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COLLEGE OF BASIC AND APPLIED SCIENCES

ASSESSMENT OF ANTIPLASMODIAL ACTIVITIES OF SELECTED HERBAL PRODUCTS USED FOR THE TREATMENT OF MALARIA IN GHANA

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL IN APPLIED PARASITOLOGY DEGREE

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DECLARATION

I hereby declare that except for references to other people's work, which has accordingly been acknowledged, this thesis is the result of my own research performed at the Department of Epidemiology, Department of Clinical Pathology and Department of Animal experimentation of the Noguchi Memorial Institute of Medical Research, University of Ghana as well as the Department of Geography, University of Ghana under the supervision of Prof. Neils Ben Quashie (Director, Center for Tropical Clinical and Pharmacology and Therapeutics, University of Ghana Medical School), Dr. Daniel Oduro (Department of Animal Biology and Conservation Science) and Dr. Nancy Quashie (Department of Epidemiology, Noguchi Memorial Institute for Medical Research).

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DEDICATION

I dedicate this thesis to Almighty God for his divine protection, guidance and knowledge throughout this thesis. I also dedicate this work to my parents Mr. and Mrs. Zoiku, my uncle Mr. Kporgbe Gershion and my siblings for their immense support, advice, love and prayers. I further dedicate this work to Daisy Dorcas Ofoli Annang for her encouragement and motivation throughout the entire work.

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Table of Contents

DECLARATION i
DEDICATION ii
ACKNOWLEDGEMENTS iii
LIST OF FIGURES x
LIST OF PLATES xi
LIST OF ABBREVIATIONS xii
ABSTRACT xv
CHAPTER ONE 1
1.0 Introduction 1
1.1 Background 1
1.2 Problem Statement 3
1.3 Justification 4
1.4 Hypothesis 5
1.5 Aim and Objectives 6
1.5.1 Aim 6
1.5.2 Specific Objectives 6
CHAPTER TWO 7
2.0 LITERATURE REVIEW 7
2.1 Overview of Malaria 7
2.2 History of Malaria 8
2.3 Transmission of malaria 10
2.3.1 Transmission intensity and endemicity 12
2.4 Life cycle of Malaria Parasite 13
..... 15
2.5 Clinical Presentation of Malaria 15
2.5.1 Severe *P. falciparum* malaria 17
2.5.2 Severe non-falciparum malaria 18
2.5.3 P. falciparum Severe Malaria Pathogenesis 19
2.6 Diagnosis and Treatment of Malaria 20
2.6.1 Microscopy 20
2.6.2 Diagnostic Rapid Tests 20
2.6.3 Molecular techniques 21
2.6.4 Malaria Treatment 22
2.7 Malaria Epidemiology 22

University of Ghana http://ugspace.ug.edu.gh

2.7.1 Global Malaria Situation 23
2.7.2 Situation of malaria cases in Ghana 26
2.8 Malaria Prevention and Control Strategies 27
2.8.1 Vector control 27
2.8.2 Parasite control 28
2.9 Challenges to Malaria Control Efforts 28
2.9.1 Environmental factors 28
2.9.2 Vector transmission factors 29
2.9.3 Humans Factors 29
2.9.4 Parasite associated Factors 30
2.10 Need for new antimalarials 30
2.10.1 Combination therapy for Malaria 31
2.11 Antimalarial drug mechanism 33
2.12 Herbal Medicines or Herbal Products 33
2.12.1 Herbal Medicine's Global Distribution and Use 35
2.12.2 Increasing use of herbal medicine in developing countries 36
2.12.3 Herbal medicine's safety, efficacy, and quality 36
2.13 The role of natural products in the treatment of malaria 37
2.13.1 Antiplasmodial agents derived from natural materials 37
2.13.2 Plant-derived antiplasmodial agents 38
2.13.3 Natural Product Classes in Malaria Treatment 38
2.13.3.1 Alkaloids 38
2.13.3.2 Terpenoids 39
2.13.3.3 Quassinoids and limonoids 40
2.13.3.4 Flavonoids and Chalcones 41
2.13.3.5 Peptides 41
2.13.3.6 Xanthones, quinones, and coumarins 42
CHAPTER THREE 43
3.0 MATERIALS AND METHODS 43
3.1 Materials 43
3.1.1 Herbal products 43
3.1.2 Plasmodium species 43
3.1.3 Antimalarial control drugs and Reagents 44
3.1.4 Consumable 44
3.1.5 Equipment 44

University of Ghana http://ugspace.ug.edu.gh

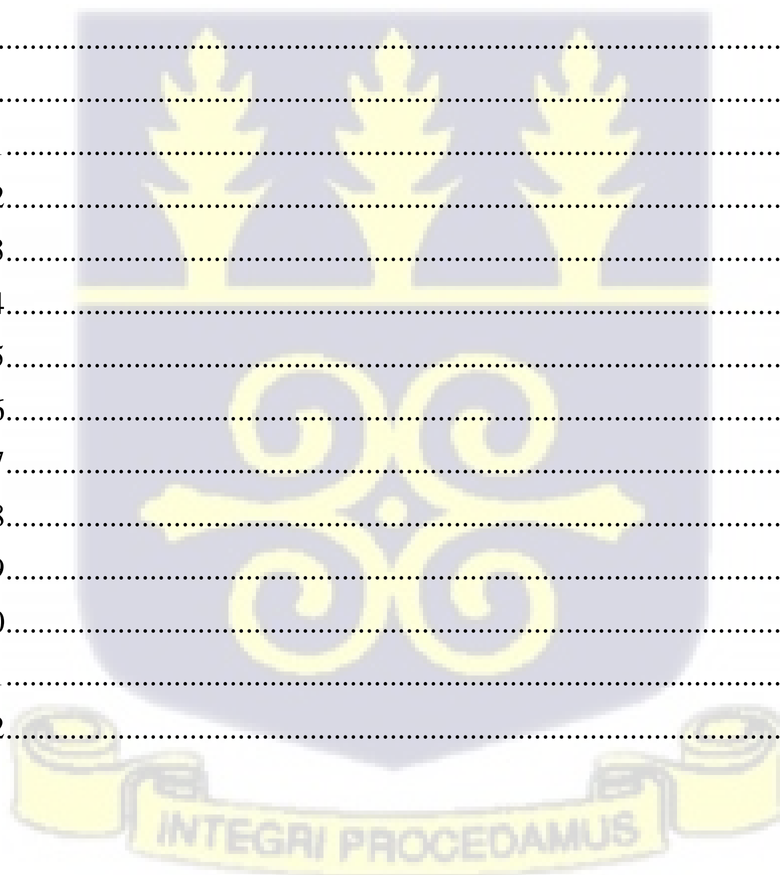
3.2 Ethics 44
3.3 Methods 45
3.3.1 General knowledge, perception and consumption of herbal use in Ghana 45
3.3.2 Freeze drying process..45
3.3.3 In vitro antiplamodial experiment 45
3.3.3.1 Preparation of medium 46
3.3.3.1.1 Incomplete medium...46
3.3.3.1.2 Complete medium 46
3.3.4 Washing of the O+ blood 46
3.3.5 Parasite culturing and preparation...46
3.3.6 Preparation of herbal extract 47
3.3.7 Herbal extract plating and assay 48
3.3.8 SYBR Green assay...49
3.3.9 Statistical Analysis 49
3.4 Ex vivo assay using P. berghei from infected mice 49
3.4.1 Statistical analysis 50
3.5 Heme crystallization inhibition mechanism using Colorimetric Inhibition Assay 50
3.5.1 Preparation of hemin chloride 51
3.5.2 Preparation of drug compounds 51
3.5.3 Evaluation of availability of BH formation induced by tween 20 and heme crystallization inhibition 51
3.5.4 Statistical Analysis 52
3.6 Cytotoxicity Assay 52
3.6.1 Statistical Analysis 52
3.7 Heavy metals and electrolyte analysis...53
3.7.1 Sample digestion 53
3.7.2 Sample analysis...53
3.7.3 Health risk assessment of heavy metals in herbal products 53
3.7.3.1 Metal Pollution Index (MPI) of Herbal Products...53
3.7.3.2 Average Daily Dose (ADD) of metals or Average Daily Intake (ADI) of metals 54
3.7.3.3 Non-carcinogenic risk assessment of heavy metals intake in the herbal products 54
3.7.3.3.1 Hazard Quotients (HQ) 54
3.7.3.3.2 Hazard Index (HI) 55
3.7.3.4 Carcinogenic risk assessment of heavy metal intake in the herbal products 55

University of Ghana http://ugspace.ug.edu.gh

3.8 High Performance Liquid Chromatography (HPLC) Analyses 55
3.9 PH contents of herbal products. 56
CHAPTER FOUR 57
4.0 RESULTS 57
4.1 General Knowledge, Perception and Consumption of Herbal Medicine 57
4.2 In vitro and ex vivo antiplasmodial activity of herbal drugs 59
4.3 Geometric mean IC_{50} values of Herbal drugs against Chloroquine-sensitive 3D7 Plasmodium falciparum Lab strain. 61
4.4 Dose response curve for herbal drugs on 3D7 Labstrain. 62
4.5 Geometric mean IC_{50} values of Herbal drugs against Chloroquine-resistant Dd2 *Plasmodium falciparum* Lab strain 63
4.6 Dose response curve for herbal drugs on Dd2 Labstrain 64
4.7 Geometric mean IC_{50} values of Herbal drugs against *Plasmodium berghei* 65
4.8: Dose response curves of herbal products on *Plasmodium berghei*. 66
4.10 Result for Mechanism of Action of various herbal products 67
4.11 Mean Cell Viability of various herbal products 68
4.12 Heavy metal levels from Atomic absorption spectrometry result of herbal drugs. 69
4.13 Metal pollution index calculated for various herbal products 72
4.14 Average daily dose (ADD) (mg/kg/day) of metals for exposure estimation assessment for herbal products 73
4.15 Hazard Quotients (HQ) and Hazard Index (HI) for Non-Carcinogen risk assessment 74
4.16 Carcinogenic risk assessment 76
4.17 Electrolyte contents from Atomic spectrometry result of herbal drugs. 77
4.18 pH levels of herbal drugs 79
4.19 HPLC chromatograms of herbal drugs 81
4.20 Peaks and respective retention times from HPLC Analysis of herbal drugs 82
4.21 Peaks and respective retention times from HPLC Analysis of some standard drugs. 83
CHAPTER FIVE 84
5.0 Discussion 84
5.1 Knowledge, Perception and Consumption of Herbal drugs 84
5.2 In vivo and ex vivo antiplasmodial activities 85
5.3 Mechanism of Action of herbal products 89
5.4 Heavy Metal Levels in Herbal Drugs 90
5.5 Electrolyte contents of the herbal drugs 93
5.6 pH Levels of the Herbal Drugs 94

University of Ghana http://ugspace.ug.edu.gh

5.7 HPLC Fingerprints of the herbal drugs 95
CHAPTER SIX 97
6.0 CONCLUSION, LIMITATION AND RECOMMENDATIONS 97
6.1 Conclusion 97
6.2 Limitations 98
6.3 Recommendation 98
REFERENCES 100
APPENDICES 129
APPENDIX 1 129
APPENDIX 2 131
APPENDIX 3 134
APPENDIX 4 137
APPENDIX 5 138
APPENDIX 6 139
APPENDIX 7 140
APPENDIX 8 141
APPENDIX 9 142
APPENIX 10 143
APPENDIX 11 144
APPENDIX 12 145
APPENDIX 13 149
APPENDIX 14 150
APPENDIX 15 152
APPENDIX 16 153
APPENDIX 17 154
APPENDIX 18 155
APPENDIX 19 156
APPENDIX 20 158
APPENDIX 21 159
APPENDIX 22 160



University of Ghana http://ugspace.ug.edu.gh

LIST OF TABLES

Table 4.1: Outcome of the priliminary survey on general knowledge, perception and consumption of herbal products in greater Accra, Ghana 58

Table 4.2: In vitro and ex vivo antimalarial activity of selected herbal drugs on Plasmodium falciparum and Plasmodium berghei and toxicity of these herbal products towards Human Red blood Cells..60

Table 4.3: Mean absorbance, f-Calculated and IC50 values for various herbal products/drugs in Drug mechanism assay 67

Table 4.4: Heavy metal contents of the selected herbal products..69

Table 4.5: Metal pollution Index (MPI) 72

Table 4.6: Average daily dose (ADD) (mg/kg/day) of metals for exposure estimation assessment for herbal product...73

Table 4.7: Hazard Quotients (HQ) and Hazard Index (HI) for Non-Carcinogen risk assessment...74

Table 4.8: Cancer risk assessment results for two heavy metals (Lead and Arsenic) 76

Table 4.9: Electrolyte content of selected herbal products...77

Table 4.10: pH content of the selected Herbal Products 79

Table 4.11: Retention times (min) of Herbal drug compounds 82

Table 4.12: Peak retention time for HPLC Analysis of pure drug 83

Table A9: Recommended daily limit for heavy metals. 142

Table A10: Toxicity response to heavy metals (Oral Reference Dose (RFD) 142

Table A12: Input parameters for Average Daily Dose (ADD)/ Estimated Daily Intake (EDI) calculation...144

Table A13: Reference values for electrolyte intake in mg/d 144

University of Ghana http://ugspace.ug.edu.gh

LIST OF FIGURES

Fig 2.0 Life cycle of malaria parasite 15

Figure 2.1 Global distribution of malaria 24

Fig 3.0 diagrammatic representation of the 96-well microtitre plate set-up used in this study. 48

Fig 4.0 Geometric mean of various IC_{50} values of herbal products for 3d7 Plasmodium falciparum Chloroquine sensitive strain. 61

Fig 4.1: Dose response curves of herbal products on 3d7 Plasmodium falciparum Chloroquine sensitive strain 62

Fig 4.2: Geometric mean of various IC_{50} values of herbal products for Dd2 Plasmodium falciparum Chloroquine resistance strain. 63

Fig 4.3: Dose response curves of herbal products on Dd2 Plasmodium falciparum Chloroquine resistance strain....................64

Fig 4.4: Geometric mean of various IC_{50} values of herbal products for Plasmodium berghei. 65

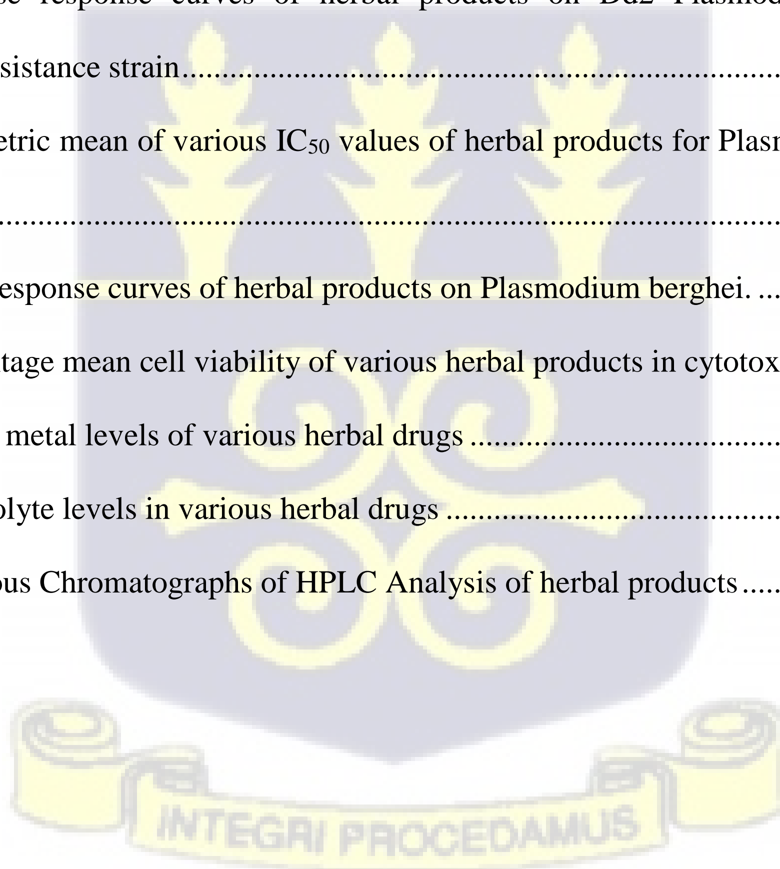
Fig 4.5: Dose response curves of herbal products on Plasmodium berghei. 66

Fig 4.6: Percentage mean cell viability of various herbal products in cytotoxicity assay.....68

Fig 4.7: Heavy metal levels of various herbal drugs 70

Fig 4.8: Electrolyte levels in various herbal drugs 78

Fig 4.10: Various Chromatographs of HPLC Analysis of herbal products....................81



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LIST OF PLATES

Plate A1: Freeze dryer Labconco 152

PlateA2: Preparation of herbal drug plates in the Biosafety hood......................................153

Plate A3: Reading drug plate with Fluostar Optima Plate Reader 153

Plate A4: Analysing herbal products for fingerprints using K15 A306 HPLC4 Machine. .154

Plate A5: Analysis of heavy metal and electrolytes contents of herbal products using Atomic absorption spectrometer...154

Plate A6: Asexual stage of Plasmodium falciparum 3D7 in a Geimsa stained slide. 155

Plate A7: Asexual stage of Plasmodium berghei in a Geimsa stained slide from maurine mice...155

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LIST OF ABBREVIATIONS

AAS	-	Atomic absorption spectrometer
ACT	-	Artemisinin-based combination therapy
AD	-	After death
ADD	-	Average Daily Dose
ADI	-	Average Daily Intake
AHM	-	Aseda Herbal Mixture
AM	-	Away Malamix
AQ	-	Amodiaquine
As	-	Arsenic
AS	-	Artesunate
AT	-	Average time
BC	-	Before Christ
BW	-	Body Weight
Ca	-	Calcium
CAM	-	Coma Acidosis Malaria
CAM	-	Complementary and Alternative Medicine
CC_{50}	-	Concentration at which 50% cytotoxicity occurred
CDC	-	Center for Disease Control
CM	-	Complete media
CO_2	-	Carbon dioxide gas
CQ	-	Chloroquine
CQR	-	Chloroquine-resistant
CQS	-	Chloroquine-sensitive
CR	-	Cancer risk
Cu	-	Copper
DDT	-	Dichlorodiphenyltrichloroethan
DMSO	-	dimethyl sulfoxide
DNA	-	Deoxyribonucleic acid
ED	-	Exposure duration

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EDTA - Ethylenediamine tetraacetic acid

EF - Exposure Frequency

FDA - Food and Drug Authority

GHM - Givers Herbal Mixture

GM - Geo Manuel

HEPES - 4 -(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid

Hg - Mercury

HI - Hazard Index

HPLC - High Performance Liquid Chromatography

HQ - Hazard Quotient

HRP2 - Histidine Rich Protein 2

IC_{50} - Concentration inhibiting 50% of growth

IPT - Intermittent preventative treatment

IR - Ingestion rate

IRB - Institutional Review Board

IRS - Indoor residual spray

ITN - Insecticide treated net

K - Potassium

KH_2PO_4 - Potassium dihydrogen phosphate

LAMP - Loop-mediated isothermal amplification

LDH - Lactate dehydrogenase

Mg - Magnesium

MICS - Multiple Indicator Cluster Survey

MPI - Metal Pollution Index

MQ - Mefloquine

MSA - Malaria Severity Assessment

MT - Mala Typhs

MTPR - Fluorescence plate reader

N_2 - Nitrogen gas

Na - Sodium

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Na_2HPO_4	-	Disodium hydrogen phosphate
NaCl	-	Sodium Chloride
NMCP	-	National Malaria Control Programme
NMIMR	-	Noguchi Memorial Institute for Medical Research
O_2	-	Oxygen gas
OPD	-	Outpatient department
OTM	-	Osompa T Malamix
Pb	-	Lead
PCR	-	Polymerase Chain Reaction
PfEMP-1	-	Plasmodium falciparum erythrocyte membrane protein 1
PH	-	Potential of Hydrogen
RBCs	-	Red blood cells
RDTs	-	Rapid diagnostic tests
RFD	-	Reference Dose
RI	-	Resistance index
RMPI	-	Roswell Park Memorial Institute Media
SI	-	Selectivity Index
SP	-	Sulfadoxine pyrimethamine
TH	-	Typhofa herbal
UNICEF	-	United Nations International Children's Fund
USEPA	-	United State Environmental Agency
WBC	-	White blood cell
WHO	-	World Health Organization
Zn	-	Zinc

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ABSTRACT

Malaria continues to be a menace in many parts of the world and remains a global public health problem, contributing to high morbidity and mortality especially in developing countries including Ghana. Even though Artermisinin-based Combination Therapies (ACTs) still remain efficacious in the treatment of malaria, most of the populace in Ghana rely heavily on medicinal plants and herbal preparations for the treatment of malaria and other infectious diseases. The seven herbal products were selected based on a preliminary survey conducted on knowledge, perception and consumption of herbal products in Greater Accra Region. The most commonly used Antimalarial herbal medications selected were Away, Givers, Osompa, Typhofa, Malatyphs, Aseda and Geo Manuel Herbal drugs. These herbal drugs were therefore assessed for their antiplasmodial activities using *in vitro* and *ex vivo* sensitivity testing on chloroquine (CQ)-sensitive (3D7), CQ-resistant (Dd2) strains of *Plasmodium falciparum* and *Plasmodium berghei.* The SYBR-Green 1 fluorescence-based method was used for the analysis. The results showed IC_{50} values ranging from 1.16µg/ml to 56 µg/ml for all the *Plasmodium* strains. However, there was a significant difference between the IC_{50} values of the standard control drugs ($p<0.05$) for both 3D7 and Dd2 strains. For the mechanism of action, a simple colorimetric inhibition of heme crystallization method was used and the IC_{50} were determined which ranged from 1.86µg/ml to 122.2µg/ml for the herbal drugs. Cytotoxicity of the herbal drugs were assessed with MTT assay using human red cells and selectivity index ranged from 4.42 to 405.60 with cell viabilities above 60% indicating no *in vitro* cytotoxic effects to the human red cells. To determine the heavy metal and electrolyte contents of the herbal drugs, atomic spectrometry method was used and the results ranged from 0.1mg/Kg to 12.8mg/kg of the heavy metals with significant differences ($p<0.05$) among all the seven herbal products. For the electrolytes, results ranged from 1.1mg/kg to 1400mg/Kg with no significant difference

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(p>0.05) among the herbal drugs. The pH of the selected herbal drugs determined were below 5 (3.56-4.61) indicating acidic nature of the herbal products. High Performance Liquid Chromatography (HPLC) was used to determine the fingerprint of the seven herbal products and the result indicated the presence of varying active compounds from 9 to 16 with no similarities in retention times (min) as compared to other standard drugs used.In conclusion, these results show that the selected herbal drugs have antiplasmodial activities with no cytotoxic effect to human red cells but slightly acidic in nature.

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CHAPTER ONE

1.0 Introduction

1.1 Background

Malaria remains the most common cause of morbidity and death in sub-Saharan Africa. Malaria caused 241 million cases in 85 malaria endemic countries and 627,000 deaths in 2020, according to the 2021 World Malaria Report, with Africa accounting for 95% of cases (228 million) and 96% of deaths. Malaria is a serious threat to children under the age of five, with children under the age of five years accounting for 80% of all malaria deaths in Africa (WHO, 2021).

Malaria is an infectious disease caused by a protozoan of the genus *Plasmodium* and is spread from person to person by the bite of an infected female Anopheles mosquito. *Plasmodium* parasites, *P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi* are known to cause malaria in humans worldwide (Kantele *et al*., 2011). *P. falciparum* is the most dangerous and widespread malaria parasite in sub-Saharan Africa. Fever, chills, and anemia are the common clinical symptoms as well as dementia, metabolic acidosis, and multi-organ system failure which can lead to coma and death (Osei-Djarbeng *et al*., 2015).

Malaria is hyper-endemic in Ghana, and the disease poses a year-round hazard to the entire population and is the leading cause of morbidity in the country (Osei-Djarbeng *et al*., 2015). The disease accounts for 38% of all out-patient illnesses, 36% of all hospital admissions and 33% of fatalities among children under the age of five years (Osei-Djarbeng *et al*., 2015). Furthermore, it is projected that 3.1 to 3.5 million cases of clinical malaria are reported to health institutions each year, including 900,000 children under the age of five. Malaria caused almost 14,000 deaths in 2008 in Ghana. (Osei-Djarbeng *et al*., 2015).

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The World Health Organization (WHO) approved the use of artemisinin-based combination therapy (ACT) as a first-line treatment for uncomplicated malaria in the early 2000s, and it has since been widely utilized to manage the condition in most disease-endemic nations. In Ghana, two regimens of ACT are used as first line medication for uncomplicated malaria, artesunate-amodiaquine (AA) andartemether-lumefantrine (AL), whilst dihydroartemisinin-piperaquine (DHAP) is used as second line drug according to 2017 Standard Treatment Guidelines by Ministry of Health. The use of the ACT in conjunction with other interventional approaches has resulted in a dramatic drop in the incidence of malaria in recent years. Despite this improvement and claims that the ACT is still effective in Ghana, commercial anti-malarial herbal treatments continue to be popular (Wilmot *et al*., 2017).

Herbal remedies have been utilized for a variety of ailments since ancient times. Medicinal plants have had a significant impact on global health. Despite significant breakthroughs in modern medicine in recent decades, plants continue to play an essential role in health care. Medicinal plants can be found throughout the world, but they are numerous in tropical areas. Interest in drugs generated from higher plants, particularly phytotherapeutic drugs, has skyrocketed in recent times. Higher plants are thought to be responsible for about 25% of all contemporary medicine, either directly or indirectly (Calixto, 2000).

Herbal antimalarials have been increasingly popular and may be chosen for their availability, relative affordability and efficacy (Willcox & Bodeker, 2004). Furthermore, for decades, plants have provided the majority of effective antimalarials, either directly or indirectly. For example, quinine, an aminoquinoline alkaloid, since its discovery in several *Cinchona* species (Rubiaceae), has been used for about 400 years (Osei-Djarbeng *et al*,

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2015). Synthetic quinine derivatives such as mefloquine, primaquine, and chloroquine have also been used to treat or prevent malaria at various stages (Saxena, 2003). Artemisinin and its derivatives from the Chinese medicinal plant *Artemisia annua* have also been identified as one of the most effective antimalarials, whether used in monotherapy or in combination with other drugs (Mercereau-Puijalon & Fandeur, 2003). Traditional antimalarials are widely used in rural communities, where people look for treatments in their surroundings. Herbal cures are significant not only in rural areas of Ghana; they are also essential in urban areas where conventional medicines are available (Asase & Oppong-Mensah, 2009).

1.2 Problem Statement

Herbal medicine use has risen dramatically in the world. Global sales of herbal goods were expected to exceed $60 billion in 2000, according to the Secretariat of the Convention on Biological Diversity (WHO, 2004). Herbal extracts in combination have been used in cultural systems for decades. Traditional Chinese medical knowledge and practice indicated the *A. annua* was used in combination with other plants for the treatment of fevers (Mojab, 2012).The dangers of natural herbal treatments are still on a rise (Ernst, 2006). The poor quality of the completed goods is responsible for the adverse events, with some of them arising from tainted raw herbal materials (WHO, 2004). Heavy metals, pesticides, microorganisms, and mycotoxins are among the contaminants most likely to be found in herbal products (Zhang, 2012).

Adulteration implies "to make impure by introducing additional, incorrect, or inferior components" and is usually deceptive. Herbal remedies have been found to be contaminated with traditional pharmaceuticals and plant ingredients on several occasions. Adulterations are divided into three categories: the addition of conventional medications to herbal remedies, substitution by the use of fake or inferior plant components and the addition of

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foreign materials (Zhang, 2012). In addition there is an ongoing problem with unexpected toxicity of herbal products due to quality issues, including use of poor quality herbal material, incorrect or misidentified herbs, incorrect processing methods, supply of adulterated or contaminated herbs or products (Shaw, 2010).

1.3 Justification

According to the WHO, roughly 65% to 80% of the world's population living in poorer nations rely on plants for basic health care due to poverty and lack of access to modern medicine (Faiz & Faiz, 2021). Medicinal plants have a long history of being used in the treatment of ailments all across the world. Traditional medicines are used by about 80% of the population in impoverished nations to cure a variety of maladies, including life-threatening ones like malaria (Komlaga *et a*l., 2015). Medical plants, which are the foundation of traditional medicine in sub-Saharan Africa, offer a wide range of applications despite the lack of scientific evidence to back them up. Plants used to treat malaria and fever make up 6% of the medicinal plants sold in Ghana and some of these plants have been turned into commercial phytomedicines (Van Andel *et al.*, 2012; Komlaga *et a*l., 2015).

The WHO reported the following issues with herbal medicines in a survey conducted in 129 countries: a lack of research data, appropriate mechanisms for herbal medicine control, education and training, expertise within national health authorities and control agencies, information sharing, safety monitoring as well as data on safety and efficacy (WHO, 2004). As the use of herbal medicines has increased, so are the reports of suspected toxicity and adverse events. The safety of herbal medicines has become an issue for the regulatory authorities, as serious effects have been reported, including hepatotoxicity, renal failure and allergic reactions (Perharic *et al.*, 199; Nortier and Vanherweghem, 2007). An investigation of the safety of 260 patented Asian medicines found that over 25% of them included

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dangerously high levels of heavy metals, while another 7% had unidentified new substances added to them to achieve the desired results (Mutua *et al.*, 2016). After taking the Chinese medicinal extract, Aristolochia Fangchi, for weight loss, 105 people in Belgium developed serious nephropathy. In the same study, 12 individuals developed urothelial cancer, 43 suffered end-stage renal failure, and 39 individuals required preventive kidney removal (Nortier *et al.*, 2000).

Even though many herbal antimalarial products are being used for the treatment of malaria and some of these herbal products are seen to be efficacious in the treatment of malaria (Cudjoe *et al.*, 2020), but their mechanism of action are still unknown. Based on the aforementioned issues, it is important to investigate these herbal preparation to ascertain their medicinal properties such as the antiplasmodial activities as well as safety and mechanisms of action. The findings from the data generated is important for stakeholders and policy makers in the health care system as well as Food and Drug Authority post market surveillance.

1.4 Hypothesis

Due to the availability of herbal medicines in the country, majority of Ghanaians may have depend on herbal treatment for malaria and other disease conditions. It is also hypothesized that due to improper control system, herbal products may not have required activity and unsafe for human use.

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1.5 Aim and Objectives

1.5.1 Aim

To assess the antiplasmodial activities of selected Ghanaian herbal products used in treating malaria in Ghana as well as their safety and their possible mechanism of action against *P. falciparum*.

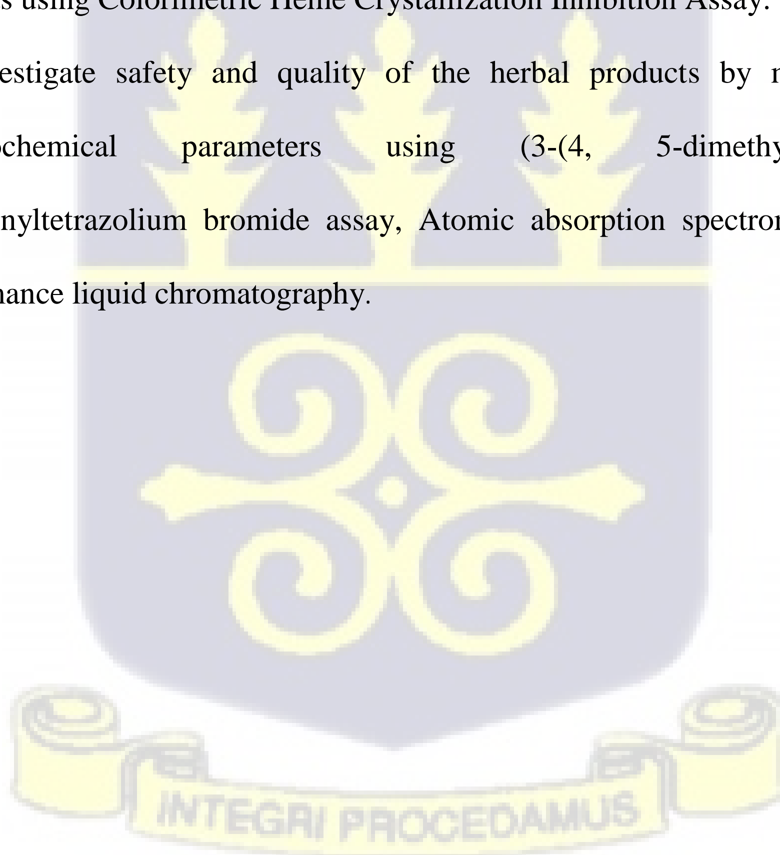
1.5.2 Specific Objectives

1. To determine the extent of herbal usage and the most commonly used herbal products for malaria treatment in Ghana.

2. To determine the antiplasmodial activities of the herbal products using *in vitro* and *ex vivo* SYBR Green assay.

3. To investigate possible heme crystallization inhibition mechanism of these herbal products using Colorimetric Heme Crystallization Inhibition Assay.

4. To investigate safety and quality of the herbal products by measuring some physicochemical parameters using (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay, Atomic absorption spectrometry and High performance liquid chromatography.



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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Malaria

Malaria is one of the most common infectious diseases in underdeveloped nations, and it is responsible for a high rate of mortality and morbidity. The disease is caused by apicomplexan protozoan parasites of the genus *Plasmodium*, which are known to possess an apicoplast, an essential organelle for the parasites' metabolic processes (Kantele *et al.*, 2011). *P. falciparum*, *P. vivax, P. ovale*, and *P. malariae* are the four *Plasmodium* parasites that can infect humans. *P. knowlesi*, which was previously only known to infect monkeys, has now been linked to human infection (Singh *et al.*, 2004). *P. falciparum* is the most dangerous parasite and is responsible for a significant portion of worldwide mortality. It is the most prevalent human parasite in Africa, and it can be found in all malaria-endemic areas worldwide (WHO, 2005). Even in malaria-endemic locations, the frequency of *P. malariae* is low, and *P. ovale*-related illness is uncommon (Mims *et al*., 2004; Mueller *et al.*, 2007).

Anemia, as well as a variety of consequences, such as hypoglycemia, cerebral malaria, metabolic acidosis, and respiratory distress, are all clinical symptoms of this condition. Fever, chills, convulsions, headache, nausea, lack of appetite, diarrhea, weakness, vomiting, and malaise are all common symptoms and complaints (English *et al*., 1996, Biersmann *et al*., 2007; Legros *et al*., 2007). Malaria affects an estimated 218- 269 million people worldwide in 2020, with a fatality rate of 583-765 million people with 77% deaths in children under five years of age (WHO, 2021). Malaria poses a serious threat to children under the age of five, as well as pregnant women (Desai *et al*., 2007). Malaria during

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pregnancy might result in a low birth weight, lowering the likelihood of survival (Rowe and Kyes, 2004). Malaria-induced anemia and jaundice can result from the rupture and lysis of red blood cells. If not treated promptly, the condition can be fatal, and it can also allow opportunistic infections to flourish (Snow *et al.*, 2005; WHO, 2005).

2.2 History of Malaria

Malaria has been documented for almost 4,000 years, and it is thought that malaria has had a significant impact on human populations and history (CDC, 2010). The disease was once thought to have originated in fetid marshes, leading to the term "mal aria," which means "foul air." The fevers are thought to be caused by miasmas rising from marshes, and it is usually assumed that the name malaria comes from the Italian “mal aria”, which means poor air. This term was coined by the Italians to describe the link between intermittent fever and marsh air exposure (CDC, 2010). The name comes from the concept that sickness begins with the collection of poorly-contaminated stagnant water (Kondrasen *et al.*, 2004). Previously, the disease was supposed to be of supernatural origin, as people who became ill were considered to have offended their gods. Hippocrates was the first to correct this erroneous assumption (CDC, 2010). He linked the parasite and its symptoms to seasonal and geographical variations (CDC, 2010).

Malaria cases have been documented in Egypt and China for over 6000 years, and in India since 1600 BC (WHO, 1986). It is a geographically restricted disease, but major migrations of migrant laborers, refugees, and non-immune travelers across borders have played a significant role in its global expansion (Rooth, 1992). The disease entered the Mediterranean Sea's beaches between 2,500 and 2,000 years ago, according to available sources, while its presence in northern Europe was around 500 to 1000 years ago (Carter and Mendis, 2002). Malaria was believed to have been introduced into the New World by

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Europeans and West Africans towards the end of the 15th century AD. *P. falciparum* was most likely introduced to the United States by African slaves brought in by Spanish colonial lords (Carter and Mendis, 2002).

By the 19th century, more than half of the world's population was at risk of malaria, and the mortality rate was rising at an alarming rate. The discovery of microorganisms by Antoni van Leeuwenhoek in 1676 heightened the hunt for the source of malaria. The germ hypothesis of infection, developed by Louis Pasteur and Robert Koch in 1878-1879, and the identification of microbes as the causative agents of infectious disorders, fueled this search even further (Cox, 2010). In 1889, a military doctor, Alphonse Laveran, named the etiologic agent as a protozoan parasite while working in Algeria (CDC, 2004). Ronald Ross discovered the female Anopheles mosquito as the carrier of these protozoan parasites eight years later (CDC, 2004; CDC, 2010). Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli, Angelo Celli, Camillo Golgi, and Ettore Marchiafava, Italian malaria researchers, established definitively in 1898 that human malaria was transmitted by anopheline mosquitoes (CDC, 2010).

