ANTIPLASMODIAL COMPOUNDS FROM GHANAIAN MEDICINAL PLANTS

A Thesis submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY (Pharmacognosy)

The Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

By

GUSTAV KOMLAGA [B. Pharm (Hons); M.Pharm (Pharmacognosy)]

December 2015

DECLARATION

I hereby declare that this submission is my own work towards the PhD and that, to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any other degree of the university, except where due acknowledgement has been made in the text.

Gustav Komlaga ….…… ……..

(PG 6958611) Signature Date

Certified by:

Dr. (Mrs) Rita A. Dickson …… …..……

(Supervisor, KNUST) Signature Date

Certified by:

Professor Philippe M. Loiseau 20 nov. 2015

(Thesis Director, Université Paris Sud)

Signature Date

Certified by:

Professor MLK Mensah …… ……..

(Supervisor, KNUST) Signature Date

Professor A. Y Mensah …… ……

(Head of Dept, Pharmacognosy, KNUST) Signature Date

DEDICATION

To my Lord, my Counsellor and my closest friend, Jesus Christ, the author and finisher of my faith whose grace and mercies saw me through this study. To my loving wife and friend, Mrs Grace Yayra Komlaga and my children, Eyram Jessica Kpodua Komlaga and Elikem Gustav Komlaga. To my dear sister, Joyce Komlaga, and my mother, Madam Adeline Komlaga of blessed memory.

ABSTRACT IN ENGLISH

Malaria is a major public health challenge in Ghana, and many indigenes employ medicinal plants, beside orthodox medicines, to treat the disease. An ethnobotanical survey was performed in the Bosomtwi and Sekyere East Districts of Ghana to identify plants used locally to manage malaria. This was done in comparison with the plant ingredients in marketed herbal antimalarial remedies in the Kumasi metropolis. The survey inventoried ninety-eight (98) plant species; twelve (12; 12.2%) reported for the first time globally, and twenty (20; 20.4%) others for the first time in Ghana for the treatment of malaria. Twenty-three (23) locally available finished, often multi/polyherbal antimalarial products examined contained aerial or underground parts of twenty-nine (29) of the plants cited in the survey as ingredients. Twenty-two (22) of these products were registered by the Ghana Food and Drugs Authority; four (4) were included in the Ghana Health Service recommended herbal medicine list for treating malaria in Ghana. The aqueous as well as serially extracted organic solvents (petroleum ether, ethyl acetate, and methanol) extracts of five plants parts, selected based on their importance in the traditional treatment of malaria and lack of the appropriate data in the literature, were studied against the chloroquine-sensitive 3D7 and chloroquine-resistant W2 *P. falciparum* parasite *in vitro*. The plant materials included the whole of *Phyllanthus fraternus*, leaves of *Tectona grandis*, *Terminalia ivorensis* and *Bambusa vulgaris*, and root of *Senna siamea*. All the aqueous extracts showed notable antiplasmodial activity (IC_{50} < 10 µg/mL), except that of *S. siamea*, against 3D7 *P. falciparum*. Only *T. ivorensis* and *S. siamea* extracts showed activity against W2 *P. falciparum* (IC_{50} < 50 µg/mL). The extracts demonstrated high selectivity index (SI) for 3D7 *P. falciparum* (SI > 3.5) but very low SI for W2 *P.*

falciparum. Resistance index (RI) was largely under 20. The organic fractions were equally active (IC_{50} < 50 µg/mL; 3D7 *P. falciparum*). The methanol extracts of the two most potent plant materials, the whole of *P. fraternus* and leaf of *B. vulgaris,* were subjected to phytochemical study to isolate and elucidate the chemical constituents, which were then assayed for antiplasmodial activity. The phytochemical study of the methanol extract of *P. fraternus* yielded six compounds; **Pf 1** to **Pf 6** identified as the lignan, phyllanthin, and five securinega alkaloids namely nirurine, *ent*-norsecurinine, *allo*-norsecurinine, bubbialine and epibubbialine. This is the first isolation of *allo* norsecurinine from a natural source and bubbialine from the *Phyllanthus* genus. The compounds displayed significant antiplasmodial activity against both 3D7 and W2 *P. falciparum* (1.14 ± 0.32 µM ≤ IC_{50} ≤ 59.00 ± 5.43 µM); *ent*-norsecurinine being the most active (IC_{50}=1.14± 0.32 µM) and against the W2 *P. falciparum*. Only **Pf2** (nirurine) and **Pf1** (phyllanthin) displayed cytotoxicity (CC_{50} < 100 µM; HUVECs). This is the first report of the antiplasmodial activity of these compounds. Similar study of the methanol extract of *B. vulgaris* yielded 6 compounds, **Bv1** to **Bv6,** identified as *p*-coumaric acid [(*E*)-3-(4-hydroxyphenyl) acrylic acid], cinnamic acid, dehydrovomifoliol [(E)-4-hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl)cyclohex2-en-1-one], 3-oxo-α-ionol [9-hydroxy megastigma-4, 7-dien-3-one], loliolide [6hydroxy-4, 4, 7a-trimethyl-5, 6, 7, 7a-tetrahydrobenzofuran-2(4H)-one] and tricin [5,7,4'-trihydroxy-3',5'-dimethoxyflavone]. The six compounds are the first everreported isolations from *B. vulgaris*. All the compounds from *B. vulgaris* displayed significant activity against 3D7 (IC_{50} < 5 µM and W2 strains of *P. falciparum* (IC_{50} < 7 µM). **Bv1** (*p*-coumaric acid) was the most active against 3D7 *P. falciparum* (IC_{50}:
0.84 ± 0.90 µM) and **Bv2** (cinnamic acid) the most active against W2 *P. falciparum* (IC_{50}: 1.41 ± 0.38 µM). The compounds displayed no cytotoxicity (CC_{50} > 100 µM; HUVECs). This is the first report of the antiplasmodial activity of the six compounds. These twelve (12) compounds with remarkable antiplasmodial activity add to the library of natural compounds with antiplasmodial activity. This study has illustrated

the potentials of Ghanaian medicinal plants as source of natural antiplasmodial compounds, and has justified the use of the plants in traditional treatment of malaria. .

ACKNOWLEDGMENTS

I give thanks, praise, glory, honour, and adoration to God Almighty for his enabling grace that saw me through this study. I am very grateful to the French Embassy in Ghana for the scholarship, to Misses Soleansky Stéphanie and Nina Amah of the embassy for facilitating my trips to and from Paris. Special thanks to the staff of Campus France for their support while in France. I am very thankful to KNUST for granting me the study leave. I am indebted to the Vice Chancellor of KNUST, Professor William Otoo Ellis, the Provost of the College of Health Sciences, Professor Tsri Agbenyega, the Dean of the Faculty of Pharmacy and Pharmaceutical Sciences, Professor Charles Ansah, for their special support. I am profoundly grateful to my thesis director at Université Paris Sud, Professor Philippe M. Loiseau, for his help especially for finding a pharmacognosy laboratory and pharmacognosy supervisor in France for me. My heartfelt appreciation to Professor Pierre Champy, my pharmacognosy supervisor at the Université Paris Sud for ensuring my work at his laboratory was without hitches and for his helpful criticisms and insightful suggestions, which greatly contributed to this work. I am grateful to Dr Sandrine Cojean of Paris Sud, for the supervision of the antiplasmodial study and her personal involvement in the experiments. I also thank my supervisors at KNUST, Kumasi, Ghana; Dr Rita A. Dickson for her valuable guidance throughout this study and Professor Merlin L.K. Mensah for his time, patience, helpful criticisms, and suggestions, which greatly contributed to the accomplishment of this study. I acknowledge Professor Bruno Figadère, the Head of BioSCIS research unit, Paris Sud, for his cordiality during the study. Thanks to Mr Sylvain Connan formerly of Maison Francaise, KNUST for linking me to Philippe for the collaboration. To Dr AhmedMehdi Beniddir, I am highly indebted for his indispensable assistance. My

appreciations to Karine Leblanc and Jean-Christophe Jullian for running the spectroscopies of the isolated compounds. I acknowledge Dr Joel Dade of Université Félix Houphouët Boigny, Côte d'Ivoire for his help in the structural elucidation of the isolated compounds. I want to make a special mention of Drs Lauran Evano, Lauran Ferrie of Laboratoire Chemie naturelle substance therapeutique (BioSCIS) and Sebastien Pomel and Vanessa Le Moal of the Antiparasitic Chemotherapy Laboratory (BioSCIS) not forgetting Adams, David, Long, Aminata, Soulaf Suyyagh-Albouz and indeed all colleagues in the BioSCIS unit for always being at hand to help. I am very thankful to Dr Christian Agyare with whom this project topic was initiated. Thanks to Mrs Helena Owusu-Ansah of the Department of Pharmacogonsy for her help. To all my friends who kept on encouraging me throughout this study, I am grateful. Finally, I am so grateful to my wife, Mrs Grace Yayra Komlaga and my children, Eyram Jessica Kpodua Komlaga and Elikem Gustav Komlaga for their support, not forgetting my sister Joyce Komlaga in Germany for her constant financial support throughout this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDICES

ABBREVIATIONS

ACTs:	Artemisinin-based combination therapies
cc:	Column chromatographed
CC_{50}:	Drug concentration that reduced the number of viable cells by 50%
$CDC1_3$:	Deuterated chloroform
COSY:	Correlation spectroscopy
CQ:	Chloroquine
DCM:	Dichloromethane
DMEM-F12:	Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12
DMSO:	Dimethyl sulfoxide
EDTA:	Ethylenediaminetetraacetic acid
EtOAc:	Ethyl acetate
FBS:	Foetal bovine serum
GPD:	Gross domestic product
HC_{50}:	Concentration of a fraction causing 50% haemolysis
HEPES:	N-2-hydroxyethylpiperazine-N'-2-ethane-sulphonic acid
HMBC:	Homonucear multiple bond correlation
HR-ESI-MS:	High-resolution electrospray ionisation mass spectrometry
HSQC:	Heteronuclear Single Quantum Coherence/correlation
HUVECs:	Human umbilical vein endothelial cells
IC_{50}:	Drug concentration that inhibits 50% *P. falciparum* cells growth
IPT:	Intermittent preventive treatment
IR:	Infrared
IRS:	Indoor residual spraying
KNUST:	Kwame Nkrumah University of Science and Technology
LLINs:	Long-lasting insecticidal nets

MeCN:	Acetonitrile
MeOD:	Deuterated methanol
MeOH:	Methanol
MTT:	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
NA:	Not applicable
NC:	Negative control
ND:	Not determined
NMR:	Nuclear magnetic resonance
NOESY:	Nuclear Overhauser Effect Spectroscopy
PCR:	Polymerase chain reaction
PfdUTPase:	*Plasmodium falciparum* 2′-deoxyuridine 5′-triphosphate nucleotidohydrolase
Prep HPLC:	Preparative high performance liquid chromatography
RDTs:	Rapid diagnostic tests
Rf:	Retardation factor
RI:	Resistance index
RPMI:	Roswell Park Memorial Institute
SI:	Selectivity index
T & CM:	Traditional and Complementary Medicines
TLC:	Thin layer chromatography
TMS:	Tetramethylsilane
USAID:	United States Agency for International Development
UV:	Ultraviolet
UV-VIS:	Ultraviolet visible
WHO AFRO:	WHO Regional Office for Africa
WHO:	World Health Organization

CHAPTER 1

1 GENERAL INTRODUCTION

1.1 CONTEXT AND OBJECTIVES

Malaria is a life-threatening disease of public health concern especially in sub-Saharan Africa where it is endemic; about 90% of the global malaria-related deaths occurs in sub-Saharan Africa. The disease is caused by protozoan parasites of the genus *Plasmodium,* which are transmitted from person to person through the bite of the female Anopheles mosquito. Such factors as hot and humid climate characteristic of the tropical regions, often coupled with poor environmental practices are conducive to the multiplication of the vector. In 2013, malaria was present in 97 countries and territories with ongoing malaria transmission, and 7 countries in the prevention or reintroduction phase; totalling 104 countries and territories in which malaria was then considered endemic (WHO, 2013b).

The control of malaria has been a multi-faceted approach involving the control of the vector and the use of drugs for treatment (White et al., 2014; WHO, 2011). Currently, artemisinin-based combination therapies (ACTs) are the major drugs for the treatment of malaria (WHO, 2010). However, the cost of ACTs is high (Mutabingwa, 2005). Unfortunately there are reports of increasing drug resistance to artemisinin and its derivatives (Jambou et al., 2005).

In developing countries where traditional medicine plays an important role in the healthcare of the citizens, medicinal plants are also employed in the management of malaria (Willcox & Bodeker, 2004; Willcox, 2011). In Ghana, the use of medicinal plants is an age-old tradition and many such plants for the treatment of malaria and

many other diseases are well-documented (Abbiw, 1990; Asase et al., 2005; Dokosi, 1998; Mshana et al., 2001). Currently, many of these plants are formulated into finished herbal products, which are sold and dispensed in herbal clinics and shops, licensed chemical shops and pharmacies especially in urban communities. This brings the traditional approach to managing malaria and other diseases to the doorstep of, especially, the urban dweller who otherwise would not have access to the plant products due to the effect of urbanisation, migration, ethnic diversification among other reasons (Soelberg et al., 2015).

However there have been challenges with traditional medicines and for that matter, the use of medicinal plants and their products; the main ones concern the issues regarding quality, safety, effectiveness, and regulation (WHO, 2013a). According to the WHO Director-General, Dr Margaret Chan (WHO, 2013a), traditional medicines, of proven quality, safety, and efficacy, contribute to the goal of ensuring global access to care. WHO, in its Traditional Medicine Strategy 2014–2023, set out, among other goals, to support Member States in promoting safe and effective use of Traditional and Complementary Medicines (T&CM) through the regulation, research and integration of T&CM products, practices and practitioners into the health system, as appropriate (WHO, 2013a). This was after the World Health Organization (WHO) realized the importance of traditional medicine in primary healthcare delivery of many countries and their increasing use worldwide (Fendall, 1978). The 49th session of the WHO Regional Committee for Africa by its resolution AFR/RC49/R5, also urged African countries to encourage and support research into medicinal plants and herbal products, and to promote their use in the health delivery system (WHO AFRO, 1999).

Ghana, over the years, had developed various policies, programmes, regulations and laws to ensure the use of safe, effective and quality healthcare (Ministry of Health, 2005; Public Health Act, 2012). These are all geared towards ensuring that quality, safe and effective herbal products are available to the patronizing communities. In the light of this, the need to scientifically study the antimalarial among other medicinal claims ascribed to various plants and their products cannot be overemphasized. Also, regulation of finished herbal products, intended for the management of various diseases including malaria, becomes very important as this ensures conformity to both national and international standards.

Additionally, plants continue to be potential reservoirs of largely untapped complex and varied compounds; some of which could serve as lead compounds in the search of new, affordable and effective antimalarials. Farnsworth et al. (1985), reported that a fairly high percentage of useful plant-derived drugs were discovered as a result of scientific follow-up on plants used in traditional medicine, and concluded that this is a good approach for discovering many other useful drugs from plants. A typical example is the discovery of quinine in 1820.

According to Newman & Cragg, (2012) almost half of the drugs approved between 1981 and 2010 are based on natural products of which plants form a significant part. Despite significant discoveries of antiplasmodial agents, the need for new chemical entities with new mechanisms of action for the treatment of malaria remains high. Drug-treatment of malaria worldwide is overwhelmed with many challenges. The development of resistance by *P. falciparum* to the first line drugs such as the quinolines and even the newer artemisinin combination therapies (ACTs) (Jambou et al., 2005;

Wichmann et al., 2004) has led to many treatment failures which is a major clinical challenge. Furthermore, the side effects of some of the products, inaccessibility and the problem of affordability, the use of substandard and counterfeit drugs especially for citizens of third world countries continue to be a hindrance to effective management of malaria (Kokwaro, 2009; Soremekun et al., 2013). The way forward is therefore a continuous search for and the development of new, safe, affordable, but effective agents and remedies for the treatment of the disease globally. Plants therefore remain important candidate in the search for new antimalarial.

Medicinal plants have been an important element of indigenous medical systems of the world and are usually regarded as part of a culture's 'traditional' knowledge (Heinrich, 2000). Ethnobotany had played important roles in the discovery of new drugs for many centuries (Heinrich, 2000); it is a recognized tool in the search for new pharmaceuticals; numerous ethnobotanical studies aimed at identifying new pharmaceutical products (Balick, 2008). Aside the usefulness of ethnobotany in the search for bioactive compounds from plants sources, it also enables the recording and documentation of traditional knowledge about the empirical uses of plants - knowledge that is fast disappearing (Hamilton et al., 2003).

In this study, selected Ghanaian medicinal plants were investigated for compounds with antiplasmodial property: an ethnobotanical study was conducted, and medicinal plants used to treat malaria in the Bosumtwi and Sekyere East Districts of Ghana, where such study had not previously been done, inventoried, From the survey results, five plant materials including the leaves of *Tectona grandis* L.f, *Terminalia ivorensis* A. Chev, Bambusa *vulgaris* Schrad., the whole plant of *Phyllanthus fraternus*

G.L.Webster, and the root of *Senna siamea* (Lam.) Irwin & Barneby were selected and evaluated for antiplasmodial activity. These plant materials had relatively high percentage citation but were insufficiently exploited for antiplasmodial compounds. The extracts of *Phyllanthus fraternus* and *Bambusa vulgaris* leaf displayed the most promising antiplasomdial activity and therefore were fractionated and the purified compounds assessed for antiplasmodial activity.

1.1.1 Problem Statement

In Sub-Saharan Africa, medicinal plants have been employed in traditional medicine to treat malaria and many other diseases by large numbers of people who find some relief in their use, though majority of these plants have no supportive scientific data. Besides, only a limited number of medicinal plants have been documented for use in treating malaria and still fewer have been researched for their antimalarial constituents.

1.1.2 Research question

Medicinal plants are used in the Bosumtwi and Sekyere East Districts of Ghana to treat malaria, and many people find some relief in their use despite the lack of scientific evidence of antimalarial activity for some of the plants. Do the extracts of these plant materials possess antiplasmodial activity? What compounds are responsible for the antiplasmodial activity?

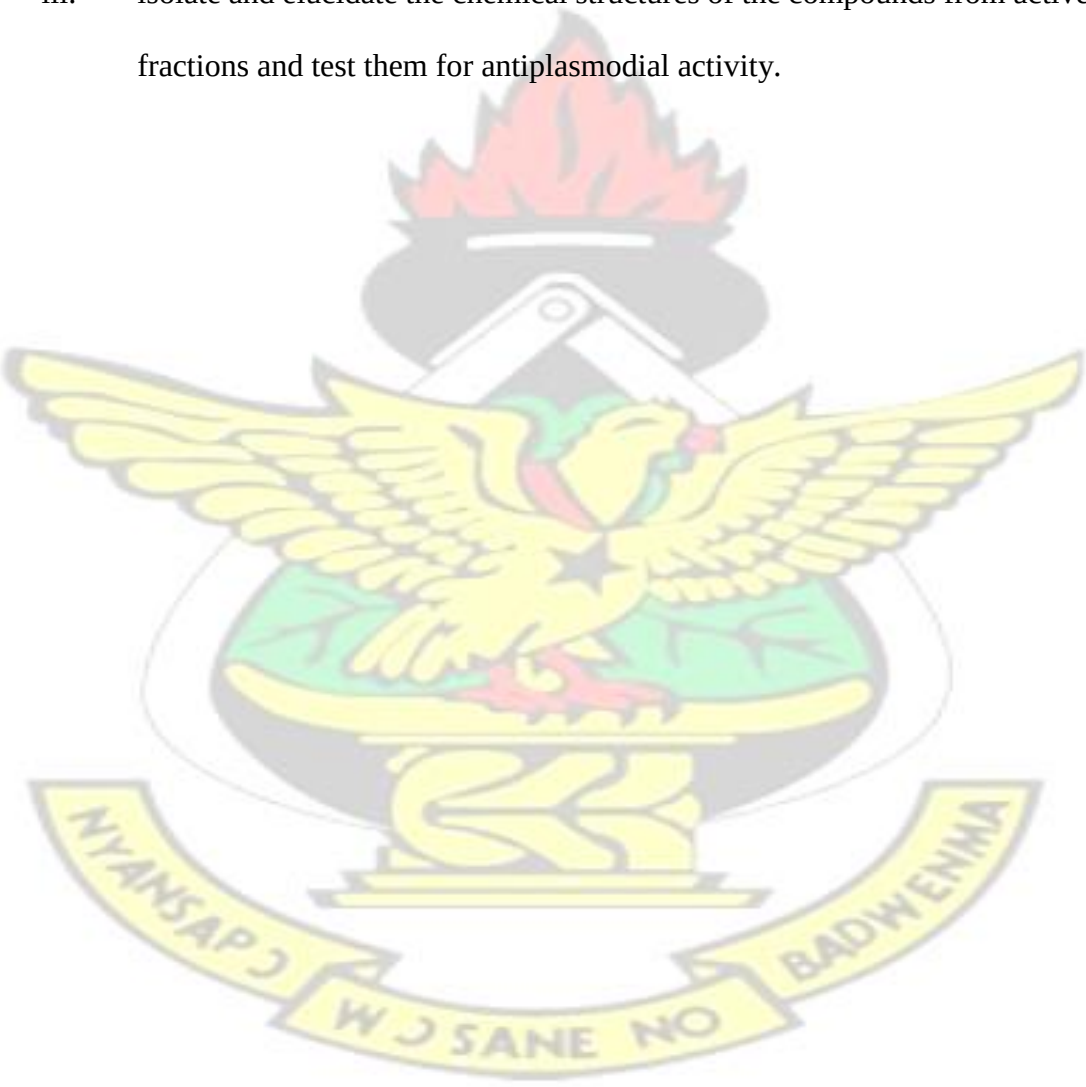
1.1.3 Research objectives

The objectives of this study included to:

i. perform an ethnobotanical study to identify medicinal plants used to treat malaria in the Bosumtwi and Sekyere East Districts of Ghana.

ii. validate the antimalarial activity of the aqueous extracts and organic fractions of selected of plants.

iii. isolate and elucidate the chemical structures of the compounds from active fractions and test them for antiplasmodial activity.



CHAPTER 2

2 LITERATURE REVIEW

2.1 MALARIA

The word malaria came from the Italian ‘mala aria’ that means ‘bad air’. It was referred to as malaria, ‘ague’ and ‘marsh fever’ by the English; the French refers to it as ‘paludisme’ - derived from the Latin word ‘paluster’ which means swampy, marshy or filthy. Other ancient names include ‘marsh fever’ and ‘fibrils intermittent’. Malaria is the name of a group of closely related severely infectious diseases, which are caused by the appearance and multiplication in the human body of the blood parasites, plasmodia (Lysenko & Semashk, 1968).

There are four main species of Plasmodium that cause malaria in humans: *Plasmodium vivax*, which causes three-day malaria; *P. falciparum*, which causes tropical malaria; *P. malariae*, which causes four-day malaria; and *P. ovale*, which causes a strain of three-day malaria. A fifth, *P. knowlesii* known to infect monkeys has been reported in humans from the forested regions of South-East Asia (White, 2008; WHO, 2010). *P. falciparum* and *P. vivax* are the most important, being the most deadly and predominant in Africa, and are the most widely distributed (WHO, 2013b). Unlike the other species, *P. vivax* is able to develop in the *Anopheles* mosquito vector at lower temperatures, and to survive at higher altitudes and in cooler climates. It also has a dormant liver stage (known as a hypnozoite) that enables it to survive during periods when *Anopheles* mosquitoes are not present to continue transmission, such as during winter months. Although *P. vivax* can occur throughout Africa, the risk of *P. vivax* infection is considerably reduced in the region by the high frequency of the Duffy

negativity trait among many African populations; in individuals without the Duffy antigen, red blood cells are resistant to infection with *P. vivax* (White et al., 2014). In many areas outside Africa, infections due to *P. vivax* are more common than those due to *P. falciparum* (WHO, 2012).

2.1.1 The economic cost of malaria

Malaria is a public health problem afflicting a community, and consists of the combined effects of the infection on the population as a whole (Koram & Molyneux, 2007). It affects the health and wealth of nations and individuals alike. (Roll Back Malaria, 2015).

Annual economic growth in countries with high malaria transmission has historically been lower than in countries without malaria. Economists believe that malaria is responsible for a 'growth penalty' of up to 1.3% per year in some African countries. When compounded over the years, this penalty leads to substantial differences in Gross domestic products (GDP) between countries with and without malaria and severely restrains the economic growth of the entire region (Roll Back Malaria, 2015).

In countries with a heavy malaria burden, the disease may account for as much as 40% of public health expenditure, 30% to 50% of inpatient admissions, and up to 50% of outpatient visits. The indirect costs of malaria include loss of productivity or income associated with illness or death. This may be expressed as the cost of lost workdays or absenteeism from formal employment and the value of unpaid work done in the home by both men and women. In the case of death, the indirect cost includes the discounted future lifetime earnings of those who die (Roll Back Malaria, 2015). Although difficult

to express in dollar terms, another indirect cost of malaria is the human pain and suffering caused by the disease. Malaria also hampers children's schooling and social development through both absenteeism and permanent neurological and other damage associated with severe episodes of the disease. The simple presence of malaria in a community or country also hampers individual and national prosperity due to its influence on social and economic decisions. The risk of contracting malaria in endemic areas can deter investment, both internal and external, and affect individual and household decision making in many ways that have a negative impact on economic productivity and growth (Roll Back Malaria, 2015).

In Ghana, the economic cost of malaria is enormous and affects the economic development of the country. The total annual costs were estimated at US$ 37.8 million nationwide and included average treatment costs of US$ 11.99 per case. Total costs per malaria episode (including direct and indirect household costs and health system costs) based on disease severity and the presence of complication and co-morbidities reportedly ranged from US$ 7.99 to $ 229.24 while health system costs per episode vary according to severity from US$ 2.89 to US$ 123. On average, up to 55% of the overall costs are said to be borne by households, 46% (US$ 1.26) of which are made of average indirect costs (Sicuri et al., 2013).

From the macroeconomic perspective, an estimated econometric model reportedly found malaria to have negative effect on real GDP growth. It was estimated that, one percentage increase in the malaria morbidity rate will slow down the rate of real GDP growth by 0.41% (Asante & Asenso-Okyere, 2003). The illness is also said to contribute to the loss of productive time not only to the economically active patients

but also to the caretakers of sick children. The value of productive time lost to the households reportedly amounted to US$ 8.92 per case. Economically, active patients lose about 9 workdays while their caretakers lose more than 5 workdays. School children also lose about four school days on the average due to the malaria illness (Asante & Asenso-Okyere, 2003).

2.1.2 The life cycle of *Plasmodium* parasites

The life cycle of the *Plasmodium* parasite involves two hosts, the vector, female *anopheline* mosquitoes, and the human host. In the human host, the parasite undergoes two developmental stages of infection. This includes the liver stage/cycle also called the exo-erythocytic (or pre-erythrocytic) stage and the erythrocytic stage/cycle.

The cycle begins when the motile sporozoites are inoculated into human host by an infected mosquito during a blood meal. The sporozoites in the blood enter the liver, each infecting a liver cell. Within the hepatocyte, a sporozoite replicates and matures into schizont from which many merozoites are produced (exo-erythrocytic schizogony).

When a schizont ruptures, it releases merozoites which invade red blood cells to begin the asexual erythrocytic cycle or stage. During the erythrocytic cycle, the merozoite undergoes asexual multiplication in the erythrocytes (erythrocytic schizogony) developing into trophozoites, schizonts and infective merozoites capable of reinfecting other erythrocytes when released and repeating the erythrocytic cycle. Some merozoites from the blood after entering a red blood cell transform into gametocytes (sexual forms) which are taken up by a feeding anopheline mosquito (Figure 2.1.1).

The parasites' multiplication in the mosquito is known as the sporogonic cycle. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinetes), which invade the midgut wall of the mosquito, where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle (White et al., 2014)

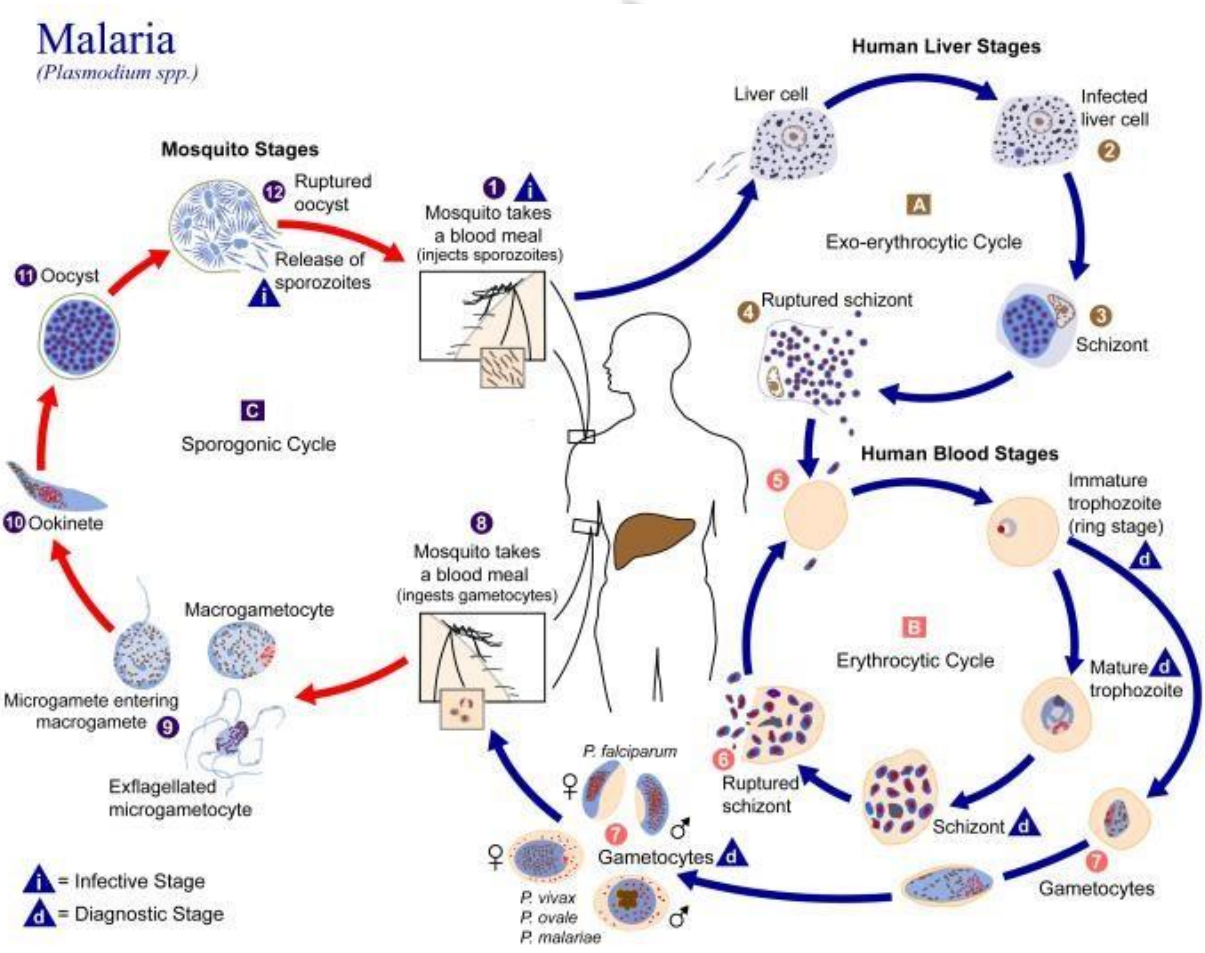


Figure 2.1.1: Overview of life cycle of *Plasmodium* (CDC, 2006)

2.1.3 Prevalence and mortality

Malaria transmission occurred in all six WHO regions in 2013. Globally, an estimated 3.2 billion people in 97 countries and territories are at risk of being infected with malaria and developing the disease (figure 2.1.2), and 1.2 billion are at high risk (>1 in 1000 chance of getting malaria in a year). According to the latest estimates, 198 million cases of malaria occurred globally in 2013 (uncertainty range 124–283 million) and the disease led to 584 000 deaths (uncertainty range 367,000–755,000). The burden of the disease is heaviest in the WHO African Region, where an estimated 90% of all malaria deaths occurred and children aged under 5 years accounted for 78% of all deaths (WHO, 2014).

In Ghana, the disease is endemic and perennial with seasonal variations that are more pronounced in the north of the country (United States Agency for International Development (USAID), 2013). Children under 5 and pregnant women are at higher risk of severe illness due to lowered immunity (USAID, 2013). According to Ghana Health Service (GHS) health facility data (cited in USAID, 2013), malaria is the number one cause of morbidity and mortality in children under five years of age, accounting for about 33 % of all hospital deaths in children under five years and about 38 % of all outpatient illnesses and about 36 % of all admissions in 2013. Between 3.1 and 3.5 million cases of clinical malaria were reported in public health facilities annually. Of this about 900,000 cases (27.27%) occurred in children under five years and 3,000-4,000 resulted in inpatient deaths (USAID, 2013).

The foregoing notwithstanding, a growing number of countries have recorded decreases in the number of confirmed cases of the disease and/ or reported admissions and deaths since 2000

(WHO, 2012). There is also reduction in the prevalence of malaria parasite infection across sub-Saharan Africa since 2000, and in the estimated malaria mortality rates by 47% worldwide and by 54% in the WHO African Region between 2000 and 2013 due to the various global control efforts and interventions

(WHO, 2014).

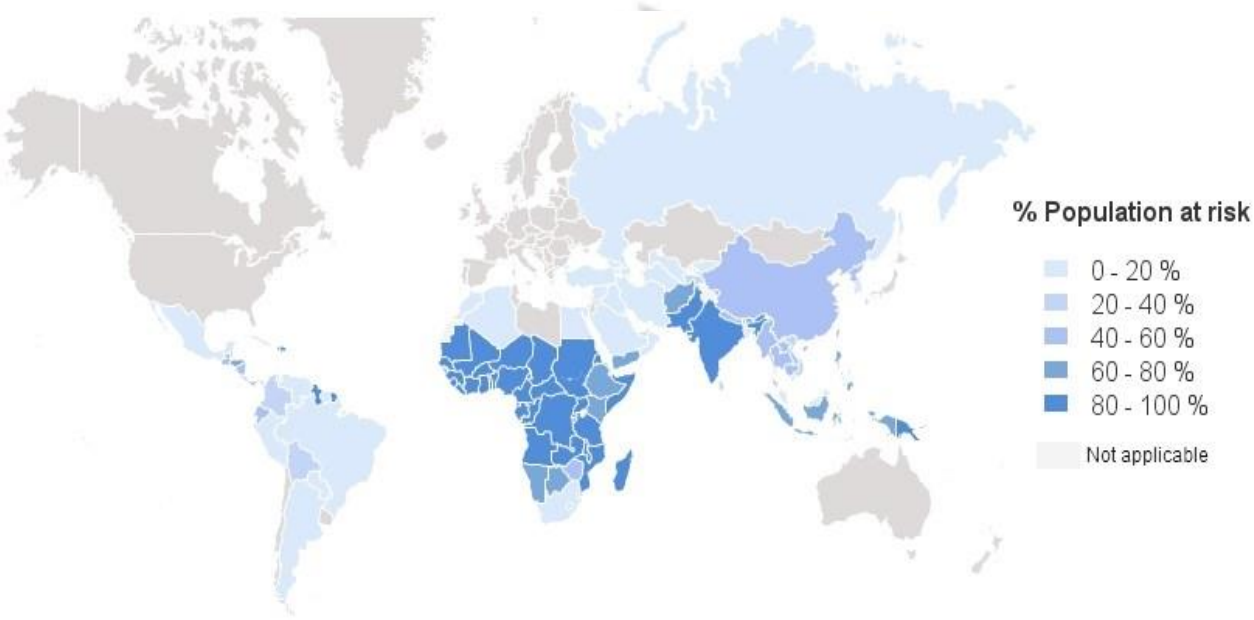


Figure 2.1.2: World malaria epidemiological profile as in 2013.

(Drawn using the 2015-Global malaria mapper at http://worldmalariareport.org/)

2.1.4 Diagnosis, prevention and treatment

Currently, the World Health Organisation has adopted an integrated approach to malarial control. The strategic approaches involve case management and prevention. Together, these strategies work against the transmission of the parasite from the mosquito vector to humans, and the development of illness and severe disease (WHO, 2012).

2.1.4.1 Diagnosis

Prompt and accurate diagnosis of malaria is part of effective disease management. The diagnosis of malaria is based on clinical assessment and on the detection of parasites in the blood (parasitological or confirmatory diagnosis). In all settings, clinical suspicion of malaria should be confirmed with a parasitological diagnosis (WHO, 2010). The two methods in routine use for parasitological diagnosis are light microscopy and rapid diagnostic tests (RDTs). The latter detect parasite-specific antigens or enzymes and some have an ability to differentiate between species (WHO, 2010).

2.1.4.2 Prevention

Preventive measures encompass malaria vector control and intermittent preventive treatment (IPT). The goals of the former are to protect individuals against infective malaria mosquito bites, and to reduce the intensity of local malaria transmission at community level by reducing the longevity, density, and human vector contact of the local vector mosquito population. The two most powerful and broadly applied vector control interventions are long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). These interventions work by reducing human-vector contact and by reducing the lifespan of female mosquitoes (so that they do not survive long enough to transmit the parasite. In a few specific settings and circumstances, the core interventions of IRS and LLINs may be complemented by other methods, such as larval source control including environmental management. On the other hand the administration of a full course of an effective antimalarial treatment at specified time targets a defined population at risk of malaria, regardless of whether the recipients are

parasitaemic or not, with the objective of reducing the malaria burden in the target population (IPT; WHO, 2012).

2.1.4.3 Treatment

The main objectives of an antimalarial treatment policy are:

i. to reduce morbidity and mortality by ensuring rapid, complete cure of *Plasmodium* infection, thus preventing the progression of uncomplicated malaria to severe and potentially fatal disease ii. to prevent chronic infection that leads to malaria related anaemia and iii. to reduce the frequency and duration of malaria infection during pregnancy and its negative impact on the foetus (WHO, 2012).

2.1.5 Drugs in malaria treatment over the years

Historically, malaria treatment has involved the use of crude plant extracts and isolated phytoconstituents to synthetic compounds. Quinine (Figure 2.1.4), an aminoquinoline alkaloid isolated from the bark of *Cinchona* species (Rubiaceae) in 1820 by Pelletier and Caventou, was the first compound used to treat malaria while methylene blue was the first synthetic drug ever used in humans against malaria in 1891. Chloroquine, a synthethic aminoquinoline, was the most effective and important antimalarial widely used throughout the world until the parasites developed resistance to it in the 1990s. Other synthetic compounds included primaquine, a standard drug for the prevention of relapses in *P. vivax* malaria, proguanil (Paludrine®) used as prophylaxis against the disease, Fansidar®, which is a combination of pyrimethamine and sulfadoxine, still in wide use, particularly in Africa, mefloquine (Lariam ®) widely used throughout the world and atovaquone, a 2-hydroxynaphthoquinones prototypes, introduced only recently (Meshnick & Dobson, 2001). Currently, the drug of choice for treatment is

artemisinin derivatives often in combination with other antimalarial drugs (ACTs) (WHO, 2010). A recent discovery, the synthetic DDD107498, is a compound with a potent and novel spectrum of antimalarial activity against multiple life-cycle stages of the *Plasmodium* parasite, with good pharmacokinetic properties and an acceptable safety profile (Baragaña et al., 2015)

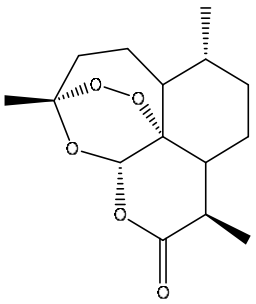
2.1.6 Drugs currently used for the treatment of malaria

Per WHO (2010) guidelines, artemisinin-based combinations therapies (ACTs) are used as the first-line treatment of malaria. These are combinations in which one of the components is artemisinin or one of its derivatives (artesunate, artemether, dihydroartemisinin and arteether; (Figure 2.1.3) with another antimalarial drug, unrelated to artemisinin, with low elimination rate, and to which the parasite had no resistance (WHO, 2010).

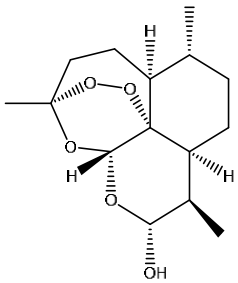
The five available ACT combinations include artemether plus lumefantrine (AL), artesunate plus amodiaquine (AS+AQ), artesunate plus mefloquine (AS+MQ), artesunate plus sulfadoxine-pyrimethamine (AS+SP) and dihydroartemsinin plus piperaquine (DHA+PPQ). These are recommended first-line antimalarial drugs for treating uncomplicated *P. falciparm* malaria. However, a combination of artesunate plus tetracycline or doxycycline or lindamycin and that of quinine plus tetracycline or doxycycline or clindamycin are reserved for very rare occasions of treatment failures (second-line of treatment) to the recommended ACTs and in some special groups, e.g. pregnant women failing ACT treatment (WHO, 2010; Figure 2.1.4).

In a recent clinical trial, a fixed-dose artesunate–pyronaridine combination showed excellent effectiveness against uncomplicated falciparum malaria in children

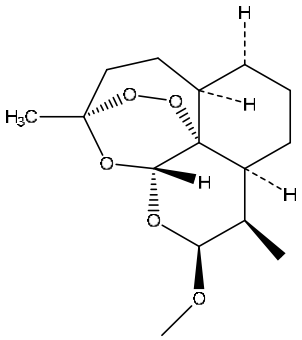
(Ramharter et al., 2008). In addition, artesunate–chlorprogunanil–dapsone – has been developed (Cui & Su, 2009), and reported to be non-inferior to the gold standard, lumefantrine- artemether, for the assessment of parasitological cure rate at Day 28 and Day 14 in children and adolescent in a phase III clinical evaluation. However, the combination possesses an intolerable safety risk in G6PD deficiency patients, as a result of the tendency of the dapsone component to cause severe haemolytic anaemia in such patients (Luzzatto et al., 2008)



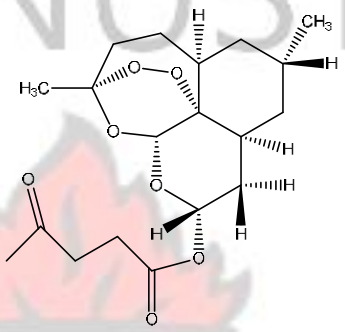
Artemisinin



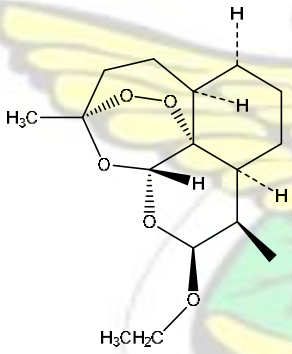
Dihydroartemisinin



Artemether



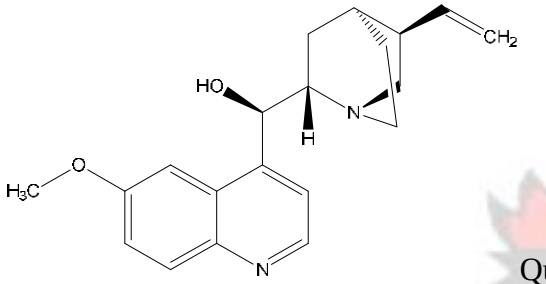
Artesunate



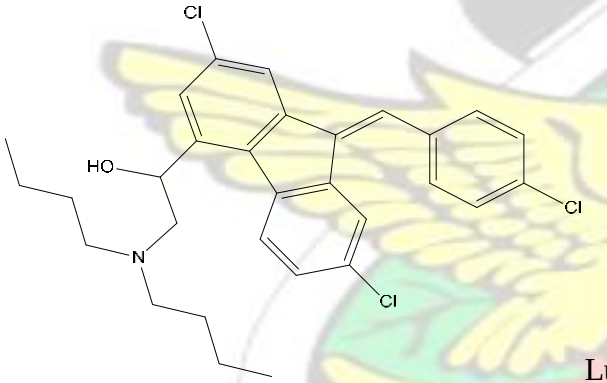
rr

Arteether

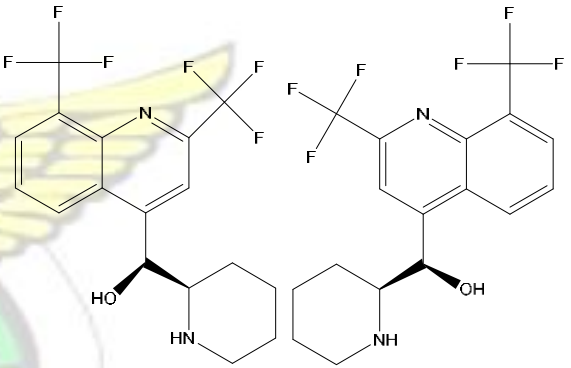
Figure 2.1.3: Artemisinin and its derivatives



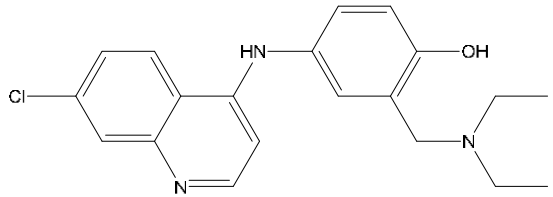
Quinine



Lumefantrine



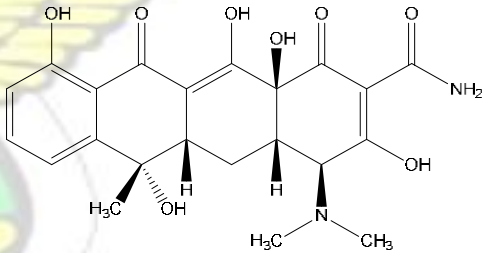
Mefloquine

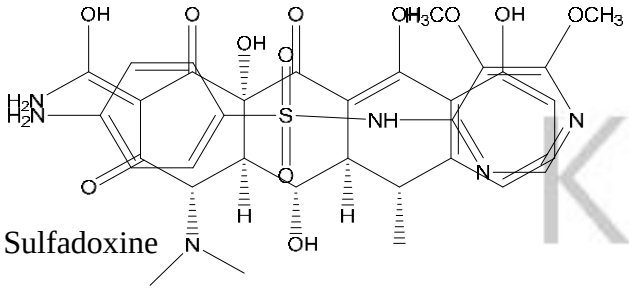


Amodiaquine

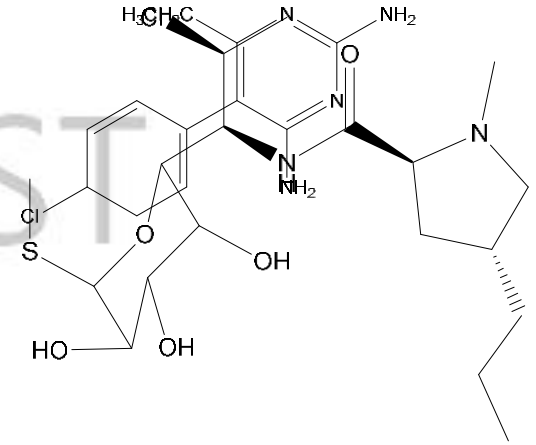
Figure 2.1.4: Antimalarial drugs other than artemisin and derivatives in ACTs in current use

Pyrimethamine

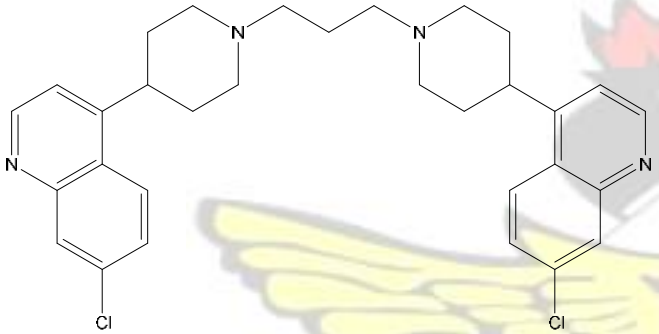




Sulfadoxine



doxycycline



Piperaquine

Tetracycline

Figure 2.1.4: Antimalarial drugs other than artemisin and derivatives in ACTs in current use continuation

Clindamycin

Figure 2.1.4: Antimalarial drugs other than artemisin and derivatives in ACTs in current use continuation

2.1.7 Malaria vaccine

According to WHO (2016), malaria vaccines are one of the most important approaches for potential prevention of malaria disease and reduction of its transmission. Research and development in this field has been an area of intense effort by many groups over the last few decades, yet there is currently no licensed malaria vaccine (WHO, 2016). RTS,S/AS01 (Mosquirix™) by GlaxoSmithKline (GSK) Pharmaceutical Company is the most advanced vaccine candidate against *Plasmodium falciparum*, which has undergone Phase III clinical trial in Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, and the United Republic of Tanzania (WHO, 2015). The vaccine offered partial protective efficacy against clinical and severe malaria after four doses in 5-17 month old children (Tinto et al., 2015).The European Medicines Agency (EMA), gave a positive opinion regarding RTS,S; indicating that the quality and the risk/benefit is favourable from a regulatory perspective. WHO's Strategic Advisory Group of Experts (SAGE) on Immunization and the Malaria Policy Advisory Committee (MPAC) recommended that pilot implementation of RTS,S/AS01 occur in moderate to high malaria transmission settings in 3-5 different sub-Saharan African countries, with ongoing malaria vector control, RDT and ACT access programmes (WHO, 2015) However, RTS,S/AS01 is yet to be licensed by any regulatory authority in sub-Saharan (WHO, 2015).

2.2 REVIEW OF ETHNOBOTANICAL SURVEYS OF MEDICINAL PLANTS USED TO TREAT MALARIA IN GHANA FROM 2005-2014.

There have been a number of ethnobotanical surveys carried out in various parts of Ghana to document medicinal plants used traditionally to treat malaria. Many of these have been published in recent years by Asase et al from 2005-2011, in various peer reviewed journals (Table 2.2.1) and in books (Abbiw, 1990; Dokosi, 1998; Mshana et al., 2001). Similar surveys have also been carried out in other parts of Africa as shown in Table 2.2.2.

Table 2.2.1. Reported ethnobotanical studies of medicinal plants used to treat malaria in Ghana

Locations of study	No. of species	No. of families	References
Wechiau Community Hippopotamus Sanctuary area southern Ghana	41	17	(Asase et al., 2005)
	29	22	(Asase & Oppong-Mensah, 2009)
Dangme West District	30	20	(Asase et al., 2010)
Bobiri Forest Reserve	42	25	(Asase & Asafo-Agyei, 2011)
Communities around Kakum National Park	33	21	(Asase et al., 2012)

Table 2.2.2. Reported ethnobotanical studies of medicinal plants used to treat malaria in other African countries

Country	Location	No. of species	No. of families	References
Guinean	-	113	46	(Traore et al., 2013)

Gabon	Ipassa - Biosphere Reserve	61	34	(Betti et al., 2013)
Burkina Faso	-	7	5	(Sanon et al., 2003)
Nigeria	Ogbomoso	40	32	(Olorunnisola et al., 2013)
Nigeria	Ogun State	38	24	(Idowu et al., 2010)
Togo	Togo Maritime Region	52	29	(Koudouvo et al., 2011)
Somalia	Shinile District	27	20	(Mesfin et al., 2012)
Nigeria	south-western Nigeria	22	18	(Dike et al., 2012)
Benin	-	9	8	(Weniger et al., 2004)
Benin	plateau of Allada	82	43	(Yetein et al., 2013)
Uganda	Nyakayojo	56	23	(Stangeland et al., 2011)
Côte d'Ivoire	-	18	15	(Ménan et al., 2006)
Côte d'Ivoire	-	5	4	(Valentin et al., 2000)
Comoro Islands	Nganzidja	44	24	(Kaou et al., 2008)
Kenya	Luo and Kuria	8		(Owuor et al., 2012)
Cameroonian	-	13		(Boyom et al., 2011)
Cameroonian	-	49	27	(Saotoing et al., 2011)

2.3 PLANTS SELECTED FOR ANTIPLASMODIAL INVESTIGATION

In order to achieve the goal of this project, an ethnobotanical study was carried out in Bosumtwi and Sekyere East Districts of the Ashanti Region to identify medicinal plants used by the local herbalists in traditional treatment of malaria. As a follow-up to the survey, five of the medicinal plants which had high percentage of citation by the herbalists for the treatment of malaria (Komlaga et al., 2015) and which were not sufficiently investigated for antiplasmodial compounds were selected for further study. The selected plants and parts included the whole of *Phyllanthus fraternus* G.L.Webster (Phyllanthaceae), the leaves of *Tectona grandis* L.f (Lamiaceae), *Bambusa vulgaris* Schrad. (Poaceae) and *Terminalia ivorensis* A. Chev (*Combretaceae*), and the root of *Senna siamea* (Lam.) Irwin & Barneby (Fabaceae)

2.3.1 *Phyllanthus fraternus* G.L. Webster

Figure 2.3.1*:* A photograph showing *Phyllanthus fraternus* plant taken at Ayeduase New Site, Kumasi, Ghana

2.3.1.1 Taxonomy and description

Phyllanthus fraternus G.L. Webster (Figure 2.3.1) belongs to the family Phyllanthaceae, (APG II, 2003; Samuel et al., 2005; Schmid & Singh, 2005). It is the second largest segregate of the Euphorbiaceae sensu lat, comprising of about 2000 species in 59 genera (Wurdack et al. 2006; Hoffmann et al., 2006). The genus *Phyllanthus* L. has a pan-tropical distribution, with more than 1000 described species (Silva, 2009) made of trees, shrubs, semi-succulents, annual herbs and even a freefloating aquatic species (Hoffmann et al., 2006; Wurdack et al., 2006). *P. fraternus* is a monoecious slender erect annual herb about 5-65 cm tall (Sen et al., 2011; Khatoon et al. 2006; Burkill 1985). The leaf is simple, numerous, somewhat imprecated, alternate, opposite, thin and almost sessile. The upper surface is green and glabrous while the lower surface is pale green and somewhat glaucous in fresh condition,, often in two rows with a whitish rachis, elliptic-oblong shaped, margin entire, apex rounded, obtuse (rarely sub-acute), base rounded, 6–13 by 3–6 mm, unicostate reticulate venations. The main lateral nerves are usually four to five pairs, petioles very short, stipules simple, minute, free-lateral, awl-shaped, lanceolate-subulate (Sen et al. 2011). The stem is herbaceous, quite smooth, erect, green, branching profuse toward the upper region, 30–60 cm in height and up to 4 mm in diameter. Stem naked below with 5–11 pairs of leaves bearing branches, pale green, angular, slender, and spreading. The internodes are small, 1–1.5 cm lon. It is often confused with both *P. amarus* and *P. niruri*. Whereas *P. fraternus* and *P. amarus* have six and five prominent tepals respectively, *P. niruri* has six very minute tepals (Sen et al. 2011; Sarin et al. 2014). The vernacular names include ‘kpavideme’ in Ewe and ‘bo mma gu w’akyi’ in Twi (Burkill, 1985; Dokosi, 1998)

2.3.1.2 Traditional medicinal uses

P. fraternus has several uses in traditional medicine. In Ghanaian traditional medicine, Ayurveda in India and in parts of the West Indies, it is used in the treatment of malaria (Burkill, 1985; Chanda et al., 2011; Khan & Khan, 2004; Sittie et al., 1998) and related symptoms such as chronic pyrexia, fever, chills, intermittent fever, painful joints, and diarrhoea (Khan & Khan, 2004). The plant is also used as spasmolytic to alleviate griping in dysentery, and other painful spasmodic affections of the intestines in Ghana (Burkill, 1985).In India, it is used to treat dysmenorrhoea (Khan & Khan, 2004). The leaf is used as a diuretic and as laxative in Ghana (Burkill, 1985). In Ayurveda, formulations containing *P. fraternus* are used to treat various conditions including: amenorrhea, anaemia, burning micturition, burning sensation in the body, chronic wounds, influenza, leucorrhoea, metrorrhagia, stomatitis, and respiratory diseases including asthma and cough with expectoration (Khan & Khan, 2004). The plant is also used to treat ulcer (Chanda et al., 2011; Burkill, 1985) and to treat gonorrhoea in Ghana and India (Burkill, 1985; Chanda et al., 2011; Khan & Khan, 2004). In Côte d'Ivoire, a leaf decoction is drunk to facilitate childbirth, and to treat oedema, costal pain, and fever. In India, Malaysia and the Philippines, it has miscellaneous usage for stomach-troubles, dropsy and urino-genital diseases; fresh roots are used to treat jaundice and are taken with milk as a galactagogue. The plant is also used in treatment of caterpillar urticarias, skin-complaints, scabies, ringworm, oedematous swellings, ophthalmia and conjunctivitis, leprosy, anuria and biliousness (Burkill, 1985; Oudhia, 2008).

2.3.1.3 Biological studies

Extracts of the plant has shown a wide range of biological activities; the aqueous extract was reported to have shown suppressive and curative antimalarial activity against *P. berghei* in infected rats (Matur et al., 2009). The alcoholic extract reduced diabetes related elevation of serum cholesterol and triacylglycerols, serum urea and creatinine, alkaline phosphate (ALP), aspartate aminotransferase (AST) and (alanine aminotranferase) ALT in alloxan-induced diabetic albino rats (Garg et al., 2010). The hydro-methanolic extract induced dose dependent diuresis in rats, resulting in increased urine and cation (sodium and potassium) output (Kalyani et al., 2010). The plant was reported to show anticoagulant effects (Koffuor & Amoateng, 2011; Upadhyay et al., 2014). Different extracts of the plant showed hepato-protective effect in experimental animals (Ahmed et al., 2002; Chandra & Sadique, 1987; Gopi & Setty,
2010; Kumari & Setty, 2012; Lata et al., 2014; Padma, 1999; Ramakrishna et al., 2012; Sailaja & Setty, 2006; Sebastian & Setty, 1999; Sharma et al., 2011; Varma & Jaybhaye, 2010). Singh (2008) reported the reduction of clinical manifestations of hepatitis B as well as reduction in levels of hepatotoxic-related enzymes in hepatitis patients, and thus concluded it was an effective hepatoprotective agent. Methanolic extract of the leaves showed antidiarrhoeal activity (Mehta et al., 2014). The plant exhibited antinociceptive activity in chronic inflammatory hyperalgesia (Chopade & Sayyad, 2013), and the aqueous and ethanolic extracts showed modest antiinflammatory activity on carrageenan-induced paw oedema in a dose dependent fashion (Oseni et al., 2013). The methanol extract showed antimicrobial activity against *P. aeruginosa* and *S. typhi B,* and the fungus, *A. niger* (Mehta et al., 2014). However, the aqueous and ethanol extracts did not show activity against the human fungi, *Trichophyton mentagrophytes* and *T. rubrum* (Bapat and Mhapsekar, 2012),

2.3.1.4 Phytochemistry

P. fraternus contains alkaloid, flavonoid, tannin, saponin, carbohydrate and resin (Matur et al., 2009; Mehta et al., 2014). It also contains phlobataninns, triterpenes, steroids and cardiac glycoside (Chanda et al., 2011). Sittie et al. (1998) isolated alkamides; E,E-2,4-octadienamide and E,Z-2,4-decadienamide, from the plant. The plant also yielded four seco-sterols: phyllanthosterol, phyllanthosecosteryl ester, phyllanthostigmasterol and fraternusterol (Gupta and Ali, 1999).

