PHYTOCHEMICAL CONSTITUENTS, ANTIMICROBIAL AND BIOCHEMICAL ACTIVITIES OF THE METHANOL EXTRACTS FROM *DURIO ZIBETHINUS*

Ph.D. THESIS

BY

ADENIYI Sunday Adegoke

B. Sc. (Ind. Chemistry), M. Sc. (Chemistry), ILORIN

MATRIC No.: 87/011733

SUBMITTED TO THE DEPARTMENT OF CHEMISTRY UNIVERSITY OF ILORIN IN PARTIAL FULFILMENT OF THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) IN CHEMISTRY

JANUARY, 2020

DECLARATION

I declare that this project is based on a study undertaken by me in the Department of Chemistry, Faculty of Physical Sciences, University of Ilorin, Ilorin, Nigeria, under the supervision of Dr O.S. Oguntoye.

This work has not been submitted for the award of any degree elsewhere. All ideas and views are products of my personal research and where the views of others have been expressed, they have been properly recognized.

I shall be totally, wholly and fully responsible for any liability that may arise or flow from this study, if any.

_____

Adeniyi Sunday Adegoke

CERTIFICATION

This is to certify that this research study has been read and approved as meeting the requirements of the Department of Chemistry, for the award of Doctor of Philosophy Degree (Ph.D) in Chemistry, Faculty of Physical Sciences, University of Ilorin, Ilorin, Nigeria.

_____ _____

Dr. O. S. Oguntoye Date

(Supervisor)

_____ _____

Prof. L. A. Usman Date

(Head of Department/Chief Internal Examiner)

_____ _____

(External Examiner) Date

_____ _____

Prof. O. M. Kolawole Date

(Internal Examiner)

_____ _____

Prof. A.C. Tella Date

(PG Coordinator)]

DEDICATION

This Thesis is dedicated to my Late Father Alhaji Buraimoh Akinpelu Adeniyi.

ACKNOWLEDGEMENT

Firstly, I am grateful to God Almighty for the gift of life, knowledge and support given me to accomplish this research work. I thank God for providing me with sufficient grace to exercise patience and endurance in the course of this research. I am indeed very much grateful for the victories over all the challenges encountered during the course of this research.

My profound gratitude goes to Prof. G. A. Olatunji, who supervised this project to an advanced stage before his retirement. His continuous support, suggestions, in-depth faith, love and care has enhanced my academic advancement. The scientific foundation, personal skills and ethical standard he impacted on me will continue to lead me through my future endeavor. His words and expressions have given me immense energy, strength and inspiration. It was a rare privilege for me to have a renowned Professor of Organic Chemistry, supervised my Ph. D. research. I shall forever remain obliged to him for his commitment to my development. The only thing I can offer him is my promise to strive hard to live up to the expectation he has set for me.

My sincere gratitude also goes to my current supervisor Dr. O. S. Oguntoye for his professional insight, mentorship, guidance and positive criticism.

I also like to express my gratitude to the Heads of Department of Chemistry, Professor L.A. Usman for his words of encouragement.

I would like to express my deep and sincere appreciation to the honourable APC

committee members; Professor J.A. Obaleye, Professor E.O. Odebunmi, Professor U.B. Eke, Professor N. Abdus-Salam, Professor A.C. Tella, Professor L.A. Usman, the Departmental PG Coordinator Dr. O.S. Oguntoye and Dr. Mrs. A.M.O. AbdulRaheem for their immense contributions and suggestions.

I am greatly indebted to Dr O. Atolani, Dr (Mrs) Fabiyi, Dr M.J. Adisa and all members of the Department of Chemistry, University of Ilorin who assisted me with valuable advice in the course of the study.

More so I would like to thank all my friends both at home and diaspora, my family members who played a special part in my work.

TABLE OF CONTENT

Title page		i
Declaration		ii
Certification		iii
Dedication		iv
Acknowledgement		v
Table of Contents		vii
List of Figures		xv
List of Tables		xvi
List of Schemes		xviii
List of Appendices		xix
Abstract		xxi
	CHAPTER ONE	
	Introduction	
1.1	Medicinal Plants	1
1.2	Traditional Medicine	2
1.2.1	Herbal Medicine	3
1.2.1.1	Herbs	3
1.3	Traditional African Medicine	4
1.4	Medicinal Chemistry Era	6
1.4.1	Natural Products and Drug Discovery	8
1.4.2	Phytochemistry in Drug Development	16

1.4.3	Traditional Way of Drug Development	18
1.4.4	Modern Method of Drug Discovery	19
1.4.4.1	Screening of Natural Products	20
1.4.4.2	Isolation and Purification	20
1.4.4.3	Structure Determination	20
1.4.4.4	Structure-activity Relationships (SARs)	21
1.4.4.5	Synthetic Analogues	22
1.4.4.6	Receptor Theories (3D Computer Modelling)	22
1.5	Classes of Bioactive Constituents in Plants	23
1.5.1	Alkaloids	24
1.5.1.1	Morphine	24
1.5.1.2	Hordenine	25
1.5.1.3	Dopamine	25
1.5.1.4	Caulerpin	25
1.5.2	Saponins	26
1.5.2.1	Classes and Chemical Structure of Saponins	29
1.5.2.2	Sources of Saponins	29
1.5.2.3	Biological Roles of Saponins	29
1.5.2.3.1	Antimicrobial Activity	30
1.5.2.3.2	Antifungal Activity	30
1.5.2.3.3	Anti-inflammatory Activity	31
1.5.2.3.4	Anticancer Activity	31

1.5.2.3.5	Adjuvants Activity	32
1.5.2.3.6	Absorption Enhancer	32
1.5.2.3.7	Anticardiovascular Activity	32
1.5.2.3.8	Haemolytic Activity	32
1.5.2.3.9	Cholesterol-lowering Activity	33
1.5.2.3.10	Virucidal activity	33
1.5.2.3.11	Hypolipidaemic activity	33
1.5.3	Tannins	34
1.5.3.1	Classes of Tannins	34
1.5.3.1.1	Gallotannins	35
1.5.3.1.2	Ellagitannins	38
1.5.3.1.3	Complex tannins	39
1.5.3.1.4	Condensed tannins	40
1.5.4	Terpenes	42
1.5.4.1	Classification of Terpenoids	43
1.5.4.1.1	Monoterpenoids	45
1.5.4.1.2	Sesquiterpenoids	46
1.5.4.1.3	Diterpenoids	47
1.5.4.1.4	Triterpenoids	48
1.5.4.1.5	Tetraterpenoids (Carotenoids)	50
1.5.5	Flavonoids and Isoflavonoids	50
1.5.6	Steroids	53

1.5.6.1	Types of steroids	54
1.5.7	Glycosides	56
1.5.8	Plant Phenolics	61
1.6	Antibiotics	62
1.6.1	Classification of Antibiotics	62
1.6.2	Modern Antibiotics Era	63
1.6.3	Antibiotics from Nature	64
1.6.4	Antibiotic Resistance	64
1.7	Antioxidants from Natural Products	65
1.8	Justification	67
1.9	Research Objectives	67
1.9.1	Summary and problem statements	67
1.9.2	Hypothesis	68
1.9.3	Aim of the Research	68
1.9.4	Objectives of the Research	68
	CHAPTER TWO	
	Literature Review	
2.1	Introduction to *Durio zibethinus* Murr.	70
2.2	Durian plant varieties	71
2.3	Durian Fruit Shape	71
2.4	Volatile Compounds in Durian	73
2.5	Polyphenols in Durian	74

2.6	The Nutritional Worth of Durian	74
2.7	Use of Durian in Traditional Medicine	75
2.8	Health Benefits of Durian	75
2.8.1	Effects of Durian on Blood Glucose	76
2.8.2	Cholesterol-Lowering Properties of Durian	77
2.8.3	Anti-Proliferative Activity	77
2.8.4	Probiotic Effects	77
	CHAPTER THREE	
	Materials and Methods	
3.1	Materials	79
3.1.1	General Laboratory Chemicals	79
3.1.2	General Solvents	79
3.1.3	Materials for Chromatography	79
3.1.4	Materials for Total Flavonoids and Phenolic Content Screening	79
3.1.5	Materials for Antioxidant Activity Studies	79
3.1.6	Materials for Biological and Anti-inflammatory Activity Studies	80
3.1.7	List of Bacteria and Fungi Strains	80
3.2	Methods	80
3.2.1	Plant Collection	80
3.2.2	Plant Preparation and Extraction	81

3.3	Preliminary Phytochemical Screening	81
3.3.1	Test for Alkaloids	81
3.3.2	Test for Saponins	82
3.3.3	Test for Tannins	82
3.3.4	Test for Flavonoids	82
3.3.5	Test for Steroidal Compounds	82
3.3.6	Test for Terpenoids	82
3.3.7	Test for Cardiac Glycosides	82
3.3.8	Test for Phenolic Compounds	83
3.4	Isolation Procedure	83
3.4.1	Column Chromatography	83
3.4.2	Thin Layer Chromatography (TLC) and Preparative TLC	84
3.5	Structure Elucidation	84
3.6	GC-MS Analysis of Phytochemical Compounds in the fractions of Stem bark Extract from *D. zibethinus*	85
3.6.1	Instruments and chromatographic conditions	85
3.6.2	Identification of phytochemical compounds	85
3.7	Energy Dispersive X-ray Fluorescence (EDXRF) elemental analysis of *D. zibethinus*	85
3.8	Estimation of Total Flavonoid Content	86
3.9	Estimation of Total Phenolic Content	87
3.10	Assessment of *In vitro* Antioxidant Activity	87

3.10.1	DPPH Radical Scavenging Activity	87
3.10.2	ABTS Radical Scavenging Activity	88
3.11	Biological Activities	89
3.11.1	Antibacterial Bioassay	89
3.11.2	Antifungal Bioassay	89
3.12	Anti-inflammatory Activity	90
3.12.1	Lipoxygenase Inhibition Assay	90
3.13	Statistical Analysis	90
	CHAPTER FOUR	
	Results and Discussion	
4.1	Preliminary Phytochemical Screening of *Durio zibethinus* extracts	91
4.2	Structure Elucidation	93
4.3	GC-MS Analysis	96
4.4	EDXRF Elemental Analysis	103
4.5	Total Flavonoid Content	107
4.6	Total Phenolic Content	108
4.7	*In vitro* Antioxidant Activity	110
4.7.1	Free Radical Scavenging Activity (DPPH)	110
4.7.2	Free Radical Scavenging Activity (ABTS)	112
4.8	Biological Activities	114
4.8.1	Antibacterial Bioassay	114

4.8.2	Antifungal Bioassay	116
4.9	Anti-inflammatory Activity	118
4.9.1	Lipoxygenase Inhibition Activity	118
4.10	Research Findings	120
4.11	Contribution to Knowledge	121
4.12	Conclusion	122
References		123

LIST OF FIGURES

Figure 2.1: Fruits and seeds of durian (*Durio zibethinus* L.) clone D24 96

Figure 4.1: Structures of the isolated compounds (A, B and C) 117

Figure 4.2: Total Flavonoid content of methanolic extracts from *D. zibethinus* M 108

Figure 4.3: Total Phenolic content of methanolic extracts from *D. zibethinus* M 110

Figure 4.4: DPPH radical scavenging activity of the methanolic extracts from *D. zibethinus* 112

Figure 4.5: ABTS radical scavenging activity of the methanolic extracts from *D. zibethinus* 114

Figure 4.6: Antibacterial activity of methanolic extracts from *D.* zibethinus 116

Figure 4.7: Antifugal activity of methanolic extracts from *D. zibethinus* 117

Figure 4.8: Lipoxygenase inhibition activity of the Stem bark extract from *D. zibethinus* 119

LIST OF TABLES

Table 1.1:	Drugs derived from plants, with their ethnomedical correlation and sources	09
Table 1.2:	Classification of Terpenoids	43
Table 2.1:	Taxonomical classification of *Durio zibethinus*	70
Table 4.1:	Qualitative phytochemical screening of methanolic extracts from *Durio zibethinus*	92
Table 4.2:	GC-MS analysis of the methanol stem-bark extract from *D. zibethinus*	97
Table 4.3:	GC-MS analysis and biological activities in methanol stem-bark extract from *D. zibethinus*	99
Table 4.4:	Metal content in leaves, stem bark and root of *Durio zibethinus*	104
Table 4.5:	Total Flavonoid content of methanolic extracts from *D. zibethinus* M	108
Table 4.6:	Total Phenolic content of methanolic extracts from *D. zibethinus* M	109
Table 4.7:	Free radical scavenging activity (DPPH) of methanolic extracts from *D. zibethinus* M	111
Table 4.8:	Free radical scavenging activity (ABTS) of methanolic extracts from *D. zibethinus* M	113
Table 4.9:	Antibacterial activity of methanolic extracts from	115

D. zibethinus

Table 4.10: Antifungal activity of methanolic extracts from *D. zibethinus* 117

Table 4.11: Anti- Lipoxygenase activity of methanolic Extracts from *D. zibethinus* 119

LIST OF SCHEMES

Scheme 1:	Classification of Saponins	27
Scheme 2:	Schematic representation of the fractionation of stem bark extract.	83
Scheme 3:	Isolation of bioactive compounds	84

LIST OF APPENDICES

Appendix I:	IR spectrum of Compound A [Maslinic acid, (2α,3β)-2,3-Dihydroxyolean-12-en-28-oic acid]	159
Appendix II:	^{1}H-NMR spectrum of Compound A [Maslinic acid, (2α,3β)-2,3-Dihydroxyolean-12-en-28-oic acid]	159
Appendix III:	IR spectrum of Compound B [3-Epimaslinic acid, (2α,3α)-Dihydroxyolean-12-en-28-oic acid]	160
Appendix IV:	^{1}H-NMR spectrum of Compound B [3-Epimaslinic acid, (2α,3 α)-2,3-Dihydroxyolean-12-en-28-oic acid]	160
Appendix V:	IR spectrum of Compound C [3-Epimaslinic acid, (2β,3α)-Dihydroxyolean-12-en-28-oic acid]	161
Appendix VI:	^{1}H-NMR spectrum of Compound C [3-Epimaslinic acid, (2β,3α)-2,3-Dihydroxyolean-12-en-28-oic acid]	161
Appendix VIIa:	GC-MS Chromatogram of fraction 1 of methanolic stem bark extract from *D. zibethinus*	162
Appendix VIIb:	Mass spectrum of Oleic acid (RT: 29.923, Area % = 91.36) from fraction 1	162
Appendix VIIIa:	GC-MS Chromatogram of fraction 2 of methanolic stem bark extract from *D. zibethinus*	162
Appendix VIIIb:	Mass spectrum of (E)-9-Octadecenoic acid (RT: 19.818, Area % = 56.10) from fraction 2	163
Appendix IXa:	GC-MS Chromatogram of fraction 3 of methanolic	163

stem bark extract from *D. zibethinus*

Appendix IXb: Mass spectrum of (Z)-6-Octadecenoic acid (RT: 26.307, Area % = 41.19) from fraction 3 163

Appendix Xa: GC-MS Chromatogram of fraction 4 of methanolic stem bark extract from *D. zibethinus* 164

Appendix Xb: Mass spectrum of (E)-9-Octadecenoic acid (RT: 19.778, Area % = 42.20) from fraction 4 164

ABSTRACT

Bacterial and viral infections represent some of the most difficult diseases to treat in humans due to resistance to most of the therapeutic agents. The emergence of drug resistance factors that have threatened the efficacy of all antimicrobial agents prompted the investigation of phytochemicals, antibacterial, antifungal and biochemical activity studies of methanol extracts from *Durio zibethinus*. The aim of this research is to analyse the phytochemical compounds of *D. zibethinus*, isolate and characterize the bioactive compounds from the stem bark extract from the plant and establish their antimicrobial, antioxidant and anti-inflammatory activities. The objectives of the research were to: (i) prepare the extracts of *Durio zibethinus* parts, using solvents of various polarities (n-hexane, ethyl acetate and methanol); (ii) determine the presence of secondary metabolites in *Durio zibethinus* parts using different standard tests; (iii) analyse the minerals in *Durio zibethinus* powders using Energy Dispersive X-Ray Fluorescence (EDXRF); (iv) fractionate and identify the constituents of the stem bark extract from *D. zibethinus* using GCMS; (v) isolate and purify the bioactive compounds from the stem bark from *D. zibethinus* through various chromatographic techniques; (vi) elucidate the structure of the isolates using various spectroscopic methods; (vii) determine the total flavonoid and phenolic contents of the stem bark extract from *D. zibethinus*; and (viii) investigate the antibacterial, antifungal, antioxidant and anti-inflammatory activities of the stem bark extract from the plant.

The methanol extracts of *D. zibethinus* parts were obtained by soxhlet extraction and concentrated using rotary evaporator. The qualitative *in vitro* screening of secondary metabolites in the extracts was done using standard methods. The bioactive compounds in stem-bark extract were isolated and purified through column chromatography and preparative thin layer chromatography; the isolates were characterized using Fourier-Transform Infrared Spectroscopy, Gas Chromatography-Mass spectrometry (GC-MS) and Nuclear magnetic resonance spectroscopy. Antimicrobial activity was determined on fresh clinical isolates using Agar Diffusion Methods. The antioxidant activity of the plant extracts was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) models. The anti-lipooxygenase bioassay was used to investigate the anti-inflammatory activity of the plant extracts.

The findings of the study were that:

(i) the presence of alkaloids, saponins, tannins, flavonoids steroids, glycosides and phenolic compounds were established in the extracts;

(ii) three isomeric triterpenoic acids (maslinic, 3-epimaslinic and bredemolic acids) were isolated from *D. zibethinus* stem-bark and their structures were confirmed using spectroscopic techniques;

(iii) GC-MS analysis of stem-bark extract revealed 17 compounds in four fractions. The principal compounds identified were (Z)-9-Octadecenoic acid (Oleic acid) (91.36 %), (E)-9-Octadecenoic acid (Elaidic acid) (56.10 %),

(Z)-6-Octadecenoic acid (Petroselinic acid) (41.19 %) and (E)-9-Octadecenoic acid (Elaidic acid) (42.20 %) in fractions 1-4 respectively;

(iv) the plant extracts exhibited antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia, Candida albicans* and *Microsporum audouinii* at a range of 10.00 – 25.00 mm relative to standard gentamicin and fluconazole antibiotics.

(v) the plant extracts showed lower DPPH antioxidant activity at a range of 10-150 μg/mL with the following IC_{50} in μmol/L (leaf- 1.467, stem-bark- 1.569 and root- 1.846) and ABTS (leaf- 1.929, stem-bark- 0.267 and root- ~ 2.002) compared to standard quercetin (DPPH- 1.490 and ABTS- 1.247; and

(vi) the plant extracts had anti-lipooxygenase activity at a range of 10-150 μg/mL with the following IC_{50} in μmol/L (leaf- 1.464, stem-bark- 1.203 and root- 1.400) as compared to indomethacin (1.660).

The IC_{50} values obtained for antioxidant and anti-lipooxygenase activities were significantly lower than the standard quercetin and indomethacin respectively at $p < 0.05$

The study concluded that the *D. zibethinus* contains some phytochemicals that exhibited higher efficacy and performance as antimicrobial and anti-lipoxygenase agents. The study recommended that the plant could be explored for the treatment of diseases caused by the studied pathogens.

CHAPTER ONE

INTRODUCTION

1.1 Medicinal Plants

Therapeutic plants, also called restorative herbs, have been found quite a long while back and utilized in folkloric prescription (Lichterman, 2004). As per Ahn (2017) a therapeutic plant is one that can be used to safeguard one's wellbeing and deal with a specific condition, either in current drug or in conventional medication. In spite of the way that natural prescriptions have been in presence before the appearance of standard medication, the majority of their defall method of activities, security limit and pharmacological potential have not been all around researched.

Singh (2016) announced that a strategy has been planned by World Health Organization on customary prescription since 1991, and rules were distributed with a progression of monographs on extensively used natural meds. In 2002, the Food and Agriculture Organization evaluated that significant measure of natural plants are utilized over the world for therapeutic purposes (Schippmann et al., 2002). The World Health Organization (WHO) has announced that huge number (about 70%) of the total populace relies basically upon conventional prescription (Smith-Hall et al., 2012).

Restorative plants may give three principle sorts of advantage, for example, wellbeing

advantages to the purchasers; money related advantages to the speculators, processors, and

wholesalers of customary medications; and society-wide advantages, for example, work creation, salary income, and a more advantageous work power (Smith-Hall et al., 2012).

Plants create optional metabolites which are associated with the plant security against bright radiation and different pathogens (Clifford, 2000). Tropical rainforest plants associated with vieing for space and light which powers species to grow increasingly proficient methods for utilizing vitality and supplements just as dispensing assets for optional mixes generation, that structures the premise of numerous restorative mixes found in the plants (Smith-Hall et al. 2012).

What's more, the fight for endurance has made plants create numerous compound safeguards to swim off assaults, for example, microorganisms, bugs, organisms and now and then warm blooded creatures, that can incapacitate the life span of a plant animal categories which may not be fundamental for their development. These auxiliary metabolites are utilized in advancing the spread and control of plant species in a natural setting (Cravotto et al. 2010). These plant metabolites structure the model (lead mixes) for medication as practically half of the items in both Europe and US are gotten from common items (Newman et al., 2003). Locale of high biodiversity contain more prominent chemo-decent variety, thus harbor more noteworthy potential for finding new mixes (Cordell, 2002).

1.2 Traditional Medicine

The World Health Organization (WHO) portrays regular drug as the summation of the data, fitness and sharpens (WHO, 2008). In any case, WHO takes note of that abuse of customary meds or practices can prompt negative or hazardous impacts, henceforth further enquiry is

expected to find the prosperity and viability of a few practices by the conventional medication frameworks (WHO, 2008).

Customary drug may fuse formalized viewpoints of society prescription, in other words longstanding fixes passed on and rehearsed by lay people. People prescription includes the mending practices and ideas of body physiology and wellbeing preservation known to some in a culture, transmitted coolly as coomon data, and rehearsed or applied by anyone inside the way of life having prior experience (Acharya and Shrivastava, 2008).

1.2.1 Herbal Medicine

Plants have been the reason for helpful prescription through quite a bit of mankind's history, and such convectional drug is still comprehensively rehearsed these days. Present day prescription perceives herbalism as a type of strange medication and pseudoscience, as the act of herbalism isn't altogether founded on evidence assembled utilizing the logical strategy (IWJS, 2007). Present day prescription utilizes various mixes got from plant as the reason for proof based pharmaceutical medications. Despite the fact that phytotherapy may apply current standard rules of ampleness testing to herbs and prescriptions derived from normal sources, barely any top notch clinical preliminaries and rules for virtue or measurements exist. The degree of home grown medication is every so often intensified to join parasitic and honey bee items, just as, minerals (IWJS, 2007).

The term, phytomedicine, may also suggest the study of pathology and harm to plants, the causes thereof, their signs, improvement, dispersal, systems for keeping up plant wellbeing and measures, used to control plant contaminations and their causes (CHABC, 2017).

1.2.1.1 Herbs

By and large use, herbs are plants with sweet-smelling properties that are used for enhancing sustenance, in pharmaceutical, or as scents. Culinary use normally perceives herbs from flavors. Herbs insinuate the verdant green pieces of a plant (either crisp or dried), while flavors are generally dried and created from different pieces of the plant, for example, seeds, berries, bark, roots and organic products (Tapsell et al., 2006).

Herbs have a collection of employments including culinary, therapeutic, and, now and again, profound. General utilization of the expression "herb" moves between culinary herbs and restorative herbs; in remedial or profound use, any pieces of the plant might be considered as "herbs", including leaves, roots, blossoms, seeds, root bark, internal bark (Tapsell et al., 2006).

1.3 Traditional African Medicine

Specialists of traditional African medicine guarantee to be equipped for giving solution for various and differing afflictions, for example, tumors, mental disarranges, hypertension, cholera, most venereal sicknesses, epilepsy, asthma, skin inflamation, fever, anxiety, hopelessness, kindhearted prostatic hyperplasia, urinary tract diseases, gout, and mending of wounds and consumes and in fact Ebola (Helwig, 2005).

Conclusion is come to through otherworldly methods and a treatment is endorsed, frequenly trading off natural cure, that is considered to have mending capacities as well as additionally representative and profound significance. Conventional African drug, accepts that infection isn't derived from chance occasions, however through profound or social divergence, fluctuates essentially from present day sensible medicine, which is really and intelligently based. Inside the 21st century, advance pharmaceuticals and remedial systems remain closed off to gigantic quantities of African individuals because of their moderately significant expense and grouping of wellbeing offices in urban focuses (Helwig, 2005). Some time as of late the establishment

of science-based prescription, routine medicine was the overwhelming helpful structure for many individuals in Africa however the section of the Europeans was a conspicuous defining moment inside the historical backdrop of this old show and culture (Abdullahi, 2011). Home grown prescriptions in Africa are commonly not comprehensively explored, and are weakly controlled (Mills et al., 2005).

As of late, the drugs and fixes used in customary African prescription have increased more gratefulness from specialists in science. Creating nations have begun to understand the significant expenses of cutting-edge prosperity care systems and the advances that are required, thus showing Africa's dependence to it (CA, 2002). Because of this, intrigue has as of late been ecommunicated in organizing customary African medication into the landmass' national prosperity care structures (Helwig, 2005). An African healer got a handle on this idea by making a 48-bed emergency clinic, the essential of its sort, in Kwa-Mhlanga, South Africa, which consolidates ordinary systems with homeopathy, iridology, and other Western mending methodologies, in any event, including a couple of conventional Asian pharmaceutical (Helwig, 2005). In any case, the significantly current development remembered for present day pharmaceutical, which is the beginning to coordinate into Africa's prosperity care structure, may possibly pulverize Africa's profound situated social qualities (CA, 2002).

At the point when an individual becomes sick, a regular expert uses chant to make a finding. Divination is regularly utilized incase the ailment isn't successfully distinguished, something else, the affliction might be immediately analyzed and given a cure. In case divination is required, at that point the expert will urge the comprehension imply a soothsyer who can help give an end and cure. Contact with the soul world through divination frequently requires medicine, yet forfeits (Borokini and Lawal, 2014).

Customary professionals use a wide combination of treatment stretching out from "charm" to biomedical systems, for example, fasting and consuming less calories, natural treatments, washing, knead, and surgeries. For instance, headaches, hacks, abscesses, and pleurisy are frequently treated utilizing the strategy for "drain measuring" after which a home developed treatment is associated with follow-up home developed medications (CA, 2002). In Africa, the locals have been known to use the fat of a boa constrictor to purportedly cure gout and stiffness, and it is thought to quiet chest torment when scoured into the skin (Gomes et al., 2011). Practically 80% of African depend on customary solutions for treat themselves for different sicknesses (WHO, 2002).

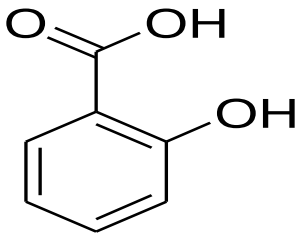
In actuality, out of the approximated 6400 plant species used in tropical Africa, more than 4000 are utilized as restorative plants (Stanley, 2011). Restorative plants are utilized inside the meds of various illnesses and diseases, the businesses and effects of which are of creating enthusiasm to Western social requests.

Not so to speak are plants used and picked for their recovering limits, yet they also routinely have regular and profound centrality. For instance, leaves, seeds, and twigs that are white, dark and red are viewed as especially run of the mill or charmed and have phenomenal properties (Helwig, 2005).

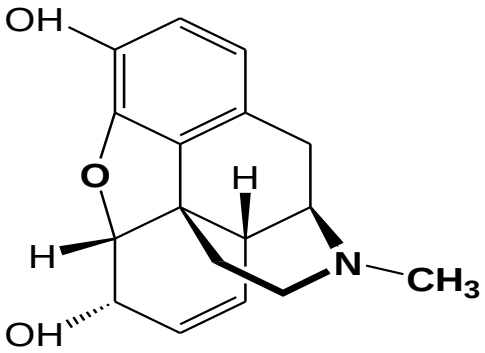
1.4 Medicinal Chemistry Era

Therapeutic science centers around the plan, disconnection, union and advancement of substance mixes for use as medications (Thomas et al., 2008). Restorative scientific experts discover and improve the particles required for comprehension, forestalling and treating sicknesses. Researchers started in the 1800′s to explore substances that figure out which explicit constituents (mixes) were answerable for remedial impacts (Patrick, 2000). The most established records of the utilization of remedial plants and minerals are gotten from the old

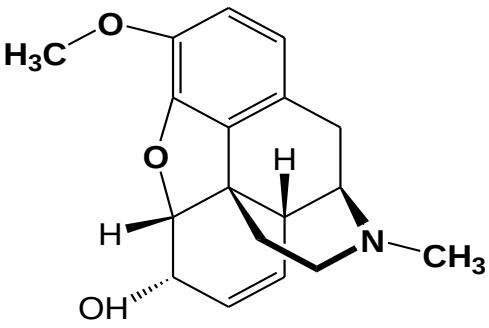
human advancement of Chinese, Hindus, Mayans of Central America, and the Mediterranean individuals of times long past (Thomas et al., 2008). Much tale therapeutics in drugs found in our market today was initially confined from plant in the old occasions. For example, salicyclic corrosive 1, the model of ibuprofen, was detached in 1874 from willow bark. Progressively powerful torment executioners, for example, morphine 2 and codeine 3, were gotten from the opium poppy. The counter malarial operator, quinine 4 was isolated from cinchona (china bark). The leaves of the purple foxglove plant gave an excellent wellspring of digitalis (digoxin 5) that was cleansed for use against coronary illness (Butler, 2004; Newman et al., 2000;Samuelsson, 2004).



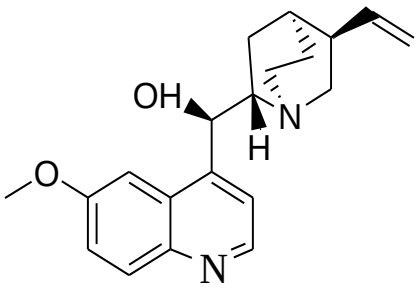
1 Salicyclic acid



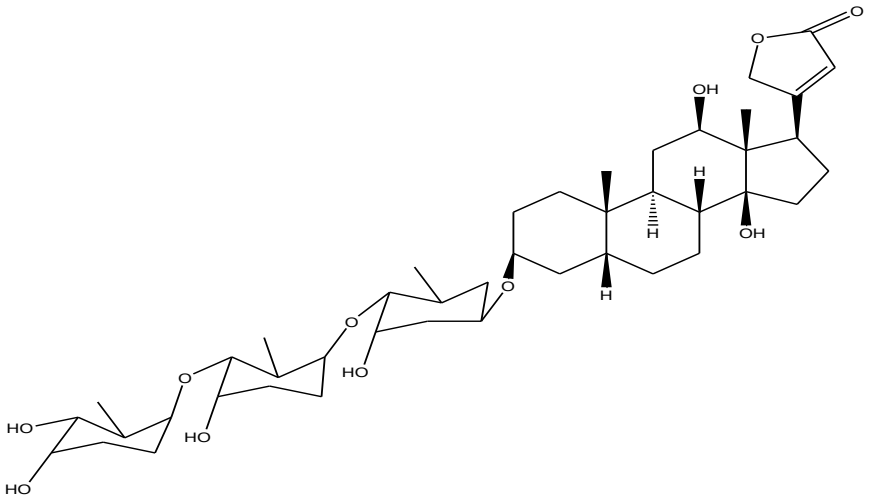
2 Morphine



3 Codeine



4 Quinine



5 Digoxin

The old strategy for separation, portrayal and clarification of regular mixes were to be sure dull. It requires a lot of test assortment, thorough confinement forms, colossal filtration and complex basic clarification ventures since it was not the time of current spectroscopic hardware. Screening technique for assurance of compound viability was created all together not to subject human's stomach muscle nitio to clinical preliminaries (Samuelsson, 2004). The examination labs of worldwide pharmaceutical ventures led the pack in propelling present day prescription as they found all the more new medications which were at some point or another circled into the medication advertise (Newman et al., 2000).

1.4.1 Natural Products and Drug Discovery

Characteristic items are concoction mixes or substances created by a whole living creatures (for example alkaloids, flavonoids, steroids and terpenoids) separated from plants, creatures or microorganisms (Cutler and Cutler,2000).The term regular items likewise suggest optional metabolites that are not fundamental for endurance, yet nonetheless, they give a few living beings that produce them (Hanson, 2003; Williams and Lemke, 2002). Various optional

metabolites are cytotoxic and have been picked and enhanced through headway for use as "compound fighting" against prey, predators, and contending creatures (Hunter, 2008). In that capacity, regular items are the dynamic constituents of numerous society prescriptions (Li and Vederas, 2009; Hunter, 2008; Brahmachari, 2010; Beghyn et al., 2008; Koehn and Carter, 2005). Besides, regular items are as often as possible used as beginning stages for sedate disclosure so as to set up their manufactured analogs with better power and security. Common item constituents have motivated various medication disclosure endeavors that in the long run picked up endorsement as new medications by the U.S. Nourishment and Drug Administration (Newman and Cragg, 2007; Gransalke, 2011). Plant realm has been a rich store of many medication lead mixes. A few examples of plant derived drugs in modern use are presented in table 1.1.

Table 1.1: Drugs derived from plants, with their ethnomedical correlation and sources

Drug	**Action or clinical use**	**Plant Source**
Acetyldigoxin	Cardiotonic	*Digitalis lanata*
Adoniside	Cardiotonic	*Adonis vernalis*
Aescin	Antiinflammatory	*Aesculus hippocastanum*
Aesculetin	Antidysentery	*Frazinus rhychophylla*
Agrimophol	Anthelmintic	*Agrimonia supatoria*
Ajmalicine	Treatment for circulatory disorders	*Rauvolfia sepentina*
Allantoin	Vulnerary	Several plants
Allyl isothiocyanate	Rubefacient	*Brassica nigra*

Anabesine	Skeletal muscle relaxant	*Anabasis sphylla*
Andrographolide	Treatment for baccillary dysentery	*Andrographis paniculate*
Anisodamine	Anticholinergic	*Anisodus tanguticus*
Anisodine	Anticholinergic	*Anisodus tanguticus*
Arecoline	Anthelmintic	*Areca catechu*
Asiaticoside	Vulnerary	*Centella asiatica*
Atropine	Anticholinergic	*Atropa belladonna*
Benzyl benzoate	Scabicide	Several plants

Source: Helmenstine (2000)

Drug	**Action or clinical use**	**Plant Source**
Berberine	Treatment for bacillary dysentery	*Berberis vulgaris*
Bergenin	Antitussive	*Ardisia japonica*
Borneol	Antipyretic, analgesic, anti-inflammatory	Several plants
Bromelain	Antiinflammatory, proteolytic	*Ananas comosus*
Caffeine	CNS stimulant	*Camellia sinensis*
Camphor	Rubefacient	*Cinnamomum camphora*
Camptothecin	Anticancerous	*Camptotheca acuminate*
(+)-Catechin	Hemostatic	*Potentilla fragarioides*
Chymopapain	Proteolytic, mucolytic	*Carica papaya*

Cissampeline	Skeletal muscle relaxant	*Cissampelos pareira*
Cocaine	Local anaesthetic	*Erythroxylum coca*
Codeine	Analgesic, antitussive	*Papaver somniferum*
Colchiceine amide	Antitumor agent	*Colchicum autumnale*
Colchicine	Antitumor, antigout	*Colchicum autumnale*
Convallatoxin	Cardiotonic	*Convallaria majalis*
Curcumin	Choleretic	*Curcuma longa*

Source: Helmenstine (2000)

Drug	**Action or clinical use**	**Plant Source**
Cynarin	Choleretic	*Cynara scolymus*
Danthron	Laxative	Cassia species
Demecolcine	Antitumor agent	*Colchicum autumnale*
Deserpidine	Antihypertensive, tranquilizer	*Rauvolfia canescens*
Deslanoside	Cardiotonic	*Digitalis lanata*
L-Dopa	Anti-parkinsonism	*Mucuna species*
Digitalin	Cardiotonic	*Digitalis purpurea*
Digitoxin	Cardiotonic	*Digitalis purpurea*
Digoxin	Cardiotonic	*Digitalis purpurea*
Emetine	Amoebicide, emetic	*Cephaelis ipecacuanha*

Ephedrine	Sympathomimetic, antihistamine	*Ephedra sinica*
Etoposide	Antitumor agent	*Podophyllum peltatum*
Galanthamine	Cholinesterase inhibitor	*Lycoris squamigera*
Gitalin	Cardiotonic	*Digitalis purpurea*
Glaucarubin	Amoebicide	*Simarouba glauca*
Glaucine	Antitussive	*Glaucium flavum*
Glasiovine	Antidepressant	*Octea glaziovii*

Source: Helmenstine (2000)

Drug	**Action or clinical use**	**Plant Source**
Glycyrrhizin	Sweetener, treatment for Addison's disease	*Glycyrrhiza glabra*
Gossypol	Male contraceptive	*Gossypium species*
Hemsleyadin	Treatment for bacillary dysentery	*Hemsleya amabilis*
Hesperidin	Treatment for capillary fragility	Citrus species
Hydrastine	Hemostatic, astringent	*Hydrastis canadensis*
Hyoscyamine	Anticholinergic	*Hyoscyamus niger*
Irinotecan	Anticancer, antitumor agent	*Camptotheca acuminate*
Kaibic acud	Ascaricide	*Digenea simplex*

Kawain	Tranquilizer	*Piper methysticum*
Kheltin	Bronchodilator	*Ammi visage*
Lanatosides A, B, C	Cardiotonic	*Digitalis lanata*
Lapachol	Anticancer, antitumor	Tabebuia species
a-Lobeline	Smoking deterrant, respiratory stimulant	*Lobelia inflata*
Menthol	Rubefacient	Mentha species
Methyl salicylate	Rubefacient	*Gaultheria procumbens*

Source: Helmenstine (2000)

Drug	**Action or clinical use**	**Plant Source**
Monocrotaline	Topical antitumor agent	*Crotalaria sessiliflora*
Morphine	Analgesic	*Papaver somniferum*
Neoandrographolide	Treatment of dysentery	*Andrographis paniculate*
Nicotine	Insecticide	*Nicotiana tabacum*
Nordihydroguaiaretic acid	Antioxidant	*Larrea divaricata*
Noscapine	Antitussive	*Papaver somniferum*
Ouabain	Cardiotonic	*Strophanthus gratus*
Pachycarpine	Oxytocic	*Sophora pschycarpa*
Palmatine	Antipyretic, detoxicant	*Coptis japonica*

Papain	Proteolytic, mucolytic	*Carica papaya*
Papavarine	Smooth muscle relaxant	*Papaver somniferum*
Phyllodulcin	Sweetener	*Hydrangea macrophylla*
Physostigmine	Cholinesterase inhibitor	*Physostigma venenosum*
Picrotoxin	Analeptic	*Anamirta cocculus*
Pilocarpine	Parasympathomimetic	*Pilocarpus jaborandi*

Source: Helmenstine (2000)

Drug	**Action or clinical use**	**Plant Source**
Pinitol	Expectorant	Several plants
Podophyllotoxin	Antitumor, anticancer agent	*Podophyllum peltatum*
Protoveratrines A, B	Antihypertensives	*Veratrum album*
Pseudoephredrine	Sympathomimetic	*Ephedra sinica*
Nor-pseudoephedrine	Sympathomimetic	*Ephedra sinica*
Quinidine	Antiarrhythmic	*Cinchona ledgeriana*
Quinine	Antimalarial, antipyretic	*Cinchona ledgeriana*
Qulsqualic acid	Anthelmintic	*Quisqualis indica*
Rescinnamine	Antihypertensive, tranquilizer	*Rauvolfia serpentine*
Reserpine	Antihypertensive, tranquilizer	*Rauvolfia serpentine*

Rhomitoxin	Antihypertensive, tranquilizer	*Rhododendron molle*
Rorifone	Antitussive	*Rorippa indica*
Rotenone	Piscicide, Insecticide	*Lonchocarpus nicou*
Rotundine	Analagesic, sedative, traquilizer	*Stephania sinica*
Rutin	Treatment for capillary fragility	Citrus species
Salicin	Analgesic	*Salix alba*

Source: Helmenstine (2000)

Drug	**Action or clinical use**	**Plant Source**
Sanguinarine	Dental plaque inhibitor	*Sanguinaria canadensis*
Santonin	Ascaricide	*Artemisia maritma*
Scillarin A	Cardiotonic	*Urginea maritima*
Scopolamine	Sedative	Datura species
Sennosides A, B	Laxative	Cassia species
Silymarin	Antihepatotoxic	*Silybum marianum*
Sparteine	Oxytocic	*Cytisus scoparius*
Stevioside	Sweetener	*Stevia rebaudiana*
Strychnine	CNS stimulant	*Strychnos nux-vomica*
Taxol	Antitumor agent	*Taxus brevifolia*
Teniposide	Antitumor agent	*Podophyllum peltatum*

Tetrahydrocannabinol (THC)	Antiemetic, decreases occular tension	*Cannabis sativa*
Tetrahydropalmatine	Analgesic, sedative, tranquilizer	*Corydalis ambigua*
Tetrandrine	Antihypertensive	*Stephania tetrandra*
Theobromine	Diuretic, vasodilator	*Theobroma cacao*
Theophylline	Diuretic, bronchodilator	*Theobroma cacao*
Thymol	Topical antifungal	*Thymus vulgaris*
Topotecan	Antitumor, anticancer agent	*Camptotheca acuminate*

Source: Helmenstine (2000)

Drug	**Action or clinical use**	**Plant Source**
Trichosanthin	Abortifacient	*Trichosanthes kirilowii*
Tubocurarine	Skeletal muscle relaxant	*Chondodendron tomentosum*
Valapotriates	Sedative	*Valeriana officinalis*
Vasicine	Cerebral stimulant	*Vinca minor*
Vinblastine	Antitumor, Antileukemic agent	*Catharanthus roseus*
Vincristine	Antitumor, Antileukemic agent	*Catharanthus roseus*
Yohimbine	Aphrodisiac	*Pausinystalia yohimbe*
Yuanhuacine	Abortifacient	*Daphne genkwa*
Yuanhuadine	Abortifacient	*Daphne genkwa*

Source: Helmenstine (2000)

1.4.2 Phytochemistry in Drug Developement

Regardless of the later charmed in calm disclosure by nuclear illustrating, combinatorial science, and other manufactured science strategies, common item inferred mixes are as yet showing to be a significant wellspring of medications for people (Salim et al., 2008). Plants keep on existing as rich wellsprings of lead mixes (for example alkaloids, for example, morphine, cocaine, digitalis, quinine, tubocurarine, nicotine, and muscarine). A great deal of these lead mixes are important medications themselves (for example the alkaloids, morphine and quinine), and others have been the reason for engineered drugs (for example neighborhood anagesics created from cocaine). In 1805, morphine goes to be the essential pharmacologically unique compound to be segregated fit as a fiddle from a plant, disregarding the its structure was not found out until 1923 (Sneader, 2005). The nineteenth century was remarkeable for the isolation of various alkaloids from plants used as medications, for instance, atropine (Atropa belladonna), caffeine (Coffea arabica) and so forth (Sneader, 2005). Following these disclosures, bioactive optional metabolites from plants were a short time later used all the more comprehensively as drugs, both in their one of a kind and balanced shapes (Samuelsson, 2004). Despite the way that for the most part barely any plant-inferred drugs have been propelled into the market in the course of the most recent couple of years, bigger number of such medications underneath clinical improvement are inside the oncological range, tallying model analogs of known anticancer (Butler, 2005).

Plants as wellsprings of medications is connected with a view focal points, for example, the choise of a competitor animal groups for assessments should be possible on the reason of a long haul use by people (ethnomedicine). This methodology depends on an assumption that the dynamic mixes confined from such plants are probably going to be secure than those gathered from plant species with no record of human use. Also, more often than not, medicate revelation

and conceivable commercialization would pressurize the asset impressively and might prompt bothersome common concerns. though blend of dynamic particle likely could be a decision, few out of every odd atom is pleasing for complete combination. Consequently, certain level of dependence on the lead asset would continue. For example, anticancer particles like etoposide, paclitaxel, docetaxel, topotecan, and irinotecan continue to rely on significantly powerless plant assets for getting the beginning material since a total combination isn't possible (Katiyar et al., 2012). One significant showdown in sedate improvement from plants remains that it is time exhausting and astoundingly costly. The technique for perceiving the structures of dynamic mixes from a concentrate may take weeks, months, or in fact quite a while, contingent upon the intricacy of the issue. Nowadays, the speed of bioassay-guided fractionation has been pushed ahead basically by headway in instrumented, for example, elite fluid chromatography (HPLC)/mass spectrometry (MS), fluid chromatography-mass spectrometry (LC-MS), higher attractive field-quality Nuclear Magnetic Resonance (NMR) instruments, and mechanical self-sufficiency to automate high-throughput bioassays (Salim et al., 2008). Screening of plant remove libraries can be dangerous because of the presence of aggravates that may either be auto-fluoresce or have UV retentions that meddle with the screen readout, yet fractionation of concentrates can be used to mitigate a portion of these sorts of issues. Likewise, most screening examine techniques have been advanced with computational sifting strategies to distinguish and expel conceivably risky intensifies that can give bogus positive outcomes (Walters and Namchuk, 2003).

1.4.3 Traditional Way of Drug Development

Conventional medication is exceptionally important to be disregarded in the innovative work of current medications. In any case, it has a perplexing nature, there are excessively wide setting for its utilization as far as non-universal helpful progression or activities. In convectional drug, a solitary herb or equation may contain various phytochemical constituents, for example,

alkaloids, terpenoids, flavonoids, and so on. By and large, these synthetic concoctions work alone or related to each other to create the ideal pharmacological impact (Parasuraman et al., 2014). It is essential that a great deal of plant-started medicates in clinical drug these days were gotten from customary prescription (Li-Weber, 2009). Likewise, it has been delineated that the numerous important medications acquired from plants were found through their application in regular prescription (Fabricant and Farnsworth, 2001).

Very nearly 20 years back, an exhaustive examination of the pharmacopeias of created and creating countries and the related world logical written works was directed as part of the WHO's Traditional Medicine Program to decide if customary medication truly had propelled current medication revelations and whether there was any connection between's the present utilization of different mixes and their application in conventional drug. The investigation was focused on various mixes utilized in drugs got from plants in totally various nations, and it developed that customary prescription had without a doubt had a significant impact in creating effective present day drugs (Fabricant and Farnsworth, 2001). The adequacy, comfort, and availability of customary medications have been, and will be, useful for new medication inquire about (Ngo et al., 2013).

The customary strategy for tranquilize revelation depends on the significant proof of action (hit) of the dynamic mixes got from the regular items. The dynamic mixes were gotten by partioning the unrefined concentrates of the normal plants, trailed by disconnecting and recognizable proof of these mixes. Each progression of fractionation and detachment is normally guided by bioassays, and the procedure is called bioassay-guided fractionation and bioassay-guided separation separately (Satyajit and Lutfun, 2007).

1.4.4 Modern Method of Drug Discovery

Present day sedate revelation approaches include High Throughput Screening (HTS) which applies full robotization and automated procedure to rapidly measure the natural or biochemical action of a colossal number of medication like mixes (Inglese and Auld, 2009; Macarron et al., 2011). So as to coordinate regular items inside the cutting edge HTS programs, a characteristic item library (for example an assortment of dereplicated regular items) should be set up (Koehn, 2008). A various number of hyphenated methods are utilized for dereplication. The coming of more current and improved advancements identified with partition, disengagement and ID of characteristic items has gotten a phenomenal deal of progress fabricating a characteristic item library, inside the later past where a ton of time are spent in building such a library from cleansed regular items (Sarker and Nahar, 2012)

A general example for novel medication advancement by detachment of bioactive constituents from common sources has been modified advance astute consequently: Screening of regular items for natural exercises

(i) Isolation and cleansing of the dynamic standards

(ii) Determination of structure

(iii) Structure-action connections (SARs)

(iv) Synthesis of simple

(v) Receptor speculations

(vi) Design and combination of novel medication structure

1.4.4.1 Screening of Natural Products

The primary target of various logical analysts and pharmaceutical organizations is the revelation of present day organically dynamic constituents got from regular items. So as to

accomplish this target by screening of regular items, the plausibility of achievement is constantly required. Utilizing plants right now, has an enormous preferred position inferable from their long haul use in medicinal services (Mouhssen, 2007).

1.4.4.2 Isolation and Purification

The breaking down of blends of mixes to acquire the unadulterated parts is of mind boggling feasible criticalness in science. Numerous manufactured responses give blends of items and it is fundamental to have a reasonably away from of how variety of mixes can be isolated in a manner of speaking with noteworthy difficulty. Advances in partition methods have brought about a grouping of chromatographic systems available to help inside the constrainment and cleaning of normal item (Altemimi et al., 2017).

1.4.4.3 Structure Determination

The errand of explaining structure of segregated mixes has been made straightforward with the work of current spectroscopic and spectrometric instruments. The most significant system for clarifying structures of characteristic items includes Nuclear Magnetic Resonance (NMR) spectroscopy. This instrument has been a significant bit of leeway in deciding the organization of obscure mixes. Different strategies for auxiliary clarification include mass spectrometry (MS) to decide the atomic mass of mixes and infrared spectroscopy (IR) to find out practical gatherings. The two systems give corresponding data to affirm the NMR-decided structure (McRae et al., 2007). The fracture design from MS can give valuable hints about the structure, however it doesn't demonstrate the structure, as a full blend may be required as definite confirmation.

1.4.4.4 Structure-Activity Relationships (SARs)

The structure–action relationship (SAR) is the relationship between the substance or 3D structure of an atom and its organic movement. The portrayal of SAR considers the assurance of the synthetic gathering responsible for inferring an objective natural impact in the creature. This permits substance alteration of a known and recently described lead compound to add to its helpfulness as a medication. This appear to be savage improving its explicitness for a specific body target area, growing its quality, making stride its rate and degree of retention, acclimating to advantage its time course inside the body, diminishing its poisonous quality, changing its physical or compound properties (like solvency) to supply hungered for features (Patidar et al., 2011). Organically dynamic substances acquired from plants have proceeded as lead mixes or pharmacological operators for blend of pharmaceuticals. Such mixes incorporate morphine 2 (pain relieving) with the engineered subsidiary, codeine 3 (gentle pain relieving and antitussive specialist). Expansion of a methylene gathering and expulsion of hydrogen iota in the chain prompts decrease of medication movement of codeine as a pain relieving (Oprea et al., 2001). Medicinal chemists use the techniques of chemical synthesis to embed new chemical groups into the biomedical compound and test the adjustments for their biological effects (McKinney *et al*., 2000).

