

**ANTI-PLASMODIAL ACTIVITY AND EFFECTS OF *SCHWENKIA AMERICANA* AND
OLAX SUBSCORPIOIDEA EXTRACTS ON CYSTEINE PROTEASE FROM
PLASMODIUM BERGHEI (NK 65) AND *PLASMODIUM FALCIPARUM***

BY

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NOVEMBER, 2018

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**DEPARTMENT OF BIOCHEMISTRY,
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AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA**

NOVEMBER, 2018

DECLARATION

I declare that this thesis entitled, '**Anti-Plasmodial Activity and Effects of *Schwenkia americana* and *Olox subscorpoidea* Extracts on Cysteine Protease from *Plasmodium berghei*(NK65) and *Plasmodium falciparum***' has been carried out by me in the Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria under the supervision of Professor H. C. Nzelibe, Dr. A. B. Sallau and Professor. K. M. Anigo. No part of this thesis, as it is, was previously presented for another degree or diploma at any University. Information and excerpts cited from the work of others have been duly acknowledged by means of references.

GABI BABA
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DATE

CERTIFICATION

This thesis entitled, **Anti-Plasmodial Activity and Effects of *Schwenkia americana* and *Olex subscorpioidea* Extracts on Cysteine Protease from *Plasmodium berghei*(NK65)and *Plasmodium falciparum*** by GABI BABA meets the regulations governing the award of the Degree of Doctor of Philosophy (PhD) in Biochemistry of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this Thesis to my parents and my family members especially my late wife Amina, Ibrahim for their uncompromised love, support and dedication toward my success in attaining what I am today and what I will become in the nearest future.

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ABSTRACT

This study investigated the *anti-plasmodial* activity and inhibitory effect of extracts of *Olex subscorpioidea*, and *Schwenkia americana* on cysteine protease from *P. falciparum* and *P. berghei*(NK65). pLDH assay method was used in the *antiplasmodial* activity, while four-day suppressive activity method was used to evaluate the *in-vivo anti-plasmodial* activity against *Plasmodium berghei* (NK 65) strain. Isolation of the lead compounds, its characterization and structural identification by UPLC/LCMS, and NMR spectra analysis were accomplished, with data generated via *anti-plasmodial* bio-assay guided fractionation. The variable from the schizont growth inhibition, IC₅₀ and the enzyme inhibition profile were analysed statistically. The *in-vitro antiplasmodial* assay showed 82% of the screened, ethnobotanical identified plants extracts to be active against *P. falciparum* 3D7 strain with *O. subscorpioidea* and *S. americana* showing more promising activity with IC₅₀ of 0.13 and 0.040 respectively. Such response led to *in-vivo study* using murine strain which indicated parasite growth inhibition of *P. berghei* NK 65 in infected mice at dose ranges of 50 to 200mg/kg body weight. *S. americana*, *O. subscorpioidea* and *S. seiberiana* exhibited parasite suppression of 71.21±4.7 to 77.15±3.36, 72.52±7.57 and 77.42±10.84 and 32.22±12.69 33.74±15.65% respectively. Expressing all the extracts to be considered active at parasite suppressions ≥ 30%. Six active fractions of *O. subscorpioidea* against *P. falciparum* with IC₅₀ range of 0.3 to 6.22µg/ml were identified, with GB003BC1.1 most active fraction with IC₅₀ of 0.33 µg/ml, which on further purification gave compounds identified to be Re1, 1β, 3α- dihydroxyl 7α, 9β dihydroxylcyclopentane (Octahydro-2-methyl-1H-indene-1, 3, 4, 6-tetraol) with IC₅₀ 1.65 µg/mol and 1,2,3,4-tetrahydro-1-(1-hydroxybutan-2-yl)-4-methylnaphthalene-2,3-diol with IC₅₀ 1.79µg/ml both of which inhibits cysteine protease of *P. berghei*. *Schwenkia americana* however, gave seven fractions active against *P. falciparum* with 010EA2.3.1 fraction most active with IC₅₀ 1.75µg/ml, on further purification gave 010EA2.3.1G identified as compounds 2,5-dihydroxy-3-(2E,6E,10E) 3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl)cyclohexa-2,5-diene-1,4-dione, with IC₅₀ 2.92µg/ml which inhibits the cysteine protease of *P. berghei* and *P. falciparum*. The crude extracts effects were comparable to chloroquine with 84.52% inhibition. LD₅₀ of the 3 plant extracts in rats and *S. seiberiana* and *S. americana* in mice were greater than 5000mg/kg body weight. While LD₅₀ of *O. Subscorpioidea* in mice was 3800mg/kg body weight. Toxicity doses were 50 -100 times higher than active doses, the extracts can be used at lower doses within the shortest time of treatment with maximum benefits achieved and deleterious effect averted effectively. While identified compounds in the study are considered good candidates as potential *anti-plasmodial* agents with their activities concentration dependent, but reduces as purification progress.

TABLE OF CONTENTS

CONTENT	PAGE
TITLE PAGE	ii
DECLARATION.....	III
CERTIFICATION.....	IV
DEDICATION.....	V
ACKNOWLEDGEMENT.....	VI
ABSTRACT.....	VIII
Table of Contents.....	X
LIST OF TABLE.....	XIII
List of Figures.....	XVI
LIST OF PLATE.....	VIII
LIST OF APPENDICES.....	XIX
List of	
Abbreviations.....	xviii
CHAPTER ONE.....	1
1.0 Introduction.....	1
1.1 Back ground of Research.....	1
1.2 Statement of Research Problem.....	4
1.3 Justification of the Study.....	4
1.4 Aim and Objectives.....	5
1.5 The specific objectives include:.....	5
CHAPTER TWO.....	6
2.0 Literature Review.....	6
2.1 Malaria.....	6
2.2 Global Threat of Malaria.....	6
2.3 Malaria and Sub-Saharan Africa.....	7
2.4 Incidence of Malaria in Nigeria.....	8
2.5 History of Malaria.....	8
2.6 Life Cycle.....	12
2.6.1 Sporozoites Invasion Mediation Surface Proteins.....	13
2.6.2 Schizogony.....	15
2.6.3 Gametocytogenesis.....	17
2.7 Resistance and the Role of Gametocytes in Drug Resistance Property of <i>Plasmodium</i> Species.....	19
2.8 Potential Drug Targets in Malaria Parasite.....	21
2.8.1 TCA Cycle as a Potential Drug Target.....	21
2.9 Current Anti-Malarial Drugs And Mechanism Of Action.....	22
2.9.1 Quinolines.....	22
2.9.2 Artemisinin.....	23
2.9.3 Antifolates.....	24
2.9.4 Atovaquone/Proguanil.....	24
2.9.5 Sulfonamides.....	24
2.9.6 Antibiotics.....	25
2.9.7 Amino Alcohols.....	25
2.9.8 Others.....	25
2.10 The Problems and the Prospects of Drug Resistance.....	26

2.11	Classification of Proteases.....	28
2.11.1	Cysteine (thiol) Protease (EC 3.4.22).....	30
2.11.2	Cysteine Protease for Infectious Diseases	30
2.11.3	Catalytic Mechanism of Cysteine Proteases	31
2.11.4	Cysteine Proteases of <i>Plasmodium</i>	33
2.12	Medicinal Plants Uses and Practices in African Ethno-pharmacopeia.....	35
2.12.1	<i>Olox subscorpioidea</i>	36
2.12.2	<i>Schwenckia americana</i>	38
CHAPTER THREE.....		40
3.0	MATERIALS AND METHODS.....	40
3.1	Materials.....	40
3.1.1	Plant Materials	40
3.1.2	Animals	40
3.1.3	Parasite	41
3.1.4	Chemicals.....	41
3.1.5	Ethno-botanical Survey Study Site	41
3.2	Methods.....	43
3.2.1	Collection, Identification and Processing of the Plants Species	43
3.2.2	Ethno-botanical Survey.....	43
3.2.3	Microscopic Determination of Parasitemia by Giemsa Stained Smear.	44
3.2.4	<i>In-vitro</i> anti- <i>plasmodial</i> assay.....	45
3.2.4.1	(pLDH) Parasite Lactate Dehydrogenase Colorimetric Enzyme Assay	45
3.2.4.2	SYBR Green I Antiplasmodial Assay Method	46
3.2.5	Cultivation and Synchronization of <i>Plasmodium falciparum</i>	47
3.2.6	Phytochemical Screening.....	48
3.2.7	Toxicity Profiling of the Active Extracts	48
3.2.7.1	Acute Toxicity Studies LD ₅₀	48
3.2.7.2	Sub-Acute Toxicity Studies	48
3.2.7.3	Determination of Biochemical Parameters	49
3.2.8	<i>In-vivo</i> Anti- <i>Plasmodia</i> Activity Test.....	49
3.2.8.1	Collection of <i>Plasmodium beghei</i> Parasite	49
3.2.8.2	Parasite Preparation and Inoculation.....	49
3.2.8.3	Animal Grouping	50
3.2.8.4	4-Day Suppressive Activity of the Extract	50
3.2.8.5	Percentage Parasitemia and Percentage Growth Suppression	51
3.2.9	Thin Layer Chromatography (TLC) Profiling of the Plant Extract	51
3.2.10	Column Chromatography.....	52
3.2.10.1	Bio- Guided Anti-Plasmodial Assay Fractionation of <i>Olox subscorpioidea</i>	52
3.2.10.2	Bio- Guided Anti-Plasmodial assay Fractionation of <i>Sckwenkia americana</i>	53
3.2.11	UPLC-MS (LC-MS) Profiling of the Fractions	56
3.2.12	NMR (Nuclear Magnetic Resonance).....	57
3.2.13	Study on Inhibition of Extract on Cysteine Protease of Parasites	57
3.2.13.1	Extraction of Cysteine Protease from the Parasites	57
3.2.13.2	Determination of Total Protein	58
3.2.13.3	Purification of Cysteine Protease using Ammonium Sulphate	58
3.2.13.4	Protease Activity Assay:.....	59
3.2.13.5	Enzyme Inhibition Studies	59
3.3	Ethical Approval.....	59

3.4	Statistical Analysis.....	60
CHAPTER FOUR.....		61
4.0	RESULTS.....	6
1		
4.1	Ethno-Botanical Survey Information and Practices of Traditional Healers in Treatment of Malaria.....	61
4.2	Percentage Yield of the Crude Extracts.....	63
4.3	In-vitro Anti-Plasmodium pLDH Assay of Some Selected Plant Extracts against <i>P. falciparum</i> 3D7 and FCR3 Strains.....	63
4.4	Phyto-chemical Analysis	64
4.4.1	The Phyto-chemical composition of <i>O. subscorpioidea</i> , <i>S. americana</i> and <i>S. seiberiana</i> Methanol Leaf Extracts.....	64
4.5	Toxicity Profiles Of The Plant Extracts	70
4.5.1	Acute Toxicity Profile of <i>O. subscorpioidea</i> , <i>S. americana</i> and <i>S. seiberiana</i>	70
4.6	In-vivo Growth Inhibition Activity of <i>O. subscorpioidea</i> , <i>S. americana</i> and <i>S. seiberiana</i> Against <i>Plasmodium berghei</i> NK 65 Strain.....	72
4.7	In-vitro <i>P. falciparum</i> asexual stage Proliferation percentage Inhibition and IC ₅₀ of Fractions and Compounds of <i>Olox subscorpioidea</i>	74
4.8	<i>In-vitro P. falciparum</i> Proliferation % Inhibition of Fractions and Compounds from <i>Schwenkia americana</i>	82
4.9	Chemical and Structural Elucidation of Compounds Isolate.....	91
4.10	¹ H NMR (Proton Nuclear Magnetic Resonance).....	91
4.11	¹³ C NMR (Carbon Nuclear Magnetic Resonance).....	91
4.12	Proposed Compound of GB003BCF2AE3.....	91
4.13	Chemical And Structural Elucidation Of Compounds Isolate.....	95
4.14	¹ HNMR (Proton Nuclear Magnetic Resonance).....	95
4.15	¹³ CNMR (Carbon Nuclear Magnetic Resonance).....	95
4.16	Proposed Compound Of GB003BCF3A1.1.....	95
4.17	Chemical And Structural Elucidation Of Compounds Isolate From <i>S. americana</i>	96
4.18	Chemical And Structural Elucidation Of Compounds Isolate From <i>S. americana</i>	96
4.19	Extraction Of <i>Plasmodium berghei</i> (NK 65) And <i>Plasmodium falciparum</i> Cysteine Protease.....	105
4.20	<i>Plasmodium berghei</i> (NK 65) And <i>Plasmodium falciparum</i> Cysteine Protease (CP) Inhibition By <i>Olox Subscopoidia</i> Fractions.....	105
4.21	<i>Plasmodium Berghei</i> (NK 65) And <i>Plasmodium Falciparum</i> Cysteine Protease Inhibition By <i>Schwenkia americana</i> Fractions.....	105
4.22	<i>Plasmodium Berghei</i> (NK 56) And <i>Plasmodium Falciparum</i> Cysteine Protease Inhibition By The Fractions Of <i>Olox subscopoidia</i> And <i>S. americana</i>	105
CHAPTER FIVE.....		114
5.0	DISCUSSION.....	114
CHAPTER SIX.....		123
6.0	Conclusion and Recommendations	123
6.1	Conclusion.....	123
References.....		125
APPENDIX		146

LIST OF TABLES

Table 2.1	Body System Regulated by Limited Proteolysis.....	29
Table 2.2:	The Nomenclature of Proteinases (Endo-proteinases) and their common inhibitors.....	34
Table 4.1:	List of Traditional Medicinal Plants Used By the Traditional Medical Practitioners for the Treatment of Malaria in Bida.....	62
Table 4.2:	Percentage Yield of the Crude Extract using methanol as solvent.....	65
Table 4.3:	In-vitro <i>Anti-Plasmodial</i> pLDH Assay and the IC ₅₀ of Some Selected Plant Extracted against of <i>P. falciparum</i> 3D7 Strains.....	66
Table 4.4:	<i>In-vitro Anti-Plasmodial</i> Activity of Some Selected Plant Extracts against <i>P. falciparum</i> 3D7 and FCR3 Strains	67
Table 4.5:	<i>In-vitro Anti-Plasmodial</i> Activity of <i>O. subscorpioidea</i> , <i>S. americana</i> and <i>S. seiberiana</i> Methanol Leaf Extracts Against <i>P. falciparum</i> 3D7 Strains.....	68
Table 4.6:	The Quantitative Phytochemical composition of <i>O. Subscorpioidea</i> , <i>S. americana</i> And <i>S. seiberiana</i> Methanol Leaf Extracts	69
Table 4.7:	Acute Toxicity Profile Of <i>O.Subscorpioidea</i> , <i>S. americana</i> And <i>S. seiberiana</i> Methanol Leaf Extracts Against Experimental Model (Mice)	71
Table 4.8	<i>In-vivo</i> Parasite Growth Inhibition Chemo-suppression and animal protection Activities of <i>O. subscorpioidea</i> , <i>S. americana</i> and <i>S. seiberiana</i> Methanol Extracts Against <i>Plasmodium berghei</i> NK 65 Strain.....	73
Table 4.9:	<i>In vitro Plasmodium falciparum</i> Asexual Stage Proliferation Percentage Inhibition and IC ₅₀ of The Crude Extract and Column Fractions of <i>Olox subscorpioidea</i>	77
Table 4.10:	<i>In vitro Plasmodium falciparum</i> Asexual Stage Proliferation Percentage Inhibition and IC ₅₀ of GB003B Fractions of <i>Olox subscoipodia</i>	78

Table 4.11:	In vitro <i>Plasmodium falciparum</i> Asexual Stage Proliferation Percentage Inhibition and IC ₅₀ of GB003C Fractions of <i>Olox subscoipodia</i>	79
Table 4.12:	<i>In vitro Plasmodium falciparum</i> Asexual Stage Proliferation percentage Inhibition and IC ₅₀ of GB003BC1.1 Fractions of <i>Olox subscorpioidea</i>	80
Table 4.13:	<i>In vitro Plasmodium falciparum</i> Asexual Stage Proliferation percentage Inhibition and IC ₅₀ of Crude Leaf Extract and Column Fractions of <i>Schwenckia americana</i>	85
Table 4.14:	<i>In vitro Plasmodium falciparum</i> Asexual Stage Proliferation percentage Inhibition and IC ₅₀ of <i>Schwenckia americana</i>	86
Table 4.15:	<i>In vitro Plasmodium falciparum</i> Asexual Stage Proliferation percentage Inhibition and IC ₅₀ GB010C3 Fractions of <i>Schwenckia americana</i>	87
Table 4.16:	<i>In vitro Plasmodium falciparum</i> Asexual Stage Proliferation percentage Inhibition and IC ₅₀ GB010EA Fractions of <i>Schwenckia americana</i>	88
Table 4.17:	<i>In vitro Plasmodium falciparum</i> Asexual Stage Proliferation percentage Inhibition and IC ₅₀ GB010EA2.3.1 Fractions of <i>Schwenckia americana</i>	89
Table 4.18:	1D and 2D Spectral Summary for Compound GB3BCF2ae3 in CDCl ₃ (400MHz, 100MHz) J= Hz.....	90
Table 4.19:	1D and 2D Spectral Summary for Compound GB3BCF3A1.1 in CDCl ₃ (400MHz, 100MHz) J = Hz.....	94
Table 4.20:	Purification Profile For <i>Plasmodium berghei</i> (NK65) Cysteine Protease.....	107
Table 4.21:	Purification Profile for <i>Plasmodium falciparum</i> cysteine protease.....	108
Table 4.22:	<i>Plasmodium berghei</i> (NK65) and <i>Plasmodium falciparum</i> Cysteine Protease Inhibition by <i>Olox Subscopoidea</i> Fractions.....	112
Table 4.23:	<i>Plasmodium berghei</i> (NK65) and <i>P.falciparum</i> Cysteine Protease Inhibition by Fractions of <i>Schwenkia americana</i>	113

LIST OF FIGURES

Figure 2.1	The life cycle of Malaria parasite	16
Figure 2.2	Catalytic Mechanism of Cysteine Proteases	32
Figure 3.1	The Map of Bida Town showing the point of Survey.....	42
Figure 3.2	Schematic Representation of Antiplasmodial Bio-assay Guided purification of active fractions from <i>Olox Subscioipodia</i> Extract showing both active and non active fractions.....	54
Figure 3.3:	Schematic Representation of Antiplasmodial Bio-assay Guided purification of active fractions from <i>Schwenkia americana</i> Extract showing both active and non active fractions.....	55
Figure 4.1:	Rel, 1 β , 3 α - dihydroxyl 7 α , 9 β dihydroxycyclopentane (Octahydro-2-methyl-1H-indene-1, 3, 4, 6-tetraol).....	93
Figure 4.2:	1,2,3,4-tetrahydro-1-(1-hydroxybutan-2-yl)-4-methylnaphthalene -2,3-diol Isolated from <i>O. Subscorpioidea</i>	97
Figure 4. 3:	UPLC-MS Chromatogram of Two Compounds With Higher Peak from the Fraction GB10EA2-3-1G In +Ve Mode.....	98
Figure 4.4:	UPLC-MS Chromatogram of Compound with M/Z 413.2651 from Fraction GB10EA2-3-1G In +Ve Mode	99
Figure 4.5:	The UPLC-MS MS/MS profile of possible Compound With M/Z 413.2651 From Fraction GB10EA2-3-1G In +Ve Mode.....	100
Figure 4.6:	Chemical Structure Of Compound 2,5-Dihydroxy-3-((2E,6E,10E)-3,7,11,15- Tetramethylhexadeca-2,6,10,14-Tetraenyl)Cyclohexa 2,5-Diene-1,4-Dione(Boviquinone 4) From <i>S. americana</i>	101

Figure 4.7: UPLC-MS Profile of Compound With M/Z of 337.33 Fraction
 GB10EA2-3-1G In + Ve Mode.....102

Figure 4.8: UPLC-MS Profile and fragments of Compound with M/Z of 337.33 Fraction
 GB10EA2-3-1G In + Ve Mode103

Figure 4.9: Chemical Structure of Compound 2-(2,5-Dimethylphenyl)-4,6-
 Diphenylpyrylium From Isolated From *S. americana*104

Figure 4.10: *Plasmodium berghei* (NK65) and *P.falciparum* Cysteine Protease Inhibition
 of the Fractions of *Olox subscopoidia*110

Figure 4.11: *Plasmodium berghei* (NK65) and *P.falciparum* Cysteine Protease Inhibition
 of the Fractions of *Schwenkia americana*111

LIST OF PLATES

Plate I:	<i>Olex subscorpioidea</i> leaf and stem part of the plant	37
Plate II:	Leaf stem and fruit parts of the <i>Schwenkia americana</i> plant.....	39
Plate III:	The TLC Chromatograms of Fractionation of <i>O. subscorpioidea</i> Crude Extract Developed With Hexane: Dichloromethane and Methanol (60:39:1) and Visualized With Phosphomolybdic Acid and UV at 256nm	75
Plate IV:	Typical result obtained from pooled fractions from the silica column of <i>S. americana</i> Crude Extract. The TLC plate were developed with Hexane: Dichloromethane and Methanol (60:39:1) and Visualized with Phosphomolybdic Acid and UV At 256nm.....	76
Plate V:	The PTLC profile of fraction GB003BCF3a1 of <i>O. subscorpioidea</i> extract under UV light at 366nm. The point circled yellow indicated the isolate of compound GB003BCF3a1.....	83
Plate VI:	The PTLC profile of fraction GB003BCF3ae3of <i>O. subscorpioidea</i> extract under UV light at 366nm, the point circled yellow indicated the isolates of compound GB003BCF1ae3.....	84

LIST OF APPENDICES

Appendix	Title	Page
I	Calculation of Inhibitory Protease activity.....	146
II	Protein Standard Curve.....	147
III	Evidence of Ethical Clearance.....	148
IV	Ethno-Botanical Survey Information and Practices of Practitioners in Ethno-Medicinal Oral Responses in the Herbs Used Specifically in Treatment of Malaria.....	149
V	Sub-Acute Toxicity Profiles of the Plant extracts.....	153
VI	NMR Spectral	159

I

List of Abbreviations

ACT-	Artemisinin –based Combination Therapy
ALT-	Alanine Aminotransferase
AL-	Artemether-lumefantrine
ANOVA-	Analysis of Variance
ARDS-	Adult Respiratory Distress Syndrome,
AST-	Aspartate Aminotransferase
BANA-	α -N-benzoyl-DL-arginine-p-nitroanilide
BSA-	Bovine Serun Albumin
CDC-	Center for Disease Control and Prevention
COSY-	Correlation spectroscopy
CQ-	Chloroquine
CPI-	Cystein Protease Inhibitor
CSP-	Circumsporozoite Protein,
DIC-	Disseminated Intravascular Coagulation ,
DCM-	Dichloromethane
DEPT-	Distortionless enhancement by polarization transfer
DHFR-	Dihydrofolate Reductase
DMSO-	Dimethylsulfoxide
DNA-	Deoxyribonucleic Acid
DTT-	Dithiotherol
EDTA-	Ethylene Diamine Tetra Acetic acid
ESI-	Electron Spray Ionization
EGF-	Epidermal Growth Factor

FBS-	Foetal Bovine Serum
FMN-	Flavin Mononucleotide
FP-2-	Falcipan -2 (<i>P. falciparum</i> cysteine protease)
FTIR-	Fourier Transform Infra-Red Spectroscopy
G6PD -	Glucose -6- phosphate dehydrogenase
HEPES -	N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid
HMBC-	Heteronuclear Multiple Bond Coherence
HPLC-	High Performance Liquid Chromatography
HSQC-	Heteronuclear Single Quantum Coherence
IC ₅₀ -	Drug concentration that inhibits the parasite growth by 50%
IMC -	Inner Membrane Complex
ITN-	Insect Treated Bed Nets
LD ₅₀ -	Lethal dose 50
MeOH-	Methanol
MS-	Mass Spectrometry
NMR-	Nuclear Magnetic Resonance
PBS-	Phosphate Buffer Saline
PfCRT -	<i>P. falciparum</i> chloroquine resistance transporter
PfMDR -	<i>P. falciparum</i> multidrug resistance gene
Pfs 25-	<i>P. falciparum</i> 25
Pfccc -	<i>P. falciparum</i> Ccp
Pf Gdv1 -	<i>Plasmodium falciparum</i> Gametocyte Development 1
PfS-	PfScreening
pLDH-	parasite Lactate Dehydrogenase
PMSF-	Phenylmethylsulfonyl Fluoride
PPV-	Percentage Parasite Viability

PPI-	Plant Protease Inhibitors
PVM-	Parasitophorous Vacuolar Membrane
RBC-	Red Blood Cells
RBC -	Red Blood Cell
RFU -	Relative Fluorescence Units
RPMI-	Roswell Park Memorial Institute
SDS-PAGE-	Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis
SSP2 -	Sporozoite Surface Protein 2
TRAP -	Thrombospondin-Related Anonymous Protein
SE-	Standard Error
SI-	Selectivity Index
TEM-	Transmission Electron Microscopy
TFA-	Trifluoroacetic Acid
TOF-	Time of Flight
USAID-	United State of Agency for International Development
WHO-	World Health Organization

Chapter One

1.0 INTRODUCTION

1.1 Back ground of Research

Malaria is caused by a blood protozoan parasite called *Plasmodium*. This notorious organism exposes an estimated 45% of the world's population to the risk of malaria infection (WHO, 2014). Five species of *Plasmodium* transmit the disease in human (WHO, 2015), four of which are *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale* being implicated in more than 98% of human malaria cases worldwide (Mukandavire *et al.*, 2009; Lee *et al.*, 2011; Lee *et al.*, 2013) *Plasmodium knowlesi* is the fifth of the *Plasmodium* species recently identified to cause malaria in humans comparable in severity of attack to *P. falciparum* (Daneshvar *et al.*, 2009; Lee *et al.*, 2013). The species was initially identified to cause malaria among the *Macaca fascicularis* (the 'kra' monkey) and *Macaca nemestrina* (pig-tail monkey) identified in certain forested area of South-East Asia (Pain *et al.*, 2008; Lee *et al.*, 2011).

Plasmodium falciparum is the most deadly and it claims the lives of more than 90% of its victims especially in Sub-Saharan Africa (WHO, 2013, 2014). World Health Organization (WHO) report had shown that both *Plasmodium falciparum* and *Plasmodium vivax* pose the greatest public health challenges which greatly corroborate the *Plasmodium falciparum* species thrives in Sub-Saharan Africa region where malaria is most endemic (Onguéné *et al.*, 2013). *P. falciparum* accounts for more than 90% of the malaria cases and is the commonest disease in Nigeria with major vectors as *Anopheles gambiaes* and *A. funestus* (USAID, 2013). Malaria is tagged the leading African child killer disease, responsible for an estimated 225,000 deaths annually in Nigeria of which 11% is the maternal mortality and 10% low birth weight (USAID, 2013).

Current resistance to the existing drugs had introduced serious complications in the disease management leading to increased cases of its morbidity and mortality (Greenberg *et al.*, 1989; Solomon *et al.*,1999; Kiszewski, 2011; De Silva and Marshall 2012; Mboowa, 2014).

Resistance to classical drugs such as chloroquine, mefloquine and sulfadoxine/pyrimethamine is well known(Weekley and Smith, 2013; Jang *et al.*, 2014). Artemisinin and its derivatives currently used as alternative had reported cases of recrudescence in Southeast Asia and parts of Africa where the expected high level of success recorded in its use is grossly frustrated (Frits *et al.*,1997; Roper *et al.*, 2004; Plouffe *et al.*, 2008; Reverter and Lima, 2010).

Resistance documented in all classes of anti-malarial attributed to the parasite genetic factors that resulted in the parasite polymorphism such as *Plasmodium. falciparum* chloroquine resistance transporter (PfCRT) (Maiga *et al.*, 2007; Roper *et al.*, 2004), *Plasmodium falciparum* multidrug resistance gene (PfMDR)(Wellems *et al.*,1991; Fidock *et al.*,2000) and DHFR residue 164, DHFR I164L implicated in non-synonymous nucleotide changes that leads to antifolate drugs resistance (Cooper *et al.*, 2005; Valderramos and Fidock, 2006; Fernandez and Hons, 2009; Adjalley *et al.*, 2011; Falkard, 2013). Such mutation result from the influence of some strenuous effect of drugs especially in children and symptomatic patients in non-endemic areas (Travassos and Laufer, 2009), the parasites thereby acquired various degrees of resistance to such drugs (Travassos and Laufer, 2009; WHO, 2010).

The mechanism of resistance expression by the parasite is yet to be elucidated, however, possible factors that influences resistance include pharmacological contribution (Watkins and Mosobo, 1993; Nyunt and Plowe, 2007; Stepniewska and White, 2008), counterfeit medication, human host genetic influences and complication with other infections such as HIV(Travassos and Laufer, 2009; Mboowa, 2014).

The emergence of *Plasmodium falciparum* resistance strain and its spread is the greatest obstacle to the effort in the control of malaria in Nigeria, Sub-Saharan Africa and the globe as a whole (WHO, 2003). This calls for urgent need for an affordable, effective and safe alternative to the existing drugs. The urgency generated by such resistance have accelerated new anti-malarial drug research, even though the synthetic compounds continue to dominate the research, attention is increasingly directed towards natural healing remedies with special focus on the plants as a source (Elujoba *et al.*, 2005; Ogbonna *et al.*, 2008).

The search for anti-malarial drugs from the medicinal screening of plants with novel action therefore, become eminent among researchers and is considered with high significance (Katiyar *et al.*, 2012). Many of the affected populace in malaria endemic areas relies on herbal medication. WHO reported up to 80% (WHO, 2003; On *et al.*, 2009; Agbon *et al.*, 2014 Ochora *et al.*, 2014) of the world populace treats themselves with traditional herbal medicines, which are often considered more available, affordable, and perceived to be more effective and safer compared to the synthetic conventional available anti-malarial drugs. The phyto-constituents of the plants extracts play key pharmacological role on the targeted biochemical pathways of the parasites (Zaidan *et al.*, 2005; Adeiza *et al.*, 2010).

The traditional background of some indigenous plants usage coupled with factors such as the increased cost of insecticides, the vector's resistance to insecticides, and lack of an effective vaccine, have resulted in reliance upon case management and effective curative chemotherapy as the primary approach to malaria control today. Hence, the development of drugs and medicinal agents from plants are necessary in the investigation, prevention and, treatment of malaria (Wang *et al.*, 2006; Denis, 2012; Ploemen, 2013; Saddler *et al.*, 2015) .

1.2 Statement of Research Problem

The consistency in the expanding problem of malaria mortality and morbidity is at an alarming rate. The emergence and spread of *Plasmodium* resistant to the existing anti-malarial drugs present a great challenge to the global effort to the control of malaria (WHO, 2007). Widespread counterfeit and indiscriminate use of anti-malaria drugs had further contributed to the high level of parasite resistance (WHO, 2010). Challenges of the increased cost of anti-malaria, the resistance of the parasite to drugs and lack of an effective vaccine had exacerbated and frustrated the global efforts in the control of malaria (WHO, 2015).

1.3 Justification of the Study

New approaches for the development of anti-malarial drugs to overcome the characteristic resistance activity of the parasite are highly needed. Many indigenous plants with folkloric history of their traditional uses in the management of malaria and its control are available, but limited numbers are characterized and fully exploited. There is a sustained interest in plant products as a cheaper and highly potential anti-malarial agent since the identification of artemisinin from a plant source (Oliveira *et al.*, 2009; Katiyar *et al.*, 2012).

The urgency generated by drug resistant strains of malaria coupled with the claim by traditional herbal healers of the treatment of malaria using herbs suggests the need for further exploration of the indigenous flora for possible anti malaria drugs development (Saddler *et al.*, 2015).

1.4 Aim and Objectives

The aim of this research was to investigate the anti-*plasmodial* activity of *Olax subscorpioidea* and *Schwenkia americana* extracts and their effects on *Plasmodium* cysteine proteases from *Plasmodium berghei* NK 65 and *Plasmodium falciparum*.

1.5 The specific objectives include:

- i. Ethnobotanical survey for the anti-malaria medicinal plants in Bida, Niger State
- ii. *In-vitro* anti-*plasmodial* screening and phytochemical analysis of the crude methanolic extracts of the plants
- iii. Determination of the toxicity profiles (LD₅₀) and Study of the *in vivo* anti-*plasmodial* potential of crude extracts of *Schwenkia americana* and *Olax subscorpioidea*
- iv. Bioguided *antiplasmodial* fractionation and Identification of the most active fractions of the active crude extracts of the plants.
- v. Chemical and Structural elucidation of the possible lead compounds.
- vi. To determine inhibitory effects of the identified compounds on *Plasmodium* cysteine protease.

Chapter Two

2.0 LITERATURE REVIEW

2.1 Malaria

Malaria is described as an alternating and intermittent fever condition caused by the invasion of red blood cells by the *Plasmodium* parasites of the genus protozoan, transmitted through the bite of female anopheles mosquito, predominantly in the tropical region of Sub-Saharan Africa (Carter and Mendis, 2002; CDC, 2012; WHO, 2013). WHO reported the common symptoms to include intermittent fever, head-ache, joint pains and vomiting, for uncomplicated malaria and usually comes up within 10 to 15 days of bite of infected mosquito (WHO, 2013). However, complications such as cerebral malaria occurred in children of age 6 month to 3 years which accounts for 80% of deaths in such patients. It involves sequestration of parasites in the brain leading to complication that manifests as repeated convulsions, coma and seizures (Chan-Kok, 2007; Martins *et al.*, 2009; WHO, 2010; Chinsembu, 2015). Additionally, the parasites sequester in various organs such as heart, kidney and placenta, thereby resulting to cases of kidney failure, cardiovascular shock, and premature delivery (Gardner *et al.*, 2002). Severe anemia, general oedema associated to kidney dysfunction especially in female children (Ogbadoyi and Gabi, 2007) are manifestations associated to malaria complications. Delay in treatment of such complications may leads to pulmonary oedema, adult respiratory distress syndrome (ARDS), hypoglycaemia, hypotension or shock, bleeding, disseminated intravascular coagulation (DIC), acidosis and or acidaemia and ultimately to death (Patel *et al.*, 2003; WHO 2014).

2.2 Global Threat of Malaria

Malaria is consistently associated with considerable morbidity and mortality with significant social and economic negative impact on most developing societies it is endemic (Ku *et al.*,

2011) WHO (2014), reported malaria to be endemic in 97 countries, predominantly in Africa, Asia and Latin America. More than 210 million clinical cases are reported annually resulting to about 600, 000 deaths. This is an amazing decline of more than 40% in the mortality rate compared to the same incidence in 2010 to 2013 (WHO, 2014; Mohammed *et al.*, 2014). However, despite the current decline in global incidence, malaria burden and its impact on Africans is still alarming, currently an African child died of malaria every 30 seconds (Mohammed *et al.*, 2014) and about 520,000 deaths occur in Africa, with 411,840 deaths in children under 5 years of age (WHO, 2014; 2015).

2.3 Malaria and Sub-Saharan Africa

The Global efforts towards the eradication of malaria including strategies such as "roll back malaria"(WHO, 2010), introduction of Insect Treated Bed nets (ITN) and the use of combine therapy such as Artemisinin Drug (ACT) yielded result in retarding the accelerating rates and death of the disease. These strategies have led to 30% decrease in global malaria infection risk and 34% drop in it's incidence in Sub-Saharan Africa region in 2013, While the rate has also declined by 47% globally and 54% in African continent during the year 2014, the impact of the parasites is still biting hard on the lives of the younger Sub-Saharan Africans. Which could be associated to factors such as climate, land use, location of rivers or sea and their altitude, proximity and or flood plain which predisposed the region to malaria devastations. Other factors that exacerbated further exposure are mosquito species, their breeding sites, human movement patterns, socioeconomic status, waste management, and local malaria intervention programs (Tabuti, 2008; Buchholz *et al.*, 2011; Haussig *et al.*, 2014). But, the most damning of all are the malaria parasite and its secondary invertebrate host, the *Anopheles* mosquito, provide the most suitable answer to the question; why malaria in Africa Sub-Saharan region?

The life cycle of *plasmodium* parasite depends mainly on its environmental temperature, between 16°C to 30°C is required for the progress of the parasite development. The warmer with higher climatic temperature the faster the development, its slower at lower temperature and ceases to grow completely if the temperature is below 16°C. The anopheles mosquitos at lower temperature stop biting. However, Sub-Saharan Africa climatic condition is indeed, favorable, since the region temperature never goes down below 16°C throughout the year. So it allows for the malarial parasites to quickly go through its life-cycles and Anopheles mosquito bite conveniently for blood meal. Therefore both the parasite and its mosquito host actively seek to infect all year-round (Sachs and Malaney, 2002).

2.4 Incidence of Malaria in Nigeria

WHO in 2014 reported higher concentration of malaria burden in Africa, with 17 countries account for 80% of the global malaria cases and 14 countries accounting for 80% of the mortality rate in African region. Nigeria, Ethiopia, Tanzania and Kenya contributed over 50% of the malaria infection rate. However, Nigeria and DRC Congo contributed 90% of the death toll (WHO, 2013). In Nigeria Malaria transmission is at its peak, with 97% of the population at risk and it account for more than a quarter of malaria cases in Africa. Malaria transmission occurs in the Southern part of the country all year round while it is considered more seasonal in the Northern part of the country (Adebayo and Krettli, 2011).

2.5 History of Malaria

Malaria is an ancient disease known to man for the past 7000 to 12,000 years (Mandal 2013). The novel historical discovery of the *plasmodium* parasite and its link to malaria was credited to Charles Louis Alphonse Laveran, an unknown French army officer working in Algeria (Sullivan 2006; CDC 2012), who in 1880 discovered parasitic protozoan he referred to as

Oscillaria malariae from blood samples of his patients, what today is referred to as *plasmodium*. He was later awarded Nobel Prize later in 1907 (Bruce-Chwatt, 1981). However, malaria symptoms were described earlier in 2700 BC by Ebers Papyrus in the Canon of Medicine, while the name malaria was coined in 1550 BC by Romans from term “mal” or bad and “aria” or air foul or bad air (Sullivan 2006; Cox, 2010; CDC, 2012), this explained the inclination of the belief, that malaria emanated from the gases of the swamp, an earlier speculation by Hippocrates when he associated nearness to stagnant water to the occurrence of fevers (Hippocrates 1923 as in Cox, 2010). In 1890 Italian researchers Grassi and Filetti, named *Plasmodium vivax*, and *Plasmodium malariae* (Sullivan, 2006; Cox, 2010) both of which were believed to attain the widest global distribution (Mohammed *et al.*, 2014), and are described as “benign tertian” (*P. vivax*) and “quartan” (*P. malariae*) characteristic of their periodic fevers, acute febrile condition, or paroxysm, recurrences every third (*P. vivax*) or fourth (*P. malariae*) day (Jones 1909; Hippocrates 1923 as mentioned in Cox, 2010 (Dobson, 1994; Carter and Mendis 2002; Cox, 2010) and both are thus referred, Tertian and quartan, fevers respectively.

In 1897 an American scientist, Welch, named *Plasmodium falciparum*, the species unlike the first two that were associated with severe and fatal manifestations, thus referred to as “subtertian, and or malignant. *P falciparum* is the most widely distributed and deadly in tropical and subtropical regions (Berger, 2015), while John Stephens was credited with the nobility of naming *P. ovale*, in 1922, while working in West Africa, and this specie is equally found in tropical region, its however, found to be the most limited in distribution amongst all, but only found to be endemic in Papua New Guinea, Philippines East Timor, Indonesia and Vietnam (Berger, 2015).

The Italian scientists were credited with the nobility of reporting the involvement of mosquito in transmission of malaria, however, Sir Ronald Ross an officer in 1897 while in the Medical Services working in India discovered that culicine mosquitoes transmitted the avian *Plasmodium relictum* from bird to bird in Calcutta. He thereby suggested that human malaria parasites might also be transmitted via the bite of mosquito as was later demonstrated in 1899, in Sierra Leone, he proved that human malaria parasites were indeed transmitted by anopheles mosquitoes and was awarded a Nobel Prize in 1902 to that effect (Carter and Mendis, 2002; Cox, 2010). The sexual stage of the parasite was discovered in 1897, by William MacCallum, and his colleague, Eugene Opie, they described the fusion of flagellated bodies with non-motile bodies to form a vermicule (now known to be ookinete) and both related their findings to that which today appeared in human *plasmodium* species (Cox, 2010), the discovery of the erythrocytic stage of *plasmodium* parasite was therefore initiated in 1893 and 1894 by Grassi and his colleagues. However their findings remains in-conclusive even after the contribution of Fritz Schaudinn in 1903, who described penetration of red blood cells by infective sporozoites of *P. vivax* and that was corroborated by Sydney James and Parr Tate experiments (James and Tate 1938 as retrieved by Cox 2010).

In 1937 the discovery of sporozoite of *P. gallinaceum* infections in birds undergo phase of multiplication between the injection of sporozoites and the appearance of parasites in the blood and that was found to occur in the reticuloendothelial system, which marked the conclusive historical background discoveries involved in the stages of *plasmodium* development in both host and vector (James and Tate 1938), it therefore took nearly forty years before the concept of the asexual state of development was comprehensively revealed. These findings were further substantiated in 1947 by Henry Shortt and Cyril Garnham, in London after the Second World War. They demonstrated different phases of sporozoites

division in the liver of primate monkey which was subsequently followed by the development of parasites in the blood (Shortt and Garnham, 1948). This stage was supported by the work of Shortt, Garnham and their Co-workers in 1949 who further demonstrated the exo-erythrocytic forms of *P. vivax* and *P. falciparum* in human volunteers infected with the parasites and in 1954 same was demonstrated in *P. ovale* 1954 (Garnham *et al.*, 1948; Shortt *et al.*,p 1949; Garnham *et al* 1954) while that of *P. malaria* was elaborated in 1960 by Robert (Bill) Bray on infected chimpanzees (Bray 1960). The sporozoites stages of development at both liver and the exo-erythrocytic stages were concluded by the contributions of Bray and Gamham in 1966. In addition Wojciech Krotoski and Garnham's team, in 1982 proffer explanations to the cause of the long observed period after infection, the appearance and reappearance of parasites in *P. vivax* species, which was associated to the existence of characteristic dormant exo-erythrocytic stages, called hypnozoites. Essentially in 1966 the most vital researches that led to a conclusive basic history of the life cycle of plasmodium species were accomplished. Therefore, the chronological development in the discovery of malaria life cycle took approximately 70 years (Cox, 2010).

In human malaria five species of plasmodium genus are implicated, which include *P. falciparum*, *P. vivax*, *P. malariae* *P. ovale* and *P. knowlesi*. However, some authors believe that even though *P. knowlesi* was found to infect human the transmission through anopheles mosquito was not established so it should not be considered human *plasmodium* yet (Ramasamy, 2014). However, much evidence had shown that *P. knowlesi* is transmitted by mosquitoes of the Leucosphyrus group of Anopheles mosquitoes in Malaysia and elsewhere in Southeast Asia (Galinski and Barnwell, 2009). More so several hundred cases of human malaria infections have been reported in Sarawak, Malaysian and Borneo (Singh *et al.*, 2004). So overwhelming evidences had shown that *P. knowlesi* is infectious to human and can be

considered among the human *plasmodium species* (Cox, *et al.*, 2008; Cox, 2010; Mohammed *et al.*, 2014). About 250 species of *plasmodium* are currently documented to infect and possibly cause malaria in vertebrates involving aves (birds) reptiles and mammals (Ramasamy, 2014) and all the species are parasitic in nature.

Sixteen sub-genera exist among plasmodium genus and six are associated with the avian malaria, earlier discovered by Ronald Ross, which include *Bennettinia*, *Giovannolaia*, *Heamameoba*, *Huffia*, *Novyella* and *Papernaia* (Bennett *et al.*, 1993; Cox, 2010; Marzal, 2012). The first rodent malaria parasite *Plasmodium berghei* was discovered by Ignace Vincke and Marcel Lips in 1948. The parasite was isolated from wild rodents in Central Africa and since then three other rodent *plasmodium* have been discovered. They include *P. yoelii*, *P. vinckei* and *P. chabaudi*, which are today adapted and maintained for laboratory use in mice, rats, hamsters and gerbils (Cox, 1998; Mota *et al.*, 2001) useful as surrogate models of human malaria studies, in different fields of biology, pathology, immunology, genetics, molecular biology and biochemistry (Cox, 1998; Mota *et al.*, 2001).