2.3.2 *Tectona grandis* L. f (Lamiaceae)

Figure 2.3.2: A photograph showing Tectona grandis plant taken on KNUST Campus, Kumasi, Ghana. (Insert: leaf)

2.3.2.1 Taxonomy and description

Tectona grandis L. (Figure 2.3.2) commonly known in English as Teak, belongs to the genus *Tectona*, tropical hardwood trees in the mint family, Lamiaceae. The plant is a large deciduous forest tree well known for its high quality timber. It occurs naturally in

only four countries: India, Myanmar, Laos and Thailand, but is often planted along roads and in large plantations throughout the tropics (Kollert & Cherubini, 2012).

2.3.2.2 Traditional medicinal uses

The leaf decoction of Teak is used to treat malaria (Asase et al., 2010) anaemia (Diallo et al., 2008; Ghaisas et al., 2009; Pooja et al, 2011) and many other diseases. Other unspecified parts are used to treat various diseases including bronchitis, hyperacidity, dysentery, verminosis, diabetes, leprosy, inflammation, skin diseases, pruritus, stomatitis, various types of ulcers, haemorrhages and haemoptysis, constipation, piles, leucoderma, headache, biliousness, anuria, urethral discharges, body swellings and menstrual disorders. It also has diuretic, anti-bacterial, anti-viral, tocolytic applications, also used as a gargle for sore throat and to facilitate childbirth (Jaybhaye et al., 2010; Khera & Bhargava, 2013; Kubo, 2014; Orwa et al., 2009; Pooja et al., 2011). The oil from the seed is applied to promote hair growth and is useful in the treatment of scabies (Orwa et al., 2009; Oudhia, 2008).

2.3.2.3 Biological studies

Formulations of the extracts possess wound healing property (Nayeem & Karvekar, 2009; Varma & Giri, 2013) and the ointment of the petroleum ether extract of the seed enhanced hair growth in rats (Jaybhaye et al., 2010). Jaybhaye et al. (2010) reported the tocolytic effect on the oxytocin induced contraction of the uterus in non-pregnant rats. The methanolic extract of the root exhibited hypoglycemic activity in diabetic rats (Pooja et al., 2011) as did the ethanol extract of the stem bark and flower which also inhibited α-amylase and α- glucosidase activities (Ramachandran & Rajasekaran, 2014; Varma & Jaybhaye, 2010). The methanol extract of the leaf and ethanol extract

of the defatted bark demonstrated antidiabetic, antihyperglycaemic and antioxidant properties in streptozotocin-induced diabetic rats. (Ghaisas et al., 2009; Ramachandran et al., 2011). Ghaisas et al. (2010) indicated that the ethanolic extract of the bark also prevented diabetic related renal damage in rats. The methanol extract of the flowers, the aqueous and ethanol extract of the stem bark showed anti-inflammatory and analgesic properties in rat models (Asif, 2011; Ramachandran et al., 2011) while the methanolic and petroleum ether extracts of the seed possessed hepatoprotective activity (Jangame & Burande, 2013; Sachan et al., 2014). The ethanol extract and butanol fraction were anti-ulcerative and displayed potent anti-secretory and cytoprotection of the gastrointestinal tract (Singh et al., 2010). The ethanol extract of the leaf demonstrated haemopoeitic effect by reversing phenylhydrazine-induced anaemic condition, through increased haemoglobin concentration, red blood cell numbers, haematocrit and reticulocytes rate, as well as enhanced osmotic resistance of the red blood cells in rats (Diallo et al., 2008). The aqueous leaf extract showed immunostimulatory properties (Egba et al., 2014). The methanol extract demonstrated *in vitro* activity against clinical isolate of uropathogenic bacteria (Mishra & Padhy, 2013).

2.3.2.4 Phytochemistry

Tectona grandis yielded terpenoids (sesquiterpenoids, diterpenes, triterpenes and abeograndinoic acid); apocarotenoids (tectoionols A and B) (Macías et al., 2008, 2010); tectograndone, (10-(2,5,8-trihydroxynaphthaquinon-3-yl)-5,12-dihydroxy2,2,8-trimethyl-2(H)-pyrano{2,3-b}anthra~n-6,11-dione) (Aguinaldo et al., 1993); 9,10-dimethoxy-2-methyl anthra-1,4-quinone, tecomaquinone I, and naptho- and anthraquinone derivatives (Singh et al., 1989); 5-hydroxylapachol, lapachol, dehydroα-

lapachone, methylquinizarin and squalene (Khan & Mlungwana, 1999); Gallic and ellagic acids (phenolic acids), rutin and quercetin (flavonoids) (Naira & Karvekar, 2010).

2.3.3 *Bambusa vulgaris* Schrad. ex J.C.Wendl. (Poaceae)

Figure 2.3.3: A photograph showing Bambusa vulgaris plant taken on KNUST Campus, Kumasi, Ghana. (Insert: leaf)

2.3.3.1 Taxonomy and description

Bambusa vulgaris Schrad. ex J.C.Wendl. (Family: Poaceae; Figure 2.3.3), is the most widely grown bamboo throughout the tropics and subtropics and the most common of all *Bambusa* species. It originated in the Old World, probably in tropical Asia (Brink,

2008) and occurs spontaneously or naturalised mostly along river banks, road sides, wastelands and open ground; generally at low altitudes (Ohrnberger, 1999). In tropical Africa, it is widely grown and also occurs sub-spontaneously (Brink, 2008). The subfamily Bambusoidaeae comprises both woody and herbaceous bamboos numbering altogether 1575 species (Barker et al., 2001). It is divided into one (1) tribe of woody bamboos (Bambuseae) and from one (1) to three (3) tribes of herbaceous bamboos (Barker et al., 2001). In total, nearly 400 species of woody bamboos are distributed in Northern, Central and South America, and in Africa (Bystriakova, Kapos, & Lysenko, 2002). *B. vulgaris* belong to the woody bamboos, and it is the only Bamboo species, which occurs in Ghana and the rest of West Africa (Bystriakova et al., 2002). At least three (3) groupings of cultivars of *B. vulgaris* can be distinguished:

(i) plants with green stems;

(ii) those with yellow stems (‘golden bamboo’) often designated as *Bambusa striata* Lodd. ex Lindl and

(iii) plants with stems up to about 3 m tall with` inflated internodes in the lower part (Buddha's belly bamboo) often referred to as *Bambusa wamin* Brandis ex E.G.Camus (Brink, 2008)

2.3.3.2 Traditional medicinal uses

In Ghana, a decoction of the leaf, solely or in combination with other plants is used in traditional medicine to treat malaria (Asase & Asafo-Agyei, 2011; Komlaga et al., 2015). In Nigeria, the leaf decoction is taken to treat venereal diseases (Brink, 2008), as an abortifacient (Yakubu & Bukoye, 2009) and as a typhoid remedy (Ajaiyeoba et al., 2003). The leaf is used to treat measles in Mauritius (Nunkoo & Mahomoodally, 2012) and in Nigeria (Sonibare et al., 2009). In DR Congo and southwest Nigeria, the plant is used to treat measles (Phalan, 2009; Sonibare et al., 2009) fever, haematuria, kidney troubles, as sudorific and febrifuge agents, as astringent and emmenagogue;

culm and internodes used to treat infantile epilepsy, (Phalan, 2009). The plant is used for stomach problems, pains and against internal parasites, cuts, injuries and swellings in Trinidad and Tobago (Lans, 2007) . It has been used to treat coughs and excess mucous and helps alleviate fever (Annonymous, 2014). The plant has been used extensively as an ingredient in skin care products and as a tooth polisher (Carey et al., 2009). The root is used in Fiji for kidney problems , fever and flu (Singh, 1986) and in Guinea to manage diabetes mellitus (Diallo et al., 2012). The root and stem are used in the treatment of hepatitis B and hookworm respectively in Indonesia (Roosita et al., 2008).

2.3.3.3 Biological studies

Various extracts of the leaf have been reported to have various biological activities. Aqueous extract of the leaf was active against the Ghanaian strain of *Plasmodium falciparum* (Valdés et al., 2010) but inactive against F32/Tanzanian strains of *Plasmodium falciparum* (Rodríguez-Pérez et al., 2006). The leaf extract was also active against *Lactobacillus spp* of bacteria (Owokotomo & Owoeye, 2011). Carey et al. (2009) reported the anti-inflammatory activity of the methanol extract of the leaf in rats and mice. The petroleum ether extract of the leaves showed antihyperglycaemic effect in streptozotocin-induced diabetic rats (Senthilkumar et al., 2011). Extracts of the plant lowered the fasting blood glucose level and improved glucose tolerance in Sprague-Dawley rats (Fernando et al., 1990). Aqueous extract of the leaf demonstrated abortifacient potential in pregnant Dutch rabbits, caused changes in the implantation site, hormone levels and partly demonstrated estrogenicity in the rabbit (Yakubu & Bukoye, 2009). It also exhibited hypotensive activity in rabbits (N'guessan et al., 2009). The extract induced changes in biomarkers of liver and kidney damage without showing histological changes in the corresponding organs (Yakubu et al.,

2009). Horses experienced clinical and pathological neurological disorders following large intake of fresh leaves (Barbosa et al., 2006).

2.3.3.4 Phytochemistry

Phytochemical studies showed the presence of phytosterols, tannins, saponins, coumarins and cyanogenic glycosides, alkaloids, flavonoids, anthraquinones, steroids, lactones, triterpenoids, phenols, quinones, anthocyanidins, and amino acids (Coffie et al., 2014; Goyal et al., 2010; Senthilkumar et al., 2011; Valdés et al., 2010; Yakubu & Bukoye, 2009). Xylooligosaccharides, a probiotic, was found in the plant (Annonymous, 2014).

2.3.4 *Terminalia ivorensis* A. Chev.

Figure 2.3.5: A photograph showing the trunk of *Terminalia ivorensis* taken at FORIG Fomesua, Ghana. (Insert: leaf)

2.3.4.1 Taxonomy and description

Terminalia ivorensis A. Chev (Figure 2.3.5) is of the family Combretaceae. The genus Terminalia is pantropical with about 200 species. In tropical mainland Africa, about 30 species occur and in Madagascar there are about 35 species (Foli, 2009). *T. ivorensis*

occurs from Guinea-Bissau to Cameroon. It has been grown in many tropical countries as a timber plantation (Foli, 2009).

2.3.4.2 Traditional medicinal uses

Different parts of the plant are used in traditional medicine to treat malaria (Foli, 2009). The plant is used to treat yellow fever, and as an anodyne in cases of rheumatism and muscular pain (Foli, 2009), also in arthritic conditions, piles and as a diuretic (OliverBever, 1986). Stem bark decoctions or the powdered bark is used to treat wounds, ulcers haemorrhoids and infections (Cobbinah, 2008; Foli, 2009; Oliver-Bever, 1986) also for stomach ulcer and burns (Agyare et al., 2009). The leaf extract is employed for colds, and the bark decoctions used as an enema to treat gonorrhoea and kidney complaints, and as an aphrodisiac (Foli, 2009). The stem bark is also used to treat infectious diseases in Guinean traditional medicine (Magassouba et al., 2010). The plant is useful in the management of age-related diseases (like cancer, trauma, stroke, asthma, hyperoxia, retinal damage, liver injury, periodontis and infections (Cobbinah, 2008).

2.3.4.3 Biological studies

Annan et al., (2012) reported the antiplasmodial activity of the ethanol extract of the stem bark. The stem bark extracts also showed activities against *E. coli, S. pneumoniae, S. aureus, and P. aeruginosa* and displayed antioxidant activity (Cobbinah, 2008). The aqueous and hydro-ethanol extracts of the bark inhibited the growth of methicillin/oxacillin-resistant strains of *Staphylococcus aureus, S. epidermidis,* coagulase-negative *S.* and reference strain of *S. aureus* ATCC 25923

(Coulibaly et al., 2014), and also demonstrated activity against clinical isolates of

Candida albicans and *Aspergillus fumigatus* (Ouattara et al., 2013). The stem and root bark showed anti-trypanocidal properties (Adewunmi et al., 2001; Oliver-Bever, 1986) and the stem bark extract caused a reduction in carrageenan-induced oedema of the rat paw, stopped diarrhoea and ameliorated all signs associated with adjuvant-induced polyarthritis in rats (Iwu & Anyanwu, 1982).

2.3.4.4 Phytochemistry

Phytochemical screening of the plant disclosed the presence of triterpenoids, cardiac and anthraquinones glycosides, tannins and flavonoids, alkaloids (Cobbinah, 2008; Johnny et al., 2014). Terminolic acid, ellagic acid, sericic acid, quercetin, Bglycyrrhetinic acid, 2-8 hydroxy 18α-glycyrrhetinic acid and β-sitosterol were isolated from the plant (King et al., 1955). Isoflavanone, 5,7,8-Trihydroxy-2′5′-methoxy-3′,4′methylenedioxyisoflavanone, was isolated from the stem bark extract (Ogundare & Olajuyigbe, 2012) and ivorenosides A, B and C and a sericoside, were isolated from the stem bark (Ponou et al., 2010).

2.3.5 *Senna siamea* (Lam.) Irwin & Barneby (Leguminosae)

Figure 2.3.4: A photograph showing *Senna siamea* plant taken at Ayeduase New Site, Kumasi, Ghana. (Insert: root)

2.3.5.1 Taxonomy and description

Senna siamea (Lam.) Irwin & Barneby (Figure 2.3.4), synonyms: *Cassia arayatensis* sensu Naves. Non Litv.; *Cassia arborea* Macfad.; *Cassia florida* Vahl, *Cassia gigantean* DC (Lim, 2014) belong to the family Fabaceae. It is a medium-sized, evergreen tree growing up to 18m tall, with a straight trunk of about 30 cm in diameter (Lim, 2014; Orwa et al., 2009). It is a very widespread medicinal and food plant cultivated in Southeast Asia and sub-Saharan Africa (Nsonde-Ntandou et al., 2010) mostly to improve quality of agricultural lands. It is native to South and Southeast Asia from India through to Malaysia and has been introduced to other humid tropical countries (Lim, 2014) such as Ghana.

2.3.5.2 Traditional medicinal uses

In West Africa, diferent parts of the plant are used to treat malaria (Kamagaté et al., 2014; Komlaga et al., 2015), insomnia, hypertension, liver disorders, constipation, microbial infections, for treating cough and stomach pains (Kamagaté et al., 2014). In La Côte d'Ivoire, the root bark decoction is taken to treat angina. In India and Laos, the leaf is used to treat anaemia and fever, and for its sedative and euphorising effects. It is used as a purgative and sedative in Thailand (Kamagaté et al., 2014). The plant is used in the treatment of intestinal worms and for preventing convulsion in children among other conditions (Kapoor et al., 1996; Orwa et al., 2009). The decoction is used against scabies (Kamagaté et al., 2014; Orwa, et al., 2009). It was also cited for the treatment of typhoid fever, jaundice, abdominal pain, menstrual pain (Bukar et al., 2009), and diabetes mellitus (Bukar et al., 2009; Nsonde-Ntandou et al., 2010). A paste

of the bark is used as dressing for ringworm and chilblains while the root is used as antipyretic and the leaves to treat hypertension, insomnia and asthma (Kamagaté et al., 2014; Nsonde-Ntandou et al., 2010). Kamagaté et al., (2014) also reported the use of the leaf in the treatment of toothache, hypertention, and constipation; the root for diabetes mellitus and sore throat and in Kenya, as antidote for snake bite. Other uses include treatment of urogenital diseases, herpes, rhinitis and scorpion sting; anxiety, rheumatism, swellings and dysuria (Kamagaté et al., 2014). Young tender pods and inflorescences are edible (Lim, 2014). The pods and seeds are good as cattle feed (Kapoor et al., 1996).

2.3.5.3 Biological studies

In a review, *C. siamea* was reported to exhibit several pharmacological activities including antimalarial, antidiabetic, antitumoral or anticancer, hypotensive, diuretic, laxative, anti-inflammatory, analgesic, antipyretic, anxiolytic, antidepressant, sedative, and antimicrobial activities (Kamagaté et al., 2014). The ethanol and chloroform extracts, and alkaloid fraction exhibited antimalarial activity against *Plasmodium falciparum* (Ekasari et al., 2009). The leaf extract is reported to possess analgesic, anti-inflammatory and bacteriostatic properties (Momin et al., 2012; Nsonde-Ntandou et al., 2010). The methanol extract of the leaf and stem bark demonstrated antidiabetic activity by lowering fasting blood sugar and reversed metabolic changes associated with diabetes in alloxan-induced diabetic rats (Mohammed & Atiku, 2012). The flower exhibited antioxidant activity (Kaisoon et al., 2011) and the methanol extract, xanthine oxidase inhibitory activity (Argulla & Chichioco-Hernandez, 2014). The methanol extract of the leaf was weakly antiproliferative against some cancer and normal cells (Tagne et al., 2014). The ethanolic extract of leaf exhibited moderate cytotoxic effect in the brine shrimp test (Abdul et al., 2012) .

2.3.5.4 Phytochemistry

Homogenous group of alkaloids were isolated from different parts of the *S. siamea.* The alkaloids: cassiarins A, B, G, H, J, and K were isolated from the leaf (Deguchi et al., 2012; Morita et al., 2007) and C, D, E from the flowers (Oshimi et al., 2009). Besides, a chromone, 10, 11-dihydroanhydrobarakol was isolated from the flowers (Oshimi et al., 2009). The anthraquinones, chrysophanol and physcion and the triterpenoids: lubenone, lupeol and betulinic acid; β-sitosterol glucoside were isolated from the stem bark (Ogbole et al., 2014). Siameaquinones A and B (Ye et al., 2014) and siamchromones A-G (Hu et al., 2012), were isolated from the stem. The leaf yielded luteolin, 5-acetonyl-7-hydroxy-2-methylchromone, 5-acetonyl-7-hydroxy-2hydroxymethyl-chromone, 4-(trans)-acetyl-3,6,8-trihydroxy-3-methyl dihydronaph thalenone, and 4-(cis)-acetyl-3,6,8-trihydroxy-3-methyldihydronaphthal-enone (Ingkaninan et al., 2000).

CHAPTER 3

3 MATERIALS AND GENERAL METHODS

3.1 PLANT MATERIALS

3.1.1 Selection, collection, and preparation of plant materials

Plant materials including the whole of *Phyllanthus fraternus*, the leaves of *Tectona grandis*, *Terminalia ivorensis* and *Bambusa vulgaris* and the root of Senna *siamea* were selected from an earlier survey conducted by Komlaga *et al.* (2015). The selected plants parts were harvested in July 2012 in Kumasi, Ghana and were authenticated by Dr G. H. Sam of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Sciences and Technology (KNUST), Kumasi, Ghana. Herbarium specimens with voucher numbers were kept in the herbarium of the Department. The harvested plant materials were washed under running tap water to remove earth materials and other unwanted materials, chopped into smaller bit and air dried under shade and ambient temperature (28-35^{o}C) between 7 days for leaves and 21 days for whole plants and roots. Each dried plant material was coarsely powdered using an electric mill and then extracted with the appropriate solvent.

3.2 MATERIALS FOR ANTIPLASMODIAL STUDY

3.2.1 Chemicals and reagents

3.2.1.1 Components of HUVECs culture medium

DMEM-F12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12), streptomycin, fetal bovine serum (FBS), Trypsin, EDTA were purchased from GIBCO/Invitrogen life Technologies, Paris, France.

3.2.1.2 Components of plasmodium culture medium

RPMI (Roswell Park Memorial Institute) 1640, hypoxanthine, HEPES (N-2hydroxyethylpiperazine-N'-2-ethane-sulphonic acid), albumax II (obtained from GIBCO/Invitrogen life Technologies, Paris, France), NaOH, and Glucose (Sigma-Aldrich, Paris, France).

3.2.2 Fresh erythrocytes

RBC stock suspensions were prepared from whole blood bags of fresh non-infected O positive erythrocytes from incomplete blood donation kindly provided by the Bichat Hospital (Paris, France). Whole blood was aliquoted and stored at 4˚C. To prepare red blood cells, whole blood was centrifuged and washed 3 times with serum-free RPMI by centrifugation (7 minutes at 2800 rpm at 20 ºC). The upper phase, containing white blood cells, was removed and washed red blood cells were kept as a 50% suspension in complete medium, and stored for a maximum of 4 days at 4˚C. The human biological samples were sourced ethically, and were used for research in compliance with the terms of the informed consents.

3.2.3 Equipment used during biological evaluation

Olympus light microscope, 5810R Eppendorf Centrifuge, Eppendorf realplex mastercycler epgradient S, UVmini-1240 UV-VIS Spectrophotometer: Shimadzu

3.3 GENERAL METHODS FOR ANTIPLASMODIAL STUDY

3.3.1 *Plasmodium* culture maintenance

Parasite cultures consisting of chloroquine-sensitive 3D7 *P. falciparum* strain and chloroquine-resistant W2 *P. falciparum* strain were maintained according to the modified method described by Trager & Jensen, (1976). The parasites were separately cultivated at 37^{o}C in a candle jar on complete culture medium of RPMI 1640, containing 2.5% hematocrit, hypoxanthine, HEPES, glucose, albumax II, and buffered with $NaHCO_3$ (Appendix 1a). Fresh culture was maintained for at least 96 hours (2 complete life cycles) before being used for assays. Cultures were synchronized with 5% D-sorbitol, and at least 90% ring forms were obtained before assays were run. Prior to the initiation of assays, the level of parasitemia of an aliquot of a stock culture was measured by light microscopy following Giemsa staining and the parasitemia determined (Appendix *2f*).

3.3.2 Determination of antiplasmodial activity

Antiplasmodial activity of test samples (extracts, fractions or purified compounds) was evaluated against 3D7 *P. falciparum* and W2 *P. falciparum*, using the fluorescencebased SYBR® Green I assay measurement in 96-well microplates as described by (Smilkstein et al. 2004) with some modifications. Negative control wells

for each assay contained no inhibitor while positive controls contained chloroquine (CQ).

Experiments were run in duplicate with both parasites.

Stock samples solutions were prepared in dimethyl sulfoxide (DMSO) and diluted with culture medium to give a maximum DMSO concentration of 0.5% in a final well volume of 200 µL culture, containing 1 % parasitemia and 2.5 % haematocrit. Samples and positive control [chloroquine (CQ)] in the wells were prepared by two-fold dilution, in a dose-titration range of 0.098 to 100 µg/mL for extracts and fractions, and 0.098 to 100 µM for purified compounds, to obtain eleven (11) concentrations each in triplicates. After 42 h incubation in a candle jar at 37^{o}C, the plates were frozen at 80^{o}C overnight. The frozen plates were then taken through a three (3) freeze-thaw cycles of 1 h thawing at 20 ^{o}C, 5 min homogenization and 1 h freezing at -80^{o}C. Five microlitres (5 µL) of homogenate was then added to 45 µL of SYBR® Green I in lysis buffer, in a PCR plate. The PCR plate was incubated at room temperature for an hour and fluorescence measured using a multi-well plate reader (Eppendorf realplex mastercycler epgradient S) with excitation and emission wavelength bands centered at 485 and 530 nm, respectively. The IC_{50} (drug concentration that inhibits the growth of 50% P. falciparum cells) was estimated using antimalarial estimator, ICEstimator at http://www.antimalarial-icestimator.net/MethodIntro.htm (Kaddouri et al., 2006; Nagard et al., 2011).

3.3.3 Determination of cytotoxicity against HUVECs

Human umbilical vein endothelial cells (HUVECs) were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM-F12) medium in the presence

of Foetal bovine serum (FBS) (10%) plus streptomycin (1%) and incubated in 5% CO_2 at 37 °C. The cytotoxicity of extracts/compounds was evaluated using MTT

(3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) colorimetric method (Mosmann, 1983). HUVECs were seeded in a 96 well plate at 15000 cells/ well and incubated for 24 h when cells reached >80% confluence. After discarding the old medium, the cells were incubated in the medium containing test samples at eight concentrations range of 0.78 to 100 µg/mL for extracts and fractions, and 0.78 to 100 µM for purified compounds. After 24 h incubation, 20 µL MTT (5 mg/mL) was added to each well and cells were incubated for another 3 h. Finally, the culture medium containing MTT solution was removed and the formazan crystals were dissolved in 100 µL of dimethylsulfoxide (DMSO). Absorbance was read with an Eppendorf plate reader at 546 nm. $CC_{50,}$ defined as drug concentration that reduced the number of viable cells by 50%, was calculated using GraphPad Prism Software (Version 5.0, San Diego, CA, USA).

3.4 GENERAL MATERIALS AND METHODS FOR THE PHYTOCHEMISTRY STUDY

3.4.1 Solvents and reagents

Solvents employed in the phytochemical experiment included methanol, acetonitrile, acetone, n-butanol, ethanol, dichloromethane, ethyl acetate, hexane, petroleum ether, chloroform and water. Solvents used for extractions and initial fractionations were of 99 %v/v purity and HPLC grade solvents (100%v/v) for HPLC analysis and separations involving preparative HPLC and for all other mass spectroscopic analysis. The water used during mass spectroscopy experiments and HPLC

separations, MilliQ water, was re-filtered on the day of manipulation in the laboratory with the Millipore Simplicity 185 device.

Reagents included Dragendorff's reagent (1.7 g bismuth nitrate and 20 mL of acetic acid in 80 ml water + 72 g of potassium iodide in 180 mL water) for alkaloids and vaniline (2 g vaniline (powder) + 5 ml conc H_2SO_4 + 100 ml ethanol) for other phytoconstituents.

3.4.2 Column chromatography (cc)

Column chromatographic fractionation of extracts were performed using columns (cc) with solvent reservoirs and sizes 46 x 8 cm, 44 x 5 cm or 42 x 2.5 cm with silica gel (silica gel 60, particle size 40-63 µm) or LH-20 Sephadex or neutral activated alumina as the adsorbent. Eluents were driven through columns by compressed air (3 bar).

3.4.3 Thin layer chromatography (TLC)

Eluates were monitored with thin layer chromatography (TLC); on silica gel precoated aluminium plates support (Merck silica gel 60 F254). Plates were visualized first under UV (254 amd 366 nm) and then spots revealed by spraying with

Dragendorff's reagent (1.7 g bismuth nitrate and 20 mL of acetic acid in 80 ml water + 72 g of potassium iodide in 180 mL water) for alkaloids or vaniline (2g vaniline + 5ml conc H_2SO_4 + 100ml ethanol).

3.4.4 Analytical high-performance liquid chromatography (HPLC)

Analytical HPLC was performed using the autopurification Alliance 2695 HPLC System (WATERS) with a chain modular pump (WATERS), a UV PDA 2996 detector (WATERS) or evaporative light scattering detector (ELSD, WATERS). Columns employed included SUNFIRE reverse phase column (C18, 3.5 µm, 2.1 x 150 mm; 5 µm, 4.6 x150 mm) and XBridge reverse phase column (C18, 3.5 µm, 2.1 x 150 mm; 5 µm, 4.6 x150 mm).

3.4.5 Preparative High-performance liquid chromatography (Prep-HPLC)

Preparative HPLC was performed on a Deltaprep (WATERS) instrument equipped with a binary pump (Waters 2525), a UV-visible diode array detector (190-600 nm, Waters 2996). Sunfire reverse phase columns (C18, 5 µm, 19 x 150 mm; Waters) and XBridge reverse phase columns (C18, 5 µm, 30 × 250 mm; Waters) were used as columns.

3.4.6 Liquid chromatography–mass spectrometry (LC-MS)

LC-MS was performed on LCT Premier time-of-flight (TOF) mass spectrometer-Waters Alliance 2695 system fitted with a diode array detector (190-600 nm, Waters 2996) and controlled by the Empower software. Columns included SUNFIRE reverse phase column (C18, 3.5 µm, 2.1 x 150 mm; 5 µm, 4.6 x150 mm) and XBridge reverse phase column (C18, 3.5 µm, 2.1 x 150 mm; 5 µm, 4.6 x150 mm).

3.4.7 Optical rotation

Optical rotations of compounds were recorded on an Optical Activity PolAAr 32 Polarimeter using a sample concentration of 10 mg/ml, unless otherwise specified.

Monochromatic sodium D line was used as the light source. The experiments were carried out in a 10 cm tube, and the compounds dissolved in methanol or dichloromethane.

3.4.8 Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance experiments were performed on Bruker Avance 400 MHz device using chloroform-*d*, DMSO-*d6* or methanol-*d4* with TMS as internal standard. Chemical shifts were measured in δ ppm and coupling constants *J* were measured in Hertz (Hz). The multiplicity of signals were given as s (singlet), d (doublet), t (triplet), m (multiplet), q (quartet), qt (quintet) or br (broad). ^{1}H and ^{13}C signals were assigned from ^{1}H and ^{13}C NMR spectra at carrier frequencies of 400 Hz and 100 Hz respectively. 2D spectra including short range correlations COSY, HSQC, HMBC and long range NOESYcorrelations spectra were obtained at 400 MHz on a Bruker Avance-400 NMR spectrometer. NMR data was analysed using NMR NoteBook® software.

3.4.9 Mass spectroscopy

High-resolution electrospray ionisation mass spectrometry (HR-ESI-MS) of compounds were determined on an LCT Premier TOF (WATERS) coupling with HPLC Alliance 2695 (Waters), and with micrOTOFq Brüker.

3.4.10 Infrared

IR absorption spectra were measured on a Brüker Vector 22. The absorption bands were given in cm^{-1}.

CHAPTER 4

4 IDENTIFICATION, SELECTION AND PRELIMINARY ANTIPLASMODIAL STUDY OF SELECTED PLANTS EXTRACTS

4.1 ETHNOBOTANICAL SURVEY OF MEDICINAL PLANTS AND FINISHED MARKETED HERBAL PRODUCTS USED FOR TO TREAT MALARIA

See Appendix 15 for published article

4.2 ANTIMALARIAL ACTIVITY OF EXTRACTS AND FRACTIONS OF SELECTED MEDICINAL PLANTS

4.2.1 Collection and preparation of plant materials

Materials of *Phyllanthus fraternus* G.L.Webster, *Tectona grandis* L.f, *Terminalia ivorensis* A. Chev, Bambusa *vulgaris* Schrad. and *Senna siamea* (Lam.) Irwin & Barneby were obtained as described in 3.1.1

4.2.2 Extraction of plant materials

4.2.2.1 Aqueous extract

For each plant material, 100 g powder was boiled in 2 L of water in accordance with traditional preparations for 30 min. The decoction was then strained through doublelayered white cotton material and subsequently through Whatmann filter paper Grade 1. The filtrate was then lyophilized and the dry material kept at 4^{o} C until needed for analysis.

4.2.2.2 Organic fractions

One kilogram (1 kg) of each powdered materials was exhaustively extracted sequentially with petroleum ether, ethyl acetate, and finally methanol using the Soxhlet

extractor to obtain the various organic fractions. The fractions were concentrated at 40 ^{o}C under reduced pressure using a rotary evaporator to a semisolid mass. The semisolid extracts were further dried under high vacuum and subsequently kept at 4 ^{o}C until needed.

4.2.3 Biological studies

4.2.3.1 Determination of antiplasmodial activity

Extracts and fractions were evaluated at a dose-titration range of 0.098 - 100 µg/ml against 3D7 and W2 *P. falciparum* as described under section 3.3.2.

4.2.3.2 Determination of cytotoxicity

Extracts and fractions were assayed at a dose-titration range of 0.78- 100 µg/mL against HUVECs as described under section 3.3.3

4.2.3.3 Assessment of chemical injury to erythrocytes

During the antiplasmodial activity study of the organic fractions, some wells turned red; possibly due to lysis of the erythrocytes. We therefore tested the organic (petroleum ether, ethyl acetate and methanol) fractions to assess if they caused haemolysis of and/or morphological changes of the erythrocytes. The method as described by Ilić et al. (2013) with some modifications was adopted. The haemolytic activity of the fractions was monitored by the measurement of released haemoglobin at 412 nm using type O positive human red blood. Erythrocytes (2.5 %) suspended in RPMI 1640 supplemented with hypoxanthine, HEPES, glucose, albumax II, and buffered with $NaHCO_3$ were incubated with fractions at concentrations from 3.125 to 200 µg/mL, in

triplicate at 37 °C for 42 h in 200 µL volume 96-well micro-plates in a candle jar. Hundred percent (100 %) haemolysis was determined by adding 100 µL distilled deionised water to 100 µL of 5 % hematocrit. Negative controls were determined by incubating erythrocytes with culture medium without test solution. At the end of incubation, 60 µl of the supernatant of each well was taken and its absorbance measured by direct spectrophotometric method at a wavelength (λ) of 412 nm using UV spectrophotometer (UVmini-1240 UV-VIS Spectrophotometer: Shimadzu). The culture medium was used as the reference. The percentage haemolysis of the various concentrations of the fractions were determined and the values plotted against concentration using GraphPad Prism Software (Version 5.0, San Diego, CA, USA) and HC50 was taken as the mean concentration of a fraction causing 50% haemolysis. Selectivity index (SI) of each fraction was then calculated as the ratio of HC_{50} to IC_{50}.

Erythrocytes treated with 200 µg/mL of extracts were examined under high-power light microscopy for any morphological modification. The morphological findings were compared with those of negative control.

4.2.4 Results

4.2.4.1 *In vitro* antiplasmodial activity and cytotoxicity of aqueous extracts

All five aqueous extracts showed activity against the chloroquine-sensitive 3D7 *P. falciparum* ($0.64\pm0.14 \leq IC_{50} \leq 28.76\pm2.65$ µg/mL) but only the aqueous extracts of *T. ivorensis* and *S. siamea* were active against the chloroquine-resistant W2 *P. falciparum* (IC_{50} <50 µg/mL). Only two extracts, *P. fraternus* and *T. ivorensis* extracts, exhibited cytotoxicity with CC_{50} < 100 µg/mL. Extracts have comparatively higher selectivity indexes (SIs) for 3D7 *P. falciparum* strain but lower SIs for W2 *P. falciparum* except *S. siamea* (SI >2.1) .The resistance indexes (RIs) of *B. vulgaris*, *P. fraternus* and *T. grandis* varied from moderate to very high; that of *T. ivorensis* was moderate and *S. siamea* had low RI (Table 4.3.1).

Table 4.3.1: *In vitro* antiplasmodial and cytotoxic activities of the aqueous extracts

Extracts	IC_{50}, 3D7	IC_{50}, W2	CC_{50}, HUVECs	SI, 3D7	SI, W2	RI
B. vulgaris	7.50±1.08	>100	> 100	>13.3	1.0	>13.3
P. fraternus	4.07±1.46	>100	31.11±3.31	7.6	<0.3	>24.5
T. grandis	7.18±1.22	>100	> 100	>13.9	1.0	>13.9
T. ivorensis	0.64±0.14	10.52±3.55	6.25±0.40	9.8	0.6	16.4
S. siamea	28.76±2.65	48.32±3.50	>100	>3.5	>2.1	1.7
CQ	0.02± 0.0024	0.13±0.023	ND	ND	ND	6.0

Keys: 3D7: 3D7 *P. falciparum*; W2: W2 *P. falciparum*; CQ: chloroquine; ND: not determined; NA: not applicable; SI: Selectivity Index, defined as the ratio of CC_{50} to IC_{50}; RI: Resistance Index, defined as the ratio of the IC_{50} of the resistant line (W2 *P. falciparum*) to that of the parent strain (3D7 *P. falciparum*); Concentration of CQ is expressed in µM; IC_{50} and CC_{50} of extracts are expressed in µg/mL ± SD

4.2.4.2 *In vitro* antiplasmodial activity of organic fractions

The fourteen fractions (14), obtained by serially exhaustive extraction with organic solvents of increasing polarity (petroleum ether, ethyl acetate and methanol), from the 5 medicinal plants materials (*P. fraternus* - whole plant; *T. grandis*- leaves; *T. ivorensis*-leaves*; S. siamea*- root and *B. vulgaris*- leaves), displayed significant antiplasmodial activity with IC_{50} value generally under 50 µg/mL against 3D7 *P. falciparum*. Four fractions comprising the methanol fractions of *P. fraternus* and *T. ivorensis*, the petroleum and ethyl acetate fractions of *B. vulgaris* exhibited the strongest antiplasmodial activity (IC_{50}<1 µg/mL). The fractions displayed varying levels of haemolysis with HC_{50} from 89.2 ±1.09 to above 200 µg/mL. Only ethyl acetate fraction of *T. ivorensis* has HC_{50} below 100 µg/mL. Besides, the petroleum ether and ethyl acetate fractions of *T. grandis*, and the ethyl acetate and methanol fractions of *P. fraternus*, showed HC_{50} between 100 and 200 µg/mL. The SI of the fractions ranged from 4 to 410 (Table 4.3.2).

Table 4.3.2. *In vitro* antiplasmodial activity and haemolytic effect of fractions

Plant	Extract type	IC_{50}(µg/mL±SD)3D7	HC_{50} (µg/mL±SD)	SI (HC_{50}// IC_{50})
Bambusa vulgaris	Petroleum ether	0.75±0.21	>200	>266.7
	Ethyl acetate	0.49±0.06	>200	>408.2
Phyllanthus fraternus	Petroleum ether	37.92±14.50	>200	>5.3
	Ethyl acetate	24.31±6.83	109.0±1.12	4.5
	Methanol	0.44±0.08	180.3±1.21	409.8
Tectona grandi	Petroleum ether	4.48±1.42	184.9±1.23	41.3
	Ethyl acetate	14.15±3.19	166.7±1.11	11.8
	Methanol	0.92±0.25	>200	>217.4
Terminalia ivorensis	Petroleum ether	14.79±2.42	>200	>13.5
	Ethyl acetate	16.01±4.35	89.2±1.09	5.6
	Methanol	5.70±1.23	>200	>35.1
Senna siamea	Petroleum ether	39.07±20.21	>200	>5.1
	Ethyl acetate	48.50±25.45	>200	>4.1
	Methanol	22.89±27.01	>200	>8.7
Chloroquine		0.021 ± 0.0024 µM		
NA				
ND				
ND				

Key: SI: Selectivity Index; ND: not determined; NA: not applicable. ND: not determined.

4.2.5 EFFECTS OF FRACTIONS ON ERYTHROCYTE INTEGRITY

Based on the external morphological features, three types of erythrocytic forms were observed under the microscope. These were categorized as ‘normal’ cells, ‘deformed’ cells and ‘ghost’ cells. ‘Normal’ cells were round to slightly ovoid and biconcave in shape with smooth surfaces. ‘Deformed’ erythrocytes included cells with opaque patches on the surfaces and those with spiky projection on their surfaces, also termed ‘virus-shaped’ cells; both were irregular in shape. ‘Ghost cells’ looked waned, faint and almost invisible.

Except for the ‘ghost cells’, all the other types also occurred in the negative control. The number of each type of cell was estimated and the percentage composition was determined. Percentage of deformed erythrocytes in extract-treated samples ranged between 2.01-16.14 %. That of the negative control was 6.14 %, and this value fell within the range for the extract-treated samples. In terms of deformation, there was therefore not much difference between the treated erythrocytes and the negative control (those not treated with fractions). On the other hand, erythrocytes treated with petroleum ether and ethyl acetate fractions of *T. grandis*, ethyl acetate and methanol fractions of *T. ivorensis*, petroleum ether, ethyl acetate and methanol fractions of *S. siamea* showed ghost cells.

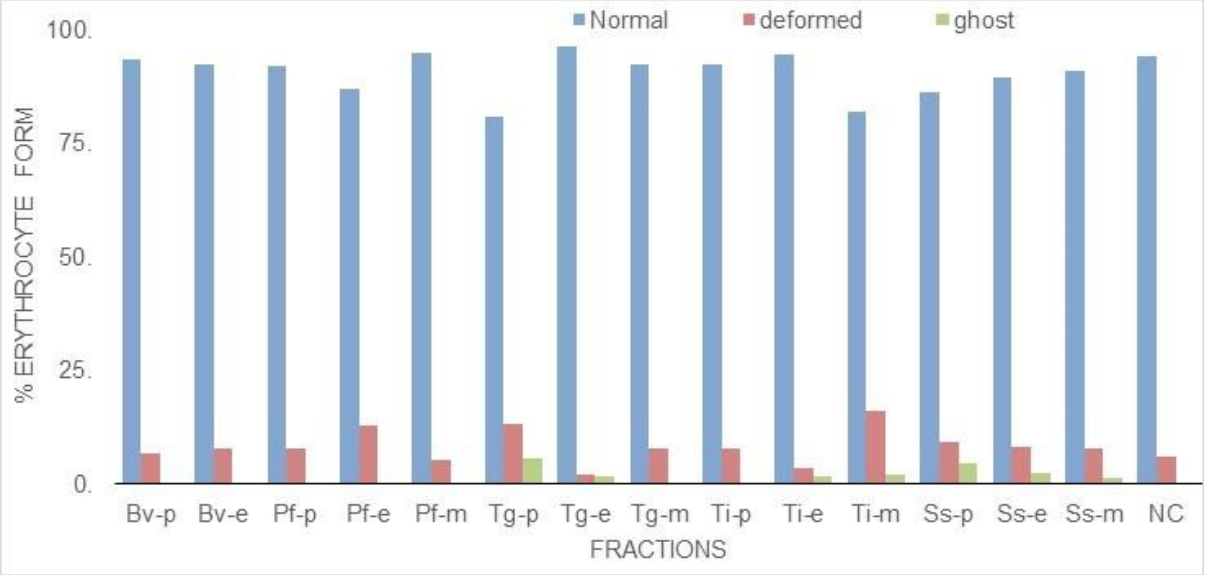


Figure 4.3.1: Composition of morphological types of erythrocytes after treatment with 200 µg/mL of test fractions.

Key: Bv-p: petroleum ether fraction of *B. vulgaris*; Bv-e: ethyl acetate fraction of *B. vulgaris*; Bv-m: methanol fraction of *B. vulgaris;* Pf-p: petroleum ether fraction of *P. fraternus*; Pf-e: ethyl acetate fraction of *P. fraternus* Pf-m: methanol fraction of *P. fraternus* ; Tg-p: petroleum ether fraction of *T. grandis*; Tg-e: ethyl acetate fraction of *T. grandis*; Tg-m: methanol fraction of *T. grandis*;Ti-p: petroleum ether fraction of *T. ivorensis*; Ti-e: ethyl acetate fraction of *T. ivorensis;* Ti-m: methanol fraction of T. ivorensis; Ss-p: petroleum ether fraction of *S. siamea*; Ss-e: ethyl acetate fraction of *S. siamea*; Ss-m: methanol fraction of *S. siamea*; NC: negative control

4.2.6 Discussion

The use of medicinal plants in the treatment of malaria and other diseases is a common tradition in many developing countries especially in Africa, and *T. grandis, P. fraternus, T. ivorensis, S. siamea* and *B. vulgaris* are some of the medicinal plants used in the traditional treatment of malaria (Komlaga et al., 2015).

The aqueous extracts of different parts of these plants demonstrated varying degrees of antiplasmodial activity against 3D7 and W2 strains of *P. falciparum*, and cytotoxicity against HUVECs (Table 2).

The antiplasmodial activities of the aqueous extracts were classified according to the classification by Philippe et al., (2005). With respect to 3D7 *P. falciparum*, the aqueous extract of *P, fraternus,* and *T. ivorensis* were considered as highly active (IC_{50}<5 µg/mL); that of *T. grandis,* and *B. vulgaris* as moderately active (5<IC_{50}<15 µg/mL) and *S. siamea* as showing weak activity (15<IC_{50}<50µg/mL). On the other hand, only two aqueous extracts, *T. ivorensis* and *S. siamea* were active against W2 *P. falciparum* and were accordingly categorized respectively as moderately (5<IC_{50}<15 µg/mL) and weakly active (15<IC_{50}<50µg/mL) in respect of W2 strain. The other extracts were considered inactive (IC_{50}>100 µg/mL) against same.

Two aqueous extracts, whole *P. fraternus* plant and *T. ivorensis* leaf, exhibited cytotoxicity against HUVECs (Table 2), suggesting their potential harmful effect.

They were, also, only selective for 3D7 *P. falciparum* ($9.8 \geq SI \geq 7.4$). According to Scholar & Pratt (2000) and Wright & Phillipson (1990), drugs demonstrating high selectivity indexes (high SI values) offer potential for safer therapy. However, these (the aqueous extracts of *P. fraternus* plant and *T. ivorensis* leaf) were poorly selective for the chloroquine-resistant W2 *P. falciparum*, and hence should be used with caution as traditional antimalarial medicines in Ghana, where chloroquine-resistant *P. falciparum* is the principal cause of malaria.

The chloroquine-resistant W2 *P. falciparum* demonstrated high levels of resistant to *B. vulgaris, P. fraternus and T. ivorensis* (resistance index, RI >13.9), moderate resistant to T. ivorensis (RI: 16.4), and low resistant to S. siamea (RI: 1.7) compared to that of the reference drug, chloroquine, at 6.0 (Table 2). According to Nzila & Mwai (2009), the value of RI is directly proportional to the level of parasite resistance to a particular agent, yet a low RI does not necessarily mean a low level of resistance; for

example, mefloquine-resistant parasite line (from W2) used to identify the mechanism of mefloquine resistance, had an RI value of only 4.6. These RI values, particularly of *B. vulgaris, P. fraternus and T. ivorensis,* give an indication of the possibility of malaria treatment failure using this plants, at least, in places endemic with chloroquine resistant strains of the parasite. Nevertheless, *in vivo,* the relationship between the level of *in vitro* resistance and the resulting therapeutic response is complex, and depends on parameters such as the host immune response, and the pharmacokinetic and pharmacodynamic properties of the drug (Houghton et al 2007; White & Pongtavornpinyo 2003). In the case of herbal medicine therapy where decoctions are taken by the oral route, some constituents could be modified into active metabolites *in vivo,* and thus contribute to the antiplasmodial action of the decoction. Therefore despite the high levels of resistance observed in some of the aqueous extracts, they (the extracts) could have the potential for effective treatment of malaria.

Besides, the extracts may also exert other effects that may alleviate the feverish state associated with malaria. For instance, the hydro-alcohol extract of *P. fraternus* has reportedly exhibited antinociceptive activity in chronic inflammatory hyperalgesia (Chopade & Sayyad 2013), and the aqueous and ethanolic extracts showed antiinflammatory activity (Oseni et al. 2013). The extracts of *T. grandis* was reported to possess anti-inflammatory and analgesic properties (Asif 2011; Ramachandran et al. 2011). Carey et al. (2009) reported the anti-inflammatory activity of the methanol extract of *B. vulgaris*. The extracts of *S. siamea* also possess analgesic and antiinflammatory properties (Momin et al. 2012; Nsonde-Ntandou et al. 2010). Extracts of *T. ivorensis* demonstrated anti-arthritis and anti-inflammatory properties (Iwu & Anyanwu 1982). It is therefore possible that aside the potential antiplasmodial property of the aqueous extracts, they might also alleviate the symptoms associated

with malaria fever, the possible reasons for their long and continuous traditional use as such in Ghana (Abbiw 1990; Asase & Oppong-Mensah 2009; Sittie et al. 1998). These findings may thus justify to some extent the use of these plants in the traditional treatment of malaria in Ghana.

The petroleum ether, the ethyl acetate and the methanol fractions, obtained by successive extraction of the various plant materials, all displayed activity against the only strain, 3D7 *P. falciparium*, tested. The methanol fractions of *P. fraternus* and *T. grandis*, the petroleum ether fractions of *T. grandis* and *B. vulgaris* and ethyl acetate fractions of *B. vulgaris* were categorized as showing important or high activity (IC_{50} <5 µg/mL), the ethyl acetate fraction of *T. grandis*, petroleum ether and methanol fractions of *T. ivorensis* are classified as having moderate activity (5 < IC_{50} <15 µg/mL) and the ethyl acetate fractions of *T. ivorensis*, petroleum ether and ethyl acetate fractions of *P. fraternus*, the 3 fractions of *S. siamea* as being weakly active (15 < IC_{50} < 50 µg/mL) (Table 4.3.2).

The antiplasmodial activity of *P. fraternus* and *B. vulgaris* increased with the polarity of the extracting solvent; implying polar constituents/metabolites may possess the antiplasmodial activity. On the whole, high antiplasmodial activity was more associated with the polar methanolic fraction than the less polar/hydrophobic (petroleum ether/ethyl acetate) fraction. Exception to this is the ethyl acetate and petroleum ether fractions of *B. vulgaris* both of which showed activity with IC_{50} below 1 µg/mL.

The fractions were also assessed for harmful activity *in vitro* on erythrocytes to rule out any injurious effect that could contribute to the observed antiplasmodial activity. In general selectivity was more towards the parasite (Table 3). The haemolytic effect of the fractions may therefore not contribute significantly to their antiplasmodial activity, especially when the activity was monitored for only one life cycle of the parasites (monitoring was within 42 h).

Comparing the antiplasmodial activity of the aqueous extracts to the corresponding most active organic fractions against *P. falciparum* 3D7 strain, it was realized that the ethyl acetate fraction of *B. vulgaris* was 29.0 times more potent while the methanol fraction of *T. grandis* and *P. fraternus* were respectively 15.5 and 1.7 times more active than the corresponding aqueous extracts. However, while the methanol fraction and the aqueous counterpart of *S. siamea* were equipotent, the aqueous extract of *T. ivorensis* was 4.8 times more potent than the corresponding methanol fraction. Since the organic fractions of *B. vulgaris* and *T. grandis* are more potent than the corresponding aqueous extract, one could recommend the use of ethanol, a suitable organic solvent in traditional medicine, for the extraction of *B. vulgaris* and *T. grandis* for enhanced activity in the traditional treatment of malaria.

Aqueous extract of *P. fraternus* has been reported to have suppressive effect on *P. berghei berghei* in mice (Matur et al. 2009). Valdés et al. (2010) reported that 80 % ethanol extract of *B. vulgaris* leaf possessed potent and specific antiplasmodial activity against the chloroquine susceptible Ghanaian strain of *P. falciparum*. Rodríguez-Pérez et al. (2006), on the other hand, reported that ethanol extract of the plant is inactive against chloroquine susceptible F32/Tanzanian strains of *P. falciparum.* In this study, the aqueous extracts of *B. vulgaris* was active against the chloroquine susceptible 3D7

P. falciparum, and this agreed with the antiplasmodial activity displayed by the hydroalcohol extract of *B. vulgaris* as reported by Valdés et al. (2010). Though, there were no report on the antiplasmodial activity of the extract(s) of *S. siamea*, alkaloids isolated from the leaves and flowers demonstrated varying degrees of antiplasmodial activity (Deguchi et al. 2012; Ekasari et al. 2009; Morita et al. 2007; Oshimi et al. 2009). Similarly, anthraquinones isolated from *T. grandis* and their derivatives were reported to exhibit antiplasmodial activity (Kopa et al. 2014). This study is the first report of the antiplasmodial activity of the extracts of both *S. siamea* and *T. grandis.* Their respective compounds tested earlier may be responsible for their antiplasmodial activity. Annan et al. (2012) reported on the antiplasmodial activity of the ethanol extract of *T. ivorensis*. Altogether, the findings of this study support the reported antiplasmodial activity of these plants. However, while this work studied the *in vitro* antiplasmodial activity and cytotoxicity of the aqueous extract and also the organic solvent (petroleum ether, ethyl acetate and methanol) fractions of *P. fraternus.* Matur et al. (2009) studied the *in vivo* activity of the aqueous extract. This study thus complements their study. In the case of *T. grandis* and *S. siamea*, this, to the best of our knowledge, is the first report of the antiplasmodial activity of the aqueous extract which mimicked the traditional preparation. Our approach to the study of *T. ivorensis* also imitated the traditional approach of use and for the first time its cytotoxicity was established.

As the search for new medicinal agents from plants to combat *P. falciparum* infestation continues, and some of the plants used in traditional medicine to treat malaria are validated for antiplasmodial activity, there is also the need to develop standardized and optimized herbal medicines, based on plants of proven safety and efficacy. Any such development should consider the normally employed approaches of the herbalists

including combination of plants and plant parts in preparing the remedies. In this regard, it is important that scientists work with herbalists to develop such products addressing safety issues, effectiveness and quality. This, in the long run, would contribute to the goal of ensuring global access to care, especially for people of the developing world, as stated by WHO Director-General, Dr Margaret Chan (WHO 2013).

5 CONCLUSION

The aqueous extracts of the leaves of *Tectona grandis*, *Terminalia ivorensis* and *Bambusa vulgaris;* the whole of *Phyllanthus fraternus* and the root of *Senna siamea* demonstrated noteworthy antiplasmodial activity against the *plasmodium* parasites with the chloroquine-sensitive 3D7 *P. falciparum* being more susceptible. The majority were however poorly selective for the chloroquine resistant W2 *P. falciparum*. These results provide rationale for the traditional use of the plants as antimalarial remedies in Ghana, but suggest potential harmful effect of some extracts. This study is the first report of the antiplasmodial activity of the aqueous extracts of both *S. siamea* and *T. grandis.* The petroleum ether, ethyl acetate, and methanol fractions showed similar but enhance antiplasmodial activity and were generally selective for the parasite (3D7 *P. falciparum*). The highly promising fractions (IC_{50} < 1 µg/mL) including the methanol extracts of *P. fraternus* and the fractions of *B. vulgaris* were selected for purification to isolate, identify, and test the isolated compounds for antiplasmodial activity.

CHAPTER 5 6 FRACTIONATION, ISOLATION, CHARACTERISATION AND

ANTIPLASMODIAL ACTIVITY OF ISOLATED COMPOUNDS FROM *P. FRATERNUS* AND *B. VULGARIS*

6.1 INTRODUCTION

Following the antiplasmodial evaluation of the extracts of *Phyllanthus fraternus; Bambusa vulgaris*; *Tectona grandis*; *Terminalia ivorensis* and *Senna siamea*, the methanol extracts of *P. fraternus* and *B. vulgaris* were identified as good candidates for further study. These were therefore subjected to chromatographic analysis which subsequently led to the isolation and characterization of their constituents, and thereafter the determination of the antiplasmodial activity of the isolated compounds.

6.2 EXTRACTION

Powdered material (4.63 kg) of *P. fraternus* was obtained as described in 3.1.1. The material was extracted cold with methanol and concentrated at 50^{o} C under vacuum using the rotavapor to obtain 425.25 g extract of *P. fraternus* extract (yield: 9.18 %) . The procedure was repeated for powdered leaf of *B. vulgaris* (4.22 k*g*) which yielded 251 g extract (yield: 5.95 %)

6.3 FRACTIONATION AND ISOLATION

6.3.1 P. FRATERNUS

Portions of extract (425.25 g) were shaken with 5% acetic acid solution to extract alkaloids. The solution was filtered and the filtrate successively partitioned with hexane to remove lipophilic substances after which the remaining solution was made alkaline with dilute ammonium hydroxide. The basified solutions were exhaustively extracted with chloroform and evaporated

to yield a total of 1.9491 g of alkaloids (% alkaloid yield: 0.46 %) (Figure 5.3.1). The alkaloid extract revealed seven (7) Dragendorff positive spots on pre-coated TLC plate (Merck silica gel 60 F254; Rf: 0.19; 0.26; 0.31; 0.46; 0.53; 0.66; 0.79) in $CHCl_3$:MeOH (12:1) system. The crude alkaloid was column chromatographed (cc) on silica gel 60 (40-63 µm) and gradient eluted with $CHCI_3$:MeOH in increasing amount of MeOH (Figure 5.3.2). The eluates were monitored using TLC and seven (7) bulk fractions (A-F) were collected after comparing the TLC profiles of the aliquots. Fraction A was cc using an isocratic system (5% MeOH in $CHCl_3$) to obtain two Dragendoff positive eluates, II and I. Both sub-fractions were separately chromatographed on prep TLC on silica gel (solvent:

DCM/EtOAc- 9/1) to yield **Pf1** (PfcAL A1B: 4.4 mg) and **Pf2** (PfcAl A2c: 20.3 mg). Fraction B was double run in prep HPLC (WATERS; Columns: XBridge C18, 5 µm, 30 x 250 mm; H2O + 0.1% formic acid / ACN isocratic 60:40 %v/v) to obtain **Pf3** (PfcAl B1A; 15mg) and **Pf4** (PfcAl B1B: 90 mg). Fraction C was cc using aluminium oxide 90 (70-230 mesh ASTM) Merck and an isocratic system of cyclohexanedichloromethane (3:2) as the mobile phase to obtain a Dragendorff positive subfraction I (PfcAl C2B) which was purified on prep. HPLC (Columns: XBridge C18, 5 µm, 19 x 150 mm; H2O + 0.1% formic acid / ACN gradient 5 – 100%) to yield compound **Pf5** (PfcAl C2B1: 4.13mg); Fraction E was cc on silica gel 60 (40-63 µm) eluted with CHCI/MeOH (7:3) to yield **Pf6** (PfcAl E2E: 99.2 mg).