1.4.4.5 Synthetic Analogues

Obviously, few natural compounds are ideal. Many have serious side-effects and there is an extraordinary advantage in finding analogues which lacks the side effect. In general, the medicinal chemist is developing drugs with three objectives, namely, increase activity, reduce side-effects and provide easy and efficient administration to the patient (Maier, 2015).

Drug development in the past has mostly been a hit or a miss affair with a huge number of compounds being synthesized at random. Luck has played a great part, but now recognized scientific strategies have evolved over the years (Teixeira *et al*., 2013). These strategies include:

(i) dissimilarity of substituents

(ii) enlarging the structure

(iii) chain extensions/contractions

(iv) ring variation

(v) simplification of the structure

(vi) rigidification of the structure

1.4.4.6 Receptor Theories (3D Computer Modelling)

The organic synthesis of a large number of analogues gives both compounds with improved activity, reduced side-effects and information about the protein with which the drugs interact. Macromolecule proteins in nature are the target sites for drugs. Most drugs have to fasten receptors to produce effects. Receptors are found generally on the cell membrane but certain intracellular receptors are found as well. There are three forms of fastening to receptors: Agonists, Antagonists and Partial agonists (Tannenbaum & Sheehan, 2014).

Agonists: Agonists are the drugs which when fasten to receptors, cause enactment of receptors. They are capable of producing chain reactions within the receptors which eventually bring about the effects. Agonists have two properties, ie. fondness for receptor, and capability to produce chain responses within the

cells having capability of natural activity or efficacy.

Antagonists: Almost of the drugs fastening receptors take after the agonists, but they cannot enact the receptors, addionally avoid agonist binding. Hence, inverse effect happens within the case of agonists and antagonists. They have two properties, i.e. fondness for receptors, but they do not have adequancy or natural activity.

Partial Antagonists: Partial antagonists have mid-levels of viability. They bind with the receptors but have exceptionally little inherent activity and efficacy. It can be seen that even on 100 percent occupation of the receptors, the response is below capacity (Baños & March, 2002).

1.5 Classes of Bioactive Constituents in Plants

While there are millions of different compounds that can exist within the thousands of plants species worldwide, they can usually be arranged into distinct classes of compounds based on similar characteristics. The main classes of bioactive constituents from plants include alkaloids, saponins, tannins, flavonoids, terpenes, steroids, glycosides, phenolics and carbohydrates (Satyajit & Lutfun, 2007).

1.5.1 Alkaloids

These are the most investigated group of plant compounds because they are highly active and are easily extracted from the plant material. Generally, alkaloids are those compounds that consist of heterocyclic ring with a nitrogen atom. They include caffeine, morphine and nicotine (Aniszewski, 2007). Many alkaloids are however toxic while some alkaloids obtained from marine algae are basically contaminants, such as the indole derivative, communesin, isolated

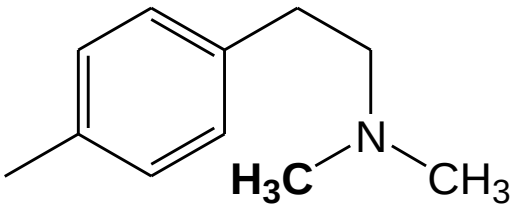
from *Penicillium* sp. found on the green alga *Enteromorpha intestinalis* and leptosins from *Leptosphaeria* polygalifolium (Guven *et al*., 2010).

1.5.1.1 Morphine:

Morphine **2** was the first alkaloid isolated from a plant (Courtwright, 2009). This is firmly believed to be the first isolation of an active ingredient from a plant (Luch, 2009). Morphine can be utilized for both acute pain and chronic pain (Stefano *et al*., 2012).

1.5.1.2 Hordenine:

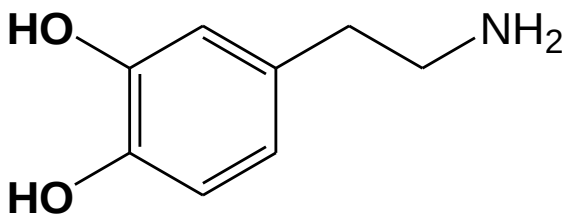
Hordenine **6** was the first alkaloid isolated from a marine alga (Sommer *et al*., 2017). Hordenine (*Anhaline)* (HORD, *4-(2-Dimethylaminoethyl*) phenol) was first isolated from terrestrial plant *Anhanolium fissuratus* (Guven *et al*., 2010) and its structure was later elucidated (Berkov *et al*., 2007).



6 Hordenine

1.5.1.3 Dopamine:

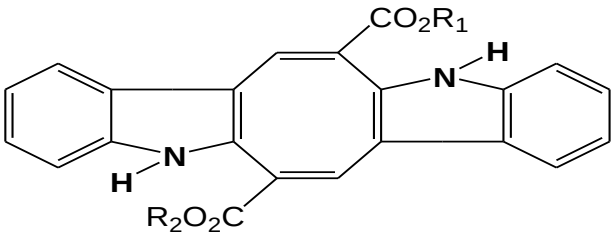
Dopamine **7** *(*DOP, 3,4-Dihydroxyphenethylamine) is an important alkaloid found in animals and several terrestrial plants (Drożak & Bryła, 2005) along with the green alga *Monostroma fuscum* (Van Alstyne *et al*., 2015). It is a hormone and a neurotransmitter. It is an adrenergic compound used for treating cardiovascular and kidney disorders (Sweetman, 2005).



7 Dopamine

1.5.1.4 Caulerpin:

Caulerpin **8** (CLP, dimethyl-6,13-dihydrodibenzo [b,i]phenazine-5,12-dicarboxylate methyl ester) have been isolated principally from green algae and partly from red algae. CLP (I) was isolated from various *Caulerpa* sp. as: *C. lamourouxii* (Guven *et al*., 2013). There are diverse opinions on the toxicity of CLP (I). Symptoms were noticed after the ingestion of *Caulerpa* genus (Guven *et al*., 2013). It exhibits low toxicity (Gorbi *et al*., 2014). *C. racemosa* extracts showed some level cytotoxicity, but CLP (I) obtained from these extracts showed no activity (Rocha *et al*., 2007). CLP (I) exhibited a moderate *in-vitro* antitumor activity on monoamine oxidase B (Scotti *et al*., 2015) and showed moderate level antibacterial activity against 8 species of bacteria isolated from algal surface (Vairappan, 2004). CLP (I) showed antifungal activity in *Laurencia majuscula* (Xu *et al*., 2006). CLP (I) has been observed to be a plant growth regulator (Huang *et al*., 2001).



8 Caulerpin

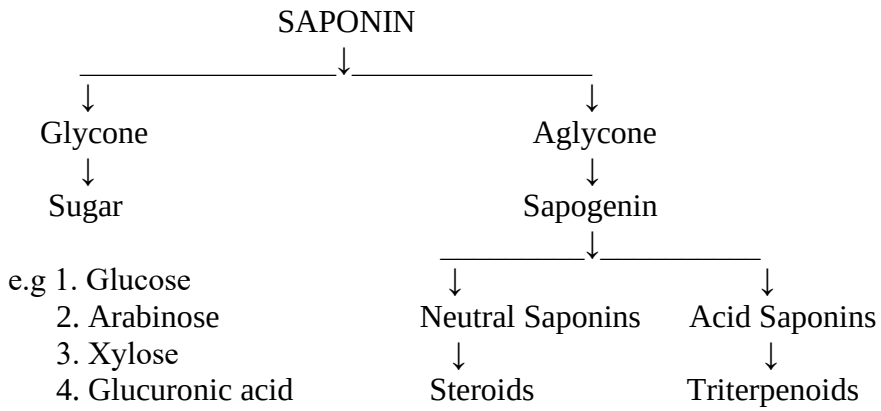
I. R_1: CH_3, R_2: CH_3 ; II. R_1: H, R_2: H ; III. R_1: CH_3, R_2: H

1.5.2 Saponins

Saponins are primarily produced by plants but also by lower marine animals and a few microscopic organisms (Francis *et al*., 2002). Saponins are secondary metabolites synthesized by numerous diverse plant species (Arif *et al*., 2009). Their name is derived from Latin word "sapo" meaning cleanser, due to their surfactant properties which permits steady stable soap-like foam upon shaking in aqueous solution (Faizal & Geelen, 2013; Hosamath, 2011). The combination of hydrophobic or fat-soluble sapogenin and hydrophilic or water-soluble sugar part facilitates the foaming ability of saponins (Sapna *et al*., 2009).

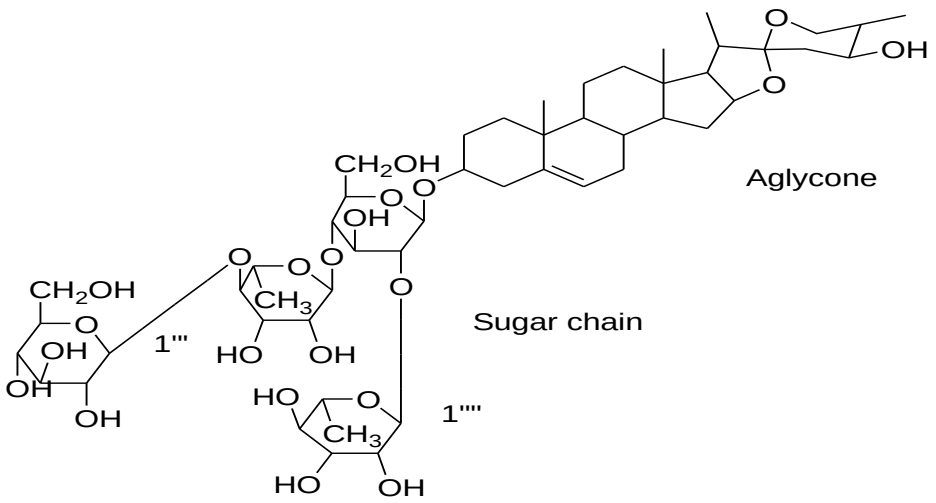
1.5.2.1 Classes and Chemical Structure of Saponins

Saponins on hydrolysis yield glycone (sugar) and aglycone (sapogenin) (Sapna *et al*., 2009). Agreeing to the structure of the aglycone or sapogenin, saponins are classified as neutral and acid type, the neutral types are the derivatives of steroids (27 carbon atoms) with spiroketal side chains which are almost exclusively present in the monocotyledonous angiosperms and the acid saponins that possess triterpenoid (30 carbon atoms) structure type, which is foremost common and occur mainly in the dicotyledonous angiosperms (Mert-Turk, 2006; Stanimirova *et al*., 2011; Faizal & Geelen, 2013).



Scheme 1: Classification of Saponins

Saponins are glycosylated compounds composed of two main parts: a water soluble glucidic chain and a generally triterpenic or steroidic liposoluble structure (aglycone) (Mert-Turk, 2006; Chaieb, 2010). The structure of saponin is shown in **9** (Chaieb, 2010).

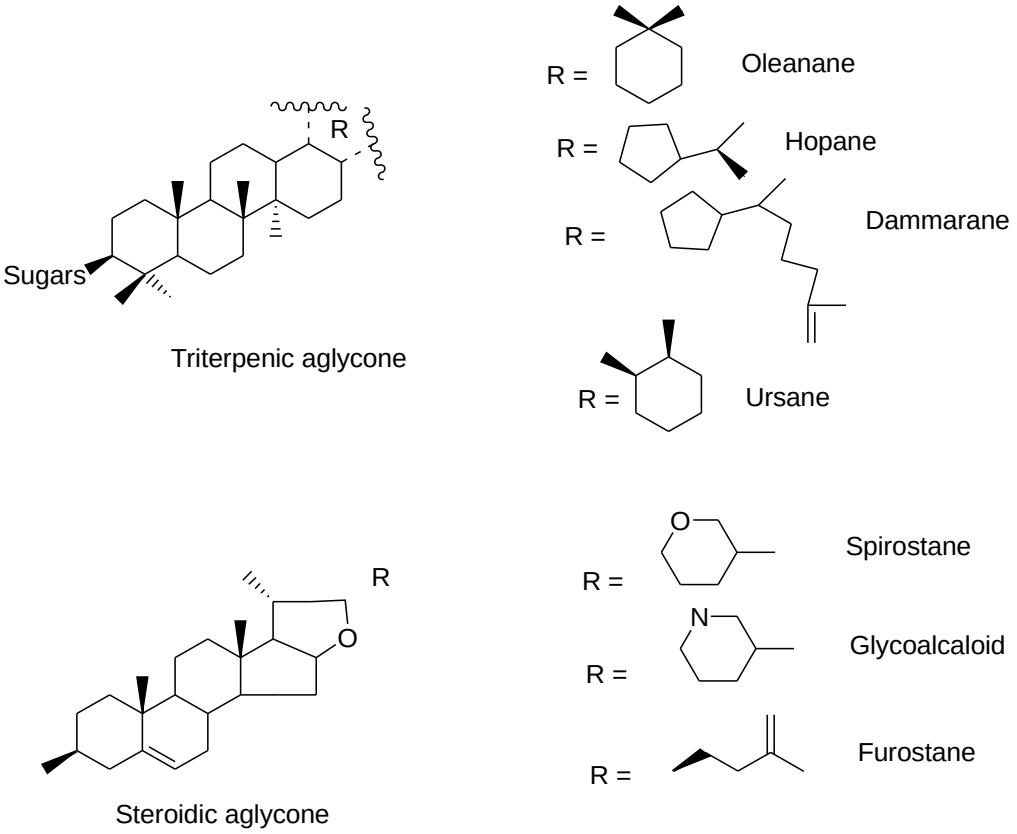


9 Saponin

The non-sugar and sugar components are called aglycone and glycone, respectively. Aglycone portion is composed of a triterpenoid or steroid backbone (Abid *et al*., 2012). L-arabinose, D-xylose, Dglucose, D-glucuronic acid, D-galactose, Lrhamnose and D-fructose are among the sugar constituents of saponins (Chaieb, 2010). The sugar moiety is connected to the aglycone through an ester or other glycosidic linkage at one or two glycosylation sites (Yücekutlu & Bildacı, 2008).

When the oligosaccharide chain is joined to C_3 position the molecule is called monodesmosidic saponin, whereas saponins which have an additional sugar moiety and joined to C_{26} or C_{28} position, are called bidesmosidic (Francis *et al*., 2002). It was observed that younger plants have higher saponin contents than matured or older plants, although some factors such as physiological state and environmental factors affect the saponin contents (Abid *et al*., 2012). Saponins are classified in two principal groups, that is, saponosides with steroidic aglycone and saponosides with triterpenic aglycone. These molecules come from an intramolecular cetalisation that intervenes after oxidation in C_{16}, C_{22} and C_{26} of a cholestanic

precursor taking into account spironature of C_{22}; this hexacyclic skeleton is usually indicated by the spirostane term. The triterpenic aglycones, move closer from the cyclization of the (3S)-2,3-epoxy-2,3-dihydrosqualene. Different possible structures of saponins are shown in **10** (Chaieb, 2010).



10 Structures of some saponins

1.5.2.2 Sources of Saponins

The steroidal saponins are principally found in monocotyledons whereas triterpene saponins are mostly present in dicotyledons (Sparg *et al*., 2004). The principal dietary sources of saponins are legumes (e.g. soybeans, chickpeas, mungbeans, peanuts, broad beans, kidney beans, lentils), but are also present in oats, allium species, asparagus, tea e.t.c (Shi *et al*., 2004).

Soap bark tree and ginseng are the principal non-food sources of saponins utilized in health and industrial applications (Ustundag & Mazza, 2007).

1.5.2.3 Biological Roles of Saponins

Saponins have been differently ascribed with a assorted extend of properties, a few of which incorporate both advantageous and hindering impacts on human wellbeing, pesticidal, insecticidal and molluscicidal action, allelopathic activity, antinutritional impacts, sweetness and bitterness, and as phyto-protectants that protect plants against attack by organisms and herbivores (Haralampidis *et al*., 2002).

1.5.2.3.1 Antimicrobial Activity

Soetan *et al.* (2006) examined the antimicrobial activity of saponins extricate of *Sorghum bicolor* against three pathogens, *Escherichia coli*, *Staphylococcus aureus* and *C. albicans*. The saponins hindered the growth of the S. aureus, but had no inhibitory impact on *E. coli* and *C. albicans*. They illustrated that incapability of the saponins from *S. bicolor* on gram-negative microscopic organisms and fungus may be as a result of the defensive impact of the microbial membranes. The saponins may not be able to enter the cell membranes of the microorganisms. Khanna and Kannabiran (2008) explored the antimicrobial movement of saponin extricated from the leaves of *Gymnema sylvestre* and *Eclipta prostrate*. According to their results, saponins showed the best antimicrobial action against *P. vesicularis* and least against *E. coli* at 50 mg/mL.

1.5.2.3.2 Antifungal Activity

Hazem *et al*. (2012) reported that saponin extracted from flowering aerial part of *Achillea fragrantissima* revealed antifungal activity against *Aspergillus, Fusarium* and *Rhizopus*. The saponin isolated from *Capsicum frutescens*, exhibited antifungal action against *Candida spp* and *Aspergillus fumigatus*, with minimum inhibitory concentrations ranging from 4.0 to 16 mg mL^{-1} (Saleem *et al*., 2010). These saponins showed

significant activity against *C. neoformans* and *A. fumigates* that was comparable to the standard control, Amphotericin B. Tsuzuki *et al*. (2007) evaluated the antifungal activity of saponin extracted from *Sapindus saponaria.* The isolated saponins revealed strong activity against *C. parapsilosis.* It was earlier reported that *C. albicans,* and *C. tropicalic* were quickly responsive to the saponins of *G. glabra* and *Q. saponaria*.

1.5.2.3.3 Anti-inflammatory Activity

Patel & Patil (2012) studied anti-inflammatory activity of saponin isolated from the *Thespesia populnea* (L.) leaves.

They demonstrated that mechanisms for anti-inflammatory activity might be associated with the inhibition of prostaglandin and histamine. Yassin *et al*. (2013) investigated the anti-inflammatory activity of a saponin-containing fraction obtained from methanolic extract from *Gleditsia caspica* fruits. They observed that the saponin could significantly inhibit the progression of the inflammation in the treated animals. They illustrated that the inhibitory effect of saponin may be due to inhibition of the enzyme cyclo-oxygenase and subsequent inhibition of prostaglandin synthesis. Saikosaponins induces anti-inflammatory impact by stifling both the DNA binding action and the nuclear translocation of atomic factor of enacted T cells (NF-AT) (Yadav *et al*., 2010).

1.5.2.3.4 Anticancer Activity

Yan et al. (2009) assessed the anticancer action of steroid saponins separated from the rhizome of *Paris polyphylla var. yunnanensis*. They illustrated that the saponins may well be respected as promising drugs for cancer treatment. Their results indicated that the *in vitro* anti-tumor impact of saponins is more potent than polysaccharides. Several reports have been demonstrated that plants saponins can lessen the risk of colorectal cancer. Their results showed saponins could inhibit HT-29 cell proliferation and induce polymerase (PARP) cleavage.

Rejinolda *et al*. (2011) prepared and evaluated saponin filled chitosan nanoparticles as a cancer therapeutic agent for an enhanced and sustained release. Many studies illustrated that the induction of cell death in cancer cells by anticancer saponins present in a dose and time-dependent manner. The result of Rejinolda *et al*. (2011) study correlates with the results obtained by Kim et al. (2008). According to their results, nanosaponin with higher concentrations of saponin may cause cancer cell death within 24 hrs. They concluded that the nanosaponin could be an efficient therapeutic agent for treatment of cancer.

Saponins of *Acacia victoriae* advanced apoptosis by invigoration of caspases and cytochrome C release. These impacts are the result of impedances with degradation of the inhibitor of NFkB, IkB (Bachran *et al.*, 2008).

1.5.2.3.5 Adjuvants activity

The saponins are used as adjuvants in vaccines (Stanimirova *et al*., 2011). *Quillaja saponaria* saponins can stimulate both the humoral and the cellular immune response against the pathogens. So, they can be utilized as adjuvants in vaccine formulations (Silva *et al*., 2005). The mechanism of immune stimulatory action of saponins have not been clear, but it is believed that production of cytokines may be induced by these compounds (Abid et al., 2012; Francis et al., 2002). Kukhetpitakwong *et al.* (2006) examined immunological adjuvant actions of extracted saponin (methanolic fraction) from the pods of *Acacia concinna* on the cellular and humoral immune response of BALB/c mice against ovalbumin. Their results showed that saponin at concentrations of 40μg may activate T and B cells.

1.5.2.3.6 Absorption enhancer

Sajadi *et al.* (2007) evaluated the enhancing impact of total saponin extracted from *Acanthophyllum squarrosum* on intranasal insulin immersion in rat. According to their results, the saponin was able to improve insulin absorption through the nose and reduce blood glucose in rat.

1.5.2.3.7 Anticardiovascular Activity

It has been reported that ingestion of saponin containing food decrease cholesterol levels in the bloodstream and as a result decrease the risk of cardiovascular diseases (Afrose *et al*., 2010; Stanimirova *et al*., 2011; Lee *et al*., 2013). It was also, reported that ginseng saponins decrease blood cholesterol levels in rabbits by increasing cholesterol excretion through bile acid formation (EL-Farok *et al*., 2013). Elekofehinti *et al*. (2012) showed that absorption of saponin from *Solanum anguivi* fruit lead to reduction in the risk of hyperlipidemic symptoms and heart diseases. It has been previously reported that the total saponins extracted from *G. glabra* and *Q. saponaria* were able to form complex with cholesterol.

1.5.2.3.8 Haemolytic Activity

The capacity of saponins to swell and break erythrocytes causing a discharge of hemoglobin (the in vitro haemolytic action) has been one of the foremost examined properties of saponins (Oda et al., 2000). However, even for this action, which has been related to the saponin structure (type of aglycone and the presence of sugar side chains), there's no clear consistency between individuals of this different group (Oda *et al*., 2000).

1.5.2.3.9 Cholesterol-lowering Activity

Cholesterol-lowering activity of saponins, which has been illustrated in animal (Matsuura, 2001) and human trials (Kim *et al*., 2003) has been ascribed to inhibition of the retention of cholesterol from the small intestine, or the reabsorption of bile acids (Vinarova et al., 2015). Feeding animals (poultry, rats, monkeys) diets containing decontaminated saponins or concentrated extracts containing saponins such as digitonin (a steroid saponin gotten from *Digitalis purpurea*), saikosaponin (triterpenoid saponins gotten from roots of *Bupleurum falcatum* L. and related plants), saponaria, soya, chick pea, yucca, alfalfa, fenugreek, quillaja, gypsohila, and garlic saponins resulted in diminishments within the plasma and in a few cases liver cholesterol concentrations (Matsuura, 2001). The cholesterol lowering impact of dietary

saponins in people is additionally upheld by ecological studies (Oakenfull, 2001). The low incidence of heart infection within the Batemi and Maasai populaces of East Africa in spite of a saturated fat/cholesterol diet, has in part been credited to the utilize of plant dietary added substances containing saponins in addition to polyphenols, phytosteroids and water-soluble dietary fiber (Oakenfull, 2001).

1.5.2.3.10 Virucidal activity

A few saponins and sapogenins are able of deactivating viruses, for example, purified saponin mixture from *Maesa lanceolata*. The triterpenoid sapogenin oleanolic acid inhibits HIV-1 virus replication probably by inhibiting HIV-1 protease activity (Mengoni *et al*., 2002).

1.5.2.3.11 Hypolipidaemic activity

Saponins have moreover been appeared to posses high degree of hypolipidaemic activity. The synergistic action of saponins and fiber content of the plant extract brings about the lessening in plasma concentration of cholesterol and the lipids, hence decreasing the conceivable event of coronary heart malady such as atherosclerosis (Raju *et al*., 2001).

1.5.3 Tannins

Tannins are one of the principal phytochemicals found in many higher plants (Pius *et al*., 2011). They are large polyphenolic compounds containing adequate hydroxyls and other suitable groups, such as, carboxyls (Praveen & Kumud, 2012). Tannins have a characteristic of unusual smell and astringent taste and may bind to proteins and subsequently precipitate the proteins (Pius *et al*., 2011).

High tannin concentrations are found in nearly every part of the plant, such as in the bark, wood, leaves, fruit, roots, and seed (Okonwu & Ugiomoh, 2015). In the bark of trees, tannins act as a barrier for micro-organisms and protect the tree (Furlan *et al*., 2011). The tannins appear as light yellow or white amorphous powders, flakes or sponge (Furlan *et al*., 2011).

Tannins are broadly conveyed in nearly all plant foods (Serrano et al., 2009; Manach et al., 2004), the essential source of tannins utilized as dynamic pharmaceutical ingredients are the medicinal plants (Okuda, 2005) suggesting that the pharmacologic impact of tannins is subordinate on plant type. Tannins may have a major impact on animal nutrition, including hindrance of growth rate digestive enzymes (Bennick, 2002).

Tannins have been involved with different pharmaco-therapeutic impacts (Ferreira et al., 2008). Tannins, within the frame of proanthocyanidins, seem to have an advantageous impact on vascular health (Pius et al., 2011). Topical applications of tannins offer assistance to deplete out all aggravations from the skin (Pius et al., 2011). They are valuable as an anti-inflammatory agent and within the treatment of burns and other wounds based on their antihemorrhagic and antiseptic potentials (Okonwu & Ugiomoh, 2015). In particular, tannins-rich cures are utilized as antihelmintics (Ketzis et al., 2006), antioxidants (Koleckar et al., 2008), antimicrobials and antivirals (Buzzini et al., 2008), in cancer chemotherapy (Tikoo et al., 2011) and to chelate dietary iron (Clauss et al., 2007).

1.5.3.1 Classes of Tannins

There are four classes of tannins (Karamali & Teunis, 2001), namely,

1.5.3.1.1 Gallotannins

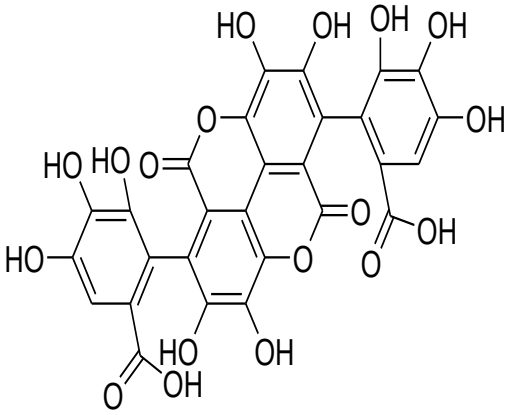
Gallotannins are the simplest hydrolysable tannins, containing a polyphenolic and a polyol residue (Sylla *et al*., 2015). Although a great variety of polyol residues are possible, most of the gallotannins separated from plants contain a polyol residue determined from D-glucose (Sylla et al., 2015).

The hydroxy capacities of the polyol residues may be mostly or completely substituted with galloyl units. In the case of fractional substitution with galloyl residues the remaining hydroxy groups may be either unsubstituted or substituted with different other residues. For example,

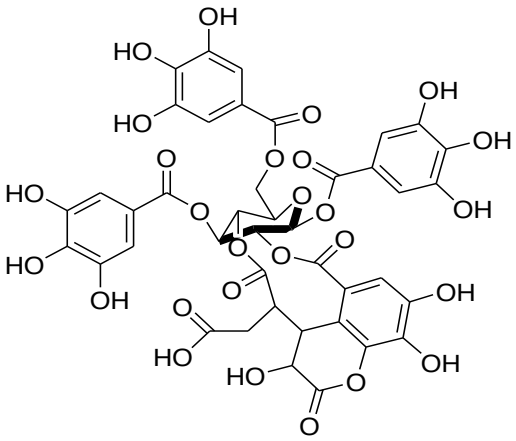
the anomeric centre of the glycosidic residues of the gallotannins may be unsubstituted (α, β mixture) or substituted, in α or β form, as ester or acetal.

The *meta*depsides (or '*syn*-gallotannins') belongs to the gallotannin group (Bee *et al*., 2017). Their galloyl residues are esterified with the polyol residue additionally with one or more linked galloyl units within the meta position relative to the galloyl units' carboxyl groups. The gallotannins 2,3,4,6-*t*etra-*O*-*g*alloyl-D-*g*lucopyranose (TGG) and 1,2,3,4,6-*penta*-*O*-*g*alloyl-β-D-*g*lucopyranose (β-PGG), found in many plant families, are the key intermediates in the biosynthesis of nearly all hydrolysable plant polyphenols (Hartzfeld *et al*., 2002). Gallotannins in which the polyol residues are coupled to cinnamoyl or coumaroyl groups are relatively scarce (Kim *et al*., 2009).

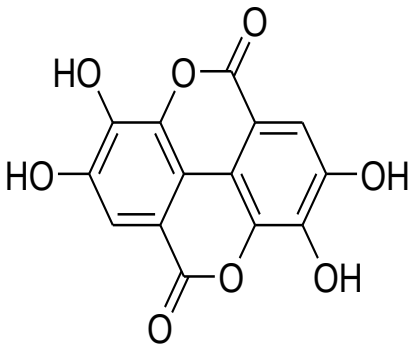
Examples of gallotannins are Gallagic acid **11** (Reddy *et al*., 2007), Chebulinic acid **12** (Quanbin *et al*., 2006), Ellagic acid **13** (Larrosa *et al.*, 2006), Corilagin **14** (Jia *et al*., 2013) and Chebulagic acid **15** (Lin *et al*., 2011).



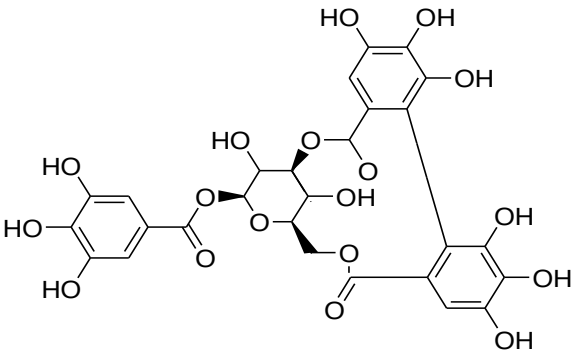
11 Gallagic acid



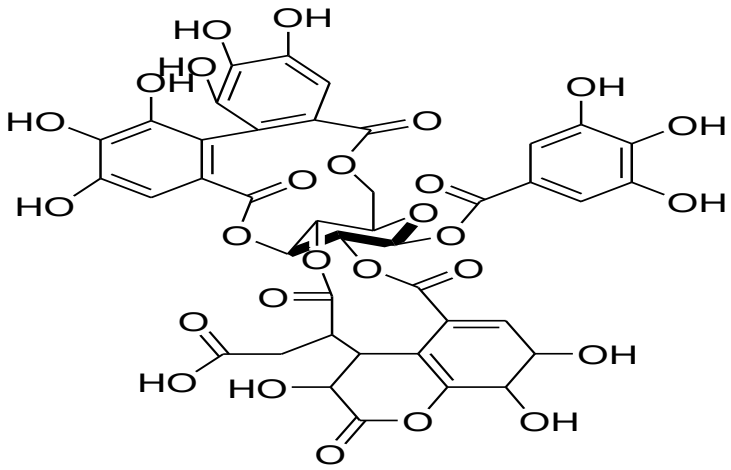
12 Chebulinic acid



13 Ellagic acid



14 Corilagin



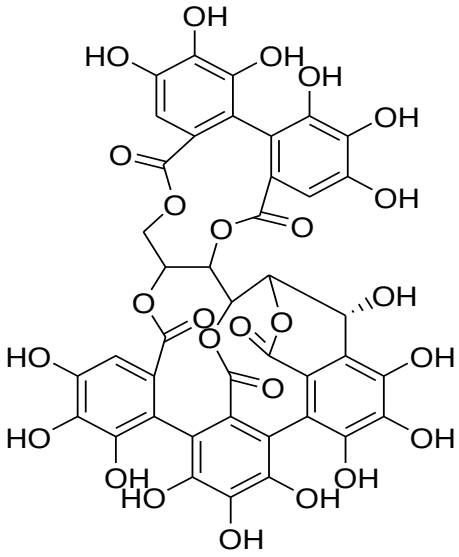
15 Chebulagic acid

1.5.3.1.2 Ellagitannins

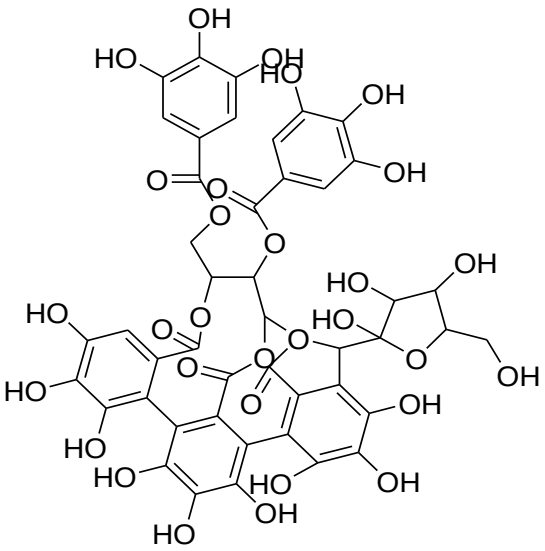
In order to decide the amount of each individual unit, the hydrolysis of the extracts with trifluoroacetic acid in methanol/water framework is performed. Hexahydroxydiphenic acid, made after hydrolysis, suddenly lactonized to ellagic acid, and sanguisorbic acid to sanguisorbic acid dilactone, while gallic acid remains intact (Yoshida *et al*., 2009; Davis & Milner, 2009).

Ellagitannins generally form macrocycles, whereas gallotannins do not (Yoshida *et al*., 2009; Davis & Milner, 2009). Ellagitannins are reported in dicotyledoneous angiospermes, and notably in species in the order Myrtales, such as the pomegranate (Yoshida *et al*., 2010; Gómez-Caravaca *et al.*, 2013). Examples of ellagitannins

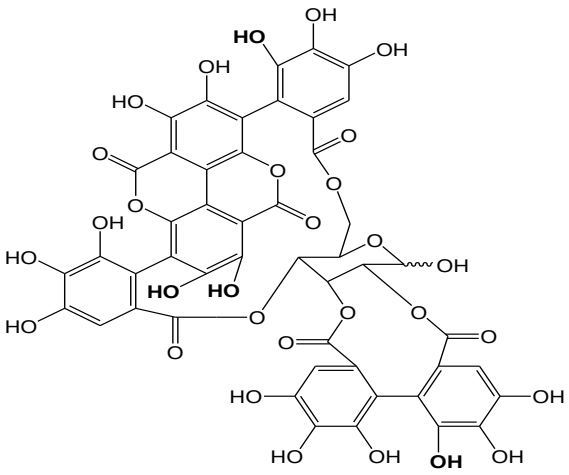
are Castalagin **16** (Shuaibu *et al*., 2008; Vivas *et al*., 2004), Gradinin **17** (Moharram *et al*., 2003; Mämmelä *et al*., 2000; Hofmann *et al*., 2006), Punicalagin **18** (Marzouk *et al*., 2002; Asres *et al*., 2001; Cerdá *et al*., 2003) and Punicalin **19** (Zhang *et al*., 2009).



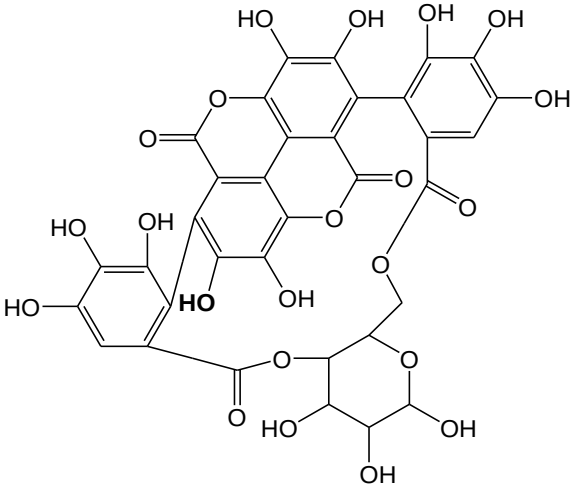
16 Castalagin



17 Gradinin



18 Punicalagin

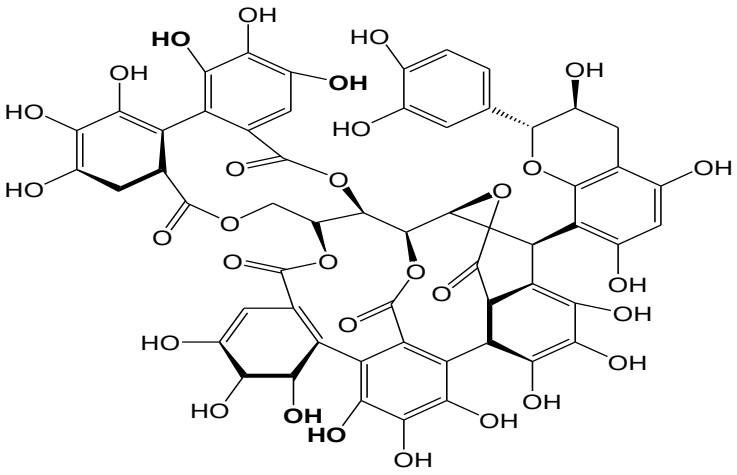


19 Punicalin

1.5.3.1.3 Complex tannins

The structures of the complex tannins are developed from a gallotannin unit or an ellagitannin unit, and a catechin unit (Yoshida et al., 2010). One illustration from this substance

class is acutissimin A **20**, having a flavogallonyl unit (nonahydroxytriphenoyl unit) bound glucosidically to C-1, and connected by means of three further hydrolysable ester bridges to the D-glucose determined polyol (Quideau *et al*., 2003).



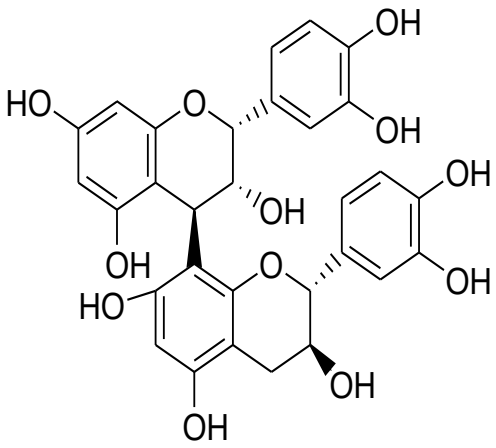
20 Acutissimin A

1.5.3.1.4 Condensed tannins

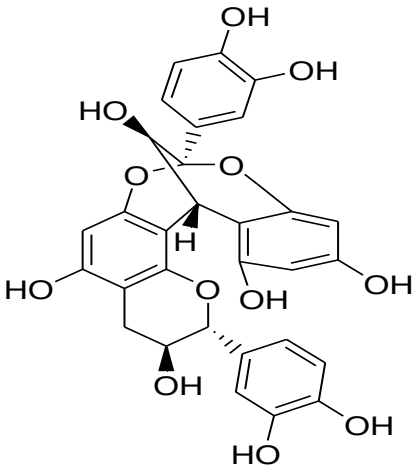
One of the striking properties of the monomeric catechins and leukoanthocyanidins, that have no tanning properties, is their capacity to be changed over into oligomers and polymers that do have tanning properties, by the activity of acids or enzymes (Okonwu & Ugiomoh, 2015; Yoshida et al., 2010). Condensed tannins are oligomeric and polymeric proanthocyanidins comprising of flavan-3-ol (catechin) units. For example, numerous condensed tannins are known where the coupling of the single units is by way of position C-4 of the primary unit connected with C-8 (or C-6) (Yoshida et al., 2010).

The tannins found in red wine (and to a lesser degree in white wine) are this sort of condensed tannins. The properties of these tannins, and particularly their significance to wine making depend on their particular response with proteins. Some typical condensed tannins with unsubstituted catechin units are procyanidin B2 **21** [epicatechin-(4β 8)-epicatechin] (Xiao *et al*., 2017), proanthocyanidin A**1** **22** [epicatechin- (4β 8,2β *O* 7)-catechin] (Sharma *et*

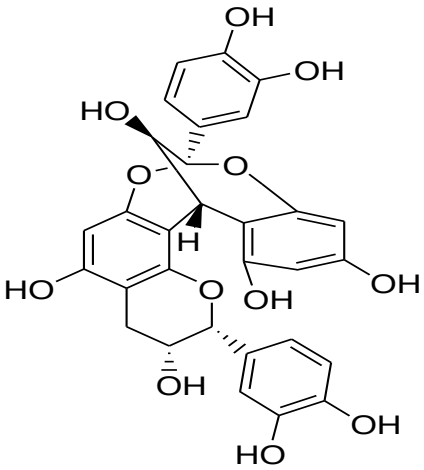
al., 2015), proanthocyanidin A**2** **23** [epicatechin-(4β 8,2β *O* 7)-epicatechin] (Sharma *et al*., 2015), and proanthocyanidin C**1** **24** [epicatechin-(4β 8)-epicatechin-(4β 8)-epicatechin] (Nakamura *et al*., 2003). Their structures are also presented below



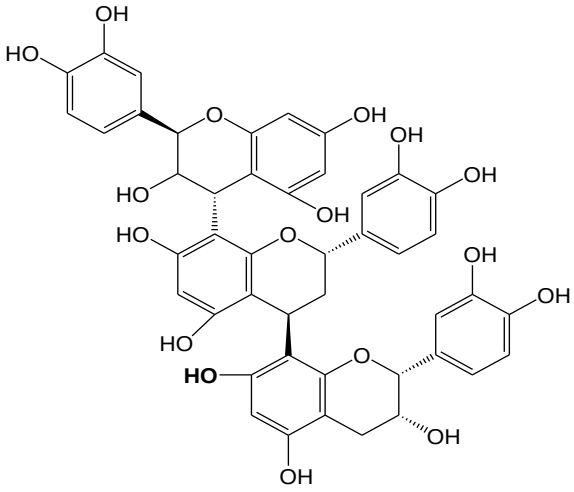
21 Procyanidin B2



22 Proanthocyanidin A**1**



23 Proanthocyanidin A**2**



24 Proanthocyanidin C**1**

1.5.4 Terpenes

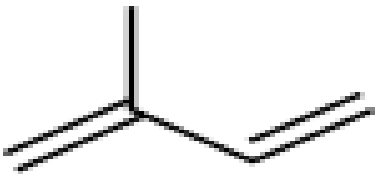
Terpenes are a large and diverse class of organic compounds, manufactured by a assortment of plants, particularly conifers and by some insects such as termites or swallowtail butterflies, which emit terpenes (Martin *et al.,* 2003). They are mostly hydrocarbons and when contained additional functional groups, they are called terpenoids.

The easier mono and sesquiterpene is chief constituent of the fundamental oils gotten from sap and tissues of certain plant and trees. The di and tri terpenoids are steam stable (i.e. not steam volatile). They are gotten from plant and tree gums and resins. Tertraterpenoids frame an isolated bunch of compounds called ‘Carotenoids’ (Yadav et al., 2014).

The term ‘terpene’ was initially utilized to portray a blend of isomeric hydrocarbons of the molecular formula $C_{10}H_{16}$ occurring in the basic oils gotten from sap and tissue of plants and trees (Fraga, 2008). But there is a tendency to use more common term ‘terpenoids’ which incorporate hydrocarbons and their oxygenated subsidiaries. Be that as it may, the term “terpene” is being utilized these days by a few authors to represent terpenoids (Connolly & Slope, 2010; Grayson, 2000).

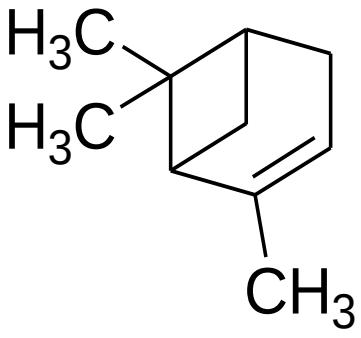
The clouds reflect sunlight, allowing the forest temperature to regulate (Steenackers *et al*., 2015).The aroma and flavor of hops comes, in part, from terpenes (mainly alpha-humulene and beta-caryophyllene), which affect beer quality (Steenackers *et al*., 2015).Terpenes are also major constituents of *Cannabis sativa* plants, which contain at least 120 identified compounds (Andre *et al*., 2016).

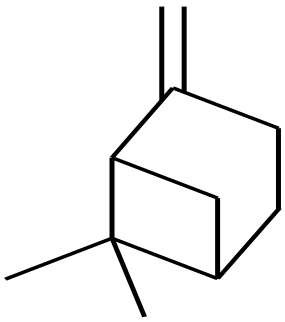
Terpenes are derived biosynthetically from units of isoprene **25**, which has the molecular formula C_5H_8 (Singh & Sharma, 2015). One can regard the isoprene unit as one of nature's regular building blocks. As chains of isoprene units gather, the deriving terpenes are classified successively by estimate as hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, and tetraterpenes (Kumari *et al*., 2017; Pazouki & Niinemets, 2016).



25 Isoprene

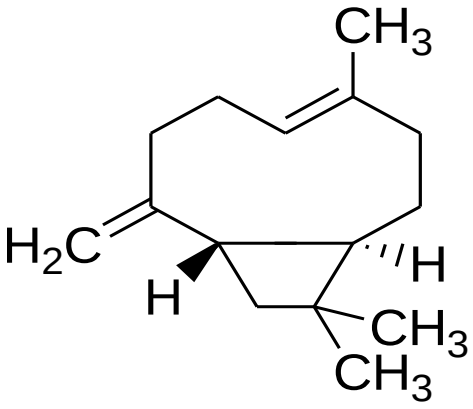
Terpenoids are the chief compounds of essential oils, which are widely used directly as flavouring agents (Reiss *et al*., 2009; Closson & Monteleone, 2009; Dewis *et al*., 2009; Franklin *et al*., 2010) or for more isolation of flavouring substances (Stroganov, 2001; Dewis *et al*., 2009; Bowen, 2006). Terpenes are also responsible for the fragrance of essential oils (Brahmkshatriya & Brahmkshatriya, 2013). They are moreover known to guarantee plant survival within the biological system since they support plant guard against biotic (herbivores, pathogens) and abiotic stretch components (Gouinguene & Turlings, 2002). They may balance interactions between competing plants (Gniazdowska & Bogatek, 2005). Blends of cotton terpenes containing constituents such as α-pinene **26**, β-pinene **27** and caryophyllene **28** have been appeared to be appealing to pests such as boll weevils (*Anthonomus grandis grandis* Boheman) (Guntert *et al*., 2001).





26 α-pinene

27 β-pinene



28 Caryophyllene

Terpenoids are of awesome interest due to the wide range of biological activities reported such as cancer preventive impacts and pain relieving, anti-inflammatory, antimicrobial, antifungal, antiviral and antiparasitic activities (Singh & Sharma, 2015).

1.5.4.1 Classification of Terpenoids

Most normal terpenoids hydrocarbon have the general formula of $(C_5H_8)_n$. They can be classified on the premise of value of 'n' or number of carbon particles present within the structure (Table 1.2) (Paul, 2001; Sun et al., 2006). Each lesson can be partitioned into subclasses according to the number of rings present within the structure.

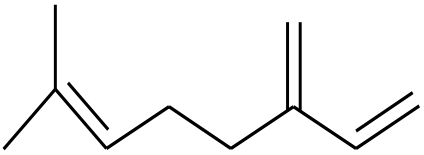
Table 1.2: Classification of Terpenoids

Number of carbon atoms	Value of n	Class
10	2	Monoterpenoids ($C_{10}H_{16}$)
15	3	Sesquiterpenoids ($C_{15}H_{24}$)
20	4	Diterpenoids ($C_{20}H_{32}$)
25	5	Sesterpenoids ($C_{25}H_{40}$)
30	6	Triterpenoids ($C_{30}H_{48}$)
40	8	Tetraterpenoids ($C_{40}H_{64}$)
>40	>8	Polyterpenoids $(C_5H_8)_n$

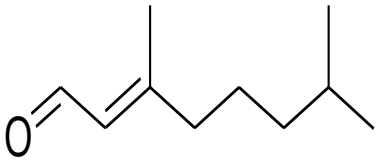
1.5.4.1.1 Monoterpenoids

Monoterpenoids consist of a 10 carbon backbone (2 isoprene units) structure and can be splitted into three subgroups based on different parameters: acyclic, monocyclic, and bicyclic (Yadav *et al*., 2014; Ludwiczuk *et al*., 2017). Common examples of acyclic monoterpenoids include myrcene **29**, citral **30**, geraniol **31**, lavandulol **32**, and linalool **33**. The important

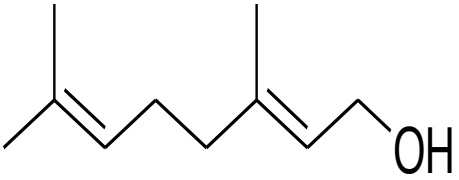
representatives of monocyclic monoterpenoids are α-terpineol **34**, limonene **35**, thymol **36**, menthol **37**, carvone **38**, eucalyptol **39**, and perillaldehyde **40**.