2.6 Life Cycle

The life cycle of *plasmodium* parasite is an embodiment of heterophasic generation stages with the sexual cycle initiated by the injection of plasmodium sporozoites, from mature oocysts within 10 to 14 days of mosquito's infectivity (Sultan, 1999), to the blood stream of the vertebrate host, through the bite of infected anophele mosquito. Within a few minutes the sporozoites migrate to the liver which mark the beginning of hepatic stage by the invasion of the hepatocytes; parenchymal cells of the liver (in mammals) or the endothelial cells (in birds) by these sporozoites (Cristin and Smith, 2013). The sporozoites therein, undergoes division and differentiation to produce Schizonts which harbor the daughter progeny called merozoites. Ultimately, the merozoites are released to enter the blood circulation, where they

invade the erythrocytes. Within an erythrocyte each parasite grows and divides asexually producing a substantial number of merozoites. Finally, on lysis of the erythrocyte, merozoites are released and reinvasion of erythrocytes can take place. The synchronous asexual development and rupturing of erythrocytes by the parasite is marked by periodic fever-chill cycles which are the hallmark of malarial infections; the asexual reproduction stage in which the nucleus undergoes multiple divisions prior to cell division (Cristin and Smith, 2013).

2.6.1 Sporozoites Invasion Mediation Surface Proteins

Plasmodium invasion of the hepatic cell is a complex sporozoite movement from the mosquito midgut to the mammalian liver which is mediated by the sporozoites surface proteins and the host cell molecules. The few proteins identified to that effect include circumsporozoite protein (CSP), which covers the entire sporozoite surface and had been the most clinically advanced malaria vaccine candidate providing significant but limited protection against malaria (Regules *et al.*, 2011). One important finding that provided the rationale for clinical testing of CSP was that antibodies against it block sporozoite motility and inhibit invasion of hepatocytes (Yoshida *et al.*, 1980; Roestenberg, 2013) Thus, identifying a novel surface proteins which could provide potential new targets for blocking sporozoite infection. Another sporozoite protein is, thrombospondin-related anonymous protein (TRAP), also known as sporozoite surface protein 2 (SSP2), identified to be essential for sporozoite motility, mosquito salivary gland invasion and hepatocyte infection (Robson *et al.*, 1995; Sultan *et al.*, 1997; Wengelnik *et al.*, 1999; Ghosh *et al.*, 2009). TRAP is released from micronemes and anchors into the sporozoite plasma membrane, bind to heparin proteoglycans of the hepatocytes, there it becomes part of the glideosome, and unique actomyosin based protein complexes which initiates and ensures merozoite motility and invasion (WHO, 2010; Chinsembu, 2015). The actomyosin motor is made up of myosin and

actin located in between the space of the plasma membrane and the underlying inner membrane complex (IMC), and it is made up of flattened vesicles that are connected to the parasite cytoskeleton. Myosin is anchored to the IMC, while actin is indirectly linked to the cytoplasmic tail of TRAP, which in turn interacts with the substrate or target cell via its extracellular adhesive domains. As the stationary myosin pulls on actin filaments, TRAP is displaced toward the posterior end of the sporozoite, resulting in forward movement (Keeley and Soldati, 2004).

Additional surface proteins associated with sporozoite invasion is sporozoite surface protein 3 (SSP3) (Harupa *et al.*, 2014), that plays a role in gliding motility necessary for the parasites' life cycle progression. SSP3 reveals complexity of the sporozoite surface protein beyond CSP important for parasite motility (Harupa *et al.*, 2014).

The sporozoites in the mammalian hepatocyte becomes dormant as in *P. vivax* and *P. ovale*, a stage described as hypnozoites (Adjalley *et al.*, 2011; Bantie and Engidawork, 2011; Smith *et al.*, 2013). It developed within 5-15 days to pre-erythrocytic stage of schizogony which results in schizonts, within which the host cell ruptured to release tens of thousands merozoites that enter the blood stream to invade erythrocytes. The schizont harbors thousands of hepatic merozoites that infects new host erythrocytes cells on their release to undergo another round of schizogony or become a gametocyte. Erythrocytic stage is initiated by the invasion and subsequent multiplication of merozoites within the red blood cells (RBCs), such initial stage is the trophozoites stages. It alter the cell cytoplasm to become ring form which eventually undergoes multiple fission to form < 32 merozoites (Adjalley *et al.*, 2011; Bantie and Engidawork, 2011; Smith *et al.*, 2013).

2.6.2 Schizogony

The schizogony in the hepatocyte is heralded by the erythrocytic stage parasite infection with very few about 80 to 100 sporozoites introduced to the blood by a mosquito bite, therefore any drug that targets the liver stage will surely be a protection against parasite infection and possibly eliminate the cryptic infection reservoirs hypnozoite (WHO, 2010;Tempera, 2013) When a mosquito feeds on a vertebrate host and ingests these gametocytes, sexual cycle is initiated in the mosquito stomach. The gametocytes are transformed into gametes, followed by fertilization and the resultant wormlike zygotes penetrate the stomach wall and come to lie on the outer surface of the stomach. Each zygote forms a cystlike body, the oocyst, harbouring thousands of sporozoites produced by asexual multiplication. Upon sporozoite maturation the oocyst bursts, and release the sporozoites, which enter the salivary glands and on the mosquito next blood meal, sporozoites are injected and the life cycle is completed (Singh *et al.*, 2009).

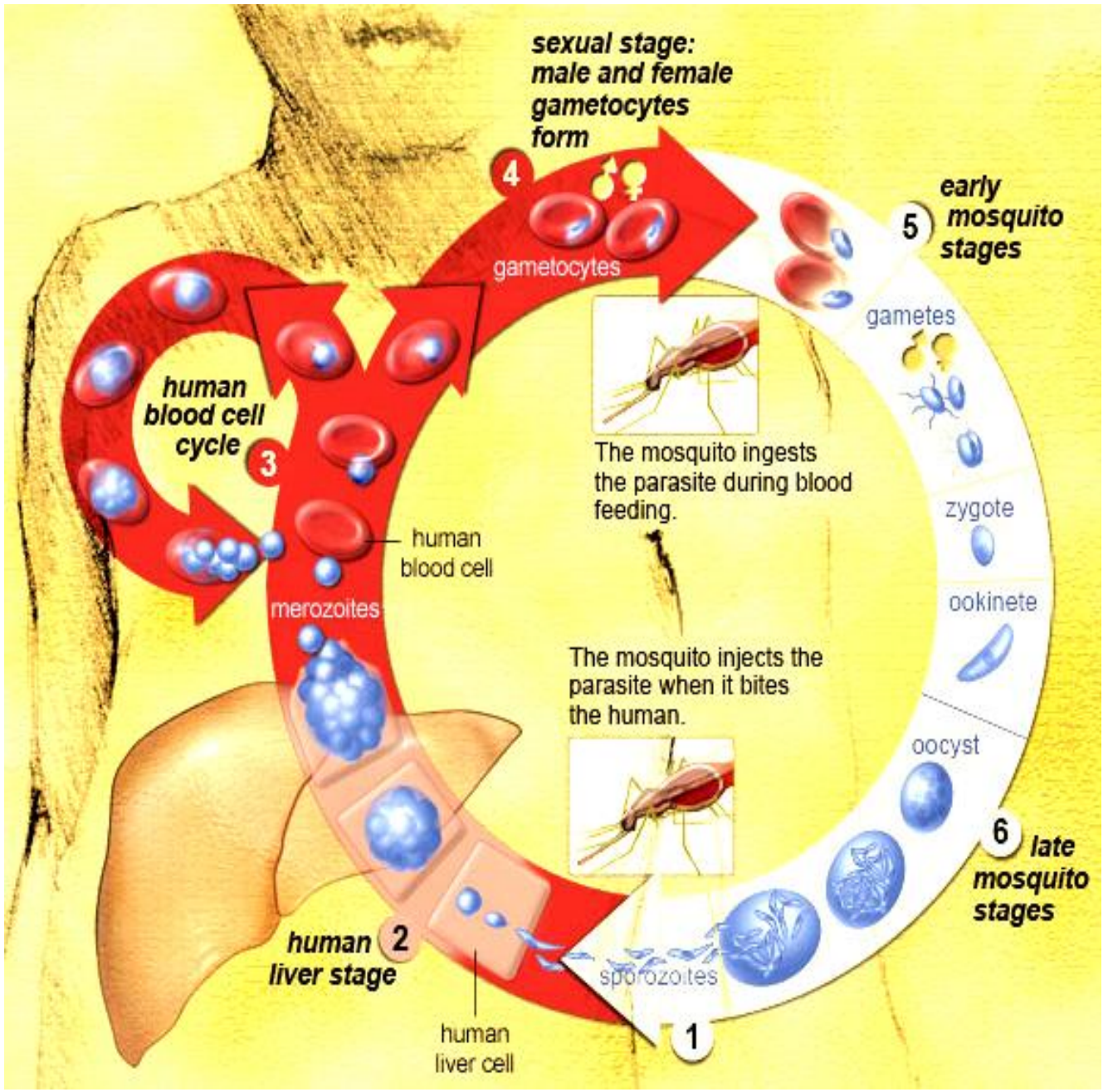


Figure 2.1: The life cycle of The Malaria parasite

(NIAID, 2007)

2.6.3 Gametocytogenesis

Gametocytogenesis is a process that leads to the development of gametocytes formation from the asexual stage parasite in the host blood. *P. falciparum* gametocyte usually appears 12 - 14 days after the first appearance of the asexual stage parasite in the blood stream (Trenholme *et al.*, 2014). It is very important land mark to the transmission of *plasmodium* parasites in human. The gametocytes erupt from the schizonts committed to sexual stage development usually less than 1 – 3% of the merozoites differentiate into gametocyte at each point of schizogony. This is a characteristic stage of cells that prelude transmission to the sexually dimorphic forming micro and macro-gametocytes which eventually produce male and female gametes by process of ex-flagellation and activation respectively (Moll *et al.*, 2008). The haploid asexual erythrocytic stage of parasite rapidly undergo differentiation to produce male gametes in less than 20 minutes of ingestion by the mosquito (Makanga, 2014). The process is induced by decrease temperature and mosquito derived gametocyte activating factor Xanthurenic acid which involves three rounds of DNA replication and axoneme assembly resulting in the release of eight male gametes (Raabe, *et al.*, 2009 ; Trenholme *et al.*, 2014). Female gametes formation involved 370 gene transcripts in translational repression controlled by DOZI, until the gametes are formed which egress from RBC and subsequently accompanied by fertilization (Makanga, 2014; Trenholme *et al.*, 2014; Vaidya *et al.*, 2014). The process described is followed by formation of zygote by fertilization process, subsequently followed by zygote differentiation to form a motile and invasive ookinete within which the diploid genome undergoes meiosis (Smith *et al.*, 2013; Makanga, 2014). It is pertinent to mention that processes of exo-flagellation, activation, fertilization and ookinete formation occur within an environment almost completely dependent on or derived from the

host blood, thus this could be an important target for a novel and ideal channel for transmission stage inhibition (Garcia *et al.*, 2006; Makanga, 2014).

Having crossed the mosquito mid-gut wall, the very few surviving ookinetes undergo differentiation to form oocyst which further undergoes endo mitosis that eventually produces thousands of daughter sporozoites which migrate to the mid-gut to the salivary glands of the mosquito. The mosquito injects the sporozoites to the blood stream and takes the gametocytes to initiate another life cycle again (Weekley and Smith, 2013; Makanga, 2014). Gametogenesis therefore represent a transition period in which parasite differentiate both morphologically and biochemically to maturation of gametocyte which involves five distinctive stages of development (stage I -V) as identified by light and electron microscopes. Stages I and early stage II are almost indistinguishable from asexual stage. The metabolic profiles of the first three stages are similar which explains why many asexual growth inhibiting drugs are active at those stages. Parasites commitment to sexual development become physiologically distinguishable only at late stage II and stage III, 72 – 96 hours after invasion of a committed asexual stage merozoite into erythrocyte. Transitions occur through stage IV to stage V as it matures and characteristically become crescent shaped (Buchholz *et al.*, 2011; Trenholme *et al.*, 2014). Gametocytaemia in *P. falciparum* is usually delayed to 7 to 10 days at the peak of the asexual stage parasite. Stages VI and V are metabolically less active than the asexual stage parasites and with the exception of primaquine the stages are insensitive to the currently available used anti-malarial drugs. Some proteins that are associated and expressed during gametogenesis but are not readily expressed during asexual stage include adhesive proteins such as EGF (Epidermal growth factor) domain containing proteins such as *P. falciparum* 25(Pfs 25) Pfs28, 6-cys proteins Pfs230 and Pfs48/45, LccI-domain containing *P. falciparum* Ccp (Pfccp) proteins (Trenholme *et al.*, 2014). Recent works

identified certain genes such as *Plasmodium falciparum* gametocyte development 1 (Pf gdv1) gene critical for gametogenesis (Trenholme *et al.*, 2014).

It is important to mention that most antimalarial drugs developed aimed at the pathogenic endo-erythrocytic asexual blood stages, the activity of such drugs are threaten by the parasite resistance does not seem to address the global target of malaria eradication. However, the goal of malarial eradication can be achieved when critical attention is giving to the parasite transmission stage blocking, together with the elimination of the hepatic and asymptomatic stage in anti malaria drugs development (Smith *et al.*, 2013).

2.7 Resistance and the Role of Gametocytes in Drug Resistance Property of *Plasmodium* Species

Resistance is the general outcome of the rate of recrudescence in the parasite level in it responds to the drug treatment. This is accompanied by the slower responses to the initial treatment and results in increase gametocytes densities of *plasmodium* species. Such densities play important role in the drive to the spread of the resistance (Gimode *et al.*, 2015) hence, increase in gametocyte carriage could be attributed to increase level of resistance. The risk factors that are associated to onset and possible increase in gametocyte densities includes; parasite age, increase age especially in children, host immune response and co infection with other pathogens. Other factors include host RBC level (anaemia), duration of parasitaemia, existence of gametocemia at enrolment, mass drug administration and insecticide spraying (Makanga 2014; Yerbanga *et al.*, 2014).

Historically, antimalarial drug discovery effort is focused on the asexual blood stages so most of the drugs developed were meant to target the pathogenic blood stages in human and to address the constant threat of resistance by the parasite. To achieve these goals and possible malaria eradication, it is pertinent that parasite transmission stage inhibition, elimination of

the asymptomatic and the cryptic hepatic forms need to be considered in the development. However, these are marred by challenges such as the population of parasite in the liver and sexual stage (Triggi, 1985 ;Yerbanga *et al.*, 2014).

The search for novel drug or improved means to control malaria, whether chemotherapeutic or immune-prophylactic is dependent on the availability of practical experimental models for preclinical investigations. Investigations on the parasite species that infect humans are hampered by the strict specificity to the host's cells (Yerbanga *et al.*, 2014). *In vivo* models have been limited to infections of selected species of South American primates that are now increasingly restricted by ethical and cost considerations. At present, routine *in vitro* cultivation is only available for the blood stages of *P. falciparum*, and that of the liver stages of this parasite is best obtained using primary human hepatocytes alone or in coculture 3–5, though hepatic development in HCO₄ cell lines has been reported albeit with much lower levels of infection (Makanga, 2014; Yerbanga *et al.*, 2014).

Cultivation of the *P. vivax* liver stages are equally well sustained when human primary hepatocytes or a HepG2 cell lines are used. In general, *Plasmodium* hepatic stage cultures are limited to 10 - 15 days of cultivation, though recent advances have extended this to a month or so. Consequently, the general knowledge of biology and immunology of the malarial liver stages is very limited and fragmented, and is minimal for the hypnozoites. The major concern about hepatic forms of the plasmodial cultivation is that it represents the initial obligatory phase of the life cycle of *plasmodium* in the human host. At the pre-erythrocytic phase the parasites are present at very low level of parasitemia and generally develop over a period 14 - 15days). This makes them an ideal target for parasite elimination. (Makanga, 2014; Yerbanga *et al.*, 2014)

2.8 Potential Drug Targets in Malaria Parasite

2.8.1 TCA cycle as a potential drug target

The parasite's mitochondrion is highly divergent from its human counterpart (Colborn *et al.*, 2008) and provides a basis for selective toxicity of antimalarial drugs. However, the tricarboxylic acid (TCA) cycle, a fundamental metabolic pathway within the parasite mitochondrion, has not been fully explored as a potential drug target. Several lines of evidence support the existence of TCA reactions in the human malaria parasite, *Plasmodium falciparum*. The parasite's genome encodes all of the TCA cycle enzymes (Timta, 2013), most of which are expressed during the asexual stages (Chan-Kok, 2007; Adjalley *et al.*, 2011). The eight TCA enzymes are exceptionally localized to the mitochondrion (Singh *et al.*, 2009; Tao *et al.*, 2011; Ramasubramanian, 2012) of the malaria parasites which bears features that are divergent from the host's mitochondria. In recent advances six of the TCA cycle enzymes were disrupted without affecting asexual stages of *Plasmodium falciparum*. The TCA cycle is adaptable and is essential in mosquito stages of the parasite. Aconitase, Specifically in KO parasites are arrested as late gametocytes, whereas α -ketoglutarate-dehydrogenase-deficient parasites failed to develop oocysts in the mosquitoes. The study describes a comprehensive analysis of TCA cycle function in the human parasite *P. falciparum* maintain an oxidative TCA cycle with the main flux supplied by glutamine (Tao *et al.*, 2011; Ramasubramanian, 2012). In DAco parasites however, maturation of gametocytes is prevented, perhaps due to accumulation of damages related to the loss of mitochondrial NADPH production and/or high levels of citrate. In the DKDH line blood-stage parasites metabolize glucose into glutamate, whereas the insect-stage parasites fail to survive. Compounds targeting the mitochondrial electron transport chain, such as atovaquone, completely block the flux of metabolites through the TCA cycle. The recent advances

however, reveal that the parasites have a flexible carbon metabolism, which may be important for making the transitions between the different environments encountered during the life cycle (Tao *et al.*, 2011). In addition, the availability of 11 different KO lines described provide an important resource for investigating biological consequences of disrupting the TCA cycle under various conditions, such as nutritional restrictions, in *P. falciparum* (Ramasubramanian, 2012).

2.9 Current Anti-Malarial Drugs and Mechanism of Action

Killing the mosquitoes that transmit malaria has been an established method for controlling and preventing malaria but it is difficult to eradicate mosquito species completely, therefore individual infections can however be prevented by methods such as insecticide-treated bed nets. However, the most effective way of controlling malaria involves the use of the classes of anti-malarials outlined below:

2.9.1 Quinolines

The quinoline antimalarials and related aryls derived from quinine, which are found in the bark of the Cinchona tree. Synthetic form of the drugs was developed. The immense use of chloroquine, the most famous drug of this group, provided well founded hopes for the eradication of malaria. The other drugs from this family include: amodiaquine, piperaquine, primaquine, quinine and mefloquine. The understanding of the mode of action of quinoline-based antimalarials has increased in the recent years, but remains incomplete (Kaur *et al.*, 2010). The drugs from this group mostly act during the blood stages of the parasite's life cycle but some target the hepatic stages as well (Almela *et al.*, 2012). The quinolones are known to inhibit the polymerization of heme and prevent disposal of polymers from the food vacuole to the cytoplasm where hemo-zoin is formed. This leads to intra parasitic

accumulation of free heme, which is highly toxic to the parasite. In addition to the important targets such as heme and phospholipids, several other targets have been postulated to be involved in the antimalarial action of quinolines such as tyrosine kinase, DNA, hemo- globin degrading proteases and phospholipases(Kaur *et al.*, 2010;Almela *et al.*, 2012).

They are of two classes

- a. 4-aminoquinoline antimalarials, this class include chloroquine, amodiaquine, mefloquine, Naphthoquine, AQ13, piperquine, Tert-butylisoquine, hydrochloroquine and pyronaridine and the structurally similar halofantrine(Almela *et al.*, 2012; Gopalakrishnan and Kumar, 2015).
- b. 8- Aminoquinolines is the second of the quinolines and it includes drugs like primaquine, diethylprimaquine, bulaquine, tafenoquine and NPC-1161B (Almela *et al.*, 2012; Gopalakrishnan and Kumar, 2015).

The success of chloroquine as a first line defence drug against malaria can be attributed to its clinical efficacy, limited host toxicity, easy use and simple cost-effective synthesis. Unfortunately, the mode of action of the quinoline compounds is not completely understood, hence the difficulty in circumventing the parasite's resistance (Gopalakrishnan and Kumar, 2015).

2.9.2 Artemisinin

This class of antimalarial drugs are also referred to as endo-peroxides which contain semisynthetic derivatives of artemisinin (originally found in the herb *Artemisia annua*), like artemether, artesunate, OZ439 and OZ277 (Almela *et al.*, 2012). These drugs are metabolized in the human into a more active compound, namely dihydroartemisinin and are known for their ability to quickly reduce the rates of parasitaemia (Gopalakrishnan and Kumar, 2015).

They can also be used for treatment of severe and uncomplicated types of malaria. There is no

reported case of clinically-relevant resistance against artemisinin or its derivatives in Africa yet (Meshnick, 2002) and that may not be unconnected to the compound's short half-life and elimination time (Gopalakrishnan and Kumar, 2015).

2.9.3 Antifolates

The most successfully used antifolate drug is the combination of 2, 4-diaminopyrimidine, proguanil, cycloquanil, dabson, chlorproquail, fansidar (pyrimethamine and sulphadoxine), which inhibits the enzymes dihydrofolate reductase (DHFR) and dihydroopteroate synthetase (DHPS), respectively. One dose of this combination is usually highly effective due to the slow elimination from the body. Unfortunately, this combination is subjected to a high emergence of drug resistance (Winstanley, 2000).

2.9.4 Atovaquone/Proguanil

Atovaquone was used in monotherapy as a selective inhibitor of parasite mitochondrial electron transport leading to the disruption of membrane potential, but a high frequency of parasite resistance occurred. Atovaquone was then combined with proguanil (a DHFR inhibitor) in a synergistic approach for therapy and prophylaxis against malaria parasite. The biggest disadvantage of using atovaquone and proguanil is the high production cost (Kiszewski, 2011).

2.9.5 Sulfonamides

This class of anti-malaria includes sulfadoxine, sulfadiazine and sulfamethoxazole (Almela *et al* 2012). Sulfadoxine and Pyrimethamine: Pyrimethamine and sulphadoxine are very useful adjuncts in the treatment of uncomplicated, chloroquine resistant, *P. falciparum* malaria. It is now used in combination with artesunate for the treatment of *P. falciparum* malaria. It is also used in intermittent treatment in pregnancy (IPTp) (Srinivas, 2015). Anti malarial activity of

Pyrimethamine inhibits the dihydrofolate reductase of plasmodia and thereby blocks the biosynthesis of purines and pyrimidines, which are essential for DNA synthesis and cell multiplication. This leads to failure of nuclear division at the time of schizont formation in erythrocytes and liver. Sulfadoxine on the other hand inhibits the utilisation of para-aminobenzoic acid in the synthesis of dihydropteroic acid. The combination of pyrimethamine and sulfa thus offers two step synergistic blockade of plasmodial division (Srinivas, 2015).

2.9.6 Antibiotics

These classes of antibiotics are effective as anti-malaria and it include the following, Azithromycin, trimethoprim, tetracycline, formidomycin, mirincamycin, doxycycline and thistrepton (Almela et al., 2012).

2.9.7 Amino Alcohols

This is a class of antimalarial that include Lumefantrine, halofantrine, mefloquine and quinine. Halofantrine was developed in the 1960s by the Walter Reed Army Institute of Research. It is a phenanthrene methanol structurally related to quinine. Its mechanism of action may be similar to that of chloroquine, quinine, and mefloquine; by forming toxic complexes with ferritoporphyrin IX that damage the membrane of the parasite. This anti malarial is effective against multi drug resistant *P. falciparum* malaria (Srinivas, 2015).

2.9.8 Others

This class of drugs contains compound of diverse functional groups that are effective anti-malaria which includes, rifloflavin, pentamidine, DHEA cyclohexamide, atovaquone, N-acetyl-D-penicillamine, deferoxamine and methylene blue (Almela et al., 2012).

2.10 The Problems and the Prospects of Drug Resistance

In the light of the overview of antimalarial drugs it is important to remember that there is no such thing as a perfect drug. Each and every drug known to man has its benefits and its liabilities. Drug resistance is an additional liability when performing research on an infectious disease; this however should be a primary motivation for continued innovation. *Plasmodium falciparum* resistance against the following antimalarial drugs: chloroquine, mefloquine, halofantrine, lumefantrine, pyrimethamine, cycloguanil, chlorocycloguanil, atovaquone, sulfonamides and sulfones have been well documented (Gopalakrishnan and Kumar, 2015). *P. falciparum* resistance to chloroquine was first and earliest reported cases in southern Asia and South America in 1957 and in 1978 drug resistant malaria parasites reached Africa. In 1989 chloroquine resistance became a major health threat in Africa. Consequently, the first line defence switched to a combination therapy of sulfadoxine and pyrimethamine. In Africa resistance against sulfadoxine-pyrimethamine occurred in the late 1980's. This combination drug acts synergistically to inhibit enzymes in the folate pathway. The molecular basis for this resistance is specific mutations in their target enzymes, and has been widely studied. Clinical resistance to quinolines occurred only sporadically in southern Asia and western Oceania and therefore, quinolones is established as a second line or third line drug against severe malaria; it is used in combination with antibiotics such as tetracycline and doxycycline to increase effectiveness (Kaur *et al.*, 2010).

Studies suggest the presence of *Plasmodium falciparum* with low mefloquine sensitivity in Africa but clinical mefloquine resistance is rare. Unfortunately the molecular basis of resistance to other antimalarials like mefloquine, amodiaquine, halofantrine and artemisinin derivatives is incompletely understood (May and Meyer, 2003; Francisco *et al.*, 2013).

Classically, multidrug resistance was defined as resistance to more than two operational anti-malarial drugs belonging to different chemical classes. For *P. falciparum* this usually means a parasite that shows resistance against 4-aminoquinolines (like chloroquine) and the antifolates (like the sulfadoxine-pyrimethamine combination). Future prospects to combat multidrug resistance include using drugs that are used to treat other diseases like antibacterial and antifungal drugs, increased usage of combination therapies and the discovery and exploitation of novel molecular targets (Sumsakul *et al.*, 2014).

A major focus for antimetabolic therapy against *P. falciparum* has been the de novo pyrimidine biosynthetic pathway. The human host can utilise and synthesise pyrimidine nucleosides but the malarial parasite is dependent solely on de novo synthesis (Webster and Hill, 2003).. A few of these enzymes have been identified as possible drug targets: carbamoyl phosphate synthetase II, aspartate transcarbamylase, dihydroopterae, CTP synthetase and phosphorybosyl pyrophosphate synthase (Fernandez and Hons, 2009; Kiszewski, 2011; Skinner-adams *et al.*, 2012). One of the promising drug targets that have recently emerged is the apicoplast. The genes found on the apicoplast genome code for enzymes that play a role in the fatty acid biosynthesis, non-mevalonate isoprenoid biosynthesis and heme synthesis pathways. It is hypothesized that some of the enzymes needed for Fe-S cluster biosynthesis are also located on the apicoplast. These enzymes make attractive drug targets for two reasons; firstly, most of these enzymes are vital for the parasites survival. Secondly, the apicoplast is a relict chloroplast; consequently the anabolic pathways coded by the apicoplast will be fundamentally different from the human host (Prasad *et al.*, 2013). It is known that after the infection of a red blood cell by a malaria parasite there is an increase in the permeability of the host erythrocyte membrane. The reason for this can be attributed to the emergence of new permeability pathways (Staines *et al.*, 2005). This pathway comprises of

channels and transporters (integral proteins that mediate the movement of ions and molecules across biological membranes). In principle, the inhibition of channels and transporters will deprive the parasite of nutrients needed for growth and proliferation, but also poison the parasite because of the accumulation of hazardous metabolic waste (Go *et al.*, 2004).

One of the most promising drug targets that have recently emerged among various potential new targets, is the cysteine protease falcipain-2 (FP-2) of *P. falciparum* which is an attractive and promising target enzyme (Shenai *et al.*, 2000; Dua *et al.*, 2001). FP-2 is a principal cysteine protease and essential hemoglobinase of erythrocytic *P. falciparum* trophozoites. Many *in vitro* studies have confirmed that inhibitors of falcipain-2 can block parasite hemoglobin hydrolysis and halt the development of culture parasites (Rosenthal *et al.*, 1996; Lee *et al.*, 2003; Shenai *et al.*, 2003). Some of them were also effective against murine malaria *in vivo* (Domínguez *et al.*, 2005; Hogg *et al.*, 2006). However, FP-2 inhibitors reported in the literature are mainly derived from peptide analogues (Rosenthal *et al.*, 1996; Lee *et al.*, 2003; Shenai *et al.*, 2003), which tend to form covalent bonds with the thiolate of the catalytic cysteine and commonly have nanomolar IC₅₀ values. Obviously, it is desirable to design non-peptidic inhibitors that would bind non-covalently to the target enzyme, in order to minimize toxicity while retaining the potential for high *in vivo* activity and selectivity.

2.11 Classification of Proteases

All proteolytic enzymes fall within only four groups based on their catalytic mechanism of a key active site amino acid and because of the requirement of a metal ion for the catalysis. Thus three of the classes, serine, cysteine and aspartic proteases are named after the active sites amino acids while metallo-proteases are named because of the metal ion requirement for the effective catalytic action (Rawlings and Barratt, 1995) Table 2.2 summarized some characteristics of the proteinases.

Table 2.1. :Body System Regulated by Limited Proteolysis

SYSTEM	EXAMPLES
Hormone Production	Insulin, Glucagon and Pro-ACTH
Regulatory Peptides	Angiotensin and Kinins
Defense, Protection	Blood coagulation, complement
Invassiveness	Connective tissue penetration, metastasis
Membrane passage	Pre-pro-proteine
Zymogen activation	Pro-enzymes
Transformation, assembly	Pro- collagen, fibrinogen, capsid virus polypeptides
Development and differentiation	Fertilization, implantation, surface proteins

Sources: Rawlings and Barratt, 1995

2.11.1 Cysteine (thiol) Protease (EC 3.4.22)

Cysteine proteases (EC 3.4 .22) (CPs) bears a catalytic cysteine and thiol ester intermediate which acted intracellulaly at an acidic pH with a broad substrate specificity (Rawlings and Barrett, 1994). Cysteine proteases are proteins with molecular mass about 21–30 kDa. They show the highest hydrolytic activity at pH 4–6.5. Because of the high tendency of the thiol group to oxidize, the environment of their catalytic domains consist of two adjacent sub-domains, which permit a V-shaped active site cleft to accommodate the active site residue Cys 25, His 159 and Asn 175. It is believed that the S2 subsite (pocket) of these enzymes is the significant determinant in the recognition and selection of the peptide substrate (Bode and Huber, 2000). They are important in several biological activities and appear to play key role in processes such as pathogenesis of many parasitic protozoa and helminth infections (Sakanari et al., 1989; Mulenga et al., 1999). The mechanism of action of cysteine proteases is shown in Figure 2.2

2.11.2 Cysteine Protease for Infectious Diseases

Cysteine proteases of many diseases causing organisms catalyse the entrance of host tissues, and in many bacterial infections are likewise involved in food digestion (Hansen *et al.*, 2011). Parasite cysteine proteases play central roles in immune evasions, activation of enzymes, emergence and molting of parasite (Sajid and McKerrow, 2002). Viral cysteine proteases are implicated in the proteolytic cleavage of precursor proteins for virus replication and in the production of new viral particles (Solomon *et al.*, 1999; Hansen *et al.*, 2011).

Protozoal cysteine proteases are also involved in the cell invasion of the host and in the metabolism of host proteins for their existence (Sundararaj *et al.*, 2012; Lehmann *et al.*, 2014). They are also implicated in the degradation of host immune molecules. They induce

diseases such as Chagas' diseases, malaria and other parasitic infections (Redzynia *et al.*, 2009).

2.11.3 Catalytic Mechanism of Cysteine Proteases

The cysteine proteases active site consists of the conserved cysteine, histidine and asparagine residues (CYS25, HIS159 and ASN175). The proteolytic activity of the enzyme is initiated by the formation of ion pair between CYS and HIS stabilized by hydrogen bond from ASN (Lecaille *et al.*, 2002). Nucleophilic attack of CYS is enhanced by the closed proximity between CYS and HIS, which stabilized the cysteine residue prior to substrate binding. The residues react with substrates and inhibitors by three main processes which include hydrolysis, acylation and deacylation (Teixeira *et al.*, 2011) as summarized in Figure 2.2. The interaction of the enzyme with substrate or inhibitor is by nucleophilic thiolate cysteine attacks on the carbonyl carbon of the substrate or inhibitor scissile bond (Teixeira *et al.*, 2011). This led to the formation of tetrahedral intermediate, which is stabilized by the oxyanion hole. The tetrahedral intermediate is then transformed into an acyl enzyme (enzyme-substrate thiol ester) and the C-terminal portion of the substrate or inhibitor is released in a process called acylation (Lecaille *et al.*, 2002). Consequently, the acyl enzyme is hydrolyzed by water and a second tetrahedral intermediate formed, which splits into the free enzyme and N-terminal protein of the substrate in a process called deacylation. The active site of this enzyme is generally known to be representative of the prime targets for therapeutic intervention (Lecaille *et al.*, 2002).

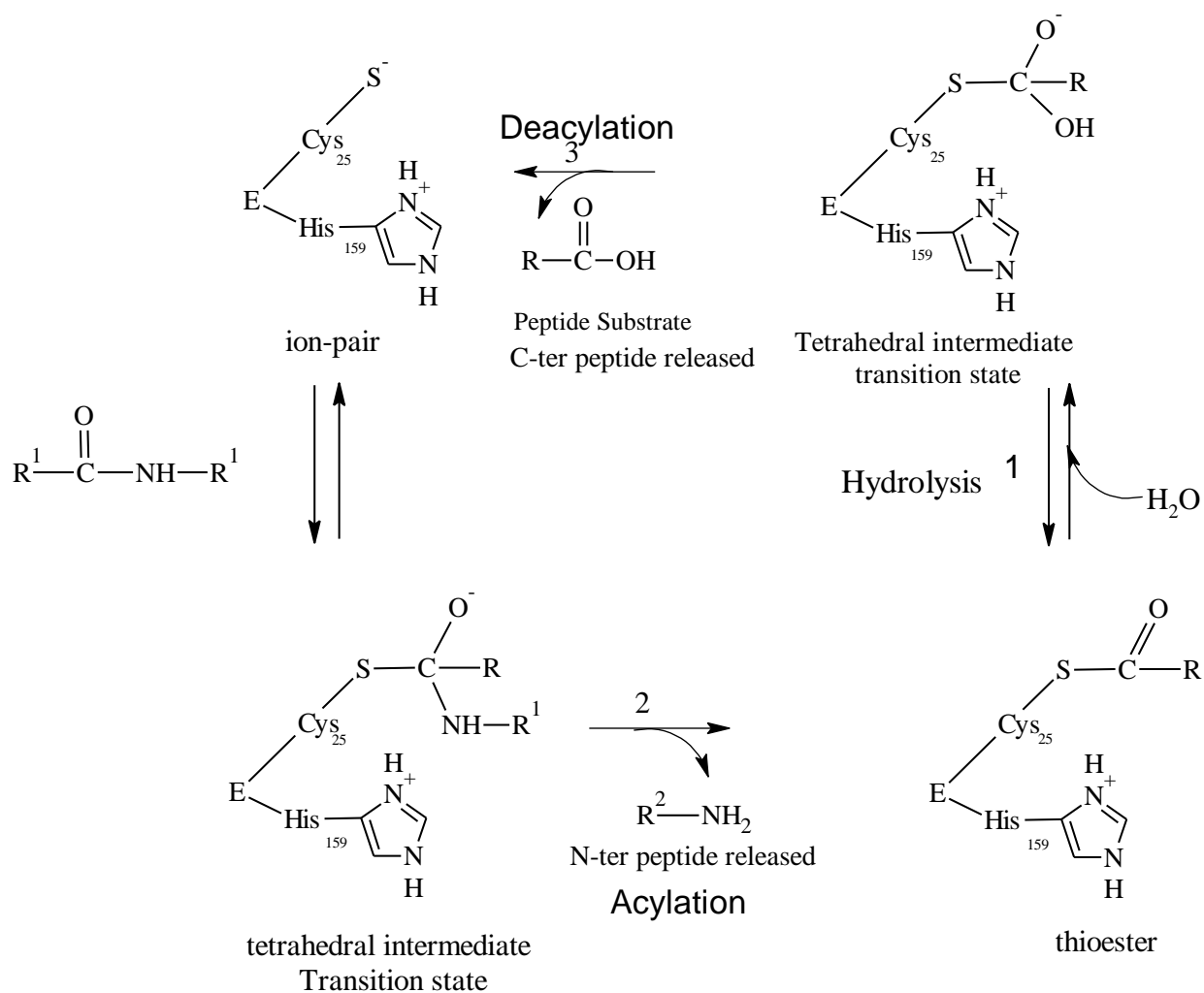


Figure 2.2: Catalytic Mechanism of Cysteine Proteases. (**1** Shows the Hydrolysis Process; **2** the Acylation, **3**-Deacylation and **E** Shows the Enzyme and **R** the Side Chain Of Peptide Substrate) (Lecaille *et al.*, 2002).

2.11.4 Cysteine Proteases of *Plasmodium*

There have been extensive *in vivo* and *in vitro* studies regarding the roles of *Plasmodium* cysteine proteases using their specific inhibitors. These studies have identified three key functions of *Plasmodium* cysteine proteases: hemoglobin hydrolysis, erythrocyte rupture and erythrocyte invasion by malaria parasites (Rosenthal, 2004). Analysis of the *P. falciparum* genome has resulted in the identification of three papain-like cysteine proteases: falcipains, dipeptidyl peptidase, a calpain homolog, and serine-repeat antigens (Pandey *et al.*, 2004). Falcipains are the most studied cysteine proteases of *P. falciparum* (Pandey *et al.* 2012). The falcipains are all expressed during the erythrocytic stage of the parasite (Rosenthal *et al.*, 2010). They share a number of features and sequence identity with papain-like cysteine proteases. There are four falcipains: falcipain-1 (FP1), two nearly identical proteases falcipain-2 (FP2) and falcipain-2' (FP2') and falcipain-3 (FP3) (Hanspal *et al.*, 2002; Jeong *et al.*, 2006; Singh *et al.*, 2006). FP1 is encoded on chromosome 14, while FP2, FP2' and FP3 are located on the chromosome 11. FP2 shares 96% sequence identity with FP2', 68% identity with FP3 and 38% identity with FP1 (Rosenthal, 2004).

Hemoglobin degradation is an essential process for parasite survival within its host and one of the main focuses for drug development against malaria (Shenai *et al.*, 2000). FP2 is the most abundant and widely studied of all the cysteine proteases (Wang *et al.*, 2006) and it often been called the principal hemoglobinase (Pandey *et al.*, 2005). The falcipains have an acidic pH optimum, they require reducing conditions for optimal activity, and are inhibited by typical cysteine protease inhibitors (Rosenthal *et al.*, 2002).

Table 2.2 :The Nomenclature of Proteinases (Endo-Proteinases) and Their Common Inhibitors

Nomenclature sub sub-group	Group Name	Essential residue in active site	Common inhibitor	Examples
EC 3.4.21	Serine Proteinase	Serine, Histidine, Aspartic acid	Fluorophoshates	Trypsin, Chymotrypsin Blood coagulation cascade, substilisin
EC.3.4.22	Cysteine proteinase	Cysteine	Mercurial	Papain, Clostripain
EC 3.4.23	Aspartic Proteinase	Aspartic acid	Diazoacetylamino acids	Pepsin, Chymosin
EC 3.4.24	Metalloprote inase	Histidine, Aspartic acid, metal	EDTA	

Source: Agarwal, 1979

2.12 Medicinal Plants Uses and Practices in African Ethno-pharmacopeia

The uses of traditional medicine; during last decade, have expanded globally and are gaining popularity. According to world health organization (WHO 2001) herbal medicine serves the health need of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries. In Africa, the practice of using medicinal plants for ailments could be traced to early civilizations which used indigenous plants and materials from plants such as the extracts, essential oil, and resins, for treatment of different ailments such as malaria, fever and skin care (Sofowora, 1993). Within this period, there were no isolations of active constituents since in most cases the whole plant were used and all the chemical components work synergistically to achieve the purpose for which the plants were being used (Tarkang *et al.*, 2014).

In Nigeria, a large percentage of the populace depends on herbal medication since the commercially available orthodox medicines are becoming increasingly expensive and out of reach to the poor (Lawal *et al.*, 2015). Anti-plasmodia resistance has become a global concern (Cui *et al.*, 2015) and the clinical efficacy of many existing anti-malaria drugs is being threatened by the emergence of multi drug resistant parasites (Sinha *et al.*, 2014). Natural products, either as pure compounds or as standardized plants extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Many effective drugs such as quinine and artemisinin were isolated from plant materials, however due to increased resistance of many pathogens, e.g. malaria parasites, towards the current drugs, investigation of the chemical compounds within traditional plants become necessary, (Sinha *et al.*, 2014; Cui *et al.*, 2015; WHO, 2017).

2.12.1 Olax subscorpioidea

These are woody plants, native throughout the tropical regions of the world. They belong to the family of flowering plants in the order Santalales. The plants' tree grows to 10 m high, bole to 60 cm girth with long thin trunk. It's widely used in Nigeria and so has many household names. The plant is referred to as Ifon in Yoruba, Aziza in Igbo, Kulanci in Nupe and Gwano kurmi or Tsamiyarbiri in Hausa (Ibrahim *et al.*, 2008). Ethnobotanical surveys revealed it's used in traditional medicine for the management of asthma (Sonibare and Gbile, 2008), cancer (Soladoye *et al.*, 2010), infectious diseases, mental illnesses (Ibrahim *et al.*, 2008) and diabetes mellitus and antinociceptive, anti-ulcer, antimicrobial (Ayandele and Adebiyi, 2007) and anti-protease activities. It is traditionally used as antidotes (venomous stings, bites, etc.); pain-killers; in treatment of venereal diseases; arthritis and rheumatism (Soladoye *et al.*, 2012).



Plate I *Olax subscorpioidea* leaf and stem part of the plant

2.12.2 *Schwenckia americana*

Schwenckia americana Linn family Solanaceae is a slender erect herb, woody at the base and grows up to 1 meter tall. It is commonly found in waste places and widespread in tropical Africa and America (Hassan *et al.*, 2012). It is known as *Dandana* (Hausa), *igbale odan* (Yoruba) and Rogogi (Nupe) in Nigeria (Mann *et al.*, 2008). In Northern Nigeria it is a common remedy for rheumatic pains, swelling, to treat arthritis and fungal infections (Asusheyi *et al.*, 2010). It is also used traditionally for the treatment of diarrhea in infant caused by breast milk (Eriyamremu and Iorliam, 2014). In Ghana it is used as treatment of cough for children, for chest complaints in Angola and In Southern Nigeria as a fish poison (Asusheyi *et al.*, 2010; Hassan *et al.*, 2012) . Figure 2.3 shows the picture of the plant *Schwenckia americana*.



Plate II. Leaf stem and fruit parts of the *Schwenkia americana* plant

Chapter Three

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Materials

The plant materials used in this research were the plants frequently identified among the traditional practitioners after oral interview, to be efficacious in treatment of malaria. These plants include: *Parinary curatellifolia*, *Chrozophora senegalensis*, *Olox subscopiodiasis*, *Acanthospermum hispidus*, *Termarindus indica*, *Blighia sapida*, *Senna seiberriana*, *Schwenkia americana*, *Senna occidentalis*, *Senna obtusifolia*, *Cymbopogon citrullus*, *Ocimum bacilicum* *Balanite aegyptica* and *Senna obtusifolia* were obtained from bida and it environs between September, 2013 to February 2014.

