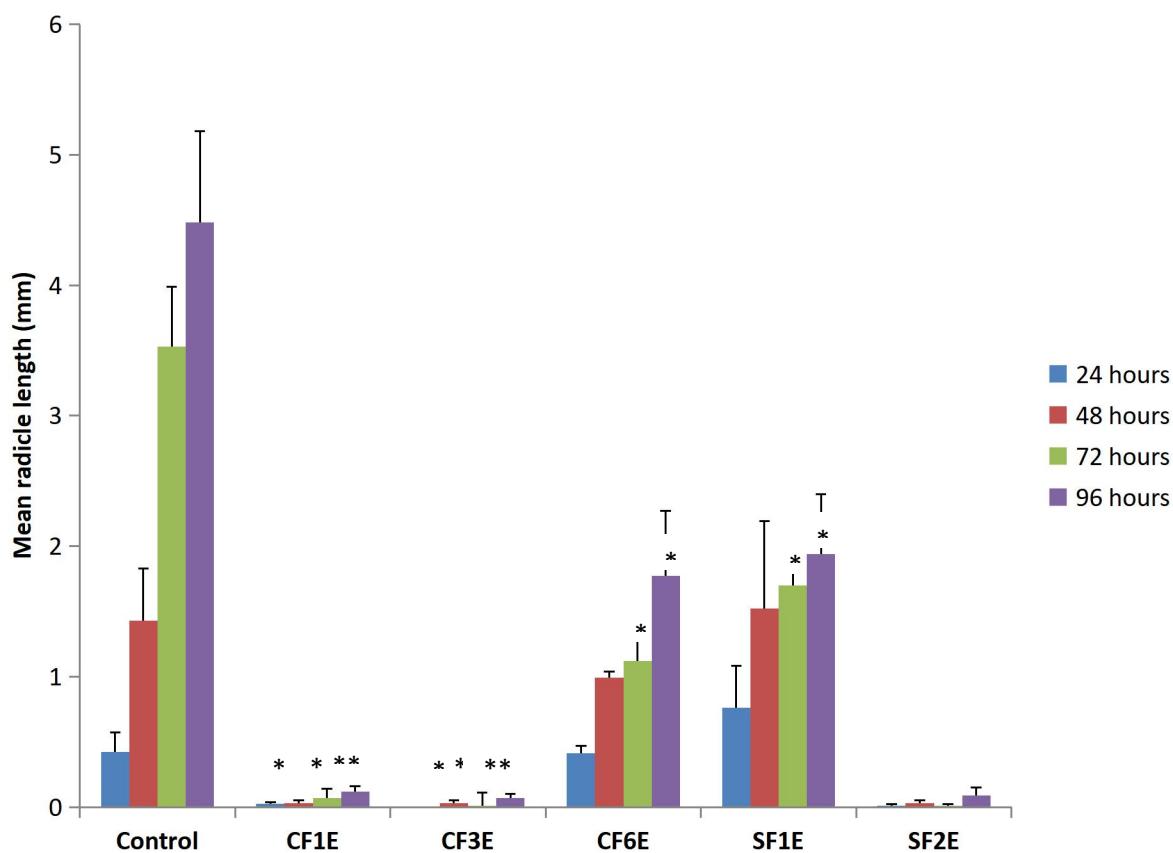


Figure 3.16: Growth inhibitory effect of *E. caryophyllus* oil column fractions and subfractions on the radicle length of *S. bicolor* seeds. Values are Mean $\pm$ SEM, n = 20. *P < 0.05 compared to control

3.23 Result of Inhibition effects of *E. caryophyllus* oil column fractions and preparative TLC sub-fractions on AU 565 and HeLa cell lines

Fraction CF₄E gave the highest inhibitory activity of +37.48% against AU 565. Preparative TLC fraction SF₂E demonstrated low activities against AU 565 and HeLa cell lines with +18.82 and -1.24% inhibitions observed respectively (Table 3.15).

Table 3.15 Inhibition/stimulation effects of *E. caryophyllus* oil column fractions and sub-fraction on AU 565 and HeLa cell lines at 50 µg/mL

Fractions	Cell line	% Inhibition/Stimulation	IC ₅₀
Column fractions			
CF ₁ E	AU 565	+22.94	-
CF ₂ E	AU 565	+7.85	-
CF ₃ E	AU 565	+19.06	-
CF ₄ E	AU 565	+37.48	-
CF ₅ E	AU 565	+25.29	-
Preparative TLC fraction			
SF ₂ E	AU 565	+18.82	-
SF ₂ E	HeLa	-1.24	-

Each value represents % mean ± SEM of three independent experiments as compared to control

3.24 Result of GC-MS analysis of preparative TLC fraction SF₂E from *E. caryophyllus* essential oil

GC-MS of SF₂E from *E. caryophyllus* essential oil showed the presence of eugenol and acetyleneugenol with relative abundance of 82.62 and 15.26% respectively (Table 3.16).

Table 3.16: GC-MS Analysis of SF₂E

S/No	Retention time (minutes)	Compound	% Concentration
1	30.31	Eugenol	82.62
2	33.24	Acetyleugenol	15.26

3.25 Result of column chromatography of *C. citrinus* oil

Test tube fractions (75) were obtained and bulked together based on similarities in their TLC profile as shown below (Figure 3.17). Fractions 3-5 and 6-14 were separately bulked and coded CF₁C and CF₂C.

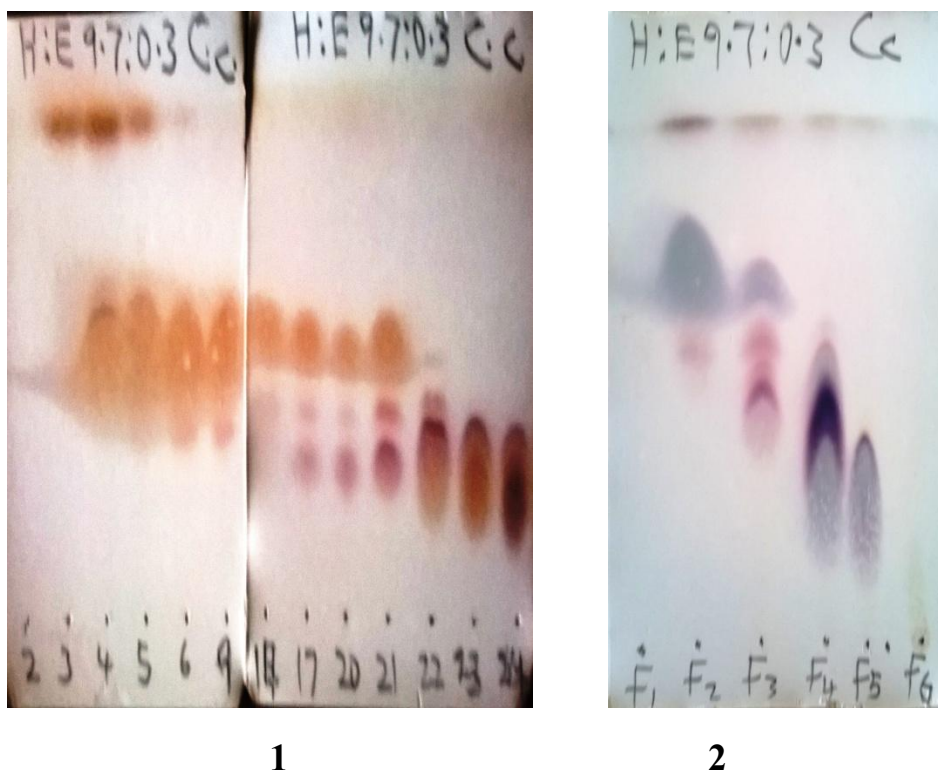


Figure 3.17: Chromatogram showing the column fractions of *C. citrinus* oil before (1) and after (2) bulking. Adsorbent- Silica gel GF₂₅₄, Solvent system: $C_6H_{14}-CH_3COOC_2H_5$ (9.7:0.3). Spray reagent: 12% H_2SO_4 , heated at $110^\circ C$. Fractions 3-5, 6-14, 15-21 were bulked.

3.26 Result of the growth inhibitory effects of the column fractions of *C. citrinus* oil

At 24 h, using 1 mg/mL concentration, the radicle length of the control was 1.89 mm while CF₁C and CF₂C produced 0.5 and 0.41 mm indicating a percentage inhibition of 73.54 and 78.31% respectively. At 96 h, the radicle length of the control was 24.05 mm while CF₁C and CF₂C gave 1.83 and 2.55mm indicating 92.39 and 89.4% growth inhibitions respectively. (Figure 3.18).

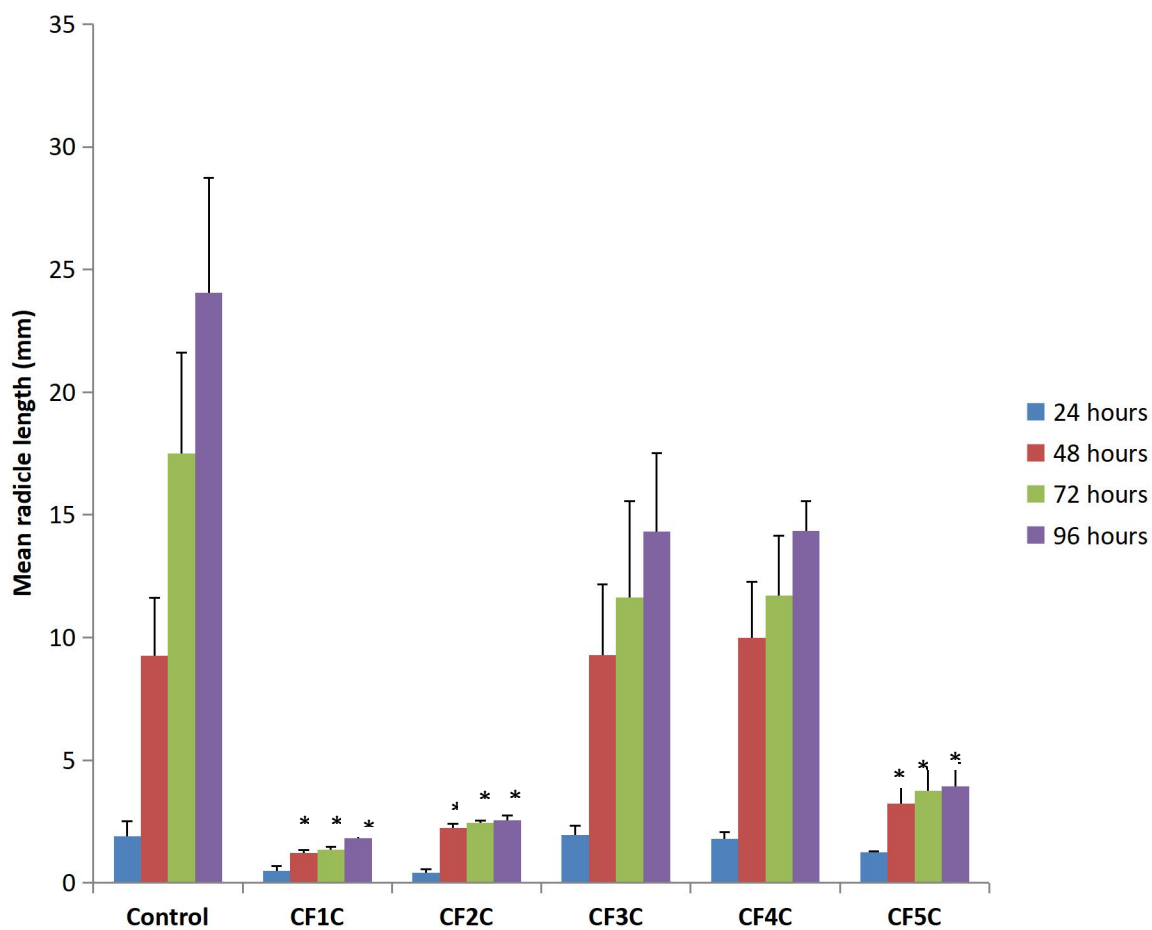


Figure 3.18: Antiproliferative effect of *C. citrinus* oil column fractions on the radicle length of *S. bicolor* seeds. Values are Mean $\pm$ SEM, n = 20. *P < 0.05 compared to control

3.27 Result of inhibition effects of *C. citrinus* oil column fractions on AU 565

The highest inhibitory activity against AU 565 was shown by fraction CF₁C with a percentage inhibition of 70.44%. CF₅C also demonstrated good inhibition of 45.05% at the same concentration. The lowest activity was produced by CF₄C having 20.07% inhibition (Table 3.17).

Table 3.17 Inhibition/stimulation effects of *C. citrinus* oil column fractions on AU 565 cell line

Fractions	Concentration ($\mu\text{g/mL}$)	% Inhibition/Stimulation	IC ₅₀
CF ₁ C	50	+70.44	15.96 $\pm$ 1.61
CF ₃ C	50	+24.91	-
CF ₄ C	50	+20.07	-
CF ₅ C	50	+45.05	-

Each value represents % mean $\pm$ SEM of three independent experiments as compared to control.

3.28 Result of GC-MS analysis of CF₁C from *C. citrinus* essential oil

Column fraction CF₁C obtained from *C. citrinus* oil revealed an increase in the quantity of 1,1'-(5-hydroxy-2,2-dimethylbicyclo[4.1.0]heptane-1,7-diyl)bis-, (1 α ,5 β ,6 α ,7 α)-ethanone from 1.13% to 24.89%. α -Phellandrene, β -Caryophyllene and Isoaromadendrene epoxide were also present with relative abundance of 2.37, 5.09, and 9.5% respectively (Table 3.18).

Table 3.18: GC-MS Analysis of CF₁C

S/No	Retention time (minutes)	Compound	% Concentration
1	22.01	α -Phellandrene	2.37
2	30.49	Geraniol acetate	1.11
3	31.52	β -Caryophyllene	5.09
4	32.14	α -Caryophyllene	1.12
5	32.30	Alloaromadendrene	4.04
6	32.93	.(+)-Ledene	3.05
7	33.81	Ethanone, 1,1'-(5-hydroxy-2,2-dimethylbicyclo[4.1.0]heptane-1,7-diyl)bis-, (1 α ,5 β ,6 α ,7 α)-	24.89
8	34.75	Palustrol	4.05
9	35.17	Isoaromadendrene epoxide	9.5
10	35.75	3-Dodecyl-2,5-furandione	5.06
11	35.92	β -Caryophyllene oxide	2.15

3.29 Result of the growth inhibitory effects of the solvent fractions of *T. tetraptera* pod extract

At 24 h, the mean radicle length of the control was 2.82 ± 0.57 mm. Those of the hexane, chloroform and aqueous fractions were 1.43 ± 0.17 , 1.06 ± 0.25 , and 1.36 ± 0.15 mm indicating 49.47, 62.41 and 51.77% inhibitions respectively. At 96 h, the growth of the radicles was greatly retarded by the solvent fractions. The mean control length was 15.82 ± 2.66 mm while that of the chloroform fraction was 2.85 ± 1.99 mm indicating 81.98% growth inhibition. Those of the aqueous and hexane fractions were 5.65 ± 1.27 and 4.47 ± 0.9 mm indicating 64.29 and 71.74% growth inhibitions respectively (Figure 3.19).

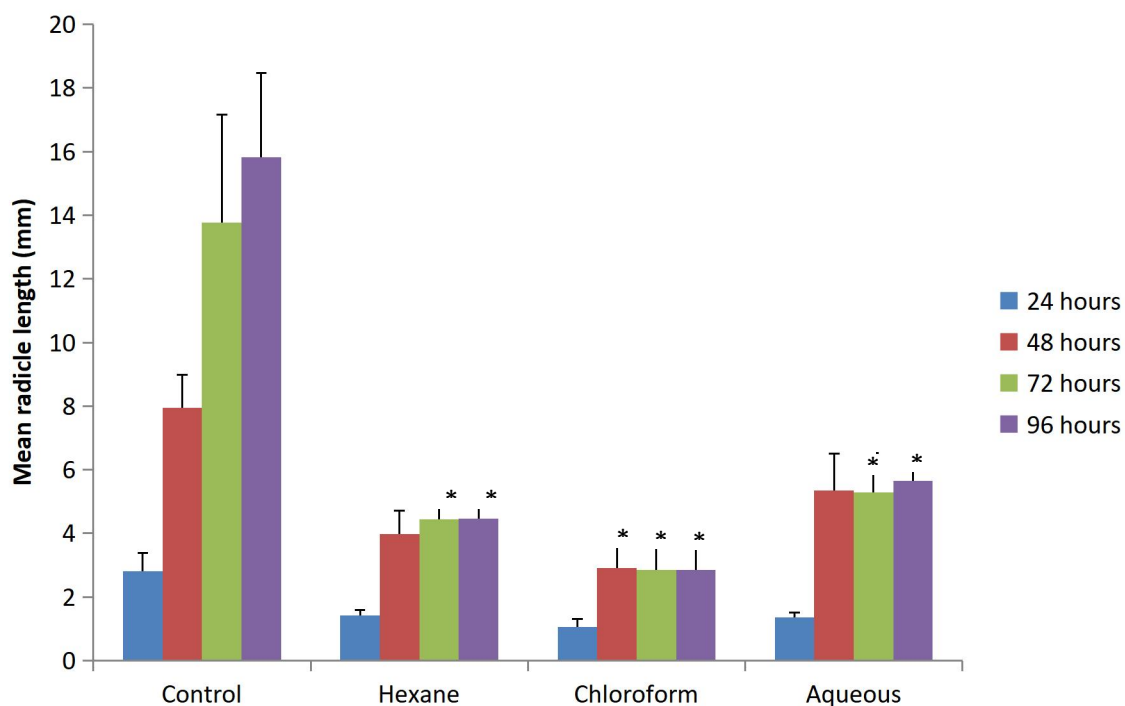


Figure 3.19: Growth inhibitory effect of the solvent fractions of *T. tetraptera* pod on the radical length of *S. bicolor* seeds at 5 mg/mL concentration. Values are Mean $\pm$ SEM, n = 20. *P < 0.05 compared to control

3.30 Result of inhibition effects of the solvent fractions on AU 565 cell line

The chloroform fraction produced the highest activity against AU 565 cell line with 82.27% inhibition. That of the hexane and aqueous fractions were 34.02 and 16.12% respectively (Table 3.19).

Table 3.19 Inhibition/stimulation effects of the solvent fractions on AU 565 cell line

Fractions	($\mu\text{g/mL}$)	% Inhibition/ Stimulation	IC ₅₀
Hexane	50	34.02	
Chloroform	50	82.27	9.63 $\pm$ 3.61
Aqueous	50	16.12	

Each value represents % mean $\pm$ SEM of three independent experiments. IC₅₀ = Concentration of extract causing 50% inhibition of cells.

3.31 Result of the growth inhibitory effects of the VLC fractions of *T. tetraptera* pod chloroform fraction

Using a concentration of 2 mg/mL, the growth of the radicles was greatly varied by the VLC fractions. Chloroform:ethylacetate fraction (D) showed the highest growth inhibitory activity at 24 h. While the control gave a mean radicle length of 4.16 ± 0.69 mm, fraction D produced a mean length of 1.58 ± 0.17 mm, indicating 62.05% reduction in growth. Chloroform (C) and ethylacetate fractions (E) were also active with 47.36 and 48.56% growth reductions respectively while methanol (G) and methanol:water (H) fractions produced 36.54 and 18.03% growth inhibitions respectively (Figure 3.20).

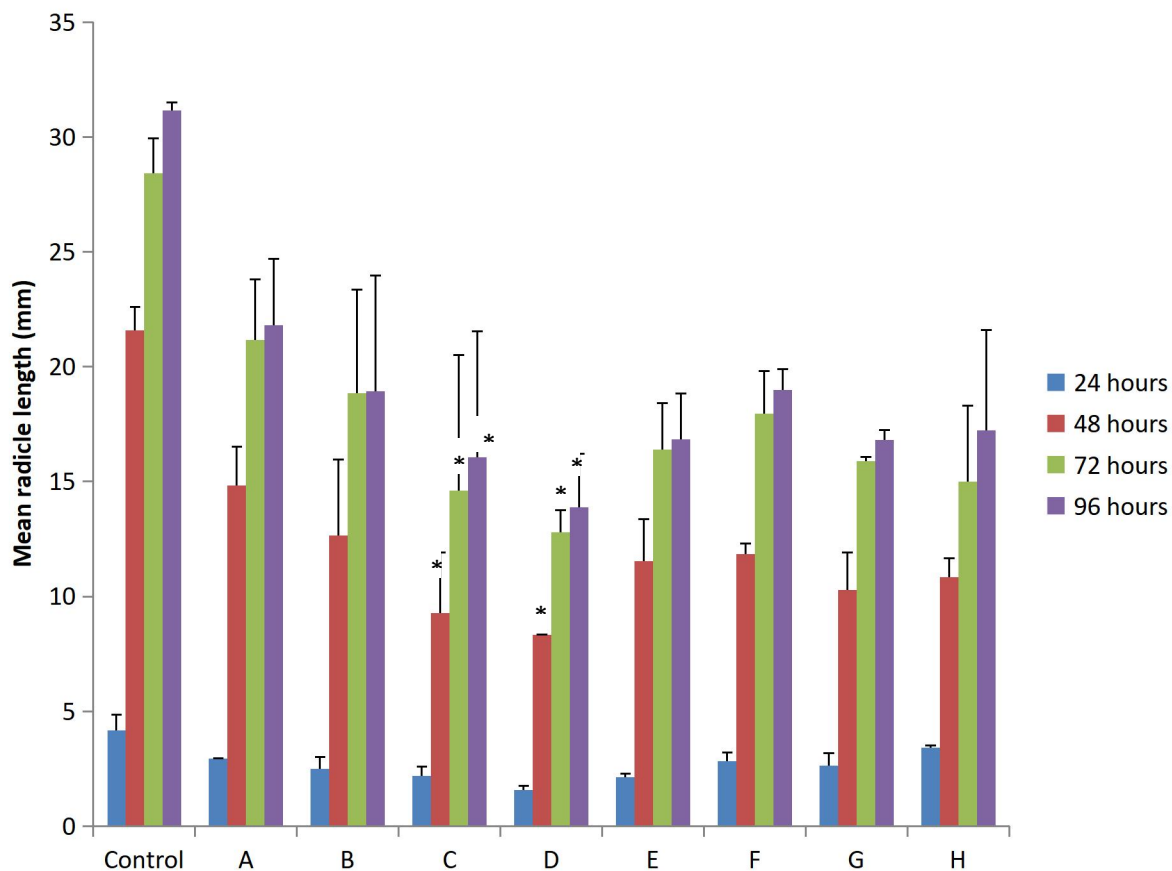


Figure 3.20: Antiproliferative effect of the VLC fractions of the chloroform fraction of *T. tetraptera* pod on the radical length of *S. bicolor* seeds at 2 mg/mL concentration. Values are Mean $\pm$ SEM, n = 20. *P < 0.05 compared to control
Fractions C and D showed the highest growth inhibitory effects.

KEY	
A = C ₆ H ₁₄ (100%)	E = CH ₃ COOC ₂ H ₅ (100%)
B = C ₆ H ₁₄ :CHCl ₃ (1:1)	F = CH ₃ COOC ₂ H ₅ :CH ₃ OH (1:1)
C = CHCl ₃ (100%)	G = CH ₃ OH (100%)
D = CHCl ₃ : CH ₃ COOC ₂ H ₅ (1:1)	H - CH ₃ OH:H ₂ O (1:1)

3.32 Result of inhibition effects of VLC fractions on AU 565 cell line

Among the VLC fractions, a combination of C and D produced the highest inhibition of 23% against AU565 cell line. Fractions G and H gave 13.38 and 20.75% inhibitions respectively while fraction B showed the lowest activity with -5.04% inhibition (Table 3.20).

Table 3.20 Inhibition/stimulation effects of the VLC fractions on AU 565 cell line

Fractions	($\mu\text{g/mL}$)	% Inhibition / Stimulation
A	50	+4.02
B	50	-5.04
C+D	50	+23.50
E	50	+15
F	50	-0.78
G	50	+13.38
H	50	+20.75

Each value represents % mean $\pm$ SEM of three independent experiments.

3.33 Result of inhibitory effects of column subfractions from VLC fractions C and D on AU 565 cell line

Column chromatographic analysis of combined VLC fractions C and D produced 96 test tubes fractions which were bulked differently based on the resolutions produced on the TLC plates. All the bulked fractions (C1 – C6) produced remarkable inhibitory effects against AU 565 cell line with C2 showing the highest activity (41.55%). Subfractions C5 and C6 produced inhibitions of 36.68 and 40.53% respectively. Fraction C3 gave the least inhibition of 28.45% (Table 3.21).

Table 3.21: Inhibition/stimulation effects of column chromatography subfractions from VLC fractions C and D on AU 565 cell line

Fractions	($\mu\text{g/mL}$)	% Inhibition / Stimulation
C1 (3-20)	50	+35.00
C2*(21-34)	50	+41.55
C3 (35-37)	50	+28.45
C4 (38-50)	50	+30.87
C5 (51-68)	50	+36.68
C6 (69-96)	50	+40.53

C2* showed the highest inhibitory activity

3.34 Result of preparative HPLC of C2* subfractions (21-34)

Twenty five fractions were obtained with fractions 11-13 and 19-22 showing single peaks and also producing white precipitates. One spot was obtained for each of them on a TLC plate using $\text{C}_6\text{H}_{14} - \text{CH}_3\text{COOC}_2\text{H}_5$ (7:3) as solvent system and R_f value of 0.48 was obtained for each (Figure 3.21). They were bulked separately and coded as TT1 and TT2. The NMR and MS results for TT2 are shown below (Figure 3.22-3.26).