Two years later, Giovanni Batista Grassi and Raimondo Filetti were the first to coin the terms *P. vivax* and *P. malariae* for two human malaria parasites (CDC, 2010). The movement of malaria parasites through pre-erythrocytic stages in the liver before entering the bloodstream was discovered by Henry Shortt and Cyril Garnham in 1948. In 1982, Wojciech Krotoski discovered the parasite's dormant stages in the liver, which he described as the parasite's ultimate stage in its life cycle (Cox, 2010; CDC, 2010). Insecticide-treated bednets, repellents, and protective clothes were recommended as preventative measures. Unfortunately, due to the development of resistance by vector species to pesticides such as

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dichlorodiphenyltrichloroethane (DDT), comprehensive malaria control has been pushed back (Subbarao, 1988; Baird, 2000; Corbel *et al.,* 2007).

The first documented therapy for malaria was in the year 1600. This can be traced back to local Peruvian Indians who employed the Cinchona tree's bitter bark to treat fever (CDC, 2010). Until the Chinese developed another effective medication from the worm wood *Artemisia annua L.*, quinine from Cinchona tree extracts was the backbone of treatment (CDC, 2010). Artemisinin (Qinghaousin) was initially isolated in 1971 from the aerial portions of *A. annua L.*, a Chinese plant used in the treatment of fever and malaria in traditional Chinese medicine (Klayman, 1985; Noedl, Teja-Isavadharm & Miller, 2004). Artemisinin's medicinal efficacy is hampered by a number of issues, including its short half-life, neurotoxicity, and low solubility, all of which reduce its bioavailability (Balint, 2001).

Malaria has been eradicated in Europe and North America, according to records (WHO, 2005). The issue of "imported malaria" from migrant workers and tourists from a variety of endemic countries, however, continues to be a source of concern. This anxiety has been heightened by the advent of drug-resistant parasites. The first report of parasite resistance to chloroquine was made in East Africa in 1978 (Atroosh *et al*., 2012). According to Huong *et al*. (2001), the sensitivity to artemisinin has decreased. The WHO has advocated the use of artemisinin-based combination therapy to combat parasite resistance to currently available antimalarials (WHO, 2006).

2.3 Transmission of malaria

The parasite is transmitted to humans through intravenous inoculation of sporozoites from an infected female anopheles mosquito. These sporozoites are not harmful in and of

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themselves; rather, they are a temporary phase whose survival and growth are required for the parasite's life cycle to be completed and subsequent transmission to occur. The sporozoites infect hepatocytes, multiplying fast for a week or two before releasing thousands of merozoites. According to Ménard (2000), each successful sporozoite has the ability to produce 20,000 merozoites. Merozoites break hepatic cells and infiltrate erythrocytes, where they multiply and infiltrate new erythrocytes. The asexual replication process continues, but some of the cells develop into gametocytes. These gametocytes are the stages of the female anopheles mosquito's blood meal that it consumes. For around two weeks, the parasite develops in the mosquito vector. Ingested gametocytes split into gametes, which combine in the midgut to generate zygotes. The zygotes, in turn, evolve into ookinetes, which pierce the gut wall and mature into oocysts. The oocytes burst, releasing sporozoites that move to the mosquito vector's salivary gland. The sporozoites are injected into the next host as the mosquito takes a blood meal, which is required for its reproductive process. This maintains the transmission. The existence of the parasite and a human host, as well as an anthropophylic (human-biting) vector, is required for successful and efficient transmission. The anthropophilic propensity of *A. gambiae* is unusual. An endophagic or exophagic (biting indoors and outdoors) female Anopheles mosquito and an exophilic (resting outside) male Anopheles mosquito are required for effective transmission (WHO, 2004). Female mosquitoes require a blood meal in order to lay eggs (Moorthy *et al*., 2004). Malaria can be passed down through the generations or transmitted directly by blood transfusion, organ transplantation, or sharing needles. These transmission mechanisms do not require a vector because the transmission is direct. In a warmer environment, the parasite extrinsic cycle shortens, whereas temperatures below 16 ^{0}C have a detrimental effect on the extrinsic cycle of malaria parasites and the biting activity of vector species (Colluzi, 1999). This could explain why the disease is endemic in tropical and warm

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climates around the world. Concerns about global warming and its implications for malaria prevalence and distribution may be linked to the temperature impacts seen. Hot weather may also lead to people sleeping outside without bed nets, exposing their skin to mosquito bites (CDC, 2010). The genetic composition of the population can influence the rate of transmission in people, and, for example, sickle cell anemia carriers are usually immune to malaria (Carter and Mendis, 2002). Variations in a population may also be caused by innate, inborn, or acquired immunity. These variances are also influenced by vectoral capability and preferences for biting and resting (WHO, 2004; CDC, 2010).

2.3.1 Transmission intensity and endemicity

The clinical manifestation of malaria is very much tied to previous exposure, which in turn depends on transmission intensity and endemicity in the area where an individual is living, as will be addressed in more detail below. Previously, the strength of transmission was determined by counting the number of children in a certain area who had enlarged spleens. The parasite rate, which measures the incidence of peripheral blood-stage infection in children aged 2 to 9 years, is now widely employed (WHO, 2012). Traditionally, four terms have been used to classify endemicity: holoendemic (> 75 percent parasite/spleen rate in 2-9-year-old children), hyperendemic (50-75 percent), mesoendemic (11-50 percent), and hypoendemic (less than 10%), which correspond to the more commonly used terms high, moderate, and low transmission (WHO, 2015). These are obviously very basic endemicity estimates, and there is a lot of variance and clustering within a country, and there can even be noticeable variances within families (Kreuels *et al.*, 2008).

Malaria transmission is defined by its consistency over time. Malaria transmission has remained consistent in wide regions of sub-Saharan Africa for number of years, and people are constantly exposed to malaria infection, though with seasonal variation. In contrast, epidemic transmission, which is more common in Asia and Latin America, is marked by

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inconsistency and significant seasonal and regional variation. The entomological inoculation rate (EIR), or the number of infected bites per person per year, is a more straightforward method for estimating transmission levels that also captures variation (Mugenyi *et al.*, 2017; Amoah *et al.*, 2021).

2.4 Life cycle of Malaria Parasite

The inoculation of *Plasmodium* sporozoites from the salivary glands of a biting female Anopheles mosquito into the bloodstream of a human, or into the subcutis, from where they trickle into the bloodstream over a few hours, invading liver cells (hepatocytes), is the first step in a malaria infection (Yamauchi *et al.*, 2007).The sporozoites are only present in the blood circulation for 60 minutes following inoculation, according to early experimental experiments in humans (Fairley, 1947, Vanderberg,1975; Yamauchi *et al.*, 2007). Sporozoites multiply asexually (exo-erythrocytic schizogony) in hepatocytes, forming pre-erythrocytic schizonts with thousands of daughter merozoites. The schizonts mature and break, releasing the merozoites into the blood stream. This maturation process can take anywhere from 6 to 21 days, depending on the species, and it correlates to the incubation time when combined with the considerably shorter cycle of erythrocytic schizogony that follows. As a result, this stage of the infection is symptomless. *P. vivax* and *P. ovale* sporozoites can evolve into hypnozoites, a latent state that can last for months to years, with relapse frequency influenced by transmission seasonality, before resuming schizogony and blood stage infection (White, 2011).

The released merozoites then invade the erythrocyte through a multi-step process involving merozoite surface proteins (MSP), apical membrane antigens (AMA), erythrocyte binding antigens (EBA), and *P. falciparum* reticulocyte binding homologue proteins (PfRh) (Cowman *et al.*, 2012), several of which have been considered as vaccine candidates.

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Asexual reproduction (erythrocytic schizogony) occurs within the erythrocyte, and merozoites evolve into trophozoites (also known as mature ring-forms, a stage easily recognizable in microscopy) and eventually erythrocytic schizonts. The erythrocyte ruptures, releasing 6-36 merozoites (most of which are seen in *P. falciparum* schizonts), which then enter into the new red blood cells. This is when the clinical infection begins. *P. knowlesi* requires 24 hours for erythrocytic replication, 48 hours for *P. falciparum, P. vivax,* and *P. ovale*, and 72 hours for *P. malariae.*

The parasite requires resources from its host through the replication stages in both hepatocytes and erythrocytes. The permeability of the human erythrocyte membrane is increased during invasion, allowing essential nutrients to be taken up more quickly (Kirk 2001). Ion, aminoacid, lipid, and vitamin fluxes were shown to be higher in infected erythrocytes than in uninfected cells (Kirk and Lehane, 2014), and glucose inflow was shown to be many times higher in infected erythrocytes than in uninfected cells (Mehta *et al*., 2005).

A complex protein machinery develops in the erythrocyte at the same time as the parasite replicates, and parasite-derived surface proteins are produced on the erythrocytic surface. The *P. falciparum* erythrocyte membrane protein 1 (*PfEMP-1*) is the most well-studied of these, as it is implicated in parasite pathogenicity by regulating the adherence of erythrocytes to endothelium walls, resulting in sequestration of infected red blood cells in numerous organs and tissues (Miller *et al*., 2002). Some of the merozoites secreted by red blood cells mature into the parasite's sexual form, gametocytes, which are consumed by female Anopheles mosquitos. *P. falciparum* gametocytes are created shortly after merozoites are released from the liver, but *P. vivax* gametocytes are created after numerous rounds of asexual erythrocyte replication. As a result, *P. vivax* can be transferred before the

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disease becomes clinical (Mueller *et al.*, 2009). The gametocytes replicate within the mosquito's gut after ingestion, resulting in diploid zygotes that mature into ookinetes, which migrate from the gut lumen to the gut wall, where they mature into oocysts. The oocysts then burst, releasing thousands of sporozoites that move to the mosquito's salivary glands, where they can infect a human once more.

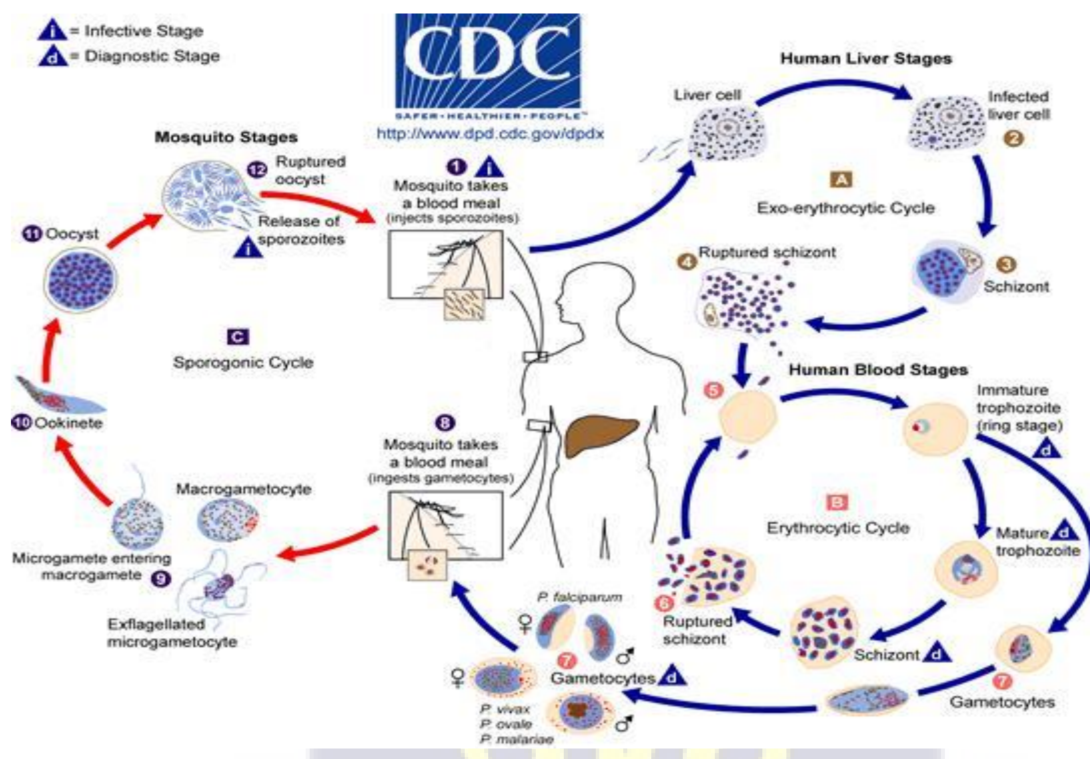

Fig 2.0 Life cycle of malaria parasite
(Source:http//www.dpd.cdc.gov/dpdx)

2.5 Clinical Presentation of Malaria

Malaria begins with non-specific symptoms that resemble the flu, such as fatigue, nausea, headache, muscle and joint pain, and is followed by a high temperature, chills, and sweating, which may be accompanied by vomiting and diarrhoea. Children and adults have different symptoms. Chills, arthralgia/myalgia, headache, GI symptoms, cough, and

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non-specific symptoms like lethargy, malaise, and poor eating are less common in children (Ladhani *et al.*, 2007). Malaria symptoms are caused by the rupture of parasite-infected erythrocytes, which sets off a chain reaction of inflammatory reactions, resulting in the typical symptoms of fever and rigors.The fever is normally irregular, but in semi-immune patients with an untreated *P. vivax* or *P. ovale* infection, the frequently stated periodicity can be found, with fever every 48 hours in *P. vivax* and every 72 hours in *P. malariae*, which is explained by synchronized erythrocytic reproduction (Greischar, Read & Bjørnstad, 2014).

Repeated infections in children living in endemic areas cause anemia, which is caused by both hemolysis of erythrocytes and parasite-induced bone-marrow depression, and this anemia can be severe (Perkins, 2011). Adults who live in high-transmission areas develop immunity to clinical malaria, and infections only seldom cause symptoms, which manifest as a flu-like sickness. Asymptomatic parasitaemia can affect more than half of the population in these locations (Gudo *et al.*, 2013), with a lower prevalence in places with lower endemicity (Golassa, 2013). The duration of an asymptomatic infection is determined by the transmission intensity (Soulama *et al.*, 2009) as well as the patient's age (Smith *et al.*, 1999). Children in Papua New Guinea, for example, reported median lengths of 9-60 days (Bruce *et al.*, 2000). Longer infection duration throughout several months of the dry season is a requirement for transmission in the following year in strictly seasonal settings. However, the length of asymptomatic infections is poorly understood, and there have been case reports of adult immigrants living in malaria-free nations who have had chronic *P. falciparum* infections for longer periods of time, in one case for 13 years (Ashley and White, 2014).

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2.5.1 Severe *P. falciparum* malaria

P. falciparum infections can escalate to severe and perhaps lethal diseases, depending on the individual's immunological status. Unrousable coma, metabolic acidosis, respiratory distress, severe anemia, hypoglycemia, acute renal failure, circulatory congestion, and hyperbilirubinaemia are all symptoms of severe malaria (for a complete list of symptoms, see "Definition of severe malaria"). However, there are many different ways to treat severe malaria. Severe malaria in children in endemic areas usually manifests as three distinct but often overlapping syndromes: cerebral malaria, severe anemia, and respiratory distress (a clinical proxy for metabolic acidosis), with the highest mortality reported in children with both respiratory distress and impaired consciousness (32%) (Gérardin *et al*., 2007). Severe malaria symptoms appear at different ages and may be affected by transmission intensity (Idro *et al*., 2006). In places with high transmission, severe anemia is the most prevalent symptom in children under the age of two (Reyburn *et al*., 2005; Obonyo *et al*., 2007), with a few studies showing a lower frequency in places with less transmission (Reyburn *et al*., 2005; Snow *et al*., 1994). In places with less transmission, a higher proportion of children with severe malaria have cerebral malaria than in places with the highest transmission, and peak rates in intermediate transmission areas are reported at 3-5 years of age (Reyburn *et al*., 2005; Snow *et al*., 1994; Okiro *et al*., 2009). Cerebral malaria is reported in all age groups in locations with low or epidemic transmission, as well as other severe malaria symptoms such as pulmonary oedema, cardiac collapse, and renal failure in non-immune travelers (Kleinschmidt and Sharp, 2001; Bruneel *et al*., 2010; Mclean *et al*, 2015).

However, healthcare standards and diagnostic capabilities may influence the manifestations observed in various clinical settings. As medical diagnostics has improved in general, a recent study found that renal failure was far more common than previously thought among

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African children with severe malaria, and that it was also significantly linked to death (Conroy *et al.*, 2016).Severe falciparum malaria is fatal in the majority of cases if left untreated (Beales *et al.*, 2000), but even with effective treatment, severe malaria is fatal in both children and adults, with mortality rates of 8-24 percent in low-resource settings (Dondorp *et al.*,2005, Dondorp *et al.*, 2010; von Seidlein *et al.*, 2012) and 10-15 percent in high-resource settings (Legros *et al.*, 2007; Seringe*et al.*,2011; Santos *et al.*, 2012).The initiation of intravenous therapy with artesunate (Sinclair *et al.,* 2012) is the intervention with the best evidence for reducing malaria mortality in individuals with severe malaria. Despite this, artesunate is not widely available in some countries, and in the United States, artesunate has just recently supplanted quinine as the first-line treatment for severe malaria (CDC, 2019). There are few randomized clinical trials examining additional therapies for patients with severe malaria (Day and Dondorp, 2007), and there is still much to be improved in the management of severe malaria patients in both low and high resource settings.

2.5.2 Severe non-falciparum malaria

Severe malaria can develop in infections of non-falciparum species, which were previously thought to be exceptional. Several reports, mostly from endemic areas, have described severe anemia, thrombocytopenia, liver dysfunction, kidney failure, and splenic rupture in *P. vivax* malaria (Tjitra *et al.*, 2008; O'Brien *et al*, 2014 ; Kenangalem *et al.*, 2016), but more recently, cases of severe *P. falciparum* malaria have been reported, including cerebral malaria, shock, and acidosis (Rahimi *et al.*, 2014; Tanwar *et al.*, 2011; Nadkar *et al.*,2012) , possibly due to increased virulence of certain strains (Anstey *et al.*, 2012). In Indonesia, severe malaria was shown to be more common in *P. vivax* patients than in *P. falciparum* patients; 23% of *P. vivax* patients had severe illness, with severe anemia being the most common consequence (Tjitra *et al.*, 2008). In recent years, *P. knowlesi* has been

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linked to high parasite densities and severe symptoms similar to *P. falciparum* (Kotepui *et al*., 2020; Daneshvar *et al*., 2009), including mortality (Cox-Singh *et al*., 2008). Severe symptoms in *P. malariae* and *P. ovale* have only lately received attention (Bellanger *et al*., 2010; Groger *et al*., 2017; Kotepui *et al*., 2020; Kotepui *et al*., 2020). However, severe cases may be underdiagnosed due to mistaken species and a lack of careful control of clinical and laboratory indicators included in the severe criteria. With the exception of a few case reports (Izri *et al*., 2019, Seilmaier *et al*., 2014; Rojo-Marcos *et al*., 2008) and observational studies based on current data (Broderick *et al*., 2015; Muhlberger *et al*., 2004), severe malaria research in Europe is primarily focused on *P. falciparum*. In a non-endemic situation, risk factors for severe *non-falciparum* malaria have not been thoroughly examined.

2.5.3 P. falciparum Severe Malaria Pathogenesis

Although the pathogenesis of severe malaria is not fully understood, various findings specific to the biology of *P. falciparum* have led to the following processes being proposed: 1) High rate of multiplication and capacity to infect erythrocytes of all ages (as opposed to *P. vivax,* which prefers young reticulocytes), resulting in a high percentage of infected erythrocytes (Chotivanich *et al*., 2000). 2) Sequestration, or the attachment of infected erythrocytes to endothelial cells in capillaries, prevents infected red blood cells from being removed by the spleen (Miller *et al.,* 2002), causes tissue hypoperfusion, anaerobic metabolism, and metabolic acidosis. This sequestration is not evenly distributed, according to studies, but is more pronounced in particular organs, such as the brain (Dondorp, 2005; White, 2017). The pathophysiology of placental malaria is similarly influenced by sequestration (Duffy and Fried, 2003). 3) Erythrocyte deformability is reduced in both infected and uninfected erythrocytes, resulting in increased blood flow blockage in the microvasculature (Dondorp *et al*., 2000). 4) Rosetting (Wahlgren *et al.,* 1994), a

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phenomenon in which uninfected erythrocytes bind to P. falciparum infected erythrocytes. 5) Increased production of pro-inflammatory cytokines (TNF-, IFN-, and IL-1) and potential mediator release, such as nitrogen oxide (Clark and Cowden, 2003).

Both sequestration and rosetting require unique surface proteins known as adhesins, the most significant of which is the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), which is expressed by infected erythrocytes and can bind to other erythrocytes or epithelial cells (Ho and White, 1999). Certain kinases found solely in *P. falciparum* control the "stickiness" of red blood cells, according to a new study (Davies *et al*., 2020). Furthermore, there is evidence that some Plasmodium strain genotypes are more likely to induce severe malaria infection (Ariey *et al*., 2001).

2.6 Diagnosis and Treatment of Malaria

2.6.1 Microscopy

Microscopy is the gold standard for diagnosing malaria since it allows for both species identification and parasite quantity. Microscopy is used to evaluate thick and thin blood films stained with Giemsa or Field's stain. If parasites are found in a thick smear, parasite count and species identification are normally done on thin films, while parasite count may be calculated in a thick smear by counting parasites against leukocytes or ocular fields in some cases. The bottom limit of detection of parasites in thick film is 1 parasite per 200 ocular fields (about 5-10 per microliter), though this varies based on the microscopist's skill (Heutmekers *et al*., 2012).

2.6.2 Diagnostic Rapid Tests

Antibody-antigen interactions on a test strip identify *Plasmodium* antigens in malaria rapid diagnostic tests (RDTs). The most often utilized antigen specific for *P. falciparum* is Histidine Rich Protein 2 (*HRP 2*), but lactate dehydrogenase (Pan LDH) can be used to

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detect both *P. falciparum* and non-falciparum species. Most RDTs used in Sweden include both antigens, allowing them to detect both *P. falciparum* and *non-falciparum* malaria (*P. vivax, P. ovale, P. malariae*). In *P. falciparum* infections, one extensively used RDT in Sweden (CareStartTM Malaria Combo Test) can identify as few as 50 parasites per microliter. The sensitivity is good for *P. falciparum* and *P. vivax* (depending on parasitaemia level), whereas sensitivities of around 30% have been recorded for *P. ovale* and *P. malaria* (Heutmekers *et al*., 2012).

Rapid diagnostic tests are only employed as a supplement to microscopy and they are only used to aid in acute diagnostics outside of office hours. Microscopy must be used to confirm both positive and negative fast tests. Microscopy is required in positive tests to determine the species and level of parasitaemia, as high parasitaemia is a criterion for severe malaria that necessitates intravenous therapy. False negative rapid tests can occur due to the so-called prozone effect (excess antigens binding to detection antibodies) in high parasitaemic infections with *P. falciparum*, or due to *HRP2* negative *P. falciparum* strains that have become increasingly detected in some parts of the world, such as Eritrea, Rwanda, and the Democratic Republic of the Congo.

2.6.3 Molecular techniques

Although polymerase chain reaction (PCR) detection of *Plasmodium* species DNA is the most sensitive method of parasite detection and species characterization, it is rarely employed in the acute clinical environment because it is less accessible, more expensive, and takes longer to analyze than microscopy. In most cases, PCR can identify as little as one parasite per microliter, but bigger blood samples and high-sensitivity PCR can identify lower amounts (Imwong *et al*., 2014). Because PCR can stay positive for weeks after a *Plasmodium* infection has been successfully treated (up to 42 days in travellers has been documented) (Homann *et al*., 2017), microscopy is required to confirm a persistent

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infection following therapy. Loop-mediated isothermal amplification (LAMP), a more recently developed molecular method with similar sensitivity to PCR (Charpentier *et al.*, 2020), amplifies nucleic acid under isothermal conditions, eliminating the need for the expensive thermocyclers used in PCR, making the method suitable for parasite detection and species determination in low-income countries. LAMP has only been used frequently in one part of Sweden, but it is set to be used for acute diagnosis at Stockholm's malaria reference laboratory.

2.6.4 Malaria Treatment

The recommended treatment for uncomplicated *P. falciparum* and *P. knowlesi malaria* is artemisinin-based combination therapy (ACT) (WHO, 2015). Chloroquine may normally treat uncommplicated *P. vivax, P. ovale* and *P. malariae* infections, but in some locations where *P. vivax* is chloroquine resistant, ACT treatment is favored. An additional two-week course of primaquine is given to *P. vivax* and *P. ovale* patients to remove hypnozoites, which are not destroyed by ACTs or chloroquine and will otherwise remain in the liver, posing a risk of relapse (WHO, 2015). Intravenous artesunate is currently the WHO's recommended first-line treatment for complex malaria, regardless of species, and in patients who are vomiting, including pregnant women in all trimesters (Sinclair *et al.*, 2012). It has a far faster parasite clearance time than quinine. In the event that aspirin is not available, quinine is delivered intravenously instead.

2.7 Malaria Epidemiology

Epidemiology is a discipline of study that analyzes the state of health or health-related incidence and distribution in a given population, as well as the application of that knowledge to control health problems (Last, 2001). Malaria epidemiology aims to gain a thorough understanding of the disease's distribution and transmission patterns, which are important for malaria control. Malaria is believed to be endemic in Africa because the

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pattern of *Plasmodium* transmission in humans has remained consistent across time. Several studies found that the global epidemiology of malaria displayed diverse transmission intensities and patterns (Snow *et al.*, 2005). The strength of transmission varies between continents and countries, as evidenced by this research (Snow *et al.*, 2005; Guerra *et al.*, 2006). In sub-tropical and tropical areas, stable malaria is characterized by a genetically heterogeneous parasite surface antigen (Khelioune et *al*., 2010).

The rate at which parasites develop resistance to antimalarial medications that are presently available is alarming. Parasite resistance may be influenced by the genetic diversity and mutations of these parasite populations (Jianbing *et al*., 2003). In areas with strong Plasmodium transmission, such as Africa and Papua New Guinea (PNG), where repeated infections are common, genetic diversity is unavoidable (Babiker *et al*., 1994; Paul *et al*., 1995; Snow *et al*., 2005). This opens up additional opportunities for alleles that benefit the parasite population to spread (Babiker *et al*., 1994; Paul *et al*., 1995; Snow *et al*., 2005). In places like Papua New Guinea, malaria epidemiology varies greatly between villages and even between homes within the same community (Cattani *et al*., 1986; Snow *et al*., 2005). The intricate interplay of environmental, vectorial, human, and parasite species could explain the varied patterns of malaria epidemiology around the world.

2.7.1 Global Malaria Situation

Sub-Saharan Africa continues to endure a disproportionately large share of the global burden of malaria. This is because, when compared to other parts of the world, these regions account for 92 percent of all malaria cases and 93 percent of malaria deaths (WHO, 2018).

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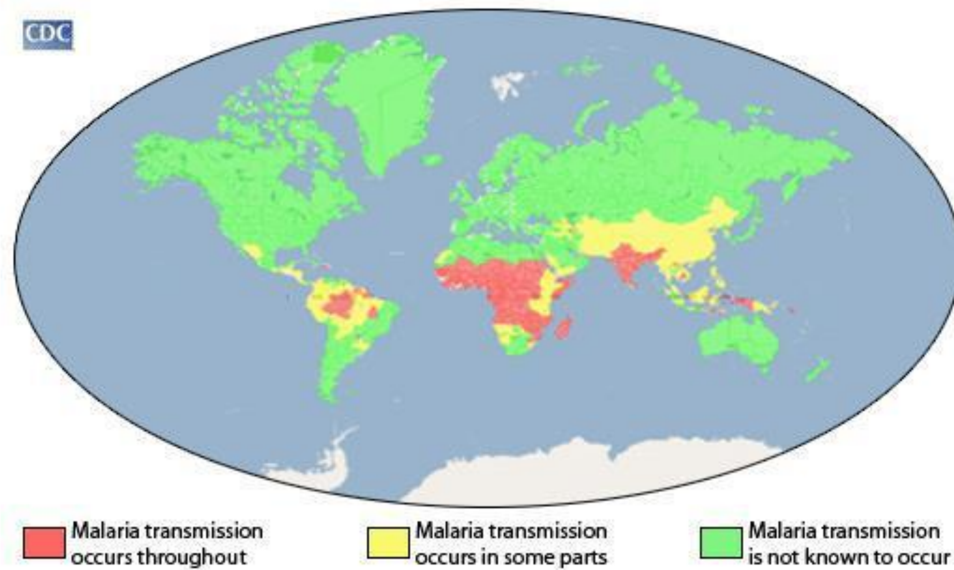

Figure 2.1 Global distribution of malaria

The map shows the sub-Saharan African region, marked in red where malaria transmission is known to persistently occur (CDC, 2013). Other parts marked in yellow have recorded malaria transmission while the parts marked in green have no record of malaria transmission (Adapted from CDC, accessed on 8th June, 2021).

With regards to *falciparum* malaria, Olupot and Maitland (2013) anticipated that the number of cases would be between 350 and 550 million. In 2010, between 655,000 and 1,240,000 people died as a result of the disease (Murray *et al*., 2012). Malaria continues to be the leading cause of death and severe complications among children and pregnant women in the sub-Saharan region, with 92 percent of cases and 93 percent of fatalities occurring there. However, an estimated 61 percent of malaria fatalities occur among children under the age of five years (WHO, 2018). The high rate of illness and mortality among children aged six months to five years can be related to a lack of maternal immunity and the underdeveloped child's subsequent immunity to infection. Annually, an estimated 125 million pregnant women globally are at risk of malaria infection. However, malaria in

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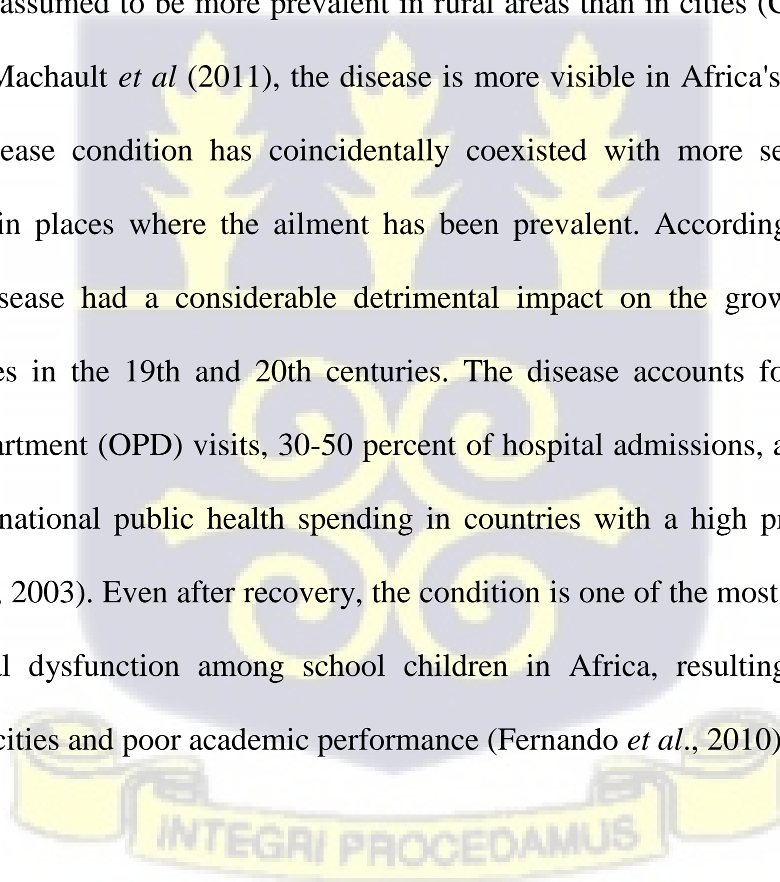
pregnancy has killed two hundred thousand (200,000) infants in the sub-Saharan African region (Hartman *et al.,* 2010).

Malaria cases are estimated to be around 10,000 in Western Europe and 1300-1500 in the United States. In the ten years between 1993 and 2003, an estimated 900 people died in Europe. The global incidence of malaria and its consequences has decreased by 60% in recent years, from a total of 985,000 deaths projected in 2000 (WHO, 2015). The fall in mortalities has been attributed to the widespread use of artemisinin-based combination therapy and insecticide-treated nets (Howitt *et al.,* 2012).

Malaria is prevalent in tropical and subtropical climates due to high temperatures, rainfall, and humidity, as well as stagnant waterways that are suitable for mosquito larvae growth. The disease is assumed to be more prevalent in rural areas than in cities (Cui *et al*., 2012). According to Machault *et al* (2011), the disease is more visible in Africa's urban and rural areas. The disease condition has coincidentally coexisted with more serious economic consequences in places where the ailment has been prevalent. According to Humphreys (2001), the disease had a considerable detrimental impact on the growth of Southern American states in the 19th and 20th centuries. The disease accounts for 50 percent of outpatient department (OPD) visits, 30-50 percent of hospital admissions, and an estimated 40 percent of national public health spending in countries with a high prevalence of the disease (WHO, 2003). Even after recovery, the condition is one of the most common causes of neurological dysfunction among school children in Africa, resulting in diminished cognitive capacities and poor academic performance (Fernando *et al*., 2010).



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2.7.2 Situation of malaria cases in Ghana

Malaria is a public health concern in Ghana, with *P. falciparum* being the most common parasite. The disease has long been a leading source of morbidity and mortality in children under the age of five, accounting for 44 percent of falciparum malaria patients seen in hospital outpatient departments (Bahaah *et al*., 2019). Sickness has a negative impact on productivity, household economic development, and the country's economy (Thuilliez *et al*., 2017; Singleton and Osei, 2014). Illness is the leading cause of a child's absence from school in the state. Approximately 20,000 children under the age of five years predominated among 3.5 million suspected malaria morbidity cases in Ghana's public hospitals during the year under review (2006), according to UNICEF Ghana. As a result, the disease was responsible for sixty-one (61) percent of hospital admissions and twenty-five (25) percent of deaths among children under the age of five (UNICEF Ghana, 2007). Malaria predominates in practically all Out-Patient Department (OPD) morbidities and mortalities in children, according to the National Malaria Control Programme, and the disease is responsible for three child fatalities every day in the country (NMCP, 2016). Ghana launched certain control mechanisms to reach the target population, such as increasing long-lasting insecticide net coverage and usage by 80% and 60% respectively by 2010, as part of a concerted effort by several nations to reduce and stop the incidence of malaria by 2015. Prompt diagnosis and therapy management with Artemisinin-based combination treatments (ACTs) are two of the measures, as is expanding the scope of intermittent preventative treatment (IPT) among pregnant women. Despite the fact that the approaches indicated above in the country have influenced the reduction of malaria morbidity among children, the country still has a large number of children under the age of five who are infected with the disease (Appiah *et al.,* 2017). According to Appiah *et al* (2017), the disease is still hyper-endemic in Ghana, with a prevalence rate ranging from 11.2 percent to 40%. Malaria

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prevalence rates in children living in rural communities are greater than in children living in urban areas, according to a study conducted by the Multiple Indicator Cluster Survey (MICS) in 2011. According to the MICS study, children under the age of five who have malaria in urban areas have a lower prevalence rate of the disease than children in rural areas: 14 percent and 44 percent, respectively (NMCP, 2013).

2.8 Malaria Prevention and Control Strategies

2.8.1 Vector control

Malaria transmission can be prevented by using vector control (WHO, 2004). Indoor residual spraying (IRS), insecticide-treated nets (ITN), ultra-low volume sprays, and house screening are all methods of vector control. Insecticide resistance management could be greatly aided by combining IRS with ITN. IRS, on the other hand, requires adequate professional vector control services, solid planning, and a thorough understanding of the vector species' behavior (Rowland & Nosten, 2001; WHO, 2006). There is no doubt that these control mechanisms have resulted in some degree of success. This, however, necessitates the vectors feeding and resting indoors. The selective exclusion of exophage vectors, the unfavourable effect that spraying may have on human, and the little knowledge of the appropriate pesticides are all downsides of these tactics. Furthermore, the personnel and financial resources available to maintain the control effort are limited (WHO, 2004). A decrease in indoor residual spraying for vector control could be connected to a significant increase in parasite comeback.

Larvicides work by killing larvae or restricting their breathing trumpets, as seen when kerosene is spilled on stagnant water surfaces (CDC, 2010). The difficulty here is evident, as kerosene is not provided free of charge. There is a limit to how long a procedure can be maintained. DDT, insecticide-treated nets (ITNs), pyrethroids, and organophosphates are all

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examples of this (WHO, 2004). Domestic sanitation, water drainage, and naturally-induced biological control techniques are frequently used by the majority of the population, yet they are woefully inadequate to control vectors. It has been proven that the oil derived from *Citrus limon L.* and *Burm.f* are efficient against mosquito larvae (Zayed *et al*., 2009). This is consistent with recommendations that plants be used as an alternate source of mosquito vector control due to their bioactive chemical content (Park *et al*., 2002). The introduction of genetically modified mosquitos has been considered, but the speed with which they replicate and spread may be insufficient to counterbalance the normally occurring vector species (WHO, 2004). Adaptation and the survival of the fittest could impede replication speed. Newcomers will need to develop unique survival skills in order to survive in the long run.The majority of the time, this is met with opposition. For efficient use and execution of existing control measures, health education and community involvement are essential strategies.