3.1.2 Animals

A total of 66 male adult Wistar rats weighing between 100 – 130 g and 114 Albino swiss mice also weighing 21-28g of male were used for the acute and sub-acute toxicity as well as the females mice for *in-vivo* anti- plasmodial activity studies. They were obtained from the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria and bred at the Department of Applied Science, Kaduna Polytechnic, Kaduna State. The animals were fed *ad libitum* with feed from the reknown company VITA feeds using the grower mash and had free access to water. They were also maintained under standard conditions of humidity, temperature and 12 h light/darkness cycle. They were allowed to acclimatize for two weeks before the commencement of the study.

3.1.3 Parasite

The *Plasmodium berghei* (NK 65) chloroquine - sensitive was obtained from National Institute of Medical Research, Lagos, Nigeria and maintained in mice by passaging. While the clinical isolate, of *P. falciparum* 3D7 and FRC3 strains were obtained from Ahmadu Bello University Teaching Hospital, Shika, Zaria and Bioscience Laboratory, Council for Scientific and Industrial Research (CSIR), Pretoria, South Africa respectively.

3.1.4 Chemicals

Ready to use assay Kits for glutamate oxaloacetate transaminase (GOT, ASAT), glutamate pyruvate transaminase (GPT, ALAT), alkaline phosphatase (ALP), total proteins, albumin, total bilirubin, urea and creatinine were used for the biochemical tests and were obtained from Biochemica and Diagnostica MBH, Germany. Other chemicals used in the course of the research were of analytical grade.

3.1.5 Ethno-botanical Survey Study Site

The ethno-botanical survey site was in Bida town which is the second largest city in Niger State with an estimated population of 185,553 (NPC, 2006) and the population growth is projected at 3.46% per year. It is located southwest of Minna, capital of Niger State, and is a dry, arid town. The town is located on the world map, between latitudes 9° 0' and 9° 9' North of the equator and longitudes 5° 56' and 6° 4' East of Greenwich meridian. The town is placed geographically, about 239km to Abuja the Nigerian capital city with area of about 51km². Bida is generally regarded as the capital of Nupe land in Nigeria (Yahaya, 2003).



Figure 3. 1 The Map of Bida Town showing the point of Survey
Indicated location of the Respodants Interview in Bida town



Source: Google Map

3.2 Methods

3.2.1 Collection, Identification and Processing of the Plants Species

The whole plants of some plant species were used, while in some plants, leaves were the parts used. In all cases fresh part of the plants were collected, identified and authenticated at the Herbarium unit, Department of Botany, Ahmadu Bello University Zaria and voucher samples were deposited.

The plant collected were shade-dried at room temperature for one week. The dried materials were then ground into powder using pestle and mortar. Hundred grams of powdered of the plants leaf of was macerated in 500mls of methanol for 48 hours with intermittent shaking. It was then filtered by the use of cheese cloth, after which it was properly suction filtered and the extracts were then concentrated by the use of rotary evaporator under reduced pressure at 40°C, and stored until required for use. The percentage yield was calculated as follows;

$$\text{Percentage yield} = \frac{\text{weight of Crude extract obtained}}{\text{Total Weight of Sample}} \times 100$$

3.2.2 Ethno-botanical Survey

The survey site was divided into 2 geo-political zones (Zone A and B) referred as Bida North and Bida South each comprising seven wards for the survey. Data generation from the ethno-botanical study was done using the structured questionnaire method as described by Mann *et al.*, (2008) and Gbolade, (2009) with some modifications and prior information obtained before the interview (Asase and Oppong-mensah, 2009; Asase *et al.*, 2010). The interview was conducted with attention focused mainly on herbalists/traditional medical practitioners in the area with the principally spoken language in the study area (Nupe). The members of the herbal association, Nigerian Association of Traditional Medical Practitioners were contacted for the identification of the practitioners and the questionnaires administered on the individual

members listed. The questionnaires are made up of two parts. The first part provides the personal bio-data of the respondents (age, sex, religion, contact number) and whether the practice was inherited or simply acquired individually. Renowned traditional practitioner healers, 30 of them in the area were interviewed orally on plant materials, its parts and preparation mode used for the traditional treatment of malaria or common symptoms of malaria such as fever, head ache, body pain were examined.

The data of the anti-malaria medicinal herbs obtained from the survey area was analyzed and ranked on the bases of their frequency in citation of the particular plant by different individual practitioner using the formulae as adopted by Salihu *et al.*, (2015)

$$\text{PFC} = \text{CN/TP} \times 100$$

PFC = percentage frequency of citation, CN=Number of Citation by different practitioner and

TP = total number of practitioner interviewed

The second part involved the ethno-pharmacological question (mode of preparation, mode of application and diagnostic method) used by the respondents in the treatment of malaria on herbal remedies and their knowledge about malaria. The survey also included field trip with some of the practitioners for practical identification of the plant material mentioned in the field. The research was conducted from December 2012 to April 2013 with the support of the branch leaders of the well-organized herbal association referred to as Nigerian Association of Traditional Medical Practitioners. The Designed concise questionnaire is as shown (appendix II).

3.2.3 Microscopic Determination of Parasitemia by Giemsa Stained Smear.

Thin blood films were made and stained with 5% giemsa, malaria parasites were counted, the percentage of parasitemia was calculated against 1,000 RBCs in thin blood films counts per μL (Adu-Gyasi *et al.*, 2012;Alves-Junior *et al.*, 2014). Parasites count for each blood film was

done. Each film was observed at three different visual fields, for the determination of the percentage inhibition. The inhibitions of parasite growth in the drug treated groups were calculated as follows:-

$$\text{Percentage inhibition} = 100 - \frac{\text{Number of schizonts in test well}}{\text{Number of schizonts in control wells}} \times 100$$

3.2.4 *In-vitro* anti-plasmodial assay

The *in vitro* antimalarial activity of test samples against the 3D7{3D7 is a drug-sensitive clone of the NF54 strainThe NF54 strain was originally derived from a clinical isolate from an individual living near Schipol Airport, Amsterdam, Netherlands. As the patient had never left the country, it is considered a case of imported malaria with unknown origin (Ponnudurai *et al.*, 1981; Walliker *et al.*, 1987)} and FCR3 strain of the malaria parasite, *Plasmodium falciparum*, was measured in this work by assessing parasite survival based on the following assay methods:

3.2.4.1 (pLDH) Parasite Lactate Dehydrogenase Colorimetric Enzyme Assay

This method is based on exposure to the drugs using a parasite lactate dehydrogenase (pLDH) colorimetric enzyme assay. Lactate dehydrogenase is an enzyme present in the cells and it catalyses the formation of pyruvate from lactate by reducing the co-enzyme NAD⁺ (nicotinamide adenine dinucleotide) to NADH. Research conducted by Knobloch and Henk likewise by Jelinek and co-workers shows that lactate dehydrogenase from malaria parasites (pLDH) metabolizes 3-acetyl pyridine NAD (APAD) much faster than human erythrocyte LDH leading to the formation of pyruvate from lactate(Calderón *et al.*, 2012). The pLDH assay is therefore principally based on the NAD⁺ analogue APAD (3-acetylpyridine adenine nucleotide) reduction to APADH and this coupled to a yellow NBT/PES (nitro blue tetrazolium/phenazine ethosulphate) reagent that is in turn converted to purple formazan crystals.

Procedure

The absorbance is read at 620 nm using a multi-well spectrophotometer (Tecan Infinite F500). Formazan formation is directly proportional to pLDH activity which in turn is indicative of the number of parasites in the cultures following drug exposure. Assay specificity is ensured by the inability of human LDH, found in the host red blood cells, to use APAD as a co-factor. Plants inhibitory activity was determined by preparing test samples in parasite culture medium in transparent 96-well flat bottom plates (Greiner Bio-one) 10 µg/mL and 1 µg/mL concentrations (n = 3 for each data point). Parasitized red blood cells are added to a final concentration of 1% haematocrit, 2% parasitaemia and the plates incubated for 48 hours before proceeding with the pLDH assay. Percentage parasite survival in each well is calculated relative to control wells that receive no drug.

3.2.4.2 SYBR Green I Antiplasmodial Assay Method

SYBR Green I Stain is a highly sensitive fluorescent stain for detecting nucleic acids. The dye exhibits a preferential affinity for DNA and its fluorescent signal is greatly enhanced when bound to DNA (with greater magnitude than the fluorescent enhancement of bound ethidium bromide). The detection limit using SYBR Green I Stain is as low as 60 pg per band of DNA using 300nm trans-illumination.

Procedure

The SYBR Green I assay was carried out as described with the modifications thus: The harvested parasite pellets culture in aliquots of 0.2 µl/ml and SYBR Green I were mixed with lysis buffer (25 mM Tris, pH 7.5, 20 mM EDTA, 0.01% saponin, 0.1% Triton X-100,) making the final haematocrit of 3%. Aliquots of 100 µl were mixed with 100 µl of ice cold SYBR Green I solution in a 96-well plate cover with black. The plate was incubated at room

temperature for 1-hr on a rotating platform. The relative fluorescent intensity (RFI) readings were measured (excitation 485 nm/emission 520 nm) using a Fluostar Optima Microplate Reader

3.2.5 Cultivation and Synchronization of *Plasmodium falciparum*

P. falciparum strain were cultivated in human red blood cells (O⁺), diluted to 2% hematocrit in RPMI 1640 medium supplemented with 25 mM HEPES and 25 mM NaHCO₃ and complemented with 10% human A⁺ serum and 5% Gentamycine). Incubations were done at 37°C 5%CO₂, 90% N₂, and 5%O₂. Hematocrit was adjusted to 5% and 0.5 -1% parasitaemia. Thin blood films were made every day from each culture flask, fixed with methanol, and stained for 30 minutes in Giemsa at 15% in buffered water pH 7.2 (Buffer tablets, Merck) or tap water. The different stages of development (early and late rings (trophozoites), and early and late schizonts) were determined and counted. Synchronization is performed as described by (Lambros and Vanderberg, 1979) which is based on the differential permeability of parasitized RBC membrane. While RBCs are naturally impermeable to sorbitol, but infected RBCs with mature stages of schizonts have a permeable membrane due to the structural modifications induced by the parasite. This property is used to selectively kill mature forms of the parasite by osmotic shock without affecting uninfected RBCs and RBCs parasitized by ring stages. The process was performed by initial Pre-warm of an aliquot of 5% D-sorbitol with complete medium at 37°C. The culture was centrifuged at 1800 rpm for 5 minutes and the supernatant discarded. The pellet is further resuspend in 5 volumes of pellet of pre-warmed sorbitol solution. Homogenated with the pipette and incubated for 5 minutes at 37°C. It is further Centrifuged for 5 minutes at 1800 rpm at room temperature. The supernatant Removed, and the residual pellet was washed twice with complete medium. Finally the

supernatant is removed and the pellet is adjusted to a hematocrit of 50%. The resultant mixture was inoculated for further cultivation in a 75 cm² culture flask, adding fresh RBCs to maintain hematocrit at 5% for the synchronous cultures.

3.2.6 Phytochemical Screening

The crude extracts of the plants were screened for the different phytochemicals following standard procedures as described by WHO (1987); Trease and Evans (1989); Sofowara (1993).

3.2.7 Toxicity Profiling of the Active Extracts

3.2.7.1 Acute Toxicity Studies LD₅₀

The acute toxicity of the plant extracts using LD₅₀ as the index was estimated orally in both mice and rats (n = 13) following Lorke's method (1983). The study was performed in two phases and the dose levels ranging from 10 to 5000 mg/kg body weight. In Phase I: 3 doses of the extracts were selected 10, 100 and 1000mg/kg for each plant extract and 3 mice/rats were treated for each dose. The number of deaths in each group within 24 h was recorded. Phase II doses depended on the outcome of Phase I and in this study four doses were selected and 1 mouse each per dose was used. The number of deaths in each group within 24 h was recorded. LD₅₀ was thus calculated as given by the square root of maximum value that did not caused death and lowest value that cause death (Lorke's, 1983)

3.2.7.2 Sub-Acute Toxicity Studies

Animals were selected by stratified randomization and then divided into four groups of five each. The first group served as control, while the remaining three groups were given 100, 200 and 400 mg/kg for mice and 10, 100, and 1000mg/kg of extracts orally for Rats for the period

of 28 days. The first day of dosing was considered as D0 whereas the day of sacrifice was designated as D28.

3.2.7.2.1 Mortality and Clinical Signs

During the four-week extract administration period, all the animals were observed daily for clinical signs and mortality patterns if any before and after extract administration and up to 4 h after dosing.

3.2.7.3 Determination of Biochemical Parameters

Biochemical parameters were determined colorimetrically using the standard ready-to-use kits. The parameters include the liver function parameters such as Glutamate oxaloacetate transaminase (GOT, AST), glutamate pyruvate transaminase (GPT, ALT), alkaline phosphatase (ALP), total proteins, albumin, total bilirubin, while others include the kidney function parameters such as serum urea, creatinine and electrolytes (Sodium, potassium, bicarbonate and chloride). The manufacturer's instruction for each biochemical parameter was adhered to in the course of the assay.

3.2.8 In-vivo Anti-*Plasmodia* Activity Test

3.2.8.1 Collection of *Plasmodium berghei* Parasite

The *Plasmodium berghei* was obtained from The National Institute for Medical Research, Yaba, Lagos, Nigeria through the Pharmacy and Pharmacology Department, Ahmadu Bello University Zaria and maintained by blood transfer in female albino Swiss mice of body weight (20-22g).

3.2.8.2 Parasite Preparation and Inoculation

The *Plasmodium berghei* was prepared and inoculated as described by Okonkon *et al.*, (2008) with modification. The parasitized blood was initially prepared from infected donor with 20 – 30% percentage parasitemia by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline (0.9%

NaCl) in proportions indicated by both determinations. Each mouse was inoculated on day 0, intraperitoneally with 0.2 mL of infected blood containing about 1×10^7 *P. berghei* NK 65 parasitized red blood cells.

3.2.8.3 Animal Grouping

Swiss Albino female mice with weight ranging between 21 -24g and of age between 6 – 8 weeks were obtained from Ahmadu Bello University Zaria. They were maintained on standard animal pellets and enough water (*ad libitum*). The animals were randomized and grouped into five groups of 5 mice each identified as Control groups (Positive control), Negative control, and three other groups for the plant extracts in different concentration of (50, 100 and 200mg/kg body weight).

3.2.8.4 4-Day Suppressive Activity of the Extract

The 4-day suppressive test described by Peters *et al.*, (1995) was used in the evaluation of the anti-malarial activity of plant extracts against chloroquine sensitive *Plasmodium berghei* NK65 strain. As described with modification, each mouse was inoculated on the first day (day 0) intra-peritoneal with 0.2cm³ of infected blood containing about 1.0×10^7 *P. berghei* parasitized RBCs. Three hours later the Control Group animals were administered Chloroquine orally 10mg/Kg/day. Equivalent volume of distilled water was given to 2nd control Group animals (Negative control) and other groups are administered 50, 100 and 200mg of the individual plant extracts orally. The drug and the extracts were then given once daily for 4 days. The parasitemia level was monitored using a prepared thin blood film obtained from the mice tail and stained with Giemsa on the third and fifth days after treatment. The mean survival time for each group was determined by arithmetic means (Average survival time/day) of the mice after inoculation in each group over a period of 15 days (day 0 to day 14) as described by Okonkon *et al.*, (2008).

3.2.8.5 Percentage Parasitemia and Percentage Growth Suppression

The percentage parasitemia was measured by preparing thin smears of blood from the tail of each mouse on day 3 and 5. The blood was applied on microscope slides and smeared and fixed with absolute methanol for 30 to 45 seconds and stained with 10% Geimsa stain at pH 7.2 for 10 min. The stained slides were then washed gently using distilled water and air dried at room temperature. Two stained slides for each mouse were examined under microscope using an oil immersion eye piece of 100× magnification. Ten different fields on each slide were examined at random and the average percentage parasitemia was determined by counting the number of parasitized RBCs out of 500 erythrocytes, and thus calculated using the formula (Bantie *et al.*, 2015):

$$\% \text{ parasitemia} = \frac{\text{Number of infected RBCs}}{\text{Total number of RBCs}} \times 100$$

The percentage parasite suppression was also determined by comparing the parasitemia suppression of the drug and extracts with respect to that of the control and thus calculated using the formulae (Bantie *et al.*, 2015):

$$\% \text{ suppression} = \frac{\text{Mean parasitemia of control} - \text{mean parasitemia of treated group}}{\text{Mean parasitemia of negative control}} \times 100$$

3.2.9 Thin Layer Chromatography (TLC) Profiling of the Plant Extract

A strip of the pre-coated silica gel was cut out and a spot of the sample applied on the plate about 1.0 cm from the edge. It was then dried using hot air dryer. The strip was lowered into a small chromatographic jar containing the solvent system. The solvent combination was Methanol/Dichloromethane/Hexane in the ratio of 0.6/5/4.4, 0.6/5.9/3.4. The jar covered with a glass lid and the solvent was allowed to ascend until the solvent front was about $\frac{3}{4}$ of the length of the strip. The strip removed and dried by a hot air dryer and viewed under UV light to identify the fluorescing spot. The fluorescent spot marked and then sprayed with

phosphomolybdic acid hydrate prepared in 70% ethanol. The colour reaction recorded and the relative Retention factor (R_f) values were then calculated based on the formula described by Patra *et al.*, (2009).

$$R_f = \frac{\text{Distance traveled by the streak from the starting point}}{\text{Distance traveled by the solvent from the starting point to the solvent front.}}$$

3.2.10 Column Chromatography

Silica gel of mesh 0.063 – 0.2mm pore was used as the stationary phase, while wet packing method was adopted in preparing the silica gel column using the solvent combinations of Methanol/Dichloromethane/Hexane in the ratio of 0.6/5.4/4. At the end of the packing process, the column was allowed 24h to stabilize; 6.6g of the crude sample was loaded to the 350g of the silica gel and increasing solvent polarity was used as the mobile phase. The column was eluted at a flow rate of 36 drops per minute. Elution of the extract was done with solvent systems of gradually decreasing polarity using Methanol/Dichloromethane/Hexane ratio as obtained from TLC. The eluted fractions were then collected in aliquots of 10 ml in test tubes. Similar fractions were pooled together according to the TLC profile to yield the possible fractions as represented in figure 3.2 and 3.3.

3.2.10.1 Bio- Guided Anti-Plasmodia Assay Fractionation of *Olox subscorpioidea*

The result of the fractionation using Methanol: Dichloromethane (DCM): hexane at the ratio of 0.1:3.9: 6.0 gave 10 fractions; the fractions were pooled to give 3 fractions 003A1, 003B1 and 003C1. 03B1 which on further purification gave 6 fractions 003B1.1 to 003B6.1 which were further pooled to obtain 3 fractions 003B1.1A, 003B2.1A and 003B3.1A. The TLC of the fractions was shown as Plate I. Fraction 003C1 was further purified using meth: DCM and

Hexane 0.1:4.9:5 which gave 6 fractions pooled to give 003CA1, 003CB1 and 003CC1. Fraction 003BC gave 003BCF-2ae and 003BCF-3a1 on separation using PTLC (Plate III)

3.2.10.2 Bio- Guided Anti-Plasmodia assay Fractionation of *Sckwenkia americana*

The fractionation of plant 010 (*Schwenkia americana*) was accomplished using solvent combination Methanol: Dichloromethane (DCM) and Hexane at ratio 0.1:3.9: 6. 13 component fractions 010A to 010M were obtained, the TLC of which is shown as Plate IV. The fractions were pooled to 5 fractions 010A1 to 010E1. Fraction 010B1 was further purified and pooled to obtain 010B1.1A and 010B2.1A. while fractions 010D1 and 010E1 were pooled to obtained 010EA. Fraction 010C1 was further purified using meth;DCM:Hex: 0.1:3.9:6 and pooled to obtained fractions 010C1.1, 010C2.1 and 010C3 which on further fractionation gives 010C3A to 010C3. Fraction 010C3C was further purified to give 010C3C1 to 010C3C9 using flash silica gel. 010EA on further purification gave 010EA1 to 010EA17 pooled to give 010EA1, 010EA2.3.1, 010EA4.16.1 and 010EA7.1.2. 010EA2.3.1 was subjected to PTLC which gave 010EA2.3.1G (Plate VI).

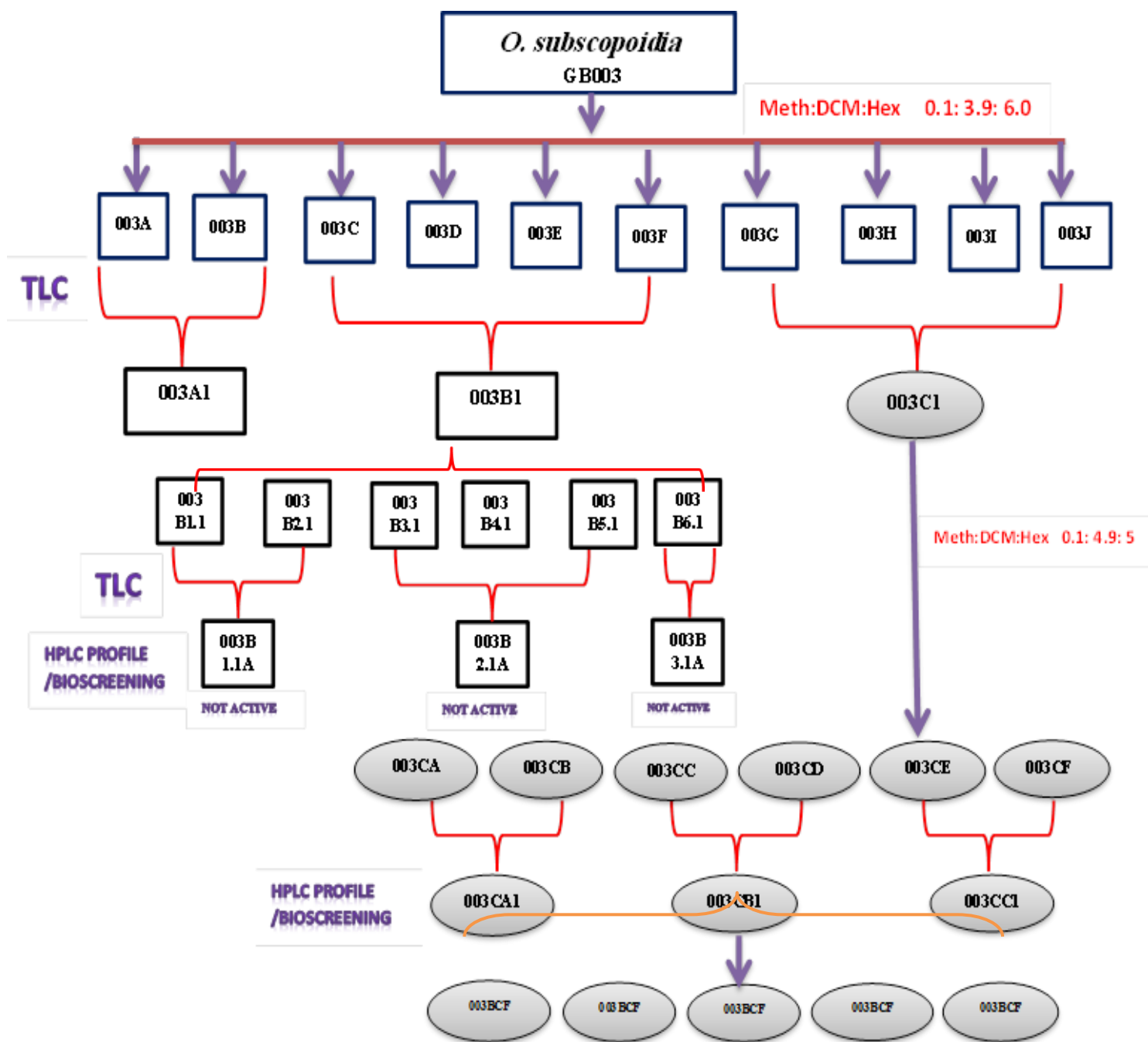


Figure 3.2: Schematic Representation of Antiplasmodial Bio-assay Guided purification of active fractions from *Olax Subscopoidia* Extract showing both active and non active fractions

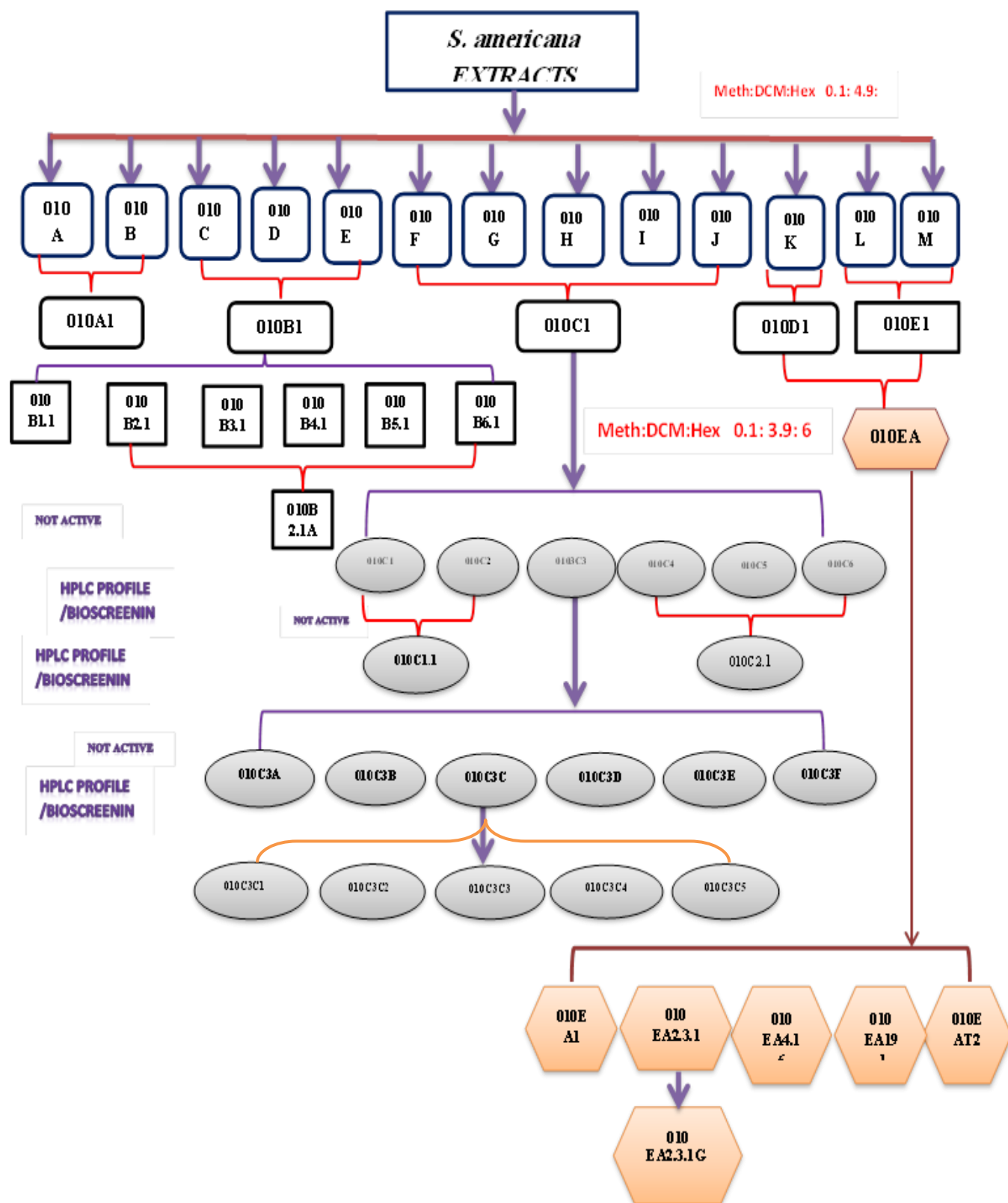


Figure 3.3: Schematic Representation of Antiplasmodial Bio-assay Guided purification of active fractions from *Schwenkia americana* Extract showing both active and non active fractions

3.2.11 UPLC-MS (LC-MS) Profiling of the Fractions

UPLC–MS data were obtained with a Waters Acquity UPLC system connected in series to a Waters photodiode array (PDA) detector as well as a SYNAPT G1 HDMS QTOF (4 kDa) mass spectrometer. The system was controlled through MassLynx v4.1 SCN639. UPLC separation was performed using an Acquity HSS T3 column (C18, 1.8 μm , 150 \times 2.1 mm, Waters) at 40 °C. Chromatographic solvents were ultra-pure water containing 0.1% (v/v) formic acid (Solvent A) and UPLC grade acetonitrile (Solvent B). The following gradient conditions were used: 5% (v/v) acetonitrile in 0.1% (v/v) aqueous formic acid increasing to 90% acetonitrile (v/v) over 13 min followed by a 2-min plateau at 90% (v/v) acetonitrile. Flow rates used were 0.35 mL/min (0–13 min) and 0.45 mL/min (13–15 min). Full scan absorption spectra (200–500 nm, 1.2 nm resolutions) were recorded in real time for each analysis with a runtime of 20 min. five μL of sample at a concentration of 1 mg/mL in water was injected. Fractions of interest were analysed by electrospray ionisation-quadrupole time-of-flight mass spectrometry (ESI-QTOF MS) using a scanning range of 100 to 1200 Da. The SYNAPT G1 mass spectrometer was used in V-optics and operated in electrospray ionisation mode. The mass spectrometer was operated in positive mode with a capillary voltage of 2.5 kV, the sampling cone at 30 V and the extraction cone at 5 V. The scan time was 0.1 s covering the 100 to 1200 Da mass ranges. The source temperature was 120 °C and the desolvation temperature was set at 400 °C. Nitrogen gas was used as the nebulization gas at a flow rate of 800 L/h. The trap collision energy was set at 6 V while the transfer optics collision energy was set to 4 V. The SYNAPT mass spectrometer was calibrated using a formic acid solution in acetonitrile to obtain a mass accuracy below 5 mDa for the scan range of 100 to 1200 Da. A linear gradient system was used for the UPLC separation as

summerized in Table 3.1 consisting of 1% formic acid in deionized water (Solvent A) and 1% formic acid in acetonitrile (solvent B).

3.2.12 NMR (Nuclear Magnetic Resonance)

NMR (Nuclear Magnetic Resonance) data were recorded on a Bruker Avance III 400 MHz magnet operating at 400.21 MHz for ¹H and 100.64 MHz for ¹³C. Chemical shifts are reported in ppm, *J* coupling constants in hertz (Hz) and the ¹H multiplicity of ¹H peaks are denoted with corresponding letters in italic (e.g., *s*: singlet, *d*: doublet, *dd*: doublet of doublets, *t*: triplet, *m*: multiplet, etc.).

3.2.13 Study on Inhibition of Extract on Cysteine Protease of Parasites

3.2.13.1 Extraction of Cysteine Protease from the Parasites

Triton X-100 temperature-induced phase separation procedures were used in the extraction of plasmodium parasite using Wannapa *et al* (2006) protocol with slight modifications as described briefly. The parasite obtained from saponin washed whole blood of 40% infected mice and 5% *in-vitro* cultivation of clinical isolate of *P. falciparum* was mixed with 0.5 % Triton X-100 in Tris-buffered saline and incubated at 4°C for 90 min. The supernatant obtained after centrifuging at 10,000 x g for 30 min at 4°C was layered on 6% sucrose containing 0.06 % Triton X-100 followed by incubation at 37°C for 5 min. At the end of the initial centrifugation at 900xg for 5 min at 37°C the cytosolic phase was collected and precipitated with cold acetone. Pellets of each preparation were suspended in 6 ml of 50 mM phosphate buffered saline at pH 7.2 (Ramos *et al.*, 2010).

3.2.13.2 Determination of Total Protein

The protein concentration was quantified according to the method described by Bradford (1976). The assay is based on an absorbance shift of the dye Coomassie Brilliant Blue G-250 when it binds to in which under acidic conditions the red form of the dye is converted to its blue form. Both the hydrophobic and ionic interactions stabilize the ionic form of the dye, causing a visible color change.

Procedure:

Coomassie Brilliant Blue G-250 (25 mg) was dissolved in 12.5 ml 95% ethanol. To this solution, 25 ml 85% (w/v) phosphoric acid was added. The resulting solution was diluted to a final volume of 250 ml. Protein solution (0.1 ml) was pipetted in test tubes. Five milliliters of protein reagent was added to the test tube and the contents mixed by vortexing. The absorbance at 595 nm was measured after 2 min and before 1 hr against a reagent blank prepared from 0.1 ml of the appropriate buffer and 5 ml of protein reagent. The concentration of protein was plotted against the corresponding absorbance resulting in a standard curve. The latter is used to determine the concentration of protein in unknown samples.

3.2.13.3 Purification of Cysteine Protease using Ammonium Sulphate

Purification with Ammonium Sulphate: Ammonium sulphate was added slowly to the crude enzyme with constant stirring starting from 40% until 70% saturation was achieved. The sample was allowed to stand overnight at 4°C, thereafter, it was centrifuged at 14,000×g for 30 minutes, the supernatant was then discarded and the pellet was suspended in the assay buffer (10mM sodium phosphate, pH 8.0, 1 mM Dithiothreitol, 1 mM EDTA, and 10% glycerol) and tested for the enzyme activity.

3.2.13.4 Protease Activity Assay:

The proteolytic activity of the enzyme was determined by colorimetric measurements using N α -Benzoyl-DL-arginine b-naphthylamide hydrochloride (BANA) as substrates as described by Ramos *et al.*, (2010) with modification. The reaction mixtures contained 20 μ L of the enzyme (1 mg/mL), which was added with or without pre-incubation with 40 μ L of 3 mM DTT for 10 min, 240 μ L of buffer plus 200 μ L of a 1% solution of azocasein. The reactions were performed at 37 °C. After 30 min, the reaction was stopped by adding 500 μ L of 2% HCl in ethanol and 500 μ L of 0.06% 4-(dimethylamino) cinnamaldehyde. After 40 min, the resulting color was measured by absorbance at 540 nm. One unit of enzymatic activity was defined as the amount of enzyme that increases the absorbance at 540 nm by 0.01.

3.2.13.5 Enzyme Inhibition Studies

The method described by Wannapa *et al* (2006) was used in the inhibition study of cysteine protease using the plant extracts as described briefly. The activity of the purified enzyme was determined in the presences of varying concentrations (50 μ g/ml, 25 μ g/ml, 12.5 μ g/ml, 6.25 μ g/ml, 3.725 μ g/ml, and 1. mg/ml) of the most active fractions. The mixture was incubated at 37⁰C and allowed to stand for 30 min prior to substrate addition. The reaction mixture was further incubated for 10 min and the absorbance was read at 540nm.

$$\% \text{ Inhibition} = \frac{A_{540\text{nm control}} - A_{540\text{nm test}}}{A_{540\text{nm control}}} \times 100$$

3.3 Ethical approval

Ethical approval was given by the research and ethical committee of the Kaduna Ministry of Health with ethical reference number MOH/ADM/744/Vol.1/196. A standard protocol was followed in accordance with the Good Laboratory Practice (GLP) and Regulations as recommended by WHO (1998).

3.4 Statistical Analysis

The results were analyzed using SPSS 20 and Prism 5 software for the analysis of the *in-vivo* anti-plasmodia screening. The data there obtained were expressed as means and standard deviation (mean \pm SD). Some of the data were analyzed as a completely randomized design using one-way analysis of variance (ANOVA) and any significant differences between means were assessed by Duncan post hoc test at 95% level of significance.

Chapter Four

4.0 RESULTS

4.1 Ethno-Botanical Survey Information and Practices of Traditional Healers in Treatment of Malaria

The findings from the survey on the ethno-botanical, medicinal uses and folkloric practices of anti-malarial plants in Bida and its environs identified 48 plants related to the traditional treatment of malaria and its common symptoms. (Appendix I). Those plants with higher response in their applications by the practitioners are represented in table 4.1. The plants were generally divided into two categories. The first categories were the first 5 plants commonly mentioned by majority of the practitioners which included *Sclerocarya birrea* (A.Rich) (40%), *Balanites aegyptica* (L) Del (40%), *Tamarindus indica* (L) (36.67%), *Azadirachta indica* A (43.33%) and *Blighia sapid konig*(36.67%)(Table 4.1). These plants were identified directly for management of malaria locally described as “Illness on bite of mosquitoes”.

The second category includes set of plants identified for the treatment of one or more common symptoms of malaria. It includes from plant 19 – 48 (Appendix I), *Alternanthera sessilis* (L) to *Ocimum bacilicum*. The most mentioned in the treatment of symptoms related to malaria by 35 – 40% practitioners includes *Acanthospermum hispidus* (36.67%), *Chrozophora senegalensis* (36.67%), *Olax subscopiodia* (41.33%), *Schwenkia americana*(40%), *Senna occidentalis*(36.67%), *Senna obtusifolia*(40%), *Senna seiberriana*(36.67%)*Parinary curatellifolia* and *Cymbopogon Citrullus*. The details of the parts used and mode of preparation as indicated in Table 4.1.

Table 4.1. List of Traditional Medicinal Plants Used By the Traditional Medical Practitioners for the Treatment of Malaria in Bida

PLANT Botanical Common and Vernacular NAME	Percentage of respondents	Pharmaceutical preparation /Usage	Plant Part Used
<i>Sclerocarya birrea</i> (A.Rich) Marula Jingere goyi	40.00	Decoction taken twice daily	Dried stem bark
<i>Balanites aegyptica</i> (L) Del Soap Berry tree desert date Aduwa	40.00	Decoction Taken ones daily	Bark and seeds
<i>Tamarindus india</i> (L) Tamarina tree Darachi	36.67	Cold infusion Taken once daily	Fresh stem bark and fruit
<i>Azadirachita india</i> A. Neem tree Nimu	43.33	Decoction steam bath and taken orally	Stem bark and leaves
<i>Blighia sapida konig</i> Akee apple Yilanchi	36.67	Decoction of stem bark taken once daily	Stem bark
<i>Acanthospermun hispidus</i> Dc Kashiyawo	36.67	Juice of leaves for palm wine potash	Leaves
<i>Chrozophora senegalensis</i> Damagi	36.67	Decoction Taken orally several times	Leaf
<i>Olox subscorpioidea</i> Kulanchi	41.33	Decoction Taken orally several times	
<i>Schwenkia americana</i> , Rogogi	40.00	Decoction steam bath and taken orally	Whole plant
<i>Senna ocidentalis</i> Gaya eba	36.67	Decoction Taken orally several times	Whole plant
<i>Senna obtusifolia</i> Gaya yiwo	40.00	Decoction Taken orally several times	Whole plant
<i>Senna seiberriana</i> Gbogi	36.67	Decoction Taken orally early in the morning	Root, leaves
<i>Parinary curatellifolia</i> , Putu bokun	36.67	Fever Decoction Taken orally several times	Leaves
<i>Cymbopogon citrullus</i> Lemon grass	30.00	Fever Decoction	Whole plant

4.2 Percentage Yield of the Crude Extracts

The percentage yields of the methanol extracts of the plants were between 2.5% the lowest and 22.4% the highest as shown in Table 4.2. *Termaridus indica* gave the highest percentage yield 22.4% then followed by *Olox subscorpioidea* with 16.1% while *Senna seiberriana*, *Senna obtusifolia*, *Balanite aegyptica*, and *Chrozophora senegalensis* were 14.5, 11.85, 10.5 and 9.6% respectively. While the percentage yield for *Acanthospermum hispidus*, *Annogeisses leicarpus*, and *Parinary curatellifolia*, were 5.9 4.6 and 4.0% respectively and *Schwenkia americana*, gave the lowest percentage yield of 2.5% (Table 4.2).

4.3 *In-vitro* Anti-Plasmodium pLDH Assay of Some Selected Plant Extracts against *P. falciparum* 3D7 and FCR3 Strains

The result of the antiplasmodial pLDH assay of different plant extracts showed that 82% of the plant extracts tested showed inhibition activity against *P. falciparum* 3D7 universal strains (Table 4.3). As the general rules implies, at an appropriate concentration a compound is considered active if the percentage parasite viability is less than $< 20\%$ and marginally active or inactive if greater than 20% and less than 50% and inactive if greater than 50% . Seventy two percent (72.7%) of the plant extracts screened were active with percentage parasite viability (PPV) less than 20% . These included *Termarindus indica*, *Senna occidentalis* and *Schwenkia americana* which were considered most active as compared to the chloroquine used as the standard drug which had PPV of $6.04 \pm 2.01\%$ lower than that of the plant extracts with 2.65 ± 0.61 , 2.95 ± 1.84 and $4.11 \pm 2.84\%$ respectively. Other plant extracts include: *Blighia sapida*, *Senna seiberriana*, *Chrozophora senegalensis* and *Olox subscorpioidea* with PPV of 10.98 ± 0.84 , 12.91 ± 1.72 , 13.41 ± 5.16 , 13.69 ± 1.26 , and $14.57 \pm 2.03\%$ respectively (Table 4.3). *Acanthospermum hispidus* is marginally active with PPV of $32.54 \pm 5.04\%$ which was $> 20\%$ but $< 50\%$. However the two plants screened and found to be inactive were *Cymbopogon*

citrullus and *Ocimum bacilicum* with PPV of 71.95 ± 3.80 and $85.06\pm 10.34\%$ respectively (Table 4.3). The screening of the plants against the resistant FCR3 strain results indicated that the plant extracts PPV were above 100%. This indicated that they were inactive with respect to this strain and even promotes the growth of the parasites in many cases; however, the standard drug had shown sensitivity with PPV -26.40 ± 8.91 (Table 4.3- 4.4). Three of the plants that showed higher potency against *P. falciparum* 3D7 universal strain were selected for further studies as represented in the Table 4.5.

4.4 Phyto-chemical Analysis

4.4.1 The Phyto-chemical composition of *O. subscorpioidea*, *S. americana* and *S. seiberiana* Methanol Leaf Extracts

The quantitative phytochemical analysis of the extracts indicated the presence saponin, tannins, flavonoids, glycosides, alkaloids and phenols. *S. seiberiana* was shown to have the highest level of saponin, tannins, glycosides, alkaloids and phenols While *O. subscorpioidea* was next in terms of saponins content however, the extract had higher flavonoids compared to others (Table 4.6)

Table 4.2. Percentage Yield of the Crude Extract Using Methanol as Solvent.

Plant Name	Percentage Yield % \pm SD
<i>Parinary curatellifolia,</i>	4.0 \pm 2.3
<i>Annogeisses leicarpus</i>	4.6 \pm 1.0
<i>chrozophora senegalensis,</i>	9.6 \pm 0.2
<i>Olax subscorpioidea</i>	16.1 \pm 0.5
<i>Acanthospermum hispidus</i>	5.9 \pm 2.1
<i>Termaridus indica,</i>	22.4 \pm 0.4
<i>Balanite aegyptica,</i>	10.5 \pm 1.2
<i>Senna seiberriana,</i>	14.5 \pm 1.0
<i>Schevenkia americana,</i>	2.5 \pm 3.1
<i>Senna occidentalis</i>	8.2 \pm 2.2
<i>Senna obtusifolia</i>	11.85 \pm 0.4

The values of the percentage yields of crude extracts of the selected plants are presented as means \pm SD (n = 3)

Table 4.3. In-vitro Anti-Plasmodial pLDH Assay and the IC₅₀ of Some Selected Plant Extracted against *P. falciparum* 3D7 Strains.