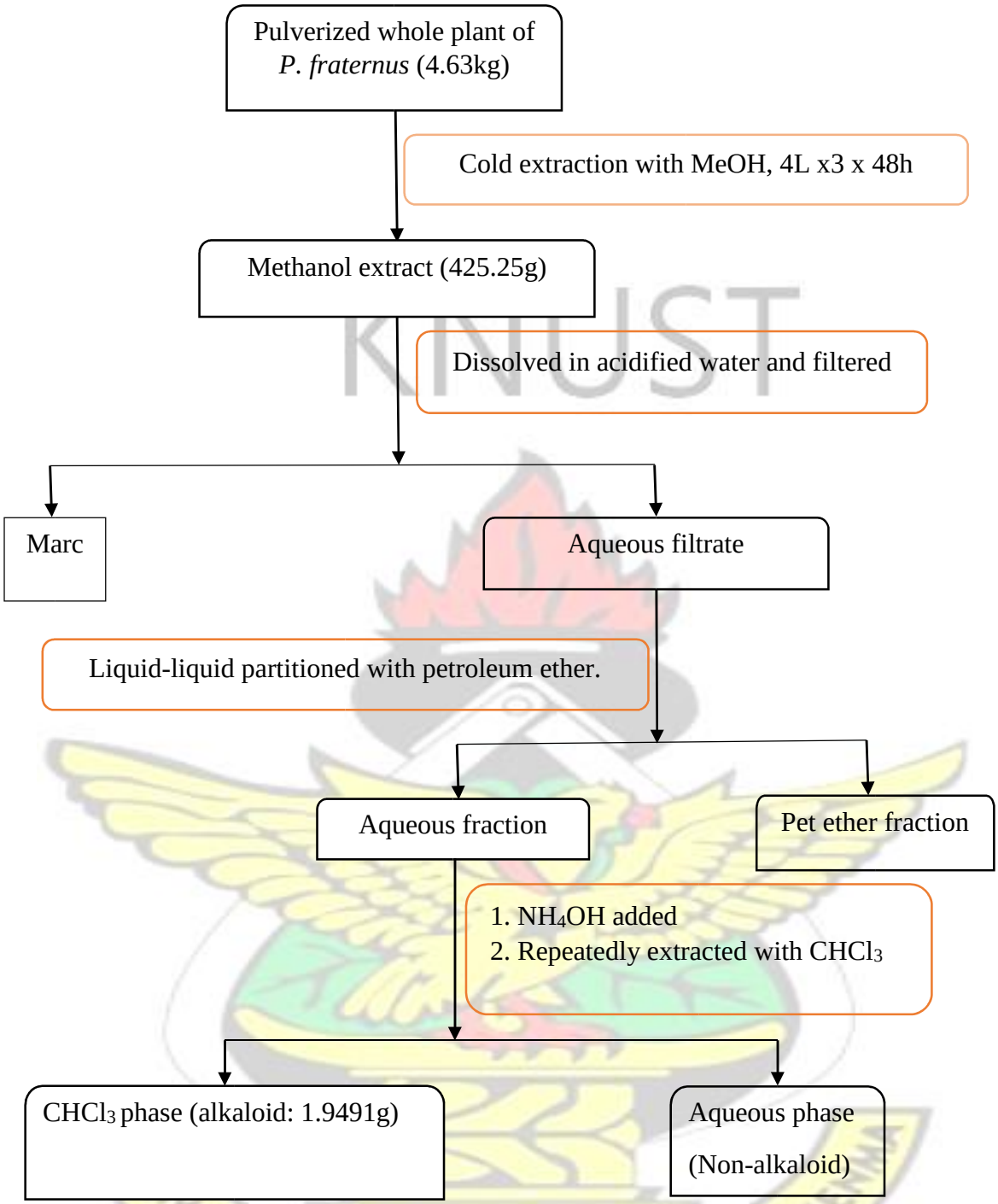


Figure 5.3.1: Schematic representation of the extraction of alkaloids from *P. fraternus* powder

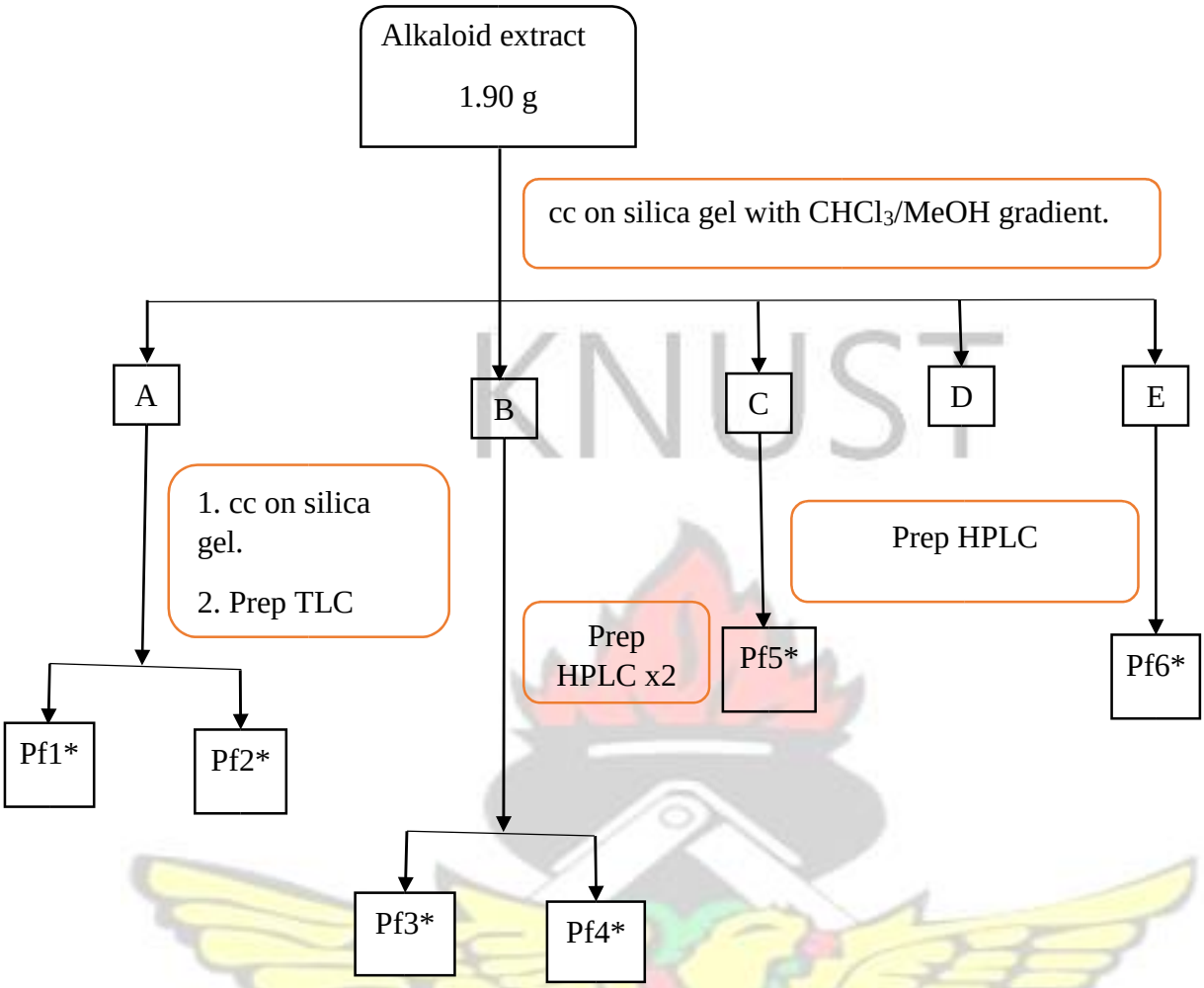


Figure 5.3.2: Schematic representation of the chromatographic fractionation of the alkaloid extract of *P. fraternus*

Key: *: isolated compounds

6.3.2 B. VULGARIS

Portions of the extract (251 g) were dissolved in water and then extracted with hexane, chloroform, and then butanol. The hexane fraction was partitioned with methanol to obtain altogether five fractions designated BvH, BvN, BvM, BvBu and BvW (Figure

5.3.2.1). Each fractions was evaporated to dryness, and three (3) including BvN, BvM and BvBu subsequently fractionated using combinations of chromatographic techniques to afford various compounds.

The chloroform fraction, BvN, was column chromatographed on silica gel 60 (40-63 µm) eluting with increasing concentration of MeOH in CH_2Cl_2. This gave two main fractions, BvN1 and BvN2 following TLC monitoring using vanillin in conc H_2SO_4 as detecting reagent. These fractions were purified on SUNFIRE C18, 5 µm, 30 x 250 mm column using isocratic MeCN:H_2O (65:35 %v/v) as mobile phase to afford BvN12, BvN15 and BvN21.

The butanol fraction, BvBu, was purified on silica gel column chromatography using a EtOAc/MeOH in a gradient elution. Fractions were monitored with TLC analysis and compounds revealed by spraying with vanillin in conc. H_2SO_4. Eight fractions (BvBu1-BvBu8) were collected. Repeated column chromatographic purification of fractions BvBu4 gave BvBu4a7 which was subsequently purified on a preparative HPLC with SUNFIRE C18, 5 µm, 30 x 250 mm column using MeCN: H_2O (40:60 %v/v) as a mobile phase which afforded BvBu4a74 and BvBu4a75. The methanol fraction, BvM, after series of column chromatographic separations with CH_2Cl_2/MeOH gradient as eluent and preparative TLC with isocratic MeCN:H_2O (60:40) yielded the pure compound, BvMC1.

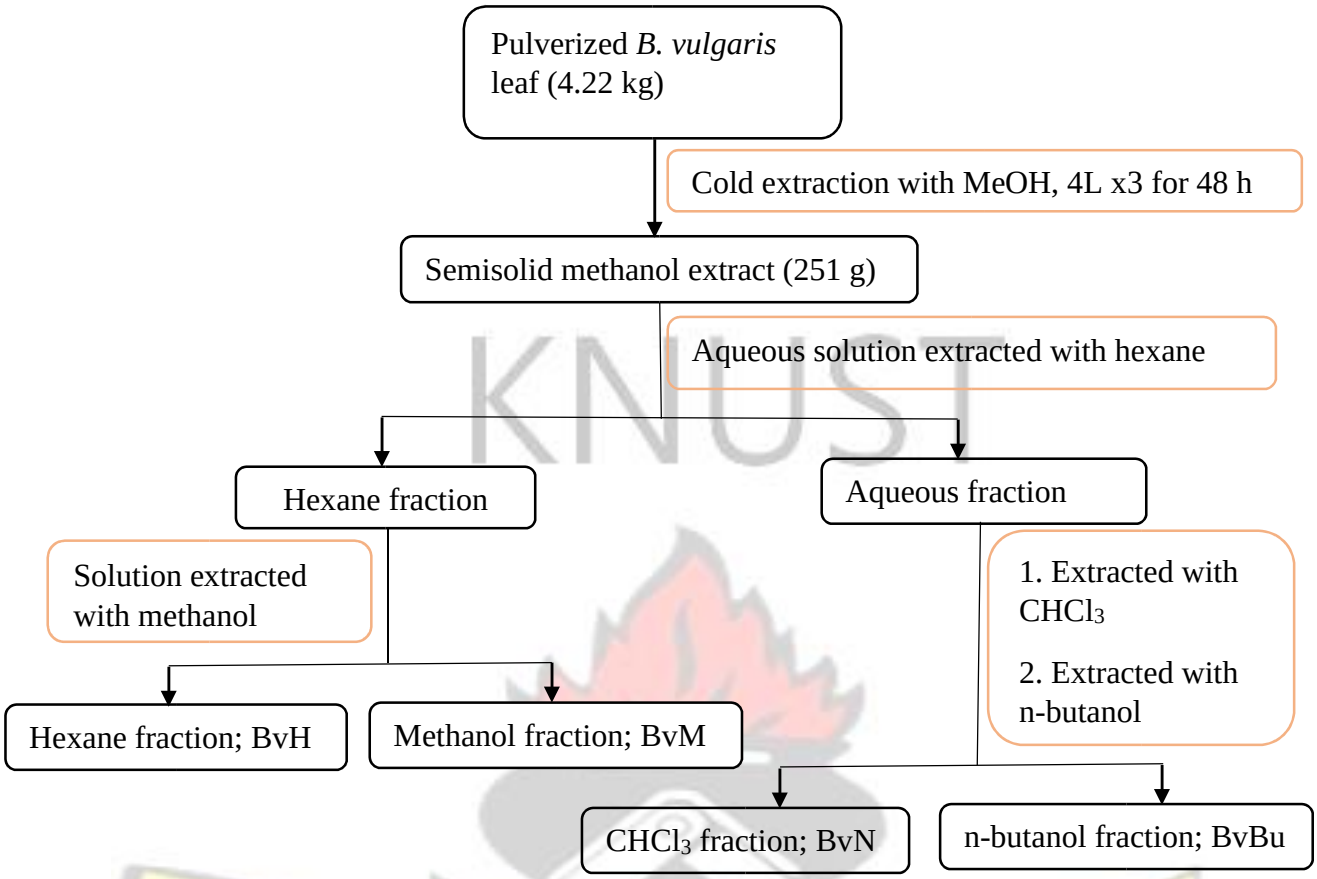


Figure 5.3.2.1: Schematic representation of successive fractionation of the methanol extracts of *B. vulgaris* leaf.

6.4 BIOLOGICAL ASSAY OF ISOLATED COMPOUNDS FROM P. *FRATERNUS AND B. VULGARIS*

6.4.1 Determination of antiplasmodial activity

Purified compounds from *P. fraternus* and *B. vulgaris* were evaluated in a dosetitration range of 0.098 - 100 µM against 3D7 and W2 *P. falciparum;* the procedure is as described under section 3.3.2.

6.4.2 Determination of cytotoxicity

Compounds isolated from *P. fraternus* and *B. vulgaris* were assayed at a dose-titration range of 0.78- 100 μM against HUVECs and the method is as described under section 3.3.3

6.5 RESULTS

6.5.1 Structural elucidation of compound isolated from *P. fraternus*

6.5.1.1 Structural elucidation of PfcAlA1B (Pf1)

Compound **Pf1** (PfcAlA1B; 4.3 mg) was obtained as a colourless crystals. The molecular formula was established as $C_{24}H_{34}O_6$ by its HR-ESI-MS (*m/z* 441.2257 $[M+Na]^+$, calcd for $C_{24}H_{34}O_6Na$: 441.2253, mDa 0.4; Appendix 3a). The UV absorption maximum at 280 nm is consistent with substituted aromatic rings.

The ^1H NMR spectrum (Appendix 3b; Table 5.5.1.1) revealed 3 aromatic signals each integrated for 2 protons at δ 6.62 (dd, *J*=8.1 Hz, 2.0 Hz; H-2), δ 6.60 (d, *J*=2 Hz; H-5) and δ 6.73 (d, *J*=8.1 Hz; H-6). The spectrum also showed 3 oxy-methyl singlets at δ 3.28, δ 3.79 and δ 3.83 each integrated for 6 protons; a methylene protons alpha to the aromatic ring (integrated for 4 protons) at δ 2.63, an oxy-methylene multiplet (integrated for 4 protons) at δ 3.29 and a methine multiplet (integrated for 2 protons) at δ 2.02. The olefinic signal at δ 6.62 *meta*- and *ortho*-coupled respectively to the doublets at δ 6.60 and δ 6.73 of the aromatic ring. The multiplet at δ 2.02 coupled with the multiplets at δ 3.29 and δ 2.63. The connectivity of the protons to the carbons and the subunits of the structure were achieved with the help of the 2D spectra including

HMBC (Table 5.5.1.1a) and HSQC spectra (Table 5.5.1.1). The NMR spectra of **Pf1** was identical to that of phyllanthin, a known lignan, isolated from *Phyllanthus niruri* (Row et al., 1964; Somanabandhu et al., 1993). **Pf1** was therefore identified as phyllanthin (Figure 5.5.1.1).

Table 5.5.1.1: ^{1}H, ^{13}C and COSY NMR for Pf1 in $CDCl_3$

Position	^{13}C (δ)	^{1}H (δ)	^{HH}J-coupling constant (Hz)	COSY coupling
1,1'	133.8			
2, 2'	112.4	6.60	dd, *J*=2.0	6.62, 6.73
3,3'	148.9			
4,4'	147.3			
5,5'	111.2	6.73	d, *J*=8.1	6.62
6,6'	121.3	6.62	d, *J*=8.1, 2.0	
7,7'	35.2	2.63	m	2.02
8,8'	41.0	2.02	m	3.29, 2.63
9,9'	72.8	3.29	m	2.02
3a, 3a'	55.1	3.83	s	
4a, 4a'	56.0	3.79	s	
9a, 9a'	59.0	3.28	s	

^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively **Table 5.5.1.1a: HMBC for PfcAlA1B**

^{1}H (δ)	*J*-Coupling ^{13}C
6.60	121.3, 35.2
6.73	133.8, 148.9
6.62	147.3, 112.4, 147.3, 148.9
2.63	40.96, 72.8, 121.3, 133.8, 112.4
3.83	147.3
3.79	148.9
3.28	72.8

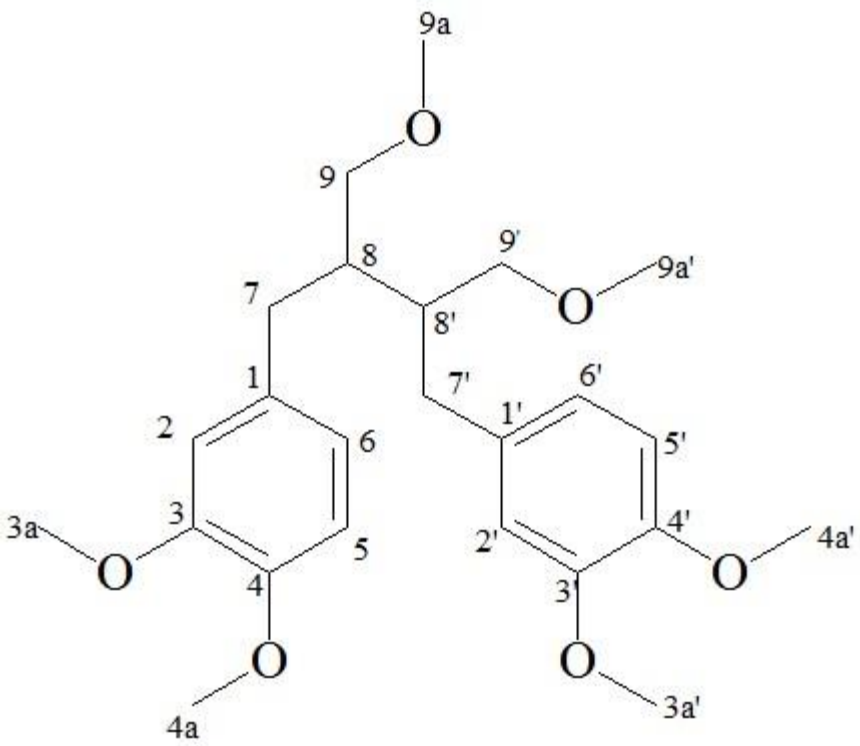


Figure 5.5.1.1: Structure of compound Pf1 (phyllanthin)

6.5.1.2 Structural elucidation of PfcAlA2C (Pf2)

Compound **Pf2** (33 mg) was obtained as a colourless crystalline substance. Optical rotation $[\alpha]^D$ 21.5: +112.5 (c=0.016g/dl; MeOH); UV absorption maximum at 241nm; IR absorption bands at 1735, 1683, 1650, 1288,-1064, 1245 cm^{-1} corresponding to α, β-unsaturated γ-lactone ring, ether bond and aliphatic amine. The HR-ESI-MS spectrum showed a peak at *m/z* 220.0985 $[M+H]^+$ (calcd. for $C_{12}H_{14}NO_3$ $[M+H]^+$, 220.0974, mDa 1.1; Appendix 4a) corresponding to the molecular formula $C_{12}H_{13}NO_3$. Seven (7) degrees of unsaturation were calculated and were assigned to two (2) double bonds and five (5) rings.

The ^1H NMR spectrum (Appendix 4b; Table 5.5.1.2) showed a triplet at δ 5.68 (t, *J*=2.0Hz) which is characteristic of the olfienic proton at position 12 of Securinega

alkaloids. This proton allylically coupled with the gem-coupled protons at δ 3.29 (dt, *J*= 20.0 Hz, 2.8 Hz) and δ 2.91(dt, *J*= 20.0 Hz, 2.2 Hz). Its triplet nature suggested it bisected the angle between the two geminal protons (Pechnaree, 1986). These two geminal protons, ascribed to position 14, coupled with the quadruple at δ 3.49 (q, *J*= 3.5 Hz; H-6) which also coupled with the doublet of doublet at δ 4.17 (dd, *J*=6.5 Hz, 4.2 Hz; H-7). This doublet of doublet, H-7, coupled to only one of the geminal protons at position H-8β (δ 1.57, ddd, *J*= 14.2 Hz, 6.3 Hz, 2.2 Hz) which also showed a Wcoupling with the doublet of doublet of doublet at δ 3.21 (ddd, *J*= 8.5 Hz, 5.7 Hz, 2.4 Hz; H-2) suggesting ∝-orientation of H-2. This orientation is confirmed by the upfield shift of the H-2 (Houghton 1996). Interestingly, H-2 also showed a NOESYcorrelation with the other geminal protons at position 8 whose chemical shift occurred at δ 2.91. The proton at δ 3.21 (H-2) couple with the gem-coupled multiplets at δ 2.17 and δ 2.06 which were attributed to position 3 (Figure 5.5.1.2; Table 5.5.1.2).These protons coupled with the geminal multiplets at position 4, only one (δ 1.97) of which coupled with the downfield doublet, at δ 4.91(*J*= 2.8 Hz).

The ^{13}C NMR spectrum (Appendix 4b; Table 5.5.1.2) showed 12 carbon signals including a carbonyl carbon at δ 173.52 (C-11), a tri-substituted double bond at δ 110.0 (C-12) and δ 172.85 (C13) and an oxygenated sp3 quaternary carbon at δ 82.66 (C-9) all of which were attributed to the ∝, β-unsaturated γ-lactone ring characteristic of securinega alkaloids. Others included the four downfield methine signals at 98.64, 63.62, 58.51, and 70.31, methylene signals at 34.08, 30.28, 23.94, and 23.44. The signal at δ 98.64 was characteristic of a carbon linked with nitrogen and oxygen (Pechnaree et al., 1986). This carbon in HMBC spectra (Table 5.5.1.2a) exhibited a ^{3}J heteronuclear multiple bond connectivity with the downfield doublet at δ_H 4.19, suggesting an ether bond between the two.

In the NOESYspectrum (Table 5.5.1.2b), the double triplet at δ 2.91 (H-14 β) coupled with the protons at δ 4.17 (H-7) and δ 1.57 (H-8). The NMR spectral data as well as the optical rotation value were in accordance with those published for nirurine previously isolated from *Phyllanthus* niruri (Petchnaree et al., 1986). Compound **Pf2** (Figure 5.5.1.2) was therefore identified as the known nirurine.

Table 5.5.1.2: ^{1}H, ^{13}C and COSY NMR for Pf2 in $CDCl_3$

Position	^{13}C (δ)	^{1}H (δ)	^{HH}J-coupling constant (Hz)	COSY coupling
2	63.6	3.21	ddd, *J*= 8.5, 5.7, 2.3	2.06, 2.17, 1.57
3	30.3	2.17	m m	2.02
		2.06		
4	30.3	1.97	m m	
		2.02		
5	98.6	4.91	d, *J*= 2.8	1.97
6	58.5	3.49	q, *J*= 3.5	2.91, 3.29
7	70.3	4.17	dd, *J*=6.5, 4.2	1.57, 3.49
8	34.2	2.23	d, *J*= 14.1	1.57
		1.57	ddd, *J*= 14.2, 6.3, 2.2	
9	82.7			
11	173.5			
12	110.0	5.68	t, *J*=2.0	2.91, 3.29
13	172.9			
14	30.3	3.29	dt, *J*= 20.0, 2.8 dt,	2.91,
		2.91	*J*= 20.0, 2.2	

^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively

Table 5.5.1.2a HMBC for Pf2

^{1}H (δ)	*J*-Coupling ^{13}C
5.68	82.7, 173.5, 23.9
4.91	23.4, 30.2, 63.6
4.17	34.2, 82.7, 98.6
3.21	34.2, 82.7, 98.6, 172.9, 173.5
3.49	23.9, 172.9
2.23	172.9, 173.5, 58.5, 63.6, 70.3, 82.7, 30.3
1.57	63.6, 82.7, 172.9, 70.3
1.97	23.4 , 98.6
2.02	23.4, 63.6, 82.7, 98.6, 30.3
3.29	58.5, 70.3, 172.9, 173.5, 110.0, 82.7
2.91	172.9, 110.0, 58.5, 82.7,
2.17	30.3, 63.6, 82.7
2.06	98.6, 82.7, 63.6, 30.3, 23.4

Table 5.5.1.2b: Important NOESY for Pf2

^{1}H (δ)	Coupling ^{1}H (δ)
4.17	2.91
1.57	2.91
3.21	2.23

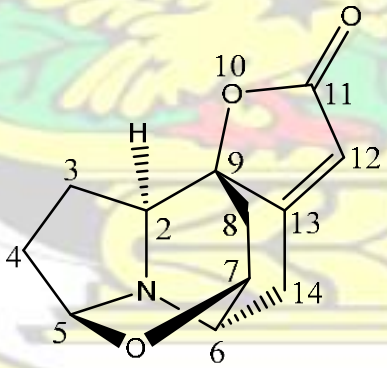


Figure 5.5.1.2: 3D structure of compound Pf2 (nirurine)

6.5.1.3 Structural elucidation of PfcAlB1A (Pf3)

Compound **Pf3** (PfcAlB1A; 33 mg) was obtained as an amorphous solid. Optical rotation $[\propto]^{D}$ 21.2: + 15.27 (c=0.010g/dl; $CHCl_3$); UV absorption maximum at 241nm;

The presence of an α, β-unsaturated γ-lactone ring was suggested by IR absorption bands at 1746 and 1633 cm^{-1}. The HR-ESI-MS spectrum showed a peak at *m/z* 204.1021 $[M+H]^+$ (calcd. for $C_{12}H_{14}NO_2$ $[M+H]^+$, 204.1025, mDa -0.4; Appendix 5a) corresponding to the molecular formula $C_{12}H_{13}NO_2$. Seven (7) degrees of unsaturation were calculated and were assigned to three (3) double bonds and four (4) rings.

The ^1H NMR spectrum (Appendix 5b; Table 5.5.1.3) revealed an olefenic proton characteristic of the ∝, β-unsaturated γ-lactone system at δ 5.75 and was assigned to H-12 (Table 5.5.1.3). This proton showed a very small ^5J dienylic and ^4J-allylic coupling with the cis-coupled olefinic signals at δ 6.75 (dd, *J*= 9.0Hz, 6.5 Hz; H-14) and δ 6.59 (d, *J*= 9.0 Hz; H-15) respectively in the ^1H-^1H COSY spectrum (Table 5.5.1.3). The doublet of doublet at δ 3.84 (dd, *J*= 6.5 Hz, 5.3 Hz; H-7) showed vicinal ^3J-coupling to the olefinic proton at δ 6.75 and methylene proton δ 2.64 (dd, *J*= 11.0 Hz, 5.3 Hz; H-8α). It also showed an allylic ^4J-coupling to the signal at δ 6.59 in the COSY spectrum. The doublet at 1.84 (d, *J*=11.0 Hz) assigned to H-8β showed long range W-coupling with the multiplet at δ 3.47 (H-2) suggesting an α-orientation of H2. This orientation was supported by the lack of NOESYcorrelation (Table 5.5.1.3b) between H-2 and H-8α.

The ^{13}C NMR spectrum (Table 5.5.1.3; Appendix 5b) showed among other peaks a carbonyl function at δ 172.0, a trisubstituted double bond at δ 166.9 and δ 109.4, a disubstituted double bond at δ 141.5 and δ 122.5, downfield methines at δ 65.94 and δ

59.7 (C-7), oxygenated quaternary carbon at δ 90.1 (C-9). The methylene carbon at δ 36.3 (C-8) forms a bridge between the C-7 and C-9. The two olefinic bond system together with the carbonyl bond formed a conjugated double bond system.

Analysis of the 2D NMR spectra including heteronuclear single quantum coherence (HSQC; Table 5.5.1.3), the heteronuclear multiple bond correlation (HMBC; Table 5.5.1.3a) correlation of the NMR spectra facilitated the connectivity between ^{1}H and ^{13}C nuclei and supported their assignment. NMR data and the optical rotation value were identical to those of *ent*-norsecurinine (Joshi et al., 1986). Compound **Pf3** was therefore identified as *ent-norsecurinine* (Figure 5.5.1.3)

Table 5.5.1.3: ^{1}H, ^{13}C and COSY NMR for Pf3 in $CDCl_3$

Position	**^{13}C (δ)**	**^{1}H (δ)**	**^{HH}J-coupling constant (Hz)**	**COSY coupling**
2	64.9	3.47	m	1.92, 2.09, 1.84
3	29.3	2.09	m m	3.47, 2.04, 1.92, 1.88
		1.92		3.47, 2.09, 1.88, 2.04
4	26.7	2.04	m m	1.84, 1.88
		1.88		
5	55.1	3.56	ddd, *J*= 9.5, 6.4, 1.8	2.62, 1.88
		2.62	m	2.04, 3.56
7	59.7	3.84	dd, *J*= 6.5, 5.3	2.64, 6.75
8	36.3	2.64	dd, *J*= 11.0, 5.3 d, *J*=11.0	1.84, 3.84
		1.84		2.64, 3.47
9	90.1			
11	172.0			

[1] H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively

12	109.4	5.75	s	
13	166.9			
14	122.5	6.59	d, *J*= 9.1	6.75, 5.75, 3.84
15	141.5	6.75	dd, *J*= 9.0, 6.5	3.84, 6.59, 5.75

Table 5.5.1.3a: HMBC for PfcAlB1A

^{1}H (δ)	***J*-Coupling ^{13}C**
6.75	36.3, 59.7, 166.9
6.59	59.7, 90.1, 166.9, 109.4
5.75	90.12, 122.5, 166.9, 172.0
3.47	166.90, 59.7, 55.1, 36.3, 29.3
3.84	55.1, 64.9, 90.1, 122.5, 141.5
3.56	29.3, 64.9
2.62	26.7, 59.7, 90.1, 141.5, 166.9
2.64	26.7, 59.7, 90.1, 141.5, 166.9
1.84	29.3, 55.1, 64.9, 90.1, 141.5, 166.9
2.09	26.7, 55.1, 64.9, 90.1
1.92	26.7, 64.9, 90.1
2.04	29.3, 64.9
1.88	64.9, 55.1

Table 5.5.1.3b: NOESY for PfcAlB1A

1	**Coupling ^{1}H (δ)**	**H (δ)**
	1.84, 3.84	6.75
6.59	5.75	
3.47	2.62	
3.84	1.84	

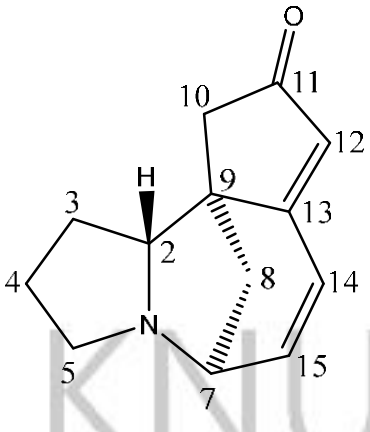


Figure 5.5.1.3: 3D structure of compound Pf3 (*ent-norsecurinine*)

6.5.1.4 Structural elucidation of PfcAlB1B (Pf4)

Compound **Pf4** (PfcAlB1B; 90 mg) was obtained as a slightly brown amorphous solid. Optical rotation, $[\propto]^{D}$ 21.2: +56 (c=0.010g/dl; $CHCl_3$); UV absorption maximum at 253nm.

Compound **Pf4** exhibited similar spectroscopic characteristics as compound **Pf3** and was considered a stereoisomer of **Pf3**. The presence of an α, β-unsaturated γ-lactone ring was suggested by the IR absorption bands at 1750 and 1635 cm^{-1}. The HR-ESIMS spectrum showed a peak at *m/z* 204.1025 $[M+H]^+$ (calcd. for $C_{12}H_{14}NO_2$ $[M+H]^+$, 204.1025, mDa 0.0; Appendix 6a) corresponding to the molecular formula $C_{12}H_{13}NO_2$. Seven (7) degrees of unsaturation were determined and were assigned to three (3) double bonds and four (4) ring systems.

The ^1H NMR spectrum (Appendix 6b; Table 5.5.1.4) showed the presence of the three olefenic protons at δ 6.78 (dd, *J*= 9.0, 5.5; H-15), δ 6.72 (d, *J*= 9.0; H-14) and δ 5.79 (s; H-12) which together with the carbonyl bond form an extended conjugated double bond system. The signal at δ 4.04 (dd, *J*= 5.5, 4.5; H-7) coupled with doublet of doublet at 6.78 and with only one proton (δ 2.90; dd, *J*= 10.2, 4.5) of the methylene protons at position 8, and this sequence of signals point to a structure similar to that observed in *ent*-norsecurinine . The ^{13}C NMR and the HSQC spectra (Table 5.5.1.4),

also agree very closely with those reported for norsecurine. However, unlike observed for *ent*-norsecurinine in the relative configuration, the methylene proton at δ 2.90 displayed strong NOESYcorrelation (Table 5.5.1.4b) with the methine proton at 4.16 (H-2) suggesting they are cofacial.

The NMR spectra together with the the optical rotation value of **Pf4** were consistent with those published for the synthetic alkaloid, *allo* -norsecurine (Medeiros & Wood, 2010a). Compound **Pf4** (Figure 5.5.1.4) was therefore identified as *allo* -

norsecurinine.

Table 5.5.1.4: ^{1}H, ^{13}C and COSY NMR for Pf4 in $CDCl_3$

Position	**^{13}C (δ)**	**^{1}H (δ)**	**^{HH}J-coupling constant (Hz)**	**COSY coupling**
11	172.1			
13	166.5			
15	147.5	6.78	dd, *J*= 9.0, 5.5	4.04, 6.72
14	124.6	6.72	d, *J*= 9.0	6.78, 5.79
12	110.8	5.79	s	
9	90.6			
2	69.0	4.16	dd, *J*= 7.9, 7.3	1.26, 1.67
7	57.8	4.04	dd, *J*= 5.5, 4.5	2.90
5	49.3	2.95	m	2.82, 1.79
		2.82	dt, *J*= 10.4, 7.2	1.79, 2.95
8	46.7	2.90	dd, *J*= 10.2, 4.5 d,	2.03, 4.04
		2.03	*J*= 10.2	2.90, 4.04
4	27.9	1.87	m, *J*= 5.8 dm,	1.79,1.26, 1.67
		1.79	*J*= 12.3, 7.6	1.26, 1.67, 1.87, 2.95, 2.82
3	25.5	1.67	dtd, *J*= 12.6, 7.3, 5.1	1.26
		1.26	ddd, *J*= 20.2, 7.5	

^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively **Table 5.5.1.4a: HMBC of PfcAlB1B (compound 4)**

^{1}H (δ)	***J*-Coupling ^{13}C**
4.16	49.3, 57.8, 90.6, 166.5

1.67	49.3, 27.9, 90.6,
1.26	27.9, 49.3, 69.0, 90.6
1.87	69.0, 25.5
1.79	69.0, 49.3, 25.5
2.95	69.0 57.8,
2.82	
4.04	124.6, 90.6, 68.95
2.90	57.79, 90.58, 147.5, 116.5
2.03	69.0, 90.6
5.79	172.1, 166.5, 124.6, 90.6
6.72	57.8, 90.6, 166.5
6.78	57.8, 166.5

Table 5.5.1.4b: NOESY for PfcAlB1B (Pf4)

1	**Coupling ^{1}H (δ)**	**H (δ)**
	2.90, 1.79	4.16
2.90	1.26	
2.03	1.67, 1.86	

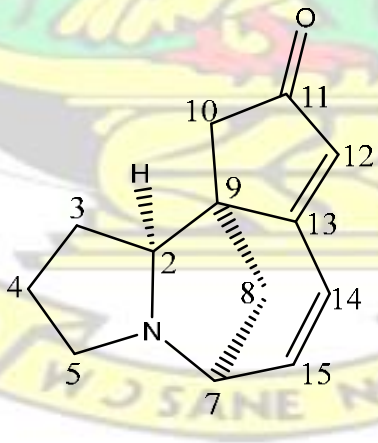


Figure 5.5.1.4: 3D structure of compound Pf4 (*allo*-norsecurinine)

6.5.1.5 Structural elucidation of PFCAL C2B1 (Pf5)

Compound **Pf5** was obtained as an amorphous substance with optical rotation [∝] ^{D}21.3: +12.94 (c=0.034g/dl; MeOH); UV absorption maximum at 241nm; The

HRESI-MS spectrum showed a peak at *m/z* 222.1122 $[M+H]^+$ (calcd. for $C_{12}H_{16}NO_3$ $[M+H]^+$, 222.1130, mDa -0.8; Appendix 7a) corresponding to the molecular formula $C_{12}H_{15}NO_3$. Six (6) degrees of unsaturation were calculated and were assigned to two (2) double bonds and four (4) rings.

The ^1H NMR spectrum (Appendix 7b; Table 5.5.1.5) revealed an olefenic proton at δ 5.75 (t, *J*=2.3 Hz), characteristic with the H-12 of securinega alkaloids (Babady-Billa et la, 1996; Houghton, 1996). This triplet coupled with two mutually coupled geminal protons at δ 2.36 (ddd, *J*=20.5, 2.3Hz; H-14 β) and δ 3.12 (m H-14 α) which also coupled with the multiplet at 3.14(H-6). The carbinol proton at δ 4.01 coupled with the methine proton at 3.14 and the gem-coupled protons at δ 2.05 and 1.99. The downfield signals at δ 3.03 and δ 3.81 are also vicinal to nitrogen (BABADY-BILA et al., 1996).

The ^{13}C NMR spectrum (Appendix 7b; Table 5.5.1.5) showed 12 carbon signals including a carbonyl carbon at δ 173.34, a trisubstituted double bond at δ 113.49 and δ 169.76 together with an oxygenated sp^3 quartenary carbon at δ 84.69 of an ∝ ,βunsaturated γ-lactone ring, characteristic of securinega alkaloids. The ^{13}C NMR spectrum also revealed 5 sp^3 methylene at δ 27.2, δ 24.9, δ 40.9, δ 50.8 and δ 24.3, three (3) sp^3 methines at δ 63.2, δ55.7 and δ 67.6. The ^{13}C NMR signals at δ 63.2 (C2), δ 50.8 (C-5), δ 55.7 (C-6) and 67.6 were indicative of carbons attached to nitrogen or oxygen. The pyrrolidine unit was established by the help of the COSY (Table 5.5.1.5), HMBC (Table 5.5.1.5a), and HSQC correlations. The relative configuration (Table 5.5.1.5b) displayed a correlation between the protons at δ 4.18 (H-7) and δ 2.78 (H-14 β) indicating they are on the same side of the molecule and were randomly assigned as ∝ oriented. The NOESY spectrum also showed correlation between 3.60 (H-2) and 2.05 (H-8∝) suggesting β orientation of H-2.

Analysis of the 2D NMR spectra including COSY, HSQC and HMBC spectra, the coupling constants of the peaks in the [1]H NMR spectrum, and the the optical rotation value resulted in the resolution of PFCALC2B1 (compound **Pf5)** as the known bubbialine (Figure 5.5.1.5). The spectra data of compound **Pf5** were identical with those published data (Ahond et al., 1990).

Table 5.5.1.5: ^{1}H, ^{13}C and COSY NMR for Pf5 in $CDCl_3$

Position	^{13}C (δ)	^{1}H (δ)	^{HH}J-coupling constant (Hz)	COSY coupling
C-2	63.2	3.60	dd, *J*=9.5, 6.4	1.07, 1.80
C-3	27.2	1.07	dddd, *J*=12.3, 10.9, 9.5, 7.2 m	1.80, 3.6, 1.74
		1.80		1.07, 3.6, 1.74
C-4	24.9	1.74	m m	2.67, 3.03
		1.74		
C-5	50.8	3.03	ddd, *J*=9.4, 6.9, 2.6 td,	2.67
		2.67	*J*=9.8, 6.2	1.74
C-6	55.7	3.14	tq, *J*=3.9, 2.1	2.36, 3.11, 4.01
C-7	67.6	4.01	dt, *J*=8.6, 2.9	2.05, 3.14
C-8	40.9	2.05	dd, *J*=13.3, 2.9 dd,	4.01
		1.99	*J*=13.3, 8.5	4.01
C-9	84.7			
C-11	173.3			
C-12	113.5	5.75	t, *J*=2.3	2.36, 3.12
C-13	169.8			
C-14	24.3	2.36	ddd, *J*=20.5, 2.3, 2.3 m	
		3.12		

Table 5.5.1.5a: HMBC for PFCALC2B1 (Pf5)

^{1}H (δ)	^{2}J-Coupling ^{13}C
3.60	40.9, 84.7, 169.8, 55.7, 50.8

[1] H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively

1.07	24.9, 63.2, 84.7
1.80	24.9, 50.8
1.74	27.2, 50.8, 63.2
3.03	27.2, 63.2
2.67	24.9, 55.7, 27.2
3.14	40.9, 63.2, 67.6, 84.7, 113.5, 169.8
4.01	84.7
2.02	169.8, 84.7, 67.6, 63.2, 55.7, 27.2
5.75	24.3, 84.7, 169.8, 173.3
2.36	169.8, 113.5, 55.7, 84.7, 173.3
3.11	40.9, 55.7, 67.6, 84.7, 113.5, 169.8, 173.3

Table 5.5.1.5b: NOESY for PFCALC2B1 (Pf5)

^{1}H (δ)	**Coupling ^{1}H (δ)**
4.01	2.36,
3.60	2.05
1.07	2.67

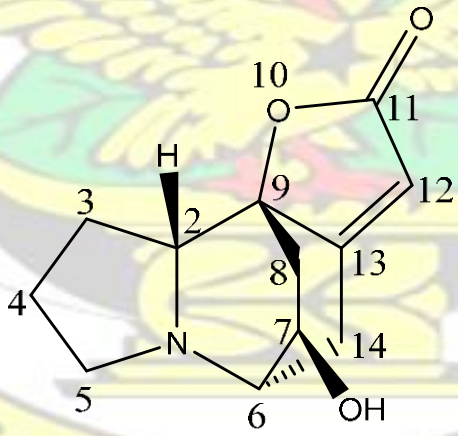


Figure 5.5.1.5: 3D structure of compound Pf5 (bubbialine)

6.5.1.6 Structural elucidation of PfcAle2e (Pf6)

PfcAl E2E (compound **Pf6**) was obtained as colourless acicular crystals. The HR-ESIMS showed a peak at *m/z* 222.1132 $[M+H]^+$ (calcd. for $C_{12}H_{16}NO_3$ $[M+H]^+$, 222.1130, mDa 0.2; Appendix 8a) corresponding to the molecular formula $C_{12}H_{15}NO_3$.

Optical rotation, $[\propto]^D$ 21.5 : -70 (c=0.16g/dl; MeOH); UV absorption maximum at 274nm; IR absorption bands at 3500- 3250 (broad), 1725, 1650, 1121, 1073 cm^{-1} corresponding to hydroxyl, α, β-unsaturated γ-lactone ring, alkenes and aliphatic amines functional groups respectively. Six (6) degrees of unsaturation were calculated and were assigned to two (2) double bonds and four (4) rings.

PfcAlE2E (**Pf6**) (Figure 5.5.1.6) exhibited similar ^1H and ^{13}C NMR pattern (Appendix 8b; Table 5.5.1.6) as compound **Pf5** (Figure 5.5.1.5), except in the relative orientation of the protons at position 2 (H-2). Compound **Pf5** was as a result considered an epimer of compound **Pf6**. Both compound **Pf5** and **Pf6** has three stereo centres located at C-2 (δ 64.89), C-6 (δ 57.60), and C-7 (δ 72.61). The triplet of triplet at δ 3.04 (tt, *J*=6.9 Hz, 2.3 Hz: H-2) exhibited a W-coupling with the doublet of doublet at 1.89 (ddd, *J*=13.4, 10.6, 2.0: H-8 α; Table 5.6.1.6) suggesting α orientation for H-2. The α orientation for H-2 is supported by the NOESY correlation between it (δ 3.04) and the proton at δ 2.95 (H-14 α). The relative configuration also showed the carbinol proton at 4.18 correlating with the proton at δ 2.78 (H-14 β), indicating the cofacial orientation of the two protons.

The ^{13}C NMR spectrum (Figure 5.5.1.6) presented 12 carbon signals including a carbonyl function at δ 177.4, tri-substituted double bond at δ 112.4 and δ 176.0 and oxygenated sp^3 quartenary signal at δ 86.4; 5 sp^3 methylene at δ 32.0, δ 33.8, δ 26.6, δ 28.1 and δ 53.7; three sp^3 methines at δ 64.9, δ 57.6 and δ 72.6. By comparing with literature data, compound **Pf6** (PfcAl E2E) was identified as the known epibubbialine (Figure 5.5.1.6) previously isolated from *Phyllanthus amarus* Schum. et Thonn.(Houghton et al., 1996)

Table 5.5.1.6: ^{1}H, ^{13}C and COSY NMR for Pf6 in MeOD

Position	^{13}C (δ)	^{1}H (δ)	^{HH}J-coupling constant (Hz)	COSY coupling
C-2	64.9	3.04	tt, *J*=6.9, 2.3	2.25; 1.95; 1.72, 1.89
C-3	26.6	1.72	dtt, *J*=11.9, 9.2, 6.6	1.95; 3.03; 3.81
		2.16	m	
C-4	28.1	1.95	mdd, *J*=6.9, 5.3, 3.60 m	1.72; 3.03; 2.24; 2.16
		2.24.		1.95; 3.03
C-5	53.7	3.03	tt, *J*=6.9, 2.3	3.81; 2.25; 1.95; 1.72
		3.81	td, *J*=9.3, 6.4	3.03; 2.16; 1.72
C-6	57.6	3.25	q, *J*= 2.3	2.78; 2.95, 4.18
C-7	72.6	4.18	ddd, *J*=10.6, 4.7, 1.5	1.89; 2.33, 3.25
C-8	33.8	1.89	ddd, *J*=13.4, 10.6, 2.0 dd,	2.33; 3.04; 4.18
		2.33	*J*=13.4, 4.6	1.89; 4.18
C-9	86.4			
C-11	177.4			
C-12	112.4	5.82	t, *J*= 2.1	2.78; 2.95
C-13	176.0			
C-14	32.0	2.78	dt, *J*=19.4, 2.3, 2.3 ddd,	2.95; 3.25, 5.82
		2.95	*J*= 19.4, 3.5, 2.0	2.78; 3.25, 5.82

Table 5.5.1.6a: HMBC for PfcAlE2E

^{1}H (δ)	*J*-Coupling ^{13}C
^{1}H	86.4, 176.0, 177.4
5.82	32.0, 86.4
4.18	26.6, 57. 6
3.25	177.4, 64.9, 33.8
3.03	28.1, 33.8, 57.6, 64.9, 86.4, 177.4
2.95	72.6, 177.4, 57.6, 112.4

^{1} H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively

2.78	72.6, 86.4, 112.4, 177.4
2.33	64.9, 72.6, 86.4, 177.4
2.24	26.6, 53.7, 64.9, 86.4
2.16	64.9
1.95	53.7, 64.9, 86.4
1.89	53.7, 64.9, 72.6, 86.4, 177.4
1.72	53.7, 64.9

TAble 5.5.1.6b: NOESY for PfcAlE2E

^{1}	**Coupling ^{1}H (δ)**	**H (δ)**
4.18	2.78	
3.04	2.95	

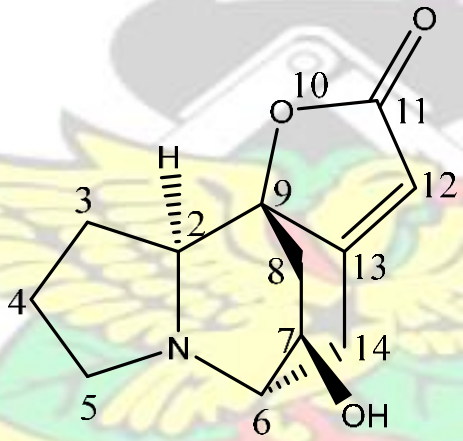


Figure 5.5.1.6: 3D structure of compound Pf6 (epibubbialine)

6.5.2 Structural elucidation of compounds isolated from *B. vulgaris*

6.5.2.1 Structural elucidation of BvBu4a74 (Bv1)

BvBu4a74 (Compound **Bv1**) was obtained as brown amorphous substance. The molecular formula was established as $C_9H_8O_3$ by its HR-ESI-MS (*m/z* 187.0362 $[M+Na]^+$, calcd for $C_9H_8O_3Na$: 245.1154, mDa -0.9; Appendices 10a). It has six (6) degrees of unsaturation which was attributed to a C=O bond, 4 C=C bonds and a ring. The ^{1}H NMR spectrum (Appendix 9b; Table 5.5.1.5) displayed trans-coupled olefinic

doublets at δ 6.28 (d, *J*=15.9) and δ 7.60 (d, *J*=15.9) and two equivalent aromatic protons at δ 6.81 and δ 7.45 each.

The ^{13}C-NMR spectrum (Appendix 9b; Table 5.5.2.1) disclosed 9 carbon resonances due to a carboxylic acid signals at δ 171.2, a C=C signals at δ 146.8 and δ 115.8 respectively α and β to an aromatic ring, two quaternary aromatic carbons at δ 161.3 and δ 127.4 respectively and two equivalent methine carbons at δ 131.2 and δ 117.0.

Analysis of the 2D spectra facilitated the resolution of compound **Bv1** as (*E*)-3-(4hydroxyphenyl) acrylic acid also known as viz *p*-coumaric acid (Figure5.5.2.1).

Table 5.5.2.1: ^{1}H, ^{13}C and COSY NMR for Bv1 in $CDCl_3$

Position	**^{13}C** (δ)	**^{1}H** (δ)	**^{HH}J-coupling constant (Hz)**	**COSY coupling**
9	171.2			
4	161.3			
7	146.8	7.60	d, *J*=15.9	6.28
2, 6	131.2	7.45	d, *J*= 8.6	6.81
1	127.4			
3,5	117.0	6.81	d, *J*=8.6	

8	115.8	6.28	d, *J*=15.9

^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively

Table 5.5.2.1a: HMBC for Bv1

^{1}H (δ)	***J*-Coupling ^{13}C**
7.60	131.2(5), 171.2 (1)
7.45	131.2 (5), 146.8 (4), 161.3 (2)
6.81	117.0 (7), 127.4 (6), 161.3 (2)
6.28	171.2 (1), 127.4 (6)

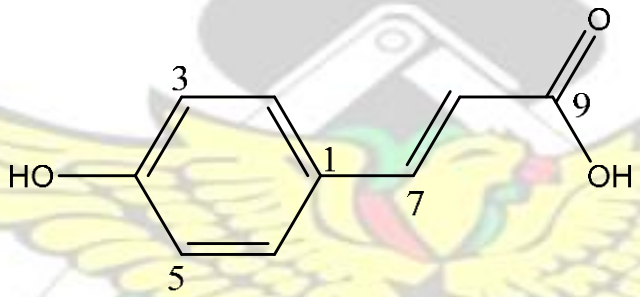


Figure 5.5.2.1: Structure of Bv1 (*p*-coumaric acid or (E)-3-(4-hydroxyphenyl) acrylic acid)

6.5.2.2 Structural elucidation of BvBu4a75 (Bv2)

BvBu4a75 (compound **Bv2**) was obtained as a colourless crystalline substance. The molecular formula was established as $C_9H_8O_2$ by its HR-ESI-MS (*m/z* 171.0416 $[M+Na]^+$, calcd for $C_9H_8O_2Na$: 245.1154, mDa 0.2; Appendices 10a). It has five (6) degrees of unsaturation attributed to a carbonyl function, four (4) π-bonds and a ring. The ^{1}H NMR spectrum (Appendix 10b; Table 5.5.2.2) showed trans-coupled olefinic doublets at 6.48 (d, *J*=16.0) and 7.67 (d, *J*=16.0) and poorly resolved aromatic protons

at δ 7.41, δ 7.40 and δ 7.59. The signals at δ 7.41 and δ 7.59 each integrated for two protons.

The ^{13}C-NMR spectrum (Appendix 10b; Table 5.5.2.2) disclosed 9 carbon resonances ascribed to a carboxylic acid signals at δ 170.5; olefinic carbon signals at δ 119.6 and δ 146.4, an aromatic quaternary carbon at 136.0 and two aromatic methine carbons at δ 130.14 and δ 129.3 each showing a single bond correlation to 2 protons (Table 5.5.2.2).

Analysis of the 2D spectra including COSY, HSQC (Table 5.5.2.2) and HMBC (Table 5.5.2.2a) together with the coupling constants of the peaks in the ^{1}H NMR spectrum resulted in the resolution of BvBu4A75 (compound **Bv2**) as cinnamic acid (Figure 5.5.2.2). It demonstrated antioxidant activity (Mousa et al., 2013).

Table 5.5.2.2: ^{1}H, ^{13}C and COSY NMR for Bv2 **in $CDCl_3$**

Position	**^{13}C** (δ)	**^{1}H** (δ)	**^{HH}J-coupling constant (Hz)**	**COSY coupling**
3, 5	129.3	7.59	dd, *J*=6.5, 2.9 (dq, *J*=7, 2.6)	
2, 6	130.1	7.41	dd, *J*=4.1, 2.2	7.59
4	131.5	7.40	dd, *J*=4.1, 2.2	7.59
1	136.0			

8	119.6	6.48	d, *J*=16.0	
7	146.4	7.67	d, *J*=16.0	6.48
9	170.5			

^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively

Table 5.5.2.2a: HMBC for Bv2

^{1}H (δ)	***J*-Coupling ^{13}C**
7.67	170.5, 129.3, 119, 136.0
7.40	136.0
7.40	129.3, 136.0
7.59	131.5
7.59	131.5
6.48	136.0

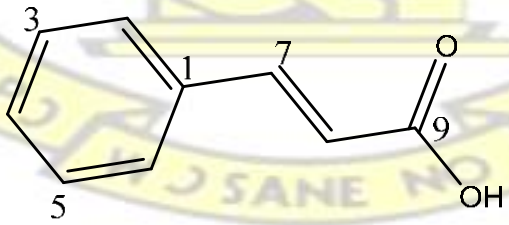


Figure: 5.5.2.2: Structure of cinnamic acid

6.5.2.3 Structural elucidation of BvN12 (Bv3)

BvN12 (compound **Bv3**) was obtained as an amorphous substance. The molecular formula was established as $C_{13}H_{18}O_3$ by its HR-ESI-MS (*m/z* 245.1156 $[M+Na]^+$, calcd for $C_{13}H_{18}O_3Na$: 245.1154, mDa 0.2; Appendix 11a). It has five (5) degrees of unsaturation which was attributed to two carbonyl functions, two double bonds and a ring.

The ^{1}H NMR spectrum (Appendix 11b Table 5.5.2.3) showed a trans-coupled olefinic doublets at δ 6.83 (d, *J*=15.8) and δ 6.47 (d, *J*=15.8); an olefinic quartet at δ 5.97 (q, *J*= 1.5) which showed an allylic coupling with the methyl doublet at δ 1.89 (d, *J*=1.5); a mutually coupled diastereotopic doublets at δ 2.50 (d, *J*= 17.1) and δ 2.34 (d, *J*= 17.1) and three (3) methyl singlets at δ 2.31, δ 1.02 and δ 1.11. The olefinic signals at δ 6.47 and δ 5.97 are characteristic of the α-protons of an α, β-unsaturated ketone (Jacobsen, 2007).

The ^{13}C-NMR spectrum (Appendix 11b; Table 5.5.2.3) disclosed 13 carbon resonances due to *α,β* unsaturated carbonyl signals at δ 197.6 and δ 197.3; disubstituted alkene at δ 130.6 and δ 145.1; a trisubstituted alkene at 160.6 and 128.0; oxygenated quaternary carbon at δ 79.5; a quaternary carbon resonance at δ 41.7; a methene carbon signal at δ 49.8, and four methyl carbon signals at δ 18.9, δ 23.2, δ 24.6 and δ 28.6 Analysis of the 2D spectra including H-H COSY, HSQC (Table 5.5.2.3) and HMBC spectra (Table 5.5.2.3a) together with the coupling constants of the peaks in the ^{1}H NMR spectrum resulted in the resolution of compound **Bv3** as (E)-4-hydroxy-3,5,5trimethyl-4-(3-oxobut-1-en-1-yl)cyclohex-2-en-1-one (Figure 5.5.2.3), a known compound also called dehydrovomifoliol isolated from the ‘kidney bean" (*Phaseolus vulgaris* L.) (Takasugi et al., 1973).

Table 5.5.2.3: ^{1}H, ^{13}C and COSY NMR for Bv3 in $CDCl_3$

Position	^{13}C (δ)	^{1}H (δ)	^{HH}J-coupling constant (Hz)	COSY
12	197.6			
1	197.3			
3	160.6			
10	145.1	6.83	d, *J*= 15.8	6.47
11	130.6	6.47	d, *J*=15.8	2.31
2	128.0	5.97	q, *J*= 1.5	1.89, 2.34
4	79.5			
6	49.8	2.50 2.34	d, *J*= 17.1 d, *J*= 17.1	1.11
5	41.7			
13	28.6	2.31	s	
8	24.6	1.02	s	1.11
9	23.2	1.11	s	
7	18.9	1.89	d, *J*=1.5	

Table 5.5.2.3a: HMBC for Bv3

^{1}H (δ)	***J*-Coupling ^{13}C**
6.83	79.5, 197.6
6.47	197.6, 79.5

[1] H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively

5.97	18.9, 49.8, 79.5
2.50	23.2, 197.2
2.34	79.5, 41.7
2.31	197.6, 130.6
1.02	23.2, 41.7, 49.8, 79.5
1.119	24.6, 41.7, 79.5, 49.8
1.89	79.5, 128.0, 160.6

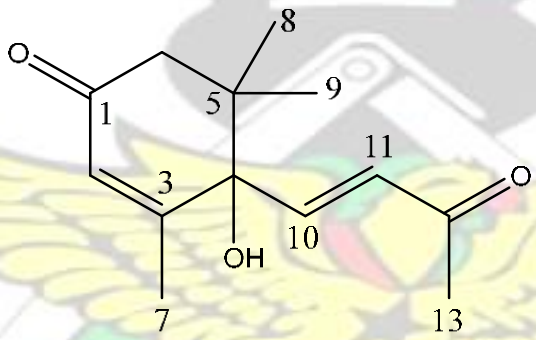


Figure 5.5.2.3: Structure of BvN12 [(E)-4-hydroxy-3, 5, 5-trimethyl-4-(3-oxobut1-en-1-yl) cyclohex-2-en-1-one or dehydrovomifoliol]

6.5.2.4 Structural elucidation of BvN15 (Bv4)

The molecular formula of **Bv4** was established as $C_{13}H_{20}O_2$ by its HR-ESI-MS (*m/z* 231.1351 $[M+Na]^+$, calcd for $C_{13}H_{20}O_2Na$: 231.1361, mDa -1.0; Appendix 12a). Four (4) degrees of unsaturation was calculated accounting for three (3) double bonds and a ring system.