2.8.2 Parasite control

The goal is to eliminate or kill the parasites. Chemotherapeutic drugs are some of the most common ways to accomplish this (Dorsey, 2000). Antimalarial or antiplasmodial medications are used to treat malaria. In Ghana and sub-Saharan Africa, the some of the population relies completely on traditional healers' herbal concoctions for the treatment of numerous maladies, including malaria (Igoli *et al*., 2005).

2.9 Challenges to Malaria Control Efforts

2.9.1 Environmental factors

A community or region has its own set of characteristics. Climate and geographical elements, such as soil type, rainfall patterns, wind speed, temperature, height, water salinity, and vegetation, are examples of such elements. These, in one way or another, affect control efforts and are natural elements that people cannot change, such as temperature variation,

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which plays a role in explaining malaria's geographic distribution (Sachs and Malaney, 2002).

2.9.2 Vector transmission factors

P. falciparum sporogony is not possible at low temperatures, while it is possible at high temperatures (Abeku *et al.*, 2003). *Plasmodium* parasites require optimal temperature conditions of 20^0C- 30^0C for successful transmission. Because parasites cannot grow at temperatures below 16 ^{0}C, transmission is delayed or altered below this temperature (Sachs and Malaney 2002). Some vector species stop biting at very low temperatures while others have evolved survival strategies over time. Others are exophagic, while some are endophagic (WHO, 2004). Endophilic and endophagic behaviors are both genetically programmed survival strategies for vectors. Indoor residual spraying (IRS) relies primarily on mosquitoes feeding and resting indoors to be effective (WHO, 2004). Most mosquitoes, on the other hand, do not do this, and the pyrethroid used in spraying eventually repels them. The spread of parasites among humans is aided by factors that favor vectors. Control measures are harmed by vectorial resistance and behavior (WHO, 2004; CDC, 2010). Resistance to insecticides can be caused by enzyme detoxification or mutations at the target location; sodium channels for DDT and pyrethroids, and acetylcholinesterase for organophosphates are two examples (CDC, 2010). When compared to endophillic species that like to rest inside, exophilic species that prefer to rest outside are less likely to acquire deadly dosages of pesticides sprayed on the walls (CDC, 2010).

2.9.3 Humans Factors

Human actions in a given area, such as deforestation and reforestation, are also key elements that work against control attempts. Dams, canals, and irrigation are examples of agricultural activities that contribute to the high prevalence of malaria in impacted areas. Malaria transmission is heavily influenced by land cover and land usage (Hay *et al.,* 2000).

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Control methods are challenged by global warming, urbanization, and their repercussions, particularly in emerging countries. Control may be possible or impossible, depending on the public's knowledge, attitudes, and perceptions. Noncompliance with appropriate treatment modalities maintains prevalence and fosters resistance. It increases infection epidemiology and pathogenicity. The lack of a consistent and appropriate supply of DDT and ITN to the population is a major impediment (CDC, 2010). Poverty is a crucial component, which may explain why malaria is known as the "poor man's illness."

2.9.4 Parasite associated Factors

The resistance of parasites to currently available antimalarial medications is a key worry in this area (Jianbing *et al.,* 2003). The rate of resistance development among these parasites is worrying. The backbone of treatment, chloroquine, has been found to be ineffective against parasites (Jianbing *et al.,* 2003). Several treatment failures have been reported in various places, putting the relief from artemisinin, its derivatives, and combinations in jeopardy. The first case of artemether-lumefantrine therapy failure has been described by a Japanese traveler (Mizuno *et al*., 2009). Artemisinin sensitivity has been observed to be reduced in China and Vietnam (Huong *et al*., 2001, Yang *et al*., 2003). As a result, new and effective antimalarials are urgently needed.

2.10 Need for new antimalarials

In the development of novel antimalarials, it is critical to utilize fresh, effective, and long-term strategies. Chloroquine resistance was first identified in non-immune visitors in East Africa in 1978. (Atroosh *et al*., 2012). Similar studies were followed in the rest of tropical Africa (Trape, 2001), where chloroquine was the first-line malaria treatment (Trape *et al*., 2002). Another extensively used antimalarial is sulphadoxinepyrimithamine (SP). It's inexpensive and readily available, yet it's been faced with opposition. In various parts of Asia, South America, and Africa, examples of SP treatment failure have been reported

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(Plowe, 2003). In various places, therapeutic failures with artemisinin combination treatments (ACTS) have been reported (Jambou *et al*., 2005; Rogers *et al*., 2009).

The absence of a variety of drug treatment options, high population migration levels, significant underdosing, the presence and use of adulterated drugs, prescription delivery by non-qualified personnel, and non-compliance with treatment are all conditions that favor the development of drug resistance by parasites. It's no surprise that malaria's endemicity, as well as treatment resistance, affects the world's impoverished, underdeveloped, and uneducated regions. Effective antimalarials against which parasites are unlikely to acquire resistance are needed. Combination therapy for malaria is one of the most effective strategies to achieve this (Jambou *et al*., 2005; Rogers *et al*., 2009).

2.10.1 Combination therapy for Malaria

The WHO recently suggested the use of ACTs as a first-line treatment in areas where parasite resistance has significantly harmed the therapeutic efficacy of currently available medications (WHO, 2007). In 15 African countries, the combination of artesunate (AS) and amodiaquine (AQ) is now used as a first-line treatment (WHO, 2006). In Senegal, the use of AS+AQ, AS+mefloquine (MQ), and AQ+SP in the treatment of uncomplicated falciparum malaria resulted in excellent clinical responses and tolerability (Faye *et al*., 2007). However, in a second trial in Burkina Faso, young people observed the beginnings of minor discomforts that went away once the dosage was completed (Barennes *et al.,* 2004). In underdeveloped nations, combination therapy is an efficient way to achieve optimal malaria control (Guerin *et al.,* 2002). Further research suggested that to prevent medication resistance pressure in Africa, a combined treatment with rapid clearance rates be used (Watkins *et al*., 1988; Watkins and Mosobom, 1993; Guerin *et al.,* 2002).

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Compared to monotherapies, combination therapy has a number of advantages. It is an effective anti-plasmodial technique for preventing or delaying resistance (White *et al*., 1996, White, 1998; Guerin *et al.,* 2002), and it successfully slows down selection pressure due to the target specificity of each component. Combination therapy extends the component drugs' useful therapeutic lives while also shortening treatment periods. It has been demonstrated to be effective in the treatment of tuberculosis and HIV/AIDS (Nyunt and Plowe, 2007). Combination therapy provides the added benefit of improving patient adherence to treatment (Nosten *et al*., 1994; Nyunt and Plowe, 2007). It reduces the overall cost of production, transportation, and dispensing, particularly in a fixed dose regimen. It also minimizes the danger of patients receiving the incorrect dosage, especially when prescribed by unqualified drug dealers, as is common in most developing nations. It also aids in the simplification of patient counseling and education. It slows or stops the progression of resistance and improves cure rates (Price *et al*., 1997; Nyunt and Plowe, 2007).

The idea behind pharmacological combination therapy is that parasite resistance is delayed if it has to evolve in multiple places with different mechanisms of action. The essence of an effective medication combination is that at least one of the drugs in the combination will remain clinically active even when resistance develops (White, 1998; Nyunt and Plowe, 2007). Because our environment is rich in natural goods, we should use them wherever possible to address our health issues and concerns. New, integrated, and multivalent treatment techniques are needed to slow the evolution of resistance, especially since treatment failures, including the use of ACTs, have been reported along the Thai-Cambodian border and in Thailand itself (Jambou *et al*., 2005; Vijaykadga *et al.,* 2006, Wongsrichanalai and Meshnick, 2008; Rogers *et al.,* 2009). This has pushed scientists to

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look for new medicine combinations by investigating our natural resources more thoroughly.

2.11 Antimalarial drug mechanism

Antimalarial drugs have different mechanisms of action for which they help cure malaria. An example is the mechanism of action of chloroquine, an antimalarial drug, which is known to become concentrated in the parasites' food vacuole, thereby preventing the polymerization of heme into hemozoin. This leads to the eliciting of parasite toxicity and eventually parasite death (Saifi, 2013). However, for the artemisinin analogs, they contain a peroxide bridge that undergoes an iron-catalyzed cleavage within the parasite's food vacuole, which results in the production of free radicals that kill the malaria parasite (Talapko *et al.*, 2019). Aside the orthodox antimalarial drugs, there are other herbal drugs that are used to cure malaria (Oteng Mintah *et al.*, 2019). Although the mechanism of action of most of these drugs are unknown, they are still being used to treat malaria.

2.12 Herbal Medicines or Herbal Products

Different people have characterized herbal medicine in different ways. "Herbal pharmaceuticals are just those traditional medicines that predominantly utilise medicinal plant preparations for their therapy," according to Kamboj (2008). Herbal medicine, according to Lucas (2010), is "the utilization of plant products to treat or prevent disease." Herbal practitioners' treatment "takes the shape of herbs, plant mixtures, and prayers," according to (Nsowah-Nuamah et *al,* 2004).

Herbal medicine is defined as "a plant-derived material or preparation with therapeutic or other human health advantages that incorporates either raw or processed elements from one or more plants," according to the World Health Organization (WHO, 2008). Traditional medicine, on the other hand, is defined by the WHO Regional Office for Africa (2004) as the use of indigenous medicinal and aromatic plants, animal parts, or organic and inorganic

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materials for preventative and therapeutic purposes. Complementary and alternative medicine, formerly known as traditional and herbal medicine, has a new name (CAM). The term "complementary and alternative medicine" (CAM) refers to therapeutic and diagnostic disciplines that exist mostly outside of institutions that provide orthodox or conventional health care (Shaikh and Hatcher, 2005).Herbal medicine, on the other hand, is defined as “plant seeds, berries, roots, leaves, bark, or flowers used for medicinal purposes” by the University of Maryland Medical Center (2010).

Medicinal herbs come in a range of shapes and sizes. Leaves, flowers, stems, roots, seeds, and berries are examples of active sections of a plant (Woolf, 2003). They can be consumed as tablets or powders, tinctures or syrups, or made as teas and mixtures. In many regions of the world, herbal medications and herbal products are widely used to cure a variety of ailments. As evidenced by Ayurveda, India has a long history of herbal therapy that could not have survived for two thousand years without a scientific foundation. The usage of herbal medicine is growing in popularity around the world (Ernst, 2006). Traditional medicines are used by 80% of the world's population for health care (Gesler, 1992). Opium, aspirin, digitalis, and quinine are just a few of the medications currently available to doctors. Approximately 25% of current medications used in the United States are derived from plants, according to the World Health Organization (Pandey *et al*., 2007).

Natural products account for 30% of the top 25 best-selling pharmaceuticals in the world today (Kong *et al.,* 2002). According to Cragg *et al.* (1997), natural compounds or derivatives accounted for 157 of the 520 medicines approved (30%). At least 7,000 pharmacological chemicals in today's pharmacopoeia are generated from plants, according to the Interactive European Network for Industrial Crops and their applications (IENICA, 2000-2005). Eighty percent of the 120 active chemicals extracted from higher plants and

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widely employed in modern medicine today demonstrate a good relationship between their modern therapeutic usage and the traditional use of the plants from which they are derived (Fabricant and Farnsworth, 2001). Approximately 74 percent of the 119 plant-based medications used in modern medicine come from plants (Fabricant & Farnsworth, 2001). The World Health Organization (WHO) has compiled a list of 21,000 medicinal plants used worldwide (WHO, 1996). Medicinal plants, minerals, and organic matter are used to make a variety of traditional remedies (Grover *et al*., 2002).

2.12.1 Herbal Medicine's Global Distribution and Use

The use of herbal supplements has increased dramatically over the past 30 years. The number of patients seeking herbal approaches for therapy is also growing exponentially (Alsculer *et al*., 1997). According to a study in America, nearly one-third of Americans use herbs. According to a survey released in May 2004 by the National Centre for Complementary and Alternative Medicine, USA herbal therapy, or use of natural products other than vitamins and mineral, was the most commonly used CAM therapy (18.9%). Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries, for health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. The World Health Organisation (WHO) estimates that 80 percent of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care (Payyappallimana, 2006).

According to the WHO (2003), the global market for herbal medicines is currently valued at over US $60 billion per year and is constantly expanding. In Western Europe, half of the population in the United Kingdom has at some point tried a natural cure. In Germany, the percentage is around 90%. The annual spending on alternative medicine in the United Kingdom is projected to be US $232 million (WHO, 2003). Over half of the people in North

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America are thought to have tried complementary or alternative medicine at least once. For example, in Canada, the figure of 70% is higher than the regional average. Between 1995 and 2000, the number of doctors with advanced training in natural remedy medicine nearly doubled, to 10,800. Complementary medications are used by 158 million adults in the United States. Traditional treatments cost $17 billion in 2000, according to the United States Commission on Alternative and Complementary Medicine (WHO, 2003). Traditional herbal preparations represent 30 percent to 50 percent of total medicinal consumption in Asia, particularly in China.The use of herbal medications at home is the first line of treatment for 60 percent of children with high fever due to malaria in African nations such as Ghana, Mali, Nigeria, and Zambia (WHO, 2003).

2.12.2 Increasing use of herbal medicine in developing countries

Herbal medicine is used for a variety of reasons, which differ from country to country (Shaikh and Hatcher, 2005). The most popular arguments for the continued use of herbal therapy are that it is more accessible, less expensive, culturally acceptable, and, most importantly, effective (Darko, 2009). The widespread use of herbal medicine in underdeveloped nations is generally linked to its availability and physical accessibility (WHO, 2002). Herbal medication is more widely available and accessible, especially in rural regions (Otieno, 2010).

2.12.3 Herbal medicine's safety, efficacy, and quality

The primary concept behind a medicine's efficacy is that it is a measure of its ability to promote health and well-being. As a result, according to Darko (2009), each medical system's functional scope is essentially determined by its ability to provide results in specific cases of illness. Despite the fact that several studies have shown an increase in the use of herbal medicine around the world, only a few have looked at how patients felt about the usefulness of this healthcare modality in certain disorders (Clement *et al.*, 2007).

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According to Clement *et al*. (2007), the belief that herbal therapies are as effective as allopathic medicines is a crucial factor contributing to their growing popularity in industrialized countries and their continued use in underdeveloped countries. This high degree of perceived efficacy supports their continued use, as well as the usage of conventional drugs in a considerable percentage of patients. Clement *et al*. (2007) discovered that 86.6 percent of users of primary health care in Trinidad believed that herbal therapies were equally or more effective than orthodox/conventional pharmaceuticals for particular symptoms and disorders in a survey.

The potency and efficiency of herbal therapy have been demonstrated through study, according to Mensah (2008). Herbal remedies have demonstrated significant success in the treatment of both acute and chronic disorders (Shaikh and Hatcher, 2005). Herbal medicine provides more effective treatments for certain health problems such as boils, tuberculosis, stroke, arthritis, epilepsy, asthma, infertility, hernia, hypertension, diabetes, malaria, depression, mental illness, disease prevention, and the elderly population, where modern medicine has either failed to produce equally good results or has simply ignored the need for systematic attention and research (Darko, 2009).

2.13 The role of natural products in the treatment of malaria

2.13.1 Antiplasmodial agents derived from natural materials

Nature continues to be a rich source of medicinally valuable chemicals and compounds. Various natural products have proven to be extremely beneficial in the treatment of a variety of ailments. In traditional therapeutic practices, the majority of these natural items are used in their natural state. The extraction, purification, isolation, and characterization of compounds with good medicinal values from natural sources has piqued the interest of industrialized societies and scientists, who want to learn more about the flora and fauna of

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our diverse ecosystems and their medicinal uses and properties. Natural products have the potential to provide fresh and innovative scaffolds for the creation of new antimalarial medicines. Alkaloids, terpenoids, flavonoids, chalcones, peptides, xanthones, quinones, coumarins, and fatty acids are among the antimalarial substances found in them.

2.13.2 Plant-derived antiplasmodial agents

Antimalarial agents have been found in plants (Farnsworth and Morris, 1976). The therapeutic characteristics of natural ingredients in herbal medicines and extracts from plants are traceable to their activities. Quinolines and artemisinins are antimalarial medications derived from plants that have made a significant contribution to the fight against malaria (CDC, 2010). Antimalarial chemicals have been identified in plants and other natural sources in many classes. Unsaturated hydrocarbons are another type of chemical that can be found in plants and food supplements. This class of hydrocarbons has been shown to have antimalarial properties. The antiplasmodial activity of Omega 3 and Omega 6 fatty acids was proven according to Okonkon *et al* (2017).

2.13.3 Natural Product Classes in Malaria Treatment

2.13.3.1 Alkaloids

One of the most important groups of natural goods is alkaloids. This category of natural goods has long been prized for its medical properties. Alkaloids have been used to treat parasite infections in the past. Quinine is an indole alkaloid derived from *Cinchona succirubra* Pav. ex Klotzsch, a plant in the *Rubiaceae* family. Quinine has been used to treat malaria for more than three centuries. Indole alkaloids derived from natural sources have demonstrated promising antiplasmodial efficacy *in vitro* and *in vivo* systems (Frederich *et al*., 2008). Naphthylisoquinolines, bisbenzylisoquinolines, protoberberines, aporphines, indoles, and manzamines are some of the antimalarial alkaloids that have been discovered.

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These were extracted from natural product extracts and showed promising anti-plasmodial efficacy against various malaria parasite strains.

In *P. berghei* infected mice, extracts of *Triphyophyllum peltatum* (Hutch. and Dalziel) Airy Shaw, of the *Dioncophyllaceae* family, which were discovered as containing naphthylisoquinoline alkaloids, demonstrated strong antiplasmodial activity (Amoa Onguéné et *al*., 2013). Korupensamine, a monomeric naphthylisoquinoline alkaloid with antiplasmodial activity (IC50=2.0 g/ml) (Hallock *et al*., 1997), and korundamine A, a heterodimeric naphthylisoquinoline alkaloid with antiplasmodial activity (IC50=1.1 g/ml) (Hallock *et al.,* 1998) were isolated from *Ancistrocla* Angerhofer *et al*. (1999) studied the antiplasmodial and cytotoxic properties of 53 bisbenzylisoquinoline alkaloids. These alkaloids had IC50 values of 29-1500nM and 59-4030nM, respectively, against the D6 and W2 clones of *P. falciparum*. Mambu *et al*. (2000) demonstrated the antiplasmodial action of chemicals from the stem bark of *Isolona qhesquierenina*, a member of the *Annonaceae* family. Wright *et al.* (2000) discovered that protoberberine alkaloids have promising antiplasmodial properties. Mendiola *et al*. (2006) have reported on the antiplasmodial actions of three ascidians. Manzamine alkaloids isolated from an Indonesian *Acantho strongylophia* sponge were found to have antimalarial properties, according to Rao *et al*. (2006). Manzamine alkaloids have also been shown to have antimalarial action in previous research (Ang *et al*., 2000; Roa *et al.,* 2004).

2.13.3.2 Terpenoids

This category includes a vast number of structurally different natural compounds (Zwenger and Basu, 2008). Sesquiterpenes, diterpenes, triterpenes, and miscellaneous terpenes are some of the other types of terpenes.*Vernonia* has a lot of sesquiterpene lactones. Numerous researchers have documented the anti-malarial activity of sesquiterpenes from *Distephanus*

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angulifolius (DC.) *H.Rob. & B.Kahn* of the Asteraceae family (Pedersen *et al*., 2009), *Tithoma diversifolia* A.Gray of the Asteraceae family (Goffin *et al*., 2002), *Drechslera dematioidea* (Bubák & W (Topcu *et al*., 2003). The Caribbean gorgonian octocoral *Eunice* species produced novel cembradiene diterpenoids with antimalarial activity (IC50 15.0 g/ml) (Wei *et al.,* 2004). Five antimalarial labdane diterpenoids were extracted and tested against an FCBI chloroquine resistant strain from *Aframomum zambesiacu*m K.Schum. Seeds of the Zingiberaceae family (Kenmogne *et al.,* 2006). Novel furanoterpenoids isolated from the ethyl acetate fraction of *Siphonochilus aethiopicus* (Schweinf.) B.L.Burtt of the *Zingiberaceae* family showed moderate antiplasmodial activity (Lategan *et al*., 2009). Artemisinin and its derivatives are sesquiterpene trioxane lactones with antimalarial activity due to the presence of an endoperoxide bridge (Martinelli *et al*., 2008).

2.13.3.3 Quassinoids and limonoids

Okunade. (2003) reported the activity of two quassinoids: ailanthone (IC50=0.03 g/ml) and 6α-tigloloxychaparrinone (IC50=0.06 g/ml). These were isolated from the Simaroubaceae family's *Ailanthus altissima* (Mill.) Swingle. Antimalarial activities of pasakbumin B, pasakbumin C, and eurycomanone were identified from *Eucalyptus longifolia* Link of the Myrtaceae family (IC50 22.6 ng/ml, 93.3 ng/ml, and 40.0 ng/ml, respectively) (Kuo *et al*., 2004; Chan *et al*., 2004). Quassinoids from the roots of *Simaba orinocensis* Kunth of the *Simaroubaceae* family demonstrated antiplasmodial action against the D6 and W2 strains (IC50 3.0 and 3.67 ng/ml vs. 3.2 and 8.5 ng/ml, respectively) (Muhammad *et al.,* 2007). Quassinoids, on the other hand, have shown *in vivo* toxicity due to protein synthesis suppression, and efforts to design selective inhibitors of parasite and host cell ribosomes have been hampered by the difficulty of distinguishing between the two. This could indicate that the quassinoids' antimalarial effect is related to their toxicity to host cells. Limonoids from the Meliaceae family's *Cedrela odorata L.* (Bray *et al*., 1990), *Khaya senegalensis*

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A.Juss. (Khalid *et al*., 1998), and *Khaya grandifoliola* C.DC. (Khalid *et al*., 1998) have been shown to be shown in vitro (Bickii *et al*., 2000). This group includes *Azadirachta indica* A.Juss. (Neem), a member of the Meliaceae family that has been widely employed as an antiplasmodial agent.

2.13.3.4 Flavonoids and Chalcones

Flavonoids have been found to have promising antimalarial properties. *Wikstroema indica* (L.) C.A.Mey. of the family Thymelaeaceae, *cikokianin B* and C (IC50 0.53 and 0.56 g/ml) (Nunome *et al*., 2004) are two biflavones that have been demonstrated to have moderate to good antiplasmodial activity. Isolates from *Ochna integerrima* (Lour.) Merr. of the *Ochnaceae* family (IC50 80 ng/ml) and *Garcinia livingstonei* T. Anderson of the *Clusiaceae* family (IC50 6.7 M) (Ichino *et al*., 2006). (Mbwambo *et al*., 2006). Licochalcone A, an antiplasmodial compound derived from the Leguminosae plant Glycyrrhiza inflata Batalin, has shown promising results. This is due to its ability to suppress *Plasmodium* protease activity (Chen *et al*., 1994). Nyasol, a chalcone isolated from *Asparagus africanus* Lam of the *Asparagaceae* family, was found to have mild antiplasmodial activity with an IC50 of 49 1M. (Oketch-Rabah *et al*., 1997).

2.13.3.5 Peptides

This group has produced a variety of chemicals having antiplasmodial properties. From the insect pathogenic fungus *Paecilomyces tenuipes*, two antiplasmodial compounds with modest activity against the K1 strain (EC50=1.60 and 12.0 g/ml) were identified. The *cyclodepsipeptides*, which include *beuvericin* and *beuvericin A*, are a class of chemicals (Nilanonta *et al.,* 2000). *P. falciparum* was successfully treated using a cyclic peptide derived from a marine sponge. The recorded activity can be linked to the apical protrusion, which appears to obstruct merozoite invasion of erythrocytes (Mizuno *et al.,* 2002). Fennel et al., (2003) revealed the antimalarial activity of dolastatin 10, a peptide microtubule

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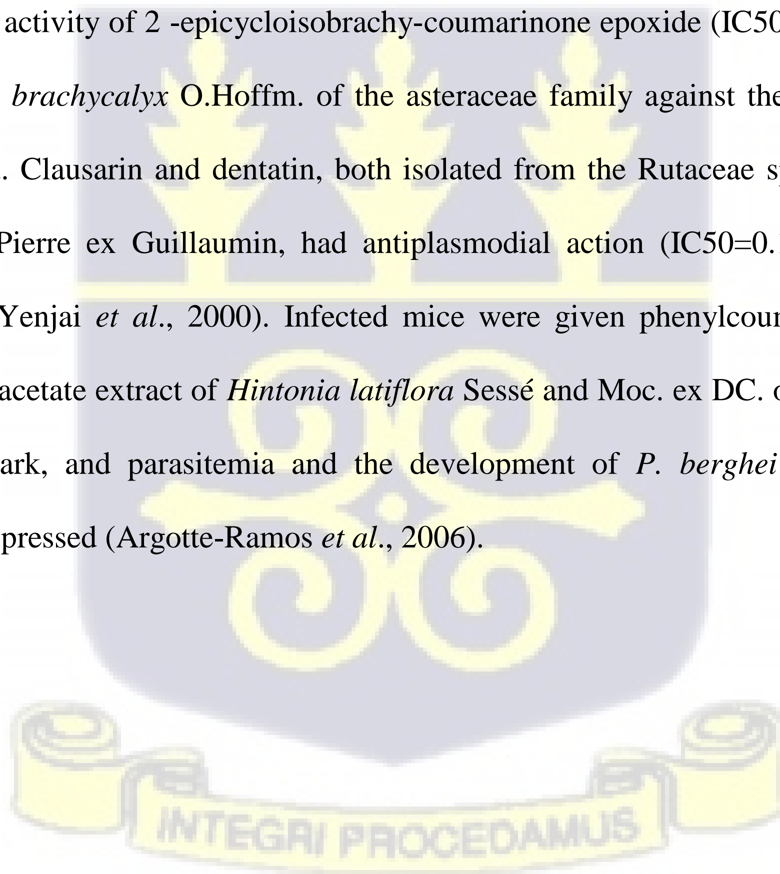
inhibitor derived from the sea hare D. auricularia. Venturamide A and B (IC50=8.2 and 5.6 M, respectively) are two cyclic hexapeptides derived from the marine *Cyanobacterium oscillatoria* showing antimalarial efficacy against the W2 strain and modest toxicity against Vero cells in mammals (Linington *et al.,* 2007).

2.13.3.6 Xanthones, quinones, and coumarins

Cowaxanthone (IC50=1.5 g/ml), calothwaitesixanthone (IC50 2.7 g/ml), and mangostin (IC50 17.0 M) were isolated from *Garcinia cowa* Roxb. of the *Clusiaceae* family, *Calophyllum caledonicum* Vieill. ex Planch. and Triana of the Clusiaceae family, and *Garcinia mangostana* L. of the (Quinone methides A and B, which were isolated from the roots of *Salasia kraussii* Harv. of the *Celastraceae* family, had high antiplasmodial action (IC50 = 94.0 and 27.6 ng/ml, respectively) (Argotte-Ramos *et al*., 2006). The antiplasmodial activity of 2 -epicycloisobrachy-coumarinone epoxide (IC50=54 M) isolated from *Vernonia brachycalyx* O.Hoffm. of the asteraceae family against the Dd2 strain has been described. Clausarin and dentatin, both isolated from the Rutaceae species *Clausena harmandiana* Pierre ex Guillaumin, had antiplasmodial action (IC50=0.1 and 8.5 g/ml, respectively) (Yenjai *et al*., 2000). Infected mice were given phenylcoumarins extracted from the ethyl acetate extract of *Hintonia latiflora* Sessé and Moc. ex DC. of the *Rubiaceae* family stem bark, and parasitemia and the development of *P. berghei* schizonts were completely suppressed (Argotte-Ramos *et al*., 2006).



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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Herbal products

Seven (7) selected herbal products were purchased from different pharmaceutical and herbal shops in Greater Accra. The selection of these herbal products were based on general information gathered from preliminary survey using questionnaire administered to 400 participants in Greater Accra. Convenience sampling method was adopted in the administration of the questionnaires. The total sample was calculated based on 95% confidence level (Z score =1.96) with 5% margin error and the total population of Greater Accra from 2010 population and housing cencus.

These selected herbal products used in this research are: **TH** (*Ocimum viride, Vernonia amygdalina, Morinda lucida, Alstonia boonei, Carica papaya, Corn still*)**, AM** (*Alstonia boonia, Naulea latifolia ,Enantia forlycarpa*) **, GM** (*Terminalia ivorensis, Pycnanthus angolensus, Alstonia boonei*)**, AHM** (*Astonia boonei, Lanea kerstingil, Mangnifera Indica*)**, GHM** (*Khaya senegalenis, Azadirachta indica*) **, MT** (*Khaya senegalensis, Cryptolepis senguinolenta , Cassia siamea, Citrus aurantium*)**, OTM** (*Carica papaya, Cassia alata*)**.**

3.1.2 Plasmodium species

Chloroquine resistance (CQR) *P. falciparum* DD2 strain and Chloroquine sensitive (CQS) *P. falciparum* 3D7 strains and *P. berghei* were obtained from Department of Immunology at Noguchi Memorial Institute for Medical Research, University of Ghana and Department of Biochemistry of University of Ghana.

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3.1.3 Antimalarial control drugs and Reagents

Drugs and reagents used are: Chloroquine (sigma), Artesunate (sigma), Amodiaquine (sigma), DMSO(sigma), HEPES, L-glutamine, Albumax, Hypoxanthine, Sodium bicarbonate, RPMI1640 powder (GIBCO) 10.43g per pack, RPMI 1640medium,Glucose, Albumax, Hypoxanthine,SYBR Green 1 (10000 in DMSO), Ethanol, Methanol, Immersion oil, Giemsa stain, EDTA, Saponin, Triton X-100,Gas mixture, Na_2HPO4, NaCl, KH_2PO4, Sorbitol, Glycerol, Gas mix (92.5% N_2, 5.5 % CO_2, 2% O_2,).

3.1.4 Consumable

The following were consumables used in the experiment: gloves, serological pipette, pipette tips, reagent reservoirs, 96 well plates, Culture flasks, Falcon tubes, Nalgene Filter unit, Weighing boat, Cryogenic vials, Aluminum foil, Frosted end microscope slides.

3.1.5 Equipment

Equipment employed in this experiments were Freeze dryer (Labconco freezone 4.6L), Class 11 Biosafety cabinet, Centrifuge, Cell Counter, Vortex, aspirators, Water bath, Modular incubator chamber, CO2 Incubator set at 37°C, Electric vacuum pump, Fluorescence plate reader (MTPR), Light Microscope, Refrigerator, Freezer, High Power Liquid Chromatography Machine, Atomic Absorption Spectrometry Machine

3.2 Ethics

Ethical clearance was obtained from Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (IRB) with approval number NMIMR-IRB CPN082/17-18 and also Noguchi Memorial Institute for Medical Research Institutional Animal Care and use Committee with approval number 2018-03Y2.

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3.3 Methods

3.3.1 General knowledge, perception and consumption of herbal use in Ghana

Four hundred (400) questionnaires were designed and administered in three (3) cities in Greater Accra (**Accra, Tema and Ashaiman**) which are highly populated cities according to 2010 Population and housing census. This tool was used to gather information on general knowledge, perception, consumption and most commonly use malaria herbal products in Greater Accra, Ghana. Seven herbal products used for treating malaria and other pathogens were carefully selected through a thorough assessment of individual responses from the questionnaires administered. These selected herbal products were approved by the Foods and Drugs Authority (FDA) of Ghana.

3.3.2 Freeze drying process

Two hundred and fifty millilitres (250ml) of each herbal product was transferred into different freeze drying cups and tightened well under sterile conditions and kept at -80°C overnight. They were then lyophilized using the LABCONCO Freezone 4.6L to preserve the quality and integrity of the herbal products (Chimsook, 2018).

3.3.3 In vitro antiplamodial experiment

The *in vitro* antiplasmodial studies involved a continuous culturing of both strains, *Plasmodium* Chloroquine resistant Dd2 and *Plasmodium* Chloroquine sensitive 3d7. The *in vitro* assay was carried out in a sterile environment in a biosafety cabinet. The inclusion of red blood cells to the *in vitro* setup was required for continuous cultivation of the erythrocytic stage. The O+ human blood was collected from volunteer individual donors and then later washed in a procedure described in 3.3.4 washing of O+ blood below (Trager and Jensen, 1976)

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3.3.3.1 Preparation of medium

Two media were used for the *in vitro* cultivation of the parasites: complete medium (CM) and incomplete medium.

3.3.3.1.1 Incomplete medium

It is made up of 10.4g/L RPMI 1640 with glutamine (GIBCO), 2g Dextrose (GIBCO), 7.15g HEPES, 0.088g/L Hypoxanthine and 1.2 ml/L (0.05 g/L) gentamycin (Sigma-Aldrich). At the desired pH the colour indicator in the RPMI appears orange. The medium was filtered through 0.22 μm filter and store in the Fridge at 4^0 C.

3.3.3.1.2 Complete medium

The addition of 5ml of 20% Albumax and 7.5% sodium bicarbonate (6.4 ml) to 200 ml to incomplete medium gave rise to a complete medium (CM). This was also filtered through 0.22 μm filter unit and then stored in the Fridge at 4^0 C until use.

3.3.4 Washing of the O+ blood

A portion of the WBC is removed during the washing process. The RBCs that are left were used to cultivate malaria parasites *in vitro*. An aliquot of blood was transferred into a sterile 5ml falcon tubes.The washing process begin by removing plasma and buffy coat. It was mixed with an equivalent amount of incomplete medium and centrifuged for 5 minutes at 2500rpm. The supernatant was aspirated after spinning and was discarded. The process was repeated with the addition of an equal volume of complete medium (CM) as before. The supernatant was removed and the RBC containing pellet was resuspended in an equal volume of complete medium (CM) and then kept in the fridge at 4°C until needed.

3.3.5 Parasite culturing and preparation

The efficacy of herbal products on asexual parasite stages was tested on the 3D7 chloroquine-sensitive strain of *P. falciparum* and Dd2 Choroquine- resistant strain of

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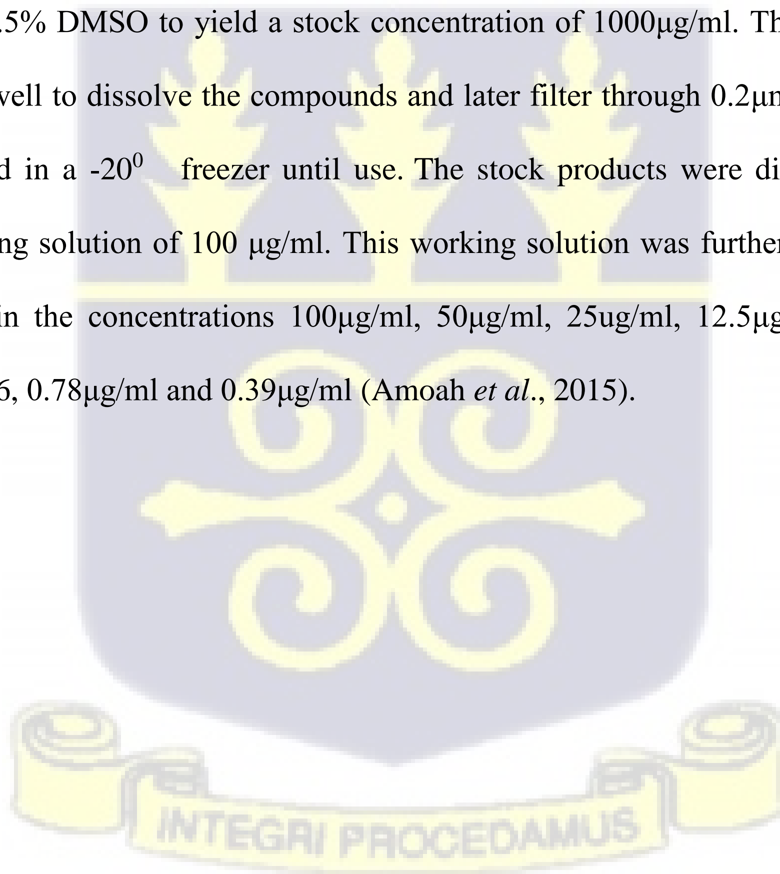
P.falciparum. Continuous *P. falciparum* asexual cultures were maintained *in vitro* using a modified method of (Trager and Jensen, 1976) in an atmosphere of 93% N2, 4% CO2, and 3% O2 at 37°C in complete medium (CM) (10.44 g/liter RPMI 1640, 5.94 g/liter HEPES, 5 g/liter AlbuMAX II, 50 mg/liter hypoxanthine, 2.1 g/liter sodium bicarbonate). Parasites were cultured in O+ RBCs and maintained in the incubator at 37°C with daily media change until a parasitemia of more than 5% ring stages were obtained. The culture was then treated with 5% sorbitol to obtain synchronized ring stage. Parasite mixture of 2% hematocrit with 1% parasitemia were prepared using uninfected blood to make a total of 14ml in a complete culture medium for the plating.

3.3.6 Preparation of herbal extract

Ten milligrams (10mg) powder of each extracts/compounds was weighed and transferred into 10ml of 0.5% DMSO to yield a stock concentration of 1000µg/ml. The stock solution was vortexed well to dissolve the compounds and later filter through 0.2µm pore filter unit and then stored in a -20^{0} freezer until use. The stock products were diluted 10 fold to obtain a working solution of 100 µg/ml. This working solution was further serially diluted 9-fold to obtain the concentrations 100µg/ml, 50µg/ml, 25ug/ml, 12.5µg/ml, 6.25µg/ml, 3.13µg/ml, 1.56, 0.78µg/ml and 0.39µg/ml (Amoah *et al*., 2015).