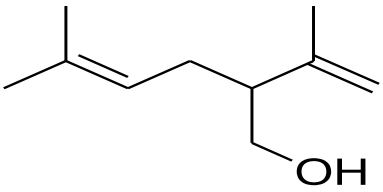
29 Myrcene



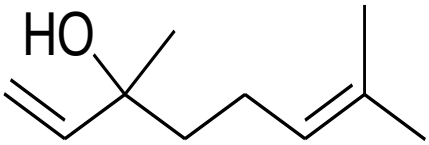
30 Citral



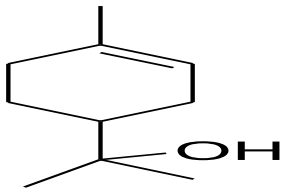
31 Geraniol



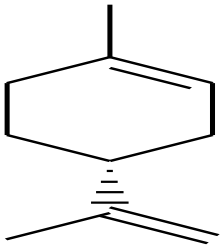
32 Lavandulol



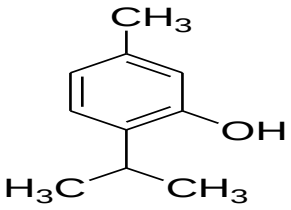
33 Linalool



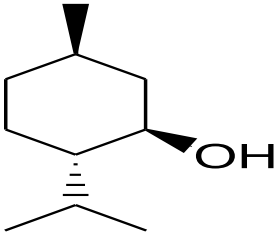
34 α-terpineol



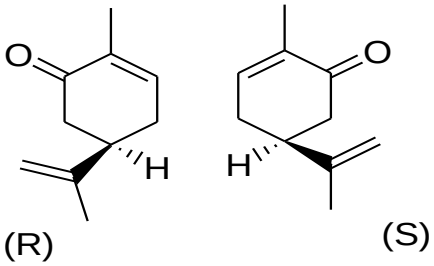
35 Limonene



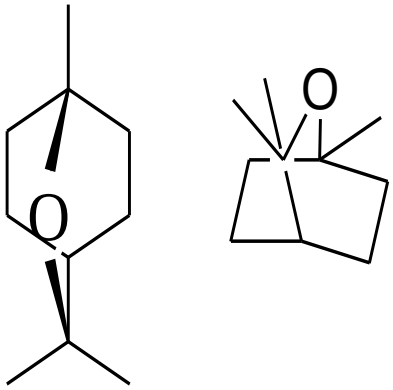
36 Thymol



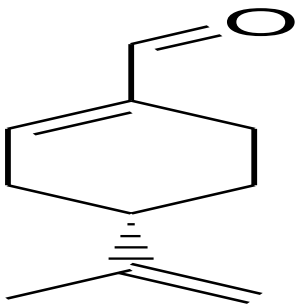
37 Menthol



38 Carvone



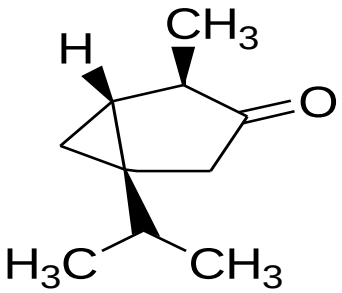
39 Eucalyptol



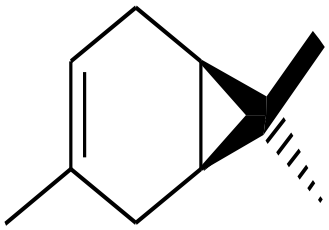
40 Perillaldehyde

The bicyclic monoterpenes may be splitted into three classes according to the size of the second ring. Thujone **41** and Carene **42** are the representatives of the group having 6+3-membered rings, α- **43** and β-pinene **27** (see pg 58) represent a 6+4 group, while borneol **44** and camphor **45**, a 6+5 group (Yadav *et al*., 2014; Ludwiczuk *et al*., 2017).

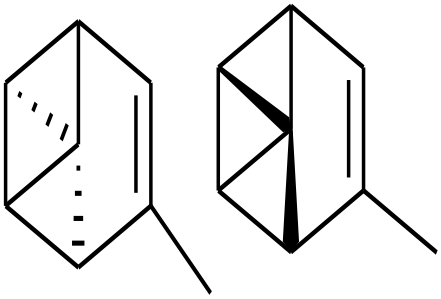
The monoterpenoids showed anti-inflammatory activity (Kotamballi *et al*., 2013), cancer chemopreventive and therapy (Itani *et al*., 2008) and insect repellent properties (April, 2016; Nesterkina *et al*., 2018).



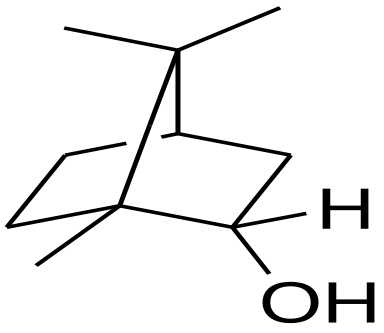
41 Thujone



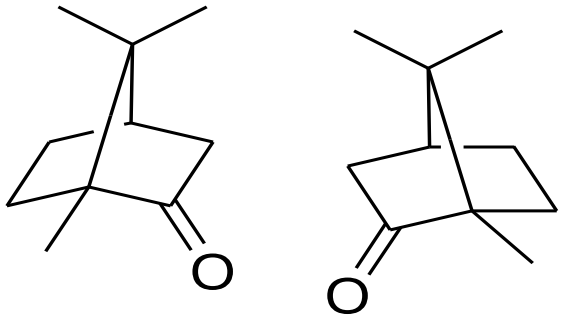
42 Carene



43 (+)-α-pinene & (-)-α-pinene



44 Borneol

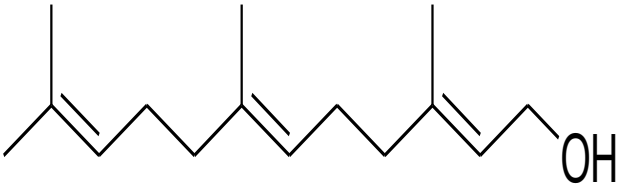


45 Camphor

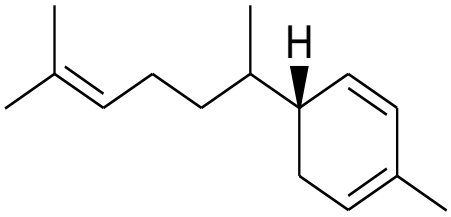
1.5.4.1.2 Sesquiterpenoids

Sesquiterpenoids are characterized as the group of 15 carbon compounds inferred by the gathering of 3 isoprenoid units and they are found primarily in higher plants and invertebrates. Sesquiterpenes, with monoterpenes, are critical constituents of essential oils in plants. They are the foremost different group of isoprenoids. In plants, they function as pheromones and juvenile hormones.

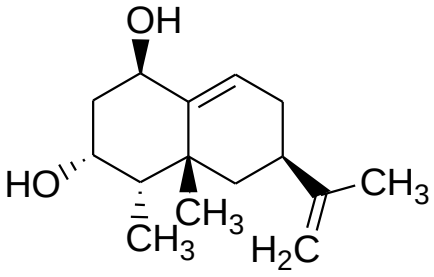
Sesquiterpenoids are further divided into several subgroups based on the kind of skeletons they possess (Angeh, 2006). Some examples of sesquiterpenoids are given below: farnesol **46**, zingiberene **47** and capsidiol **48** (Angeh, 2006).



46 Farnesol



47 Zingiberene

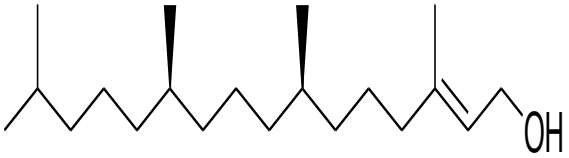


48 Capsidiol

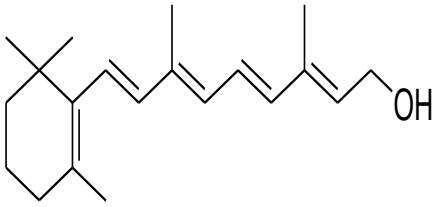
1.5.4.1.3 Diterpenoids

Diterpenoids comprise of chemically heterogeneous group of compounds, all with a carbon skeleton built on four isoprene units; most of them have a very limited distribution. Many classes of diterpenes are known, and among them are resin diterpenes, toxic diterpenes and gibberelins. The resin compounds are protective in nature and are exuded from wood trees or latex of plants. The toxic diterpenes occur in the greenery of the leaves of some plants and are credited for the poisonous nature of the foliage. The gibberellins are a group of hormones, which genrally stimulate growth and are known to be widespread in plants (Hanson, 2003; Xiao-Rong *et al*., 2011).

Diterpenoids can be splitted into acyclic diterpenoids e.g. phytol **49** and monocyclic diterpenoids e.g. vitamin A **50** (Xiao-Rong *et al*., 2011).



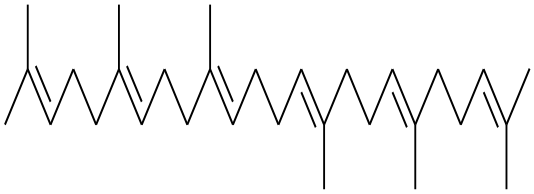
49 Phytol



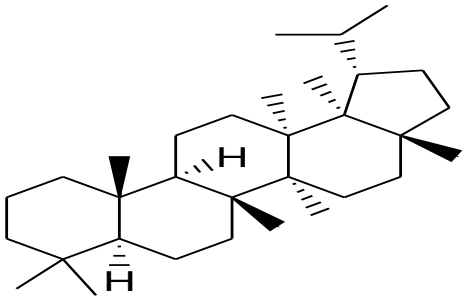
50 Vitamin A

1.5.4.1.4 Triterpenoids

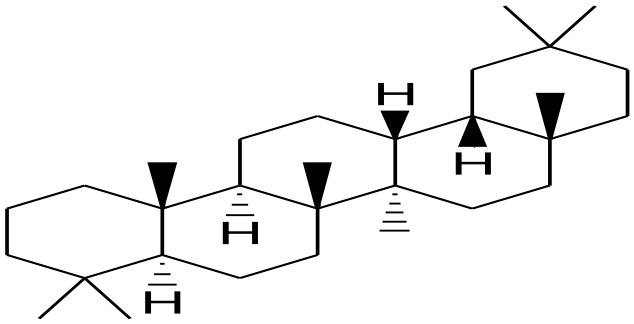
Triterpenes are hydrocarbons and possess no heteroatoms; functionalized triterpenes should alternatively be called triterpenoids (Xu & Matsuda, 2004).Triterpenes are a class of chemical compounds comprised of three terpene units with the molecular formula of $C_{30}H_{48}$; they could also be thought of as consisting of six isoprene units. Animals, plants and fungi all create triterpenes, with arguably the most important example being squalene **51** as it forms the basis of almost all steroids (Xu & Matsuda, 2004). Lupane **52**, oleanane **53** and ursanes **54** show specific promise as anti-cancer agents (Laszczyk, 2009).



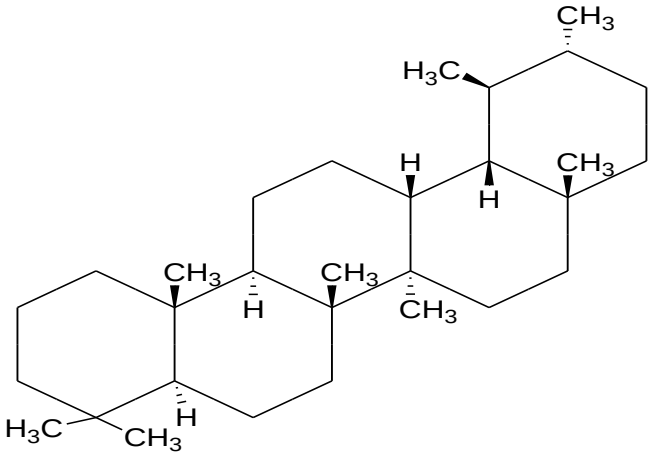
51 Squalene



52 Lupane



53 Oleanane

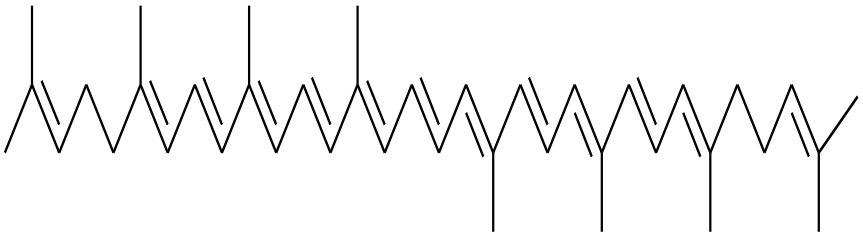


54 Ursanes

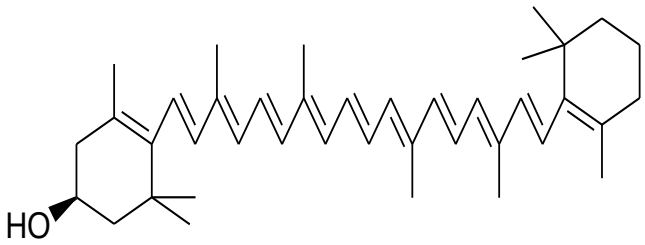
1.5.4.1.5 Tetraterpenoids (Carotenoids)

Carotenoids that are C-40 tetraterpenoids are extensive group of lipid-soluble pigments, find in all kind of plants. In plants, carotenoids posses two principal functions: as accessory pigments in photosynthesis and as colouring constituents in flowers and fruits. In flowers, their presence is indicated by yellow pigments, while in fruits, they may, in addition, give rise to orange or red colouration (Lavoie *et al*., 2012). Well known carotenoids are either simple

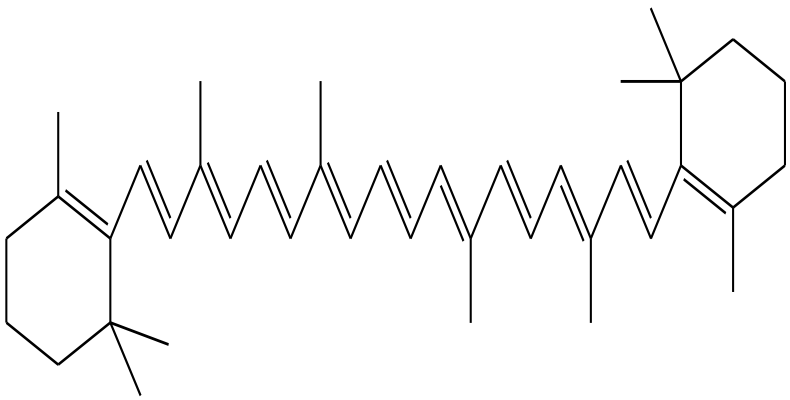
unsaturated hydrocarbon based on lycopene **55** or their oxygenated derivatives known as xanthophylls **56** (Lavoie *et al*., 2012). The chemical structure of lycopene comprises of a long chain of eight isoprene units joined head to tail to form a totally conjugated framework of substitute twofold bonds which is the chromophore responsible for its colour (Ono *et al*., 2015). Cyclization of lycopene at one end produces γ-carotene while cyclization at both ends gives the bicyclic hydrocarbon beta-carotene. β-carotene **57** is the most frequent of all these pigments. Triterpenoids are the most ubiquitous non-steroidal secondary metabolites in terrestrial and marine flora and fauna (Ono *et al*., 2015).



55 Lycopene

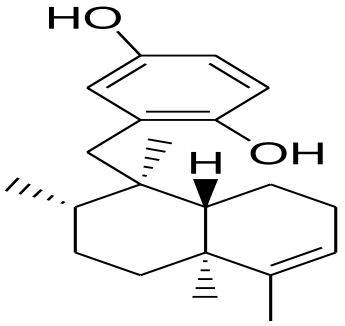


56 Xanthophylls

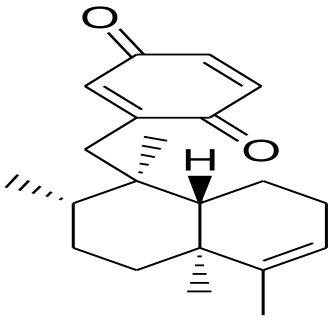


57 β-carotene

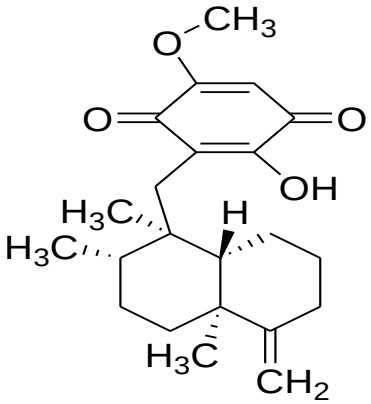
Terpenes, such as avarol **58**, avarone **59**, illimaquinone **60**, nakijiquinone **61** and bolinaquinone **62** (Motti *et al*., 2007; Cragg & Newman, 2002).



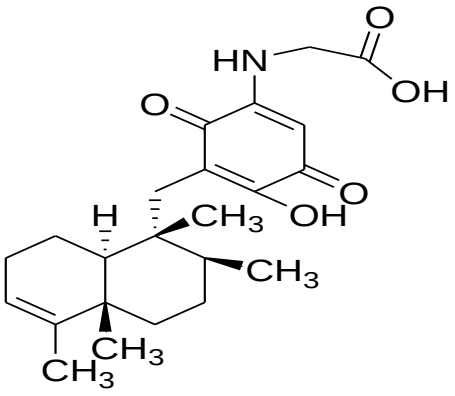
58 Avarol



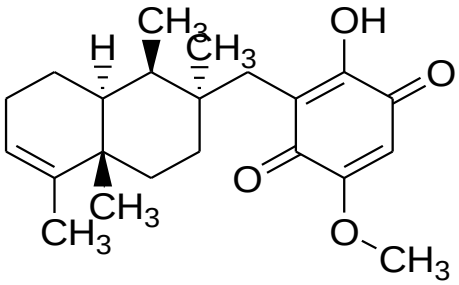
59 Avarone



60 Illimaquinone



61 Nakijiquinone



62 Bolinaquinone

1.5.5 Flavonoids and Isoflavonoids

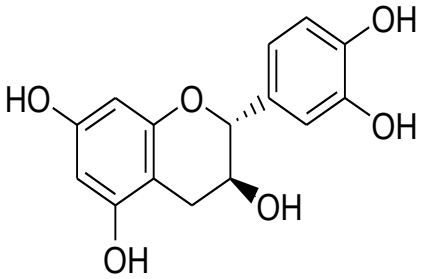
Flavonoids are polyphenolic compounds that produce the flavor of fruits and

vegetables in addition to the red and blue pigments of plants, and have been used in taxonomic studies of angiosperms (Panche *et al*., 2016). The core structure comprises of a C_{15} skeleton with two aromatic rings joined by a chromane ring (Aruoma, 2003).

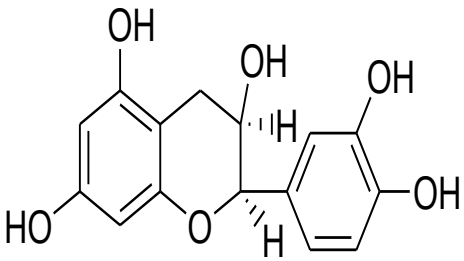
The flavan-3-ol [(+)-catechin **63**, (-)-epicatechin **64**, (-)-epigallocatechin **65**] and their gallate esters are extensively distributed in plants (Aron & Kennedy, 2008). Flavan oligomers (proanthocyanidins) are present in apples, grapes, berries, persimmon, blackcurrant, sorghum and barley grains (Vinha *et al*., 2015).

Flavonoids are divided into three main groups namely,

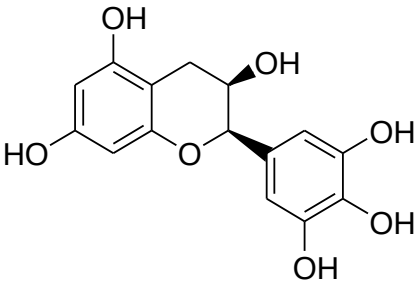
1. Flavonoids from 2-phenylchromen-4-one (2-phenyl-1,4-benzopyrone structure e.g. quercetin **66**, rutin **67**;
2. Isoflavonoids from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure e.g. Genistein **68** and
3. Neoflavonoids, derived from 4-phenylcoumarine (4-phenyl-1,4-benzopyrone) structure e.g. 4-arylcoumarins **69** (Brodowska, 2017).



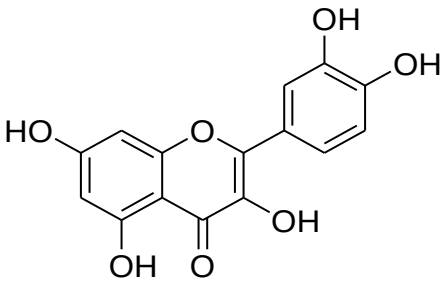
63 (+)-catechin



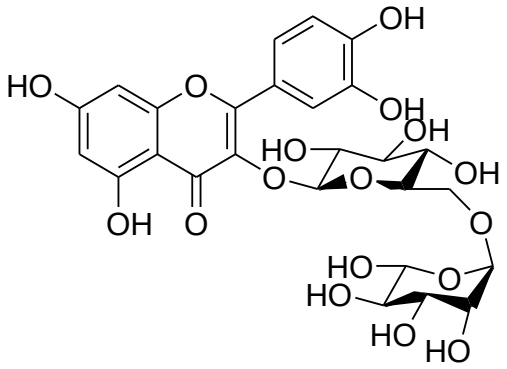
64 (-)-epicatechin



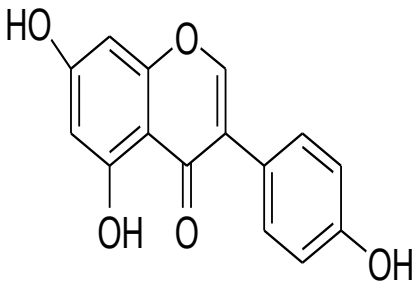
65 (-)-epigallocatechin



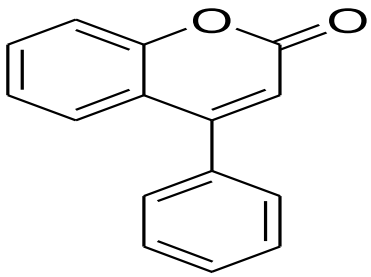
66 Quercetin



67 Rutin



68 Genistein



69 4-arylcoumarins

Flavonoids have been alluded to as natures 'biological response modifiers' because of strong experimental prove of their inherent capacity to modify the body's response to allergens, viruses and carcinogens (Yamamoto & Gaynor, 2001). They play different roles in the ecology of plants. Because of their astringency, cathechins and other flavonols can represent a defense system against insects harmful to the plant (Kumar & Pandey, 2013). Cyanidin-3-O-glucoside has been reported to hinder the growth of larvae of tobacco budworm *Heliothis virescen*, an important pest of cotton and other crops (Nix *et al*., 2017). Anthocyanins serve as antifungal agents in red coloured tropical young leaves as well as assist in heavy metal tolerance of plants (Wen *et al*., 2016).

Parasitic plants often use chemicals released by their host plant to stimulate seed germination, to locate the host, or for development. Many different compounds are involved,

including strigolactones, quinines, coumarins, flavonoids, and other phenolics. Flavonoids contribute to signalling in some species (Andersen & Markham, 2005).

Flavonoids give protection against UV radiation. UV light is a potential hazard to plants because it can damage DNA and impair various physiological processes. Plants often respond to UV light by the activity flavonoid biosynthetic genes with favorable UV absorbing properties. Flavonoids therefore protect plants from UV (especially UV-B) radiation of the sun and scavenge UV-generated reactive oxygen species (ROS) (Andersen & Markham, 2005).

Anthocyanins, flavonols, or chalcones which often have attractive colours accumulate in both male and female sex organs in a flower, including the pollen grains (Farrant 2000).

Flavonoids have been more often used than any other group of plant substances in chemotaxonomic studies (Mohy-Ud-Din *et al*., 2009). For example, flavonols, such as quercitin, with two hydroxyls in the ring (resulting into a catechol group) hinder IAA oxidase whereas flavonols with one hydroxyl (e.g. kaempferol) promote the enzyme's activity. Other flavonoids, especially certain of the isoflavonoids, serve as phytoalexins (Harbourne and Williams 2000).

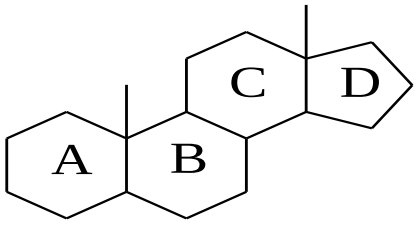
In vitro investigations have revealed that some flavonoids have anti-allergenic, anti-inflammatory (Yamamoto & Gaynor 2001), antimicrobial (Cushnie & Lamb 2005), anticancer (Chahar *et al*., 2011) as well as antidiarrheal (Yao *et al*., 2011) activities. Some flavonoids show antihistaminic (Park *et al*., 2008), antiviral (Seo *et al*., 2016), antioxidant (Procházková *et al*., 2011) with antimutagenic properties (Okuno *et al*., 2019).

1.5.6 Steroids

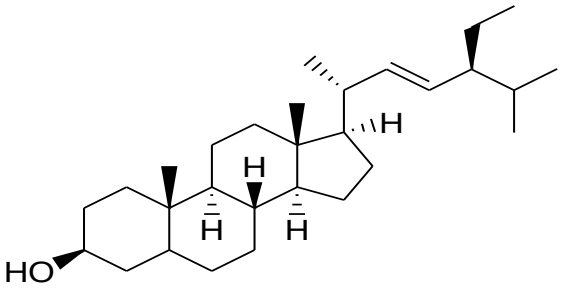
Steroids are lipids characterized by a prevalent basic ring structure: three fused cyclohexane rings (phenanthrene part) blended to a cyclopentane ring system. This is referred to as the cyclopentaphenanthrene ring **70** (Satyajit & Lutfun, 2007).

Previously, steroids were considered to be mainly animal substances (sex hormones) but in recent years such compounds have been detected in plant tissues e.g. stigmasterol **71** is a phytosterol where they act as chemical messengers, also known as hormones. They are synthesized in glands and delivered by the bloodstream or plant fluid to target tissues to stimulate or inhibit some process. Steroids as lipids are nonpolar. Their nonpolar character allows them to cross cell membranes, so they can leave the cells in which they are synthesized and enter their target cells (Satyajit & Lutfun, 2007).

Many steroids have methyl groups at C-10 and C-13 positions. These are called the angular methyl groups. Many steroids possesing an alcoholic hydroxyl attached to the ring system are known as sterols. The most frequent sterol is the cholesterol which occurs in most animal tissues. There are many different steroids hormones, in which cholesterol is the precursor for all of them. Cholesterol is also the precursor of vitamin D.



70 Cyclopentaphenanthrene ring



71 Stigmasterol

1.5.6.1 Types of steroids

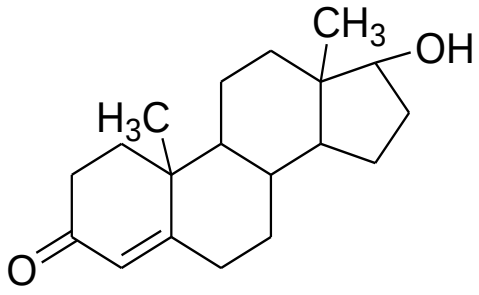
(**a**) Anabolic steroids or anabolic androgenic steroids: These are categories of natural and synthetic steroids that interact with androgen receptors to promote cell growth and division, resulting in growth of several types of tissues especially muscles and bones. There are natural and synthetic anabolic steroids e.g. testosterone **72**, nandrolone **73** and methandrotenolone **74** (Mhilaj *et al*., 2015).

(**b**) Corticosteroids (glucocorticoids and mineralocorticoids): Glucocorticoids are one of the categories of steroidal hormones characterized by the ability to bind with the cortisol receptor. They regulate many aspects of metabolism and immune functions, and are often prescribed as a remedy for inflammatory states such as asthma and arthritis e.g. Cortisol **75**. Mineral corticoids are corticosteroids that help maintain blood volume and manage renal excretion of electrolytes e.g. Aldosterone **76** (Brown & Chandler, 2001).

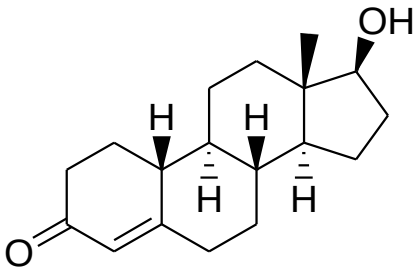
(**c**) Sex steroids or gonadal steroids: These are a subset of sex hormones that collaborate with vertebrate androgen or oestrogen receptors to produce sex differences (primary and secondary sex characters) and support reproduction. e.g. androgens **77**, oestrogens **78**, and progestagens **79** (Young & Becker, 2009).

(**d**) Phytosterols or plant sterols: They are steroid alcohols that occur naturally in plants. e.g. beta-sitosterol **80** (Cabral & Klein, 2017).

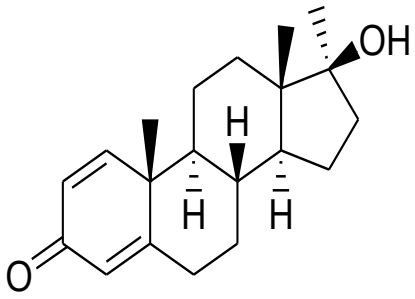
(**e**) Ergosterols: These are steroids that take place in fungi. They include a few vitamin D supplements (Zhang & Rao, 2010).



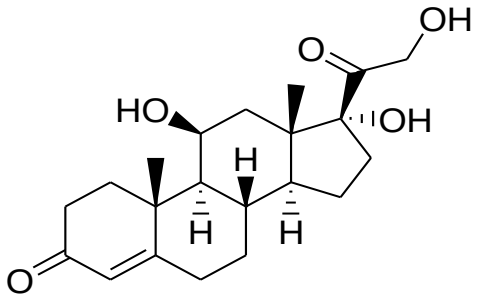
72 Testosterone



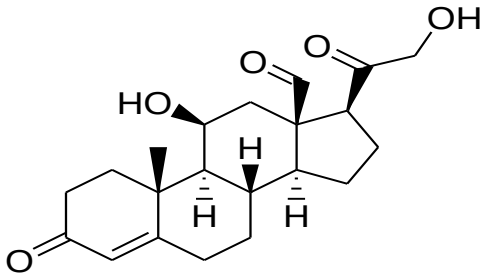
73 Nandrolone



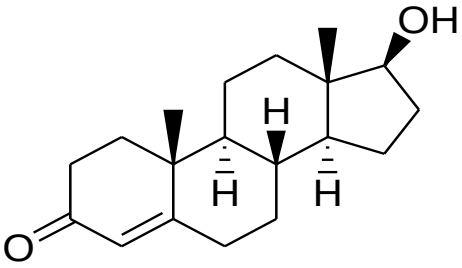
74 Methandrotenolone



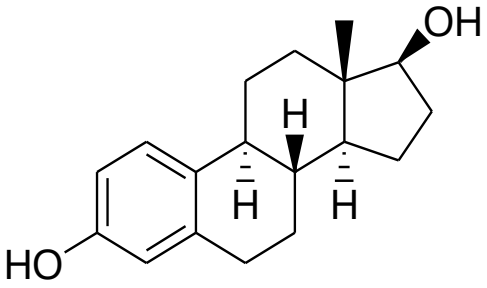
75 Cortisol



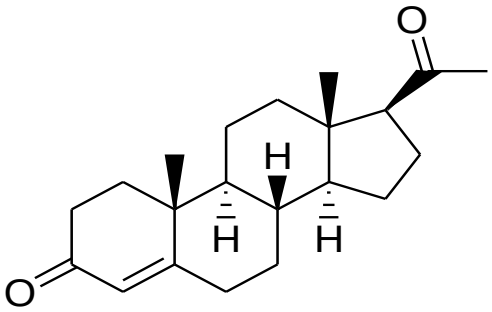
76 Aldosterone



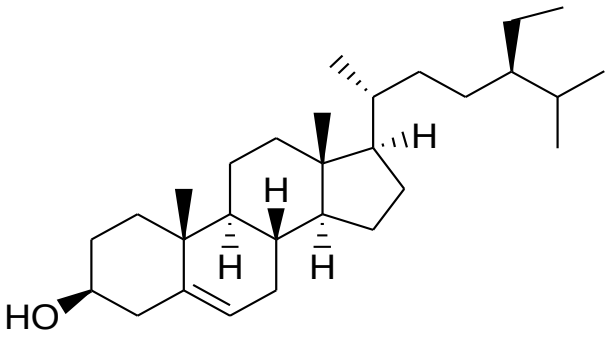
77 Androgens



78 Oestrogens



79 Progestagens



80 Beta-sitosterol

1.5.7 Glycosides

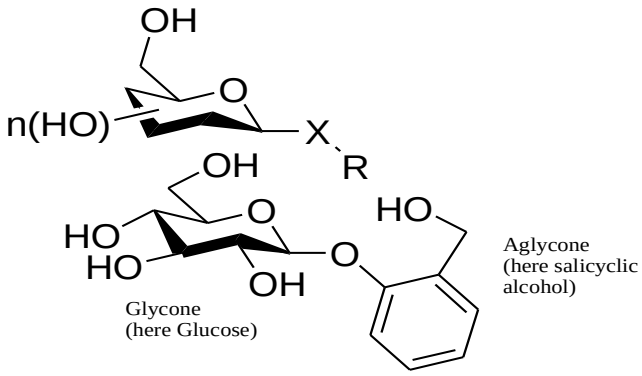
They produce one or more sugars upon hydrolysis (Kren & Martínková, 2001). A few of glycosides are formed from polyphenols, phenols, steroidal and terpenoidal alcohols through glycosidic linkages to sugars. Among the sugars appearing in normal glycoside, D-glucose is the foremost predominant one but L-rhamnose, D- and L- fructose and L- arabinose also occur very regularly. Of the pentoses, L-arabinose is more common than D-xylose (Sarker & Nahar, 2007).

Glycosides can also be alluded to as any molecule in which a sugar group is bonded through its anomeric carbon to another group by means of a glycosidic bond. Glycosides can be connected by an O- (an *O*-glycoside), N- (a glycosylamine), S-(a thioglycoside), or C- (a *C*-glycoside) glycosidic bond **81** (Sarker & Nahar, 2007).

The classification of glycosides depends on the properties or functions of their sugar or aglycone portion. If the classification depends on the sugar group, a number of rare sugars are involved e.g. rhamnosides (i.e. afzelin) **82**, if the aglycone group is utilized as a basis of classification, all class of natural product will be involved e.g. lignan glycosides (i.e. lyoniside) **83**, alkaloidal glycosides (i.e. α-solanine) **84** etc (Sarker & Nahar, 2007). Also, glycosides that have soap like properties are called saponin glycosides (i.e. digitonin) **85** (Podolak *et al*., 2010). Similarly, glycosides that liberate hydrocyanic acid (HCN) on hydrolysis are known as cyanogenic glycosides (i.e. amygdalin) **86** (Balcerek & Szopa, 2012). Other types of glycosides are cardiac glycosides (i.e. oleandrin) **87** (Prassas & Diamandis, 2008), phenolic glycosides (i.e. syringin) **88** (Chang & Case, 2005), aldehyde glycosides (i.e. glucovanillin) **89** (Sarker & Nahar, 2007) and anthraquinone glycosides (i.e. cascaroside) **90** (Wang *et al*., 2013).

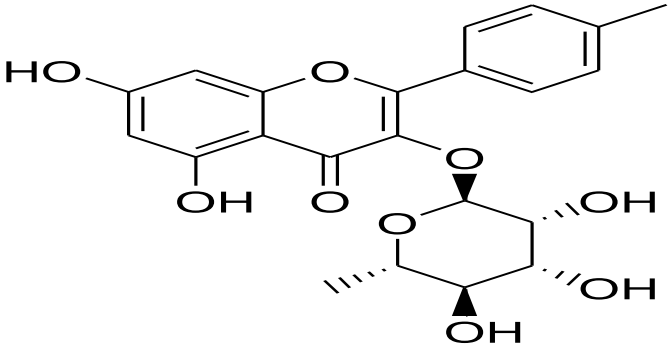
Glycosides play numerous important roles in living organisms. Most plants store chemicals in the form of inactive glycosides. These can be enacted by enzyme hydrolysis which causes the sugar portion to be broken off, making the chemical accessible for utilize. In animals and

humans, poisons are frequently bound to sugar molecules as part of their disposal from the body (Chang & Case, 2005).

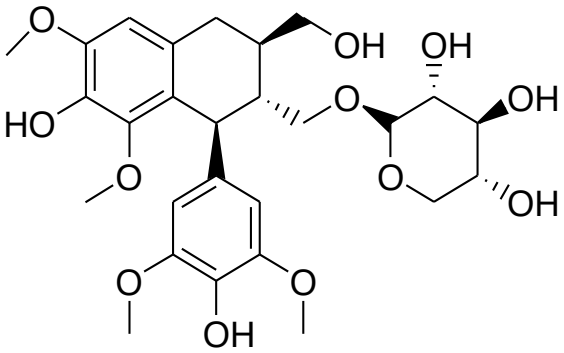


81 Glycosidic bond

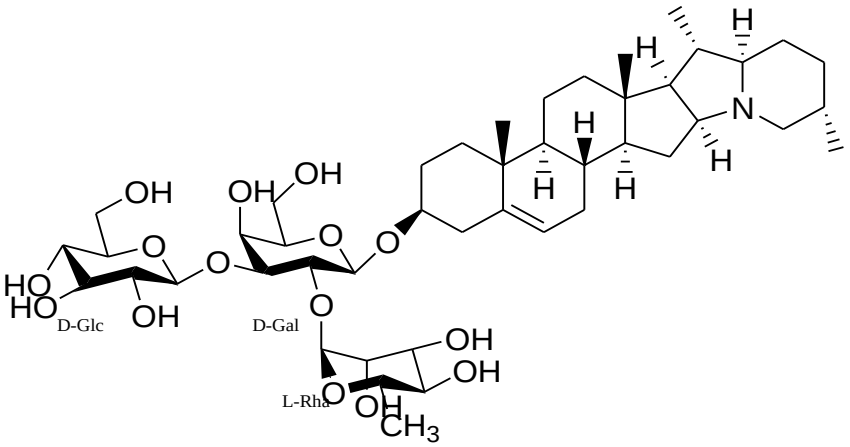
X = O (glycoside), S (thioglycoside), N (N-glycoside), CR'R" (C-glycoside)



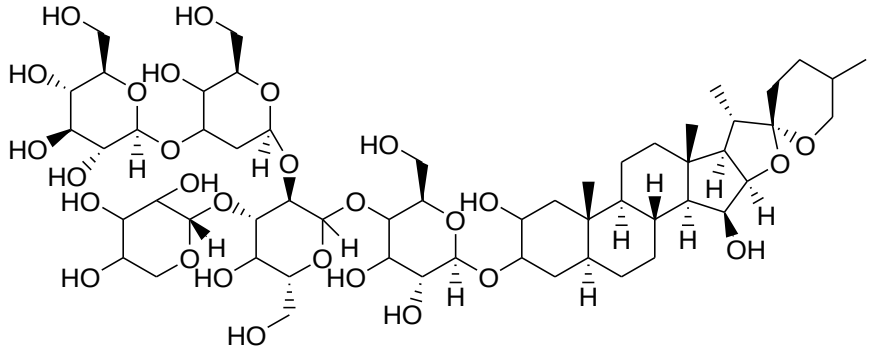
82 Afzelin (rhamnoside)



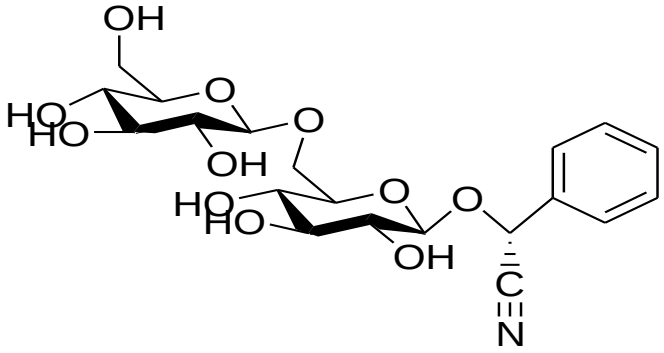
83 Lyoniside (Lignan glycoside)



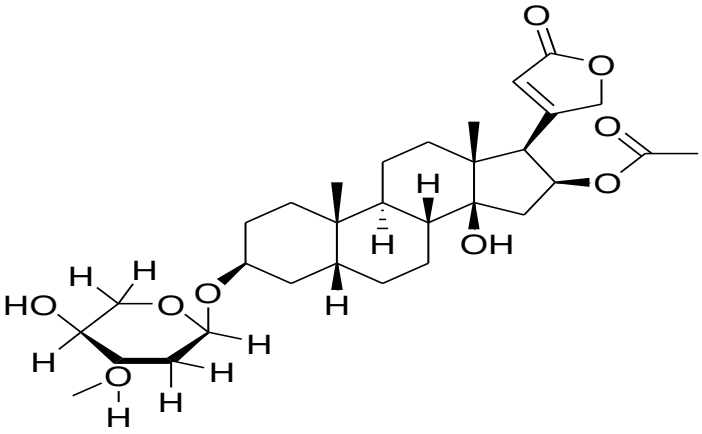
84 α-solanine (Alkaloidal glycoside)



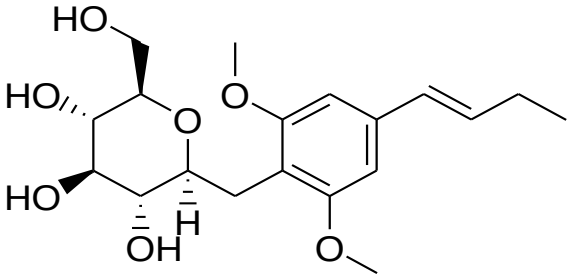
85 Digitonin (Saponin glycoside)



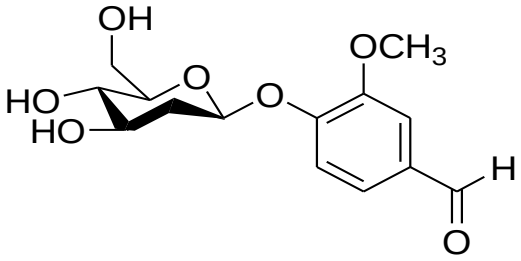
86 Amygdalin (cyanogenic glycoside)



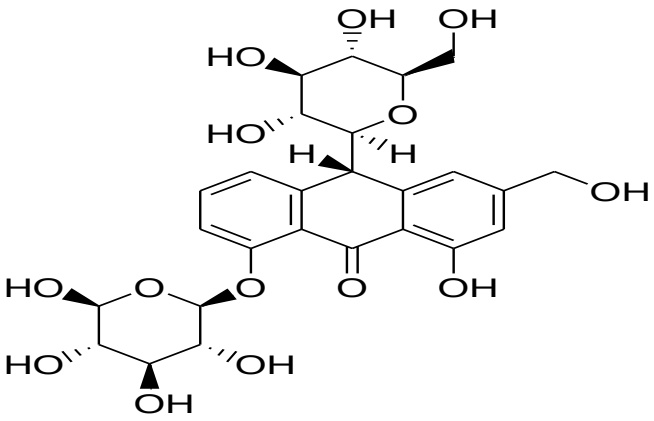
87 Oleandrin (Cardiac glycoside)



88 Syringin (Phenolic glycoside)



89 Glucovanillin (Aldehyde glycoside)

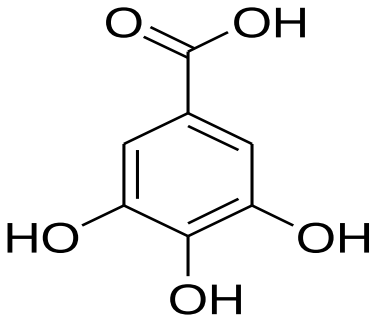


90 Cascaroside (Anthraquinone glycoside)

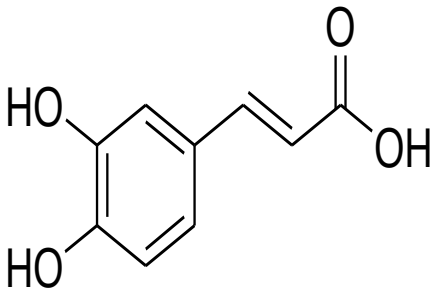
1.5.8 Plant Phenolics

Phenolics are the most articulated secondary metabolites found in plants, and their distribution appeared all through the entire metabolic process. Examples are, Gallic acid **91**, Caffeic acid **92**, Protocatechuic acid **93**, Coumaric acid **94**, Ellagic acid **13**, Rasveratrol **95**, Quercetin **66** and Rosmarinic acid **96** (Velderrain-Rodríguez *et al*., 2014).

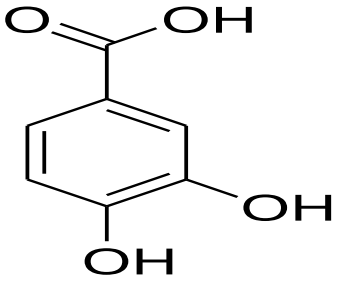
These phenolic compounds are ordinarily related to defense responses in the plant. Phenolic compounds, including stress-linked phytochemicals, have been related to favorable influences, which are caused by the consumption of fruits and vegetables (Heima *et al*., 2002).



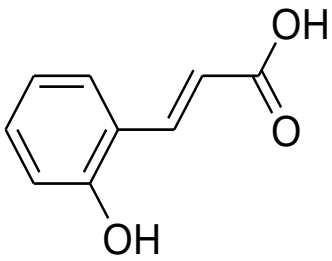
91 Gallic acid



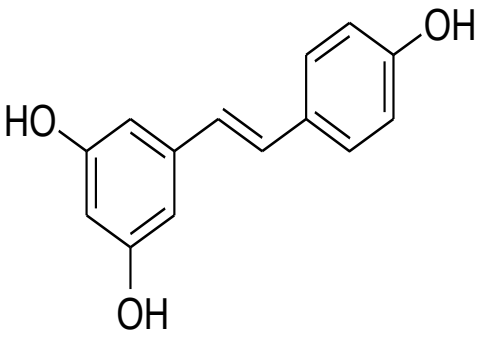
92 Caffeic acid

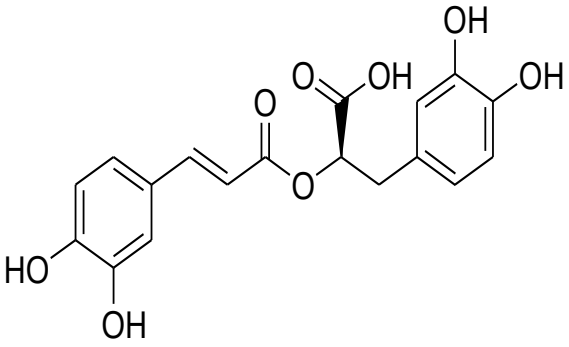


93 Protocatechuic acid



94 Coumaric acid





95 Rasveratrol

96 Rosmarinic acid

1.6 Antibiotics

An antibiotic is a chemical compound derived from or manufactured by one microorganism that selectively hinders the growth of or destroys certain microorganisms. Antibiotics belong to a class of antimicrobials, a larger group which also includes anti-virals, anti-fungals, and anti-parasitic drugs (Mboya *et al*., 2018). Antibiotics are used to treat an assortment of bacterial infections. Antibiotics remedy irresistible illnesses by murdering or harming microbes. Microbes are single-celled organisms, natural and normal part of life. Pathogenic bacteria cause disease when they are able to gain access to vulnerable parts of the body and multiply rapidly (Mboya *et al*., 2018).

It has become evident (González-Lamothe *et al*., 2009; Obeidat *et al*., 2012; Ahmad & Wajid, 2013) that higher plants are potential sources of antibiotic agents. Several investigators (Mostafa *et al*., 2017; Gonelimali *et al*., 2018; Manandhar *et al*., 2019) have reported antibiotic effects of juices and of crude extracts of plants on various microorganisms.

1.6.1 Classification of Antibiotics

The serviceable antibiotics is based on chemical structure. Antibiotics within a structural class will generally have comparative designs of viability, harmfulness and unfavorably potential.

The principal classes of antibiotics are:

1. Beta-Lactams e.g. Penicillins, Cephalosporins (Kong *et al*., 2010)

2. Macrolides e.g. Erythromycin, Clarithromycin (Crosbie & Woodhead, 2009).

3. Fluoroquinolones (fluoridated quinolones) a synthetic antibiotic e.g. Ciprofloxacin, Levofloxacin (Briasoulis *et al*., 2011; Kim, 2010; Kushner *et al*., 2001).

4. Tetracyclines.e.g. Tetracycline, Oxytetracycline (Sloan & Scheinfeld, 2008).

5. Aminoglycosides (derived from various species of *Streptomyces*) (Lopez-Novoa *et al*., 2011).

While each class is composed of multiple drugs, each drug is unique in some way.

1.6.2 Modern Antibiotics Era

The systematic screening program adopted by Paul Ehrlich in 1904 driven to discovery of a drug (Salvarsan) against syphilis. The precise screening approach introduced by Paul Ehrlich got to be foundation of drug search strategies within the pharmaceutical industry and brought about in thousands of drugs distinguished and interpreted into clinical practice, including, of course, a variety of antimicrobial drugs.

During the earlier days of antibiotics research, this approach led to the discovery of sulfa drugs, namely sulfonamide chrysoidine (KI-730, Prontosil), which was blended by Bayer chemists Josef Klarer and Fritz Mietzsch and tested by Gerhard Domagk for antibacterial activity in a number of diseases (Aminov, 2010).

Prontosil, nonetheless, appeared to be a precursor to the active drug, and the active part of it, sulfanilamide, was thus not patentable as it had already been in use in the dye industry for some years (Bentey, 2009). Also, in 1928, Alexander Fleming discovered Penicillin (Bentey, 2009).

The uncovering of these first three antimicrobials, Salvarsan, Prontosil, and Penicillin, was exemplary, as those studies set up the paradigms for future drug discovery project. The

timeframe of 1950s and 1970s was indeed the golden era of discovery of novel antibiotics classes, with no new classes discovered since then. (Chopra *et al*., 2002).

1.6.3 Antibiotics from Nature

Plants have served as a profitable source of fixings for conventional drugs for centuries. Plants are able to produce an endless cluster of fundamentally different compounds, each of which serves a particular part for the plant itself (e.g., defense against phytopathogens). Some of the time, these compounds are moreover dynamic against human pathogens. There are four principal groups of antimicrobial compounds made by plants: phenolics and polyphenols, terpenoids and essential oils, lectins and polypeptides, and alkaloids. In most cases, bioactive plant extricates contain complex mixtures of these groups, and their synergistic activity can yield an enhanced effect (Brahmkshatriya & Brahmkshatriya, 2013).

These compounds act on microbes through a number of mechanisms, counting inactivation of proteins, adhesins and enzymes, among other targets. When it comes to the search for new antimicrobials, and particularly the logical assessment of conventional drugs, there is much that an ethnobotanical approach to medicate disclosure can offer (Ghorbani *et al*., 2006). Ethnobotany, or the study of the interactions between plants and people, is a field that has also been described as “the science of survival” (Prance, 2007).

1.6.4 Antibiotic Resistance

Antimicrobials are amazingly imperative in medication, but shockingly microscopic organisms are able to create resistance to them. Antibiotic-resistant microscopic organisms are organisms that are not killed effectively by antibiotics. When bacteria are exposed to the same medication over and over, the bacteria change and are no longer affected by the drug (Ventola, 2015).