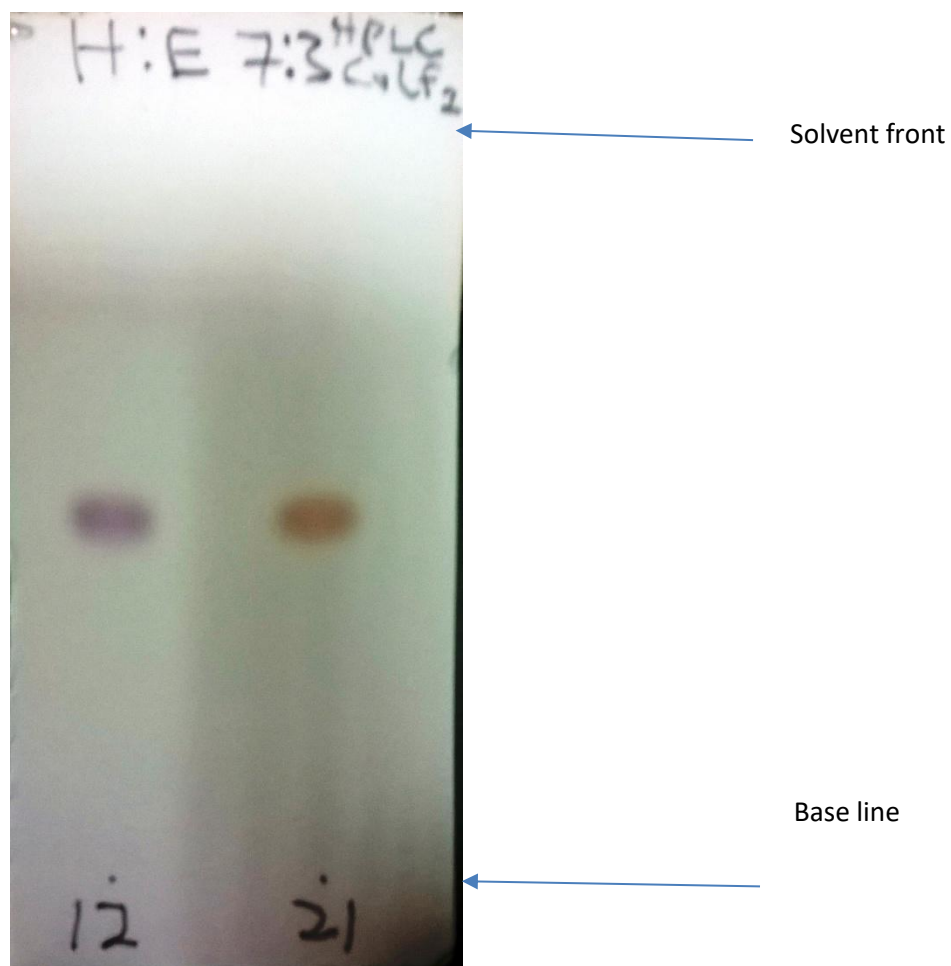


Figure 3.21: Chromatogram showing preparative HPLC fractions 12 and 21 obtained from column fraction C2*. Adsorbent- Silica gel GF₂₅₄, Solvent system: C₆H₁₄ – CH₃COOC₂H₅ (7:3). Spray reagent: 20% 1N H₂SO₄, 12 = TT1, 21 = TT2

ICCBS

8/6/2018 3:53:07 PM

File: TT2

Sample: ROSE /DR. IQBAL

Instrument: JEOL JMS 600H-1

Inlet: Direct Probe

Date Run: 08-06-2018 (Time Run: 15:43:45)

Run By: HEJ MASS LAB104

Ionization mode: EI+

Scan: 31

Base: m/z 189; 4.1%FS TIC: 373770

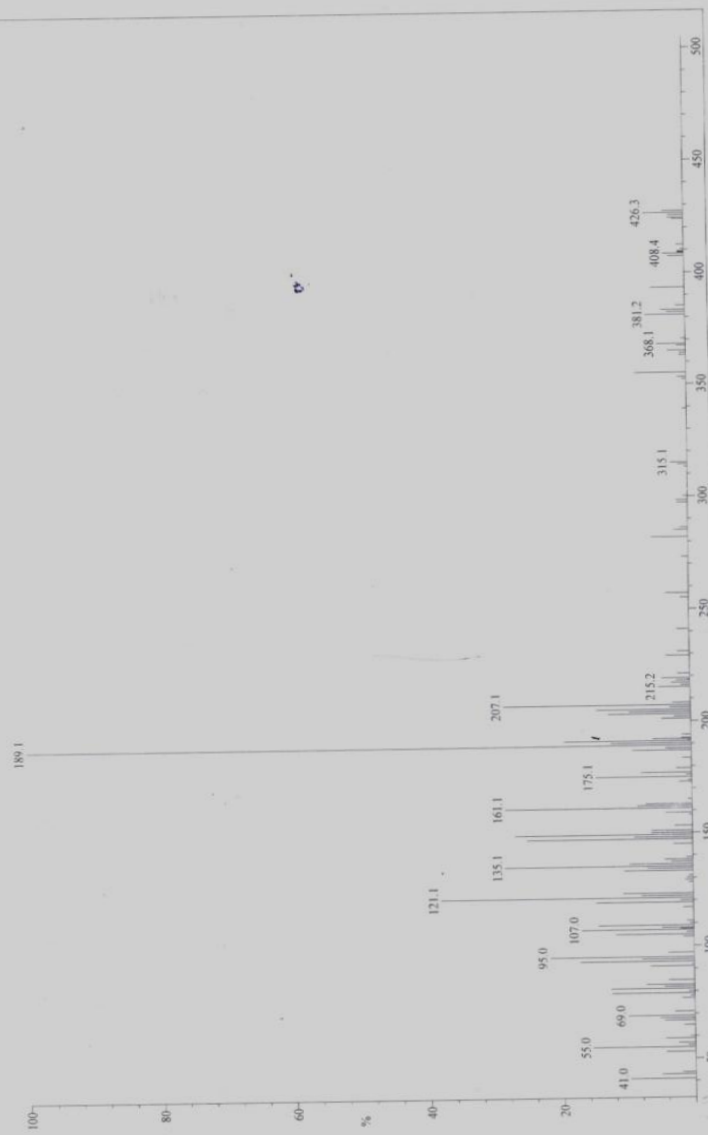


Figure 3.22: Mass Spectral data of Compound TT2 (22-hydroxyisohopane)

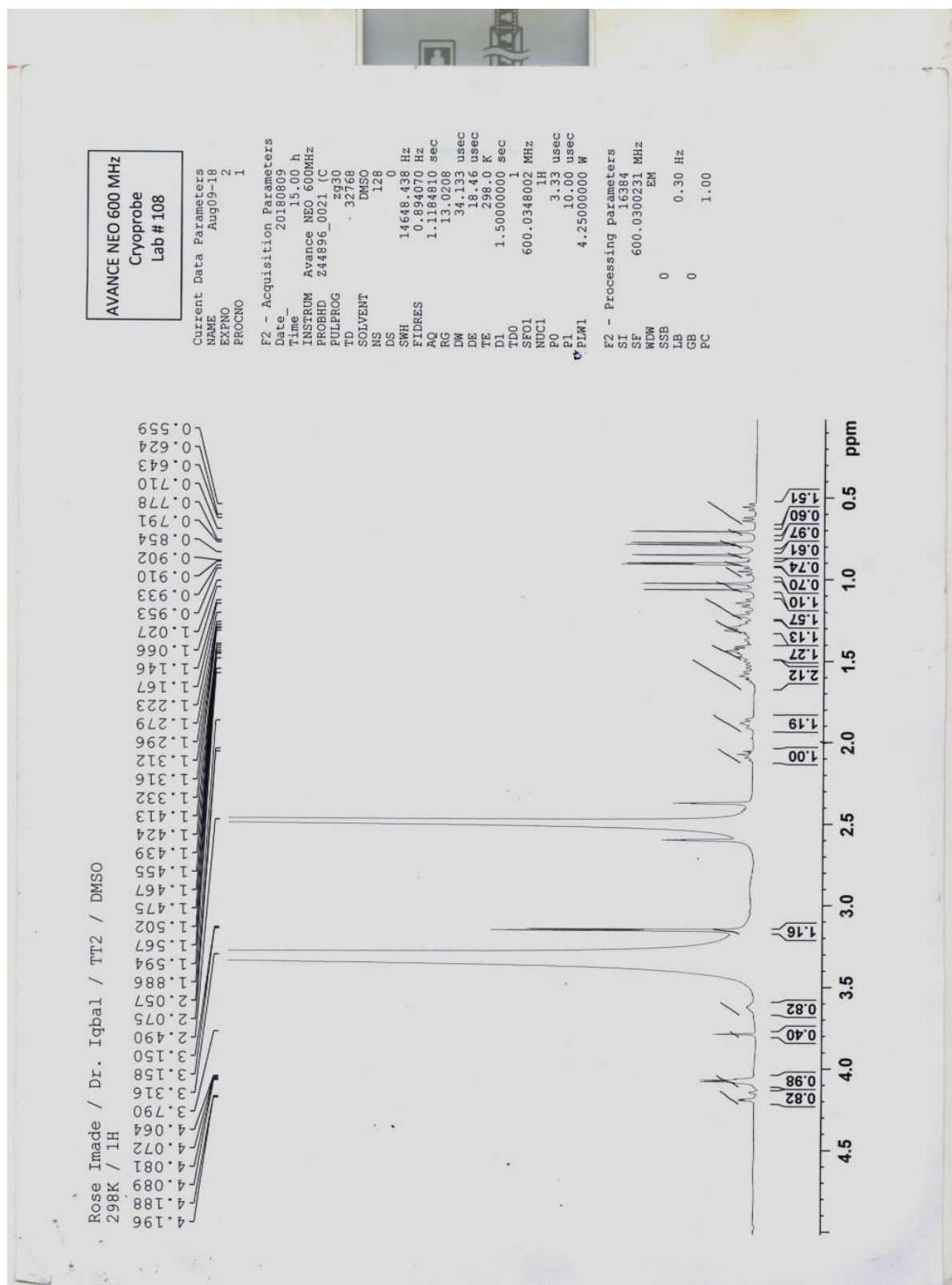
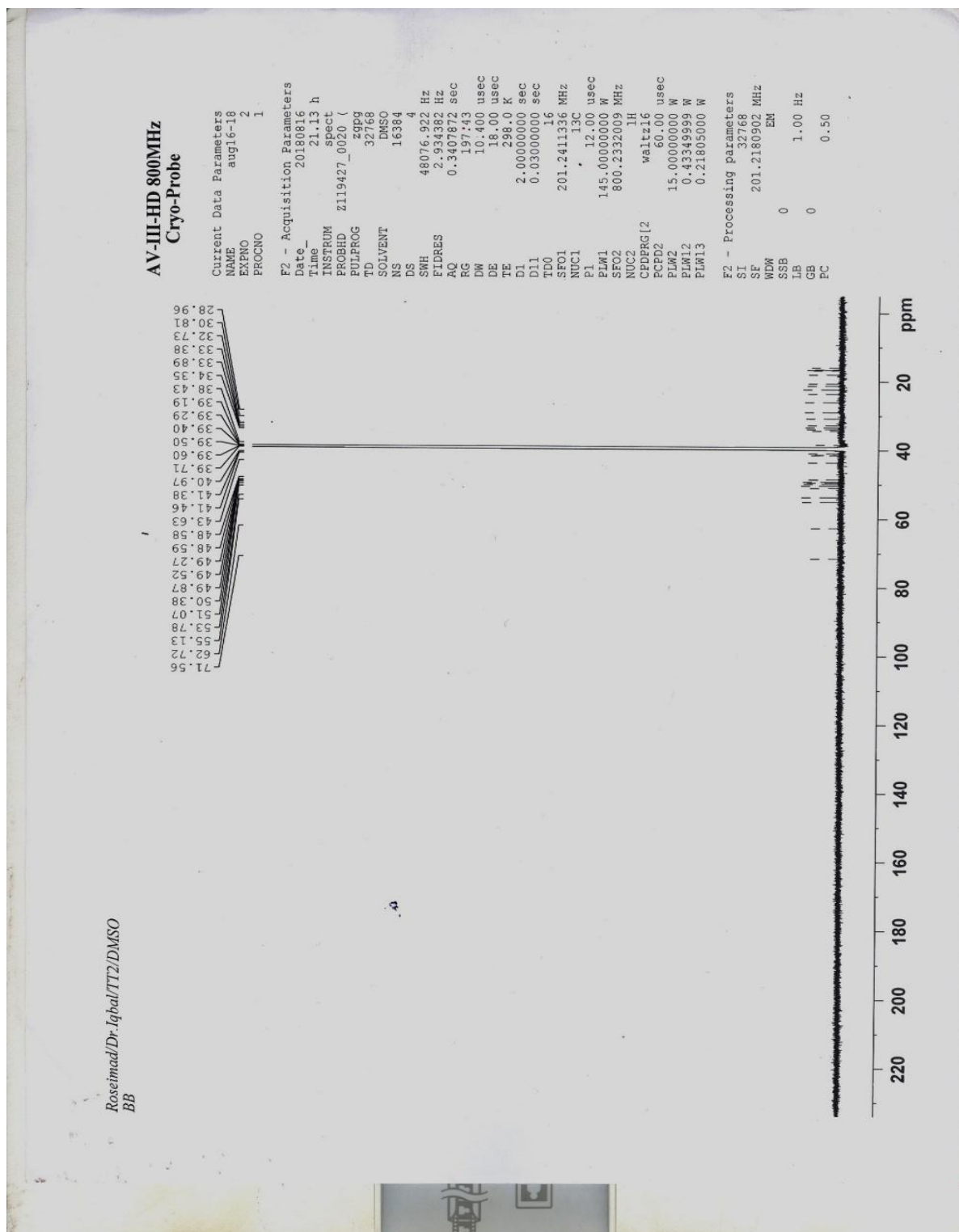


Figure 3.23: ^1H NMR Spectral data of Compound TT2 (22-hydroxyisohopane)



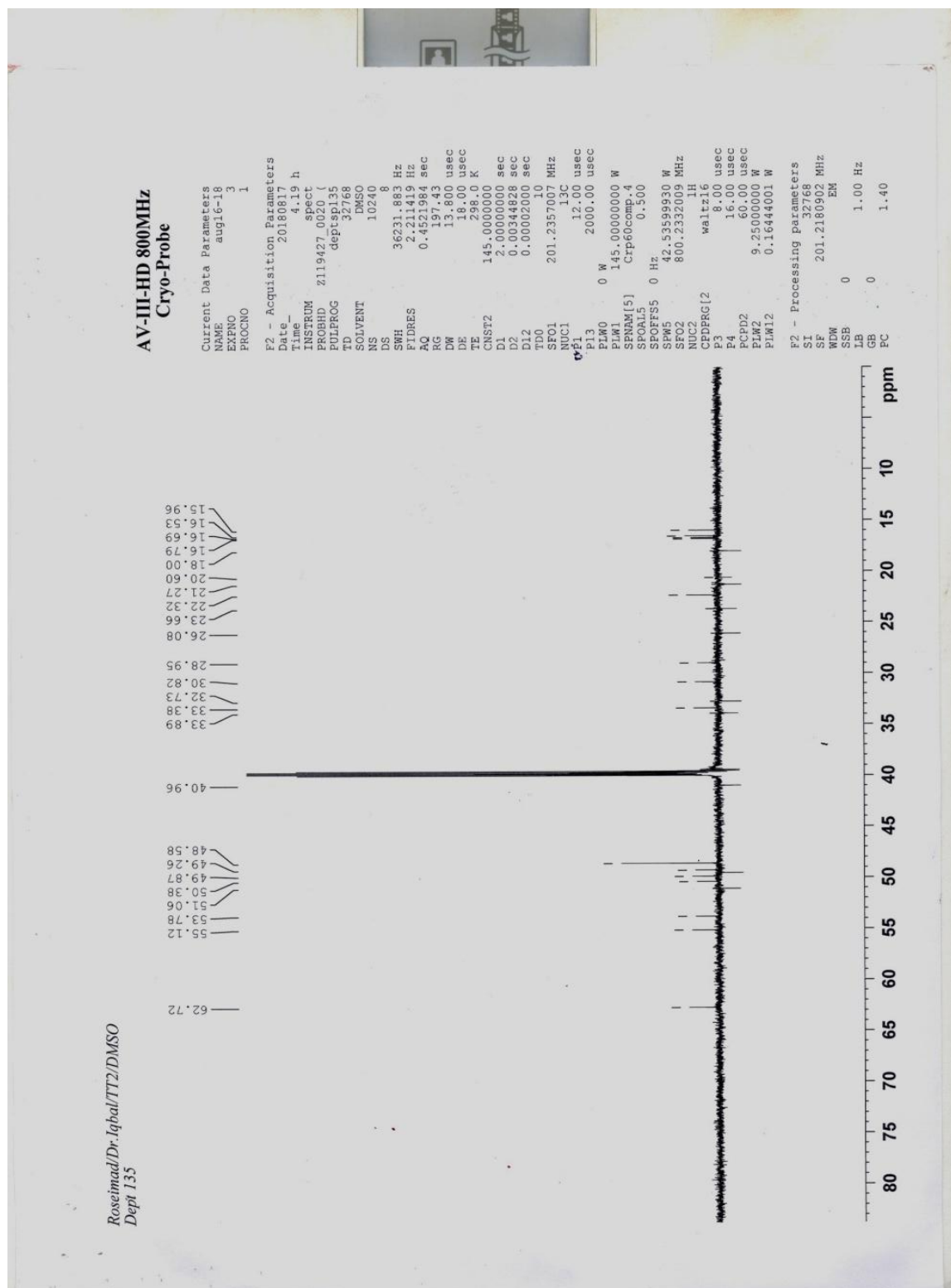


Figure 3.25: ^{13}C Spectral data (DEPT 135) of Compound TT2 (22-hydroxyisohopane)

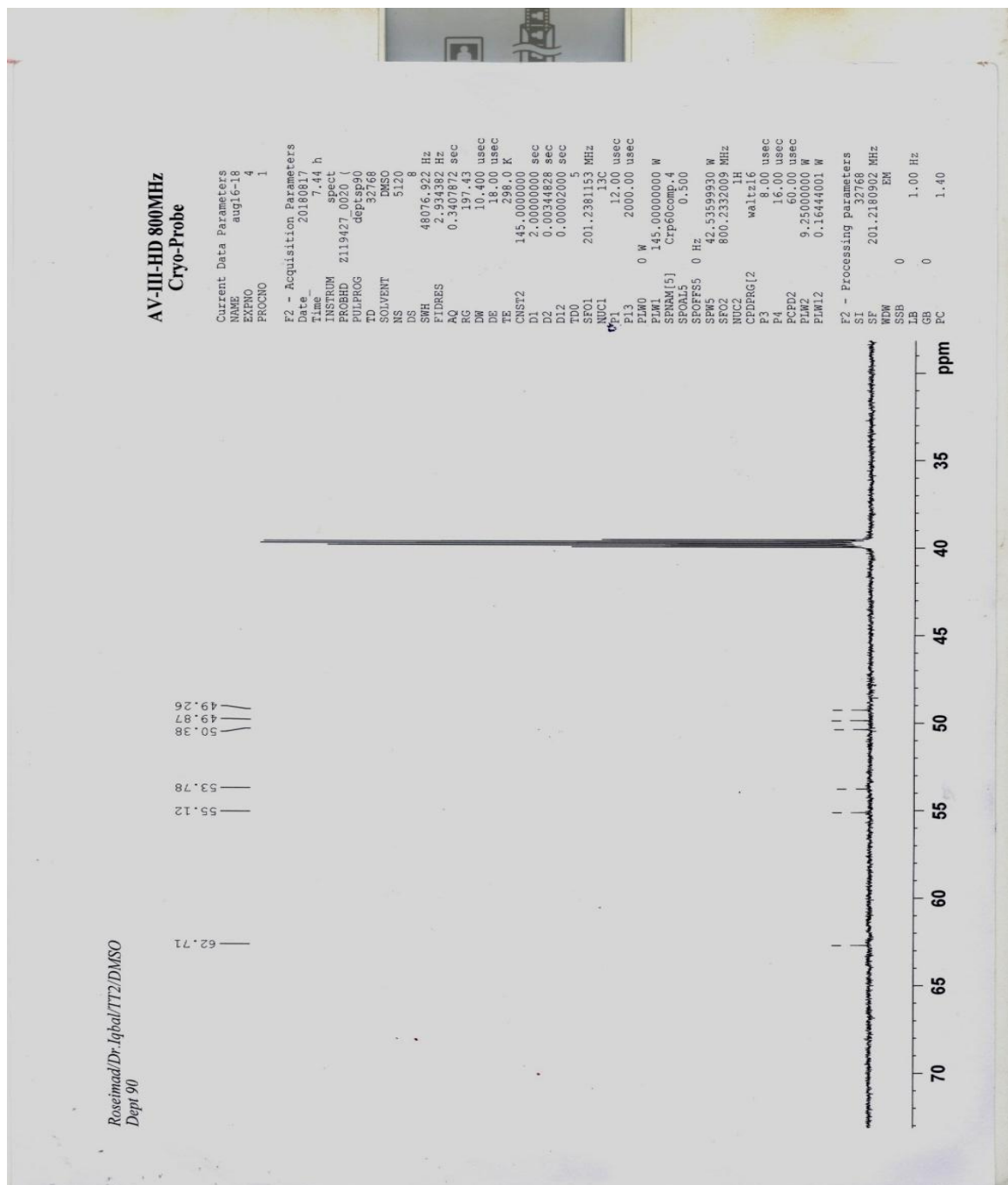


Figure 3.26: ^{13}C Spectral data (DEPT 90) of Compound TT2 (22-hydroxyisohopane)

Table 3.22: NMR Data of Compound TT2 (600 MHz for ^1H and 150 MHz for ^{13}C) in DMSO

Carbon No.	δ_{C} (ppm)	DEPT	δ_{H} (ppm)	Multiplicity, J (Hz)
1	51.1	CH_2	1.15	m
2	32.7	CH_2	1.57	m
3	40.9	CH_2	1.33	m
4	49.5	C	-	-
5	55.1	CH	1.89	m
6	23.7	CH_2	1.52	m
7	33.9	CH_2	1.41	m
8	43.6	C	-	-
9	53.8	CH	2.07	m
10	34.4	C	-	-
11	18.0	CH_2	1.42	m
12	21.3	CH_2	1.44	m
13	48.6	CH	1.91	m
14	41.5	C	-	-
15	33.9	CH_2	1.31	q
16	26.1	CH_2	1.54	m
17	49.9	CH	1.88	m
18	38.4	C	-	-
19	40.2	CH_2	1.22	m
20	20.6	CH_2	1.59	m
21	62.7	CH	1.62	m
22	71.6	C	-	-
23	33.4	CH_3	1.07	s
24	16.5	CH_3	0.79	d
25	16.7	CH_3	0.91	d
26	16.8	CH_3	0.85	s
27	15.9	CH_3	1.07	s
28	22.3	CH_3	1.03	m
29	30.8	CH_3	1.07	s
30	28.9	CH_3	0.85	S
-OH	-	-	4.08	q

3.35 Result of column chromatographic fractions of VLC fractions F and G

Column chromatographic fractions of VLC fractions F and G yielded 78 test tube fractions which were variously bulked based on the resolutions on the plates (Table 2.10). Test tubes 58-62, 63-66, 67-69, and 70-74 were bulked separately and coded F11, F12, F13, and F14 respectively (Figure 3.28).

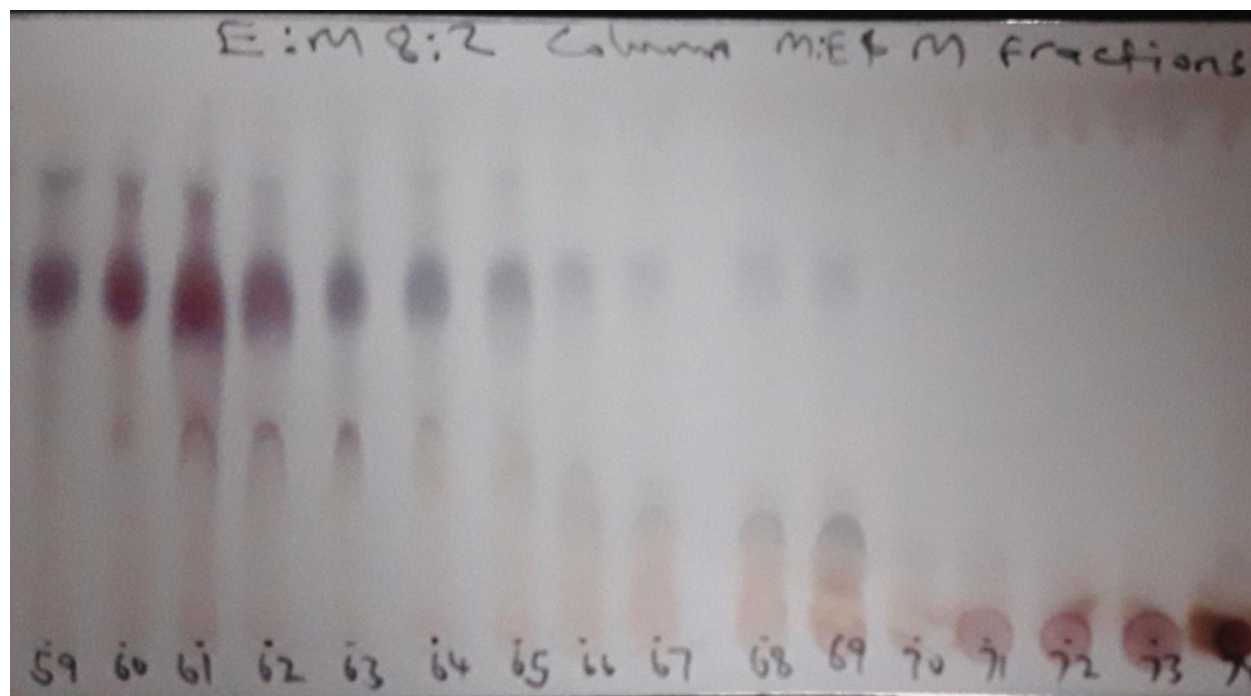


Figure 3.28: Chromatogram showing the column fractions of *T. tetraptera pod* obtained from VLC fraction F and G. Adsorbent- Silica gel GF₂₅₄, Solvent system: CH₃COOC₂H₅-CH₃OH (8:2). Spray reagent: 20% 1N H₂SO₄

3.36 Result of reversed phase preparative HPLC of bulked fraction F11 obtained from column chromatography of F and G (2.14, 2.14.1, Table 2.10)

Seven fractions (7) were obtained with fractions 5 and 6 showing one spot on TLC plate with R_f value of 0.29 using $\text{CH}_3\text{Cl}-\text{CH}_3\text{OH}$ (8:2) as solvent system (Figure 3.29) with a weight of 35 mg. It was coded TRA and its NMR and MS results are shown below (Figure 3.30-3.34).