The H NMR spectrum (Appendix 12b; Table 5.5.2.4) showed a trans-coupled olefinic doublet of doublets at δ 5.66 (dd, *J*=15.3, 5.8) and δ 5.54 (dd, *J*=15.3, 9.0); a methine doublet at δ 2.51 (d, *J*=9.0) which showed allylic coupling to the signal at δ 5.54; a carbinol triplet of triplets at δ 4.34 (tt, *J*= 6.4, 5.8) which showed a vicinal coupling with the methyl doublet at δ 1.27 (d, *J*=6.4) and allylic coupling with the olefinic signal at δ 5.66; an olefinic doublet at δ 5.90 (d, *J*=1.0), characteristic of the *α* proton of an *α,β*-unsaturated ketone (Jacobsen, 2007) showed allylic coupling to the methyl doublet at δ 1.88 (d, *J*=1.0); mutually coupled geminal doublets at δ 2.08 (d, *J*=16.8) and δ 2.32(d, *J*=16.8) and two methyl singlets at δ 0.96 and δ 1.02.

The ^{13}C-NMR (Appendix 13b) disclosed 13 carbon resonances due to an *α,β* unsaturated carbonyl signal at δ 199.8; a trisubstituted alkene at δ 162.5 and δ 126.0; a disubstituted alkene at δ 126.9 and δ 138.7; a quaternary carbon at δ 36.4; a methene carbon signal at δ 47.6, a carbinol and methine carbons at δ 68.7 and δ 55.7 respectively and four methyl carbon signals at δ 23.8, δ 23.9, δ 27.4 and δ 28.1

Analysis of the 2D spectra including H-H COSY , HSQC (Table 5.5.2.4) and HMBC (Table 5.5.2.4a) together with the coupling constants of the peaks in the ^{1}H NMR spectrum resulted in the resolution of compound **Bv4 (**Figure 5.5.2.4) as (E)-4-(3hydroxybut-1-en-1-yl)-3,5,5-trimethylcyclohex-2-en-1-one, also known as 3-oxo-α-ionol (Aasen et al., 1971: Figure 5.6.2.4)

It was previously isolated from tobacco (Aasen et al., 1971) and grape juice (Strauss et al., 1987). The spectra data of Bvn15 were identical with published data (Aasen et al., 1971). 3-oxo-α-ionol had a moderate inhibitory profile on root and shoot growth (D'Abrosca et al., 2004).

Table 5.5.2.4: ^{1}H, ^{13}C and COSY NMR for Bv4 in $CDCl_3$

Position	^{13}C (δ)	^{1}H (δ)	^{HH}J-coupling constant (Hz)	COSY coupling
1	199.8			
3	162.5			
11	138.7	5.66	dd, *J*=15.3, 5.8	4.34, 5.54
10	126.9	5.54	dd, *J*=15.3, 9.0	2.51
2	126.0	5.90	d, *J*=1.0	1.88
12	68.7	4.34	tt, *J*= 6.4, 5.8	1.27
4	55.7	2.51	d, *J*=9.0	-
6	47.6	2.08 2.32	d, *J*=16.8 d, *J*= 16.8	2.32
5	36.4	-	-	
9	28.1	1.02	s	-
8	27.4	0.96	s	-
13	23.9	1.27	d, *J*=6.4	-
7	23.8	1.88	d, *J*=1.0	-

Table 5.5.2.4a: HMBC for Bv4

^{1}H (δ)	*J*-Coupling ^{13}C
5.66	55.7, 126.9, 68.7

[1] H and ^{13}C NMR spectra were recorded in at 400 MHz and 100 MHz respectively

5.54	68.7,138.7
5.90	23.8, 55.7
4.34	126.9
2.51	126.9, 162.5, 138.7, 47.6, 36.4
2.08	199.8, 55.7, 36.4, 28.1
2.32	199.8, 28.1, 36.4, 55.7
1.02 (3H)	27.4, 36.4, 47.6, 55.7
0.96 (3H)	28.1, 36.4, 47.6, 55.7
1.27 (3H)	68.7, 138.7
1.88 (3H)	162.5, 126.0, 55.7

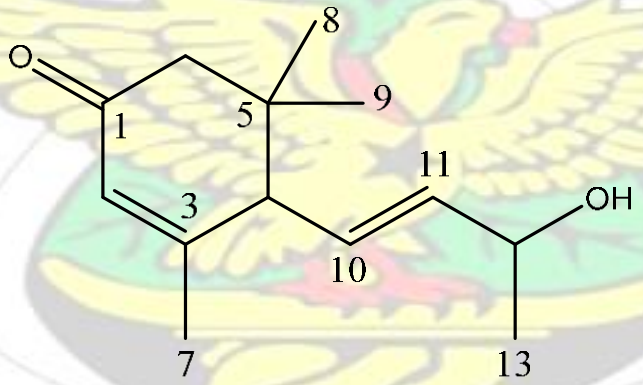


Figure 5.5.2.4: Structure of Bv4 [(E)-4-(3-hydroxybut-1-en-1-yl)-3, 5, 5trimethylcyclohex-2-en-1-one]

6.5.2.5 Structural elucidation of BvN21 (Bv5)

Compound **Bv5** was obtained as a brown solid substance. The HR-ESI-MS showed a peak at m/z219.0990 $[M+H]^+$ (calcd. for $C_{11}H_{16}O_3Na$ $[M+Na]^+$, 219.0997, mDa -0.7; Appendix 13a) corresponding to the molecular formula $C_{11}H_{16}O_3$. Four (4) degrees of

unsaturation were calculated and were assigned to two (2) double bonds and two (2) rings.

The H NMR spectrum (Appendix 13b; Table 5.5.2.5) showed an olefinic singlet at δ 5.70 characteristic of the α-proton of an α, β-unsaturated γ-lactone ring; A carbinol proton at δ 4.12 which coupled with four methene protons at δ 2.02, δ 1.32, δ 2.52 and δ 1.49 and three (3) methyl singlets at δ 1.29, δ 1.56 and δ 1.24 (Tabel 5.5.2.5). The methene proton at δ 2.02 showed a long range coupling with that at δ 2.52.

The ^{13}C-NMR spectra (Appendix 13b; Table 5.5.2.5) disclosed 11 carbon resonances due to a carbonyl signal at δ 181.4; a trisubstituted alkene at δ 113.4 and δ 172.3; a carbinol carbon at δ 65.3; two quaternary carbons at δ 35.3 and δ 87.1; two methylene carbons at δ 49.9 and δ 48.0 and three (3) methyl signals at δ 30.1, δ 25.7 and δ 25.2.

Careful analysis of the 2D spectra including H-H COSY, HSQC (Table 5.5.2.5) and HMBC spectra (Table 5.5.2.5a) together with the coupling constants of the peaks in the 1H NMR spectrum resulted in the resolution of compound **Bv5** as 6-hydroxy-4, 4, 7a-trimethyl-5, 6, 7, 7a-tetrahydrobenzofuran-2(4H)-one, trivially known loliolide (Figure 5.5.2.5) and was previously isolated from plants including *Bunias orientalis*, *Acanthosyris Paulo-alvinii*, *Xanthoxyllum setulosum* and *Eucommia ulmoides* and *Lolium perenne*, *A. cephalotes*. (Chavez et al., 1997; Dietz & Winterhalter, 1996; Hodges & Porte, 1964; Okada et al., 1994; Okumade & Wiemer, 1985). The spectral data of BvN21 were in agreement with those published (Chavez et al., 1997; Dietz & Winterhalter, 1996; Hodges & Porte, 1964; Okada et al., 1994; Okumade & Wiemer,
1985). Loliolide was also isolated from the Opisthobranch mollusc (Pettit et al., 1955)

The biological properties reported for this compound include allelochemical property (Dietz & Winterhalter, 1996), ant-repellent potentials (Okumade & Wiemer, 1985), immunosuppressive activity against mice lymphocytes (Okada et al., 1994) and cancer activity (Pettit et al., 1955).

Table 5.5.2.5: ^{1}H, ^{13}C and COSY NMR for BV5 in $CDCl_3$

Position	^{13}C (δ)	^{1}H (δ)	^{HH}J-coupling constant (Hz)	COSY coupling ^{1}H
6	181.4			
8	172.3			
7	113.4	5.70	s	

5	87.1			
3	65.3	4.12	dddd, *J*= 12.0, 10.3, 4.7, 3.2	1.32, 1.49, 2.02, 2.52
2	49.9	2.02, 1.31	ddq, *J*=13.8, 4.6 , 2.3 t, *J*=11.4	1.32, 2.52
4	48.0	2.52, 1.49	dqq, *J*=11.6, 2.8, 1.5 td, *J*=11.4, 2.1	1.49 2.52, 4.12
1	35.3			
9	30.1	1.29	s	
11	25.7	1.56	s	
10	25.2	1.24	S	

^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively **Table 5.5.2.5a: HMBC for Bv5**

^{1}H (δ)	***J*-Coupling ^{13}C**
2.02	65.3
5.70	35.3, 87.1, 172.3, 181.4
1.32	65.3, 35.3
1.49	65.3, 87.1
1.29	181.4, 25.2, 35.3, 49.9
1.56	48.0, 87.1, 181.4
1.24	181.4, 30.1, 35.3, 49.9

Table 5.5.2.5b: NOESY for Bv5

^{1}H (δ)	Coupling ^{1}H
5.70	1.32
4.12	1.24, 1.56

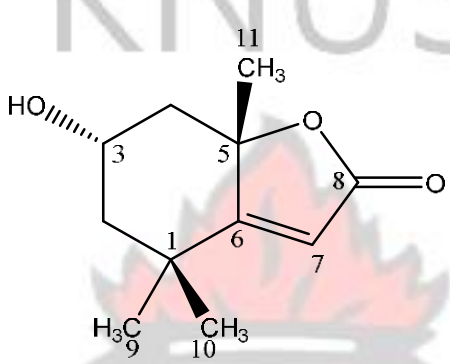


Figure 5.5.2.5: Structure of Bv5 [6-hydroxy-4, 4, 7a-trimethyl-5, 6, 7, 7atetrahydrobenzofuran-2(4H)-one or loliolide]

6.5.2.6 Structural elucidation of BvM2C1 (Bv6)

BvM2C1, Compound **Bv6,** (33 mg) was obtained was obtained as a yellow substance. IR absorption bands at 3414, 1121, 1658, 1121, 1263, 1609 and 1658 were ascribed to phenol OH, methoxy, ketone, oxygen bridge and C=C bonds. The HR-ESI-MS showed a peak at *m/z* 331.0825 $[M+H]^+$ (calcd. for $C_{17}H_{15}O_7$ [M+H]+, 331.0818, mDa 0.7; Appendix 14a) corresponding to the molecular formula $C_{17}H_{14}O_7$. Eleven (11) degrees of unsaturation were calculated and were assigned to a carbonyl function, seven (7) C=C bonds and three (3) rings.

The ^{1}H NMR spectrum (Appendix 14b; Table 5.5.2.6) showed two equivalent aromatic singlets at δ 7.33 (s) and δ 6.98 (s), two doublets at δ 6.20 (d, *J*=2.1) and δ 6.55 (d,

J=2.1), two equivalent methoxy singlet at δ 3.88 and a singlet at δ 12.96 typically characteristic of extended H-bonding signal for 5-hydroxyisoflavones in aprotic solvents (Santos et al., 1995).

The ^{13}C-NMR spectrum (Appendix 14b; Table 5.5.2.6) revealed 17 carbon resonances due to a carbonyl signal at δ 181.8; five sp^2 methine peaks at δ 104.4, δ 103.6, δ 98.8 and δ 94.2; seven oxygenated aromatic peaks at δ 163.62, δ 161.4, δ 164.2, δ 157.3, δ 139.9 and δ 148.2, and a methoxy signal at 56.4. Two equivalent carbons at δ 104.4, δ 148.2, and δ 56.4 each.

Careful analysis of the 2D spectra including H-H COSY HSQC (Table 5.5.2.6) and HMBC spectra (Table 5.5.2.6a) with the coupling constants of the peaks in the ^{1}H NMR spectrum resulted in the resolution of compound **Bv6** as 5,7,4'-trihydroxy-3',5'dimethoxyflavone (Figure 5.5.2.6), trivially known as tricin. The spectra data of Bvm2c11 were in accordance with the literature data (Bhattacharyya et al., 1978) Tricin has been isolated from other members of the Poacea family including *Spartina cynosuroides*, *Poa ampla* and rice. It was also isolated from *Seriphidium santolium* Pol*J*ak (compositae) (Bhattacharyya et al., 1978; Deng et al., 2004; *J*u et al., 1998; Kong et al., 2004) and from the fern *Lycopodium Japonicum* (Yan et al., 2005). It displayed allelochemical properties, inhibited the spore germination of fungal pathogens *Pyricularia oryzae* and *Rhizoctonia solani* (Kong et al., 2004) and demonstrated moderate antitumor activity (Yan et al., 2005)

Table 5.5.2.6: ^{1}H, ^{13}C, and COSY NMR forBv6 in DMSO

Position	^{13}C (δ)	^{1}H (δ)	COSY coupling
4	181.8		
7	164.2		
2	163.622		
5	161.4		
10	157.3		
3',5'	148.2		
4'	139.9		
1'	120.4		
2', 6'	104.4	7.32 (s)	
9	103.7		
3	103.6	6.98(s)	
6	98.8	6.20 (d, *J*=2.1)	6.55
8	94.2	6.55 (d, *J*=2.1)	
OCH_3	56.4	3.88 (s)	
HO-5	-	12.96 (s)	

Table 5.5.2.6a: HMBC for Bv6

^{1}H (δ)	*J*-Coupling ^{13}C
7.32	104.4, 120.4, 139.9, 148.2, 163.6
6.98	103.7, 120.4, 163.6, 181.8

[1] H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively

6.20	94.2, 103.7, 161.4, 164.1
6.55	98.8, 103.6, 164.2, 157.4
3.88	148.2, 104.4
12.96	98.8, 103.6, 103.7, 161.4

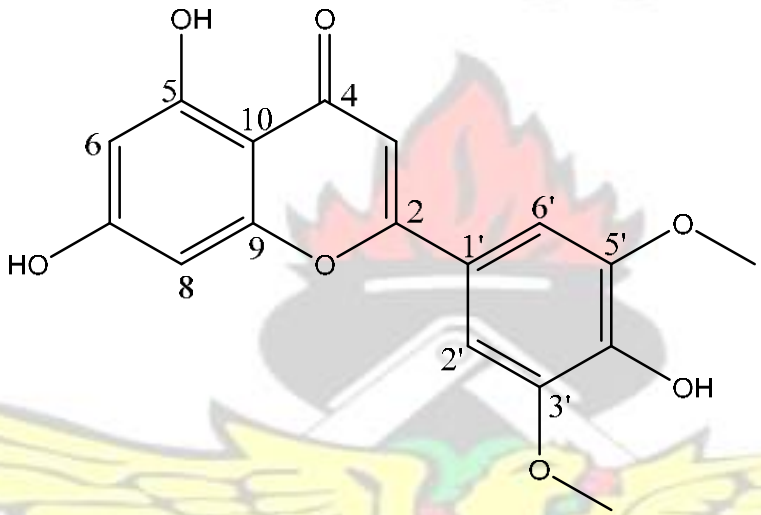


Figure 5.5.2.6: Structure of BvM2C1 [5, 7, 4'-trihydroxy-3', 5'-dimethoxyflavone /tricin]

6.5.3 *In vitro* antiplasmodial and cytotoxic activities of *P. fraternus* compounds

The six (6) compounds isolated from *P. fraternus* exhibited antiplasmodial activity against *P. falciparum* strains 3D7 and W2 (IC50 < 28 µM and IC50 < 60 µM respectively; Table 5.5.3). **Pf2** (nirurine) exhibited the highest activity (IC_{50}: 4.19±1.13 µM) against 3D7 *P. falciparum* but was weakest (IC_{50}: 59 ± 5.43 µM) against W2 *P. falciparum*. **Pf3** (norsecurine) showed the strongest activity (IC_{50}:

1.14±0.32 µM) against W2 *P. falciparum* but was weak (IC_{50}: 21.24 ± 2.21 µM) against 3D7 *P. falciparum*. Only two compounds (**Pf1**; Phyllanthin**,** and **Pf2**: nirurine) showed cytotoxicity against HUVECs with CC_{50} <22 µM. **Pf1** and **Pf2** displayed low selective indexes. The levels of resistance to the compounds were generally low except **Pf2**, to which resistance was moderate (Table 5.5.3).

Table 5.5.3: *In vitro* antiplasmodial activity and cytotoxicity of compounds from *P. fraternus*

COMPOUND	IC_{50}; 3D7	IC_{50}; W2	CC_{50}; HUVECs	SI 3D7	SI W2	RI
Pf1	26.23 ± 3.47	5.65 ± 1.48	5.89 ± 0.77	0.2	1.0	0.2
Pf2	4.19 ± 1.13	59.00 ± 5.43	21.10 ± 2.94	5.0	0.4	14.1
Pf3	21.24 ± 2.21	1.14 ± 0.32 [a]	>100	>4.7	>87.7	0.1
Pf4	42.53 ± 6.42	2.57 ± 0.53 [a]	>100	>2.4	>38.9	0.1
Pf5	6.24 ± 0.58	27.69 ± 1.75	>100	>16.0	>3.6	4.4
Pf6	27.23 ± 2.97	23.67 ± 1.80	>100	>3.7	>4.2	0.9
CQ	0.02 ± 0.00	0.11± 0.00	ND	ND	ND	5.5

Keys: IC_{50}: W2: W2 strain of *P. falciparum*; 3D7: 3D7 strain of *P. falciparum*; CQ: chloroquine; ND: not determined; SI: Selectivity Index; RI: Resistance Index; Concentration of CQ was expressed in µM; IC_{50} and CC_{50} are expressed in µM ± SD; [a]: the µg/mL equivalent of the IC_{50} is less than 1µg/mL (1.14 µM =0.23 µg/mL and 2.57 µM = 0.52 µg/mL**.**

6.5.4 *In vitro* antiplasmodial and cytotoxic activities of *B. vulgaris* compounds

The six (6) compounds exhibited antiplasmodial activity with IC_{50} generally less than 5 µM against 3D7 *P. falciparum* and less than 7 µM against W2 strain. **Bv1** (*p*coumaric acid) was the most active against 3D7 (IC_{50}: 0.84 ± 0.90 µM) but **Bv2** (cinnamic acid) was the most active against W2 *P. falciparum* (IC_{50}: 1.41 ± 0.38 µM; Table 5.5.4). The compounds did not exhibit toxicity against HUVECs (CC_{50}s > 100 µM). They were selective towards the parasite (Selectivity index (SI) > 16.0; Table 5.5.4). The levels of resistance to the compounds were generally low (Table 5.5.4).

Table 5.5.4: *In vitro* antiplasmodial activity and cytotoxicity of compounds from *B. vulgaris*

COMPOUND	IC_{50}; 3D7	IC_{50}; W2	CC_{50}; HUVECs	SI, 3D7	SI, W2	RI
Bv1	0.84 ± 0.90	1.54 ± 0.60	>100	>119.0	>64.9	1.8
Bv2	0.92 ± 0.10	1.41 ± 0.38	>100	>108.7	>70.9	1.5
Bv3	1.23 ± 0.12	2.5 ± 1.40	>100	>81.3	>40.0	2.0
Bv4	2.89 ± 1.25	3.11 ± 1.08	>100	>34.6	>32.2	1.1
Bv5	4.32 ± 1.25	6.24 ± 1.81	>100	>23.1	>16.0	1.4
Bv6	4.54 ± 0.37	5.93 ± 1.13	>100	>22.0	>16.9	1.3
CQ	0.02 ± 0.00	0.11± 0.00	ND	ND	ND	ND

Keys: see keys for Table 5.6.3.1.

6.6 DISCUSSION

6.6.1 P. fraternus

In Chapter 4, the antiplasmodial activity of the water and various organic solvents comprising methanol, ethyl acetate, and petroleum ether extracts of *P. fraternus* (whole plant), *B. vulagaris* (leaf), *T. grandis* (leaf), *T. ivorensis* (leaf), *and S. siamea* (root) were established. The methanol fraction of *P. fraternus* was one of the fractions showing the promising antiplasmodial activity (IC50 < 1µg/mL) and so was subjected to chromatographic separation. This led to the isolation of five (5) securinega alkaloids; three (3) neonor-securinine-type identified as nirurine (**Pf2**), bubialine (**Pf5**) and epibubialine (**Pf6**) and two (2) norsecurinine –type namely *ent*-norsecurinine (**Pf3**) and *allo*-norsecurine (**Pf4**) (Chirkin et al, 2015), and a lignan, phyllanthin (**Pf1**) (Figures 5.5.1.1-6). Norsecurinine-type alkaloids differ from the securinine-type alkaloids by their 5-membered pyrrolidine ring A instead of the piperidine ring A in securinines.

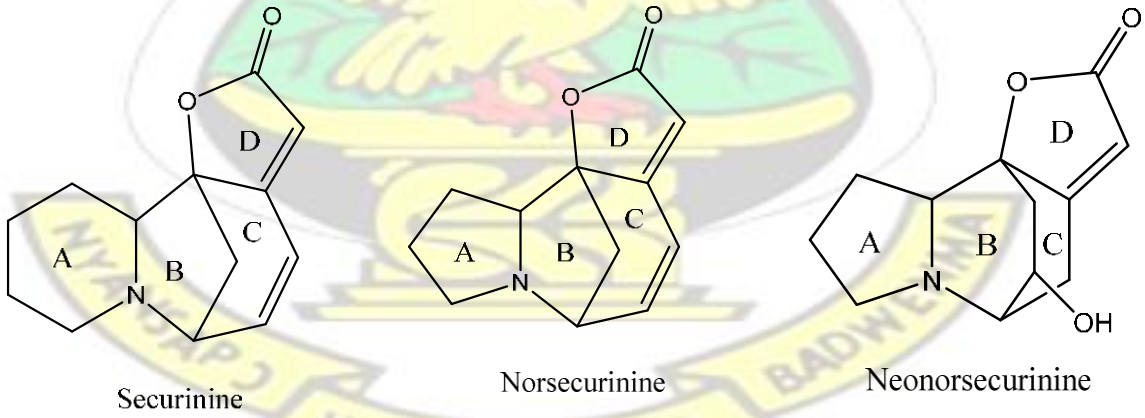


Figure 5.6.1: Types of securinega alkaloid

Both norsecurine- and neonorsecurinine-type securinega alkaloids are analogues of securinine with a 5-membered pyrrolidine ring, A. Securinine has a 6-membered

piperidine ring, A (Figure 5.6.1; Chirkin et al., 2015). Norsecurinine-type alkaloids possesses a tetracyclic structure with a methylene bridge, an azabicyclo[3.2.1]octane system as five-membered cyclopentyl B ring and cyclohexenyl C rings, pyrrolidine ring A and a conjugated dienic system (Chirkin et al., 2015). Neonor-securinine-type securinega alkaloid is a subgroup of nor-securinine-type alkaloids, and possesses a hydroxy-ethylene bridge, a 2-azabicyclo [2.2.2] octane unit as cyclohexyl B and C rings (Chirkin et al, 2015).

Ent-norsecurinine and *allo* -norsecurinine are epimers; they differ from each other by the relative configuration of the methylene bridge and the proton at position 2. Entnorsecurinine has α-oriented proton at position 2 and β-oriented methylene bridge (Figure 5.5.1.3). In *Allo*-norsecurinine, the two groups are α-oriented (Figure 5.5.1.4).

For the neonorsecurine-type alkaloids, Epibubbialine (Figure 5.5.1.6) differs in its 3D structure from bubbialine (Figure 5.5.1.5). In epibubbialine, the proton at position 2 is in α-orientation and the hydroxy-ethylene bridge is in a β-orientation. On the other hand, in bubbialine both the proton at position 2 and the hydroxy-ethylene bridge are β-oriented. Also, nirurine, which is epibubbialine -derivative, differs from epibubbialine by the presence of the ether linkage between the oxy-ethylene bridge and C-5 of the pyrrolidine ring A.

Bubialine and epibubialine are epimers, and were previously isolated from *Zygogynum pauciflorum* by Ahond et al (1990) and *Phyllanthus amarus* by Houghton et al (1996) respectively. *Ent*-norsecurinine was isolated from *Phyllanthus niruri* (Joshi et al.,

1986) whereas *allo* –norsecurinine had never been isolated from natural source but was synthesied (Medeiros & Wood, 2010b)

The securinega alkaloids are particularly common in the Phyllanthaceae family and appear limited to this family (Chirkin et al., 2015). They are more specific to the genera *Securinega, Phyllanthus, Breynia,* and *Margaritaria* which are largely endemic in tropical and subtropical regions from Africa to Asia (Chirkin et al., 2015). Interestingly, the neonorsecurine-type securinega alkaloid, bubbialine occurs also in the non-homologous species, *Zygogynum pauciflorum* of the family, Winteraceae (Ahond et al., 1990)

This is the first isolation of bubbialine from the genus *Phyllanthus* though the epimer, epibubbialine was isolated from *Phyllanthus amarus* (Houghton et al., 1996). This is also the first isolation of *allo* -norsecurinine from a natural source. In addition and to the best of my knowledge, this is the first isolation of alkaloids from *P. fraternus* though these have been reported in related species of the *Phyllanthus* genus including *P. amarus*, *P. niruri*. *P. simplex, P. discoideus* and *Phyllanthus discoides* (Chirkin et al., 2015).

From the results of the antiplasmodial assay, all five alkaloids and phyllanthin demonstrated interesting antiplasmodial activity. The norsecurinine-type alkaloids (*ent*-norsecurinine and *allo* -norsecurinine) and neonorsecurinine-type alkaloids (bubbialine and nirurine except epibubbialinine) exhibited contrasting activity against the two strains, 3D7 and W2 *P. falciparum* (Table 5.5.3). Though the mechanism of action was not determined, this difference in activity suggested a possible relationship

between the chemical structures of the molecules and their activity on the particular strain (3D7 or W2) of *P. falciparum*. The norsecurinine-type alkaloids (*ent*norsecurinine and *allo*-norsecurinine) largely showed higher activity against W2 (1.14

± 0.32 and 2.57 ± 0.53) but low activity against 3D7 (21.24 ± 2.21 and 42.53 ± 6.42). On the other hand, the neonorsecurines (nirurine, and bubbialine) showed weaker activity against W2 (59 ± 5.43 and 27.69 ± 1.75 respectively) but were stronger against 3D7 (4.19 ± 1.13 and 6.24 ± 0.58) except epibubbialine which exhibited generally the same level of activity against the two strains. These differences in the levels of activity of the norsecurinine-type and that of the neonorsecurinine-type alkaloids against the two strains (W2 and 3D7) of *P. falciparum* may be accounted for by the differences in the chemical structures of the two related groups. The norsecurinine-type alkaloids isolated possess a methylene bridge, a cylohexenyl ring B and a conjugated dienic system as part of the molecule whereas the neonorsecurinine-types have hydroxyethylene bridge and cyclohexyl ring B system as part of the molecule (Figure 5.6.1). The molecules bearing methylene bridge, the five-membered ring B and the conjugated dienic systems (*ent*-norsecurinine and *allo* -norsecurinine) were more active against W2 *P. falciparum* compared to those with hydroxy-ethylene bridge and cyclohexyl ring B system (the neonorsecurinine: bubbialine and nirurine) except epibubbialine which was more active against W2 *P. falciparum*. The reverse is the case for 3D7 strain of the parasites. The methylene bridge, the seven-membered ring, B and/or the conjugated dienic system of the norsecurinines may be responsible for the higher activity against W2 *P. falciparum*, while the hydroxy-ethylene bridge and/or cyclohexyl rings B might have induced the high activity of the neonorsecurinines against 3D7 P. falciparum.

For the norsecurinine-type alkaloids, though activity seemed to reside in the conjugated dienic system of these compounds, the 3D structure of the compounds may also contribute to their overall activity. The activity exhibited by *ent*-norsecurinine is twice as effective as that of the epimer, *allo*-norsecurinine (Table 5.5.3), and this could be explained on the bases of the differences in their 3D structures. In noresecurinine, the proton at position 2 (H-2) (Figure 5.5.1.3) is opposite in orientation to the methylene bridge about the plane of the molecule whereas in *allo*-norsecurinine, it is on the same side of the plane as the the methylene bridge (Figure 5.5.1.4). Therefore the opposite orientation of the proton at position 2 (H-2) to the methylene brigde about the plane of the molecule, probably enhances antiplasmodial activity.

The neonorsecurinine-type alkaloids also exhibited a structure-activity relationship regarding their chemical and 3D structures and the antiplasmodial activity they showed. Bubbialine exhibited higher activity against 3D7 when compared to the epimer, epibubbialine. This difference in activity can be explained by the 3D structure of these neonorsecurinines. When the proton at position 2 (H-2) is in the same orientation as the hydroxy-ethylene bridge about the plane of the molecule, as in bubbialine (Figure 5.5.1.5), antiplasmodial activity is enhanced. However, activity is low when the proton is in opposite orientation to the hydroxy-ethylene bridge as in epibubbialine (Figure 5.5.1.6).

Nirurine (Figure 5.5.1.2) which differs from epibubbialine (Figure 5.5.1.6) only by the ether linkage between the oxy-ethylene bridge and the pyrrolidine ring A also showed high activity when compared to epibubbialine, suggesting the presence of the ether (R-O-R) linkage resulted in the potentiation of its activity.

There was an earlier report of the antiplasmodial activity of the analogue of norsecurine-type securinega alkaloid, Securinine (Weenen et al., 1990). Securinine and related compounds including norsecurinine, the stereoisomer of the *ent*norsecurinine and *allo* –norsecurinine, bound to *Plasmodium falciparum* 2′deoxyuridine 5′-triphosphate nucle-otidohydrolase (PfdUTPase) thereby enhancing the enzyme activity and inhibiting viability of both *P. falciparum* gametocyte (sexual) and blood (asexual) stage parasites (Vu et al., 2013). The antiplasmodial activity of the 5 securinega alkaloids namely, *ent*-norsecurinine, *allo* -norsecurinine, bubbialine, epibubbialine and nirurine, isolated from *P. fraternus*, could be explained by this mechanism.

The antiplasmodial classification of the compounds was as follows: IC_{50} < 1 μM, excellent/potent activity; IC_{50} of 1-20 μM, good activity; IC_{50} of 20-100 μM, moderate activity; IC_{50} of 100-200 μM, low activity; and IC_{50} > 200 μM, inactive (Batista et al., 2009). Thus, from the results, **Pf1** (phyllanthin), **Pf2** (nirurine), **Pf3** (*ent*norsecurinine), **Pf4** (*allo* –norsecurinine), and **Pf5** (bubbialine) were designated as having good activity ($1 \leq IC_{50} \leq 20$ μM) and **Pf6** (epibubbialine) (IC_{50}: 23.67 ± 1.8 W2 and 27.23 ± 2.97; 3D7) had generally moderate activity ($20 \leq IC_{50} \leq 100$ μM).

Of the three (3) neonorsecurine alkaloids, nirurine was the most active against 3D7 (IC_{50}: 4.19 ± 1.13) but the weakest against W2 (IC_{50}: 59 ± 5.43). It was, also, the only alkaloid with cytotoxicity (CC_{50} <100 μM) against HUVECs (CC_{50} = 21.1±2.94 μM). The ether bridge may be responsible for its cytotoxicity, since the derivative, epibubbialine did not show cytoxicity. Thus, the norsecurinine-types alkaloids may be

showing (chemical) structure-related cytotoxicity. Apart form nirurine and phyllanthin which displayed cytotoxicity (CC_{50} < 100 μM) against HUVECs, the other molecules did not show cytotoxicity. The lignan, phyllanthin, the only non-alkaloid of the compounds displayed the highest cytotoxicity against HUVECs and was about 4 times as cytotoxic as nirurine (Table 5.5.3). Phyllanthin enhanced the cytotoxic response mediated by vinblastine against multidrug-resistant KB cells (Somanabandhu et al., 1993). The relatively high cytotoxicity of nirurine among the alkaloids could be attributed to the ether-linkage, which also appeared to have influenced the antiplasmodial activity of this epibubbialine derivative (nirurine) against 3D7 strain of *P. falciparum*.

The selectivity index (SI) of the compounds with respect to 3D7 ranged from 0.2 to 16.0 or more with phyllanthin having the lowest selectivity index of 0.2. In the case of W2, the compounds showed SI range of 0.4 to >87 with nirurine showing the lowest SI of 0.4 for W2. In general, the neonorsecurines seem to be more selective towards 3D7 while the norsecurinines are towards W2. Phyllanthin generally exhibited low SI against both strains of the parasite.

The microgram per mille (μg/mL) equivalent of the IC_{50} values of **Pf3** (*ent*norsecurinine) and **Pf4** (*allo* –norsecurinine) were less than 1 μg/mL (respectively 0.2 μg/mL and 0.5 μg/mL; Table 5.5.3) against W2 *P. falciparum*. Their selectivity indexes (SI) were above 10 (SI > 87.72 and SI > 38.91 respectively). According to Pink et al, (2005), compounds that are active against whole parasite with IC_{50} < 1 μg/mL and SI>10, are defined as 'hit', and can be considered for further testing in animal models of the disease. **Pf3** (*ent*-norsecurinine) and **Pf4** (*allo* –norsecurinine)

having met this criteria should be considered for *in vivo* assessment in animal models of *plasmodium* parasite infection, and eventual optimization for efficacy and good pharmaceutical properties.

Resistance index (RI) of the compounds ranged from 0.05 to 14.1 whereas the reference drug, chloroquine, exhibited RI of 5.5 (Table 5.5.3). The norsecurinine-type alkaloids (*ent*-norsecurinine and *allo*-norsecurinine) demonstrated the lowest resistance index (RI: 0.1). On the other hand, the neonorsecurines (nirurine, bubbialine and epibubbialine) displayed low to moderate levels of resistance ($0.9 \leq RI \leq 14.1$). According to Nzila & Mwai, (2009), the higher the RI, the higher the level of resistance of the resistant strain of the parasite to the particular agent. Lower RI could be an indication of lower level of resistance of the parasite to the particular molecule. The norsecurines, with their low RI, may provide a more effective therapy against malaria than the neonorsecurinine analogues, since the resistant strain of the plasmodium parasite may not exhibit resistance to them (norsecurinines).

Securinega alkaloids exhibited many biological activities including neurological, antineoplastic, and antibacterial activities. Some bound to *Plasmodium falciparum* 2'deoxyuridine 5'-triphosphate nucleotidohydrolase (PfdUTPase) in a fragment-based screening using mass spectrometry (Vu et al., 2013). *Ent* -norsecurinine inhibited the spores of many fungi including *Alternaria brassicae, Curvularia penniseti, Curvularia spp., Erysiphe pisi, Fusarium udum, and Helminthosporium frumentacei.* It was also reported to have prevented the development of phytopathogen *Erysiphe pisi* on *Pisum sativum* plant. (Chirkin et al., 2015). Phyllanthin showed antioxidant potential in both human fibroblasts and keratinocytes and inhibited the growth of *Staphylococcus*

aureus (Lam et al., 2012). It also exhibited cytotoxicity against K-562 cells and Lucena-1 (Leite et al., 2006). *P. fraternus* is used for the traditional treatment of malaria and the various extracts were established to possess activity against *P. falciparum* (Chapter 5; Sittie et al., 1998). The antiplasmodial activity of the plant can therefore be explained on the basis of the antiplasmodial activity of the six (6) isolated compounds and probably other unknown constituents. Finally, Phyllanthin and nirurine may contribute to the cytotoxicity of the aqueous extracts of *P. fraternus* against HUVECs

6.6.2 ***B. vulgaris***

Six (6) compounds; **Bv1** to **Bv6** identified as *p*-coumaric acid [(E)-3-(4hydroxyphenyl) acrylic acid], cinnamic acid, dehydrovomifoliol, 3-oxo-α-ionol, loliolide and tricin were isolated from the methanol extract of *B. vulgaris*. This is the first reported isolation of compounds from *B. vulgaris* though the same compounds have been isolated from different plants and organisms.

Some of the compounds bear chemical similarities; *p*-coumaric acid acid is a parahydroxy derivative of cinnamic acid so is dehydrovomifoliol (Figure 5.5.2.3) and 3oxo-α-ionol (Figure 5.5.2.4) where the former have a ketone function instead of hydroxyl function at position 12 of the side chain and hydroxy substitution at position 4 of the cyclohexenone ring.

The compounds from *B. vulgaris* displayed remarkable activity against both strains of *P. falciparum* with $IC_{50} < 5$ (five) µM against 3D7 and less than 10 µM against W2 (Table 5.5.4). For related compounds, activity appeared to be influenced by the chemical differences. For instance, **Bv1** (*p*-coumaric acid) is a para-hydroxy derivative of **Bv2** (cinnamic acid) and is more active than **Bv2.** The relatively higher activity of **Bv1** (Table 5.5.4) could only be explained by the presence of the hydroxyl function. This phenomenon seems to have played a role in the activity of **Bv3 (**dehydrovomifoliol) and **Bv4 (**3-oxo-α-ionol) where **Bv3** was more active. **Bv3** possesses a ketone function instead of hydroxyl function at position 12 of the side chain and a hydroxy substitution at position 4 of the cyclohex-2-en-1-one ring and this appeared to have enhanced its activity.

Altogether, the compounds demonstrated either excellent/potent (IC_{50} < 1 μM) or good (IC_{50} of 1-20 μM) activity. The non-cytotoxicity shown by the aqueous and organic extracts of *B. vulgaris* (as reported in section 4.2) was reflected in the constituent isolated. From the literature, the compounds isolated from *B. vulgaris* have demonstrated various biological activities including phytotoxicity by 5, 7, 4'trihydroxy-3',5'-dimethoxyflavone (tricin); 6-hydroxy-4,4,7a-trimethyl-5,6,7,7atetrahydrobenzofuran-2(4H)-one (loliolide); (E)-4-(3-hydroxybut-1-en-1-yl)-3,5,5trimethylcyclohex-2-en-1-one (3-oxo-α-ionol) and (E)-4-hydroxy-3,5,5-trimethyl-4(3-oxobut-1-en-1-yl) cyclohex-2-en-1-one (dehydrovomifoliol) (DellaGreca et al,

2004). Indeed the leaf extract of *B. vulgaris* showed allelopathicity (Alencar et al., 2015). The antiplasmodial activity of the extracts of the plant is thus confirmed by the antiplasmodial activity of the isolated compounds.

6.7 CONCLUSION

Six (6) compounds; five sercurinega alkaloids and a lignan were isolated from the methanol extract of *P. fraternus*. The lignan, **Pf1 [**phyllanthin], and the five alkaloids comprising **Pf2** [nirurine]; **Pf3** [*ent*-norsecurinine]; **Pf4** [*allo* –norsecurinine]; **Pf5** [bubbialine] and **Pf6** [epibubbialine] displayed antiplasmodial activity against both W2 and 3D7 strains of *P. falciparum* with norsecurinine-type alkaloids being more active against W2 and the neonorsecurinine alkaloids being more active against 3D7 strain. Apart from phyllanthin and nirurine, the compounds were non-toxic to HUVECs and were more selective for the parasites.

The methanol extract of *B. vulgaris* yielded six (6) compounds; two aromatic acids viz **Bv1** [*p*-coumaric acid or (E)-3-(4-hydroxyphenyl) acrylic acid] and **Bv2 [**cinnamic acid]; **Bv3 [** (E)-4-hydroxy-3, 5, 5-trimethyl-4-(3-oxobut-1-en-1-yl) cyclohex-2-en-1one or dehydrovomifoliol]; **Bv4** [(E)-4-(3-hydroxybut-1-en-1-yl)-3,5,5-trimethylcyclohex-2-en-1-one or 3-oxo-α-ionol]; **Bv5 [**6-hydroxy-4, 4, 7a-trimethyl-5, 6, 7, 7atetrahydrobenzofuran-2(4H)-one or loliolide]; and **Bv6 [**5,7,4'-trihydroxy-3',5'dimethoxyflavone, trivially known as tricin]. All six compounds displayed antiplasmodial activity against both W2 and 3D7 *P. falciparum.* The compounds did not show any cytotoxicity to HUVECs and were more selective for the parasites.

CHAPTER 6

7 GENERAL DISCUSSION, CONCLUSION, AND RECOMMENDATIONS.

7.1 GENERAL DISCUSSION

The ethnobotanical survey component of this study amply demonstrated the existence of undocumented knowledge of medicinal plants used to treat malaria in Ghana. In the study, twenty (20) plant species were for the first time recorded in Ghana with twelve (12) of these recorded for the first time worldwide. The use of these plants in the preparation of commercial and often regulated finished herbal products for the treatment of malaria points to their perceived efficacy against malaria.

The aqueous extracts and various organic solvent fractions of the selected plant materials; the whole of *Phyllanthus fraternus* G.L.Webster (Phyllanthaceae), the leaves of *Bambusa vulgaris* Schrad. (Poaceae), *Tectona grandis* L.f (Lamiaceae), *Terminalia ivorensis* A. Chev (*Combretaceae*) and root of *Senna siamea* (Lam.) Irwin & Barneby (Fabaceae), display remarkable *in vitro* antiplasmodial activity (Tables 4.3.1 and 4.3.2) and only two extracts, the aqueous extracts of *T. ivorensis* and *P. fraternus* showing toxicity against HUVECs with all extracts showing high selectivity indexes towards the *plasmodium* parasites (Tables 4.3.1). These may be an indicative of their potential usefulness in the traditional treatment of malaria in Ghana.

The organic extracts of *B. vulgaris*, *T. grandis* and *P. fraternus* were more active than the corresponding aqueous extracts. This suggests the possibility of such organic extracts containing higher amount of the active constituents or constituent with higher antiplasmodial activity.

Altogether, twelve (12) compounds were isolated from the methanol extracts of the two plants materials; *P. fraternus* (whole plant) and *B. vulgaris* (leaf). This is the first isolation of **Pf4** (*allo* -norsecurinine) from a natural source, the first isolation of **Pf5**

(bubbialine) from the Phyllanthus genus, the first isolation of the sercurinega alkaloids from *P. fraternus,* the first ever-reported isolation of phytocontituents from *B. vulgaris* and the first report of antiplasmodial assay on these compounds.

7.2 CONCLUSION AND RECOMMENDATION

The project has demonstrated that, Ghanaian medicinal plants used to treat malaria in traditional medicine could be a potential source of antiplasmodial compounds. Ninetyeight (98) medicinal plants are used to treat malaria in Bosomtwe and Sekyere East Districts of Ghana were inventoried. The aqueous extracts of the five (5) selected plant materials displayed antiplasmodial activity. Twelve (12) compounds; **Pf1** (phyllanthin); **Pf2** (nirurine); **Pf3** (*ent*-norsecurinine); **Pf4** (*allo* -norsecurinine); **Pf5** (bubbialine) and **Pf6** (epibubbialine) from *P. fraternus,* and **Bv1** (p-coumaric acid); **Bv2** (cinnamic acid); **Bv3** (dehydrovomifoliol); **Bv4** (3-oxo-α-ionol or 9hydroxymegastigma-4, 7-dien-3-one); **Bv5** (loliolide) and **Bv6** (tricin) from *B. vulgaris* were isolated and demonstrated activity against the chloroquine-sensitive 3D7 and chloroquine-resistant W2 *P. falciparum*. all twelve compounds, six (6) from *P. fraternus* and six (6) from *B. vulagaris,* are for the first time reported for antiplasmodial activity. This is the first isolation of these compounds from *P. fraternus* except phyllanthin, and the first isolation of *allo*-norsecurinine from a natural source. This is also the first reportedisolation from *B. vulgaris.* Not only did the search for antiplasmodial compounds from Ghanaian medicinal plant yield twelve (12) +antiplasmodial compounds that would be added to the library of natural compounds with antiplasmodial activity, but the presence of these compounds and other similar ones in the respective plants explains the antimalarial activity of the plants as used in traditional medicine.

It was realized from the study that many of the medicinal plants cited in this study are reported for the first time, and this may mean many such plants are yet to be recorded in Ghana. It may therefore be relevant to carry out ethnobotanical surveys in other locations in Ghana. This will ensure a comprehensive inventory of medicinal plants used to treat malaria in Ghana.

The organic extracts of some plants were more active than the corresponding aqueous extracts and the use of suitable organic solvents for the extraction of such plants in traditional medicine for enhanced activity should be a subject of further studies. In such studies, toxicity evaluation should be given an equal attention.

Some of the compounds in this study clearly exhibited structure-related activity and are worthy of further study, and perhaps chemical modifications to enhance the benefit/risk ratio. The mechanism of action of the compounds with interesting activity should be studied and this may help in any chemical modification of the respective compound. In addition, the purified compounds could be evaluated in drug combinations with themselves and with classical antiplasmodial drugs to identify possible synergistic effects against *P. falciparum in vitro* model. Herbal antimalarial products containing *P. fraternus* or/and *B. vulgaris* should be standardized using the purified compounds as chemical markers.

REFERENCES

Aasen, A. J., Kimland, B., Enzell, C. R., Sørensen, N., Lindberg, A., & Ehrenberg, L. (1971). Tobacco Chemistry. 7. Structure and Synthesis of 3-Oxo-alpha-ionol, a New Tobacco Constituent. *Acta Chemica Scandinavica*, *25*(4), 1481–1482. http://doi.org/10.3891/acta.chem.scand.25-1481

Abbiw, D. K. (1990). *Useful Plants of Ghana*. London: Royal Botanic Gardens, KEW.

Abdul, M., Momin, M., Bellah, S. F., Afrose, A., Urmi, K. F., Hamid, K., & Rana, S. (2012). Phytochemical Screening and Cytotoxicity Potential of Ethanolic Extracts of *Senna siamea* Leaves, *4*(8), 1877–1879.

Adewunmi, C. O., Agbedahunsi, J. M., Adebajo, A. C., Aladesanmi, A. J., Murphy, N., & Wando, J. (2001). Ethno-veterinary medicine: screening of Nigerian medicinal plants for trypanocidal properties. *Journal of Ethnopharmacology*, *77*(1), 19–24. http://doi.org/10.1016/S0378-8741(01)00247-1

Aguinaldo, A. M., Ocampo, O. P. M., Bowden, B. F., Gray, I., & Peter, G. (1993). Tectograndone, an anthraquinone-naphthoquinone pigment from the leaves of *Tectona grandis*. *Phytochemistry*, *33*(4), 933–935.

Agyare, C., Asase, A., Lechtenberg, M., Niehues, M., Deters, A., & Hensel, A. (2009). An ethnopharmacological survey and *in vitro* confirmation of ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima-Kwanwoma area, Ghana. *Journal of Ethnopharmacology*, *125*(3), 393–403. http://doi.org/10.1016/j.jep.2009.07.024

Ahmed, B., Al-Howiriny, T. A., & Mathew, R. (2002). Antihepatotoxic activity of *Phyllanthus fraternus*. *Die Pharmazie*, *57*(12), 855–6.

Ahond, A., Guilhem, J., Hamon, J., Hurtado, J., Poupat, C., Pusset, J., Pusset, M.

Sevenet, T. & Potier, P. (1990). Bubbialine et bubbialidine, alcaloides nouveaux extraits de Zygogynum pauciflorum. *Journal of Natural Products*, *53*(4), 875–881.

Ajaiyeoba, E. ., Oladepo, O., Fawole, O. ., Bolaji, O. ., Akinboye, D. ., Ogundahunsi, O. A. ., Falade, C.O., Gbotosho, G.O., Itiola, O.A., Happi, T.C., Ebong, O.O., Ononiwu, I.M., Osowole, O.S., Oduola, O.O., Ashidi, J.S. & Oduola, A. M. . (2003). Cultural categorization of febrile illnesses in correlation with herbal remedies used for treatment in Southwestern Nigeria. *Journal of Ethnopharmacology*, *85*(2-3), 179–185. http://doi.org/10.1016/S03788741(02)00357-4

Alencar, S. R., Silva, M. a. P., Figueiredo, M. F., Santos, M. a. F., Generino, M. E. M., Torquato, I. H. S., & Crispim, M. K. M. (2015). Biological Activity of *Bambusa vulgaris* Schrad. ex J.C. Wendl. (Poaceae). *Journal of Agricultural Science*, *7*(6), 150–159. http://doi.org/10.5539/jas.v7n6p150

Annan, K., Sarpong, K., Asare, C., Dickson, R., Amponsah, K., Gyan, B. & Gbedema, S. (2012). *In vitro* anti-plasmodial activity of three herbal remedies for malaria in Ghana: *Adenia cissampeloides* (Planch.) Harms., *Termina liaivorensis* A. Chev, and *Elaeis guineensis* Jacq. *Pharmacognosy Research*, *4*(4), 225–9. http://doi.org/10.4103/0974-8490.102270

Annonymous. (2014). *Bambusa Vulgaris* extract health benefit. Retrieved December 10, 2014, from http://www.alphalipoic-acid.org/*bambusavulgaris*.html

APG II. (2003). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: *Botanical Journal of the Linnean Society*, *141*(4), 399–436. http://doi.org/10.1046/j.1095-8339.2003.t01-100158.x

Argulla, L. E., & Chichioco-Hernandez, C. L. (2014). Xanthine oxidase inhibitory activity of some Leguminosae plants. *Asian Pacific Journal of Tropical Disease*, *4*(6), 438–441. http://doi.org/10.1016/S2222-1808(14)60602-2

Asante, F. A. F., & Asenso-Okyere, K. (2003). *Economic Burden of Malaria in Ghana*. *A Technical Report Submitted to the World Health Organisation (WHO), African Regional Office (AFRO).* Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Economic+Burden+of+Malaria+in+Ghana#0

Asase, A., Akwetey, G. A., & Achel, D. G. (2010). Ethnopharmacological use of herbal remedies for the treatment of malaria in the Dangme West District of Ghana. *Journal of Ethnopharmacology*, *129*(3), 367–76. http://doi.org/10.1016/j.jep.2010.04.001

Asase, A., & Asafo-Agyei, T. (2011). Plants Used for Treatment of Malaria in Communities Around the Bobiri Forest Reserve in Ghana. *Journal of Herbs, Spices & Medicinal Plants*, *17*(2), 85–106. http://doi.org/10.1080/10496475.2011.581132

Asase, A., Hesse, D. N., & Simmonds, M. S. J. (2012). Uses of multiple plants prescriptions for treatment of malaria by some communities in southern Ghana. *Journal of Ethnopharmacology*, *144*(2), 448–52. http://doi.org/10.1016/j.jep.2012.09.028

Asase, A., & Oppong-Mensah, G. (2009). Traditional antimalarial phytotherapy remedies in herbal markets in southern Ghana. *Journal of Ethnopharmacology*, *126*(3), 492–9. http://doi.org/10.1016/j.jep.2009.09.008

Asase, A., Oteng-Yeboah, A. A., Odamtten, G. T., & Simmonds, M. S. J. (2005). Ethnobotanical study of some Ghanaian anti-malarial plants. *Journal of Ethnopharmacology*, *99*(2), 273–9. http://doi.org/10.1016/j.jep.2005.02.020

Asif, M. (2011). *In vivo* analgesic and antiinflammatory effects of *Tectona grandis* Linn . stem bark extracts. *Malaysian Journal of Pharmaceutical Sciences*, *9*(1), 1–11.

Babady-Billa, Gedris, T. E., & Herz, W. (1996). Niruroidine, a norsecurinine-type alkaloid from Phyllanthus niruroides. *Phytochemistry*, *41*(5), 1441–1443. http://doi.org/10.1016/0031-9422(95)00738-5

Balick, M. J. (2008). Ethnobotany, drug development and biodiversity conservationexploring the linkages. In *Ethnobotany and the Search for New Drugs* (pp. 4– 18). John Wiley & Sons.

Bapat, U. C., & Mhapsekar, D. (2012). Antifungal activity study of the extracts of *Homonoia riparia, Pedilanthus tithymaloides* and *Phyllanthus fraternus*. *Ancient Science of Life*, *32*(Suppl 1), S49.

Baragaña, B., Hallyburton, I., Lee, M. C. S., Norcross, N. R., Grimaldi, R., Otto, T. D., … Gilbert, I. H. (2015). A novel multiple-stage antimalarial agent that inhibits protein synthesis. *Nature*, *522*(7556), 315–320. http://doi.org/10.1038/nature14451

Barbosa, J. D., de Oliveira, C. M. C., Duarte, M. D., Riet-Correa, G., Peixoto, P. V., & Tokarnia, C. H. (2006). Poisoning of horses by bamboo, *Bambusa vulgaris.*

Journal of Equine Veterinary Science, *26*(9), 393–398. http://doi.org/10.1016/j.jevs.2006.07.003

Barker, N., Clark, L., Davis, J., Duvall, M. R., Guala, G., Hsiao, C., … Spangler, R. (2001). Phylogeny and subfamilial classification of the grasses (Poaceae). *Annals of the Missouri Botanical Garden*, *88*(3), 373–457.

Betti, J., Iponga, D., Yongo, O. D., Mbomio, D., Yobo, C. M., & Ngoy, A. (2013). Ethnobotanical study of medicinal plants of the Ipassa-Makokou Biosphere Reserve, Gabon: Plants used for treating malaria. *Journal of Medicinal Plants Research*, *7*(31), 2300–2318. http://doi.org/10.5897/JMPR11.1754

Bhattacharyya, J., Stagg, D., Mody, N. V., & Miles, D. H. (1978). Constituents ofspartina cynosuroides: Isolation and13C-NMR analysis of tricin. *Journal of Pharmaceutical Sciences*, *67*(9), 1325–1326. http://doi.org/10.1002/jps.2600670938

Boyom, F. F., Fokou, P. V. T., Yamthe, L. R. T., Mfopa, A. N., Kemgne, E. M., Mbacham, W. F., … Rosenthal, P. J. (2011). Potent antiplasmodial extracts from Cameroonian Annonaceae. *Journal of Ethnopharmacology*, *134*(3), 717–24. http://doi.org/10.1016/j.jep.2011.01.020

Brink, M. (2008). *Bambusa vulgaris* Schrad. ex J.C.Wendl. In *:Louppe, D., Oteng-Amoako, A.A. & Brink, M. (Editors). Prota 7(1): Timbers/Bois d'œuvre 1. [CDRom]. PROTA, Wageningen, Netherlands*. Retrieved from http://database.prota.org/PROTAhtml/B*ambusa vulgaris*_En.htm

Bukar, A., Mukhtar, M., & Hassan, A. (2009). Phytochemical screening and antibacterial activity of leaf extracts of *Senna siamea* (LAM) on *Pseudomonas aeruginosa*. *Bayero Journal of Pure and Applied Sciences*, *2*(1), 139–142. Retrieved from http://www.ajol.info/index.php/bajopas/article/view/58528

Burkill, H. M. (1985). *The useful plants of west tropical Africa* (Vol. 2). Royal Botanic Gardens, Kew.

Bystriakova, N., Kapos, V., & Lysenko, I. (2002). *Potential distribution of woody bamboos in Africa and America (Working Paper)*. UNEP World Conservation Monitoring Centre (UNEP-WCMC), Cambridge. Retrieved from http://www.inbar.int/sites/default/files/resources/inbar_working_paper_no43.pdf

Carey, W., Dasi, J. B., Rao, N., & Gottumukkala, K. (2009). Anti-inflammatory activity of methanolic extract of *Bambusa vulgaris* leaves. *International Journal of Green Pharmacy*, *3*(3), 234. http://doi.org/10.4103/0973-8258.56282

CDC. (2006). Overview of life cycle of Plasmodium. Retrieved April 2, 2015, from http://www.cdc.gov/malaria/about/biology/

Chanda, S., Keneria, M., & Vaghesiya, Y. K. (2011). Evaluation of antimicrobial potential of some Indian medicinal plants against some pathogenic microbes. *Indian Journal of Natural Products and Resources*, *2*(June), 225–228. Retrieved from http://nopr.niscair.res.in/handle/123456789/12147

Chandra, T., & Sadique, J. (1987). A new receipt for liver injury. *Ancient Science of Life*, *7*(2), 99–103. Retrieved from

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3331389&tool=pmcentrez&rendertype=abstract

Chavez, J. P., Dos Santos, I. D., Cruz, F. G., David, J. M., Yang, S.-W., & Cordell, G. A. (1997). A Quinoline Alkaloid From Acanthosyris Paulo-alvinii. *Phytochemistry*, *46*(5), 967–968.

Chirkin, E., Atkatlian, W., & Porée, F.-H. (2015). Chapter One – The Securinega Alkaloids. *The Alkaloids: Chemistry and Biology*, *74*, 1–120. http://doi.org/10.1016/bs.alkal.2014.11.001

Chopade, A. R., & Sayyad, F. J. (2013). Antinociceptive effect of *Phyllanthus fraternus* extract in complete Freund's adjuvant induced chronic pain in mice. *Biomedicine & Aging Pathology*, *3*(4), 235–240. http://doi.org/10.1016/j.biomag.2013.09.001

Cobbinah, E. A. (2008). Antioxidant and antibacterial activities of the chemical constituents of *Terminalia ivorensis* chev. Retrieved December 13, 2014, from http://ir.knust.edu.gh:8080/handle/123456789/664

Coffie, G. Y., Antwi-Boasiako, C., & Darkwa, N. A. (2014). Phytochemical constituents of the leaves of three bamboo (Poaceae) species in Ghana . *Journal of Pharmacognosy and Phytochemistry*, *2*(6), 34–38.