There exist several ways of how bacteria become antibiotic-resistant. For example, their internal mechanism makes them change their structures, so as to render the antibiotic inactive,

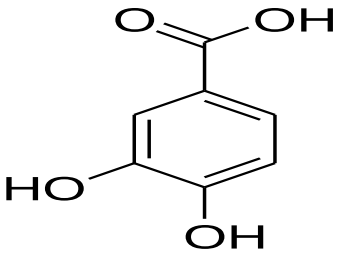
hence developing new ways to inactivate or neutralize the antibiotic. Moreover, microscopic organisms can exchange the qualities coding for antimicrobial resistance between them, making it conceivable for microscopic organisms to obtain resistance. The issue of antimicrobial resistance gets to be more genuine when antimicrobials are utilized to treat clutters for which they are unfit (Lushniak, 2014).

Resistance to antibiotics represents a serious and growing problem, since a few irrestible illmess are getting to be difficult to treat. Some safe microscopic organisms can be treated with more capable drugs, but a few infections are difficult to cure even with novel drugs (Ventola, 2015).

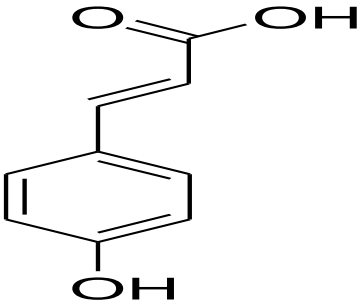
1.7 Antioxidants from Natural Products

Since plant tissues are living, they are beneath consistent oxidative stress from free radicals, reactive oxygen species, and prooxidants resulted in both exogenously and endogenously. For this reason, numerous tissues have antioxidant frameworks to control free radicals, lipid oxidation catalysts, oxidation intermediates, and secondary breakdown products (Agati *et al*., 2007; Brown & Kelly 2007; Chen 2008; Iacopini *et al*., 2008).and in therapeutic blends for their physiological impacts, regularly contain high concentrations of phenolic compounds that have powerful H-donating activity (Muchuweti *et al*., 2007). Some plant-derived compounds (carnosol, rosmanol, rosmariquinone, and rosmaridiphenol) are better antioxidants than synthetic antioxidant [butylated hydroxyanisole (BHA)] (Carvalho *et al*., 2005).

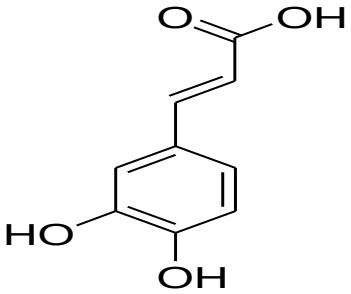
The major antioxidative plant phenolics can be splitted into 4 general groups: phenolic acids (gallic acid **91**, protochatechuic acid **97**, p- Coumaric acid **98**, caffeic acid **99**, and rosmarinic acid **96**), phenolic diterpenes (carnosol **100**, carnosic **101**, rosmanol **102** and rosmarinic acid **96**), flavonoids (epicatechin **64**, quercetin **66**, epicatechin gallate **103**, epigallocatechin gallate **104** and rutin **67**), and volatile oils (eugenol **105**, carvacrol **106**, thymol **36**, and menthol **37**) (Shan *et al*., 2005).



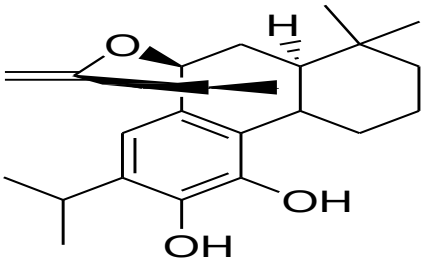
97 Protochatechuic acid



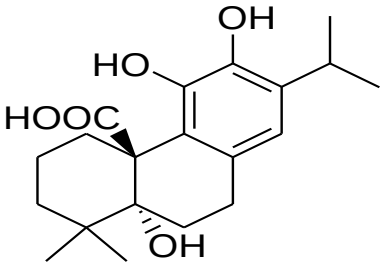
98 p- Coumaric acid



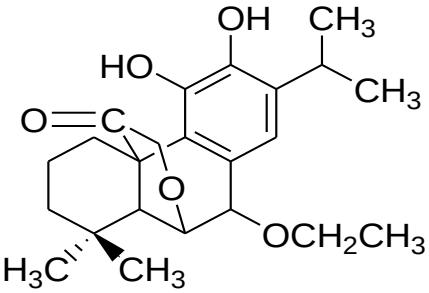
99 Caffeic acid



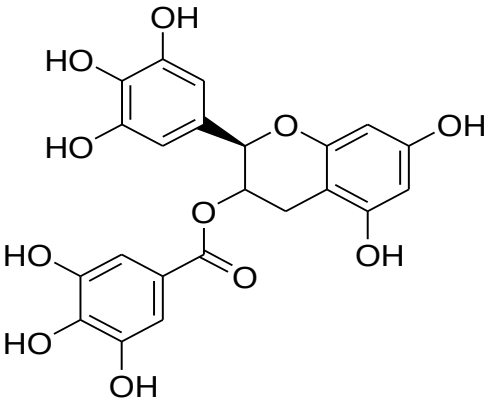
100 Carnosol



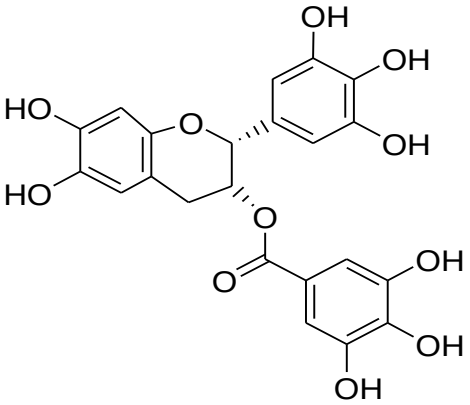
101 Carnosic



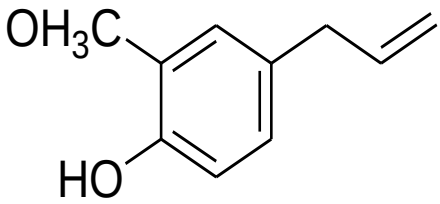
102 Rosmanol

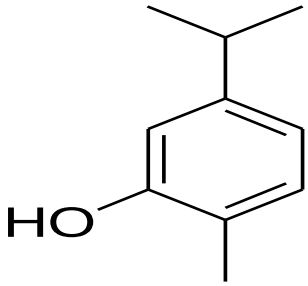


103 Epicatechin gallate



104 Epigallocatechin gallate





105 Eugenol **106** Carvacrol

Proanthocyanidins also contain multiple −OH groups that can donate hydrogen, quench $^{\bullet}O_2^-$, and chelate metals (Fukumoto & Mazza 2000). Free radical-scavenging ability increases as the number of phenolic −OH groups increases (Kondo *et al*., 2001). The fruits (apples and cherries), a few berries (rosehips, raspberries, blackberries, and strawberries), as well as, the leaves (tea), seeds (grape, sorghum, soy, and cocoa bean), and bark of numerous plants contain proanthocyanidins that have demostrated antioxidant activity and potential biologically therapeutic effects (Dixon *et al*., 2005; Buricova & Reblova_2008; Bak *et al*., 2010).

1.8 Justification

Due to the high cost and increased bacterial and viral resistance of synthetic drugs, it is necessary to look for alternative therapy from our indigenous plants which will provide cheap drugs and eradicate the problems of resistance connected with the use of synthetic drugs.

1.9 Research Objectives

1.9.1 Summary and problem statements

Plants have provided humans with many of their essential needs, including life-saving pharmaceutical agents. Few years ago, some new plant-derived drugs have been introduced into the market, and many more are currently going through clinical trials. As an endless extent of the accessible higher plant species have not however been screened for

biologically active compounds, medicate revelation from plants ought to remain an essential component within the explore for modern drugs, especially with the improvement of profoundly touchy and flexible explanatory strategies.

However, the active principles of *D. zibethinus* stem-bark have not been thoroughly investigated. Also, the biological activities of the stem-bark from *D. zibethinus* have not been thoroughly studied scientifically.

1.9.2 Hypothesis

In view of the alarming resistance to anti-infective drugs and unfavourable side-effect of antimicrobial therapy, *D. zibethinus* stem-bark with excellent folkloric antimicrobial, antioxidant and anti-inflammatory activities may produce lead compounds that will treat or prevent microbial infections, cardiovascular related imbalances such as inflammations as well as free radicals related diseases etc.

1.9.3 Aim of the Research

The aim of this research is to analyse the phytochemical compounds of *D. zibethinus*, isolate and characterize the bioactive compounds from the stem bark extract from the plant and establish their antimicrobial, antioxidant and anti-inflammatory activities.

1.9.4 Objectives of the Research

The specific research objectives were to:

(i) prepare the extracts from *Durio zibethinus* parts, using solvents of various polarities (n-hexane, ethyl acetate and methanol).

(ii) determine the presence of secondary metabolites in *Durio zibethinus* parts using different standard tests.

(iii) analyse the minerals in *Durio zibethinus* powders using Energy Dispersive X-Ray Fluorescence (EDXRF).

(iv) fractionate and identify the constituents of the stem bark extract from

D. zibethinus using GCMS.

(v) isolate and purify the bioactive compounds from the stem bark from *D. zibethinus* through various chromatographic techniques.

(vi) elucidate the structure of the isolates using various spectroscopic methods.

(vii) determine the total flavonoid and phenolic contents of the stem bark extract from *D. zibethinus*; and

(viii) investigate the antibacterial, antifungal, antioxidant and anti-inflammatory activities of the stem bark extract from the plant.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction to *Durio zibethinus* Murr.

Durio zibethinus Murr. is a genus Durio, belonging to the family of Bombacaceae. It is a seasonal tropical fruit grown in Southeast Asian countries such as Malaysia, Indonesia, Thailand, and the Philippines. There are nine edible Durio species, namely, *D. lowianus, D. graveolens* Becc., *D. kutejensis* Becc., *D. oxleyanus* Griff., *D. testudinarum* Becc., *D. grandiflorus* (Mast.) Kosterm. ET Soeg., *D. dulcis* Becc., Durio sp., and also *D. zibethinus* (Idris, 2011). However, only *Durio zibethinus* species have been extensively grown and harvested, thus commonly known as durian. The taxonomical classification of *Durio zibethinus* is as shown in table 2.1 (Brown, 1997).

Table 2.1: Taxonomical classification of *Durio zibethinus*

Classification	**Taxonomy**
Domain	*Eukaryota* - eukaruotes
Kingdom	*Plantae* - Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Magnoliopsida* - Dicotyledons
Subclass	*Dilleniidae*
Order	*Malvales*
Family	Bombacaceae (or Durionaceae) – Kapok-tree family
Tribe	*Durioneae* (is characterized by simple entire

	penninervate leaves)
Genus	*Durio* Adanson - durio
Species	*Durio zibethinus* Murry - durian

2.2 Durian plant varieties

There are 15 varieties of durians that are endorsed for commercial planting such as D24, D99 (kob kecil), D123 (Chanee), D145 (Beserah), D158 (Kan yau), D159 (Mon Thong), D169, D168 (IOI, Ma Muar), D175 (Udang merah, An He), D197 (Raja Kunyit, Musang King), D198 (Kim Hong) and D199 (Bola 828). Three additional hybrid durian clones that are produced using (MARDI) are named as MDUR78, MDUR79 and MDUR88 (Hean, 2007).

Accorrding to the Thai Agricultural Standard (TAS 3-2013), seven commercial varieties of durian are readily available in Thailand. These seven varieties are named as Chanee, Karnyao, Monthong, Kradoomthong, Puangmanee, Longlublae, and Nualthongchan (Husin et al., 2018; Tirtawinata *et al*., 2016). Based on commercial basis, the most popular and widely cultivated among all the varieties are Monthong and Chanee.

2.3 Durian Fruit Shape

Fruits are essential part of our daily diet because of their nutritious attributes. Tropical fruits can be classified into two groups namely major and minor. Durian fruit is belonging to the minor group (Bhat and Paliyath, 2015). In Malaysia, the durian fruit is considered as the "King of Tropical Fruit" due to its high dietary status and with its appearance that take after the prickly positions of royalty of Asian rulers (Subhadrabandhu and Ketsa, 2001; Norjana and Noor, 2011). It is an iconic, expensive and seasonal fruit in many parts of Southeast Asian countries, especially in Malaysia, Indonesia, Thailand, and the Philippines (Ernest, 2011; Leontowicz et al., 2008). Mature durian trees are known to produce fruits at a duration of 5-7 years after the

germination of their seeds (Figure 1A). In every fruiting season, each durian tree bears around 15-800 fruits. The fruit weight is by and large within the extend of 1-3 kg with the distance across within the range of 14-18 cm, and the length may well be within the range of 19-32 cm **(**Figure 1B**).** The eatable part (also called aril) is the tissue of durian fruits **(**Figure 1C**)**. Durian fruit is circular or oval, with spiky outer parts either light green or brownish**.** The fruit contains 3-5 longitudinal sutures that cover from its apical to basal end, and it is known to open after total aging of the fruit. The shape of the durian seeds **(**Figure 1D) is similar to chestnuts, and the length and breadth of the seeds range from 2-6 cm, and 2-3 cm, respectively and seeds are light brownish (Ernest, 2011).

In general, durian fruits have a particular and vigorous aroma. The infamous odour (that resembles to rotten eggs or onions) is caused by volatile sulphur compounds (VSC) controlled by methionine gamma lyases (MGL). Because of its strong smell, durian fruit is prohibited in airplane terminals, lodgings and there are confinements on its transport and storage (Voon *et al*., 2007). The most Asian producers of durian are Malaysia and Thailand (Li, *et al*., 2012; Siew *et al*., 2018). Indonesia, Philippines, and a couple of other Asian nations moreover do cultivate the durian but in small scale and mostly for domestic uses only. The major merchants of durian fruits are Taiwan, Hong Kong, and Singapore, whereas a total of 90% exports come from Malaysia, Thailand, and Indonesia (Ho & Bhat, 2015).

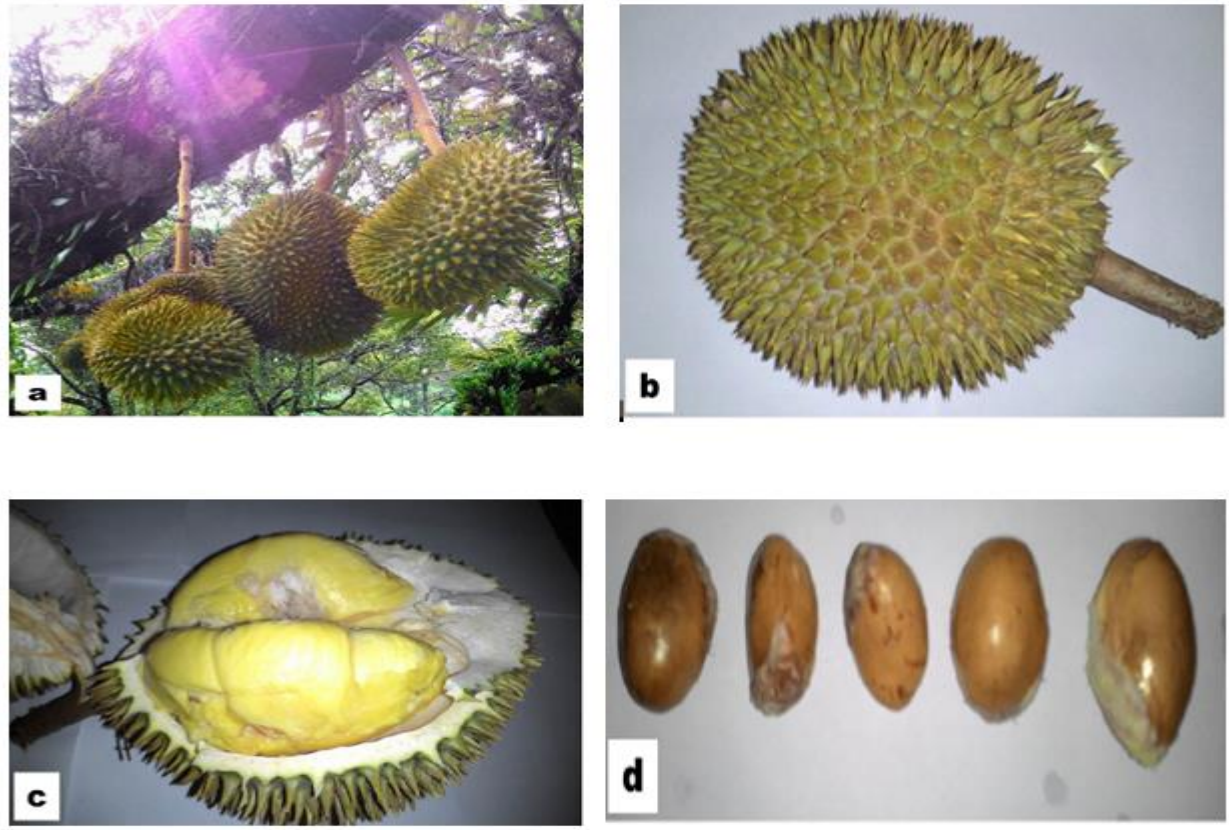


Figure 2.1 Fruits and seeds of durian (*Durio zibethinus* L.) clone D24. (a) Fruits bearing branch of a durian plant, (b) closer view of a fruit showing its spiny surface, (c) opened fruit showing fruit pulp, (d) seeds

2.4 Volatile Compounds in Durian

Durian is abundant in volatiles esters, alcohols, aldehydes, ketones and sulphur. These volatile constituents gave durian a distinctive flavour and taste. The most unstable sulphur compounds (VSCs) are ethanethiol, propanethiol, diethyl disulphide, ethyl propyl disulphide and diethyl trisulphide (Weenen *et al*., 1996). The odour of volatile sulphur compounds (VSCs) resembles that of onion (Li *et al*., 2017).

The most abundant ester compounds in durian varieties were identify as 2-methylbutanoate, methyl propanoate, ethyl propanoate, propyl propanoate, 2-methyl propanoate and 2-methylbutanoate (Chin *et al*., 2007; Belgis *et al*., 2017). Ethanol was the most important

alcoholic compound in Malaysian and Indonesian assortments as reported by Jalil and Aziz (2019).

The two major ketones identified in durian varieties are 3-Hydroxy-2-butanone and 2-hydroxy-3-pentanone (Belgis *et al*., 2017). 3-Hydroxy-2-butanone (common name: acetoin) incorporates a wonderful yogurt velvety smell and a greasy butter taste (Xiao and Lu, 2014). In any case, 2-hydroxy-3-pentanone has a troublesome scent like fishy and earthy (Chang *et al*., 2016). Meanwhile, acetaldehyde is the only aldehyde that has been detected in most durian varieties; which contributed to the fruity and sweet aroma in durian (Li *et al*., 2012).

2.5 Polyphenols in Durian

Durian is additionally rich in polyphenols such as flavonoids, phenolic acids tannins, and other bioactive components such as carotenoids (Haruenkit *et al*., 2010; Gorinstein *et al*., 2011; Charoenkiatkul *et al*., 2015; Isabelle *et al*., 2010).

Epidemiological studies have recommended that polyphenols reduce the chance of unremitting infections such as cardiovascular diseases, cancers and diabetes (Oba *et al*., 2010; Schmit *et al*., 2016; Costa *et al*., 2017; Mostofsky *et al*., 2017). In any case, polyphenols might act synergistically with other phytochemicals (Costa *et al*., 2017).

2.6 The Nutritional Worth of Durian

Durian pulp contains various nutrients that are noteworthy within the human being diet. Devalaraja *et al*. (2011) have detailed that the durian fruit pulp is an excellent source of supplements as it contains proteins (1.47%), carbohydrates (27%), dietary fat (5.33%) and fibers (3.1%). It also contains substantial quantity of vitamins and minerals such as vitamin A, vitamin B6, vitamin C, folic acid, thiamin, riboflavin, niacin, potassium, iron, calcium, magnesium, sodium, zinc and phosphorus (Ho and Bhat, 2015). It also contains linoleic acid

(2.20%), myristic acid (2.52%), oleic acid (4.68%), 10-octadecenoic acid (4.86%), palmitoleic acid (9.50%), palmitic acid (32.91%), and stearic acid (35.93%) (Ho and Bhat, 2015).

2.7 Use of Durian in Traditional Medicine

Traditionally, in Asia, the durian leaf and root decoctions are acknowledged to show antipyretic impact; its decoctions are also utilized as a febrifuge and anti-malarial agent. It is additionally utilized to treat phlegm, relieve colds, and treat skin diseases, jaundice, and swellings (Bhore *et al*., 2018). The medicinal and therapeutic properties of Durian fruit have been reported and this incorporate its capacity to boost the immune system and wound healing (Chansiripornchai and Pongsamart, 2008). Authors have reported that durian possess anti-cancer, anti-cardiovascular, anti-diabetic (Leontowicz *et al*., 2007; Oba *et al*., 2010; Costa *et al*., 201COS; Leifert and Abeywardena, 2008; Mostofsky *et al*., 2017), anti-oxidant (Ashraf *et al*., 2010) agents and antiobesity properties (Leontowicz *et al*., 2008), and can enhance digestion, cure insomnia, lower blood pressure and relieve symptoms of depression, anxiety, and stress disorders (Bhat and Paliyath , 2015; Haruenkit *et al*., 2007). It is also widely believed that durian pulp posesses strong aphrodisiac effects (Venkatesh *et al*., 2010) Previous studies have moreover reported the prospective use of durian fruit pulp as fertility enhancing agent and studies were conducted to find out its efficacy in treating infertility in PCOS (polycystic ovarian syndrome) (Ansari, 2016). Durian fruits have also shown anti-proliferative activities as being reported by Bhat and Paliyath (2015)

2.8 Health Benefits of Durian

Durian is endowed with macronutrients (sugars and fat) and micronutrients (potassium), dietary fibres, and bioactive and volatile compounds. The consumption of one serving size of durian aril (155 g) contributes 130 to 253 kcal and is comparable to one expansive pear and

four little apples without skin, respectively (Belgis *et al*., 2016). Durian is energy-dense due to the sugar and fat content and hence, might contribute to daily energy intake and will also increase postprandial blood glucose.

2.8.1 Effects of Durian on Blood Glucose

Durian has an exceptionally high sugar, and additive of 5% freeze-dried Monthong (Thailand variety) in 1% cholesterol-enriched diets in rats (Leontowicz *et al*., 2007). In humans, Robert *et al*. (2008) revealed that durian had the lowest glycaemic index (GI = 49) comparable to watermelon (GI = 55), papaya (GI = 58), and pineapple (GI = 90).

The smaller value of GI for durian may be the anti-atherosclerotic properties due to the presence of fibre and fat. Fibre slows digestion in the stomach related to tract and will slow down the change carbohydrate to glucose, thus lower the GI of food (Ma´ckowiak *et al*., 2016). Fat does not have a coordinate impact on blood glucose response, but it may influence glycaemic response indirectly by delaying gastric emptying, and thus slowing the rate of glucose absorption (Hu *et al*., 2001).

Durian is rich in potassium and is comparable to potassium-rich natural product, i.e., banana (Jalil and Aziz, 2019). A study revealed that there was a linear dose-response between low serum potassium and risk of type 2 *Diabetes mellitus* (Peng *et al*., 2017). Chatterjee *et al*. (2017) illustrated that potassium chloride supplementation reduced the worsening effect of fasting glucose in African-Americans compared with placebo. Collectively, the evidence has been seen that potassium content in durian might play a role in the regulation of blood glucose. The impart of durian on blood glucose has not been altogether investigated both in animal and human studies, and hence, warrants further investigation. Potassium might play a role in glucose homeostasis but might also have negative implications in certain conditions. For example, those with chronic kidney disease (CKD), *Diabetes mellitus* (DM), and heart failure (HF) or on pharmacological therapies may develop hyperkalaemia (Lakkis and Weir, 2018).

2.8.2 Cholesterol-Lowering Properties of Durian

Gorinstein *et al*. (2011) revealed a reduction in the levels of plasma TC (12.1%), LDL-C (13.3%), and triglycerides (TG) (14.1%) compared with the control group. The results were correlated with other durian from Thailand varieties (Chanee and Kan Yao) as compared with control.

Histological analysis illustrated that ripe durian protected the liver and aorta from exogenous cholesterol loading and protected the surface area of the aorta (Leontowicz *et al*., 2011). Durian also illustrated the ability to hinder postprandial plasma lipids compared with snake fruit and mangosteen (Haruenkit *et al*., 2007). Previous studies showed that propionate inhibited fatty acid and cholesterol synthesis in isolated rat hepatocytes (Park *et al*., 2018).

2.8.3 Anti-Proliferative Activity

The mechanisms of action of polyphenols emphatically relates to their antioxidant activity.

There are numerous studies pointing out an important role of polyphenolic compounds as inferred from vegetables, fruits, or herbs in the regulation of epigenetic modifications, leading to the antiproliferative protection (Stefanska et al., 2012). Their results have proven that durian fruit can be regarded as potential source of polyphenols with protective effects against nitric oxide-induced proliferation of MCF-7 cells, which is an oestrogen receptor-positive human breast cancer cell line (Jayakumar and Kanthimathi, 2011).

2.8.4 Probiotic Effects

These could be the most favourable condition for bacteria fermentation. In Malaysia, a product known as Tempoyak was produced by fermenting (spontaneous and uncontrolled) underutilised durian aril (Chuah et al., 2016). According to Leisner et al. (2001) lactic acid bacteria (LAB) are the principal microorganisms (namely *Lactobacillus plantarum*) in

Tempoyak. Khalil *et al*. (2018) and Ahmad *et al*. (2018) recently illustrated the potential of Tempoyak as a source of probiotics. The potential of Tempoyak as a source of probiotics was recently illustrated by Khalil et al. (2018) and Ahmad et al. (2018). These strains were able to create exopolysaccharide (EPS) and had awesome strength to resist the extraordinary conditions of either low pH in bile salts. Ahmad *et al*. (2018) isolated *Lactobacillus plantarum* from Tempoyak and manifested good probiotic properties including the tolerance of acid and bile salt, antioxidative, antiproliferative effects, and extraordinary adhesion on colon adenocarcinoma cell line (HT-29 cell lines) (Korcz *et al*., 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 General Laboratory Chemicals

The common laboratory chemicals (Merck products) used in this work include Acetic anhydride, Aluminium chloride, Ammonium hydroxide, Anhydrous magnesium sulphate, Ferric chloride, Glacial acetic acid, Hydrochloric acid, Lead acetate, Potassium acetate, Potassium persulphate ($K_2S_2O_8$), Sodium carbonate, Sodium hydroxide, Sulphuric acid and Linoleic acid. Others are; Mayer's and Draggendoff's reagent.

3.1.2 General Solvents

Common solvents (Sigma Products) used include Acetone, Chloroform, Dichloromethane, Ethyl acetate, Hexane, Methanol, Deuterated methanol Uvasol, Water Uvasol and Distilled water. Most of the solvents were reagent grade which were distilled before use and analytical grade solvents were used for spectroscopic measurements.

3.1.3 Materials for Chromatography

The materials (Merck products) used for chromatography are Pre-coated TLC plates, Silica Gel 60, 40−63 μm mesh size and Aluminium Foil.

3.1.4 Materials for Total Flavonoids and Phenolic Content Screening

These materials were utilized for the determination of total flavonoids and

phenolic contents are Folin–Ciocalteu reagent, Quercetin and Gallic acid.

3.1.5 Materials for Antioxidant Activity Studies

The following reagentswere used for the antioxidant activity experiment:

1,1-diphenyl-2-picryl hydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS) and Butylated hydroxytoluene (BHT).

3.1.6 Materials for Biological and Anti-inflammatory Activity Studies

Listed below are the reagents employed to establish anti-inflammatory activity: Borate buffer, Nutrient broth, Filter paper strips, Sabouraud Dextrose Agar (SDA), Fluconazole, Sterile cork borer, Gentamicin, Sterile forceps, Indomethacin, Sterile nutrient agar, Innoculating loops, Sterile petri dishes, Inoculated plates, Tween 20, Lipoxidase enzyme, Tween 65, Mueller-Hinton agar (MHA) and Wax pencil

3.1.7 List of Bacteria and Fungi Strains

The bacteria and fungi strains used are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans*, *Microsporum audouinii*. All the bacteria and fungi are clinical strains, obtained from Department of Microbiology & Parasitology, Igbinedion University Teaching Hospital, Okada, Nigeria.

3.2 Methods

3.2.1 Plant Collection

Fresh plant materials (leaves, stem-bark and root) of *Durio zibethinus* were collected from the premises of Crown Estate, Okada in Ovia North-East Local Government Area of Edo State, Nigeria. The identification and authentication of the plant materials were carried out at the Department of Plant Biology, University of Ilorin. The Voucher No. UILH/001/1371 was assigned.

3.2.2 Plant Preparation and Extraction:

The fresh plant materials were air-dried at room temperature (26°C), after which they were milled into uniform powder with the aid of an electrical grinder. Extracts were processed by soaking 2.0 kg of the powdered plant material in 10.0 L of methanol at ambient temperature for 72 hr. The extracts were filtered with Whatmann filter paper. Each extract was concentrated *in vacuo*, chromatographed and the chromatographic fractions obtained were subjected to purification processes prior to spectroscopic analyses that facilitated the elucidation of structures of isolated individual conmponents (Prabhavathi *et al*., 2016).

The crude extracts were coded Dz/LF/MeOH for leaf extract; Dz/SB/MeOH for stem-bark extract and Dz/RT/MeOH for root extract.

3.3 Preliminary Phytochemical Screening

Standard screening test on the extracts were carried out. The crude extracts were screened for the presence or absence of secondary metabolites such as alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, cardiac glycosides and phenolic compounds using standard procedures with minor modifications where necessary.

3.3.1 Test for Alkaloids

A 100 mg of each plant extract was diluted with 5 mL of hydrochloric acid (1M), boiled and filtered. To 2 mL of the filtrate, 1 mL of dilute ammonia and 2 mL of chloroform was incorporated. The mixture was shaken delicately to extract the alkaloidal base. The chloroform layer was extracted with 5 mL of acetic acid. This was partitioned into two portions. Mayer's reagent was introduced to one portion and Draggendoff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish-brown precipitate (with Draggendoff's reagent) was taken as positive test for the presence of alkaloids (Edrah, 2017).

3.3.2 Test for Saponins

Froth test: Each plant extract (0.5 g) was liquefied in 10 mL of purified water in a test tube, stoppered, shaken strenuously for 20 seconds, permitted to stand in a vertical position and watched over a 30 minute period of time for a "honey comb" foam over the surface of fluid after 30 min was taken as a positive test for the presence of saponins (Ejikeme et al., 2014).

3.3.3 Test for Tannins

Ferric chloride test: A portion of each plant extract liquefied in purified water and clarified by filtration. A bluish-black colouration after the addition of 10 % ferric chloride solution indicated the presence of tannins (Santhi and Sengottuvel, 2016).

3.3.4 Test for Flavonoids

Each plant extract (0.5 g) was liquefied in dilute NaOH and HCl was introduced. A yellow solution that turned colourless indicated the presence of flavonoids (Gothandam et al, 2010

3.3.5 Test for Steroidal Compounds

Liebermanʹs test: Each plant extract (0.5 g) was liquefied in 2 mL of acetic anhydride and cooled in an ice-bath. A colour change from purple to blue to green when concentrated sulphuric acid was carefully introduced, indicating the presence of a steroid nucleus (Seema and Parwez, 2011).

3.3.6 Test for Terpenoids

Salkowskiʹs test: Each plant extract (0.5 g) was liquefied in 2 mL of chloroform. Concentrated H_2SO_4 (3 mL) was carefully introduced to form a layer. A reddish-brown colouration at the interface indicated the presence of terpenoids (Rahman et al., 2017).

3.3.7 Test for Cardiac Glycosides

Keller-Killianiʹs test: Each plant extract (0.5 g) was liquefied in water and 2 mL of glacial acetic acid containing one drop of ferric chloride solution was introduced. 1 mL of concentrated sulphuric acid was carefully added to make a brown ring at the interface showing the presence

of Deoxysugar characteristic of Cardenolides. A violet ring will show up below the brown ring, whereas within the acetic acid layer a greenish ring will form just over the brown ring and steadily spread all through this layer (Aiyelaagbe and Osamudiamen, 2009).

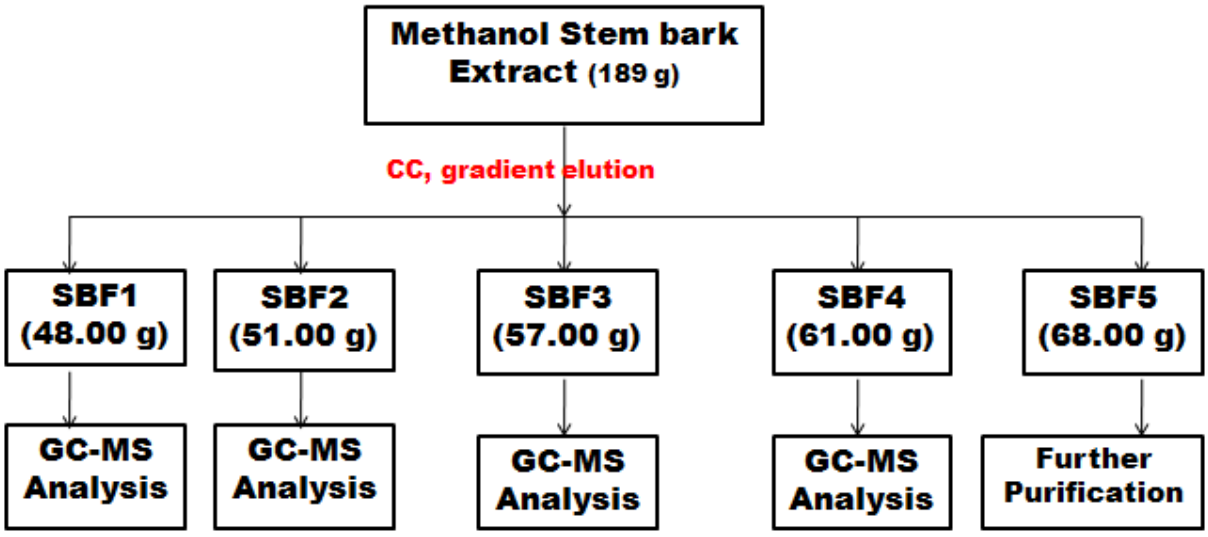
3.3.8 Test for Phenolic Compounds

Each plant extract was liquefied in purified water, and a number of drops of 1% lead acetate was added to form a bulky white precipitate which shown the presence of phenolic compounds (Prabhavathi et al., 2016).

3.4 Isolation Procedure

3.4.1 Column Chromatography

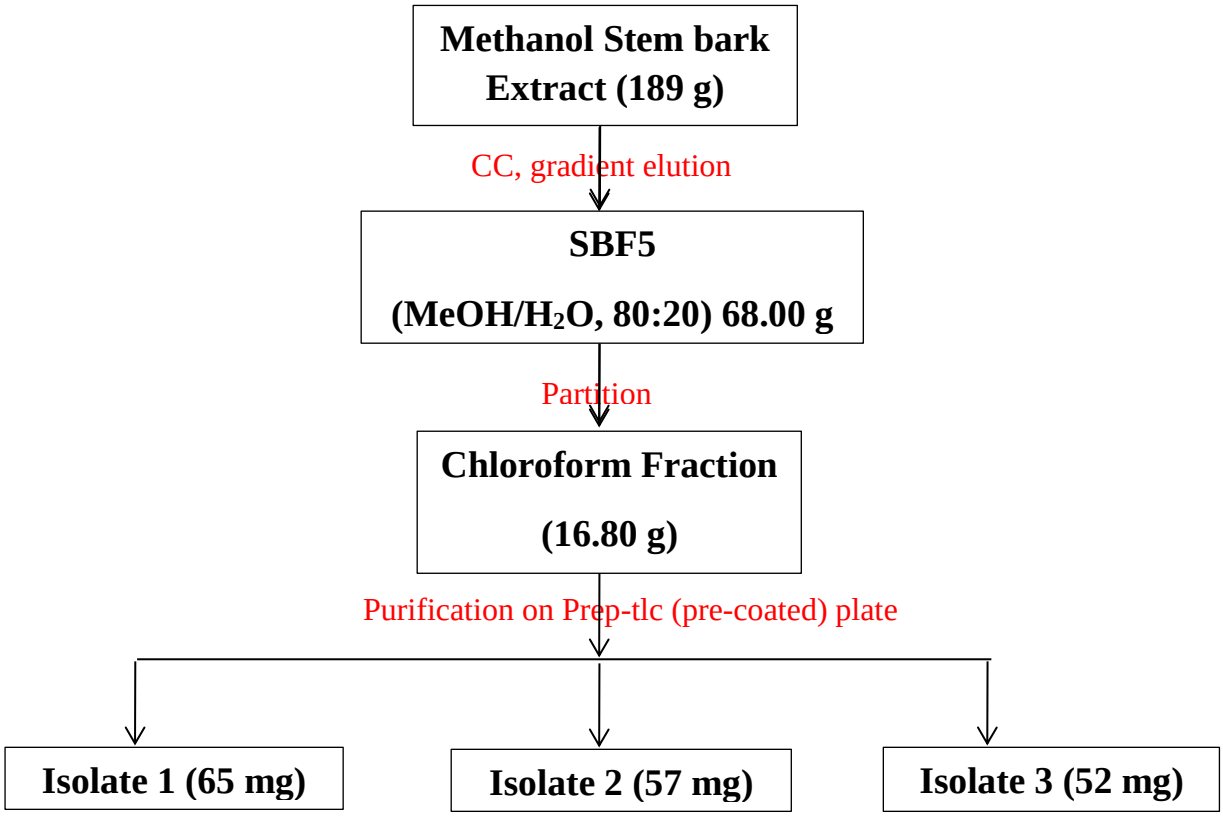
The stem bark extract was liquefied in small volume of methanol admixed into small quantity of Sigel, vacuo-dried and fractionated using n-hexane with progress addition of ethyl acetate and methanol. The fractions were examined under UV lamp and combined appropriately. Five combined fractions were obtained coded SBF1-SBF5. The first four fractions were analyzed with GC-MS while the fifth fraction (SBF5) was partitioned with chloroform. The schematic diagram of the fractionation of stem-bark extract is presented in Scheme 2.



Scheme 2: Schematic representation of the fractionation of stem bark extract.

3.4.2 Thin Layer Chromatography (TLC) and Preparative TLC

TLC analysis was accomplished on the little volume of the chloroform fraction using solvent mixture of dichloromethane and n-hexane (8:1). A preparative thin layer chromatography (PTLC) plate was used to isolate three constituents/compounds (isolates 1-3) from chloroform fraction. The schematic diagram of the isolation of bioactive compounds from fraction 5 (SBF5) of the stem back extract is presented in Scheme 3.



Scheme 3: Isolation of bioactive compounds from SBF5

3.5 Structure Elucidation

The structure elucidation of the three isolates from the chloroform fraction was carried out. The characterization of the constituents/compounds (isolate 1-3) was based on spectroscopic analyses (IR and 1D- ^{1}H nmr).

3.6 GC-MS Analysis of Phytochemical Compounds in the fractions of Stem bark Extract from *D. zibethinus*

3.6.1 Instruments and chromatographic conditions

The GC-MS analysis was performed on an Agilent Technologies interfaced [Model: 7890A (GC)] with Mass Selective Detector model: 5975C (MSD). The electron ionization was at a 7 eV with an ion source temperature at 250 °C. Highly pure helium gas (99.9 % purity) was used as carrier gas, while HP-5ms (30mm X 0.25mm X 0.320µm) was utilized as the stationary phase. The oven temperature program initial temperature was 140 °C held for 5 minutes at 4 °C per minute to the final temperature of 240 °C to hold for 15 minutes at the rate of 3.5 °C/minute holding for 6 minutes.

3.6.2 Identification of phytochemical compounds

Identification of phytochemical compounds and interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components using computer searches on a NIST Ver.2.1 MS data library. Hence the name, molecular weight and structure of the components of the test materials were established.

3.7 Energy Dispersive X-ray Fluorescence (EDXRF) Elemental Analysis of *D. zibethinus*

The Energy Dispersive X-ray Fluorescence (EDXRF) spectrometry (Model: ECLIPSE III, AMTEK INC. MA; USA) at the Centre for Energy Research and Development (CERD), Obafemi Awolowo University, Ile-Ife, Nigeria was used to analyze the trace elements in the plant parts (leaves, stem bark and the root) using standard method (Yao *et al*., 2015). A powder pellet was prepared by mixing 2 g of plant powder with 0.2 g of pure wax (Hoechst

Cridust wax) and homogenising the mixture by using an agate mortar. The prepared sample was pressed at 20 ton for 120 sec. to obtain a cylindrical pellet 40 mm in diameter. The standard solution with known content of elements was deposited onto a pellet of pressed plants using micropipette. After drying at room temperature, this pellet was homogenized and again pelletized under the same conditions as for the analysed sample. The quantitative analysis of the sample was carried out using the X-Ray Fluorescence with Fundamental Parameters (XRF-FP) Software package which converts elemental peak intensities to elemental concentrations.

3.8 Estimation of Total Flavonoid Content

The total flavonoid content (TFC) of each methanolic plant extract was investigated using the aluminum chloride colorimetric method described by Chang *et al*., (2002) with slight modifications. In this method, quercetin was used as a suitable reference. Therefore, quercetin solutions of various concentrations were used to make the standard calibration curve. Quercetin (10 mg) was dissolved in 100 mL methanol and then diluted to 6.25, 12.5, 25, 50, 80, and 100 μg/mL using methanol. Stock solution of plant extracts was prepared by dissolving 100 mg of the methanolic extract from each plant part in 5 mL methanol and transferred to 10 mL volumetric flask and made up the volume with methanol. 10% aluminium chloride and 1M potassium acetate were prepared using distilled water. The assay was determined using 0.5 mL of each extract stock solution (1 mg/mL) and each dilution of standard quercetin solution (10-100 μg/mL) taken separately in test tubes. To each test tube 1.5 mL methanol, 0.1 mL aluminium chloride solution, 0.1 mL potassium acetate solution and 2.8 mL distilled water were added and well shaken. Sample blank for all the dilution of standard quercetin was prepared in similar manner by replacing aluminium chloride solution with distilled water. All the prepared solutions were filtered through Whatmann filter paper before measuring their absorbance. Absorbance was taken at 510 nm using a UV-VIS spectrophotometer against the

suitable blank (Akbay *et al*., 2003). TFC was expressed as milligram quercetin equivalent per gram of dried extract (mg QCE/g DW). The sample was analyzed in three replicates.

3.9 Estimation of Total Phenolic Content

Total phenol content (TPC) of each methanolic plant extract was determined using the Folin-Ciocalteu (FC) method described by Dewanto *et al*. (2002). An aliquot of diluted extract was added to 0.5 mL of distilled water and 0.125 mL of Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before the addition of 1.25 mL of 7% Sodium carbonate (Na_2CO_3). The solution was then adjusted with distilled water to a final volume of 3 mL, mixed thoroughly and incubated in the dark. Sample blank for all the dilution of standard gallic acid was prepared in similar manner by replacing Folin-Ciocalteu solution with distilled water, filtered and absorbance was spectrophotometrically (UV-VIS spectrophotometer V-550 model, Japan) at 760 nm, measured against the suitable blank (Faten *et al*., 2014). TPC was expressed as milligram gallic acid equivalent per gram of dried extract (mg GAE/g DW). The sample was analyzed in triplicate.

3.10 Assessment of *In vitro* Antioxidant Activity

D. zibethinus extracts were screened for antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH•) quenching assay and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS•+) cation decolorization test.

3.10.1 DPPH Radical Scavenging Activity

DPPH free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability using stable DPPH radical, method described by Shekhar & Anju (2014). One mL of plant extract solution (10, 20, 50, 100 and 150 μg/mL in methanol) was added to 1mL of a DPPH solution (0.2 mM in methanol). After a 30 min of reaction at room temperature, the absorbance of the solution was measured at 517 nm. Lower absorbance in-

dicates the higher free radical scavenging activity. The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of a blank solution (no sample). The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. Mean values were obtained from triplicate of experiment values.

3.10.2 ABTS Radical Scavenging Activity

Scavenging activity of extracts was tested by using ABTS assay and quercetin was used as standard. This method was described by Jamuna *et al*. (2012) with a slight modification. The ABTS•+ cation radical was produced by the reaction between 5 mL of 14 mM ABTS solution and 5 mL of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 h. Before use, this solution was diluted with methanol to get an absorbance of 0.700 ± 0.020 at 734 nm. The plant extract at various concentrations with 1mL of ABTS solution was homogenized and its absorbance was recorded at 734 nm. Methanol blanks were run in each assay, and all measurements were done after at least 6 min. Similarly, the reaction mixture of standard group was obtained by mixing 950 µL of ABTS•+ solution and 50 µL of Buthylated Hydroxytoluene (BHT). As for the antiradical activity, ABTS scavenging ability was expressed as IC_{50} (µg/mL). The inhibition percentage of ABTS radical was calculated using the following formula:

$$\text{ABTS scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample. Mean values were obtained from triplicate of experiment values.

3.11 Biological Activities

3.11.1 Antibacterial Bioassay

Different extracts of *D. zibethinus* M were screened for antibacterial activities using two Gram-positive bacteria strains (*Staphylococcus aureus* and *Staphylococcus epidermis*) and three Gram-negative bacteria strains (*Klebsiella pneumonia*, *Escherichia coli* and *Salmonella typhi*) that are clinical strains, obtained from Department of Microbiology & Parasitology, Igbinedion University Teaching Hospital, Okada, Nigeria.

The agar diffusion test followed the procedure described by Aiyelabola *et al*. (2012). The bacteria strains were grown in 50 mL of nutrient broth at 37 °C and maintained in nutrient agar slant at 4 °C. An overnight suspension culture of the five bacteria strains were spread on the Sabouraud Dextrose Agar (SDA) media. Sterile discs were prepared and placed on the culture spread agar media. The disc was impregnated with 20 µL of 150 mg/mL of the plant extract in triplicates per treatment. Methanol was included as a negative control and the commercial antibiotic gentamicin was included as a positive control to determine the sensitivity of the bacteria strains. The inoculated plates were incubated at 37 °C for 24 hrs. The antibacterial activity was evaluated by measuring diameter of the inhibition zone around the disc.

3.11.2 Antifungal Bioassay

Different extracts of *D. zibethinus* M were screened for antifungal activities using two fungi strains, that is, *Candida albicans* and *Microsporum audouinii* (both are clinical strains) obtained from Department of Microbiology & Parasitology, Igbinedion University Teaching Hospital, Okada, Nigeria.

The agar diffusion test followed the procedure described in Aiyelabola *et al*. (2012). The fungi strains were grown in 50 mL of nutrient broth at 37 °C and maintained in nutrient agar slant at 4 °C. An overnight suspension culture of the two fungi strains were spread on the Mueller-Hinton agar (MHA) media. Sterile discs were prepared and placed on the culture

spread agar media. The discs were impregnated with 20 µL of 150 mg/mL of the respective plant extracts in triplicates per treatment. Methanol was included as a negative control and the commercial antibiotic fluconazole was included as a positive control to determine the sensitivity of the fungi strains. The inoculated plates were incubated at 37 °C for 24 h. The antifungal activity was evaluated by measuring diameter of the inhibition zone around the disc.

3.12 Anti-inflammatory Activity

3.12.1 Lipoxygenase Inhibition Assay

Anti-Lipoxygenase activity was studied utilizing linoleic acid as substrate and lipoxidase as enzyme (Eshwarappa *et al*., 2016). Test solution was dissolved in 0.25 mL of 2 M borate buffer pH 9.0 and added 0.25 mL of lipoxidase enzyme solution (20,000 U/mL) and incubated for 5 min at 25 °C. After which, 1.0 mL of linoleic acid solution (0.6 mM) was added, mixed well and absorbance was measured at 234 nm. Indomethacin was used as reference standard. The percent inhibition was calculated from the following equation:

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample. Mean values were obtained from triplicate of experiment values.

3.13 Statistical Analysis

Results obtained were evaluated for IC_{50} on GraphPad Prism 5 (San Diego, CA), and the one-way ANOVA using IBM SPSS statistics version 25. Dunnett test was used as Post Hoc to determine the significant pairs. Triplicate values were represented as mean ± standard deviation (± SD).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Preliminary Phytochemical Screening of *Durio zibethinus* extracts

Qualitative determination of phytochemicals as seen in (Table 4.1) showed various plant secondary metabolites detected in the extracts. These are alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, glycosides and phenolic compounds in both stem bark and root extracts of *D. zibethinus*. The results also revealed the absence of steroids in the leaf extract. This variation may be due to environmental conditions, which can alter the phytoconstituents of the plant leaves. By comparison, both the stem bark and root extracts contained more secondary metabolites than the leaf extract and usually a sign that the stem bark and root have the potential as source of valuable herbal drugs conjointly to improve the wellbeing status of the consumers as a consequence of the presence of more compounds that are crucial for good health.

Plants have shown to contain secondary metabolites that can also protect humans against diseases (Doughari *et al*., 2009). To these impacts most of the phytochemicals identified have been proclaimed by some authors for particular capacities. For occurence, Agu and Thomas (2012) reported that alkaloids have defense mechanisms through which plants ward off pests. This proposes the therapeutic properties (such as analgesic, antispasmodic and bactericidal effects) of alkaloids from plants (Doherty *et al.*, 2010; Kigigha *et al*., 2015; Epidi *et al*., 2016; Osuntokun and Oluwafoise, 2015). Alkaloids also exhibit potency on cardiovascular and kidney disorders (Sweetman, 2005). It was also reported that alkaloids have a broad spectrum of pharmacological activities including antimalarial (e.g. quinine), anticancer (e.g. homoharringtonine) (Kittakoop *et al.,* 2014), antibacterial (e.g., chelerythrine) (Cushnie *et al*., 2014), and antihyperglycemic activities (e.g. piperine) (Qiu *et al*., 2014).