Plants Name	Percentage viability (%) 3D7 strain	IC ₅₀ 3D7 strain µg/ml
<i>Parinary curatellifolia,</i>	14.57±2.03	0.14
<i>Chrozophora senegalensis</i>	13.41±5.16	0.15
<i>Olox subscorpioidea</i>	13.69±1.26	0.13
<i>Acanthospermum hispidus</i>	32.54±5.04	7.52
<i>Termaridus indica,</i>	2.65±2.61	0.73
<i>Blighia sapida</i>	10.98±0.84	4.50
<i>Senna seiberriana,</i>	12.91±1.72	0.80
<i>Schevenkia americana,</i>	4.11±2.84	0.040
<i>Senna occidentalis</i>	2.95±1.84	4.40
<i>Cymbopogon citrullus</i>	71.95±3.80	NA
<i>Ocimum bacilicum</i>	85.06±10.34	NA
<i>Chloroquine</i>	6.04±2.01	0.00803
Negative Control	100.00±2.41	

The values of the *in-vitro* anti-plasmodial pLDH Assay and the IC₅₀ of the crude extracts of the selected plants against *P. falciparum* 3D7 strain were presented as means ±SD (n = 3)

NT – Not Active

REFERENCE ACTIVITIES

As a general rule, compounds can be classified as:

Inactive: % parasite viability > 50% at a compound concentration

Marginally active or Inactive: % parasite viability > 20% at a compound concentration

Active : % parasite viability < 20% at a compound concentration

Table 4.4. In-vitro Anti-Plasmodial Activity of Some Selected Plant Extracts against *P. falciparum* 3D7 and FCR3 Strains

Plants Name	Percentage viability (%) 3D7 strain	Percentage viability (%) FCR3 strain
<i>Parinary curatellifolia</i>	14.57±2.03 ^b	157.20±11.72 ^c
<i>Chrozophora senegalensis,</i>	13.41±5.16 ^b	159.03±3.58 ^c
<i>Olox subscorpioidea</i>	13.69±1.26 ^{ab}	166.44±11.38 ^c
<i>Acanthospermum hispidus</i>	32.54±5.04 ^b	86.83±14.50 ^{bc}
<i>Termaridus indica,</i>	2.65±0.61 ^{ab}	160.37±8.11 ^c
<i>Blighia sapida</i>	10.98±0.84 ^b	172.70±2.64 ^c
<i>Senna seiberriana,</i>	12.91±1.72 ^b	175.99±21.08 ^c
<i>Schevenkia americana,</i>	4.11±0.84 ^b	168.99±14.12 ^c
<i>Senna occidentalis</i>	2.95±1.04 ^a	174.22±20.45 ^c
<i>Cymbopogon citrullus</i>	71.95±3.80 ^b	134.89±13.80 ^c
<i>Ocimum bacilicum</i>	85.06±10.34 ^{bc}	149.29±1.85 ^c
<i>Chloroquine</i>	6.04±2.01 ^a	-26.40±8.91 ^{ab}
Negative Control	100.00± 2.41 ^a	100±0.66 ^a

Values of *In-vitro* Anti-Plasmodial Activity of Extracts against *P. falciparum* 3D7 and FCR3 Strains were presented as mean ±SD (n = 3). Values within the same row with different superscripts differ significantly at (P<0.05)

Table 4.5. In-vitro Anti-Plasmodial Activity of *O. subscorpioidea*, *S. americana* and *S. seiberiana* Methanol Leaf Extracts Against *P. falciparum* 3D7 Strains

Plants Name	Percentage viability (%) 3D7 strain	IC ₅₀ 3D7 strain µg/ml
<i>Olax subscorpioidea</i>	13.69±1.26 ^{ab}	0.13
<i>Senna seiberiana</i> ,	12.91±1.72 ^b	0.80
<i>Schevenkia americana</i> ,	4.11±0.84 ^b	0.0040
Chloroquine	6.04±2.01	0.0080
Negative Control	100.00± 2.41	

The values of *invitro anti-plasmodial* activity of *O. subscorpioidea*, *S. americana* and *S. seiberiana* against *P. falciparum* 3D7 strain percentage viability with their IC₅₀ were presented as means ±SD (n = 3)

Table 4.6. The Quantitative Phytochemical composition of *O. subscorpioidea*, *S. americana* and *S. seiberiana* Methanol Leaf Extracts

<i>Phytochemicals</i>	<i>O. subscorpioidea</i> (mg/g)	<i>S. americana</i> (mg/g)	<i>S. seiberiana</i> (mg/g)
Saponins	1.06±0.02 ^a	0.22±0.01 ^b	2.20±0.21 ^{ab}
Tannins	0.29±0.03 ^a	0.18±0.05 ^a	0.34±0.04 ^a
Flavonoids	0.80±0.02 ^a	0.78±0.04 ^a	0.17±0.20 ^b
Glycosides	0.78±0.11 ^a	0.20±0.02 ^{ab}	1.03±0.11 ^c
Alkaloids	0.53±0.03 ^a	0.96±0.03 ^a	1.27±0.20 ^{ab}
Phenols	0.12±0.01 ^a	0.05±0.010 ^b	0.17±0.01 ^a

Values of the quantitative phytochemicals composition present in the extracts of *O. subscorpioidea*, *S. americana* and *S. seiberiana* are presented as mean ±SD (n = 3). Values in the same row with different superscripts differ significantly at (P<0.05)

4.5 Toxicity Profiles of the Plant Extracts

4.5.1 Acute Toxicity Profile of *O. subscorpioidea*, *S. americana* and *S. seiberiana*

The acute toxicity of crude extracts as measured by LD₅₀ indicates, LD₅₀ of the extracts of *O. subscorpioidea*, *S. americana* and *S. seiberiana* administered on rats and *S. americana* and *S. seiberiana* on mice were greater than 5000mg/kg body weight. While LD₅₀ for *O. subscorpioidea* on mice as given by the square root of maximum value that did not cause death and lowest value that causes death was approximately 3800mg/kg body weight (Lorke, 1983) as calculated thus: LD₅₀ (*O. Subscorpioidea*) in mice = $\sqrt{2900 \times 5000} = 3807.89$ mg/kg body weight {Table 4.7). The study also look into the longer term effect, the sub-acute effect, of the extracts on the experinmental animals especially on the organs (liver and kidney). The results generally were statistically insignificant when the results of the treated groups are compared with that of the control groups. The overall effect of the plants extracts expressed no deleterious influence of the on both the liver and kidney. The detailed results are shown in the Appendix.

Table 4.7. Acute Toxicity Profile of *O. Subscorpioidea*, *S. americana* and *S. seiberiana* Methanol Leaf Extracts Against Experimental Model (mice)

Doses (mg/kg)	<i>O. subscorpioidea</i>		<i>S. americana</i>		<i>S. seiberiana</i>	
	Mice	Rats	Mice	Rats	Mice	Rats
10	0/3*	0/3	0/3	0/3	0/3	0/3
100	0/3	0/3	0/3	0/3	0/3	0/3
1000	0/3	0/3	0/3	0/3	0/3	0/3
Doses (mg/kg)	Survival rate (phase II)					
1000	0/1*	0/1	0/1	0/1	0/1	0/1
1600	0/1	0/1	0/1	0/1	0/1	0/1
2900	0/1	0/1	0/1	0/1	0/1	0/1
5000	1/1	0/1	0/1	0/1	0/1	0/1

* Survival rate numerator is the number of death animal recorded and denominator is the number of used animals.

LD₅₀ is given by the square root of maximum value that did not cause death and lowest value that cause death according to Lorke's, 1983. The LD₅₀ values of extracts of *O. subscorpioidea*, *S. americana* and *S. seiberiana* orally in rats model and *S. americana* and *S. seiberiana* in mice model are greater than 5000mg/kg body weight.

LD₅₀ for *O. subscorpioidea* in mice = $\sqrt{2900 \times 5000} = 3807.89$ mg/Kg.

4.6 In-vivo Growth Inhibition Activity of *O. subscorpioidea*, *S. americana* and *S. seiberiana* Against *Plasmodium berghei* NK 65 Strain

The result of the *in-vivo* anti-plasmodial growth inhibition activity of the *S americana*, *S. seiberiana* and *O. subscorpioidea* demonstrated considerable inhibition activity against the parasitemia of the *P. berghei* NK 65 in infected mice. The percentage parasitemia and chemo-suppression activity of the extracts based on the 4 and 6 days post infection smear according to Mustofa *et al.*,(2007) were summarized in Table 4.8 at the concentration ranges at 50 to 200mg/kg body weight of the extracts. *S. americana* exhibited parasite suppression with mean average percentage of 34.32 ± 18.99 , 38.66 ± 9.02 to $43.51\pm 10.99\%$ at day four and 56.46 ± 9.11 , 77.15 ± 3.36 and $71.21\pm 4.70\%$ at day 6 post infection smear for the 3 concentration respectively. *Olax subscorpioidea* demonstrated the parasite growth inhibition with 77.42 ± 10.84 , 72.52 ± 7.57 and $77.26\pm 12.97\%$ at day six of post infection smear. While *Senna seiberiana* showed the least inhibition activity at the sixth days of infection with mean percentages of 33.74 ± 15.65 , 33.34 ± 6.68 and $32.22\pm 12.69\%$ for the three doses (Table 4.8).

Plants are generally considered active when the parasite suppression level are greater than 30% (Vale *et al.*, 2015). *O. subscorpioidea* and *S. americana* expresses the highest level of suppression at dose of 50 and 100mg/kg body weight respectively and *S. seiberiana* show the least suppression at dose of 200mg/kg body weight (Table 4.8). The suppression activities of the extracts were practically not dose-dependency and could be compare to the standard drug that have $84.52\pm 7.89\%$ inhibition at day six of the smear.

Table 4.8. In-vivo Parasite Growth Inhibition Chemo-suppression and animal protection Activities of *O. subscorpioidea*, *S. americana* and *S. seiberiana* Methanol Extracts Against *Plasmodium berghei* NK 65 Strain

Drugs	Ave % Parasitamia		Ave % chemo suppression		% Protection	
	Day 4	Day 6	Day 4	Day 6	N*	%
Control(Normal Saline)	20.16±4.14	28.39±5.28			0	0
Chloroquine(Positive Control)	7.66±2.33	4.46±2.21	61.97±7.1	84.52±7.89	5	100
<i>S. americana</i> 50mg/kg	12.73±2.09	12.28±3.10	34.32±18.99	56.46±9.11	3	60
<i>S. americana</i> 100mg/kg	12.14±1.64	6.40±1.01	38.66±9.02	77.15±3.36	4	80
<i>S. americana</i> 200mg/kg	11.02±0.57	8.13±1.70	43.51±10.99	71.21±4.70	4	80
<i>O. subscorpioidea</i> 50mg/kg	15.13±2.56	6.03±2.58	25.78±18.54	77.42±10.84	4	80
<i>O. subscorpioidea</i> 100mg/kg	9.85±2.59	7.65±1.87	48.52±21.20	72.52±7.57	3	60
<i>O. subscorpioidea</i> 200mg/kg	10.18±0.49	4.94±1.89	47.58±11.85	77.26±12.97	4	80
<i>S. seiberiana</i> 50mg/kg	3.092±1.18	11.58±2.55	32.05±24.99	33.74±15.65	2	40
<i>S. seiberiana</i> 100mg/kg	2.86±1.54	8.82±1.05	21.51±22.89	33.34±6.68	2	40
<i>S. seiberiana</i> 200mg/kg	2.45±1.54	10.77±1.99	28.02±15.98	32.22±12.69	2	40

Values of average percentage Parasite growth inhibition, chemo-suppression and percentage protection of experimental animal are presented as mean ±SD (n = 5).

*N= Number of mice that survived after 20 days of post treatment with the corresponding extracts and the standard drug

4.7 *In-vitro* P. falciparum asexual stage Proliferation percentage Inhibition and IC₅₀ of Fractions and Compounds of *Olex subscorpioidea*

The plant *O. subscorpioidea* methanol extract obtained, fractionated as represented in (Plate III) and evaluated for its antimalarial potential using dual point *in-vitro* asexual stage proliferation inhibition and IC₅₀ assay showed that three fractions at concentration of 25 µg/ml inhibited the parasite viability \geq 50%. Fraction GB003 (crude extract) showed the highest percentage proliferation inhibition > 80 % (IC₅₀ 0.127 µg/ml). While the fraction GB003B was active against clinical strain at 25 µg/ml with IC₅₀ 6.12 µg/ml (Table 4.9). All of the fractions were generally inactive at 5 µg/ml concentration. On further fractionation of GB003B fraction GB003B2.1 and 003B3.1 were obtained and found to be moderately active with 50% inhibition with IC₅₀ 33.73 and 36.75 µg/ml respectively (Table 4.10). Fraction GB003C on the other hand gave 3 fractions GB003CA1, GB003BC1.1 and GB003CC1 all of which are active with IC₅₀ 2.51, 0.33 and 6.22 µg/ml respectively with GB003BC1.1 having the highest activity (Table 4.11). Further purification of GB003BC1.1 using PTLC gave two compounds GB003BCF2AE3 and GB003BCF3A1.1 as shown in Plate IV, both of which were active against *Plasmodium falciparum* with IC₅₀ 1.79 and 1.65 µg/ml respectively (Table 4.12).

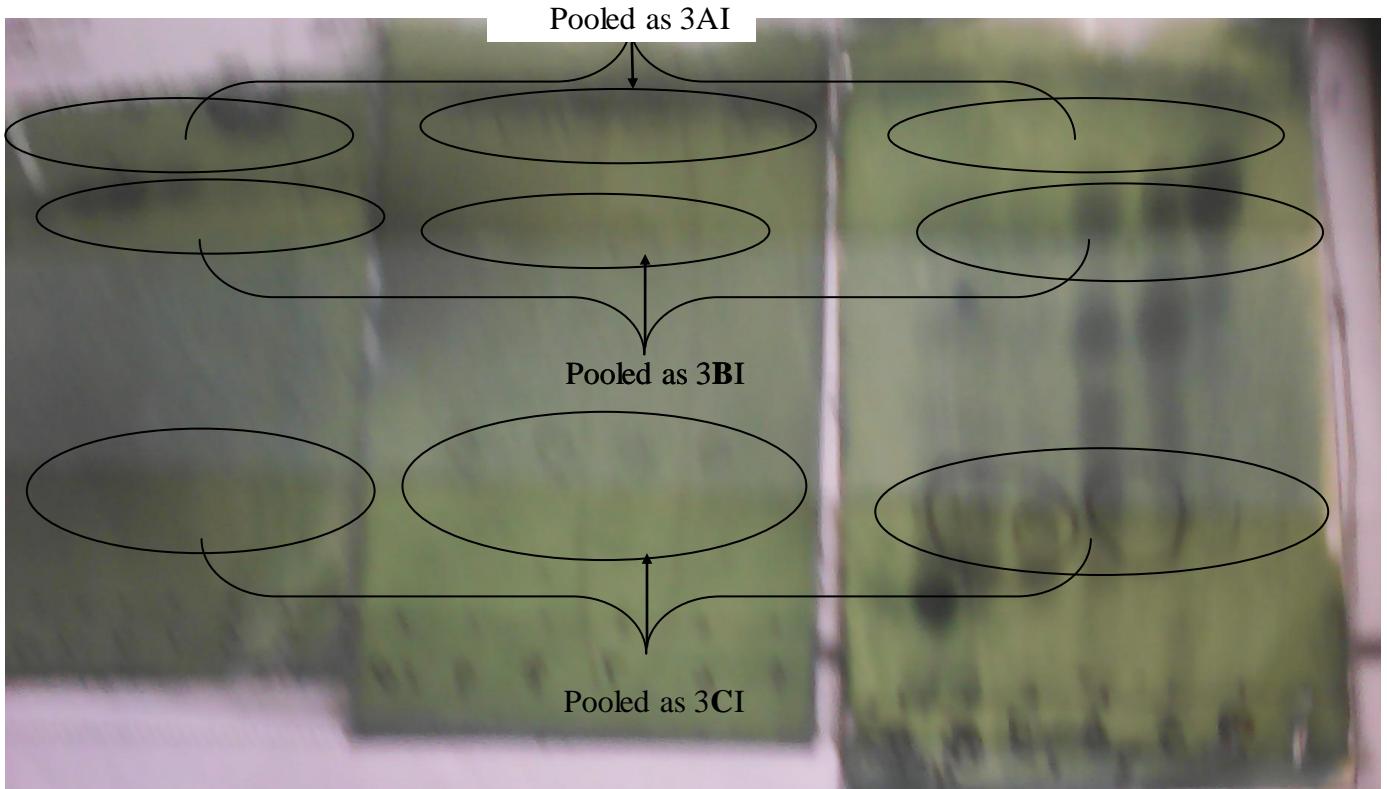


Plate III: The TLC Chromatograms of Fractionation of *O. subscorpioidea* Crude Extract Developed With Hexane: Dichloromethane and Methanol (60:39:1) and Visualized With Phosphomolybdic Acid and UV at 256nm
 Fractions at top most level were pooled as 3AI
 The set of pool next lower were pooled as 3BI
 The pool lower down are pooled as 3CI

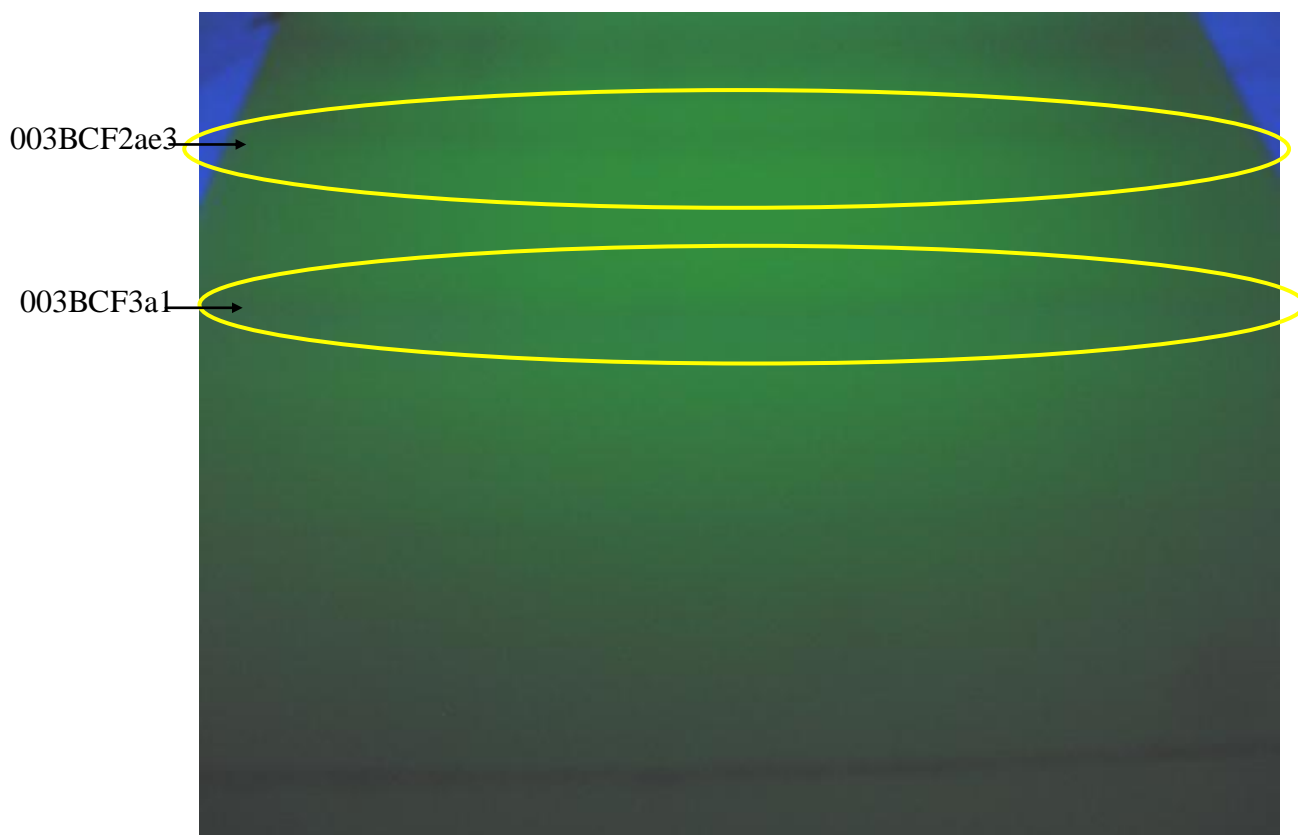


Plate IV: The PTLC Profile of Fraction GB003BCF3a1 of *O. subscorpioidea* Extract Under UV Light at 366nm
The Point Circled Yellow Indicated The Isolates of Compound GB003BCF3a1
PTLC – Preparatory Thin Layer Chromatography
UV- Ultra violet Light

Table 4.9. *In vitro Plasmodium falciparum* Asexual Stage Proliferation percentage Inhibition and IC₅₀ of The Crude Extract and Column Fractions of *Olox subscorpioidea*

Crude extract/Fractions	Asexual proliferation (% inhibition)		
	5 µg/ml	25 µg/ml	
	% inhibition±SD	% Inhibition±SD	IC ₅₀ µg/ml
GB003(Crude)	32.53±5.4	87.73±1.62	0.13
GB003A	19.2±3.1	31.15±0.40	43.12
GB003B	0.02±2.3	49.82±2.04	6.12
GB003C	19.3±3.1	50.49±2.1	3.22
Chloroquine (1 µg/ml)	93.96±2.01		0.0080
Negative Control	0.00±3.74	0.00±2.41	

The values of the invitro asexual stage proliferation % inhibition and the IC₅₀ of extract and column fraction of *Olox Subscorpioidea* presented as mean ±SD (n= 3) technical replicate

Table 4.10. *In vitro Plasmodium falciparum* Asexual Stage Proliferation percentage Inhibition and IC₅₀ of GB003B Fractions of *Olox subscopioidea*

Fractions	Asexual proliferation (% inhibition)		IC ₅₀ µg/ml
	5 µg/ml	25 µg/ml	
	% inhibition ±SD	% inhibition ±SD	
GB003B1.1	0.47±4.5	30.43±4.80	79.60
GB003B2.1	16.3±5.2	59.42±0.73	33.73
GB003B3.1	21.88±5.3	54.9±1.30	36.75
Chloroquine (1 µg/ml)	93.96±2.01		0.0080
Negative Control	0.00±0.00	0.00±0.00	

The values of the invitro asexual stage proliferation % inhibition and the IC₅₀ of extract and column fraction of *Olox Subscopioidea* presented as mean ±SD (n= 3) technical replicate

Table 4.11. *In vitro Plasmodium falciparum* Asexual Stage Proliferation percentage Inhibition and IC₅₀ of GB003C Fractions of *Olox subscoipodia*

Fractions	Asexual proliferation (% inhibition)		
	5 µg/ml	25 µg/ml	IC ₅₀ µg/ml
	% inhibition ±SD	% inhibition ±SD	
GB003BC1.1	24.7±7.4	61.38±0.07	0.33
GB003CA1	21.8±1.6	59.42±4.2	2.51
GB003CC1	17.1±2.2	51.50±3.3	6.22
Chloroquine (1 µg/ml)	95.75±4.11		0.0066
Negative Control	0.00±0.00	0.00±0.00	

The values of the invitro asexual stage proliferation % inhibition and the IC₅₀ of column fraction of *Olox Subscorpioidea* presented as mean ±SD (n= 3) technical replicate

Table 4.12. *In vitro Plasmodium falciparum* Asexual Stage Proliferation percentage Inhibition and IC₅₀ of GB003BC1.1 Fractions of *Olox subscorpioidea*

Fractions	Asexual proliferation (% inhibition)		IC ₅₀ µg/ml
	5 µg/ml	25 µg/ml	
	% inhibition ±SD	% inhibition ± SD	
GB003BCF2AE3	19.3±2.3	50.49±1.7	1.79
GB003BCF3A1.1	15.21±1.4	60.23±3.3	1.65
Chloroquine (1 µg/ml)	95.75±4.11		0.0066
Negative Control	0.00±0.00	0.00±0.00	

The values of the invitro asexual stage proliferation % inhibition and the IC₅₀ of column fraction of *Olox Subscorpioidea* presented as mean ±SD (n= 3) technical replicate

4.8 *In-vitro P. falciparum* Proliferation % Inhibition of Fractions and Compounds from *Schwenkia americana*

The plant *S. americana* initially extracted with methanol fractionated as represented in Plate V and evaluated for its antimalarial potential using *invitro P. falciparum* asexual proliferation inhibition assay. Four fractions were obtained with two of the fractions GB010C, GB010EA and the crude extract active at concentration of 25 µg/ml with IC₅₀ 2.61, 4.42 and 0.004 µg/ml respectively (Table 4.13). Fraction GB010C on further purification gave three fractions, two of the which were GB010C1.1 and GB010C3 and showed activity against 3D7 strain of the parasite with respective IC₅₀ 15.32 and 2.65 µg/ml (Table 4.14). Fraction GB010C3 most active was further purified to obtained three fractions, out of which two were GB010C3C1 and GB010C3C3.1 both fractions were active against the parasite at 25 µg/ml with respective IC₅₀ 60.32 and 23.42 µg/ml (Table 4.15). All of the fractions were generally inactive at lower concentration 5 µg/ml. Further fractionation of GB010EA gave only one active fraction GB010EA2.3.1 exhibited >50% inhibition with IC₅₀ 1.75 µg/ml (Table 4.16). The purification of GB010EA2.3.1 using PTLC gave an active fraction GB010EA2.3.1G (Plate VI) against *Plasmodium falciparum* with IC₅₀ 2.92 µg/ml (Table 4.17).

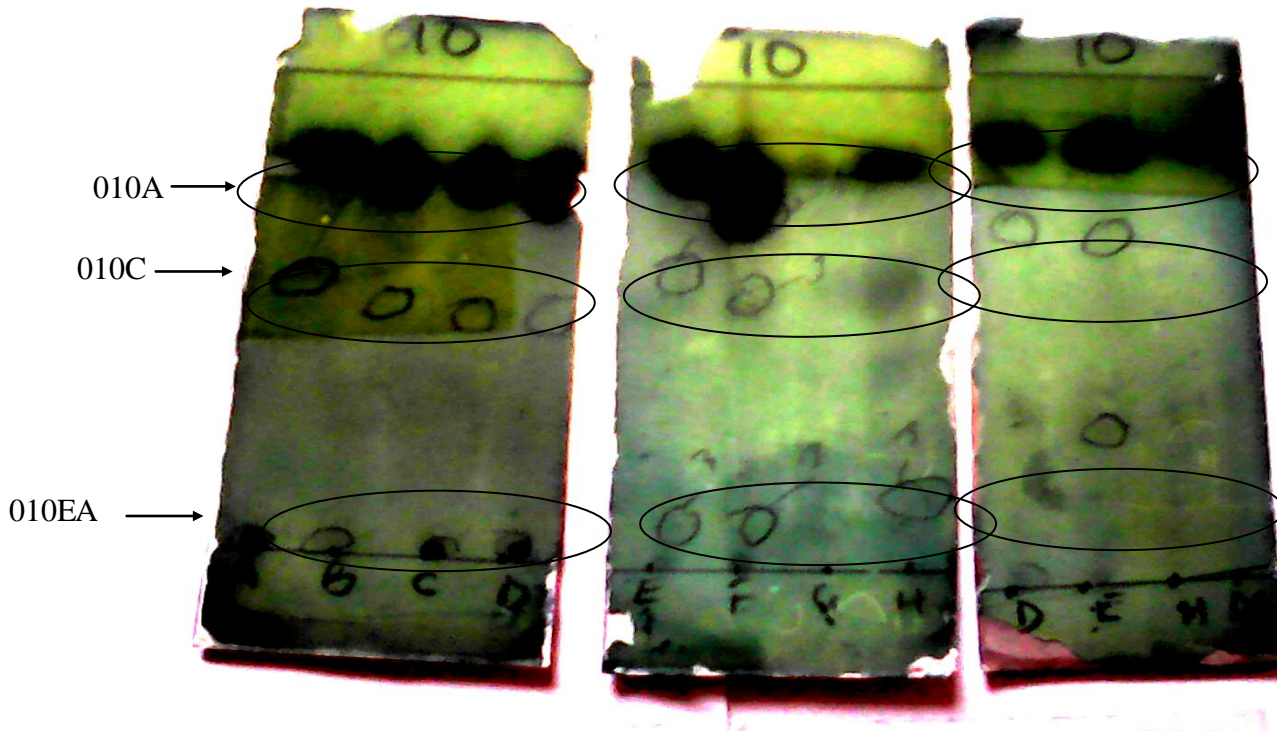


Plate V: Typical result obtained from pooled fractions from the silica column of *S. americana* Crude Extract. The TLC plate were developed with Hexane: Dichloromethane and Methanol (60:39:1) and Visualized with Phosphomolybdic Acid and UV At 256nm

GB010EA2.3.1

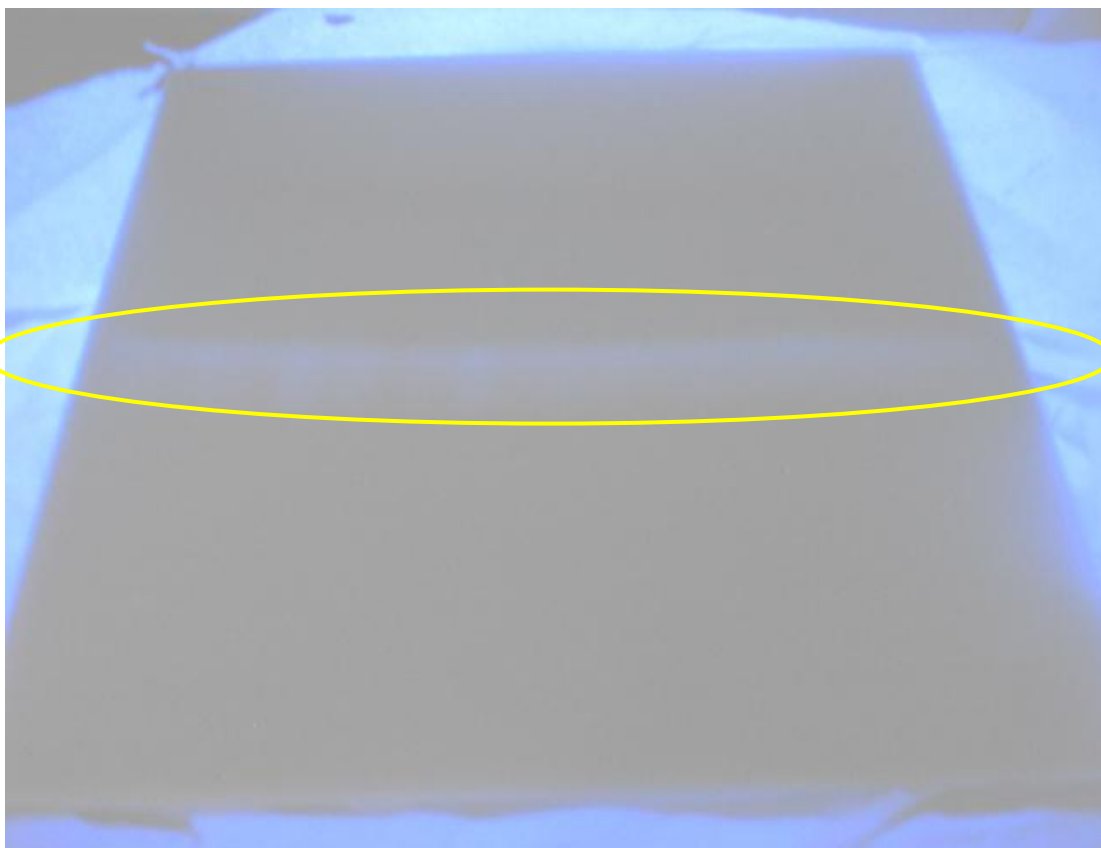


Plate VI: The PTLC Profile of Fraction of *O. Subscorpioidea* Extract Under UV Light at 366nm.
The Point Circled Yellow Indicated the Isolate of Compound GB003BCF2ae3
PTLC – Preparatory Thin Layer Chromatography
UV- Ultra violet Light

Table 4.13. *In vitro Plasmodium falciparum* Asexual Stage Proliferation percentage Inhibition and IC₅₀ of Crude Leaf Extract and Column Fractions of *Schwenckia americana*

Compound	Asexual proliferation (% inhibition)		
	5 µg/ml	25 µg/ml	IC ₅₀ µg/ml
	% inhibition ±SD	% inhibition ±SD	IC ₅₀ µg/ml
GB010(Crude)	50.6±1.7	95.89±2.84	0.004
GB010A 1	4.0±3.3	30.54±6.40	68.11
GB010B1.1	5.3±4.3	40.20±5.04	56.23
GB010C	27.1±4.5	58.61±3.50	2.61
GB010EA	11.21±3.1	50.49±2.1	4.42
Chloroquine (1 µg/ml)	93.96±2.01		0.0080
Negative Control	0.00±0.00	0.00±0.00	

The values of the invitro asexual stage proliferation % inhibition and the IC₅₀ of column fraction of *Schwenckia americana* presented as mean ±SD (n= 3) technical replicate

Table 4.14. *In vitro Plasmodium falciparum* Asexual Stage Proliferation percentage Inhibition and IC₅₀ of *Schwenckia americana*

Compound	Asexual proliferation (% inhibition)		IC ₅₀ µg/ml
	5 µg/ml	25 µg/ml	
	% inhibition ±SD	% inhibition ±SD	
GB010C1.1	18.4±5.2	52.32±4.44	15.32
GB010C2.1	10.3±5.3	9.90±3.50	173.01
GB010C3	12.8±3.2	56.90±1.21	2.65
Chloroquine (1 µg/ml)	93.96±2.01		0.0080
Negative Control	0.00±0.00	0.00±0.00	

The values of the invitro asexual stage proliferation % inhibition and the IC₅₀ of column fraction of *Schwenckia americana* presented as mean ±SD (n= 3) technical replicate

Table 4.15. *In vitro Plasmodium falciparum* Asexual Stage Proliferation percentage Inhibition and IC₅₀ GB010C3 Fractions of *Schwenckia americana*

Compound	Asexual proliferation (% inhibition)		IC ₅₀ µg/ml
	5 µg/ml	25 µg/ml	
	% inhibition ±SD	% inhibition±SD	
GB010C3C1	18.8±6.2	48.90±3.3	60.32
GB010C3C3.1	30.7±3.3	50.39±1.7	23.42
GB010C3 C5.1	9.3±1.4	10.23±3.2	157.32
Chloroquine (1 µg/ml)	95.75±4.11		0.0066
Negative Control	0.00±0.00	0.00±0.00	

The values of the invitro asexual stage proliferation % inhibition and the IC₅₀ of column fraction of *Schwenckia americana* presented as mean ±SD (n= 3) technical replicate

Table 4.16. *In vitro Plasmodium falciparum* Asexual Stage Proliferation percentage Inhibition and IC₅₀ GB010EA Fractions of *Schwenckia americana*

Compound	Asexual proliferation (% inhibition)		IC ₅₀ μg/ml
	5 μg/ml	25 μg/ml	
GB010EA1	% inhibition ±SD 11.21±3.1	% inhibition ±SD 20.49±2.1	117.02
GB010EA2.3.1	5.8±1.6	59.42±4.2	1.75
GB010EA4.16	5.8±5.4	10.31±8.6	137.11
GB010EA19.1	27.6±4.4	48.5±5.0	63.62
GB010EAT2	12.07±0.5	20.5±5.5	119.17

The values of the invitro asexual stage proliferation % inhibition and the IC₅₀ of column fraction of *Schwenckia americana* presented as mean ±SD (n= 3) technical replicate

Table 4.17. *In vitro Plasmodium falciparum* Asexual Stage Proliferation percentage Inhibition and IC₅₀ GB010EA2.3.1 Fractions of *Schwenckia americana*

Compound	Asexual proliferation (% inhibition)		
	5 µg/ml % inhibition ±SD	25 µg/ml % inhibition ±SD	IC ₅₀ µg/ml
GB010EA 2.3.1G	31.7±4.7	65.35±3.3	2.92
Chloroquine (1 µg/ml)	95.75±4.11		0.00663
Negative Control	0.00±0.00	0.00±0.00	

The values of the invitro asexual stage proliferation % inhibition and the IC₅₀ of column fraction of *Schwenckia americana* presented as mean ±SD (n= 3) technical replicate

Table 4.18. 1D and 2D Spectral Summary for Compound GB3BCF2ae3 in CDCl₃ (400MHz, 100MHz) J= Hz

Position	δ_H(ppm)	δ_C(ppm)	HSQC	COSY
1	7.72	77.34	H-1	H-0.9
2	2.40	29.71	H-2	
3	7.10	77.22	H-3	H-4.3
4	2.30	29.38	H-4	
5	2.00	23.00	H-5	
6	3.40	31.94	H-6	H-4.3
7	7.00	77.02	H-7	
8	1.40	22.00	H-8	
9	7.00	76.70	H-9	H-6
10	0.9	14.03	H-10	H-7.2

Key: CDCl₃ = Deuterated Chloroform

GB3BCF2ae3 = Purified fraction from *O. subscorpioidea*

Ppm = part per million

HSQC = Heteronuclear Single Quantum Coherence

COSY = Correlation spectroscopy

4.9 Chemical and Structural Elucidation of Compounds Isolate

The results of the isolation and purification of two compounds from *O. subscorpioidea* yield GB003BCF2AE3 and GB003BCF3A1.1 both of which were found to be active at concentration 25 µg/ml against *Plasmodium falciparum* with IC₅₀ 1.79±0.88 and 1.65±4.31 µg/ml respectively. The NMR spectral analysis was employed for the structural elucidation which further identified compound GB003BCF2AE3 as thus;

4.10 ¹H NMR (Proton Nuclear Magnetic Resonance)

The Proton NMR spectrum of GB003BCF2AE3 in CDCl₃ (0.0 – 8.0ppm) exhibited signal at δ_H 0.9-1.03, δ_H 1.40, δ_H 2.00, δ_H 2.30, δ_H 2.40, δ_H 3.40, δ_H 7.00, δ_H 7.00 δ_H 7.10 and δ_H 7.72 as represented in Table 4.18 and the spectral are shown in appendix V.

4.11 ¹³C NMR (Carbon Nuclear Magnetic Resonance)

The ¹³C NMR spectrum of GB003BCF2AE3 in CDCl₃ (δ ppm 400MHz CDCl₃) analysis revealed signal at δ_C 77.34 (C-1), δ_C 77.22(C-3), δ_C 77.02 (C-7), δ_C 76.70 (C-9), δ_C 31.94 (C-6), δ_C 29.71 (C-2), δ_C 29.38 (C-4), δ_C 23.00 (C-5), δ_C 22.00 (C-8) and δ_C 14.03 (C-10) as summarized in table 4.18 and the spectral are shown in appendix V

4.12 Proposed Compound of GB003BCF2AE3

The 1D and 2D spectral of the compound is represented in Table 4.18, the molecular formular for compound was found to be C₁₀H₁₉O₄ established by FABMS. The UV absorbance at (344 cm) could be attributed to the hydroxyl groups that are attached to different position of the compound. The ¹H NMR spectrum exhibited signals for methyl group at δ_H 0.9, while the two methylene protons signals at H-2 and H-4 positions were also exhibited. Six methine proton signals were found to be at H-1, H-3, H-5, H-6, H-7 and H-8. The ¹³C NMR experiment spectrum exhibited a total of 10 carbon signals corroborating a methyl group, two- methylene group and six methine

carbon signals. The COSY and HSQC spectral correlation of compound GB003BCF2AE3 as in table 4.25 were also used to further ascertain the structure of this compound. Consequently based on that the compound GB003BCF2AE3 was determined as **Rel, 1 β , 3 α - dihydroxyl 7 α , 9 β dihydroxylcyclopentane** in figure 4.1.

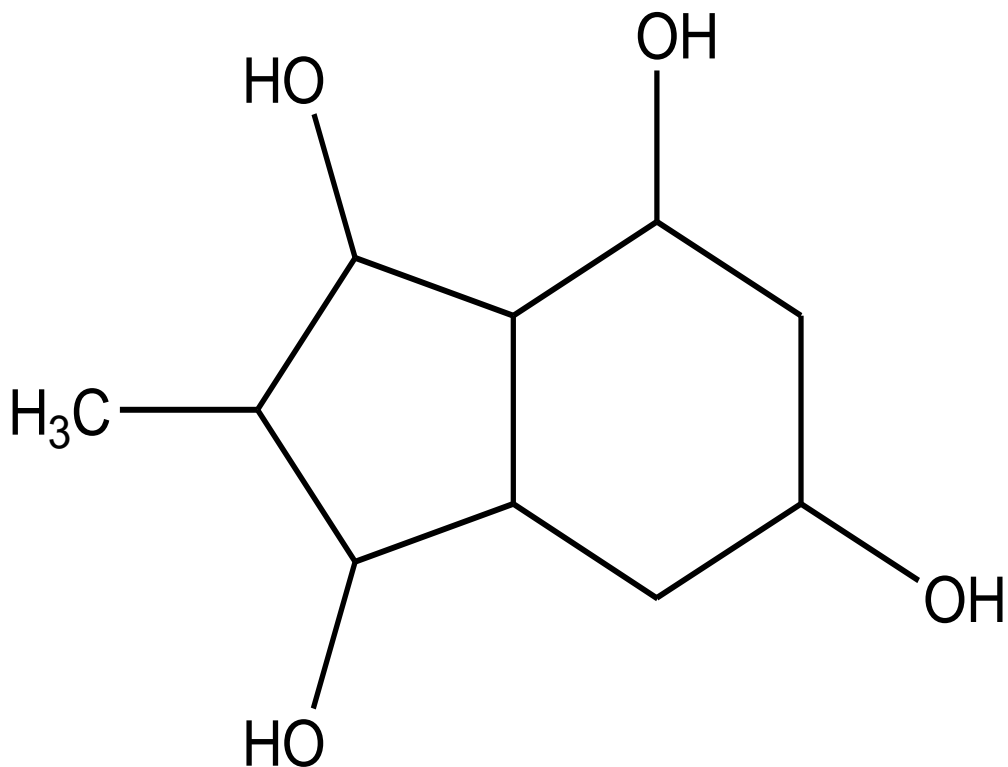


Figure 4.1: Rel, 1 β , 3 α - dihydroxyl 7 α , 9 β dihydroxylcyclopentane
(Octahydro-2-methyl-1H-indene-1, 3, 4, 6-tetraol)

Table 4.19. 1D and 2D Spectral Summary for Compound GB3BCF3A1.1 in CDCl₃ (400MHz, 100MHz) J= Hz

Position	δ_C	δ_H	DEPT	HSQC	COSY
1	128.10	7.18	CH	H-1	-
2	114.48	-	C	-	-
3	37.11	1.20	CH	H-3	-
4	77.34	6.40	CH	H-4	H-5, H-4
5	77.23	6.23	CH	H-5	-
6	37.43	4.30	CH	H-6	-
7	114.48	-	C	-	-
8	114.15	7.20	CH	H-8	-
9	127.18	7.10	CH	H-9	H-8, H-9
10	127.00	7.11	CH	H-10	-
11	14.13	0.90	CH ₃	H-11	H-11
1'	21.71	1.20	CH	H-1'	-
2'	19.70	2.10	CH ₂	H-2'	H-3'
3'	23.71	1.70	CH ₃	H-3'	H-3'
4'	77.02	3.60	CH ₂	H-4'	-

Key: CDCl₃ = Deuterated Chloroform

GB3BCF2ae3 = Purified fraction from *O. subscorpioidea*

Ppm = part per million

HSQC = Heteronuclear Single Quantum Coherence

COSY = Correlation spectroscopy

DEPT- Distortionless enhancement by polarization transfer

4.13 Chemical and Structural Elucidation of Compounds Isolate

The NMR spectral analysis further identified compound GB003BCF3A1.1 as **1,2,3,4-tetrahydro-1-(1-hydroxybutan-2-yl)-4-methylnaphthalene-2,3-diol** isolated from *O. subscorpioidea* (Figure 4.2), on the basis of comparison within the spectral of the Proton ^1H , ^{13}C , DEPT, COSY and HSQC data summarized in Table 4.19 and the spectral are shown in the appendix V.