Coulibaly, K., Zirihi, G. N., Guessennd-Kouadio, N., Oussou, K. R., & Dosso, M. (2014). Antibacterial properties of trunk barks of *Terminalia ivorensis* , a commercial and medicinal species on methicillin-resistant *Staphylococci* species strains. *African Health Sciences*, *14*(3), 8–10. http://doi.org/http://dx.doi.org/10.4314/ahs.v14i3.35

Cui, L., & Su, X. (2009). Discovery, mechanisms of action and combination therapy of artemisinin. *Expert Review of Anti-Infective Therapy*, *7*(8), 999–1013. http://doi.org/10.1586/eri.09.68

D'Abrosca, B., DellaGreca, M., Fiorentino, A., Monaco, P., Oriano, P., & Temussi, F. (2004). Structure elucidation and phytotoxicity of C13 nor-isoprenoids from Cestrum parqui. *Phytochemistry*, *65*(4), 497–505. http://doi.org/10.1016/j.phytochem.2003.11.018

Deguchi, J., Hirahara, T., Hirasawa, Y., Ekasari, W., Widyawaruyanti, A., Shirota, O., … Morita, H. (2012). New tricyclic alkaloids, cassiarins G, H, J, and K from leaves of *Cassia siamea*. *Chemical & Pharmaceutical Bulletin*, *60*(2), 219–22. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22293481

DellaGreca, M., Di Marino, C., Zarrelli, A., & D'Abrosca, B. (2004). Isolation and phytotoxicity of apocarotenoids from Chenopodium album. *Journal of Natural Products*, *67*(9), 1492–5. http://doi.org/10.1021/np049857q

Deng, Y.-R., Song, A.-X., & Wang, H.-Q. (2004). Chemical Components of Seriphidium Santolium Poljak. *Journal of the Chinese Chemical Society*, *51*(3), 629–636. http://doi.org/10.1002/jccs.200400094

Diallo, A., Gbeassor, M., Vovor, A., Eklu-Gadegbeku, K., Aklikokou, K., Agbonon, A., … Akpagana, K. (2008). Effect of *Tectona grandis* on phenylhydrazineinduced anaemia in rats. *Fitoterapia*, *79*(5), 332–6. http://doi.org/10.1016/j.fitote.2008.02.005

Diallo, A., Traore, M. S., Keita, S. M., Balde, M. A., Keita, A., Camara, M., … Balde, A. M. (2012). Management of diabetes in Guinean traditional medicine: an ethnobotanical investigation in the coastal lowlands. *Journal of Ethnopharmacology*, *144*(2), 353–61. http://doi.org/10.1016/j.jep.2012.09.020

Dietz, H., & Winterhalter, P. (1996). Phytotoxic constituents from Bunias orientalis leaves. *Phytochemistry*, *42*(4), 1005–1010. http://doi.org/10.1016/00319422(96)00020-9

Dike, I. P., Obembe, O. O., & Adebiyi, F. E. (2012). Ethnobotanical survey for potential anti-malarial plants in south-western Nigeria. *Journal of Ethnopharmacology*, *144*(3), 618–26. http://doi.org/10.1016/j.jep.2012.10.002

Dokosi, O. B. (1998). *Herbs of Ghana*. Accra: Ghana University Press.

Egba, S. I., Njoku, O. U., & Igwe, C. D. (2014). Immunostimulatory Effect of Aqueous Leaf Extract of *Tectona grandis* Linn. on Male Wistar Albino Rat. *Advances in Biological Research*, *8*(1), 49–52. http://doi.org/10.5829/idosi.abr.2014.8.1.8318

Ekasari, W., Widyawaruyanti, A., Zaini, N. C., Syafruddin, D., Honda, T., & Morita, H. (2009). Antimalarial Activity of Cassiarin A from the Leaves of *Cassia siamea*. *Heterocycles*, *78*(7), 1831–1836. http://doi.org/10.3987/COM-09-11680

Farnsworth, N. R., Akerele, O., Bingel, A. S., Soejarto, D. D., & Guo, Z. (1985). Medicinal plants in therapy. *Bulletin of the World Health Organization*, *63*(6), 965–981. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2536466/

Fendall, N. R. (1978). Declaration of Alma-Ata. *Lancet*, *2*(8103), 1308. http://doi.org/10.1016/S0140-6736(79)90622-6

Fernando, M. R., Thabrew, M. I., & Karunanayake, E. . (1990). Hypoglycaemic activity of some medicinal plants in Sri-Lanka. *General Pharmacology*, *21*(5), 779–782.

Foli, E. . (2009). *Terminalia ivorensis* A.Chev. In *Lemmens, R.H.M.J., Louppe, D. & Oteng-Amoako, A.A. (Editors). Prota 7(2): Timbers/Bois d'œuvre 2. [CD-Rom]*. *PROTA, Wageningen, Netherlands.* Retrieved from http://database.prota.org/PROTAhtml/*Terminalia ivorensis*_En.htm

Garg, M., Garg, C., Dhar, V. J., & Kalia, A. N. (2010). Standardized alcoholic extract of *Phyllanthus fraternus* exerts potential action against disturbed biochemical parameters in diabetic animals. *African Journal of Biochemistry Research*, *4*(July), 186–190. Retrieved from http://www.academicjournals.org/journal/AJBR/article-abstract/4402F3611513

Ghaisas, M. M., Navghare, V. V., Takawale, A. R., Zope, V. S., & Phanse, M. A. (2010). Antidiabetic and Nephroprotective effect of *Tectona grandis* linn . In Alloxan induced Diabetes. *ARS Pharmaceutica*, *51*(4), 195–206.

Ghaisas, M., Navghare, V., Takawale, A., Zope, V., Tanwar, M., & Deshpande, A. (2009). Effect of *Tectona grandis* Linn. on dexamethasone-induced insulin resistance in mice. *Journal of Ethnopharmacology*, *122*(2), 304–7. http://doi.org/10.1016/j.jep.2009.01.008

Gopi, S., & Setty, O. H. (2010). Protective effect of *Phyllanthus fraternus* against bromobenzene induced mitochondrial dysfunction in rat liver mitochondria. *Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association*, *48*(8-9), 2170–5. http://doi.org/10.1016/j.fct.2010.05.024

Goyal, Arvind Kumar; Middha, Sushil Kumar; Sen, A. (2010). Evaluation of the DPPH radical scavenging activity, total phenols and antioxidant activities in Indian wild *Bambusa vulgaris* "Vittata" methanolic leaf extract. *Journal of Natural Pharmaceuticals*, *1*(1), 40.

Hamilton, A. C., Shengji, P., Kessy, J., Khan, A. A., Lagos-Witte, S., & Shinwari, Z. K. (2003). *The Purposes and Teaching of Applied Ethnobotany*. *People and plants working paper-II*. WWF, Godalming, UK.

Heinrich, M. (2000). Ethnobotany and its role in drug development. *Phytotherapy Research : PTR*, *14*(July), 479–488. http://doi.org/10.1002/1099-1573(200011)14:7<479::AID-PTR958>3.0.CO;2-2

Hodges, R., & Porte, A. L. (1964). the Structure of Liliolide, A terpene from Lolium perenne. *Tetrahedron*, *20*(1938), 1463–1467.

Hoffmann, P., Kathriarachchi, H., & Wurdack, K. J. (2006). A phylogenetic classification of Phyllanthaceae (Malpighiales; Euphorbiaceae sensu lato). *Kew Bulletin*, *61*(1), 37–53.

Houghton, P. J., Howes, M. J., Lee, C. C., & Steventon, G. (2007). Uses and abuses of *in vitro* tests in ethnopharmacology: Visualizing an elephant. *Journal of Ethnopharmacology*, *110*(3), 391–400. http://doi.org/10.1016/j.jep.2007.01.032

Houghton, P. J., Woldemariam, T. Z., O'Shea, S., & Thyagarajan, S. P. (1996). Two securinega-type alkaloids from Phyllanthus amarus. *Phytochemistry*, *43*(3), 715–717. http://doi.org/10.1016/0031-9422(96)00345-7

Hu, Q., Zhou, B., Gao, X., Yang, L., Shu, L., Shen, Y., … Yang, G. (2012). Antiviral Chromones from the Stem of *Cassia siamea*. *Journal of Natural Products*, *75*, 1909–1914. http://doi.org/org/10.1021/np300395m

Idowu, O. A., Soniran, O. T., Ajana, O., & Aworinde, D. O. (2010). Ethnobotanical survey of antimalarial plants used in Ogun State, Southwest Nigeria. *African Journal of Pharmacy and Pharmacology*, *4*(2), 055–060. Retrieved from http://www.academicjournals.org/journal/AJPP/article-abstract/18D5A0F32826

Ilić, N., Novković, M., Guida, F., Xhindoli, D., Benincasa, M., Tossi, A., & Juretić, D. (2013). Selective antimicrobial activity and mode of action of adepantins, glycine-rich peptide antibiotics based on anuran antimicrobial peptide sequences. *Biochimica et Biophysica Acta*, *1828*(3), 1004–12. http://doi.org/10.1016/j.bbamem.2012.11.017

Ingkaninan, K., Ijzerman, A. P., & Verpoorte, R. (2000). Luteolin , a Compound with Adenosine A 1 Receptor-Binding Activity , and Chromone and

Dihydronaphthalenone Constituents from *Senna siamea*. *Journal of Natural Products*, *63*(3), 315–317. http://doi.org/10.1021/np9904152 CCC

Iwu, M. M., & Anyanwu, B. N. (1982). Phytotherapeutic profile of Nigerian herbs I: Anti-inflammatory and anti-arthrictic agents. *Journal of Ethnopharmacology*, *6*, 263–274.

Jacobsen, E. N. (2007). *NMR spectroscopy explained: Simplified theory, applications and examples for organic chemistry and structural biology*. A John Wiley and Sons, Inc., Publications.

Jambou, R., Legrand, E., Niang, M., Khim, N., Lim, P., Bouchier, C., … Mercereaupuijalon, O. (2005). Resistance of Plasmodium falciparum field isolates to invitro artemether and point mutations of the SERCA-type. *The Lancet*, *366*(9501), 1960–1963.

Jangame, C. M., & Burande, M. D. (2013). Hepatoprotective activity of methanolic and petroleum ether extracts of *Tectona grandis* against paracetamol induced hepatotoxicity. *International Journal of PharmTech Research*, *5*(1), 249–253.

Jaybhaye, D., Varma, S., Bonde, V., & Gite, A. (2010). Effect of *Tectona grandis* stem extract on estradiol benzoate injected uterus of female albino wistar rats. *Asian Journal of Pharmaceutical and Clinical Research*, *3*(2), 123–125.

Johnny, I. I., Ekong, N. J., & Okon, J. E. (2014). Phytochemical Screening and Antihyperglycaemic Activity of Ethanolic Extract of *Terminalia ivorensis* A . Chev . Leaves on Albino Wistar Rats. *Global Advanced Research Journal of Medicine and Medical Science*, *3*(8), 186–189.

Joshi, B. S., Gawad, D. H., Pelletier, S. W., Kartha, G., & Bhandary, K. (1986). Isolation and structure (x-ray analysis) of ent-norsecurinine, an alkaloid from Phyllanthus niruri. *Journal of Natural Products*, *49*(4), 614–620.

Ju, Y., Sacalis, J. N., & Still, C. C. (1998). Bioactive Flavonoids from EndophyteInfected Blue Grass (Poa ampla). *Journal of Agricultural and Food Chemistry*, *46*(9), 3785–3788. http://doi.org/10.1021/jf980189m

Jyoti Gupta, & Ali, M. (1999). Four new seco-sterols of *Phyllanthus fraternus* roots. *Indian Journal of Pharmaceutical Sciences*, *61*(2), 90–96.

Kaddouri, H., Nakache, S., Houzé, S., Mentré, F., & Le Bras, J. (2006). Assessment of the drug susceptibility of Plasmodium falciparum clinical isolates from africa by using a Plasmodium lactate dehydrogenase immunodetection assay and an inhibitory maximum effect model for precise measurement of the 50-percent inhibitory conc. *Antimicrobial Agents and Chemotherapy*, *50*(10), 3343–9. http://doi.org/10.1128/AAC.00367-06

Kaisoon, O., Siriamornpun, S., Weerapreeyakul, N., & Meeso, N. (2011). Phenolic compounds and antioxidant activities of edible flowers from Thailand. *Journal of Functional Foods*, *3*(2), 88–99. http://doi.org/10.1016/j.jff.2011.03.002

Kalyani, B., K., K., Santosh, P., & S, S. R. (2010). Evaluation of diuretic activity of *Phyllanthus fraternus* arial parts On albino rats. *International Journal of Pharmaceutical & Biological …*, *1*(4), 389–392.

Kamagaté, M., Koffi, C., Kouamé, N. M., Akoubet, A., Alain, N., Yao, R., & Die, H. M. (2014). Ethnobotany , phytochemistry , pharmacology and toxicology profiles of *Cassia siamea* Lam . *The Journal of Phytopharmacology*, *3*(1), 57–76.

Kaou, A. M., Mahiou-Leddet, V., Hutter, S., Aïnouddine, S., Hassani, S., Yahaya, I., … Ollivier, E. (2008). Antimalarial activity of crude extracts from nine African medicinal plants. *Journal of Ethnopharmacology*, *116*(1), 74–83. http://doi.org/10.1016/j.jep.2007.11.001

Kapoor, V. P., Milas, M., Taravel, F. R., & Rinaudo, M. (1996). Rheological properties of a seed galactomannan from *Cassia siamea* Lamk. *Food Hydrocolloids*, *10*(2), 167–172. http://doi.org/10.1016/S0268-005X(96)80031-2

Khan, A. A., & Khan, A. V. (2004). Medico-ethnobotanical uses of *Phyllanthus fraternus* Webst . (Family- Euphorbiaceae) from western Uttar Pradesh , India. *Journal of Natural Remedies*, *4*(1), 73–76.

Khan, R. M., & Mlungwana, S. M. (1999). 5-Hydroxylapachol: a cytotoxic agent from *Tectona grandis*. *Phytochemistry*, *50*(3), 439–442. http://doi.org/10.1016/S0031-9422(98)00478-6

Khatoon, S., Rai, V., Rawat, A. K. S., & Mehrotra, S. (2006). Comparative pharmacognostic studies of three Phyllanthus species. *Journal of Ethnopharmacology*, *104*(1-2), 79–86. http://doi.org/10.1016/j.jep.2005.08.048

Khera, N., & Bhargava, S. (2013). Phytochemical and pharmacological evaluation of *Tectona grandis* Linn. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*(3).

King, F. E., King, T. J., & Ross, J. M. (1955). The chemistry of extractives from hardwoods. Part XXIII. The isolation of a new triterpene (terminolic acid) from *Terminalia ivorensis*. *Journal of the Chemical Society (Resumed)*, 1333. http://doi.org/10.1039/jr9550001333

Koffuor, G., & Amoateng, P. (2011). Antioxidant and anticoagulant properties of *Phyllanthus fraternus* GL Webster (Family: Euphorbiaceae). *Journal of Pharmacology and Toxicology*, *6*(7), 624–636. http://doi.org/10.3923/jjpt.2011.624.636

Kokwaro, G. (2009). Ongoing challenges in the management of malaria. *Malaria Journal*, *8 Suppl 1*(Suppl 1), S2. http://doi.org/10.1186/1475-2875-8-S1-S2

Kollert, W., & Cherubini, L. (2012). *Teak resources and market assessment 2010 (Tectona grandis Linn. F.), Working Paper FP/47/E*. *Food and Agriculture Organization, Rome* (Vol. 2010). Rome, Italy. Retrieved from http://www.fao.org/3/a-an537e.pdf

Komlaga, G., Agyare, C., Dickson, R. A., Mensah, M. L. K., Annan, K., Loiseau, P. M., & Champy, P. (2015). Medicinal plants and finished marketed herbal products used in the treatment of malaria in the Ashanti region, Ghana. *Journal of Ethnopharmacology*, *172*, 333–346. http://doi.org/10.1016/j.jep.2015.06.041 Kong, C., Liang, W., Xu, X., Hu, F., Wang, P., & Jiang, Y. (2004). Release and activity of allelochemicals from allelopathic rice seedlings. *Journal of Agricultural and Food Chemistry*, *52*(10), 2861–5. http://doi.org/10.1021/jf035467i

Kong, C., Xu, X., Zhou, B., Hu, F., Zhang, C., & Zhang, M. (2004). Two compounds from allelopathic rice accession and their inhibitory activity on weeds and fungal pathogens. *Phytochemistry*, *65*(8), 1123–8. http://doi.org/10.1016/j.phytochem.2004.02.017

Koram, K., & Molyneux, M. (2007). When is "malaria" malaria? The different burdens of malaria infection, malaria disease, and malaria-like illnesses. *The American Journal of Tropical Medicine and Hygiene*, *77*(Suppl 6), 1–5.

Koudouvo, K., Karou, D. S., Kokou, K., Essien, K., Aklikokou, K., Glitho, I. A., … Gbeassor, M. (2011). An ethnobotanical study of antimalarial plants in Togo Maritime Region. *Journal of Ethnopharmacology*, *134*(1), 183–90. http://doi.org/10.1016/j.jep.2010.12.011

Kubo, B. (2014). *Tectona grandis* Linn. Retrieved December 7, 2014, from http://www.stuartxchange.com/Tekla.html

Kumari, K. K., & Setty, O. H. (2012). Protective effect of *Phyllanthus fraternus* against mitochondrial dysfunction induced by co-administration of cisplatin and cyclophosphamide. *Journal of Bioenergetics and Biomembranes*, *44*(1), 179–88. http://doi.org/10.1007/s10863-012-9423-6

Lam, P.-L., Gambari, R., Yip, J., Yuen, M. C.-W., Lam, K.-H., Wong, R. S.-M., … Chui, C.-H. (2012). Development of phyllanthin containing microcapsules and their improved biological activity towards skin cells and Staphylococcus aureus. *Bioorganic & Medicinal Chemistry Letters*, *22*(1), 468–71. http://doi.org/10.1016/j.bmcl.2011.10.097

Lans, C. (2007). Comparison of plants used for skin and stomach problems in Trinidad and Tobago with Asian ethnomedicine. *Journal of Ethnobiology and Ethnomedicine*, *3*, 3. http://doi.org/10.1186/1746-4269-3-3

Lata, S., Singh, S., NathTiwari, K., & Upadhyay, R. (2014). Evaluation of the antioxidant and hepatoprotective effect of *Phyllanthus fraternus* against a chemotherapeutic drug cyclophosphamide. *Applied Biochemistry and Biotechnology*, *173*(8), 2163–73. http://doi.org/10.1007/s12010-014-1018-8

Leite, D. F., Kassuya, C. A., Mazzuco, T. L., Silvestre, A., de Melo, L. V, Rehder, V. L., … Calixto, J. B. (2006). The cytotoxic effect and the multidrug resistance reversing action of lignans from Phyllanthus amarus. *Planta Medica*, *72*(15), 1353–8. http://doi.org/10.1055/s-2006-951708

Lim, T. K. (2014). *Edible Medicinal And Non-Medicinal Plants*. Dordrecht: Springer Netherlands. http://doi.org/10.1007/978-94-007-7395-0

Luzzatto, L., Richardson, N., Carter, N., Duparc, S., Premji, Z., Tiono, A., & Pamba, A. (2008). A multi-centre, randomised, double-blind, double dummy study comparing the efficacy and safety of chlorproguanil-dapsone-artesunate versus artemether-lumefantrine in the treatment of acute uncomplicated Plasmodium falciparum malaria in children and adoles. Retrieved December 18, 2015, from https://gsk.sylogent.com/files/20583.pdf

Lysenko, A. Y., & Semashk, I. N. (1968). *Medical geography: A medicalgeographical study of an ancient disease*. Academy of Sciences, USSR,. http://doi.org/911.3:616.936(100)

Macías, F. a, Lacret, R., Varela, R. M., Nogueiras, C., & Molinillo, J. M. G. (2008). Bioactive apocarotenoids from *Tectona grandis*. *Phytochemistry*, *69*(15), 2708–15. http://doi.org/10.1016/j.phytochem.2008.08.018

Macías, F. a, Lacret, R., Varela, R. M., Nogueiras, C., & Molinillo, J. M. G. (2010). Isolation and phytotoxicity of terpenes from *Tectona grandis*. *Journal of Chemical Ecology*, *36*(4), 396–404. http://doi.org/10.1007/s10886-010-9769-3

Magassouba, F. B., Diallo, a., Kouyaté, M., Mara, F., Mara, O., Bangoura, O., … Baldé, a. M. (2010). Corrigendum to "Ethnobotanical survey and antibacterial activity of some plants used in Guinean traditional medicine" [Journal of Ethnopharmocology 114(1) (2007) 44–53]. *Journal of Ethnopharmacology*, *128*(3), 705–708. http://doi.org/10.1016/j.jep.2008.10.024

Matur, B., Matthew, T., & Ifeanyi, C. (2009). Analysis of the phytochemical and *in vivo* antimalaria properties of *Phyllanthus fraternus* webster extract. *New York Sci. J*, *2*(5), 12–19.

Medeiros, M. R., & Wood, J. L. (2010a). An Enantioselective Approach to the Securinega Alkaloids: The Total Synthesis of (+)-Norsecurinine and (+)Allonorsecurinine. *Tetrahedron*, *66*(26), 4701–4709. http://doi.org/10.1016/j.tet.2010.03.015

Medeiros, M. R., & Wood, J. L. (2010b). An Enantioselective Approach to the Securinega Alkaloids: The Total Synthesis of (+)-Norsecurinine and (+)Allonorsecurinine. *Tetrahedron*, *66*(26), 4701–4709. http://doi.org/10.1016/j.tet.2010.03.015

Mehta, K., Patel, B. N., & Jain, B. K. (2014). Antibacterial and antifungal potentiality of leaf extract of *Phyllanthus fraternus* Webster: an ethnomedicinal. *American Journal of Microbiological Research*, *2*(2), 74–79.

Ménan, H., Banzouzi, J.-T., Hocquette, A., Pélissier, Y., Blache, Y., Koné, M., … Valentin, A. (2006). Antiplasmodial activity and cytotoxicity of plants used in West African traditional medicine for the treatment of malaria. *Journal of Ethnopharmacology*, *105*(1-2), 131–6. http://doi.org/10.1016/j.jep.2005.10.027

Mesfin, A., Giday, M., Animut, A., & Teklehaymanot, T. (2012). Ethnobotanical study of antimalarial plants in Shinile District, Somali Region, Ethiopia, and *in vivo* evaluation of selected ones against Plasmodium berghei. *Journal of Ethnopharmacology*, *139*(1), 221–7. http://doi.org/10.1016/j.jep.2011.11.006

Meshnick, S., & Dobson, M. (2001). The history of antimalarial drugs. In P. J. Rosenthal (Ed.), *Antimalarial chemotherapy* (pp. 15–26). Humana Press Inc., Totowa, NJ.

Ministry of Health, R. of G. (2005). *Policy guidelines on traditional medicine development*. Accra: Ministry of Health.

Mishra, M. P., & Padhy, R. N. (2013). *In Vitro* antibacterial efficacy of 21 Indian timber-yielding plants against multidrug-resistant bacteria causing urinary tract infection. *Osong Public Health and Research Perspectives*, *4*(6), 347–57. http://doi.org/10.1016/j.phrp.2013.10.007

Mohammed, A., & Atiku, M. K. (2012). Antihyperglycemic and antihyperglycemic effect of leaves and stem bark methanol extract of *Senna siamea* in alloxaninduced diabetic rats. *Current Research in Cardiovascular Pharmacology.*, *1*(1), 10–17. http://doi.org/10.3923/crcpaj.2012.10.17

Momin, M. A. M., Rana, M. S., Khan, M. R., Emran, T. Bin, & Hosen, S. M. Z. (2012). Antimicrobial and peripherally acting analgesic activity of *Senna* `. *Molecular & Clinical Pharmacology*, *3*(2), 149–157.

Morita, H., Oshimi, S., Hirasawa, Y., Koyama, K., Honda, T., Ekasari, W., … Zaini, N. C. (2007). Cassiarins A and B , Novel Antiplasmodial Alkaloids from *Cassia siamea*. *Organic Letters*, *9*(18), 3691–3693. http://doi.org/10.1021/ol701623n CCC

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, *65*(1-2), 55–63. http://doi.org/10.1016/0022-1759(83)90303-4

Mousa, N. K., Khudier, S. H., Mohammad, A. N., Sabbar, S. S., & Ahmed, I. A. (2013). Antioxidant activity of Cinnamic acidagainst Trichlorfon in mice. *Iraqi Journal of Biotechnology*, *12*(2), 54–62.

Mshana, R. N., Abbiw, D. K., Addae-Mensah, I., Adjanouhoun, E., Ahyi, M. R. A., Ekpere, J. ., … Tackie, A. . (2001). *Traditional medicine and Pharmacopoeia; Contribution to the revision of ethnobotanical and floristic studies in Ghana*. Accra: Science and Technology Press, CSIR.

Mutabingwa, T. K. (2005). Artemisinin-based combination therapies (ACTs): best hope for malaria treatment but inaccessible to the needy! *Acta Tropica*, *95*(3), 305–15. http://doi.org/10.1016/j.actatropica.2005.06.009

N'guessan, K., Zirihi, G. N., & Etien, D. T. (2009). Hypotensive Effect of aqueous extract of *Bambusa vulgaris* sheets on the arterial pressure of rabbits. *American Journal of Scientific Research*, *2*, 60–72.

Nagard, H. Le, Vincent, C., Mentré, F., & Le Bras, J. (2011). Online analysis of *in vitro* resistance to antimalarial drugs through nonlinear regression. *Computer Methods and Programs in Biomedicine*, *104*(1), 10–8. http://doi.org/10.1016/j.cmpb.2010.08.003

Naira, N., & Karvekar, M. (2010). Isolation of phenolic compounds from the methanolic extract of *Tectona grandis*. *Research Journal of Pharmaceutical , Biological and Chemical Sciences*, *1*(2), 221–225.

Nayeem, N., & Karvekar, M. (2009). Preliminary phytochemical analysis and wound healing activity of various extracts of the frontal leaves of *Tectona grandis*. *Pharmacologyonline*, *2*, 402–412.

Newman, D. J., & Cragg, G. M. (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. *Journal of Natural Products*, *75*(3), 311–35. http://doi.org/10.1021/np200906s

Nsonde-Ntandou, F. G., Banzouzi T, J., Mbatchi, B., Elion-Itou, R. D. G., EtouOssibi, A. W., Ramos, S., … Ouamba, J. M. (2010). Analgesic and antiinflammatory effects of *Cassia siamea* Lam. stem bark extracts. *Journal of Ethnopharmacology*, *127*(1), 108–11. http://doi.org/10.1016/j.jep.2009.09.040

Nunkoo, D. H., & Mahomoodally, M. F. (2012). Ethnopharmacological survey of native remedies commonly used against infectious diseases in the tropical island of Mauritius. *Journal of Ethnopharmacology*, *143*(2), 548–64. http://doi.org/10.1016/j.jep.2012.07.013

Nzila, A., & Mwai, L. (2009). *In vitro* selection of Plasmodium falciparum drugresistant parasite lines. *Journal of Antimicrobial Chemotherapy*, *65*(3), 390–398. http://doi.org/10.1093/jac/dkp449

Ogbole, O., Adeniji, J., Ajaiyeoba, E., Kamdem, R., & Choudhary, M. (2014). Anthraquinones and triterpeniods from *Senna siamea* (Fabaceae) Lam inhibit poliovirus activity. *African Journal of Microbiology Research.*, *8*(31), 2955–2963. http://doi.org/10.5897/A

Ogundare, A. O., & Olajuyigbe, A. O. (2012). Bioactivity guided isolation of the antifungal components in sawdust extracts of *Piptadeniatrum africanum* , and *Terminalia ivorensis*. *Malaysian Journal of Microbiology*, *8*(1), 34–41.

Ohrnberger, D. (1999). *The bamboos of the world: annotated nomenclature and literature of the species and the higher and lower taxa.* Elsevier.

Okada, N., Shirata, K., Niwano, M., Koshino, H., & Uramoto, M. (1994). Immunosuppessive activity of a monterpene from Eucommia ulmoides. *Phytochemistry*, *37*(1), 281–282.

Okumade, A. L., & Wiemer, D. F. (1985). (-) -Loliolide , an antirepplent compound from Xanthoxyllum setulosum. *Journal of Natural Pharmaceuticals*, *48*(3), 472–473.

Oliver-Bever, B. (1986). *Medicinal plants in tropical West Africa*. Cambridge: Cambridge University Press.

Olorunnisola, O. S., Adetutu, A., Balogun, E. A., & Afolayan, A. J. (2013). Ethnobotanical survey of medicinal plants used in the treatment of malaria in Ogbomoso, Southwest Nigeria. *Journal of Ethnopharmacology*, *150*(1), 71–8. http://doi.org/10.1016/j.jep.2013.07.038

Orwa, C., Muta, A., Kindt, R., Jamnads, R., & Anthony, S. (2009). *Senna siamea*. Retrieved December 25, 2014, from htp:/ww.orldagrofrestry.org/sites/tredbs/tredatbase.asp

Oseni, L. A., Amiteye, D., Antwi, S., Tandoh, M., & Aryitey, G. M. (2013). Preliminary *in vivo* evaluation of anti-inflammatory activities of aqueous and ethanolic whole plant extracts of P*hyllanthus fraternus* on Carrageenan-induced Paw Oedema in Sprague-Dawley Rats. *Journal of Applied Pharmaceutical Science*, *3*(03), 62–65. http://doi.org/10.7324/JAPS.2013.30312

Oshimi, S., Zaini, N. C., Deguchi, J., Hirasawa, Y., Shirota, O., Ekasari, W., … Morita, H. (2009). Cassiarins C− E, Antiplasmodial Alkaloids from the Flowers of *Cassia siamea*. *Journal of Natural …*, *72*(10), 1899–1901. http://doi.org/10.1021/np9004213 CCC

Ouattara, S., Kouassi, K. E., Kra, K. A. M., Zirihi, G. N., N' guessan, J. D., Coulibaly, A., & Djaman, A. J. (2013). Ethnopharmacology Antifungal activities of *Terminalia ivorensis* A . Chev . bark extracts against *Candida albicans* and *Aspergillus fumigatus*. *Journal of Intercultural Ethnopharmacology*, *2*(1), 49–52. http://doi.org/10.5455/jice.20121205083931

Oudhia, P. (2008). *Phyllanthus fraternus G.L.Webster*. *In: Schmelzer, G.H. & GuribFakim, A. (Editors). Prota 11(1): Medicinal plants/Plantes médicinales 1. [CDRom]*. *PROTA, Wageningen, Netherlands.*

Owokotomo, I. A., & Owoeye, G. (2011). Proximate analysis and antimicrobial activities of *Bambusa vulgaris* L . leaves ' beverage. *African Journal of Agricultural Research*, *6*(21), 5030–5032. http://doi.org/10.5897/AJAR10.1106

Owuor, B. O., Ochanda, J. O., Kokwaro, J. O., Cheruiyot, A. C., Yeda, R. A., Okudo, C. A., & Akala, H. M. (2012). *In vitro* antiplasmodial activity of selected Luo and Kuria medicinal plants. *Journal of Ethnopharmacology*, *144*(3), 779–81. http://doi.org/10.1016/j.jep.2012.09.045

Padma, P. (1999). Protective effect of *Phyllanthus fraternus* against carbon tetrachloride-induced mitochondrial dysfunction. *Life Sciences*, *64*(25), 2411–2417.

Petchnaree, P., Bunyapraphatsara, N., Cordell, G. a., Cowe, H. J., Cox, P. J., Howie, R. A., & Patt, S. L. (1986). X-Ray crystal and molecular structure of nirurine, a novel alkaloid related to the securinega alkaloid skeleton, from Phyllanthus niruri(Euphorbiaceae). *Journal of the Chemical Society, Perkin Transactions 1*, 1551. http://doi.org/10.1039/p19860001551

Pettit, G. R., Herald, C. L., Ode, R. H., Brown, P., Gust, D. J., & Michel, C. (1955). A sieve for t h e isolation of cancer cells and other large cells from t h e blood. *Journal of Natural Products*, *43*(6), 752–755.

Phalan, B. (2009). Ecology of *Bambusa vulgaris*. Retrieved December 10, 2014, from http://www.issg.org/database/species/ecology.asp?si=1399

Philippe, G., Angenot, L., De Mol, P., Goffin, E., Hayette, M.-P., Tits, M., & Frédérich, M. (2005). *In vitro* screening of some Strychnos species for antiplasmodial activity. *Journal of Ethnopharmacology*, *97*(3), 535–9. http://doi.org/10.1016/j.jep.2004.12.011

Pink, R., Hudson, A., Mouriès, M.-A., & Bendig, M. (2005). Opportunities and challenges in antiparasitic drug discovery. *Nature Reviews. Drug Discovery*, *4*(9), 727–40. http://doi.org/10.1038/nrd1824

Ponou, B. K., Teponno, R. B., Ricciutelli, M., Quassinti, L., Bramucci, M., Lupidi, G., … Tapondjou, L. A. (2010). Dimeric antioxidant and cytotoxic triterpenoid saponins from *Terminalia ivorensis* A. Chev. *Phytochemistry*, *71*(17-18), 2108–15. http://doi.org/10.1016/j.phytochem.2010.08.020

Pooja, Sharma, V., & Samanta, K. C. (2011). Hypoglycemic activity of methanolic extract of *Tectona grandis* linn . root in alloxan induced diabetic rats. *Journal of Applied Pharmaceutical Science*, *01*(04), 106–109.

Public Health Act. The 851 Act of the Parliament of the Republic of Ghana (2012). Ghana.

Ramachandran, S., & Rajasekaran, A. (2014). Blood glucose-lowering effect of *Tectona grandis* flowers in type 2 diabetic rats: a study on identification of active constituents and mechanisms for antidiabetic action. *Journal of Diabetes*, *6*(5), 427–37. http://doi.org/10.1111/1753-0407.12121

Ramachandran, S., Rajini, kanth B., Rajasekaran, A., & Manisenthil, K. K. T. (2011). Evaluation of anti–inflammatory and analgesic potential of methanol extract of *Tectona grandis* flowers. *Asian Pacific Journal of Tropical Biomedicine*, *1*(2), S155–S158. http://doi.org/10.1016/S2221-1691(11)60146-9

Ramakrishna, V., Gopi, S., & Setty, O. H. (2012). Protective effect of *Phyllanthus fraternus* against bromo- benzene-induced mitochondrial dysfunction in rat kidney. *Chinese Journal of Natural Medicines*, *10*(5), 328–333. http://doi.org/10.1016/S1875-5364(12)60066-1

Ramharter, M., Kurth, F., Schreier, A. C., Nemeth, J., Glasenapp, I. von, Bélard, S., … Kremsner, P. G. (2008). Fixed-dose pyronaridine-artesunate combination for treatment of uncomplicated falciparum malaria in pediatric patients in Gabon. *The Journal of Infectious Diseases*, *198*(6), 911–9. http://doi.org/10.1086/591096

Rodríguez-Pérez, M., Martínez, J. M., Rivero, L. R., Álvarez, H. M. H., Valdez, A. F. C., Rodríguez, D. A., … Payrol, J. A. (2006). Evaluación de la actividad antimalárica de algunas plantas utilizadas en la medicina tradicional cubana. *Revista de Ciências Farmacêuticas Básica e Aplicada*, *27*(3), 197–205.

Roll Back Malaria. (2015). Economic Costs of Malaria. Retrieved September 14, 2015, from http://www.rollbackmalaria.org/files/files/toolbox/RBM Economic Costs of Malaria.pdf

Roosita, K., Kusharto, C. M., Sekiyama, M., Fachrurozi, Y., & Ohtsuka, R. (2008). Medicinal plants used by the villagers of a Sundanese community in West Java, Indonesia. *Journal of Ethnopharmacology*, *115*(1), 72–81. http://doi.org/10.1016/j.jep.2007.09.010

Row, L. R., Srinivasulu, C., Smith, M., & Rao, G. S. R. S. (1964). New lignans from Phyllanthus niruri Linn. *Tetrahedron Letters*, 1557–1567.

Sachan, K., Singh, P., Aswalayan, V., & Singh, R. (2014). Evaluation of hepatoprotective activity of *Tectona grandis* Linn. *International Journal of Pharmaceutical and Medicinal Research*, *2*(3), 105–108.

Sailaja, R., & Setty, O. H. (2006). Protective effect of *Phyllanthus fraternus* against allyl alcohol-induced oxidative stress in liver mitochondria. *Journal of Ethnopharmacology*, *105*(1-2), 201–9. http://doi.org/10.1016/j.jep.2005.10.019

Samuel, R., Kathriarachchi, H., Hoffmann, P., Barfuss, M. H. J., Wurdack, K. J., Davis, C. C., & Chase, M. W. (2005). Molecular phylogenetics of Phyllanthaceae: evidence from plastid MATK and nuclear PHYC sequences. *American Journal of Botany*, *92*(1), 132–41. http://doi.org/10.3732/ajb.92.1.132

Sanon, S., Ollivier, E., Azas, N., Mahiou, V., Gasquet, M., Ouattara, C. ., … Fumoux, F. (2003). Ethnobotanical survey and in vitro antiplasmodial activity of plants used in traditional medicine in Burkina Faso. *Journal of Ethnopharmacology*, *86*(2-3), 143–147. http://doi.org/10.1016/S03788741(02)00381-1

Santos, S. A. dos, Carvalho, M. G. de, & Braz-Filho, R. (1995). Unambiguous H and

C-NMR assigmemts of isoflavononeS from Virola caducifolia. *Journal of Brazilian Chemical Society*, *6*(4), 349–352.

Saotoing, P., Toua, V., Tchobsala, Tchuenguem, F.-N. F., Alexandre-Michel, N. N., & Jean, M. (2011). Medicinal plants used in traditional treatment of malaria in Cameroon. *Journal of Ecology and the Natural Environment*, *3*(March), 104–117.

Sarin, B., Verma, N., Martín, J. P., & Mohanty, A. (2014). An Overview of Important Ethnomedicinal Herbs of Phyllanthus Species : Present Status and Future Prospects. *E -Scientific World Journal*, *2014*. http://doi.org/http://dx.doi.org/10.1155/2014/839172

Schmid, R., & Singh, G. (2005). *Plant Systematics: An Integrated Approach*. *Taxon* (Vol. 54). http://doi.org/10.2307/25065339

Scholar, E. M., & Pratt, W. B. (2000). *The Antimicrobial Drugs*. Oxford University Press. Retrieved from https://books.google.com/books?id=ZQ6KNRLWHLQC&pgis=1

Sebastian, T., & Setty, O. H. (1999). Protective Effect of *P. fraternus* Against Ethanol-Induced Mitochondrial Dysfunction. *Alcohol*, *17*(1), 29–34. http://doi.org/10.1016/S0741-8329(98)00029-9

Sen, B., Dubey, S. D., & Tripathi, K. (2011). Pharmacognostical study of Tamalaki (*Phyllanthus fraternus* Webster), a herb used in Tamaka-svasa. *Ayu*, *32*(3), 398–401. http://doi.org/10.4103/0974-8520.93924

Senthilkumar, M. K., Sivakumar, P., Changanakkattil, F., Rajesh, V., & Perumal, P. (2011). Evaluation of anti-diabetic activity of *Bambusa vulgaris* leaves in streptozotocin induced diabetic rats. *International Journal of Pharmaceutical Sciences and Drug Research*, *3*(3), 208–210.

Sharma, Kumar, S., Arogya, M. S., Bhaskarmurthy, D. H., Agarwal, A., & Velusami, C. C. (2011). Hepatoprotective activity of the Phyllanthus species on tert-butyl hydroperoxide (t-BH)-induced cytotoxicity in HepG2 cells. *Pharmacognosy Magazine*, *7*(27), 229–33. http://doi.org/10.4103/0973-1296.84237

Sicuri, E., Vieta, A., Lindner, L., Constenla, D., & Sauboin, C. (2013). The economic costs of malaria in children in three sub-Saharan countries: Ghana, Tanzania and Kenya. *Malaria Journal*, *12*(1), 307. http://doi.org/10.1186/1475-2875-12307

Silva, M. J. da. (2009). Neotropical Phyllanthaceae. Retrieved December 20, 2014, from http://www.kew.org/science/tropamerica/neotropikey/families/Phyllanthaceae.htm.

Singh, H. (2008). Hepatoprotective effect of Bhumyamalki (*Phyllanthus fraternus* Webster) and Phaltrikadi decoction in patients of acute viral hepatitis. *Indian Journal of Traditional Knowledge*, *7*(4), 560–565.

Singh, N., Shukla, N., Singh, P., Sharma, R., Rajendran, S. M., Maurya, R., & Palit, G. (2010). Verbascoside isolated from *Tectona grandis* mediates gastric protection in rats via inhibiting proton pump activity. *Fitoterapia*, *81*(7), 755–61. http://doi.org/10.1016/j.fitote.2010.03.019

Singh, P., Jain, S., & Bhargava, S. (1989). A 1,4-anthraquinone derivative from *Tectona grandis**. *Phytochemistry*, *28*(4), 1258–1259. http://doi.org/doi:10.1016/0031-9422(89)80224-9

Singh, Y. N. (1986). Traditional medicine in Fiji: Some herbal folk cures used by Fiji Indians. *Journal of Ethnopharmacology*, *15*(1), 57–88. http://doi.org/10.1016/0378-8741(86)90104-2

Sittie, A. A., Lemmich, E., Olsen, C. E., Hviid, L., & Brøgger Christensen, S. (1998). Alkamides from *Phyllanthus fraternus*. *Planta Medica*, *64*(2), 192–3. http://doi.org/10.1055/s-2006-957405

Soelberg, J., Asase, A., Akwetey, G., & Jager, A. K. (2015). Historical versus contemporary medicinal plants uses in Ghana. *Journal of Ethnopharmacology*, *160*, 109–131.

Somanabandhu, A., Nitayangkura, S., Mahidol, C., Ruchirawat, S., Likhitwitayawuid, K., Shieh, H., … Cordell, G. A. (1993). 1 H- and 13 C-Nmr Assignments of Phyllanthin and Hypophyllanthin: Lignans That Enhance Cytotoxic Responses with Cultured Multidrug-Resistant Cells. *Journal of Natural Products*, *56*(2), 233–239. http://doi.org/10.1021/np50092a008

Sonibare, M. A., Moody, J. O., & Adesanya, E. O. (2009). Use of medicinal plants for the treatment of measles in Nigeria. *Journal of Ethnopharmacology*, *122*(2), 268–72. http://doi.org/10.1016/j.jep.2009.01.004

Soremekun, R. O., Ogunbanjo, O. A., & Ogbo, P. U. (2013). Accessibility of Antimalarials in Secondary Health Care Facilities and Community Pharmacies in Lagos State – A Comparative Study. *Journal of Community Medicine and Primary Health Care*, *25*(2), 19–27.

Stangeland, T., Alele, P. E., Katuura, E., & Lye, K. A. (2011). Plants used to treat malaria in Nyakayojo sub-county, western Uganda. *Journal of Ethnopharmacology*, *137*(1), 154–66. http://doi.org/10.1016/j.jep.2011.05.002

Strauss, C. R., Wilson, B., & Williams, P. J. (1987). 3-oxo-α-ionol, vomifoliol and roseoside in Vitis vinifera fruit. *Phytochemistry*, *26*(7), 1995–1997. http://doi.org/10.1016/S0031-9422(00)81745-8

Tagne, R. S., Telefo, B. P., Nyemb, J. N., Yemele, D. M., Njina, S. N., Goka, S. M. C., … Farooq, A. D. (2014). Anticancer and antioxidant activities of methanol extracts and fractions of some Cameroonian medicinal plants. *Asian Pacific Journal of Tropical Medicine*, *7S1*(Suppl 1), S442–7. http://doi.org/10.1016/S1995-7645(14)60272-8

Takasugi, M., Anetai, M., Katsui, N., & Masamune, T. (1973). the Occurrence of Vomifoliol, Dehydrovomifoliol and Dihydrophaseic Acid in the Roots of “Kidney Bean” (*Phaseolus Vulgaris* L.). *Chemistry Letters*, (3), 245–248. http://doi.org/10.1246/cl.1973.245

Tinto, H., D’Alessandro, U., Sorgho, H., Valea, I., Tahita, M., Kabore, W., … Schellenberg. (2015). Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *The Lancet*, *386*(9988), 31–45. http://doi.org/10.1016/S0140-6736(15)60721-8

Traditional Medicine Practice Act, Pub. L. No. Act 575 (2000). Ghana. Retrieved from http://www.wipo.int/wipolex/en/text.jsp?file_id=225616

Trager, W., & Jensen, J. B. (1976). Human malaria parasites in continuous culture. *Science*, *193*, 673–675.

Traore, M. S., Baldé, M. A., Diallo, M. S. T., Baldé, E. S., Diané, S., Camara, A., … Baldé, A. M. (2013). Ethnobotanical survey on medicinal plants used by Guinean traditional healers in the treatment of malaria. *Journal of Ethnopharmacology*, *150*(3), 1145–53. http://doi.org/10.1016/j.jep.2013.10.048

United States Agency for International Development (USAID). (2013). *President's malaria initiative, liberia malaria operational plan FY 2013*. Retrieved from http://www.pmi.gov/countries/mops/fy13/liberia_mop_fy13.pdf

Upadhyay, R., Chaurasia, J. K., Tiwari, K. N., & Singh, K. (2014). Antioxidant property of aerial parts and root of *Phyllanthus fraternus* Webster, an important medicinal plant. *TheScientificWorldJournal*, *2014*, 692392. http://doi.org/10.1155/2014/692392

Valdés, A. F.-C., Martínez, J. M., Lizama, R. S., Gaitén, Y. G., Rodríguez, D. A., & Payrol, J. A. (2010). *In vitro* antimalarial activity and cytotoxicity of some selected cuban medicinal plants. *Revista Do Instituto de Medicina Tropical de São Paulo*, *52*(4), 197–201.

Valentin, A., Benoit-Vical, F., Pélissier, Y., Koné-Bamba, D., & Mallié, M. (2000). Antiplasmodial activity of plant extracts used in west African traditional medicine. *Journal of Ethnopharmacology*, *73*(1-2), 145–151. http://doi.org/10.1016/S0378-8741(00)00296-8

Varma, S. B., & Giri, S. P. (2013). Study of wound healing activity of *Tectona grandis* Linn. leaf extract on rats. *Ancient Science of Life*, *32*(4), 241–4. http://doi.org/10.4103/0257-7941.131984

Varma, S. B., & Jaybhaye, D. L. (2010). Antihyperglycemic activity of *Tectona grandis* Linn. bark extract on alloxan induced diabetes in rats. *International Journal of Ayurveda Research*, *1*(3), 163–6. http://doi.org/10.4103/09747788.72488

Vu, H., Roullier, C., Campitelli, M., Trenholme, K. R., Gardiner, D. L., Andrews, K. T., … Quinn, R. J. (2013). Plasmodium Gametocyte Inhibition Identi fi ed from a Natural- Product-Based Fragment Library.

Weenen, H., Nkunya, M. H. H., Bray, D. H., Mwasumbi, L. B., Kinabo, L. S., Kilimali, V. A. E. B., & Wijnberg, J. B. P. A. (1990). Antimalarial Compounds Containing an α, β-Unsaturated Carbonyl Moiety from Tanzanian Medicinal Plants. *Planta Medica*, *56*(4), 371–373. http://doi.org/10.1055/s-2006-960985

Weniger, B., Lagnika, L., Vonthron-Sénécheau, C., Adjobimey, T., Gbenou, J., Moudachirou, M., … Sanni, A. (2004). Evaluation of ethnobotanically selected Benin medicinal plants for their *in vitro* antiplasmodial activity. *Journal of Ethnopharmacology*, *90*(2-3), 279–84. http://doi.org/10.1016/j.jep.2003.10.002

White, N. J. (2008). Plasmodium knowlesi: the fifth human malaria parasite. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *46*(2), 172–3. http://doi.org/10.1086/524889

White, N. J., & Pongtavornpinyo, W. (2003). The de novo selection of drug-resistant malaria parasites. *Proceedings. Biological Sciences / The Royal Society*, *270*(1514), 545–554. http://doi.org/10.1098/rspb.2002.2241

White, N. J., Pukrittayakamee, S., Hien, T. T., Faiz, M. A., Mokuolu, O. A., & Dondorp, A. M. (2014). Malaria. *Lancet*, *383*(9918), 723–35. http://doi.org/10.1016/S0140-6736(13)60024-0

WHO. (2010). *Guidelines for the treatment of malaria. Second edition*. *WHO Press*. Geneva: World Health Organization.

WHO. (2012). *World malaria report 2011*. *WHO Press*. Geneva: World Health Organization.

WHO. (2013a). *WHO Traditional Medicine Strategy 2014-2023*. Geneva: World Health Organization.

WHO. (2013b). *World Malaria Report: 2013*. *WHO Press*. Geneva.

WHO. (2014). *World Malaria Report 2014*. Geneva: WHO Press.

WHO. (2015). Questions and answers on malaria vaccines. Retrieved January 5, 2016, from http://www.who.int/immunization/research/development/malaria_vaccine_qa/en/

WHO. (2016). Tables of malaria vaccine projects globally. Retrieved January 5, 2016, from http://www.who.int/immunization/research/development/Rainbow_tables/en/

WHO AFRO. (1999). *Forty-ninth session of the WHO regional committee for Africa held in Windhoek, Namibia, from 30 August to 3 September 1999*. Windhoek, Namibia,. Retrieved from http://apps.who.int/iris/bitstream/10665/1965/1/AFRRC49-INF DOC-1 EN.pdf

Wichmann, O., Muehlen, M., Gruss, H., Mockenhaupt, F. P., Suttorp, N., & Jelinek, T. (2004). Malarone treatment failure not associated with previously described mutations in the cytochrome b gene, *3*, 4–6.

Willcox, M. (2011). Improved traditional phytomedicines in current use for the clinical treatment of malaria. *Planta Medica*, *77*(6), 662–671.

Willcox, M., Benoit-Vical, F., Fowler, D., Bourdy, G., Burford, G., Giani, S., … Rasoanaivo, P. (2011). Do ethnobotanical and laboratory data predict clinical safety and efficacy of anti-malarial plants? *Malaria Journal*, *10*(Suppl 1), S7. http://doi.org/10.1186/1475-2875-10-S1-S7

Willcox, M. L., & Bodeker, G. (2004). Traditional herbal medicines for malaria. *BMJ (Clinical Research Ed.)*, *329*(7475), 1156–9. http://doi.org/10.1136/bmj.329.7475.1156

Wright, C. W., & Phillipson, J. D. (1990). Natural products and the development of selective antiprotozoal drugs. *Phytotherapy Research*, *4*(4), 127–139. http://doi.org/10.1002/ptr.2650040402

Wurdack, K. J., Bruijn, A. De, Hoffmann, P., Samuel, R., Bank, M. Van Der, &

Chase, M. W. (2006). Molecular phylogenetic analysis of Phyllanthaceae (Phyllanthoideae pro parte, Euphorbiaceae sensu lato) using plastid rbcl DNA sequences. *American Journal of Botany*, *91*(11), 1882–1900.

Yakubu, M. T., & Bukoye, B. B. (2009). Abortifacient potentials of the aqueous extract of *Bambusa vulgaris* leaves in pregnant Dutch rabbits. *Contraception*, *80*(3), 308–13. http://doi.org/10.1016/j.contraception.2009.03.003

Yakubu, M. T., Bukoye, B. B., Oladiji, A. T., & Akanji, M. A. (2009). Toxicological implications of aqueous extract of *Bambusa vulgaris* leaves in pregnant Dutch rabbits. *Human & Experimental Toxicology*, *28*(9), 591–8. http://doi.org/10.1177/0960327109106975

Yan, J., Sun, L., Zhang, X., & Qiu, M. (2005). A new flavone from Lycopodium japonicum. *Heterocycles*, *65*(3), 661–666.

Ye, Y.-Q., Xia, C.-F., Li, Y.-K., Wu, X.-X., Du, G., Gao, X.-M., & Hu, Q.-F. (2014). Anthraquinones from *Cassia siamea* and their Cytotoxicity. *Chemistry of Natural Compounds*, *50*(5), 819–822. http://doi.org/10.1007/s10600-014-1091-9

Yetein, M. H., Houessou, L. G., Lougbégnon, T. O., Teka, O., & Tente, B. (2013). Ethnobotanical study of medicinal plants used for the treatment of malaria in plateau of Allada, Benin (West Africa). *Journal of Ethnopharmacology*, *146*(1), 154–63. http://doi.org/10.1016/j.jep.2012.12.022

APPENDICES

Appendix 1a: Formula for plasmodium culture medium

Formula	
RPMI 1640	20.86g (2 tins)
Hypoxanthine	100 mg
$NaHCO_3$	4g
HEPES	11.88 g
Glucose solution (2g/l)	25 mL
Albumax II	10g
Sterile double-distilled, deionized water to	2L
pH:	7.34

Appendix 1b: Formula of cryo solution

3 g D-mannitol

0.65g NaCl

28 mL glycerol

Double distilled water to 100 mL in volumetric flask

Filter solution through 0.2µm sterile filter with a syringe of 50 mL and pack into aliquots in 14 mL Falcon tubes. Store at 4^oC

Appendix 1c: Determining amount of components of malaria culture medium

Parasitemia : 1%

Haematocrit: 5 %

Preparation of blood samples:

Available parasitemia = 7.02%

Available haematocrit =100%

Volume of culture per well =100 µL

Number of drugs = 18

Number of wells in duplicate for 12 wells = 18 x 2 x 12 = 432

Volume of culture needed = 100 µl x 432 = 43200 µL =43.2 ml ≈ 45ml of culture

Volumes of infected and non-infected erythrocyte to be taken. Dilution

factor for non-infected erythrocyte = $\frac{\%}{\%} = 20$

Volume of culture needed by: $\frac{}{} = 2250$ µL

Determination of amt of infected blood

Dilution factor = $\frac{\%}{\%} = 7.02$

Volume of infected blood required is given by, X = $\frac{}{} = 320.5$ µL

Determination of amt of complete medium

Volume of complete medium needed = 45000 – (1929.5 + 320.5) = 42750 µL

The culture was prepared by adding 320.5 µL of infected erythrocytes to 1929.5 µL of non-infected erythrocyte and toping up with 42750 µL of wash medium to obtain 45000 µL culture with a starting 5% haematocrit and 1% parasitemia using the above determinations.

Appendix 2a: Preparation of 5% haematocrit

20ml of 5% haematocrit was prepared by adding 19 mL culture medium to 1 mL erythrocyte pellet (100% haematocrit).

Appendix 2b: Preparation of packed red blood cells or pellet

Erythrocytes were used in the dilution of culture in order to lower parasitaemia and maintain haematocrit concentration; Plasmodium cultural medium without erythrocytes (wash medium) was warmed in water bath to 37ºC. Fresh blood from the Hospital in 10 mL centrifuge tubes was centrifuged at 2800 rpm at 20º C for 7 minutes. The serum and "Buffy-coat" was aspirated using a Pasteur pipette. The volume was then supplemented with 2x "Wash medium" and mixed well. The mixture was centrifuged at 2800 rpm for 7 min, at 20º C. The washing was repeated two times and the washed erythrocytes re-suspended in wash medium and stored at 4 ºC

Appendix 2c: Thawing of culture (Clones)

Clones were stored in cryotube containing glycerolyte at -196 ºC in liquid nitrogen. A sterile NaCl solution (3.5% w/v) was prepared and stored at 4º C. Wash medium was preheated in water bath to 37ºC. A vial was removed from the nitrogen tank and thawed under warm running water (37º C) for 2 minutes and transferred into twice its volume of the NaCl solution using a Pasteur pipettes. This was agitated gently for 7 minutes on a shaker at room temperature. It was then centrifuged at 2800rpm at 20º C for 7 minutes and the supernatant removed with Pasteur pipettes. The pellet was washed two more times using culture medium in a ratio of 1:9 v/v.

Appendix 2d: Setting up culture in 25cm^3- capacity tissue-culture flask.

RPAL medium was preheated to 37º C in a water-bath. Eight (8) drops (1drop= 0.05mL) of haematocrit (6 drops uninfected haematocrit plus 2 drops infected

haematocrit) was added to 8 ml of medium to obtain 5% haematocrit in the 25cm^3 tissue-culture flask. The content was stirred gently by swirling the flask in a circular motion while holding it at an angle of about 45^o and ensuring the content did not touch or climb the neck. The screw caps were loosely closed (initially closed tightly and then opened through 180^o). The flask was placed in a candle jar and incubated at 37 ^{o}C. The medium was replaced daily except the the day after subcultivation. The culture was subcultured 2-3 times per week.

Appendix 2e: Replacement of culture medium on culture

The culture medium was preheated to 37 ^{o}C in water-bath. The candle jar was carefully removed from the incubator onto the hood. With the help of a Pasteur pipette and pipette aid, the medium on the culture (about 7 ml) was aspirated while tilting the culture flask to avoid removing the erythrocytes. Four drops of non-infected erythrocytes and 7 ml of culture medium were then added using Pasteur pipette and 25ml-capacity pipette respectively. The flask was placed back in the candle jar and incubated at 37^o C as usual.

Appendix 2f: Preparation of thin smear (frottis)

Using a pencil, a slide was labelled with date, the culture flask numbered and then initialled. Four microliter (4 μL) of culture was taken from the horizontal corner and the base of the flask while tilted, and placed on the slid. A second slide was placed on the first and moved, in a single back and fro, to spread the drop of culture sideways and across the first slid in order to form a film or smear of blood on the latter. The smear was allowed to dry, and the slide dropped into a container with methanol to fixe it, for 1 minute. The slid was removed, dried in air and then dipped in eosin, where it

was moved up and down ten times (10x). It was taken out, washed under running tap and dried and then dropped in methylene blue for 1 minute, rinsed and dried. The fixed and stained slid was observed under x100 (oil immersion). The parasitemia was determined by counting both uninfected and parasite-infected erythrocytes. The count was repeated for 10 fields on the slide and the % parasitemia calculated as below.

$$\% = \frac{(No\ of\ ring\ forms + No\ of\ trophozoites + No\ of\ schizonts) \times 100}{\text{(h \#(}}$$

$$\% = \frac{\times 100}{+ \quad h" \quad + \quad \#h" \quad)}$$

Appendix 2g: Preparation of 3.5% NaCl sterile solution.