TABLE 4.1: Qualitative phytochemical screening of methanolic extracts from *Durio zibethinus*

Secondary Metabolites	Leaf	Stem bark	Root
Alkaloids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Steroids	-	+	+
Terpenoids	+	+	+
Glycosides	+	+	+
Phenolic compounds	+	+	+

Key: Present: +; Absent: −

Saponins in *D. zibethinus* indicates that the plant could be used as expectorant, cough suppressant, as well as, the hemolytic activity (Kigigha *et al*., 2015; Okwu, 2005; Osuntokun & Oluwafoise, 2015; Epidi *et al*., 2016). Saponins have been reported to posses antimicrobial (Soetan *et al*., 2006), antifungal (Hazem *et al*., 2012) and anti-inflammatory properties (Patel & Patil, 2012). Brusotti *et al*. (2013) also detailed that saponin has been illustrated to be a great candidate for the control of a pathogen of rice blast disease, *Pyricularia grisea*.

Tannins are known to occur abundantly in the bark of trees where they act as a barrier to micro-organisms. Tannin is contracting in nature and has the ability to fasten or precipitate proteins and various other organic compounds making them inaccessible for absorption. Plants rich in Tannin are said to posses some medical applications (Okuda, 2005; Doughari *et al*, 2009). Tannins have health significants such as wound healing, varicose ulcers, hemorrhoids, frostbite and burns, and it has the capacity to recover skin, in addition to anti-inflammatory and anti-diuretics activity (Okwu &Okwu, 2004; Khanbabaee & van Ree, 2001).

Flavonoids have been proclaimed to possess antioxidant, anticarcinogenic, antimicrobial antitumor, allergenic, anti-inflammatory and antidiarrheal properties (Yamamoto & Gaynor

2001; Cushnie & Lamb 2005; Chahar *et al*., 2011; Yao *et al*., 2011). Flavonoids are also important in the treatment of ulcer (Ateufack *et al*., 2015).

Naturally occurring terpenoids frequently exhibit an assortment of biological activities such as anti-inflammatory, anti-HIV, anti-tumour-promoting, ichthyotoxic and antimycobacterial activities (Cantrell *et al*., 2001; Rajic *et al*., 2000; Villar *et al*., 2003).

Reports have ascertained the anti-proliferative and apoptotic effects of cardiac glycosides in several cancer cell lines, including breast (Kometiani *et al*., 2005; Bielawski *et al*., 2006; Lopez-Lazaro *et al*., 2005), prostate (McConkey *et al*., 2000; Huang *et al*., 2004; Yeh *et al.*, 2003), melanoma (Newman *et al.*, 2006), pancreatic (Newman *et al*., 2007), lung (Mijatovic *et al*., 2006; Frese *et al*., 2006), leukaemia (Raghavendra *et al*., 2007; Daniel *et al*., 2003) and renal adenocarcinoma (Lopez-Lazaro *et al*., 2005)78. Cardiac glycosides is essential in treating heart diseases (Ahmed *et al*., 2008).

Phenols have been detailed to possess antioxidant (Heima *et al*., 2002), antibacterial and antifungal (Alasalvar *et al*., 2001; Acamovic & Brooker, 2005; Edreva *et al*., 2008). All these activities may be associated to the biological roles of wood bark from *D. zibethinus*. From these results, it is obvious that *Durio zibethinus* possess significant phytochemicals indicating that the plant is a notable source of vital phytochemicals with potential therapeutic benefits.

4.2 Structure Elucidation

Three compounds (A, B and C) were isolated from methanol extract of stem-bark from *D. zibethinus* by column chromatography and purified by PTLC. The identification of the compounds was further confirmed by their corresponding melting points, retention factor and IR / ^{1}H-NMR spectral data in conjuction with reported data in literature.

The compound A isolated from methanol extract of stem-bark from

D. zibethinus appeared as white crystalline powder with retention factor (R_f) 0.53 in hexane: dichloromethane (1:8). The melting point of compound A, 249.5 - 250.4°C, correlated with the melting point of maslinic acid (249 - 250°C). The IR spectrum showed absorption band at 3430.08 cm^{-1} due to merged O-H stretching with C-H stretching at 2923.48 cm^{-1} and 2863.32 cm^{-1}. Absorption band at 1704.49 cm^{-1} indicated the presence of C=O stretching. The absorption band at 1641.16 cm^{-1} indicates the presence of an olefinic (C=C) group. The absorption band at 1457.52 cm^{-1} can be attributed to a C-H bending of a $-CH_2$ group, at 1267.55 cm^{-1} is characteristic of C-O stretching and absorption at 720.49 cm^{-1} peak due to CH_2 group. The ^{1}H-NMR spectrum of compound A showed integration of proton signals at δ 3.27 (1H, dd, H-2) and 2.92 (1H, d, H-3) of maslinic acid. One olefinic hydrogen signal appeared at δ 5.28 (1H, t, H-12). The signals for seven methyl groups appeared at δ 1.30 (3H, s, Me-23), 1.30 (3H, s, Me-24), 0.93 (3H, s, Me-25), 0.93 (3H, s, Me-26), 1.36 (3H, s, Me27), 1.30 (3H, s, Me-29), 1.30 (3H, s, Me-30). Hence the structure was identified and confirmed by comparison with the reported melting point, retention factor and spectral data (Hossain and Ismail, 2013). The presence of maslinic acid, (2α,3β)-2,3-Dihydroxyolean-12-en-28-oic acid, manifest that the plant has the potential to exhibit antitumor, anti-inflammatory, antimicrobial, antiparasitic, antihypertensive, and antioxidant properties (Mokhtari *et al*., 2015). The IR and ^{1}H-NMR spectral of the compound A are shown in Appendix I and II.

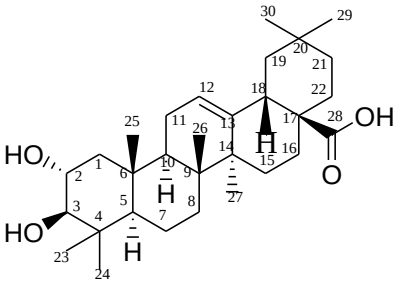
The compound B isolated from methanol extract of stem-bark from *D. zibethinus* appeared as white powder with retention factor (R_f) 0.40 in hexane: dichloromethane (1:8). The melting point of compound B, 192.1 - 193.4°C, was in correlation with melting point of 3- epimaslinic acid (191 - 194°C). The IR spectrum showed absorption band at 3392.08 cm^{-1} due to merged O-H stretching with C-H stretching at 2957.15 cm^{-1} and 2837.99 cm^{-1}. Absorption band at 1701.32 cm^{-1} revealed the presence of C=O stretching, at 1653.83 cm^{-1} displayed the presence of olefinic (C=C) group. The absorption band at 1457.52 cm^{-1} was showing the presence of C-

H bending due to -CH_2 group, at 1270.71 cm^{-1} is characteristic of C-O stretching and absorption at 745.12 cm^{-1} peak due to CH_2 group. The ^1H NMR spectrum of compound B showed integration of proton signals at δ 3.89 (1H, dd, H-2) and 3.05 (1H, d, H-3) of 3-epimaslinic acid. One olefinic hydrogen signal appeared at δ 5.32 (1H, t, H-12). The signals for seven methyl groups appeared at δ 1.30 (3H, s, Me-23), 1.30 (3H, s, Me-24), 0.91 (3H, s, Me-25), 0.91 (3H, s, Me-26), 1.36 (3H, s, Me27), 1.30 (3H, s, Me-29), 1.30 (3H, s, Me-30). Hence the proposed structure for compound B was affirmed by comparison with the reported melting point, retention factor and spectral data such as IR and 1H-NMR for 3-epimaslinic acid (Ferhat *et al*., 2017). The presence of 3-epimaslinic acid, (2α,3α)-Dihydroxyolean-12-en-28-oic acid, in the plant indicated that the plant can exhibit anti-inflammatory property (Nelson *et al*., 2015). The IR and ^1H-NMR spectral of the compound B are shown in Appendix III and IV.

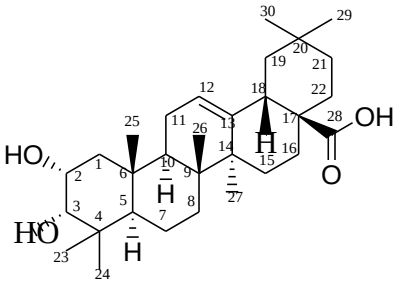
The compound C isolated from methanol extract of stem-bark from *D. zibethinus* appeared as white powder with retention factor (R_f) 0.22 in hexane: dichloromethane (1:8). The melting point of compound C, 266.7 - 267.2°C, correlated with melting point of bredemolic acid (267 - 269°C). The IR spectrum showed absorption band at 3423.75 cm^{-1} due to merged O-H stretching with C-H stretching at 2926.65 cm^{-1} and 2847.49 cm^{-1}. Absorption band at 1717.15 cm^{-1} indicated the presence of C=O stretching, at 1650.66 cm^{-1} showed the presence of olefinic (C=C) group. The absorption band at 1460.69 cm^{-1} showed the presence of C-H bending due to -CH_2 group, at 1267.55 cm^{-1} is characteristic of C-O stretching and absorption at 732.45 cm^{-1} peak due to CH_2 group. The ^1H NMR spectrum of compound 3 showed integration of proton signals at δ 3.51 (1H, dd, H-2) and 2.92 (1H, d, H-3) of bredemolic acid. One olefinic hydrogen signal appeared at δ 5.28 (1H, t, H-12). The signals for seven methyl groups appeared at δ 1.29 (3H, s, Me-23), 1.29 (3H, s, Me-24), 0.91 (3H, s, Me-25), 0.91 (3H, s, Me-26), 1.36 (3H, s, Me27), 1.29 (3H, s, Me-29), 1.29 (3H, s, Me-30). Hence the structure was identified and confirmed by comparison with the reported melting point, retention factor and spectral data for

bredemolic acid (Cheng *et al*., 2008). The presence of bredemolic acid, (2β,3α)-Dihydroxyolean-12-en-28-oic acid, in the plant has the potential to exhibit hypoglycemic property (Suresh & Ganesh, 2013). The IR and ^{1}H-NMR spectral of the compound C are shown in Appendix V andVI.

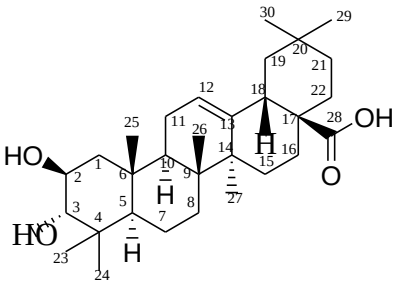
The structures of the isolated compounds (A, B and C) are represented in Figures 4.1.



Compound A



Compound B



Compound C

Figure 4.1: Structures of the isolated compounds (A, B and C)

4.3 GC-MS Analysis

GC-MS is one of the leading techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc. The GC-MS analysis of fractions of methanolic stem bark extract from *D. zibethinus* revealed the presence of two, six, five and four compounds (phytochemical constituents) in fractions SBF1, SBF2, SBF3, and SBF4 respectively. The peaks in the chromatogram were integrated and compared with the database of spectrum of known components stored in the GC-MS NIST library. The chemical

constituents identified in fraction SBF1are Heptadecanoic acid, heptadecyl ester (8.64 %) and (Z)-9-Octadecenoic acid, Oleic acid (91.36 %). Fraction SBF2 consists of Furo [2,3-c] pyridine, 2,3-dihydro-2 ,7-dimethyl- (0.90 %), Hexadecanoic acid, methyl ester (8.28 %), 3-Pyrrolidinol (0.58 %), Hexadecanoic acid, ethyl ester (13.13 %), (Z)-6-Octadecenoic acid, methyl ester (21.01 %) and (E)-9-Octadecenoic acid, Elaidic Acid (56.10 %). Fraction SBF3 has (E)-9-Octadecenoic acid, Elaidic Acid (14.78 %), 2-Ethylacridine (9.79 %), (Z)-6-Octadecenoic acid, Petroselinic acid (41.19 %) and trans-13-Octadecenoic acid, methyl ester (29.34 %) and 2,3-Dihydroxypropyl (E)-9-Octadecenoate (4.89 %) while fraction SBF4 consists of Hexadecanoic acid, methyl ester (17.89 %), Hexadecanoic acid, ethyl ester (21.33 %), (Z)-9-Octadecenoic acid, methyl ester (18.56 %) and (E)-9-Octadecenoic acid, Elaidic acid (42.20 %).

GC-MS chromatograms of the peak of the compounds detected in fractions SBF1-SBF4 were shown in Appendix VIIa, VIIIa, IXa and Xa respectively. The identification of the phytochemical compounds was confirmed based on the fragmentation pattern and molecular formula.

The active principles with their retention time (RT), retention index (RI), percentage composition and mass spectral data are presented in Tables 4.2.

Table 4.2: GC-MS analysis of the methanol stem-bark extract from *D.zibethinus*

S/N	Retention Time (RT)	Retention Index (RI)	Name of Compound	% Compo-sition	Mass Spectral Data
Fraction SBF1					
1	19.557	NA	Heptadecanoic acid, heptadecyl ester	8.64	43,**57**,68,83,97
2	29.923	2161	(Z)-9-Octadecenoic acid (Oleic Acid)	91.36	15,29,**41**,55,60

Fraction SBF2					
3	16.356	NA	Furo [2,3-c] pyridine, 2,3-dihydro-2,7-dimethyl-	0.90	39,65,79,120,**149**
4	16.968	1927	Hexadecanoic acid, methyl ester	8.28	15,43,55,**74**,87
5	17.644	NA	3-Pyrrolidinol	0.58	15,28,30,42,**43**
6	17.730	1993	Hexadecanoic acid, ethyl ester	13.13	14,29,43,**57**,71
7	19.063	2104	(Z)-6-Octadecenoic acid, methyl ester	21.01	29,41,55,**74**,84
8	19.818	2102	(E)-9-Octadecenoic acid (Elaidic Acid)	56.10	18,29,41,**55**,69
Fraction SBF3					
9	24.527	2102	(E)-9-Octadecenoic acid (Elaidic Acid)	14.78	18,29,41,**55**,60
10	24.579	NA	2-Ethylacridine	9.79	62,76,89,**96**,101
11	26.307	2102	(Z)-6-Octadecenoic acid (Petroselinic acid)	41.19	15,29,41,50,**55**
12	26.410	2119	trans-13-Octadecenoic acid, methyl ester	29.34	15,29,41,**55**,60
13	29.946	NA	2,3-Dihydroxypropyl (E)-9-Octadecenoate	4.89	15,29,41,55,61
Fraction SBF4					
14	16.877	1927	Hexadecanoic acid, methyl ester	17.89	15,29,43,55,**74**
15	17.712	1993	Hexadecanoic acid, ethyl ester	21.33	14,29,32,43,**57**
16	19.028	2104	(Z)-9-Octadecenoic acid, methyl ester	18.56	18,29,32,41,**55**
17	19.778	2102	(E)-9-Octadecenoic acid (Elaidic Acid)	42.20	18,29,41,**55**,60

NA = Not Available

The phytochemicals identified through GC-MS analysis showed many biological activities pertinent to this study as listed in Table 4.3. The biological activities listed are sourced from Dr. Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA. The mass spectra of the compound with the highest percentage peak areas in the four fractions were also represented in Appendix VIIb, VIIIb, IXb and Xb.

Table 4.3: GC-MS analysis and biological activities of the methanol stem-bark extract from *D. zibethinus*

S/N	R/T	% Composition	Name of Compound	Compound Class	Activity
Fraction SBF1					
1	19.557	8.64	Heptadecanoic acid, heptadecyl ester	Fatty acid	Acidifier, acidulant, arachidonic acid-inhibitor, inhibit production of uric acid.
2	29.923	91.36	(Z)-9-Octadecenoic acid (Oleic Acid)	Fatty acid	Anticancer, Anemiagenic, Insectifuge, Antiandrogenic, Dermatitigenic.
Fraction SBF2					
3	16.356	0.90	Furo [2,3-c] pyridine, 2,3-dihydro-2,7-dimethyl-	Pyridine derivative	Use in dyes, flavor, fragrances, Pharmaceuticals, agrochemicals and veterinary products

4	16.968	8.28	Hexadecanoic acid, methyl ester	Fatty acid ester	Antioxidant, hypocholesterolemic, antiandrogenic, flavor, nematicide, hemolytic, 5-alpha reductase inhibitor
5	17.644	0.58	3-Pyrrolidinol	Pyrrolidine derivative	No activity reported
6	17.730	13.13	Hexadecanoic acid, ethyl ester	Fatty acid ester	Antioxidant, flavor, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, hemolytic, 5-alpha reductase inhibitor
7	19.063	21.01	(Z)-6-Octadecenoic acid, methyl ester	Fatty acid ester	Antioxidant, Antimicrobial.
8	19.818	56.10	(E)-9-Octadecenoic acid (Elaidic Acid)	Fatty acid	Acidifier, acidulant, anticancer, antitumor, arachidonic acid-inhibitor.

Fraction SBF3					
9	24.527	14.78	(E)-9-Octadecenoic acid (Elaidic Acid)	Fatty acid	Acidifier, acidulant, anticancer, antitumor, arachidonic acid-inhibitor.
10	24.579	9.79	2-Ethylacridine	Heterocyclic compound	No activity reported
11	26.307	41.19	(Z)-6-Octadecenoic acid (Petroselinic acid)	Fatty acid	Cancer preventive, Insectifuge.
12	26.410	29.34	trans-13-Octadecenoic acid, methyl ester	Fatty acid ester	Acidifier, acidulant, arachidonic acid-inhibitor, inhibit production of uric acid.
13	29.946	4.89	2,3-Dihydroxypropyl (E)-9-Octadecenoate	Fatty acid ester	Anticancer, antitumor,ectoparasiticide, edemagenic
Fraction SBF4					
14	16.877	17.89	Hexadecanoic acid, methyl ester	Fatty acid ester	Antioxidant, hypocholesterolemic, antiandrogenic, flavor, nematicide, hemolytic, 5-alpha reductase inhibitor

15	17.712	21.33	Hexadecanoic acid, ethyl ester	Fatty acid ester	Antioxidant, flavor, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, hemolytic, 5-alpha reductase inhibitor
16	19.028	18.56	(Z)-9-Octadecenoic acid, methyl ester	Fatty acid ester	Acidifier, acidulant, arachidonic acid-inhibitor, increase zinc bioavailability.
17	19.778	42.20	(E)-9-Octadecenoic acid (Elaidic Acid)	Fatty acid	Acidifier, acidulant, anticancer, antitumor, arachidonic acid-inhibitor.

**Source: Dr. Duke's Phytochemical and Ethnobotanical Databases

Most phytochemical constituents identified in *D. zibethinus* stem bark are fatty acids and fatty acid esters that contribute to the antioxidant (Pinto *et al*., 2017; Khan *et al*., 2018), antimicrobial (Sati *et al*., 2017), antitumor (Yong-Kim & Byung-Zun, 2003; Tolnai & Morgan, 2011), cancer-preventive (Xu & Qian, 2014; Jóźwiak *et al*., 2019), hypocholesterolemic (Attia *et al*., 2015), nematicide (Machado *et al*., 2015), pesticide (Dalia, 2011; Abay *et al*., 2013), antiandrogenic (Liu *et al*., 2009), hemolytic (Dorantes-Aranda *et al*., 2009), 5-alpha reductase inhibitor (Sermakkani &Thangapandian, 2012), lubricant (Aoshima *et al*., 2005; Yoshiaki *et*

al., 2015) and flavor (Guler, 2005) activities. Hence, the stem bark of *D. zibethinus* Murr is worthy for further investigation on isolation of individual phytochemical constituents and toxicological aspects for the development of new lead of therapeutic interest.

4.4 EDXRF elemental analysis

The results of EDXRF measurements for the determination of the concentration of essential macro and micro elements in various plant extracts from *D. zibethinus* are presented in Table 4.4. Quantitative analysis of two macroelements: calcium (Ca) and potassium (K) and seven microelements: iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), arsenic (As), rubidium (Rb), strontium (Sr) and bromine (Br) was done. These results revealed the levels of the two macroelements in *Durio zibethinus* leaves, stem bark and roots as 3.58 ± 0.04 wt.%, 6.13 ± 0.06 wt.% and 3.42 ± 0.05 wt.% respectively for Ca while K levels were 4.53 ± 0.07 wt.%, 3.48 ± 0.07 wt.% and 5.06 ± 0.10 wt.% respectively. The levels of the microelements in the leaves, stem bark and roots of the plant range from 1 ± 1.0 ppm to 219 ± 8.0 ppm. Many of these elements are of cardinal importance in human metabolism. They are considered essential for the growth of living organisms.

Table 4.4: Metal content in leaves, stem bark and root of *Durio zibethinus*

Elements	Conc. in Leaves ± SD	Conc. in Stem bark ± SD	Conc. in Root ± SD
Ca (wt. %)	3.5775 ± 0.04	6.1283 ± 0.06	3.4240 ± 0.05
K (wt. %)	4.5343 ± 0.07	3.4796 ± 0.07	5.0641 ± 0.10
Fe (ppm)	219 ± 8.0	69 ± 3.0	622 ± 14.0
Mn (ppm)	92 ± 2.0	58 ± 1.0	108 ± 3.0
Cu (ppm)	26 ± 1.0	21 ± 1.0	30 ± 1.0
Zn (ppm)	75 ± 3.0	40 ± 3.0	96 ± 4.0
Rb (ppm)	9 ± 1.0	7 ± 1.0	10 ± 1.0
Sr (ppm)	16 ± 1.0	7 ± 1.0	34 ± 2.0
Br (ppm)	7 ± 1.0	1 ± 1.0	6 ± 1.0

All data were expressed as mean ± SD (n =3).

Plants have the capacity to uptake metals as supplement from the soil and its environment which are so basic for their physiological and biochemical development and hence the constract within the levels of the trace elements from different countries may well be due to the variety in soil sorts, agricultural and industrial activities, and local growing conditions such as differences in water, plant interactions, and weather (Anal, 2014; Kabata-Pendias, 2011). The role of inorganic elements in human metabolism has long been accepted. Macro and microelements impact numerous biochemical processes to maintain good health. Study of elements with regards to therapeutic plants uncovers that major and trace elements play noteworthy parts in combatting diseases (Pandey *et al*., 2006). A few metals in wellbeing items are as a result of adulteration. Many herbs are metal accumulators and absorb both essential and non-essential metals from soil and environment during the growth development. Thus, the understanding of metal content is also essential to assess the quality of herbal products.

Calcium (Ca) is accountable for keeping bones strong and control many vital cellular activities such as nerve and muscle function, hormonal actions, blood clotting and cellular mortality. It also lowers the risk of osteoporosis and its insufficiency produces skeletal muscle spasms and irregularity in heartbeat (Khan *et al*., 2011). According to a report by FAO/WHO, the calcium intake varies from 300 mg to 1200 mg per day depending on the age and physical health of the individual (Preet *et al*., 2017).

Potassium is an essential mineral micronutrient and is the principal intracellular ion for all types of cells. It is significant in maintaining fluid and electrolyte balance in the bodies of humans and animals (Pohl *et al.,* 2013*;* Clausen & Poulsen 2013). Potassium is fundamental for the work of all living cells, and is in this way display in all plant and animal tissues. A high potassium intake reduces the risk of overall mortality by 20 percent. It moreover diminishes the chance of stroke, drops blood pressure, safeguard against loss of muscle mass, maintain bone mineral density, and lessen the formation of kidney stones (Cogswell *et al*., 2012). Potassium deficiency can cause severe muscle weakness and paralysis, respiratory failure, painful obstructions in the gut, tingling, crawling, numb, or itchy sensations in the hands, feet, legs, or arms and intermittent muscle spasms (Ekinci *et al*., 2004; Cogswell *et al*., 2012).

Iron is a carrier of oxygen from the lungs to the tissues by red blood cells hemoglobin. It is additionally required for the improvement of healthy brain and immune system. Its recommended daily requirement is from 8 to 11 mg per day depending on the age of the person (Ptyakowska *et al*., 2012; Swaminathan *et al*., 2007).

It has been detailed that manganese (Mn) is involved in normal immune functions, regulation of blood sugar and cellular energy, and the defence mechanisms against free radicals (Koh *et al*., 2014). Manganese enacts certain enzymes that play critical parts in the metabolism of carbohydrates, amino acids, and cholesterol. Manganese is additionaly required for typical normal insulin synthesis, its secretion, and a change in its metabolism has been embroiled in

diabetes development (Kazi *et al*., 2008). Mn deficiency can result in impeded glucose resilience, altered carbohydrate and lipid metabolism, impeded insulin secretion, and skeletal abnormalities (Nicoloff *et al*., 2004). It plays a significant part within the normal growth, skeleton formation and reproductive function (Wang *et al*., 2008).

Copper is a prerequisite trace element (i.e. micronutrient) that is required for plant, animal, and human health (Scheiber *et al*., 2013). It is additionally required for the typical working of aerobic (oxygen-requiring) microorganisms. Copper is consolidated into assortment of proteins and metalloenzymes which perform basic metabolic functions; the micronutrient is fundamental for the proper growth, development, and maintenance of bone, connective tissue, brain, heart, and numerous other body organs. It reinforces connective tissue and is involved in energy production, within the protection of cells from free radical harm and in brain neurotransmitters. (Fox, 2003).

Zinc is a fundamental metal for the normal functioning of various enzymes. It plays a vital role in the storage and secretion of insulin, which subsequently increases the uptake of glucose (Li, 2014). The role of Zn in the production of insulin and catalysis of various enzymatic responses has been detailed (Maret, 2013). The low level of Zn in plasma unfavourably influences the ability of islet cells to produce and secrete insulin (Brender *et al*., 2010). In addition, it may aggravate the insulin resistance in type 2 diabetes and this way causes other complications (Hashemipour *et al*., 2009). Zn deficiency can lead to food aversion, growth retardation, weakness and even stagnation of sexual development. Zinc has been reported for beneficial effects on vision, hair loss and increased milk production by pregnant women (Saracoglu *et al*., 2009).

The metabolism of rubidium is closely related to that of potassium, and it shows eguivalence with potassium in a variation of biological systems with small prove for any harmfulness (McCall *et al*., 2014). In biological systems, calcium is substituted in a little degree by

strontium and within the human body; most of the absorbed strontium is kept within the bones (Pors, 2004). The stem bark containing a fairly good amount of Bromine (Br) may be used for the preparation of the drugs in curing natural diuretic, phlegm eliminating and stomach invigorating diseases (McCall *et al*., 2014). From EDXRF analysis results, it is obvious that *Durio zibethinus* accumulates essential elements in significant concentrations indicating that the plant is a notable source of nutrient elements with potential therapeutic benefits.

4.5 Total Flavonoid Content

The results of total flavonoid content of various plant extracts from *D. zibethinus* are presented in Table 4.5. Total flavonoid content in quercetin equivalent gave the highest levels of 2.74 ± 0.66, 4.71 ± 2.10 and 7.16 ± 0.76 mg QE/g sample weight for the leaf, stem bark and root extracts from *D. zibethinus* respectively.

Flavonoids (including flavones and flavanols) are plant secondary metabolites, the antioxidant activity of which depends on the existence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidant agents *in vivo* (Geetha *et al*., 2003). Flavonoids are exceedingly compelling scavengers of most oxidizing molecules, including singlet oxygen, and different other free radicals embroiled in a few infections (Trem & Smejkal, 2016). Flavonoids stifle reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species and up-regulate and protect antioxidant defenses (Agati *et al*., 2012). In this study, the order of decreasing total flavonoid content among the methanolic extracts of the plant was found (Figure 4.2) to be root extract > stem bark extract > leaf extract.

Table 4.5: **Total Flavonoid content of methanolic extracts from *D. zibethinus* M**

Extract	Total Flavonoids (mg QE/g DW)
Leaf	2.74 ± 0.66
Stem bark	4.71 ± 2.10
Root	7.16 ± 0.76

The values are means ± SD of three replicates. QE = Quercetin equivalent, DW = Dried weight.

This result is represented graphically in Fig. 4.2 below:

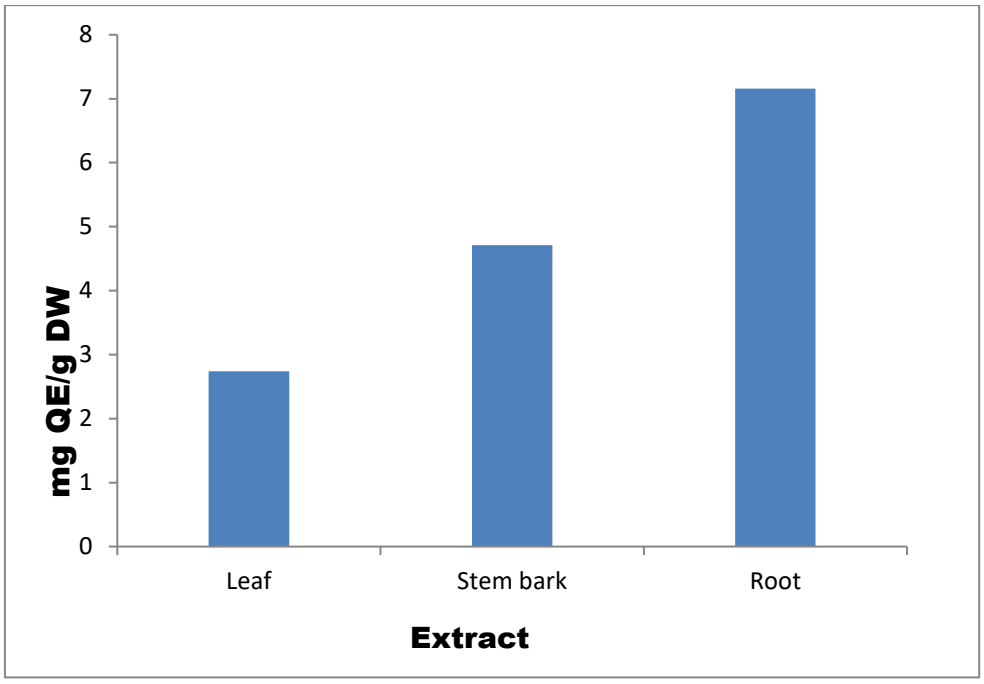


Figure 4.2: Total Flavonoid content of methanolic extracts from *D. zibethinus* M

4.6 Total Phenolic Content

The results of total phenolic content of various plant extracts from *D. zibethinus* are summarized in Table 4.6. The amount of total phenolics varied widely between 6.88 ± 1.54 and 29.00 ± 3.43 mg GAE/g sample weight. Total phenolic content in gallic acid equivalent gave the highest levels of 6.88 ± 1.54, 23.32 ± 1.73 and 29.00 ± 3.43 mg GAE/g sample weight for the leaf, stem bark and root extracts from *D. zibethinus* respectively.

Phenolic compounds have properties, which permit them to act as antioxidants (Soobrattee *et al*., 2005). Their free radical scavenging capacity is encouraged by their hydroxyl groups, the total phenolic concentration can be utilized as a premise for rapid screening of antioxidant activity. Several reports have shown a close relationship between total phenolic content and high antioxidant activity, and numerous researchers have illustrated that phenolic compounds are one of the foremost successful antioxidants in marine green growth (Luo *et al*., 2010; Zakaria *et al*., 2011). Phenolics bestow oxidative stress tolerance on plants. Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolics are progressively being utilized in the food industry for their antioxidative properties and wellbeing benefits (Shoib and Shahid, 2015).

Phenols have been reported to have a wide range of biological activity, including antithrombotic, cardio-protective, vasodilator and antimicrobial activities (García-Lafuente et al., 2009). Consequently, the root of *D. zibethinus* could be a great source of antioxidant phenolics. In this study, the order of decreasing total phenolic content among the studied plant part extracts was found (Figure 4.3) to be root extract > stem bark extract > leaf extract.

Table 4.6: **Total Phenolic content of methanolic extracts from *D. zibethinus* M**

Extract	Total Phenolics (mg GAE/g DW)
Leaf	6.88 ± 1.54
Stem bark	23.32 ± 1.73
Root	29.00 ± 3.43

The values are means ± SD of three replicates. GAE = Gallic acid equivalent, DW = Dried weight.

The graphical presentation is represented in Fig. 4.3 below:

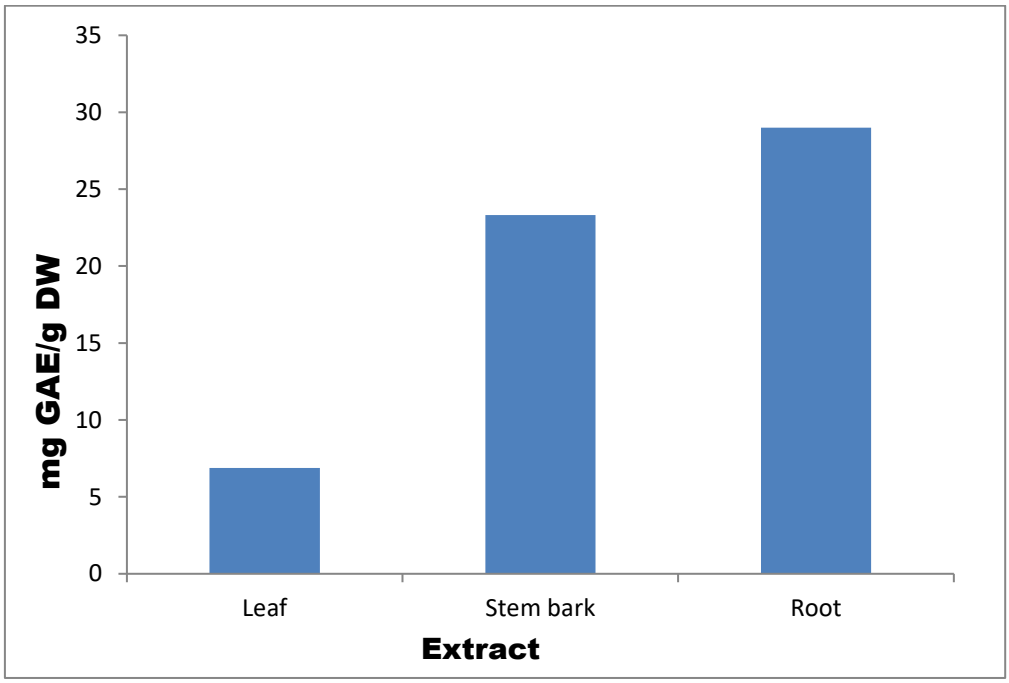


Figure 4.3: Total Phenolic content of methanolic extracts from *D. zibethinus* M

4.7 *In vitro* Antioxidant Activity

4.7.1 Free radical scavenging activity (DPPH)

The scavenging activity of the plant part extracts are summarized in Table 4.7. Percentage scavenging activity at 150 µg/mL concentrations of standard quercetin, leaf, stem-bark and root extracts from *D. zibethinus* were found to be 62.22 ± 0.02% (IC_{50} = 1.490 µg/mL), 14.74 ± 0.02% (IC_{50} = 1.467 µg/mL), 16.20 ± 0.02% (IC_{50} = 1.569 µg/mL) and 19.67 ± 0.03% (IC_{50} = 1.846 µg/mL) respectively. From Figure 4.4, it was noted that DPPH scavenging activity increased with increase in concentration for the standard and the methanolic extracts from *D. zibethinus* parts.

Table 4.7: Free radical scavenging activity (DPPH) of methanolic extracts from *D. zibethinus* M

S/ No.	Sample concentration (µg/mL)	Leaf % inhibition	Stem bark % inhibition	Root % inhibition	Quercetin % inhibition
1	10	1.80 ± 0.01	0.09 ± 0.02	0.31 ± 0.01	16.55 ± 0.01
2	20	6.10 ± 0.01	6.48 ± 0.01	1.19 ± 0.01	25.11 ± 0.01
3	50	10.81 ± 0.01	7.18 ± 0.01	4.77 ± 0.02	53.55 ± 0.01
4	100	13.81 ± 0.01	13.23 ± 0.01	14.52 ± 0.02	59.57 ± 0.01
5	150	14.74 ± 0.02	16.20 ± 0.02	19.67 ± 0.03	62.22 ± 0.02

Quercetin was used as reference standard. Values were performed in triplicates and represented as mean ± SD.

Antioxidant capacity may be associated with high phenol content, as Mansouri *et al*. (2005) reported that most of the antioxidant activity of plants is derived from phenols. However, the root extract of *D. zibethinus* was found to be the most potent scavenger among the three extracts. Hence, the order of decreasing antioxidant activity among the studied plant part extracts was found to be root extract > stem bark extract > leaf extract. This order is comparable to the phenolic contents of the extracts that showed that the degree of antioxidant action of the extract is in understanding with the sum of phenolics present in the extracts (Abdille *et al*., 2005).

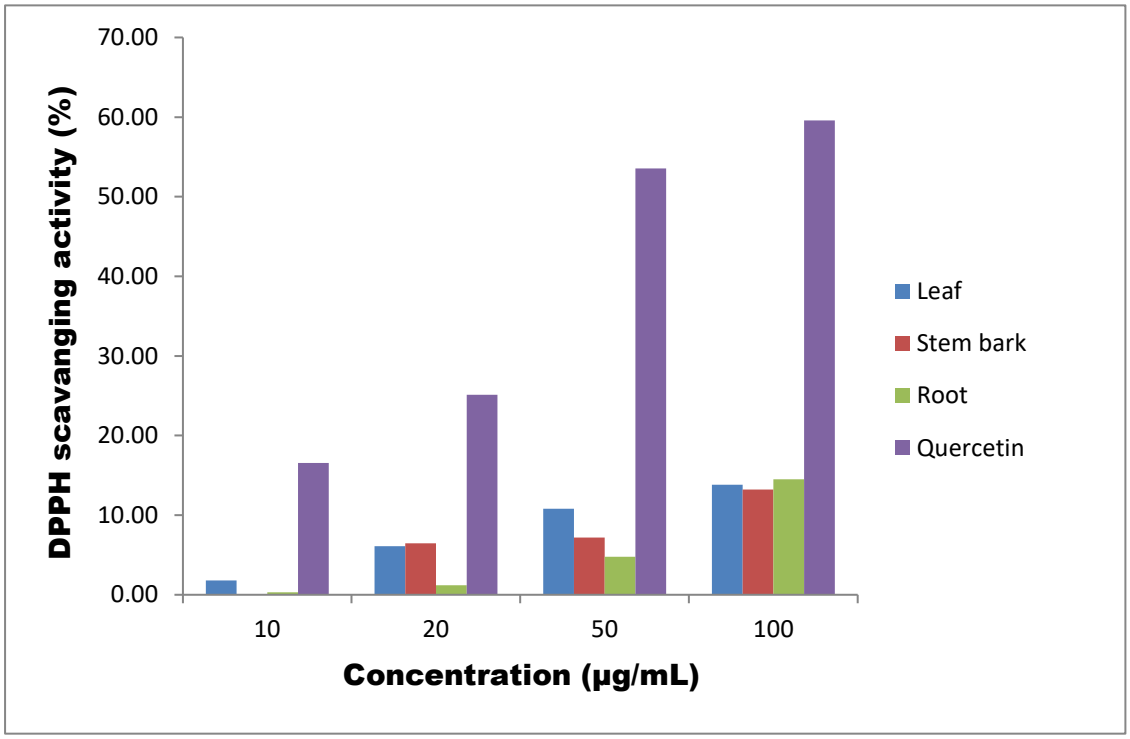


Figure 4.4: DPPH radical scavenging activity of the methanolic extracts from *D. zibethinus*

4.7.2 Free radical scavenging activity (ABTS)

The free radical scavenging activity of methanolic extracts of *D. zibethinus* along with reference standard, quercetin was determined by ABTS assay and the results are shown in Table 4.8. Percentage scavenging activity at 150 µg/mL concentrations of standard quercetin, leaf, stem bark and root extracts of *D. zibethinus* were found to be 62.87 ± 0.02% (IC_{50} = 1.247 µg/mL), 18.79 ± 0.01% (IC_{50} = 1.929 µg/mL), 19.69 ± 0.01% (IC_{50} = 0.267 µg/mL) and 40.30 ± 0.06% (IC_{50} = ~ 2.002 µg/mL) respectively. From Figure 4.5, *D. zibethinus* extracts were found to be effective in scavenging radicals and was concentration dependent.

ABTS assay is commonly adopted to assess radical scavenging or antioxidant activity. ABTS assay is a decolourization method, the ABTS oxidant generated from 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) by persulfate oxidation. The antioxidant activity of

extracts of plant part in this method is due to either inhibiting or scavenging the radicals. Both the inhibition and scavenging activity of ABTS++ was reported earlier (Abdille *et al*., 2005).

Table 4.8: Free radical scavenging activity (ABTS) of methanolic extracts from *D. zibethinus* M

S/ No.	Sample concentration (µg/mL)	Leaf % inhibition	Stem bark % inhibition	Root % inhibition	Quercetin % inhibition
1	10	7.08 ± 0.04	5.13 ± 0.01	10.83 ± 0.01	32.59 ± 0.02
2	20	7.95 ± 0.03	8.32 ± 0.01	10.91 ± 0.01	55.18 ± 0.02
3	50	8.65 ± 0.03	9.42 ± 0.02	13.40 ± 0.03	61.86 ± 0.02
4	100	14.26 ± 0.03	13.17 ± 0.01	24.36 ± 0.05	62.01 ± 0.05
5	150	18.79 ± 0.01	19.69 ± 0.01	40.30 ± 0.06	62.87 ± 0.02

Quercetin was used as reference standard. Values were performed in triplicates and represented as mean ± SD.

All the extracts had antioxidant abilities below the control, quercetin. The difference in the antioxidant activity profiles is in accordance with previous reports (Amin *et al*., 2004). Phenolic compounds scavenge free radicals by forming a stable ABTS-H (Abdille *et al*., 2005). The order of antioxidant potency of

D. zibethinus extracts in this study was found to be root extract > stem bark extract > leaf extract.

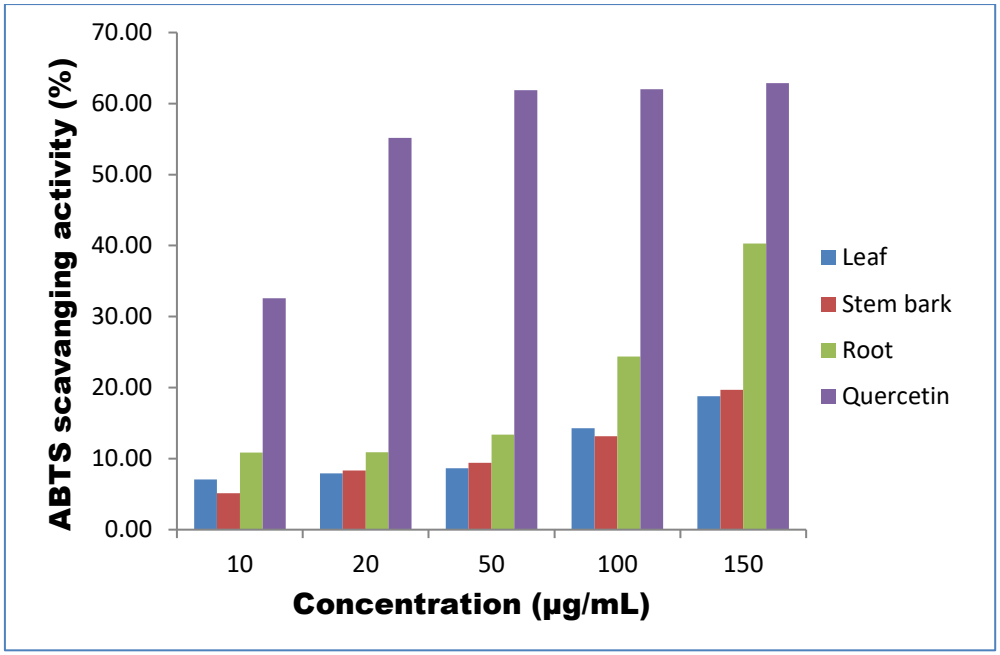


Figure 4.5: ABTS radical scavenging activity of the methanolic extracts from *D. zibethinus*

4.8 Biological Activities

4.8.1 Antibacterial Bioassay

The results of antibacterial activity studies of the methanol extracts of *D. zibethinus* are presented in Tables 4.9. The results revealed that the methanolic leaf extract exhibited antibacterial activity against *S. aureus* **(**16.00 ± 0.26 mm) and *E. coli* (14.07 ± 0.06 mm) while the growth of other bacteria were not inhibited by this extract. Maximum activity was observed against *S. aureus* (14.03 ± 0.06 mm), *S. epidermidis* (14.07 ± 0.06 mm) and *E. coli* (16.07 ± 0.06 mm), while other bacteria were resistant to methanolic stem bark extract. The results obtained for the methanolic stem bark extract (Table 4.9) of *D. zibethinus* against *E. coli* suggests that the antimicrobial activity may be favorably matched with the commercially available antibiotic Gentamicin drug of (14.00 mm). This manifest that purified extracts of *D. zibethinus* stem bark may be used in treating dieseases caused by *E. coli*. The methanolic root extract showed maximum antibacterial activity against *S. aureus* (24.00 ± 0.10 mm), *S. epidermidis* (12.07 ± 0.06 mm), *K. pneumoniae* (16.03 ± 0.06 mm) and *S. typhi* (14.03 ± 0.06

mm) but showed no activity against *E. coli*. The results obtained for the methanolic root extract of *D. zibethinus* (Table 4.9) against *K. pneumoniae* (16.03 ± 0.06 mm) suggests that the antimicrobial activity may match that of the commercially available antibiotic Gentamicin drug of (16.00 mm), an indication that purified extracts of *D. zibethinus* root may be used in treating diseases caused by *K. pneumoniae*. The results obtained were in assention with the report of Doughari *et al*. (2008).

Table 4.9: Antibacterial activity of methanolic extracts of *D. zibethinus*

Bacteria	Zone of Inhibition (mm)			
	Leaf	Stem bark (Ctr)	Root	Gentamicin
S. aureus	16.00 ± 0.26	14.03 ± 0.06	24.00 ± 0.10	___
S. epidermidis	___	14.07 ± 0.06	12.07 ± 0.06	22.00
K. pneumonia	___	___	16.03 ± 0.06	16.00
E. coli	14.07 ± 0.06	16.07 ± 0.06	___	14.00
S. typhi	___	___	14.03 ± 0.06	22.00

- = No zone of inhibition, Values expressed as mean ± SD, Ctr = Control

The graphical presentation is represented in Fig. 4.6 below:

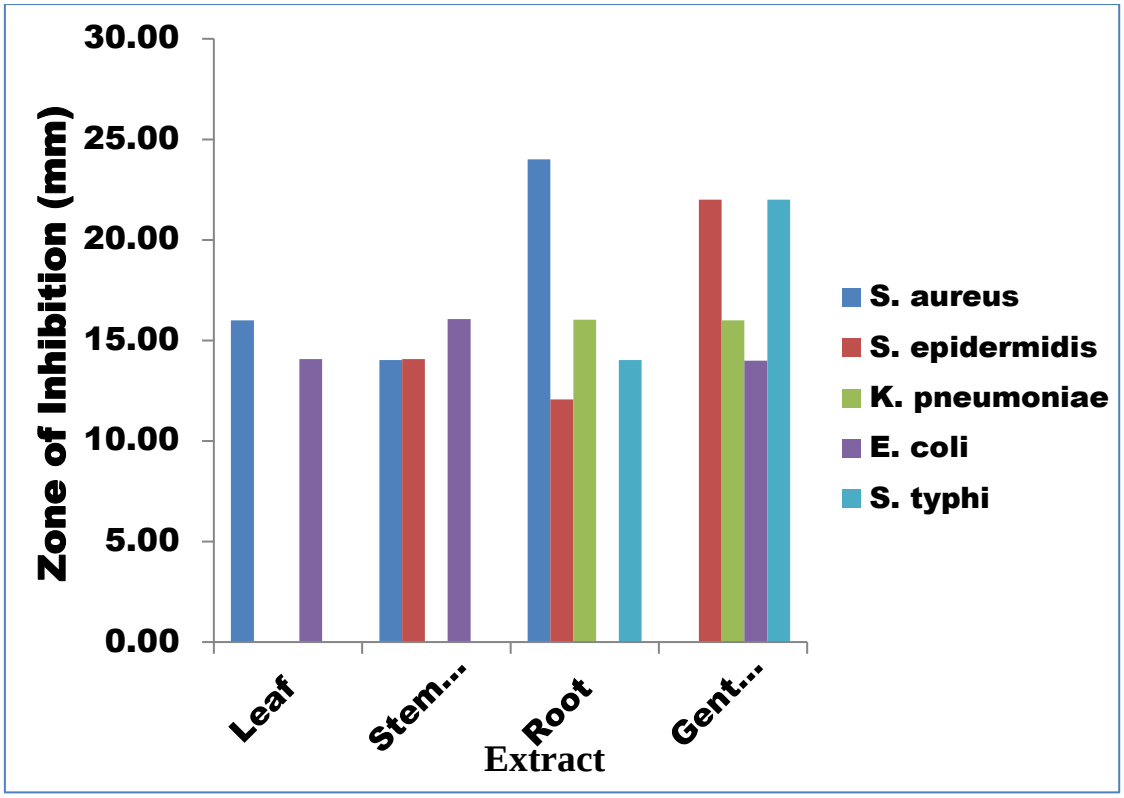


Figure 4.6: Antibacterial activity of methanolic extracts from *D. zibethinus*

4.8.2 Antifungal Bioassay

The results of antifungal activity studies of methanol extracts of *D. zibethinus* are presented in Table 4.10. The results revealed that methanolic extracts of leaf, stem bark and root exhibited higher antifungal activity against *C. albicans* as zones of inhibition were given as **(**10.33 ± 1.53 mm), (25.67 ± 2.08 mm) and (12.00 ± 1.00 mm) respectively. The zones of inhibition of *M. audouinii* were observed as (10.67 ± 2.08 mm), (15.00 ± 2.00 mm) and (11.67 ± 2.08 mm) for methanolic extracts of leaf, stem bark and root respectively. The results of antifungal activity studies showed that methanolic stem bark extract exhibited higher antifungal activity against *C. albicans* followed by methanolic root extract and methanolic leaf extract. Similarly, when comparing the antifungal activity against *M. audouinii*, the methanolic stem bark extract is highly sensitive followed by methanolic root extract and methanolic leaf extract. The antifungal activity of methanolic stem bark extract is significantly higher compared to the commercial antibiotic Fluconazole (20 mm) against *C. albicans* (25.67 ± 2.08 mm). Also, the antifungal activity of methanolic stem bark extract may compare favorably with the commercial antibiotic

Fluconazole (14 mm) against *M. audouinii* (15.00 ± 2.00 mm). The antifungal activities of the root and leaf extracts may be expressed as moderate when compare with the commercially available drug Fluconazole. Hence, the purified methanolic stem-bark extract *D. zibethinus* may be used in treating diseases caused by both *C. albicans* and *M. audouinii*. The data obtained in this study agreed with the report of Fayemi *et al*, (2012).