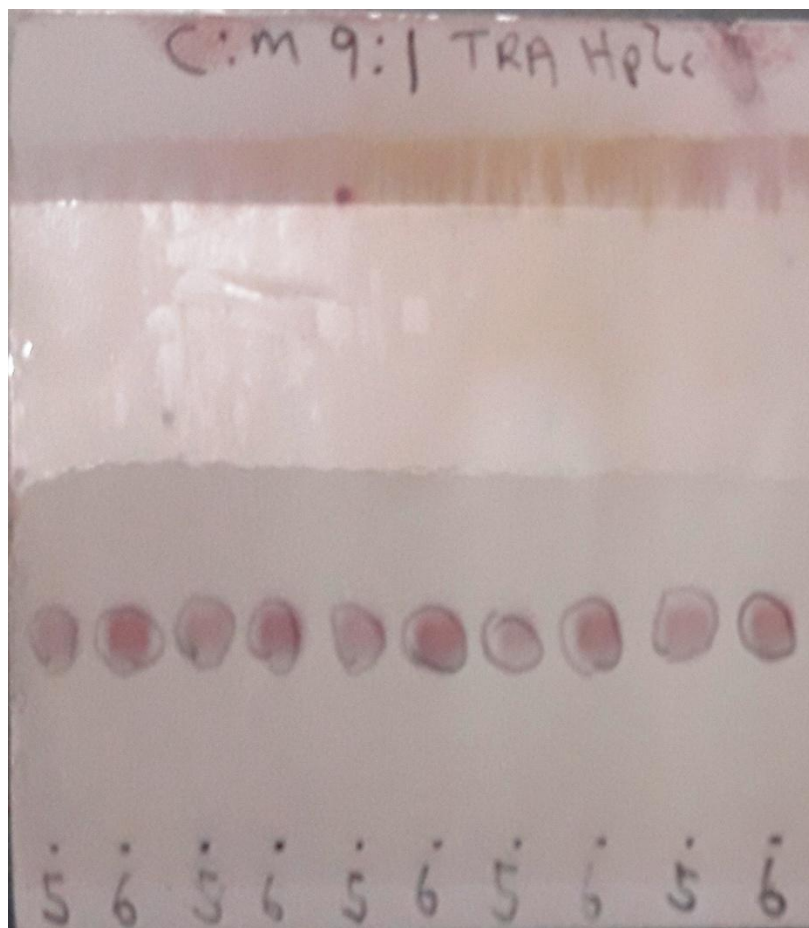


Figure 3.29: Chromatogram showing preparative HPLC fractions 5 and 6 obtained from column fraction F11*. Adsorbent- Silica gel GF₂₅₄, Solvent system: CHCl₃-CH₃OH (8:2). Spray reagent: 20% 1N H₂SO₄. Fraction 6 = TRA

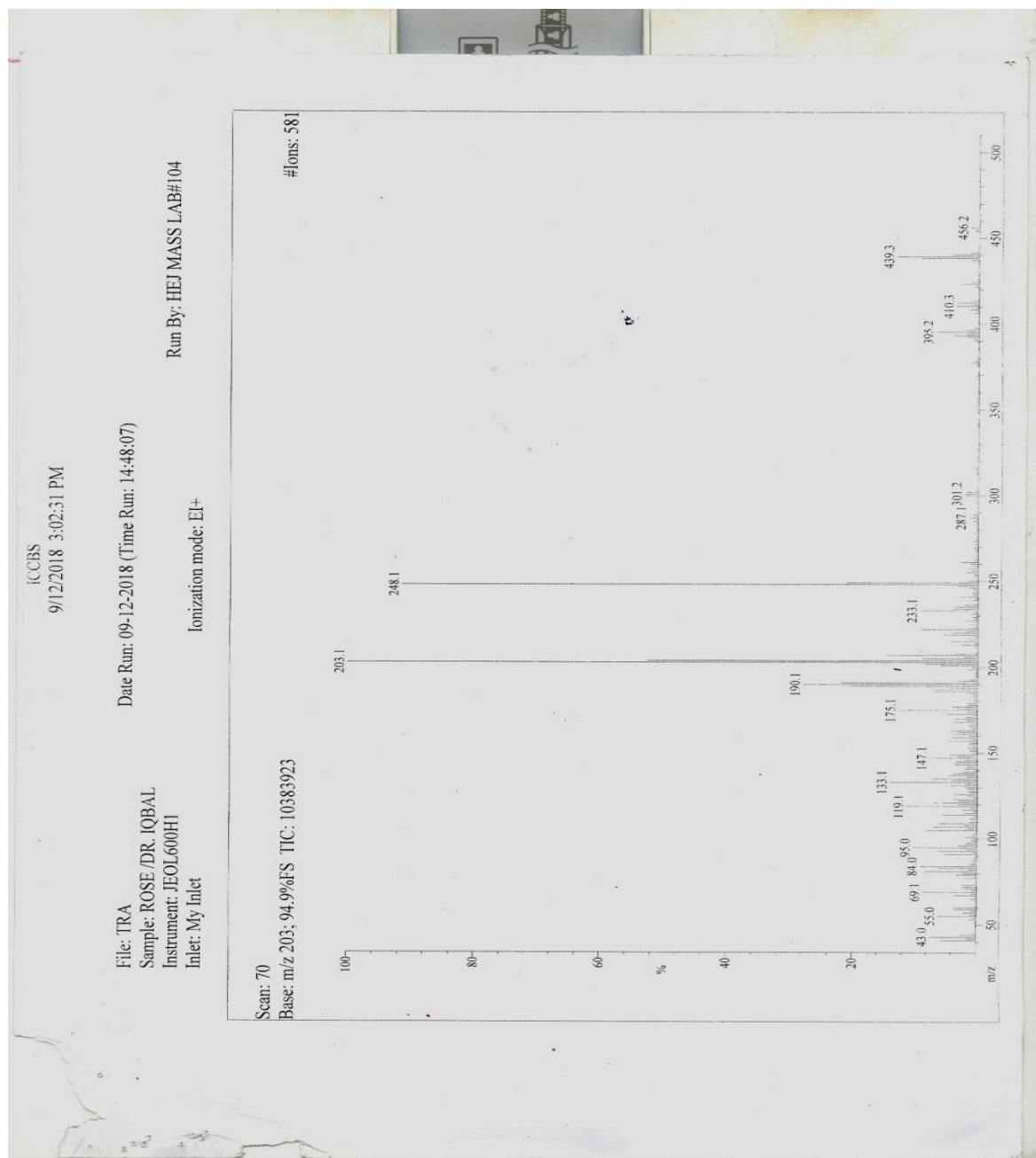


Figure 3.30: Mass Spectral data of Compound TRA (3-O-[β -D-glucopyranosyl]-2'-acetamido-2'-deoxy]-oleanolic acid (Aridanin))

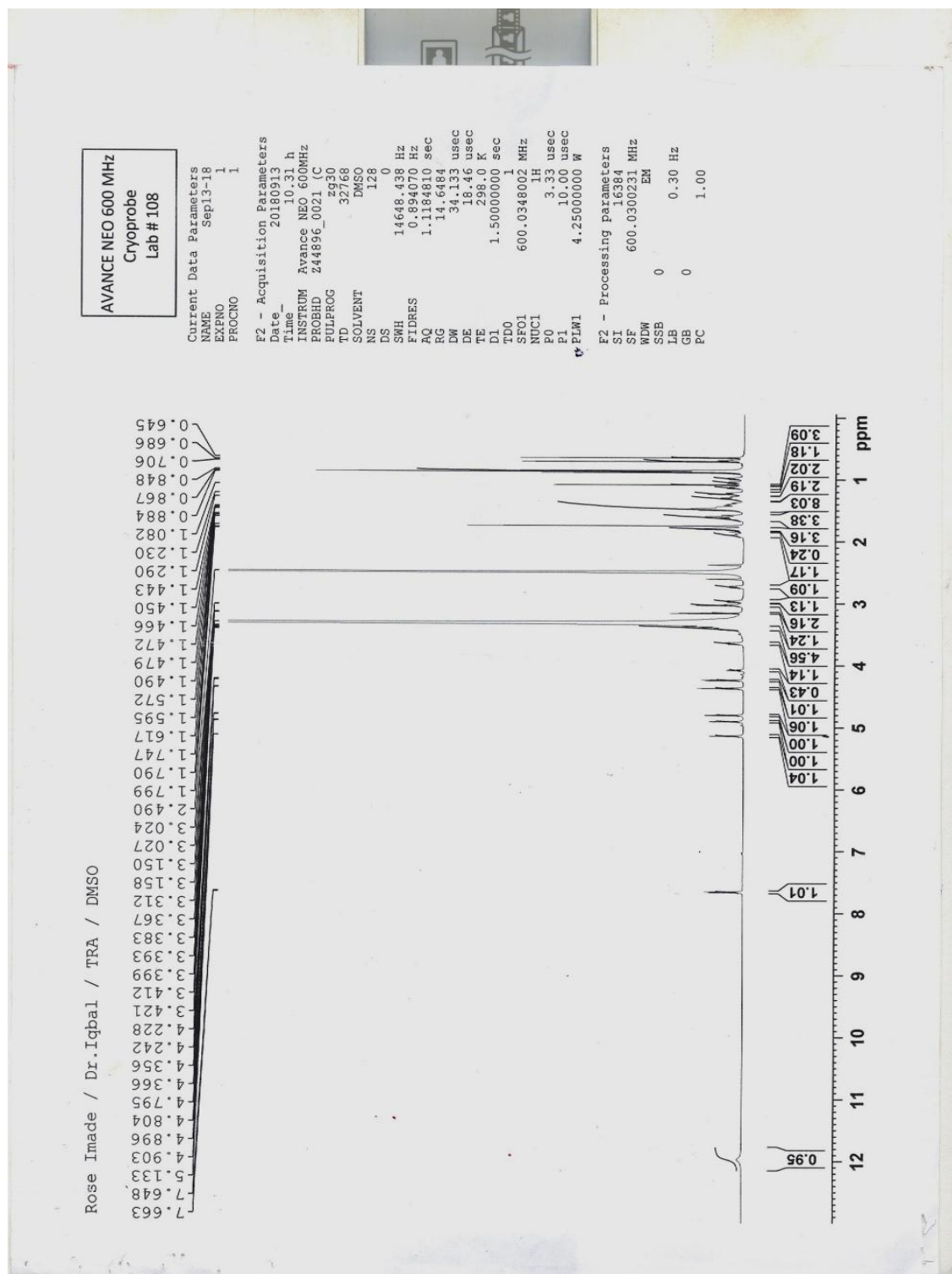


Figure 3.31: ^1H NMR Spectral data of Compound TRA ((3-O-[β -D-glucopyranosyl]-2'-acetamido-2'- deoxy]-oleanolic acid (Aridanin))

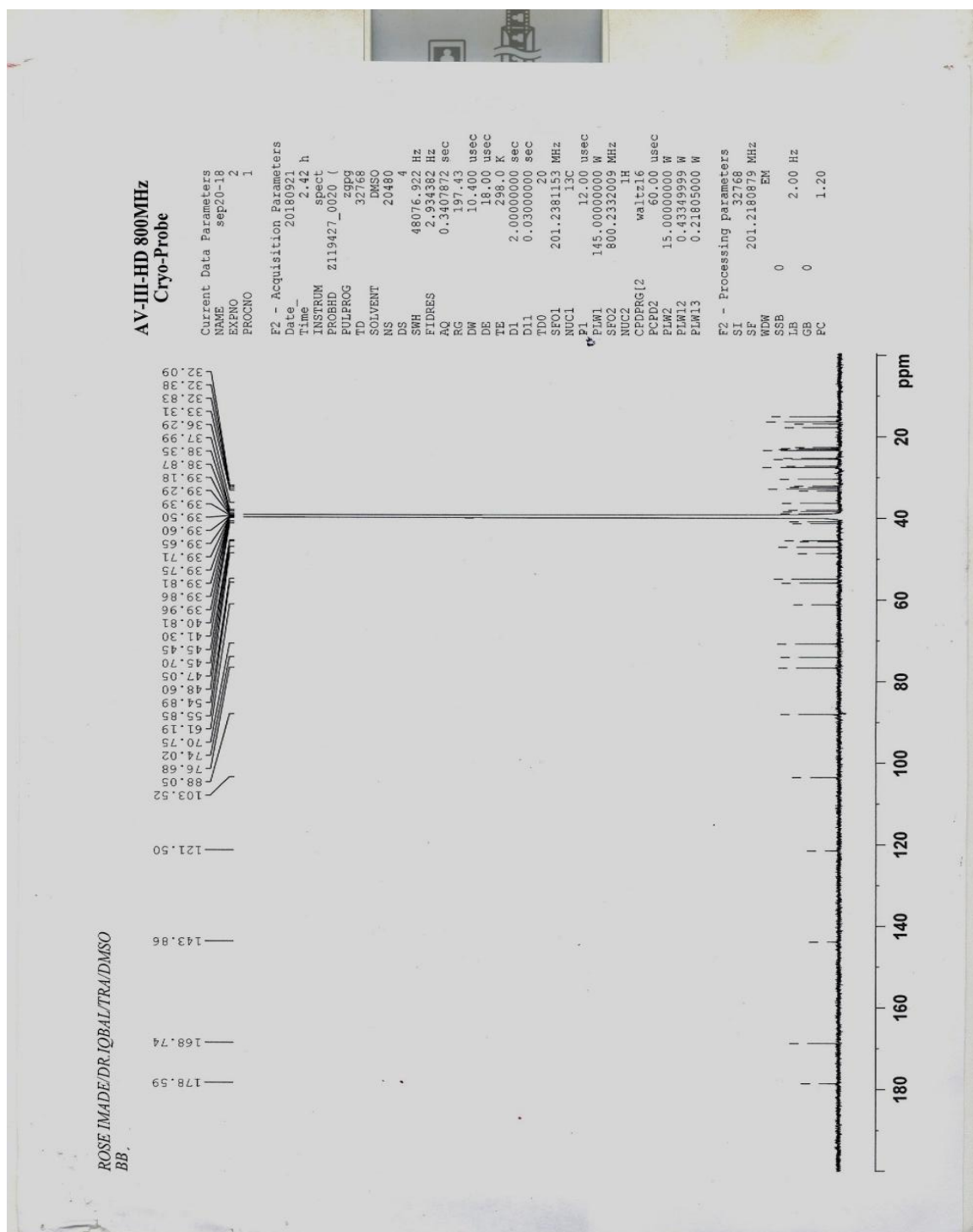


Figure 3.32: ^{13}C Spectral data (BB) of Compound TRA (3-O-[β -D-glucopyranosyl]-2'-acetamido-2'-deoxy]-oleanolic acid (Aridanin))

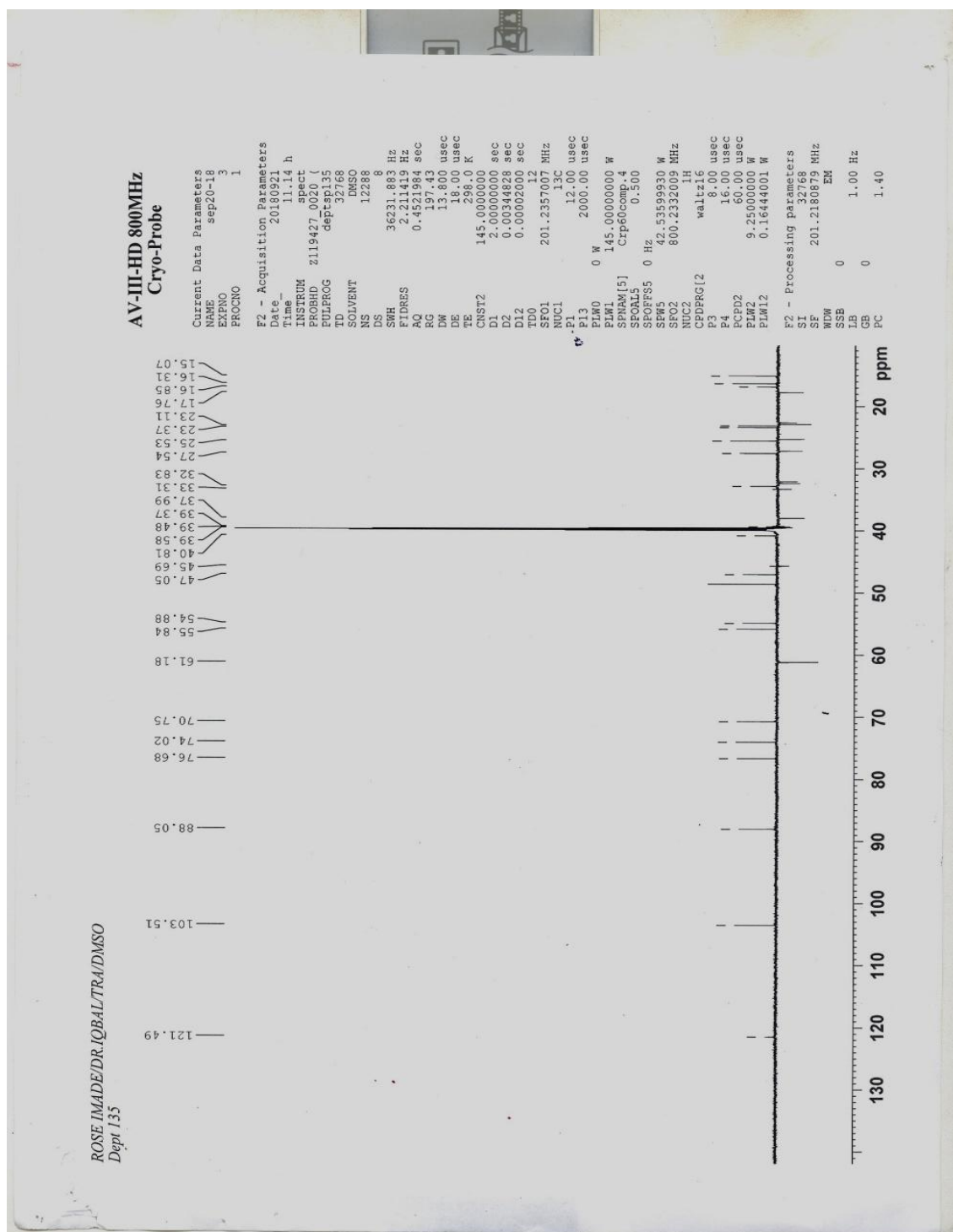


Figure 3.33: ^{13}C Spectral data (DEPT 135) of Compound TRA (3-O-[β -D-glucopyranosyl]-2'-acetamido-2'-deoxy]-oleanolic acid (Aridanin))

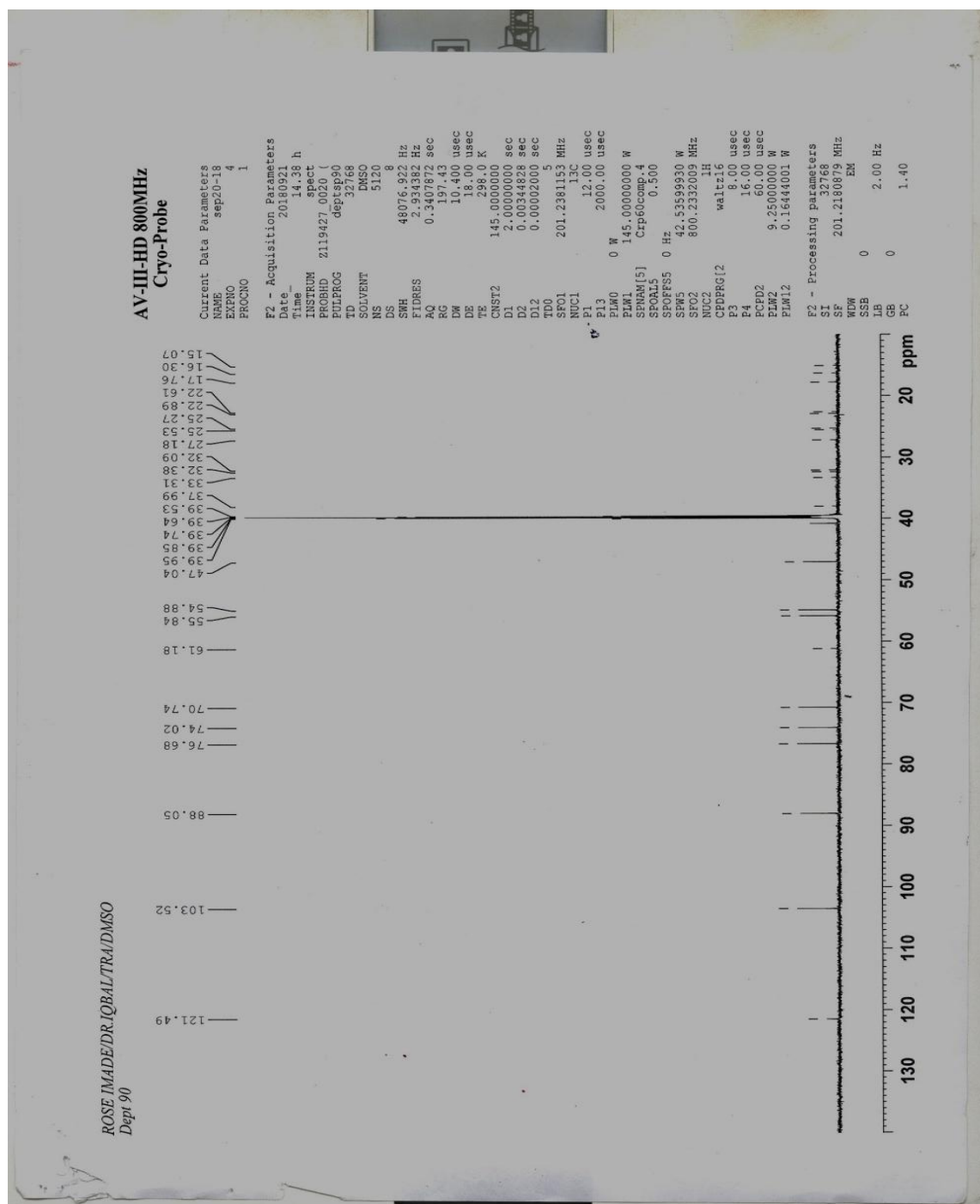


Figure 3.34: ^{13}C Spectral data (DEPT 90) of Compound TRA (3-O-[β -D-glucopyranosyl-2'-acetamido-2'- deoxy]-oleanolic acid (Aridanin))

Table 3.23: NMR Data of Compound TRA (600 MHz for ^1H and 150 MHz for ^{13}C) in DMSO

Carbon No.	δ_{C} (ppm)	DEPT	δ_{H} (ppm)	Multiplicity, J (Hz)
1	17.8	CH_2	1.23	m
2	33.3	CH_2	1.29	m
3	76.7	CH	3.40	m
4	39.7	C	-	-
5	45.7	CH	1.89	m
6	22.9	CH_2	1.40	m
7	23.1	CH_2	1.45	m
8	41.3	C	-	-
9	47.1	CH	2.72	dd (10.2)
10	39.8	C	-	-
11	38.0	CH_2	2.96	dd (3.6, 10.8)
12	121.5	CH	5.13	s
13	143.9	C	-	-
14	48.6	C	-	-
15	25.3	CH_2	1.47	m
16	25.5	CH_2	1.47	m
17	45.5	C	-	-
18	54.9	CH	3.03	m
19	27.2	CH_2	1.49	m
20	40.0	C	-	-
21	32.4	CH_2	1.61	m
22	32.8	CH_2	1.65	m
23	178.6	C	-	-
24	25.5	CH_3	0.87	s
25	27.5	CH_3	0.87	s
26	23.4	CH_3	0.88	s
27	23.1	CH_3	1.08	s
28	16.9	CH_3	0.85	s
29	15.1	CH_3	0.71	s
30	16.3	CH_3	0.65	s
1'	103.5	CH	7.65	d (9.0)
2'	88.1	CH	4.80	d (5.4)
3'	74.0	CH	3.64	dd (5.4, 11.4)
4'	70.8	CH	3.15	d (4.8)
5'	55.8	CH	4.07	q (4.8)
6'	61.2	CH_2	3.39	m
1''	168.7	C	-	-
2''	32.8	CH_3	1.80	s

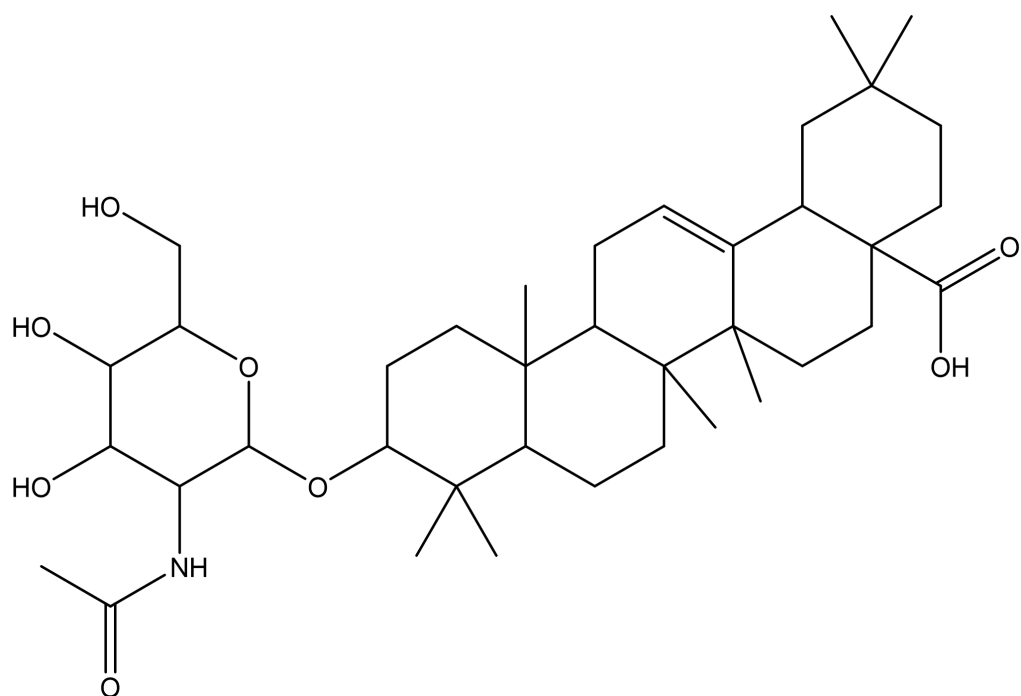


Figure 3.35: Structure of Compound TRA (3-O-[β -D-glucopyranosyl-2'-acetamido-2'- deoxy]-oleanolic acid (Aridanin))

3.37 Result of the inhibition/stimulation effects of TT2 and TRA on breast cancer cell lines

TT2 and TRA both produced minimal inhibitory effects against breast cancer with TT2 showing 14.36% inhibition and TRA giving 19% inhibition against AU 565 and MDA-MB231 cell lines respectively (Table 3.24).

Table 3.24 Inhibition/stimulation effects of TT2 and TRA on breast cancer cell lines

Compound	Concentration (μ M)	% Inhibition/Stimulation
TT2 (AU 565)	50	+14.36
TRA (MDA-MB231)	50	+19
Doxorubicin	50	+75.5

CHAPTER FOUR

4.1 DISCUSSION

Higher plants have proved their value as traditional medicines, dietary supplements, spices and foods and they synthesize a large number of secondary metabolites with an enormous diversity of chemical structures (Clark, 1996; Rates, 2001).