Sodium chloride (ref. A4890951 labosi lot No 0898037) (3.5g) was dissolved in 100 ml volumetric flask using sterile double distilled water. The solution was filterred with 0.2 µm filter and aliquoted into 14 mL falcon tubes and store at 4ºC

Appendix 2h: Preparation of Stock test solution

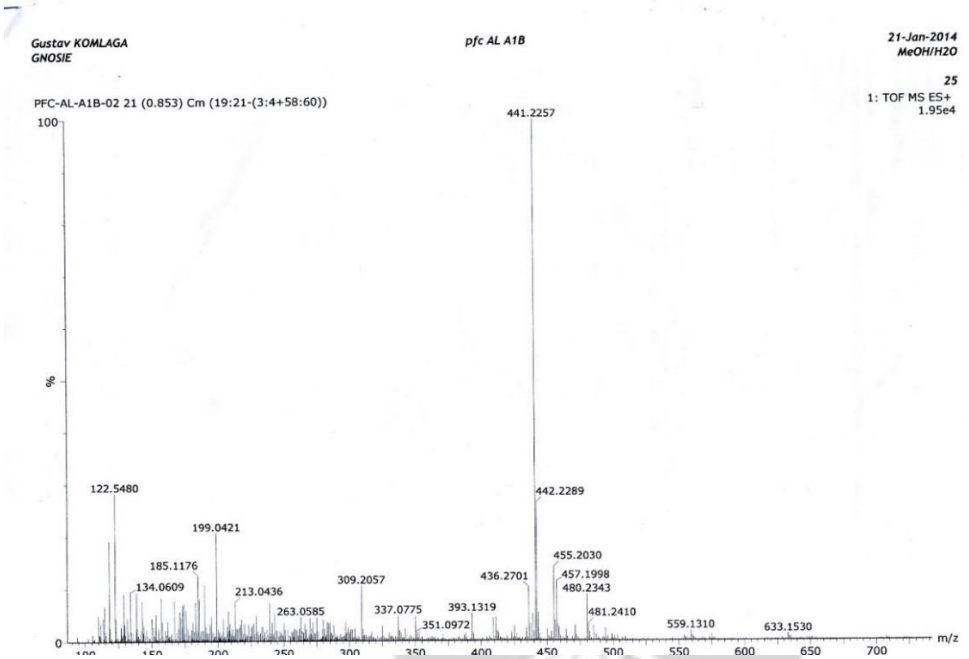
Stock concentrations (20 mg/mM) of extracts were prepared in DMSO at room temperature using the sonicator. The solutions were appropriately labelled and kept at 4 ºC until needed.

Appendix 2i: Preparation of test solutions from stock solution

One thousand micro litres (1000 µL) of test solutions (200 µg/mL) were prepared by adding 990 µL of culture medium to 10 µL of stock solution (10 mg/mL) of extract to give a test solution of concentration, of 200 µg/mL Crude extract:

1000µl of 200 µg/mL =10 µL (10 mg/mL) + 990µL culture medium

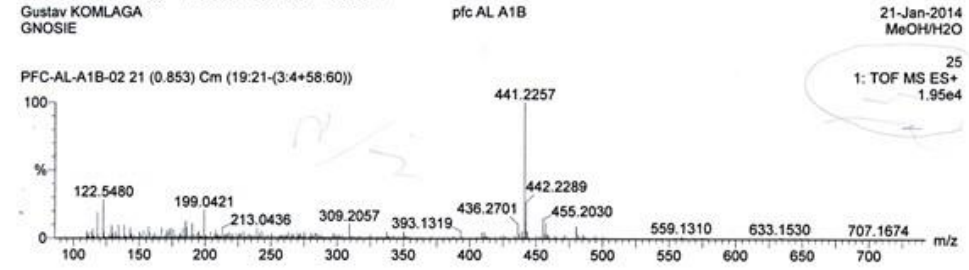
*Appendix 3a: Mass spectrum and elemental composition report for PfCAlA1B (**Pf 1**)*



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -2.0, max = 100.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
626 formula(e) evaluated with 5 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-80 H: 1-100 N: 0-6 O: 0-8 Na: 0-1

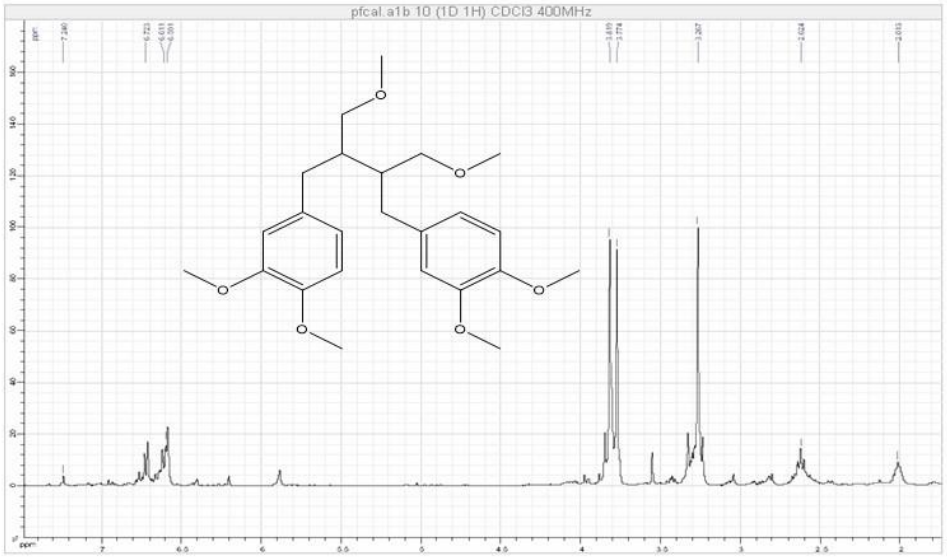


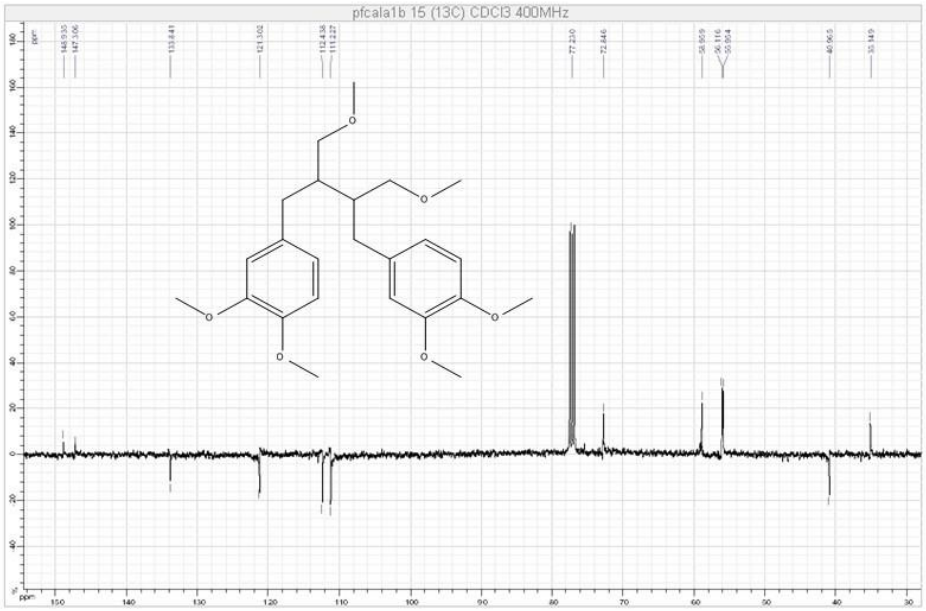
Minimum:				-2.0		
Maximum:		200.0	5.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
441.2257	441.2253	0.4	0.9	7.5	8.6	C24 H34 O6 Na ✓
	441.2250	0.7	1.6	11.5	19.6	C22 H29 N6 O4
	441.2266	-0.9	-2.0	12.5	43.5	C25 H30 N4 O2 Na
	441.2277	-2.0	-4.5	10.5	31.2	C26 H33 O6
	441.2237	2.0	4.5	6.5	16.9	C21 H33 N2 O8

m/z = 441,2257 ; calculated for $C_{24}H_{34}O_6Na^+$: 441,2253 (δ = 0.

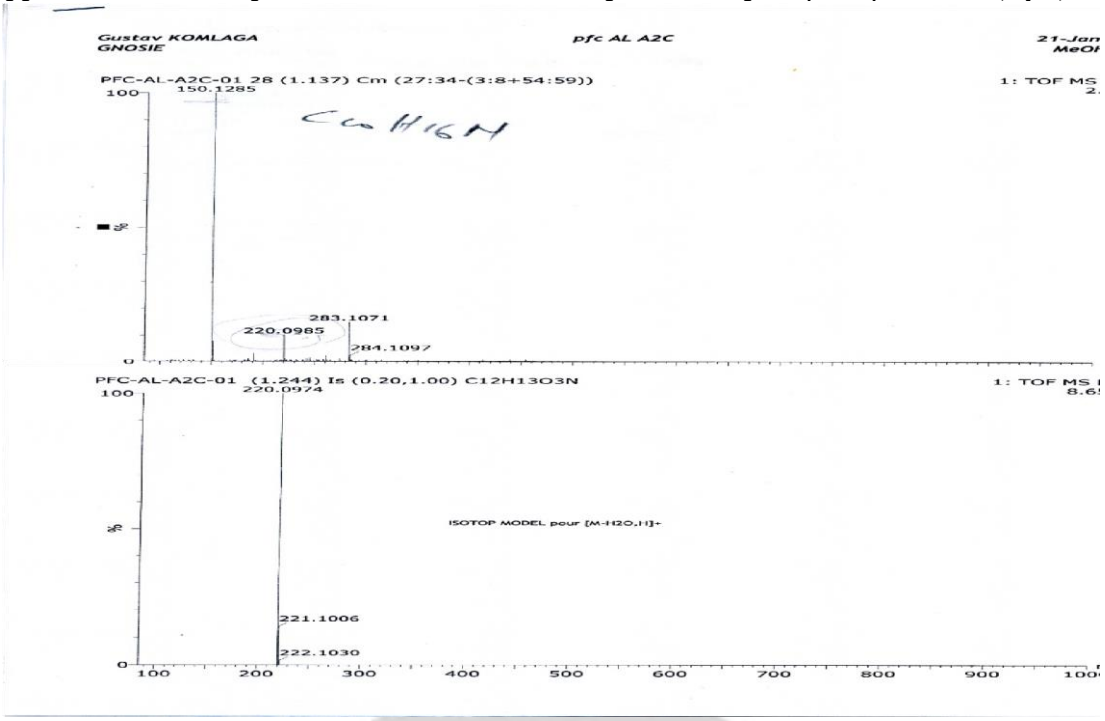
^{1}H and ^{13}

*Appendix 3b: C NMR OF PfCAlA1B (**Pf 1**) at 400Mhz in $CDCl_3$*





*Appendix 4a. Mass spectrum and elemental composition report for PfCAlA2C (**Pf 2**)*



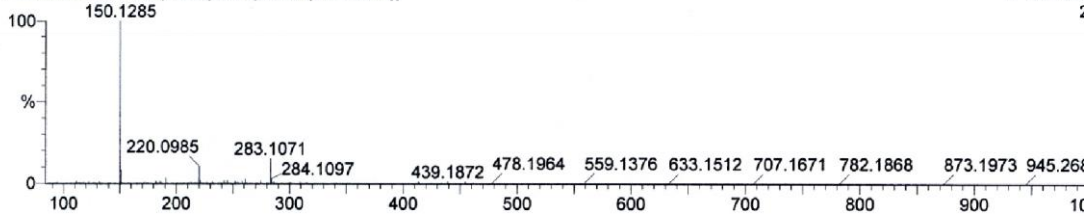
Elemental Composition Report

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -2.0, max = 100.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
406 formula(e) evaluated with 2 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-80 H: 1-100 N: 0-10 O: 0-10 Na: 0-1

Gustav KOMLAGA
GNOSIE
pfc AL A2C
21-Jan
MeOH

PFC-AL-A2C-01 28 (1.137) Cm (27:34-(3:8+54:59))
1: TOF MS

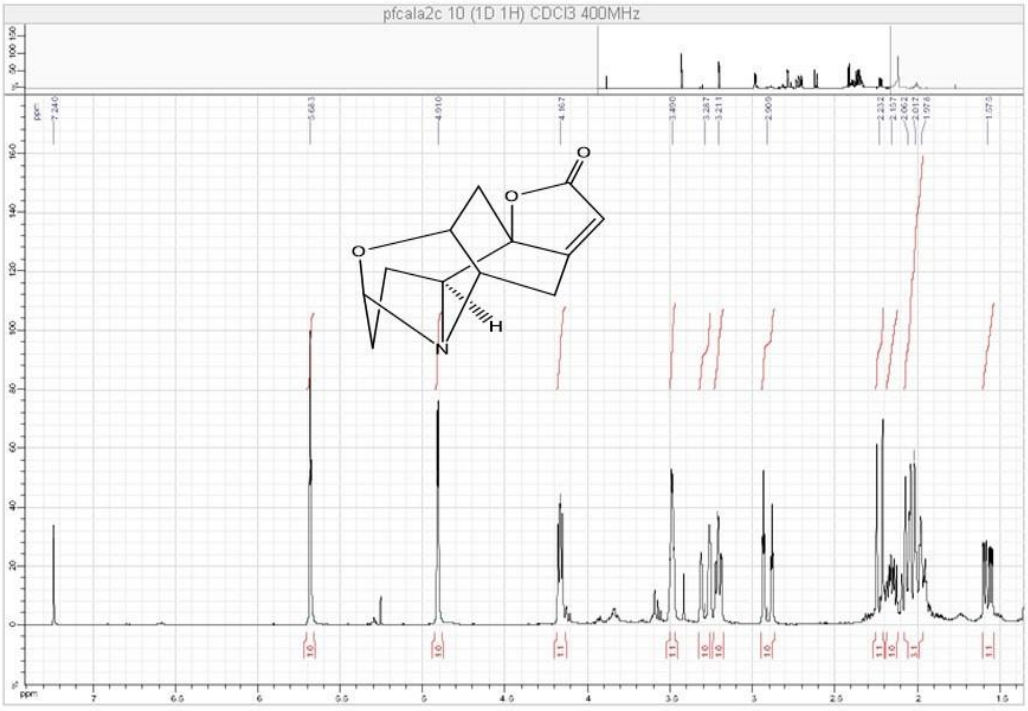


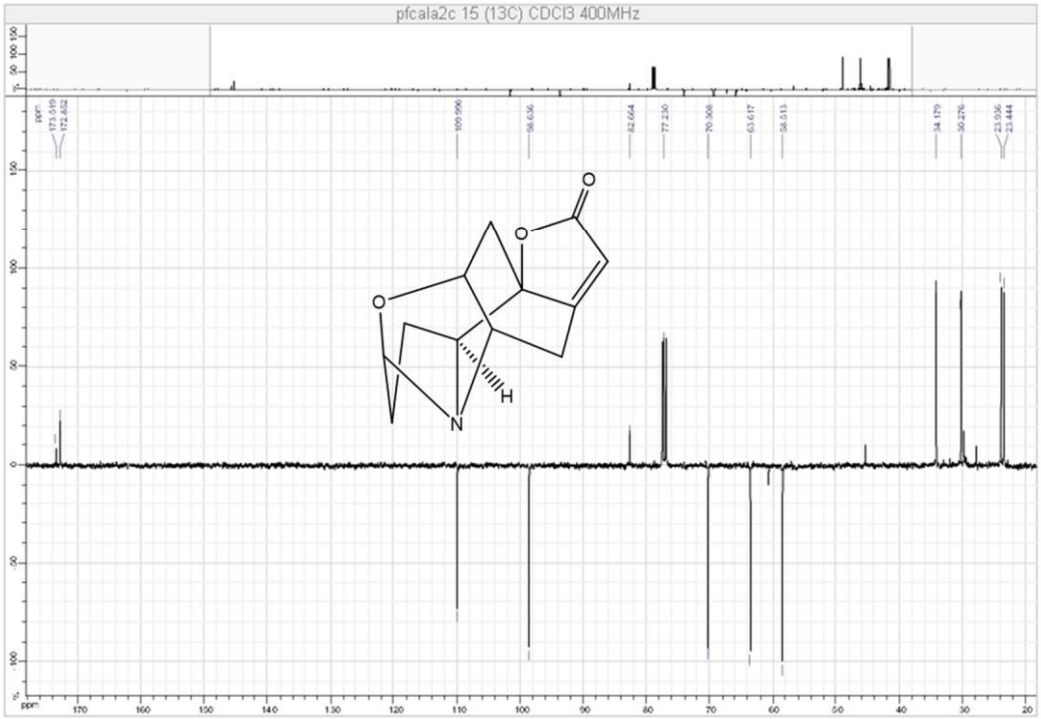
Minimum:				-2.0		
Maximum:		200.0	10.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
220.0985	220.0974	1.1	5.0	6.5	171.6	C12 H14 N O3
	220.1006	-2.1	-9.5	-1.5	1377.6	C H14 N7 O6

(M-H2O)

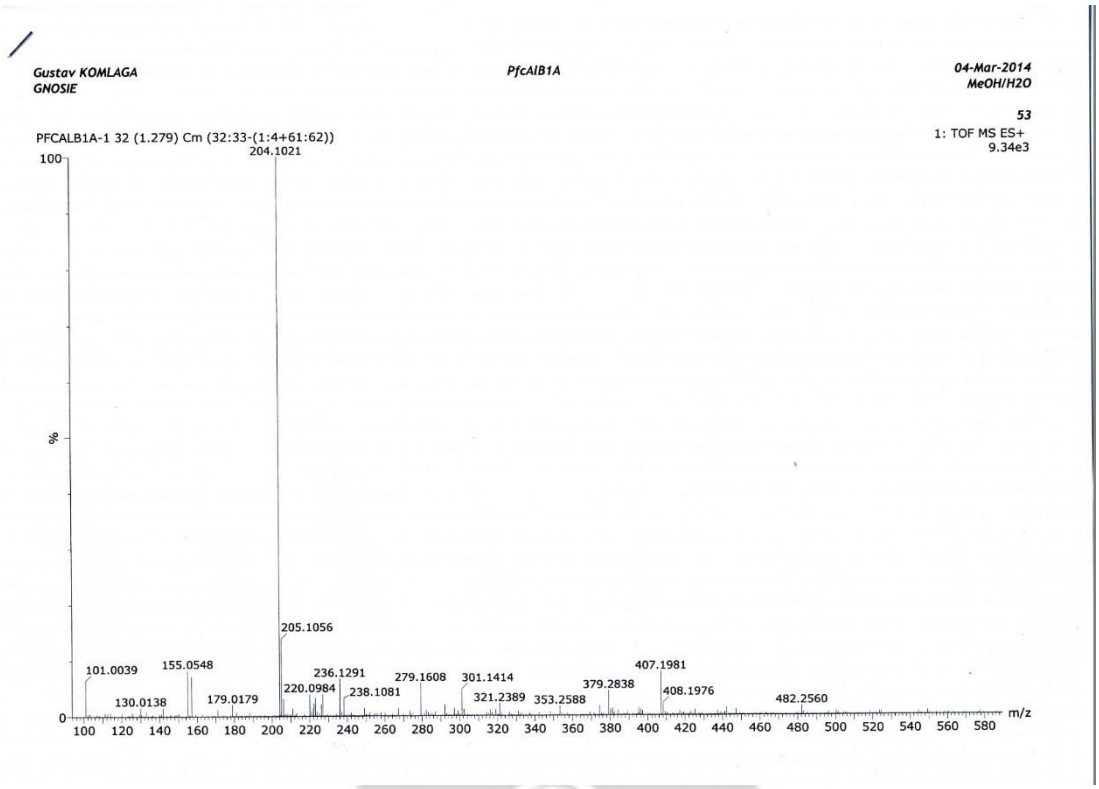
*Appendix 4b: C NMR of PfCAlA2C (**Pf 2***

^{1}H and ^{13}) at 400Mhz in $CDCl_3$





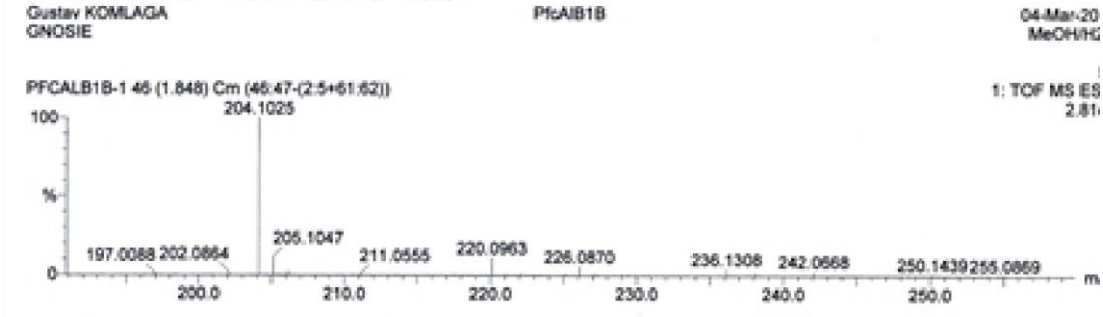
*Appendix 5a Mass spectrum and elemental composition of PfCB1A (**Pf 3**)*



Elemental Composition Report **Page**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -2.0, max = 100.0
Selected filters: None

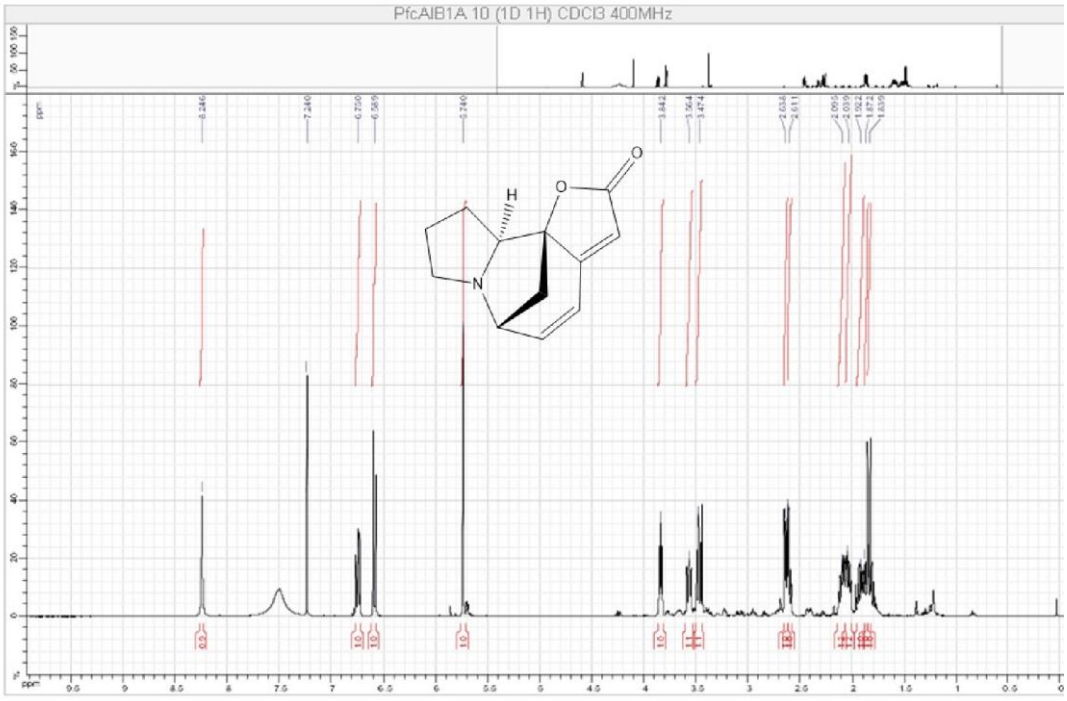
Monoisotopic Mass, Even Electron Ions
353 formula(e) evaluated with 1 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-70 H: 1-100 N: 0-10 O: 0-13 Na: 0-1

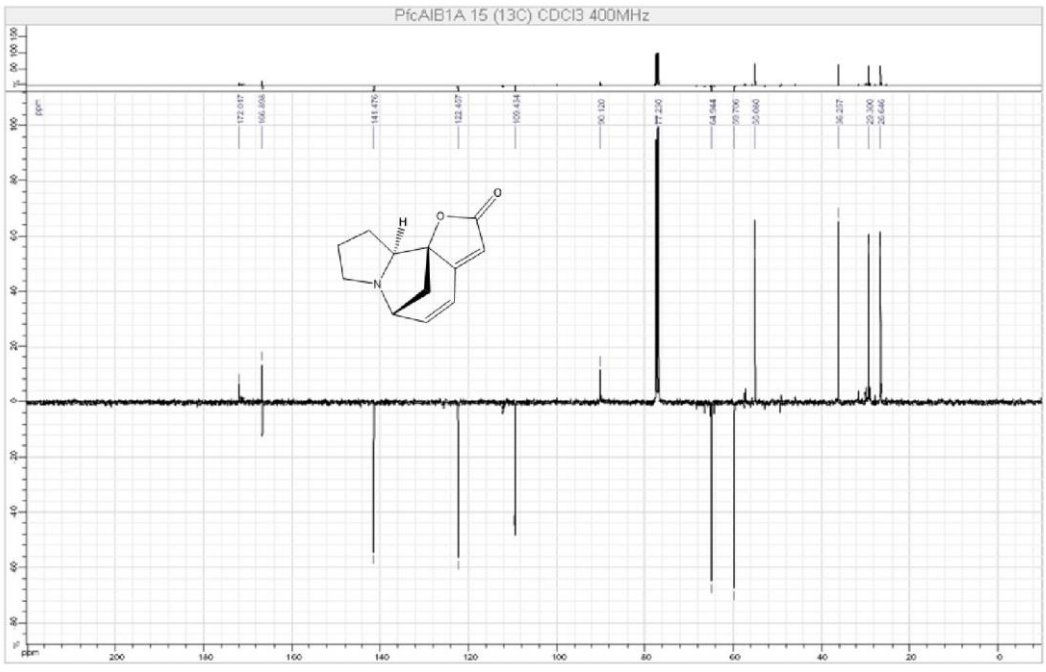


Minimum:				-2.0		
Maximum:		200.0	5.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
204.1025	204.1025	0.0	0.0	6.5	18.8	C12 H14 N O2

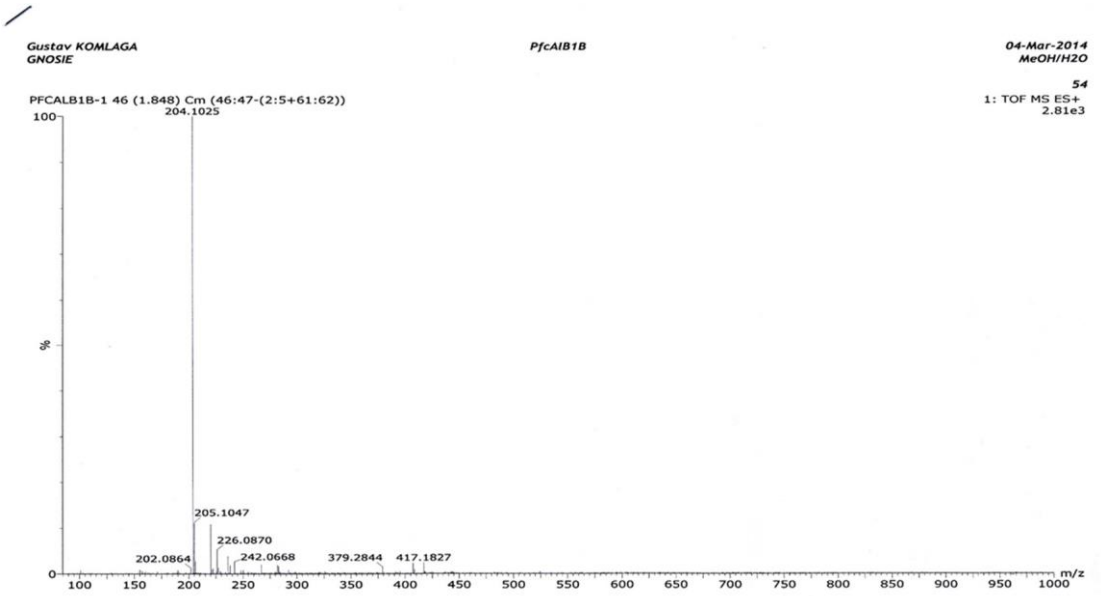
*Appendix 5b:: C NMR of PfCAlB1A (**Pf 3***

^{1}H and ^{13}) at 400Mhz in $CDCl_3$





*Appendix 6a Mass spectrum and elemental composition of PfCB1B (**Pf 4**)*

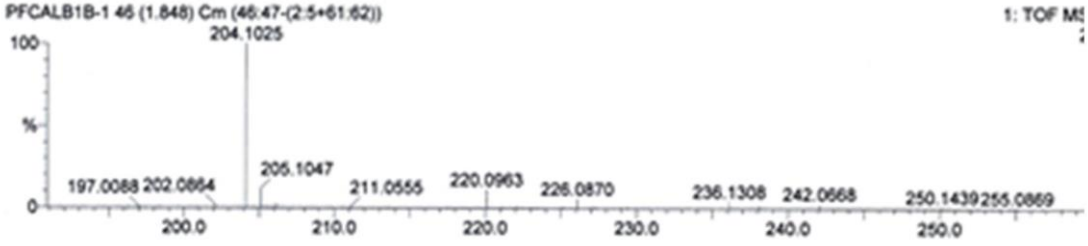


Elemental Composition Report Pa

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -2.0, max = 100.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
353 formula(e) evaluated with 1 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-70 H: 1-100 N: 0-10 O: 0-13 Na: 0-1

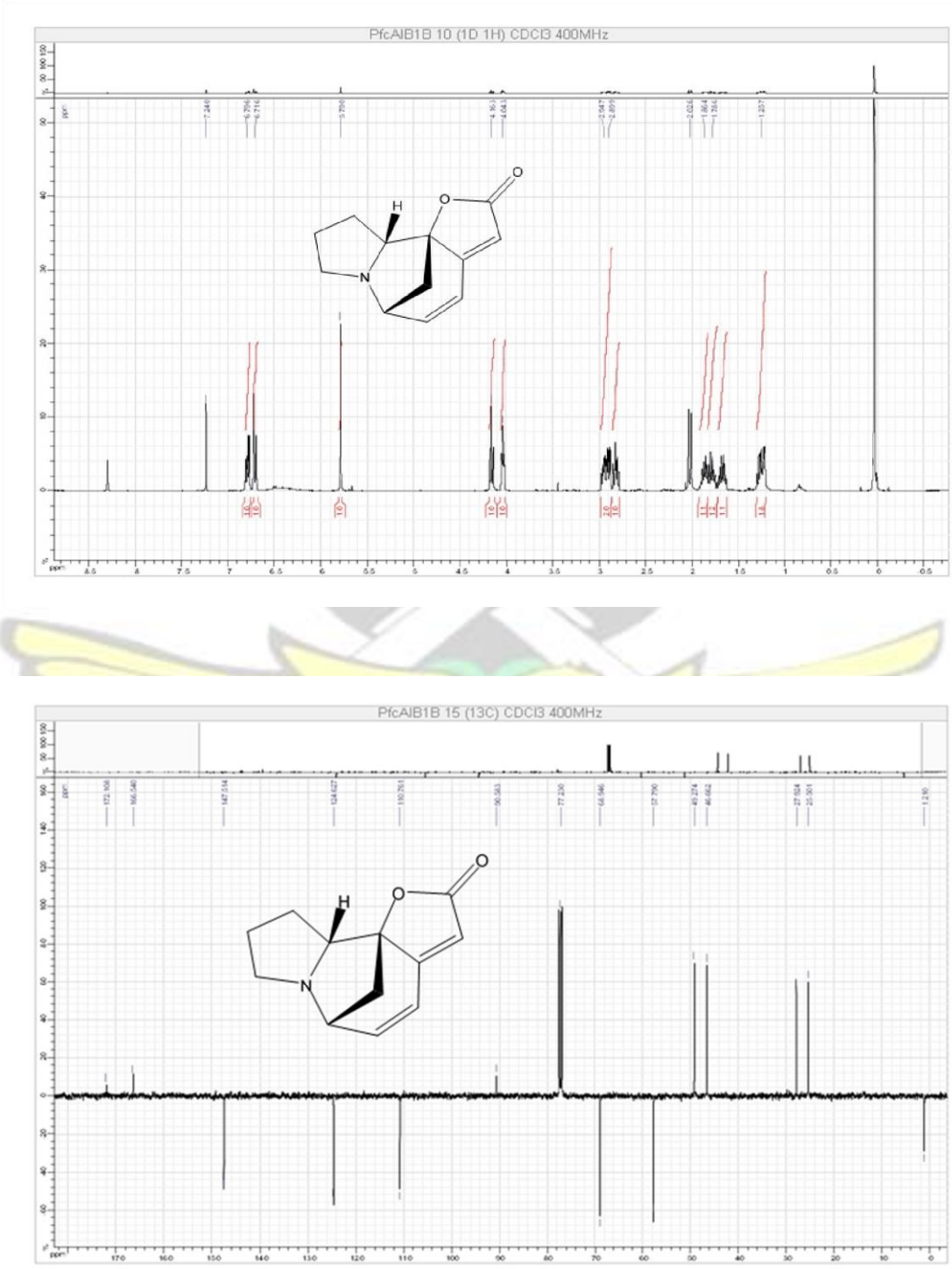
Gustav KOMLAGA
GNOSIE
PfcAlB1B
04-Ma
MeO



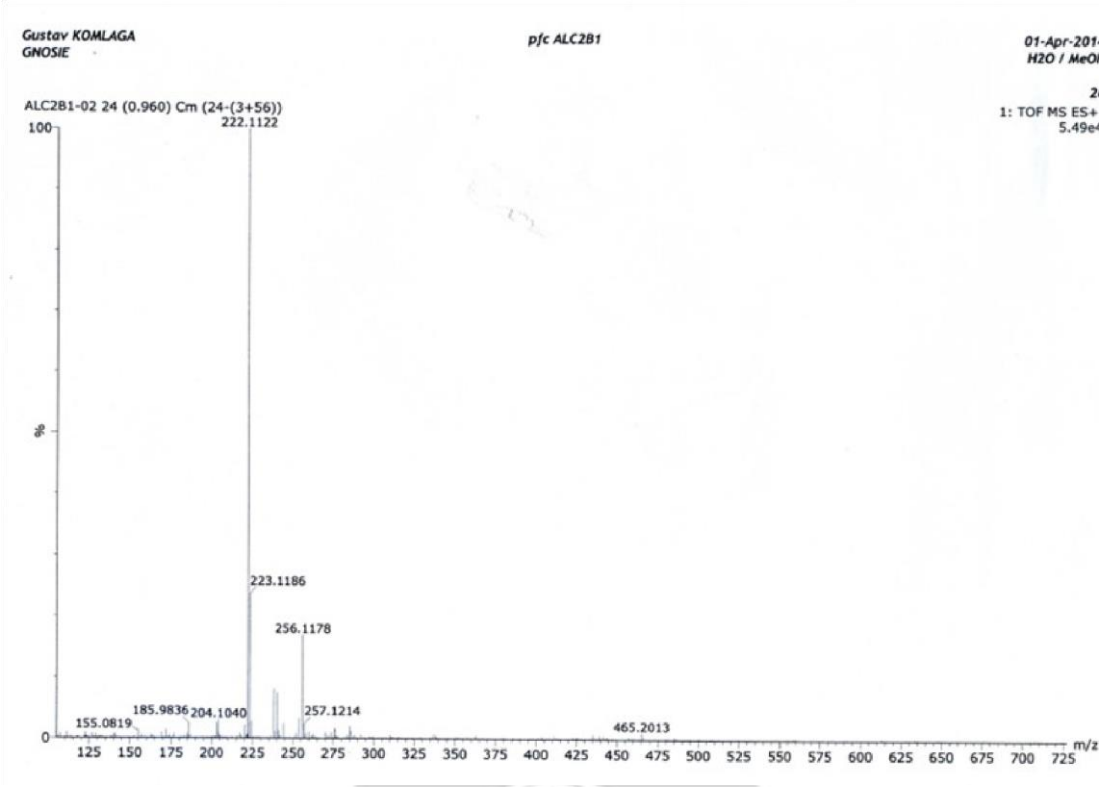
Minimum:				-2.0		
Maximum:		200.0	5.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
204.1025	204.1025	0.0	0.0	6.5	18.8	C12 H14 N O2

Appendix 6b: *C NMR of PfCAlB1B (**Pf 4***

^{1}H and ^{13}) at 400Mhz in $CDCl_3$



*Appendix 7a: Mass spectrum and elemental composition report of PfCAlC2B1 (**Pf 5**)*



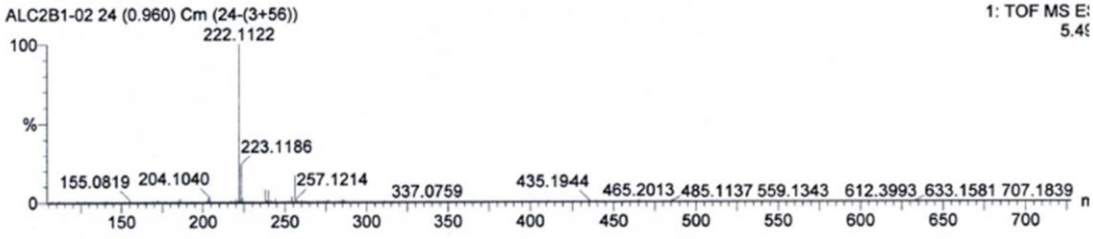
Elemental Composition Report **Page**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -2.0, max = 100.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
417 formula(e) evaluated with 1 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-70 H: 1-100 N: 0-10 O: 0-10 Na: 0-1

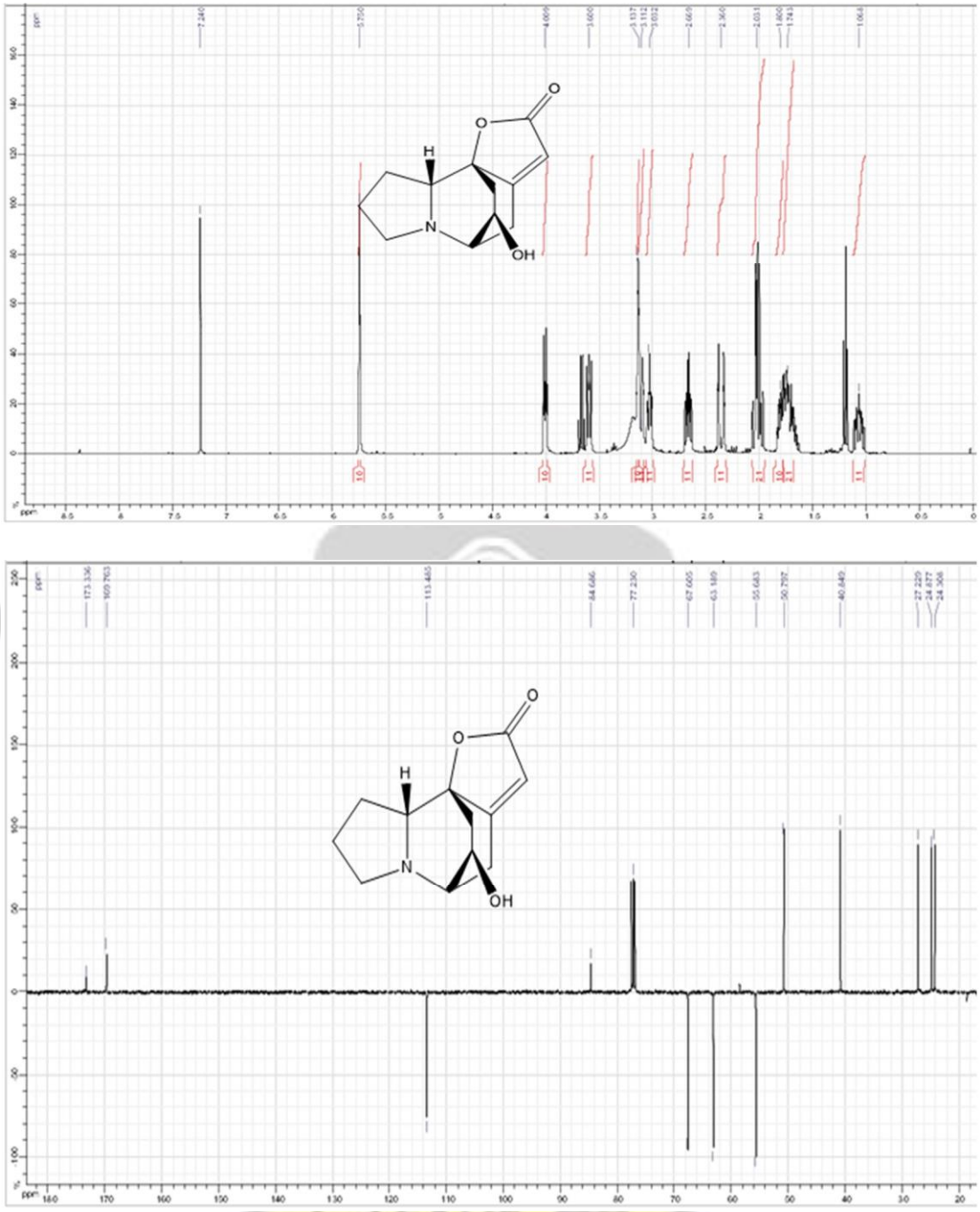
Gustav KOMLAGA
GNOSIE

pfc ALC2B1

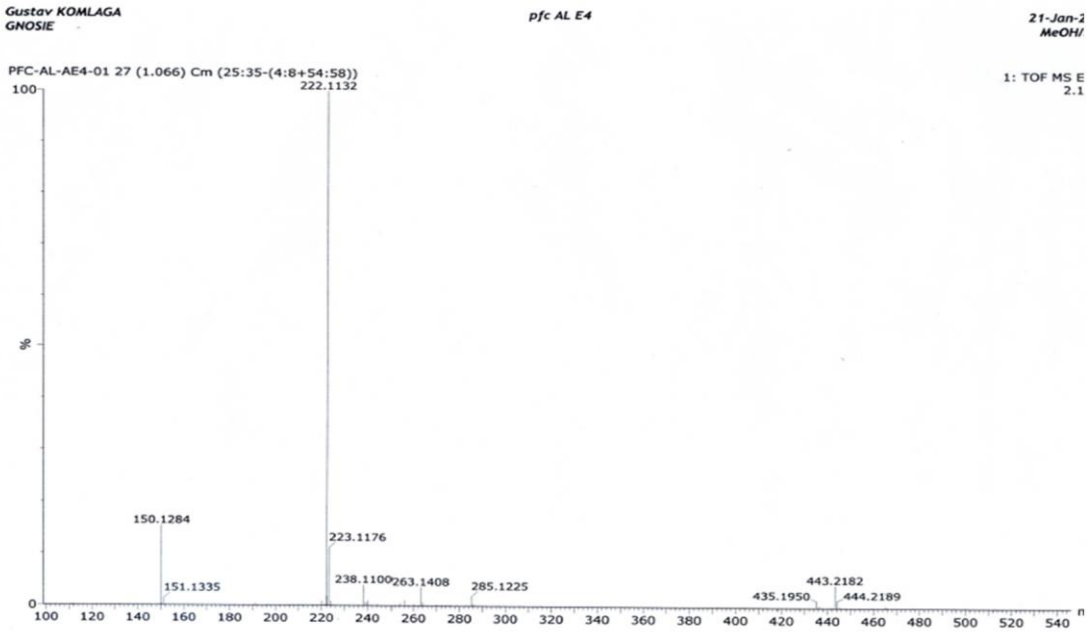


Minimum:				-2.0		
Maximum:		200.0	5.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
222.1122	222.1130	-0.8	-3.6	5.5	1129.6	C12 H16 N O3

*Appendix 7b: ^{1}H and ^{13}C NMR of PfCAlC2B1 (**Pf 5**) at 400Mhz in $CDCl_3$*



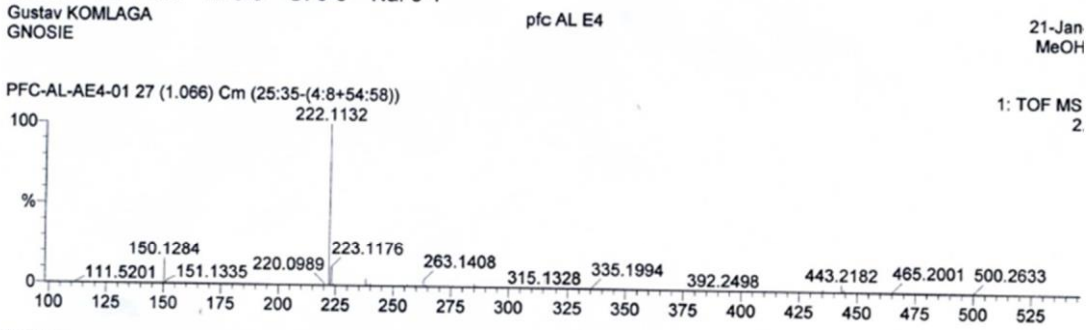
*Appendix 8a: Mass spectrum and elemental composition report of PfCAlE2E (**Pf 6**)*



Elemental Composition Report

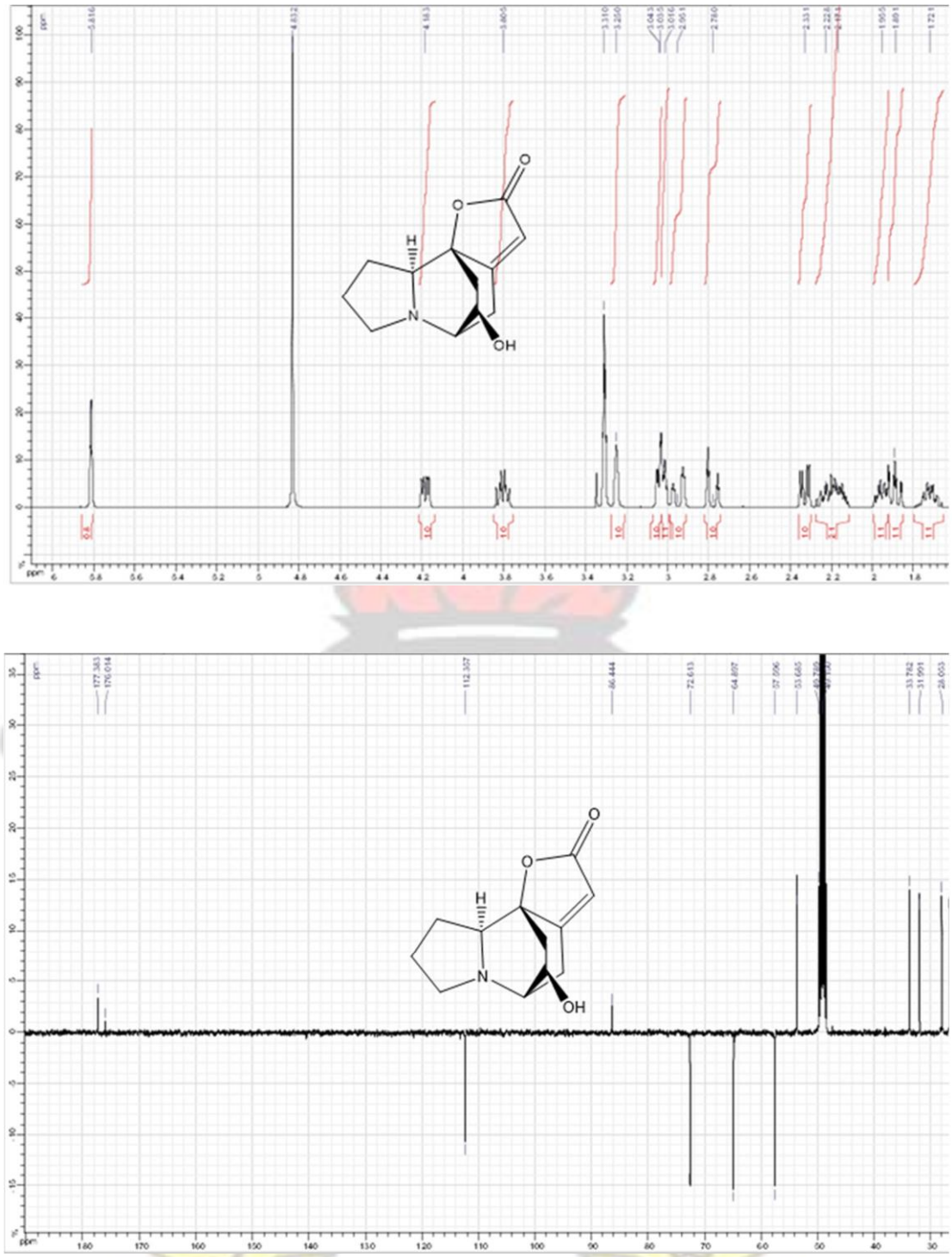
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -2.0, max = 100.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
291 formula(e) evaluated with 1 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-80 H: 1-100 N: 0-6 O: 0-8 Na: 0-1

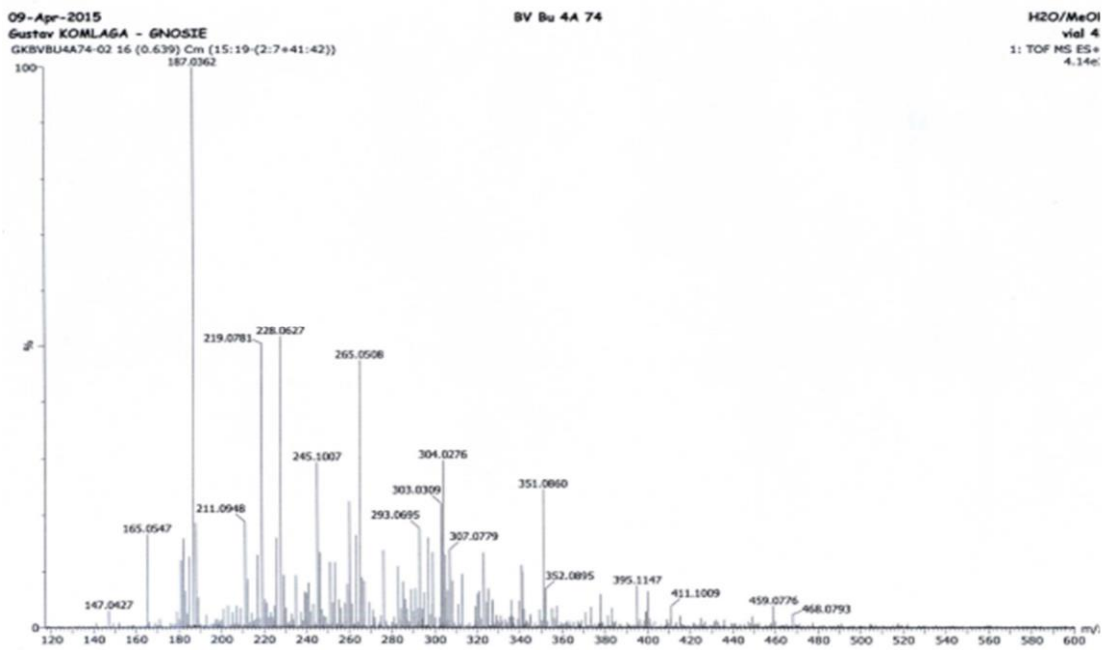


Minimum:				-2.0		
Maximum:		200.0	5.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
222.1132	222.1130	0.2	0.9	5.5	13467.4	C12 H16 N O3

*Appendix 8b: ^{1}H and ^{13}C NMR of PfCAlE2E (**Pf 6**) at 400Mhz in CD_3OD*



*Appendix 9a: Mass spectrum and elemental composition report of BvBu4a74 (**Bv1**)*

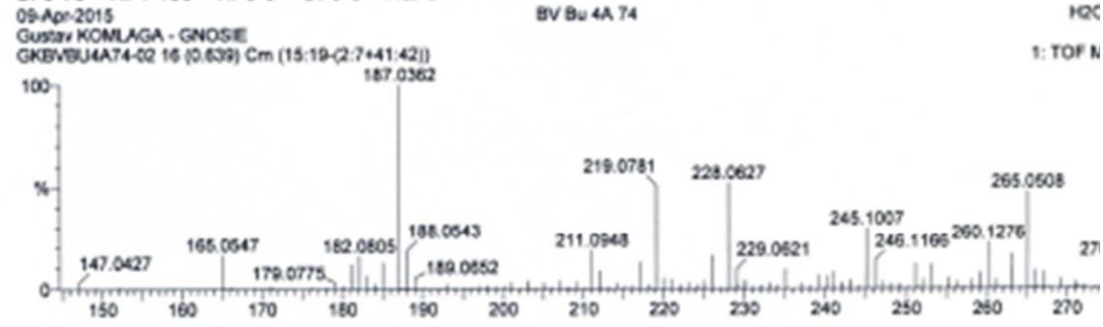


Elemental Composition Report

Pa

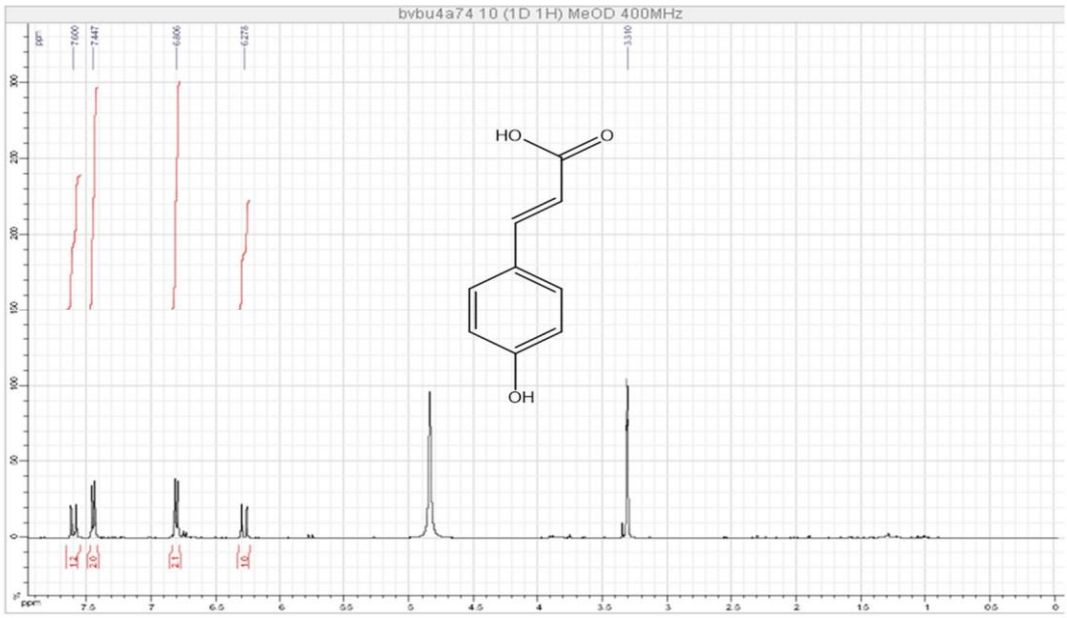
Tolerance = 5.0 PPM / DBE: min = -2.0, max = 100.0
Selected filters: None

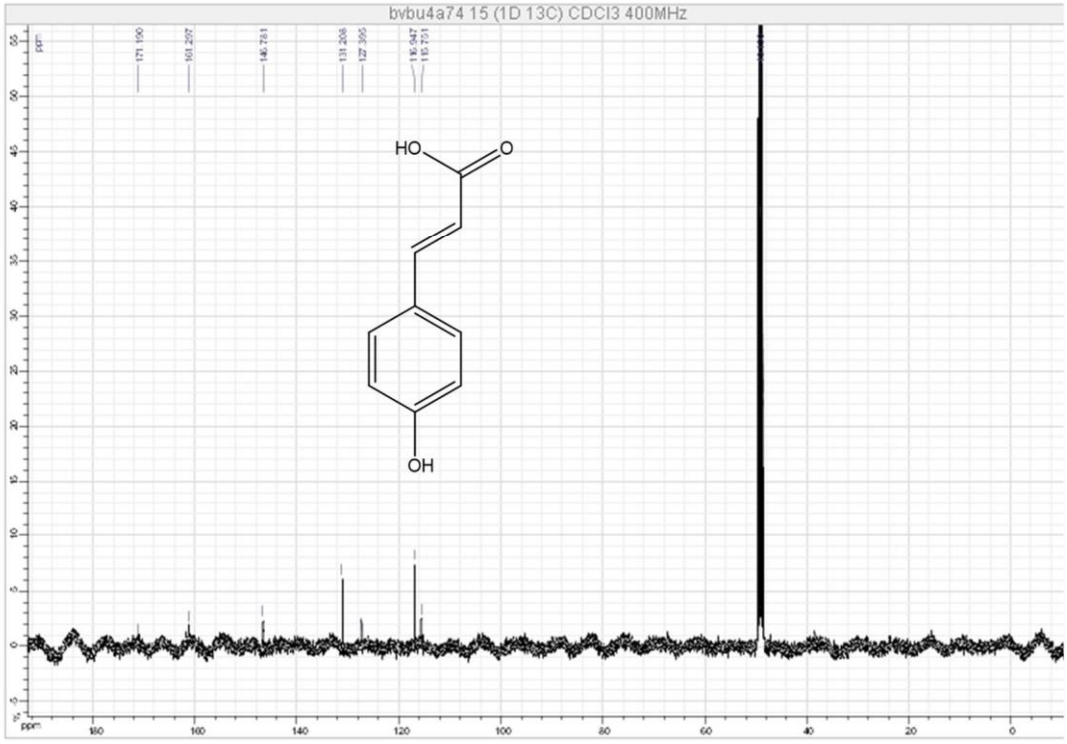
Monoisotopic Mass, Even Electron Ions
203 formula(e) evaluated with 2 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-70 H: 1-130 N: 0-5 O: 0-8 Na: 0-1