Table 4.10: Antifungal activity of methanolic extracts from *D. zibethinus*

Fungi	Zone of Inhibition (mm)			
	Leaf	**Stem bark**	**Root**	**Fluconazole (Ctr)**
C. albicans	10.33 ± 1.53	25.67 ± 2.08	12.00 ± 1.00	20.00
M. audouinii	10.67 ± 2.08	15.00 ± 2.00	11.67 ± 2.08	14.00

- = No zone of inhibition, Values expressed as mean ± SD, Ctr = Control

The graphical presentation is represented in Fig. 4.7 below:

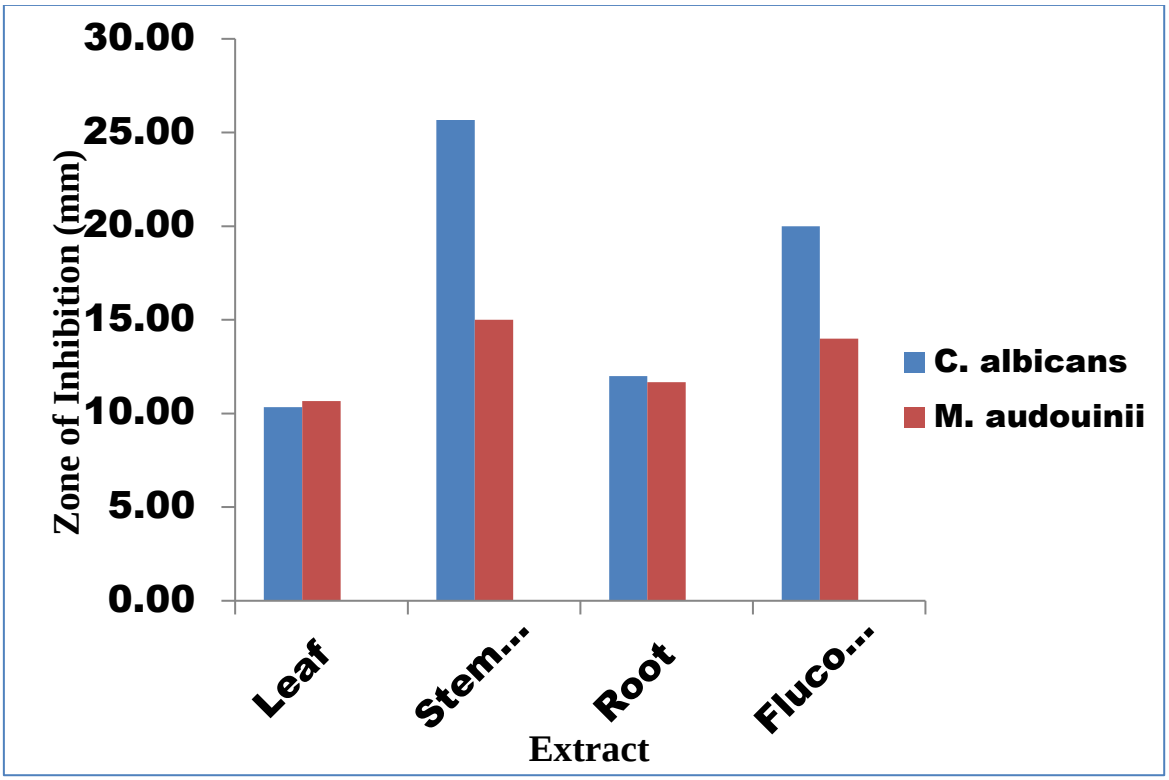


Figure 4.7: Antifugal activity of methanolic extracts from *D. zibethinus*

4.9 Anti-inflammatory activity

4.9.1 Lipoxygenase inhibition activity

The results of lipoxygenase (LOX) inhibition activity of methanolic extracts are summarized in Table 4.11 and Figure 4.8. Percentage lipoxygenase inhibition activity at 150 μg/mL concentrations of standard indomethacin, leaf, stem bark and root extracts from *D. zibethinus* were found to be 83.29 ± 0.01% (IC_{50} = 1.660 μg/mL), 62.97 ± 0.01% (IC_{50} = 1.464 μg/mL), 75.06 ± 0.01% (IC_{50} = 1.203 μg/mL) and 86.31 ± 0.01% (IC_{50} = 1.400 μg/mL) respectively. From Figure 4.8, it was noted that lipoxygenase inhibition activity increased with increase in concentration for the standard indomethacin and the methanolic extracts from *D. zibethinus* parts. The plant lipoxygenase pathway is in many respects equivalent to the 'arachidonic acid cascades' in animals (Bigby, 2002). For this reason, the *in vitro* inhibition of lipoxygenase constitutes a great show for the screening of plants with anti-inflammatory potential (Butovich & Lukyanova, 2008). LOXs are touchy cancer prevention agents and the foremost of their activity may comprise inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy or lipid peroxy- radical shaped in course of enzyme peroxidation. This could constrain the accessibility of lipid hydroperoxide substrate fundamental for the catalytic cycle of LOX.

Table 4.11: Anti- Lipoxygenase activity of methanolic Extracts from *D. zibethinus*

S/ No.	Sample concentration (µg/mL)	Leaf % inhibition	Stem bark % inhibition	Root % inhibition	Indomethacin % inhibition
1	10	29.15 ± 0.03	44.23 ± 0.01	34.16 ± 0.02	44.31 ± 0.01
2	20	37.61 ± 0.01	55.17 ± 0.01	57.67 ± 0.02	52.09 ± 0.01
3	50	56.48 ± 0.05	55.40 ± 0.01	70.82 ± 0.01	62.65 ± 0.01
4	100	60.31 ± 0.01	63.40 ± 0.01	83.87 ± 0.01	80.05 ± 0.01
5	150	62.97 ± 0.01	75.06 ± 0.01	86.31 ± 0.01	83.29 ± 0.01

Indomethacin was utilized as reference standard. Values were performed in triplicates and represented as mean ± SD.

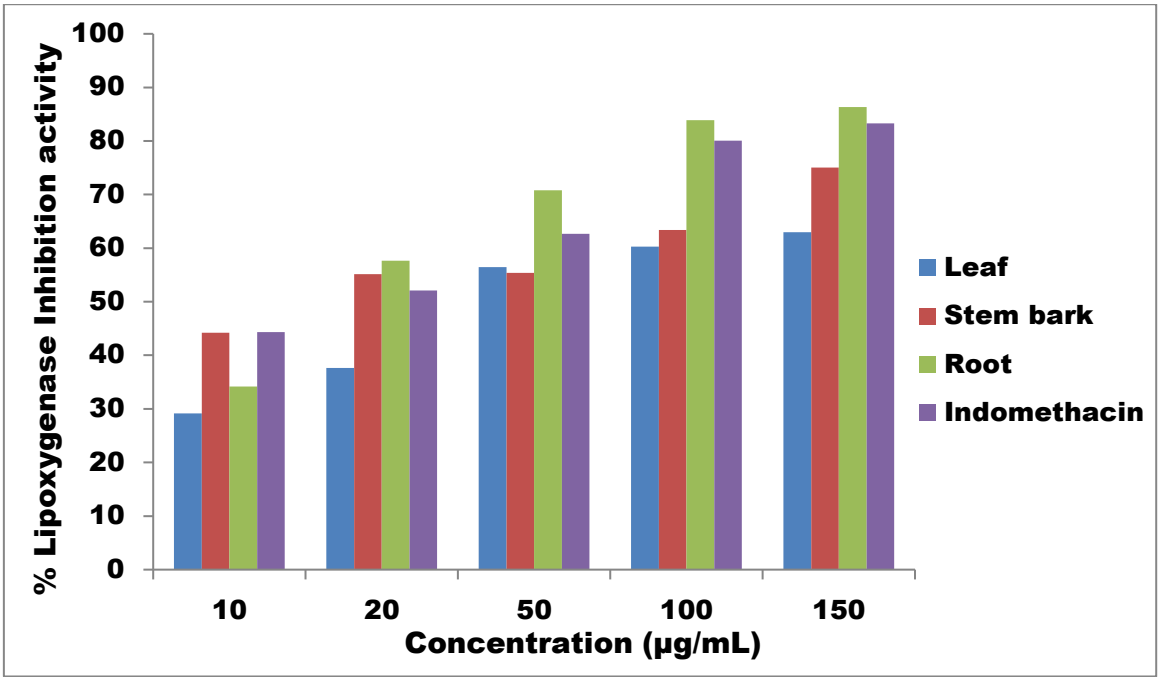


Figure 4.8: Lipoxygenase inhibition activity of the Stem bark extract from *D. zibethinus*

In later a long time, the search for phytochemicals having anti-inflammatory properties has been on the rise due to their potential utilize within the therapy of different incessant and irresistible infections. Epidemiology and experimental studies have involved oxidative cellular harm emerging from an awkwardness between free radical generating and scavenging systems

as essential cause of cardiovascular, diseases, cancer, aging etc (Celermajer *et al*., 2012). High total phenolic content values found in methanol extract from *D. zibethinus* implies the role of phenolic compounds in contributing these activities. Plant phenolic compounds have been established to have potent anti-inflammatory activity (Sakat *et al*., 2010; Roy *et al*., 2010; Garg *et al*., 2010). The results obtained from the studies on *D. zibethinus* have displayed a potential anti-inflammatory activity. The *D. zibethinus* extracts inhibited the lipoxygenase enzyme activity. This indicates that *D. zibethinus* plant is more useful in studies of inflammation and in various related physiological studies, aging and diseases such as cancer, neurological disorder etc.

4.10 Research Findings

It was discovered that

(i) *D. zibethinus* is very rich in phytochemicals that have great medicinal values.

(ii) Three isomeric triterpenoic acids (maslinic, 3-epimaslinic and bredemolic acids) were isolated from *D. zibethinus* stem bark and characterised based on IR and ^{1}H nmr analyses.

(iii) The major chemical constituents in the stem bark fractions (SBF1-4) are oleic acid [(Z)-9-Octadecenoic acid] (91.36 %), elaidic acid [(E)-9-Octadecenoic acid] (56.10 %), petroselinic acid [(Z)-6-Octadecenoic acid] (41.19 %) and elaidic acid [(E)-9-Octadecenoic acid] (42.20 %) respectively, based on GC-MS analysis.

(iv) The plant contained appreciable amount of minerals (Ca, K, Fe, Mn etc.) that can boost human immunity.

(v) The flavonoid contents (4.71 ± 2.10 mg QCE/g DW) is relatively lower

than phenolic contents (23.32 ± 1.73 mg QCE/g DW) in the plant.

(vi) The antioxidant activity of the stem bark extract cannot be favourably compared with the standard drug (quercetin) and this could be attributed to the lower quantity of flavonoids in the stem bark.

(vii) The plant extracts showed moderate to high antimicrobial activity against *K. pneumoniae, E. coli, C. albicans* and *M. audouinii*.

(viii) The anti-inflammatory activity of stem bark extract can be favourably compared with the standard drug (indomethacin) and this could be attributed to the substantial quantity of phenolics in the stem bark.

4.11 Contribution to Knowledge

The chemistry of *D. zibethinus* leaves, stem bark and roots has been poorly investigated. Most of the works reported in the literature are limited to pharmacological investigations of fruit and the rinds of *D. zibethinus*, with little or no attempt on the characterization of the bioactive compounds from this plant species.

The present work is thus the first attempt on the systematic isolation, purification and structure elucidation of bioactive compounds from the stem bark of *D. zibethinus*. We report in this thesis the isolation and structure elucidation of three (3) isomeric triterpenoic acids, which to the most excellent of our information, have not been previously isolated from the plant parts.

This work is also the first attempt at validating the antioxidant, antimicrobial and anti-lipooxygenase activities of *D. zibethinus*.

4.12 Conclusion

In conclusion, *D. zibethinus* Murr is rich in bioactive phytochemicals and minerals that are critical for human health and well-being. The plant extracts exhibited high efficacy as anti-lipoxygenase agents. The results suggest that the plant materials may be useful in the formulation of nutritional and/or therapeutic products. This research has contributed to the global effort to encourage use of herbal drugs from natural sources which has been generally accepted as safer drugs to synthetic ones.

REFERENCES

Abay, G., Altun, M., Karakoc, O. C., Gul, F., and Demirtas, I. (2013). Insecticidal action of unsaturated fat rich Turkish bryophyte extricates against Sitophilus granarius (Coleoptera: Curculionidae). Combinatorial science and high throughput screening, 16(10), 806-816.

Abdille, M. H., Singh, R. P., Jayaprakasha, G. K., and Jena, B. S. (2005). Cancer prevention agent movement of the concentrates from Dillenia indica organic products. Nourishment science, 90(4), 891-896.

Abdullahi, A. A. (2011). Patterns and difficulties of conventional drug in Africa. African diary of conventional, correlative and elective drugs, 8(5S).

Acamovic, T., and Brooker, J. D. (2005). Organic chemistry of plant auxiliary metabolites and their belongings in creatures. Procedures of the Nutrition Society, 64(3), 403-412.

Acharya, D., and Shrivastava, A. (2008). Indigenous natural prescriptions. Aavishkar Publishers, Distributors.

Afrose, S., Hossain, M., Salma, U., Miah, A. G., and Tsujii, H. (2010). Dietary karaya saponin and Rhodobacter capsulatus apply hypocholesterolemic impacts by concealment of hepatic cholesterol combination and advancement of bile corrosive blend in laying hens. Cholesterol, 2010.

Agati, G., Azzarello, E., Pollastri, S., and Tattini, M. (2012). Flavonoids as cancer prevention agents in plants: area and utilitarian essentialness. Plant science, 196, 67-76.

Agati, G., Matteini, P., Goti, An., and Tattini, M. (2007). Chloroplast-located flavonoids can rummage singlet oxygen. New Phytologist, 174(1), 77-89.

Agu, G. C., and Thomas, B. T. (2012). Antibacterial exercises of ethanol and fluid concentrates of five Nigerian restorative plants on some twisted pathogens. Nature and Science, 10(2), 78-84.

Ahmad, M., and Wajid, M. (2013). Plants as potential wellspring of antimicrobial operators. J Pharm Altern Med, 2, 18-25.

Ahmed, A., Pitt, B., Rahimtoola, S. H., Waagstein, F., White, M., Love, T. E., and Braunwald, E. (2008). Impacts of digoxin at low serum focuses on mortality and hospitalization in cardiovascular breakdown: an inclination coordinated investigation of the DIG preliminary. Worldwide diary of cardiology, 123(2), 138-146.

Ahn, K. (2017). The overall pattern of utilizing organic medications and techniques for creating worldwide medications. BMB reports, 50(3), 111.

Aiyelaagbe, O. O., and Osamudiamen, P. M. (2009). Phytochemical screening for dynamic mixes in Mangifera indica leaves from Ibadan, Oyo State. Plant Sci Res, 2(1), 11-13.

Akbay, P., Basaran, A. An., Undeger, U., and Basaran, N. (2003). In vitro immunomodulatory action of flavonoid glycosides from Urtica dioica L. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 17(1), 34-37.

Alasalvar, C., Grigor, J. M., Zhang, D., Quantick, P. C., and Shahidi, F. (2001). Correlation of volatiles, phenolics, sugars, cancer prevention agent nutrients, and tangible nature of various shaded carrot assortments. Diary of Agricultural and Food Chemistry, 49(3), 1410-1416.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., and Lightfoot, D. A. (2017). Phytochemicals: Extraction, detachment, and distinguishing proof of bioactive mixes from plant removes. Plants, 6(4), 42.

Amin, I., Norazaidah, Y., and Hainida, K. E. (2006). Cancer prevention agent movement and phenolic substance of crude and whitened Amaranthus species. Nourishment science, 94(1), 47-52.

Aminov, R. I. (2010). A short history of the anti-infection period: exercises learned and challenges for what's to come. Wildernesses in microbiology, 1, 134.

Andersen, O. M., and Markham, K. R. (2005). Flavonoids: science, natural chemistry and applications. CRC press.

Andre, C. M., Hausman, J. F., and Guerriero, G. (2016). Cannabis sativa: the plant of the thousand and one atoms. Boondocks in plant science, 7, 19.

Angeh, J. E. (2007). Separation and portrayal of hostile to bacterial mixes present in individuals from Combretum area, Hypocrateropsis (Doctoral paper, University of Pretoria).

Aniszewski, T. (2007). Alkaloids-Secrets of Life:: Aklaloid Chemistry, Biological Significance, Applications and Ecological Role. Elsevier.

Ansari, R. M. (2016). Potential utilization of durian natural product (Durio zibenthinus Linn) as an extra to treat barrenness in polycystic ovarian disorder. Diary of integrative prescription, 14(1), 22-28.

Aoshima, H., Miyagisnima, A., Nozawa, Y., Sadzuka, Y., and Sonobe, T. (2005). Glycerin unsaturated fat esters as another oil of tablets. Global diary of pharmaceutics, 293(1-2), 25-34.

April, U. (2016). What are Essential Oils with Monoterpenes and how might they advantage your wellbeing? Fundamental oil princess, pp 12-16.

Arif, T., Bhosale, J. D., Kumar, N., Mandal, T. K., Bendre, R. S., Lavekar, G. S., and Dabur, R. (2009). Common items antifungal specialists got from plants. Diary of Asian normal items investigate, 11(7), 621-638.

Aruoma, O. I. (2003). Methodological contemplations for portraying potential cell reinforcement activities of bioactive segments in plant nourishments. Transformation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 523, 9-20.

Ashraf, M. A., Maah, M. J., and Yusoff, I. (2010). Estimation of cancer prevention agent phytochemicals in four unique assortments of durian (Durio zibethinus murray) organic product. Center East J Sci Res, 6(5), 465-71.

Asres, K., Bucar, F., Knauder, E., Yardley, V., Kendrick, H., and Croft, S. L. (2001). In vitro antiprotozoal action of concentrate and mixes from the stem bark of Combretum molle. Phytotherapy Research, 15(7), 613-617.

Ateufack, G., Mokam, E. C. D., Mbiantcha, M., Feudjio, R. B. D., David, N., and Kamanyi, A. (2015). Gastroprotective and ulcer mending impacts of piptadeniastrum Africanum on tentatively instigated gastric ulcers in rodents. BMC correlative and elective drug, 15(1), 214.

Attia, Y. An., Al-Harthi, M. A., Korish, M. An., and Shiboob, M. M. (2015). Unsaturated fat and cholesterol profiles and hypocholesterolemic, atherogenic, and thrombogenic lists of table eggs in the retail showcase. Lipids in wellbeing and malady, 14(1), 136.

Babbar, N., Oberoi, H. S., Sandhu, S. K., and Bhargav, V. K. (2014). Impact of various solvents in extraction of phenolic mixes from vegetable deposits and their assessment as common wellsprings of cancer prevention agents. Diary of nourishment science and innovation, 51(10), 2568-2575.

Bachran, C., Bachran, S., Sutherland, M., Bachran, D., and Fuchs, H. (2008). Saponins in tumor treatment. Scaled down surveys in therapeutic science, 8(6), 575-584.

Bak, I., Lekli, I., Juhasz, B., Varga, E., Varga, B., Gesztelyi, R., ... and Tosaki, A. (2010). Segregation and investigation of bioactive constituents of harsh cherry (Prunus cerasus) seed bit: a rising useful nourishment. Diary of restorative nourishment, 13(4), 905-910.Food. Diary of Medicinal Food, 13(4): 905–910.

Balcerek, M., and Szopa, J. (2012). Ethanol biosynthesis and hydrocyanic corrosive freedom during organic product pounds maturation. Czech Journal of Food Sciences, 30(2), 144-152.

Baños Díez, J.E. and March, P.M. (2002). Farmacología visual (in Spanish) (2ndEditions) UPC. p. 87. (ISBN 8483016478).

Beghyn, T., Deprez-Poulain, R., Willand, N., Folleas, B., and Deprez, B. (2008). Regular mixes: leads or thoughts? Bioinspired particles for sedate disclosure. Substance science and medication plan, 72(1), 3-15.

Belgis, M., Wijaya, C. H., Apriyantono, A., Kusbiantoro, B., and Yuliana, N. D. (2016). Physicochemical contrasts and tangible profiling of six lai (Durio kutejensis) and four

durian (Durio zibethinus) cultivars indigenous Indonesia. Universal Food Research Journal, 23(4).

Belgis, M., Wijaya, C. H., Apriyantono, A., Kusbiantoro, B., and Yuliana, N. D. (2017). Volatiles and fragrance portrayal of a few lai (Durio kutejensis) and durian (Durio zibethinus) cultivars developed in Indonesia. Scientia Horticulturae, 220, 291-298.

Bennick, A. (2002). Association of plant polyphenols with salivary proteins. Basic Reviews in Oral Biology and Medicine, 13(2), 184-196.

Bentley, R. (2009). Various streets to disclosure; Prontosil (consequently sulfa medications) and penicillin (subsequently β-lactams). Diary of modern microbiology and biotechnology, 36(6), 775-786.

Berkov, S., Reyes-Chilpa, R., Codina, C., Viladomat, F., and Bastida, J. (2007). Changed NMR information for incartine: an alkaloid from Galanthus elwesii. Atoms, 12(7), 1430-1435.

Bhat, R. and Paliyath, G. (2015). In "Reference book of Food and Health", first Edition, Academic Press, United State of America. 4006 pages. ISBN: 9780123849533.

Bhore, S.J., Husin, N.A., Rahman, S., Karunakaran, R. (2018). A Review on The Nutritional, Medicinal, and Genome Attributes of Durian (Durio zibethinus L.), the King of Fruits in Malaysia, Bioinformation, 14(6): 265-270.

Bielawski, K., Winnicka, K. and Bielawska, A. (2006). Hindrance of DNA Topoisomerases I and II, and Growth Inhibition of Breast Cancer MC 7 Cells by Ouabain, Digoxin and Proscillaridin A. Organic and Pharmaceutical Bulletin, 29: 1493–1497.

Bigby, T.D. (2002). The Yin and the Yang of 5-Lipoxygenase PathwayActivation. Atomic Pharmacology, 62(2): 200-202.

Borokini, T.I. and Lawal, I.O. (2014). Conventional drug rehearses among the Yoruba individuals of Nigeria: A chronicled point of view. Diary of therapeutic plants examines, 2(6): 20-33.

Bowen, H.R. (2006). Particular decontamination of mono-terpenes for evacuation of oxygen containing species. pp: 1-10. US20060100470.

Brahmachari, G. (2010). Handbook of Pharmaceutical Natural Products.Weinheim: Wiley-VCH. 496 pages. (ISBN 978-3-527321483).

Brahmkshatriya, P.P. and Brahmkshatriya, P.S. (2013). Terpenes: Chemistry, Biological Role, and Therapeutic Applications. Normal Products, pp 2665-2691.

Brender, J.R., Hartman, K. and Nanga, R.P.R. (2010). Job of zinc in human islet amyloid polypeptide collection. Diary of the American Chemical Society, 132(26): 8973–8983.

Briasoulis, An., Agarwal, V. and Pierce, W.J. (2011). QT prolongation and torsade de pointes incited by fluoroquinolones: inconsistent reactions from normally utilized drugs. Cardiology, 120(2): 103-110.

Brodowska, K.M. (2017). Normal flavonoids: arrangement, potential job, and utilization of flavonoid analogs. European Journal of Biological Research, 7 (2): 108-123.

Brusotti, G., Tosi, S., AldoTava, An., Anna, M., Picco, A.M., Pietro, G.P., Cesari, I. and Gabriele, C.G. (2013). Antimicrobial and phytochemical properties of stem-bark removes from Piptadeniastrum africanum (Hook f.) Brenan. Mechanical Crops and Products, 43: 612-616.

Buricova, L. and Reblova, Z. (2008). Czech Medicinal Plants as Possible Sources of cell reinforcements. Czech Journal of Food Science, 26(2): 132–138.

Butovich, I.A. and Lukyanova, S.M. (2008). Hindrance of Lipoxygenases and Cyclooxygenases by Linoleyl Hydroxamic Acid: Comparative In Vitro Studies. Diary of Lipid Research, 49(6): 1284–1294.

Butt-centric, J. M. H. (2014). Follow and basic components examination in Cymbopogon citratus (DC.) stapf tests by graphite heater nuclear assimilation spectroscopy and its wellbeing concern. Diary of toxicology, 2014.

Buzzini, P., Arapitsas, P., Goretti, M., Branda, E., Turchetti, B., Pinelli, P., Ieri, F. and Romani, A. (2008). Antimicrobial and antiviral action of hydrolysable tannins. Smaller than expected Reviews in Medicinal Chemistry, 8: 1179-1187

CA. (Preserve Africa) (2002). Africa: Overview on Medicinal Plants and Traditional Medicine. Moderate Africa. Pambazuka News.

Cabral, C.E. and Klein, M.R.S.T. (2017). Phytosterols in the Treatment of Percholesterolemia and Prevention of Cardiovascular Diseases. Arquivos Brasileiros de Cardiologia, 109(5): 475–482.

Cantrell, C.L., Franzblau, S.G. and Fischer, N.H. (2001). Antimycobacterial Plant Terpenoids. Planta Medica, 67: 685-694.

Carvalho, R.N., Moura, L.S., Rosa, P.T.V. and Meireles, M.A.A. (2005). Supercritical liquid extraction from Rosemary (Rosmarinus officinalis): Kinetic information, concentrate's worldwide yield, arrangement and cell reinforcement action. Diary of Supercritical Fluids, 35: 197–204.

Celermajer, D.S., Chow, C.K., Marijon, E., Anstey, N.M. and Woo, K.S. (2012). Cardiovascular Disease in the creating scene: Prevalences, designs, and the capability of early infection location. Diary of the American College of Cardiology, 60(14): 1207-1216.

Cerdá, B., Cerón, J.J., Tomás-Barberán, F.A. and Espín, J.C. (2003). "Rehashed Oral Administration of High Doses of the Pomegranate Ellagitannin Punicalagin to Rats for 37 Days isn't Toxic". Diary of Agricultural and Food Chemistry. 51(11): 3493–501.

CHABC. (Canadian Herbalist Association of British Columbia) (2017). What is herbalism?

Chahar, M.K., Sharma, N., Dobhal, M.P. and Joshi, Y.C. (2011). Flavonoids: AVersatile Source of Anticancer Drugs. Pharmacognosy Reviews, 5(9): 1-12.

Chaieb, I. (2010). Saponins As Insecticides: A Review. Tunisian Journal of Plant Protection, 5: 39-5.

Chang, C.C., Yang, M.H. and Wen, H.M. (2002). Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. Diary of Food and Drug Analysis, 10: 178-182.

Chang, J. and Case, R. (2005). Phenolic glycosides and ionone glycoside from the stem of Sargentodoxa cuneata. Phytochemistry, 66(23): 2752-2758.

Chang, Y., Hou, H. and Li, B. (2016). Distinguishing proof of unstable mixes in Codfish (Gadus) by a blend of two extraction strategies combined with GC-MS Analysis. Diary of Ocean University of China, 15: 509 514.

Chansiripornchai, P. and Pongsamart, S. (2008). Treatment of contaminated open injury on two canines utilizing a film dressing of polysaccharide separated from the frames of Durian (Durio Zibethinus Murr.): case report, Thai Journal of Veterinary Medicine, 38(3): 55-61.

Charoenkiatkul, S., Thiyajai, P. and Judprasong, K. (2015). Supplements and bioactive mixes in famous and indigenous durian (Durio zibethinus murr.). Nourishment Chemistry, 193: 181–186.

Chatterjee, R., Slentz, C., Davenport, C.A., Johnson, J., Lin, P., Muehlbauer, M., D'Alessio, D., Svetkey, L.P. and Edelman, D. (2017). Impacts of otassium supplements on glucose digestion in African Americans with prediabetes: A pilot preliminary. American diary of clinical sustenance, 106(6): 1431–1438.

Chen, Z. (2008). Research of antioxidative limit in fundamental oils of plants. China Condiment, 11:40–43.

Cheng, K., Zhang, P., Liu, J., Xie, J. and Sun, H. (2008). Down to earth Synthesis of Bredemolic Acid, a Natural Inhibitor of Glycogen Phosphorylase. Diary of Natural Products, 71(11): 1877-1880.

Cho, J.G., Song, N.Y., Nam, T.G., Shrestha, S., Park, H.J., Lyu, H.N., Kim, D.O., Lee, G., Woo, Y.M., Jeong, T.S. and Baek, N.I. (2013). Flavonoids from the Grains of C1/R-S Transgenic Rice, The Transgenic Oryza Sativa spp. Japonica, and Their Radical

Scavenging Activities. Diary of farming and nourishment science, 61(43): 10354-10359.

Chopra, I., Hesse, L. and O'Neill, A. (2002). "Disclosure and advancement of new enemy of bacterial medications," in Pharmacochemistry Library, Vol 32, Trends in Drug Research III, ed. van der Goot H., editorial manager. (Amsterdam: Elsevier), 213–225.

Chuah, L., Shamila-Syuhada, A.K., Liong, M.T., Rosma, A., Thong, K.L. and Rusul, G. (2016). Physio-substance, microbiological properties of Tempoyak and atomic characterisation of Lactic Acid microscopic organisms Isolated from Tempoyak. Nourishment Microbiology, 58: 95–104.

Clausen, M.J.V. and Poulsen, H. (2013). Section 3 Sodium/Potassium Homeostasis in the Cell. In Banci, Lucia (Ed.). Metallomics and the Cell. Metal Ions in Life Sciences, 12. Springer. ISBN 978-94-007-5560-4.

Clauss, M., Castell, J.C., Kienzle, E., Dierenfeld, E.S., Flach, E.J., Behlert, O., Ortmann, S., Streich, W.J., Hummel, J. and Hatt, J.M. (2007). Th Influence of Dietary Tannin Supplementation on Digestive Performance in Captive Black Rhinoceros (Diceros bicornis). Diary of Animal Physiology and Animal Nutrition (Berl), 91(11-12): 449-458.

Clifford, M.N. (2000). Chlorogenic Acids and Other Cinnamates—Nature, Occurrence, Dietary Burden, Absorption and Metabolism. Diary of the Science of Food and Agriculture, 80: 1033–1043.

Closson, A. and Monteleone, M.G. (2009). Organoleptic mixes. pp: 1-13. US20090156456.

Cogswell, M.E., Zhang, Z., Carrquiry, A.L., Gunn, J.P., Kuklina, E.V., Saydah, S.H. and Moshfegh, A.J. (2012). Sodium and potassium admissions among U.S. grown-ups: NHANES 2003-2008. American Journal of Clinical Nutrition, 96(3): 647-657.

Connolly, J.D. and Hill, R.A. (2010). Triterpenoids, Natural Product Reports, 27: 79-132.

Cordell, J. (2002). Common Products in Drug Discovery Creating a New Wisdom. Phytochemistry Review, 1: 261-273.

Costa, C., Tsatsakis, A., Mamoulakis, C., Teodoro, M., Briguglio, G., Caruso, E. Tsoukalas, D., Margina, D., Efthimious, D., Kouretas, D. and Fenga, C. (2017). Ebb and flow proof on the impact of dietary polyphenols consumption on incessant illnesses. Nourishment and compound toxicology, 110: 286–299.

Courtwright, D.T. (2009). Powers of propensity drugs and the creation of the cutting edge world (first Ed.). Cambridge, Mass.: Harvard University Press. pp. 36-37. (ISBN 978-0-674-02990-3).

Cragg, G.M. and Newman, D.J. (2002). Modern applications normal items for restorative purposes. Medications from nature: Present advancement and future possibilities. In

Natural items in the new millenium: Prospects and Industrial Applications; Rauter A.P, Palma F.B, Justino J, Araújo M.E and Dos Santos S.P (2002) Eds. Kluwer: Dordrecht, The Netherlands, 441-461.

Cravotto, G., Boffa, L., Genzini, L. and Garella, D. (2010). "Phytotherapeutics: an assessment of the capability of 1000 plants". Diary of Clinical Pharmacy and Therapeutics, 35 (1): 11–48.

Crosbie, P.A.J. and Woodhead, M.A. (2009). Long haul macrolide treatment in ceaseless fiery aviation route sicknesses. European Respiratory Journal, 33: 171-181.

Cushnie, T.P.T. and Lamb, A.J. (2005). Antimicrobial movement of flavonoids International Journal of Antimicrobial Agents, 26: 343-356.

Cushnie, T.P.T., Cushnie, B. and Lamb, A.J. (2014) "Alkaloids: An Overview of Their Antibacterial, Antibiotic-Enhancing and Antivirulence exercises," International Journal of Antimicrobial Agents, 44(5): 377–386.

Cutler, S. and Cutler, H.G. (2000). Organically Active Natural Products: Pharmaceuticals. CRC Press. p. 5. (ISBN 978-0-8493-1887-0).

Dalia, A.B. (2011). Insecticidal and antifeedant exercises and compound organization of Casimiroa edulis La Llave and Lex (Rutaceae) leaf concentrate and its divisions against Spodoptera Littoralis Larvae. Australian Journal of Basic and Applied Sciences, 5(9): 693-703.

Daniel, D., Susal, C., Kopp, B., Opelz, G. and Terness, P. (2003). Apoptosis-Mediated Selective Killing of Malignant Cells via Cardiac Steroids: Maintenance of Cytotoxicity and Loss of Cardiac Activity of Chemically Modified Derivatives. Universal Immunopharmacology, 3: 1791–1801.

Dark colored, E.S. and Chandler, P.A. (2001). Disposition and psychological changes during fundamental corticosteroid treatment. Tidy Care Companion. Diary of Clinical Psychiatry, 3(1): 17-21.

Dark colored, J.E. and Kelly, M.F. (2007). Hindrance of lipid peroxidation by anthocyanins, anthocyanidins and their phenolic corruption items. European Journal of lipid science and Technology, 109(1): 671.

Darker, M.J. (1997). Durio – A Bibliographic Review. Worldwide plant hereditary assets establishment (IPGRI): New Delhi, India, pp. 2–87, ISBN 92-9043-3-18-3.

Davis, C.D. and Milner, J.A. (2009). Gastrointestinal microflora, nourishment segments and colon malignancy avoidance. Diary of Nutritional Biochemistry, 20(10): 743-752.

de Roode, J.C., Lefèvre, T. and Hunter, M.D. (2013). Self-drug in creatures. Science, 340(6129): 150-151.

Devalaraja, S., Jain, S. and Yadav, H. (2011). Colorful natural products as restorative supplements for diabetes, corpulence and metabolic disorder. Nourishment Research International, 44: 1856-1865.

Dewanto, X., Wu, K., Adom, K. and Liu, R.H. (2002). Warm Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. Diary of Agricultural and Food Chemistry, 50: 3010–3014.

Dewis, M.L., Huber, M.E., Agyemang, D.O. and Conklin, G. (2009). Pieces of oxo-terpene subsidiaries and procedure for setting up the equivalent. US20090180973.

Dixon, R.A., Xie, D.Y. and Sharma, S.B. (2005). Proanthocyanidins—A Final Frontier in Flavonoid investigate? New Phytologist, 165: 9–28.

Doherty, V.F., Olaniran, O.O. and Kanife, U.C. (2010). Antimicrobial exercises of Framomum melegueta (Alligator pepper). Global Journal of Biology, 2(2): 126 – 131.

Dorantes-Aranda, J.J., Garcia-de la Parra, L.M., Alonso-Rodriquez, R. and Morquecho, L. (2009). Hemolytic Activity and Fatty Acids Composition in the Ichthyotoxic Dinoflagellate Cochlodinium polykrikoides Isolated from Bahía de La Paz, Gulf of California. Marine Pollution Bulletin, 58(9): 1401-1405.

Doughari, J.H., Human, I.S., Bennade, S. and Ndakidemi, P.A. (2009). Phytochemicals as chemotherapeutic operators and cancer prevention agents: Possible answer for the control of anti-microbial safe Verocytotoxin delivering microscopic organisms. Diary of restorative plants look into, 3(11): 839-849.

Drozak, J. and Bryla, J. (2005). Dopamine: not only a synapse. Postepy Higieny I Medycyny Doswiadczalnej, (on the web), 59: 405-420.

Edrah, S.M., Alfalluos, K.A., Alnade, H.S., Kollab, W.A. and Alafid, F. (2017). Subjective and quantitative phytochemical investigation and antimicrobial movement of "Retama" remove developed in Zliten Libya, International Journal of Medical Science and Clinical Inventions, 4(4): 2861-2866.

Edreva, A., Velikova, V., Tsonev, T., Dagnon, S., Gürel, A.L. and Aktas, L. (2008). Stress-defensive job of optional metabolites: Diversity of capacities and instruments. General and Applied Plant Physiology, 34: 67–78.

Ejikeme, C.M., Ezeonu, C.S. and Eboatu, A.N. (2014). "Assurance of Physical and Phytochemical Constituents of Some Tropical Timbers Indigenous to Niger Delta Area of Nigeria". European Scientific Journal, 10(18): 247–270.

Ekinci, N., Ekinci, R., Polat, R. and Budak, G. (2004). Examination of follow elementsin therapeutic plants with Energy Dispersive X-Ray Fluorescence. Diary of Radioanalytical and Nuclear Chemistry, 260: 127-131.

Elekofehinti, O.O., Adanlawo, I.G., Saliu, J.A. and Sodehinde, S.A. (2012). Saponins From Solanum anguivi Fruits display hypolipidemic potential in Rattus novergicus. Der Pharmacia Lettre, 4(3): 811-814.

EL-Farok, D.H.M., EL-Denshry, E.S., Mahmou, M., Asaaf, N. and Emam, M. (2013). Lipid bringing down impact of ginseng and alpha-lipoicacid in Hypercholesterolemic patients. Worldwide Journal of Pharmacology, 7(3): 298-306.

Epidi, J.O., Izah, S.C. and Ohimain, E.I. (2016). Antibacterial and synergistic adequacy of concentrates of Alstonia boonei tissues. English Journal of Applied Science, 1(1): 21–26.

Ernest, S. (2011). Top 100 Exotic Food Plants, first Edition, CRC Press, Taylor and Francis Group, Boca Raton, Florida, USA. 708 pages.

Eshwarappa, R.S.B., Ramachandra, Y.L., Subaramaihha, S.R., Subbaiah, S.G.P., Austin, R.S. and Dhananjaya, B.L. (2016). Hostile to Lipoxygenase action of leaf nerve concentrates of Terminalia chebula (Gaertn.) Retz. (Combretaceae). Pharmacognosy Research, 8(1): 78–82.

Fabricant, D.S. and Farnsworth, N.R. (2001). The estimation of plants utilized in customary medication for tranquilize revelation. Natural Health Perspectives, 109: 69–75.

Faizal, A. and Geelen, D. (2013). Saponins and their job in organic procedures in plants. Phytochemistry Reviews, 12: 877–893.

Farrant, J.M. (2000). A Comparison of Mechanisms of Desiccation Tolerance Among Three Angiosperm Resurrection Plants. Plant biology, 151(29): 121-125.

Farrant, J.M. (2000). A correlation of instruments of drying up resilience among three Angiosperm restoration plants. Plant Ecology, 151(29): 121-125.

Faten, M., Hanen, F., Riadh, K. and Chedly, A. (2014). Complete phenolic, flavonoid and tannin substance, cell reinforcement, antimicrobial exercises of natural concentrates of shoots of the plant Limonium delicatulum. Diary of Taibah University for Science, 8: 216–224.

Fayemi, S.O., Osho, A. and Atolani, O. (2012). In Vitro Anticandidal and Antioxidant Potential of Mezoneuron benthamianum, Journal of Acute Disease; pp: 120-125.

Ferhat, M., Zahia, K., Yoshinori, F. and Hiroshi, A. (2017). Two New Triterpenes and different Compounds from Mentha aquatica (Lamiaceae). Characteristic item interchanges, 12(4): 483-486.

Ferreira, D., Gross, G.G., Hagerman, A.E., Kolodziej, H. and Yoshida, T. (2008). Tannins and related polyphenols: Perspectives on their science, science, natural impacts, and human wellbeing security. Phytochemistry, 69: 3006-3008.

Fox, P.L. (2003). The copper-iron accounts: the narrative of a close connection. BioMetals, 6: 9-40. Fraga, B.M. (2008). Common sesquiterpenoids, Natural Product Reports, 25: 1180-1209.

Francis, G., Kerem, Z., Makkar, H.P.S. and Becker, K. (2002). The Biological Action of Saponins in Animal Systems: An audit. English Journal of Nutrition, 88: 587–605.

Franklin, L., Cloud, E., Knapp, L., Ostroff, G. and Harman, G. (2010). Terpene-containing sytheses and strategies for making and utilizing them. pp: 1-137. US20100136102.

Frese, S., Frese-Schaper, M., Andres, A.C., Miescher, D., Zumkehr, B. and Schmid, R.A. (2006). Cardiovascular glycosides start Apo2L/TRAIL-prompted apoptosis in non-little cell lung and disease cells by up-guideline of death receptors 4 and 5. Malignancy Research, 66: 5867–5874.

Fukumoto, L. and Mazza, G. (2000). Evaluating cell reinforcement and master oxidant exercises of phenolic mixes. Diary of Agricultural and Food Chemistry, 48: 3597–3604.

Furlan, C.M., Motta, L.B. and Santos, D. (2011). Tannins: What do they representin vegetation? In: Tannins: Types, Foods Containing, and Nutrition, Chapter 10, Nova science distributers, Inc. New York, pp: 1-13.

García-Lafuente, A., Villares, A., Guillamón, E., Rostagno, M.A. and Martínez, J.A. (2009). Flavonoids as calming operators: Implications in malignant growth and cardiovascular infection. Aggravation Research, 58: 537–552.

Garg, V.K.R., Jain, M., Sharma, P.K.R. and Garg, G. (2010). Calming movement of Spinacia oleracea. Global Journal of Pharma.Professional's Research, 1(1): 1-4.

Geetha, S., Sai-Ram, M., Mongia, S.S., Singh, V. and Ilavazha-gan, G. (2003). Assessment of Antioxidant Activity of Leaf Extract of Sea Buckthorn (Hippophae rhamnoides L.) on Chromium (VI) Induced Oxidative Stress in Albino Rats, Journal of Ethnopharmacology, 87: 247–251.

Geldof, N. and Engeseth, N.J. (2002). Cancer prevention agent Capacity of Honeys from Various Floral Sources Based on the Determination of Oxygen Radical Absorbance Capacity and Inhibition of In Vitro Lipoprotein Oxidation in Human Serum Samples. Diary of Agricultural and Food Chemistry, 50:3050–3055.

Ghorbani, A., Naghibi, F. and Mosaddegh, M. (2006). Ethnobotany, Ethnopharmacology and Drug Discovery. Iranian Journal of Pharmaceutical Sciences, 2(2): 109-118.

Gniazdowska, A. and Bogatek, R. (2005). Allelopathic connections between plants. Multi-site activity of allelochemicals. Acta Physiologiae Plantarum, 27(3): 395-407.

Gomes, An., Alam, A., Bhattacharya, S., Dasgupta, S.C., Mukherjee, S., Bhattacharya, S. and Gomes, A. (2011). Ethno natural utilization of zoo items in rheumatoid joint inflammation. Indian diary of trial science, 49(8): 565-573.

Gómez-Caravaca, A.M., Verardo, V., Toselli, M., Segura-Carretero, A., Fernández-Gutiérrez, A. and Caboni, M.F. (2013). "Assurance of the Major Phenolic Compounds in Pomegranate Juices by HPLC-DAD-ESI-MS". Diary of Agricultural and Food Chemistry, 61(22): 5328–5337.

Gonelimali, F.D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M. and Hatab, S.R. (2018). Antimicrobial properties and instrument of activity of some plant removes against nourishment pathogens and waste microorganisms. Outskirts in Microbiology, 9: 1639-1647.

González-Lamothe, R., Mitchell, G., Gattuso, M., Diarra, M.S., Malouin, F. and Bouarab, K. (2009). Plant antimicrobial specialists and their impacts on plant and human pathogens. Universal Journal of Molecular Sciences, 10(8): 3400–3419.

Gorbi, S., Giuliani, M.E., Pittura, L., d'Errico, G., Terlizzi, A., Felline, S., Grauso, L., Mollo, E., Cutignano, A. and Regoli, F. (2014). Could Molecular Effects of Caulerpa racemosa Metabolites Modulate the Impact on Fish Populations of Diplodus sargus? Marine Environmental Research, 96: 2-11

Gorinstein, S. (2010). Examination of bioactive mixes, cell reinforcement and antiproliferative exercises of Mon Thong durian during aging. Nourishment Chemistry, 118: 540–547.

Gorinstein, S., Poovarodom, S., Leontowicz, H., Leontowicz, M., Namiesnik, J., Vearasilp, S., Haruenkit, R., Ruamsuke, P., Katrich, E. and Tashma, Z. (2011). Cancer prevention agent properties and bioactive constituents of some uncommon colorful Thai leafy foods with customary organic products. In vitro and in vivo examinations. Nourishment Research International, 44, 2222–2232.

Gothandam, K.M., Aishwarya, R. and Karthikeyan, S. (2010). Starter screening of antimicrobial properties of hardly any restorative plants. Diary of Phytology. 2(4): 1-6.

Gouinguene, S. and Turlings, T. (2002). The Effects of Abiotic Factors on Induced Volatile Emissions in Corn Plants. Plant physiology, 129(3): 1296-307.

Gransalke, K. (2011). The compelling force of nature's Drug Cabinet. Lab Times, 11(1): 16–19. Grayson, D.H. (2000). Monoterpenoids. Characteristic Product Reports, 17: 385-419.

Guler, Z. (2005). Measurement free unsaturated fats and flavor attributes of Kasar cheeses. Diary of Food Lipids, 12(3): 209-221.

Guntert, M., Kindel, G., Koppe, V., Lambrecht, S. and Surburg, H. (2001). Mint as well as organic product enhance arrangements. pp: 1-10. US6322838.

Guven, K.C., Coban, B., Sezik, E., Erdugan, H. and Kaleagasioglu, F. (2013). Alkaloids of Marine Macroalgae. Common Products, pp 25-37.

Guven, K.C., Percot, A. and Sezik, E. (2010). Alkaloids in Marine Algae. Marine Drugs, 8(2): 268-284.

Hanson, J.R. (2003). Common Products: The Secondary Metabolite. Cambridge:Royal Society of Chemistry. 147 pages. (ISBN 0-85404-490-6).

Hanson, J.R. (2003). Diterpenoids. Common Product Reports. 20(1): 70-78. Haralampidis, K., Trojanowska, M. and Osbourn, A.E. (2002). Biosynthesis of triterpenoid saponins in plants. Advances in Biochemical Engineering/Biotechnology. 75: 31-49.

Harbourne, J.B. what's more, Williams, C. A. (2000). Advances in Flavonoid researchsince 1992, Phytochemistry; 55(6): 481-504.

Hartzfeld, P.W., Forkner, R., Hunter, M.D. and Hagerman, A.E. (2002). Assurance of Hydrolyzable Tannins (Gallotannins and Ellagitannins) after Reaction with Potassium Iodate. Diary of Agricultural and Food Chemistry, 507: 1785-1790.

Haruenkit, R., Poovarodom, S., Kruszewska, H., Leontowicz, M., Sajewicz, M., Kowalska, T., Delgado, E., Rocha, N., Gallegos-Infante, J.A., Trakhtenberg, S. and Gorinstein, S. (2007). Similar Study of Health Properties and Nutritional Value of Durian, Mangosteen, and Snake Fruit: Experiments In Vitro and In Vivo, Journal of horticultural and nourishment science, 55(14): 5842-5849.

Haruenkit, R., Poovarodom, S., Vearasilp, S., Namiesnik, J., Sliwka-Kaszynska, M., Park, Y., Heo, B., Cho, J., Jang, H.G. and

Hashemipour, M., Kelishadi, R. and Shapouri, J. (2009). Impact of ZincSupplementation on Insulin Resistance and Components of the Metabolic Syndrome in Prepubertal Obese Children, Hormones, 8(4): 279–285.

Hazem, A., Fawzia, A.C. and Dina, G. (2012). Biochemical, antibacterial and antifungal action of concentrates from Achillea fragrantissima and assessment of unstable oil organization. Der Pharmacia Sinica. 3(3), 349-356.

Hean, L.B. (2007). Direction for the Authentication and Characterization of Durian Tree, Department of Agriculture, Malaysia (In Malay), pp: 25.

Heima, K.E., Tagliaferroa, A.R. and Bobilya, D.J. (2002). Flavonoid cancer prevention agents: Chemistry, digestion and structure-movement connections. Diary of Nutritional Biochemistry, 13: 572–584.

Helmenstine, A.M. (2000). Leslie Taylor's Plant Based Drugs and Medicines. Downpour Tree Nutrition. 12(4): 234-245.

Helwig, D. (2005). "Conventional African drug". Storm Encyclopedia of Alternative Medicine. 10 pages.

Ho, L.H. and Bhat, R. (2015). Investigating the Potential Nutraceutical Values of Durian Durio zibethinus L.)– An Exotic Tropical Fruit. Nourishment Chemistry, 168: 80-89.

Hofmann, T., Glabasnia, A., Schwarz, B., Wisman, K.N., Gangwer, K.A. and Hagerman, A.E. (2006). "Protein Binding and Astringent Taste of a Polymeric Procyanidin, 1,2,3,4,6-Penta-O-galloyl-ß-d-glucopyranose, Castalagin and Grandinin". Diary of rural and nourishment science, 54 (25): 9503–9509.

Honey bee, G., Silacci, P., Ampuero-Kragten, S., Čandek-Potokar, M., Wealleans, A. L., Litten-Brown, J., ... and Mueller-Harvey, I. (2017). Hydrolysable tannin-based eating regimen rich in gallotannins minimally affects pig execution however altogether diminishes salivary and bulbourethral organ size. creature, 11(9), 1617-1625.

Hosamath, P.V. (2011). Assessment of antibacterial action of Litsea glutinosa. Worldwide Journal of Pharmaceutical Applications, 2(1): 105-114.

Hossain, M.A. and Ismail, Z. (2013). Confinement and Characterization of Triterpenesfrom the Leaves of Orthosiphon stamineus. Bedouin Journal of Chemistry,6(3): 295-298.