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3.3.7 Herbal extract plating and assay

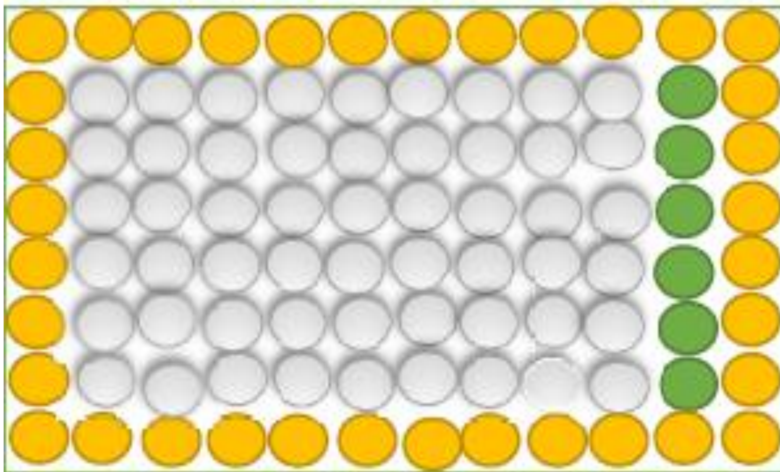

Fig 3.0 diagrammatic representation of the 96-well microtitre plate set-up used in this study.

KEY: Yellow colour= Represent the incomplete media filled in the outer well to prevent edging effect, Green = Negative control (Parasite mix without drugs), Ash = Column 2-10th (Parasite mix + drugs in duplicates for 3 different herbal products).

One hundred microliters of each nine dilutions was plated in duplicates wells of 96 well plate shown in the **Fig 3.0** above. Artesunate (200ng/ml) and Chloroquine (1000ng/ml) was serially diluted and plated alongside with the herbal products as control standard antimalarial drugs. One hundred microlitres of parasite mix with 2% hematocrit and 1% parasitemia of each parasite strains were added to each treated well starting from the 2nd well to the tenth well represented by the ash colour in **Fig 3.0** above.

One hundred microliters of parasite mix with same 2% hematocrit and 1% parasitemia were added to the 11th wells as a negative control as shown in the colour green in Fig **3.0** above and the procedure was repeated for the rest of other extract/compounds and the plates were arranged in a modular Chamber and gassed for 5min with gas mixture of 5% Oxygen, 5% Carbon dioxide and 90% Nitrogen and then kept at 37 °C for 72 hrs.

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3.3.8 SYBR Green assay

The plates were harvested after 72hrs and and the assay were paused by adding 100ul lysing buffer containing SYBR Green to each well and was thoroughly and gently mixed to avoid production of bubbles. The plates were then incubated in the dark for 30minutes before reading the assay using FLUOstar OPTIMA Fluorometer plate reader with control software version 2.20 at 470nm and 520nm wavelengths.

3.3.9 Statistical Analysis

Each product was tested in a duplicate and herbal product concentration that inhibits asexual *Plasmodium falciparum* parasite by 50% (IC_{50}) were estimated from dose-response curves by non-linear regression analysis using Graph pad Prism version 7.0 Software (Graph Pad software, San Diego, CA, USA). A non parametric test (Kruska Wallis H test) was perfomed to find out significant differences among the performance of the seven herbal products at 95% confidence level using R 4.0.5 studio software package.

3.4 Ex vivo assay using P. berghei from infected mice

Four (4) mice (25 to 30 g) were obtained from the Animal Experimentation Department of Noguchi Memorial Institute for Medical Research, University of Ghana and were randomly assigned to cages. The mice were allowed to acclimatize before the initiation of the experiments. Heparinized blood from infected mice (*P. berghei* GFP ANKA malaria strain was obtained from Department of Immunology of Noguchi Memorial Institute for Medical Research.

Ex vivo antimalarial activity was assessed using protocol by (Lakshminarayana *el al*, 2015) with modifications using two mice (20 to 22 g) intravenously infected with 2×10^7 erythrocytes parasitized with *P. berghei* ANKA malaria strain and then monitored for establishment of infection within 3-4 days. Parasite was monitored until 9% parasitemia obtained using prepared thin film smear stained with 10% working giemsa. Infected blood

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from the mice was drawn aseptically from the mice using sterile syringe and needle into ACD tube and later diluted in 14ml complete culture medium with washed red blood cells (RBCs) from uninfected mice to a hematocrit of 2% and a parasitemia of 1 % as a parasite mix for plating. One hundred microliters of each nine dilutions were plated in duplicates wells of 96 well coastal plate. Artesunate (200ng/ml) and Chloroquine (1000ng/ml) was serially diluted and plated alongside with the extract/compounds as a control standard antimalarial drugs. One hundred of parasite mix with 2% hematocrit and 1% parasitemia parasite mix was added to each treated well starting from the 2nd well to the 10th well and with 11th well as negative control with only parasite mix without any drug. The procedure was repeated for the rest of other extract/compounds and the plates were arranged in a modular Chamber and gassed for 5min with gas mixture of 5% Oxygen, 5% Carbon dioxide and 90% Nitrogen and then kept at 37 °C for 72 hrs.

3.4.1 Statistical analysis

Each product was tested in a duplicate and herbal product concentration that inhibits asexual *Plasmodium falciparum* parasite by 50% (IC_{50}) were estimated from dose-response curves by non-linear regression analysis using Graph pad Prism version 7.0 Software (Graph Pad software, San Diego, CA, USA). A non parametric test (Kruskal Wallis H test) was perfomed to find out significant differences among the performance of the seven herbal products at 95% confidence level using R 4.0.5 studio software package.

3.5 Heme crystallization inhibition mechanism using Colorimetric Inhibition Assay

Simple colorimetric inhibition assay of heme crystallization by Huy *et al.* (2007) was used with some modification.

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3.5.1 Preparation of hemin chloride

Hemin chloride (16.3 mg; Sigma) was dissolved in 1 ml of dimethyl sulfoxide. The solution was passed through a 0.2- m-pore membrane filter to remove insoluble particles. The solution was kept at 4°C as a stock solution (25) and later diluted to obtain 111.1 M of heme with 1 M acetate buffer of pH 4.8, just before being used (Huy *et al.,* 2007).

3.5.2 Preparation of drug compounds

Chloroquine (Sigma), amodiaquine (from MP Biomedical Inc.), and quinacrine (from Calbiochem, Canada) were dissolved in dis-tilled water. Quinine sulfate (Q; Sigma), quinidine (from Wako, Osaka, Japan), and 8-hydroxyquinoline (Sigma) were dissolved in 20 mM sulfuric acid. Mefloquine (from Sigma) was prepared in methanol. pH was controlled at 4.8 in all assays. Tween 20 (1 g/100 ml) was dissolved in distilled water (Huy *et al.,* 2007).

3.5.3 Evaluation of availability of BH formation induced by tween 20 and heme crystallization inhibition

Antimalarial control drugs and the herbal products were prepared at various concentrations using appropriate diluents in a 96 well plate. Ninety microliter of heme solution (50 uM), freshly buffered by 1 M acetate buffer (pH 4.8) from dimethyl sulfoxide stock, was added into the plate. Tween 20 was added into each well at 0.012 g/liter and the plate was incubated for 250 min at 37°C. The samples were mixed by being pipetted three times after incubation and then the plate was read at 415/630 nm. The fraction (*f*) of heme converted to BH was calculated as in a previous study (Trang *et al*., 2006).

$$f = (A_{control} - A_{sample}) / (A_{control} - A_{min})$$

where $A_{control}$ is the absorbance of the heme without Tween 20 or an antimalarial at 415/630 nm, while A_{sample} represents the absorbance of the heme in the presence of both Tween 20 and drugs and A_{min} is the absorbance of the heme with Tween 20 in the absence of an antimalarial at 415/630 nm.

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3.5.4 Statistical Analysis

The values obtained from duplicate assays were analysed and the IC_{50} values (the concentrations inhibiting 50% of heme crystallization) were calculated using GraphPad Prism.

3.6 Cytotoxicity Assay

The compounds were tested for toxicity to red blood cells using a modified version of the tetrazolium-based colorimetric technique by (Appiah-Opong *et al.*, 2011). One hundred microliters of each diluted compounds with concentrations ranging from 6.25 μg/ml to 100 μg/ml were put in triplicate wells of a 96-well microtiter plate. Following that, 100 μl of uninfected red blood cells were put into each well. Compound, culture medium, and uninfected red blood cells were subtracted from the optical densities by running control experiments for each parameter independently alongside the main experiment. The plates were then incubated for 72 hours at 37°C in a humidified incubator with 5% O2 and CO2 before 20 μl of 7.5mg/ml MTT (in phosphate buffered saline) solution was added to each well and the plate was incubated for another 2 hours. After incubation, aliquots of culture medium (150 μl) were taken and discarded from each well, and 200 μl of Triton X-100 in acidified isopropanol was added to each well to dissolve any formazan produced. The plates were then maintained at room temperature in the dark for 24 hours before the optical densities of the wells were measured using a plate reader at 570 nm.

3.6.1 Statistical Analysis

The concentrations at which 50% cytotoxicity occurred (CC_{50} values) were obtained by using Graphpad Prism. The CC50 values were compared to standard values.

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3.7 Heavy metals and electrolyte analysis

3.7.1 Sample digestion

Seven (7) commercial herbal products of various brands were analyzed for their toxic metal and electrolyte contents. They were available in liquid dosage forms. For these liquid dosage forms, 1 ml of the liquid sample each was taken in a 100 ml flask and made up to 100 ml with deionized water (Gomez *et al.,* 2007). For viscous suspensions, a few drops of nitric acid (65 %) were added to digest the particles and this was done to eliminate bacteria and sediment particles from the solution, to reduce sorption losses to the container walls and also stabilize ions in solution since most metals are soluble at low pH. The solution was then filtered through Whattman filter paper no. 42 and later kept in a transparent bottle until analyzed by atomic absorption spectrometer (AAS).

3.7.2 Sample analysis

The FAAS (pinAAcle 900t) was turned on, calibrated with the various calibration standards and the samples automatically ran in triplicate. Mean concentration of the analyte was recorded on the software interface on a computer (Perkin-Ekmer Corporation, 1996). A non parametric test (Kruska Wallis H test) was perfomed at 95% confidence level to find out significant differences of each heavy metals and electrolytes among the seven herbal products using R 4.0.5 studio software package.

3.7.3 Health risk assessment of heavy metals in herbal products

3.7.3.1 Metal Pollution Index (MPI) of Herbal Products.

The overall heavy metal concentration of the herbal products was calculated by computing metal pollution index (MPI) by using the following formula:

MPI (mg/kg) = Cf1 x Cf2 x Cf3 …… x Cfn)$^{1/n}$ ………………………………… (1)

Where Cfn = Concentration of metal, n= Number of metals analyzed according to (Eze *et al*., 2018).

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3.7.3.2 Average Daily Dose (ADD) of metals or Average Daily Intake (ADI) of metals

Average Daily Dose was calculated by using the concentrations of heavy metals and reference input parameters from (Wongsasuluk *et al*., 2014). Reference input parameters were: Ingestion rate (IR) = 2.2 d/day, Exposure frequency (EF) = 365 days/year, Exposure duration (ED) = 70years, Adult BW (Body weight) = 70Kg, Child BW (Body weight) = 16 Kg, Average time (AT) = 25,550 days.

The following equation was used:

$$ADD = \frac{C \times IR \times EF \times ED}{BW \times AT} \quad \text{...} \ (2)$$

C = Metal concentration in the herbal products, **EF**= Exposure Frequency, **ED**= Exposure duration, **BW**= Body weight of consumer, **AT** = Average time. (George *et al.,* 2017; Sultana *et al.,* 2019).

3.7.3.3 Non-carcinogenic risk assessment of heavy metals intake in the herbal products

This was conducted to estimate the potential health risks of heavy metal pollutants in the herbal products by using target hazard quotients (HQ) and Hazard Index (HI).

3.7.3.3.1 Hazard Quotients (HQ)

Hazard Quotients (HQ) is a proportion of the probable exposure to an elements/ chemicals and level at which no negative impacts are expected and this was calculated by using the following formula:

$$\text{Hazard Quotients (HQ)} = \frac{ADD}{RFD} \quad \text{...} \ (3)$$

Where ADD = Average Daily Dose of Heavy metal calculated in equation 2.

RFD = Oral Reference Dose from was gotten from literature (USEPA, 2010; George *et al.*, 2017; Sultana *et al.,* 2019).

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3.7.3.3.2 Hazard Index (HI)

Hazard Index (HI) is an exposure to more than one pollutant results in additive effects and it was calculated by using the following formula**:**

Hazard Index (HI) = (HQ1 + HQ2+ HQ3 + …+HQ6) ………………………… (4)

Where HQ = Hazard Quotients of each heavy metals in the herbal products. (Sultana *at al.,* 2019; George *et al*., 2017; Ametepey *et al*., 2018)

3.7.3.4 Carcinogenic risk assessment of heavy metal intake in the herbal products

Carcinogenic risk (CR) indicated an incremental probability of an individual of developing cancer over a lifetime due to exposure to a potential carcinogen. Cancer risk over a lifetime exposure to Lead (Pb) and Arsenic (As) using the cancer slope factor provided by USEPA and the formula as follow:

CR = CSF X ADD ………………………………………………………………. (5)

CR = Carcinogenic Risk, CSF= Cancer Slope factor, ADD= Average Daily Dose

But CSF FOR Pb = 0.085 mg/kg/day, CSF for As = 1.5 mg/kg/day (USEPA, 2010; Ullah *et al*., 2017; Luo *at al*., 2020)

3.8 High Performance Liquid Chromatography (HPLC) Analyses

The HPLC analyses was preformed using an Agilent 1100 system (Santa Clara, CA, USA) made up of an autosampler, quaternary pump and diode array detector. The column used was C18 (Tskgel ODS, with 5 µm diameter, length x width 150 mm x 4.6 mm). Twenty microliters (20 µL) of sample was injected and the wavelength monitored at 254 nm. The eluents were: water (0.1% phosphoric acid) (A) and methanol (B) at a flow rate of 0.7 mL/min. The gradient system used was as follows; 0 -5 min, 5-10 % B; 5-10 min, 10-20% B; 10-12 min, 20-50 %B; 12-15 min, 50-80% B; 15-20 min, 80-80 % B; 20-23 min, 80-5% B,23-25 min, 5-5% B (Soares *et al*., 2012).

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3.9 PH contents of herbal products.

The pH of a solution is a measure of hydrogen ion concentration, which in turn is a measure of its acidity. HANNA digital pH meter was used to determine the pH contents of the various herbal products (Macharáčková *et al*., 2021).

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CHAPTER FOUR

4.0 RESULTS

4.1 General Knowledge, Perception and Consumption of Herbal Medicine

From the **Table 4.0** below, a total of four hundred (400) participants were interviewed in a preliminary survey on knowledge, perception and consumption of herbal medicine including antimalarial herbal drugs in some part of Greater Accra. This was done to determine the extent of herbal use and also the most commonly used antimalarial herbal drugs in Greater Accra, Ghana. Out of this 194 (48.5%) were females and 206 (51.5%) were males. Eight out of 400 representing 2% have no formal education, 87 (21.8%) have basic education, 150(37.8%) have secondary education and 155(38.8) have tertiary education. About 385 representing 99.5% have heard about herbal medicine in Ghana while two people representing 0.5% have not heard about herbal medicine before.

Out of 400 respondents, 287(71.8%) have used herbal medicine before while 113(28.3%) have not used herbal medicine for treatment of any disease condition before. For the common diseases treated with herbal medicine, 174(43.5%) used herbal for malaria, 28(2.8%) used for the treatment of Typhoid, 11(2.8%) for ulcer while 187 people representing 46.8% used it for treatment of other diseases. Three hundred and sixty-six (366, 91.5%) think herbal medicines are effective while 34 (8.6%) think otherwise. For treatment of malaria, 30(7.6%) used Taabia herbal medicine, 34(8.5%) used Time herbal Mixture, 24(6%) used Rooter Mixture and 312(78%) used other herbal medicine such as Away Mala Mix, Givers Herbal Mixture, Osompa, Typhofa, Geo Manuel, Mala typhs, Aseda for malaria treatment. One hundred and eighty-eight (188) representing 47% believed that Herbal medicines are better than orthodox treatment while 101(25.3%) do not think so and 111(27.8%) do not have any idea about its effectiveness as compared to orthodox medicines.

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Table 4.1: Outcome of the priliminary survey on general knowledge, perception and consumption of herbal products in greater Accra, Ghana

CHARACTERISTICS/QUESTIONS	N(400) (%)
SEX	
Female	194 (48.5%)
Male	206 (51.5%)
Education	
No Education	8 (2%)
Basic Education	87 (21.8%)
Secondary Education	150(37.8%)
Tertiary	155(38.8%)
Heard about herbal medicine	
Yes	398(99.5%)
No	2(0.5%)
Use of herbal medicine	
Yes	287 (71.8%)
No	113(28.3%)
Herbal treatment for common diseases	
Malaria	174 (43.5%)
Typhoid	28(7%)
Ulcer	11(2.8%)
Others	187(46.8)
How often herbal products are used	
More often	60 (15%)
Sometimes	137 (34.3%)
Not often	104 (26%)
Not at all	99(24.8%)
Effectiveness of herbal treatment	
Yes	366 (91.5%)
No	34(8.6%)
Use of one herbal to treat multiple condition	
Yes	88(22%)
No	312(78%)
Use of following herbal for malaria treatment	
Taabia	30 (7.6%)
Time Herbal Mixture	34(8.5%)
Rooter Mixture	24(6%)
Others (Away, Givers, Osompa, Typhofa, Geo manuel, Mala typhs, Aseda etc).	312(78%)
Is herbal treatment better than orthodox	
Yes	188(47%)
No	101 (25.3%)
Not sure	111(27.8%)

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4.2 In vitro and ex vivo antiplasmodial activity of herbal drugs

From the (**Table 4.2**) bellow, using *Plasmodium falciparum* Chloroquine sensitive laboratory strain, AM (Aseda Herbal Mixture) have the highest IC_{50} values as 56.00 µg/ml followed by TH (Typhofa Herbal) with IC_{50} values as 39.51 µg/ml. GHM (Givers Herbal Mixture) and AHM(Aseda Herbal Mixture) have IC_{50} values as 9.40 µg/ml and 9.06 µg/ml respectively and MT (Mala Typhs) recorded the least IC_{50} value as 2.18 µg/ml. Artesunate (ART), Chloroquine (CQ) and Amodiaquine (ADQ) were standard antimalarial drugs used as controls and recorded an IC_{50} values as 0.0001µg/ml, 0.12 µg/ml and 0.0144 µg/ml respectively.

For *Plasmodium falciparum* Chloroquine-resistance strain, the highest IC_{50} values was recorded by TH (Typhofa Herbal) as 51.34 µg/ml followed by GM (Geo Manuel) and AM (Away Malamix) with 26.17 µg/ml and 24.72 µg/ml respectively. OTM (Osompa T Malamix) and AHM (Aseda Herbal Mixture) also recorded 19.85 µg/ml and 18.78 µg/ml respectively. Finally, GHM (Givers Herbal Mixture) recorded 8.47 µg/ml as the least IC_{50} values. Artesunate (ART) and Chloroquine (CQ) were standard antimalarial drugs used as controls and recorded an IC_{50} values as 0.0023 µg/ml and 0.0005 µg/ml respectively.

The IC_{50} values of the various herbal products and standard control drugs tested against *Plasmodium burghei* in the *ex vivo* antimalarial drug experiment in this study. GM (Geo Manuel) recorded the highest IC_{50} values as 10.96 µg/ml followed by MT (Mala Typhs) with 10.20 µg/ml. TH (Typhofa Herbal), OTM (Osompa T Malamix) and AHM (Aseda Herbal Mixture) also recorded IC_{50} values as 9.02 µg/ml, 5.65 µg/ml and 3.20 µg/ml respectively. Finally, AM (Away Malamix) recorded the least IC_{50} values as 1.16 µg/ml. Artesunate (ART) and Chloroquine (CQ) were standard antimalarial drugs used as controls and recorded an IC_{50} values as 0.00089µg/ml and 0.01µg/ml respectively.

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For the cytotoxicity, the CC_{50} values were all above 100 and their selectivity indices were above 4. The resistivity index for all the herbal products ranged from 0.44 to 12.06.

Table 4.2: In vitro and ex vivo antimalarial activity of selected herbal drugs on Plasmodium falciparum and Plasmodium berghei and toxicity of these herbal products towards Human Red blood Cells

In vitro and *ex vivo* antimalarial activity of selected herbal drugs and their cytotoxicity results.

Herbal products/drug	IC_{50} 3D7 (µg/ml) ± std error	IC_{50} DD2 (µg/ml) ± std Error	IC_{50} P. berghei (µg/ml) ± std error	CC_{50} RBCs (µg/ml) ± std error	SI 3D7	SI DD2	SI P.berghei	RI
MT	2.18±0.03	9.02±0.02	10.20±0.12	263.00±0.97	120.64	29.16	25.78	4.14
GHM	9.40±0.04	8.47±0.02	0.50±0.02	202.80±0.70	21.57	23.94	405.6	0.90
AM	56.00±15.59	24.72±0.05	1.16±0.02	247.30±2.14	4.42	10.00	213.19	0.44
OTM	4.21±0.08	19.85±0.03	5.65±0.02	104.10±0.25	24.73	5.24	18.42	4.71
AHM	9.06±0.03	18.78±0.03	3.20±0.02	307.40±2.94	33.93	16.37	96.06	2.07
GM	2.17±0.07	26.17±0.26	10.96±0.04	223.70±0.84	103.08	8.55	20.41	12.06
TH	39.51±0.02	51.34±0.01	9.02±0.02	504.90±2.94	12.78	9.83	55.98	1.30
ART	0.00011 ± 0.00005	0.0022687± 0.00024	0.000089± 0.00072	51.27±0.14	>100	>100	>100	ND
CQ	0.12 ± 0.00003	1.05 ± 0.00050	0.01 ± 0.00003	ND	ND	ND	ND	ND
ADQ	0.0144± 0.00020	ND	ND	ND	ND	ND	ND	ND

IC50 Values are given as the mean of two replicate of the experiments.

Key: **MT**=Mala Typhs, **GHM**= Givers Herbal Mixture, **AM**= Away Malamix ,**OTM**=Osompa T Malamix ,**AHM**=Aseda Herbal Mixture, **GM**= Geo Manuel, **TH**= Typhofa Herbal ,**CQ**= Chloroquine ,**ART**= Artesunate, **ADQ** = Amodiaquine , **RBCs**= Red blood cells, **ND**= Not determine , **SI** = Selectivity index = cytotoxic antiplasmodial ratio(

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CC_{50} RBCs/IC_{50} *P. falciparum* or *P. berghei*) ,**RI** = Resistance index = IC_{50} DD2/IC_{50} 3D7, **CC_{50}**= The 50% cytotoxic concentration =the herbal product/drug concentration that reduced the cell viability by 50% when compared to untreated controls, **IC_{50}**= the 50% inhibitory concentration= concentration of herbal products/drug that inhibit 50% of parasite growth when compared to untreated control.

4.3 Geometric mean IC_{50} values of Herbal drugs against Chloroquine-sensitive 3D7 Plasmodium falciparum Lab strain.

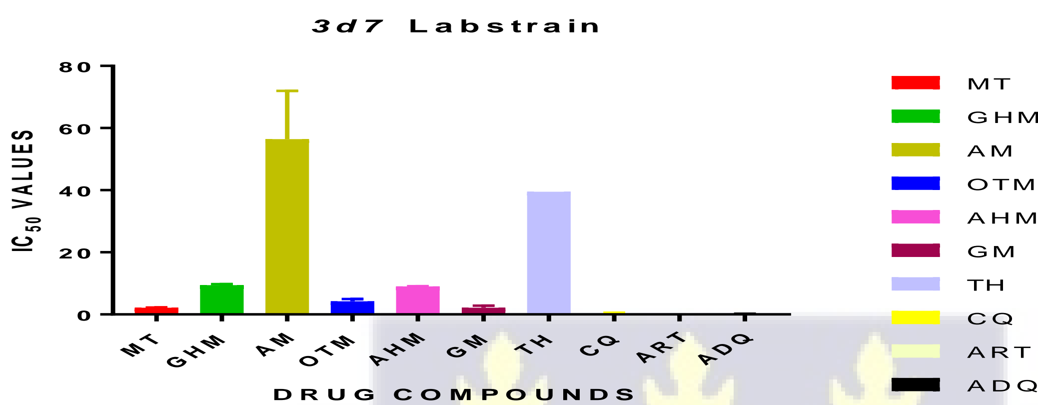

Fig 4.0 Geometric mean of various IC_{50} values of herbal products for 3d7 Plasmodium falciparum Chloroquine sensitive strain.

Key: **MT**=Mala Typhs, **GHM=** Givers Herbal Mixture, **AM**= Away Malamix, OTM=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM**= Geo Manuel, **TH**= Typhofa Herbal, **CQ**= Chloroquine, **ART**= Artesunate, **ADQ** = Amodiaquine

From the (**Fig 4.0**) above, using *Plasmodium falciparum* Chloroquine sensitive laboratory strain, AM (Aseda Herbal Mixture) have the highest IC_{50} values as 56.00 µg/ml followed by TH (Typhofa Herbal) with IC_{50} values as 39.51 µg/ml. GHM (Givers Herbal Mixture) and AHM(Aseda Herbal Mixture) have IC_{50} values as 9.40 µg/ml and 9.06 µg/ml respectively and MT (Mala Typhs) recorded the least IC_{50} value as 2.18 µg/ml. Artesunate (ART), Chloroquine (CQ) and Amodiaquine (ADQ) were standard antimalarial drugs used as

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controls and recorded an IC_{50} values as 0.0001µg/ml, 0.12 µg/ml and 0.0144 µg/ml respectively. **Fig 4.1** bellow show dose-response curves for the various herbal drugs used.

4.4 Dose response curve for herbal drugs on 3D7 Labstrain.

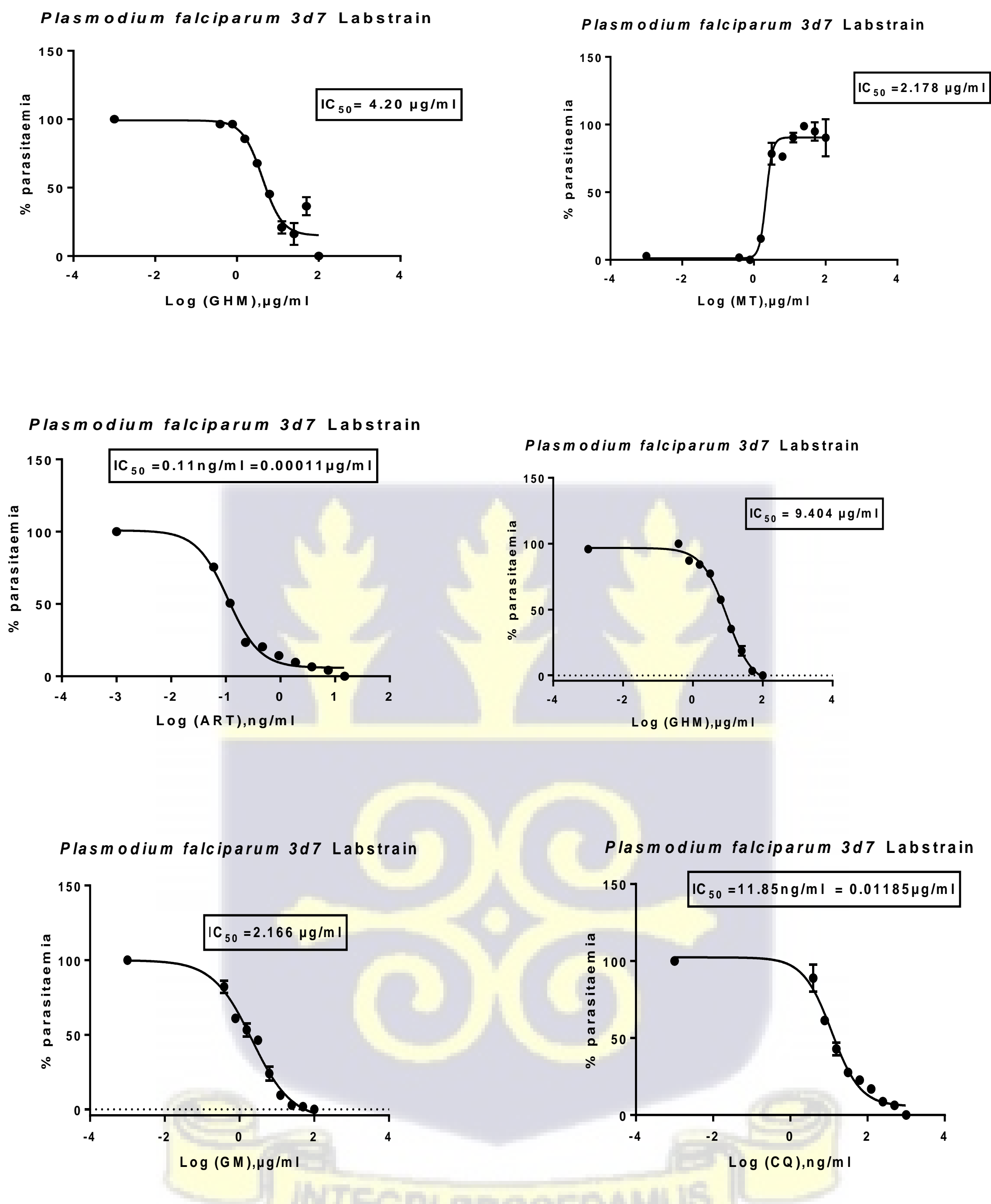

Fig 4.1: Dose response curves of herbal products on 3d7 Plasmodium falciparum Chloroquine sensitive strain

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Key: **MT**=Mala Typhs, **GHM=** Givers Herbal Mixture, **AM**= Away Malamix, **OTM**=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM**= Geo Manuel, **TH**= Typhofa Herbal, **CQ**= Chloroquine, **ART**= Artesunate.

4.5 Geometric mean IC_{50} values of Herbal drugs against Chloroquine-resistant Dd2 *Plasmodium falciparum* Lab strain

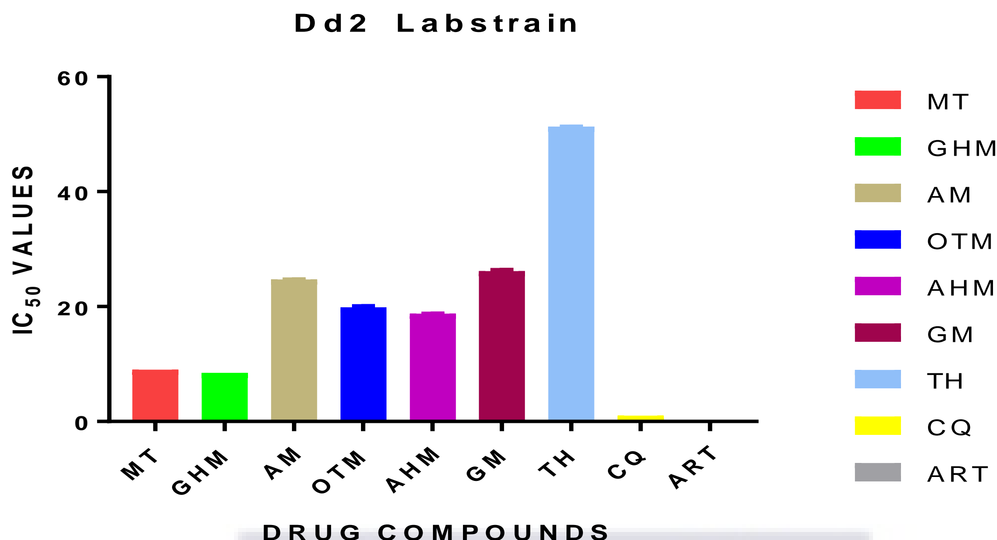

Fig 4.2: Geometric mean of various IC_{50} values of herbal products for Dd2 *Plasmodium falciparum* Chloroquine resistance strain.

Key: **MT**=Mala Typhs, **GHM=** Givers Herbal Mixture, **AM**= Away Malamix, **OTM**=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM**= Geo Manuel, **TH**= Typhofa Herbal, **CQ**= Chloroquine, **ART**= Artesunate.

From **Fig 4.2** above, the highest IC_{50} values was recorded by TH (Typhofa Herbal) as 51.34 µg/ml followed by GM (Geo Manuel) and AM (Away Malamix) with 26.17 µg/ml and 24.72 µg/ml respectively. OTM (Osompa T Malamix) and AHM (Aseda Herbal Mixture) also recorded 19.85 µg/ml and 18.78 µg/ml respectively. Finally, GHM (Givers Herbal Mixture) recorded 8.47 µg/ml as the least IC_{50} values. Artesunate (ART) and Chloroquine (CQ) were standard antimalarial drugs used as controls and recorded an IC_{50} values as 0.0023 µg/ml and 0.0005 µg/ml respectively. **Fig 4.3** bellow show dose-response curves for the various herbal drugs used.

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4.6 Dose response curve for herbal drugs on Dd2 Labstrain

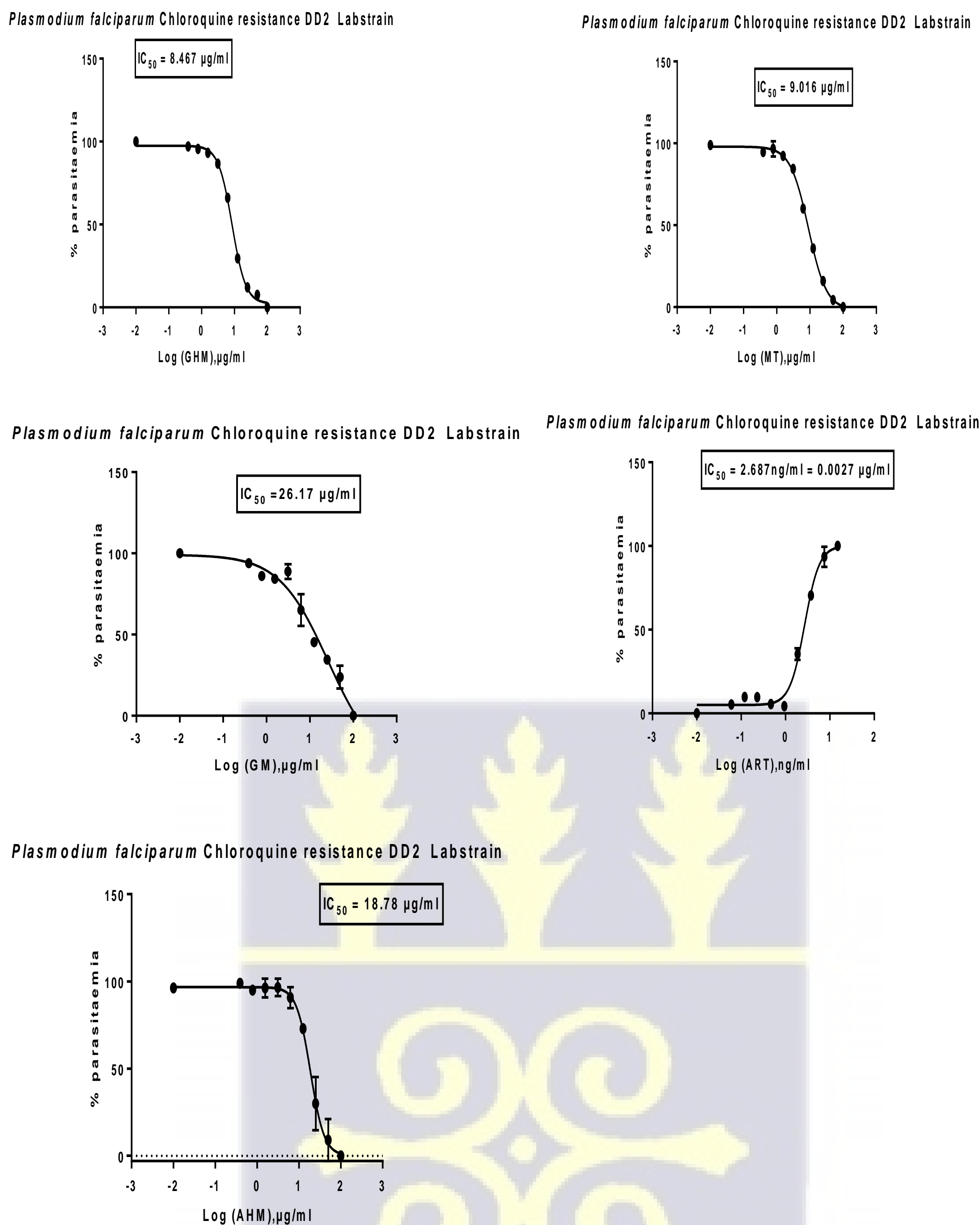

Fig 4.3: Dose response curves of herbal products on Dd2 Plasmodium falciparum Chloroquine resistance strain

Key: **MT**=Mala Typhs, **GHM**= Givers Herbal Mixture, **AM**= Away Malamix, **OTM**=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM**= Geo Manuel, **TH**= Typhofa Herbal, **CQ**= Chloroquine, **ART**= Artesunate.