4.14 ^1H NMR (Proton Nuclear Magnetic Resonance)

The Proton NMR spectrum of GB003BCF3A1.1 in CDCl_3 (0.0 – 8.0ppm) exhibited signal at δ_{H} 0.8- , δ_{H} 1.20, δ_{H} 4.30, δ_{H} 6.70, and aromatic signals at δ_{H} 7.18, δ_{H} 7.17, δ_{H} 7.16 and δ_{H} 7.20 as represented in Table 4.19

4.15 ^{13}C NMR (Carbon Nuclear Magnetic Resonance)

The ^{13}C NMR spectrum of GB003BCF3A1.1 in CDCl_3 (δ_{C} ppm 400MHz CDCl_3) analysis revealed signal at δ_{C} 128.10 (C-1), δ_{C} 127.00(C-2), δ_{C} 114.48 (C-3), δ_{C} 127.18 (C-4), δ_{C} 114.55 (C-5), δ_{C} 114. 48 (C-6), δ_{C} 77.34 (C-7), δ_{C} 77.23 (C-8), δ_{C} 37.43(C-9), δ_{C} 77.02 (C-10), δ_{C} 37.11 (C-11), δ_{C} 23.71 (C-12), δ_{C} 21.71 (C-13), δ_{C} 19.70 (C-14) and δ_{C} 14.13 (C-15) as summarized in Table 4.19.

4.16 Proposed Compound of GB003BCF3A1.1

The 1D and 2D spectral of the compound GB003BCF3A1.1 is represented in figure 4.2, the molecular formular for the compound was found to be $\text{C}_{15}\text{H}_{22}\text{O}_3$ as established by FABMS. The UV absorbance of 344cm could be attributed to the hydroxyl groups attached to different position of the compound. The ^1H NMR spectrum exhibited signals for two methyl groups at δ_{H} 0.8, while two methylene protons signals at H-2 and H-4 positions were exhibited. Six methine proton signals were found to be H-1, H-3, H-5, H-7and H-8 and 3 hydroxyl group signals were identified at H-4, H-5 and H-4'. The ^{13}C NMR experiment spectrum exhibited a total of 15 carbon signals

consisting of two quaternary carbon, two methyl groups, two- methylene groups and six methine carbon signals. The DEPT, COSY, and HSQC spectral correlation of compound were also use to ascertain the structure of this compound, consequent upon this, the compound was determined as 1,2,3,4-tetrahydro-1-(1-hydroxybutan-2-yl)-4-methylnaphthalene-2,3-diol and the structure is represented as in figure 4.2.

4.17 Chemical and Structural Elucidation of Compounds Isolate From *S. americana*

A partly purified isolate GB010EA 2.3.1G with IC_{50} 2.92 ± 0.73 was obtained from *S. americana* and with mass spectrometry the chemical profile was elucidated. Time of flight mass spectrometry, using electrospray ionization in positive mode (TOF MS ES+) gave a $[M+H]^+$ of the two most prominent spectral the compound with m/z 413.2651 represented in Figure 4.3 was through MassLynx v.4.1 soft ware and searching the mass bank and chemspider was identified as Boviquinone 4 and molecular formula were calculated to have the $C_{25}H_{36}O_4$ the spectral of the MS and MS/MS and the structural representation and name of the compound is represented in Figure 4.3, 4.4 and 4.6 respectively.

4.18 Chemical and Structural Elucidation of Compounds Isolate from *S. americana*

The spectral of the MS and MS/MS, the structural and name are represented in Figure 4.7, 4.8 and 4.9 respectively for the second compound with m/z of 337.3334 with calculated molecular formula $C_{25}H_{21}O$ through MassLynx v.4.1.and on chemical search through the chemspider the compound was identified as 2-(2,5-Dimethylphenyl)-4,6-diphenylpyrylium..

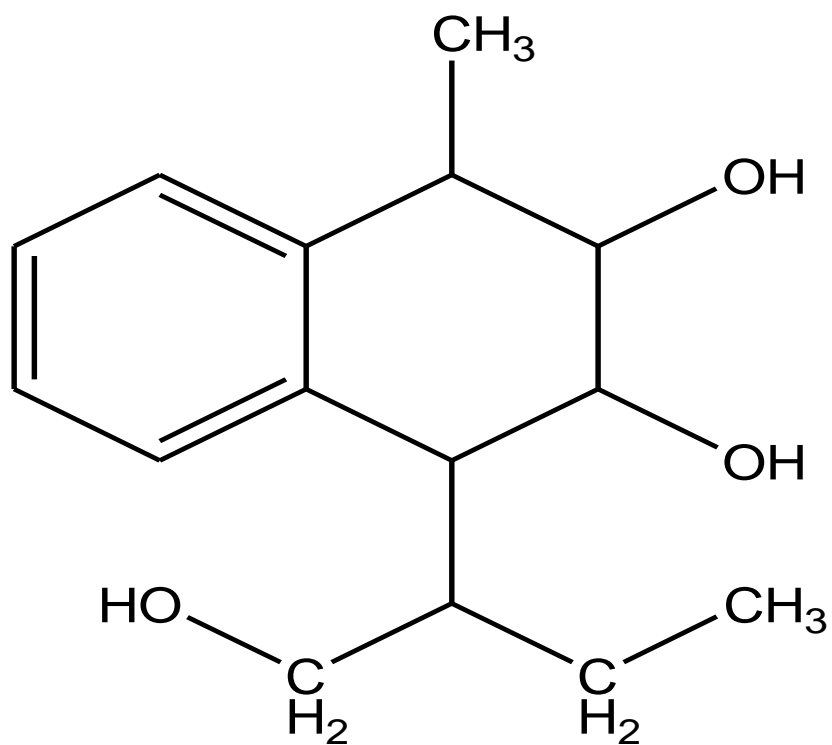


Figure 4.2 1,2,3,4-tetrahydro-1-(1-hydroxybutan-2-yl)-4-methylnaphthalene-2,3-diol
Isolated from *O. subscorpioidea*

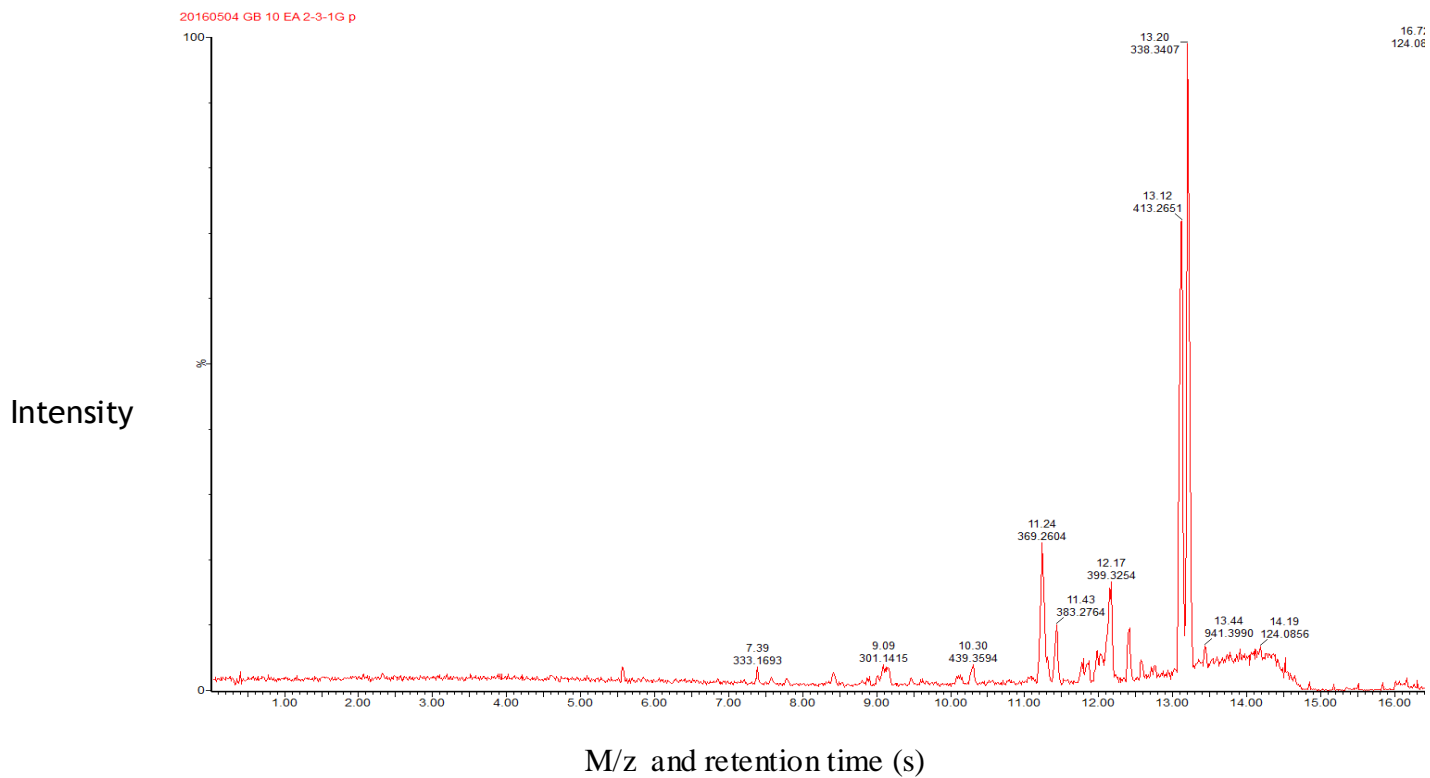


Figure 4.3: UPLC-MS Chromatogram of Two Compounds With Higher Peak from the Fraction GB10EA2-3-1G In + Ve Mode

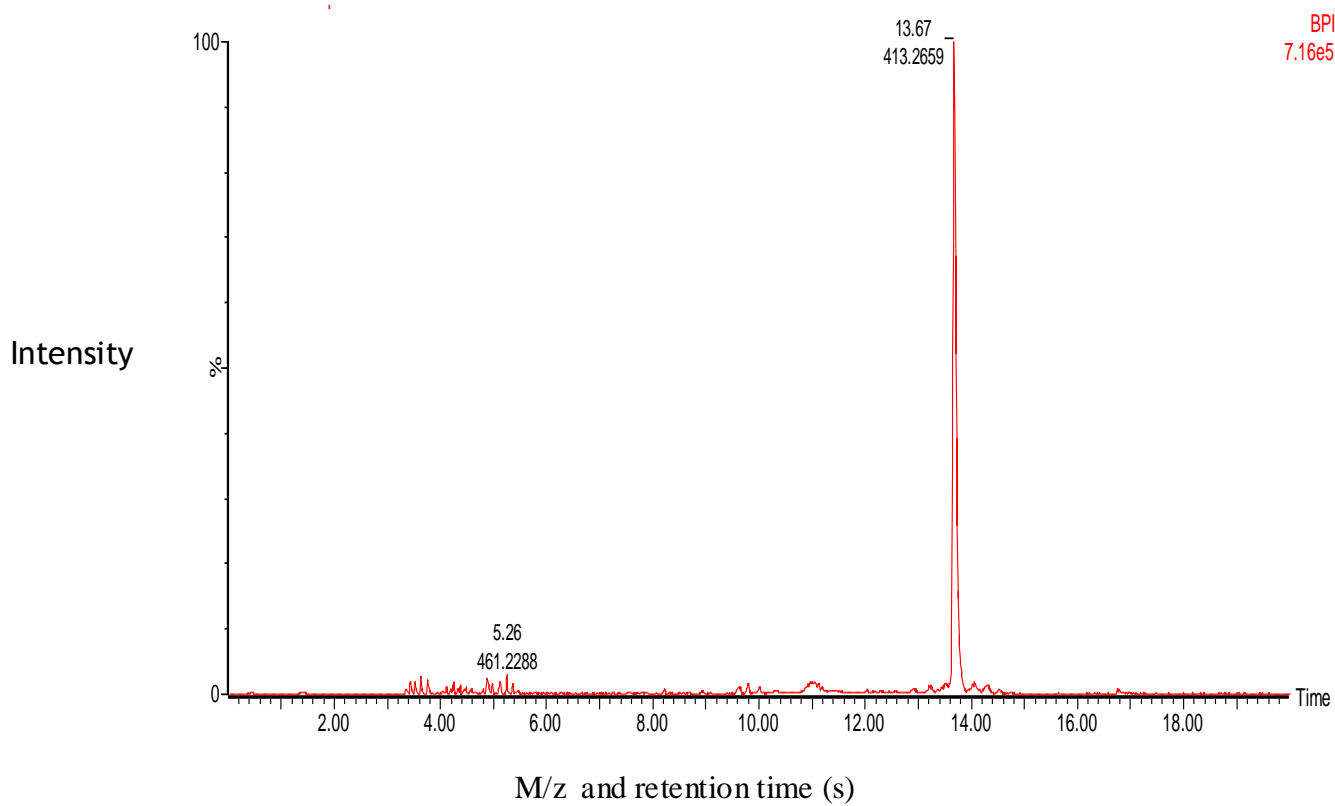


Figure 4.4: UPLC-MS Chromatogram Of Compound With M/Z 413.2651 From Fraction GB10EA2-3-1G In +Ve Mode

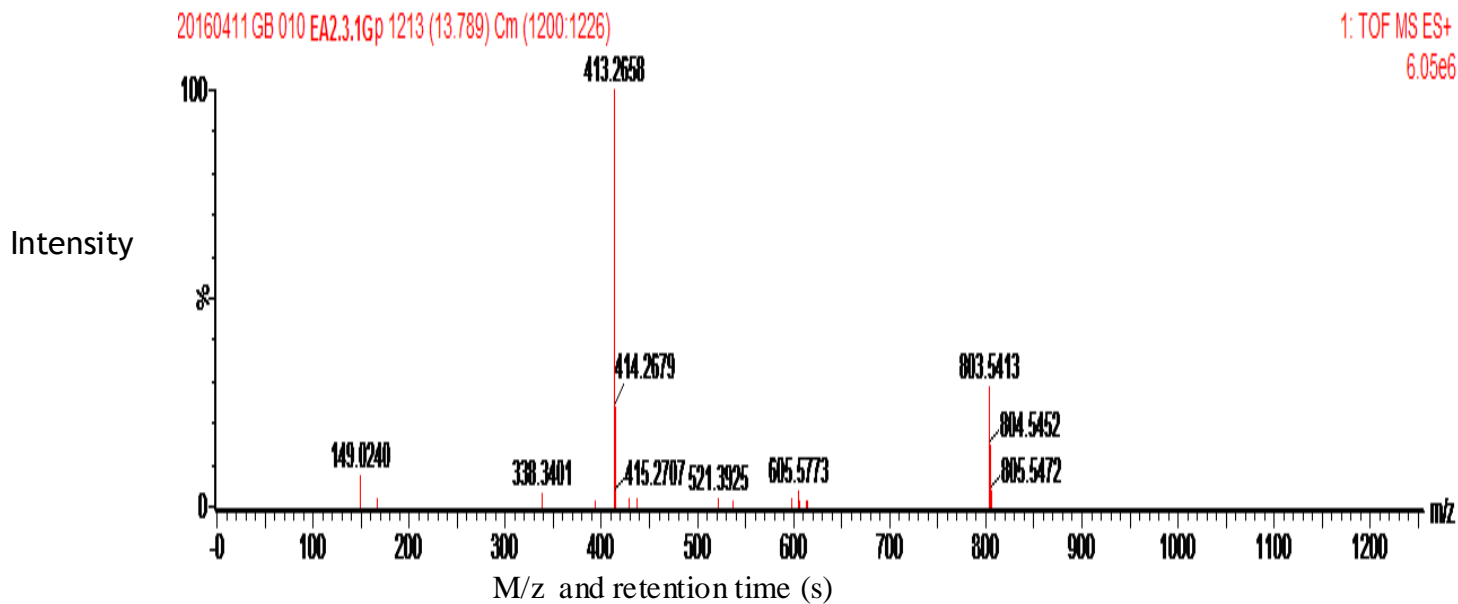


Figure 4.5 ; : The UPLC-MS MS/MS profile of possible Compound With M/Z 413.2651 From Fraction GB10EA2-3-1G In +Ve Mode

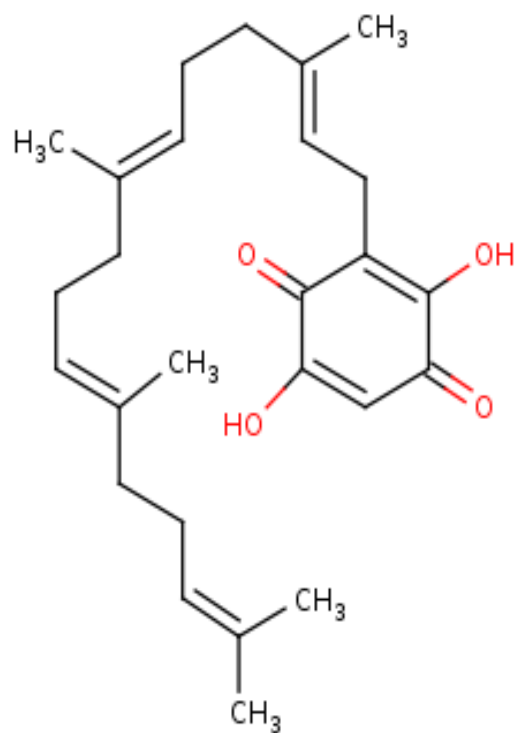


Figure 4.6. Chemical Structure Of Compound 2,5-Dihydroxy-3-((2E,6E,10E)-3,7,11,15-Tetramethylhexadeca-2,6,10,14-Tetraenyl)Cyclohexa-2,5-Diene-1,4-Dione(Boviquinone 4) From *S. americana*

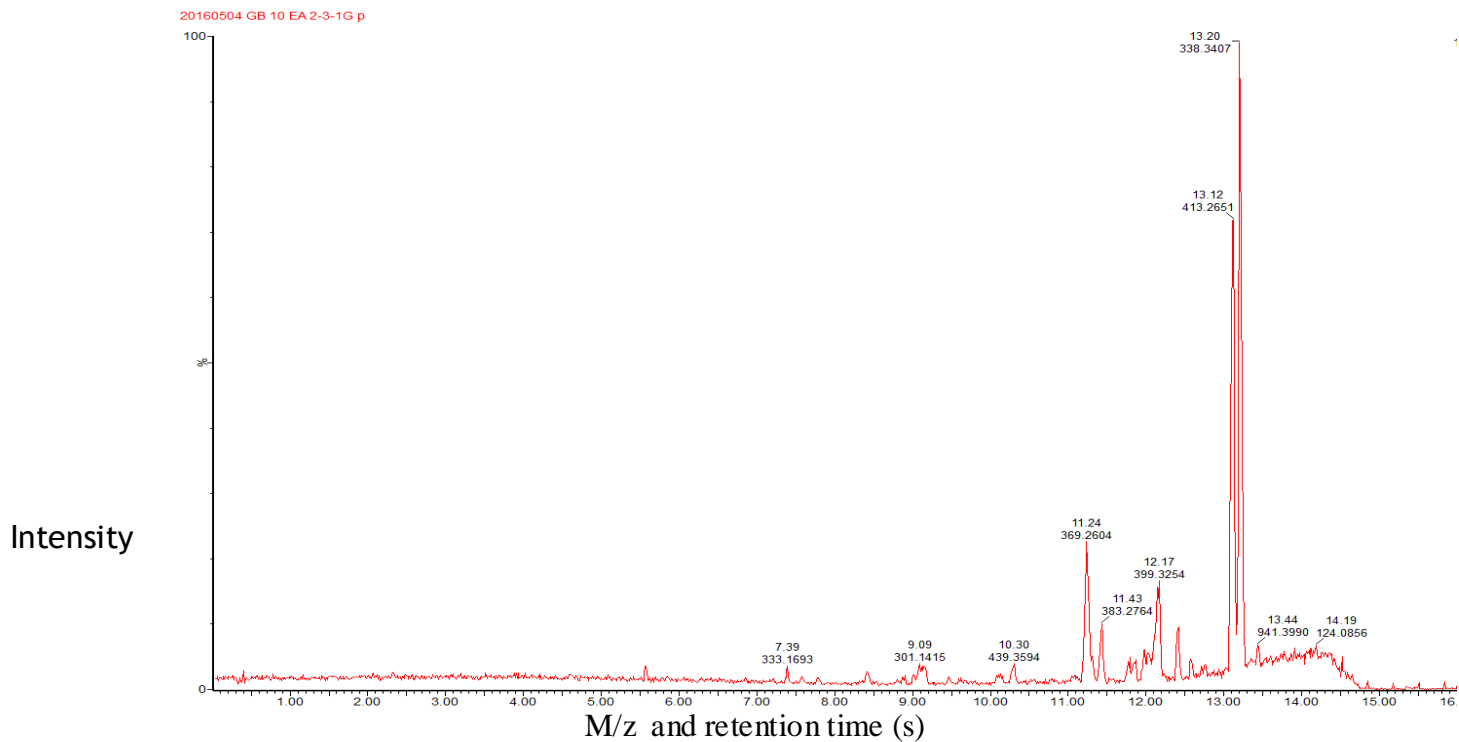


Figure 4.7 ; UPLC-MS Profile of Compound With M/Z of 337.33 Fraction GB10EA2-3-1G In +Ve Mode

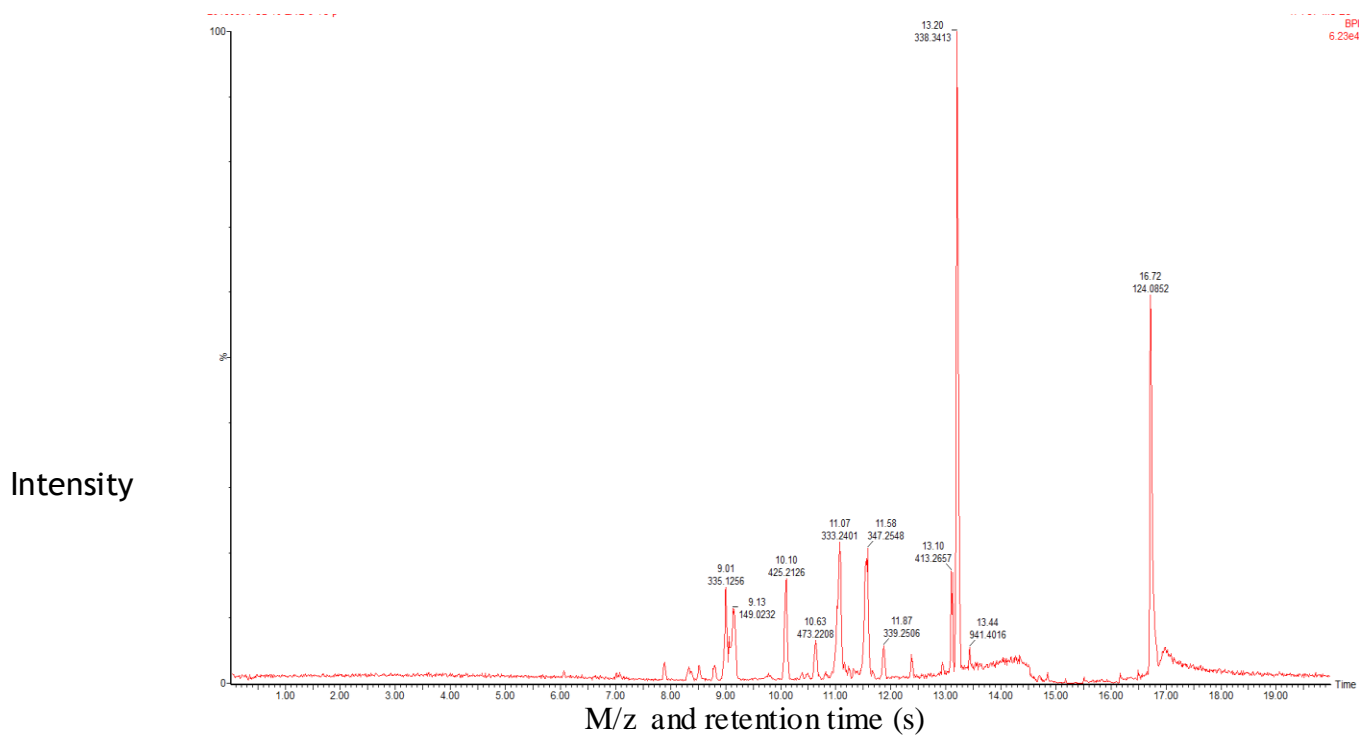


Figure 4.8. UPLC-MS Profile and fragments of Compound With M/Z of 337.33 Fraction GB10EA2-3-1G In +Ve Mode

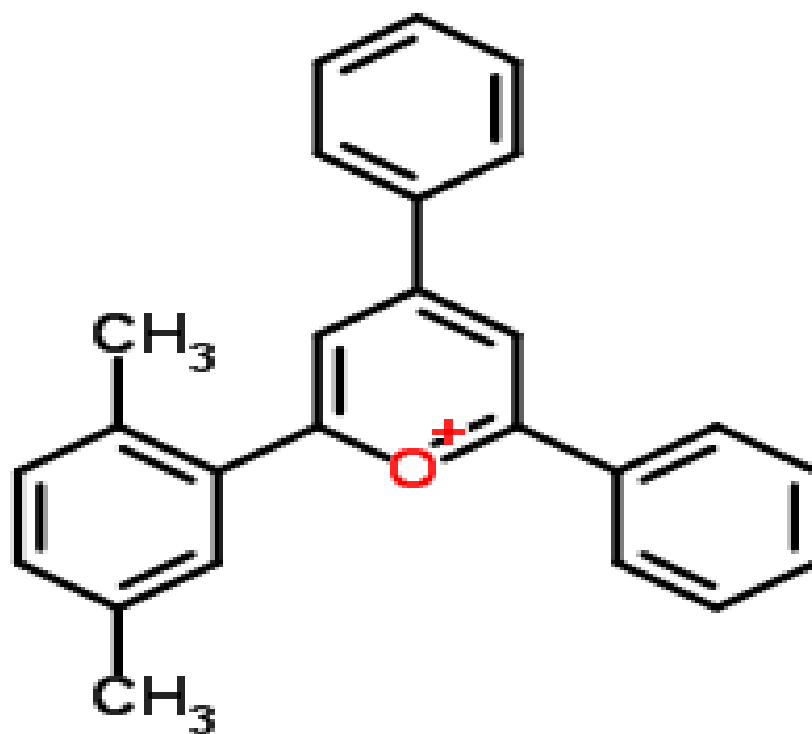


Figure 4.9. Chemical Structure Of Compound 2-(2,5-Dimethylphenyl)-4,6-Diphenylpyrylium
From Isolated From *S. americana*

4.19 Extraction of *Plasmodium berghei* (NK 65) and *Plasmodium falciparum* Cysteine Protease

Results of the partially purified *P. berghei*(NK 65) and *P. falciparum* cysteine protease are summarized in Table 4.20 and 4.21. The crude protein for *P. berghei*(NK 65) and *P. falciparum* contained approximately 1.84 and 2.75mg/ml total protein with a specific activity of 9.4 and 4.64 μ mol/min/mg respectively. Precipitation of the crude protein with ammonium sulphate yield a purification fold of 1.05 and 2.79 with percentage yield of 56.30 and 46.63% for *P. berghei*(NK 65) and *P. falciparum* respectively Table 4.20 and 4.21.

4.20 *Plasmodium berghei* (NK 65) and *Plasmodium falciparum* Cysteine Protease (CP) Inhibition by *Olox subscopoidia* Fractions

The result of the inhibition activity of isolates from *O. subscopioidea* is represented in figure 4.10. It indicates that crude (GB003) inhibited *P. berghei*(NK 65) cysteine protease (CP) by 75% while *P. falciparum* (CP) was inhibited by barely 50%. Fraction GB003BCF2ae3 and GB003BCF3A1.1 on the other hand inhibited *P. berghei* (NK 65) cysteine protease (CP) by 60%. However, *P. falciparum* was barely inhibited above 40%. Figure 4.10

4.21 *Plasmodium berghei* (NK 65) and *Plasmodium falciparum* Cysteine Protease Inhibition by *Schwenkia americana* Fractions

The result of the inhibition of the cysteine protease activity by the crude and active fractions of *S. americana* is represented by Figure 4.11. It indicated that the crude extract (GB010) inhibited CP of both *P. berghei*(NK 65) and *P. falciparum* with percentage inhibition of 80% and 60% respectively Figure 4.10. While the fraction GB010EA 2.3.1G inhibited both *P. berghei*(NK 65) and *P. falciparum* CP by 65% and 58% respectively Figure 4.11.

4.22 *Plasmodium berghei*(NK 56) and *Plasmodium falciparum* Cysteine Protease Inhibition by the Fractions of *Olox subscopoidia* and *S. americana*

The result indicated *O. subscopioidea* crude extract inhibited *P. berghei* with $1C_{50}$ 4.17 μ g/ml as compared to $1C_{50}$ 4.31 μ g/ml of *P. falciparum*, while isolates GB003BCF2ae3 inhibited both *P.*

berghei and *P. falciparim* (CP) With IC_{50} 5.22 and 8.38 μ g/ml. Fraction GB003BCF3A1 on the other hand inhibited *P. berghei*(NK 65) and *P. falciparim* (CP) (falcipain and bergheipain) with IC_{50} of 6.01 and 12.55 μ g/ml. (Table 4.22). This makes the crude extract a better inhibition for both falcipain and bergheipain. *S. americana* crude extract however inhibited the both *P. berghei*(NK 65) CP (bergheipain) and *P. falciparum* (falcipain) with IC_{50} of 1.97 and 2.93 μ g/ml (Table 4.22). While the fraction GB010EA2.3.1G exhibited good inhibition with IC_{50} of 4.099 and 5.21 μ g/ml for bergheipain and falcipain respectively. (Table 4.23).

Table 4.20. Purification Profile For *Plasmodium berghei*(NK 65) Cysteine Protease

Purification Step	Total Protein (mg) Pb A	Total Activity ($\mu\text{mol}/\text{min}$) B	Specific Activity ($\mu\text{mol}/\text{min}/\text{mg}$) B/A	Purification Fold	Percentage Yield
Crude Protein	1.84	17.41	9.46	1.00	100
Ammonium Sulphate Fractionation	1.036	16.60	17.2	1.048	56.30

Table 4.21. Purification Profile for *Plasmodium falciparum* cysteine protease

Purification Step	Total Protein (mg) Pf	Total Activity ($\mu\text{mol}/\text{min}$)	Specific Activity ($\mu\text{mol}/\text{min}/\text{mg}$)	Purification Fold	Percentage Yield
Crude Protein	2.75	12.76	4.64	1.00	100
Ammonium Sulphate Fractionation	0.46	5.95	12.63	2.79	46.63

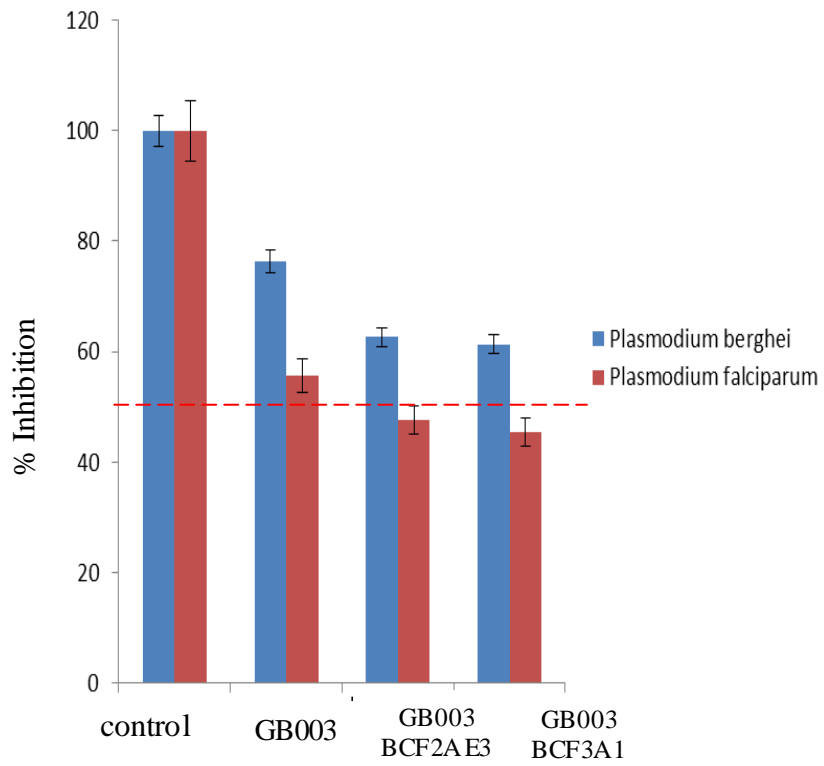


Figure 4.10: *Plasmodium berghei*(NK 65) and *Plasmodium falciparum* Cysteine Protease Inhibition of the Fractions of *Olax subscopoidia*

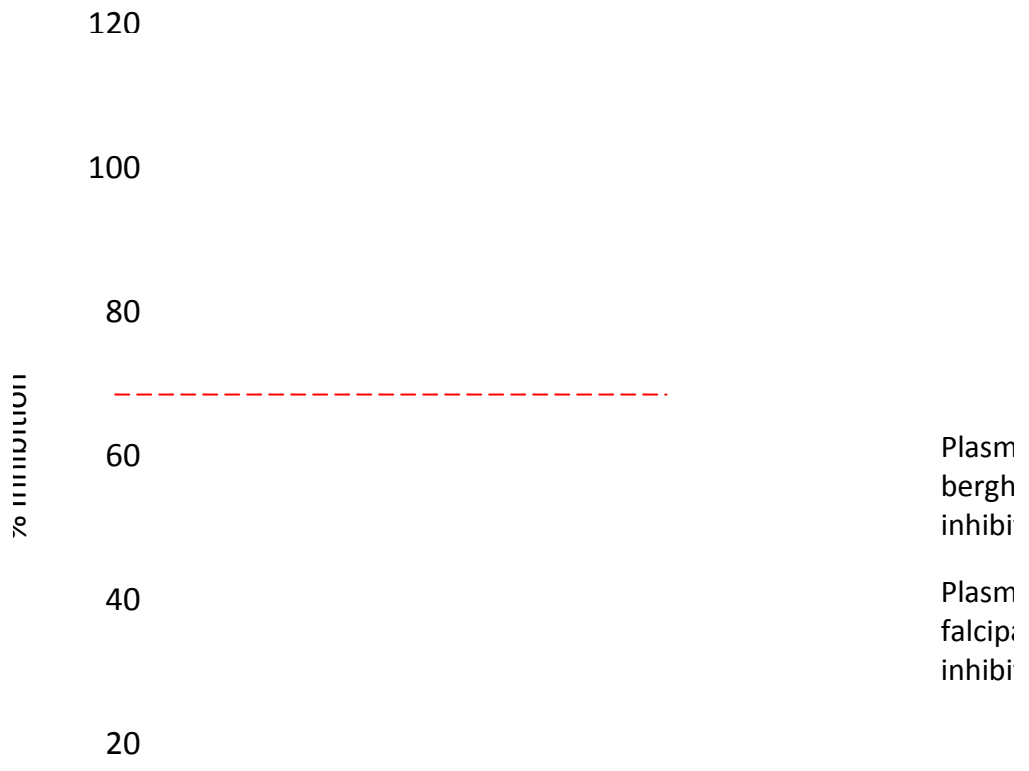


Figure 4.11: *Plasmodium berghei*(NK 65) and *P.falciparum* Cysteine Protease Inhibition of the Fractions of *Schwenkia americana*

Table 4.22. *Plasmodium berghei* (NK 65) and *Plasmodium falciparum* Cysteine Protease Inhibition by *Olox Subscopoidia* Fractions

Compound	Cysteine protease $\mu\text{g/ml}$ (% inhibition)	
	<i>Plasmodium berghei</i>	<i>Plasmodium falciparum</i>
	IC ₅₀	IC ₅₀
GB003	4.17	4.311
GB003BCF2AE1	5.22	8.38
GB003BCF3A1	6.011	12.55

Table 4.23. *Plasmodium berghei* (NK 65) and *Plasmodium falciparum* Cysteine Protease Inhibition by Fractions Of *Schwenkia americana*

Compound	Cysteine protease $\mu\text{g/ml}$ (% inhibition)	
	<i>Plasmodium berghei</i> (NK 65)	<i>Plasmodium falciparum</i>
	IC ₅₀	IC ₅₀
GB010	1.97	2.93
GB010EA 2.3.1G	4.096	5.21

Chapter Five

5.0 DISCUSSION

Plants are natural source of medicinal agents since the existence of man on earth and most of the orthodox drugs were from it products (Bantie *et al.*, 2015), with more than 80% of the world population relying on traditional medicines, plant therefore played essential roles in primary health care (Omonkhehin *et al.*, 2007). The isolation of quinine and artemisinin arose new resurgence in the use of plant materials to fight malaria disease (Rosenthal, 2001).

The ethnobotanic survey study in this work was for the first time in Nupeland, research work that identified unexplored herbs with folkloric history of anti-malaria potential in Bida, the headquarter of Nupeland, Niger State. The research work has contributed to the exploration of potential phyto-*antiplasmodial* bioefficient plants basically considered and used as agents of anti-inflammatory and antipyretic properties. It further validated the traditional claims of herbal practitioners of the antimalarial activity through the *antiplasmodial* screening activity of the plants extracts against 2 strains of malaria parasites, *Plasmodium falciparum* and *Plasmodium berghei*(NK 65 strain).

The method of the survey employed for this work had been used successfully in the same area by Mann *et al.*, (2008) although his work was based on respiratory diseases, also by Gbolade (2009) in Nigeria and Asase *et al.*, (2010) in Ghana in study of Ghanaian plants.

The 48 plants species identified were related to the treatment of malaria and or its common symptoms such as fever, cough, and general body pain (Asase and Opong-Mensah, 2009)(Alves-Junior *et al.*, 2014). From the survey the most important symptoms local practitioners associated with malaria are inflammation and pyrexia which could be as a result of their little knowledge on the concept of modern malaria (Asase *et al.*, 2010). Most of them even though are renown in the area, basically inherited the knowledge of the sciences of their practices

(Mann *et al.*, 2008; Mir *et al.*, 2013). The achieved identification and classification of the plants was based on whether the plants were directly used for the treatment of malaria or plants identified for their use in the treatment of one or more common symptoms of malaria such as headache, fever, nausea and vomiting which may not be strictly used for malaria treatment, but are rather used as antipyretic and anti-inflammatory..

Most commonly mentioned plants by practitioners (36.67 – 43.33%) from the former category includes *Sclerocarya birrea* (A.Rich) also known as marula, which possess, anti-plasmodial and anti-malarial (Gathirwa *et al.*, 2008) Its *anti-plasmodial* activity was established against *P. falciparum* with IC_{50} $5.91 \pm 0.36 \mu\text{g/ml}$. while its *in-vivo* anti-plasmodial activity indicated its chemo-suppression potential above 60% according to Gathirwa *et al.*, (2008). The activities were generally associated to the organic acids, phenolic acids, flavonoids, polyphenols, and fatty acid derivatives (Holanda *et al.*, 2014). Methanol and aqueous extracts of *S. birrea* both exhibited chemo-suppression of 63.49 and 66.51%, respectively (Gathirwa *et al.*, 2008).

Balanites aegyptica (L) Del is *Balanitaceae* widely grown and referred to as desert plant with multiuser potential with lots of different biological activities, attributed to its saponin constituents (Kusch *et al.*, 2011). Its *in vitro* anti-plasmodial activity has been determined in extract from *B. aegyptiaca* stem bark with an IC_{50} of $55 \mu\text{g/mL}$ other report shows aqueous extract *in vitro* inhibition of the chloroquine susceptible *P. falciparum* NF54 strain, with IC_{50} value of $68.26 \mu\text{g/mL}$ (Kusch *et al.*, 2011).

Tamarindus indica (L) is evergreen tree that is 24 m height and 7 m girth which belongs to the family Leguminosae (Fabaceae), commonly known as Tamarinds. The plant is found especially in Indian subcontinent, however, Africa, Pakistan, Bangladesh, Nigeria and most tropical countries were identified to harbour the plant. Traditionally *T. indica* had been identified to be used in the

treatment of abdominal pains, diarrhea, dysentery, bacterial, parasitic infections, treatment of wound, constipation and inflammations (Kuru, 2014). The claim by traditional healers of the plant potentials as antimalarial agent was confirmed by this research work with more than 98% inhibition of *P. falciparum* 3D7 strain growth, showing the highest activity among the screened plants with IC_{50} 0.73 μ g/mL. Even though very few studies on the *anti-plasmodial* activity of *T. indica* reported.(Koudouvo *et al.*, 2011), this present study result differs with the earlier literature which reported failure of methanol leaf extract of *T. indica* to inhibit the growth of *P. falciparum*. However, same literature reported aqueous extract of its fruits to be most active with IC_{50} 2.04 μ g/mL on *P. falciparum* strains (Koudouvo *et al.*, 2011). The variations could be associated to climatic and topographical differences that may have influenced the phyto-constituents of the extracts (Koudouvo *et al.*, 2011; Kamagaté and Koffi, 2014).

The later category of the plants identified for their use in the treatment of common symptoms based on the ethnobotanical findings, identified *Schwenckia americana*, *O. subscorpioidea* and *Senna seberiana* extracts to be potential antimalarial agents. These plants were reported to have folkloric background of their use traditionally as potential anti-malaria agents as corroborated from ethnobotanical survey findings. The plants were employed as analgesic, anti-pyretic and anti-inflammatory agents in the treatments of edema, rheumatism, arthritis (Hassan *et al.*, 2012). While *O. subscorpioidea* was used in the treatment of jaundice, hepatitis and skin infection (Eriyamremu and Iorliam, 2014). *Senna sieberiana* was employed as anti-fungal, antiviral and diuretics (Kamagaté and Koffi, 2014). The plants were generally used as anti-rheumatic pains, swelling and anti-fungal infections (Asusheyi *et al.*, 2010).

Traditionally the plants are prepared as decoction for malaria treatment, although there was little or no scientific validation from the literature on the antimalarial activity claims by the traditional healer as evidenced from the survey, the *in-vitro anti-plasmodial* finding of this research work

identified and confirmed the activity of the plants against *Plasmodium. falciparum* (3D7 strain) . *Schwenckia americana* exhibited the highest activity in both *in-vivo* and *in-vitro* screening of the plants. For the first time the *anti plasmodial* activity of *Schwenckia americana* is reported, it showed an amazing pattern of higher activity, with IC₅₀ of 0.040 µg/ml against *P. falciparum* (3D7 strains). Activity that compared favourably with chloroquine on the same parasite strain and is associated to the phytoconstituents like phenols and polyhydric compounds isolated there in. However, the screened plants does not showed any considerable activity against the resistant FCR3 strain of *P. falciparum*, which can be associated to the instability of the active phytoconstituents of the plants which decompose with time and by the influence of the solvent. It's also an indication that the response of the *parasite* to plants extracts has no regards to the host factors that may have likely influenced the drug efficacy *in-vivo*. Further to that, the failure of the extract sensitivity to FCR3 strain demonstrated that even though there was high level of potency relative to the universal 3D7 strain there is clear warning of impending resistance to the component of the extracts. (Tano *et al.*, 2015).

An interesting *anti-plasmodial* potential activity observed in the extracts of *Olex subscopoidia* and *Sena seberiana* with IC₅₀ 0.128 and 0.0797 µg/ml respectively which compare propitiously with the IC₅₀ value of chloroquine found in the assay within standard range as reported by (Wahome *et al.*, 2014). The results of this study showed that *O. subscopioidea* gave the highest percentage yield on extraction with methanol, followed by *Senna sieberiana* and *S. americana* which gave the least percentage yield. However, despite the low yield, the extract exhibited reasonable response to the activity they are subjected to. This result is in conformity with the idea that alcohols have the potential phytochemicals extraction capability (Joana Gil-Chávez *et al.*, 2013; Zlotek *et al.*, 2016). Hence methanol could be considered to have extracted the active principles of the plants, giving it an edge over the other solvents. The traditional practice involves the use of aqueous as

solvent of preparation as against the use of methanol in this work, coupled with it been considered as a toxic solvent and may not be fit for consumption. However, methanol use in this work is valid, since the focus of the research was towards Isolation and purification of the lead compounds and moreover toxicity profiles study established that extracts were relatively safe.