Hu, F.B., Dam, R.M.V. and Liu, S. (2001). Diet and Risk of Type II diabetes: Therole of kinds of fat and sugar. Diabetologia, 44: 805–817.

Huang, L., Cen, Y., Xu, S., Li, Y., Xu, S. and Wu, Q. (2001). Research progress on caulerpin: plant development controller from green growth. Tianran Chanwu Yanjiu Yu Kaifa, 13: 74–78.

Huang, Y.T., Chueh, S.C., Teng, C.M. and Guh, J.H. (2004). Examination of ouabain-instigated anticancer impact in human androgen-free prostate malignant growth PC-3 cells. Biochemical Pharmacology, 67: 727–733. Tracker, P. (2008). Tackling Nature's astuteness. Going to Nature forInspiration and keeping away from her habits. EMBO Reports, 9(9): 838–840.

Husin, N.A., Rahman, S., Karunakaran, R. and Bhore, S.J. (2018). A Review on the Nutritional, Medicinal, Molecular and Genome Attributes of Durian (Durio zibethinus L.), The King of Fruits in Malaysia. Bioinformation, 14: 265–270.

Idris, S. (2011). Durio of Malaysia, first ed.; Malaysian Agricultural Research and Development Institute (MARDI): Kuala Lumpur, Malaysia, pp: 1–130,

Inglese, J. and Auld, D.S. (2009). Use of high throughput screening (HTS) strategies: Applications in substance science in Wiley Encyclopedia of Chemical Biology (Wiley and Sons, Inc., Hoboken, NJ), pp: 260–274.

Isabelle, M., Lee, B.L., Koh, W., Huang, D., Ong, C.N. (2010). Cancer prevention agent action and profiles of regular organic products in Singapore. Nourishment Chemistry, 123: 77–84.

Itani, W.S., El-Banna, S.H., Hassan, S.B., Larsson, R.L., Bazarbachi, A. &Gali-Muthasib, H.U. (2008). Against colon malignancy parts from Lebanese sage (Salvia libanotica) basic oil: unthinking premise. CancerBiology and Therapy, 7: 1765-1773.

IWJS. (Worldwide Weekly Journal of Science) (2007). Difficult to accept: Is itpossible to measure the genuine capability of customary Chinese prescription? Nature, 448(7150): 105–106.

Jalil, A.M.M., Aziz, N.A.A. (2019). Bioactive mixes, dietary benefit, and potential medical advantages of indigenous durian (Durio zibethinus Murr.): A Review. Nourishments, 8(96): 1-18.

Jamuna, S., Paulsamy, S. and Karthika, K. (2012). Screening of In Vitro Antioxidant Activity of Methanolic Leaf and Root Extracts of Hypochaeris radicata L. (Asteraceae). Diary of Applied Pharmaceutical Science, 2(07): 149-154.

Jaw, S.T., Nazimah, S.A.H., Quek, S.Y., Man, Y.B.C., Rahman, R.A. and Hashim, D.M. (2007). Investigation of Volatile Compounds from Malaysian Durians (Durio zibethinus) Using Headspace SPME Coupled to Fast GC-MS. Diary of Food Composition and Analysis, 20: 31–44.

Jayakumar, R. and Kanthimathi, M.S. (2011). Inhibitory Effects of Fruit Extractson Nitric Oxide-Induced Proliferation in MCF-7 Cells. Nourishment Chemistry,126: 956–960.

Jia, L., Jin, H., Zhou, J., Chen, L., Lu, Y., Ming, Y. and Yu, Y. (2013). A Potential Anti-Tumor Herbal Medicine, Corilagin, Inhibits Ovarian Cancer Cell Growth Through Blocking the TGF-ß Signaling Pathways. BMC Complementary and Alternative Medicine, 13: 33-44.

Jozwiak, M., Struga, M., Roszkowski, P., Filipek, A., Nowicka, G. and Olei, W. (2019). Anticancer Effects of Alloxanthoxyletin and Fatty Acids Esters-In Vitro Study on Cancer HTB-140 and A549 Cells. Biomedicine and Pharmacotherapy, 618-630.

Kabata-Pendias, A. (2011). Follow Elements in Soils and Plants, CRC Press, Boca Raton, Fla, USA. 548 pages.

Karamali, K. and Teunis, V.R. (2001). Tannins: Classification and Definition. Normal Product Reports, 18: 641–649.

Katiyar, C.K., Gupta, A., Kanjilal, S. and Katiyar, S. (2012). Medication Discovery from Plant Sources: An Integrated Approach. Ayu Journal, 33(1): 10-18.

Kazi, T.G., Afridi, H.I. and Kazi, N. (2008). "Copper, Chromium, Manganese, Iron, Nickel, and Zinc Levels in Biological Samples of Diabetes Mellitus Patients". Natural Trace Element Research, 122(1): 1–18.

Ketzis, J.K., Vercruysse, J., Stromberg, B.E., Larsen, M., Athanasiadou, S. and Houdijk, J.G. (2006). Assessment of Efficacy Expectations for Novel and Non-Chemical Helminth Control Strategies in Ruminants. Veterinary Parasitology, 139: 321-335.

Khalil, E.S., Manap, M.Y.A., Mustafa, S., Alhelli, A.M. and Shokryazdan, P. (2018). Probiotic Properties of Exopolysaccharide-Producing Lactobacillus Strains Isolated from Tempoyak. Particles, 23: 398.

Khan, I.T., Nadeem, M., Imran, M., Ajmal, M. and Ali, S. (2018). Cell reinforcement Activity, Fatty Acids Characterization and Oxidative Stability of Gouda Cheese Fortified with Mango (Mangifera indica L.) Kernel Fat. Diary of Food Science and Technology, 55(3): 992–1002.

Khan, K.Y., Khan, M.A., Niamat, R., Munir, M., Fazal, H., Mazari, P., Seema, N., Bashir, T., Kanwal, A. and Ahmed, S.N. (2011). Component Content Analysis of Plants of Genus Ficus utilizing Atomic Absorption Spectrometer. African Journal of Pharmacy and Pharmacology, 5 (3): 317-321.

Khan, M. M. A. A., Naqvi, T. S., and Naqvi, M. S. (2012). Recognizable proof of phytosaponins as novel biodynamic operators: a refreshed review. Asian J Exp Biol Sci, 3(3), 459-67.

Khanbabaee, K. and van Ree, T. (2001). "Tannins: Classification and Definition," Natural Product Reports, 18(6): 641–649.

Khanna, V.G. and Kannabiran, K. (2008). Antimicrobial Activity of Saponin Fractions of the Leaves of Gymnema sylvestre and Eclipta prostrate. World Journal of Microbiology and Biotechnology, 24: 2737-2740.

Kigigha, L.T., Izah, S.C. and Ehizibue, M. (2015). Exercises of Aframomum melegueta Seed against Escherichia coli, S. aureus and Bacillus species. Point Journal of Botany and Microbiology Research, 1(2): 23–29.

Kim, G.K. (2010). The Risk of Fluoroquinolone-initiated Tendinopathy and Tendon Rupture. Diary of Clinical and Esthetic Dermatology, 3(4): 49-54.

Kim, J.Y., Park, K.W., Moon, K.D., Lee, M.K., Choi, J., Yee, S.T., Shim, K.H.& Seo, K.I. (2008). Acceptance of apoptosis in HT-29 colon malignancy cells byCrude saponin from Platycodi radix. Nourishment and Chemical Toxicology, 46: 3753–3758.

Kim, M.S., Park, S.B., Suk, K., Kim, I.K., Kim, S.Y., Kim, J.A., Lee, S.H. and Kim, S.H. (2009). Gallotannin Isolated from Euphorbia Species, 1,2, 6-tri-O-galloyl-beta-D-allose, diminishes nitric oxide generation through hindrance of atomic factor-kappa>b

and downstream inducible nitric oxidesynthase articulation in Macrophages. Organic and Pharmaceutical Bullettin, 32(6):1053-1056.

Kim, S.H. (2008). Flavonoids Inhibit Histamine Release and Expression of Proinflammatory Cytokines in Mast Cells. Files of Pharmacal Research, 31(10): 1303-1311.

Kim, S.W., Park, S.K., Kang, S.I., Kang, H.C., Oh, H.J., Bae, C.Y. and Bae, D.H. (2003). Hypocholesterolemic property of Yucca schidigera and Quillaja saponaria separates in human body. Files of Pharmacal Research, 26: 1042–1046.

Kittakoop, P., Mahidol, C. and Ruchirawat, S. (2014). "Alkaloids As Important Scaffolds in Therapeutic Drugs for The Treatments of Cancer, Tuberculosis, and smoking end". Current themes in therapeutic science, 14(2): 239–252.

Koehn, F.E. (2008). High Impact Technologies for Natural Products Screening. Normal Compounds as Drugs, 1: 175-210.

Koehn, F.E. and Carter, G.T. (2005). The Evolving Role of Natural Products in Drug Discovery. Nature Reviews. Medication Discovery. 4(3): 206–220.

Koh, E.S., Kim, S.J. and Yoon, H.E. (2014). "Relationship of Blood Manganese Level with Diabetes and Renal Dysfunction: A Cross-Sectional Study of the Korean General Population," BMC Endocrine Disorders, 14(24): 1-22.

Koleckar, V., Kubikova, K., Rehakova, Z., Kuca, K., Jun, D., Jahodar, L. and Opletal, L. (2008). Dense and Hydrolysable Tannins as Antioxidants Influencing the Health. Small scale Reviews in Medicinal Chemistry, 8(5): 436-447.

Kometiani, P., Liu, L. and Askari, A. (2005). Digitalis-Induced Signaling by Na+/K+-ATPase in Human Breast Cancer Cells. Sub-atomic Pharmacology, 67: 929–936.

Kondo, K., Kurihara, M. and Fukuhara, K. (2001). System of cell reinforcement impact of catechins. Techniques in Enzymology, 335:203–217.

Kong, K.F., Schneper, L. and Mathee, K. (2010). Beta-lactam Antibiotics: From Antibiosis to Resistance and Bacteriology. APMIS, 118(1): 1-36.

Korcz, E., Kerényi, Z. and Varga, L. (2018). Dietary Fibers, Prebiotics, and Exopolysaccharides Produced by Lactic Acid Bacteria: Potential Health Advantages with Special Regard to Cholesterol-Lowering Effects. Nourishment and Function, 9: 3057–3068.

Kotamballi, N., Chidambara, M., Jayaprakasha, G.K. and Bhimanagouda, S.P.(2013). Mitigating interceded utilizations of monoterpenes found in natural products. Tropical and Subtropical Fruits: Flavors, Color, and Health Benefits. Part 7, pp 121–131.

Kren, V. and Martínková, L. (2001). Glycosides in Medicine: "The Role of Glycosidic Residue in Biological Activity". Current Medicinal Chemistry, 8(11): 1303-1328.

Kukhetpitakwong, R., Hahnvajanawong, C., Homchampa, P., Leelavatcharamas, V., Satra, J. and Khunkitti, W. (2006). Immunological Adjuvant Activities of Saponin Extracts from the Pods of Acacia concinna. Worldwide of Immunopharmacology, 6: 1729–1735.

Kumar, S. and Pandey, A.K. (2013). Science and Biological Activities of Flavonoids: An Overview. The Scientific World Journal, 2013(162750): 1-16.

Kumari, I., Ahmed, M. and Akhter, Y. (2017). "Development of Catalytic Microenvironment Governs Substrate and Product Diversity in Trichodiene Synthase and Other Terpene Fold Enzymes". Biochimie, 144: 1-9.

Kushner, J.M., Peckman, H.J. and Snyder, C.R. (2001). Seizures related with fluoroquinolones. Archives of Pharmacotherapy, 35(10): 1194-1198.

Lacopini, P., Baldi, M., Storchi, P. and Sebastiani, L. (2008). Catechin, Epicatechin, Quercetin, Rutin, and Resveratrol in Red Grapes: Content, In Vitro Antioxidant Activity and Interactions. Diary of Food Composition and Analysis, 21(8): 589–598.

Lakkis, J.I. and Weir, R.W. (2018). Hyperkalemia in the Hypertensive Patient. Current Cardiology Reports, 20(2): 12.

Larrosa, M., González-Sarrías, A., García-Conesa, M.T., Tomás-Barberán, F.A. and Espín, J.C. (2006). "Urolithins, Ellagic Acid-Derived Metabolites Produced by Human Colonic Microflora, Exhibit Estrogenic and Antiestrogenic Activities". Diary of Agricultural and FoodChemistry, 54 (5): 1611–20.

Laszczyk, M. (2009). "Pentacyclic Triterpenes of The Lupane, Oleanane and Ursane Group as Tools In Cancer Therapy". Planta Medica, 75 (15): 1549–1560.

Lavoie, S., Legault, J., Gauthier, C., Mshvildadze, V., Mercier, S. and Pichette, A. (2012). Abibalsamins An and B, Two New Tetraterpenoids from Abies balsamea Oleoresin. Natural Letters, 14(6): 1504-1507.

Lee, M.R., Chan Kim, B., Kim, R., Oh, H.I., Kyoung Kim, H., Choi, K.J. Sung, C.K. (2013). Antiobesity Effects of Black Ginseng Extract in High Fat Diet-nourished Mice. Diary of Ginseng Research, 37(3): 308-314.

Leifert, W.R. and Abeywardena, M.Y. (2008). Cardioprotective Actions of Grape Polyphenols. Sustenance Research, 28(11): 729-737.

Leisner, J.J., Vancanneyt, M., Lefebvre, K., Vandemeulebroecke, K., Hoste, B., Euras, V.N., Rusul, G. and Swings, J. (2002). Lactobacillus Durianis sp. nov, Isolated from an Acid-Fermented Condiment (tempoyak) in Malaysia. Universal Journal of Systematic and Evolutionary Microbiology, 52: 927–931.

Leontowicz, H., Leontowicz, M., Haruenkit, R., Poovarodom, S., Jastrzebski, Z., Drzewiecki, J., Ayala, M.L.A., Jesion, I., Trakhtenberg, S. and Gorinstein, S. (2008). Durian (Durio

zibethinus Murr.) Cultivars as Nutritional Supplementation to rodent's weight control plans. Nourishment and Chemical Toxicology, 46: 581-589.

Leontowicz, H., Leontowicz, M., Jesion, I., Bielecki, W., Poovarodom, S., Vearasilp, S., González-Aguilar, G., Robles-Sánchez, M., Trakhtenberg, S. and Gorinstein, S. (2011). Constructive outcomes of Durian Fruit at Different Stages of Ripening on the Hearts and Livers of Rats Fed Diets High in Cholesterol, European Journal of Integrative Medicine, 3: e169–e181.

Leontowicz, M., Leontowicz, H., Drzewiecki, J., Jastrzebski, Z., Haruenkit, R., Poovarodom, S., Park, Y.S., Kang, S.G., Trakhtenberg, S. and Gorinstein, S. (2007). Two Exotic Fruits Positively Affect Rat's Plasma Composition, Food Chemistry, 102(1): 192-200.

Leontowicz, M., Leontowicz, H., Jastrzebski, Z., Jesion, I., Haruenkit, R., Poovarodom, S., Katrich, E., Tashma, Z., Drzewiecki, J., Trakhtenberg, S., and Gorinstein, S. (2007). The Nutritional and Metabolic Indices In Rats Fed Cholesterol-Containing Diets Supplemented With Durian at Different Stages of Eipening. BioFactors, 29: 123–136.

Li, J., Schieberle, P. and Steinhaus, M. (2012). Portrayal of The Major Odor-Active Compounds in Thai Durian (Durio zibethinus L. 'Monthong') by Aroma Extract Dilution. Investigation and Headspace Gas Chromatography-Olfactometry. ". Diary of Agricultural and Food Chemistry, 60: 11253–11262.

Li, J., Schieberle, P. and Steinhaus, M. (2017). Experiences into the Key Compounds of Durian (Durio zibethinus L. 'Monthong') Pulp Odor by Odorant Quantitation and Aroma Simulation Experiments. ". Diary of Agricultural and Food Chemistry, 65: 639–647.

Li, J.W. and Vederas, J.C. (2009). Medication Discovery and Natural Products: End of An Era or An Endless Frontier? Science, 325(5937): 160-165.

Li, Y.V. (2014). Zinc and Insulin in Pancreatic Beta-Cells. Endocrine, 45(2): 178–189.

Lichterman, B.L. (2004). "Ibuprofen: The Story of a Wonder Drug". English Medical Journal, 329 (7479): 1408.

Lin, L.T., Chen, T.Y., Chung, C.Y., Noyce, R.S., Grindley, T.B., McCormick, C., Lin, T.C., Wang, G.H., Lin, C.C. and Richardson, C.D. (2011). Hydrolyzable Tannins (Chebulagic Acid and Punicalagin) Target Viral Glycoprotein-lycosaminoglycan Interactions to Inhibit Herpes SimplexVirus 1 Entry and Cell-to-Cell Spread, Journal of Virology, p. 4386–4398.

Liu, J., Shimizu, K. and Kondo, R. (2009). Hostile to androgenic Activity of Fatty Acids.Chemistry and Biodiversity, 6(4): 503-512.

Li-Weber, M. (2009). New Therapeutic Aspects of Flavones: The Anticancer Properties of Scutellaria and Its Main Active Constituents Wogonin, Baicalein and Baicalin. Malignant growth Treatment Reviews, 35: 57–68.

Lopez-Lazaro, M., Pastor, N., Azrak, S.S., Ayuso, M.J., Austin, C.A. and Cortex, F. (2005). Digitoxin Inhibits the Growth of Cancer Cell Lines at Concentrations Commonly Found in Cardiac Patients. Diary of Natural Products, 68: 1642–1645.

Lopez-Novoa, J.M., Quiros, Y., Vicente, L., Morales, A.I. and Lopez-Hernandez, F.J. (2011). New Insights into The Mechanism of Aminoglycoside Nephrotoxicity. Kidney International, 79(1): 33-45.

Luch, A. (2009). Sub-atomic, Clinical and Environmental Toxicology. Springer.p. 20. ISBN 3-7643-8335-6.

Ludwiczuk, A., Skalicka-Wozniak, K. and Georgiev, M.I. (2017). Essentials, Applications and Strategies. Pharmacognosy, Pages 233–266.

Luo, H.Y., Wang, B., Yu, C.G., Qu, Y.L. and Su, C.L. (2010). Assessment of Antioxidant Activities of Five Selected Brown Seaweeds from China. Diary of Medicinal Plants Research, 4: 2557-2565.

Lushniak, B.D. (2014). Anti-infection Resistance: A Public Health Crisis. General Health Reports, 129(4): 314–316.

Macarron, R., Banks, M.N., Bojanic, D., Burns, D.J., Cirovic, D.A., Garyantes, T., Green, D.V., Hertzberg, R.P., Janzen, W.P., Paslay, J.W., Schopfer, U. and Sittampalam, G.S. (2011). "Effect of High-Throughput Screening in Biomedical Research". Normal Reviews Drug Discovery, 10: 188–195.

Machado, A.R.T., Ferreira, S.R., Medeiros, F.D., Ricardo, T., Fujiwara, R.T., Filho, J.D.D. and Pimenta, L.P.S. (2015). Nematicidal Activity of Annona crassiflora Leaf Extract on Caenorhabditis Elegans. Parasite Vector, 8: 113-117.

Ma'ckowiak, K., Torli'nska-Walkowiak, N. and Torli' nska, B. (2016). DietaryFibre As an Important Constituent of the Diet. Postepy Hig Med Dosw, 70,104–109.

Maier, M.E. (2015). Plan and Synthesis of Analogs of Natural Products. Natural and Biomolecular Chemistry, 13: 5302-5343.

Mämmelä, P., Savolainen, H., Lindroos, L., Kangas, J. and Vartiainen, T. (2000). "Examination of Oak Tannins by Liquid Chromatography-Electrospray Ionization Mass spectrometry". Diary of Chromatography A, 891(1):75–83.

Manach, C., Scalbert, A., Morand, C., Remesy, C., and Jimenez, L. (2004). Polyphenols: Food Sources and Bioavailability. American Journal of Clinical Nutrition, 79: 727-747.

Manandhar, S., Luitel, S. and Dahal, R.K. (2019). In vitro Antimicrobial Activity of Some Medicinal Plants Against Human Pathogenic Bacteria. Diary of Tropical Medicine, Article ID 1895340, 5 pages.

Mansouri, An., Embared, G., Kokkalou, E. and Kefalas, P. (2005). Phenolic Profile and Antioxidant Activity of the Algerian Ripedate Palm Fruit (Phoenix dactylifera). Nourishment Chemistry, 89: 411–420. Maret, W. (2013). Zinc Biochemistry: From a Single Zinc Enzyme To A key Element of Life. Advances in Nutrition, 4(1): 82–91.

Martin, D.M., Gershenzon, J. and Bohlmann, J. (2003)." Induction of VolatileTerpene Biosynthesis and Diurnal Emmision by Methyl Jasmonate in Foliage of Norway Spruce". Plant Physiology, 132(3): 1586–1599.

Marzouk, M.S.A., El-Toumy, S.A.A., Moharram, F.A., Shalaby, N.M. and Ahmed, A.A. (2002). "Pharmacologically Active Ellagitannins fromTerminalia myriocarpa". Planta Medica, 68(6): 523–527.

Matsuura, H. (2001). Saponins in Garlic as Modifiers of the Risk of Cardiovascular Disease. Diary of Nutrition, 131:1000S–1005S.

Mboya, E.A., Sanga, L.A. and James, S.N.J.S. (2018). Unreasonable Use of AntibioticsIn the Moshi Municipality Northern Tanzania: A Cross Sectional Study. Dish African Medical Journal, 31: 165-174.

McCall, A.S., Cummings, C.F., Bhave, G., Vanacore, R., Page-McCaw, An, and Hudson, B.G. (2014). Bromine is an Essential Trace Element for Assembly of Collagen IV Scaffolds in Tissue Development and Architecture. Cell, 157(6): 1380–1392.

McConkey, D.J., Lin, Y., Nutt, L.K., Ozel, H.Z. and Newman, R.A. (2000). Cardiovascular Glycosides Stimulate Ca2+ Increases and Apoptosis in Androgen-Independent, Metastatic Human Prostate Adenocarcinoma Cells. Malignant growth Research, 60: 3807–3812.

McGaw, L.J. and Eloff, J.N. (2008). Ethnoveterinary Use of Southern African Plants and Scientific Evaluation of Their Medicinal Properties. Diary of Ethnopharmacology, 119(3): 559–574.

McKinney, J.D, Richard, A., Waller, C., Newman, M.C. and Gerberick, F. (2000). The Practice of Structure Activity Relationships (SAR) in Toxicology. Toxicological Sciences, 56(1): 8–17.

McRae, J., Yang, Q., Crawford, R. and Palombo, E. (2007). Audit of The Methods Used for Isolating Pharmaceutical Lead Compounds from Traditional Medicinal Plants. Hippie, 27: 165-174.

Mengoni, F., Marzi, M., Mastroianni, C.M., Lichtner, M., Battinelli, L., Vullo, V. and Mazzanti, G. (2002). In Vitro Anti-HIV Activity of Oleanolic Acid on Infected Human Mononuclear Cells. Planta Medica, 68: 111-114.

Mert-Turk, F. (2006). Saponins Versus Plant Fungal Pathogens. Diary of Celland Molecular Biology, 5:13-17.

Mhilaj, E., Morgese, M.G., Tucci, P., Bove, M., Schiavone, S. and Trabace, L. (2015). Impacts of Anabolic-Androgens on Brain Reward Function. Boondocks in Neuroscience, 9(295): 1-13.

Mijatovic, T., Anne, O.O.P., Quaquebeke, E.V., Dewelle, J., Darro, F.,de Launoit, Y. and Kiss, R. (2006). The Cardenolide UNBS1450 Is Able to Deactivate Nuclear Factor KB-Mediated Cytoprotective Effects in Human Non-Small Cell Lung Cancer Cells. Sub-atomic Cancer Therapeutics, 5: 391–399.

Moharram, F.A., Marzouk, M.S., El-Toumy, S.A.A., Ahmed, A.A.E. &Aboutabl, E.A. (2003). Polyphenols of Melaleuca quinquenervia Leaves – Pharmacological Studies of Grandinin. Phytotherapy Research, 17(7): 767-773.

Mohy-Ud-Din A, Khan Z, Ahmad M, Kashmiri MA, Yasmin S and Mazhar H (2009). Chemotaxonomic Significance of Flavonoids in the Solanum nigrum Complex. Diary of the Chilean Chemical Society, 54(4): 486-490.

Mokhtari, K., Rufino-Palomares, E.E., Perez-Jimenez, A., Rayes-Zurita, F.J., Figuera, C., Garcia-Salguero L, Medina PP, Peragon J and Lupiez JA (2015). Maslinic Acid, A Triterpene from Olive, Affects the Antioxidant and Mitochondrial Status of B16F10 Melanoma Cells Grown Under Stressful Conditions. Proof Based Complementary and Alternative Medicine, 2015: 272457.

Mostafa, A.A., Abdulaziz, An., Askar, An., Almaary, K.S., Dawoud, T.M., Sholkamy, E.N. and Bakri, M.M. (2017). Antimicrobial Activity of Some Plant Extracts Against Bacterial Strains Causing Food Poisoning Diseases. Saudi Journal of Biological Sciences, 25(2): 361-366.

Mostofsky E, Tjønneland MA, Chahal HS, Johansen MB, Mittleman MA and Overvad K (2017). Chocolate Intake and Risk of Clinically Apparent Atrial Fibrillation: The Danish Diet, Cancer and Health Study. Heart, 103: 1163–1167.

Motti C.A, Bourguet-Kondracki M.L, Longeon A, Doyle J. R, Llewellyn L.E, Tapiolas D.M and Yin P (2007). Examination of Biological Properties of Several Marine Sponge-Derived Sesquiterpenoid Quinines. Particles, 12: 1376-1388.

Mouhssen, L. (2007). Screening of Natural Products for Drug Discovery. Master Opinion on Drug Discovery, 2(5): 697-705.

Muchuweti, M., Kativu, E., Mupure, C.H., Chidewe, C., Ndhlala, A.R. and Benhura, M.A.N. (2007). Phenolic Composition and Antioxidant Properties of Some Spices. American Journal of Food Technology. 2(5): 414–420.

Nakamura, Y., Tsuji, S. and Tonogai, Y. (2003). Investigation of Proanthocyanidins in Grape Seed Extract, Health Foods and Grape Seed Oils. Diary of Health Science, 49(1): 45-54.

Nelson, A.T., Camelio, A.M., Claussen, K.R., Cho, J., Tremmel, L., DiGiovanni, J. and Siegel, D. (2015). Union of Oxygenated Oleanolic and Ursolic Acid Derivatives with Anti-Inflammatory Properties. Bioorganic and Medicinal Chemistry Letters, 25(19): 4342-4346.

Nesterkina, M., Bernier, U.R., Tabanca, N. and Kravchenko, I. (2018). Repellent Activity of Monoterpenoid Esters with Neurotransmitter Amino AcidsAgainst Yellow Fever Mosquito, Aedes aegypti. Open Chemistry Journal, 16(1): 95–98.

Newman, D.J. and Cragg, G.M. (2007). "Normal Products as Sources of New Drugs Over the Last 25 Years. Diary of Natural Products, 70(3): 461–477.

Newman, D.J., Cragg, G.M. and Snader, K.M. (2000). The Influence of Natural Products Upon Drug Discovery. Normal Product Reports, 17(3): 215 – 234.

Newman, D.J., Cragg, G.M. and Snader, K.M. (2003). Normal Products as a Source of New Drugs Over the Period of 1981-2002. Diary of Natural Products, 66: 1022-1037.

Newman, R.A., Kondo, Y., Yokoyama, T., Dixon, S., Cartwright, C., Chan, D., Johansen, M. and Yang, P. (2007). Autophagic Cell Death of Human Pancreatic Tumor Cells Mediated by Oleandrin, A Lipid-Soluble Cardiac Glycoside. Integrative Cancer Therapies, 6: 354–364.

Newman, R.A., Yang, P., Hittelman, W.N., Lu, T., Ho, D.H., Ni, D., Chan, D. Vijjeswarapu, M., Cartwright, C., Dixon, S., Felix, E. and Addington, C. (2006). Oleandrin-Mediated Oxidative Stress in Human Melanoma Cells. Diary of Experimental Therapeutics and Oncology, 5: 167–181.

Ngo, L.T., Okogun, J.I. and Folk, W.R. (2013). 21st century normal item Research and Drug Development and Traditional Medicines. Normal Product Reports, 30: 584–592.

Nicoloff, G., Mutaftchiev, K., Strashimirov, D. and Petrova, C. (2004), "Serum Manganese in Children with Diabetes Mellitus Type 1". Diabetologia Croatica, 33(2): 47–51.

Nix, A., Paull, C. and Colgrave, M. (2017). Flavonoid Profile of the Cotton Plant, Gossypium hirsutum: A Review. Plants, 6(4): 43-56.

Norjana, I., Noor, A.A.A. (2011). Quality Attributes of Durian (Durio zibethinus Murr) Juice after Pectinase Enzyme Treatment. Global Food Research Journal, 18(3): 1117-1122.

Oakenfull, D. (2001). Soy Protein, Saponins and Plasma Cholesterol. Diary of Nutrition, 131: 2971.

Oba, S., Nagata, C., Nakamura, K., Fujii, K., Kawachi, T., Takatsuka, N. and Shimizu, H. (2010). Utilization of Coffee, Green Tea, Oolong Tea, Black Tea, Chocolate Snacks and the Content in Relation to Risk of Diabetes in Japanese Men and Women. English Journal of Nutrition, 103: 453–459.

Obeidat, M., Shatnawi, M., Al-Alawi, M., Al-Zu'bi, E., Al-Dmoor, H., Al-Qudah, M., El-Qudal, J. and Otri, I. (2012). Antimicrobial Activity of Crude Extracts of Some Plant Leaves. Research Journal of Microbiology,7(1): 59-67.

Oda, K., Matsuda, H., Murakami, T., Katayama, S., Ohgitani, T., and Yoshikawa, M. (2000). Adjuvant and Haemolytic Activities of 47Saponins Derived from Medicinal and Food Plants. Organic Chemistry, 381: 67–74.

Okonwu, K. and Ugiomoh, I.G. (2015). Tannin Contents of Some Economic Plantsin Nigeria. Diary of Plant Sciences, 10(4): 159-166.

Okuda, T. (2005). Systematics and Health Effects of Chemically Distinct Tannins in Medicinal Plants. Phytochemistry, 66: 2012-2031.

Okuno, Y., Marumoto, S. and Mitsuo, M.M. (2019). Antimutagenic action of Flavonoids from Sozuku. Regular Product Research, 33(6): 862-865.

Okwu, D.E. (2005). Phytochemicals, Vitamins and Mineral Contents of Two Nigeria Medicinal Plants. Worldwide Journal of Molecular Medicineand Advance Sciences, 1(4): 375-381.

Okwu, D.E. and Okwu, M.E. (2004). Concoction sythesis of Spondias mombiaLinn plant parts. Diary of Sustainable Agriculture and Environment, 6(2): 140-147.

Ono, M., Takeshima, M. and Nakano, S. (2015). Component of the AnticancerEffect of Lycopene (Tetraterpenoids). Catalysts, 37: 139-166.

Oprea, T.I., Davis, A.M., Teague, S.J., and Leeson, P.D. (2001). Is There a Difference among Leads and Drugs? A Historical Perspective. Diary of Chemical Information and Computer Sciences, 41: 1308-1315.

Osuntokun, O.T. and Oluwafoise, B.O. (2015). Phytochemical Screening of TenNigerian Medicinal Plants. Worldwide Journal of Multidisplinary Research and Development, 2(4): 390-396.

Ozsoy, N., Candoken, E. and Akev, N. (2009). Suggestions for Degenerative Disorders: Antioxidative Activity, Total Phenols, Flavonoids, Ascorbic Acid, Beta-Carotene and Beta-Tocopherol in Aloe vera. Oxidative Medicine and Cellular Longevity, 2(2): 99–106.

Panche, A.N., Diwan, A.D. and Chandra, S.R. (2016). Flavonoids: An Overview.Journal of Nutritional Science, 5(e47): 1-15.

Pandey, M.A.B., Abidi, S. and Singh, R.P. (2006). Dietary assessment of verdant vegetables Paratha. Diary of Human Ecology, 19: 155-156.

Parasuraman, S., Thing, G.S. and Dhanaraj, S.A. (2014). Polyherbal Formation: Concept of Ayurveda. Pharmacognosy Reviews, 8: 73–80.

Park, H.H., Lee, S., Son, H.Y., Park, S.B., Kim, M.S., Choi, E.J., Singh, T.S., Ha, J.H., Lee, M.G., Kim, J.E., Hyun, M.C., Kwon, T.K., Kim, Y.H. and

Park, S., Kang, J., Choi, S., Park, H., Hwang, E., Yoongu Kang, Y., Kim, A., Holzapfel, W. and Ji, Y. (2018). Amendment: Cholesterol-Lowering Effect of Lactobacillus rhamnosus BFE5264 and Its Influence on the GutMicrobiome and Propionate Level In a Murine Model. PLOS ONE, 13(11): e0208294.

Patel, P.P. and Patil, P.H. (2012). Calming Activity of Saponin Rich Fraction Isolated From the Thespesia populnea (L.) leaves. Universal Journal of Biomedical and Pharmaceutical Sciences, 3(4): 1526-1532.

Patidar, A.K., Selvam, G., Jeyakandan, M., Mobiya, A.K., Bagherwal, A., Sanadya, G. and Mehta, R. (2011). Lead Discovery and Lead Optimization: A Useful Strategy in Molecular Modification of Lead Compound in Analog Design. Worldwide Journal of Drug Design and Discovery, 2(2):458-463.

Patrick, T.B. (2000). Natural and Biological Chemistry. first release. Rinton Press Inc. USA. Section 18: 336-340.

Paul, M.D. (2001). In: Medicinal Natural Products: A Biosynthetic Approach.2nd Edition, John Wiley and Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, England. 546 pages.

Pazouki, L. and Niinemets, Ü. (2016). "Multi-Substrate Terpene Synthases: Their Occurrence and Physiological Significance". Outskirts in Plant Science, 7: 1019.

Peng, Y., Zhong, G., Mi, Q., Li, K., Wang, A., Li, L. and Liu, H. (2017). Potassium Measurements and Risk of Type 2 Diabetes: A Dose-Response Meta-Analysis of Prospective Cohort Studies. Oncotarget, 8: 100603–100613.

Pinto, M.E.A., Araújo, S.G., Morais, M.I., Sá, N.P., Lima, C.M., Rosa, C.A. Siqueira, E.P., Johann, S. and Lima, L.A.R.S. (2017). Antifungal and Antioxidant Activity of Fatty Acid Methyl Esters from Vegetable Oils. Anais da Academia Brasileira de Ciencias, 89(3): 1671-1681.

Pius, O.U., Egbuonu, A.C., Cemaluk, O.L.N. and Ejikeme, P.M. (2011). Tannins and Other Phytochemical of The Samanaea saman Pods and Their Antimicrobial Activities. African Journal of Pure and Applied Chemistry, 5(8): 237-244.

Plants, E., Cooper, C., Seely, D. and Kanfer, I. (2005). African Herbal Medicine in the Treatment of HIV: Hypoxis and Sutherlandia. An Overview of Evidence and Pharmacology. Sustenance Journal, 4: 19.

Podolak, I., Galanty, A. and Sobolewska, D. (2010). Saponins as cytotoxic operators: A survey. Phytochemistry Reviews, 9(3): 425-474.

Pohl, H.R., Wheeler, J.S. and Murray, H.E. (2013). Part 2. Sodium and Potassium in Health and Disease. In Astrid Sigel, Helmut Sigel and Roland K. O. Sigel. Interrelations Between Essential Metal Ions and Human Diseases. Metal Ions in Life Sciences, 13: 29–47.

Poovarodom, S., Haruenkit, R., Vearasilp, S., Ruamsuke, P., Leontowicz, H.,Leontowicz, M., Namiesnik, J., Trakhtenberg, S. and Gorinstein, S. (2013). Nourishing and Pharmaceutical Applications of Bioactive Compounds in Tropical Fruits. In International Symposium on Mineral Nutrition of Fruit Crops, ninth ed.; Poovarodom S, Yingjajaval, Eds.; International Society for Horticultural Science: Korbeek-Lo, Belgium, 1: 77–86. Pors, N.S. (2004). "The Biological Role of Strontium". Bone, 35(3): 583–588.

Prabhavathi, R.M., Prasad, M.P. and Jayaramu, M. (2016). Concentrates on Qualitative and Quantitative Phytochemical Analysis of Cissus quadrangularis, Advances in Applied Science Research, 7(4): 11-17. Dance, G. (2007). Ethnobotany, the Science of Survival: A Declaration from Kaua'i. Financial Botany, 61(1): 1-2.

Prassas, I. and Diamandis, E.P. (2008). Novel Therapeutic Applications of Cardiac Glycosides. Nature Reviews Drug Discovery, 7: 926–935.

Praveen, K.A. and Kumud, U. (2012). Tannins are Astringent. Diary of Pharmacognosy and Phytochemistry, 1(3): 45-50.

Preet, R., Gupta, R.C. and Pradhan, S.K. (2017). Natural Analysis and BiologicalStudies of Physalis angulata L. utilizing Wavelength-Dispersive X-beam Fluorescence Technique, Wavelength Dispersion X-beam Fluorescence, From Rajasthan, Asian Journal of Pharmaceutical and Clinical Research, 10(8): 220-224.

Procházková, D., Boušová, I. and Wilhelmová, N. (2011). Cancer prevention agent andProoxidant Properties of Flavonoids. Fitoterapia, 84(4): 513-523.

Ptyakowska, K., Kita, A., Janoska, P., Polowniak, M. and Kozik, V. (2012). Multi-Element Analysis of Mineral and Trace Elements in Medicinal Herbs and Their Infusions. Nourishment Chemistry, 135: 494-501.

Qiu, S., Sun, H., Zhang, A. H., Xu, H.Y., Yan, G.L., Han, Y. and Wang, X.J. (2014). "Common Alkaloids: Basic Aspects, Biological Roles, and Future Perspectives," Chinese Journal of Natural Medicines, 12(6): 401–406.

Quanbin, H., Jingzheng, S., Chunfeng, Q., Lina, W. and Hongxi, X. (2006). Preparative Isolation of Hydrolysable Tannins Chebulagic Acid and Chebulinic Acid from Terminalia chebula by High-Speed Counter-current chromatography. Diary of Separation. Science, 29: 1653–1657.

Quideau, S., Jourdes, M., Saucier, C., Glories, Y., Pardon, P. and Baudry, C. (2003). DNA Topoisomerase Inhibitor Acutissimin An and Other Flavano-Ellagitannins in Red Wine, Angewandte Chemie International, 42(48): 6012-6014.

Raghavendra, P.B., Sreenivasan, Y., Ramesh, G.T. and Manna, S.K. (2007). Heart Glycoside Induces Cell Death Via Fast by Activating Calcineurin and NF-AT, But Apoptosis Initially Proceeds Through Activation of Caspases. Apoptosis, 12: 307–318.

Rahman, G., Syed, U.J., Syed, F., Samiullah, S. and Nusrat, J. (2017). Starter Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan, The Scientific World Journal, 2017: 1-7.

Rajic, A., Kweifio-Okai, G., Macrides, T., Sandeman, R.M., Chandler, D.S. and Polya, G.M. (2000). Hindrance of Serine Proteases by Anti-Inflammatory Triterpenoids. Planta Medica, 66: 206-210.

Raju, J., Gupta, D., Rao, A., Yadava, P. and Baquer, N. (2001). Trigonella foenum graecum (fenugreek) Seed Powder Improves Glucose Homeostasis in Alloxan Diabetic Rat Tissues by Reversing the Altered Glycolytic, Gluconeogenic and Lipogenic Enzymes. Atomic and CellularBiochemistry, 224: 45-51.

Randhir, R., Lin, Y.T. and Shetty, K. (2004). Incitement of Phenolics, Antioxidant and Antimicrobial Activities in Dark Germinated Mung Bean Sprouts in Response to Peptide and Phytochemical Elicitors. Procedure Biochemistry,39: 637–646.

Reddy, M.K., Gupta, S.K., Jacob, M.R., Khan, S.I. and Ferreira, D. (2007). Cancer prevention agent, Antimalarial and Antimicrobial Activities of Tannin-Rich Fractions, Ellagitannins and Phenolic Acids from Punica granatum L. Planta medica, 73 (5): 461-467.

Reiss, I., Erfurt, H., Ott, F., Kindel, G. and Gabriel, B. (2009). Procedure for The Preparation of a Flavoring Concentrate, and a Flavoring Concentrate, pp: 1-22. US20090155446.

Rejinolda, N.S., Muthunarayanan, M., Muthuchelian, K., Chennazhi, K.P., Nair, S.V. and Jayakumar, R. (2011). Saponin-Loaded Chitosan Nanoparticles and their Cytotoxicity to Cancer Cell Lines In Vitro. Starch Polymers, 84: 407–416.

Robert, S.D., Ismail, A.A., Winn, T. and Thomas, M.S. (2008). Glycemic Index of Common Malaysian ruits. Asia Pacific Journal of Clinical Nutrition, 17(1): 35-39.

Rocha, F.D., Soares, A.R., Houghton, P.J., Pereira, R.C., Kaplan, M.A.C. and Teixeira, V.L. (2007). Potential Cytotoxic Activity of Some Brazilian Seaweeds on Human Melanoma cells. Phytotherapy Research, 21: 170–175.

Rockwood, C.A. (2009). Rockwood and Wilkins' cracks in youngsters (seventh Ed.). Philadelphia, Pa.: Lippincott Williams and Wilkins. p. 54. (ISBN 978-1-58255-784-7).

Roy, S.P., Niranjan, C.M., Jyothi, T.M., Shankrayya, M.M., Vishawanath, K.M., Prabhu, K., Gouda, V.A. and Setty, R.S. (2010). Antiulcer and Anti-provocative Activity of Aerial Parts of Enicostemma littorale Blume. Pharmacology, 2(4): 369-373.

Rustam, I.A. (2010). A Brief History of The Antibiotic Era: Lessons Learned and Challenges for The Future. Outskirts in Microbiology, 1:134.

Sajadi, T.S.A., Hosseinzadeh, H., Ramezani, M., Moghimipour, E. and Mohajer, S.A. (2007). Disengagement and Characterization and Study of Enhancing Effect on Nasal Absorption of Insulin in Rat of the Total Saponin from Acanthophyllum squarrosum. Indian Journal ofPharmacology, 39(5): 226-230.

Sakat, S., Juvekar, A.R. and Gambhire, M.N. (2010). In Vitro Antioxidant and Anti-provocative Activity of Methanol Extract from Oxalis corniculata Linn. Global Journal of Pharma and Pharmacological Sciences, 2(1): 146-155.

Saleem, M., Nazir, M., Shaiq, A.M., Hussain, H., Sup Lee, Y., Riaz, N. and Jabbar, A. (2010). Antimicrobial Natural Products: An Update on Future Antibiotic Drug Candidates. Common Product Reports, 27: 238–254.

Salim, A.A., Chin, Y.W. and Kinghorn. A.D. (2008) In: Bioactive MedicinalPlants. Ramawat KG, Merillon JM (eds.), Springer, pp: 1-24.

Samuelsson, G. (2004). Medications of Natural Origin: A Textbook of Pharmacognosy, fifth Swedish Pharmaceutical Press, Stockholm. 620 pages.

Santhi, K., Sengottuvel, R. (2016). Subjective and Quantitative Phytochemical Analysis of Moringa concanensis Nimmo, International Journal ofCurrent Microbiology and Applied Sciences, 5(1): 633-640.

Sapna, D., Dhruv, G.D. and Harmeet, K. (2009). Saponins and their Biological Activities. Pharma Times, Vol 41 - No. 3

Saracoglu, S., Tuzen, M. and Soylak, M. (2009). Assessment of Trace Element Contents of Dried Apricot Samples from Turkey. Diary of Hazardous Materials, 156: 647-652.

Sarker, S.D. and Nahar, L. (2007). General Organic and Natural Product Chemistry – TiERA Chemistry for Pharmacy Students. ©John Wiley and Sons, Ltd. 398 pages.

Sarker, S.D. and Nahar, L. (2012). Hyphenated Yechniques and Their Applications in Natural Products Analysis. Techniques in Molecular Biology, 864:301-340.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M. and Yoga, L.L. (2011). Extraction, Isolation and Characterization of Bioactive Compounds from Plants Extracts. African Journal of Traditional, Complementary and Alternative Medicine, 8(1): 1–10.

Sati, A.S., Sati, S.C., Sati, N. and Sati, O.P. (2017). Synthetic Composition and Antimicrobial Activity of Fatty Acid Methyl Ester of Quercus leucotrichophora organic products. Common Product Research, 31(6): 176-184.

Satyajit, D.S. and Lutfun, N. (2007). Science for Pharmacy Students: General Organic and Natural Products Chemistry. John Wiley and Sons Ltd. ISBN p. 282-320.

Scheiber, I., Dringen, R. and Mercer, J.F.B. (2013). Copper Effects of Deficiency and Overload. In Sigel, Astrid; Sigel, Helmut; Sigel, Roland K.O. Interrelations Between Essential Metal particles and Human Diseases. Metal Ions in Life Sciences, 13: 359–87.

Schippmann, U., Leaman, D.J. and Cunningham, A.B. (2002). "Effect of Cultivation and Gathering of Medicinal Plants on Biodiversity: GlobalTrends and Issues 2. A few Figures to begin with… " Biodiversity and The Ecosystem Approach in Agriculture, Forestry and Fisheries. Satellite Event on The Occasion of The Ninth Regular Session of The Commissio on Genetic Resources for nourishment and Agriculture. Rome, 12–13 October 2002. Between Departmental Working Group on Biological Diversity for Food And Agriculture. Rome. Nourishment and Agriculture Organization. Recovered 25 September2017.

Schmit, S.L., Rennert, H.S., Gruber, S.B. (2016). Espresso Consumption and The Risk of Colorectal Cancer. Malignant growth Epidemiology, Biomarkers and Prevention, 25: 634–639.

Scotti, M.T., Lorenzo, P.V., Filho, J.M.B. and Scotti, L. (2015). Joined Structure-and Ligand-Based Virtual Screening to Evaluate Caulerpin Analogs with Potential Inhibitory Activity Against Monoamine Oxidase B. Revista Brasileira de Farmacognosia, 25: 690–697.

Seema, F., Parwez, A. (2011). "Phytochemical Investigation of Extract of Amorphophallus campanulatus tubers", International Journal of Phytomedicine, 3: 32-35.

Sermakkani, M. and Thangapandian, V. (2012). GC-MS Analysis of Cassia italica Leaf Methanol Extract. Asian Journal of Pharmaceutical and Clinical Research, 5(2): 90-94.

Serrano, J., Puupponen-Pimia, R., Dauer, An., Aura, A.M., Saura-Calixto, F. (2009). Tannins: Current Knowledge of Food Sources, Intake, Bioavailability and Biological impacts. Sub-atomic Nutrition and FoodResearch, 53(Suppl. 2): S310-329.

Shan, B., Cai, Y.Z., Sun, M. and Corke, H. (2005). Cell reinforcement Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents. Diary of Agricultural and Food Chemistry, 53(2):7749–7759.

Sharma, P.K., Romanczyk, L.J., Kondaveti, L., Reddy, B., Arumugasamy, J., Lombardy, R., Gou, Y. and Schroeter, H. (2015). All out Synthesis of Proanthocyanidin A1, A2 and Their Stereoisomers. Natural Letters, 17(10): 2306-2309.

Shekhar, T.C. and Anju, G. (2014). Cell reinforcement Activity by DPPH Radical Scavenging Method of Ageratum conyzoides Linn. Leaves. American Journal of Ethnomedicine, 1(4): 244-249.

Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine. J.R., Cole, P.A., Casero, R.A. and Shi, Y. (2004). Histone Demethylation Mediated by The Nuclear Amine Oxidase Homolog lsd1. Cell. 119(7): 941-953.

Shoib, A.B. and Shahid, A.M. (2015). Assurance of Total Phenolic and Flavonoid Content, Antimicrobial and Antioxidant Activity of a Root Extract of Arisaema jacquemontii Blume, Journal of Taibah University for Science, 9: 449–454.

Shuaibu, M.N., Pandey, K. and Wuyep, P.A. (2008). "Castalagin from Anogeissus leiocarpus Mediates The Killing of Leishmania In Vitro". Parasitology Research, 103(6): 1333–1338.

Siew, G.Y., Ng, W.L., Tan, S.W., Alitheen, N.B., Tan, S.G., Yeap, S.K. (2018). Hereditary Variation and DNA Fingerprinting of Durian Types in Malaysia Using Simple Sequence Repeat (SSR) Markers, Peer J, 6: e4266.

Silva, B.P., Correa Soares, J.B.R., Souza, E.P., Palatnikc, M., Sousa, C.B.P. and Parente, J.P. (2005). Pulcherrimasaponin, From the Leaves of Calliandra pulcherrima, as Adjuvant for Immunization in the Murine Model of Visceral Leishmaniasis. Immunization, 23: 1061–1071.

Singh, A. (2016). Administrative and Pharmacological Basis of Ayurvedic Formulations. CRC Press. pp. 4–5.

Singh, B. and Sharma, R.A. (2015). Plant Terpenes: Defense Responses, Phylogenetic Analysis, Regulation and clinical Applications.Biotechnology, 5(2): 129–151.

Sloan, B. and Scheinfeld, N. (2008). The Use and Safety of Doxycycline hyclate and Other Second-Generation Tetracyclines. Master Opinion on Drug Safety, 7 (5): 571–577.

Smith-Hall, C., Larsen, H.O. and Pouliot, M. (2012). "Individuals, Plants and Health: A Conceptual Framework for Assessing Changes in Medicinal Plant Consumption. Diary of Ethnobiology and Ethnomedicine, 8: 43.

Sneader, W. (2005) Drug Discovery: A History, Wiley, Chichester, UK. 468 pages. ISBN:9780470015537, DOI:10.1002/0470015535

Soetan, K.O., Oyekunle, M.A., Aiyelaagbe, O.O. and Fafunso, M.A. (2006). Assessment of The Antimicrobial Activity of Saponins Extract of Sorghum Bicolor L. Moench. African Journal of Biotechnology, 5: 2405-2407.