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4.7 Geometric mean IC_{50} values of Herbal drugs against *Plasmodium berghei*

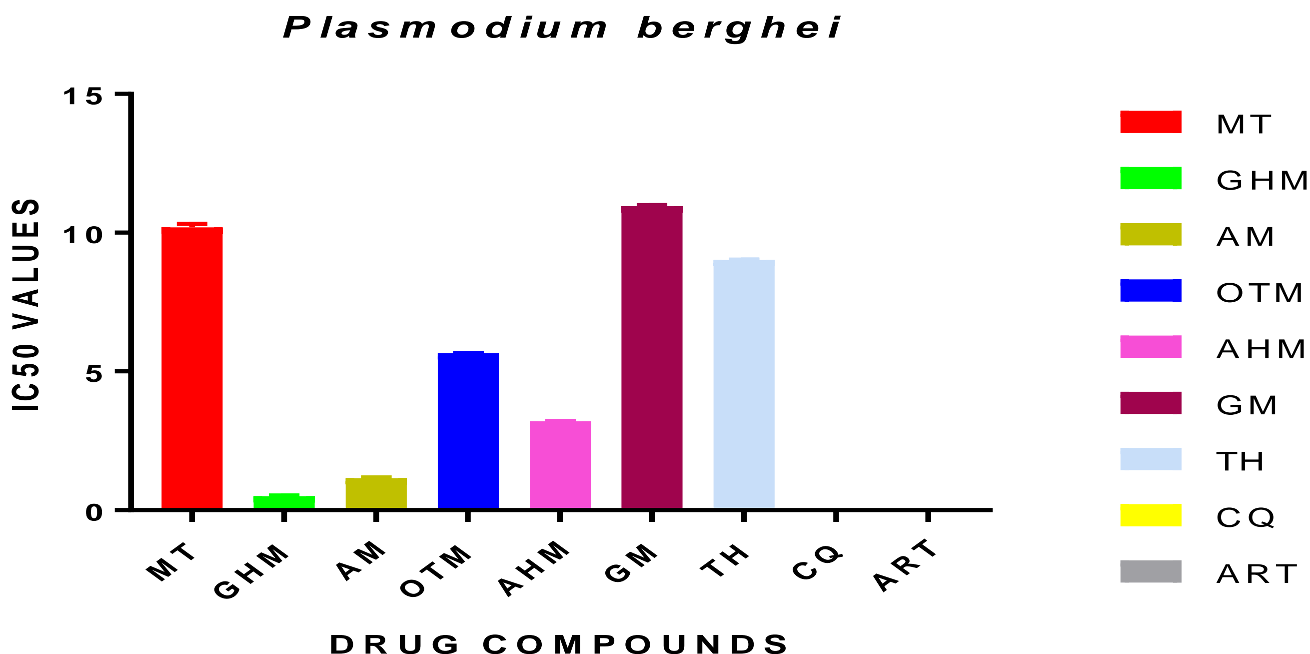

Fig 4.4: Geometric mean of various IC_{50} values of herbal products for *Plasmodium berghei.*

Key: **MT**=Mala Typhs, **GHM=** Givers Herbal Mixture, **AM**= Away Malamix, **OTM**=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM**= Geo Manuel, **TH**= Typhofa Herbal, **CQ**= Chloroquine, ART= Artesunate

Fig 4.4 shown IC_{50} values of the various herbal products and standard control drugs tested against *Plasmodium burghei* in the *ex vivo* antimalarial drug experiment in this study. GM (Geo Manuel) recorded the highest IC_{50} values as 10.96 µg/ml followed by MT (Mala Typhs) with 10.20 µg/ml. TH (Typhofa Herbal), OTM (Osompa T Malamix) and AHM (Aseda Herbal Mixture) also recorded IC_{50} values as 9.02 µg/ml, 5.65 µg/ml and 3.20 µg/ml respectively. Finally, AM (Away Malamix) recorded the least IC_{50} values as 1.16 µg/ml. Artesunate (ART) and Chloroquine (CQ) were standard antimalarial drugs used as controls

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and recorded an IC_{50} values as 0.00089µg/ml and 0.01µg/ml respectively. **Fig 4.5** bellow show dose-response curves for the various herbal drugs used.

4.8: Dose response curves of herbal products on *Plasmodium berghei*.

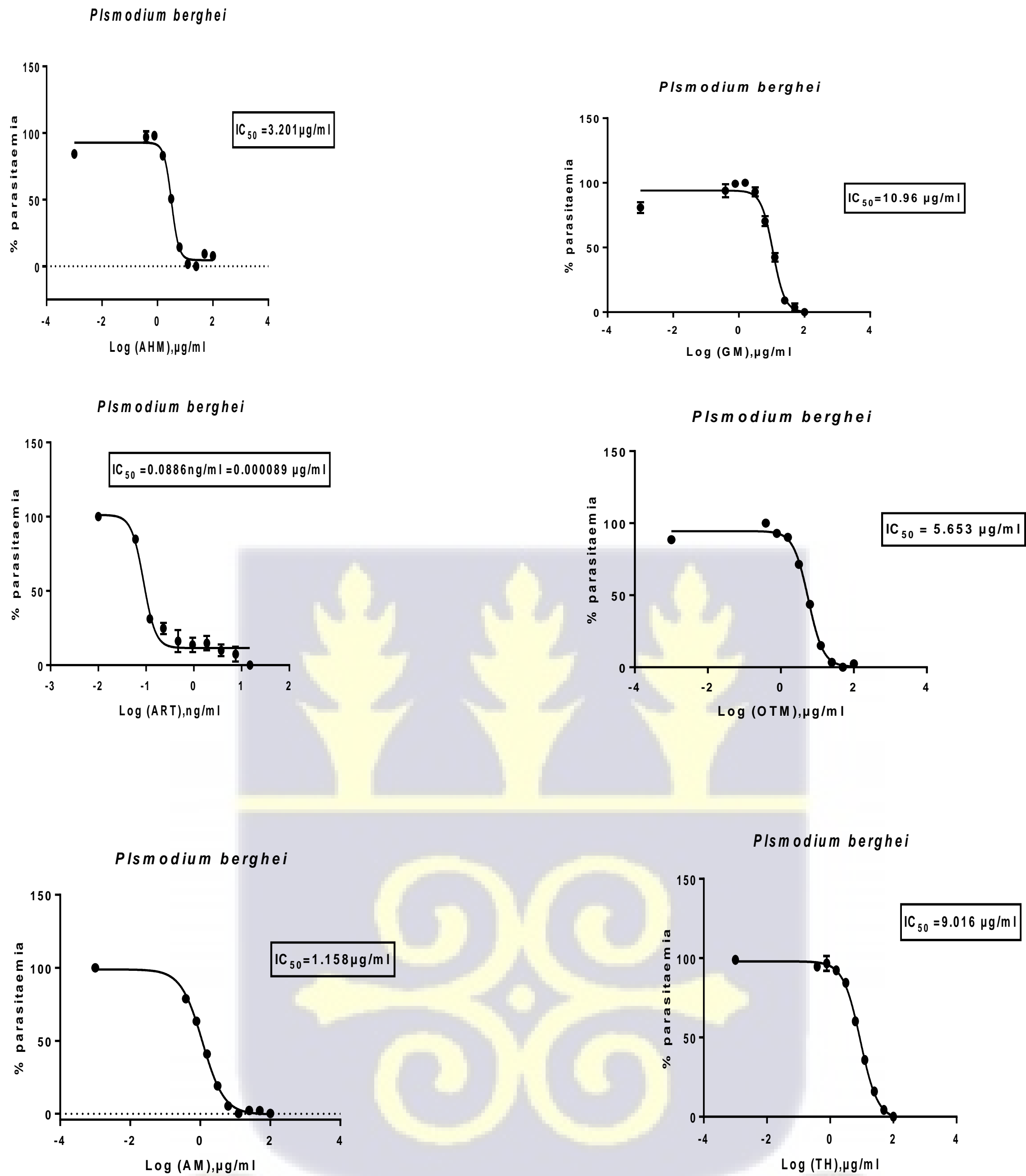

Fig 4.5: Dose response curves of herbal products on *Plasmodium berghei*.

Key: **MT**=Mala Typhs, **GHM=** Givers Herbal Mixture, **AM=** Away Malamix, **OTM**=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM=** Geo Manuel, **TH=** Typhofa Herbal, **CQ=** Chloroquine, **ART=** Artesunate.

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4.10 Result for Mechanism of Action of various herbal products

Table 4.3: Mean absorbance, f-Calculated and IC50 values for various herbal products/drugs in Drug mechanism assay

Drug/Herbal products	Mean Absorbance	Fraction of heme converted to BH	IC50 µg/ml
Heme (Acontrol)	938		
Heme with Tween 20 (Amin)	10683.5		
CQ (Asample)	31235	3.1088	0.1507
ADA (Asample)	20444	2.0015	0.03128
MQ (Asample)	24368.5	2.4042	0.09555
MT (Asample)	48128.5	4.8423	1.859
GHM (Asample)	41587.5	4.1711	2.38
AM(Asample)	8823	0.8091	46.87
OTM(Asample)	25003.5	2.4694	1.67
AHM (Asample)	11083	1.0410	49.94
GM (Asample)	40928	4.1034	12.6
TH (Asample)	42047	4.2183	122.2

Formula for calculating the fraction, f = (Acontrol – Asample)/ (Acontrol- Amin) where

f = fraction of heme converted to BH, Acontrol is the absorbance of heme without Tween20 initiator or an antimalarial at 415/630 nm, Asample is the absorbance of heme in the presence of both Tween 20 initiator and drug, Amin is absorbance of the heme with Tween 20 in the absence of an antimalarial drugs at 415/630 nm. **IC_{50}** = (Concentration of the drugs that inhibit 50% of heme crystalization).

Key: **BH**= Beta Hematin, **MT**=Mala Typhs, **GHM=** Givers Herbal Mixture, **AM**= Away Malamix, **OTM**=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM**= Geo Manuel, **TH**= Typhofa Herbal, **CQ**= Chloroquine, **ADQ** = Amodiaquine, **MQ**= Mefloquine.

Table 4.3 shown various herbal drugs including standard antimalarial drugs with their absorbance, Fraction of heme converted to BH by Tween 20(initiator of heme crystallization) and IC_{50} values (Concentrations of the drugs that inhibit 50% of heme crystalization). MT (Mala Typhs) recorded highest absorbance as 48128.5 with fraction of heme converted to BH as 4.8423 and 1.859 µg/ml as IC_{50} value followed by TH (Typhofa Herbal) with absorbance as 4204, fraction of heme converted BH as 4.2183 and IC_{50} values as 122.2 µg/ml. OTM recorded the lowest IC50 value (1.67 µg/ml) with 2.4694 as a fraction of heme converted by Tween 20.

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4.11 Mean Cell Viability of various herbal products

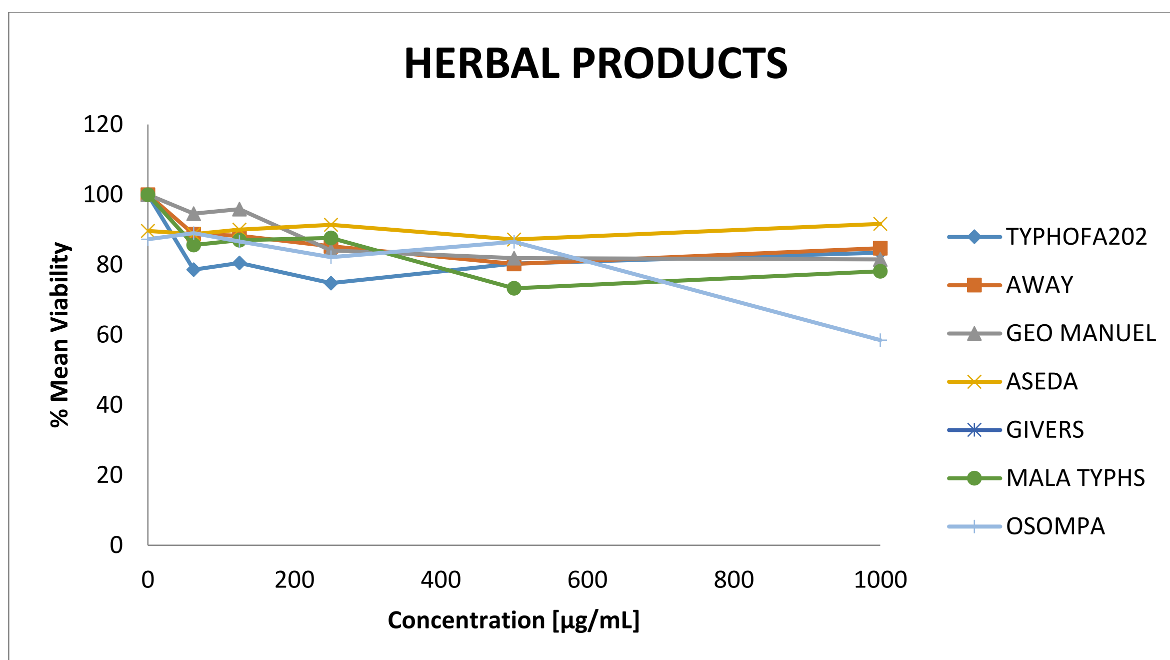

Fig 4.6: Percentage mean cell viability of various herbal products in cytotoxicity assay

Key: **MT**=Mala Typhs, **GHM=** Givers Herbal Mixture, **AM**= Away Malamix, **OTM**=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM**= Geo Manuel, **TH**= Typhofa Herbal.

Fig 4.6 above represent percentage cell viability demonstrated in the cytotoxicity assay with varying concentrations of various herbal drugs. The mean cell viability of the herbal drugs are above 60% demonstrating their non-cytotoxic effect to the human red cells. AHM (Aseda Herbal Mixture) have the highest mean cell viability followed by AM (Away Malamix) and TH (Typhofa Herbal). The least mean cell viability was recorded by OTM (Osompa T Malamix).

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4.12 Heavy metal levels from Atomic absorption spectrometry result of herbal drugs.

Table 4.4: Heavy metal contents of the selected herbal products

Herbal products/Drugs	Heavy Metals					
	Cu Mg/Kg	**Pb Mg/Kg**	**Zn Mg/Kg**	**Fe Mg/Kg**	**As Mg/Kg**	**Hg Mg/Kg**
TH	3.1	0.1	0.4	12.8	0.1	0.2
AM	1.1	0.2	0.6	4.4	0.1	0.1
MT	1.8	0.1	0.1	0.7	0.3	0.1
GHM	7	0.1	0.7	2.8	0.2	0.2
AHM	0.2	0.2	0.3	3.4	0.1	0.1
OTM	0.6	0.1	0.2	2.9	0.2	0.3
GM	0.8	0.1	0.8	2.6	0.1	0.1

Key: **MT**=Mala Typhs, **GHM=** Givers Herbal Mixture, **AM**= Away Malamix ,**OTM**=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM**= Geo Manuel, **TH**= Typhofa Herbal, **Cu** = Cupper, **Pb**= Lead, **Zn** = Zinc, **Fe** = Iron, **As**= Arsenic, **Hg**= Mercury.

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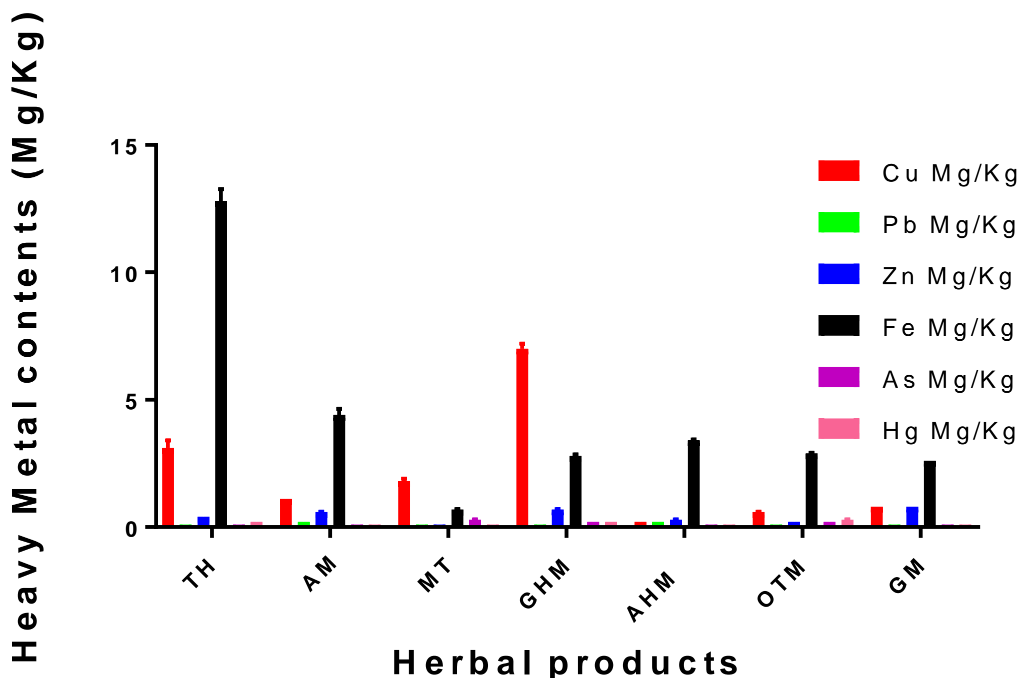

Fig 4.7: Heavy metal levels of various herbal drugs

Key: **MT**=Mala Typhs, **GHM**= Givers Herbal Mixture, **AM**= Away Malamix, **OTM**=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM**= Geo Manuel, **TH**= Typhofa Herbal, **Cu** = Cupper, **Pb**= Lead, **Zn** = Zinc, **Fe** = Iron, **As**= Arsenic, **Hg**= Mercury.

Table 4.4 illustrated various heavy metal contents of selected herbal drugs used in this study. Heavy metals such as Copper (**Cu**), Lead (**Pb**), Zinc (**Zn**), Iron (**Fe**), Arsenic (**As**) and Mercury (**Hg**) were assessed in seven herbal drugs. GHM recorded the highest Cupper content (7Mg/Kg) and next was TH with 3.1Mg/Kg followed by MT with 1.8 Mg/Kg. AM recorded 1.1 Mg/Kg of the Copper level while GM and OTM recorded 0.8 and 0.6 respectively with AHM having the least Copper content (0.2Mg/Kg). There was a significant difference in the Copper contents among the various herbal drugs ($p<0.05$). For Lead (Pb) level, MT and AHM recorded the highest as 0.2Mg/Kg and the rest have lowest Lead level as 0.1Mg/Kg. There was no significant level in the Lead content of the various herbal drugs ($p=0.2$). GM had 0.8Mg/Kg of Zinc (Zn) which was the highest followed GHM and AM which recorded 0.7 Mg/Kg and 0.6Mg/Kg respectively. TH , AHM, OTM

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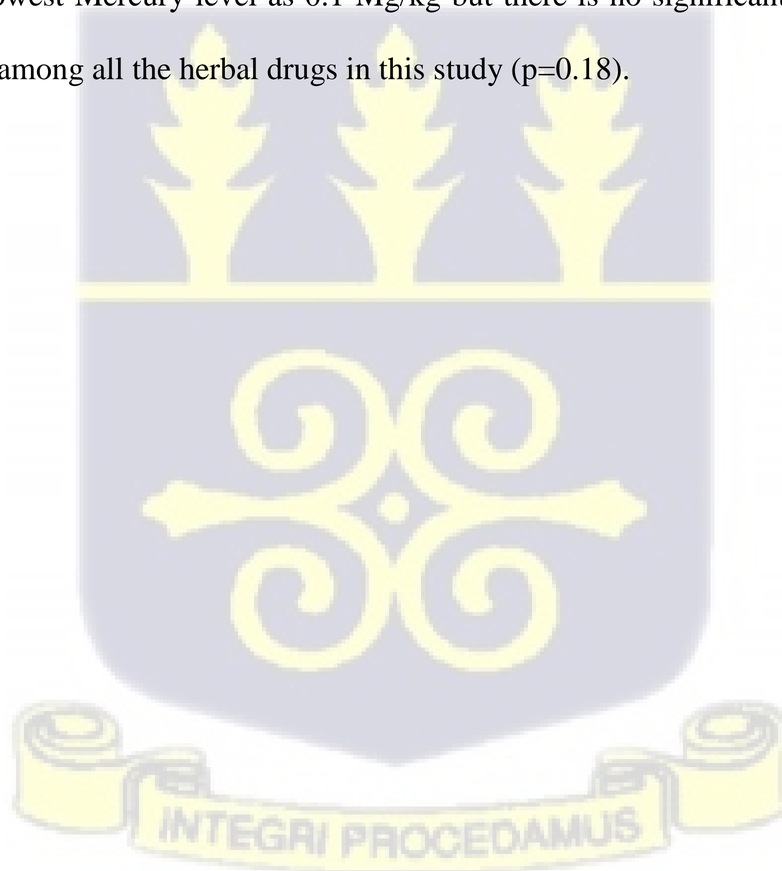
also recorded Zinc level as 0.4Mg/Kg, 0.3Mg/Kg, 0.2Mg/Kg respectively with MT having the lowest level of Zinc as 0.1Mg/Kg. There is no significant level in the Zinc (Zn) contents for the various herbal products (p=0.059). TH had 12.8Mg/Kg as highest Iron (Fe) level recorded followed by AM, AHM, OTM, GHM and GM which were 4.4Mg/Kg, 3.4Mg/Kg, 2.9Mg/Kg, 2.8Mg/Kg and 2.6Mg/Kg respectively with MT having the lowest level as 0.7Mg/Kg. There is a significant level of Iron (Fe) level in the various herbal drugs ($p<0.05$). MT recorded the highest level of Arsenic(As) level as 0.3Mg/Kg followed by OTM and GHM which recorded 0.2Mg/Kg with the rest of the herbal drugs recorded 0.1Mg/Kg as the lowest Arsenic (As) level.There was no significant level of Arsenic contents in the various herbal drugs (p=0.097). OTM recorded the highest level of Mercury (Hg) as 0.3Mg/kG followed by GHM and TH as 0.2 Mg/kg. GM, AHM, MT and AM recorded the lowest Mercury level as 0.1 Mg/kg but there is no significant different in the Mercury level among all the herbal drugs in this study (p=0.18).



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4.13 Metal pollution index calculated for various herbal products

Table 4.5: Metal pollution Index (MPI)

HERBAL PRODUCTS	MPI
TH	0.56
AM	0.42
MT	0.27
GHM	0.61
AHM	0.27
OTM	0.36
GM	0.34

Table 4.5 show Metal Pollution Index (MPI) for various herbal products used in the study. **GHM** herbal recorded the highest as 0.61 followed by **TH** herbal as 0.51. **AM** recorded 0.42, **OTM** and **GM** recorded 0.36 and 0.34 respectively. **MT** and **AHM** herbal recorded the lowest Metal Pollution Index as 0.27.

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4.14 Average daily dose (ADD) (mg/kg/day) of metals for exposure estimation assessment for herbal products

Table 4.6: Average daily dose (ADD) (mg/kg/day) of metals for exposure estimation assessment for herbal product

Heavy metals	Herbal products						
	TH	AM	MT	GHM	AHM	OTM	GM
Cu (Adult)	0.0974	0.0346	0.566	0.22	0.0063	0.0189	0.0251
Cu (Child)	0.4263	0.1513	0.2475	0.9625	0.0275	0.0825	0.11
Pb(Adult)	0.0031	0.0063	0.0031	0.0031	0.0063	0.0031	0.0031
Pb(Child)	0.0138	0.0275	0.0138	0.0138	0.0275	0.0138	0.0138
Zn(Adult)	0.0126	0.0189	0.0031	0.022	0.0094	0.0063	0.0251
Zn(Child)	0.055	0.0825	0.0138	0.0963	0.0413	0.0275	0.11
Fe(Adult)	0.4023	0.1383	0.022	0.088	0.1069	0.0911	0.817
Fe(Child)	1.76	0.605	0.0963	0.385	0.4675	0,3988	0.3575
As(Adult)	0.0031	0.0031	0.094	0.0063	0.0031	0.0063	0.0031
As(Child)	0.0138	0.0138	0.0413	0.0275	0.0138	0.0275	0.0138
Hg(Adult)	0.0063	0.0031	0.0031	0.0063	0.0031	0.0094	0.0031
Hg(Child)	0.275	0.0138	0.0138	0.275	0.0138	0.0413	0.0138

Table 4.6 show the average daily dose (**ADD**) (mg/kg/day) of heavy metals esposure estimated for various herbal products in the study. Estimated daily dose of Cupper (**Cu**) for both Adult and Children ranged from 0.0063mg/kg/day to 0.9625mg/kg/day while that of Lead (**Pb**) ranged from 0.003mg/kg/day to 0.0275mg/kg/day, Zinc (**Zn**) ranged from

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0.0031mg/kg/day to 0.11mg/kg/day, Iron (**Fe**) ranged from 0.022mg/kg/day to 1.76mg/kg/day/ Arsenic (**As**) ranged from 0.094mg/kg/day, Mercury (**Hg**) ranged from 0.0031mg/kg/day to 0.275mg/kg/day.

4.15 Hazard Quotients (HQ) and Hazard Index (HI) for Non-Carcinogen risk assessment

Table 4.7: Hazard Quotients (HQ) and Hazard Index (HI) for Non-Carcinogen risk assessment

Heavy metals	Hazard Quotients (HQ) for Herbal products						
	TH	AM	MT	GHM	AHM	OTM	GM
Cu (Adult)	2.43	0.87	1.42	5.50	0.16	0.47	0.63
Cu (Child)	10.66	3.78	6.19	24.06	0.69	2.06	2.75
Pb(Adult)	0.09	0.18	0.09	0.09	0.18	0.09	0.09
Pb(Child)	0.39	0.79	0.39	0.39	0.79	0.39	0.39
Zn(Adult)	0.04	0.06	0.01	0.07	0.03	0.02	0.08
Zn(Child)	0.18	0.28	0.05	0.32	0.14	0.09	0.37
Fe(Adult)	0.57	0.20	0.03	0.13	0.15	0.13	0.12
Fe(Child)	2.51	0.86	0.14	0.55	0.67	0.57	0.51
As(Adult)	10.33	10.33	31.33	21	10.33	21	10.33
As(Child)	46	46	137.67	916.67	46	916.67	46
Hg(Adult)	21	10.33	10.33	21	10.33	31.33	10.3
Hg(Child)	916.67	46	46	916.67	46	137.67	46
Hazard Index (HI)							
Adult	**34.46**	**21.97**	**43.21**	**47.79**	**21.18**	**53.04**	**21.55**
Child	**976.41**	**97.7**	**190.44**	**1858.66**	**94.29**	**1057.45**	**96.02**

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Table 4.7 show the estimated hazard Quotients (HQ) and Hazard Index (HI) calculated for non-carcinogen risk assessment of both Adult and children exposure based on the heavy metals present in the various herbal products.**OTM** recorded the highest Hazard Index (HI) for adult exposure as 53.04 followed by **GHM** as 47.79. **MT** recorded 43.21 and **TH** recorded 34.46. **AHM**, **GM** and **AM** recorded 21.18, 21.55 and 21.97 respectively.

For Hazard Index (HI) for child exposure, GHM recorded highest as 1858.66followed by **OTM** as 1057.45. **TH** recorded 976.91, **MT** recorded 190.44. **AHM**, **GM** and **AM** recorded 94.29, 96.02 abd 97.7 respectively.

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4.16 Carcinogenic risk assessment

Table 4.8: Cancer risk assessment results for two heavy metals (Lead and Arsenic)

HERBAL PRODUCTS	Cancer Risk Values of two heavy Metals	
	Arsenic (As)	**Lead (Pb)**
TH		
Adult	4.65×10^{-3}	2.64×10^{-5}
Child	2.1×10^{-2}	1.17×10^{-4}
AM		
Adult	4.65×10^{-3}	5.36×10^{-5}
Child	2.1×10^{-2}	2.34×10^{-4}
MT		
Adult	1.4×10^{-1}	2.64×10^{-5}
Child	6.2×10^{-2}	1.17×10^{-4}
GHM		
Adult	9.45×10^{-3}	2.64×10^{-5}
Child	4.1×10^{-2}	1.17×10^{-4}
AHM		
Adult	4.65×10^{-3}	5.36×10^{-5}
Child	2.1×10^{-2}	2.34×10^{-4}
OTM		
Adult	9.45×10^{-3}	2.64×10^{-5}
Child	4.1×10^{-2}	1.17×10^{-4}
GM		
Adult	4.65×10^{-3}	2.64×10^{-5}
Child	2.1×10^{-2}	1.17×10^{-4}

Table 4.8 show the results for cancer risk assessment of Lead and Arsenic exposure in the various herbal products. The values recorded for both Adult and child exposure to possible Arsenic in various herbal products ranged from 2.1×10^{-2} to 9.45×10^{-3}. For the Lead, the values recorded for both Adult and Child exposure to possible Lead in various herbal products ranged from 1.17×10^{-4} to 5.36×10^{-5}.

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4.17 Electrolyte contents from Atomic spectrometry result of herbal drugs.

Table 4.9: Electrolyte content of selected herbal products

Herbal Products/ Drug	Electrolytes			
	K Mg/Kg	**Ca Mg/Kg**	**Mg Mg/Kg**	**Na Mg/Kg**
TH	13.9	1.1	0.1	0.2
AM	11.2	0.9	0.9	0.8
MT	40.5	102.7	0.3	0.3
GHM	1400	89.6	0.1	0.7
ASM	1.1	22.2	0.2	0.2
OTM	51.8	113.8	0.3	0.1
GM	1.9	78	0.1	0.4

Key: **MT**=Mala Typhs, **GHM=** Givers Herbal Mixture, **AM**= Away Malamix, OTM=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM**= Geo Manuel, **TH**= Typhofa Herbal, **K= Potassium**, **Ca**= Calcium, **Mg** = Magnesium, **Na** = Sodium.

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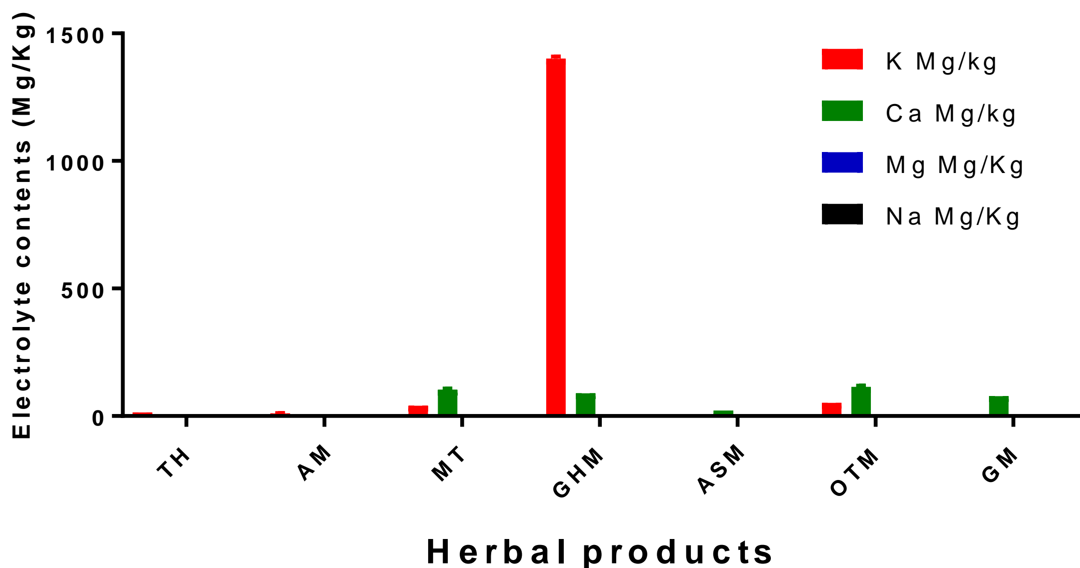

Fig 4.8: Electrolyte levels in various herbal drugs

Key: **MT**=Mala Typhs, **GHM=** Givers Herbal Mixture, **AM**= Away Malamix, **OTM**=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM**= Geo Manuel, **TH**= Typhofa Herbal, **K= Potassium**, **Ca**= Calcium, **Mg** = Magnesium, **Na** = Sodium.

From Table 4.9 and **Fig 4.8**, Electrolyte levels determined from various herbal drugs shown that Potassium (**P**) level was very high in GHM with 1400Mg/Kg recorded followed by OTM and MT which recorded 51.8 Mg/Kg and 40.5Mg/Kg respectively. TH and AM also recorded 13.9 Mg/Kg and 11.2 Mg/Kg respectively. GM recorded 1.9 Mg/Kg while ASM had the lowest Potassium level as 1.1 Mg/Kg. There is a significant different in the Potassium level among the seven herbal drugs ($p=0.046$). Calcium content was high in OTM, MT, GHM and GM with 113.8Mg/Kg, 102.7 Mg/Kg, 89.6Mg/Kg and 78Mg/Kg respectively. ASM and AM recorded 22.2Mg/Kg and 0.9Mg/Kg respectively With TH having the lowest level of Calcium as 1.1Mg/Kg. There is a significant different in the Calcium level among the herbal drugs ($p<0.05$). AM recorded the highest Magnesium (Mg) level with 0.9 Mg/Kg followed by MT and OTM as 0.3Mg/Kg respectively. ASM recorded 0.2Mg/Kg and TH, GHM and GM recorded lowest Magnesium (Mg) level as 0.1Mg/Kg respectively. There is no significant level in Magnesium (Mg) level among the various

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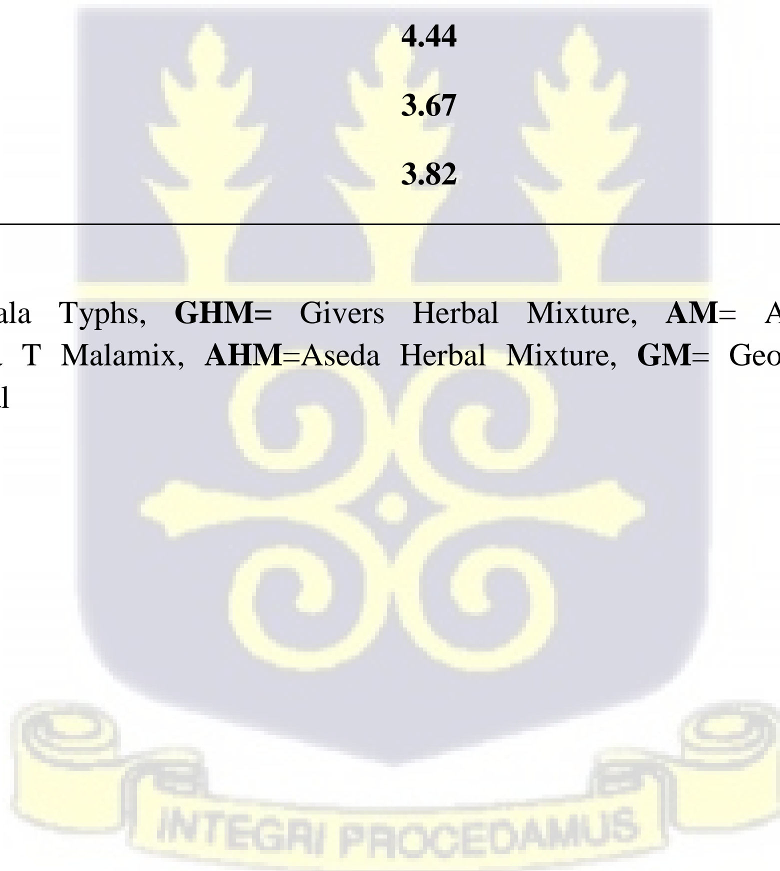
herbal drugs (p=0.063). AM and GHM recorded high level of Sodium (Na) as 0.8 Mg/Kg and 0.7Mg/Kg respectively followed by GM and MT with 0.4 Mg/Kg and 0.3 Mg/Kg respectively. TH and ASM recorded 0.2 Mg/Kg respectively and OTM with the lowest Sodium content. There is no significant level in Sodium (Na) content among the various herbal drugs (p=0.052).