The phytochemical profile of the 3 extracts in this research work identified all the three plants to contain alkaloids, flavonoids tanines, phenols and saponins which are the principle bioactive compounds associated to the general medicinal characteristic of the extracts (Toma, *et al.*, 2009; Daniel, 2015). The study further showed that LD₅₀ of *O. subscorpioidea* crude extract was 3807.89mg/kg body weight in mice, while for *S. americana* and *S. sieberiana* in mice and all the three plants in rats have their LD₅₀ to be greater than 5000mg/kg body weight showing that the extracts are relatively safe according to Lorkes (1983). It could be inferred that all the extracts are practically nontoxic on the Allain, (2000) scale, who considered compounds with (LD₅₀) > 2000mg/kg body weight to be non-toxic. Further to that Lorke, (1983) considered such extracts with LD₅₀ >5000mg/kg to be nontoxic and relatively safe. It equally indicates that the doses of 50 – 200mg/kg body weight given to mice in *invivo anti-plasmodial* treatment was by far 25 -100 times less than the LD₅₀ and can rarely interferes with the animal status and response to the outcome of the treatment. Similarly nontoxic status outcome of these plants was recorded, even though different parts and solvents were used. Abdulrazak *et al.*, (2015) reported LD₅₀ of the *S. sieberiana* ethanol extracts of root and stem bark in mice to be 2000mg/kg body weight. However, this present study suggested possible amplification of toxic effect on long time treatment at higher concentration on the mice using *S. sieberiana*. No literatures on the sub-chronic toxicity of the *S. americana* as at the time the report of this study, however *O. subscorpioidea* hepato-protective activity was reported (Konan *et al.*, 2015). So *O. subscorpioidea* is not only safe from the LD₅₀ but identified to protect liver from initial damage (Konan *et al.*, 2015).

The *in-vivo* anti-plasmodial activity results of *S americana*, *S. seiberiana* and *O. subscorpioidea* extracts for this study demonstrated considerable chemo-suppression of the extracts which equally translated to proportional reduction of the parasitemia level thereby increasing the survival period of the mice up to 20 days post infection (Vale *et al.*, 2015). *Oxalis subscorpioidea* demonstrated highest range of the parasite growth inhibition with 72.52 to 77.42% and *S. americana* exhibited $77.26 \pm 12.97\%$ at day five of post infection smear. While *Senna seiberiana* showed the least chemo suppressive activity. However all the plant extracts were generally active since all extracts with potential to reduce parasitemia level above 30% is considered active (Vale *et al.*, 2015).

The study further identified the activities of the extracts to be dose independent, because the activity at 50mg/kg body weight is as higher as that of 200mg/kg body weight. The two plant extracts demonstrated outstanding performance with parasite suppression as high as 77.5% at crude level, compare to the standard potent drug chloroquine with 84.5%, further purification of which could give as much higher to that of the standard drug. This result can be interpreted to have validated the ethno-pharmacological utilization of the plant extracts under study in malaria treatment (Ochora *et al.*, 2014). In *in-vivo* studies, the orally treated animals with crude extracts essentially have their parasitemia level lowered, as evident from the 4-days chemosuppressive test. The parasite suppressive potential of all the plant extracts was established. However, increases in the parasitemia level observed after 14 days for treated groups with *S. seiberiana* extract, could be as a result of the metabolic enzymes interference which might degraded the extract making it unavailable for the expected chemosuppression effect. Enough effective dose level is required for access to infected erythrocytes and the parasite's food vacuole where degradation of haemoglobin can be prevented and to further inhibit parasites' access amino acid, eventually preventing protein synthesis and stopping the growth process of the parasites. Interestingly, the extract-treated mice, survived longer than control (the infected and untreated mice) group, which evidently

exhibited delayed progression of the parasites in the blood, thereby extending the life span of the treated mice. This therefore, signifies the supporting effect of the extracts protecting animals from early death from anemia complication of *P. berghei*. Additionally, the extracts might possibly be involved in the immune modulation and response of the treated animals thereby altering the impact of infection by preventing the events that leads to early death in *P. berghei* infection (Feng *et al.*, 2012). Furthermore, the initial suppression of the parasite and post treatment survival of the animals could be associated to extracts' potential in inhibition of cysteine protease at different stages of parasite developments, the trophozoite, schizont and the merozoite. The ring stage produces little or no cysteine protease and so escape the inhibitory action of the extracts (Rosenthal, 2011; Karthik *et al.*, 2014). However, the inhibition impact on the other stages could have contributed significantly to the growth hindrance of the parasite.

The outcome of the crude extracts on preliminary fractionation of *Olox subscopodia* and *S.americana* using column chromatography gave fractions GB003B, GB003B2.1 and 003B3.1 which anti-plasmodial bio-assay screening showed moderate activity thereby inhibiting up to 50% parasite population with IC_{50} 33.73, 36.75 μ g/ml respectively, while fraction GB003C was identified to be more potent against *Plasmodium falciparum* with IC_{50} 3.22 μ g/ml. However the fraction gave active sub-fractions with IC_{50} of 1.65 and 1.79 μ g/ml.

A remarkable trend of this research findings, is the considerable declined in the activity of the fractions as the fractionation progress with the IC_{50} growing higher, an indication of the possible loss of efficacy, which could be associated to the characteristic synergistic effect demonstrated by different components of the sub-fractions. Same observations were inherent in the activity of the compounds isolated even though the compounds were active, the activity seem to have declined considerably. *S. americana* fractions were not as effective as the crude extract. The activity declined as the fractionation progress. The crude extract was almost more active than the

chloroquine with IC_{50} of $0.0004\mu\text{g/ml}$, but the IC_{50} of the most active fractions were between 1.75 to $4.42\mu\text{g/ml}$. the observed trend could be explained in 2 perspectives. The solvents effect and the complex nature of fractions in terms of chemical composition. It is believed that solvent of extraction play vital roles on the isolated compounds, as it long exposure to the solvent after isolation could grossly decompose the compound of interest (Dailey and Vuong, 2015; Zlotek *et al.*, 2016). The isolates could undergo auto-oxidation or become decomposed as they are left to stand for a longer time in organic solvents (Joana Gil-Chávez *et al.*, 2013; Raut *et al.*, 2013; Sanders *et al.*, 2014).

The complex nature of fractions in terms of chemical composition and presence of colossal amount of compounds can be an important factor. The interaction of the complexes might lead to steric hindrance effect, coupled with photosensitive characteristics of some compounds, could played significant role in the depletion and subsequent loss of complexity, consequently leading to the loss of biological efficacy. Large numbers of organic compounds aggregated as complex phytochemicals which activity could be additive and or synergy as related to their biological roles. Loss of such synergy will eventually lead to depreciation of the activity as was observed. However the IC_{50} of 1.75 to $6.22\mu\text{g/ml}$ are surely a very good candidate for antimalarial drugs.

Accordingly antimalarial activity for plants, based on the best laboratory practices compounds/extracts are generally considered as strongly active if the $IC_{50} < 5\mu\text{g/ml}$, active with $IC_{50} 5 - 50\mu\text{g/ml}$, while IC_{50} of $50\mu\text{g/ml} < IC_{50} < 100\mu\text{g}$ is considered weakly active and compounds/ extracts are inactive if the $IC_{50} > 100\mu\text{g/ml}$ (Chinchilla *et al.*, 2012).

Detailed investigation of promising fractions afforded 1,2,3,4-tetrahydro-1-(1-hydroxybutan-2-yl)-4-methylnaphthalene-2,3-diol which showed inhibitory effects, with IC_{50} values of $1.79\mu\text{g/ml}$ against *Plasmodium falciparum* and 5.22 and $8.38\mu\text{g/ml}$ against bergheipain and falcipain respectively. While 3-(1-hydroxybutane-2-yl) cyclohexane-1,2,4-triol on the other hand exhibited

inhibitory effects, with IC₅₀ values of 6.011 and 12.55 µg/ml. *S. americana* crude extract however inhibited both *P. berghei* CP (bergheipain) and *P. falciparum* (falcipain) with IC₅₀ of 1.97 and 2.93 µg/ml, while the promising fraction from the extract 2-(2,5-Dimethylphenyl)-4,6-diphenylpyrylium had IC₅₀ of 4.099 ± 2.21 and 5.21 ± 1.7 µg/ml for bergheipain and falcipain respectively. Plants produced secondary metabolites that serve as protease inhibitors in order to modulate the activity of proteases, Consequently, such advantages of these plants secondary metabolites that potentially inhibit this enzyme could be another source of antiparasitic agents and in extension as antimalarial agents.

In the present investigation, cysteine protease inhibition were exhibited by Rel, 1β, 3α- dihydroxyl 7α, 9β dihydroxylcyclopentane (Octahydro-2-methyl-1H-indene-1, 3, 4, 6-tetraol) and 1,2,3,4-tetrahydro-1-(1-hydroxybutan-2-yl) -4- methyl naphthalene-2,3- diol compounds isolated from *O. subscorpioidea* and 2-(2,5-Dimethylphenyl)-4,6-diphenylpyrylium from *S. americana* with loss in activity. This might be based on reports on interference of various solvents structural integrity and stability on the molecules which are due to various forces aimed at isolating the compounds (Bijina *et al.*, 2011). The potency exerted by the purified compounds confirms the preliminary results obtained with fractions, suggesting potent anti-malarial activity of natural products from *Oxalis subscorpioidea* and *S. americana* against *Plasmodium berghei* (NK 65) and *Plasmodium falciparum*

Chapter Six

6.0 Conclusion and Recommendations

6.1 Conclusion

In this research finding all the plant extracts were active against *Plasmodium berghei* NK 65 strain, since the suppression of the parasite growth of extracts greater than 30% is considered bench mark for plant extracts as active *anti-plasmodial* agents (Vale *et al.*, 2015). *O. subscorpioidea* and *S. americana* expressed the highest level of suppression at doses 50 and 100mg/kg body weight respectively and *S. seiberiana* showed the least suppression at 200mg/kg body weight. The plants contain phytochemicals that are of medicinal values that provide insight to the lead active compounds against the parasites. The extracts were relatively safe at concentrations of 25 -100 times higher than the active doses. All the plants extracts were therefore considered as potential *anti-plasmodial* agents with their activities dose independence. Hence, administered at a lower doses without any fear of losing maximum effective and benefits of the drug. Furthermore, 1 α , 1 β , 3 α - dihydroxyl 7 α , 9 β dihydroxycyclopentane (Octahydro-2-methyl-1H-indene-1, 3, 4, 6-tetraol) and 1,2,3,4-tetrahydro-1-(1-hydroxybutan-2-yl)-4-methylnaphthalene-2,3-diol compounds are isolated from *O. subscorpioidea* and 2-(2,5-Dimethylphenyl)-4,6-diphenylpyrylium from *S. americana* and identified as good candidates for potential *anti-plasmodial* agents. However, activities of the compounds are concentration dependent and reduce as purification progress.

6.2 Recommendation

However further cytotoxicity studies of the isolated compounds are required to depict the safety profile of the compounds. Different methods of isolation of the active anti-malarial compounds could preserve the compounds for maximum benefit.

Summary

This research work aims at evaluation of the anti-*plasmodial* activity, toxicity profile and the inhibitory effect on cysteine protease of the parasites by the anti-malaria plant extracts of folkloric history in the treatment of malaria, with special attention to *Olax subscopodia*, and *Schwenkia americana*. The significant portion of the studies includes Isolation and Characterization of the bioactive components, Structural elucidation of the most effective compounds/fractions by UPLC/LCMS, NMR spectral analysis. The data used in this study were generated through anti-*plasmodial* bio-assay guided fractionation. The response variable for the experiment include the shizont growth inhibition, IC₅₀ and the enzyme inhibition profile. The activities of the plants extracts/fractions at different concentrations were evaluated.

Ethno-botanical survey was undertaken and 48 plants identified and related to malaria treatment traditionally. The work through ethno-botanical survey had identify un-explore potential natural heals with folkloric history of anti-malaria potential. This work has contributed to exploration of potential phyto-*antiplasmodial* bio efficacy of plants basically considered as agents of anti-inflammatory antipyretic properties been identified as highly potential anti-*plasmodial* agents. With 77.7% of the 11 plants with higher respondents base on the traditional claims were validated as active against 2 strains of *Plasmodium* parasites. Anti-*plasmodial* potential of *Olax Subscorpioidea* and *S. americana* was further established by In-*vivo* screening against *P. berghei*. The bio safety of the selected indigenous floral beyond the recommendable effective doses used was established, with the effective doses far below the deleterious concentration if any . 3 compounds which include Rel, 1 β , 3 α - dihydroxyl 7 α , 9 β dihydroxylcyclopentane (Octahydro-2-methyl-1H-indene-1, 3, 4, 6-tetraol), 1,2,3,4-tetrahydro-1-(1-hydroxybutan-2-yl)-4-methyl-naphthalene-2,3-diol and 2-(2,5-Dimethylphenyl)-4,6-diphenylpyrylium were Isolated were all active against antiplamodia and are equally inhibitors of cysteine protease

References

- Abdulrazak, N., Asiya, U., Usman, N., Unata, I., and Farida, A. (2015). Antiplasmodial activity of ethanolic extract of root and stem bark of *Cassia sieberiana* DC on mice. *Journal of Intercultural Ethnopharmacology*, 4(2),
- Adebayo J.O., and Krettli A.U. (2011), Potential antimalarials from Nigerian plants: a review. *J Ethnopharmacol*, 133:289–302.
- Adeiza, A. A., Mohammed, A., and Mamman, M. (2010). Comparative in vivo evaluation of the trypanocidal activities of aqueous leaf, stem-bark and root extracts of *Khaya senegalensis* on *Trypanosoma evansi*, 4, 1770–1777.
- Adjalley, S. H., Johnston, G. L., Li, T., Eastman, R. T., Ekland, E. H., and Eappen, A. G. (2011). Quantitative assessment of *Plasmodium falciparum* sexual development reveals potent transmission-blocking activity by methylene blue, 108(47).
- Adeoluwa, O.A., Aderibigbe A.O., and Olonode, E.T., (2014). Antinociceptive property of *Olex subscorpioidea* Oliv (Olacaceae) extract in mice. *J. Ethnopharmacol.*, 156: 353-357.
- Adu-Gyasi, D., Adams, M., Amoako, S., Mahama, E., Nsoh, M., Amenga-Etego, S., and Owusu-Agyei, S. (2012). Estimating malaria parasite density: assumed white blood cell count of 10,000 / μ l of blood is appropriate measure in Central Ghana. *Malaria Journal*, 11(1), 1.
- Agbon, A. N., Kwanashie, H. O., Hamman, W. O., and Sambo, S. J. (2014). Toxicological Evaluation of Oral Administration of *Phoenix dactylifera* L. Fruit Extract on the Histology of the Liver and Kidney of Wistar Rats. *International Journal of Animal and Veterinary Advances*, 6(4), 122–129.
- Ajayi CO., Elujoba AA., Bejide RA., Akinloye JA., and Omonisi AE(2015). Toxicity and pharmacognostic standards for laxative properties of Nigerian *Cassia sieberiana* and *Senna obtusifolia* roots. *European Journal of Medicinal Plants.*;6(2): 110-123.
- Allain P (2000). *Les médicaments*. CdM Éditions; 3^e édition. p. 500
- Almela, M. J., Lozano, S., Miguel, C., Franco, V., and Leroy, D. (2012). Activity of Clinically Relevant Antimalarial Drugs on *Plasmodium falciparum* Mature Gametocytes in an ATP Bioluminescence “Transmission Blocking” Assay, 7(4), 1–8.
- Alves-Junior, Eduardo R. Luciano, T. G., Ribatski-Silva, D., Clebson, R. J., Mendes, Fabio A. Leal-Santos Luciano, R. S., Mello, M. B. C., and Cor Jesus, F. F. (2014). Assumed White Blood Cell Count of 8,000 Cells / mL Overestimates Malaria Parasite Density in the Brazilian Amazon. *PLOS ONE*, 9(4), 8–12.
- Asase, A., Akwetey, G. A., and Achel, D. G. (2010). Ethnopharmacological use of herbal remedies for the treatment of malaria in the Dangme West District of Ghana. *Journal of Ethnopharmacology*, 129(3), 367–376.
- Asase, A., and Oppong-mensah, G. (2009). Traditional antimalarial phytotherapy remedies in

herbal markets in southern Ghana, *I26*, 492–499.

- Asusheyi, B. I., Ndukwe, G. I., and Audu, O. T. (2010). Phytochemical studies and antimicrobial screening of *Schwenkia americana* Linn. *Journal of Medicinal Plants Research*, 4(9), 844–846.
- Audu, J.A., 1995. Studies on the effectiveness of medicinal herbs used as anthelmintics by traditional medical practitioners in South of Bauchi State II. *Journal of Economic and Taxonomic Botany* 19, 653–661.
- Ayandele, A.A., and Adebisi, A.O., (2007). The phytochemical analysis and antimicrobial screening of extracts of *Olox subscorpioidea*. *Afr. J. Biotechnol.*, 6: 868-870.
- Bantie, L., and Engidawork, E. (2011). *In vivo antimalarial activity of the leaf extract of Croton macrostachys (Euphorbiaceae) against Plasmodium berghei in mice*. Addis Ababa University School.Pp2-280
- Bantie, M. L., Assefa, S., Teklehaimanot, T., and Engidawork, E. (2015). *In vivo antimalarial activity of the crude root and fruit extracts of Croton macrostachyus (Euphorbiaceae) against Plasmodium berghei in mice*. *Journal of Traditional and Complementary Medicine*, 14(79), 168–173.
- Batra, S., Sabnis, Y.A., Rosenthal, P.J., and Avery, M.A. (2003), Structure-based approach to falcipain-2 inhibitors: synthesis and biological evaluation of 1,6,7-trisubstituted dihydroisoquinolines and isoquinolines. *Bioorg. Med. Chem.* 11, 2293-2299.
- BioMed Central (2009). Determinants of relapse periodicity in *Plasmodium vivax* malaria. *Malar. J.* 10, 297.
- Berger, S. (2015). Malaria in Philippines Gideon infectious disease of Philipines Gideon informatics
- Bode, W., and Huber, R. (2000). Structural basis of the endoproteinase-protein inhibitor interaction. *Biochemica Biophysica Acta* 1477.241-252.
- Bradford M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254.
- Bray, R.S. (1960). Studies on malaria in chimpanzees. VIII. The experimental transmission and pre-erythrocytic phase of *Plasmodium malariae* with a note on the host range of the parasite. *Amer J Trop Med Hyg*, 9:455-65.
- Bruce-Chwatt, L. J. (1981). Alphonse Laveran's discovery 100 years ago and today's global fight against malaria. *J Roy Soc Med*, 74:531-536.
- Buchholz, K., Burke, T. A., Williamson, K. C., Wiegand, R. C., Wirth, D. F., and Marti, M. (2011). Major Article A High-Throughput Screen Targeting Malaria Transmission Stages

Opens New Avenues for Drug Development, 203, 1445–1453.

- Calderón, A. I., Simithy-williams, J., and Gupta, M. P. (2012). Antimalarial natural products drug discovery in Panama, 50(June 2011), 61–71.
- Carter, R., and Mendis, K. N. (2002), Evolutionary and Historical Aspects of the Burden of Malaria *Clinical Microbiology Reviews*, 15(4), 564–594.
- CDC. (2012). CDC - Malaria - Malaria Worldwide - How Can Malaria Cases and Deaths Be Reduced - Diagnosis and Treatment.
- Chan-Kok, L. M. (2007). Functional Analyses of *Plasmodium falciparum* Primary Metabolic Genes Chan.
- Childs, R. A., Miao, J., Gowda, C., and Cui, L. (2013). An alternative protocol for *Plasmodium falciparum* culture synchronization and a new method for synchrony confirmation, 1–6.
- Chinchilla, M., Valerio, I., Sánchez, R., Mora, V., Bagnarello, V., Martínez, L., Gonzalez, A., Vanegas, J. C., and Apestegui, A. (2012). In vitro antimalarial activity of extracts of some plants from a biological reserve in Costa Rica. *Revista de biología tropical*;60(2):881-91.
- Chinsembu, K. C. (2015). Plants as antimalarial agents in Sub-Saharan Africa. *Acta Tropica*, 152, 32–48.
- Chipeleme, A., Gut, J., Rosenthal, P.J., and Chibale, K. (2007). Synthesis and biological evaluation of phenolic Mannich bases of benzaldehyde and (thio) semicarbazone derivatives against the cysteine protease falcipain-2 and a chloroquine resistant strain of *Plasmodium falciparum*. *Bioorg. Med. Chem.*, 15, 273-282.
- Chiyanzu, I., Hansell, E., Gut, J., Rosenthal, P.J., Mckerrow, J.H., and Chibale, K. (2003). Synthesis and evaluation of isatins and thiosemicarbazone derivatives against cruzain, falcipain-2 and rhodesain. *Bioorg. Med. Chem. Lett.*, 13, 3527-3530.
- Clark, B. D. E. (2001). Peroxides and peroxide- forming compounds. *Division of Chemical Health and Safety of the American Chemical Society Published by Elsevier Scienc*, 9098(1), 1–22.
- Colborn, J. M., Byrd, B. D., Koita, O. A., and Krogstad, D. J. (2008). Estimation of Copy Number using SYBR Green : Confounding by AT-rich DNA and by Variation in Amplicon Length, 79(6), 887–892.
- Cooper, R.A., Hartwig, C.L., and Ferdig, M.T.(2005), pfcrt is more than the *Plasmodium falciparum* chloroquine resistance gene: a functional and evolutionary perspective. *Acta Trop* 94:170 –180
- Cox F., (2010). History of the discovery of the malaria parasites and their vectors. *Parasit Vectors*, 3:5.
- Cox F.E.G. (1988). Major animal models in malaria research: rodent. *Malaria: Principles and Practice of Malariology* Edinburgh: Churchill LivingstoneWernsdorfer WH, McGregor I, 2:1503-1543

- Cox-Singh, J., Davis, T.M., Lee, K.S., Shamsul, S.S., Matusop, A., Ratnam, S., and Singh, B. (2008). *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. *Clinical Infectious Diseases* 46(2):165-171.
- Cristin, W., and Smith, D. S. (2013). Malaria : the clinical basics Learning Overview (Clinical). *Global Health Education Consortium*, (February), 1–67.
- Cui, L., Mharakurwa, S., Ndiaye, D., Rathod, P. K., and Rosenthal, P. J. (2015). Antimalarial drug resistance: Literature review and activities and findings of the ICEMR network. *American Journal of Tropical Medicine and Hygiene*, 93(Suppl 3), 57–68.
- Dailey, A., and Vuong, Q. V. (2015). Effect of extraction solvents on recovery of bioactive compounds and antioxidant properties from macadamia (*Macadamia tetraphylla*) skin waste. *Cogent Food and Agriculture*, 7(October), 1–10.
<http://doi.org/10.1080/23311932.2015.1115646>
- Daneshvar, C., Davis, T.M., Cox-Singh, J., Rafa'ee, M. Z., Zakaria, S. K., Divis, P. C., and Singh, B. (2009). Clinical and laboratory features of human *Plasmodium knowlesi* infection. *Clinical Infectious Diseases* 49(6):852-860
- Daniel, A. (2015). Comparative Chemical Constituents of some Cassia Species and their Pharmacognostic Importance in South Eastern Nigeria. *Science Journal of Chemistry*, 3(3), 40.
- Denis, M. (2012). *Real time imaging of the glutathione redox potential and import of host peroxiredoxin 2 in the malarial parasite Plasmodium falciparum*.
- Deressa, W., and Ali, A. (2009). “Malaria-related perceptions and practices of women with children under the age of five years in rural Ethiopia.” *BMC Public Health*. 9: 259.
- Delacollette, C., Van der Stuyft, P., Molima, K., Delacollette-Lebrun, C., and Wery, M. (1989). Etude de la mortalité globale et de la mortalité liée au paludisme dans le Kivu montagneux, Zaire. *Rev. Epidem. Sante Publ.* 37:161–166.
- Desai, P.V., Patny, A., Sabnis, Y., Tekwani, B., Gut, J., Rosenthal, P., Srivastava, A., and Avery, M. (2004). Identification of novel parasitic cysteine protease inhibitors using virtual screening. 1. The ChemBridge database. *J. Med. Chem.*, 47, 6609-6615.
- Desai, P.V., Patny, A., Gut, J., Rosenthal, P.J., Tekwani, B., Srivastava, A., and Avery, M. (2006). Identification of novel parasitic cysteine protease inhibitors by use of virtual screening. 2. The available chemical directory. *J. Med. Chem.*, 49, 1576-1584.
- De-Silva, P. M., and Marshall, J. M. (2012). Factors Contributing to Urban Malaria Transmission in Sub-Saharan Africa : A Systematic Review. *Journal of Tropical Medicine*, 2012, 1–9.

- Dobson, M. J. (1994). Malaria in England: a geographical and historical perspective. *Parassitologia* 36:35–60.
- Domínguez, J.N., López, S., Charris, J., Iarruso, L., Lobo, G., Semenov, A., Olson, J.E., and Rosenthal, P.J. (1997). Synthesis and antimalarial effects of phenothiazine inhibitors of a *Plasmodium falciparum* cysteine protease. *J. Med. Chem.*, 40, 2726-2732.
- Domínguez, J.N., León, C., Rodrigues, J., Gamboa de Domínguez, N., Gut, J., and Rosenthal, P.J. (2005). Synthesis and evaluation of new antimalarial phenylurenyl chalcone derivatives. *J. Med. Chem.*, 48, 3654-3658.
- Dua, M., Raphael, P., Sijwali, P.S., Rosenthal, P.J., and Hanspal, M. (2001). Recombinant falcipain-2 leaves erythrocyte membrane ankyrin and protein 4.1. *Mol. Biochem. Parasitol.*, 116, 95-99.
- Elujoba, A. A., Odeleye, O.M., and Ogunyemi, C. M. (2005). Traditional medicine development for medical and dental primary health delivery systems in Africa. *African Journal of Traditional Medicine* 21:46-61
- Eriyamremu, G., and Iorliam, B. (2014). Biochemical evaluation of ulcer curative effect of ethanolic and aqueous extracts of *Schwenkia americana* Linn on experimental rats. *Journal of Pharmacognosy and ...*, 3(4), 125–130. Retrieved from http://www.phytojournal.com/vol3Issue4/Issue_no_v_2014/3-3-25.1.pdf
- Falkard, B. (2013). Investigations Into The Metabolic Requirements For Lipoic Acid And Lipid Species During The Life Cycle Of The Malarial Parasite.
- Fall, B., Madamet, M., Camara, C., Amalvict, R., and Fall, M. (2016). In Vitro Resistance to, 22(5), 841–845.
- Feng, Y., Zhu, X., Wang, Q., Jiang, Y., Shang, H., Cui, L., and Cao, Y. (2012). Allicin Enhances Host Proinflammatory Immune Responses and Protects Against Acute Murine Malaria Infection. *Malaria Journal*. 11(1),1-7.
- Fernandez, L. S., and Hons, B. (2009). Identification of Novel Natural Product Antimalarial Compounds Statement of Originality.
- Fotio, A.L., Dimo, T., Nguélefack, T.B., Dzeufiet, P.D., Ngo, Lemba, E., Temdie, R.J., Nguéguim, F., Olleros, M.L., Vesin, D., Dongo, E., Kamtchouing, P. and Garcia, I., (2009) Acute and chronic anti-inflammatory properties of the stem bark aqueous and methanol extracts of *Sclerocarya birrea* (Anacardiaceae). *Inflammopharmacology*, 17, 229–237.
- Fidock, D. A., Nomura, T., Talley, A. K., Cooper, R. A., Dzekunov, S.M., Ferdig, M.T., Ursos, L.M., Sidhu, A. B., Naude, B., Deitsch, K. W., Su, X. Z., Wootton, J. C., Roepe, P. D., and Wellems, T. E. (2000). Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. *Mol Cell* 6:861– 871

- Francisco, L., Costa, S., Lopes, P., & Moraes, C. B. (2013). Amazonian Plant Natural Products: Perspectives for Discovery of New Antimalarial Drug Leads, *6594*(August), 9219–9240.
- Frits, F., Franssen, J., Louis, J. J., Smeijsters, W., Ingeborg, B., Beatriz, E., and Medinilla, A. (1997), In Vivo and In Vitro Antiplasmodial Activities of Some Plants Traditionally Used in Guatemala against Malaria. *Antimicrob. Agents Chemother. Journal* 4(7) 1500-1503
- Galinski, M.R., and Barnwell, J.W.(2009) Monkey malaria kills four humans. *Trends Parasitol*, 25:200-204.
- Garcia, J. E., Puentes, A., and Patarroyo, M. E. (2006). Developmental Biology of Sporozoite-Host Interactions in Plasmodium falciparum Malaria : *Implications for Vaccine Design*, 19(4), 686–707.
- Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, Nelson KE, Bowman S, Paulsen IT, James K, Eisen JA, Rutherford K, Salzberg SL, Craig A, Kyes S, Chan M-S, Nene V, Shallom S, Suh B, Peterson J, Angiuoli S, Pertea M, Allen J, Selengut J, Haft D, Mather MW, Vaidya AB, Martin DM, Fairlamb AH, Fraunholz MJ, Roos DS, Ralph SA, McFadden GI, Cummings LM, Subramanian GM, Mungall C, Venter JC, Carucci DJ, Hoffman SL, Newbold C, Davis RW, Fraser CM, and Barrell B, (2002). Genome sequence of the human malaria parasite Plasmodium falciparum. *Nature* 419: 498–511.
- Garnham, P.C.C., Shortt, H.E., Covell, G., and Shute, P.G. (1948). The pre-erythrocytic stage of human malaria Plasmodium vivax. *Brit Med J*, 1:547.
- Garnham, P. C. C., Bray, R. S., Cooper, W., Lainson, R., Awad, F. I., and Williamson, J. (1954). Preerythrocytic stages of human malaria: Plasmodium ovale. A preliminary note. *Brit Med J*, 1:257.
- Gathirwa, J. W., Rukunga, G. M., Njagi, E. N. M., Omar, S. A., Mwitari, P. G., Guantai, A. N., and Ndiege, I. O. (2008). combinations of some medicinal plants used traditionally for treatment of malaria by the Meru community in Kenya, *115*, 223–231.
- Gbolade, A. A. (2009). Inventory of antidiabetic plants in selected districts of Lagos State, Nigeria. *Journal of Ethnopharmacology*, 121(1), 135–9.
- Ghosh, A. K., Devenport, M., Jethwaney, D., Kalume, D. E., Pandey, A., Anderson, V.E., Sultan, A. A., Kumar, N., and Jacobs-Lorena, M. (2009). Malaria parasite invasion of the mosquito salivary gland requires interaction between the Plasmodium TRAP and the Anopheles saglin proteins. *PLoS Pathog*. 5:e1000265.
- Gimode, W. R., Kiboi, D. M., Kimani, F. T., Wamakima, H. N., Burugu, M. W., and Muregi, F. W. (2015). Fitness cost of resistance for lumefantrine and piperazine-resistant *Plasmodium berghei* in a mouse model. *Malaria Journal*, 14(1), 38.
- Go M.L., Liu M., Wilairat P., Rosenthal P.J., Saliba K.J., and Kirk K. (2004). Antiplasmodial chalcones inhibit sorbitol-induced hemolysis of Plasmodium falciparum-infected

- erythrocytes. *Antimicrob Agents Chemother* 48:3241–3245.
- Gopalakrishnan, A. M., and Kumar, N. (2015). Antimalarial Action of Artesunate Involves DNA Damage Mediated by, *59*(1), 317–325.
- Greenbaum, D.C., Mackey, Z., Hansell, E., Doyle, P., Gut, J., Caffrey, C.R., Lehrman, J., Rosenthal, P.J., McKerrow, J.H., and Chibale, K. (2004). Synthesis and structure-activity relationships of parasiticidal thiosemicarbazone cysteine protease inhibitors against *Plasmodium falciparum*, *Trypanosoma brucei*, and *Trypanosoma cruzi*. *J. Med. Chem.*, *47*, 3212-3219.
- Greenberg, A., Ntumbanzondo, E., Ntula, M., Maway, L., Howel J., and Davachi, F. (1989). Hospital-based surveillance of malaria-related pediatric morbidity and mortality in Kinshasa, Zaire. *Bull and World Health Org.*, *67*(2): 189-196.
- Hansen, G., Heitmann, A., Witt, T., Li, H., Jiang, H., Shen, X., and Hilgenfeld, R. (2011). Article Structural Basis for the Regulation of Cysteine-Protease Activity by a New Class of Protease Inhibitors in Plasmodium. *Structure/Folding and Design*, *19*(7), 919–929.
- Hanspal, M., Dua, M., Takakuwa, Y., Chishti, A.H. and Mizuno, A. (2002). Plasmodium falciparum Cysteine Protease Falcipain-2 Cleaves Erythrocyte Membrane Skeletal Proteins at Late Stages of Parasite Development. *Blood*, *100*(3),1048-1054.
- Harupa, A., Sack, B. K., Lakshmanan, V., Arang, N., Douglass, A. N., Oliver, B. G., and Kappe, H. I. (2014). SSP3 Is a Novel Plasmodium yoelii Sporozoite Surface Protein with a Role in Gliding Motility, *82*(11), 4643–4653.
- Hassan, A. H., Sule, M. I., Musa, A. M., Musa, K. Y., and Hassan, M. S. A. and A. (2012). Anti-Inflammatory Activity Of Crude Saponin Extracts From Five Nigerian Medicinal Plants ., *Afr J Tradit Complement Altern Med*, *9*(2), 250–255.
- Haussig, J. M., Burgold, J., Hafalla, J. C. R., Matuschewski, K., and Kooij, T. W. A. (2014). Signatures of malaria vaccine efficacy in ageing murine immune memory. *Parasite Immunology*, *36*(5), 199–206.
- Hippocrates. (English translation by W. H. S. Jones). 1923. *Airs, waters and places VII*, p. 83–85 and *XXIV*, p. 133–137 and *Epidemics I*, p. 181–211 and *Epidemics III*, p. 251–257 and vol. *IV*, *Aphorisms II*, p. 115 and *Aphorisms III*, p. 125–153. Heinemann, London, United Kingdom and Putnam, New York, N.Y.
- Hogg, T., Nagarajan, K., Herzberg, S., Chen, L., Shen, X., Jiang, H., Wecke, M., Blohmke, C., Hilgenfeld, R., and Schmidt, C.L. (2006). Structural and functional characterization of Falcipain-2, a hemoglobinase from the malarial parasite Plasmodium falciparum. *J. Biol. Chem.*, *281*, 25425-25437
- Holanda, C. M. D. C. X., Barbosa, D. A., Demeda, V. F., Bandeira, F. T. M., de Medeiros, H. C. S., Pereira, K. R. S. G., and Medeiros, A. C. (2014). Influence of Annona muricata

- (soursop) on biodistribution of radiopharmaceuticals in rats. *Acta Cirúrgica Brasileira / Sociedade Brasileira Para Desenvolvimento Pesquisa Em Cirurgia*, 29(3), 145–50.
- Huang, L., Lee, A., and Ellman, J.A. (2002). Identification of potent and selective mechanism-based inhibitors of the cysteine protease cruzain using solid-phase parallel synthesis. *J. Med. Chem.*, 45, 676-684
- Ibrahim, J.A., Muazzam, I., Jegede, I.A., Kunle O.F., and Okogun J.I., (2008). Ethno-medicinal plants and methods used by Gwandara tribe of Sabo Wuse in Niger state, Nigeria, to treat mental illness. *Afr. J. Tradit. Complement. Altern. Med.*, 4: 211-218.
- Jang, J. W., Kim, J. Y., Yoon, J., Yoon, S. Y., Cho, C. H., Han, E. T., and Lim, C. S. (2014). Flow Cytometric Enumeration of Parasitemia in Cultures of *Plasmodium falciparum* Stained with SYBR Green I and CD235A,
- James, S.P., and Tate, P. (1935). Exo-erythrocytic schizogony in *Plasmodium gallinaceum* Brumpt,. *Parasitology* 30:128-139.
- Jin Zhu , T., Chen , J. L., Ruoqun, M., Weiqiang , L., Jin, H., Honglin, L., Jian, L., and Hualiang J. (2009). 2-(3,4-Dihydro-4-Oxothieno[2,3-*d*]pyrimidin-2-ylthio) Acetamides as a New Class of Falcipain-2 Inhibitors. 3. Design, Synthesis and Biological Evaluation *Molecules* 2009, 14, 785-797
- Jimoh, A. O., Chika, A., Umar, M. T., Adebisi, I., and Abdullahi, N. (2011). Analgesic effects and anti-inflammatory properties of the crude methanolic extract of *Schwenckia americana* Linn (Solanaceae). *Journal of Ethnopharmacology*, 137(1), 543–546.
- Jeong, J.J.; Kumar, A.; Hanada, T.; Seo, P.-S.; Li, X.; Hanspal, M. and Chishti, A. H. (2006). Cloning and Characterization of *Plasmodium falciparum* Cysteine Protease, Falcipain-2B. *Blood Cells Molecular Diseases*, 36(3), 429-435.
- Joana Gil-Chávez, G., Villa, J. A., Fernando Ayala-Zavala, J., Basilio Heredia, J., Sepulveda, D., Yahi, E. M., and González-Aguilar, G. A. (2013). Technologies for Extraction and Production of Bioactive Compounds to be Used as Nutraceuticals and Food Ingredients: An Overview. *Comprehensive Reviews in Food Science and Food Safety*, 12(1), 5–23
- Jones, W. H. S. (1909). *Malaria and Greek history*. Manchester University Press, Manchester, United Kingdom.
- Kamagaté, M., and Koffi, C. (2014). Ethnobotany, phytochemistry, pharmacology and toxicology profiles of *Cassia siamea* Lam. *The Journal of Phytopharmacology*, 3(1), 57–76.
- Katiyar, C., Gupta, A., Satyajyoti Kanjilal, A., and Katiyar, S. (2012). Drug discovery from plant sources: An integrated approach. *An International Quarterly Journal of Research in Ayurveda*, 33(1), 10–19.
- Karthik, L., Kumar, G., Keswani, T., Bhattacharyya, A., Chandar, S.S, (2014). Protease Inhibitors from *Marine Actinobacteria* as a Potential Source for Antimalarial Compound. *Plos One*, 9(3),1-13.

- Kaur, K., Jain, M., Reddy, R. P., & Jain, R. (2010). European Journal of Medicinal Chemistry Quinolines and structurally related heterocycles as antimalarials. *European Journal of Medicinal Chemistry*, 45(8), 3245–3264.
- Keeley, A., and Soldati, D. (2004). The glideosome: a molecular machine powering motility and host-cell invasion by Apicomplexa. *Trends Cell Biol.* 14:528–532.
- Kiszewski, A. E. (2011). Current and Prospective Antimalarials, 44–68.
- Konan, K., Justin, N. K., Lydie, B., Souleymane, M., Francis, Y. A., and David, N. J. (2015). Hepatoprotective and in vivo antioxidant activity of *Olax subscorpioidea* Oliv. (Olapaceae) and *Distemonathus benthamianus* Baill. (Caesalpiniaceae). *Pharmacognosy Magazine*, 11(41), 111–116.
- Koudouvo, K., Karou, S. D., Ilboudo, D. P., Kokou, K., Essien, K., Souza, C., and De, G. M. (2011). In vitro antiplasmodial activity of crude extracts from Togolese medicinal plants, 129–132.
- Krotoski, W. A., Collins, W. E., Bray, R.S., Garnham, P.C.C., Cogswell, F. B., Gwadz, R., Killick-Kendrick, R., Wolf, R.H., Sinden, R. L., Koontz, C., and Stanfill, P.S. (1982). Demonstration of hypnozoites in sporozoite-transmitted *Plasmodium vivax* infection. *Amer J Trop Med Hyg*, 31:1291-1293.
- Ku, M.J., Dossin, F.M., Choi, Y., Moraes, C.B., Ryu, J., Song, R., and Freitas-Junior, L.H. (2011) Quantum dots: A new tool for anti-malarial drug assays. *Malar. J.*, 10, 118.
- Kuru, P. (2014). *Tamarindus indica* and its health related effects. *Asian Pacific Journal of Tropical Biomedicine*, 4(9), 676–681. <http://doi.org/10.12980/APJTB.4.2014APJTB-2014-0173>
- Kusch, P., Deininger, S., Specht, S., Maniako, R., Haubrich, S., Pommerening, T., and Kaiser, A. (2011). In Vitro and In Vivo Antimalarial Activity Assays of Seeds from *Balanites aegyptiaca* : Compounds of the Extract Show Growth Inhibition and Activity against Plasmodial Aminopeptidase, '
- Lambros, C. and Vanderberg, J.P. (1979) Synchronization of *Plasmodium falciparum* Erythrocytic Stages in Culture. *Journal of Parasitology*, 65, 418-420.
- Lawal, B., Shittu, O. K., Kabiru, A. Y., Jigam, A. A., Umar, M. B., Berinyuy, E. B., and Alozieuwa, B. U. (2015). Potential Antimalarials from African Natural Products: A review. *Journal of Intercultural Ethnopharmacology*, 4(4), 318-343
- Lecaille, F., Kaleta, J. and Bro, D. (2002). Human and Parasitic Papain-Like Cysteine Proteases: Their Role in Physiology and Pathology and Recent Developments in Inhibitor Design. *Chemistry Review*, 102,4459-4488

- Lee, B.J., Singh, A., Chiang, P., Kemp, S.J., Goldman, E.A., Weinhouse, M.I., Vlasuk, G.P., and Rosenthal, P.J., (2003). Antimalarial activities of novel synthetic cysteine protease inhibitors. *Antimicrob. Agents Chemother.*, 47, 3810-3814.
- Lee, K. S., Divis, P. C., Zakaria, S. K., Matusop, A., Julin, R. A., Conway, D. J., and Singh B. (2011). Plasmodium knowlesi: reservoir hosts and tracking the emergence in humans and macaques. *PLoS Pathogens*. 7(4).
- Lee, W. C., Chin, P.W., Lau, Y.L., Chin, L.C., Fong, M. Y., Yap, C. J., and Mahmud, R. (2013). Hyperparasitaemic human Plasmodium knowlesi infection with atypical morphology in peninsular Malaysia. *Malaria Journal*. 12(1):88.
- Lehmann, C., Heitmann, A., Mishra, S., Burda, P., Singer, M., Me, R., ... Heussler, V. (2014). A Cysteine Protease Inhibitor of Plasmodium berghei Is Essential for Exo-erythrocytic Development. *PLOS Pathogens*, 10(8), 1–19.
- Lorke, D. (1983). A New Approach for Acute Toxicity Testing. *Archive of Toxicology*, 54, 275-289.
- MacCallum, W.G. (1938). On the flagellated form of the malarial parasite. *Lancet* 1897, ii:1240-1241.
- Maiga, O., Djimde, A. A., Hubert, V., Renard, E., Aubouy, A., Kironde, F., Nsimba, B., Koram, K., Doumbo, O.K., Le Bras, J., and Clain, J., (2007). A shared Asian origin of the triple-mutant dhfr allele in Plasmodium falciparum from sites across Africa. *J Infect Dis* 196:165–172
- Makanga, M. (2014). A review of the effects of artemether-lumefantrine on gametocyte carriage and disease transmission, *I3(1)*, 1–15.
- Mandal, A. M.D. (2013). Malaria History, News medical (<http://www.news-medical.net>)
- Mann, A., Amupitan, J. O., Oyewale, A. O., Okogun, J. I., and Kolo, I. (2008). An Ethnobotanical survey of indigenous flora for treating tuberculosis and other respiratory diseases in Niger State. *Journal of Phytomedicine And Therapeutics*, 12(January 2016), 1–21.
- Mariod, A.A., Abdelwahab, S.I., 2012. Sclerocarya birrea (Marula), an African tree of nutritional and medicinal uses: a review. *Food Rev. Int.* 28, 375–388.
- Martins, Y. C., Smith, M. J., Pelajo-machado, M., Loureiro, G., Lenzi, H. L., Daniel-ribeiro, C. T., and Jose, L. (2009). Characterization of cerebral malaria in the outbred Swiss Webster mouse infected by Plasmodium berghei ANKA, 119–130.
- Marzal Alfonso (2002). recent "advances in Studies on "avian malaria parasites, and malaria parasites.