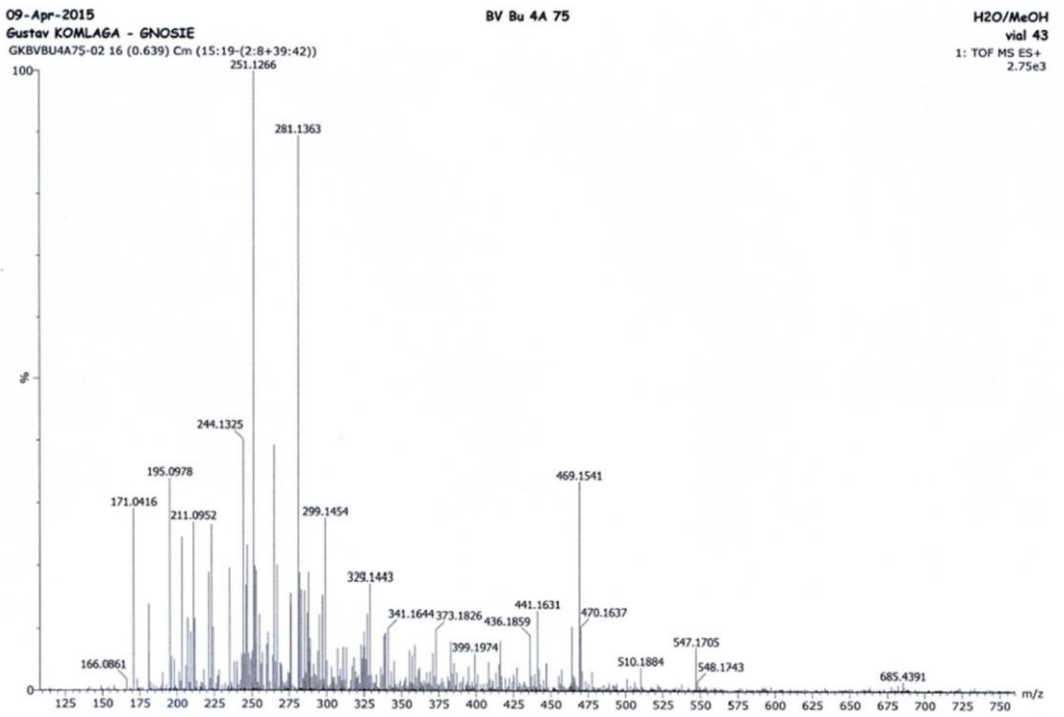
Minimum:	72.00				-2.0		
Maximum:	100.00		200.0	5.0	100.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
187.0362	100.00	187.0371	-0.9	-4.8	5.5	2773805.8	C9 H8 O3 Na
		187.0355	0.7	3.7	4.5	2774030.8	C6 H7 N2 O5

*Appendix 9b: ^{1}H and ^{13}C NMR of BvBu4a75 (**Bv1**) at 400Mhz in CD_3OD*





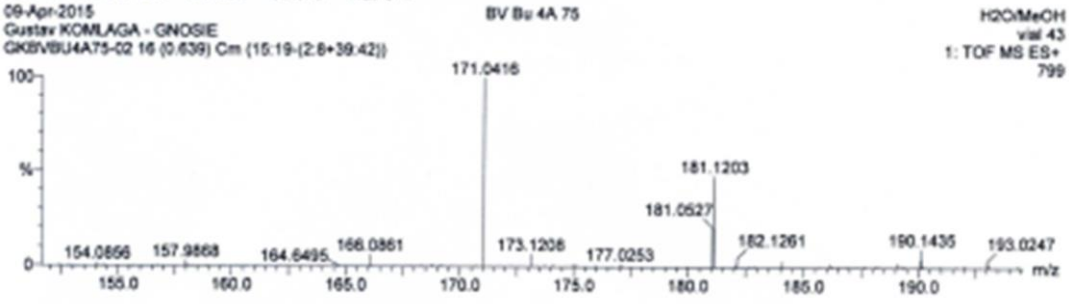
*Appendix 10a: Mass spectrum and elemental composition report of BvBu4a75 (**Bv2**)*



Elemental Composition Report **Page 1**

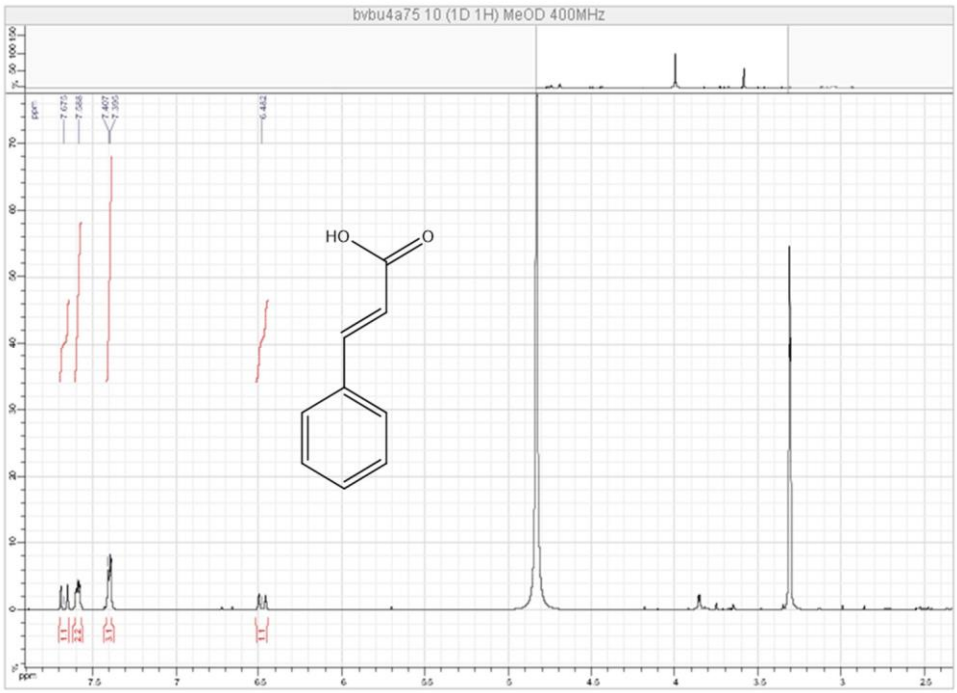
Tolerance = 5.0 PPM / DBE: min = -2.0, max = 100.0
Selected filters: None

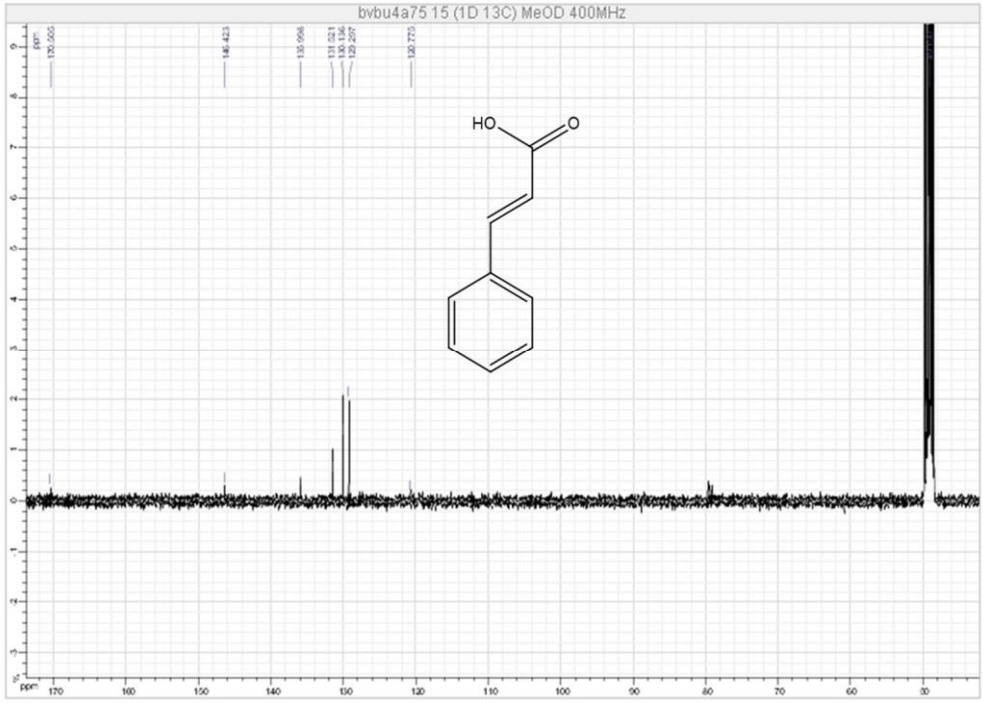
Monoisotopic Mass, Even Electron Ions
177 formula(e) evaluated with 1 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-70 H: 1-130 N: 0-5 O: 0-8 Na: 0-1



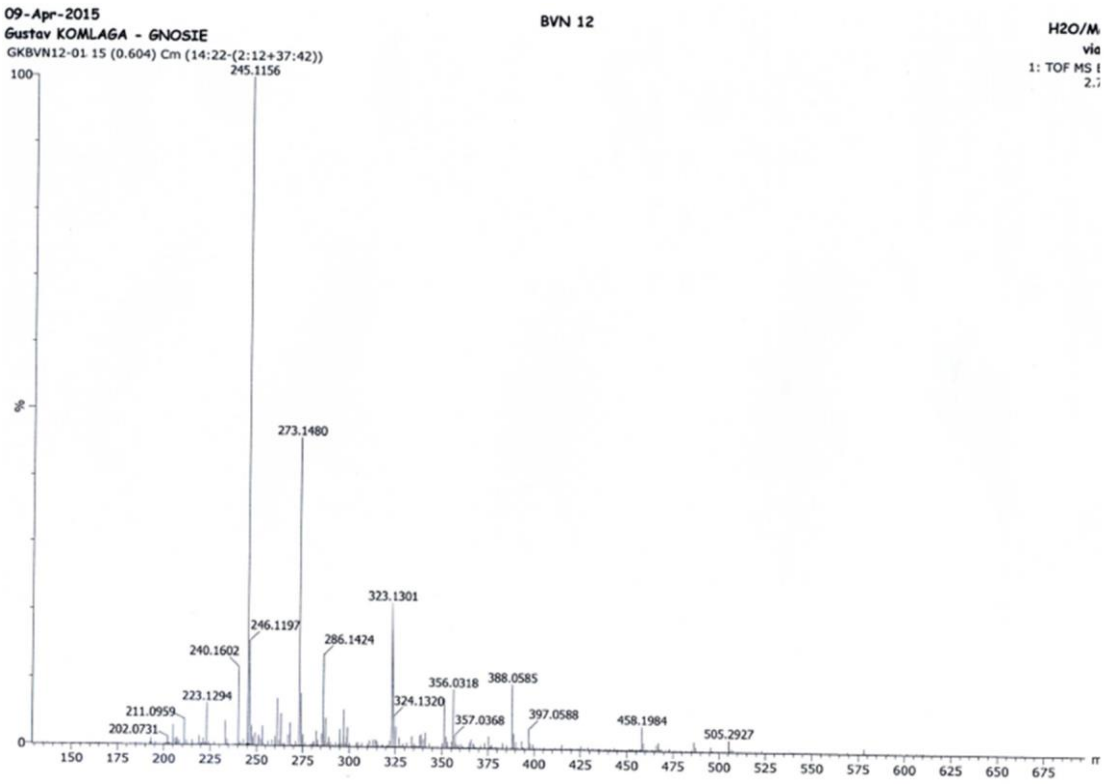
Minimum:	72.00				-2.0		
Maximum:	100.00		200.0	5.0	100.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
171.0416	100.00	171.0422	-0.6	-3.5	5.5	5546373.0	C9 H8 O2 Na

*Appendix 10b: ^{1}H and ^{13}C NMR of BvBu4A75 (**Bv2**) at 400Mhz in CD_3OD*





*Appendix 11a: Mass spectrum and elemental composition report of BvN12 (**Bv3**)*

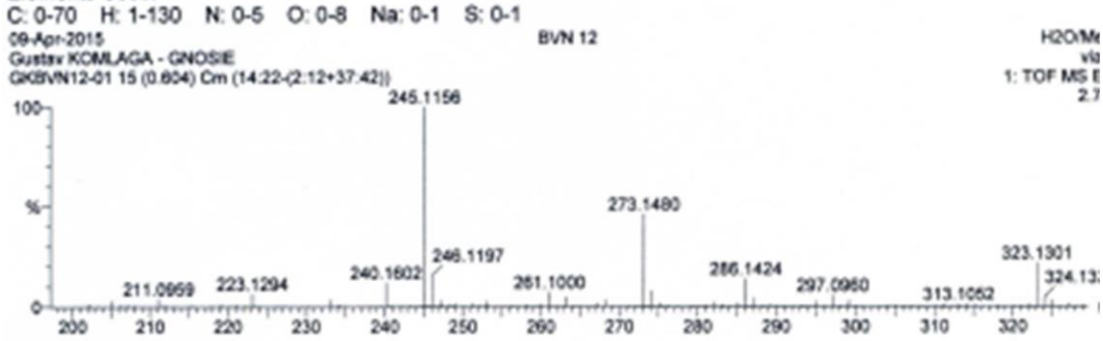


Elemental Composition Report

Page

Tolerance = 5.0 PPM / DBE: min = -2.0, max = 100.0
Selected filters: None

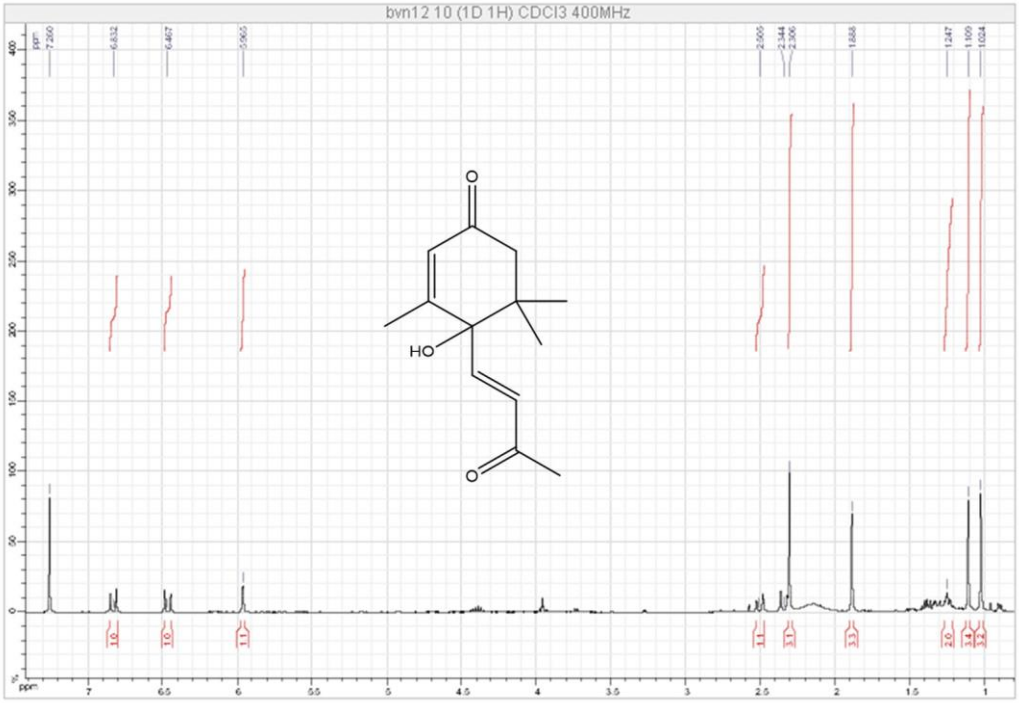
Monoisotopic Mass, Even Electron Ions
527 formula(e) evaluated with 1 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-70 H: 1-130 N: 0-5 O: 0-8 Na: 0-1 S: 0-1

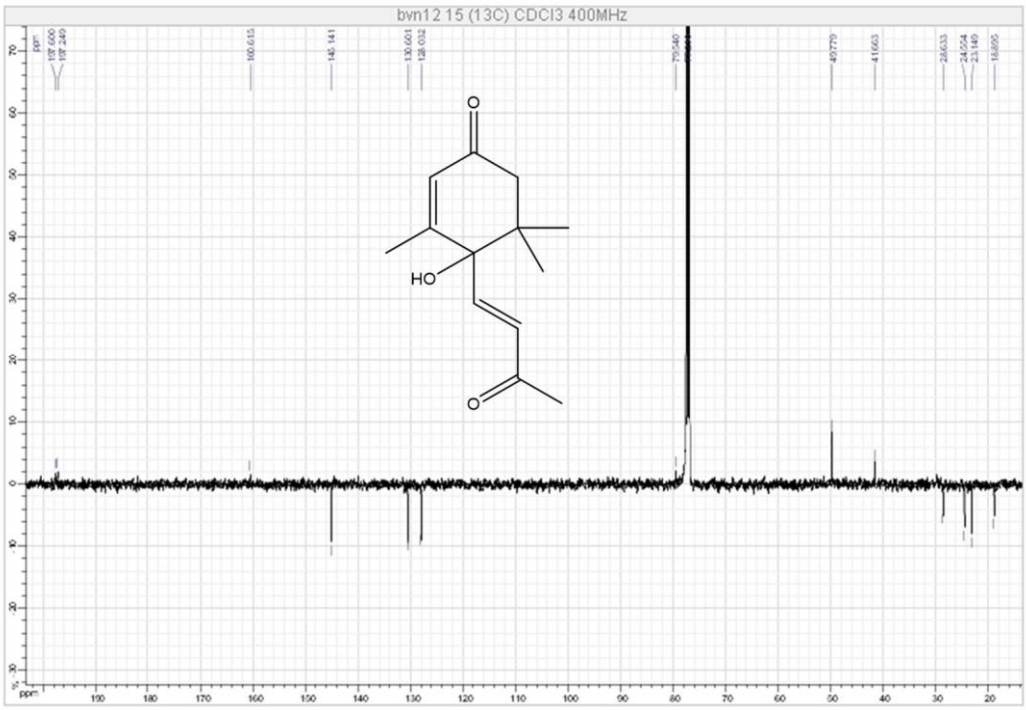


Minimum:	50.00				-2.0		
Maximum:	100.00		200.0	5.0	100.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
245.1156	100.00	245.1154	0.2	0.8	4.5	87.0	C13 H18 O3 Na

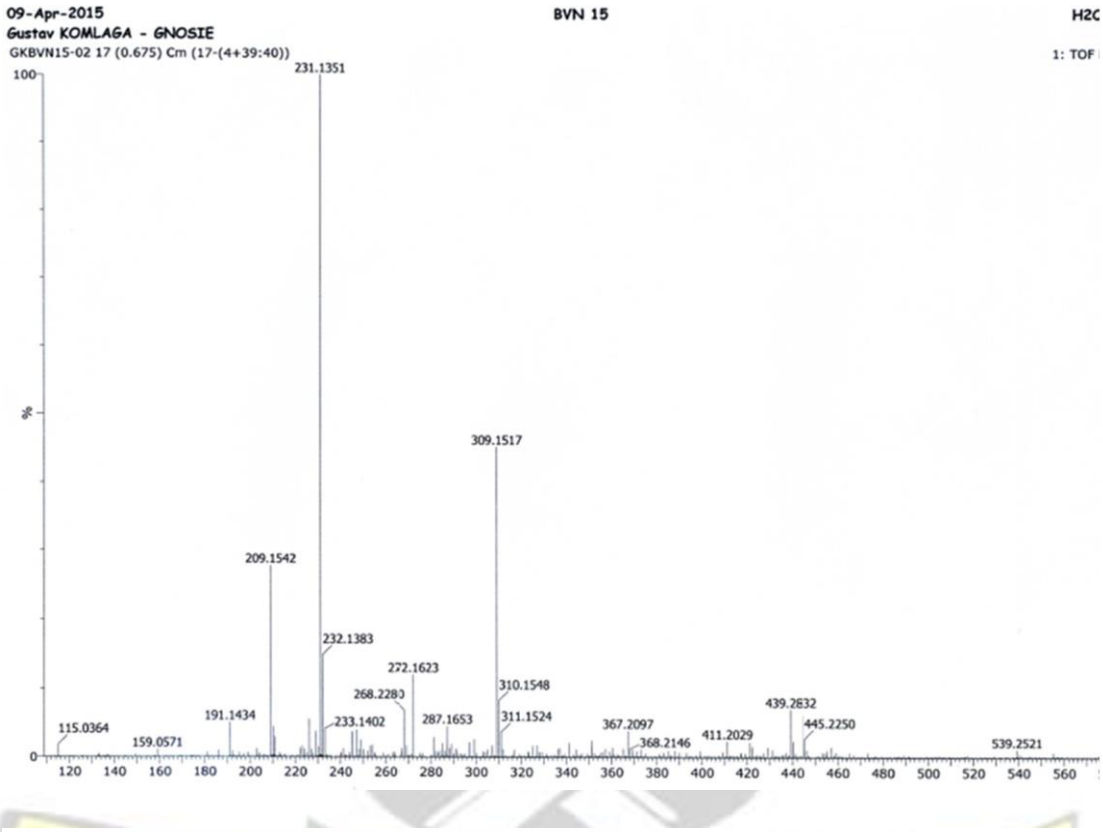
*Appendix 11b: C NMR of Bv12 (**Bv3**) at 400Mhz in $CDCl_3$*

^{1}H and ^{13}





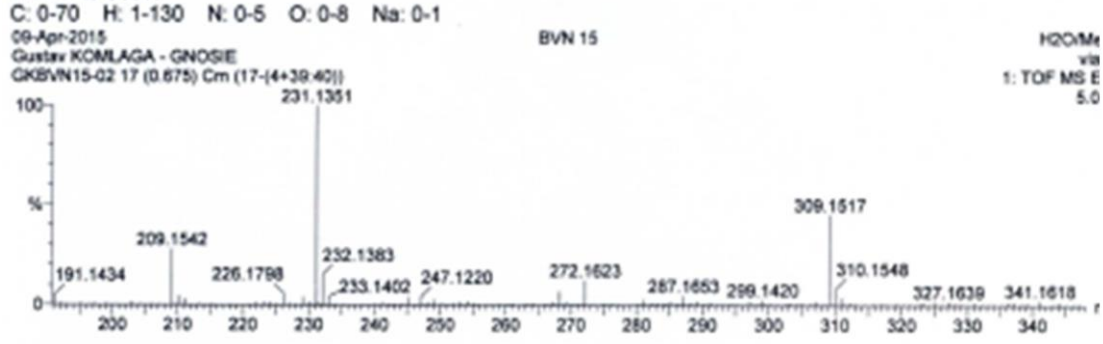
*Appendix 12a: Mass spectrum and elemental composition report of BvN15 (**Bv4**)*



Elemental Composition Report Page

Tolerance = 5.0 PPM / DBE: min = -2.0, max = 100.0
Selected filters: None

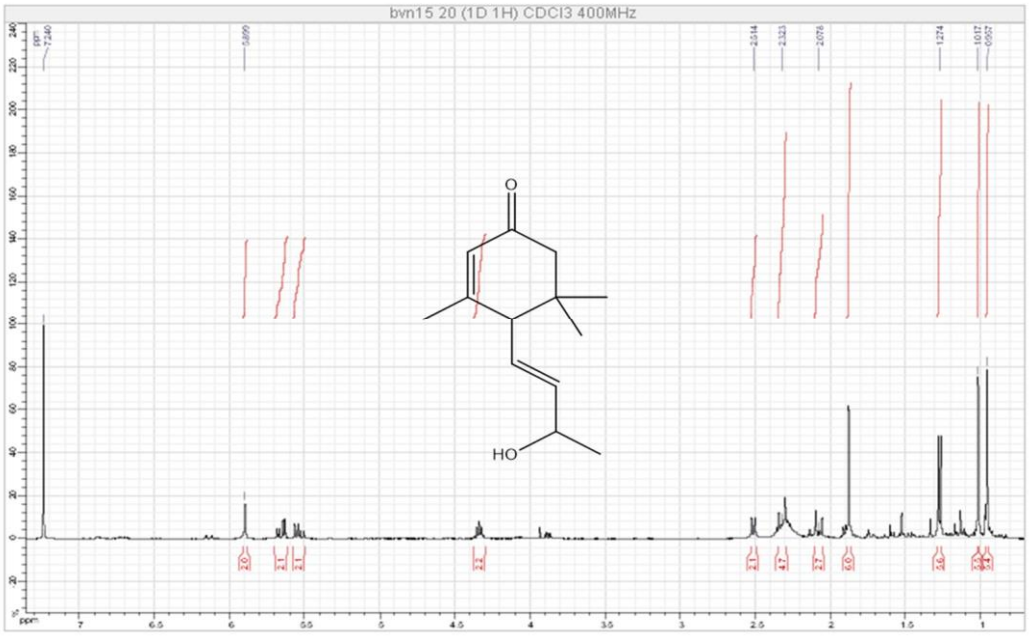
Monoisotopic Mass, Even Electron Ions
267 formula(e) evaluated with 2 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-70 H: 1-130 N: 0-5 O: 0-8 Na: 0-1

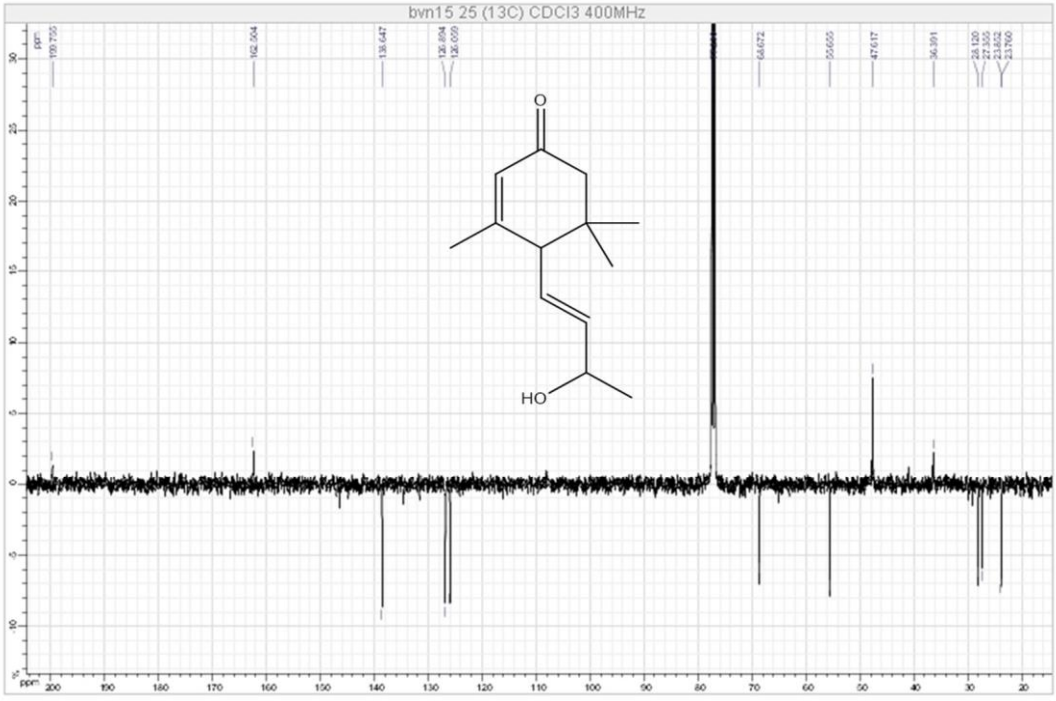


Minimum:	72.00				-2.0		
Maximum:	100.00		200.0	5.0	100.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
231.1351	100.00	231.1361	-1.0	-4.3	3.5	45.2	C13 H20 O2 Na
		231.1345	0.6	2.6	2.5	52.4	C10 H19 N2 O4

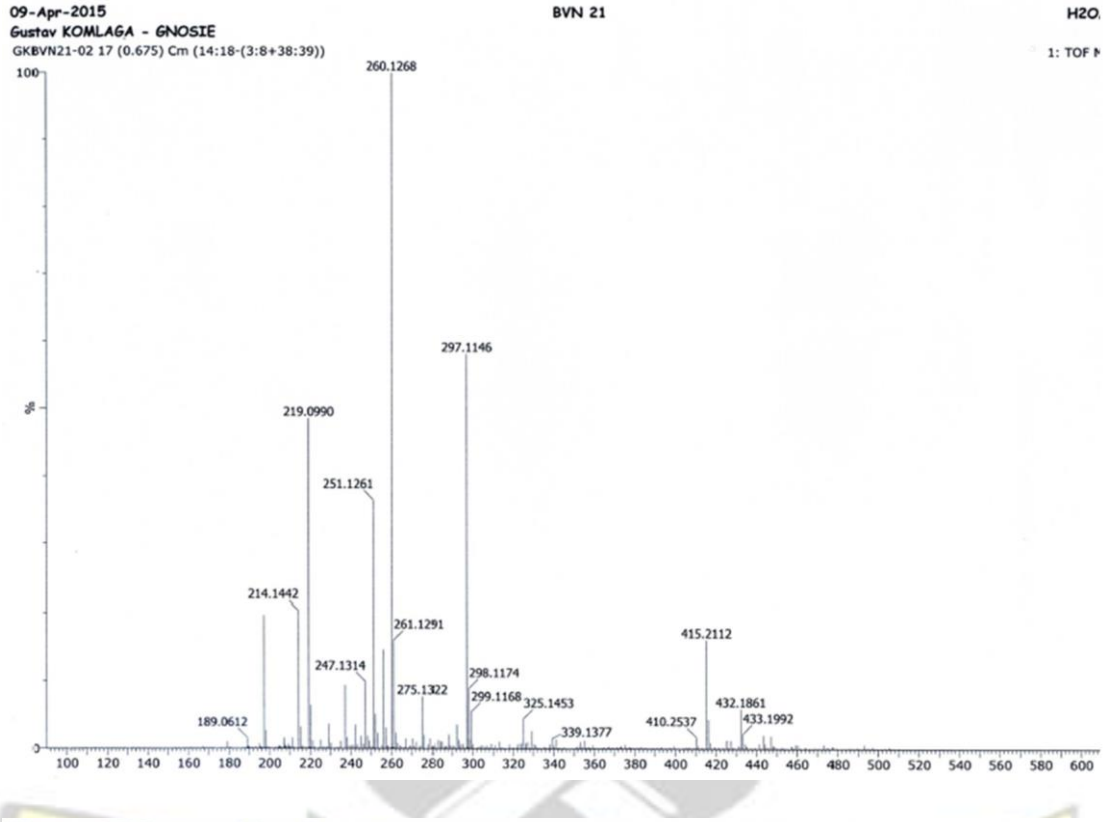
*Appendix 12b: C NMR of BvN15 (**Bv4**) at 400Mhz in $CDCl_3$*

^{1}H and ^{13}





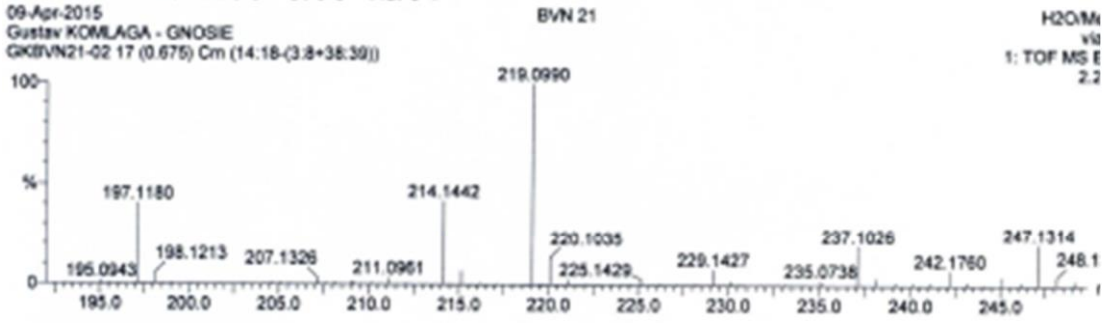
*Appendix 13a: Mass spectrum and elemental composition report of BvN21 (**Bv5**)*



Elemental Composition Report Page

Tolerance = 5.0 PPM / DBE: min = -2.0, max = 100.0
Selected filters: None

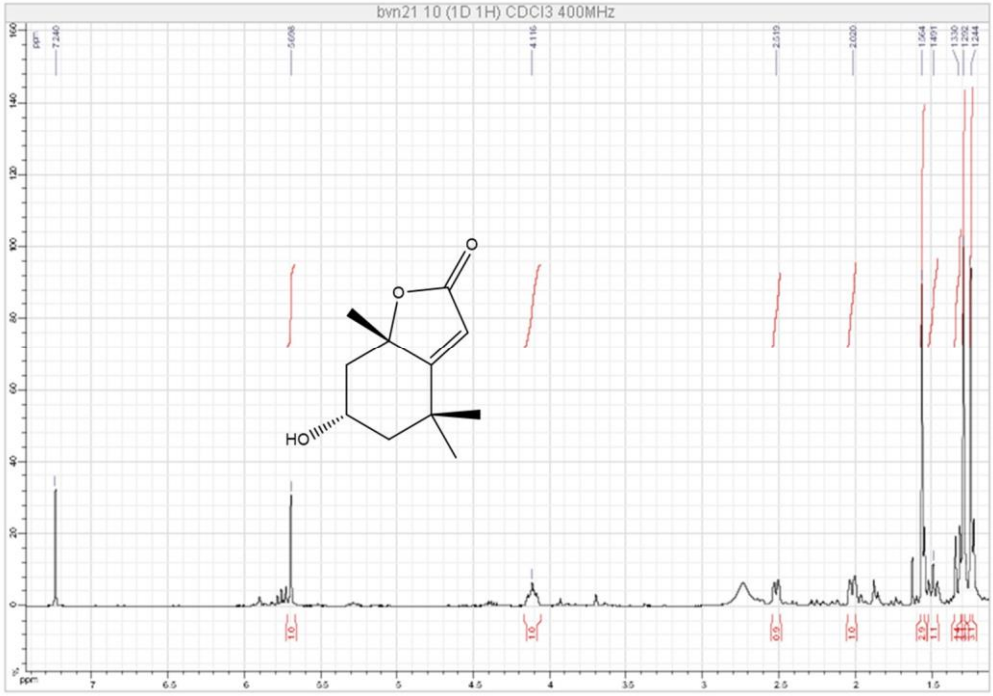
Monoisotopic Mass, Even Electron Ions
252 formula(e) evaluated with 2 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-70 H: 1-130 N: 0-5 O: 0-8 Na: 0-1

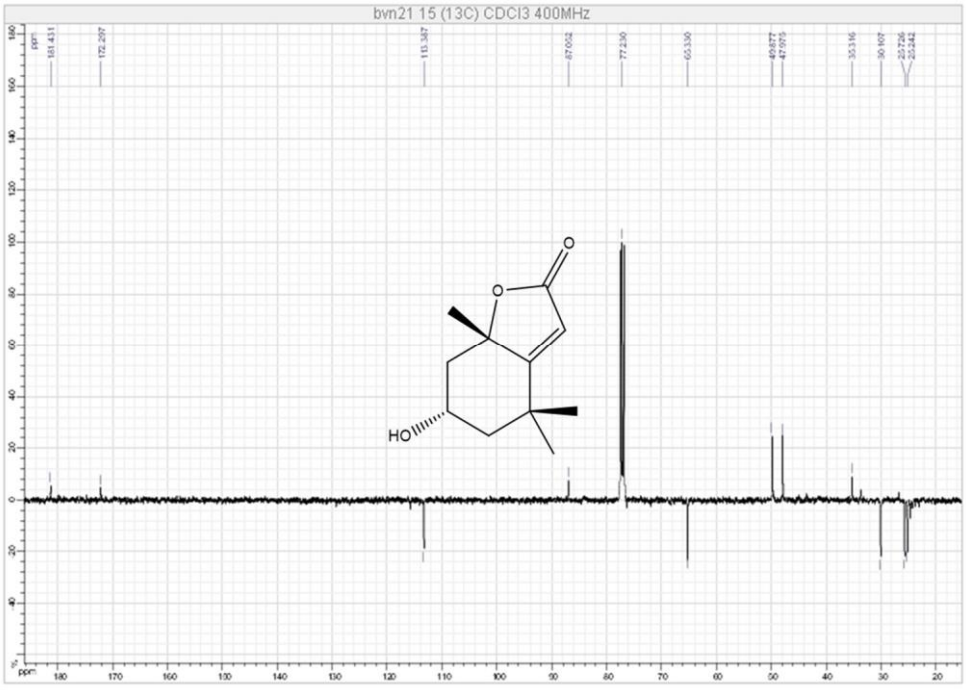


Minimum:	72.00				-2.0		
Maximum:	100.00			200.0	5.0	100.0	
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
219.0990	100.00	219.0997	-0.7	-3.2	3.5	53.6	C11 H16 O3 Na
		219.0981	0.9	4.1	2.5	119.1	C8 H15 N2 O5

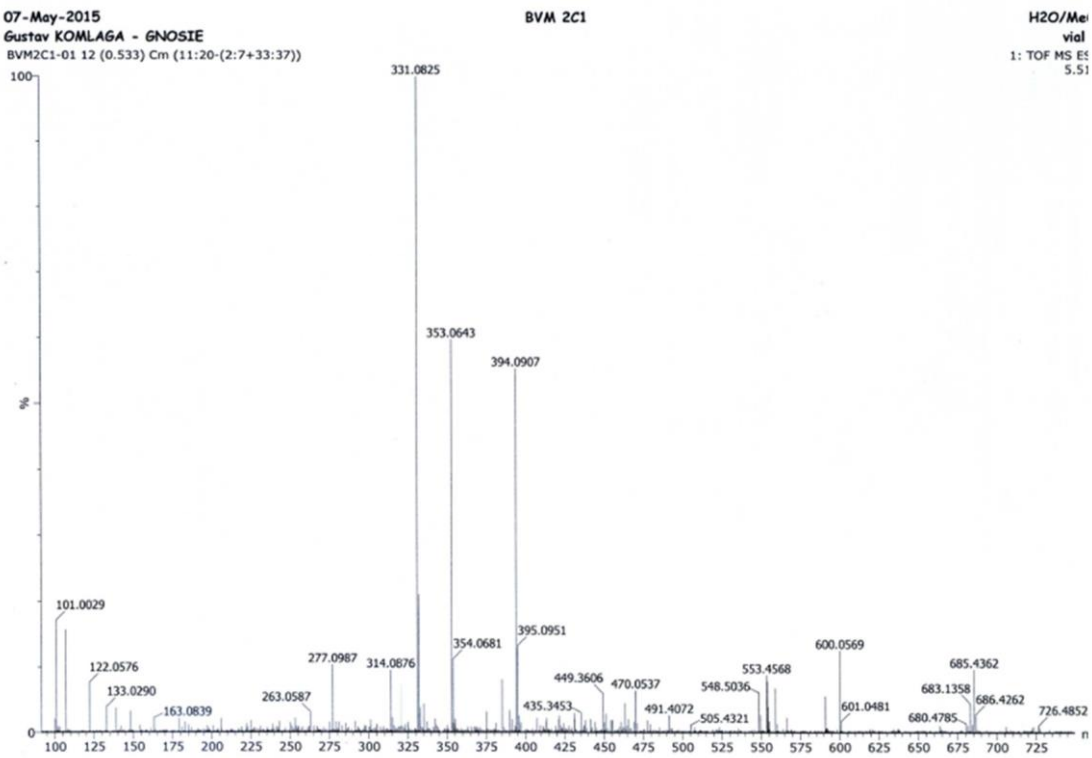
*Appendix 13b: C NMR of BvN21 (**Bv5**) at 400Mhz in $CDCl_3$*

^{1}H and ^{13}





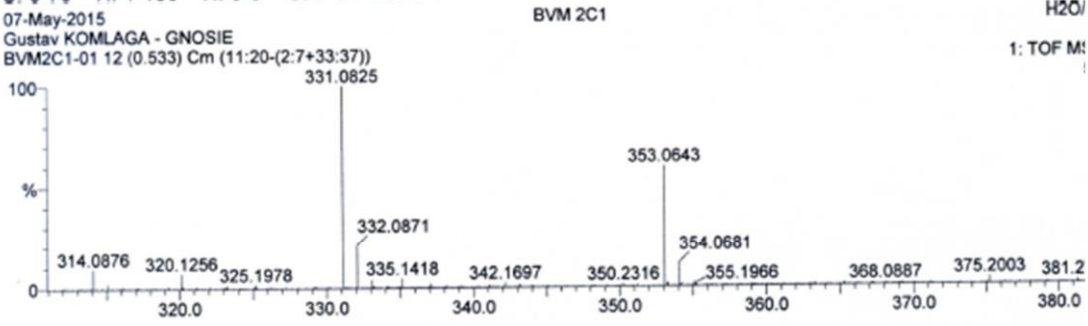
*Appendix 14a: Mass spectrum and elemental composition report of BvM 2C1 (**Bv6**)*



Elemental Composition Report

Multiple Mass Analysis: 2 mass(es) processed
Tolerance = 5.0 PPM / DBE: min = -2.0, max = 100.0
Selected filters: None

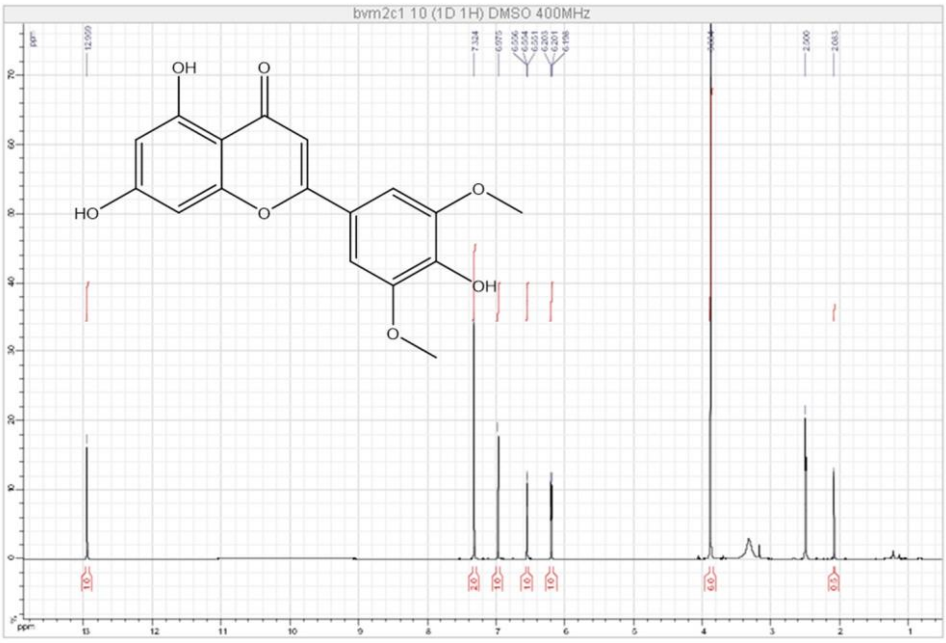
Monoisotopic Mass, Even Electron Ions
220 formula(e) evaluated with 2 results within limits (up to 3 best isotopic matches for each mass)
Elements Used:
C: 0-70 H: 1-130 N: 0-5 O: 7-9 Na: 0-1

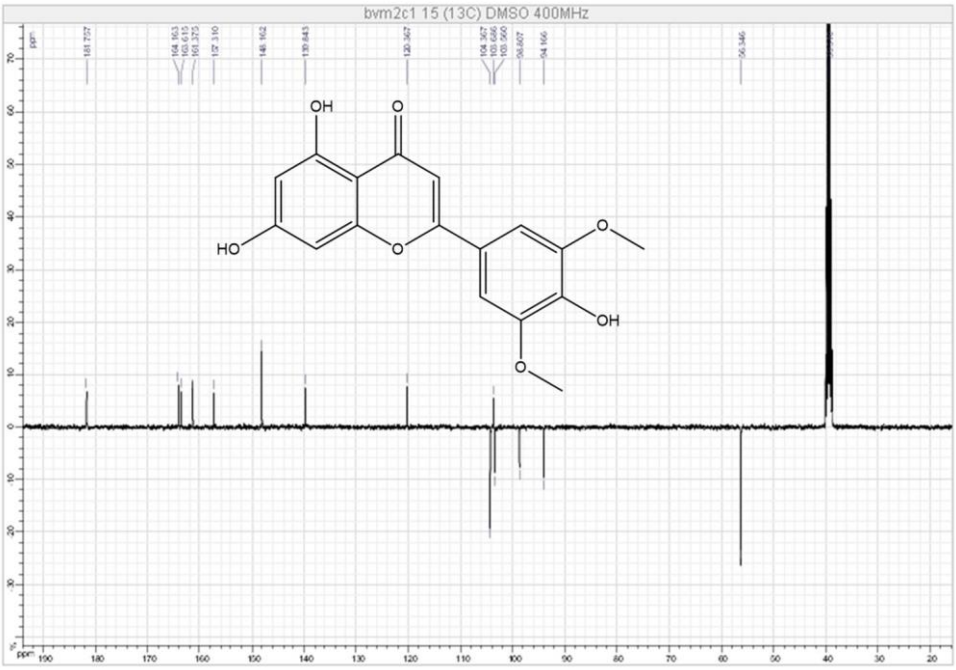


Minimum:	50.00				-2.0		
Maximum:	100.00		200.0	5.0	100.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-PIT	Formula
331.0825	100.00	331.0818	0.7	2.1	10.5	5.5	C17 H15 O7
353.0643	59.78	353.0637	0.6	1.7	10.5	0.7	C17 H14 O7 Na

Appendix 14b: *C NMR of BvM2C11 (**Bv6**) at 400Mhz in $CDCl_3$*

^{1}H and ^{13}





Appendix 15: Ethnobotanical survey of medicinal plants and finished marketed herbal products used to treat malaria in Bosomtwe and Sekyere East Districts of Ghana. **See next page for published article**

Journal of Ethnopharmacology 172 (2015) 339–346

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Medicinal plants and finished marketed herbal products used in the treatment of malaria in the Ashanti region, Ghana

Gustav Komlaga [a,d,e,n], Christian Agyare [b], Rita Akosua Dickson [a], Merlin Lincoln Kwao Mensah [c], Kofi Annan [c], Philippe M. Loiseau [d], Pierre Champy [e,nn]

[a] Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana [b] Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana [c] Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana [d] Chimiothérapie Antiparasitaire, CNRS UMR 8076 BioCIS, Faculty of Pharmacy, Université Paris-Sud, France [e] Laboratoire de Pharmacognosie, CNRS UMR 8076 BioCIS, Faculty of Pharmacy, Université Paris-Sud, France

articleinfo

Article history:
Received 19 February 2015
Received in revised form
20 June 2015
Accepted 23 June 2015 Available
online 4 July 2015

Keywords: Malaria
Antimalarial
Treatment
Medicinal plants
Finished marketed herbal products

abstract

Ethnopharmacological relevance: Ethnobotanical survey was performed to document medicinal plants employed in the management of malaria in the Bosomtwe and Sekyere East Districts of the Ashanti Region (Ghana), in comparison with the plant ingredients in herbal antimalarial remedies registered by the Ghana Food and Drug Administration.

Materials and methods: Two hundred and three (203) herbalists from 33 communities within the two districts were interviewed on medicinal plants they use to manage malaria. A literature search was made to determine already documented plants. In addition, 23 finished marketed herbal products indicated for the management of malaria were identified and their labels examined to find out which of the plants mentioned in our survey were listed as ingredients and whether these products are in anyway regulated. Results: Ninety-eight (98) species of plants were cited for the management of malaria. In comparison with literature citations, 12 (12.2%) species were reported for the management of malaria for the first time and 20 (20.4%) others for the first time in Ghana. Twenty-three (23) finished marketed herbal antimalarial products examined contained aerial or underground parts of 29 of the plants cited in our survey as ingredients. Twenty-two (22) of these products have been registered by the Ghana Food and Drugs Authority, four (4) of which were included in the recommended herbal medicine list for treating malaria in Ghana.

Conclusion: This study provides new additions to the inventory of medicinal plants used for the management of malaria and reports the commercial availability and regulation of finished marketed labelled herbal products intended for the treatment of malaria in Ghana.

Abbreviations: ACT, artemisinin-based combination therapy; BD, Bosomtwe District; FDA, Food and Drug Authority; GDP, Gross Domestic Products; KNUST, Kwame Nkrumah University of Science and Technology; PRK, the percentage of respondent with knowledge about a particular plant species; SED, Sekyere East District; WHO, World Health Organization

[n] Corresponding author at: Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

[nn] Corresponding author.

E-mail addresses: gustkomla@yahoo.com (G. Komlaga), chrisagyare@yahoo.com (C. Agyare), radickson.pharm@knust.edu.gh (R.A. Dickson), mlkmensah.pharm@knust.ed.gh (M.L.K. Mensah), annankofi@yahoo.com (K. Annan), philippe.loiseau@u-psud.fr (P.M. Loiseau), pierre.champy@u-psud.fr (P. Champy).

http://dx.doi.org/10.1016/j.jep.2015.06.041 0378-8741/

1. Introduction

Malaria is a life-threatening disease caused by protozoan parasites of the genus Plasmodium, whose transmission through the Anopheles mosquito is affected by climate and geography (Snow et al., 2005). Approximately half of the world's population is at risk. The greatest impact of the disease is felt in Sub-Saharan Africa, where most malaria cases and deaths occur (WHO, 2014). However, Asia, Latin America, and to a lesser extent the Middle East and parts of Europe are also affected. In 2013, 97 countries and territories had ongoing malaria transmission (WHO, 2014). In Ghana, there were about 8.4 million suspected cases and 2500 attributed deaths in 2013 (Ghana population: 25.9 million) (WHO, 2014), the main parasite responsible being Plasmodium falciparum (WHO, 2013b). The disease places significant financial hardships on both households and the national economy. It was estimated that 1% increase in the malaria morbidity rate will slow down the rate of real gross domestic products (GDP) growth by 0.41% (Asante and Asenso-Okyere, 2003).

WHO's approaches to malaria control are multi-faceted, involving prevention and case management (WHO, 2011). Among the prevention methods are control of the malaria vector using longlasting insecticidal nets and indoor residual spraying: These interventions work by reducing both the human–vector contact and the lifespan of female mosquitoes (White et al., 2014). The other is the intermittent preventive treatment which is the administration of a full course of an effective antimalarial treatment at specified time points to a defined population at risk of malaria, regardless of whether the recipients are parasitaemic, with the objective of reducing the malaria burden in the target population (WHO, 2011). Case management, on the other hand, involves the treatment of confirmed malaria cases using WHO recommended chemotherapy for malaria (WHO, 2011). Currently, treatment with artemisininbased combination therapies (ACTs) are the major drugs for the treatment of malaria (WHO, 2010b). However, ACTs are faced with some challenges, among which are their high cost (Mutabingwa, 2005) and the emerging parasite resistance to artemisinin-based drugs (WHO, 2010b).

In Ghana, where malaria is a major developmental challenge, many people use medicinal plants for treatment, especially in rural communities. Plants used in malaria and fever account for 6% of the medicinal plants of the Ghanaian domestic market (Van Andel et al., 2012). Many of these plants have been used in the management of the disease for centuries and numerous studies have documented this indigenous knowledge in some localities (Abbiw, 1990; Asase et al., 2005, 2010; Asase and Asafo-Agyei, 2011; Asase and Oppong-Mensah, 2009; Dokosi, 1998; Mshana et al., 2001). However, some sites are yet to be studied, leaving a knowledgegap in the documentation of these medicinal plants. Besides, the face of herbal medicine practice has changed over the years; some practitioners have moved from the hitherto extemporaneous preparations of home-made remedies to commercial production of finished/mixture marketed herbal products, made of a single or combination of medicinal plants.

We surveyed the medicinal plants used by the indigenes of the Bosomtwe and Sekyere East Districts of the Ashanti region of Ghana to manage or treat malaria. The data obtained were compared with similar studies from other parts of Ghana, Africa and the world. We also examined marketed herbal antimalarial products distributed in some health facilities in Kumasi, the Ashanti Regional capital of Ghana, to ascertain which plants mentioned in our survey are ingredients and to assess the regulatory status of these products.

2. Materials and method

2.1. Study areas

The study areas encompass 2 districts of the Ashanti Region, Ghana: Bosomtwe and Sekyere East Districts (Fig. 1). Bosomtwe District lies within latitudes 6° 43′ North and longitudes 1° 46′ West and it spreads over a land area of 68,179 km^2. It has an estimated population of about 94,000 (Ghana Statistical Service, 2010). The Sekyere East district, on the other hand, lies between latitudes 6° 45′ and 7° 32′ North and longitude 0° 22′ West. It has a land area of about 4231.4 km^2 and an estimated population of about 62,000 (Ghana Statistical Service, 2010). These areas share common climatic conditions; the equatorial zone with a rainfall regime typical of the moist semi-deciduous forest zone of Ghana. The communities are mostly rural. The residents are typically farmers and majority of the towns lack public healthcare facilities. The population is mainly of the Akan-speaking ethnic group of Ghana.

2.2. Data collection

Prior to data collection, informants' consent was sought by administering informed consent forms after explaining the purpose of the study to participants. The forms were filled and

Table 1

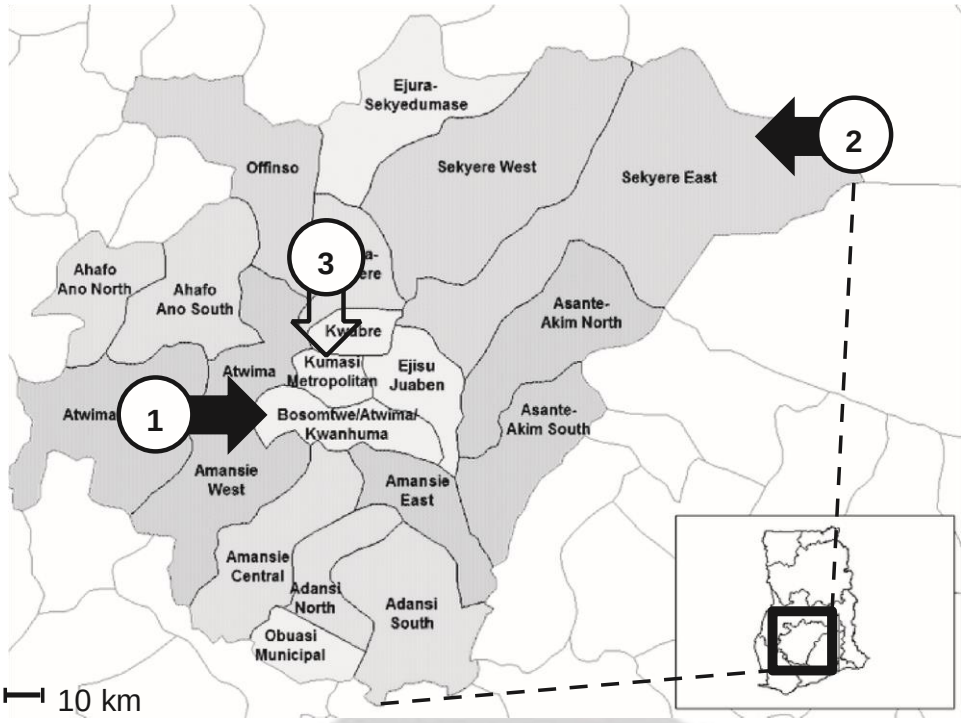


Fig. 1. District map of Ashanti region, Ghana, showing the study areas: (1) Bosomtwe and (2) Sekyere East Districts; (3) Kumasi Metropolitan Area. Source: Adapted from Wikimedia Creative commons.

Medicinal plants used by herbalists in Bosomtwe and Sekyere East Districts (Ghana) to treat malaria.