4.18 pH levels of herbal drugs

Table 4.10: pH content of the selected Herbal Products

Herbal products/ Drug	pH Values
TH	**4.61**
AM	**4.21**
MT	**4.48**
GHM	**3.56**
AHM	**4.44**
OTM	**3.67**
GM	**3.82**

Key: **MT**=Mala Typhs, **GHM=** Givers Herbal Mixture, **AM**= Away Malamix, OTM=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM**= Geo Manuel, **TH**= Typhofa Herbal



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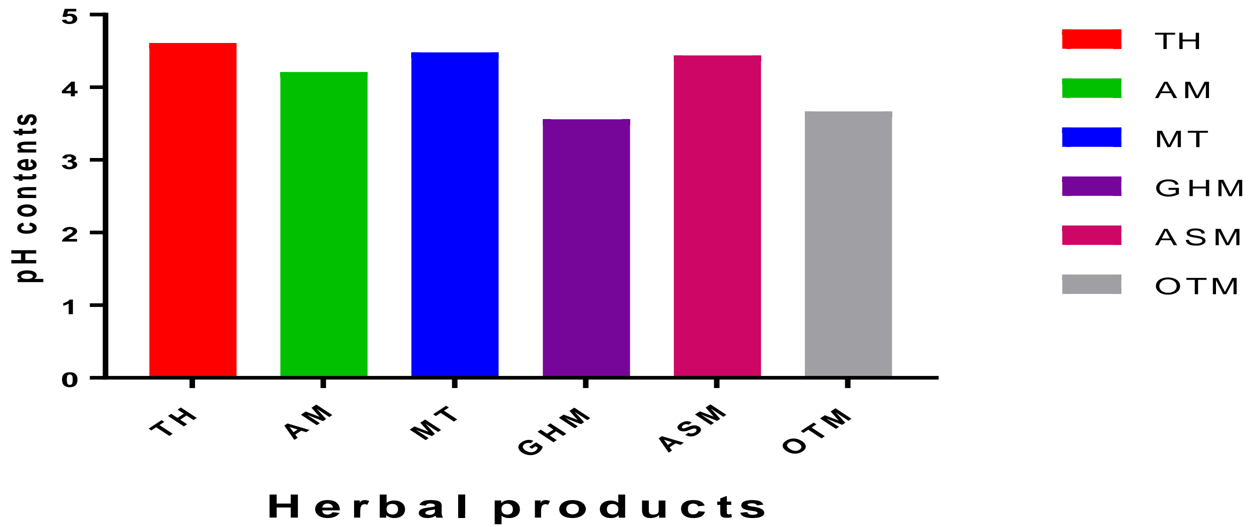

Fig 4.9: pH of the herbal drugs measured

Key: **MT**=Mala Typhs, **GHM=** Givers Herbal Mixture, **AM**= Away Malamix, **OTM**=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM**= Geo Manuel, **TH**= Typhofa Herbal

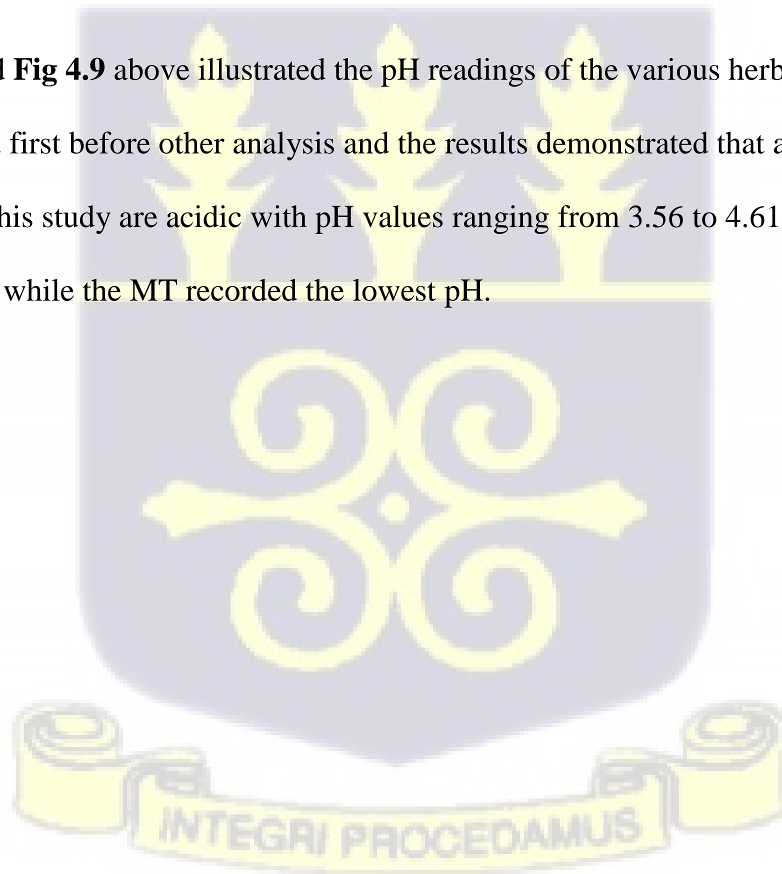

Table 4.10 and **Fig 4.9** above illustrated the pH readings of the various herbal drugs which were measured first before other analysis and the results demonstrated that all the herbal drugs used in this study are acidic with pH values ranging from 3.56 to 4.61. TH recorded the highest pH while the MT recorded the lowest pH.

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4.19 HPLC chromatograms of herbal drugs

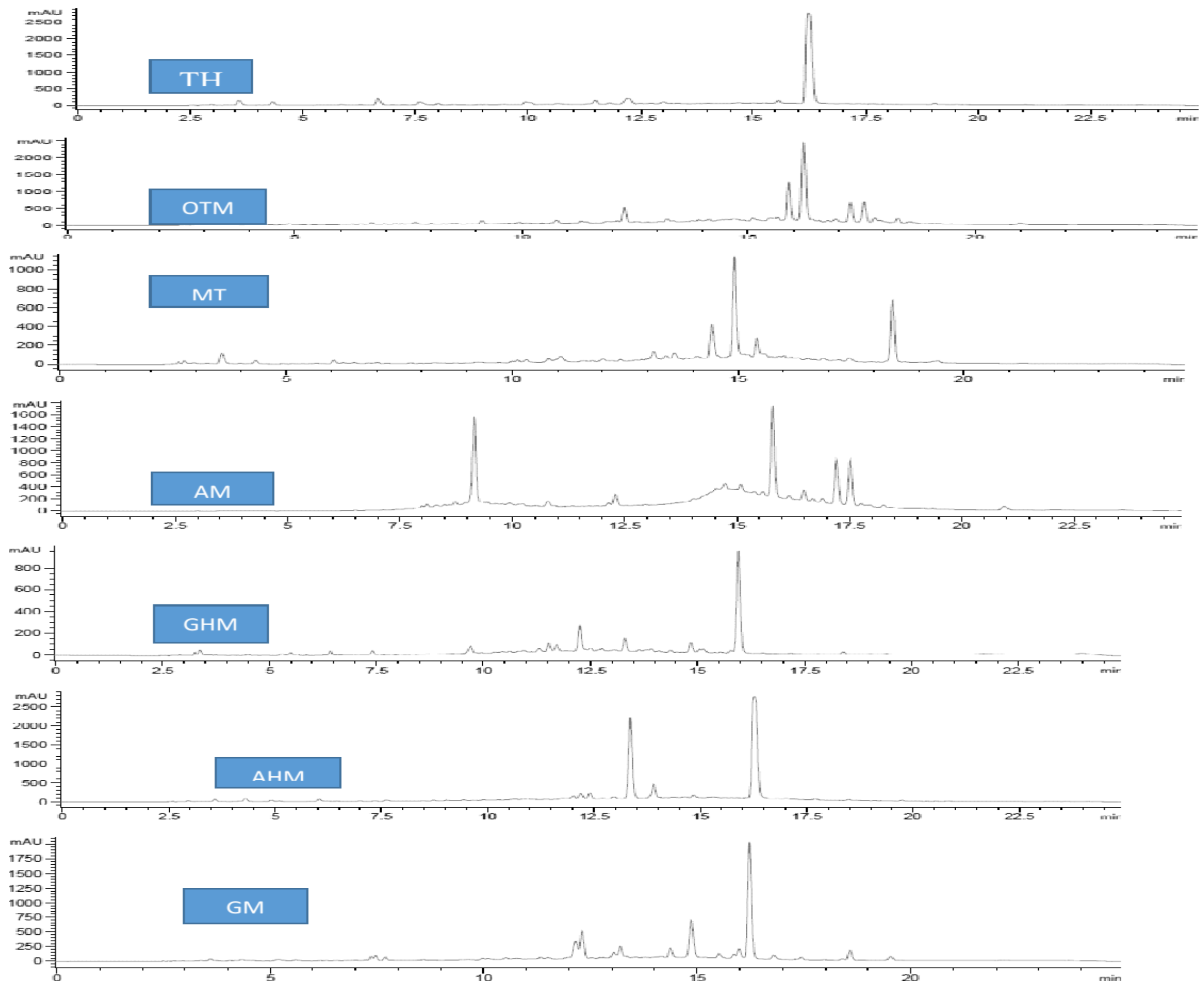
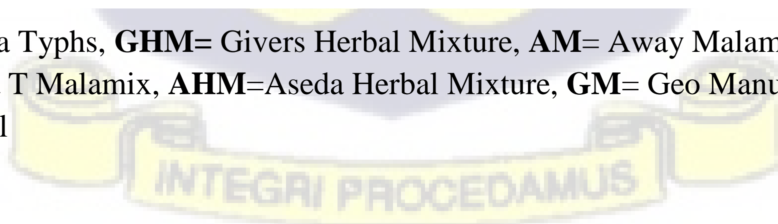


Fig 4.10: Various Chromatographs of HPLC Analysis of herbal products

Key: **MT**=Mala Typhs, **GHM=** Givers Herbal Mixture, **AM**= Away Malamix, OTM=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM**= Geo Manuel, **TH**= Typhofa Herbal



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4.20 Peaks and respective retention times from HPLC Analysis of herbal drugs

Table 4.11: Retention times (min) of Herbal drug compounds

PEAKS	HERBAL DRUG COMPOUNDS WITH RETENTION TIME (MIN.)						
	MT	**GHM**	**AHM**	**OTM**	**TH**	**GM**	**AM**
1	3.58	3.38	3.59	3.55	3.58	3.60	8.73
2	4.32	5.49	4.31	4.29	4.32	5.60	9.16
3	11.08	6.42	6.04	9.13	6.67	7.49	10.80
4	13.14	7.40	12.19	10.77	7.60	7.69	12.30
5	13.59	9.69	12.41	12.27	9.96	12.15	14.34
6	14.42	11.52	13.36	15.90	11.50	12.30	15.09
7	14.92	11.71	13.91	16.22	12.23	13.81	15.37
8	15.43	12.25	14.85	17.26	15.57	14.37	15.80
9	18.43	13.30	16.27	17.56	16.23	14.86	16.17
10	-	14.84	-	17.71	19.05	15.52	16.49
11	-	18.41	-	18.30	-	15.98	16.68
12	-	23.98	-	-	-	16.22	16.90
13	-	-	-	-	-	16.79	17.22
14	-	-	-	-	-	17.44	17.52
15	-	-	-	-	-	18.58	20.95
16	-	-	-	-	-	19.54	-

Key: **MT**=Mala Typhs, **GHM=** Givers Herbal Mixture, **AM=** Away Malamix, OTM=Osompa T Malamix, **AHM**=Aseda Herbal Mixture, **GM=** Geo Manuel, **TH=** Typhofa Herbal

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Table 4.11 and **Fig 4.10** above show the High Performance Liquid Chromatographic results of the Seven (7) herbal drug compounds used in this study.MT and AHM recorded 9 different peaks each with varying retention times which is an indication of nine (9) different active compounds present in each of these herbal drugs. TH had ten (10) peaks, OTM recorded eleven (11) peaks, and GHM recorded twelve (12) peaks. AM and GM recorded 15 and 16 peaks respectively. MT and AHM have the least active compounds while Gm have the highest active compounds in this study.

4.21 Peaks and respective retention times from HPLC Analysis of some standard drugs

Table 4.12: Peak retention time for HPLC Analysis of pure drug

NO	PURE DRUG COMPOUNDS	PEAK RETENTION TIME (MIN)
1.	AMODIAQUINE	9.74
2.	LUMIFANTRINE	21.33
3.	CHLOROQUINE	9.27
4.	DICLOFENAC SALT	22.83
5.	PARACETAMOL	9.28

Five (5) different pure standard drug compounds (**table 4.12**) were analysed using the High Performance Liquid Chromatography (HPLC). All the compounds show single resolution peaks indicating their nature of purity. Dichlofenac salt have one peak with 22.83 min retention time, Lumifantrine recorded 21.33min retention time, Amodiaquine recorded 9.74 min, Paracetamol recorded 9.28min and lastly, Chloroquine recorded 9.27min.

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CHAPTER FIVE

5.0 Discussion

5.1 Knowledge, Perception and Consumption of Herbal drugs

In this study, four hundred (400) participants were interviewed on the knowledge, perception and consumption of herbal products in Accra, Ghana to dertermine the extent of herbal use and most commonly antimalarial herbal drug patronised in Ghana. Two hundred and six (206) male representing 51% and one hundred and ninety-four (194) representing 48.5% were involved (**Table 4.1**). The high number of male representation in this study is similar to a study conducted by Bhat *et al*. (2019). Similar work by Asmelashe Gelayee *et al.* (2017) on Herbal medicines also had higher male participation compared to female. The high participation of male in this study could not be linked to consumption of herbal medicine among men than women however, another study by Aina *et al.* (2020) in South-west Nigeria showed that more men ($p<0.001$) used herbal medicine than women.

This present study also revealed that high number of respondents have heard about herbal medicine in Greater Accra, Ghana and have used herbal medicines before. This high percentage about knowing and using herbal products in Ghana was not surprising because a similar study by Ameade *et al*. (2015) in Tamale of Ghana showed that 88.1% were aware about herbal medicine and 54.75 % have used herbal medicine before and 77.5% were satisfied with the result. A work done by Johnson and Blanchard (2006) on alternative medicine recorded 88.1% and 59.9% respectively which agreed with the current findings and it is not surprising because 80% of Africans use traditional medicine, which involes the use of herbs or herbal products (Mahomoodally, 2013).

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This study also found that average number of the respondents have used herbal medicines for the treatment of malaria and some other diseases conditions. The appreciable percentage of people using herbal treatment for malaria in this study is in agreement with a research conducted in 2012 by Mensah and Gyasion on use of herbal medicine and have found out that about 50% of the malaria subjects used herbal medicine in managing the disease. This also agreed with the estimate reported by Tabi *et al*. (2006).

On effectiveness and preferable choice of herbal over orthodox or convensional medicine, more than half of participants think that herbal medicine are more effective. Again, they also think herbal medicines are better than orthodox or convensional therapies. This result is in line with a research work done by Aina *et al*.(2020). Finally, this current study has shown that majority of Ghanaians use herbal medicine in treatment of malaria and other disease conditions. A total of 30(7.6%) used Taabia herbal medicine, 34(8.5%) used Time herbal Mixture, 24(6%) used Rooter Mixture and 312(78%) used other herbal medicine such as Away Mala Mix, Givers Herbal Mixture, Osompa, Typhofa, Geo Manuel, Mala typhs, Aseda for malaria treatment.

5.2 In vivo and ex vivo antiplasmodial activities

TH (*Ocimum viride, Vernonia amygdalina, Morinda lucida, Alstonia boonei, Carica papaya, Corn still*)**, AM** (*Alstonia boonia, Naulea latifolia, Enantia forlycarpa*)**, GM** (*Terminalia ivorensis, Pycnanthus angolensus, Alstonia boonei*)**, AHM** (*Astonia boonei, Lanea kerstingil, Mangnifera Indica*)**, GHM** (*Khaya senegalenis, Azadirachta indica*) **, MT** (*Khaya senegalensis, Cryptolepis senguinolenta ,Cassia siamea, Citrus aurantium*)**, OTM** (*Carica papaya, Cassia alata*) were seven herbal drugs/products selected for the *in vitro* and *ex vivo* antiplasmodial activities based on the preliminary survey on knowledge, perception and consumption of herbal drugs in Greater Accra of Ghana. Each of these

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herbal products/drugs have a combination of two or more different plants. Based on the plant components of the selected herbal drugs, *Alstonia boonia* was found to be a common plant in four of the herbal products such as TH, AM, GM and AHM while *Khaya senegalensis* was common plant in GHM and MT herbal drugs. TH and OTM have *Carica papaya* common to them and MT and OTM having *Cassia* species such as *Cassia siamea* and *Cassia alata* common to them. These common plants found in the selected herbal products for this study agreed with a study in Ghana by Osei-Djarbeng *et al.* (2015) on Medicinal plants constituting antimalarial herbal preparations.

In vitro antiplasmodial activity of these seven herbal products were investigated in this study using Chloroquine-sensitive 3D7 laboratory of *Plasmodium falciparum* and Chloroquine-resistance Dd2 of *Plasmodium falciparum* laboratory strains. For the *ex vivo* antiplasmodial activity, the herbal products were tested against *Plamodium berghei*. According to Cudjoe, E., *et al.*(2020), antiplasmodial activity of herbal extracts against asexual disease causing parasite using the standard 72-hours has been classified as '**good**' (IC_{50} < 10 µg/ml), ' **moderate**' (IC_{50} values from 10 µg/ml to 50 µg/ml), '**low**' (IC50 values from 50 µg/ml to 100 µg/ml) and '**inactive**' (IC_{50} values >100 µg/ml).

This means that all the herbal products investigated in this study show antiplasmodial activity varying from good to moderate but just low for AM herbal product against 3D7 and TH herbal product against DD2 (**Table 4.2**). GM, MT, OTM, AHM and GHM herbal products showed good antiplasmodial activities against 3D7 which is an indication of good antimalarial herbal drug for malaria treatment. GHM and MT showed good activity against Dd2 with the rest having moderate activity. Good activity was also seen in GHM, AM, AHM, OTM and TH herbal products against *Plasmodium berghei*. Only MT and GM herbal

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products showed moderate activity against *Plasmodium berghei* which means all the herbal products tested on *Plamodium berghei* performed well in terms of parasite strains as compared to performance against 3D7 and Dd2 lab strain and differences observed might be due to different genetic make up of these parasites.

Comparing activity of the seven herbal drugs including all the standard antimalarial control drug with respect to 3D7 *P. falciparum* strain, Dd2 *P. falciparum* strain and *Plasmodium berghei*, there is significant different in performance ($p<0.05$). A work done in Nigeria by Ikem *et al*. (2020) on six selected commercial herbal formulations tested against 3D7 showed IC50 values > 100 µg/ml. This disagreement in the results could be due to different plant components of these herbal products used in the two studies. **TH** herbal product has *Vernonia amygdalina* as one of plant components of the herbal mixture and the antiplasmodial activities recoreded agreed with what Irungu *et al. (*2007) reported on antiplasmodial activities of extract from *Vernonia species.* The work of Muregi *et al*. (2003) also recorded similar findings on the antiplasmodial activities of *Vernonia species* investigated against CQ-sensitive K39 strain. **TH** herbal products exhibited moderate to low activity towards CQ-sensitive 3D7 parasite strain and CQ-resistant Dd2 parasite strain but showed good activity against *Plasmodium berghei*. This could mean that even though these plants constituents have some level of antiplasmodial activity, their performance may vary differently with respect to parasite strains.

Tajbakhsh *et al.* (2021) reported the following plant species: *Ocimum species, Vernonia species, Morinda lucida, Alstonia boonei, Naulea latifolia,Enantia species, Terminalia ivorensis, Pycnanthus angolensus, Lanea kerstingil, Mangnifera Indica, Azadirachta indica, Khaya species, Cryptolepis species, Citrus aurantium,* and *Cassia species* having IC_{50}

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values between 0.3 µg/ml to 28.12 µg/ml in a systematic review of Literature on antiplasmodial, antimalarial activities and toxicity of African medicinal plants. This correlates well with this current study since each herbal product used in this present study has a combination of two or more plant components and their overall IC_{50} values ranged between 0.5 µg/ml to 56 µg/ml similar to previously reported and similarities observed could be due to common plants components present in herbal products.For cytotoxicity experiment (**Table 4.2**), all the CC_{50} values (µg/ml) recorded for all the herbal products were greater than hundred (100) with Selectivity Index (SI) > 4. A work done by Dadzie *et al.,* (2020) on Cytotoxic and antioxidant effects of antimalarial herbal mixtures in Ghana showed CC_{50} values > 100 for three of the herbal mixtures out of five with plant components similar to selected herbal products used in this current study.

From **Table 4.2,** Resistivity Index (RI) defined as IC_{50} for Dd2/ IC_{50} for 3D7 *P. falciparum* strains. Low resistivity index with good antimalarial or antiplasmodial activity of an herbal product is promising and potential drug that can be used in combination to fight resistant parasite strains. AM herbal products recorded the lowest resistivity index followed by GHM herbal and GM herbal with the highest resistivity value. AM herbal product contained three major plant components which were *Alstonia boonia, Naulea latifolia* and *Enantia forlycarpa* but various extracts from these plants have shown good antiplasmodial activity according to a systematic review of literature done by Tajbakhsh *et al* (2021). This suggests that AM herbal with 0.44 resistivity index recorded in this present study may be a good candidate to enhance performance of Chloroquine against Chloroquine-resistant *Plasmodium* strains in a fight against malaria infections.

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5.3 Mechanism of Action of herbal products

Heme crystallization or polymerization is the process of turning free heme to hemozoin and was noted as one of the targets for antimalarials' mechanisms (Zakiah *et al*., 2021). Antimalarial medications function by preventing the formation of hemozoin crystals, which is one of the main ways antimalarial treatments kill malaria parasites. Colorimetric Inhibition of heme crystallization assay carried out in this study showed a varying ranges of IC_{50} values from 1.67 µg/ml to 122.2 µg/ml for the selected herbal products used in the study as compared to the three standard control antimalarial drugs in the experiment (**Table 4.3**).

Drug mechanisms are believed to relate to heme crystallization; they bind heme and inhibit hemozoin formation in the lysosomal digestive vacuole of the parasite (Huy *et al*., 2007). The IC_{50} values recorded for standard antimalarial quinolone drugs including herbal products in this current study were far lower than the results obtained by Huy *et al*. (2007) using the same method for studying drug action of mefloquine, amodiaquine, quinine and quinacrine respectively. The reason was not clear but the differences could be due to variation in abilities of antimalarial drugs to bind to heme in the presence of inducers used in this current assay and also the difference in the mechanism of initiation of heme crystallization under different environmental laboratory conditions. A research work conducted in Nigeria by Olanlokun *et al.* (2021) recorded similar observation to the current findings suggesting some level of agreement but the slight differences could be due to the methods used in the two experiments and also the different photochemical components of the various herbal extract tested.

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Another work done by Safarianti *et al*.(2020) on *in vitro* effect of 96% ethanol extract of bitter herbs (*Andrographis paniculata Nees*) on heme detoxification process of *Plasmodium*

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falciparum parasites showed that bitter herbs extract inhibits the formation of β-hematin equal to 61,07 ± 4,69%. An inhibition activity of heme polymerization was also seen in *1, 6, 8 trihydroxyxanthone* when tested *in vitro* in study conducted by Zakiah *et al*. (2021). The correlation between the present study and the previous findings are clear indication that herbal products with antimalarial activities could inhibit heme crystallization in the parasite detoxification processes as one of their mechanism of action.

5.4 Heavy Metal Levels in Herbal Drugs

Many studies have been carried out to evaluate the amounts of heavy metals in therapeutic plants and plant-based products. High quantities of potentially harmful heavy metals have been found in products available to the general public in both industrialized and developing countries (Street, 2012). Seven herbal products were tested for some heavy metals such as Copper (**Cu**), Lead (**Pb**), Zinc (**Zn**), Iron (**Fe**) Arsenic (**As**) and Mercury (**Hg**) in this study and all the samples were found to contain all the six metals in varying concentrations. According to Selvi *et al*. (2019), significant concentration of these toxic heavy metals are contaminants in the soil and surface waters and have been reported in several countries.For the concentration of six heavy metals analysed in the selected herbal products, Copper and Iron contents varied significantly in all the seven herbal products($p<0.05$) but Lead, Zinc, Arsenic and Mercury contents do not have any significant variation when compared individually with the seven herbal products($p>0.05$).

Generally, comparing all the heavy metals analysed (**Table 4.4**) in the various herbal products to the daily recommended limit, Lead and Zinc were found to be within the limit, Arsenic and Mercury were outside the daily recommended limit. Iron contents were within the daily recommended limit except for the TH herbal which was out of the limit. Various copper concentrations were outside the daily limit except for AHM herbal which was within

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the limit. Again, comparing various heavy metals tested (**Table 4.4**) to oral reference dose, all concentrations were outside the range except MT, OTM and AHM herbal which were found to have Zinc concentration within the oral reference dose.

A study conducted by Dghaim *et al*. (2015) found out that copper, zinc, lead and cadium were all above WHO permissible limits, suggesting unsafe levels of metals they have analysed in the herbs. The current study perfectly agreed with their findings. Also, the current findings were in agreement with a research work done by Samali *at al*. (2015).

A study conducted by Nkansah, *et al*. (2015) found the Iron, Lead, Zinc, and Cadmium contents all within the permissible limits which was contrary to the current study and the differences could be due to the locations where these herbs were harvested for the herbal preparations. A similar research on heavy metal assessment by Saeed *et al.* (2015) also found out that all the selected herbal products tested were having heavy metal content within the WHO toxic limit with the exception of two herbal products which were above the limit. The differences in the findings could be due to geographical locations of the plants used in preparing the herbal products or the preparation procedures which might limit the heavy metal concentrations.

Metal Pollution index (MPI) were calculated for all the herbal products in **Table 4.5** to assess the metal pollution level. According to George *et al*. (2017), the metal pollution index values were classified as MPI > 5 (heavily contaminated with heavy metals), MPI 1–5 (slightly contaminated with heavy metals) and MPI < 1 (not contaminated). Metal Pollution Index results from **Table 4.5** were all below 1, which means that the herbal products were not heavily polluted even though there were certain level of metal presence in them. A work done in Abuja, Nigeria by Eze *et al*. (2018) suggested that metal pollution index for all the

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vegetables were high and above the threshold limit, presenting health risk to consumers. The disagreement of the current findings to their study were obvious since the vegetables assessed were harvested from waste- water irrigated site.

Also, hazard index (HI) was calculated in **Table 4.7** using average daily dose previously calculated in **Table 4.9**. The hazard quotients (HI) values in **Table 4.7** to assess long term non-carcinogenic health risk effects of heavy metals in the herbal products on the population. But according to Sultana *et al*. (2019) and Ametepey *et al*. (2018), hazard index (HI) >1 suggests that there are significant health effects from consuming heavy metal pollutants contained in a herbal products or there is unacceptable risk of non-carcinogenic effects on health. However, hazard index (HI) <1 suggest no significant health effects from consuming pollutants contained in the herbal products. Results obtained from this study showed that hazard index calculated for all the herbal products were far above 1 for both adults and children indicating potential risks of non-cariogenic health effects to the public who heavily depends on such herbal products for malaria treatments in a long term.

Finally, carcinogenic risk (CR) assessment was done on the various herbal products using Lead (Pb) and Arsenic (As) as indicators since they are proven to have certain level of oral slope factor of cancer severity (USEPA, 1989; USEPA 2009; USEPA 2012; Liu *et al*., 2013; Farmer *et al*., 2019). According to Ullah, *et al*.(2017), acceptable risk levels for carcinogens ranges from 10^{-4} (meaning a risk of developing cancer over a human lifetime is 1 in 10000) to 10^{-6} (meaning a risk of developing cancer over a human lifetime is 1 in 1000000). The values recorded for Arsenic (As) in this study (**Table 4.8**) suggested that 1 in 10 to 1 in 1000 could risk of developing cancer over human lifetime when depending on these herbal products throughout their lifetime. Also the values for Lead (Pb) suggested that 1 in 10000 to 1 in 100000 could risk of developing cancer over human

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lifetime when depending on such herbal products for consistent treatment of malaria. This finding agreed with research done by Luo *et al*. (2020) on heavy metal contaminations in herbal medicines and comprehensive risk assessments, and the results revealed that a total of 25 herbs assessed had an unacceptable risk to the population based on their calculated hazard quotients and hazard index values. The slight disagreement in carcinogenic assessment reported in their study could be due to different herbal products used in the two studies or difference source of medicinal plants used in the herbal preparations.

5.5 Electrolyte contents of the herbal drugs

Electrolytes are involved in a variety of bodily functions, including fluid balance, acid base balance (pH), nerve conduction, blood clotting, and muscular contraction. Electrolytes, commonly known as sodium, potassium, calcium, and magnesium, have a variety of biological actions in the human body, and their imbalances have a significant impact on personal health (Schiefermeier-Mach *et al*., 2020).

For the electrolytes values recorded, Potassium and Calcium content varied significantly across the seven herbal products ($p<0.05$) but Magnesium and Sodium were seen not to have any significant variation among the seven herbal products ($p>0.05$). Comparing the tested values recorded to a reference daily mineral intake values in **Table A13 (Appendix 11)**, all the electrolytes values were found to be below the standard values for both male and female. The overall levels of electrolytes (elements) tested in all the seven herbal products were K> Ca> Na> Mg. This was rather a reverse to a findings from a research work done in Nigeria where they found out that the overall levels of elements tested were Mg>Na>K>Ca. The variation could be due to different plant components and also geographical locations of the plants used. Eventhough this present work revealed that electrolyte values were below the standard reference intake values, people with conditions such as diabetes, hypertension,

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stroke and kidney diseases must be advised in using herbal treatment for malaria since slight variation of electrolytes in blood concentration can lead to serious health problems and even increased mortality (Larsson *et al*., 2008; Kear, 2017; Dhondup & Qian, 2017).

5.6 pH Levels of the Herbal Drugs

From **Table 4.10**, all the pH values recorded for the selected herbal products in this study were below 5.0. These low pH values are an indication of acidic nature of all the herbal products in the study. A research work by Tetteh *et al.* (2020) on Ghanaian herbal medicines for malaria recorded pH value =5.7 ± 0.16. Also Tanna *et al*. (2011) recorded pH of 4.58 in their study. All the pH values recorded in this current study perfectly agreed with the previous findings but the slight difference observed could be due to some differences in the plant components of the herbal products used in those studies.

Acidic nature of these herbal products could be advantageous since low pH control or suppressed most microbial growth in food (Booth & Stratford 2003). Another research work done by Kim & Ndegwa, (2018) on Influence of pH and temperature on growth characteristics of leading foodborne pathogens showed lower growth rates in acidic environments than in alkaline environments. This could mean that all the selected herbal products with these low pH level recorded in this present study may last longer as compared to those herbal preparations that may be neutral or slightly alkaline in nature. The recommended normal water pH ranges from 6 to 8.5 and low pH level tend to be toxic (Mohsin, 2013; WHO, 2004). Human life requires a tightly controlled pH level in the serum of about 7.4 to survive (Waugh and Grant, 2007). More consumption of acid-forming products can lead to a tremendous pressure on the body's defense against acidosis-acidaemia (Suthar and Verma, 2014). Since the pH levels recorded in the present

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study were all below the range of 6-8.5, this could mean that, the selected herbal products used in the experiment may last longer but could be a bit toxic to the consumers.

5.7 HPLC Fingerprints of the herbal drugs

Fingerprint construction is an important quality control tool for herbal samples in the light of constantly growing interest in natural origin of medicines and has been accepted by WHO as a methodology for the quality control of herbal samples (WHO, 2000). From **Table 4.11** and **Figure 4.10**, the High Performance Liquid Chromatography (HPLC) fingerprint results showed that selected herbal products have multiple peaks ranging from 9 to 16 which may represent different pharmacological active components of the herbal combinations used in their preparations. MT and AHM herbal have 9 peaks, TH herbal has 10 peaks, OTM herbal has 11 peaks, GHM herbal has 12 peaks, AM and GM herbal had 15 and 16 peaks respectively.

Some pure standard control drugs such as Amodiaquine, Lumifantrine, Chloroquine, Paracetamol, Diclofenac salts were also analysed to compare their retention times (min.) to that of herbal products for any similarities that could raise concern on adulteration and the results indicated that each of these compounds recorded predominant single peaks with either one or no minor peaks (**Table 4.12**). This means herbal preparations used in this study contained a multiple ingredients or compounds from different plant materials used in their formulations and these may collectively act to represent their activity (Xie *et al.*, 2006).

Also both single and combinations of herbal medicines contain a myriad of compounds, and multiple constituents of them represent their therapeutic effects and may be diverse due to various harvest seasons, plant origins, processing and other factors (*Liang et al.*,

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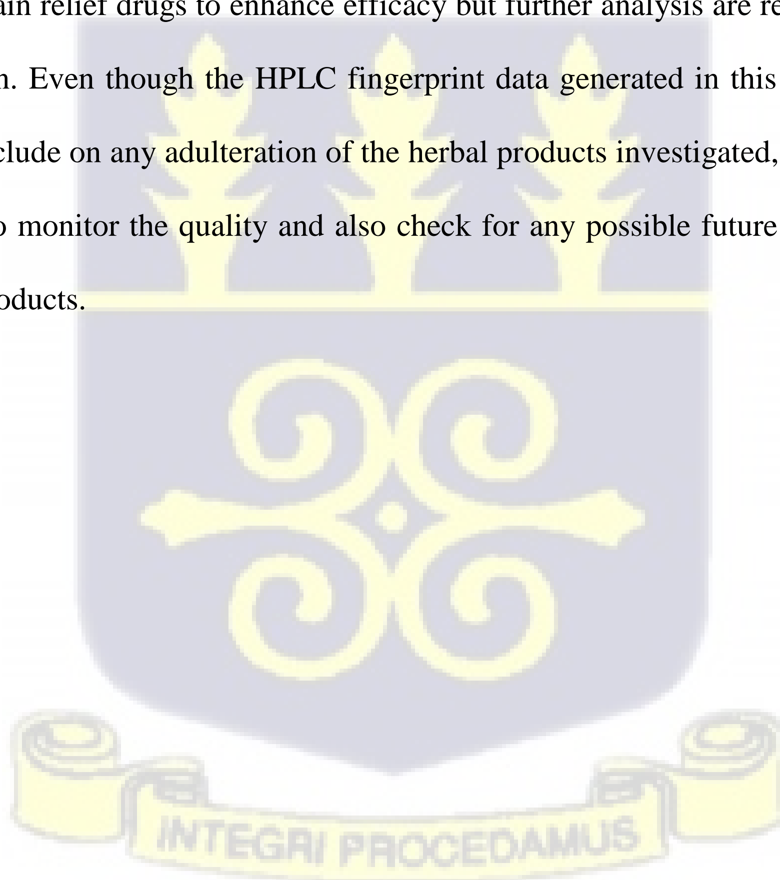
2004).Evaluation of fingerprints on 15 samples of Chinese Yiqing herbal preparations by Yongyu *et al.* (2011) observed major similarities in sample 1 to 9th sample, suggesting that similar chemical components were present in these samples regardless of manufacturer. This could mean that similar retention times observed in this present study could suggest similar chemical or pharmacological active components of herbal products but this may not be 100% conclusive since further analysis on those similar peaks need to be done to confirm them.

No similarities recorded by comparing the retention time of the herbal products and some of the standard antimalarial and painkiller drugs used in this experiment and this could mean that these herbal manufacturers do not adulterate the preparations with standard antimalarial drugs or any pain relief drugs to enhance efficacy but further analysis are required to justify this assumption. Even though the HPLC fingerprint data generated in this study could not be used to conclude on any adulteration of the herbal products investigated, it can serve as a primary data to monitor the quality and also check for any possible future adulterations of these herbal products.



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CHAPTER SIX

6.0 CONCLUSION, LIMITATION AND RECOMMENDATIONS

6.1 Conclusion

This study has demonstrated that majority of study participants depend on herbal products for the treatment of malaria and some other diseases and have believe that herbal medications are effective and better alternative to the conventional treatment for malaria. The study also revealed that the selected herbal products used for treatment of malaria have good antiplasmodial activity with no cytotoxic effects to the human red cells and one of their mechanisms of action could relate to heme crystallization inhibition. The results from this study could help in further identification and validation of active compounds from plant components in these herbal products thus facilitating the development of a new generation of efficacious antimalarial drugs as has been seen in quinine and artemisinin which are products based on the documented use of herbal plants in treating malaria.

Also, the HPLC fingerprint data generated in this study could be used as a baseline data to safeguard the quality of these herbal products from any possible future adulterations. The data will also help the scientific community who may be interested in further exploration of new antimalarial drugs from the plant components of these herbal preparations since their original components has been documented through the HPLC fingerprints results.

This study also demonstrated that these herbal products are not heavily contaminated with heavy metals but long term dependence on these herbal products could pose danger including carcinogenic risk to the consumers. The low pH levels of all the herbal products recorded in this present study is an indication that these herbal products are very acidic and could last longer without microbial deterioration but may not be good for those who have problem with high organic acid foods.

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The electrolyte values recorded in this present study were below the threshold level but could be dangerous to people with diabetes, hypertensions, strokes and kidney diseases and it means people with such conditions must seek advice from their doctors before using any of such herbal products for malaria treatment.

6.2 Limitations

Three major limitations were noted in this study. Firstly, no *in vivo* experimental set up was used to better understand the herbal activity directly in the living host. Secondly, inability of studying the mechanism of the herbal products directly with the parasite to ascertain the true mode of action. Thirdly, there is no molecular experiment to record any possible mutations that might have occurred in the parasite due to the herbal drug exposure.

6.3 Recommendation

It is recommended that researchers and pharmaceutical companies should exhaustively explore the active ingredients in these plant components of these herbal products for the discovery and development of new efficacious antimalarial drugs to support the existing ones which are currently threatened by emerging parasite resistance. The study revealed some question about *in vivo* efficacy and mechanisms of these herbal products that could not be answered by this current research. Thus, future research should study the activity and mechanisms of these herbal products using a comprehensive animal model or human clinical trial approach.

This present study could not also reveal any possible mutations in the *Plasmodium* parasites relating to resistance that might results from long use of herbal products among the population overtime. Future study is recommended to design a simple *in vitro* study using continuous parasite culture to understand any possible mutations in the parasite after herbal drug exposure overtime.

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Hazard and cancer risk index of heavy metal risk assessment of the herbal products in this present work revealed that long use of these herbal products in one's lifetime could lead to some health issues including cancer. It is therefore recommended that people who solely depend on herbal products for treatment of malaria should reduce the consumption or occasionally switch to conventional treatment to avoid any future health complications from long term usage of herbal therapies.