Sommer, T., Hübner, H., Ahmed El-Kerdawy, A., Gmeiner, P., Pischetsrieder, M. and Clark, T. (2017). Recognizable proof of The Beer Component Hordenine As Food Derived Dopamine D2 Receptor Agonist by Virtual Screening a 3D Compound Database. Logical Reports, 7(44201): 1-12.

Soobrattee, M.A., Neergheen, V.S., Luximon-Ramma, An., Aruoma, O.I. and Bahorun, O.T. (2005). Phenolics as Potential Antioxidant Therapeutic Agents: Mechanism and Actions, Mutation Research, 579: 200–213.

Sparg, S.G., Light, M.E. and van Staden, J. (2004). Natural Activities and Distribution of Plant Saponins. Diary of Ethnopharmacology, 94: 219–243.

Stanimirova, R., Marinova, K., Tcholakova, S., Denkov, N.D., Stoyanov, S. and Pelan, E. (2011). Surface Rheology of Saponin Adsorption Layers. Langmuir, 27: 12486–12498.

Stanley, B. (2011). Acknowledgment and Respect for African Traditional Medicine. Canada's International Development Research Center. https://www.idrc.ca/en/article/acknowledgment andrespect-african-custom prescription.

Steenackers, B., De Cooman, L. and De Vos, D. (2015). "Synthetic Transformations of Characteristic Hop Secondary Metabolites in Relation to Beer Properties and The Brewing Process: A survey". Nourishment Chemistry, 172: 742–756.

Stefano, G.B., Ptácek, R., Kuželová, H. what's more, Kream, R.M. (2012). "Endogenous Morphine: Up-to-Date Review". Folia Biologica. (Praha), 58 (2): 49–56.

Stefanska, B. Karlic, H., Varga, F., Fabianowska-Majeska, K. and Haslberger, A.G. (2012). Epigenetic Mechanisms in Anti-Cancer Actions of Bioactive Food Components—The Implications in Cancer Prevention. English Journal of Pharmacology, 167: 279–297.

Steward, M.S. (2004). The Role of Natural Product Chemistry in Drug Discovery. Diary of Natural Products, 67 (12): 2141 – 2153.

Steward, M.S. (2005) Natural Products to Drugs: Natural Product Derived Compounds in Clinical Trials. Regular Product Reports, 22(2): 162.

Stroganov, I.A. (2001). Technique for Production of Special Vodka. RU2171281. Su, X., Xue, C., Li, X., Gao, X., Lou, Y. and Ding, J. (2011). Antitumor Activity of Polysaccharides and Saponin Extracted from Sea Cucumber. Clinical and Cellular Imunology, 2(1): 1-5.

Subhadrabandhu, S. and Ketsa, S. (2001). Durian: King of Tropical Fruit. D. Brasell Associates Press, New Zealand, 178 pages.

Sun, H.D., Huang, S.X. and Han, Q.B. (2006). Diterpenoids from Isodon Species and Their Biological Activities, Natural Product Reports, 23: 673-698.

Suresh, R.N. and Ganesh, R.K. (2013). Improvement and Discovery Avenues in Bioactive Natural Products for Glycemic Novel Therapeutics. Concentrates in Natural Products Chemistry, 39: 431-466.

Swaminathan, S., Fonseca, V.A., Alam, M.G. and Shah, S.V. (2007). "The Role of Iron in Diabetes and Its Complications," Diabetes Care, 30(7): 1926–1933.

Sweetman, S.C. (2005). Martindale In the Complete Drug Reference. Pharmaceutical Press, Williams Clowes; Suffock, UK. p. 907.

Sylla, T., Pouységu, L., Da Costa, G., Deffieux, D., Monti, J.P. and Quideau, S. (2015). Gallotannins and Tannic Acid: First Chemical Syntheses and In Vitro Inhibitory Activity on Alzheimer's Amyloid ß-Peptide Aggregation. Angewandte Chemie International Edition Engl, 54(28): 8217-8221.

Tannenbaum, C. and Sheehan, N.L. (2014). Comprehension and Preventing Drug-Drug and Drug-Gene Interactions. Master Review of Clinical Pharmacology, 7(4): 533–544.

Tapsell, L.C., Hemphill, I., Cobiac, L., Sullivan, D.R., Fenech, M., Patch, C.S., Roodenrys, S., Keogh, J.B., Clifton, P.M., Williams, P.G., Fazio, V.A. and Inge, K.E. (2006). "Medical advantages of Herbs and Spices: The Past, the Present, the Future". Restorative Journal of Australia, 185(4): S1–S24.

Tee, L.K. (2001). Distinguishing proof of Lactic Acid Bacteria Constituting the Predominating Microflora in An Acid-Fermented Condiment (Tempoyak) well known in Malaysia. Universal Journal of Food Microbiology, 63: 149–157.

Teixeira, R.R., Pereira, W.L., Tomaz, D.C., de Oliveira, F.M., Giberti, S. and Forlani, G. (2013). Combination Analogs of the Natural Compound Cryphonectric Acid Interfer With Photosynthetic Machinery Through Two Different Mechanisms. Diary of Agricultural and Food Chemistry, 61(23): 5540-5549.

Temitayo, An., Isaac, O., and Olugbengab, A. (2012). Basic and antimicrobial investigations of coordination mixes of phenylalanine and glycine. Worldwide Journal of Chemistry, 4(2), 49.

Thomas, L.L., David, A.W., Victoria, F.R. and William, S.Z. (2008). Foye?s Principles of Medicinal Chemistry. sixth ed. Lippincott W and Wilkins, a wolter Kluwer Press. Philadephia Baltimore. New York. London. p 1.

Tikoo, K., Sane, M.S. and Gupta, C. (2011). Tannic Acid Ameliorates Doxorubicin-Induced Cardiotoxicity and Potentiates Its Anti-Cancer Activity: Potential Role of Tannins in Cancer Chemotherapy. Toxicology and Applied Pharmacology, 251(3): 191-200.

Tirtawinata, M.R., Santoso, P.J. and Apriyanti, L.H. (2016). DURIAN.Pengetahuan Dasar Untuk Pencinta Durian, first ed.; Agriflo (Penebar Swadaya Grup): Jakarta, Indonesia, pp: 31, ISBN 978-979-002-703-9.

Tolnai, S. and Morgan, J.F. (2011). Concentrates on the In Vitro Antitumor Activity of Fatty Acids. V. Unsaturated Acids. Organic chemistry and Cell Biology, 40(7): 869-875.

Trem, J. and Smejkal, K. (2016). Flavonoids as Potent Scavengers of Hydroxyl Radicals. Exhaustive Reviews in Food Science and Food Safety, 15: 720-738.

Tsuzuki, J.K., Svidzinski, T.I.E., Shinobu, C.S., Silva, L.A., Rodrigues-Filho, E., Cortez, D.A.G. and Ferreira, I.C.P. (2007). Antifungal Activity of the Extracts and Saponins from Sapindus saponaria L. Anais da Academia Brasileira de Ciencias, 79(4): 577–583.

Ustundag, O.G. and Mazza, G. (2007). Saponins: Properties, Applications and Processing. Basic Reviews in Food Science and Nutrition, 47(3): 231-258.

Vairappan, C.S. (2004). Antibacterial Activity of Major Secondary Metabolities: Found in Four Species of Edible Green Macroalgae Genus Caulerpa. Asian Journal Microbiology, Biotechnolgy and Environmental Science, 6: 197–201.

Van Alstyne, K.L., Nelson, T.A. and Ridgway, R.L. (2015). Natural Chemistry and Chemical Ecology of "Green Tide" Seaweed Blooms. Integrative and Comparative Biology, 55(3): 518–532.

Velderrain-Rodríguez, G.R., Palafox-Carlos, H., Wall-Medrano, An., Ayala-Zavala, J.F., Chen, C.Y.O., Robles-Sanchez, M., Astiazaran-García, H., Alvarez-Parrilla, E. and González-Aguilar, G.A. (2014). Phenolic Compounds: Their Journey After Intake. Nourishment and Function, 5: 189–197.

Venkatesh, P., Hariprasath, K., Soumya, V., Francis, P.M. and Sankar, S. (2010). Detachment and Aphrodisiac Screening of the Fruits of Durio zibethinus Linn. Asian Journal of Biological Sciences, 3(1):1-17.

Ventola, C.L. (2015). The Antibiotic Resistance Crisis Part 1: Causes and Threats.Pharmacy and Therapeutics, 40(4): 277-283.

Villar, A.M., de las Heras, B., Rodríguez, B. and Boscá, L. (2003). Terpenoids: Sources, Structure Elucidation andTtherapeutic Potential in Inflammation, Current Topics in Medicinal Chemistry, 3: 53-67.

Vinarova, L., Vinarov, Z., Atanasov, V., Pantcheva, I., Tcholakova, S., Denkov, N. and Stoyanov, S. (2015). Bringing down of Cholesterol Bioaccessibility and Serum

Concentrations by Saponins: In Vitro and In vivo Studies. Nourishment and Function, 6(2): 501-512.

Vincken, J.P., Heng, L., de Groot, A. and Gruppen, H. (2007). Saponins, Classification and Occurrence in the Plant Kingdom. Phytochemistry, 68(3): 275-297.

Vinha, A.F., Guido, L.F., Costa, A.S., Alves, R.C. and Oliveira, M.B. (2015). Monomeric and Oligomeric Flavan-3-ols and Antioxidant Activity of Leaves from Different Laurus sp. Nourishment and Function, 6(6):1944-1949.

Vivas, N., Laguerre, M., Pianet de Boissel, I., Vivas de Gaulejac, N. and Nonier, M.F. (2004). "Conformational Interpretation of Vescalagin andCastalagin Physicochemical Properties". Diary of Agricultural and Food Chemistry, 52 (7): 2073–2078.

Voon, Y.Y., Sheik, A.H.N., Rusul, G., Osman, A. and Quek, S.Y. (2007). Unpredictable Flavor Compounds and Sensory Properties of Minimally Processed Durian (Durio zibethinus cv. D24) Fruit During Storage at 4 oC. Postharvet Biology and Technology, 46: 76–85.

Walters, W.P. and Namchuk, M. (2003). A Guide to Drug Discovery: Designing Screens: How to make your Hits a Hit. Nature Reviews Drug Discovery, 2: 259.

Wang, D., Du, X. and Zheng, W. (2008). Change of spit and serum Concentrations of Manganese, Copper, Zinc, Cadmium, and Lead among Career Welders. Toxicology Letters, 176: 40-47.

Wang, Z., Ma, P., Xu, L., He, C., Peng, Y. and Peigen, X.P. (2013). Assessment of the Content Variation of Anthraquinone Glycosides in Rhubarb by UPLC-PDA. Science Central Journal, 7: 170.

Wen, H., Kang, J., Li, D., Wen, W., Yang, F., Hu, H. and Liu, C. (2016). AntifungalSctivities of Anthocyanins from Purple Sweet Potato in the Presence of Food Preservatives. Nourishment Science and Biotechnology, 25(1): 165–171.

Williams Wilkinsp. 25. (ISBN 0-683-30737-1). W.H.O (World Health Organization) (2002). Conventional Medicine Strategy. p. 1 W.H.O (World Health Organization) (2008). "Customary Medicine Definitions". p. 15.

Williams, D.A. and Lemke, T.L. (2002). Section 1: Natural Products. Foye's Principles of Medicinal Chemistry (fifth ed.). Philadelphia: Lippincott

Xiao, Y., Hu, Z., Yin, Z., Zhou, Y., Liu, T., Zhou, X. and Dawei, C.D. (2017). Profiling and Distribution of Metabolites of Procyanidin B2 in Mice by UPLC-DAD-ESI-IT-TOF-MSn Technique. Boondocks in Pharmacology, 8(231): 1-12.

Xiao, Z. and Lu, J.R. (2014). Age of Acetoin and Its Derivatives in Foods. Diary of Agricultural and Food Chemistry, 62: 6487–6497.

Xiao-Rong, H., Lei-Lei, W., Zha-Jun, Z., Cheng-Ping, L. and Wei-Guang, S. (2011). Examination and Determination of Diterpenoids in Unprocessed and Processed Euphorbia lathyrism Seeds by HPLC-ESI-MS. Diary ofPharmaceutical Analysis, 1(3): 197–202.

Xu, R., Fazio, G.C. and Matsuda, S.P.T. (2004). "On the Origins of Triterpenoid Skeletal Diversity". Phytochemistry, 65(3): 261–291.

Xu, X.H., Zhu, Y.D., Gao, W. and Kong, C.H. (2006). Horticultural leadcompounds from Laurencia majuscula. In Proceedings of International Forum on Green Chemical Science and Engineering and Process Systems Engineering, Tianjin, China. p. 538–541.

Xu, Y. and Qian, S.Y. (2014). Hostile to disease Activities of 6 Polyunsaturated Fatty Acids. Biomedical Journal, 37(3): 112-119.

Yadav, N., Yadav, R. and Goyal, A. (2014). Science of Terpenoids. Global Journal of Pharmaceutical Science Review and Research, 27(2): 272-278.

Yadav, V.R., Prasad, S., Sung, B., Kannappan, R. and Aggarwal, B.B. (2010). Focusing on Inflammatory Pathways by Triterpenoids for Prevention and Treatment of Cancer. Poisons, 2: 2428-2466.

Yamamoto, Y. and Gaynor, R.B. (2001). Helpful Potential of Inhibition of the NF Kappa B Pathway in the Treatment of Inflammation and Cancer. Diary of Clinical Investigation, 107(2): 135-142.

Yan, L.L., Zhang, Y.J., Gao, W.Y., Man, S.L. and Wang, Y. (2009). In vitro and In Vivo Anticancer Activity of Steroid Saponins of Paris polyphylla var. yunnaensis. Test Oncology, 31(1): 27–32.

Yang, C.R., Zhang, Y., Jacob, M.R., Khan, S.I., Zhang, Y.J. and Li, X.C. (2006). Hostile to parasitic action of C-27 steroidal saponins. Antimicrobial Agents and Chemotherapy, 50(5): 1710–1714.

Yao, M., Wang, D. and Zhao, M. (2015). Component Analysis Based on Energy-Dispersive X-Ray Fluorescence. Advances in Materials Science and Engineering, 2015: 7 pages.

Yao, W.R., Wang, H.Y., Wang, S.T., Sun, S.L., Zhou, J. and Luan, Y.Y. (2011). Evaluation of the Antibacterial Activity and the Antidiarrheal Function of Flavonoids from Bayerry Fruit. Diary of rural and nourishment science, 59(10): 5312-5317.

Yassin, N.Z., Melek, F.R., Selim, M.A. and Kassem, I.A.A. (2013). Pharmacological Activities of Saponin Containing Fraction Derived from Gleditsia capsica Desf. Methanolic Fruit Extract. Der Pharmacia Lettre, 5(2):247-253.

Yeh, J.Y., Huang, W.J., Kan, S.F. and Wang, P.S. (2003). Impacts of Bufalin and Cinobufagin on the Roliferation of Androgen Dependent and Independent Prostate Cancer Cells. Prostate, 4: 112–124.

Yong-Kim, Y.Y. and Byung-Zun Ahn, N.H.N. (2003). Antitumor Activity of unsaturated fat Esters of 4 '- demethyldeoxypodophyllotoxin. Bioorganic and Medicinal Chemistry Letters, 13(16): 2629-2632.

Yoshiaki, Y., Tatsuro, K., Toshiya, M. and Yoshimune, N. (2015). Oil and Bactericidal Properties of Calcium Salts of Fatty Acids: Effect of level of Unsaturation. Cleansers, Surfactants, Interface and Colloid, 64(10):1095-1100.

Yoshida, T., Amakura, Y. and Yoshimura, M. (2010). Basic Features and Biological Properties of Ellagitannins in Some Plant Families of the Order Myrtales, International Journal of Molecular Sciences, 11(1): 79–106.

Yoshida, T., Hatano, T.S., Ito, H., Okuda, T. and Quideau, S. (2009). Science and Biology of Ellagitannins, World Scientific Publishing, Singapore, pages 55–93.

Youthful, E.A. and Becker, J.B. (2009). Sex Matters: Gonadal Steroids and the Brain.Neuropsychopharmacology, 34: 537–538.

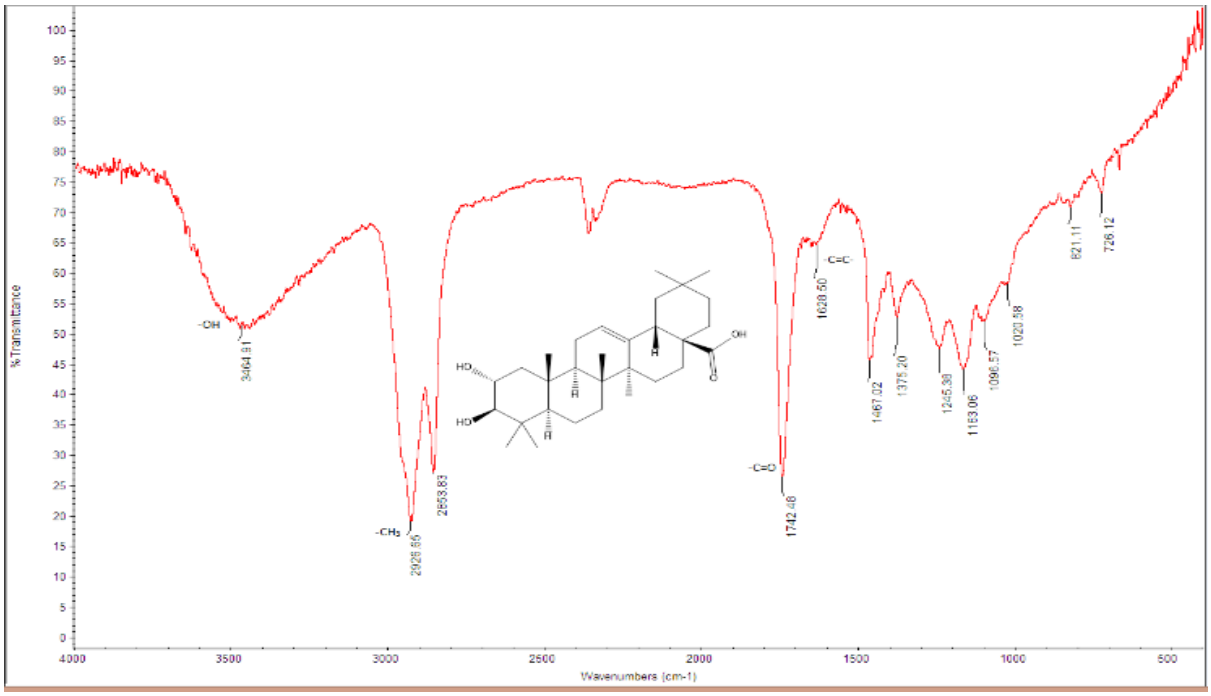
Yücekutlu, A.N. and Bildaci, I. (2008). Assurance of Plant Saponins and Some of Gypsophila Species: A Review of the Literature. Hacettepe Journal of Biological Chemistry, 36(2): 129-135.

Zakaria, N.A., Ibrahim, D., Sulaiman, S.F. and Supardy, A. (2011). Appraisal of Antioxidant Activity, Total Phenolic Content and In-Vitro Toxicity of Malaysian Red Seaweed, Acanthophora spicifera. Diary of Chemical and Pharmaceutical Research, 3: 182-191.

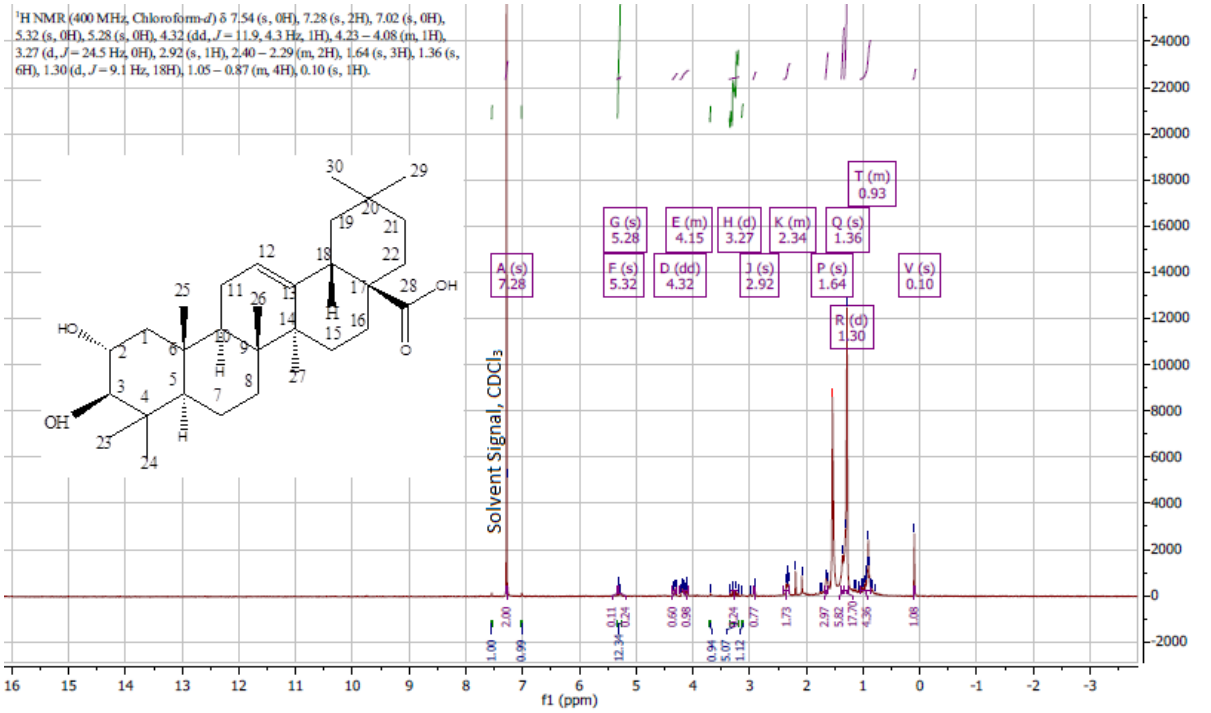
Zhang, Y., Wang, D., Lee, R.P., Henning, S.M. and Heber, D. (2009). "Nonappearance of Pomegranate Ellagitannins in the Majority of Commercial Pomegranate Extracts: Implications for Standardization and Quality Control". Diary of Agricultural and Food Chemistry, 57 (16): 7395–7400.

Zhang, Y.Q. and Rao, R. (2010). Past ergosterol: connecting pH to antifungal components. Destructiveness, 1: 551–554.

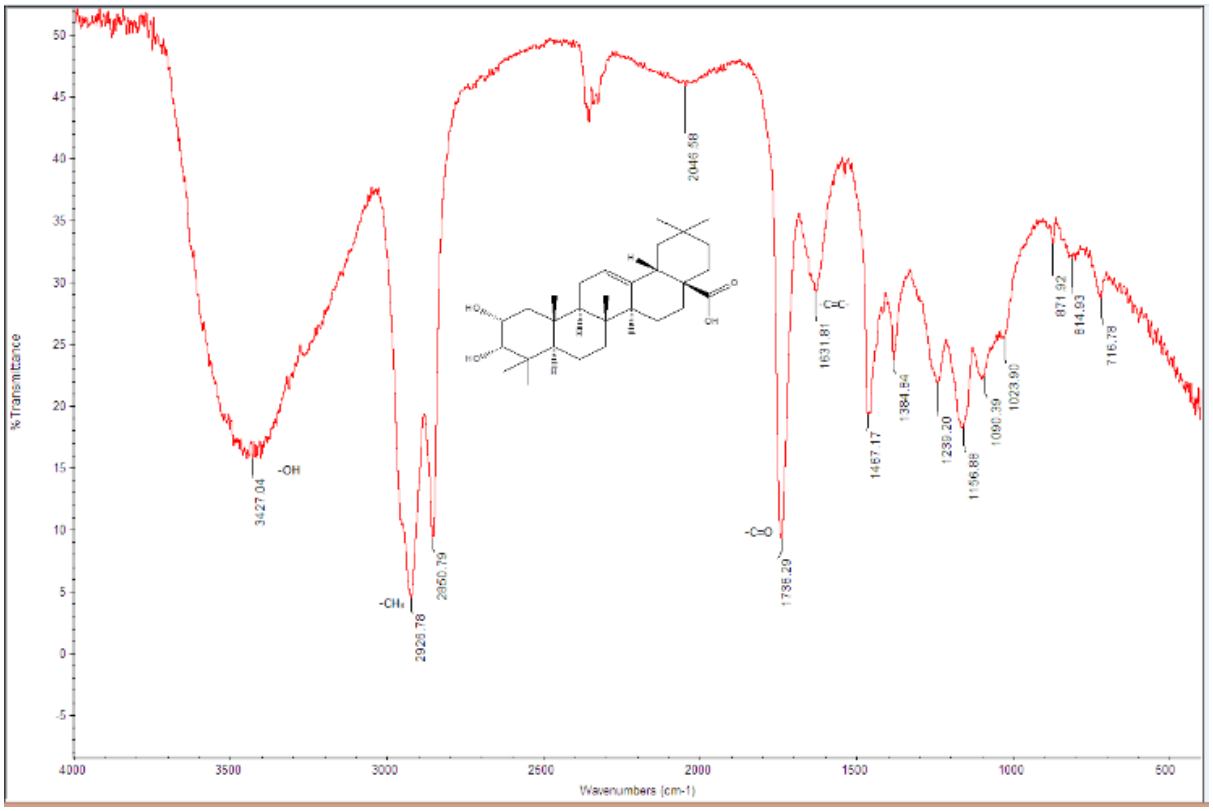
APPENDICES



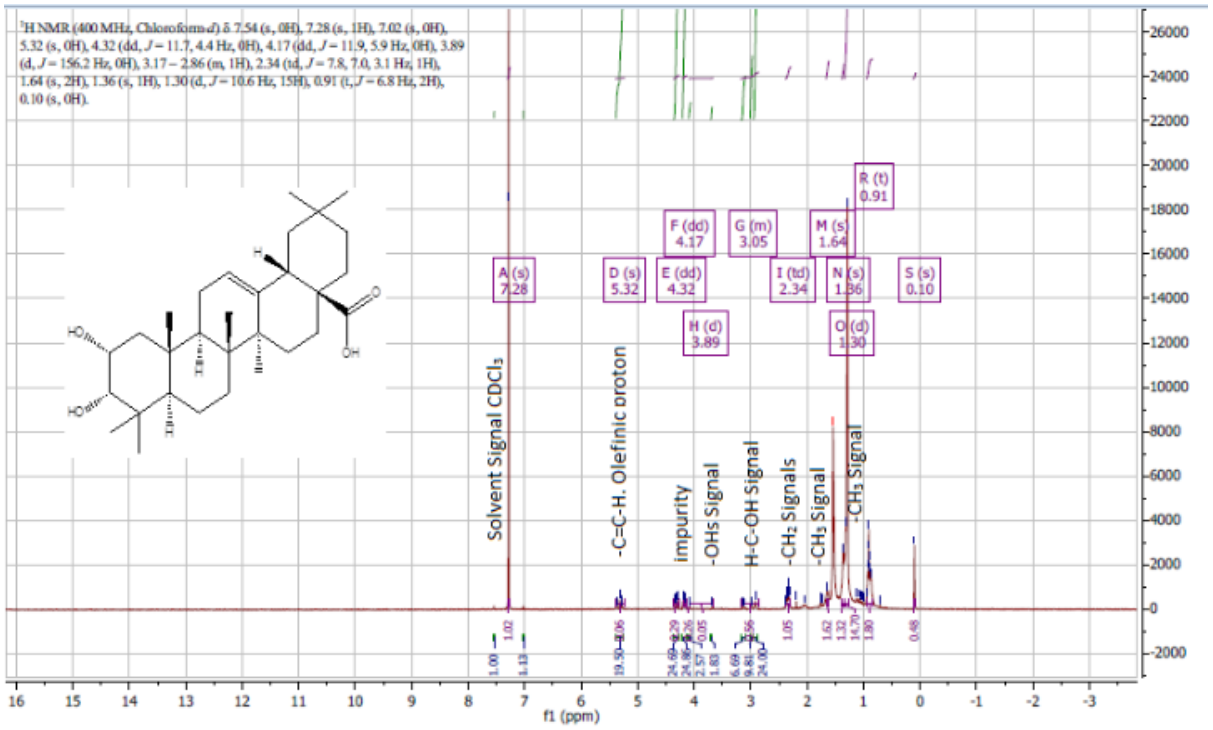
Appendix I: IR spectrum of Compound A [Maslinic acid, (2α,3β)-2,3-Dihydroxyolean-12-en-28-oic acid]



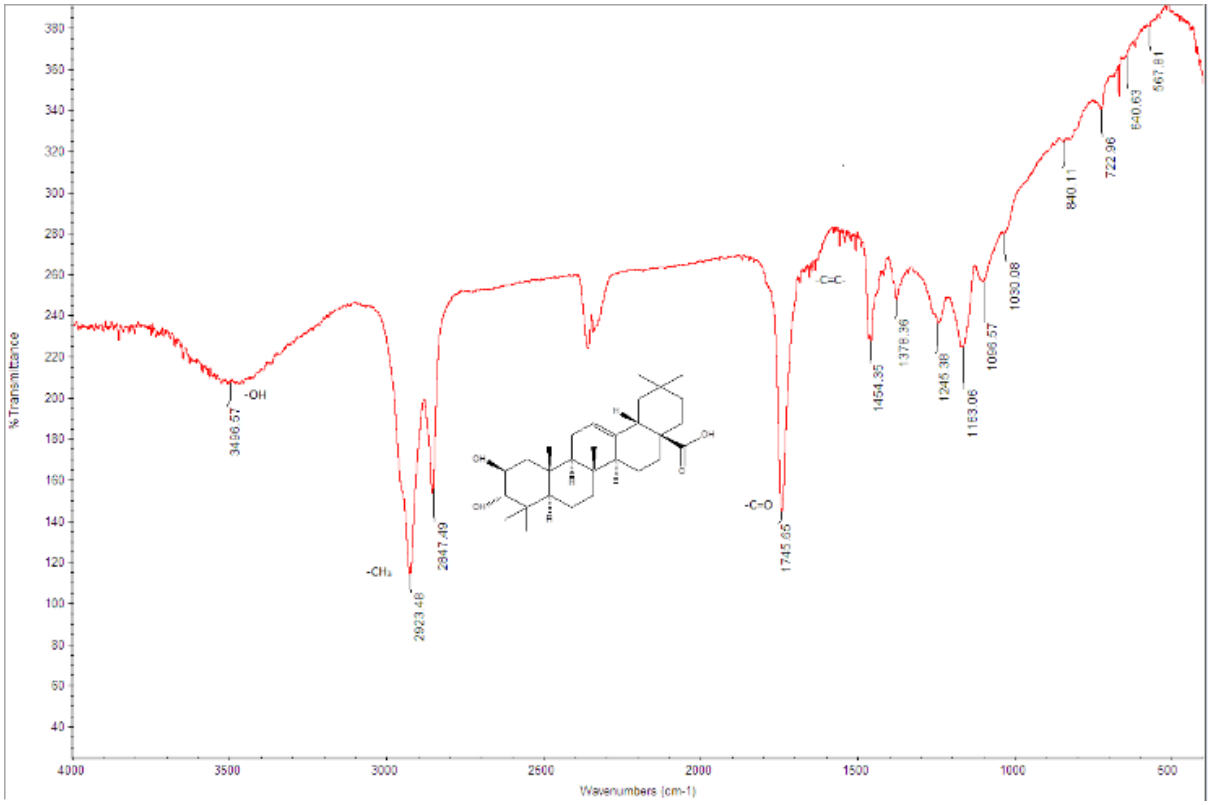
Appendix II: ^{1}H-NMR spectrum of Compound A [Maslinic acid, (2α,3β)-2,3-Dihydroxyolean-12-en-28-oic acid]



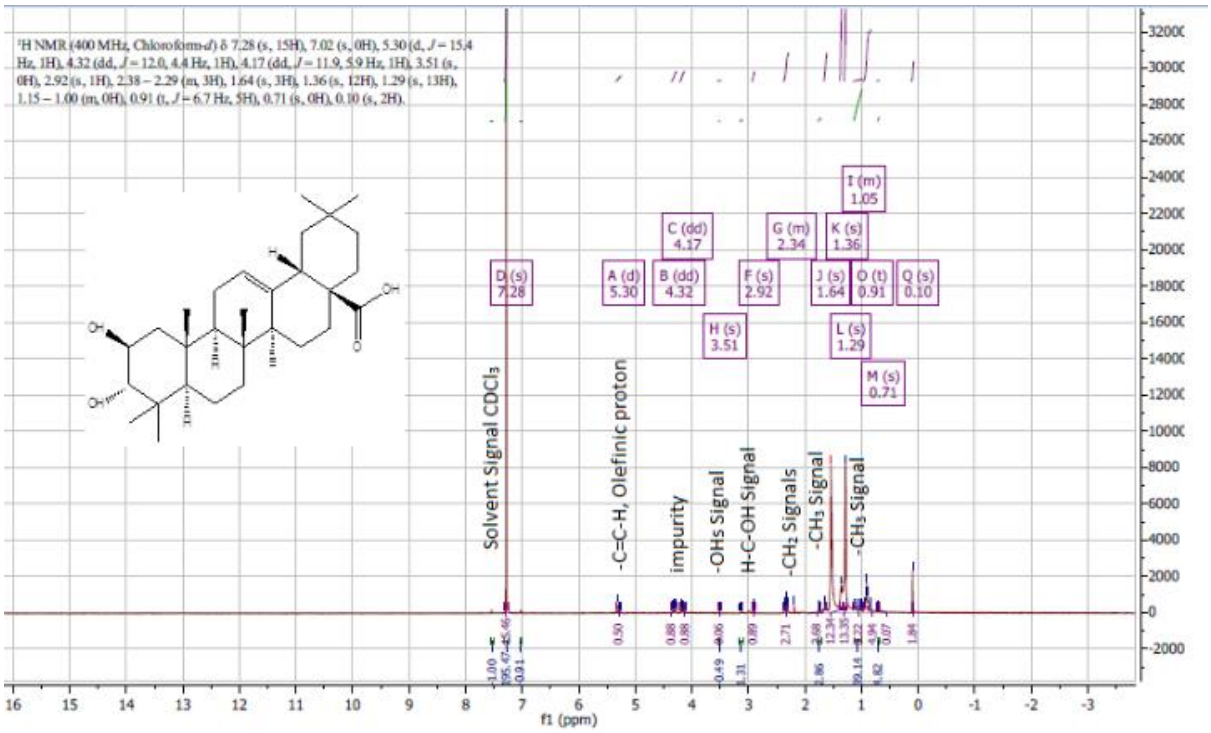
Appendix III: IR spectrum of Compound B [3-Epimaslinic acid, (2α,3α)-Dihydroxyolean-12-en-28-oic acid]



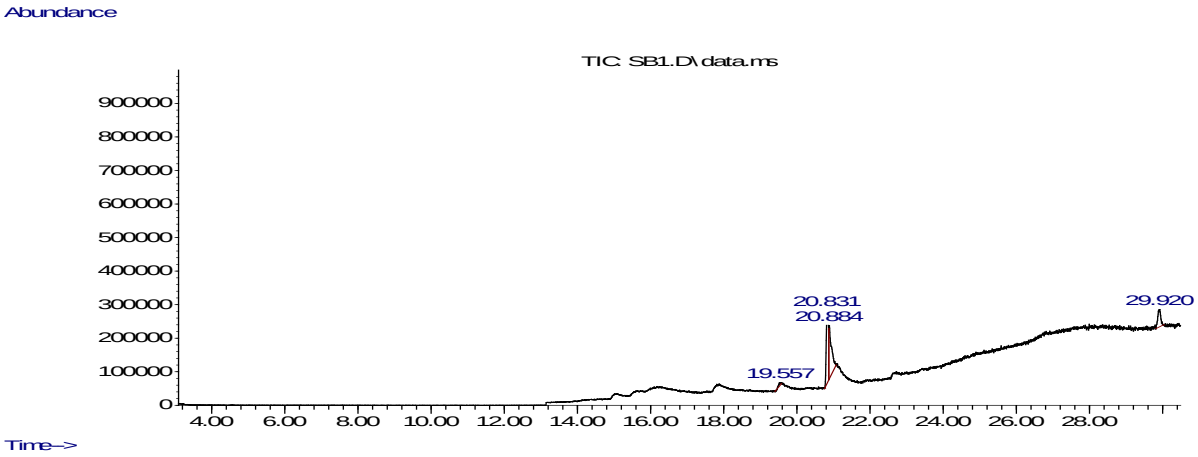
Appendix IV: ^{1}H-NMR spectrum of Compound B [3-Epimaslinic acid, (2α,3 α)-2,3-Dihydroxyolean-12-en-28-oic acid]



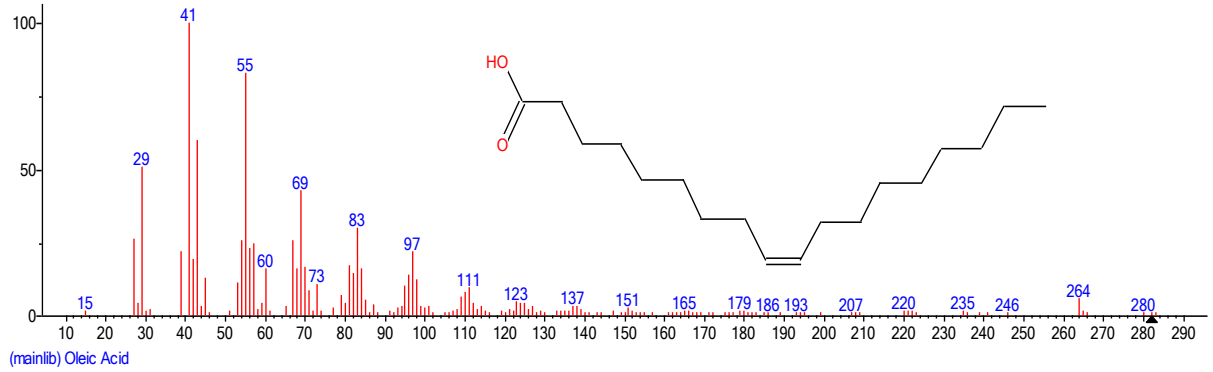
Appendix V: IR spectrum of Compound C [3-Epimaslinic acid, (2β,3α)-Dihydroxyolean-12-en-28-oic acid]



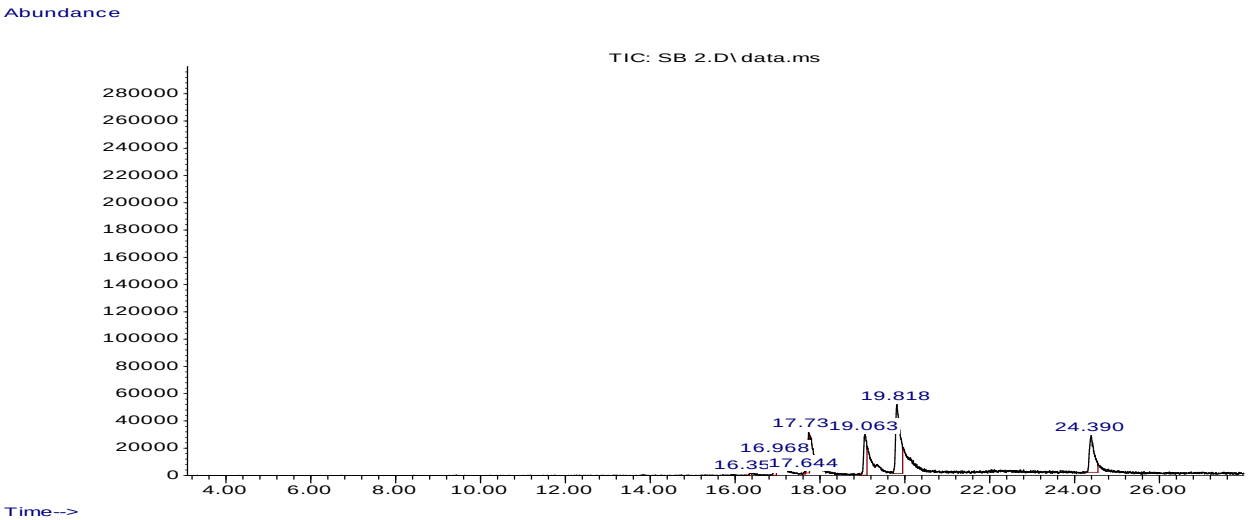
Appendix VI: ^{1}H-NMR spectrum of Compound C [3-Epimaslinic acid, (2β,3α)-2,3-Dihydroxyolean-12-en-28-oic acid]



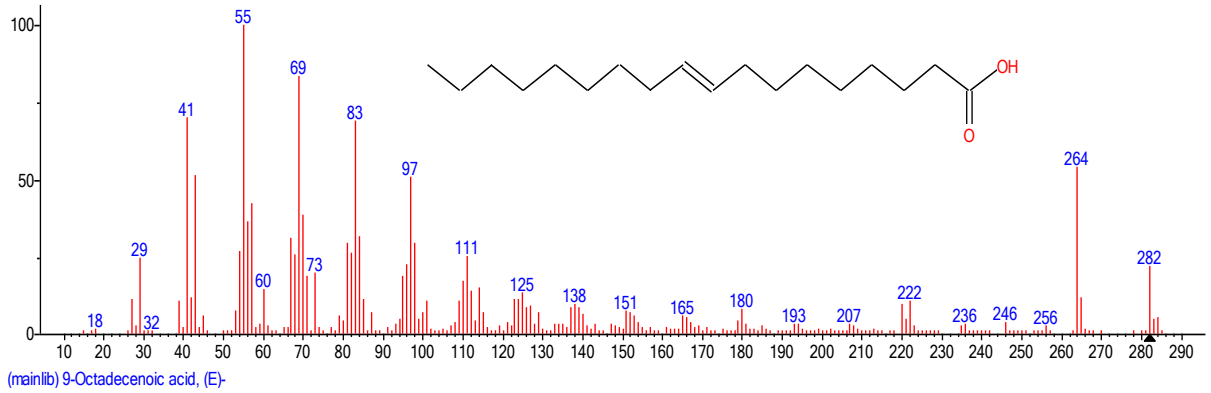
Appendix VIIa: GC-MS Chromatogram of fraction SBF1 of methanolic stem bark extract from *D. zibethinus*



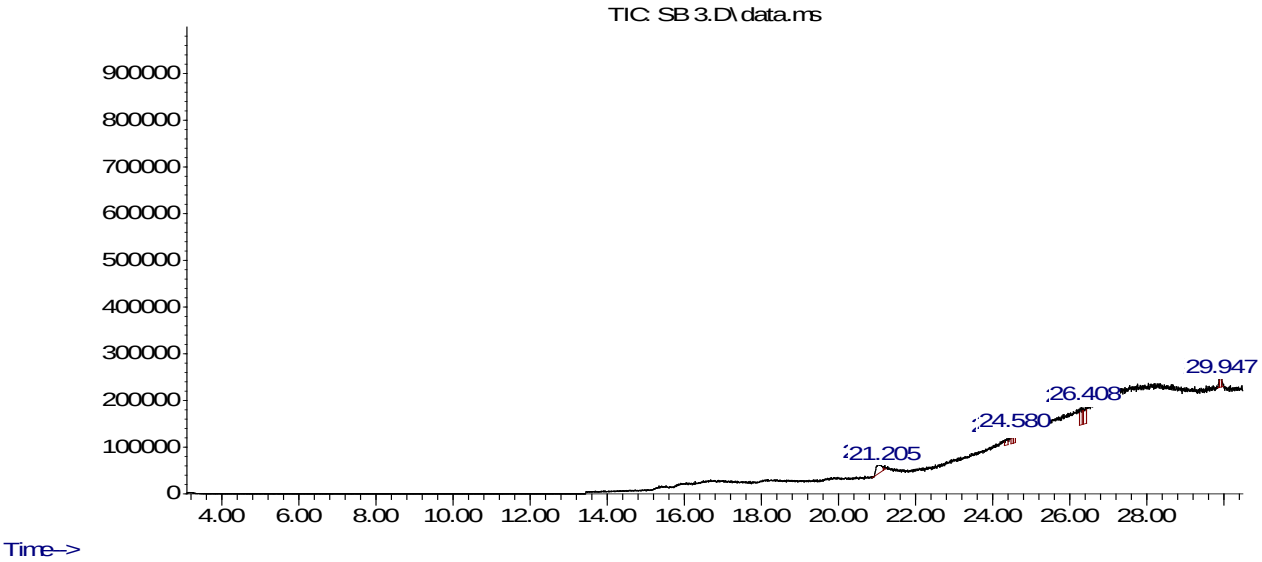
Appendix VIIb: Mass spectrum of Oleic acid (RT: 29.923, Area % = 91.36) from fraction SBF1



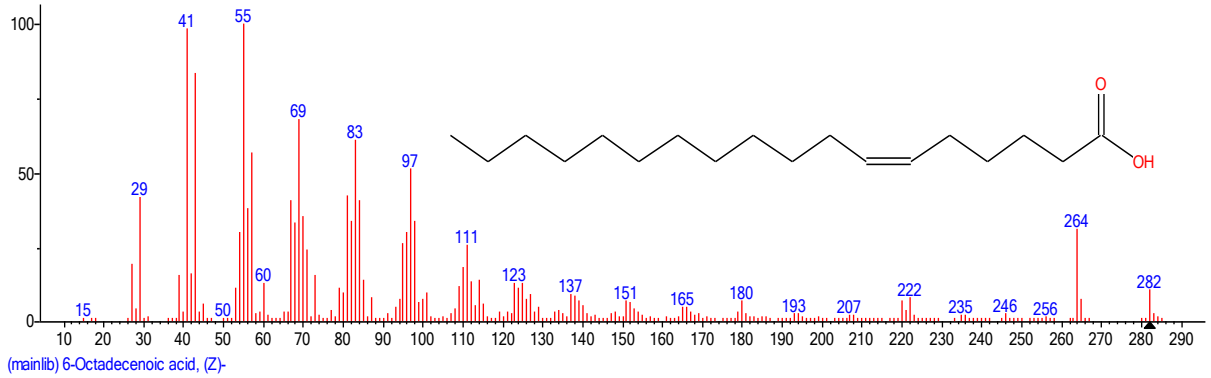
Appendix VIIIa: GC-MS Chromatogram of fraction SBF2 of methanolic stem bark extract from *D. zibethinus*



Appendix VIIIb: Mass spectrum of (E)-9-Octadecenoic acid (RT: 19.818, Area % = 56.10) from fraction SBF2

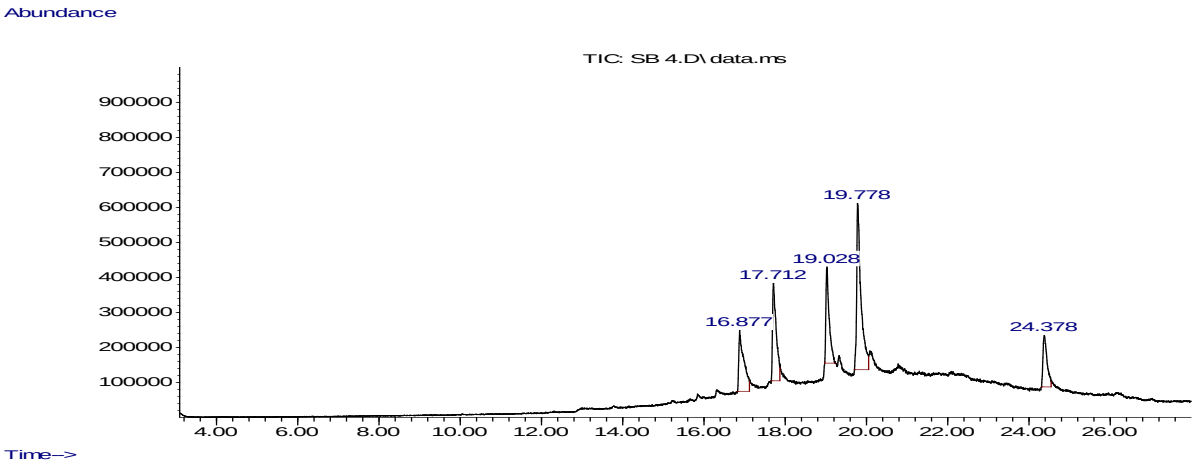


Appendix IXa: GC-MS Chromatogram of fraction SBF3 of methanolic stem bark extract from *D. zibethinus*

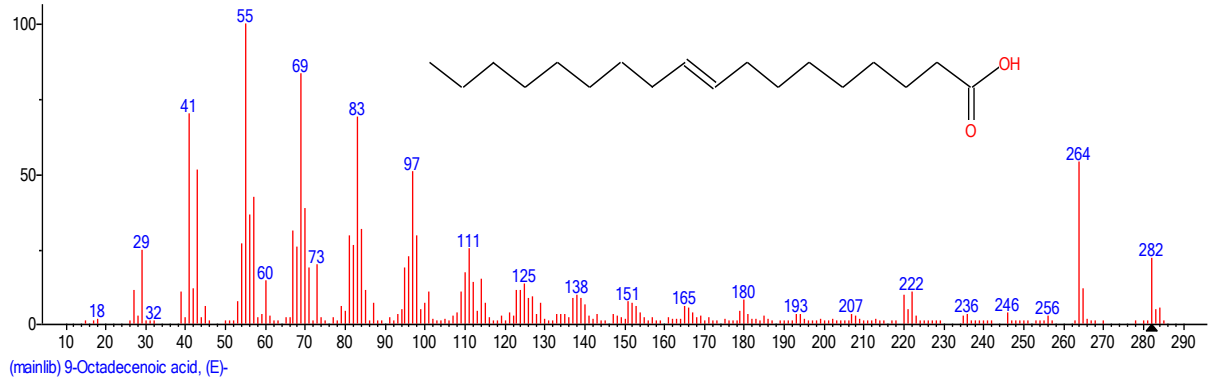


Appendix IXb: Mass spectrum of (Z)-6-Octadecenoic acid (RT: 26.307,

Area % = 41.19) from fraction SBF3



Appendix Xa: GC-MS Chromatogram of fraction SBF4 of methanolic stem bark extract from *D. zibethinus*



Appendix Xb: Mass spectrum of (E)-9-Octadecenoic acid (RT: 19.778, Area % = 42.20) from fraction SBF4