- May, J., and C. G. Meyer., (2003). Chemoresistance in falciparum malaria. *Trends Parasitol.* 19:432–435.
- Mboowa, G., (2014). Genetics of Sub-Saharan African Human Population: Implications for HIV/AIDS, Tuberculosis, and Malaria *International Journal of Evolutionary Biology* (2014), 1-8
- Ménarda, D. B., Céline B., Christiane B., Henry-Halldinc C., Laurie, R., Grayc, A., Ratsimbasoae, V. T., Jean-François, C, Olivier D., Yves, C., Olivier , Bertrandg, J.P., Christopher, L., Kingc, H., Brian T. Grimbergc, O., Mercereau-Puijalonb., and Peter A.Z., (2010). Plasmodium vivax clinical malaria is commonly observed in Duffy-negative Malagasy people *PNAS* 107(13) 5967–5971
- Meshnick SR., Taylor TE., Kamchonwongpaisan S. (2002) Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. *Microvas.Rev.* 60:301–15
- Mir, S., Bosco, S., Sunooj, K., Mir, S. A., Bosco, S. J. D. and Sunooj, K. V., (2013). Evaluation of physical properties of rice cultivars grown in the temperate region of India. *International Food Research*, 20(4), 1521–1527.
- Mohammed B. A., Yahya A. H., and Farah A. A., (2014). Optimal Control of *Plasmodium knowlesi* Malaria in Human and Macaques *British Journal of Mathematics and Computer Science* 4(2): 271-287
- Moll, K., Ljungström, I., Perlmann, H., Scherf, A., and Wahlgren, M. (2008). *Methods in Malaria Research, Fifth Edit.*
- Mota, M.M., Vandana, T., Ruth, S., and Nussenzweig, V. N. (2001). Gene targeting in the rodent malaria parasite Plasmodium yoelii *Molecular and Biochemical Parasitology* 113 (01) 271–278
- Mukandavire, Z., Gumel, A.B., Garira, W., and Tchenche, J.M., (2009), Mathematical analysis of a model for HIV-malaria co-infection. *Math. Biosci. Eng.* 6(2):333-359.
- Mulenga, A., Sugimoto, C., and Onuma, M., (1999). Characterization of proteolytic enzymes expressed in the midgut of Haemaphysalis longicornis. *Japanese Journal of Veterinary Research* 46. 179-184.
- Musabayane, C.T., (2012). The effects of medicinal plants on renal function and blood pressure in diabetes mellitus. *Cardiovasc. J. Afr.* 23, 462–468.
- Mustofa, Sholikhah, E. N., and Wahyuono, S. (2007). In vitro and in vivo antiplasmodial activity and cytotoxicity of extracts of Phyllanthus niruri L. herbs traditionally used to treat malaria in Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health*, 38(4), 609–615.
- NIAID. (2007). *Understanding Malaria Fighting an Ancient Scourge*. National Institute of

- Nicholas, E. T., Charles, A., Kevin, D., Faustina, A., Denis, C., Ambroise, W., and Collet, D. (2016). Inhibition of CYP2B6 by Medicinal Plant Extracts : Implication for Use of Efavirenz and Nevirapine-based Highly Active Anti-retroviral, Therapy (HAART) in Resource-Limited Settings. *Molecules Article*, 21(211), 1–15.
- NPC. (2006). Population and Housing Census of the Federal Republic of Nigeria, I (Volume I).
- Nyunt, M.M., and Plowe, C.V. (2007). Pharmacologic advances in the global control and treatment of malaria: combination therapy and resistance. *Clin Pharmacol Ther* 82:601–605
- Ochora, D. O., Fidahusein, D. S., Nguta, J. M., and Akunda, E. M. (2014). Antimalarial activity and acute toxicity of four plants traditionally used in treatment of malaria in Msambweni District of Kenya, 3(7), 31–40.
- Ogbadoyi, E. O. and Gabi, B., (2007). Assessment of renal function in malaria patients in Minna, North Central Nigeria. *African Journal of Infectious Diseases* 1(1): 57-64.
- Ogbonna D.N., Sokari ,T.G., and Agomuoh, A.A., (2008). Antimalarial activities of some selected traditional herbs from Southeastern Nigeria against Plasmodium species. *Research Journal of Parasitology* 3(1):25-31
- Okonkon, J.E., Etebong E. and Bassey , A., (2008). In vivo antimalarial activity of ethanolic leaf extract of *Stachytarpheta cayennensis*. *Indian J. Pharmacol.*,40(3): 111-113.
- Oliveira, A. B., Dolabela, M. F., Braga, F. C., Jácome, R. L. R. P., Varotti, F. P., & Póvoa, M. M. (2009). Plant-derived antimalarial agents: New leads and efficient phythomedicines. Part I. alkaloids. *Anais Da Academia Brasileira de Ciencias*, 81(4), 715–740.
- Omonkhelin J., Owolabi, Eri K. L., Omoghai, and Osahon O. (2007). Antifungal and antibacterial activates of the cethanolic and aqueous extract of kigelia Africana (Bignoniaceae) stem bark. *African Journal of Biotechnology*. 6(4): 1671-1680.
- On, I., (2009). Short Communication Report Investigation on The Antibacterial Activity Of The Aqueous And Ethanolic Extracts Of The Leaves Of Boerhavia Diffusa L ., 4(2), 15–18.
- Onguéné, A., Onguéné, P. A., Ntie-kang, F., Lifongo, L. L., Ndom, J. C., and Sippl, W. (2013). The potential of anti-malarial compounds derived from African medicinal plants , part I : a pharmacological evaluation of alkaloids and terpenoids The potential of anti-malarial compounds derived from African medicinal plants , part I : a pharmacological
- Onguéné, P. A., Ntie-kang, F., Mbah, J. A., Lifongo, L. L., Ndom, J. C., Sippl, W., and Meva, L. (2014). The potential of anti-malarial compounds derived from African medicinal plants , part III : an in silico evaluation of drug metabolism and pharmacokinetics profiling. *Organic and Medicinal Chemistry Letters*, 4(6), 1–9.

- Pain A, Böhme U, Berry AE, Mungall K, Finn R. D., Jackson, A. P., and Kocken C.H.M. (2008) The genome of the simian and human malaria parasite *Plasmodium knowlesi*. *Nature*.and455(7214):799-803.
- Pandey, K.C., Barkan, D.T., Sali, A. and Rosenthal, P.J. (2009). Regulatory Elements within the Prodomain of Falcipain- 2, a Cysteine Protease of the Malaria Parasite *Plasmodium falciparum*. *Plos One*, 4(5), 1-9.
- Pandey, K.C., Sijwali, P.S., Singh, A., Na, B. and Rosenthal, P.J. (2004). Independent Intramolecular Mediators of Folding, Activity, and Inhibition for the *Plasmodium falciparum* Cysteine Protease Falcipain-2. *Biochemistry*, 279(5), 3484-3491.
- Pandey, K.C., Wang, S.X., Sijwali, P.S., McKerrow, J.H. and Rosenthal, P.J. (2005). The *Plasmodium falciparum* Cysteine Protease Falcipain-2 Captures its Substrate, Hemoglobin, via a Unique Motif. *Proceedings of National Academic of Science*,102(26),27-30
- Pandey, K.C and Rajnikant Dixit, (2012). Structure-function of Falcipains; Malarial Cysteine Proteases. Invited Review article, *Journal of Tropical Medicine*, 34(5), 193-99.
- Patra, C.R., Moneim, S.S.A., Wang, E., Dutta, S., Patra, S., Eshed, M., Mukherjee, P., Gedanken, A., Shah, V.H., Mukhopadhyay, D., (2009). In vivo toxicity studies of europium hydroxide nanorods in mice. *Toxicology and Applied Pharmacology* 240, 88–98
- Patel, D.N., Pradeep, P., Surti, M.M., and Agarwal, S.B. (2003). clinical manifestations of complicated malaria – an overview *Journal, Indian Academy of Clinical Medicine _ Vol. 4, No. 4* Cox F EG 2010 History of the discovery of the malaria parasites and their vectors Parasites and Vectors
- Peters, W.J., Portus, J.H., and Robinson, BL (1995). The chemotherapy of rodent malaria XXII. The value of drug-resistant strains of *P. berghei* in screening for blood schizonticidal activity. *Ann Trop Med Parasitol*; 69: 155-71.
- Ploemen, I. (2013). *Development and Demise of Plasmodium Liver Stage Parasites*.
- Plouffe, D., Brinker, A., Mcnamara, C., Henson, K., Kato, N., Kuhen, K., and Winzeler, E. A. (2008). In silico activity profiling reveals the mechanism of action of antimalarials discovered in a high-throughput screen,.
- Ponnudurai T., Leeuwenberg A.D. and Meuwissen J.H. (1981)Chloroquine sensitivity of isolates of *Plasmodium falciparum* adapted to in vitro culture. *Trop Geogr Med*;33(1):50- 54.
- Prasad, R., Kolla, V. K., Legac, J., Singhal, N., Navale, R., Rosenthal, J., and Sijwali, P. S. (2013). Blocking *Plasmodium falciparum* Development via Dual Inhibition of Hemoglobin Degradation and the Ubiquitin Proteasome System by MG132, 8(9), 1–14.
- Raabe, A. C., Billker, O., Vial, H. J., and Wengelnik, K. (2009). Molecular and Biochemical Parasitology Quantitative assessment of DNA replication to monitor microgametogenesis in *Plasmodium berghei*. *Continued Cytoadherence of Plasmodium Falciparum Infected Red*

- Ramasamy, R., (2014). Zoonotic malaria – global overview and research and policy needs
frontiers in public health 2(123) 1-7
- Ramasubramanian, S. K. (2012). Biochemical and Structural Characterization of RCR3-AVR2 : a
model for Protease-Inhibitor Interactions at the Plant-Pathogen Interface.
- Ramos, M.V., Grangeiro, T. B., Freire, E. A., Sales, M.P., Souza, D .P. (2010). The Defensive
Role of Latex in Plants: Detrimental Effects on Insects. *Arthropod-Plant Interactions*, 4,57–
67.
- Raut, G. R., Chakraborty, S., Chopade, B. A., and Kokare, C. R. (2013). Isolation and
characterization of organic solvent stable protease from alkaliphilic marine
saccharopolyspora species. *Indian Journal of Marine Sciences*, 42(1), 131–138.
- Rawlings, N. D., and Barrett, A. J. (1994). Families of cysteine peptidases. In: *Methods in
Enzymology* (Ed. M. P. Deutscher) Academic Press, Inc: 244, 461-486.
- Rawlings, N. D. and A. J. Barrett (1995). *Methods in Enzymology*. 248.
- Redzynia, I., Ljunggren, A., Bujacz, A., Abrahamson, M., Jaskolski, M., and Bujacz,
G.(2009). Crystal structure of the parasite inhibitor chagasin in complex with papain allows
identification of structural requirements for broad reactivity and specificity determinants for
target proteases. *FEBS J.* 276, 793–806.
- Regules JA., Cummings, J.F., Ockenhouse, C.F. (2011). The RTS,S vaccine candidate for malaria.
Expert Rev. Vaccines 10:589–599.
- Reverter, D., and Lima¹, C. D. (2010). *NIH Public Access*, (4), 1–13.
- Robson, K.J., Frevert, U., Reckmann, I., Cowan, G., Beier J, Scragg IG, Takehara K, Bishop DH,
Pradel, G., Sinden, R. (1995). Thrombospondin-related adhesive protein (TRAP) of
Plasmodium falciparum: expression during sporozoite ontogeny and binding to human
hepatocytes. *EMBO J.* 14:3883–3894.
- Roestenberg, M. (2013). *Experimental infection and protection against Plasmodium falciparum
malaria in humans*. ris Spaink: Ridderprint .Pp 1- 268
- Roper C, Pearce R, Nair S, Sharp B, Nosten F., Anderson, T. (2004). Intercontinental spread of
pyrimethamine-resistant malaria. *Science* 305:1124
- Rosenthal, P.J., Mckerrow, J. H., Aikawa, M., Nagasawa, H. and Leech, J.H. (1988). A Malarial
Cysteine Proteinase Is Necessary for Hemoglobin Degradation by *Plasmodium falciparum*.
Journal of Clinical Investigation, 82(11), 1560-1566.
- Rosenthal, P.J., Wollish, W.S., Palmer, J.T. and Rasnick, D. (1991). Antimalarial Effects of
Peptide Inhibitors of a *Plasmodium falciparum* Cysteine Proteinase. *Journal of Clinical
Investigation*, 88(11), 1467-1472

- Rosenthal, P.J. and Wollish, W.S. and Palmer, J.T. and Rasnick, D. (1991). Antimalarial effects of peptide inhibitors of a Plasmodium falciparum cysteine proteinase. *J. Clin. Invest.*, 88, 1467-1472.
- Rosenthal, P.J. and Nelson, R.G. (1992). Isolation and Characterization of a Cysteine Protease Gene of Plasmodium falciparum. *Parasite*, 51, 143-152.
- Rosenthal, P.J. and Lee, G.K. and Smith, R.E. (1993). Inhibition of a Plasmodium vinckei cysteine proteinase cures murine malaria. *J. Clin. Invest.*, 91, 1052-1056.
- Rosenthal, P.J. and Olson, J.E. and Lee, G.K. and Palmer, J.T. and Klaus, J.L. and Rasnick, D. (1996). Antimalarial effects of vinyl sulfone cysteine proteinase inhibitors. *Antimicrob. Agents Chemother.*, 40, 1600-1603.
- Rosenthal, P.J., Olson, J.E., Lee, G.K., Palmer, J.T., Klaus, J.L. and Rasnick, D. (1996). Antimalarial Effects of Vinyl Sulfone Cysteine Proteinase Inhibitors. *Microbiology*, 40(7), 1600-1603.
- Rosenthal, P.J., Kim, K., McKerrow, J.H., Leech, J.H. (1999). Identification of Three stage-specific proteinases from Plasmodium falciparum. *Journal of Experimental. Medicine*, 166, 816-821.
- Rosenthal, P.J. (1998). Proteases of Malaria Parasites: New Targets for Chemotherapy. *Emergence Infectious Diseases*, 4(1), 49-57.
- Rosenthal, P.J. (2001). *Antimalarial Chemotherapy: Mechanisms of Action, Resistance and New Directions in Drug Discovery*. Totowa, New Jersey: Humana Press. (7):243-257
- Rosenthal, P.J., Sijwali, P.S., Singh, A. and Shenai, B.R. (2002). Cysteine Proteases of Malaria Parasites: Targets for Chemotherapy. *Current Chemotherapy*, 1659-1672 .
- Rosenthal, P.J., Press, H. and Chiodini, P.L. (2003). Antimalarial Chemotherapy. Mechanism of Action, Resistance and New Directions in Drug Discovery. *Journal of Antimicrobial Chemotherapy*, 1053, 3736-3741.
- Rosenthal, P.J. (2003). Review Antimalarial Drug Discovery: Old and New Approaches. *Journal of Experimental Biology*, 3735-3744.
- Rosenthal, P.J. (2004). Cysteine Proteases of Malaria Parasites. *International Journal Parasitology*, 34, 1489-1499.
- Rosenthal, P.J. (2011). Cysteine Proteases of Malaria Parasites. In *Cysteine Proteases of Pathogenic Organisms* (Ed. Mark, W. R and John, P. D.). Landes Bioscience., pp208-289
- Sachs J, and Malaney P, (2002). The economic and social burden of malaria" *Nature Magazine*. 415, 680-685

- Saddler, A., Burda, P.-C., and Koella, J. C. (2015). Resisting infection by *Plasmodium berghei* increases the sensitivity of the malaria vector *Anopheles gambiae* to DDT. *Malaria Journal*, 14(1), 134.
- Sajid, M. and McKerrow, J. (2002). Cysteine proteases of parasitic organisms. *Mol. Biochem. Parasitol.*, 120, 1-21.
- Sakanari, J. A., Stauton, C.E., Eakin, A. E., Craik, C.S. and Mckerrow, J.H., (1989). Serine protease from nematode and protozoan parasites: Isolation of sequence homologs using generic molecular probes. *Proceedings of the national Academy of Sciences USA*, 86, 4863 - 4867.
- Salihu, T., Bello, L., Wara, S., and Ali, S. (2015). An ethnobotanical survey of antidiabetic plants used by Hausa – Fulani tribes in Sokoto , Northwest Nigeria, 172, 91–99.
- Sanders, N. G., Meyers, D. J., and Sullivan, D. J. (2014). Derivatives, 58(2), 820–827.
- Sharma, R., Lall, N., 2014. Antibacterial, antioxidant activities and cytotoxicity of plants against *Propionibacterium acnes*. *S. Afr. J. Sci.* 110, 1–8.
- Sharp, K. a. (2001). Water : Structure and Properties. In *ENCYCLOPEDIA OF LIFE SCIENCES* (pp. 1–7). John Wiley and Sons, Ltd. Retrieved from www.els.net
- Shenai, B.Rand Lee, B.J.and Alvarez-Hernandez, A.and Chong, P,Y,and Emal, C.D.and Neitz, R.J.and Roush W.R.and Rosenthal, P J.,(2003). Structure-activity relationships for inhibition of cysteine protease activity and development of *Plasmodium falciparum* by peptidyl vinyl sulfones. *Antimicrob. Agents Chemother.*, 47, 154-160.
- Shenai, B.R.and Sijwali, P.S.and Singh, A.and Rosenthal, P.J., (2000). Characterization of native and recombinant falcipain-2, a principal trophozoite cysteine protease and essential hemoglobinase of *Plasmodium falciparum*. *J. Biol. Chem.*, 275, 29000-29010.
- Shillcutt, Morel C ., (2008). “Cost-effectiveness of malaria diagnostic methods in sub-Saharan Africa in an era of combination therapy.” *Bull World Health Organ* vol.86 no.2 Geneva'
- Shortt, H.E., Fairley, N.H., Covell, G., Shute, P.G., Garnham , P.C.C. (1949). A preliminary note on the pre-erythrocytic stage of *Plasmodium falciparum*. *Brit Med J*, 2:1006-8.
- Shortt, H.E., and Garnham, P.C.C. (1948). Pre-erythrocytic stages in mammalian malaria parasites. *Nature*, 161:126.
- Silvie O., (2004). A role for apical membrane antigen 1 during invasion of hepatocytes by *Plasmodium falciparum* sporozoites. *J. Biol. Chem.* 279: 9490–9496.
- Singh B, Lee KS, Matusop A, Radhakrishnan A, Shamsul SSG, Cox-Singh J, Thomas A, Conway DJ., (2004). A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. *Lancet*, 363:1017-24.

- Singh, N., Sijwali, P.S., Pandey, K.C. and Rosenthal, P.J. (2006). Plasmodium falciparum: Biochemical Characterization of the Cysteine Protease Falcipain-2 .*Experimental Parasitology*, 112,187-192.
- Singh, A. P., Surolia, N., and Surolia, A. (2009). Triclosan Inhibit the Growth of the Late Liver-stage of Plasmodium, *61*(September), 923–928.
- Sinha, S., Medhi, B., and Sehgal, R. (2014). Challenges of drug-resistant malaria. *Parasite*, 21(61), 1–15.
- Skinner-adams, T. S., Peatey, C. L., Trenholme, K. R., Krige, D., Brown, C. L., Stack, C., ... Brown, C. L. (2012). The Aminopeptidase Inhibitor CHR-2863 Is an Orally Bioavailable Inhibitor of Murine Malaria. *Antimicrob. Agents Chemother*, 56(6), 3244.
- Smith, P. W., Diagana, T. T., and Yeung, B. K. S. (2013). Progressing the global antimalarial portfolio : finding drugs which target multiple Plasmodium life stages, 66–76.
- Sofowara A (1993). Medicinal plants and traditional medicines in Africa. 2nd edition, spectrum books, Ibadan, Nigeria.
- Sofowora, A., (1995). Recent trends in research into African medicinal plants. *Journal of Ethnopharmacology*, 38(2-3), 197–208.
- Soladoye, M.O., Amusa, N.A., Raji-Esan, S.O., Chukwuma, E.C., and Taiwo, A.A., (2010). Ethnobotanical survey of anti-cancer plants in Ogun State, Nigeria. *Ann. Biol. Res.*, 1: 261-273.
- Soladoye, M.O., Chukwuma, E.C., and Owa, F.P., (2012). An avalanche of plant species for the traditional cure of *Diabetes mellitus* in South-Western Nigeria. *J. Nat. Prod. Plant Resour.*, 2: 60-72.
- Solomon, M., Belenghi, B., Delledonne, M., Menachem, E., and Levine, A. (1999). The Involvement of Cysteine Proteases and Protease Inhibitor Genes in the Regulation of Programmed Cell Death in Plants, *11*(March), 431–443.
- Sonibare, M.A. and Gbile, Z.O., (2008). Ethnobotanical survey of anti-asthmatic plants in South Western Nigeria. *Afr. J. Tradit. Complement. Alternat. Med.*, 5: 340-345.
- Srinivas, M. (2015). Antimalarial drugs. *Roll Back Malaria Site*, 54. Retrieved from <http://slideplayer.com/slide/5861681>
- Staines, H. M., Ellory, J. C. and Kirk, K. (2005) Perturbation of the pump-leak balance for Na(p) and K(p) in malaria-infected erythrocytes. *Am. J. Physiol. Cell Physiol.* 280, C1576–C1587.
- Stangeland, T., Alele, P.E., Katuura, E., and Lye, K.A., (2011). Plants used to treat malaria in Nyakayojo sub-county, Western Uganda. *J. Ethnopharmacol.* 137 (1), 154–166.

- Stepniewska, K., White, N.J., (2008). Pharmacokinetic determinants of the window of selection for antimalarial drug resistance. *Antimicrob Agents Chemother* 52:1589 –1596
- Stewart, M.J., Nawrot, R.J., Schulman, S., and Vanderberg, J.P., (1986). Plasmodium berghei sporozoite invasion is blocked in vitro by sporozoite immobilizing antibodies. *Infect. Immun.* 51:859–864
- Sullivan, D.M.D.,(2006). *Malaria: Overview History, Lifecycle, Epidemiology, Pathology, and Control*, Johns Hopkins University and David Sullivan Sargent Et, Sargent Ed: La Decouverte de Laveran Paris: Masson 1929
- Sultan, A. A., Thathy, V., Frevert, U., Robson, K. J., Crisanti, A., Nussenzweig, V., Nussenzweig R.S., and Ménard, R., (1997). TRAP is necessary for gliding motility and infectivity of plasmodium sporozoites. *Cell* 90:511–522.
- Sultan AA. (1999). Molecular mechanisms of malaria sporozoite motility and invasion of host cells. *International Microbiology : The Official Journal of the Spanish Society for Microbiology*;2:155-60.
- Sumsakul, W., Plengsuriyakarn, T., Chaijaroenkul, W., Viyanant, V., and Karbwang, J. (2014). Antimalarial activity of plumbagin in vitro and in animal models. *BMC Complementary and Alternative Medicine*, 14(15), 2–6.
- Sundararaj, S., Singh, D., Saxena, A. K., Vashisht, K., Sijwali, P. S., Dixit, R., & Pandey, K. C. (2012). The Ionic and Hydrophobic Interactions Are Required for the Auto Activation of Cysteine Proteases of Plasmodium falciparum. *PLOS ONE*, 7(10), 1–9.
- Tabuti, J. R. S. (2008). Herbal medicines used in the treatment of malaria in Budiope county, Uganda. *Journal of Ethnopharmacology*, 116(1), 33–42.
- Taha, E., Mariod, A., Abouelhawa, S., El-Geddawy, M., Sorour, M., and Matthäus, B., (2010). Antioxidant activity of extracts from six different Sudanese plant materials. *Eur. J. Lipid Sci. Technol.* 112, 1263–1269.
- Tanih, N.F., and Ndip, R.N., (2013). The acetone extract of Sclerocarya birrea (Anacardiaceae) possesses antiproliferative and apoptotic potential against human breast cancer cell lines (MCF-7). *Sci. World J.* 2013, 1–7, Article number 956206.
- Tano, K. D., Yavo, W., Trebissou, J. N. D., Offianan, A. T., and Dable, M. T. (2015). Ex vivo Antiplasmodial Activity of Plant Extracts Used in Traditional Medicine in Côte d'ivoire, 3(April), 172–179.
- Tao, Z., Zhou, H., Xia, H., Xu, S., Zhu, H., Culleton, R. L., and Lu, F. (2011). Adaptation of a visualized loop-mediated isothermal amplification technique for field detection of Plasmodium vivax infection. *Parasites & Vectors*, 4(1), 115.
- Tarkang, P. A., Franzoi, K. D., Lee, S., Lee, E., Vivarelli, D., Freitas-junior, L., and Guantai, A. N. (2014). In Vitro Antiplasmodial Activities and Synergistic Combinations of Differential Solvent Extracts of the Polyherbal Product , Nefang, 2014.

- Teixeira, C., Gomes, J.R.B. and Gomes, P. (2011). Falcipains, *Plasmodium falciparum* Cysteine Proteases as Key Drug Targets against Malaria. *Current Medicinal Chemistry*, 18, 1555-72.
- Tempera, C. I. G. (2013). Novel Applications of a Flow Cytometric Sensitivity Assay For *Plasmodium* Spp .: Compound Screening And Gametocyte Novel Applications Of A Flow Cytometric Sensitivity Assay For *Plasmodium* Spp .: Compound Screening And Gametocyte.
- Timta, M. Z. (2013). Bioinformatic Analysis and In Vitro Expression Of Malaria Parasite Translocon And Ribonuclease Binding–Like Rhopty Genes.
- Toma I, Karumi Y, Geidam MA (2009). Phytochemical screening and toxicity studies of the aqueous extract of the pods pulp of *Cassia sieberiana* DC. (*Cassia kotchiana* Oliv.). *Afr. J. Pure Appl. Chem.* 3(2): 026 – 030
- Trape, J. F., (1998). Impact of chloroquine resistance on malaria mortality. *Comptes Rendus de l'Académie des Sciences Série III—Sciences de la Vie*, , 321:689–697.
- Travassos M A. and Laufer M K. (2009) Resistance to Antimalarial Drugs: Molecular, Pharmacologic, and Clinical Considerations and International Pediatric Research Foundation, Inc Vol. 65, No. 5, Pt 2
- Trease, G.E and Evans, W C. (1989). Pharmacognsy ,11th ed, Brailliar Tindall Can. Macmillan, London.
- Trenholme, K., Marek, L., Duffy, S., Pradel, G., Fisher, G., Hansen, F. K., and Andrews, T. (2014). Lysine Acetylation in Sexual Stage Malaria Parasites Is a Target for Antimalarial Small Molecules. *Antimicrobial Agents and Chemotherap*, 58(7), 3666–3678.
- Triggi, P. I. (1985). Recent advances in malaria parasite cultivation and their application to studies on host-parasite relationships : a review, 63(2), 387–398.
- USAID (2013) President’s Malaria Initiative Nigeria Malaria operational plan FY 2
- Valderramos, S.G., and Fidock, D.A., (2006). Transporters involved in resistance to antimalarial drugs. *Trends Pharmacol Sci* 27:594 – 601
- Vale, V. V., Vilhena, T. C., Trindade, R. C. S., Ferreira, M. R. C., Percario, S., Soares, L. F. and De- Vasconcelos, F. (2015). Anti-malarial activity and toxicity assessment of *Himatanthus articulatus*, a plant used to treat malaria in the Brazilian Amazon. *Malaria Journal*, 14(1), 132. <http://doi.org/10.1186/s12936-015-0643-1>
- Vaidya, A. B., Morrisey, J. M., Zhang, Z., Das, S., Daly, T. M., Otto, T. D., and Bergman, L. W. (2014). that target Na⁺ homeostasis in intraerythrocytic, (May), 1–10.
- Wahome M.M., Nyataya J.N., Akala H.M., Awinda G.O., B.K. Mutai, R. Waihenya, E. Kamau, S.A. Omar, J.N. Waitumbi, (2014) *American Journal of Biology and Life Sciences*, , 2(5): 100-107

- Walliker D, Quakyi I.A., Wellem's T.E., McCutchan T.F., Szarfman A., London W.T., Corcoran L.M., Burkot T.R., and Carter R. (1987) Genetic analysis of the human malaria parasite *Plasmodium falciparum*. *Science*;236(4809):1661-1666.
- Wannapa, S., Gregory, P., William, F., Siems, C.A., Ryan, R.W. and Dhirayos, W. (2006). Isolation and Characterization of Isoinhibitors of the Potato Protease Inhibitor I Family from the Latex of the Rubber Trees, *Hevea brasiliensis*, *Phytochemistry*, 67, 1644-1650.
- Wang, S.X., Pandey, K.C., Somoza, J.R., Sijwali, P.S., Kortemme, T., Brinen, L.S., Fletterick, R.J., Rosenthal, P.J., and Mckerrow, J.H., (2006). Structural basis for unique mechanisms of folding and hemoglobin binding by a malarial protease. *Proc. Natl. Acad. Sci. USA*, 103, 11503-11508.
- Watkins, W.M., and Mosobo, M., (1993). Treatment of *Plasmodium falciparum* malaria with pyrimethamine-sulfadoxine: selective pressure for resistance is a function of long elimination half-life. *Trans R Soc Trop Med Hyg* 87:75-78
- Webster, D., and Hill, A. V. S. (2003). Progress with new malaria vaccines, 81(3), 902-908.
- Weekley, C., and Smith, D. S. (2013). *Malaria : the clinical basics Learning Overview (Clinical)*.
- Wellem's, T.E., Walker-Jonah, A., and Panton, L. J., (1991). Genetic mapping of the chloroquineresistance locus on *Plasmodium falciparum* chromosome 7. *Proc Natl Acad Sci USA* 88:3382-3386
- Wengelnik, K., Spaccapelo, R., Naitza, S., Robson, K.J., Janse, C. J., Bistoni, F., Waters, A.P., and Crisanti, A., (1999). The A-domain and the thrombospondin-related motif of *Plasmodium falciparum* TRAP are implicated in the invasion process of mosquito salivary glands. *EMBO J.* 18:5195-5204.
- Winstanley, P. A., (2000). Chemotherapy for *falciparum* malaria: the armoury, the problems and the prospects. *Parasitol. Today* 16: 146-153
- WHO. (2010). *Guidelines for the treatment of malaria.*
- WHO. (2010). *Guidelines for the treatment of malaria.*
- WHO. (2007). *Report of the WHO Interregional Workshop on the Use of Traditional Medicine in Primary Health Care.*
- WHO. (2013). *universal access to malaria diagnostic testing An Operational Manual.*
- WHO. (2014). *World Health Statistics. WHO.*
- WHO. (2015). *World Health Statistics.*
- WHO., (2003). Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated *falciparum* malaria. Geneva, World Health Organization, 2003

- WHO. (2017). Responding to antimalarial drug resistance.
- WHO., (2010). World Health Organization Fact Sheet WHO.
- WHO., (2016). *MALARIA PARASITE COUNTING*.
- Yahaya, M. K., (2003). The Nupe People of Nigeria, *I*(2), 95–110.
- Yerbanga, R. S., Lucantoni, L., Ouédraogo, R. K., Da, D. F., Yao, F. A., Yaméogo, K. B. and Habluetzel, A. (2014). Transmission blocking activity of *Azadirachta indica* and *Guiera senegalensis* extracts on the sporogonic development of *Plasmodium falciparum* field isolates in *Anopheles coluzzii* mosquitoes, 1–10.
- Yoshida, N., Nussenzweig, R.S., Potocnjak, P., Nussenzweig, V., and Aikawa, M., (1980). Hybridoma produces protective antibodies directed against the sporozoite stage of malaria parasite. *Science* 207:71–73.
- Zainal-Abidin, B. A. H., and Abdulelah, H.A.A., (2007). *In Vivo* Anti-malarial Tests of *Nigella sativa* (Black Seed) Different Extracts *American Journal of Pharmacology and Toxicology* 2 (2): 46-50
- Zaidan, M., Rain, N., and Badrul, A., (2005). *In vitro* screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Trop. Biomed.* 22: 165-170.
- Zlotek, U., Mikulska, S., Nagajek, M., and Swieca, M. (2016). The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (*Ocimum basilicum* L.) extracts. *Saudi Journal of Biological Sciences*, 23(5), 628–633.

APPENDICES

Appendix 1 Calculation of Inhibitory Protease activity

$$\text{Protease activity } (\mu\text{mol pNA released}) = A_{410\text{nm}} \times d / \epsilon \times t \times v$$

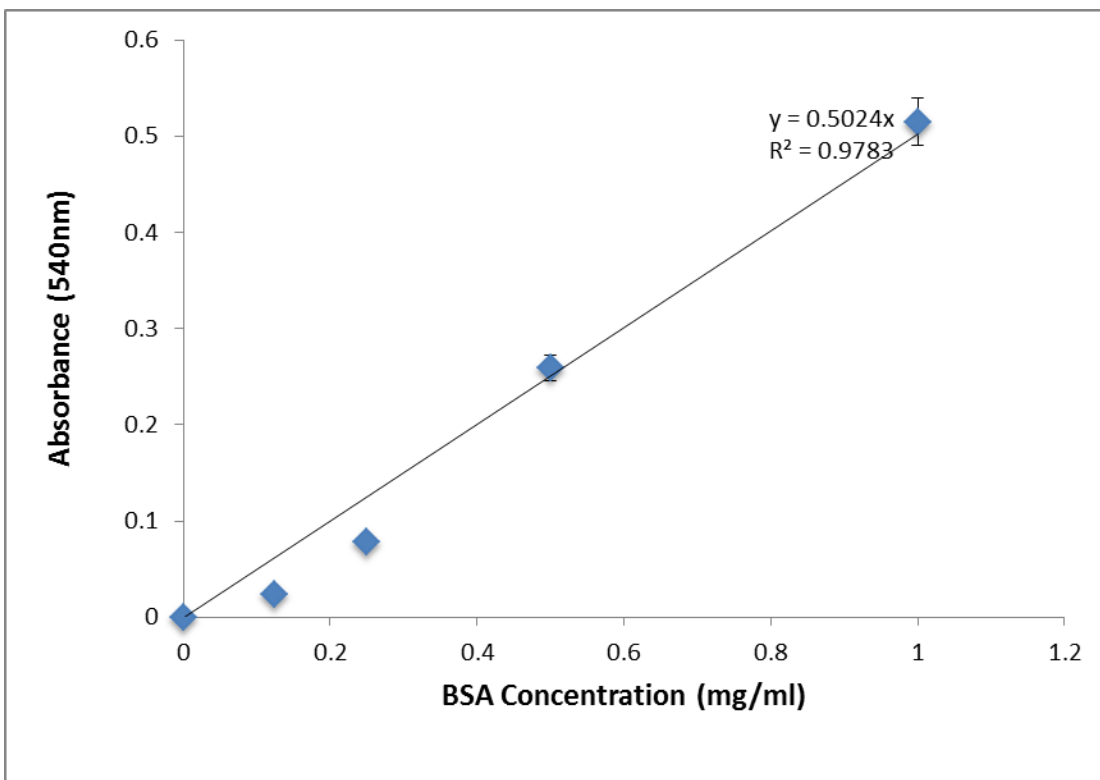
ϵ =Extinction coefficient of p-nitroaniline (8800 /cm/M), V-vol of sample, d-dilution factor, t-rxn time in min.

$$\text{Protease Inhibitory activity} = (A_{410\text{nm}} \text{c} - A_{410\text{nm}} \text{t}) \times d / \epsilon \times t \times v$$

$$\% \text{ Inhibition} = (A_{410\text{nm}} \text{c} - A_{410\text{nm}} \text{t}) / A_{410\text{nm}} \text{c} \times 100$$

Where $A_{410\text{nm}} \text{c}$ and $A_{410\text{nm}} \text{t}$ are Absorbance at 410nm of the control and the test sample, respectively

Appendix II Protein Standard curve



Protein Standard curve

Appendix III Evidence of Ethical clearance



MINISTRY OF HEALTH AND HUMAN SERVICES
KADUNA STATE, NIGERIA

MOH/ADM/744/VOL.1/459

8TH SEPTEMBER, 2016


NOTICE OF APPROVAL AFTER FULL COMMITTEE REVIEW
ANTI-PLASMODIAL ACTIVITY AND INHIBITORY STUDY OF MALARIA MEDICINAL
PLANT EXTRACTS ON CYSTEINE PROTEASE FROM NK 65 CHLOROQUINE
SENSITIVE PLASMODIUM FALCIPARUM

Name of Principal Investigator: **GABI BABA**
Address of Principal Investigator: **Dept. of Biochemistry**
A.B.U-Zaria
Date of receipt of Application **5th August, 2016**
Date of Ethical Approval **17th August, 2016**

This is to inform you that the Research described in the submitted Protocol, the Consent forms, advertisements and other participant information materials have been reviewed and given full approval by the Health Research Ethics Committee (HREC).

If there is delay in starting the research or any change, inform the HREC so that the dates of approval can be adjusted accordingly.

However, Researcher is kindly requested to submit a copy of his/her findings to the State Ministry of Health, please.


DR. B. M. JATAU
CHAIRMAN

KADUNA STATE MINISTRY OF HEALTH
OPEN REGISTRY
DISPATCHED
SIGN - [Signature] - DATE - 9/9/16

Appendix IV: Ethno-botanical survey information and practices of Practitioners in Ethno-medicinal oral responses in the herbs used specifically in treatment of malaria

S/NO	PLANT Botanical Common and Vernacular NAME	Percentage of respondents	Pharmaceutical preparation /Usage	Plant Part Used
1	<i>Anacardium occidentale</i> Cashew Kashium (Eko Nasara)	26.67	Malaria fever Decoction Taken daily	Stem bark
2	<i>Mangifera indica (L)</i> Mango Mungoro	23.33	Decoction with other additives Taken often	leaves and stem bark
3	<i>Sclerocarya birrea</i> (A.Rich) Marula Jingere goyi	40.00	Decoction taken twice daily	Dried stem bark
4	<i>Balanites aegyptica (L)</i> Del Soap Berry tree desert date Aduwa	40.00	Decoction Taken ones daily	Bark and seeds
5	<i>Newboldia leaveis</i> New boldia levis Aduruko (dimbere Chyamile)	33.33	Infusion Taking once daily	Root and leaves
6	<i>Carica papaya (L)</i> Pawpaw Konkeni	26.67	Cold infusion or decoction ½ a cup twice daily	Leave, seeds and roots
7	<i>Anogessus leocapus</i> Shici	23.33	Decoction with <i>Xylopi aethopic</i> and <i>Capsicum</i> fruit Taken orally	Leaves
8	<i>Tamarindus india (L)</i> Tamarina tree Darachi	36.67	Cold infusion Taken once daily	fresh stem bark and fruit
9	<i>Sida acuta</i> acuta broomuced, Horn beam leaved sida Sangi ekoti	26.67	Decoction Taken once daily	(whoe harb)
10	<i>Azadirachita india A.</i> Neem tree <u>Nimu</u>	43.33	Decoction steam bath and taken orally	stern back and leaves

Ethno-botanical survey information and practices of Practitioners in Ethno-medicinal oral responses in the herbs used specifically in treatment of malaria

S/NO	Plant Botanical Common And Vernacular Name	Percentage of respondents	Pharmaceutical Preparation /Usage	Plant Part Used
11.	<i>Trichilia emetic</i> (vahl) <i>Trichilia roka</i> <u>Yilanchi lati</u>	26.67	Decoction and taken orally	Leaves
12.	<i>Musanga cercropioides</i> Umbrella tree cork wood <u>Chigbankata</u>	30.00	Decoction Taken orally	Leaves
13.	<i>Psidium guajava</i> L. Guava <u>Goyiba guayaba</u>	23.33	Decoction taken 3 times daily	(leaves)
14.	<i>Piper guineense</i> sachum and thonn Guinea fruits West African black papper Guinea black papper Mansoro	33.33	Additive and taken orally	Fruits
15.	<i>Neocaya macropylla</i> Neou oil tree (sabine) fruit, kernels and twig sprance Putu	26.67	Decoction take when necessary	Stem bark, leaves and root
16.	<i>Blighia sapida konig</i> Akee apple Yilanchi	36.67	Decoction of stem bark taken once daily	Stem bark
17.	<i>Trema orientalis</i> (Linn)DC <i>Trema guineensis</i> Charcoal tree Nyanyachi	26.67	Decoction	whole plant
18.	<i>Achyranthes aspect</i> Prickly-chaff Egyagi	20.00	Fever Decoction, stem bath	whole plant

Ethno-botanical survey information and practices of Practitioners in Ethno-medicinal oral responses in the herbs used in the treatment of malaria symptoms

S/No	<i>Plant Botanical Common And Vernacular Name</i>	Percentage of Respondents	Pharmaceutical Preparation /Usage	Plant Part Used
19.	<i>Atternanthera Sessilis</i> (L) DC flower Sessile joyweed Masantogi	13.33	Fever decoction taken orally	whole plant
20.	<i>Pupalia lappacea</i> (L) Mamarigbo	20.00	Decoction taken orally	
21.	<i>Spondias mombine</i> (L) Hog plum Jinjerechi	13.33	Decoction taken once daily	stem bark
22.	<i>Ceiba pentadre</i> (L) Cotton seed <u>Kuchi</u>	10.00	Decoction drink often	leaves and bark
23.	<i>Commiphora kerstingii</i> England <u>Enagunbochi</u>	16.67	Decotion with potash Taken many times daily	stem bark
24.	<i>Cassia rotundifolia</i> (pers) <u>Dzamma</u>	13.33	Decoction Taken orally	whole plant
25.	<i>Piliostigma thonniiglii</i> <u>Bafin</u>	10.00	Decoction Taken orally several times	fresh leaves stern bark and root bark
26.	<i>Cleome viscose</i> (L) Chiken weed Timigi, Emigi, Egyagi	16.67	Decoction Taken orally	whole plant
27.	<i>Commelina diffusa.</i> (Burum f). Water grass Roko roko, Gofin o Naokin	10.00	Yellow fever juice of the leaves for fever	whole herb
28.	<i>Acanthospermun hispidus</i> Dc Kashiyawo	36.67	Juice of leaves for palm wine potash	Leaves
29.	Tridax, coat bur Biyennalelu or Kungbaa	16.67	Cold infusion with potash and drink	whole plant
30	<i>Chrozophora senegalensis</i> Damagi	36.67	Decoction Taken orally several times	Leaf
31.	<i>Vernonia cinerea</i> (linn) Little iron weed fleabae Tsula bishe	10.00	Decoction	whole plant
32.	<i>Vernonia colorata</i> (wild) prake Sumaba	13.33	Decoction Taken once	whole plant

Ethno-botanical survey information and practices of Practitioners in Ethno-medicinal oral responses in the herbs used in the treatment of malaria symptoms

S/NO	PLANT Botanical COMMON and Vernacular NAME	Percentage of respondents	Pharmaceutical preparation /USAGE	Plant Part Used
33.	<i>Olox scopiodiasis</i> Kulanchi	41.33	Decoction Taken orally several times	
34.	<i>Vernonia perottetti</i> <i>schultz-bip</i> Doko chintara	16.67	Decoction	whole plant
35.	<i>Euphorbia hirta l.</i> Kinkere chintara	13.33	Decoction	whole plant (fresh shoot)
36.	<i>Cleome viscosa (L)</i> Chicken weed Timigi, Emigi, Egyagi	16.67	Decoction Taken orally	whole plant
37.	<i>Schwenkia americana,</i> Rogogi	40.00	Decoction steam bath and taken orally	Whole plant
38.	<i>Senna occidentalis</i> Gaya eba	36.67	Decoction Taken orally several times	Whole plant
39.	<i>Senna obtusifolia</i> Gaya yiwo	40.00	Decoction Taken orally several times	Whole plant
40.	<i>Physalis angulata L.</i> Wildcape Goose berry Chinese kanthera Alasangi	20.00	Fever Decoction and taken twice daily	(whole plant)
41.	<i>Steculia setigera Del</i> Bokochi Kokongiga	13.33	Decoction taken thrice daily with pap	(stem bark and seeds)
42.	<i>Waltheria indica L</i> Ankufa or Gbarufu mubo	16.67	Cold in fusion or Decoction taken daily	Whole plant
43.	<i>Senna seiberriana</i> Gbogi	36.67	Decoction Taken orally early in the morning	Root, leaves
44.	<i>Annogeisses leicarpus</i> Shici	30.00		
45.	<i>Achyranthes aspect</i> Prickly-chaff Egyagi	13.33	Fever Decoction, steam bath	whole plant
46.	<i>Parinary curatellifolia,</i> Putu bokun	36.67	Fever Decoction Taken orally several times	Leaves
47.	<i>Cymbopogon Citrullus</i> Lemon grass	30.00	Fever Decoction	Whole plant
48.	<i>Ocimum bacilicum</i> Basil plant Saint leaf	26.67	Fever Decoction	Leaves