Many of the most effective phytomedicines on the market are whole extracts of plants and practitioners believe that synergistic interactions between the components of individual or mixtures of medicinal plants are vital for their therapeutic efficacy (Williamson, 2001). Consequently, it is no longer necessary to isolate the active constituents of a medicinal plant, although it remains necessary to determine the identity of the principal active compounds so that chemical profiling and establishment of phytoequivalence can be more precise (Tyler, 1999).

Bioassays play a crucial role in the isolation of new bioactive natural products. In the first approach, a bioassay-guided isolation of a pharmacologically active plant extract leads to the purification and structure elucidation of bioactive compound(s). Structure activity relationship (SAR) studies may result in more active and/or less toxic substances with improved bioavailability properties. These leads, after scale up, become normal competitors of synthetic leads for developing a new drug (Gupta *et al.*, 2006).

In the second approach, active constituents are not isolated but are identified using several chromatographic and other techniques. Chemical profiling and quantitative determination of these active constituents afford a standardised extract, which can be screened for toxicity and safety before carrying out clinical tests to develop them into herbal drugs (Gupta *et al.*, 2006).

Research into antitumor agents usually involves a series of complex procedures that sometimes produces non-encouraging results after much time and materials have been expended. In addition, paucity of research funds has made the development and acceptance of simple bench-top assays imperative (Mc Laughlin, 1991). These bioassays are simple, rapid, reproducible, inexpensive, and are adaptive to a large number of samples.

Growth inhibition test using *S. bicolor* seeds, tadpole mortality test and brine shrimp lethality test were the bench-top assay methods used in this study due to their availability. Meristematic tissues of seeds have the tendency to proliferate when exposed to favourable conditions and the extent of proliferation is shown in the increase in length of the radicles produced in the control seeds. Seeds of *S. bicolor* were found to be more convenient due to their small size. Also, the availability is high and up to 90% can germinate within 24 hours (Ayinde and Agbakwuru, 2010). The radicle growth inhibition of the extracts is tied to the suppression of the cell division (Ray-Gallet *et al.*, 2013) thereby by affecting its elongation (Fusconi *et al.*, 2006), probably through reduction in mitotic index. The inhibition of the radicles by the extracts could also be explained by changes in osmotic potential which prevents the development of turgor pressure (in the seeds cells) which is one of the key factors required for the initiation of radicle growth during seed germination (Maia and Rainer, 2001). It is also possible that the constituents of the extracts interfere with the production and activities of amino acids necessary for the proliferation of cells in germinating seeds (Alabadi and Carbonell, 1999).

E. caryophyllus, *Z. officinale*, and *O. gratissimum* oil produced the highest growth inhibitory activities. 5 mg/mL concentration of *E. caryophyllus* oil completely inhibited the growth of the radicles (Figure 3.1 – 3.4). *O. basilicum* oil and *T. tetraptera* pod extract were also observed to

retard the growth of the seed radicles. The extent of sensitivity could be due to the biochemical resistance/agents the cells produce as a response to the constituents of the oils or the extract.

In vivo lethality in simple zoologic organisms can be used as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products (Mc Laughlin, 1991). *H. suaveolens*, *T. tetraptera* pod and *E. caryophyllus* oil demonstrated the highest activity in the tadpole mortality test.

Lethality of substances to brine shrimp nauplii has been linked to the probable ability of such compounds to kill cancer cells in cell cultures (Babajide, 2010). It has been demonstrated that Brine Shrimp Cytotoxicity Test (BSCT) correlates reasonably well with cytotoxicity and other biological properties (Mc Laughlin, 1991). In toxicity evaluation of plant extracts by brine shrimp lethality bioassay, LC₅₀ values lower than 1000 µg/mL are considered bioactive (Meyer *et al.*, 1982). The activities of *Z. officinale*, *H. suaveolens*, *E. caryophyllus* and *O. gratissimum* oil were comparable with LC₅₀ of 157.75, 188.67, 143.17, and 200.34 µg/mL respectively. *T. tetraptera* pod extract and *O. basilicum* oil showed the least activity with LC₅₀ values greater than 1000 µg/mL (Table 3.2).

MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay is widely used for measuring cell proliferation with respect to cellular metabolism, involving a reduction of tetrazolium salts by dehydrogenase enzymes. This chemical reaction yielding a purple product (formazan) is displayed only in metabolically active cells thereby eliminating non-proliferating cells (Ravi *et al.*, 2016). The more the number of metabolically active cells, the darker the purple colour.

H. suaveolens and *Z. officinale* showed the highest cytotoxicity using MTT assay on AU 565 cells at 50 µg/mL with 86.74 and 43% inhibitions respectively. An IC₅₀ of 34.75 µg/mL was obtained for *H. suaveolens* oil. Lower percentage inhibitions of 17.82, 16.52, and 11.80% were obtained for *O. gratissimum*, *E. caryophyllus* and *C. citrinus* oil at the same concentration while *O. basilicum* and *T. tetraptera* pod extract produced no inhibition of the cancer cells.

Z. officinale oil produced the highest potency against HeLa with 50% inhibition and IC₅₀ of 29.90 µg/mL. The efficacy of *O. basilicum* and *H. suaveolens* were commensurate with 23.02 and 21.80% inhibitions respectively while *O. gratissimum*, *E. caryophyllus* and *C. citrinus* oil showed no activity against this cancer cell line.

Z. officinale essential oil has been reported to exhibit strong cytotoxicities at a concentration of 0.01% (v/v) towards human ovarian cancer cell line (HO-8910) and human hepatocellular liver carcinoma cell line (Bel-7402) (Wang *et al.*, 2012). Its methanol extract has previously been reported to potently inhibit the proliferation of HeLa at a concentration of 100 µg/mL with 84.88% growth inhibition (Nalbantsoy *et al.*, 2010).

Generally, biological activities of essential oils are related to their chemical compositions i.e. to the major constituents of the oils. However, the less present compounds could also be of importance as the various molecules could synergistically act with the major compounds (Bakkali *et al.*, 2006).

Zingiberene, a major component of *Z. officinale* has been cited to possess anti-proliferative activity against N2a-NB cells at concentrations over 50 µg/mL (Togar *et al.*, 2015). It also showed activity with IC₅₀ values of 60.6 ± 3.6, 46.2 ± 0.6, 172.0 ± 6.6 and 80.3 ± 6.6 (µg/mL) against HeLa, SiHa, MCF7 and HL-60 cells respectively (Togar *et al.*, 2015).

1, 8-cineole, another compound found in *Z. officinale* oil has been reported to induce specific apoptosis on human colon cancer cell lines HCT116 and RKO. Also, geraniol has *in vitro* and *in vivo* antitumor activity against several cell lines (Crespo *et al.*, 2013). It was found to alter several lipid metabolic pathways of HepG2 cells which resulted in cell growth inhibition, cell cycle arrest occurring at the G0/G1 interphase, and increased apoptosis (Crespo *et al.*, 2013).

H. suaveolens essential oil on the other hand, has been reported to be active against human breast cancer cell line (MCF-7) with IC₅₀ value of 90.6 µg/ml (Bayala *et al.*, 2020). Its leaf ethanolic and water extracts have also been previously investigated for cytotoxic effect and apoptotic cell death induction in human T-leukemia cell line and Jurkat cells with IC₅₀ values of 553.52 and 912.06 µg/mL respectively, recorded. (Musika and Indrapichate, 2014).

D-Limonene, a compound found in *H. suaveolens*, has been reported to be toxic to V79 cells in a dose-dependent manner (Mauro *et al.*, 2013). β-caryophyllene oxide, a sesquiterpene also found in *H. suaveolens*, was cited to show activity against multiple myeloma, breast and prostate cancer cell lines (Kim *et al.*, 2014).

These major constituents may be responsible for the observed activities of the oils.

Although *O. basilicum*, *O. gratissimum*, *E. caryophyllus*, and *C. citrinus* oil at 50 µg/mL concentration produced low inhibitions against AU 565 and HeLa, they have been reported to possess cytotoxic activities against a wide array of other cell lines. For example SF-767 glioblastoma (Bayala *et al.*, 2014) and P388 murine leukemia (Marotti *et al.*, 1996) cell lines have been reported to be sensitive to *O. basilicum* oil. Essential oil of *O. gratissimum* was documented to have cytotoxic effect on the human epidermic cell line HaCat (IC₅₀: 2400

µg/mL). Pure commercial thymol reportedly showed an IC₅₀ of 1800 µg/mL on the same cell line (Koba *et al.*, 2007). Crude extract of *O. gratissimum* and its hydrophobic and hydrophilic fractions also differentially inhibited breast cancer cell *in vitro* and retarded tumor growth and temporal progression of MCF10ADCIS.com xenografts (Nangia-Makker *et al.*, 2013).

E. caryophyllus oil on the other hand, has been cited to show anticarcinogenic activity because of its strong free radical activity (Miyazawa and Hisama, 2001), particularly in cases of lung, skin and digestive cancers (Schonfelder and Schonfelder, 2004). *C. citrinus* leaves oil also showed high cytotoxicity on A549 cells (Kumar *et al.*, 2015). Murata *et al.* in 2013, found out that 1, 8-cineole (the main component of *C. citrinus*) suppresses the proliferation of colon cancer cells by inducing apoptosis. The antiproliferative activity of another species, *C. viminalis*, against MCF-7 (IC₅₀: 25.15 ± 0.3), HeLa (IC₅₀: 18.75 ± 1.5), Jurkat (IC₅₀: 53.10 ± 3.5), and HT-29 (IC₅₀: 10.51 ± 1.0) has been reported (Elansary *et al.*, 2018). *T. tetraptera* pod on the other hand has also been reported to demonstrate cytotoxic and antiproliferative effects *in vivo* and *invitro* against ehrlich ascites carcinoma cells with an IC₅₀ of 250 µg/mL (Ozaslan *et al.*, 2016).

The chemical compositions of the various essential oils have been carried out in various parts of the world. Over 300 different compounds have been identified in essential oils and they fall into three groups (Pichersky *et al.*, 2006). The main group is composed of terpenes and terpenoids which are thought to be responsible for their observed activities. The others include aromatic components and aliphatic compounds which are generally in trace (Bakkali *et al.*, 2008).

The major components in *O. basilicum* oil from the results obtained were estragole (52.8%) and β-linalool (16.1%) (Table 3.9) while the major constituents of *O. basilicum* oil isolated in Cameroon were limonene (10.40%) and linalool (50.80%) (Zollo *et al.*, 1998). For *H. suaveolens*,

the major constituent was sabinene (10.64%) and (-)-4-terpineol (7.27%) (Table 3.11) while 1, 8-cineole (32%) and β -caryophyllene (29%) were found majorly in *H. suaveolens* oil harvested in Australia (Peerzad, 1997). In an earlier report by Joshi, (2017) the compounds eugenol (75.1%) and terpinolene (14.2%) were reported in high amount from cultivated *O. gratissimum* plant collected in India. The same author in 2017, identified eugenol (57.1%), α -bulnesene (15.6%) and β -caryophyllene (14.2%) as the prominent compounds in *O. gratissimum* growing desolately in India. From the results obtained in this study, thymol (22.49%), p-cymene (13.47%), and γ -terpinene (11.59%) were of the highest abundance in *O. gratissimum* oil (Table 3.10).

The quantitative and qualitative differences in the composition of the oils may be due to the geographical, climatic and soil conditions as well as the level of maturity of the plants at the point of harvest (Barra, 2009). It can be stated that the variations in the anti-cancer activities of these oils can be traced to the variations in their chemical compositions.

Separation through column chromatographic procedures remarkably improved the growth inhibitory effects of the volatile oils on the radicles. For example at 96 h, the radicle length of the control was 6.18 mm while CF₁B (obtained from *O. basilicum* oil) gave 4.07 mm indicating 34.14% growth inhibition. SF₂B obtained by preparative TLC of CF₁B, gave a radicle length of 1.28 mm indicating a growth reduction of 79.29% (Figure 3.3). CF₁G produced 81.27% growth inhibition of the seed radicles while SF₂G gave 92.03% growth inhibition (Figure 3.9). CF₁C gave 92.39% growth inhibition (Figure 3.11). This shows that fractionation further increased the growth inhibitory activities of the oils.

Inhibitions of AU 565 cells by the fractions were varied with CF₁C from *C. citrinus* giving the highest inhibition of 70.44%. The GCMS revealed an increase in the presence of 1,1'-(5-

hydroxy-2,2-dimethylbicyclo[4.1.0]heptane-1,7-diyl)bis-, (1 α ,5 β ,6 α ,7 α)-ethanone from 1.13% (in the oil) to 24.89% in the fraction. As stated earlier, chromatographic separation enhanced the biological activity. Ethanone derivatives have been documented to possess cytotoxic activities. For example a series of novel 1-(3-substituted-5-phenyl-4,5-dihydropyrazol-1-yl)-2-thio-ethanone derivatives reportedly demonstrated antiproliferative activity against SGC-7901, MGC-803, Bcap-37 and HEPG-2 cell lines (Wu *et al.*, 2013).

Application of preparative TLC led to the isolation of SF₂B (obtained from *O. basilicum* oil), SF₂G (obtained from *O. gratissimum* oil), and SF₂E (obtained from *E. caryophyllus* oil), which were found to have 33.19, 85.07 and 18.82% inhibitions against AU 565 cell line respectively. IC₅₀ of 15.96 μ g/mL was obtained for SF₂G. As earlier stated, the inhibitory activities of the whole forms of the oils against this cell line were: *O. basilicum* oil (-1.02%), *O. gratissimum* oil (17.82%), and *E. caryophyllus* oil (16.52%). Isolation of the major constituent therefore resulted in a general increase in the activity of the oils.

GCMS revealed that SF₂G was majorly thymol (94.31%, Table 3.15). Thymol has been reported to show cytotoxic effect against Hep-2 cells (Stammati *et al.*, 1999), Caco-2 human colonic cells, and V79 hamster lung cells (Slamenova *et al.*, 2007). Jayakumar and collaborators (Jayakumar *et al.*, 2012; Ozkan and Erdogan, 2011) demonstrated that thymol is cytotoxic against HepG2 human hepatoma cells, and K562 cells, via a mechanism that may be related to antioxidant activity and not associated with a DNA-damaging effect. The cytotoxic effect of thymol was also reportedly tested against murine B16-F10 melanoma cells and an IC₅₀ value of 88.5 μ g/mL was obtained (Paramasivam *et al.*, 2012). Yin and his team (Yin *et al.*, 2012) demonstrated that thymol induced cell cycle arrest at G0/G1 phase. It was also documented that thymol induced apoptosis in HL-60 cells via caspase-dependent and caspase-independent pathways (Deb *et al.*,

2011). Against human osteosarcoma (MG63) cells, thymol induces a cytosolic Ca^{2+} rise by inducing phospholipase C- and protein kinase C-dependent Ca^{2+} release from the endoplasmic reticulum and Ca^{2+} entry via protein kinase C-sensitive store-operated Ca^{2+} channels (Chang *et al.*, 2011).

Thymol occurs naturally in *Thymus vulgaris* L. (Lamiaceae), *Monarda didyma* (Lamiaceae), *Origanum vulgare* (Lamiaceae), and *Euphrasia rostkoviana* (Orobanchaceae) (Salehi *et al.*, 2018)

SF₂B obtained from *O. basilicum*, gave the highest inhibition of 89.3% with IC₅₀ of 18.70 µg/mL against HeLa. SF₂G and SF₂E on the other hand were inactive with inhibitions of -29.20 and -1.24% respectively. Estragole was found to be the major component in SF₂B with relative abundance of 88.61%, as revealed by GCMS (Table 3.14). It occurs naturally in *Artemisia dracunculus* L. (Asteraceae), *Foeniculum vulgare* (Apiaceae), *Pinus longifolia* Roxb (Pinaceae), *Croton zehntneri* (Euphorbiaceae), and *Pimpinella anisum* (Apiaceae)

O. basilicum oil has been previously reported to have *invitro* cytotoxic activity against HeLa cells with IC₅₀ value of 90.5 µg/mL (Wierdak *et al.*, 2013). The lower IC₅₀ value of estragole against HeLa reported here, indicates that it may be responsible for the observed anticancer activity. Howes *et al.*, in 1990 demonstrated the genotoxic activity of estragole via UDS assay, in which estragole induced dose-dependent increases in UDS up to 2.7 times that of the control in rat hepatocytes in primary culture. Estragole has been tested against MCF-7 (4.62%), HEP-2 (0%) and NCI-H292 (7.36%) human cancer cell lines but the inhibitions were not rewarding (Andrade *et al.* 2015).

Chromatographic separation of eugenol content of *E. caryophyllus* oil through preparative TLC did not improve its activity as demonstrated by SF₂E. Indeed, a molecule could have an effect on

one type of tumor and not on others. For example, Murata *et al.*, in 2013 showed that 1,8-cineole induces apoptosis of human colon cancer cells but it has no effect on prostate cancer and glioblastoma cell survival (Bayala *et al.*, 2014).

Eugenol has been reported to induce cell death in several tumors and cancer cell types (Park *et al.*, 2005; Jaafari *et al.*, 2012). Breast adenocarcinoma (Jaafari *et al.*, 2012), melanoma cells (Satooka and Kubo, 2009; Pisano *et al.*, 2007), leukemia (Satooka and Kubo, 2009; Fujisawa *et al.*, 2000), and colon carcinoma (Slameňová *et al.*, 2009) are examples.

Plants are known to contain many constituents of varying polarities and molecular mass. Using two appropriate immiscible solvents ensures the separation of the constituents based on their relative solubilities in the solvents. Partitioning of methanol extract of *T. tetraptera* pod improved the activity greatly.

At 96 h, with a concentration of 5 mg/mL, the mean control radicle length of 15.82 ± 2.66 mm was reduced to 2.85 ± 1.99 mm by the chloroform fraction indicating 81.98% growth inhibition. The aqueous and hexane fractions gave 64.29 and 71.74% growth inhibitions respectively (Figure 3.28). The chloroform fraction also produced the highest inhibition of 82.27% against AU565 cell line with IC_{50} of 9.63 μ g/mL. The hexane and aqueous fractions gave 34.02 and 16.12% inhibitions respectively.

Vacuum Liquid Chromatography (VLC) was employed to further separate the chloroform fraction to rapidly yield bioactive constituents.

Among the VLC fractions, the chloroform-ethylacetate subfraction (D) showed the highest activity with a growth inhibition of 62.05% on the seed radicles. Chloroform (C) and Ethylacetate (E) fractions were also active with 47.36 and 48.56% growth reductions

respectively (Figure 3.29). C* which is a combination of C, D and E produced the highest inhibition of 23% against AU565 cell line while Methanol (G) and Methanol-Water (H) subfractions gave 13.38 and 20.75% inhibitions respectively.

The chemical structures of the isolated compounds were determined by means of spectroscopic methods. The data were obtained from 1D NMR (^1H NMR and ^{13}C NMR).

Compound TT2 -(5a,5b,8,8,11a,13b-Hexamethyl-icosahydro-cyclopenta[a]chrysen-3-yl)-propan-2-ol (22-hydroxyisohopane) was isolated as a white powder (7 mg).

The ^{13}C -NMR spectrum showed that there are 30 carbon atoms in the molecule which makes it most likely a triterpenoid. The signals at δ 51.1, 32.7, 40.9, 23.7, 33.9, 18.0, 21.3, 33.9, 26.1, 40.2, 20.6, indicate the presence of the methylenes (CH_2) in the molecule. Five methine (CH) resonances (55.5, 53.8, 48.6, 49.9, 62.7), eight methyl resonances (33.4, 16.5, 16.7, 16.8, 15.9, 22.3, 30.8, 28.9) and six quaternary carbons (49.5, 43.6, 34.4, 41.5, 38.4, 71.6) were also observed in the spectrum. Due to the presence of an hydroxyl group at position 22, ^{13}C NMR showed two signals downfield at 62.7 (C-21) and 71.6 (C-22) which are a methine and a quaternary carbon respectively.

The broad band has a signal at 71.6. This signal did not show in both dept 90 and 135 suggesting that it's a quaternary carbon which supports the proposed structure.

The presence of a signal downfield at 4.089 shows that a hydroxyl group is present as revealed by the ^1H NMR spectra. The proton NMR shows the presence of tall signals which are indicative of methyl signals.

The above data shows that compound TT2 is a triterpene with a hydroxyl group attached at position 22. The molecular ion peak (M^+) at 426.3 ($M \pm 2$) is consistent with the molecular formula $C_{30}H_{52}O$.

The triterpenoid 22-hydroxyhopane has previously been isolated from leaves of the fern *Adiantum latifolium* and was revealed to possess larvicidal activity against the larvae of the coconut pest *Oryctes rhinoceros* (Kumar *et al*, 2019).

The compound TRA (3-O-[β -D-glucopyranosyl-2'-acetamido-2'-deoxy]-oleanolic acid) was isolated as a white powder (8 mg) which gave a positive reaction to the test for amines. A carboxylic acid signal was obtained at δ 178.6 (C-23) and a double bond signal at δ 121.5 (C-12) and δ 143.9 (C-13). The absence of a carbon signal in DEPT at C-23 and C-13 further confirms this. An O- glycosidic bond is present at position 3 of the steroid nucleus with a resonance of δ 76.7. An anomeric carbon signal at δ 103.5 indicated the presence of a single monosaccharide moiety. This indicates a β -glucose anomeric carbon. The chemical shift for α -glucose is between 97-101 while β anomeric could be up to 105. An amide group is present in the sugar portion (C-7', δ 168.7) which is further confirmed by the absence of a carbon in the DEPT. EIMS did not show the sugar portion due to its polarity and bulky nature. The molecular ion peak (M^+) at 456.2 is consistent with the molecular formula $C_{30}H_{48}O_3$ which is the aglycone portion of the structure thus confirming the presence of an extra mass of 204 g/mol which is obviously from a hexose sugar (glucose) in addition to a nitrogen containing moiety. The chemical shift for Fructose was ruled out because of the absence of a carbonyl signal in the ^{13}C -NMR spectrum. The ^{13}C -NMR spectrum of compound TRA showed that there are 38 carbon atoms in the molecule. With these data, compound TRA is likely a glycoside of a triterpene. The signals at δ 17.8, 33.3, 22.9, 23.1, 38.0, 25.3, 25.5, 27.2, 32.4, 32.8, and 61.2 indicate the presence of the methylenes in the

molecule. The ten methine resonances at δ 76.7, 45.7, 47.1, 121.5, 54.9, 103.5, 88.1, 74.0, 70.8, 55.8 as well as the methyl resonance at δ 25.5, 27.5, 23.4, 23.1, 16.9, 15.1, 16.3 and 32.8 were due to C-3, C-5, C-9, C-12, C-18, C-1' , C-2' , C-3' , C-4' , C-5' and C-24, C-25, C-26, C-27, C-28, C-29, C-30, C-8' respectively on the molecule. Signals at δ 39.7 (C-4), 41.3 (C-8), 39.8 (C-10), 143.9 (C-13), 48.6 (C-14), 45.5 (C-17), 40.0 (C-20), 178.6 (C-23), 168.7 (C-7') correspond to the quaternary carbons of the moiety. The fragmentation of the molecule is shown by the EIMS. For example the difference between peak 456.2 and 439.3 shows that an hydroxyl group has been lost while the difference between 456.2 and 410.3 shows that a carboxylic acid group has been lost.

After comparing with the information in literature, the compound was identified to be similar to aridanin. It has been reported to be found in *T. tetraptera* fruit where it was cited to have mulluscicidal activity against schistosomiasis-transmitting snails *Biomphalaria glabrata*, *Schistosoma mansoni* and *S. bovis* miracidia (Aladesanmi, 2007; Adewunmi and Furu, 1989). It has also been reported to have anticonvulsant, analgesic, and hypothermic activities (Aderibigbe *et al.*, 2007a).

CHAPTER FIVE

5.1 CONCLUSION

The results have justified the use of bench-top assay methods in screening medicinal plants reputed to have anti-tumor activities. The volatile oils were screened *in vitro* against AU 565 and HeLa cancer cell lines while *T. tetraptera* pod extract was screened against AU 565 cell line, and the final isolate also screened against MDA-MB231 cell line using MTT assay. *H. suaveolens* and *Z. officinale* volatile oils were observed to show significant growth inhibitory potency. Oils of *O. basilicum*, *O. gratissimum*, *E. caryophyllus*, and *C. citrinus* were chosen for further studies. They were processed with multistep chromatographic methods (Column chromatography and preparative TLC) to isolate the major components. SF₂B (estragole), SF₂G (thymol) and SF₂E (eugenol) were obtained and their identity determined by GCMS. Estragole possesses significant growth inhibitory activity against HeLa while thymol showed active inhibition against AU 565 cell line. Eugenol demonstrated little effect against these cell lines.

Methanol extract of *T. tetraptera* pod underwent various chromatographic procedures (VLC, CC and HPLC). Two compounds were isolated and their structure analysed by NMR and mass spectroscopy. Compounds TT2 and TRA showed little growth inhibitory activities against AU565 and MDA-MB231 cell lines respectively.

These results have shown the potency and justified the probable use of these plants in ethnomedicine in treating tumor-related ailments. However further studies are necessary using *in vivo* methods.

5.2 CONTRIBUTIONS TO KNOWLEDGE

The study has contributed to knowledge in the following ways:

1. It has established the probable cytotoxic and antiproliferative effects of these volatile oils and extract on tadpoles, *S. bicolor* radicle, brine shrimp using bench-top assay methods.
2. It has further validated the predictive methods used in this work.
3. It has established that the cytotoxic and antiproliferative effects of *T. tetraptera* on tadpoles, *S. bicolor* radicle and the cell line resides more in the chloroform fraction.
4. The presence of 1,1'-(5-hydroxy-2,2-dimethylbicyclo[4.1.0]heptane-1,7-diyl)bis-, (1 α ,5 β ,6 α ,7 α)-ethanone in *C. citrinus* oil and its cytotoxic effect on the cell line has been reported here for the first time.
5. The effects of thymol, estragole, eugenol, 22-hydroxyisohopane and 3-O-[[β -D-glucopyranosyl-2'-acetamido-2'- deoxy]-oleanolic acid on the cell lines have been established here for the first time.