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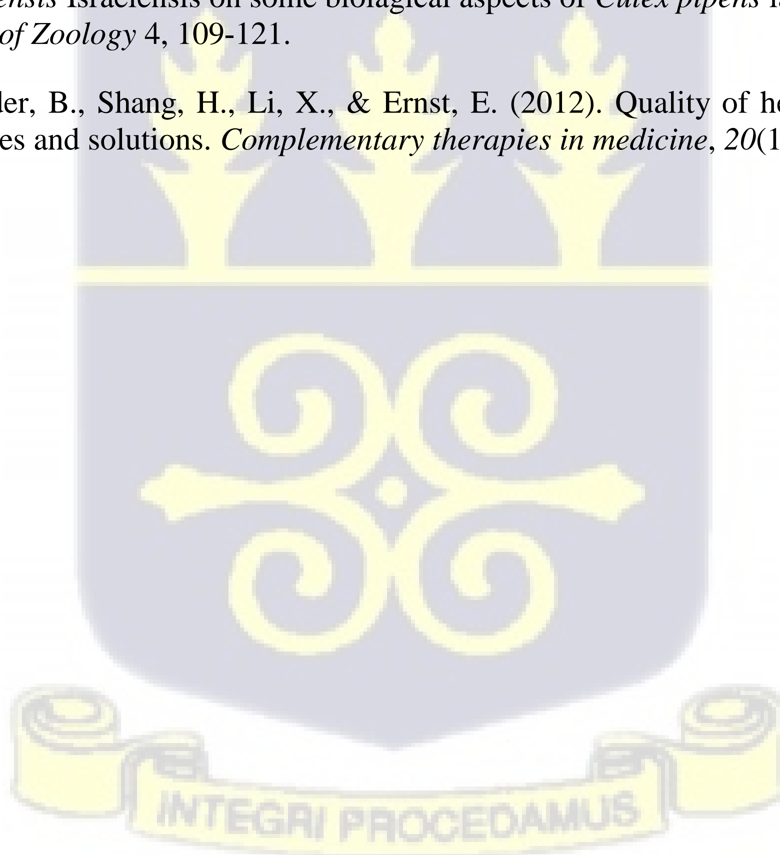

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APPENDICES

APPENDIX 1

REAGENTS PREPARATION

Preparation of Albumax

100g of Albumax powder (GIBCO) was dissolved in 500 ml of incomplete RPMI medium using a magnetic stirrer and was later filtered using 0.2 micro pore filter and 5mL aliquoted into 15ml falcon tubes and then stored at -20°C.

Preparation of Sodium Hydrogen Carbonate

37.5g of $NaHCO_3$ powder was in 500 ml of distilled water and later filtered and stored at 4°C.

Preparation of Sorbitol

25g of powder was dissolved in 500 ml of distilled water and later filtered and stored at room temperature for later use.

Preparation of Incomplete RPMI 1640

10.4g (1 pack) of RPMI 1640 powder (GIBCO) was dissolved in about 850 ml of distilled water in a large conical flask using a magnetic stirrer.7.15g HEPES,2g Dextrose,2ml hypoxanthine (25mg/ml in NaOH) was added and then mixed well with magnetic stirrer and adjust the pH to 7.2 with NaOH or HCl. The media was then top up with distilled water to 1L and filtered sterilize with 0.2µm filter in a laminar flow hood and later stored at 4°C

Preparation of Complete RPMI 1640

200ml of complete RPMI was prepared by transferring 188.6ml of incomplete RPMI into 500ml sterilized bottle and 5ml of 20% Albumax and 6.4 ml of 7.5% NaHCo3 were added then top up to the mark. The medial was then stored at 4°C.

Preparation of Giemsa buffer

3g of Na_2HPO_4 powder and 0.6g of KH_2PO_4 powder was dissolved in 1 L of distilled water and the pH to 7.4 was adjusted using a pH meter and then later filtered and stored at 4°C.

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Preparation of lysis buffer for SYBR Green 1 assay (1L)

2.423g Tris base was dissolved in 1L cell culture water using magnetic stirrer and pH to 7.5 using conc HCl.10ml 0.5M EDTA (5mM final conc.), 80mg saponin (0.008% w/v final), 0.8ml Triton X-100 (0.08% w/v final) was then added and mixed thoroughly to avoid production of bubbles. Solution was then filtered and stored at room temperature.

Preparation of MSF Lysis Buffer containing SYBR Green 1 (10 ml)

SYBR Green1 aliquot vial was thawed and 2 µl SYBR Green was added to 10 ml MSF lysis buffer (0.2 µl SYBR Green 1/ml of lysis buffer) and mixed well before using.

Processing of O+ blood

The blood was first collected from donor aseptically into centrifuge tubes and then centrifuge at 2500 rpm for 5 minutes and the plasma was removed. An equal volume of cold incomplete RPMI was to the cells and mixed well and later entrifuged at 2500 rpm for 5 minutes again. The process was repeated three (3) times the cells were re suspended in an equal volume of complete RPMI and stored at 4°C.

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APPENDIX 2

RESULTS FROM *IN VITRO* AND *EX VIVO* ANTIPLASMODIAL ACTIVITIES OF HERBAL PRODUCTS

Table A1: Various drug compounds and their IC50 values using 3D7 Labstrains.

NO	ANTIMALARIAL DRUG/HERBAL PRODUCT	IC50 VALUES	STD ERROR VALUES	R-SQUARE VALUES
1	MALA TYPHS	2.178 ug/ml	0.03217	0.9745
2	GIVERS	9.404 ug/ml	0.03653	0.9938
3	AWAY	56.000 ug/ml	15.5900	0.9907
4	OSOMPA	4.208 ug/ml	0.0752	0.9410
5	ASEDA	9.060 ug/ml	0.0292	0.9816
6	GEO MANUEL	2.166 ug/ml	0.06744	0.9843
7	TYPHOFA	39.510 ug/ml	0.01907	0.9907
8	CHLOROQUINE	11.85 ng/ml (0.1185 ug/ml)	0.05362	0.9787
9	ARTESUNATE	0.11 ng/ml (0.00011ug/ml)	0.03373	0.9863
10	AMODIAQUINE	14.4 ng/ml (0.0144ug/ml)	0.2081	0.9715
11				

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Table A2: Various drug compounds and their IC50 values using *Plasmodium berghei*

NO	ANTIMALARIAL DRUG/HERBAL PRODUCT	IC50 VALUES	STD ERROR VALUES	R-SQUARE VALUES
1	MALA TYPHS	10.2 ug/ml	0.1209	0.8434
2	GIVERS	0.503 ug/ml	0.0239	0.5015
3	AWAY	1.158 ug/ml	0.01821	0.9968
4	OSOMPA	5.653 ug/ml	0.02003	0.9944
5	ASEDA	3.201 ug/ml	0.02361	0.9860
6	GEO MANUEL	10.96 ug/ml	0.0363	0.9784
7	TYPHOFA	9.016 ug/ml	0.01667	0.9976
8	CHLOROQUINE	10.52 ng/ml (0.01052 ug/ml)	0.7209	0.9150
9	ARTESUNATE	0.0886 ng/ml (0.000089 ug/ml)	0.03483	0.9631

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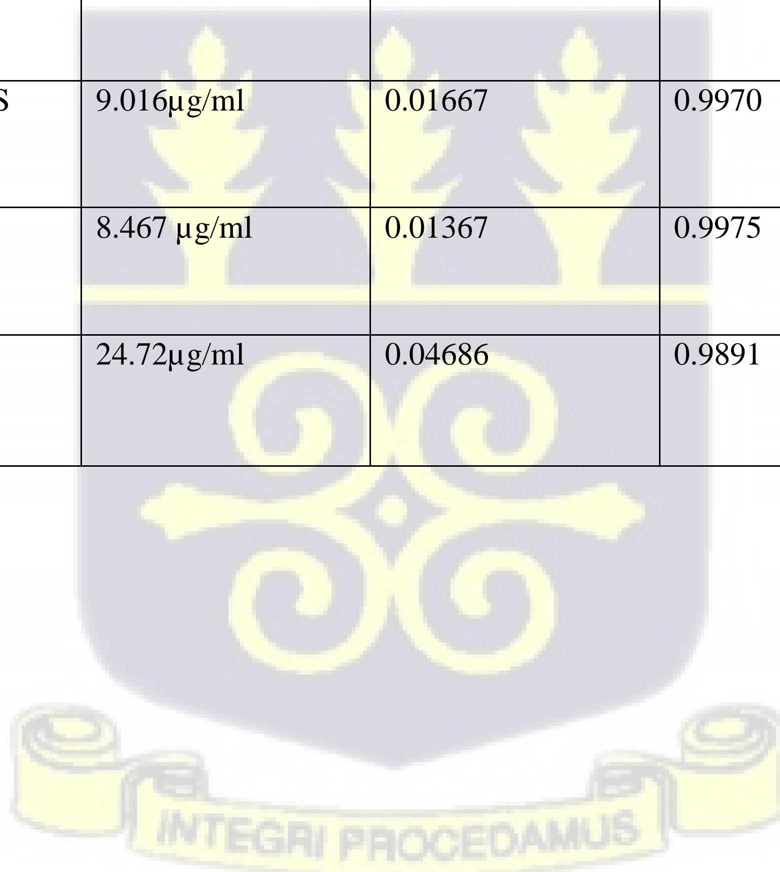
Table A3: Various drug compounds and their IC50 values using Dd2 Labstrain.

SAMPLE NAME	IC50	STANDARD ERROR	R-SQUARE
ARTESUNATE	2.687ng/ml 0.002687µg/ml	0.2463	0.9890
CHLOROQUINE	1046ng/ml 1.046µg/ml	0.5448 0.0005	0.9516
OSOMPA	19.85µg/ml	0.02697	0.9518
ASEDA	18.78µg/ml	0.0314	0.9821
GEO MANUEL	26.17 µg/ml	0.2618	0.9691
MALA TYPHS	9.016µg/ml	0.01667	0.9970
GIVERS	8.467 µg/ml	0.01367	0.9975
AWAY	24.72µg/ml	0.04686	0.9891



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APPENDIX 3

GEOMATRIC MEAN OF VARIOUS IC50 VALUES

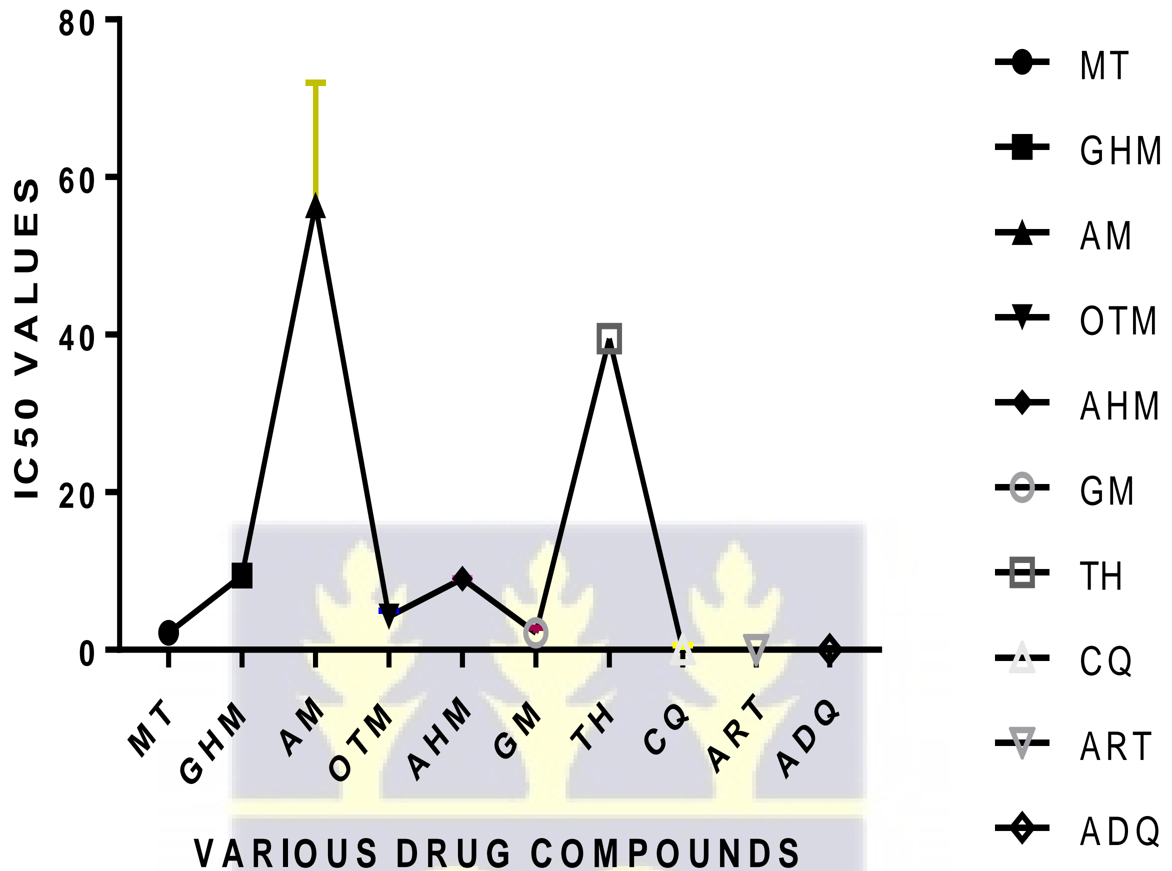

Fig A1: Geometric mean IC 50 Values recorded from drug compounds using 3D7 Lab strains.

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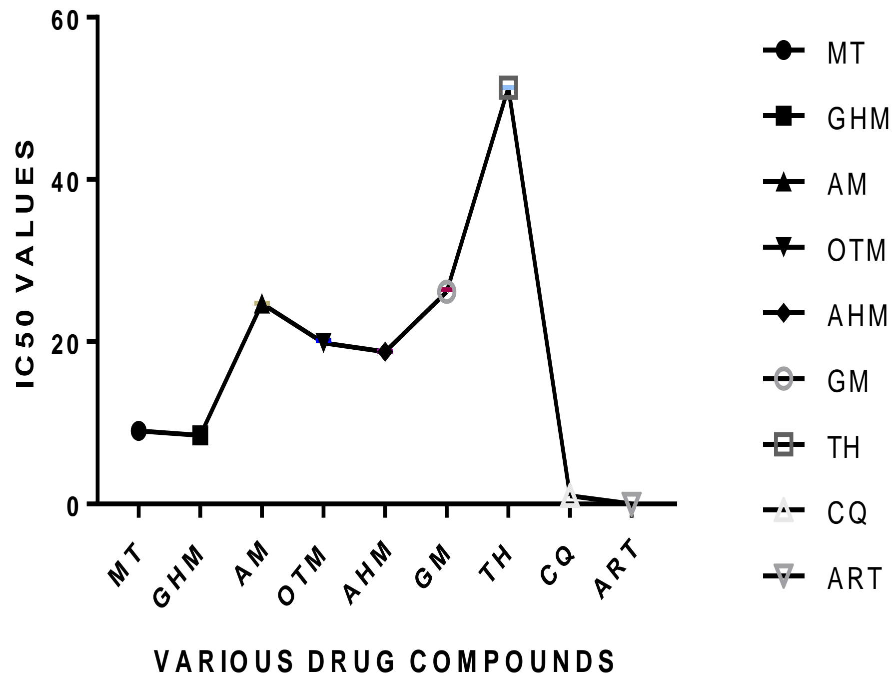

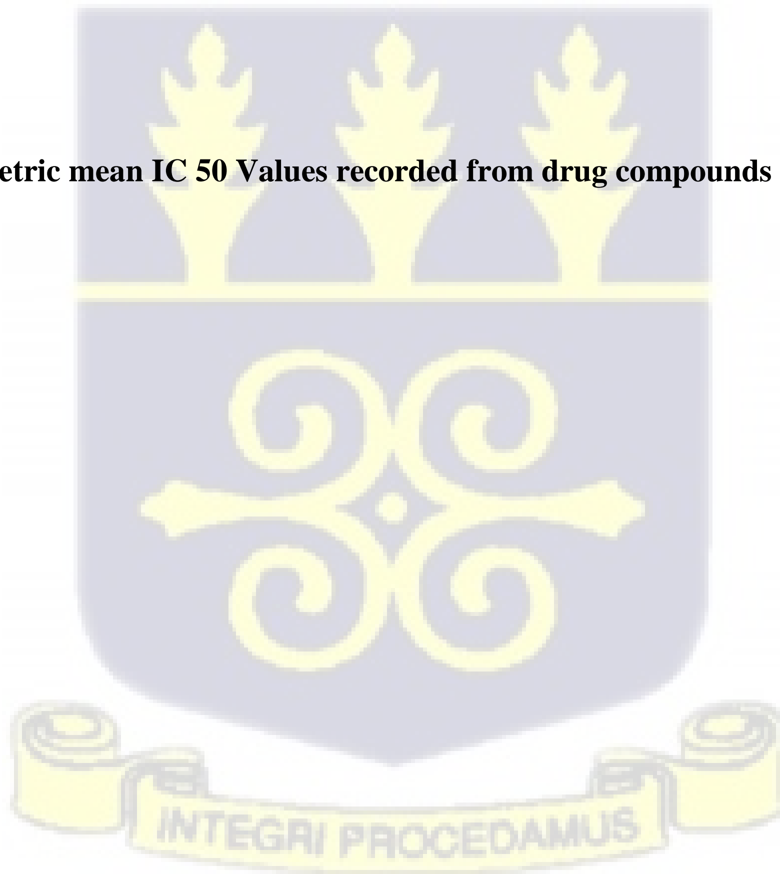

Fig A2: Geometric mean IC 50 Values recorded from drug compounds using Dd2 Lab strain.

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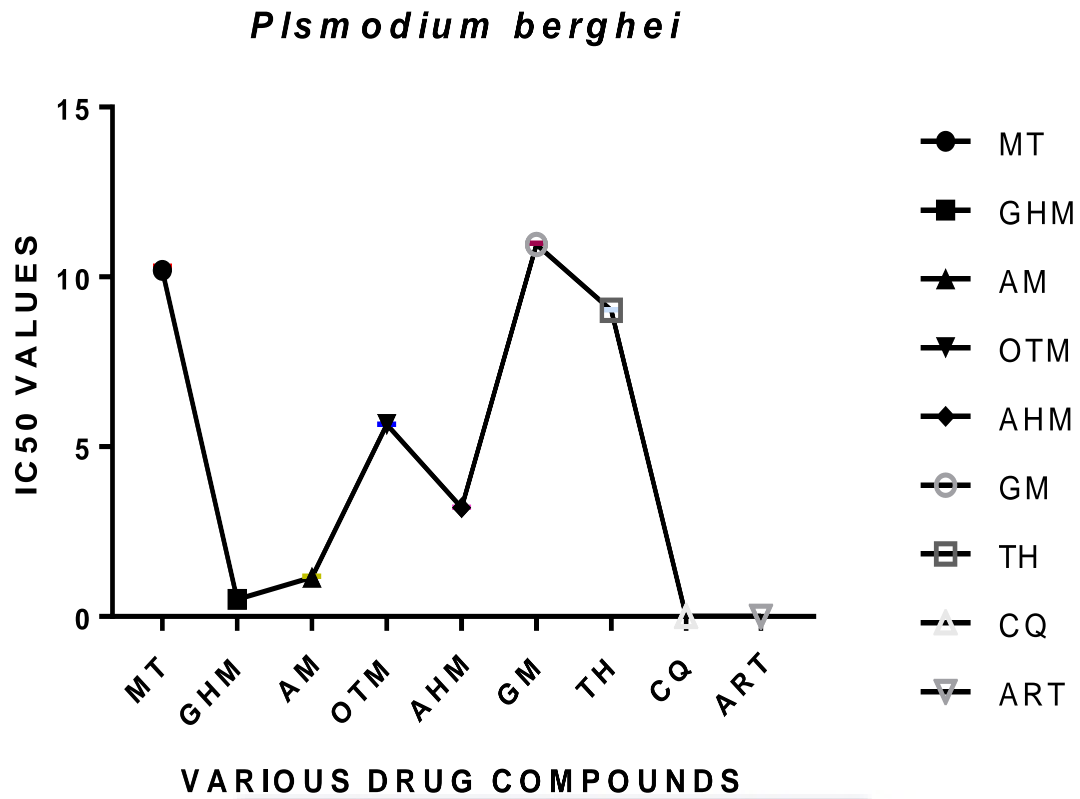

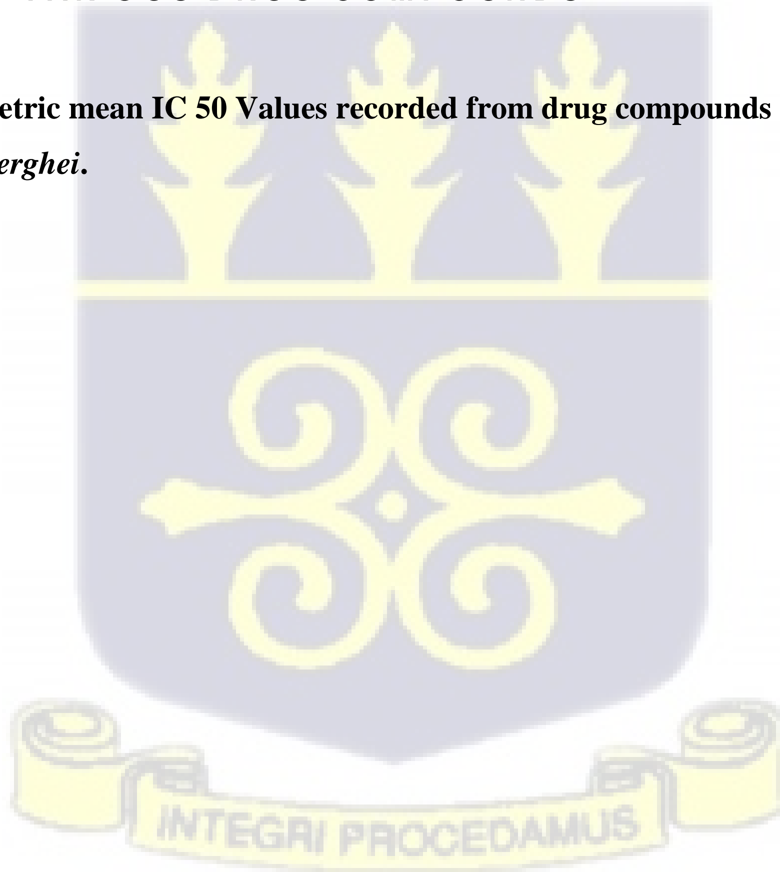
INTEGRI PROCEDAMUS

Fig A3: Geometric mean IC 50 Values recorded from drug compounds using *Plasmodium berghei*.

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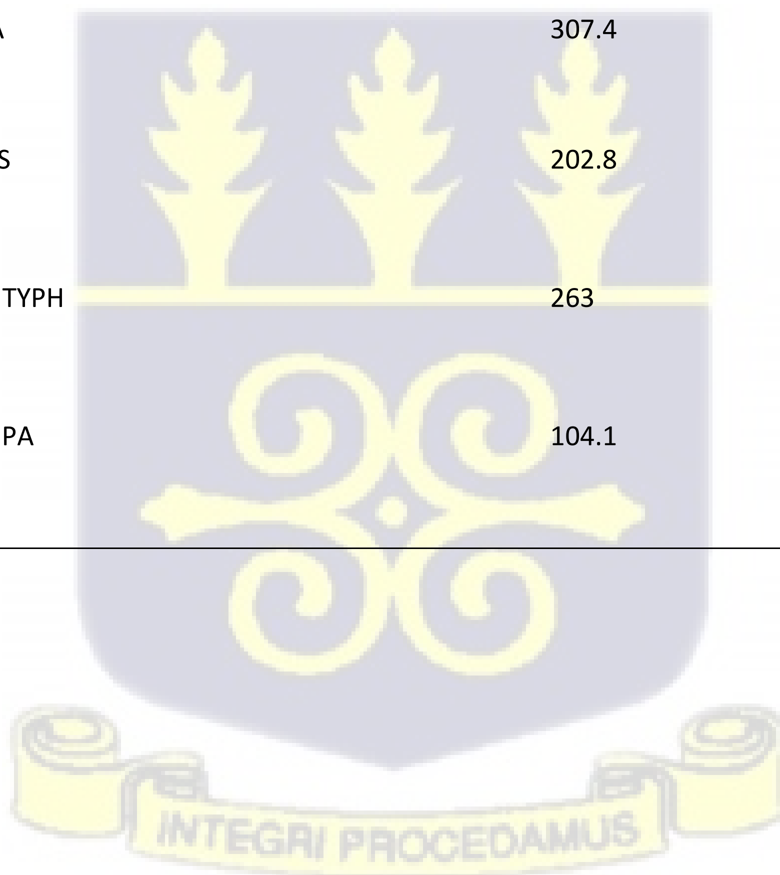
APPENDIX 4

CYTOTOCIXITY ASSAY

Table A4: Cytotoxicity CC50 Results

NO	DRUG/ HERBAL PRODUCTS	CC50 VALUES
1	ARTESUNATE	51.27
2	TYPHOFA	504.9
3	AWAY	247.3
4	GEO MANUEL	223.7
5	ASEDA	307.4
6	GIVERS	202.8
7	MALA TYPH	263
8	OSOMPA	104.1



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APPENDIX 5

Table A5: Heavy metal contents of the herbal products.

NO	HERBAL PRODUCTS	Cu ppm	Cu Mg/Kg	Pb ppm	Pb Mg/Kg	Zn ppm	Zn Mg/Kg	Fe ppm	Fe Mg/Kg	As ppm	As Mg/Kg	Hg ppm	Hg Mg/Kg
1	TYPHOFA 202	0.031	3.1	0.001	0.1	0.004	0.4	0.128	12.8	0.001	0.1	0.002	0.2
2	AWAY MALAMIX	0.011	1.1	0.002	0.2	0.006	0.6	0.044	4.4	0.001	0.1	0.001	0.1
3	MALA TYPHS	0.018	1.8	0.001	0.1	0.001	0.1	0.017	1.7	0.003	0.3	0.001	0.1
4	GIVERS HERBAL MIXTURE	0.07	7	0.001	0.1	0.007	0.7	0.028	2.8	0.002	0.2	0.001	0.1
5	ASEDA HERBAL	0.002	0.2	0.002	0.2	0.003	0.3	0.034	3.4	0.001	0.1	0.001	0.1
6	OSOMPAT-MALA MIX	0.006	0.6	0.001	0.1	0.002	0.2	0.029	2.9	0.002	0.2	0.003	0.3
7	GEO MANUEL	0.008	0.8	0.001	0.1	0.008	0.8	0.026	2.6	0.001	0.1	0.001	0.1
	PERCENT RECOVERIES		99.7		99.5		100.1		100		99.4		98.9

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APPENDIX 6

Table A6: Electrolyte contents of the herbal products.

NO	HERBAL PRODUCTS		k	K Mg/Kg	Ca	Ca Mg/Kg	Mg	Mg Mg/Kg	Na	Na Mg/Kg	P	% P
1	TYPHOFA 202		0.139	13.9	0.011	1.1	0.001	0.1	0.002	0.2	7.4	0.0074
2	AWAY MALAMIX		0.113	11.3	0.009	0.9	0.009	0.9	0.008	0.8	2.8	0.0028
3	MALA TYPHS		0.405	40.5	1.027	102.7	0.003	0.3	0.003	0.3	8.3	0.0083
4	GIVERS HERBAL MIXTURE		14	1,400	0.896	89.6	0.001	0.1	0.007	0.7	6.6	0.0066
5	ASEDA HERBAL		0.011	1.1	0.222	22.2	0.002	0.2	0.002	0.2	7.1	0.0071
6	OSOMPA T-MALAMIX		0.518	51.8	1.138	113.8	0.003	0.3	0.001	0.1	3.9	0.0039
7	GEO MANUEL		0.019	1.9	0.78	78	0.001	0.1	0.004	0.4	6.2	0.0062

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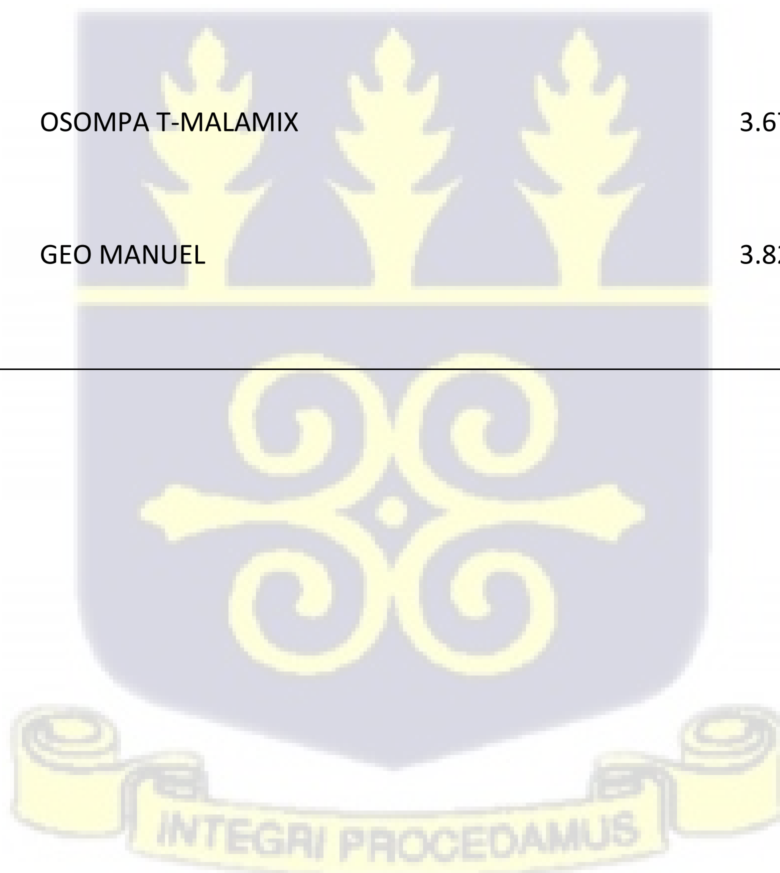
APPENDIX 7

Table A7: pH content of the herbal products:

NO	HERBAL PRODUCTS	pH
1	TYPHOFA 202	4.61
2	AWAY MALAMIX	4.21
3	MALA TYPHS	4.48
4	GIVERS HERBAL MIXTURE	3.56
5	ASEDA HERBAL	4.44
6	OSOMPA T-MALAMIX	3.67
7	GEO MANUEL	3.82



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APPENDIX 8

Table A8: HPLC ANALYSIS OF PURE DRUG COMPOUNDS

NO	PURE DRUG COMPOUNDS	PEAK RESOLUTION TIME (MIN)
1.	AMODIAQUINE	9.740
2.	LUMIFANTRINE	21.325
3.	CHLOROQUINE	9.266
4.	AMOXICILLIN	12.126
5.	TETRACYCLINE	11.217
6.	PYRAZINAMIDE	7.077
7.	METRONIDAZOLE	7.779
8.	DICLOFENAC SALT	22.830
9.	ASCOBIC ACID	2.837
10.	PARACETAMOL	9.278
11.	ASPIRIN	14.916
12.	CAFFEINE	11.983
13.	DIPHENYLHYDRAMINE	15.020
14.	PHENOBARBITAL	15.638
15.	SALICYCLIC ACID	17.079

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APPENDIX 9

Table A9: Recommended daily limit for heavy metals.

METAL	RECOMMENDED DAILY LIMIT	SOURCE (REFERENCE
Lead (Pb)	20-514 ug	Seed *et al*. (2011)
Copper(Cu)	340-400 ug (child) 900 ug (adult)	Seed *et al*. (2011)
Zinc (Zzn)	3-8 mg	Seed *et al*. (2011)
Iron (Fe)	8-10 mg	Seed *et al*. (2011)
Arsenic (As)	15-25 ug (adult)	Ezeabara *et al*. (2014)
Mercury (Hg)	0.1 to 0.3 ug/kg	Passos *et al*. (2008)

Table A10: Toxicity response to heavy metals (Oral Reference Dose (RFD)

HEAVY METALS	ORAL REFERENCE DOSE (RFD) Mg/kg/day	REFERENCE
Lead (Pb)	3.5×10^{-2}	Luo *et al*. (2020)
Cupper (Cu)	$4.o \times 10^{-2}$	George *et al*. (2017)
Zinc (Zn)	0.3	USEPA IRIS. (2011)
Iron (Fe)	0.7	USEPA IRIS. (2011).
Mercury (Hg)	3×10^{-4}	Sultana, M., at al.(2019)
Arsenic (As)	3×10^{-4}	WHO/FAO.(2013)

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APPENIX 10

TABLE 11A: HPLC analysis of herbal products

NO	**HERBAL PRODUCTS**	**PEAK RESOLUTION TIME (MIN)**
1.	MALA TYPH	3.582, 4.320, 11.081, 13.139, 13.592, 14.424, 14.924, 15.425, 18.427.
2.	GIVERS	3.375, 5.488, 6.419, 7.402, 9.690, 11.521, 11.707, 12.248, 13.300, 14.843, 18.405, 23.982.
3.	ASEDA	3.594, 4.305, 6.041, 12.190, 12.411, 13.357, 13.905, 14.853, 16.274.
4.	OSOMPA	3.549, 4.291, 9.134, 10.771, 12.270, 15.898, 16. 216, 17.255, 17.555, 17.707, 18.299
5.	TYPHOFA	3.577, 4.320, 6.668, 7.600, 9.962, 11.500, 12.227, 15.571, 16.233, 19.045.
6.	GEO MANUEL	3.595, 5.594, 7.489, 7.688, 12.152, 12.300, 13.81, 14.370, 14.862, 15.515, 15.984, 16.224, 16.792, 17.441, 18.582, 19.535.
7.	AWAY MALAMIX	8.731, 9.160, 10.797, 12.299, 14.739, 15.088, 15.373, 15.800, 16.166, 16.490, 16.683, 16.903, 17.215, 17.518, 20.953.

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APPENDIX 11

Table A12: Input parameters for Average Daily Dose (ADD)/ Estimated Daily Intake (EDI) calculation

Exposure parameters	Symbol	Units	Values	Reference
Concentration of heavy metal	C	Mg/kg	-	Ametepey *et al.* (2018)
Ingestion rate	IR	g/day	2.2	
Exposure frequency	EF	Days/year	365	Wongsasuluk *et al.*(2014)
Exposure duration	ED	Years	70	
Adult BW	BW	Kg	70	
Child BW	BW	Kg	16	
Average time	AT	years	25550	

Table A13: Reference values for electrolyte intake in mg/d

ELECTROLYTES	WOMEN	MEN	REFERENCE
Sodium	1500	1500	WHO.(2004)
Potassium	4000	4000	WHO.(2004)
Calcium	1000	1000	Lewis. (2019)
Magnesium	300-310	350-400	DA-CH. (2008)

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APPENDIX 12

Fig A4: Dose response curves for various herbal drugs.