APPENDIX V : Sub-Acute Toxicity Profiles of the Plant extracts

Table 1 Effects of the *S. seiberiana* Methanol Extract on Some Serum Biochemical Parameters for Liver Function Assessment of Mice After Treatment

Dose of Extracts (mg/Kg body weight)	Parameters				
	Total protein(58-80g/L)	Albumin(35-50 g/L)	ALAT(UI/mg) (up to 22UI/mg)	AST(UI/mg) (Up to 218)	ALP(UI/mg) (60 - 170)
Control (Distiled water)	55.00 ± 8.22 ^a	23.00 ± 4.64 ^a	1.42 ± 0.70 ^a	2.34 ± 0.75 ^a	68.40 ± 3.85 ^{ab}
100mg/Kg body wt	64.20 ± 4.15 ^a	41.00 ± 16.58 ^b	0.90 ± 0.92 ^a	1.52 ± 0.95 ^a	67.20 ± 4.66 ^{ab}
200mg/Kg body wt	54.75 ± 6.85 ^a	25.25 ± 2.50 ^a	0.53 ± 0.33 ^a	1.35 ± 0.50 ^a	60.25 ± 5.38 ^a
400mg/Kg body wt	57.67 ± 1.53 ^a	30.33 ± 5.77 ^{ab}	1.00 ± 0.26 ^a	1.97 ± 0.40 ^a	71.33 ± 9.02 ^b

Values are serum biochemical parameters for liver function test expressed as mean ±SD for (n = 5). Data in the same column with different super scripts differ significantly at (P<0.05)

ALAT: alanine aminotransferase

AST: aspartate aminotransferase

ALP: Alkaline phosphatase

Body wt = Body weight

Table 2 Effects of the *S. seiberiana* Methanol Extract on Some Serum Biochemical Parameters for Kidney Function Assessment of Mice After Treatment

Dose (mg/kg body weight)	Parameters					
	Urea (mmol/l) 2.5-6.5	Creatinine (mmol/l) 90.0-126	Sodium (Na ⁺) (mmol/l) 135-150	Potassium (K ⁺) (3.4-5.3mmol/l)	Chloride (Cl ⁻) (mmol/l) 95-110	Bicarbonate (HCO ₃ ⁻) (24-32mmol/l)
Control	6.82 ± 0.90 ^a	76.60 ±14.77 ^a	153.40 ±14.98 ^a	8.12 ± 1.56 ^a	115.20 ± 5.97 ^a	40.20 ± 1.79 ^a
100mg/Kg body wt	7.14 ± 1.76 ^a	74.60 ±10.06 ^a	148.60 ±18.89 ^a	9.20 ± 2.52 ^a	109.00 ± 10.95 ^a	39.20 ± 2.17 ^a
200mg/Kg body wt	7.08 ± 1.20 ^a	84.00 ±9.97 ^{ab}	152.25 ±8.96 ^a	26.83 ± 36.80 ^a	113.00 ± 5.89 ^a	40.00 ± 1.83 ^a
400mg/Kg body wt	10.10 ± 1.31 ^b	101.33 ±3.06 ^b	192.00 ±7.55 ^b	10.23 ± 1.01 ^a	150.00 ± 28.58 ^b	39.33 ± 2.08 ^a

Values are serum biochemical parameters for kidney function test expressed as mean ±SD for (n = 5). Data in the same column with different super scripts differ significantly at (P<0.05)

Na⁺: Sodium ion

K⁺: Potassium ion

Cl⁻: Chloride ion

HCO₃⁻: Biocarbonate

Body wt = Body weight

Table 3 Effects of The *S. americana* Methanol Extract on some Serum Biochemical Parameters for Liver Function Assessment of Rats After Treatment

Dose (mg/kg body weight)	Parameters						
	Total protein (58-80g/L)	Albumin(35- 50g/L)	ALAT(UI/mg) (up to 22UI)	ASAT(UI/mg) (Up to 218)	ALP(UI/mg) (60 - 170)	T. Bilirubin ($\mu\text{mol/l}$) (1.7- 17.1)	C. Bilirubin ($\mu\text{mol/l}$) (1.7- 8.5)
Control (Distiled water)	69.60 \pm 1.52 ^b	45.20 \pm 0.45 ^a	7.60 \pm 2.60 ^{ab}	8.40 \pm 02.3 ^{ab}	87.00 \pm 2.24 ^{ab}	8.60 \pm 0.55 ^b	3.60 \pm 0.55 ^a
10mg/Kg body wt	69.60 \pm 1.14 ^b	46.20 \pm 2.17 ^a	10.20 \pm 4.60 ^b	11.40 \pm 4.16 ^b	88.00 \pm 0.71 ^{ab}	7.00 \pm 1.58 ^a	4.00 \pm 0.00 ^{ab}
100mg/Kg body wt	72.20 \pm 5.12 ^b	49.00 \pm 1.22 ^a	5.20 \pm 4.09 ^a	6.00 \pm 4.12 ^a	89.40 \pm 2.5 ^b	8.20 \pm 0.84 ^{ab}	3.40 \pm 0.55 ^a
1000mg/kg body wt	64.40 \pm 3.91 ^a	46.40 \pm 1.52 ^a	5.20 \pm 3.80 ^a	6.00 \pm 2.92 ^a	85.80 \pm 2.05 ^a	8.40 \pm 0.55 ^b	3.20 \pm 0.84 ^a

Values are serum biochemical parameters for liver function test expressed as mean \pm SD for (n = 5). Data in the same column with different super scripts differ significantly at (P<0.05)

ALAT: alanine aminotransferase

AST: aspartate aminotransferase

ALP: Alkaline phosphatase

T. Bilirubin: Total Bilirubin

C. Bilirubin: Conjugated

Body wt = Body weight

Table 4: Effects of the *S. americana* Methanol Extract on some Serum Biochemical Parameters for Kidney Function Assessment of Rats After Treatment

Dose (mg/kg body weight)	Parameters					
	Urea (mmol/L) 3.0-6.6	Creatinine (mmol/l) (72.0-125)	Sodium (Na+) (mmol/l) (135-150)	Potassium (K+) (3.4-5.3mmol/l)	Chloride (Cl) (97-108 mmol/l)	Bicarbonate (HCO ₃ ⁻) (24-32mmol/l)
Control (Distilled water)	7.78±0.82 ^{cd}	89.20±10.13 ^{bcd}	149.40±1.34 ^a	5.12±0.16 ^a	113.40±1.67 ^b	28.40±0.89 ^{ab}
10mg/Kg body wt	4.18±0.79 ^a	91.20±17.24 ^{cd}	148.60±2.60 ^a	5.14±0.19 ^a	115.00±1.22 ^b	26.80 ±1.09 ^a
100mg/Kg body wt	4.94±1.94 ^{ab}	96.00±5.78 ^d	149.80±10.57 ^a	4.76±0.47 ^a	112.60±3.51 ^b	28.60±2.60 ^{ab}
1000mg/kg body wt	4.64 ±0.49 ^{ab}	74.20±5.76 ^{ab}	148.40±2.88 ^a	4.82±0.23 ^a	113.60±0.55 ^b	29.40±0.55 ^b

Values are serum biochemical parameters for kidney function test expressed as mean ±SD for (n = 5). Data in the same column with different super scripts differ significantly at (P<0.05)

Na+: Sodium ion

K+: Potassium ion

Cl: Chloride ion

HCO₃⁻: Biocarbonate

Body wt = Body weight

Table 5: Effects of the *O. subscorpioidea* Methanol Extract on some Serum Biochemical Parameters for Liver Function Assessment of Rats After Treatment

Dose (mg/kg body weight)	Parameters						
	Total protein (58-80g/L)	Albumin (35-50g/L)	ALAT(UI/mg) (up to 22UI)	ASAT(UI/mg) (Up to 218)	ALP(UI/mg) (60 -170)	T. Bilirubin (μ mol/l) (1.7- 17.1)	C. Bilirubin (μ mol /l) (1.7- 8.5)
Control (Distiled water)	69.60 \pm 1.52 ^b	45.20 \pm 0.45 ^a	7.60 \pm 2.60 ^{ab}	8.40 \pm 2.30 ^{ab}	87.00 \pm 2.24 ^{ab}	8.60 \pm 0.55 ^b	3.60 \pm 0.55 ^a
10mg/Kg body wt	68.60 \pm 0.89 ^{ab}	47.60 \pm 2.30 ^{ab}	7.40 \pm 2.88 ^{ab}	8.00 \pm 2.12 ^{ab}	88.20 \pm 2.95 ^{ab}	7.40 \pm 1.34 ^{ab}	4.20 \pm 0.45 ^{ab}
100mg/Kg body wt	70.60 \pm 1.95 ^b	45.60 \pm 2.19 ^a	5.40 \pm 2.30 ^a	6.80 \pm 1.78 ^a	89.60 \pm 1.67 ^b	7.60 \pm 0.55 ^{ab}	4.80 \pm 1.09 ^b
1000mg/kg body wt	68.60 \pm 5.27 ^{ab}	46.40 \pm 1.52 ^a	7.40 \pm 2.17 ^{ab}	8.80 \pm 2.05 ^a	88.80 \pm 2.94 ^{ab}	7.40 \pm 0.55 ^{ab}	4.00 \pm 1.22 ^{ab}

Values are serum biochemical parameters for liver function test expressed as mean \pm SD for (n = 5). Data in the same column with different super scripts differ significantly at (P<0.05)

ALAT: alanine aminotransferase

AST: aspartate aminotransferase

ALP: Alkaline phosphatase

T. Bilirubin: Total Bilirubin

C. Bilirubin: Conjugated

Body wt = Body weight

Table 6: Effects of the *O. subscorpioidea* Methanol Extract on some Serum Biochemical Parameters for Kidney Function Assessment of Rats After Treatment

Dose (mg/kg body weight)	Parameters					
	Urea (mmol/L) 2.5 -6.5	Creatinine (mmol/L) 90.0-126	Sodium (Na) (mmol/L) 135-150	Potassium (K) (3.4- 5.3mmol/L)	Chloride (Cl) (95-110 mmol/L)	Bicarbonate(HCO ₃ ⁻) (24-32mmol/L)
Control (Distiled water)	7.78±0.82 ^{cd}	89.20±10.13 ^{bcd}	149.40±1.34 ^b	5.12±0.16 ^a	113.40±1.67 ^b	28.40±0.89 ^{ab}
10mg/Kg body wt	6.24±1.00 ^{bc}	79.40±14.41 ^{abc}	145.60±4.04 ^{ab}	4.70±0.42 ^a	113.60±1.67 ^b	27.60 ±0.55 ^{ab}
100mg/Kg body wt	4.14±0.29 ^a	64.60±7.79 ^a	140.40±2.51 ^b	5.02±0.30 ^a	105.40±4.16 ^a	28.40±1.140 ^{ab}
1000mg/kg body wt	8.84 ±2.34 ^d	97.40±15.43 ^d	147.20±2.77 ^b	4.90±0.19 ^a	112.80±1.30 ^b	28.40±1.14 ^{ab}

Values are serum biochemical parameters for kidney function test expressed as mean ±SD for (n = 5). Data in the same column with different super scripts differ significantly at (P<0.05)

Na+: Sodium ion

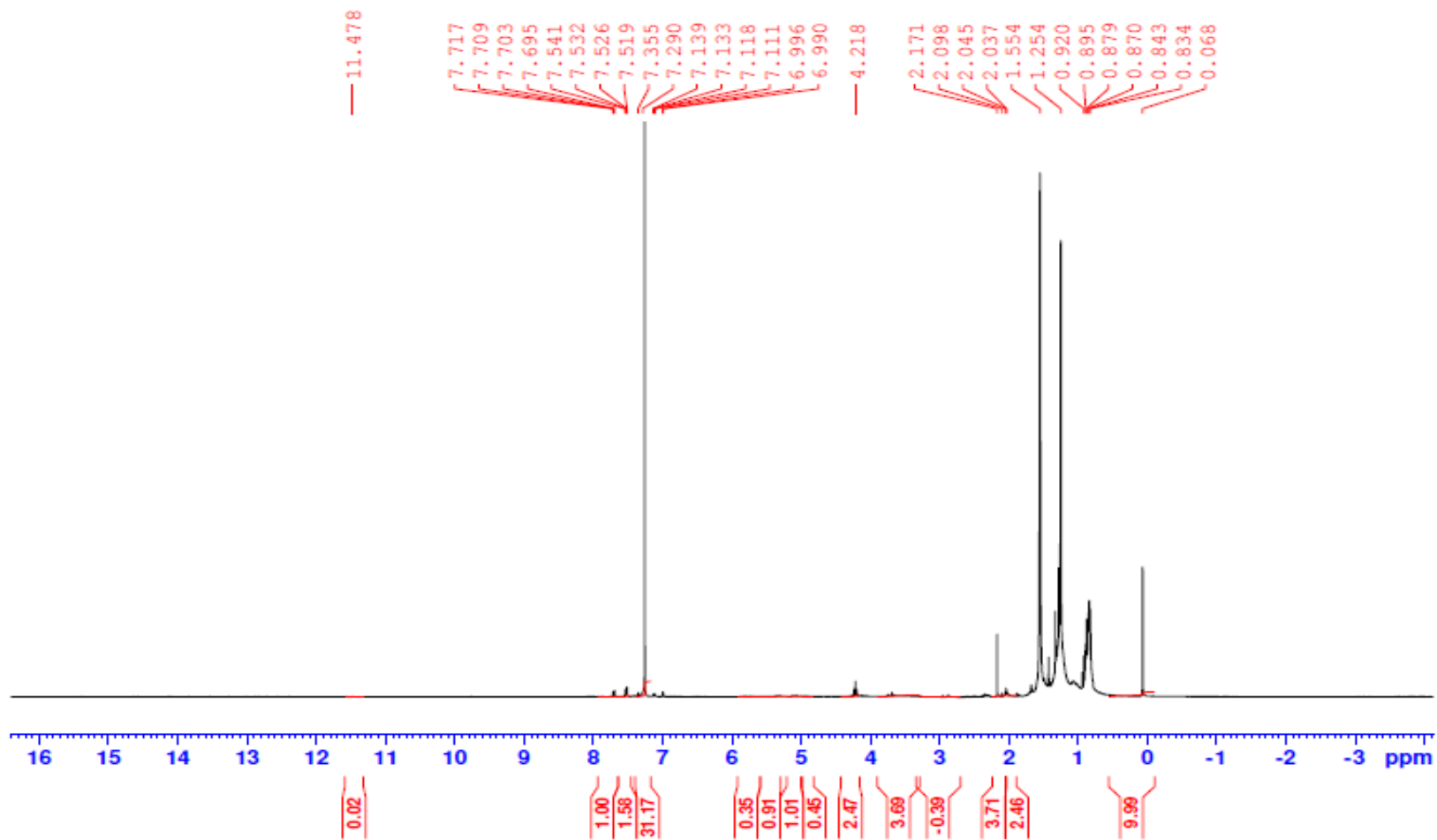
K+: Potassium ion

Cl: Chloride ion

HCO₃⁻: Biocarbonate

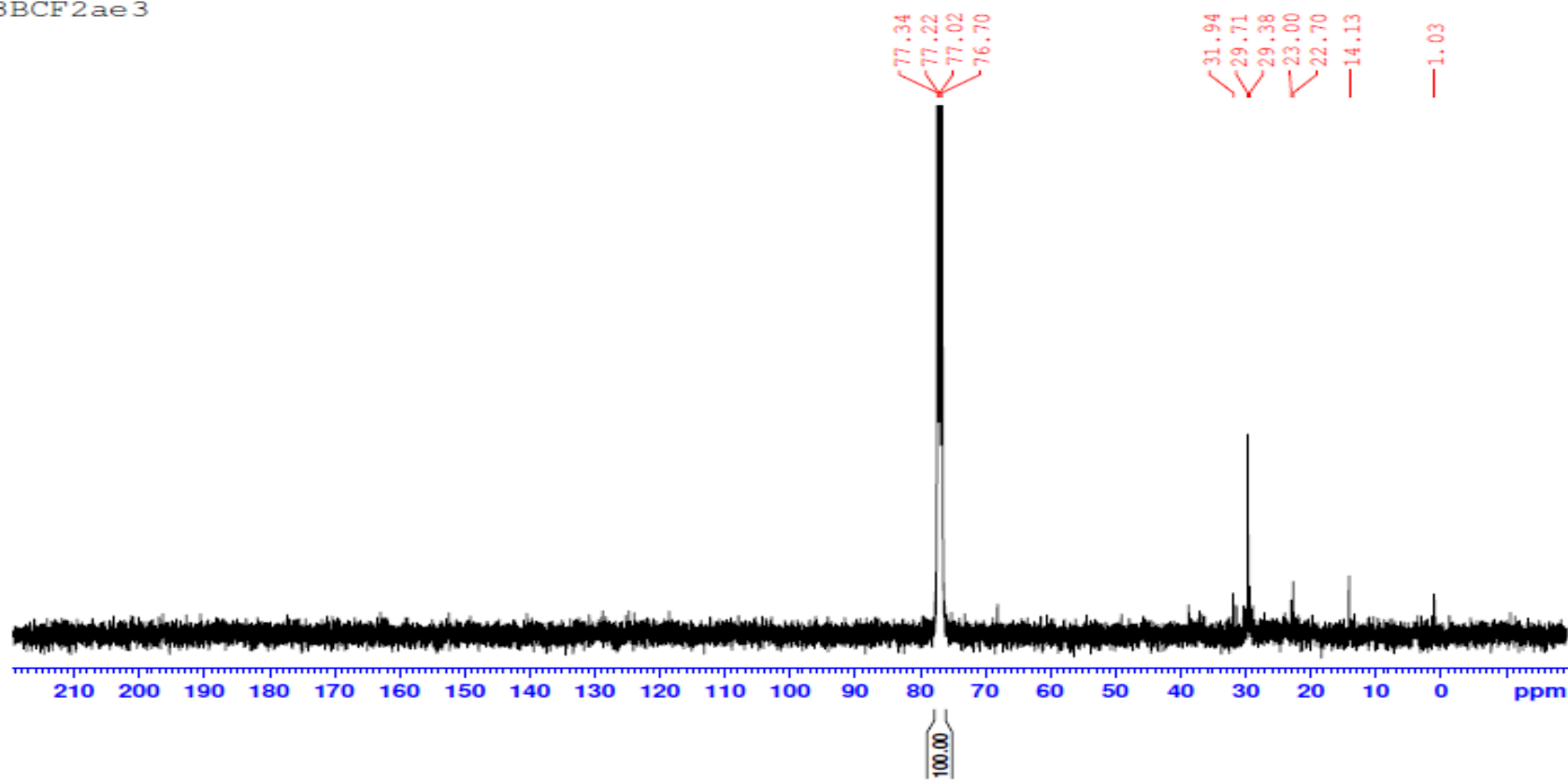
Body wt = Body weight

APPENDIX VI NMR Spectral



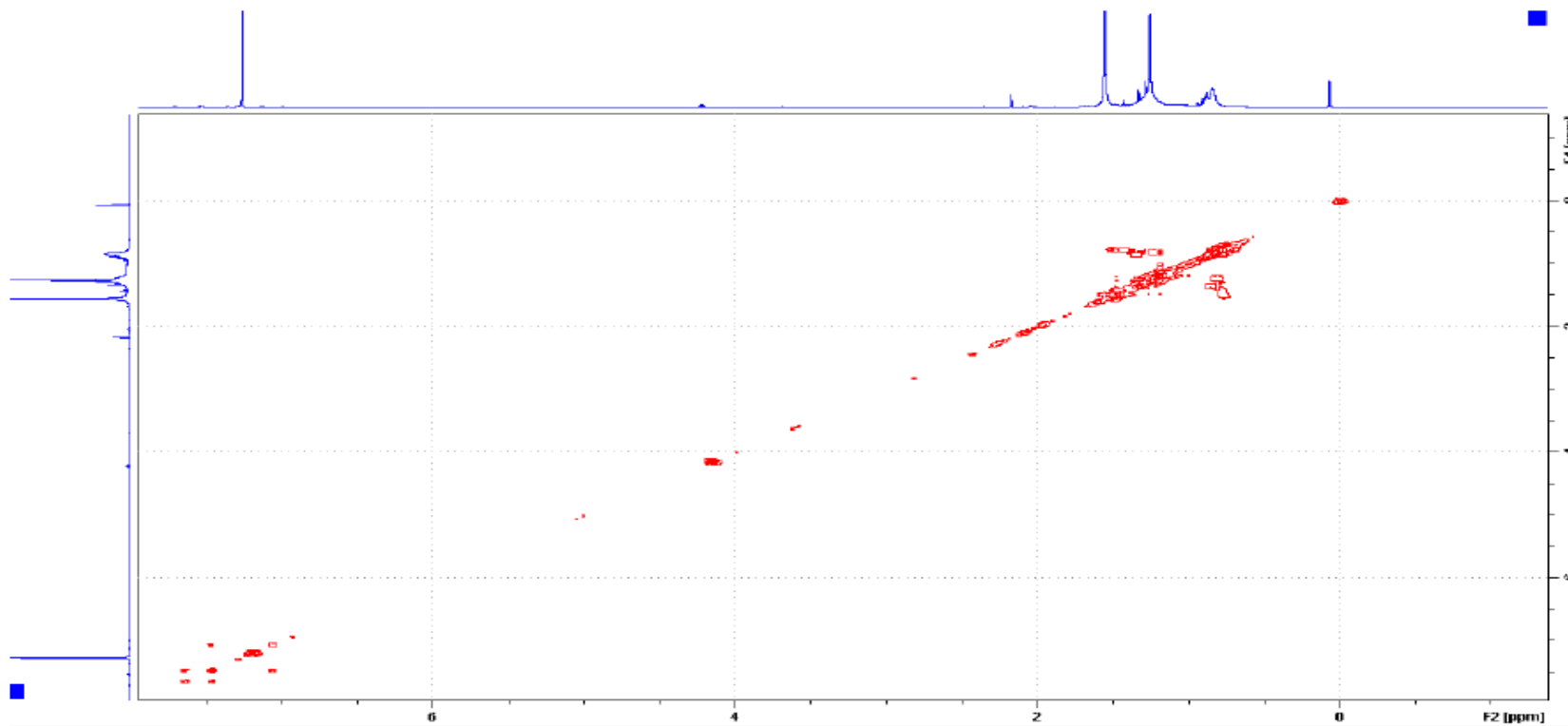
Proton ^1H NMR Spectra (400MHz, 100MHz CDCl_3) For the Compound GB003BCF2ae3

3BCF2ae3



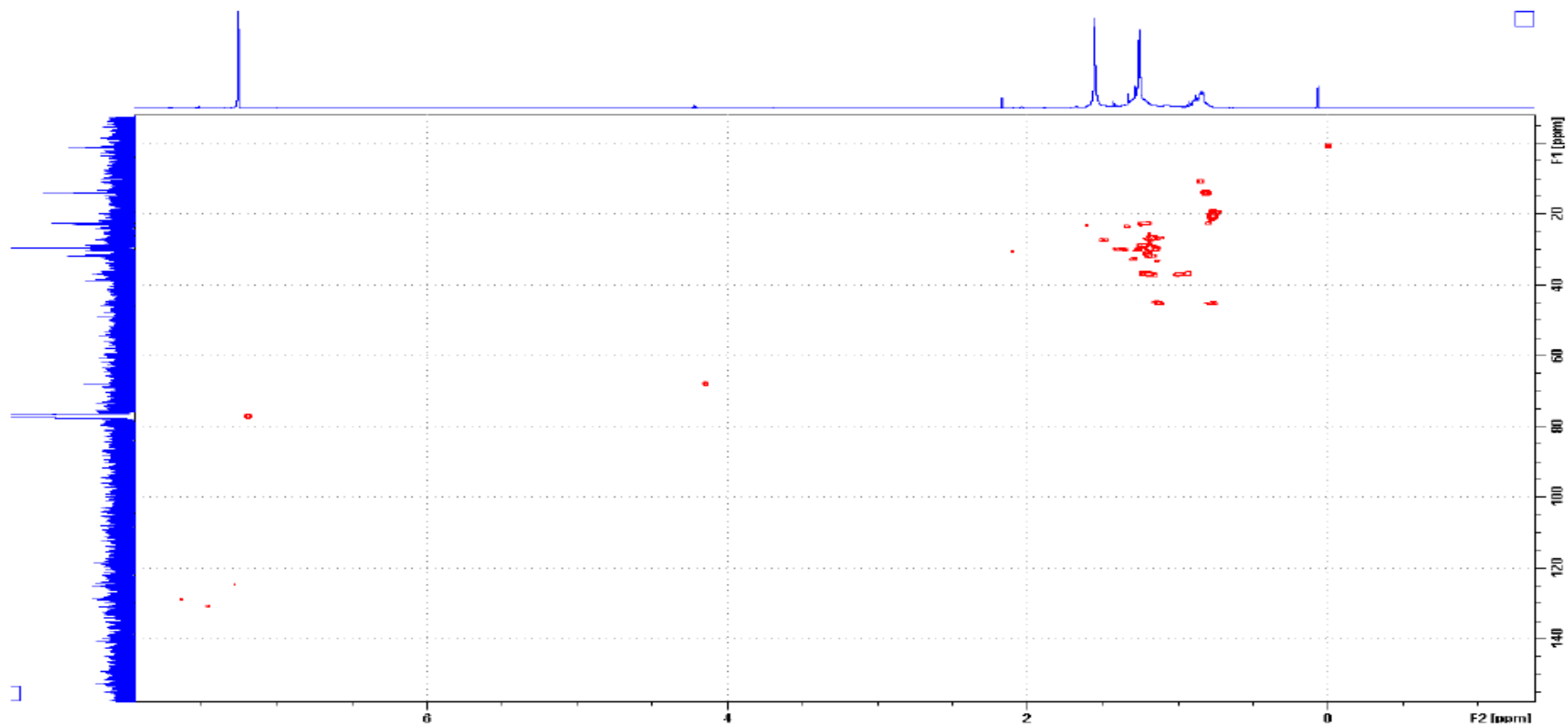
^{13}C NMR Spectra (400MHz, 100MHz CDCl_3) For the Compound GB3BCF2ae3

Cosy 3BCF2ae3



COSY NMR Spectra (400MHz, 100MHz CDCl₃) For the Compound GB3BCF2ae3

HSQC-3BCF2ae3



1

HSQC NMR Spectra (400MHz, 100MHz CDCl_3) For the Compound GB3BCF2ae3

1

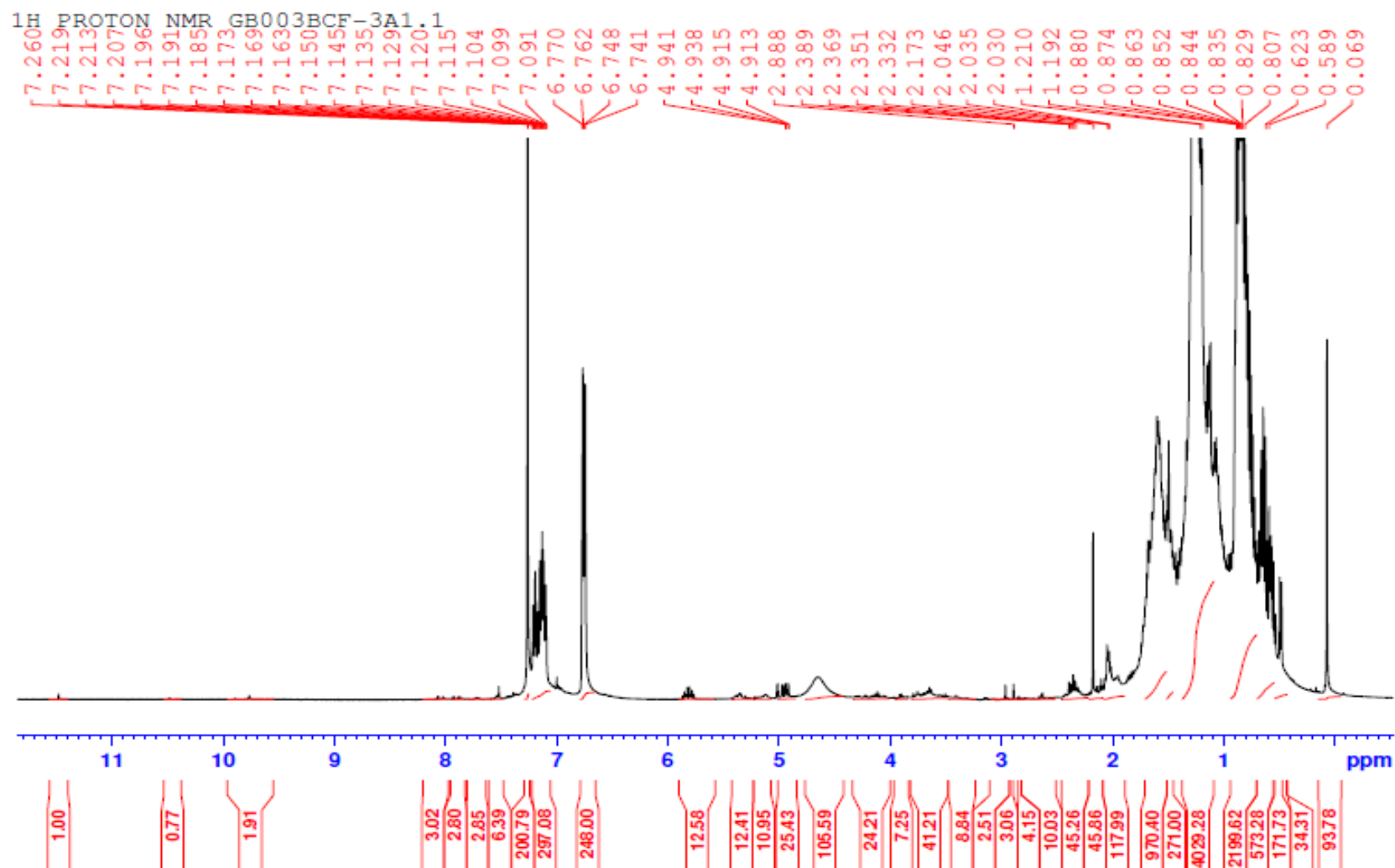
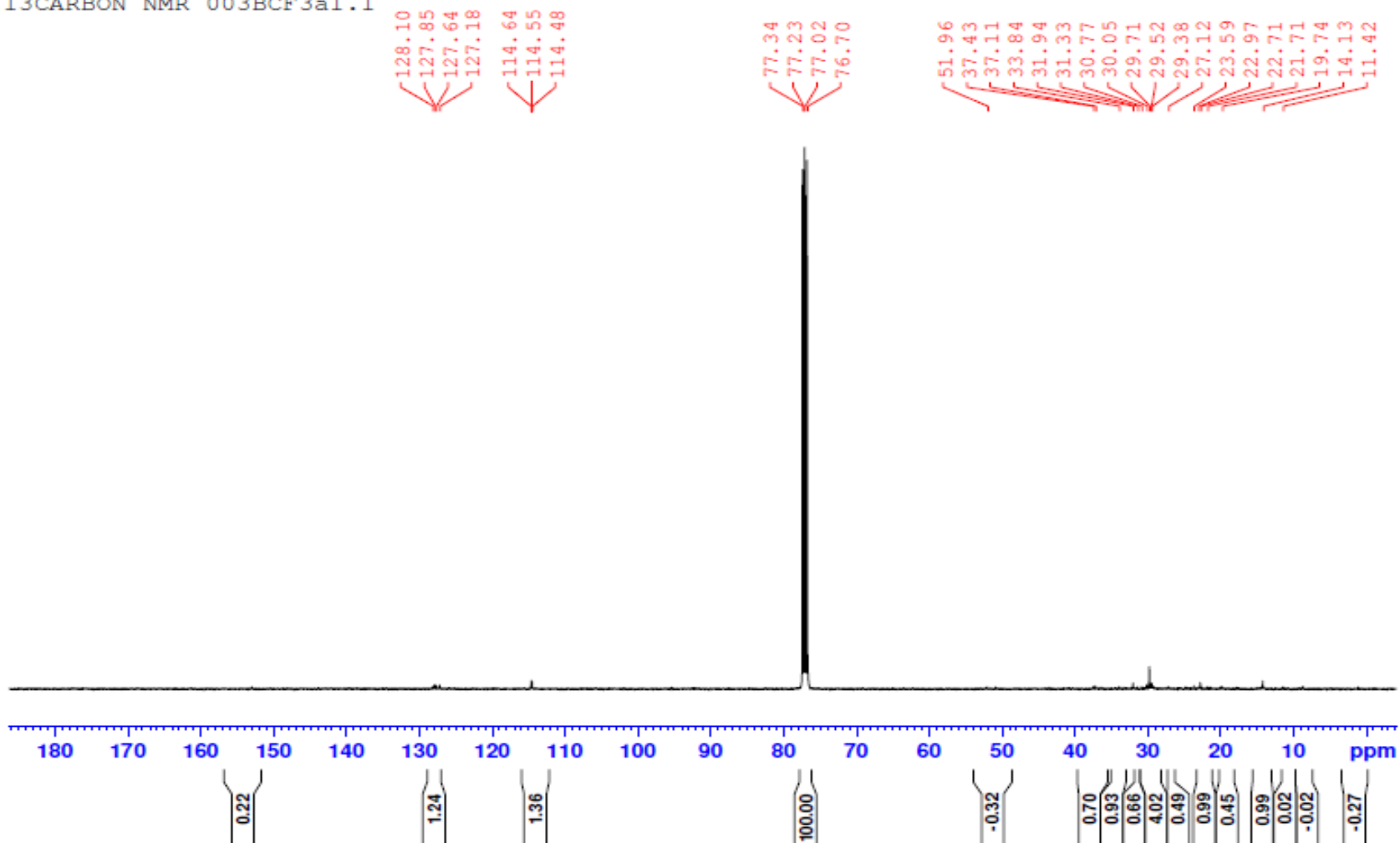
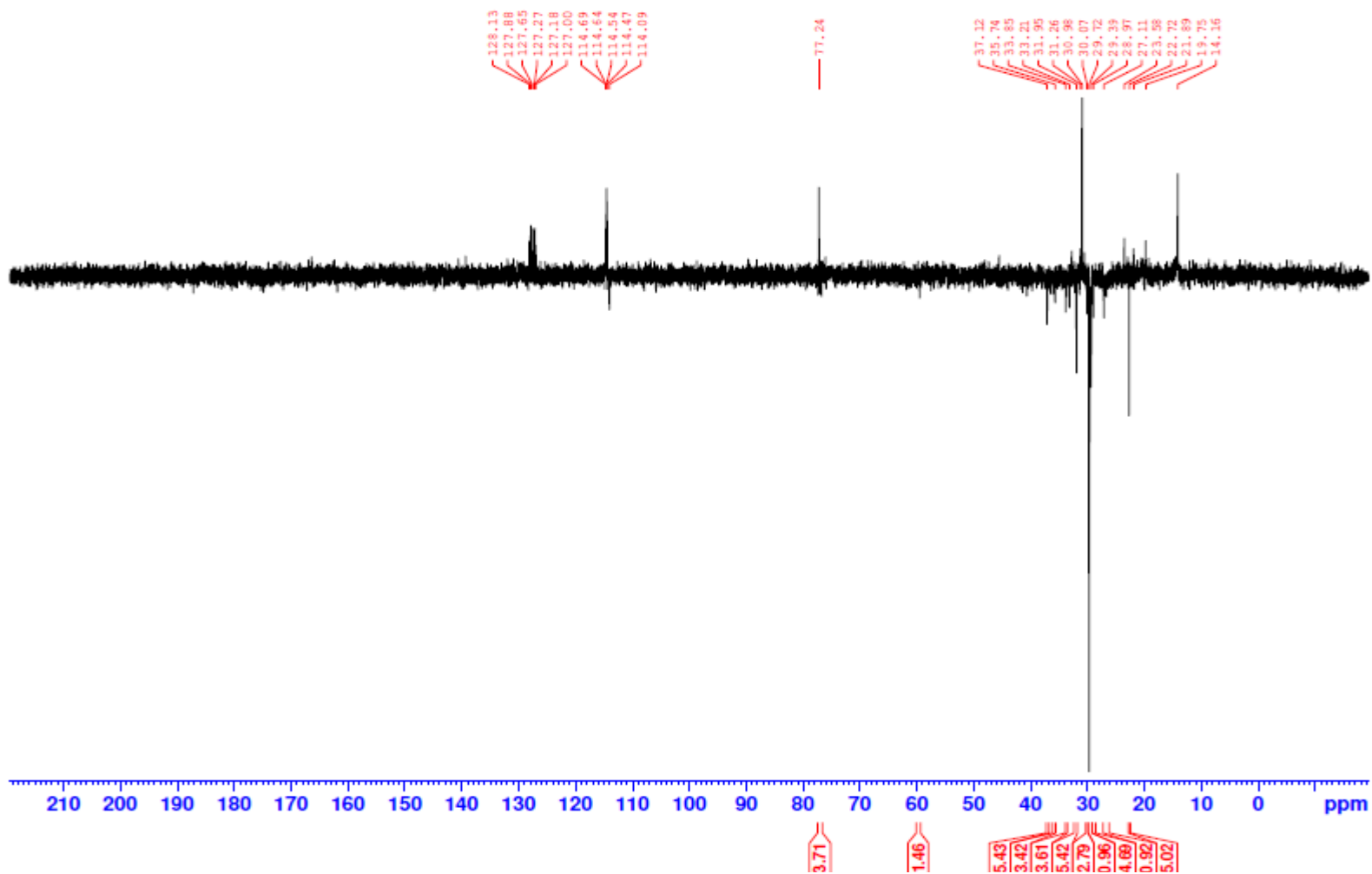


Figure 4.7 Proton 1H NMR Spectra (400MHz, 100MHz CDCl₃) For the Compound GB003BCF3a1.1

¹³CARBON NMR 003BCF3a1.1

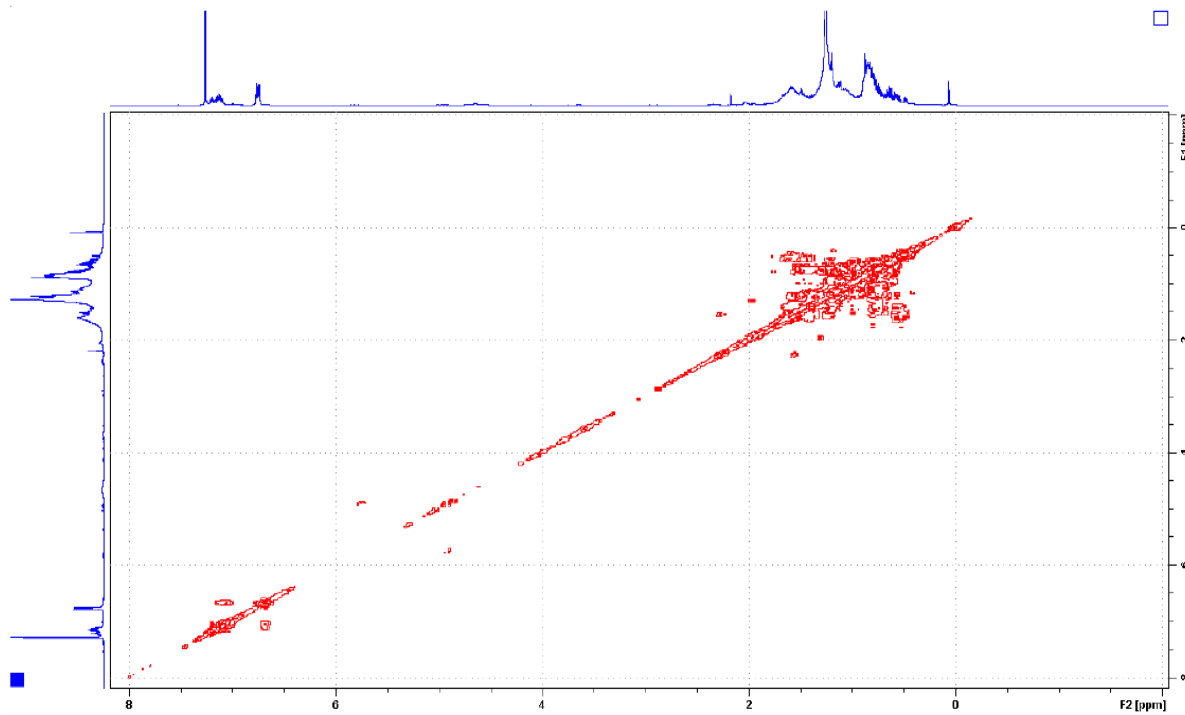


¹³C NMR Spectra (400MHz, 100MHz CDCl₃) For the Compound GB3BCF3a1.1



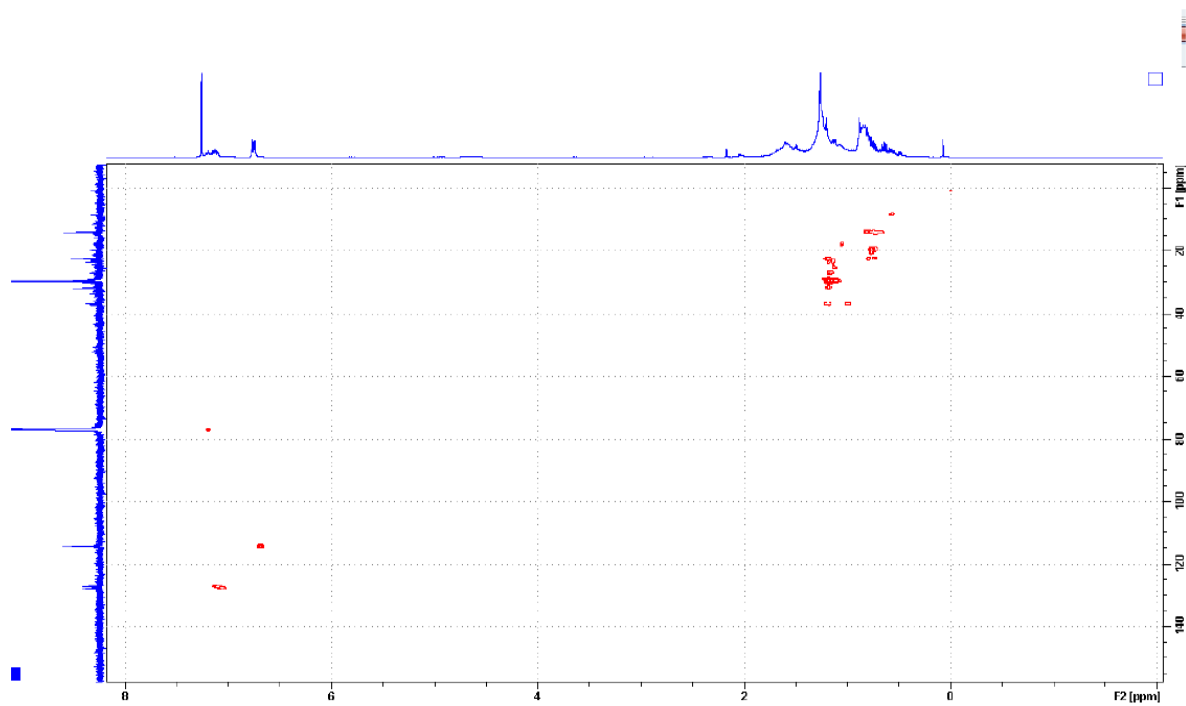
DEPT NMR Spectra (400MHz, 100MHz CDCl₃) For the Compound GB3BCF3A1.1

COSY GB003BCF3a1.1



COSY NMR Spectra (400MHz, 100MHz CDCl₃) For the Compound GB3BCF3A1.1

HSQC GB003BCF3a1.1



HSQC NMR Spectra (400MHz, 100MHz CDCl₃) For the Compound GB3BCF3A1.1