REFERENCES

- Abdallah HM** and Ezzat SM. (2011). Effect of the method of preparation on the composition and cytotoxic activity of the essential oil of *Pituranthos tortuosus*. *Zeitschrift fur Naturforschung C*. 66:143–8.
- Adaikan PG**, Gauthaman K and Prasad RNV. (2001). History of herbal medicines with an insight on the pharmacological properties of *Tribulus terrestris*. *The Aging Male*. 4(3): 163-169.
- Adebolu T** and Oladimeji SA. (2005). Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in southwestern Nigeria. *African Journal of Biotechnology*. 4(7): 682-4.
- Aderibigbe AO**, Iwalewa EO, Adesina SK, Adebajo AO and Ukponmwan OE. (2007a). Anticonvulsant, analgesic and hypothermic effects of aridanin isolated from *Tetrapleura tetrapetra* fruit in mice. *Journal of Biological Sciences*, 7: 1520-1524.
- Aderibigbe AO**, Iwalewa EO, Adesina SK, Ukponmwan OE and Adebajo AO. (2007b). Neuropharmacological evaluation of aridanin, a glycoside Isolated from *Tetrapleura tetraptera* fruit. *Discovery and Inovation*. 19 (3). Pp 177-181.
- Adesina SK**. (1978). Anticonvulsant properties of the extracts of some Nigerian medicinal plants. *The Nigerian Journal of Pharmaceutical Research*. 9(5): 211-212:224.
- Adesina SK**, Adewunmi CO and Marquis VO. (1980). Phytochemical investigations of the molluscicidal properties of *Tetrapleura tetraptera* Taub. *African Journal of Traditional, Complementary and Alternative Medicines*. 3:7-15.
- Adesina SK**, Iwalewa EO and Imoh IJ. (2016). *Tetrapleura tetraptera* Taub- Ethnopharmacology, chemistry, medicinal and nutritional values- A review. *British Journal of Pharmaceutical Research* 12(3): 1-22.
- Adesina SK** and Reisch J. (1985). A triterpenoid glycoside from *Tetrapleura tetraptera* fruit. *Phytochemistry*. 24(12):3003-3006.

Adewunmi CO and Furu P. (1989). Evaluation of aridanin, a glycoside, and an aqueous extract of *Tetrapleura tetraptera* fruit, on *Schistosoma mansoni* and *S. bovis*. *Journal of ethnopharmacology*. (27) 3: 277-283.

Afoulous S, Ferhout H, Raoelison EG, Valentin A, Moukarzel B, Couderc F and Bouajila J. (2013). Chemical composition and anticancer, antiinflammatory, antioxidant and antimalarial activities of leaves essential oil of *Cedrelopsis grevei*. *Food and Chemical Toxicology Journal*. 56:352–62.

Ahmad RS, Hussain MB, Sultan MT, Arshad MS, Waheed M, Shariati MA, Plygun S, and Hashempur MH (2020). Biochemistry, safety, pharmacological activities, and clinical applications of turmeric: A mechanistic review. *Hindawi Evidence-Based Complementary and Alternative Medicine*; 2020 Article ID 7656919

Akgül A. (1989). Volatile oil composition of sweet basil (*Ocimum basilicum* L.) cultivating in Turkey. *Nahrung*. 33:87–8.

Akpan OU, Bassey RB, Agba BS and Edegha IA. (2014). Elevation of serum pancreatic amylase and distortion of pancreatic cyto-architecture in type 1 diabetes mellitus rats treated with *Ocimum gratissimum*. *Nigerian Journal of Medicine*. 55(1): 34.

Alabadi D and Carbonell J. (1999). Differential expression of two spermidine synthase genes during early fruit development and in vegetative tissues of pea. *Plant Molecular Biology*. 39: 933-943.

Aladesanmi AJ. (2007). *Tetrapleura tetraptera*: Molluscicidal activity and chemical constituents. *African Journal of Traditional complementary and alternative medicine* 4: (1).

Albuquerque UP, Medeiros PM, Almeida ALS, Monteiro JM, Neto EM and Melo JG. (2007). Medicinal plants of the Caatinga (semi-arid) vegetation of NE Brazil: A quantitative approach. *Journal of Ethnopharmacology*. 114(3): 325-54.

Altman SA, Randers L and Rao G. (1993). Comparison of trypan blue dye exclusion and fluorometric assays for mammalian cell viability determinations. *Biotechnology Progress*. 9(6): 671-74.

Amla MH, Ertas M, Nitz S and Kollmannsberger H. (2007). Chemical composition and content of essential oil from the bud of cultivated Turkish clove (*Syzygium aromaticum* L.). *BioResources*. 2(2):265-269.

Andrade TCB, De Lima SG, Freitas RM, Rocha MS, Islam T, Da Silva TG and Militao GCG. (2015). Isolation, characterization and evaluation of antimicrobial and cytotoxic activity of estragole, obtained from the essential oil of *Croton zehntneri* (Euphorbiaceae). *Anais da Academia Brasileira de Ciências*. (87):1

Angiosperm Phylogeny Group (2009). An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society*. 161 (2): 105-121.

Atta-ur-Rahman, Choudary MI and Thomsen WJ. (2001) Bioassay techniques for drug development. 1st edition, informa health care, United Kindom, ISBN-13: 978-9058230515. Pp 1-14.

Awogbindin IO, Tade OG, Metibemu SD, Olorunsogo OO and Farombi EO. (2014). Assessment of flavonoid content, free radical scavenging and hepatoprotective activities of *Ocimum gratissimum* and *Spondias mombin* in rats treated with dimethylnitrosamine. *Archives of Basic and Applied Medicine*. 2(1): 45-54.

Ayinde BA and Agbakwuru U (2010). Cytotoxic and growth inhibitory effects of the methanol extract of *Struchium sparganophora* Ktze (Asteraceae) leaves. *Pharmacognosy Magazine* 6(2):12-21.

Baba G, Lawal AO and Shariff HB. (2012). Mosquito repellent activity and phytochemical characterization of essential oils from *Striga hermonthica*, *Hyptis spicigera* and *Ocimum basilicum* leaf extracts. *British Journal of Pharmacology and Toxicology*. 3:43-8.

Babajide OJ, Mabusela WT, Green IR , Ameer F , Weitz F and Emmanuel I. Iwuoha EI (2010). Phytochemical screening and biological activity studies of five South African indigenous medicinal plants. *Journal of Medicinal Plants Research* Vol. 2(18), pp. 1924-1932.

Bakkali F, Averbeck S, Averbeck D, Zhiri A, Baudoux D and Idaomar M. (2006). Antigenotoxic effects of three essential oils in diploid yeast (*Saccharomyces cerevisiae*) after treatments with UVC radiation, 8-MOP plus UVA and MMS. *Mutation Research*. 606:27–38

Bakkali F, Averbeck S, Averbeck D and Idaomar M. (2008). Biological effects of essential oils-a review. *Food and Chemical Toxicology*. 46:446–75.

Barra A. (2009). Factors affecting chemical variability of essential oils: a review of recent developments. *Natural Product Communications*. Vol 4, no 8: 1147-1154.

Barton K, Randall G and Sargon AL Jnr. (1989). The effects of the antitumor agent mezerein on the cytotoxic capacity and oxidative metabolism of human blood cells. *PubMed*; 7(2-3): 179-188.

Bayala B, Bassole IHN, Gnoula C, Nebie R, Yonli A, Morel L, Figueredo G, Nikiema JB, Lobaccaro JMA and Simpore J. (2014). Chemical composition, antioxidant, anti-inflammatory and anti-proliferative activities of essential oils of plants from Burkina Faso. *Plos One*. 9(3): e92122. Doi:10.1371.

Bayala B, Nadembega C, Guenne S, Bunay J, Zohoncon TM, Djigma FW, Yonli A, Baron S, Figueredo G, Lobaccaro JA and Simpore J (2020). Chemical composition, antioxidant and cytotoxic activities of *Hyptis suaveolens* (L.) Poit. essential oil on prostate and cervical cancer cells. *Pakistan Journal of Biological Sciences*; 23(9): 1184-1192.

Baykara M, Buyukberber S and Ozturk B. (2013). Efficiency and safety of concomitant chemoradiotherapy with cisplatin and docetaxel in patients with locally advanced squamous cell head and neck cancers. *Asian Pacific Journal of Cancer Prevention*. 14: 2557-2561.

Beg T, Siddique YS, Ara G, Gupta J and Afzal M. (2008). Antigenotoxic effect of genistein and gingerol on genotoxicity induced by norethandrolone and oxandrolone in cultured human lymphocytes. *International Journal of Pharmacology*. 4: 177-183.

Berman B and Cockerell CJ. (2013). Pathobiology of actinic keratosis: ultraviolet dependent keratinocyte proliferation. *Journal of the American Academy of Dermatology*. 68: 10-19.

Bertino JR. (1997). Irinotecan for colorectal cancer. *Seminars in oncology*. 24: 18-23.

Boik J. (2001). *Natural compounds in cancer therapy*. Promising non-toxic antitumor agents from plants and other natural resources. First edition. University of Texas, Oregon. Medical Press, Princeton, Page 2-4.

Bou DD, Lago JH, Figueiredo CR, Matsuo AL, Guadagnin RC, Soares MG and Sartorelli P. (2013). Chemical composition and cytotoxicity evaluation of essential oil from leaves of *Casearia sylvestris*, its main compound α -zingiberene and derivatives. *Molecules*. 18:9477–87.

Braun AC and Stonier T. (1958). Morphology and physiology of plant tumors. *Protoplasmatologia*; 10:1-93.

Brophy JJ, Forster PI, Goldsack RJ, Hibbert DB and Punruckvong A. (1997). Variation in *Callistemon viminalis* (Myrtaceae): New evidence from leaf essential oils. *Australian Systemic Botany*. 10(1), 1-13.

Buatone S and Indrapichate K. (2011). Protective effects of mintweed, kitchen mint and kaffir lime leaf extracts against rice weevils, *Stitophilus oryzae* L., in stored, milled rice. *International Journal of Agricultural Science*, 2011; 3(3): 133-139.

Buchbauer G. (2012). A review on recent research results (2008-2010) on essential oils as antimicrobials and antifungals. A review. *Flavour and Fragrance Journal*. 27:13-39.

Buckle J. (1999). Use of aromatherapy as a complementary treatment for chronic pain. *Alternative Therapy in Health and Medicine*. 5:42–51.

Burns MB, Temiz NA and Harris RS. (2013). Evidence for APOBEC3B mutagenesis in multiple human cancers. *Nature Genetics*. 45: 977-983.

Cai L, Ye H, Li X, Lin Y, Yu F, Chen J, Li H and Liu X. (2013). Chemical constituents of volatile oil from *Pyrolae herba* and antiproliferative activity against SW1353 human chondrosarcoma cells. *International Journal of Oncology*. 42:1452–8.

Carnesecchi S, Schneider Y, Ceraline J, Duranton B, Gosse F, Seiler N, Raul F. (2001). Geraniol, a component of plant essential oils, inhibits growth and polyamine biosynthesis in human colon cancer cells. *Journal of Pharmacology and Experimental Therapeutics*. 298:197–200.

Celline JX, Jiahui L, Heng L, Shihui L, Meigui L and Yueyu G. (2015). The antitumor effect of tanshinone II A on anti- proliferation and decreasing VEGF/VEGFR2 expression on the human non-small cell lung cancer A549. *Acta Pharmaceutica Sinica B*. 5(6), 554–63.

Chang HT, Hsu SS, Chou CT, Cheng JS, Wang JL, Lin KL, Fang YC, Chen WC, Chien JM, Lu T, Pan CC, Cheng HH, Huang JK, Kuo CC, Chai KL and Jan CR. (2011). Effect of thymol on Ca²⁺ homeostasis and viability in MG63 human osteosarcoma cells. *Pharmacology*. 88(3-4):201–212.

Chen H and Nalbantoglu J. (2014). Ring cell migration assay identifies distinct effects of extracellular matrix proteins on cancer cell migration. *BMC Research Notes*. 7:183.

Chen HC. (2005). Boyden chamber assay. Methods. *Molecular Biology*. 294:15-22.

Chidambara MKN, Jayaprakasha GK and Patil BS. (2012). D-limonene rich volatile oil from blood oranges inhibits angiogenesis, metastasis and cell death in human colon cancer cells. *Life Sciences*. 91:429–39.

Chistokhodova N, Nguyen C, Calvino T, Kachirskaia I, Cunningham G, Howardmiles D. (2002). Antithrombin activity of medicinal plants from central Florida. *Journal of ethnopharmacology*. 81:(2), 277-280.

Christenhuz MJM and Byng JW. (2016). The number of known plant species in the world and its annual increase. *Phytotaxa*. Magnolia Press. 261 (3): 201-217.

Chukwujekwu JC, Smith P, Coombes PH, Mulholland DA, Van SJ. (2005). Antiplasmodial diterpenoid from the leaves of *Hyptis suaveolens*. *Journal of ethnopharmacology*. 102(2): 295 - 297.

Clark AM. (1996). Natural products as a resource for new drugs. *Pharmaceutical Research*. 13: 1133-1144.

Collins AR. (2004). The comet assay for DNA damage and repair: principles, applications, and limitations. *Molecular Biotechnology*. 26(3): 249-61.

Cooper KL, King BS, Sandoval MM, Liu KJ and Hudson LG. (2013). Reduction of arsenite-enhanced ultraviolet radiation-induced DNA damage by supplemental zinc. *Toxicology and Applied Pharmacology*. 269: 81-88.

Cragg GM, Grothaus PG and Newman DJ. (2009). Impact of natural products on developing new anti-cancer agents. *Chemical Reviews*. 109: 3012-3043.

Cragg GM and Newman DJ. (2005a). Biodiversity: A continuing source of novel drug leads. *Pure and applied chemistry*; 77 (1): 7-24.

Cragg GM and Newman DJ. (2005b). “Plants as a source of anticancer agents”. *Journal of ethnopharmacology*. 100:72-9.

Creemers JW, Vey M, Schafer W, Ayoubi TA, Roebroek AJ, Klenk HD, Garten W and Van de Ven WJ. (1995). Endoproteolytic cleavage of its propeptide is a prerequisite for efficient transport of furin out of the endoplasmic reticulum. *Journal of Biological Chemistry*; 270: 2695-2702.

Crespo R, Montero VS, Abba MC, DeBravo MG and Polo MP. (2013). Transcriptional and posttranscriptional inhibition of HMGCR and PC biosynthesis by geraniol in 2 Hep-G2 cell proliferation linked pathways. *Biochemistry and Cell Biology*. 91:131–9.

Daferera DJ, Tarantilis PA and Polissiou MG. (2002). Characterization of essential oils from Lamiaceae species by Fourier transform Raman spectroscopy. *Journal of Agricultural and Food Chemistry*; 50: 5503–5507.

Deb DD, Parimala G, Saravana DS and Chakraborty T. (2011). Effect of thymol on peripheral blood mononuclear cell PBMC and acute promyelotic cancer cell line HL-60. *Chemico-Biological Interactions*. 193(1):97–106.

Deshpande RS and Tipnis HP. (1997). Insecticidal activity of *Ocimum basilicum* L. *Pesticides*. 11:1–12.

Do N Fontes JE, Ferraz RPC, Britto ACS, Carvalho AA, Moraes MO, Pessoa C, Costa EV and Bezerra DP. (2013). Antitumor effect of the essential oil from leaves of *Guatteria pogonopus* (Annonaceae). *Chemistry and Biodiversity*. 10:722–9.

Dossus L, Allen N, Kaaks R, Bakken K, Lund E, Tjonneland A, Olsen A and Overvad K. (2010). Reproductive risk factors and endometrial cancer: the european prospective investigation into cancer and nutrition. *International Journal of Cancer*;127:442–51.

Einhellig FA, Rasmussen MS and Schon MK. (1985). Effects of allelochemicals on plant-water relationship. In: *The chemistry of allelopathy*. (Ed): Thompson A. C. American chemical society, Washington D. C. pp 170-195.

Ekoh SN, Akubugwo EI, Ude VC and Edwin N. (2014). Anti-hyperglycemic and anti-hyperlipidemic effect of spices (*Thymus vulgaris*, *Murraya koenigii*, *Ocimum gratissimum* and *Piper guineense*) in alloxan-induced diabetic rats. *International Journal of Biosciences*. 4(2): 179-87.

Erasto P, Grierson DS and Afolayan AJ. (2006). Bioactive sesquiterpene lactones from the leaves of *Vernonia amygdalina*. *Journal of Ethnopharmacology*. 106:117-120.

Essien EE, Adesina SK and Reisch J. (1983). Quantitative analysis of scopoletin in the fruit of *Tetrapleura tetraptera* fruit (Mimosaceae). *Scientia Pharmaceutica*. 51:397402.

Essien EE, Ogunwande IA, Setzer WN and Ekundayo O. (2012). Chemical composition, antimicrobial, and cytotoxicity studies on *S. erianthum* and *S. macranthum* essential oils. *Pharmaceutical Biology*. 50:474–80.

Essien EU, Izunwane BC, Aremu CY, and Eka OU. (1994). Significance for humans of the nutrient contents of the dry fruit of *Tetrapleura tetraptera*. *Plant Food for Human Nutrition*. 45(1):47–51.

Evans WC (2002). Trease and Evans Pharmacognosy. Elsevier science limited, 15th edition; pp 1-549.

Ferlay J (2010) Estimates of worldwide burden of cancer in 2008. *International Journal of Cancer*. 127, 2893-2917.

Ferraz RPC, Cardoso GMB, da Silva TB, Fontes JE do N, Prata AP do N, Carvalho AA, Moraes MO, Pessoa C, Costa EV and Bezerra DP. (2013). Antitumour properties of the leaf essential oil of *Xylopia frutescens* Aubl. (Annonaceae) *Food Chemistry*. 141:196–200.

Fleischer TC, Komlaga AY, Mensah MLK, Mensah E, Wood E, Sawyer IK and Gray AI. (2006). Flavonoid constituents of the mature fruit of *Tetrapleura tetraptera* Schum. et Thonn. *Journal of Science and Technology*. (Ghana). 26(1):4147.

Fogang HPD, Maggi F, Tapondjou LA, Womeni HM, Papa F, Quassinti L, Bramucci M, Vitali L A, Petrelli D, Lupidi G, Vittori S and Barboni L. (2014). *In vitro* biological activities of seed essential oils from the Cameroonian spices *Afrostryax lepidophyllus* Mildbr. and *Scorodophloeus zenkeri* Harms rich in sulfur-containing compounds. *Chemistry and Biodiversity*. 11:161–9.

Francis KMC, Kenta M and María JDR. (2013). Detection of necrosis by release of lactate dehydrogenase (LDH) activity. *Methods in Molecular Biology*. 979: 65–70.

Fuhrman B, Rosenblat M, Hayek T, Coleman R and Aviram M. (2000). Ginger extract consumption reduces plasma cholesterol, inhibits LDL oxidation and attenuates development of atherosclerosis in atherosclerotic, apolipoprotein e-deficient mice. *The Journal of Nutrition*. 130(5): 1124-1131.

Fujisawa S, Atsumi T and Satoh K. (2000). Radical generation, radical-scavenging activity, and cytotoxicity of eugenol-related compounds. *In Vitro and Molecular Toxicology*. 13(4): 269–279.

Funk JL, Frye JB, Oyarzo JN and Timmermann BN. (2009). Comparative effects of two gingerol-containing *Zingiber officinale* extracts on experimental rheumatoid arthritis. *Journal of Natural Products*. 72: 403-407.

Fusconi A, Repetto O, Bona E, Massa N, Gallo C and Dumas-Gaudot E. (2006). Effects of cadmium on meristem activity and nucleus ploidy on roots of *Pisum sativum* L. cv. Frisson seedlings. *Environmental and Experimental Botany*. 58: 253-260.

Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM, Haugen-Strano A, Swensen J and Miki Y. (1994). BRCA1 mutations in primary breast and ovarian carcinomas. *Science*. 266: 120-122.

Galsky AB, Wilsey JP and Powell RG. (1980). Crown gall tumor disc bioassay: a possible aid in the detection of compounds with antitumor activity. *Plant Physiology*; 65: 184-185.

Garg S and Kasera H. (1982). Anthelmintic activity of the essential oil of *Callistemon viminalis*. *Fitoterapia*. 53(5/6), 179-181.

Gege-Adebayo GI, Igbokwe VU, Shafe MO, Akintayo CO and Mbaka DI. (2013). Anti-ulcer effect of *Ocimum gratissimum* on indomethacin induced ulcer and percentage of superoxide dismutase on wistar rats. *International Journal of Medical Sciences*. 4(1): 8-12.

Ghasemi V, Yazdi AK, Tavallaie FZ and Sendi JJ. (2014). Effect of essential oils from *Callistemon viminalis* and *Ferula gummosa* on toxicity and on the hemocyte profile of *Ephesia kuehniella* Lep. (Pyralidae). *Archives Phytopathology and Plant Protection*. 47(3), 268-278.

Ghosh R, Nadiminty N, Fitzpatrick JE, Alworth WL, Slaga TJ and Kumar AP. (2005). Eugenol causes melanoma growth suppression through inhibition of E2F1 transcriptional activity. *Journal of Biological Chemistry*. 280 (7): 5812-9.

Gibellini L, Pinti M, Nasi M, De Biasi S, Roat E, Bertoncelli L and Cossarizza A. (2010). Interfering with ROS metabolism in cancer cells: The potential role of quercetin. *Cancers*. 2(2): 1288-1311.

Gmelin R and Larson O. (1967). L- γ -ethylideneglutamic acid and L- γ -methyleneglutamic acid in seeds of *Tetrapleura tetraptera* (Schum et Thonn) Taub. (Mimosaceae). *Biochimica et Biophysica Acta*. 136:572-573.

Goni MG, Roura SI, Ponce AG and Moreira MR. (2016). Clove (*Syzygium aromaticum*) oils In: *Essential oils in food preservative, flavor and safety*. Edited by Preedy V. R. ISBN: 978-0-12-416641-7. Pp 349.

Govaerts R. (2004). World checklist of monocotyledons database in ACCESS: 1-54382. The board of trustees of the royal botanic gardens, Kew.

Grassi P, Urias-Reyes TS, Sosa S, Tubaro A, Hofer O and Zitterl-Eglseer K. (2006). Antiinflammatory activity of two diterpenes of *Hyptis suaveolens* from El Salvador. *Zeitschrift fur Naturforschung B*; 61c, 61: 165-170.

Greaves M and Marley CC. (2012) Clonal Evolution in Cancer; *Nature*; 481, 306-313.

Gupta MP, Handa SS, Vasisht K. (2006). Biological screening of plant constituents training manual: *International centre for science and high technology*, Trieste, Page 1.

Gupta S, Kumar A, Srivastava K, Santosh K. Srivastava, luqman S, Maurya A, Darokar MP, Syamsundar KV and Khanuja SPS (2008) Antimicrobial activity and chemical composition of *Callistemon comboyensis* and *C. citrinus* leaf essential oils from the northern plains of India. *Natural Product Communications*. 3(11): 1931 -1934

Gurunagarajan S and Pemaiah B. (2011). Comparative studies on cytotoxic effect of *Hyptis suaveolens* Poit. and *Leonotis nepeatefolia* R.Br. against EAC cell lines. *Journal of Pharmacy Research*, 4(4): 1222-1224.

Harley RM, França F, Santos EPJ. (2010). Lamiaceae. In: *Catálogo de plantas e fungos do Brasil*. In: Labaque MC, editor. p. 1130-46.

Heywood VH, Brummitt RK, Seberg O and Culham A. (2007). *Flowering plant families of the world*. Ontario, Canada: Firefly Books. ISBN 978-1-55407-206-424

Hossain MA, Al-Hashmi RA, Weli AM, Al-Riyami Q and Al-Sabahib JN. (2012). Constituents of the essential oil from different brands of *Syzigium caryophyllatum* L. by gas chromatography-mass spectrometry. *Asian Pacific Journal of Tropical Biomedicine*. S1446-1449.

Howes AJ, Chan VSW, and Caldwell J. (1990). Structure-specificity of the genotoxicity of some naturally occurring alkenylbenzenes determined by the unscheduled DNA synthesis assay in rat hepatocytes. *Food and Chemical Toxicology*. 28(8): 537–542.

Iqbal Z, Lateef M, Akhtar MS, Ghayur MN and Gilani A H (2006). *In vivo* anthelmintic activity of ginger against gastrointestinal nematodes of sheep. *Journal of Ethnopharmacology*. 106, 285–287.

Iwami M, Shiina T, Hirayama H, Shima T, Takewaki T and Shimizu Y (2011). Inhibitory effects of zingerone, a pungent component of *Zingiber officinale* Roscoe, on colonic motility in rats. *Journal of Natural Medicine*; 65: 89–94.

Jaafari A, Tilaoui M, Mouse HA, Mbark LA, Aboufatima R, Chait A, Lepoivre M and Zyad A. (2012). Comparative study of the antitumor effect of natural monoterpenes: relationship to cell cycle analysis. *Brazilian Journal of Pharmacognosy*. 22(3): 534–540.

Jacqueline (2017). *Syzygium antisepticum* with distinctive reddish-brown, papery and flaky bark. Plants database. Wikipedia. Accessed 5th October, 2018.

Javanmardi J, Khalighi A, Kashi A, Bais HP and Vivanco JM. (2002). Chemical characterization of basil (*Ocimum basilicum* L.) found in local accessions and used in traditional medicines in Iran. *Journal of Agricultural and Food Chemistry* 50:5878-83.

Jayakumar S, Madankumar A, Asokkumar S, Raghunandhakumar S, Gokula K, Kamaraj S, Divya MGJ and Devaki T. (2012). Potential preventive effect of carvacrol against diethylnitrosamine-induced hepatocellular carcinoma in rats. *Molecular and Cellular Biochemistry*. 360:51–60.

Jedi-Agba E, Curado MP, Ogunbiyi O, Oga Emmanuel, Fabowale T, Igbinoba F, Osubor G, Olu T, Kumai H, Koechlin A, Osinubi P, Dakum P, Blattner W and Adebamowo CA (2012). Cancer incidence in Nigeria: a report from population-based cancer registries;36(5):e271-8

Jemal A, Bray F, Melissa M, Ferlay J, Elizabeth W and Forman D. (2011). Global cancer statistics. *Cancer Journal for Clinicians*. 61:69.

Jirovetz L and Buchbauer G. (2001). Analysis, chemotype and quality control of the essential oil of a new cultivated basil (*Ocimum basilicum* L.) plant from Bulgaria. *Scientia Pharmaceutica* 69:85-9.

Jo JR, Park JS, Park YK, Chae YZ, Lee GH, Park GY, Jang BC. (2012). *Pinus densiflora* leaf essential oil induces apoptosis via ROS generation and activation of caspases in YD-8 human oral cancer cells. *International Journal of Oncology*. 40:1238–45.

Joshi RK. (2017). GC–MS Analysis of the Essential Oil of *Ocimum gratissimum* L. Growing Desolately in South India. *Acta Chromatographica*. 291: 111–119.

Kadam SB, Shyama SK and Almeida VG. (2013). Evaluation of the *in vivo* genotoxic effects of gamma radiation on the peripheral blood leukocytes of head and neck cancer patients undergoing radiotherapy. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 752: 42-46.

Kamaliroostaa Z, Kamaliroosta L, Elhamirad AH. (2013). Isolation and identification of Ginger essential oil. *Journal of Food Biosciences and Technology*. 3, 73-80.