Plasmodium facilparum 3d7 Labstrain

IC50= 4.20 µg/ml

% parasitaemia

Log (GHM),µg/ml

Plasmodium facilparum 3d7 Labstrain

ic50 =0.11ng/ml =0.00011µg/ml

% parasitaemia

Log (ART),ng/ml

Plasmodium facilparum 3d7 Labstrain

IC50 =2.166 µg/ml

% parasitaemia

Log (GM),µg/ml

Plasmodium facilparum 3d7 Labstrain

IC50 = 9.404 µg/ml

% parasitaemia

Log (GHM),µg/ml

Plasmodium facilparum 3d7 Labstrain

IC50 =2.178 µg/ml

% parasitaemia

Log (MT),µg/ml

Plasmodium facilparum 3d7 Labstrain

IC50 =14.4ng/ml =0.0144 µg/ml

% parasitaemia

Log (ADQ),ng/ml

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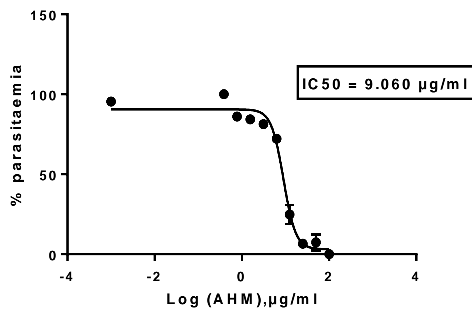
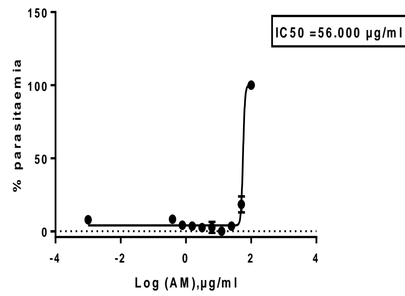
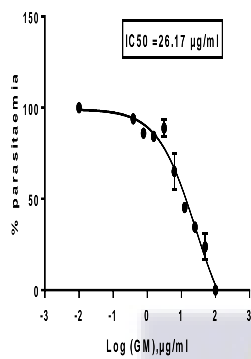
Plasmodium facilparum 3d7 Labstrain



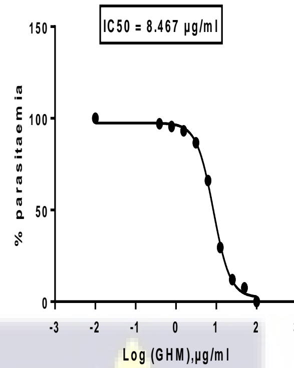
Plasmodium facilparum 3d7 Labstrain



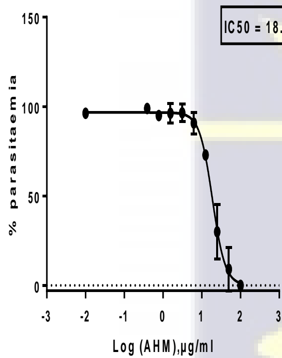
Plasmodium facilparum Chloroquine resistance DD2 Labstrain



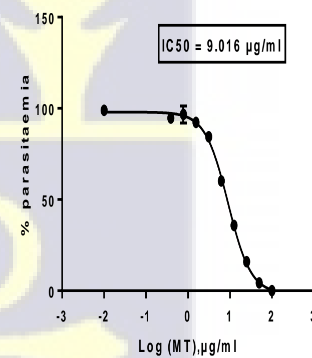
Plasmodium facilparum Chloroquine resistance DD2 Labstrain



Plasmodium facilparum Chloroquine resistance DD2 Labstrain



Plasmodium facilparum Chloroquine resistance DD2 Labstrain



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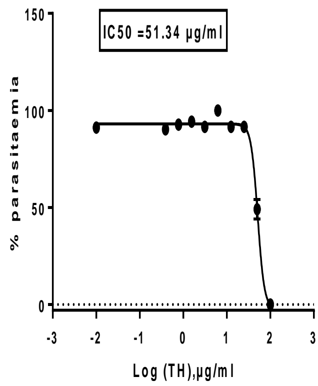
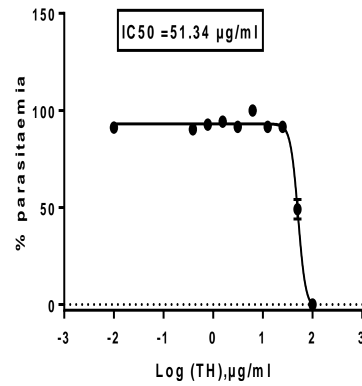
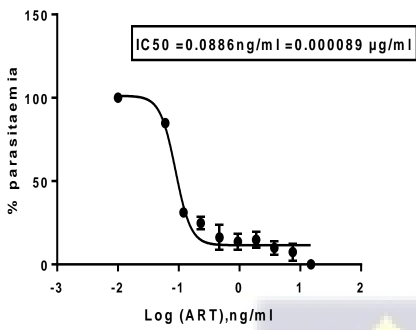
Plasmodium facilparum Chloroquine resistance DD2 Labstrain



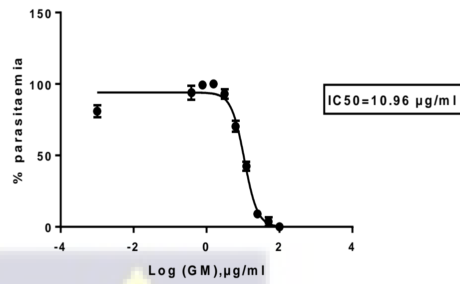
Plasmodium facilparum Chloroquine resistance DD2 Labstrain



Plsmodium berghei



Plsmodium berghei



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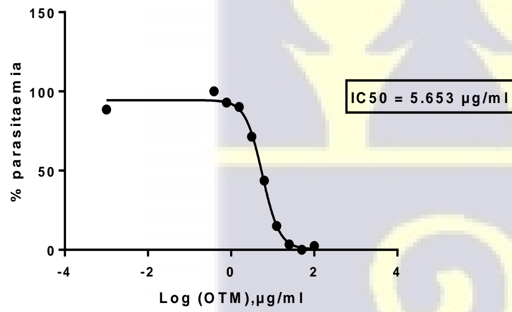

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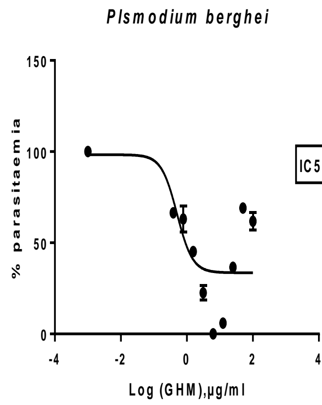

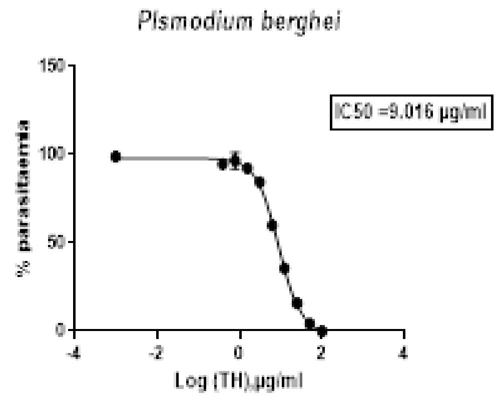

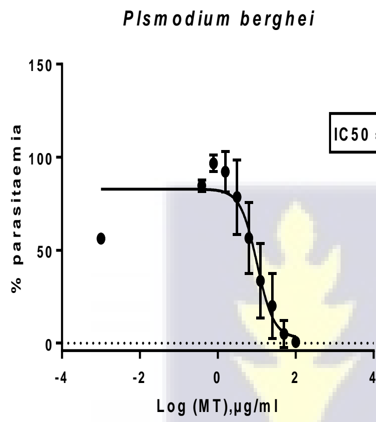

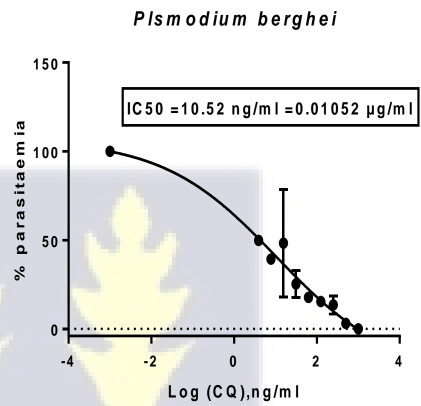

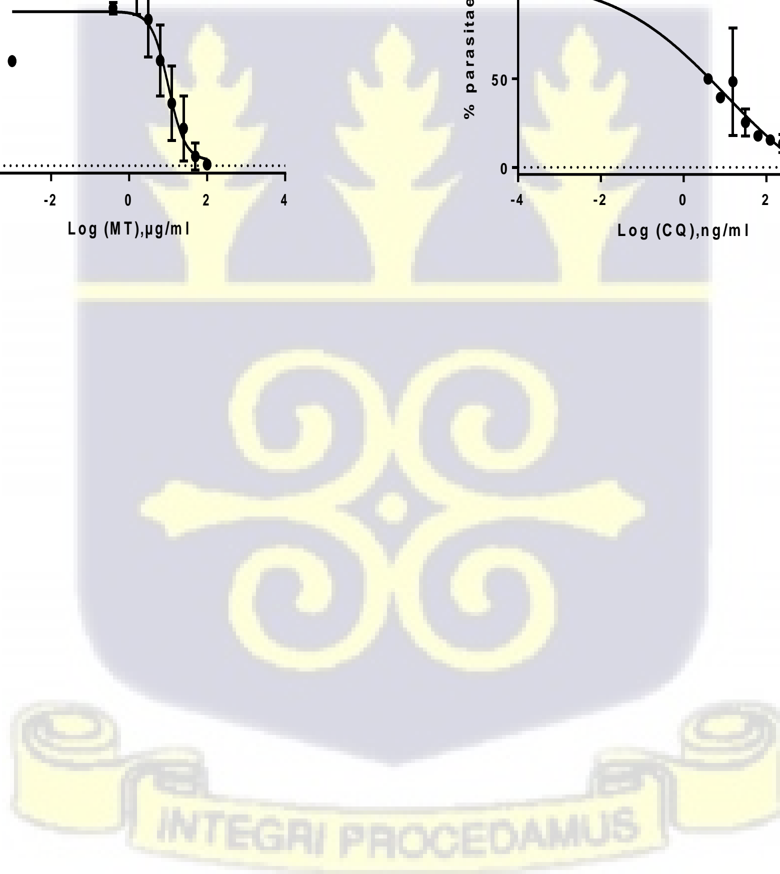

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APPENDIX 13

Table A14: Details of herbal products

ID	ACTIVE INGREDIENTS(COMMON NAME)	VOLUME	FDA No	MANUFACTORY DATE	EXPIRY DATE	BATCH No	ILLMENTS TREATED BY DRUG	DOSAGE
AHM	Astonia boonei,Lanea kerstingil,Magnifera Indica	1000ml	FDA / HD-14-8132	12/6/20	12/6/22	0023	Malaria and Typhoid fever	Malaria-4tbs 3 times(adult) for a week.Typhoid-same for 2 weeks
TH	Ocimum viride,Vernonia amygdalina,Morinda lucida,Alstonia boonei,Carica papaya,Corn still	500ml	FDA/HD-19-07227	March 2020	March 2020	001	Malaria fever	10tbs 3 times daily after meals
GHM	Khaya Senegalenis,Azaridachta indica	500ml	FDA/HD-18-04183	-	-	-	Malaria fever	4tbs(adults) and 2tbs(children),3 times daily after meals
GM	Terminalia ivorensis, Pycnanthus angolensus,Alstonia booonei	1000ml	FDA/HD-19-02063	February 2020	February 2020	GMTM	Malaria fever and Typhoid	2tbs(adults),1tbs(children) 3 times daily
AM	Alstonia boonia,Naulea latifolia,Enantia forlycarpa	500ml	-	12/02/20	12/02/23	-	Stomach ulcer,Diabetetis,Malaria fever,Typhoid fever,Stomach troubles and Hernia	2tbs(adults),1tbs(children) 2 times daily
OTM	Carica papaya,Cassia alata	1000ml	FDA/HD2-16-05177	7/01/20	7/01/23	OHC/TM001	Typhoid fever and Jaundice and Malaria	4tbs(adult) 3 times daily,2tbs(children)2 times daily
MT	Khaya senegalensis,Cryptolepis senguinolenta,Cassia siamea and Citrus aurentium	750ml	FDA/HD-13-1013	02/01/20	29/12/22	001	Strong typhoid and all types of fever	4tbs(adult) 3tbs(children)3 times daily

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APPENDIX 14

SAMPLE QUESTIONAIRE

ASSESSING GENERAL KNOWLEDGE, PERCEPTION AND CONSUMPTION ABOUT HERBAL PRODUCTS IN GHANA.

Thank you very much for agreeing to participate in the study Please, during the interview, if you have any question or require additional explanations, please feel free to ask.

Please kindly use your initials as your participant ID with respect to A3 below.

BACKGROUND DETAILS		RESPONSE
A1	Date of interview	
A2	District/Region	
A3	Participant ID.	

Indicate the preferred answer by ticking or circling the appropriate code.

QUESTIONS		RESPONSE	CODE
Q1	Sex	Male	1
		Female	2
Q2	Age	Write in years	
Q3	Education	No Education	1
		Basic school	2
		Secondary school	3
		Tertiary	4
Q4	Have you heard about herbal medicine in Ghana before?	Yes	1
		No	2
Q5	If yes, have you ever used any herbal medicine/products in treating any disease condition before?	Yes	1
		No	2
Q6	If yes, have you used it for treating the following disease conditions?	Malaria fever	1
		Typhoid fever	2
		Ulcer	3
		Other	4
Q7	Based on the above questions how often have you been using these herbal medicines?	More often	1
		Sometimes	2
		Not often	3

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Q8	Do these herbal medicines work for you?	Yes	1
		No	2
Q9	Have you ever used one herbal product to treat multiple disease conditions before?	Yes	1
		No	2
Q10	Have you ever used any of the following herbal products to treat either Malaria or Typhoid fever before?	Taabia	1
		Time Herbal Mixture	2
		Rooter Herbal Mixture	3
		Others (Away, Givers, Osompa, Typhofa, Geo manuel, Mala typhs, Aseda etc).	
Q11	Do you think herbal treatment of Malaria and Typhoid is ideal or better than orthodox treatments?	Yes	1
		No	2

In case of further enquiries please contact the Researcher:

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Tel: 0242739616/ 0276246114

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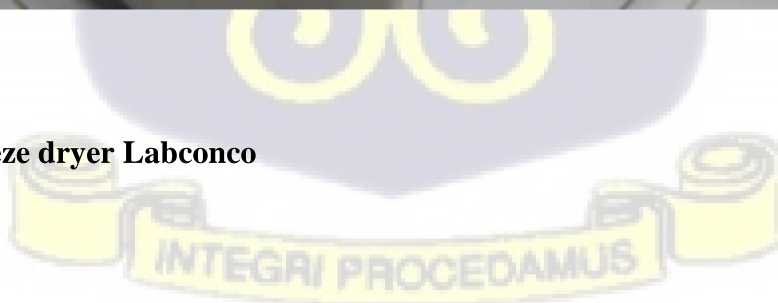
APPENDIX 15

Plate A1: Freeze dryer Labconco



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APPENDIX 16

PlateA2: Preparation of herbal drug plates in the Biosafety hood

Plate A3: Reading drug plate with Fluostar Optima Plate Reader

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APPENDIX 17

Plate A4: Analysing herbal products for fingerprints using K15 A306 HPLC4 Machine.

Plate A5: Analysis of heavy metal and electrolytes contents of herbal products using Atomic absorption spectrometer

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APPENDIX 18

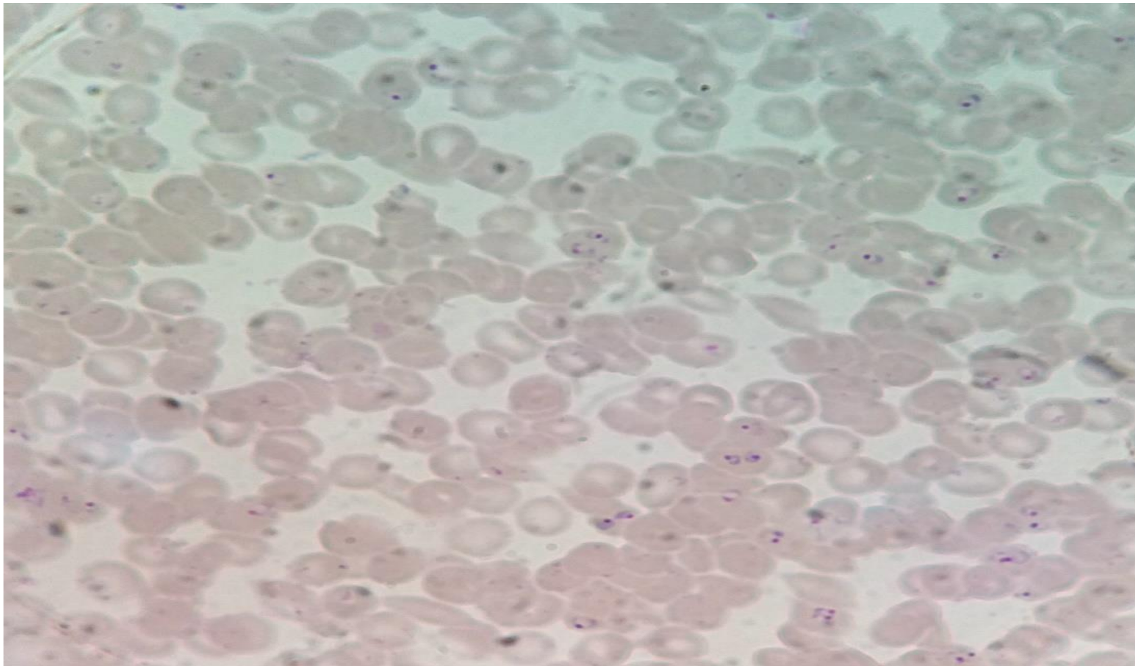

Plate A6: Asexual stage of *Plasmodium falciparum* 3D7 in a Geimsa stained slide.

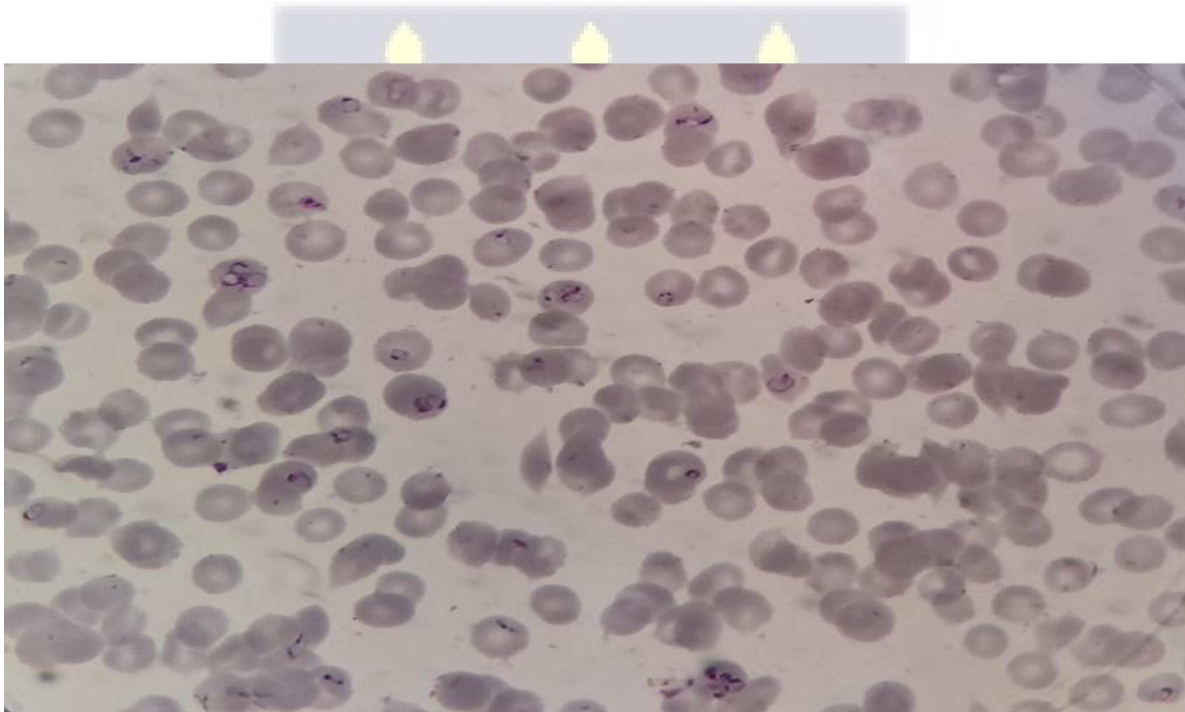

Plate A7: Asexual stage of *Plasmodium berghei* in a Geimsa stained slide from maurine mice.

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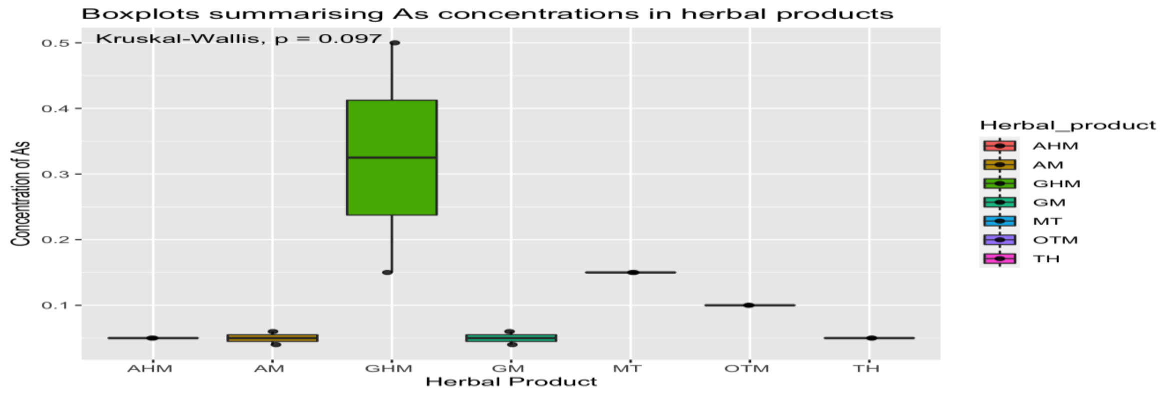
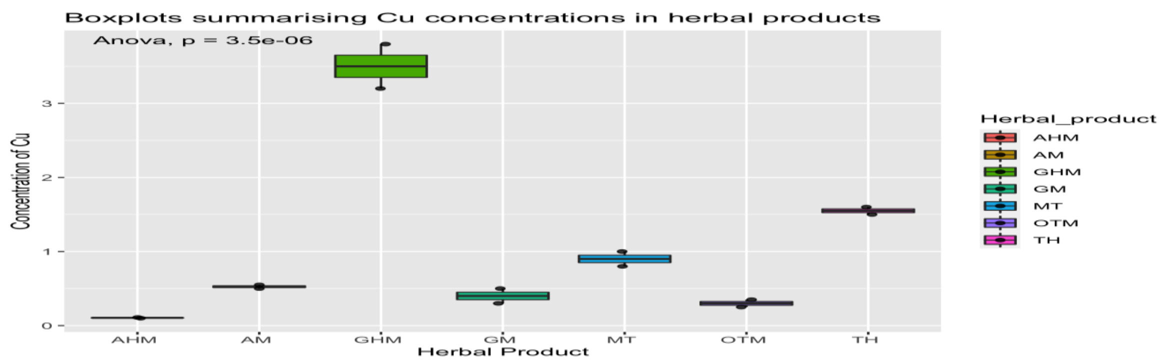
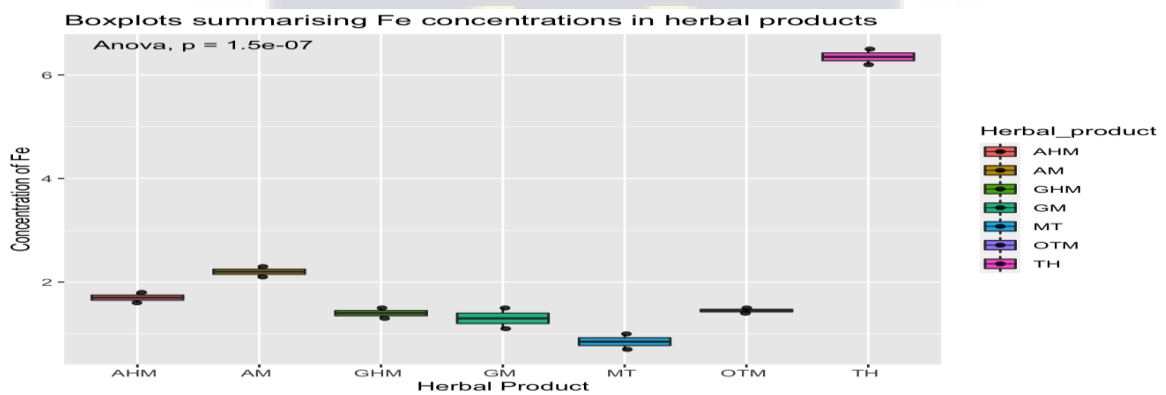
APPENDIX 19

Boxplots summary of heavy metal contents in herbal drugs analysed using Kruskall-wallis.







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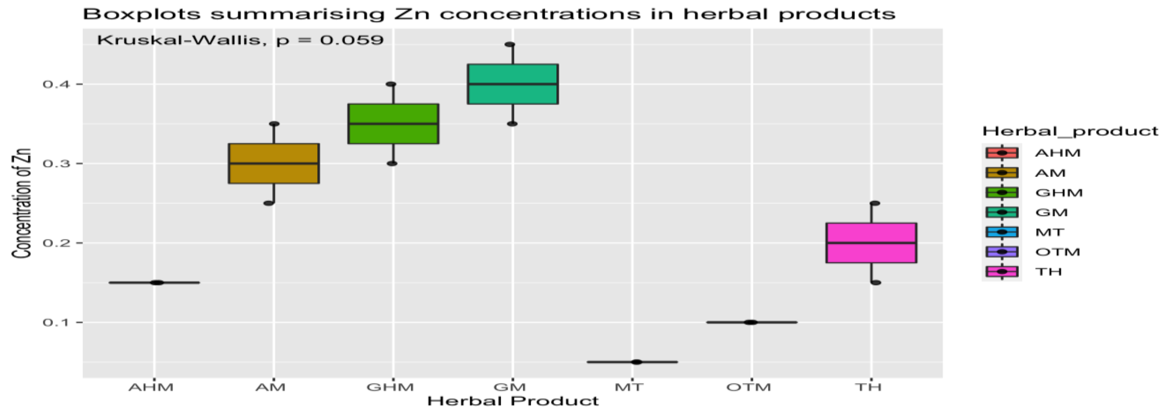

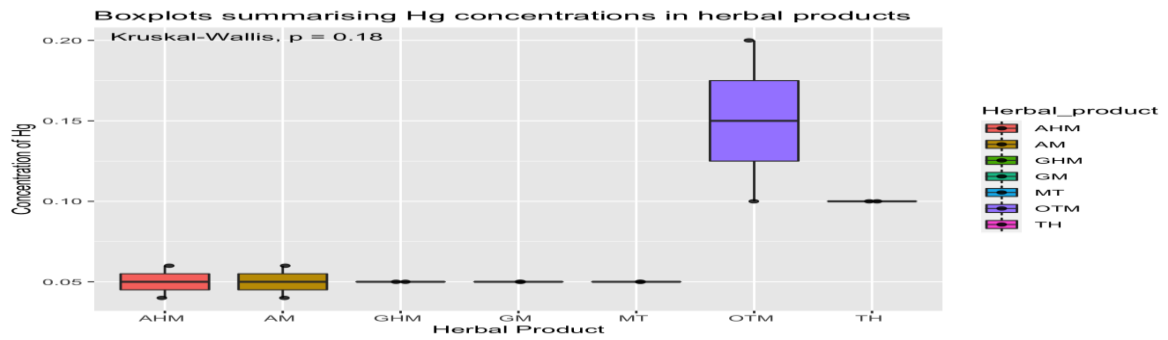

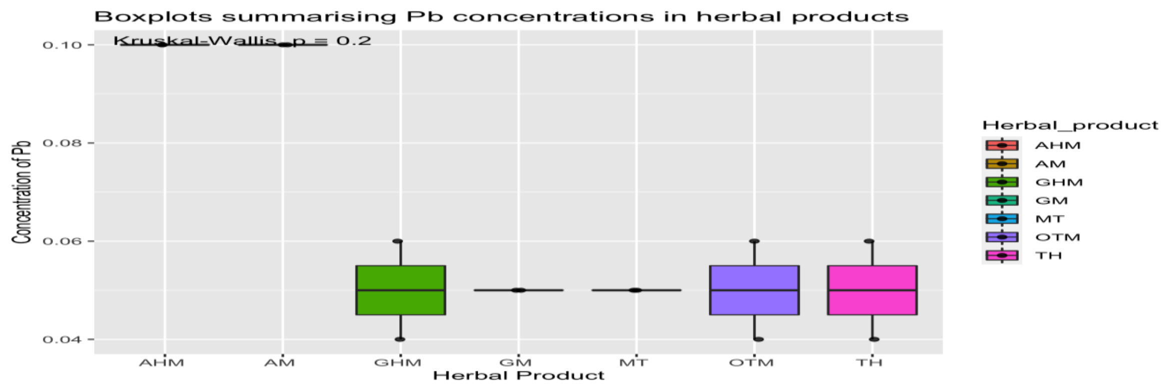

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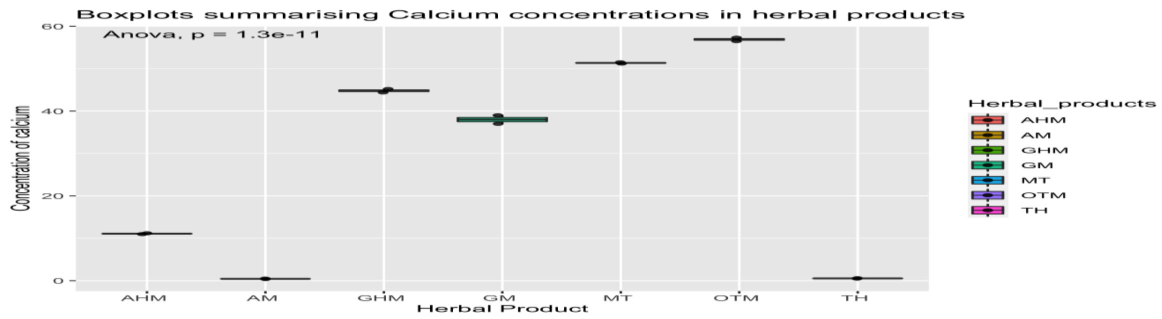
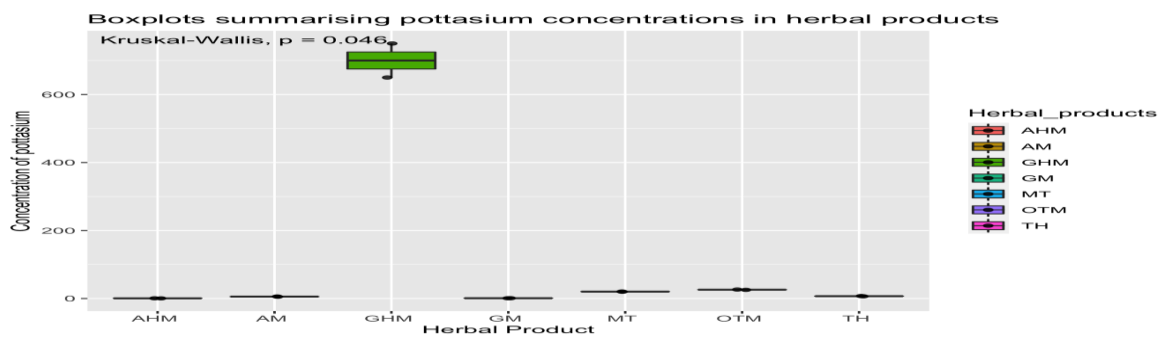
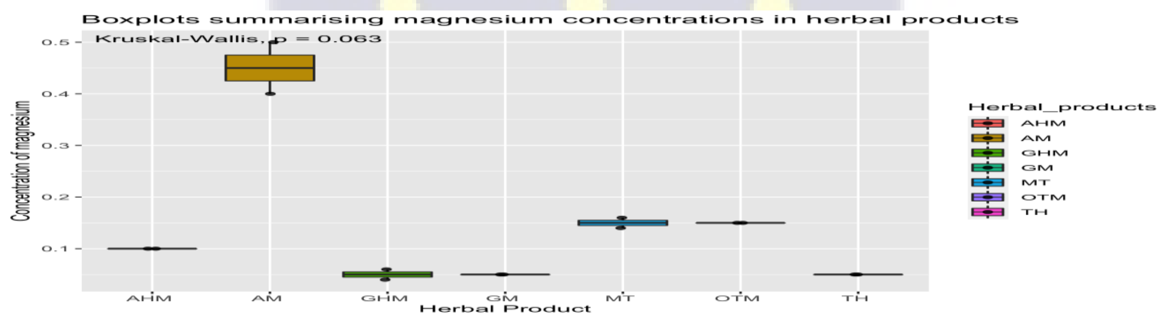
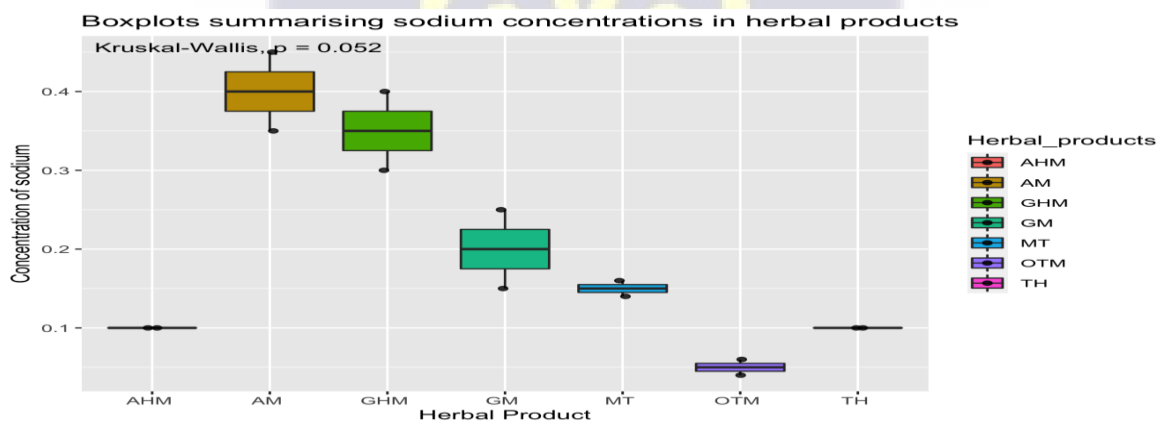
APPENDIX 20

Boxplots summary of electrolyte contents of various herbal products in Kruskal-wallis analysis.









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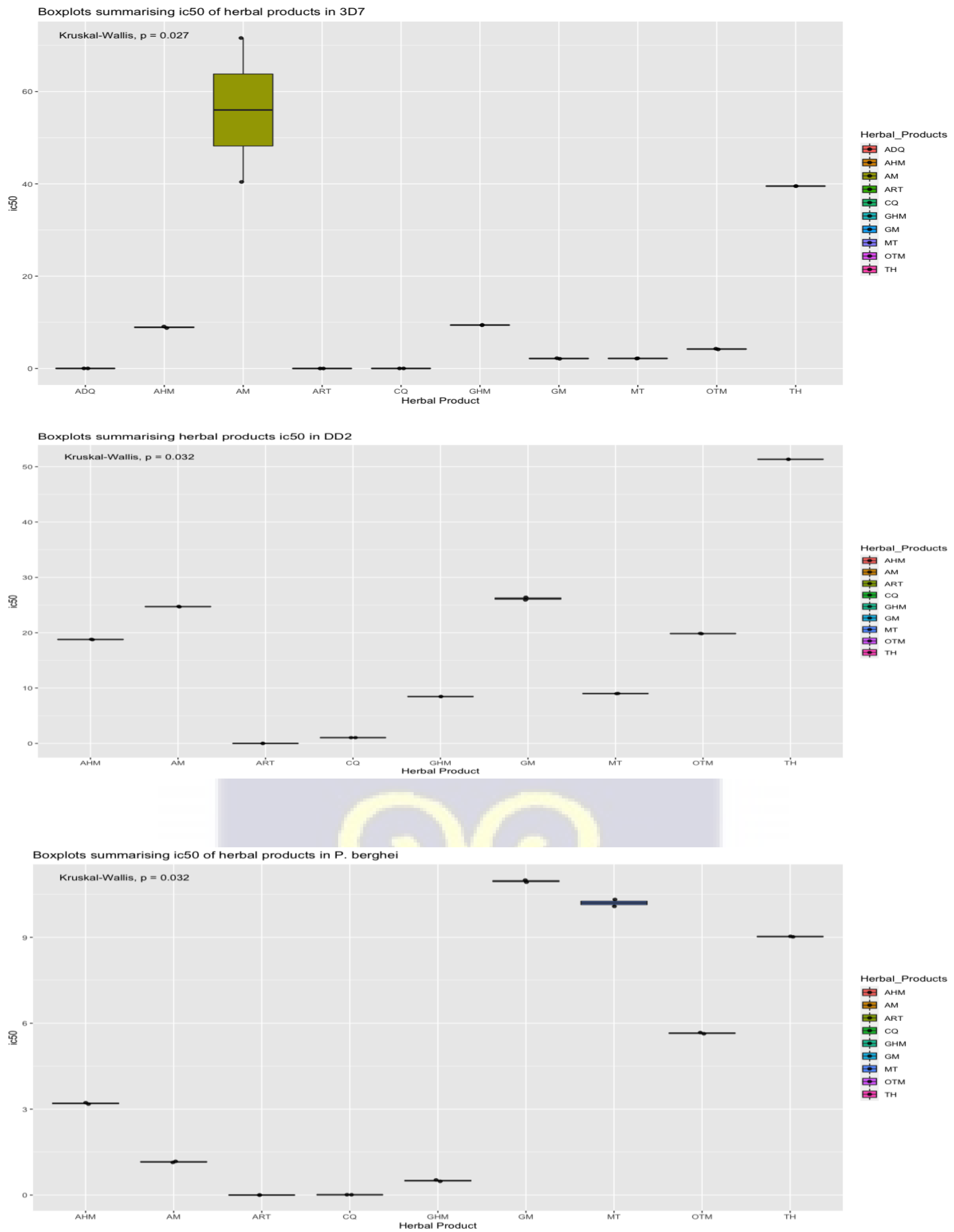
APPENDIX 21

Boxplot summary of IC_{50} values 3D7, Dd2 and *Plasmodium berghei*



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APPENDIX 22

SAMPLE SIZE CALCULATION FORMULA

Unlimited population:

$$CI = \hat{p} \pm z \times \sqrt{\frac{p(1-p)}{n}}$$

Finite population:

$$CI' = \hat{p} \pm z \times \sqrt{\frac{\hat{p}(1-\hat{p})}{n'} \times \frac{N-n'}{N-1}}$$

Where:

z is the z score at 95% is 1.96

ε is the margin of error is 5%

N is the population size (calculated from unlimited population)

$\hat{p}$ is the population proportion at 50% or 0.5