Keawsa-ard S, Liawruangrath B, Liawruangrath S, Teerawutgulrag A and Pyne SG. (2012). Chemical constituents and antioxidant and biological activities of the essential oil from leaves of *Solanum spirale*. *Nat Product Communication*. 7:955–8.

Khorasanizadeh F, Hassanloo J, Khaksar N, Taheri SM, Marzaban M, Rashidi BH, Sari AA and Zendehtdel K. (2013). Epidemiology of cervical cancer and human papilloma virus infection among Iranian women – Analysis of national data and systemic review of the literature. *Gynaecologic Oncology*. 128: 277-281.

Kim C, Cho SK, Kapoor S, Kumar A, Vali S, Abbasi T, Kim SH, Sethi G and Ahn KS. (2014). β -Caryophyllene oxide inhibits constitutive and inducible STAT3 signaling pathway through induction of the SHP-1 protein tyrosine phosphatase. *Molecular Carcinogenesis*. 53:793–806.

Kim EC, Min JK, Kim TY, Lee SJ, Yang HO, Han S, Kim YM and Kwon YG. (2005). [6]-Gingerol, a pungent ingredient of ginger inhibits angiogenesis *in vitro* and *in vivo*. *Biochemical and Biophysical Research Communications*. 335: 300–308.

Kim HM, Lee EH, Hong SH, Song HJ, Shin MK, Kim SH and Shin TY. (1998). Effect of *Syzygium aromaticum* extract on immediate hypersensitivity in rat. *Journal of Ethnopharmacology*. 60:125-131.

Kim JK, Kim Y, Na KM, Surh YJ and Kim TY. (2007). [6]-Gingerol prevents UVB-induced ROS production and COX-2 expression *in vitro* and *in vivo*. *Free Radical Research*. 41: 603-614.

Koba K, Sanda K, Guyon C, Raynaud C, Millet J, Chaumont JP and Nicod L. (2007). Chemical composition and *in vitro* cytotoxic activity of essential oils from two tropical Lamiaceae: *Aeollanthus pubescens* Benth. and *Ocimum gratissimum* L. *Journal of Essential Oil Bearing Plants*. 10:1.

Kobayashi CI and Suda T. (2012). Regulation of reactive oxygen species in stem cells and cancer stem cells. *Journal of Cellular Physiology*. 227: 421-430.

Kritsanawong S, Innajak S, Moto M, Watanapokasin R. (2016,). Antiproliferative and apoptosis induction of α -mangostin in T47D breast cancer cells. *International Journal of Oncology*. 48: 2155-2165.

Kugelman M, Lui WC, Axelrod M, McBride TJ and Rao KV. (1976). Indicine-N-oxide: The antitumor principle of *Heliotropium indicum*. *Lloydia*; 39: 125-128.

Kumar D, Sukapaka M, Babu GDK and Padwad Y. (2015). Chemical composition and *in vitro* cytotoxicity of essential oils from leaves and flowers of *Callistemon citrinus* from Western Himalayas. *Plos One*. 10(8).

Kumar NA, Sivadasan M and Ravi N. (2005). Flora of Pathanamthitta. Daya Publishing House, New Delhi, India, 1st Edition . 1-649.

Kumar P, Dinesh Babu KV and Evans DA. (2019). Isolation, characterization and mode of action of a larvicidal compound, 22-hydroxyhopane from *Adiantum latifolium* Lam. against *Oryctes rhinoceros* Linn. *Pesticide biochemistry and physiology*. Vol 153 pp 161-170

Kunkongkapan S, Sriwanthana B, Chavalittumrong P and Warachit P. (2007). Efficacy and safety of *Hyptis suaveolens* extract in HIV-infected individuals: A preliminary study. *Lanna Public Health Journal*; 3(3): 255-262.

Lachowicz KJ, Jones GP, Briggs DR, Bienvenu FE, Palmer MV and Ting SST. (1996). Characteristics of essential oil from basil (*Ocimum basilicum* L.) grown in Australia. *Journal of Agricultural and Food Chemistry*. 44:877–81.

Langner E, Greifenberg S and Gruenwald J. (1998). Ginger: history and use. *Advanced Therapeutics*. 15; 25-44.

Lawrence BM and Reynolds RJ. (1993). Progress in essential oils. *Perfum Flavor*. 19:31-44.

Lim SD, Sun C, Lambeth JD, Marshall F, Amin M and Chung L. (2005). Increased Nox 1 and hydrogen peroxide in prostate cancer. *Prostate*. 62: 200-207.

Lin CC, Chao PY, Shen CY, Shu JJ, Yen SK and Huang CY. (2014). Novel target genes responsive to apoptotic activity by *Ocimum gratissimum* in human osteosarcoma cells. *The American Journal of Chinese Medicine*. 42(03): 743-67.

Lin RJ, Chen CY, Chung LY and Yen CM. (2010). Larvicidal activities of ginger (*Zingiber officinale*) against *Angiostrongylus cantonensis*. *Acta Tropica*; 115: 69-76.

Li YL, Yeung CM, Chiu LCM, Cen YZ and Ooi VEC. (2009). Chemical composition and antiproliferative activity of essential oil from the leaves of a medicinal herb, *Schefflera heptaphylla*. *Phytotherapy Research*. 23:140–2.

Loizzo MR, Tundis R, Menichini F, Saab AM, Statti GA and Menichini F. (2008). Antiproliferative effects of essential oils and their major constituents in human renal adenocarcinoma and amelanotic melanoma cells. *Cell Proliferation*. 41:1002–12

Loizzo MR, Tundis R, Menichini F, Saab AM, Statti GA, Menichini F. (2007). Cytotoxic activity of essential oils from labiatae and lauraceae families against *in vitro* human tumor models. *Anticancer Research*. 27:3293–9.

Mahesh S. (2001). Comparison of antiinflammatory activity of diclofenac sodium with leaf essential oils of *Hyptis suaveolens* Poit. *The Antiseptic*. 98(3): 90.

Maia A. and Rainer L. (2001). Changes in water relations, solute leakage and growth characters during seed germination and seedling development in *Trigonella coerulea* (Fabaceae). *Journal of Applied Botany*. 75:144-151.

Maillard M, Adewunmi CO, Hostettman K. (1989). New triterpenoid N-acetylglycosides with molluscicidal activity from *Tetrapleura tetraptera* Taub. *Helvetica Chimica Acta*. 72(4):668-674.

Maillard M, Adewunmi CO and Hostettman K. (1992). A triterpene glycoside from the fruit of *Tetrapleura tetraptera*. *Phytochemistry*. 31(4):1321-1323.

Manda G, Nechifor MT and Neagu TM. (2009). Reactive oxygen species, cancer and anticancer therapies. *Current Chemical Biology*. 3: 342-366.

Mandal SM, Mondal KC, Dey S and Pati BR. (2007). Antimicrobial activity of the leaf extracts of *Hyptis suaveolens* (L.) Poit. *Indian Journal of Pharmaceutical Sciences*; 69(4): 568-569.

Manjamalai A, Kumar MJM and Grace VMB. (2012). Essential oil of *Tridax procumbens* L induces apoptosis and suppresses angiogenesis and lung metastasis of the B16F-10 cell line in C57BL/6 mice. *The Asian Pacific Journal of Cancer Prevention*. 13:5887-95.

Manosroi A, Dhumtanom P and Manosroi J. (2006). Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. *Cancer Letters*. 235:114-20.

Marotti M, Piccaglia R and Giovanelli E. (1996). Differences in essential oil composition of basil (*Ocimum basilicum* L.) Italian cultivars related to morphological characteristics. *Journal of Agricultural and Food Chemistry*. 44:926-9.

Mascotti K, McCullough J and Burger SR. (2000). HPC viability measurement: trypan blue versus acridine orange and propidium iodide. *Transfusion*. 40(6): 693-06.

Matasyoh LG, Matasyoh JC, Wachira FN, Kinyua MG, Muigai AWT and Mukiama TK. (2008). Antimicrobial activity of essential oils of *Ocimum gratissimum* L. from different populations of Kenya. *African Journal of Traditional, Complementary and Alternative Medicines*. 5(2): 187-93.

Matasyoh LG, Matasyoh JC, Wachira FN, Kinyua MG, Muigai AWT and Mukiama TK. (2007). Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. growing in Eastern Kenya. *African Journal of Biotechnology*. 6(6): 760-5.

Matias EF, Katiucia Santos K, Almeida TS, Costa JG and Coutinho HD. (2010). Atividade antibacteriana *In vitro* de *Croton campestris* A, *Ocimum gratissimum* L. *Cordia* Verbenacea DC. *Revista Brasileira de Biologia*. 8(3): 294-8.

Mauro M, Catanzaro I, Naselli F, Sciandrello G and Caradonna F. (2013). Abnormal mitotic spindle assembly and cytokinesis induced by D-Limonene in cultured mammalian cells. *Mutagenesis*. 28:631-5.

McLaughlin JL, Chang CJ and Smith DL. (1991). "Bench-top" bioassays for discovery of bioactive natural products: An update. (Atta-Ur-Rahman), *Studies in Natural Products Chemistry*. Elsevier Science; 9: 101-103.

Merlo LM, Pepper JW, Reid BJ and Marley CC (2006). Cancer as an evolutionary and ecological process. *National review of Cancer*. P6, 924-935.

Meyer BN, Ferrigni NR, Putnam JT, Jacobsen LB, Nichols DE and McLaghlin JL. (1982). Brine shrimp: A convenient general bioassay for active plant constituent. *Planta Medica*. 45:31-34.

Mimica-Dukic N, Bozin B, Sokovic M and Simin N. (2004). Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. *Journal of Agricultural and Food Chemistry*. 52: 2485–2489.

Miri P, Bae J and Lee DS. (2008). Antibacterial activity of [10]gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. *Phytotherapy Research*. 22: 1446-1449.

Mishra SB, Verma A, Mukerjee A and Vijayakumar M. (2011). Anti-hyperglycemic activity of leaves extract of *Hyptis suaveolens* L. Poit in streptozotocin induced diabetic rats. *Asian Pacific Journal of Tropical Medicine*; 4(9): 689-693.

Miyazawa M and Hisama M. (2001). Suppression of chemical mutagen induced SOS response by alkylphenols from clove (*Syzygium aromaticum*) in *Salmonella typhimurium* TA153/pSK1002 umu test. *Journal of Agricultural and Food Chemistry*. 49:4019-25.

Moeller LC and Fuhrer D. (2013). Thyroid hormone, thyroid hormone receptors and cancer: a clinical perspective. *Endocrine-Related Cancer*. 20:19-29.

Mohammed NH, Ahmed MH, and Hussien MO (2015). Qualitative analysis of the essential oil of *Syzygium aromaticum* (L.) (Clove) using gas chromatography-mass spectrometry (GC-MS). *International Journal of Research in Pharmacy and Chemistry*; 5(2), 350-354.

Mondal S, Mahapatra S, Naik S and Mirdha B. (2007). Antimicrobial activities of essential oils obtained from fresh and dried leaves of *Ocimum sanctum* (L.) against enteric bacteria and yeast. *Acta Horticulturae*. 756(2007): 267-70.

Moshen ZH, Jawad AL, Al-Chalabi BM and Al-Naib A. (1990). Biological activity of *Callistemon lanceolatus* against *Culex quinquefasciatus*. *Fitoterapia* 31, 191-196.

Mulyaningsih S, Youns M, El-Readi MZ, Ashour ML, Nibret E, Sporer F, Herrmann F, Reichling J and Wink M. (2010). Biological activity of the essential oil of *Kadsura longipedunculata* (Schisandraceae) and its major components. *Journal of Pharmacy and Pharmacology*. 62:1037–44.

Murata S, Shiragami R, Kosugi C, Tezuka T, Yamazaki M, Hirano A, Yoshimura Y, Suzuki M, Shuto K, Ohkohchi N and Koda K. (2013). Antitumor effect of 1, 8-cineole against colon cancer. *Oncology Reports*. 30:2647–52.

Musika S and Indrapichate K. (2014). Cytotoxicity and apoptic induction of Mintweed (*Hyptis suaveolens* L. Poit) leaf extracts on human T-Leukemia cell line, Jurkat cells. *World Journal of Pharmacy and Pharmaceutical sciences*. (3):3, 304-317.

Nalbantsoy A, Tamis DA, Akgun IH, Öztürk T, Gurhan ID and Karaboz I (2010). Antimicrobial and cytotoxic activities of *Zingiber officinalis* extracts. *Journal of Pharmaceutical Sciences*; 33, 76–85.

Nangia-Makker P, Raz T, Tait L, Shekhar MPV, Li H, Balan V, Makker H, Fridman R, Maddipati K, and Raz A. (2013). *Ocimum gratissimum* retards breast cancer growth and

progression and is a natural inhibitor of matrix metalloproteases. *Cancer Biology and Therapy* 14:5, 417-427.

Nantitanon W, Chowwanapoonpohn S and Okonogi S. (2007). Antioxidant and antimicrobial activities of *Hyptis suaveolens* essential oil. *Scientia Pharmaceutica*, 75: 35-46.

Nayak PS, Nayak S, Kar DM and Das P. (2010). *In vitro* antihelminthic activity of whole plant extracts of *Hyptis suaveolens*. *International Journal of Current Pharmaceutical Research*; 2(2): 50-51.

Niwano Y, Saito K, Yoshizaki F, Kohno M and Ozawa T. (2011). Extensive screening for herbal extracts with potent antioxidant properties. *Journal of Clinical Biochemistry and Nutrition*. 48(1):78-84.

Ni X, Suhail M M, Yang Q, Cao A, Fung K M, Postier R G, Woolley C, Young G, Zhang J and Lin H K. (2012). Frankincense essential oil prepared from hydrodistillation of *Boswellia sacra* gum resins induces human pancreatic cancer cell death in cultures and in a xenograft murine model. *BMC Complement Alternative Medicine*. 12:253.

Noamesi BK, Mensah JF, Bogale M, Dagne E, and Adotey J. (1994). Antiulcerative properties and acute toxicity profile of some African medicinal plant extracts. *Journal of Ethnopharmacology*. 1: 13-18.

Nogueira de Melo GA, Grespan R, Fonseca JP, Farinha TO, da Silva EL, Romero AL, Bersani-Amado CA and Cuman RKN (2011). Inhibitory effects of ginger (*Zingiber officinale* Roscoe) essential oil on leukocyte migration *in vivo* and *in vitro*. *Journal of Natural Medicines*, 65: 241–246.

Noté OP, Mitaine-Offer AC, Miyamoto T, Paululat T, Pegnyemb DE and Lacaille-Dubois MA. (2009). Tetrapterosides A and B, two new oleanane-type saponins from *Tetrapleura tetraptera*. *Magnetic Resonance in Chemistry MRC*. 47(3):277-282.

Nurtjahja-Tjendraputra E, Ammit AJ, Roufogalis BD, Tran VH and Duke CC. (2003). Effective anti-platelet and COX-1 enzyme inhibitors from pungent constituents of ginger. *Thrombosis Resrarch*. 111(4-5): 259-265.

Nwaiwu JI, and Akali PA. (1986). Anti-convulsant activity of the volatile oil from the fruit of *Tetrapleura tetraptera*. *Journal of Ethnopharmacology*. 18:103–107.

Obuotor EM and Onajobi FD. (2000). Preliminary evaluation of cytotoxic properties of *Raphia hookeri* fruit mesocarp. *Fitoterapia*. 71: 190-192.

Ojewole JAO and Adewunmi CO. (2004). Anti-inflammatory and hypoglycaemic effects of *Tetrapleura tetraptera* (Taub.) (Fabaceae) fruit aqueous extract in rats. *Journal of Ethnopharmacology*. 95: 177-182.

Ojewole JAO and Adeshina SK. (1983). Cardiovascular and neuromuscular actions of scopoletin from fruit of *Tetrapleura tetraptera*. *Planta Medica* 48: 99-102.

Ojo OA, Oloyede O, Tugbobo O, Olarewaju O and Ojo A. (2014). Antioxidant and inhibitory effect of scent leaf (*Ocimum gratissimum*) on Fe²⁺ and sodium nitroprusside induced lipid peroxidation in rat brain *in vitro*. *Advanced Biomedical Research*. 8(1): 08-17.

Okochi VI, Gbenle GO, Kazeem AA, Fagbenro-Bayioku AF, Igbodudu HE and Arukwe U, (1999). Effect of water extract of *Tetrapleura tetraptera* (Aidon) on haematological and biochemical parameters in rats infected with *Trypanosoma brucei*, *Nigerian Quarterly Journal of Hospital Medicine*. 9: (1): 66-70.

Okoye FB, Obonga WO, Onyegbule FA, Ndu OO, Okechukwu O, and Ihekwereme CP. (2014). Chemical composition and anti-inflammatory activity of essential oils from the leaves of *Ocimum basilicum* L. and *Ocimum gratissimum* L. (Lamiaceae). *International Journal of Pharmaceutical Sciences and Research*. 5(6): 2174-80.

Okwu DO, (2001). Evaluation of the chemical composition of indigenous spices and flavouring agents. *Global Journal of Pure and Applied Science*. 7:445-459.

Oliveiraa JS, Porto LA, Estevam CS, Siqueira RS, Alves PB, Niculau ES, and Blank AF. (2009). Phytochemical screening and anticonvulsant property of *Ocimum basilicum* leaf essential oil. *Latin American and Caribbean Bulletin of Medicinal and Aromatic Plants*. 8:195-202.

Oluwagbemiga LA, Oluwole A and Kayode AAR. (2012). Seventeen years after BRCA1: what is the BRCA mutation status of the breast cancer patients in Africa? - a systematic review. *Springer plus*: 1(1): 83-93.

Orwa CA, Mutua KR, Jamnadass R and Anthony S. (2009). Agroforestry Database: a tree reference and selection guide version 4.0. Available from <http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp>. Accessed 13th February, 2018.

Oyedeeji OO, Lawal OA, Shode FO and Oyedeeji AO. (2009). Chemical composition and antibacterial activity of the essential oils of *Callistemon citrinus* and *Callistemon viminalis* from South Africa. *Molecule*. 14(6), 1990-1998.

Ozkan A and Erdogan A. (2011). A comparative evaluation of antioxidant and anticancer activity of essential oil from *Origanum onites* (Lamiaceae) and its two major phenolic components. *Turkish Journal of Biology*. 35:735–742

Ozcan M and Chalchat J. C. (2002): Essential oil composition of *Ocimum basilicum* L. and *Ocimum minimum* L. in Turkey. *Czech Journal of Food Sciences*; 20: 223–228.

Ozaslan ID, Karagoz RA, Lawal IH, Kilic A, Cakir OS, Odesanmi IG and Ebuehi OAT. (2016). Cytotoxic and anti-proliferative activities of the *Tetrapleura tetraptera* fruit extract on ehrlich ascites tumor cells. *International Journal of Pharmacology*. 12: 655-662.

Paik SY, Koh KH, Beak SM, Paek SH, and Kim JA. (2005). The essential oils from *Zanthoxylum schinifolium* pericarp induce apoptosis of HepG2 human hepatoma cells through increased production of reactive oxygen species. *Biological and Pharmaceutical Bulletin*. 28:802–7.

Palliyaguru D and Wu F. (2013). Global geographical overlap of aflatoxin and hepatitis C: Controlling risk factors for liver cancer worldwide. Food additives and contaminants. Chemistry, Analysis, control, exposure and risk assessment. 30: 534-540.

Paramasivam A., Sambantham S., Shabnam J., Raghunandhakumar S., Anandan B., Rajiv R., Vijayashree P. J. and Jayaraman G. (2012). Anti-cancer effects of thymoquinone in mouse neuroblastoma (Neuro-2a) cells through caspase-3 activation with down-regulation of XIAP. *Toxicology Letters*. 213:151–159.

Park BS, Song YS, Yee SB, Lee BG, Seo SY, Park YC, Kim JM, Kim HM and Yoo YH. (2005). Phospho-ser 15-p53 translocates into mitochondria and interacts with Bcl-2 and Bcl-xL in eugenol-induced apoptosis. *Apoptosis*. 10(1): 193–200.

Park IK and Shin SC. (2005). Fumigant activity of plant essential oils and components from garlic (*Allium sativum*) and clove bud (*Eugenia caryophyllata*) oils against the Japanese termite (*Reticulitermes speratus* Kolbe) *Journal of Agricultural Food Chemistry*. 53:4388–4392.

Parnell J and Curtis T. (2012). *Webbs an Irish Flora*. Cork University Press ISBN 978-185918-4783.

Peerzad N. (1997). Chemical composition of the essential oil of *Hyptis suaveolens*. *Molecules*. 2:165–168.

Pichersky E, Noel JP and Dudareva N. (2006).. Biosynthesis of plant volatiles: nature's diversity and ingenuity. *Science*. 311:808–11.

Pisano M, Pagnan G, Loi M, Mura ME, Tilocca MG, Palmieri G, Fabbri D, Dettori MA, Delogu G, Ponzoni M and Rozzo C. (2007). Antiproliferative and pro-apoptotic activity of eugenol-related biphenyls on malignant melanoma cells. *Molecular Cancer*. 6(8).

Poonkodi K. (2016). Chemical composition of essential oil of *Ocimum basilicum* L. (BASIL) and its biological activities—an overview. *Journal of Critical Reviews*. 3(3):56-62.

Poonkodi K, Jaganathan K, Velusamy T, Ramasamy A and Sundaramoorthi V. (2017). Chemical composition of essential oil of *Hyptis suaveolens* (L.) POIT and its *in vitro* anticancer activity. *Journal of Pharmacy Research*. 11(5):410-413.

Portmeisel M and Pinedo H. (1995). *Camptothecins: new anticancer agents*. Boca Raton, Florida. CRC Press. Pp 149-150.

Powell RG, Bajaj R and McLaughlin JL. (1987). Bioactive stilbenes of *Scirpus maritimus*. *Journal of Natural Product*. 50: 293-296.

Powell RG, Weisleder D, Smith CR Jr. and Rohwedder WK. (1970). Structures of harringtonine, isoharringtonine and homoharringtonine. *Tetrahedron Letters*. 11: 815-818.

Puig T, Aguilar H, Cufi S, Oliveras G, Turrado C and Ortega-Gutiérrez S. (2011). A novel inhibitor of fatty acid synthase shows activity against HER2+ breast cancer xenografts and is active in anti-HER2 drug-resistant cell lines, *Breast Cancer Research*. 13: R131.

Quassinti L, Lupidi G, Maggi F, Sagratini G, Papa F, Vittori S, Bianco A and Bramucci M. (2013). Antioxidant and antiproliferative activity of *Hypericum hircinum* L. subsp. majus (Aiton) N. Robson essential oil. *Natural Product Research*. 27:862–8.

Rabelo M, Souza E, Soares P, Miranda A, Matos F, and Criddle D. (2003). Antinociceptive properties of the essential oil of *Ocimum gratissimum* L. (Labiatae) in mice. *Brazilian Journal of Medical and Biological Research*. 36(4): 521-4.

Rang HP, Dale MM, Ritter JM and Moore PK. (2003). *Pharmacology*. Fifth edition. Pp 694-696.

Rashid S, Rather MA, Shah WA and Bhat BA. (2013). Chemical composition, antimicrobial, cytotoxic and antioxidant activities of the essential oil of *Artemisia indica* Willd. *Food Chemistry*. 138:693–700.

Rates S. M. (2001). Plants as source of drugs. *Toxicon*. 39(5): 603-613

Ravi SBE, Ramachandra YL, Rajan N, Ganapathy PSS, Yarla NS, Richard SA, Dhananjaya BL (2016). Evaluating the anticancer potential of ethanolic gall extract of *Terminalia chebula* (Gaertn.) Retz. (combretaceae) *Short communication*; 8(3): 209-212

Ray-Gallet D, Quivy JP, Scamps C, Martini EM, Lipinski M and Almouzni G. (2013). HIRA is critical for a nucleosome assembly pathway independent of DNA synthesis. *Molecular Cell*. 9:1091-1100.

Riss TL, Moravec RA, and Niles AL. (2011). Cytotoxicity testing: measuring viable cells, dead cells, and detecting mechanism of cell death. *Methods in Molecular Biology*. 740: 103-14.

Rokhaya F, Saliou N, Dicnaba S, Mbacke S and Abdoulaye S. (2017). Chemical characterization of essential oil from the leaves of *Callistemon viminalis* (D.R.) and *Melaleuca leucadendron* (Linn.) *Asian pacific journal of tropical biomedicine*. 7(4): 347-351.

Rowinsky EK, Onetto N, Canetta RM and Sarbuck SG. (1992). Taxol-the first of the texanes, an important new class of antitumor agents. *Seminars in Oncology*. 19: 646-662.

Salehi B, Mishra AP, Shukla I, Sharifi-Rad M, Contreras MDM, Segura-Carretero A, Fathi H, Nasrabadi NN, Kobarfard F, and Sharifi-Rad J. (2018). Thymol, thyme, and other plant sources: Health and potential uses. *Phytotherapy Research*. 32(9):1688-1706.

Santana JS, Sartorelli P, Guadagnin RC, Matsuo AL, Figueiredo CR, Soares MG, da Silva AM and Lago JHG. (2012). Essential oils from *Schinus terebinthifolius* leaves - chemical composition and *in vitro* cytotoxicity evaluation. *Pharmaceutical Biology*. 50:1248–53.

Santos TC, Marques MS., Menezes IAC, Dias KS, Silva ABL, Mello ICM, Carvalho ACS, Cavalcanti SCH, Antonioli AR and Marçal R. M. (2007). Antinociceptive effect and acute toxicity of the *Hyptis suaveolens* leaves aqueous extract on mice. *Fitoterapia*. 78: 333-336.

Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, and Rehder VLG. (2004). Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Brazilian Journal of Microbiology*. 35(4): 275-80.

Sasco AJ, Secretan MB and Straif K. (2014). Tobacco smoking and cancer: a brief review of recent epidemiological evidence. *Lung cancer*. 45(2): 3-9.

Satooka H and Kubo I. (2009). Effects of eugenol on murine B16-F10 melanoma cells in *Proceedings of the 238th ACS national meeting*. pp. 16–20, Washington, DC, USA.

Saud A, Daoud A, Saad A, Maqsood AS, and Bahy AA. (2013). Arsenic trioxide-mediated oxidative stress and genotoxicity in human hepatocellular carcinoma cells. *Onco Targets and Therapy*. 6, 75–84.

Schonfelder I and Schonfelder P. (2004). *Syzygium aromaticum* (L.) Merr & Perr (*Eugenia caryophyllata* Thunb *Jambosa caryophyllus* (Spreng.) (Niedenzu). *J Das neue Handbuch der Heilpflanzen*, Kosmos-Verlag; 431-2.

Seal S, Chatterjee P, Bhattacharya S, Pal D, Dasgupta S, Kundu R, Mukherjee S, Bhattacharya S, Bhuyan M, Bhattacharyya PR, Baishya G, Barua NC, Baruah PK, Rao PG and Bhattacharya S. (2012). Vapor of volatile oils from *Litsea cubeba* seed induces apoptosis and causes cell cycle arrest in lung cancer cells. *PLoS One*. 7:e47014.

Sethi S, Prakash O, Chandra M, Punetha H, Pant AK. (2013). Antifungal activity of essential oils of some *Ocimum* species collected from different locations of Uttarakhand. *Indian Journal of Natural Products and Resources*; 4:392-7.

Shaikat ZH, Hossain T, and Azam G. (2012). Phytochemical screening and antidiarrhoeal activity of *Hyptis suaveolens*. *International Journal of Applied Research in Natural Product*; 5(2):1-4.

Shamon LA, Pezzuto JM and Graves JM. (1997). Evaluation of the mutagenic, cytotoxic, and antitumor potential of tripdiolide, a highly oxygenated diterpene isolated from *Tripterygium wilfordii*. *Cancer Letters*. 112: 113-117.

Shatha AH and Malek Z. (2013). Gene expression alterations in doxorubicin resistant MCF-7 breast cancer cell line. *Genomics*. 101: 213–20.

Silva MGV, Vieira IGP, Mendes FNP, Albuquerque IL, Santos RN, and Silva FO. (2008). Variation of ursolic acid content in eight *Ocimum* species from northeastern Brazil. *Molecules* 13:2482-7.

Simon JE, Quinn J and Murray R G. (1990). Basil: a source of essential oils. In: Janik J, Simon J E *Advances in New Crops*. Eds. Timber Press: Portland. OR. p. 484-9.

Slameňová D, Horváthová E, Wsóllová L, Šramková M, and Navarová J. (2009). Investigation of anti-oxidative, cytotoxic, DNA-damaging and DNA-protective effects of plant volatiles eugenol and borneol in human-derived HepG2, Caco-2 and VH10 cell lines. *Mutation Research—Genetic Toxicology and Environmental Mutagenesis*. 677(1-2):46–52.

Slamenova D, Horvathova E, Sramkova M and Marsalkova L. (2007). DNA-protective effects of two components of essential plant oils carvacrol and thymol on mammalian cells cultured in vitro. *Neoplasma*. 54:108–112.

Spencer RD and Lumley PF (1991). In: Harden GJ *Callistemon: Flora of New South Wales*. Vol 2. New South Wales University Press; Sydney, Australia. pp. 168–173.

Srivastava HC, Shukla P, Tripathi S, and Shanker B. (2014). Antioxidant and antimicrobial activities of sweet basil oils. *International Journal of Pharmaceutical Sciences and Research*. 5:279-85.

Srivastava KC. (1984). Aqueous extracts of onion, garlic and ginger inhibit platelet aggregation and alter arachidonic acid metabolism. *Biomedica Biochimica Acta*. 43: 335-S346.

Srivastava SK, Ahmad A, Jain N, Aggarwal KK and Syamasunder KV (2001). Essential oil composition of *Callistemon citrinus* leaves from the lower region of Himalayas. *Journal of Essential Oil Research*;13:359–361.

Stahelin H. (1973). Activity of a new glycosidic lignin derivative (VP 16-213) related to podophyllotoxin in experimental tumors. *European Journal of Cancer*; 9: 215-221.

Stammati A, Bonsi P, Zucco F, Moezelaar R, Alakomi HL and Von WA. (1999). Toxicity of selected plant volatiles in microbial and mammalian short-term assays. *Food and Chemical Toxicology*. 37(8):813–823.

Suhail MM, Wu W, Cao A, Mondalek FG, Fung KM, Shih PT, Fang YT, Woolley C, Young G, and Lin HK. (2011). *Boswellia sacra* essential oil induces tumor cell-specific apoptosis and suppresses tumor aggressiveness in cultured human breast cancer cells. *BMC Complementary and Alternative Medicine*. 11:129.

Sun XY, Zheng YP, Lin DH, Zhang H, Zhao F, and Yuan CS. (2009). Potential anti-cancer activities of Furanodiene, a sesquiterpene from *Curcuma wenyujin*. *The American Journal of Chinese Medicine*. 37:589–96.

Sutar N, Sutar R and Kumar M. (2014). *Callistemon citrinus* (bottle brush) an important medicinal plant: a review of its traditional uses, phytoconstituents and pharmacological properties. *Indian Research Journal of Pharmacy & Science*; 1:68–71.

Su YC, Hsu KP, Wang EIC and Ho CL. (2013). Composition and *in vitro* anticancer activities of the leaf essential oil of *Neolitsea variabilissima* from Taiwan. *Natural Product Communications*. 8:531–2.

Tabuti JRS, Kukunda CB and Waako PJ. (2010). Medicinal plants used by traditional medicine practitioners in the treatment of tuberculosis and related ailments in Uganda. *Journal of Ethnopharmacology*;127:130–136.

Tankam JM and Ito M. (2014). Sedative, anxiolytic and antidepressant-like effects of inhalation of the essential oil of *Ocimum gratissimum* L. from Cameroon in mice. *Journal of Pharmacognosy and Phytochemistry*. 2(5): 1-9.

Terry LR, Richard AM, Andrew LN, Helene AB, Tracy JW and Lisa M. (2015). *Cell Viability Assays*. *Assay Guidance Manual*. 29.

Titanji VPK, Zofou D and Ngemenya MN. (2008). The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian folk medicine. *African Journal of Traditional, Complementary and Alternative Medicines*; 5 (3): 302 – 321

Togar B, Turkez H, Tatar A, Hacimuftuoglu A and Geyikoglu F. (2015). Cytotoxicity and genotoxicity of zingiberene on different neuron cell lines *in vitro*. *Cytotechnology*. 67:939–946

Tyler VE. (1999). Phytomedicines: back to the future. *Journal of Natural Products*. 62: 1589-1592.

Vanden BNR and Letsky EA. (2000). Etiology of anaemia in pregnancy in south Malawi. *The American Journal of Clinical Nutrition*; 72: 47–56.

Venuprasad M, Kandikattu HK, Razack S and Khanum F. (2014). Phytochemical analysis of *Ocimum gratissimum* by LC-ESI-MS/MS and its antioxidant and anxiolytic effects. *South African Journal of Botany*. 92(1): 151-8.

Vina A and Murillo E. (2003). Essential oil composition from twelve varieties of basil (*Ocimum* spp) grown in Colombia. *Journal of the Brazilian Chemical Society*. 14:744-9.

Vogelstein B. and Kinzler K (2002). *The genetic basis of human cancer*. McGraw Hill Professional, Medical pp 1-821

Walia M, Mann TS, Kumar D, Agnihotri VK and Singh B. (2012). Chemical composition and *in vitro* cytotoxic activity of essential oil of leaves of *Malus domestica* growing in western Himalaya (India) *Evidence-Based Complementary and Alternative Medicine*. :649727.

Wang CC, Chen LG, Lee LT, and Yang LL. (2003). Effects of 6-gingerol, an antioxidant from ginger, on inducing apoptosis in human leukemic HL-60 cells. *In Vivo* (Athens Greece). 17(6): 641-645.

Wang W, Zhang L, Li N and Zu Y. (2012). Chemical composition and *in vitro* antioxidant, cytotoxicity activities of *Zingiber officinale* Roscoe essential oil. *African Journal of Biochemistry Research*. 6(6): 75-80.

Wierdak RN, Borowski B, Dzida K, Zawiślak G and Kowalski R. (2013). Essential oil composition of sweet basil cultivars as affected by nitrogen and potassium fertilization. *Turkish Journal of Agriculture and Forestry*. 37:427-36.

Williamson EM. (2001). Synergy and other interactions in phytomedicines. *Phytomedicine*. 8: 401-409.

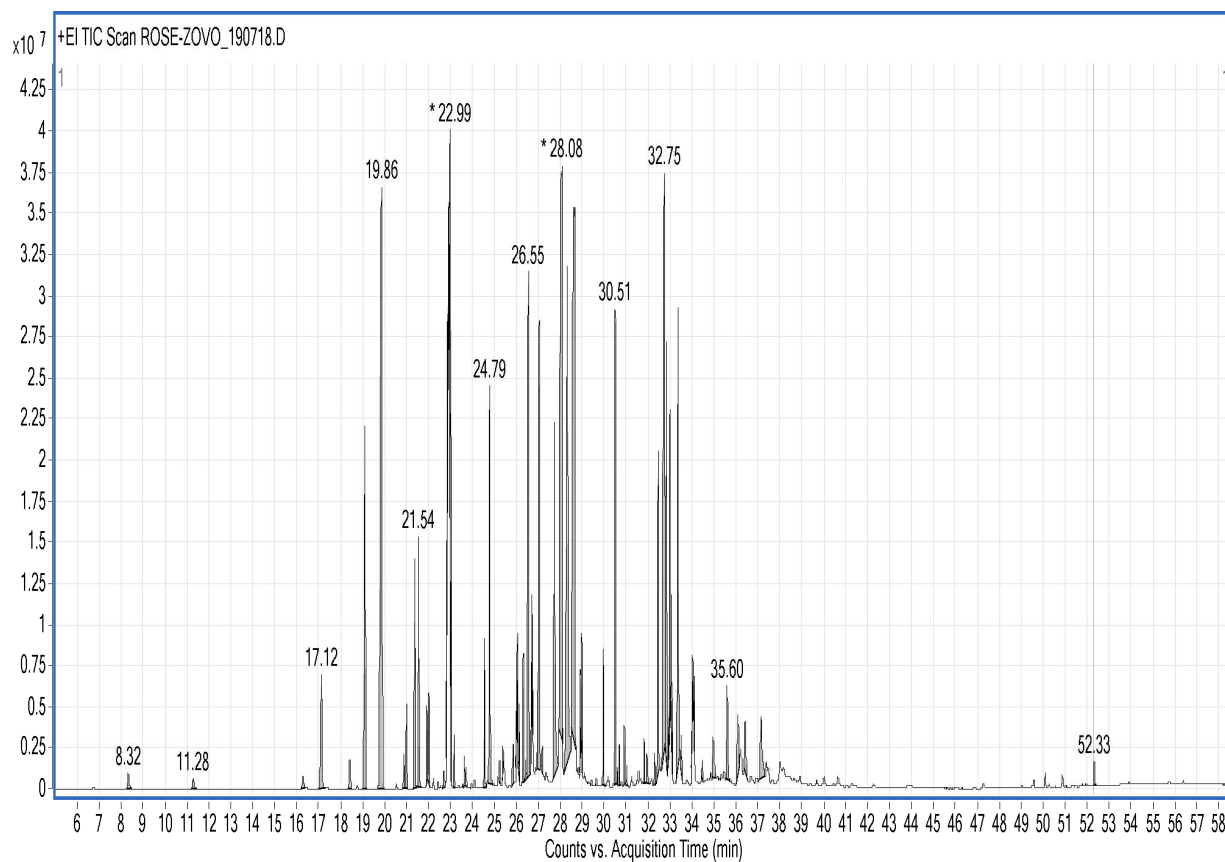
Winston TA, Man SF, Hull M., Montaner JS and Sin DD. (2013). Epidemic of lung cancer in patients with HIV infection. *Chest*. 143 (2): 305-314.

Woerdenbag HJ, Malingre TM, Lemstra W and Konings AWT. (1987). Cytostatic activity of eupatoriopicrin in fibrosarcoma bearing mice. *Phytotherapy Research*. 1:76-79.

Wu S, Wei FX, Li HZ, Liu XG, Zhang JH and Liu JX. (2013a). Chemical composition of essential oil from *Thymus citriodorus* and its toxic effect on liver cancer cells. *Zhong Yao Cai*. 36:756–9.

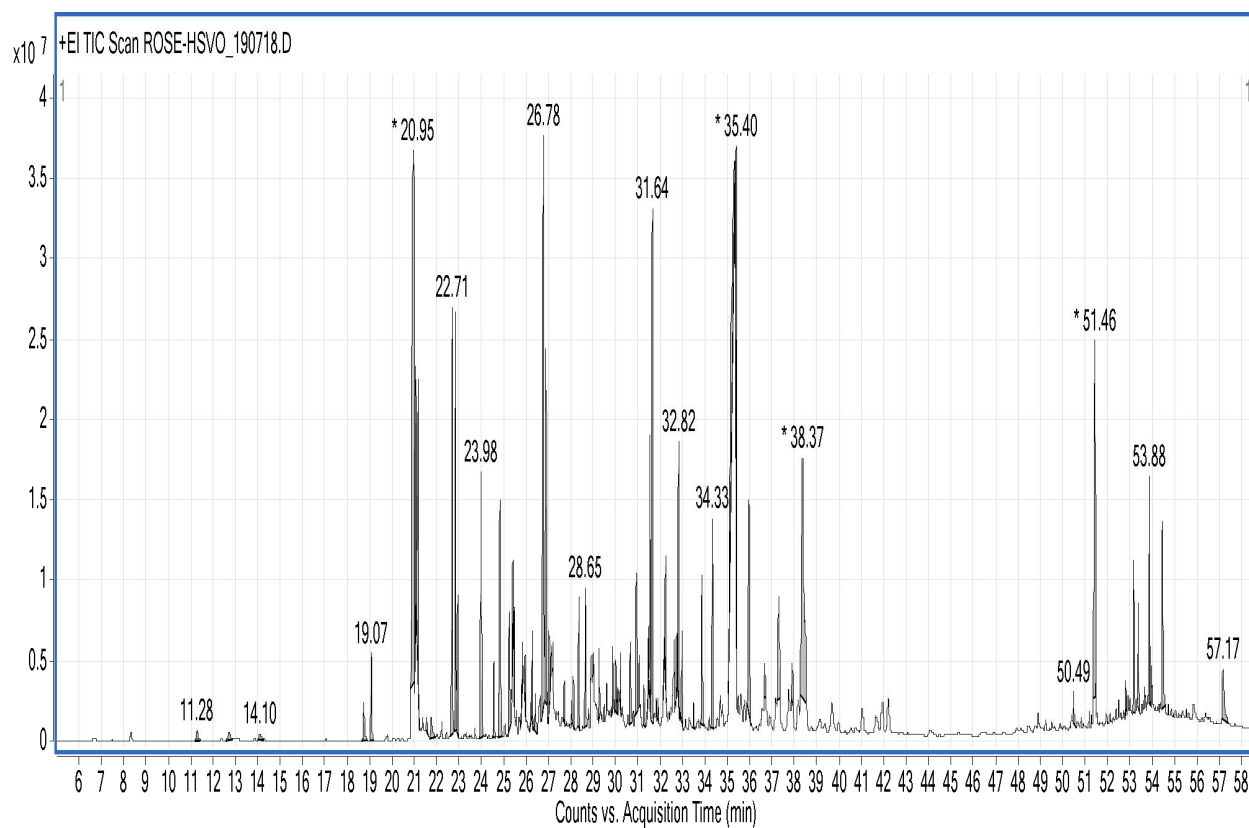
- Wu XQ**, Huang C, Jia YM, Song BA, Li J and Liu XH. (2013b). Novel coumarin-dihydropyrazole thio-ethanone derivatives: design, synthesis and anticancer activity. *European Journal of Medicinal Chemistry*. 74:717-25.
- Yin QH**, Yan FX, Zu XY, Wu YH, Wu XP, Liao MC., Deng SW, Yin LL and Zhuang YZ. (2012). Anti-proliferative and pro-apoptotic effect of carvacrol on human hepatocellular carcinoma cell line HepG-2. *Cytotechnology*. 64:43–51.
- Yongkhamcha B** and Indrapichate K. (2012). Insecticidal efficacy of mintweed, yam bean and celery seed extracts on *Aedes aegypti* L. *International Journal of Agricultural Science*; 4(3): 207-212.
- Yousefzadi M**, Riahi-Madvar A, Hadian J, Rezaee F, Rafiee R and Biniiaz M. (2014). Toxicity of essential oil of *Satureja khuzistanica*: *In vitro* cytotoxicity and anti-microbial activity. *Journal of Immunotoxicology*. 11:50–5
- Yousuf DM**, Shah WA, Mubashir S and Rather MA. (2012). Chromatographic analysis, anti-proliferative and radical scavenging activity of *Pinus wallichina* essential oil growing in high altitude areas of Kashmir, India. *Phytomedicine*. 19:1228–33.
- Zheljazzkov VD**, Callahan AN and Cantrell CL. (2007). Yield and oil composition of thirty-eight basil (*Ocimum basilicum* L.) accessions grown in Mississippi. *Journal of Agricultural and Food Chemistry*. 56:241-5.
- Zheng GQ**, Kenny PM. and Lam KT. (1992). Sesquiterpenes from clove (*Eugenia carrophyllata*) as potential anticarcinogenic agents. *Journal of Natural Products*. 55:999-1003.
- Zhou Y**, Hileman EO, Plunkett W, Keating MJ and Huang P. (2003). Free radical stress in chronic lymphocytic leukemia cells and its role in cellular sensitivity to ROS generating anticancer agents. *Blood*. 15: 101-106.
- Ziegler HL**, Jensen TH, Christensen J, Staerk D, Hagerstrand H, Sittie AA, Olsen CE, Staalso T, Ekpe P and Jaroszewski W. (2002). Possible artifacts in the *in vitro* determination of antimalarial activity of natural products that incorporate into lipid bilayer: Apparent antiplasmodial activity of dehydroabietinol, a constituent of *Hyptis suaveolens*. *Planta Medica*. 68(6): 547-549.
- Zollo PHA**, Biyiti L, Tchoumboungang F, Menut C, Lamat G and Bouchet PH. (1998). Aromatic plants of tropical central Africa. Part XXXII. Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of Cameroon. *Flavour and Fragrance Journal*; 13(2): 107-114.
- Zu YG**, Yu HM, Liang L, Fu YJ, Efferth T, Liu X and Wu N. (2010). Activities of Ten Essential Oils towards *Propionibacterium acnes* and PC-3, A-549 and MCF-7 cancer cells. *Molecules*. 15: 3200-3210.

APPENDIX 1



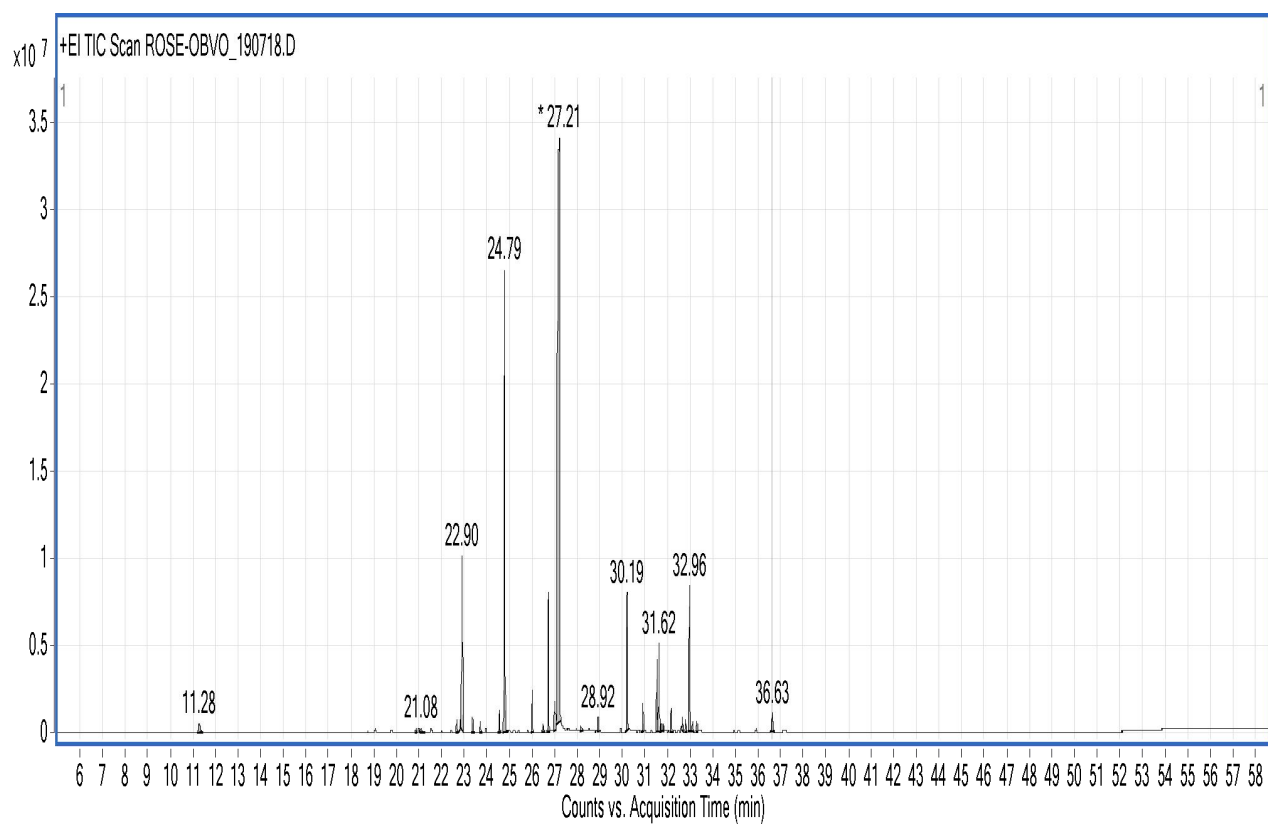
GC-MS chromatogram of essential oil of *Z. officinale*

APPENDIX 2



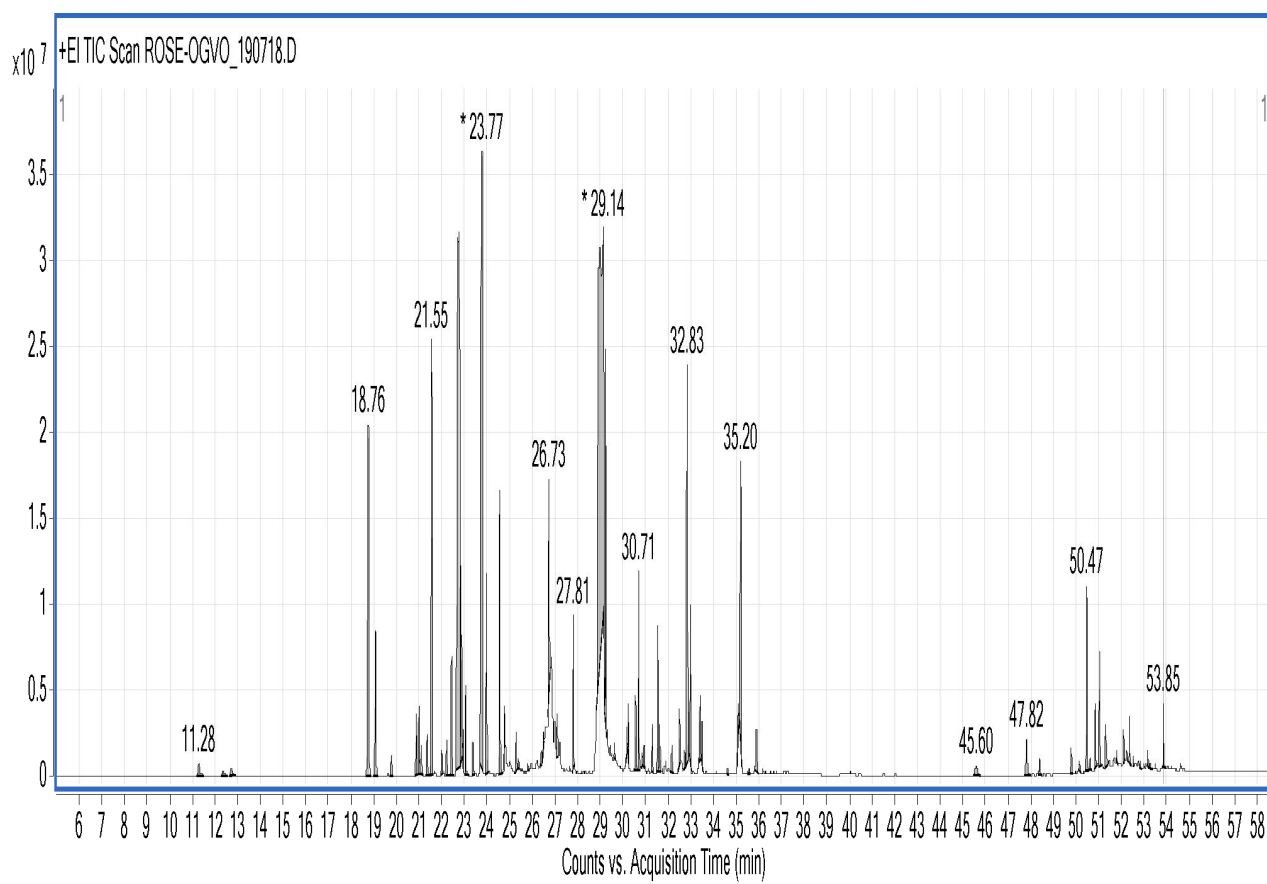
GC-MS chromatogram of essential oil of *H. suaveolens*

APPENDIX 3



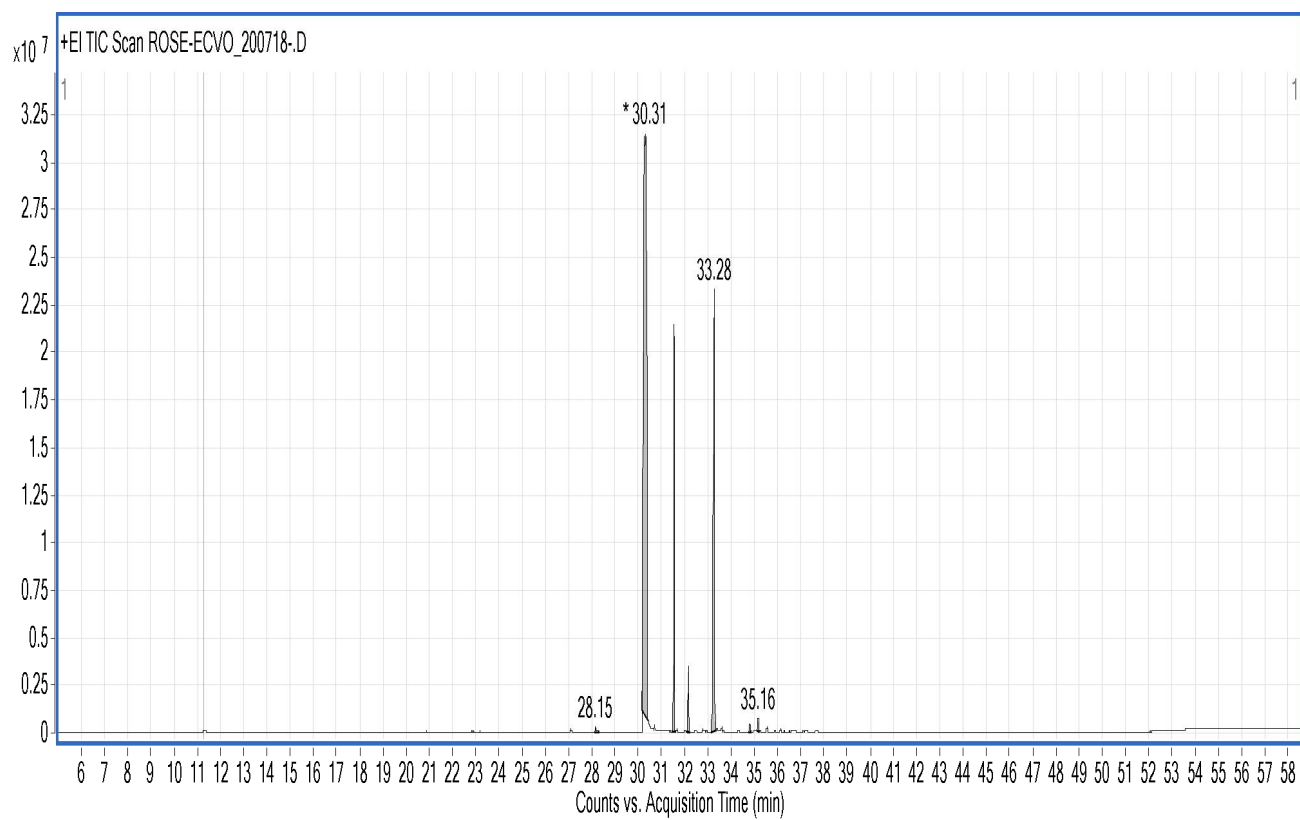
GC-MS chromatogram of essential oil of *O. basilicum*

APPENDIX 4



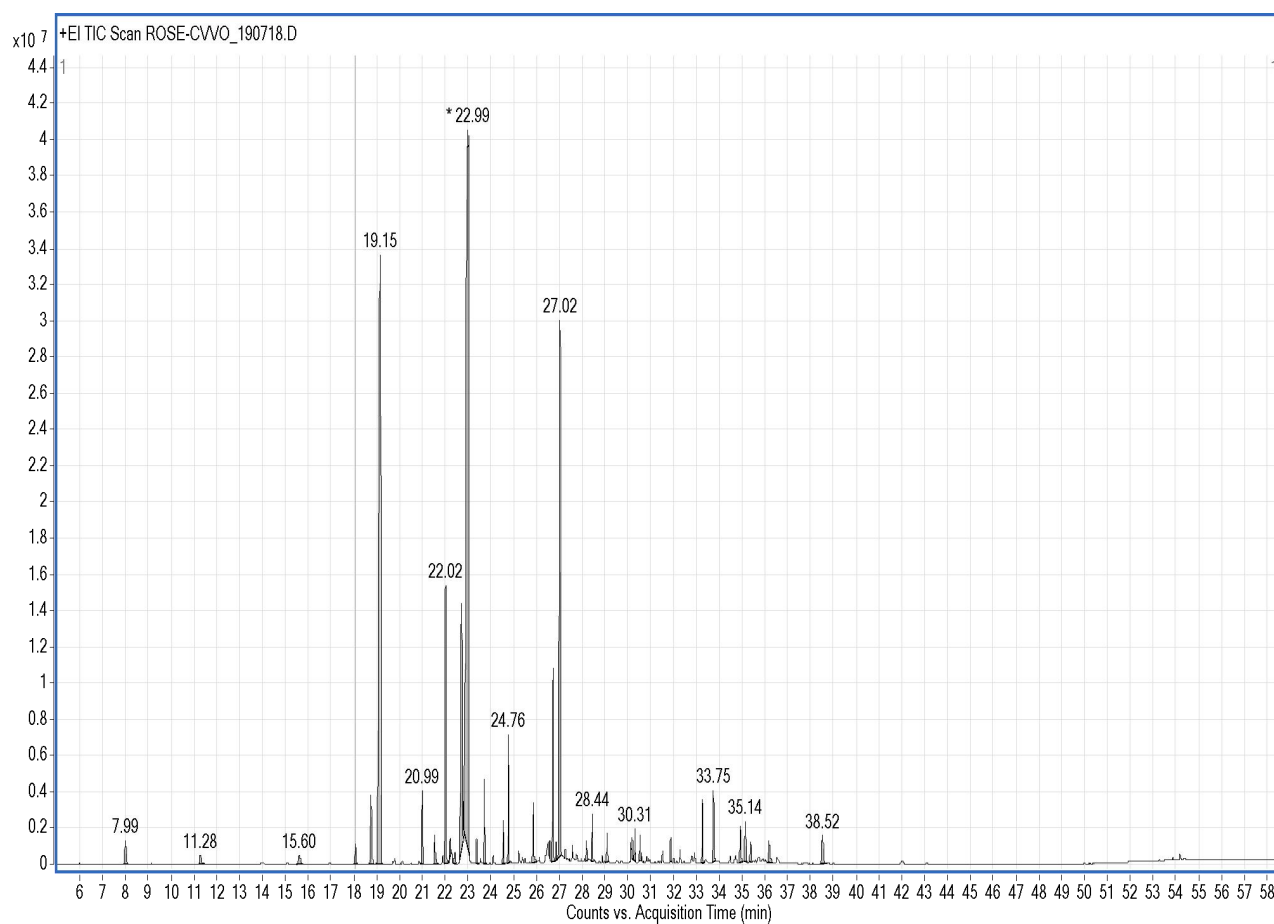
GC-MS chromatogram of essential oil of *O. gratissimum*

APPENDIX 5



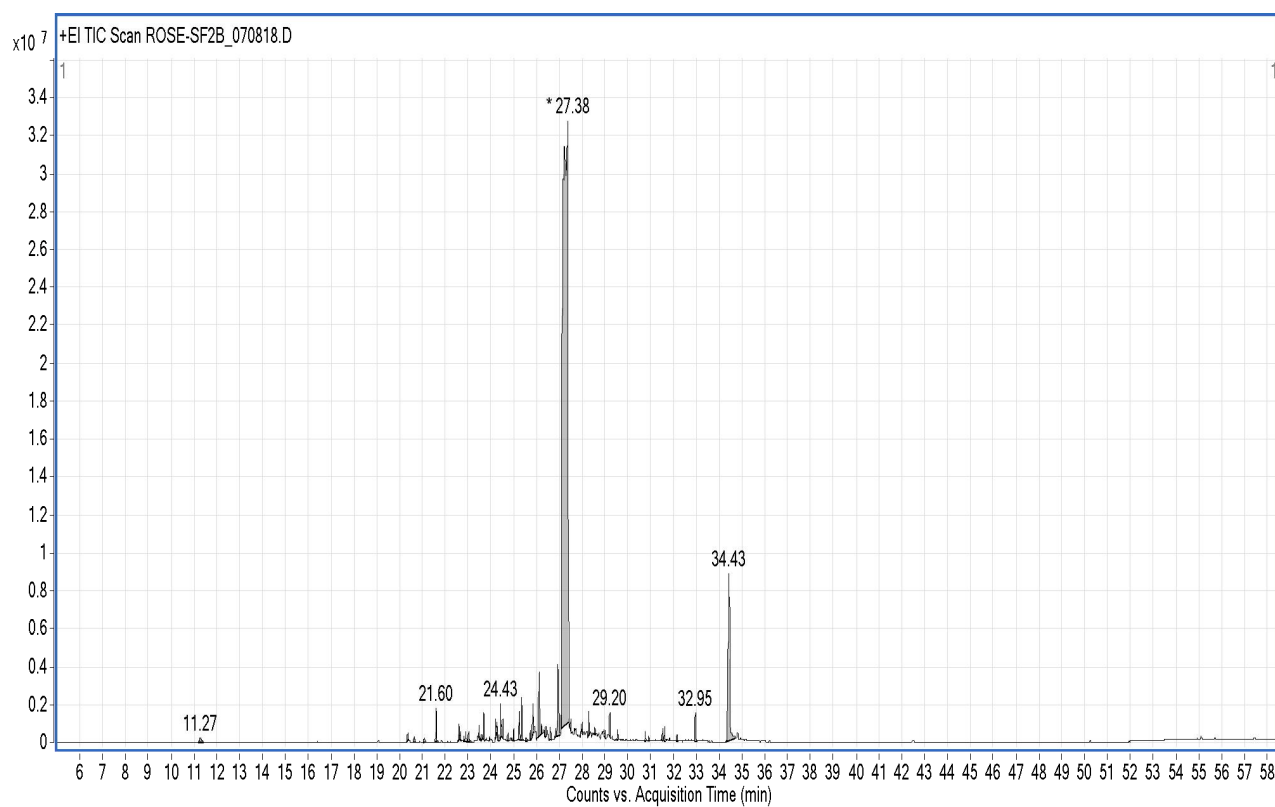
GC-MS chromatogram of essential oil of *E. caryophyllus*

APPENDIX 6



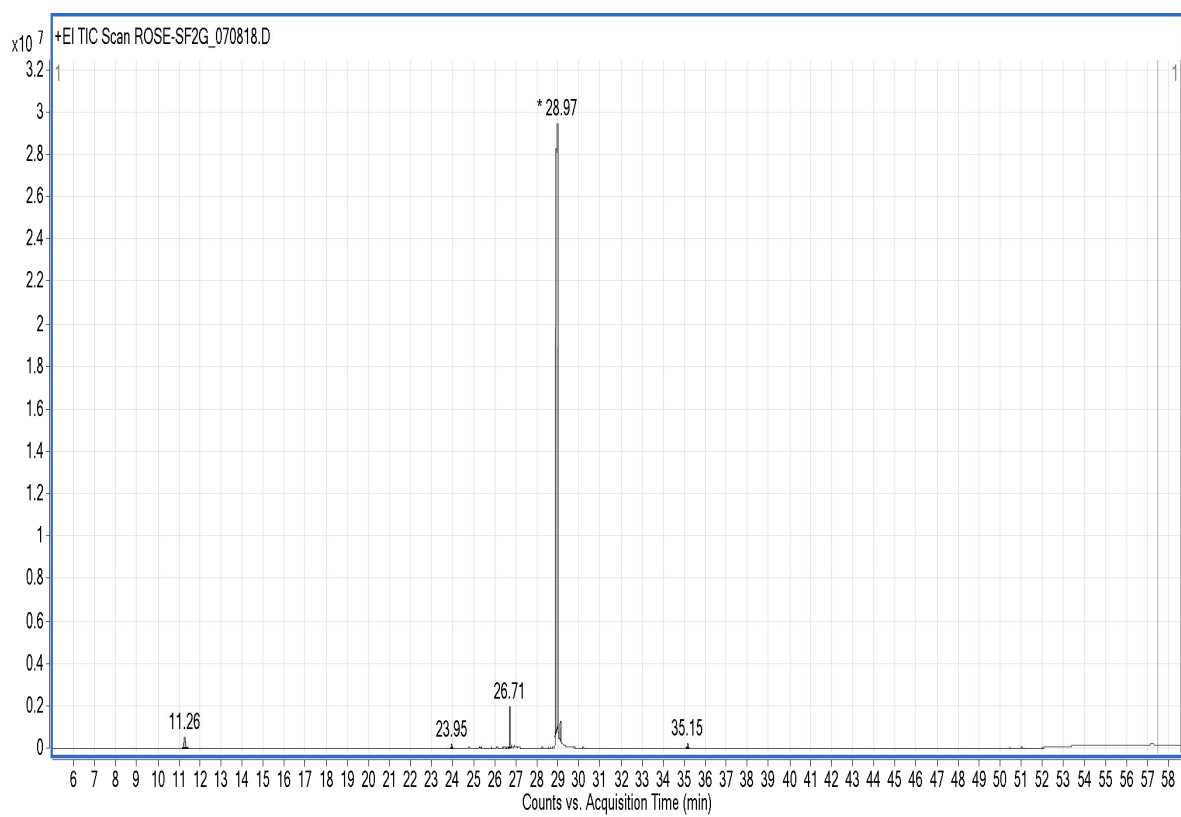
GC-MS chromatogram of essential oil of *C. citrinus*

APPENDIX 7



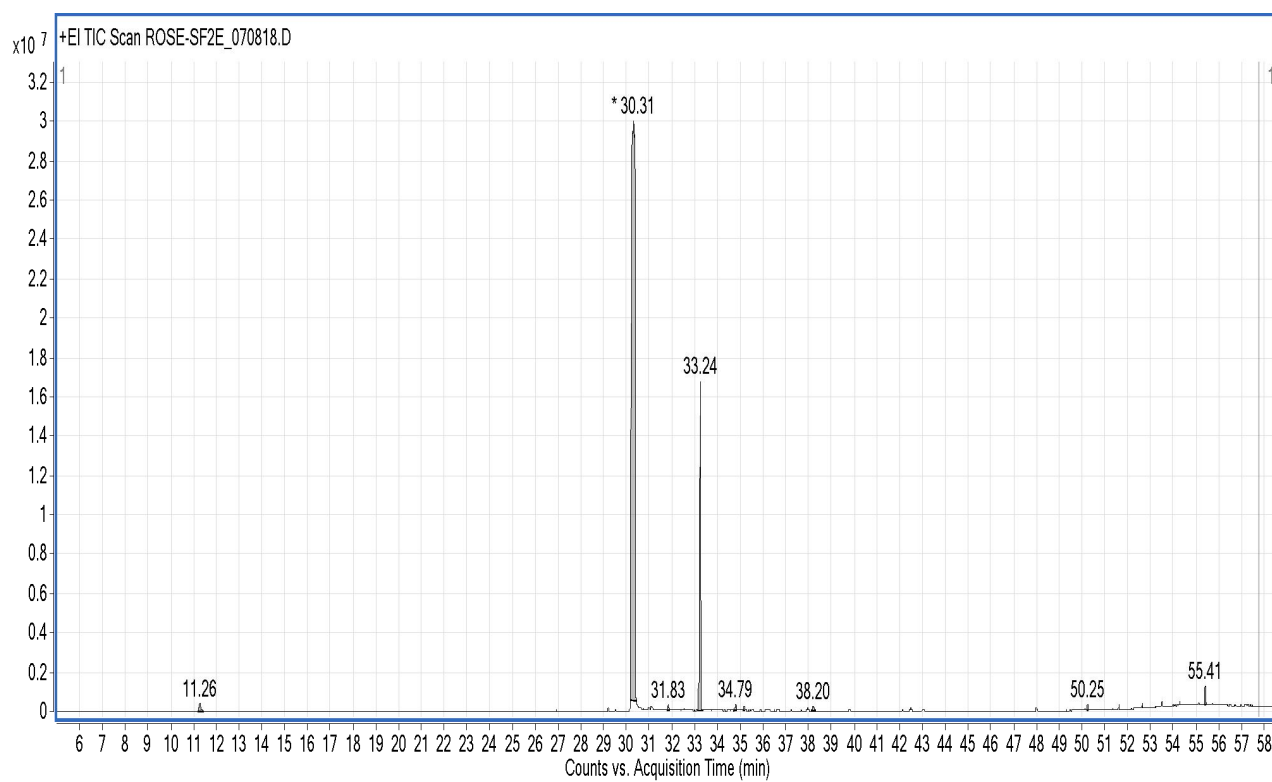
GC-MS chromatogram of SF₂B

APPENDIX 8



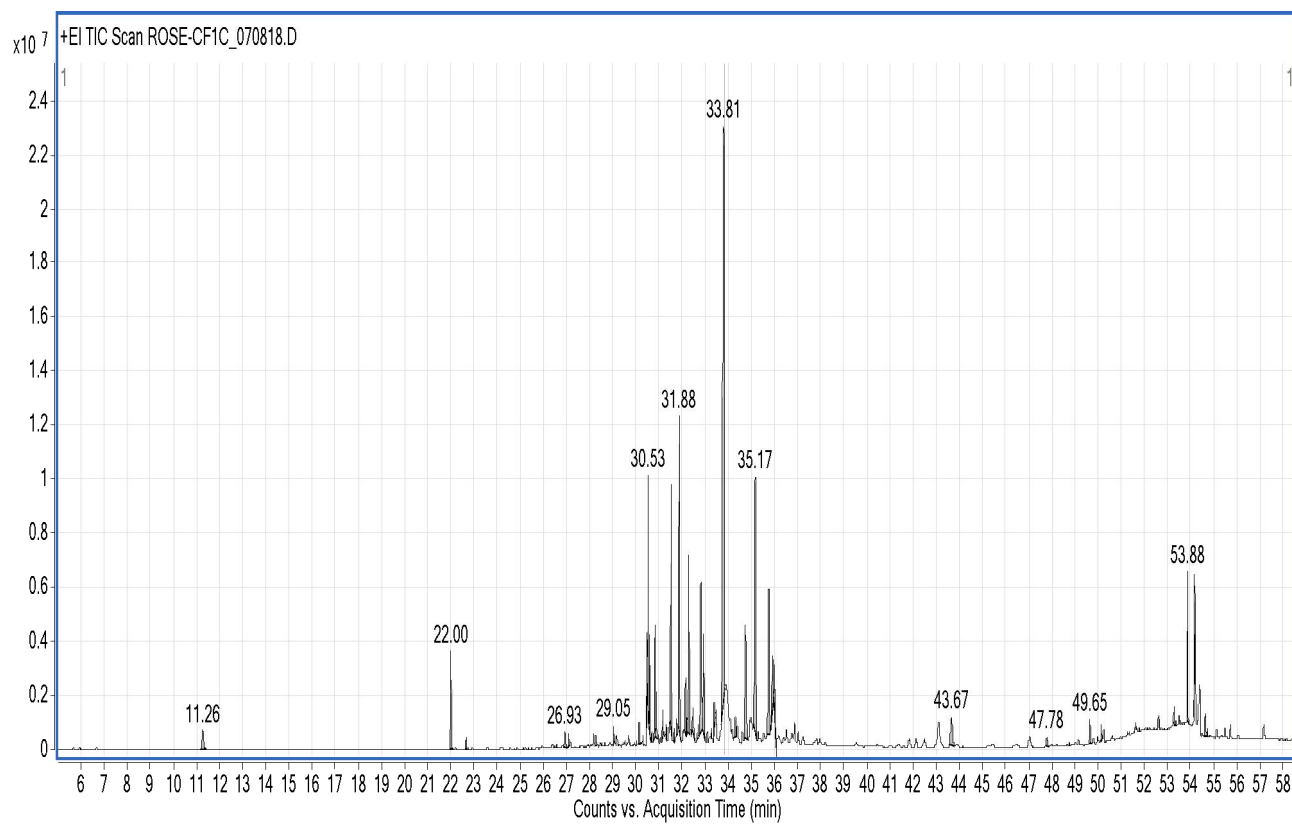
GC-MS chromatogram of SF₂G

APPENDIX 9



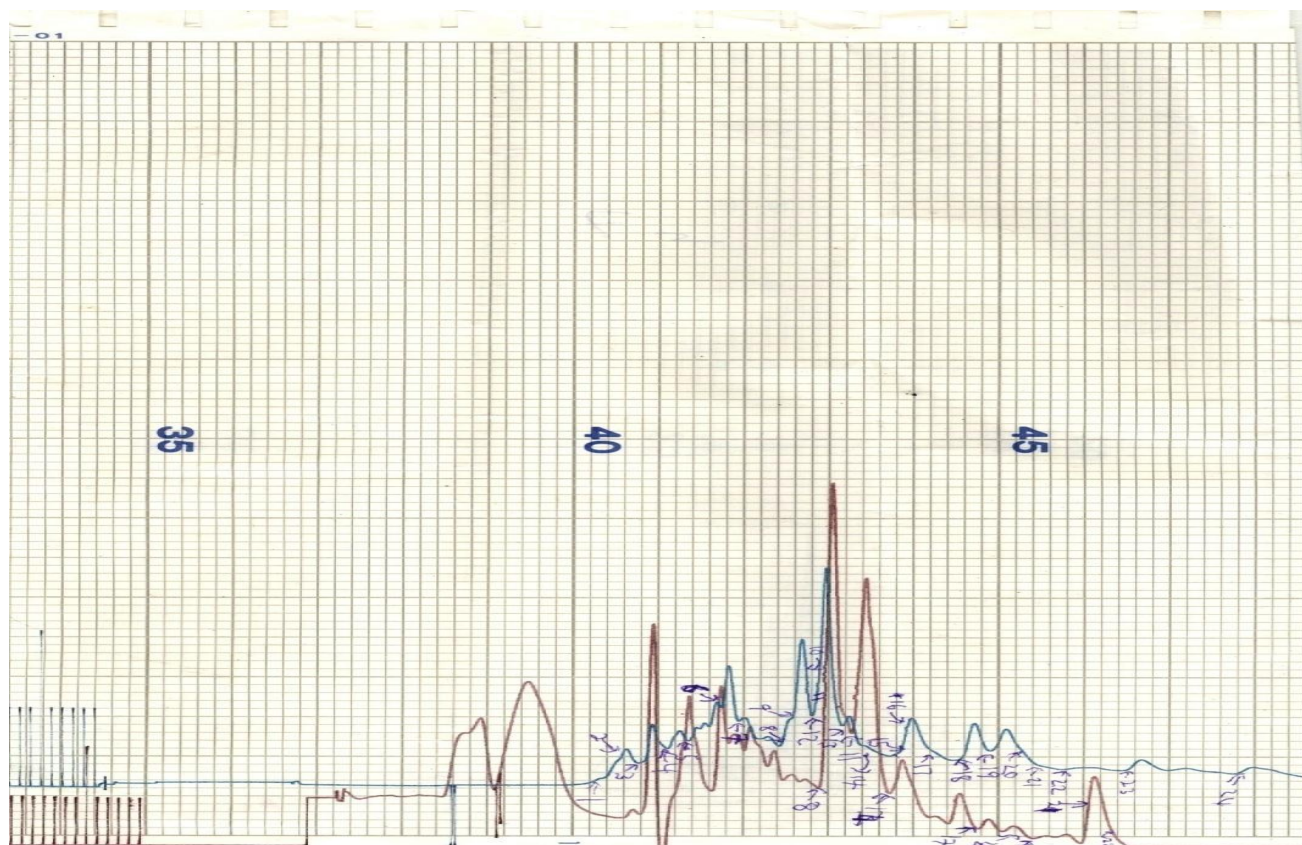
GC-MS chromatogram of SF₂E

APPENDIX 10



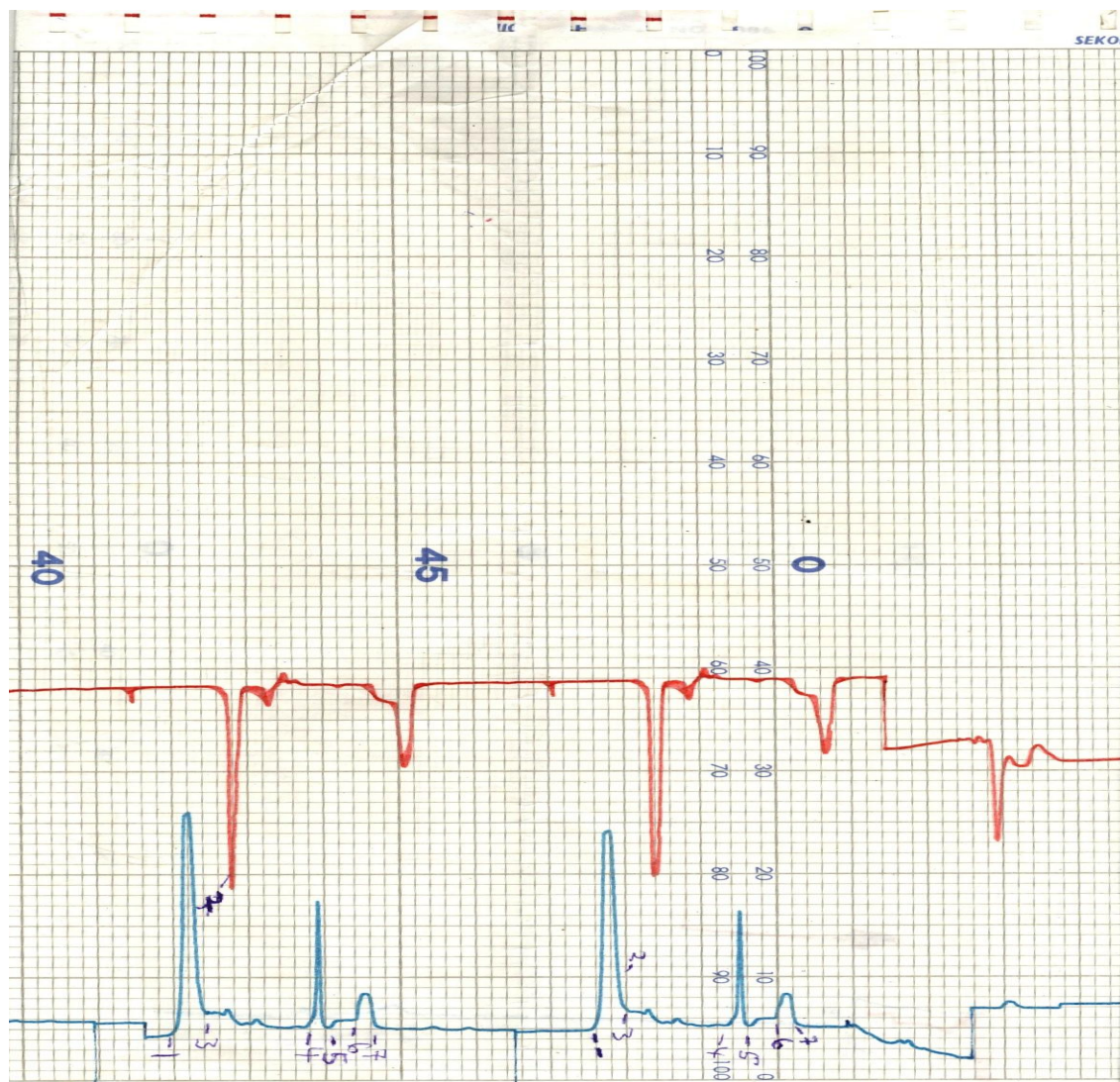
GC-MS chromatogram of CF₁C from *C. citrinus* essential oil

APPENDIX 11



Tracing of normal phase preparative HPLC carried out on C2* using $\text{C}_6\text{H}_{14} - \text{CH}_3\text{COOC}_2\text{H}_5$ (7:3). Compounds TT1 and TT2 were obtained from fractions 11-13 and 19-22 respectively.

APPENDIX 12



Tracing of reverse phase preparative HPLC carried out on F11 using $\text{CH}_3\text{OH}-\text{H}_2\text{O}$ (9:1), Compound TRA was obtained from fraction 6.