Family/Botanical name	Local name	Part used	State	Preparation[a]/association	SED	[c]BD	[c]PRK (%)	Previous reports
Acanthaceae								
Justicia carnea Lindl.	Ntumunum	L	f	With T. procumbens and C. odorata leaves.	þ		0.99	
Amaranthaceae								
Amaranthus spinosus L.[b]	Yaa asantewaa	L	d	With Z. officinale, V. amydalina leaves and X. aethiopica seeds.		þ	0.49	[5]
Cyathula prostrata (L.) Blume	Mpupua	R	d	Sole component, boiled or infused in water.		þ	0.49	[25]
Gomphrena celosioides Mart.[b]	Nkasɛnkasɛ	L, SB	d, f	1. Stem bark steeped in water withcrushed Z. officinale rhizome, used as enema. 2. With S. siamea root.	þ	þ	0.99	[20]
Amaryllidaceae								
Allium cepa L.	Gyene	B	f, d	Steeped with A. comosus leaves and peel, and V. amygdalina leaves and drink as required.		þ	0.49	[16;32;34]
Allium sativum L.	Gyenekankan	B	f	1. With A. indica: drunk, used as a bath or as enema. 2. With P. fraternus, A. comosus peels and V. amygdalina leaves.	þ	þ	1.97	[3;34]
Anacardiaceae								
Mangifera indica L.[b]	Mango	L	f	With S. alata and T. tetraptera leaves.	þ	þ	11.82	[9;40;13]
Spondias mombin L.[b]	Atoa	L, SB	f, d	Leaves and stem bark together with L. camara and M. lucida leaves.		þ	2.96	[2;38;39]
Annonaceae								
Cleistopholis patens (Benth.) Engl. and Diels [b]	Ngonenkye-ne	L	d, f	With C. papaya and M. indica leaves and S. torvum fruits.	þ	þ	3.94	[2;38]
Polyalthia longifolia (Sonn.) Thwaites[b]	Weeping willow	L	f, d	With A. indica leaves.	þ	þ	4.43	[15]

Xylopia aethiopica Dunal[b]	Hwentia	S	d	1. With dried C. papaya leaves. 2. With M. indica stem bark.		þ	1.48	[9;11;39]
Apocynaceae								
Alstonia boonei De Wild [b]	Emee/Nyamedua	L	f	Sole component.	þ	þ	10.34	[9;26]
Periploca nigrescens Afzel.	Abakamo	L	f	With N. laevis leaves.	þ		1.97	
Rauvolfia vomitoria Afzel.[b]	Kakapenpen	L	f	With A. zygia and L. camara leaves.	þ	þ	4.43	[2;40]
Secamone afzelii (Roem.and Schult.) K. Schum.	Kwatemaa	A	f, d	1. With S. campanulata stem bark. 2. With M. indica and S. occidentalis leaves. 3. With Ficus spp. and saltpetre.		þ	1.48	
Arecaceae								
Cocos nucifera L.[b]	Kube	SB, L	f	Stem bark with leaves.	þ		1.97	[9;22;39]
Elaeis guineensis Jacq.[b]	Abɛ	L	d	With T. orientalis leaves.	þ		1.48	[8;3;39]
Asteraceae								
Ageratum conyzoides L.[b]	Guakro	L	f	With V. amygdalina leaves.	þ		2.46	[2;3;11]
Bidens pilosa L.[b]	Gyinantwi	L	d	With dried T. cacao leaves.	þ	þ	3.94	[5]
Chromolaena odorata L.[b]	Acheampong	L	f	1. Boiled with C. limon fruit then added with sugar. 2. With C. citratus leaves.	þ	þ	7.39	[3;5;16]
Emilia sonchifolia (L.) DC.[b]	Guakoro	L	f, d	With M. pudica and E. hirta leaves.		þ	0.49	[2]
Launaea taraxacifolia (Willd.) Amin ex C. Jeffrey	Mmrobo	L	f	With C. odorata and T. procumbens leaves.	þ	þ	2.96	
Tridax procumbens L.b	Nantwi bini	L	f	Sole component.	þ	þ	7.39	[28]
Vernonia amygdalina Delile (accepted name: Gymnanthemum amygdalinum (Delile) Sch. Bip. ex Walp.)[b]	Awonwono	L, ST	f	1. Stem chewed, swallowing the liquid. 2. Leaves steeped in water with A. sativum bulb.	þ	þ	10.84	[7;11;39]
Bignoniaceae								
Newbouldia laevis (P.Beauv.) Seem.[b]	Sesemɛsa	L, SB	f, d	Leaves or stem bark with M. indica dried leaves.	þ	þ	8.37	[2;3;39]
Spathodea campanulata P. Beauv.[b]	Akuakuoninsuo	L, SB	f	1. Stem bark with S. alata leaves and of C. aurantifolia fruits. 2. Leaves with P. guajava and C. smeatmannii leaves.	þ	þ	3.94	[3;6]
Boraginaceae								
Heliotropium indicum L.[b]	Kɔmfemtikorɔ	L	f	With M. indica and C. odorata leaves, drunk for 7 days.		þ	0.49	[2;32;39]
Bromeliaceae								
Ananas comosus L. Merr.[b]	Aborobɛ	FP, L	f	1. Leaves or fruit peels with P. fraternus and T. cacao leaves. 2. Fruit peels with A. boonei, L. camara and C. smeatmanii leaves.	þ	þ	5.42	[9;39]
Cannabaceae								
Trema orientalis (L.) Blume	Sesea	L	f	With F. exasperata leaves.		þ	0.99	[11]
Caricaceae								
Carica papaya L.[b]	Bofire	L	f	With T. grandis, T. cacao, A. indica leaves and C. aurantifolia fruit.	þ	þ	9.36	[9;11;39]
Chrysobalanaceae								

Table 1 (continued)

Family/Botanical name	Local name	Part used	State	Preparation[a] /association	SED [c]	BD [c]	PRK (%)	Previous reports

Table 1

Family/Botanical name	Local name	Part used	State	Preparation[a]/association	SED[c]	BD[c]	PRK (%)	Previous reports
Parinari excelsa Sabine	Kwaed/Afamua	L, SB	f	Leaves or stem bark.[a]	þ		0.49	[10]
Combretaceae								
Combretum ghasalense Engl. and Diels[b]	Kwahinkwagya	S	d, f	Ground small piece of seeds, added to moistened M. puberula leaves, instilled into nose.		þ	0.49	[7]
Combretum mucronatum Schumach. and Thonn.[b]	Hwerɛmo	L	d	With F. sycomorus stem bark and C. aurantifolia fruitthen added with honey.		þ	1.48	[2]
Terminalia catappa L.[b]	Abrofo nkateɛ	L	f	With A. boonei leaves.	þ	þ	7.39	[5;16]
Terminalia ivorensis A. Chev.[b]	Emire	L, SB	f, d	Leaves and stem bark together with E. mildbraedii leaves.	þ	þ	4.43	[6]
Cucurbitaceae								
Momordica charantia L.[b]	Nyanyaa	L	f	Sole component, ground, added with palm wine.	þ	þ	3.45	[2;5;26]
Dioscoreaceae								
Dioscorea dumetorum (Kunth) Pax	Nkamfoɔ	L	f	1. Leaves boiled in strained corn (Zeamays L., Poaceae) dough liquid. 2. With P. longifolia leaves. 3. With T. catapa and S. alata leaves. 4. Added with sugar.		þ	0.49	[13]
Euphorbiaceae								
Alchornea cordifolia (Schumach. and Thonn.) Müll.Arg.[b]	Ogyama	L	f	With R. vomitoria and L. camara leaves.	þ	þ	7.39	[9;29]
Euphorbia hirta L.[b]	Kakaweadwe	L	f	With T. ivorensis leaves and stem bark.		þ	0.49	[14;40]
Grossera vignei Hoyle	Dubrafoo	L	f	Sole component, boiled with the filtrate from strained corn (Z. mays L.) dough.	þ		0.49	
Jatropha curcas L.[b]	Nkrandedua	L	f, d	With P. fraternus, O. gratissimum leaves and C. limon fruit.	þ	þ	4.43	[7;29;27]
Manihot esculenta Crantz[b]	Bankye	L	f, d	1. With P. americana and T. cacao leaves. 2. With P. americana and A. boonei leaves.		þ	0.49	[2;11;39]
Mareya micrantha (Benth.) Müll. Arg.b	Dubrafo	L	f	With O. gratissimum and A. boonei leaves.	þ	þ	1.48	[9;40]
Fabaceae								
Albizia ferruginea (Guill. and Perr.) Benth.	Awiemfo-samina	L	f	With P. fraternus and C. citratus leaves.		þ	0.99	[40]
Albizia zygia (DC.) J.F.Macbr.[b]	Okoro	L	f	Crushed, strained and used as enema.		þ	0.99	[2;37;38]
Amphimas pterocarpoides Harms	Yaya	L	f	With P. fraternus leaves, drunk or used as enema.	þ		1.97	
Erythrina mildbraedii Harms	Eya	L	d, f	With C. sanguinolenta root.		þ	0.49	
Mimosa pudica L.	Nana aberewa	L	f	Ground with Z. officinale rhizome and P. guineense seeds, strained and used as enema.		þ	0.49	[1]
Senna alata (L.) Roxb.[b]	Simpe	L	f, d	1. Boiled, allowed to cool and used as enema. 2. Dried leaves with X. aethiopica.	þ	þ	6.4	[9;40]
Senna occidentalis (L.) Link[b] (syn. Cassia occidentalis L.)	Nkwadaa borɔdeɛ	L	f	1. With B. pilosa and M. lucida leaves. 2. With T. daniellii, T. tetraptera and C. prostrata leaves.	þ	þ	4.93	[9;3;31]
Senna siamea (Lam.) H.S. Irwin and Barneby[b]	Acacia	R	f	Sole component.		þ	2.96	[4;8;32]

(continued)

Tetrapleura tetraptera (Schumach. and Thonn.) Taub.[b]	Prekesɛ	L, F, SB	f, d	1. Leaves with C. anisata leaves. 2. Fruits with A. indica leaves. 3. Chopped stem bark.	þ	þ	8.37	[2;3]
Gentianaceae								
Anthocleista nobilis G.Don [b]	Wudini kɛtɛ (Bontodie)	L, SB	f	1. Leaves with T. grandis and A. indica leaves. 2. Stem bark with A. indica leaves.		þ	0.99	[6]
Huaceae								
Afrostyrax lepidophyllus Mildbr.	Duagyanne	S	d	Seven crushed seeds with U. guineensis stem bark, steeped in hot water, drunk.	þ		5.91	[29]
Lamiaceae								
Ocimum canum L.	Akokobesa	L	f	With M. oleifera and A. vera leaves.	þ		0.49	[22]
Ocimum gratissimum L.[b]	Nunum	L	f	1. With M. oleifera leaves. 2. With P. americana leaves.	þ	þ	8.87	[9;3;29]
Tectona grandis L. f. [b]	Teak	L	f, d	With P. capitata leaves.	þ	þ	7.88	[8;22]
Lauraceae								
Persea americana Mill.[b]	Paya	L	f, d	With M. indica, S. occidentalis and M. oleifera leaves.	þ	þ	6.4	[5;11]
Loranthaceae								
Phragmanthera capitata (Spreng.) Balle	Nkranpan	L	f, d	1. With T. grandis leaves. 2. With C. sanguinolenta roots.	þ	þ	2.96	[17]
Malvaceae								
Bombax buonopozense P.Beauv.[b]	Akata	SB	f, d	With P. fraternus leaves.	þ	þ	5.91	[6]
Cola nitida (Vent.) Schott and Endl.	Bese	L	f	With P. longifolia leaves and P. fraternus, drunk for three days.		þ	0.49	
Cola gigantea A. Chev.[a]	Watapuo	L	f	With T. grandis leaves.	þ		1.48	[2]
Pterygota macrocarpa K. Schum.	Kyerere	L	f	With T. grandis and L. camara leaves.	þ		0.99	
Sida cordata (Burm.f.) Borss. Waalk.	Tentene	L	f	With O. canum and C. citratus leaves.		þ	0.49	
Sida cordifolia L.	Akyerekyere	A	f, d	Sole component.	þ		0.99	[21]
Theobroma cacao L.[b]	koko	L	d	With B. vulgaris leaves.	þ	þ	7.88	[6;16]
Marantaceae								
Thaumatococcus daniellii (Benn.) Benth. and Hook. f.[b]	Aworomo	L	f, d	With S. alata, T. tetraptera and C. prostrata leaves.		þ	0.49	[9]
Meliaceae								
Azadirachta indica A. Juss.[b]	Neem	L	f	Sole component.	þ	þ	20.2	[4;9;39]
Khaya senegalensis (Desr.) A.Juss.[b]	Kuntunkuri	SB	f	With B. buonoponzense stem bark and leaves and B. vulgaris leaves.		þ	0.99	[7;39]
Moraceae								
Ficus exasperata Vahl.	Nyankyerɛne	L	f	With M. pudica leaves.	þ	þ	3.45	[22]
Ficus sycomorus L.	Odoma	L	f	With T. orientalis leaves.		þ	0.99	[33]
Moringaceae								
Moringa oleifera Lam.[b]	Moringa	L, S	f, d	1. Leaves boiled in strained corn (Z.mays L.) dough liquid. 2. Seed chewed, swallowing of theliquid.	þ	þ	6.4	[8;37;39]
Musaceae								
Musa paradisiaca L.[b]	Borɔdeɛ	R, L	f, d	1. Petiole with P. fraternus and G. amygdalinum leaves. 2. Leaves steeped in water. 3. Roots steeped in water with Z.		þ	1.48	[5;36;19]

Table 1
Table 1 (continued)

Family/Botanical name	Local name	Part used	State	Preparation[a] /association	SED [c]	BD [c]	PRK (%)	Previous reports
				officinale rhizome, used as enema.				
Musa sapientum L.[b]	Kodu	L	f	Petiole with P. fraternus and G. amygdalinum leaves.	þ	þ	3.45	[8]
Myristicaceae								
Pycnanthus angolensis (Welw.) Warb[b]	Otie	L	f	With R. vomitoria and M. indica leaves.	þ		2.96	[6;40;24]
Myrtaceae								
Psidium guajava L.[b]	Guava	L	f	Boiled with N. laevis leaves and added with fruits juice of C. aurantifolia.	þ	þ	5.91	[6;13]
Pandaceae								
Microdesmis puberula Hook.f. ex Planch.	Ofemma	L	f	Note: induces vomiting which is stopped once a meal is taken.	þ	þ	2.46	[11]
Papaveraceae								
Argemone mexicana L.	Akusiribie	A	f, d	1. Sole component. 2. With M. charantia and M. oleifera leaves.	þ		0.49	[16;18;39]
Periplocaceae								
Cryptolepis sanguinolenta (Lindl.) Schltr.[b]	Nibima	R	d	1. With A. boonei and L. camara leaves and of A. comosus peels. 2. Powdered root infused in hotwater.	þ		4.43	[3;28]
Phyllanthaceae								
Phyllanthus fraternus G.L. Websterb	Bɔ womma gu wakyi	WP	f, d	1. Boiled with C. citratus leaves in strained corn dough liquid. 2. With C. papaya, P. americana, O. gratissimum and G. amygdalinum leaves.	þ	þ	14.78	[35]
Uapaca guineensis Müll. Arg.	Kontan	SB	d	Dried stem bark crushed, added with seven A. melegueta seeds, steeped in hot water.		þ	0.49	[23]
Piperaceae								
Piper guineense Schumach. and Thonn.[b]	Soro wisa	S	d	Seeds crushed with M. puberula leaves and Z. officinale rhizome, strained and used as enema.	þ	þ	1.97	[9;29]
Poaceae								
Bambusa vulgaris Schrad.[b]	Pamporo	L	d, f	1. With C. aurantifolia leaves. 2. With P. longifolia leaves.	þ	þ	5.42	[5]
Cymbopogon citratus (DC) Stapf.[b]	Esrɛ (lemon grass)	L	f	With T. catapa, N. laevis and B. buonopozense leaves.	þ	þ	10.84	[9;36;39]
Saccharum officinarum L.[b]	Ahwedeɛ	ST, L	f	Stem and leaves with N. laevis leaves.	þ	þ	3.45	[8;11;39]
Rubiaceae								
Morinda lucida Benth.[b]	Konkroma	L, SB	f	Leaves and stem bark.	þ	þ	6.9	[9;16]
Rutaceae								
Citrus aurantiifolia (Christm.) Swingle[b]	Ankagyua	L, F	f	1. Leaves with O. gratissimum and T. catapa leaves. 2. Leaves with J. curcas and T. procumbens leaves, added with juice of the fruit.	þ	þ	7.88	[9;38]
Citrus limon (L.) Osbeck	Ankagyua	F, L	f	Fruit with G. amygdalinum, S. occidentalis and T. grandis leaves.	þ	þ	5.91	[11;34;39]

Clausena anisata (Willd.) Hook.f. ex Benth.[b]	Saman nobi	F, L	f	1. Fruits with S. mombin and S. campanulata leaves. 2. Fruits or leaves with P. longifolia, T. catapa and C. odorata leaves.	þ	0.99	[28;39]
Zanthoxylum leprieurii Guill. and Perr.	Oyaa	L, SB	f, d	Leaves or stem bark with Khaya spp. stem bark.	þ	0.49	
Sapindaceae							
Paullinia pinnata L.[b]	Tuantini	L	f	1. Leaves boiled: steam-bath with thehot decoction. 2. Poultice applied on the body.		0.99	[7;38;39]
Solanaceae Solanum torvum Sw.[b]	Nsusuwa	F, L	f	1. Leaves with sugar added afterdecoction. 2. Fruits with of M. indica stem bark and S. officinarum stem.	þ	2.46	[8]
Solanum tuberosum L.	Potato	L	f	With T. procumbens and P. guajava leaves.	þ	0.99	[12]
Thelypteridaceae Cyclosorus afer Ching	Mmɛyaa (abɛya)	L	d	With S. alata, T. tetraptera and T. daniellii leaves.	þ	2.96	
Verbenaceae Lantana camara L.[b]	Ananse dokono	L	d	With Z. leprieurii leaves.	þ	1.97	[5;18;37;38]
Xanthorrheaceae Aloe vera (L.) Burm. f	Aloe vera	L	f	1. Sole component. 2. With A. indica leaves.	þ	1.48	[31;34]
Zingiberaceae Aframomum melegueta (Roscoe) K. Schum.[b]	Fom wisa	L	f, d	With E. hirta leaves.		0.49	[9;11;38]
Zingiber officinale Roscoe[b]	Akakaduro	Rh	f, d	With C. papaya leaves and M. indica stem bark.	þ	3.45	[9;16;30]

(continued)

Family/Botanical name	Local name	Part used	State	Preparation[a]/association	SED [c]	BD [c]	PRK (%)	Previous reports

Plant parts: A: aerial part; F: fruit; L: leaves; R: root; RB: root bark; RH: rhizome; S: seed; SB: stem bark; ST: stem; WP: whole plant. State: f: fresh; d: dried. PRK: Percentage of the respondents with knowledge about the use of the plant for the management of malaria.

[d] Previous reports of traditional use: [1] (Aarthi and Murugan, 2011); [2] (Abbiw, 1990); [3] (Adebayo and Krettli, 2011); [4] (Al-Adhroey et al., 2010); [5] (Asase and AsafoAgyei, 2011); [6] (Asase and Oppong-Mensah, 2009); [7] (Asase et al., 2005); [8] (Asase et al., 2010); [9] (Asase et al., 2012); [10] (Attioua et al., 2012); [11] (Betti et al., 2013); [12] (Chen et al., 2010); [13] (Dike et al., 2012); [14] (Dokosi, 1998); [15] (Gbedema, 2014); [16] (Idowu et al., 2010); [17] (Jiofack et al., 2009); [18] (Kamaraj et al., 2012); [19] (Kaou et al., 2008); [20] (Köhler et al., 2002); [21] (Konaté et al., 2012); [22] (Koudouvo et al., 2011); [23] (Macfoy, 2013); [24] (Madureira et al., 2002); [25] (Mbatchi et al., 2006); [26] (Ménan et al., 2006); [27] (Mesfin et al., 2012); [28] (Mshana et al., 2001); [29] (Muganza et al., 2012); [30] (Nagendrappa et al., 2013); [31] (Nguta et al., 2010); [32] (Olorunnisola et al., 2013); [33] (Sanon et al., 2003); [34] (Saotoing et al., 2011); [35] (Sittie et al., 1998); [36] (Stangeland et al., 2011); [37] (Tabuti, 2008); [38] (Traore et al., 2013); [39] (Yetein et al., 2013); [40] (Zirihi et al., 2005).

[a] Otherwise indicated: Oral route; decoction alone or in combination, drunk as required.

[b] Plants already documented in Ghana.

[c] þ: mentioned in studied District (Sekyere East District: SED; Bosomtwe District: BD).

willingly signed or thumb printed (by respondents who were illiterate) prior to the administration of questionnaires. The protocol for the study was approved by the Faculty of Pharmacy and Pharmaceutical Sciences Research Ethic Review Committee, and the research was performed in consultation with the chiefs and opinion leaders of the various communities who facilitated the identification of recognized herbalists.

Two hundred and three (203) herbalists in the Bosomtwe (100) and Sekyere East (103) districts respectively were interviewed using a semi-structured validated questionnaire (see Supplementary material S1). Purposive sampling technique was employed in selecting the respondents, members of the communities helped to identify the respondents who were known herbalists. Data collection involved both home and field interviews. Questions on practitioners' demography covered name/ID, gender, age, level of formal education, profession or career, duration of practice and how traditional healing skill or knowledge was acquired. Questions related to malaria included respondent's knowledge in malaria, ability to diagnose it, symptoms of the disease, experience with malaria cases and treatment, and if their assistance was sought by people suffering from malaria. Questions on medicinal plants used to treat malaria included local name(s) and if known, scientific name(s) of plant(s) employed, part/organ of the plant, the form in which it is used, the method of preparation and administration, known side effects and contraindications. Oral and informal interviews were also carried out with both closed and open ended questions.

During the field interview, the respondents took the interviewer to the field where they normally collected their medicinal plants. Samples of the plant species were collected to prepare herbarium specimens. The identities of plant species were authenticated by Dr. G.H. Sam of Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana in comparison with reference materials of the herbarium of the same Department and with

Table 1

database sources at the Forest Research Institute of Ghana (FORIG), Fumesua, Ghana. Specimens with voucher numbers (see Supplementary material S2) were deposited at the Herbarium of KNUST. The taxonomic validity of the plant names were checked using the plant database; www. thePlantList.org (accessed January, 2015).

2.3. Survey of finished marketed herbal products for the management of malaria

A survey was also conducted in 3 herbal medicine wholesale shops, 3 herbal medicine retail shops, 3 community pharmacies and 2 herbal clinics within the Kumasi Metropolitan area of the Ashanti Region of Ghana to identify finished marketed herbal antimalarial products on sale, examine their active plant component (see Supplementary material S3) and also assess their regulatory status within the national and international framework.

2.4. Statistical analysis

The knowledge on medicinal plants used in the treatment of malaria among the herbalists in the study areas was analysed using the percentage of respondent with knowledge about a particular plant species (PRK) (Asase et al., 2005). PRK was calculated as the percentage of respondents who mentioned the plant in comparison with the total number of respondents in both districts.

3. Results

3.1. Respondents' demographics

The study was done in 33 towns and villages in the 2 districts, the Bosomtwe and the Sekyere East Districts of the Ashanti Region of Ghana, over a period of six months, from June to November 2012. The respondents were traditional healers above 20 years of age, living in the study areas. They were known in the communities as people who have knowledge of medicinal plants, sought by users of medicinal plants for assistance and have experience in treating malaria. The respondents included herbalists from herbal clinic settings, practitioners with shrines and other people who practice herbal medicine as pastime. The former two offer their services for fees and the later often receive payment in the form of cash or kind for their services. The latter constitute 162 (79.8%) of the respondents. Of the 203 respondents, 134 (66.0%) had practiced for more than 10 years. While 51 (25.1%) of the respondents had no western-type education, the majority (152; 74.8%) had education, ranging from basic to tertiary level. Ninety-four (94; 46.3%) were females. All the 203 herbalists interviewed in the 2 districts said they employed various medicinal plants species for the treatment of malaria. According to the herbalists, anyone with malaria may demonstrate one or more of the following symptoms: elevated body temperature, bitterness in the mouth, nausea and vomiting, chills, headache, joint pains, restlessness, body weakness, loss of appetite, insomnia,

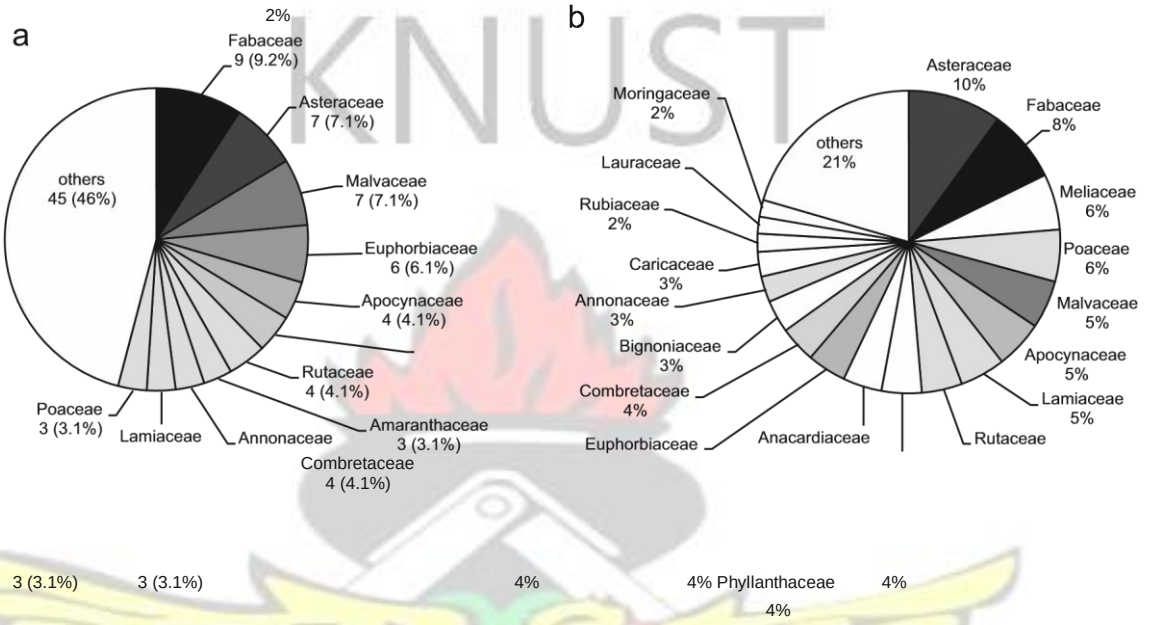


Fig. 2. (a) Number/percentage of plant species cited for major family and (b) percentage representation of plant families cited in the survey.

intense amber urine colour, yellowish eye colour and stomach upset. The most commonly reported of these symptoms are headache, nausea and vomiting, bitterness in the mouth, loss of appetite, high temperature and chills. The herbalists considered malaria treatment as a remedy which relieves these clinical signs and symptoms and they use this to judge the effectiveness of their remedies since there is no laboratory facility within these communities to facilitate the confirmation or absence of parasitaemia.

3.2. Plant species employed in the management of malaria

Ninety-eight (98) plants species belonging to 85 genera in 48 families were cited for the treatment or management of malaria in the survey (Table 1). Eighty (80) species were recorded in Bosomtwe and sixty-five (65) in Sekyere East District. Forty-seven (47; 47.9%) of the 98 were cited in both districts. Eighteen (18; 18.4%) of the species mentioned in Sekyere East District were not encountered during the survey in Bosomtwe District, while 33 (33.7%) of species were cited only in Bosomtwe. Thirty-five (35) of the

65 Species cited in only a district have PRK less than 1 (PRKo1). The plants were mostly flowering-species (1 fern was cited). The predominant family was the Fabaceae with 9 species (3 of which belong to the genus, Senna) followed by Asteraceae and Malvaceae, and then Euphorbiaceae, Combretaceae and Rutaceae among others (Fig. 2). Eleven genera including Allium, Albizia, Citrus, Cola, Combretum, Ficus, Musa, Ocimum, Sida, Solanum and Terminalia had 2 members each. Azadirachta indica was the most frequently cited (PRK¼20.20%), followed by Phyllanthus fraternus (PRK¼14.78%), Mangifera indica (PRK¼11.82%), Cymbopogon citratus, Vernonia amygdalina (PRK¼10.84%) and Alstonia boonei (PRK¼10.34%). The PRK for the rest of the species ranged from

0.49% to 9.36% (Table 1).

In terms of growth habit, trees predominate (45%) while herbs formed 34%, shrubs, 15% and climbers, 6%. The main part employed was the leaf followed by the stem bark, root, fruit and seed, whole plant and bulb (Fig. 3). Recipes were mostly decoctions and plant species were employed either as sole or multi-components in different combinations. Often, the choice of combination of plants for a recipe depended on the individual herbalist. The literature review revealed that 12 of the identified plant species including Amphimas pterocarpoides, Cola nitida, Secamone afzelii, Cyclosorus afer, Erythrina mildbraedii, Grossera vignei, Justicia carnea, Launaea taraxacifolia, Periploca nigrescens, Pterygota macrocarpa, Sida cordata and Zanthoxylum leprieurii are being reported for the first time for the traditional management of malaria and another 20 for the first time in Ghana (see Supplementary material S4).

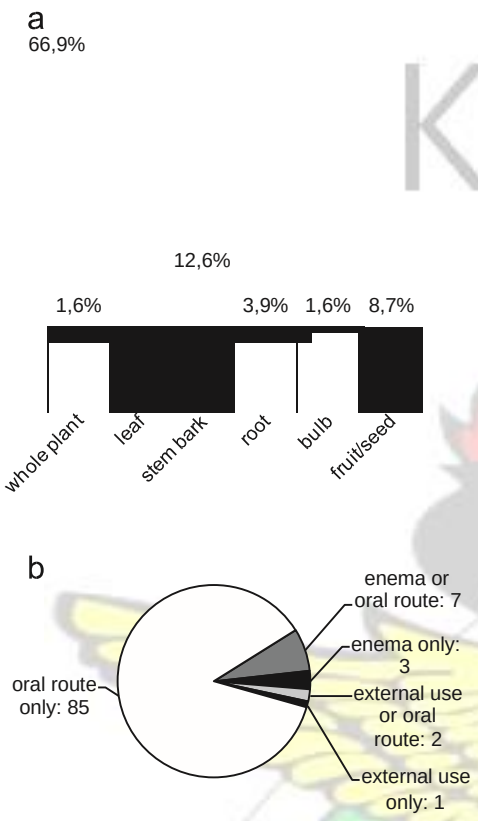


Fig. 3. (a) Plant parts employed in the treatment of malaria, as percentage of all parts use. (b) Route of administration of the various preparations.

3.3. Finished/mixture marketed labelled herbal antimalarial products

Twenty-three (23) finished/mixture marketed labelled herbal products, formulated as decoctions and intended for the treatment of malaria, were identified in a survey carried out in community pharmacies, herbal wholesale and retail shops, and herbal clinics in Kumasi, the Ashanti Regional capital of Ghana (Table 2). Twenty-two (22, 95.7%) of the products surveyed were registered by Ghana's food and drug regulatory authority (FDA) and therefore bear registration numbers. These products contained 29 (29.6%) of the medicinal plants cited in the ethnobotanical survey (Table 1)

Table 2

as plant constituents, the majority of which have PRK higher than 4. Five (5) of the identified finished marketed products were prepared from a single plant, while the others contained a combination of two (2) to seven (7) medicinal plants. These products are indicated for malaria and sometimes related conditions such as jaundice, typhoid fever, fatigue and loss of appetite. Four (4, 13.8%) of the finished marketed remedies surveyed formed part of the recommended herbal medicine list for treating malaria in Ghana (Ministry of Health, 2008). These products were dispensed in both public and private herbal clinics, sold in herbal shops, community pharmacies and licensed chemical stores as over the counter products for the treatment of malaria in Ghana. However, only those on the recommended herbal medicine list for treating malaria are dispensed in public herbal clinics. The dosage regimen usually covers 7 days or more. The herbal clinics are often operated by traditional herbalists (private herbal clinics) as well as medical herbalists (both public and private herbal clinics). The medical herbalists hold the BSc degree in Herbal medicine from the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The herbal shops are often supervised by traditional herbalists or by lay persons while the community pharmacies are supervised by pharmacists.

The most employed of the cited plants in the finished/mixture herbal products for managing malaria was Cryptolepis sanguinolenta. It occurred in 11 (47.8%) of the remedies, followed by Morinda lucida, Vernonia amygdalina and Azadirachta indica which were used in 5 products each; Alstonia boonei, Senna siamea and Xylopia aethiopica in 3 products each. Nine (9) plants including Alchornea cordifolia, Carica papaya, Cola gigantea, Khaya senegalensis, Paullinia pinnata, Solanum torvum, Spathodea campanulata, Tetrapleura tetraptera and Theobroma cacao were active components of 2 products each. Ten (10) plants comprising Ageratum conyzoides, Anthocleista nobilis, Bombax buonopozense, Citrus aurantifolia, Cymbopogon citratus, Mangifera indica, Moringa oleifera, Phyllanthus fraternus, Pycnanthus angolensis, Rauvolfia vomitoria and Momordica charantia were each used in a single
(1) product.

Twenty-two (22) of the active plants components in the registered products have been investigated for antimalarial activity (Table 3). Most have shown moderate to strong activity, including aqueous extracts which can be considered as relevant in regard to

Plants species used in the marketed herbal antimalarial products examined in Kumasi, Ghana.

Ghana FDA registration number of product	Medicinal plant components
FDB/HD 02-1007	Khaya senegalensis, Cryptolepis sanguinolenta
FDB/HD 02-7046	Cryptolepis sanguinolenta, Alstonia boonei, Azadirachta indica, Morinda lucida, Xylopia aethiopica
FDB/HD 05-10083	Cryptolepis sanguinolenta
FDB/HD 05-8060	Cryptolepis sanguinolenta

FDB/HD 05-9075	Cola gigantea, Solanum torvum, Spathodea campanulata, Vernonia amygdalina
FDB/HD 06-7058	Alstonia boonei, Vernonia amygdalina, Xylopia aethiopica
FDB/HD 07-5054	Alstonia boonei, Azadirachta indica
FDB/HD 07-7096	Cryptolepis sanguinolenta
FDB/HD 09-4071	Cryptolepis sanguinolenta, Momordica charantia, Vernonia amygdalina
FDB/HD 10-5095	Anthocleista nobilis, Phyllanthus fraternus
FDB/HD 11-9128	Cryptolepis sanguinolenta, Morinda lucida
FDB/HD 12-10154	Cryptolepis sanguinolenta
FDB/HD 12-10169	Cola gigantea, Solanum torvum, Spathodea campanulata, Bombax buonopozense, Vernonia amygdalina
FDB/HD 12-12213	Azadirachta indica, Paullinia pinnata, Theobroma cacao, Tetrapleura tetraptera, Cymbopogon citratus, Moringa oleifera
FDB/HD 12-2011	Mangifera indica, Paullinia pinnata, Pycnanthus angolensis, Rauvolfia vomitoria
FDB/HD 12-2018	Morinda lucida
FDB/HD 12-8101	Carica papaya, Xylopia aethiopica, Alchonea cordifolia
FDB/HD 12-9121	Tetrapleura tetraptera, Theobroma cacao, Carica papaya, Ageratum conyzoides, Alchonea cordifolia, Senna siamea
FDB/HD 12-9141	Cryptolepis sanguinolenta, Morinda lucida
FDB/HD 13-1020	Azadirachta indica, Cryptolepis sanguinolenta, Vernonia amygdalina
FDB/HD: 10-5092	Cryptolepis sanguinolenta, Azadirachta indica
FDB/HD: 11-6035	Citrus aurantifolia, Senna siamea, Morinda lucida
Unregistered	Senna siamea, Khaya senegalensis

Table 3
Plants species contained in the marketed herbal antimalarial products whose extract(s) reportedly showed antimalarial activity.

Cited medicinal plant contained in registered products and investigated	Extracts studied
Ageratum conyzoides[a,b,c]	Dichloromethane extracts of the whole plant (Owuor et al., 2012), aqueous extracts of the whole plant (Ukwe et al., 2010)
Alchornea cordifolia[a]	Ethanol, chloroform and ether extracts of leaves. (Banzouzi et al., 2002); Aqueous extracts of leaves (Valentin et al., 2000)
Alstonia boonei[a,b]	Aqueous and ethanol extracts of leaves and stem bark (Adepiti et al., 2014; Ménan et al., 2006)
Anthocleista nobilis[a]	Aqueous, methanol and dichloromethane extracts of leaves (Sanon et al., 2003)
Azadirachta indica[a,b,d]	Aqueous extract of leaves (Adepiti et al., 2014)
Bombax buonopozense[b]	Methanol/water extract of leaves (Akuodor et al., 2012)
Carica papaya[a]	Aqueous extract of leaves (Bhat and Surolia, 2001)
Cryptolepis sanguinolenta[a,c]	Ethanol and dichloromethane extracts of root (Ansah and Gooderham, 2002; Tona et al., 1999)
Cymbopogon citratus[a,b]	Essential oil from aerial parts (Bidla et al., 2004; Tchoumbougnang et al., 2005)
Mangifera indica[a]	Ethanol extract of stem bark (Zirihi et al., 2005); aqueous extract of leaves (Adepiti et al., 2014)
Morinda lucida[a,b]	Aqueous, ethanol and dichloromethane extracts of leaves (Makinde and Obih, 1985; Tona et al., 1999)
Moringa oleifera[a]	Acetone extract of leaves (Patel et al., 2010)
Momordica charantia[a,b]	Aqueous extract of leaves (Gbeassor et al., 1990; Ueno et al., 1996)
Paullinia pinnata[a]	Methanol extracts of leaves, stem and root (Okpekon et al., 2004)
Phyllanthus fraternus[a]	Isolated alkamides and aqueous extract of whole plant (Sittie et al., 1998)
Pycnanthus angolensis[a]	Aqueous, methanol and ethanol extracts of stem bark (Abrantes et al., 2008; Ancolio et al., 2002; Zirihi et al., 2005)
Rauvolfia vomitoria[a]	Ethanol extract of root bark (Zirihi et al., 2005)
Senna siamea[a]	Aqueous, methanol and chloroform extracts of leaves (Sanon et al., 2003)
Spathodea campanulata[b]	Hexane and chloroform extracts of stem bark (Makinde et al., 1988)
Tetrapleura tetraptera[b]	Ethanol extract of fruit (Okokon et al., 2007)
Vernonia amygdalina[a,b,c]	Aqueous, ethanol and dichloromethane extracts of leaves (Abosi and Raseroka, 2003; Tona et al., 2004)
Xylopia aethiopica[a]	Ethanol extract of leaves and stem bark (Boyom et al., 2011)

[a] Plants tested by in vitro method.
[b] Plants tested by in vivo method.
[c] Plant tested in clinical trials (Willcox, 2011). [d] Plant with established antiplasmodial activity recorded in WHO monograph on selected medicinal plants (WHO, 2007).

traditional use.

4. Discussion

Altogether, ninety-eight (98) plant species were recorded for the treatment or management of malaria in the two study areas (Table 1). The two districts are culturally and ethnically homogenous communities in the Ashanti Region of Ghana, with the population being mainly of the Asante-Twi ethnic group. Since these two survey areas share common vegetation and climate, it was expected that they would employ common medicinal species for the management of various diseases including malaria. However, only 46.5% of plants were cited in both districts for the management malaria, thirteen (13) having a PRK410%. Species mentioned in one district only generally have a poorly significant use (35 have a PRKo1%), as expected. Exceptions to this are Afrostyrax lepidophyllus and Cryptolepis sanguinolenta, which though mentioned in only one district, have PRKs as high as 5.91 and 4.43 respectively. The latter species is a well-known and studied plant for its anti-malarial property in Ghana (Abbiw, 1990; Ansah and Gooderham, 2002; Asase et al., 2005). Its PRK therefore appears relatively low in regard to its assessed clinical efficacy (Bugyei et al., 2010) and

presence in nearly half of surveyed Ghana FDA-registered herbal antimalarial products. Nevertheless, the availability of this species in the study areas might account for this observation. Other plants that were cited in only one district but having relatively high PRK, comparable to those mentioned in both districts, include Pycnanthus angolensis, Senna siamea, Solanum torvum and Spondias mombin. Their widespread use within one locality only also illustrates a local discrepancy in their perceived efficacy.

According to the respondents, the herbal antimalarial preparations from these plants were safe for use by all classes of people and have long history of use without any demonstrable harm. However, a few contraindications and side effects were mentioned: Carica papaya and Senna siamea were said to be contraindicated in pregnancy. Side effects stated included frequent urination from the use of Tridax procumbens, diarrhoea from Senna alata and vomiting from the use of Mareya micrantha, especially when taken without food. However, for some plants with established toxicities, no specific warnings were recorded during the survey. Typical examples being the hepatotoxicity reported with Lantana camara in several animal species, which could yield concern regarding its chronic use (Sharma et al., 2007), and Momordica charantia, which is also used as an abortifacient in Ghana (van Andel et al., 2012) and which yielded fatal hypoglycaemia in children (Raman and Lau, 1996).

Based on literature search, only 66 (67.3%) of the 98 plants we recorded have already been documented in Ghana. Altogether 86 (87.8%) were documented worldwide (Table 1). Azadirachta indica which was the most frequently cited species (PRK¼20.2%) in our study was also the most common species encountered in all other such surveys examined. The frequent encounter of the use of these plants for the management of malaria across geographical boundaries strongly suggests their perceived efficacy. Indeed, many have been reported to possess antiplasmodial property (see Table 3).

Twelve (12; 12.2%) of the plants recorded in our study are reported for the first time for the traditional treatment of malaria (Table 1). However, antiplasmodial activity was reported for some related species. For example, Sida acuta was successfully tested by Karou et al. (2003) against fresh clinical isolates of Plasmodium falciparum, in vitro. The presence of cryptolepine, a powerful antimalarial alkaloid justifying the use of Cryptolepis sanguinolenta, has been mentioned in the Sida genus. However, the use of Sida spp. might also be related to the presence of ephedrine, a powerful stimulant (Chatterjee et al., 2013). Similarly, an aqueous extract of Cola caricaefolia was reported to exhibit significant antiplasmodial activity against both chloroquine sensitive and chloroquine-resistant strains of P. falciparum, in vitro (Ménan et al., 2006). On the other hand, the genus is a well-known source of the stimulant compound, caffeine, which might alleviate some symptoms of the disease. Whereas the leaf of C. nitida is used to manage malaria in Ghana as we report here, interestingly, high intake of the seeds mimicked malaria symptoms among healthy volunteers in Nigeria (Alaribe et al., 2003). The leaf, however, may not demonstrate the same malaria-like effect since the two organs may differ in their secondary metabolic contents which are responsible for any biological activity. It would, nevertheless, worth it to investigate the leaf for such effect. The Erythrina species, E. fusca, E. variegata and E. abyssinica exerted significant antimalarial activity against chloroquine-sensitive (D1) and various chloroquine/multi-drugresistant strains of P. falciparum (Herlina et al., 2009; Khaomek et al., 2008; Yenesew et al., 2004). Other species belonging to genera reported here, such as Justicia betonica (Bbosa et al., 2013) and Secamone Africana, often confused with Secamone afzelii, (Schmelzer and Gurib-Fakim, 2013) showed in vitro antiplasmodial properties. Hence the traditional use of Sida cordifolia, Erythrina mildbraedii, Cola nitida, Justicia carnea and Secamone afzelii for the treatment of malaria may be justified by an actual antiplasmodial potential. Also, acridone alkaloids from the fruit of Zanthoxylum leprieurii, a species inventoried during this survey have been found to possess antiplasmodial activity against 3D7 strain of P. falciparum (Tchinda et al., 2009). Other species of this genus are used as such in other areas, due to their alkaloidic contents (Jullian et al., 2006; Randrianarivelojosia et al., 2003). Convergence of use for traditional treatment of malaria at the generic level can also be noticed for Cyclosorus afer, in regard to C. interruptus from Papua New Guinea (Oyen, 2010).

Of the 98 plant species mentioned in the survey, 29 (29.6%) served as active plant components in 23 finished marketed herbal products intended for the treatment of malaria (Table 2). The 29 are widely used species for the treatment of malaria in Ghana and have been used since time immemorial (Abbiw, 1990; Asase et al., 2010, 2012, 2005; Asase and Asafo-Agyei, 2011; Asase and Oppong-Mensah, 2009; Dokosi, 1998; Mshana et al., 2001).

Most of the registered herbal remedies were multi-plant combinations containing between 2 and 7 different medicinal plant materials. The use of such combinations in the management of malaria is common and widespread in Ghana and is also observed in neighbouring countries (Asase et al., 2012, 2005; Dike et al., 2012). However, only few antimalarial evaluation of combinations were conducted in vitro or in vivo (Ankrah et al., 2003; Bertania et al., 2005; Martey et al., 2013; Tepongning et al., 2011; Willcox, 2011) but some have proved synergistic as in the case of a mixture of Azadirachta indica, Alstonia boonei, Mangifera indica and Morinda lucida in P. berghei infested mice (Adepiti et al., 2014).

Cryptolepis sanguinolenta was the most employed, occurring in 11 products, 4 in which it was used alone. It was one of the few species of the 98 to have reportedly undergone clinical trials for efficacy and safety evaluation (Willcox, 2011, Table 3). Bugyei et al. (2010) indeed demonstrated that the tea bag formulation of the root powder was non-toxicand highly effective in the treatment of acute uncomplicated malaria on relatively short treatment regimens, with parasite clearance similar to that induced by usual doses of Artemisia annua leaves and Cinchona sp. bark herbal teas in similar conditions (for a comparison of these trials, see Willcox (2011)). The genotoxic potential of the active principle of C. sanguinolenta, cryptolepine, is reported not to be associated with severe side effects (Appiah, 2009) but warrants surveillance.

Azadirachta indica, though the most frequently cited species for managing malaria in our and other Ghanaian field studies, was employed in as few as 5 finished marketed herbal antimalarial products. It is noteworthy that the in vitro antimalarial property of Azadirachta indica has been reported in WHO monographs on selected medicinal plants (WHO, 2007). The leaves of Vernonia amygdalina, which are included in 5 of the registered combinations, also proved clinically effective (Willcox et al., 2011). It was however observed that plants such as Argemone mexicana, which was inventoried in our and other field surveys and has clinical data on its antimalarial activity (Willcox, 2011; Willcox et al., 2011) and preclinical evidence of safety (Njan, 2012), was not included in any of the marketed products identified.

The manufacture and the sale of these products are regulated under an Act of the Ghanaian Parliament, Act 851 (Public Health Act, 2012). Additionally, the herbal products are registered by Ghana's FDA in accordance with national guidelines (Ghana, 2013b), which largely conform to WHO African region's guidelines for registration of herbal medicinal products (WHO, 2010a). However, there is no clinical data in support of these products and their use therefore is fundamentally based on anecdotal evidence of efficacy. Such products, therefore, fall under "Category 2" herbal products as defined by WHO (2010a). While many of the plants used in these product are reported safe and did not show significant treatment-related toxicity in treated animals (Grover and Yadav, 2004; Moura et al., 2005; Oduola et al., 2010) and on cells (Sanon et al., 2013), others have been reported for some levels of toxicity. For example, Alstonia boonei stem bark extract is reported to show reversible antifertility toxicity in treated rats (Raji et al., 2005) and Paullinia pinnata root exhibited moderate cytotoxicity against some cell lines (Ayim et al., 2008). The root of Rauvolfia serpentina is known for its cardiac and central nervous system side effects, linked to its content in reserpine and other

indolomonoterpenes (Klyushnichenko et al., 1995). Such issues are of prime concern regarding clinical use and registration (Willcox et al., 2011).

The labels on the finished marketed herbal antimalarial products generally conformed to the Ghana FDA's labelling requirements (Ghana, 2013a), indicating product names, names of active plant constituents (but without mentioning the parts used nor proportions), therapeutic indications, mode of administration, dosage regimen and sometimes dosages for children, duration of use, contraindications, warnings and precautions, manufacturing and expiry dates, batch numbers and name of manufacturer or company with full address. However, the packages do not contain information leaflets. Due to lack of data, information on drug interactions and dosage for the elderly were absent, though they might be appropriate. It is therefore important that scientists work in tandem with the herbalists in the development of these remedies, in order to address these challenges, in respect of national and WHO standards. Such cooperation shall ideally be supportive of pharmacovigilance.

Many medicinal plants used as home-remedies to manage malaria are currently used for large scale production of finished marketed herbal products in Ghana, providing accessibility and quality-products to many patients, especially in urban areas. This, thus contributes to WHO's goal of promoting universal health coverage through accessibility to medication (WHO, 2013a). However, large scale commercial production of finished herbal products coupled with the extensive harvest and informal sales of medicinal plants in the Ghanaian markets (Asase and OppongMensah, 2009) is prone to lead to unsustainable exploitation. This is a potential threat to survivability of medicinal plants used for these preparations and might particularly be the case for several of the species identified with high PRK in our survey, when compared to sales volumes estimated by Van Andel et al. (2012) for the country. Indeed, Afrostyrax lepidophyllus, Morinda lucida or Rauvolfia vomitoria are wild plants accounting among the most frequently encountered species at the Ghanaian markets, with high sales volumes. Efforts towards the conservation of such plants are crucial (Cordell, 2014). Fortunately, in Ghana, cultivation of some medicinal plants by farmers and herbalists (e.g. Moringa oleifera, Khaya senegalensis, Xylopia aethiopica) has started on a large scale, for direct use and retail.

Also, with the increasing availability of finished remedies in commercial quantities and the fact that some of these products have not been authorized by the drug regulatory authority of Ghana's FDA (Table 2), it is important to ensure strict regulation in order not to compromise the health and safety of the people who patronize them. It is also important that scientists work with herbalists to conduct clinical trials on these products as recommended by the WHO (2010a). Case-control and retrospective studies could be conducted (Graz, 2013) to ascertain the claims of registered herbal products whose access to the market did not rely on specific clinical studies.

5. Conclusion

Despite the various documentations on the medicinal plants used to manage malaria in Ghana, many such plants remain undocumented. Also, many medicinal plants are currently used for commercial production of finished marketed herbal antimalarial products in the country.

This study thus adds to the existing literature on plants used to treat malaria and has thrown light on the development of herbal antimalarial products in Ghana.

Competing interest

The authors declare no conflict of interest.

Acknowledgements

We are most grateful to the herbalists in the Bosomtwe and Sekyere East Districts of the Ashanti region, Ghana, for freely sharing their knowledge and time with us during the study. We also feel indebted to the chiefs and the opinion leaders of the various communities and many citizens of the two Districts who helped us in identifying the herbalists. We recognize the contributions of Misses Amanda Akomah-Donkor and Bertha Ewune Ekosse in conducting the survey. We are grateful to Dr. G.H. Sam of Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, for the authentication of the plants. We acknowledge the staff of Forestry Research Institute of Ghana (FORIG) especially Mr. M.F. Mensah for their technical assistance in the identification of some of the plants. Map (Fig. 1) was adapted from Wikimedia Creative commons. We acknowledged the referees for their meticulous evaluation of the manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2015.06.041.

References

Aarthi, N., Murugan, K., 2011. Antimalarial activity and phytochemical screening of ethanolic leaf extract of Phyllanthus nururi and Mimosa pudica. Int. J. Pharm. Res. Dev. 3 (3), 198–205.

Abbiw, D.K., 1990. Useful Plants of Ghana. Royal Botanic Gardens, KEW, London, pp. 154–157.

Abosi, A.O., Raseroka, B.H., 2003. In vivo antimalarial activity of Vernonia amygdalina. Br. J. Biomed. Sci. 60, 89–91.

Abrantes, M., Mil-Homens, T., Duarte, N., Lopes, D., Cravo, P., Madureira, M., Ferreira, M.-J., 2008. Antiplasmodial activity of lignans and extracts from Pycnanthus angolensis. Planta Medica 74 (11), 1408–1412.

Adebayo, J.O., Krettli, A.U., 2011. Potential antimalarials from Nigerian plants: a review. J. Ethnopharmacol. 133 (2), 289–302.

Adepiti, A.O., Elujoba, A.A., Bolaji, O.O., 2014. In vivo antimalarial evaluation of MAMA decoction on Plasmodium berghei in mice. Parasitol. Res. 113 (2), 505–511.

Akuodor, G., Mbah, C., Megwas, U., Ikoro, N., Akpan, J., Okwuosa, B., Osunkwo, U., 2012. In vivo antimalarial activity of methanol leaf extract of Bombax buonopozense in mice infected with Plasmodium berghei. Int. J. Biol. Chem. Sci. 5 (5), 1790–1796.

Al-Adhroey, A.H., Nor, Z.M., Al-Mekhlafi, H.M., Mahmud, R., 2010. Ethnobotanical study on some Malaysian anti-malarial plants: a community based survey. J. Ethnopharmacol. 132 (1), 362–364.

Alaribe, A.A.A., Ejezie, G.C., Ezedinachi, E.N.U., 2003. The role of Kola Nut (Cola Nitida) in the etiology of malaria morbidity. Pharm. Biol. 41 (6), 458–462.

Ancolio, C., Azas, N., Mahiou, V., Ollivier, E., Di Giorgio, C., Keita, A., Timon-David, P., Balansard, G., 2002. Antimalarial activity of extracts and alkaloids isolated from six plants used in traditional medicine in Mali and Sao Tome. Phytother. Res. 16 (7), 646–649.

Ankrah, N.-A., Nyarko, A.K., Addo, P.G.A., Ofosuhene, M., Dzokoto, C., Marley, E., 2003. Evaluation of efficacy and safety of an herbal medicine used for the treatment of malaria. Phytother. Res. 17 (6), 697–701.

Ansah, C., Gooderham, N.J., 2002. The popular herbal antimalarial, extract of Cryptolepis sanguinolenta, is potently cytotoxic. Toxicol. Sci. 70 (2), 245–251.

Appiah, A.A., 2009. The golden roots of Cryptolepis sanguinolenta. In: Juliani, H.R., Simon, J.E., Ho, C.-T. (Eds.), African Natural Plant Products: New Discoveries and Challenges in Chemistry and Quality vol. 1021. ACS Symposium Series, pp. 231–239.

Asante, F., Asenso-Okyere, K., 2003. Economic Burden of Malaria in Ghana. Final Report, ISSER. Univ. of Ghana (November).

Asase, A., Akwetey, G.A., Achel, D.G., 2010. Ethnopharmacological use of herbal remedies for the treatment of malaria in the Dangme West District of Ghana. J. Ethnopharmacol. 129 (3), 367–376.

Asase, A., Asafo-Agyei, T., 2011. Plants used for treatment of malaria in communities around the Bobiri Forest reserve in Ghana. J. Herbs Spices Med. Plants 17 (2), 85–106.

Asase, A., Hesse, D.N., Simmonds, M.S.J., 2012. Uses of multiple plants prescriptions for treatment of malaria by some communities in southern Ghana. J. Ethnopharmacol. 144 (2), 448–452.

Asase, A., Oppong-Mensah, G., 2009. Traditional antimalarial phytotherapy remedies in herbal markets in southern Ghana. J. Ethnopharmacol. 126 (3), 492–499.

Asase, A., Oteng-Yeboah, A.A., Odamtten, G.T., Simmonds, M.S.J., 2005. Ethnobotanical study of some Ghanaian anti-malarial plants. J. Ethnopharmacol. 99 (2), 273–279.

Attioua, B., Yeo, D., Lagnika, L., Harisolo, R., Antheaume, C., Weniger, B., Kaiser, M., Lobstein, A., Vonthron-Sénécheau, C., 2012. In vitro antileishmanial, antiplasmodial and cytotoxic activities of a new ventiloquinone and five known triterpenes from Parinari excelsa. Pharm. Biol. 50 (7), 801–806.

Ayim, J.A., Bayor, M.T., Phillips, R.M., Shnyder, S.D., Wright, C.W., 2008. The evaluation of selected Ghanaian medicinal plants for cytotoxic activities. J. Sci. Technol. 27 (3), 16–22.

Banzouzi, J.-T., Prado, R., Menan, H., Valentin, A., Roumestan, C., Mallie, M., Pelissier, Y., Blache, Y., 2002. In vitro antiplasmodial activity of extracts of Alchornea cordifolia and identification of an active constituent: ellagic acid. J. Ethnopharmacol. 81 (3), 399–401.

Bbosa, S.G., Kyegombe, D.B., Lubega, A., Musisi, N., Ogwal-Okeng, J., Odyek, O., 2013. Anti-Plasmodium falciparum activity of Aloe dawei and Justicia betonica. Afr. J. Pharm. Pharmacol. 7 (31), 2258–2263.

Bertania, S., Bourdy, G., Landau, I., Robinson, J.C., Esterre, P., Deharo, E., 2005. Evaluation of French Guiana traditional antimalarial remedies. J. Ethnopharmacol. 98 (1–2), 45–54.

Betti, J., Iponga, D., Yongo, O.D., Mbomio, D., Yobo, C.M., Ngoy, A., 2013. Ethnobotanical study of medicinal plants of the Ipassa-Makokou Biosphere Reserve, Gabon: Plants used for treating malaria. J. Med. Plants Res. 7 (31), 2300–2318. http://dx.doi.org/10.5897/JMPR11.1754.

Bhat, G.P., Surolia, N., 2001. In vitro antimalarial activity of extracts of three plants used in the traditional medicine of India. Am. J. Trop. Med. Hyg. 65 (4), 304–308.

Bidla, G., Titanji, V.P.K., Joko, B., El-Ghazali, G., Bolad, A., Berzins, K., 2004. Antiplasmodial activity of seven plants used in African folk medicine. Indian J. Pharmacol. 36 (4), 245–246.

Boyom, F.F., Fokou, P.V.T., Yamthe, L.R.T., Mfopa, A.N., Kemgne, E.M., Mbacham, W.F., Tsamo, E., Zollo, P.H.A., Gut, J., Rosenthal, P.J., 2011. Potent antiplasmodial extracts from Cameroonian Annonaceae. J. Ethnopharmacol. 134 (3), 717–724.

Bugyei, K.A., Boye, G.L., Addy, M.E., 2010. Clinical efficacy of a tea-bag formulation of Cryptolepis sanguinolenta root in the treatment of acute uncomplicated falciparum malaria. Ghana Med. J. 44 (1), 3–9.

Chatterjee, A., Kumar, S., Chattopadhya, S.K.A., 2013. Validated HPLC-PDA method for identification and quantification of two bioactive alkaloids, ephedrine and cryptolepine, in different Sida species. Biomed. Chromatogr. 27, 1720–1725.

Chen, Y., Li, S., Sun, F., Han, H., Zhang, X., Fan, Y., Tai, G., Zhou, Y., 2010. In vivo antimalarial activities of glycoalkaloids isolated from Solanaceae plants. Pharm. Biol. 48 (9), 1018–1024.

Cordell, G.A., 2014. Phytochemistry and traditional medicine—the revolution continues. Phytochem. Lett. 10, xxviii–xl.

Dike, I.P., Obembe, O.O., Adebiyi, F.E., 2012. Ethnobotanical survey for potential antimalarial plants in south-western Nigeria. J. Ethnopharmacol. 144 (3), 618–626.

Dokosi, O.B., 1998. Herbs of Ghana. Ghana University Press, Accra, pp. 173–174.

Gbeassor, M., Kedjagni, A.Y., Koumaglo, K., de Souza, C., Agbo, K., Aklikokou, K., Amegbo, K.A., 1990. In vitro antimalarial activity of six medicinal plants. Phytother. Res. 4 (3), 115–117.

Gbedema, S.Y., 2014. Antiplasmodial Evaluation of Extracts of Selected Ghanaian Medicinal Plants and Other Bioactivities of Isolated Compounds of Polyalthia longifolia Variety Pendua (Annonaceae) (Ph.D. theses). Department of Pharmaceutics, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana.

Ghana, F.D.A., 2013a. FDA Labelling Template Guideline. Accra. Ghana. Retrieved July 10, 2014, from ⟨http://www.fdaghana.gov.gh/images/stories/pdfs/down loads/drugs guidelines/TEMPLATE LABELLING.pdf⟩.

Ghana F.D.A., 2013b. Food and Drugs Authority Guideline for Registration of Herbal Medicines. Accra. Ghana. Retrieved July 14, 2014, from ⟨http://www.fdaghana.gov.gh/images/stories/pdfs/downloads/drugs guidelines/GUIDELINE FOR REGIS TRATION OF HERBAL MEDICINAL. PRODUCTS.pdf⟩.

Ghana Statistical Service, 2010. 2010 Population and Housing Census (pp. 98–99). Accra, Ghana.

Graz, B., 2013. What is "clinical data"? Why and how can they be collected during field surveys on medicinal plants?. J. Ethnopharmacol. 150 (2), 775–779.

Grover, J.K., Yadav, S.P., 2004. Pharmacological actions and potential uses of Momordica charantia: a review. J. Ethnopharmacol. 93 (1), 123–132.

Herlina, T., Supratman, U., Soedjanaatmadja, M.S., Subarnas, A., Sutardjo, S., Abdullah, N.R., Hayashi, H., 2009. Anti-malarial compound from the stem bark of Erythrina variegata. Indones. J. Chem. 9 (2), 308–311.

Idowu, O.A., Soniran, O.T., Ajana, O., Aworinde, D.O., 2010. Ethnobotanical survey of antimalarial plants used in Ogun State, Southwest Nigeria. Afr. J. Pharm. Pharmacol. 4 (2), 50–60.

Jiofack, T., Fokunang, C., Guedje, N., Kemeuze, V., 2009. Ethnobotany and phytomedicine of the upper Nyong valley forest in Cameroon. Afr. J. Pharm. Pharmacol. 3 (4), 144–150.

Jullian, V., Bourdy, G., George, S., Maurel, S., Sauvain, M., 2006. Validation of use of a traditional antimalarial remedy from French Guiana, Zanthoxylum rhoifolium Lam. J. Ethnopharmacol. 106 (3), 348–352.

Kamaraj, C., Kaushik, N.K., Rahuman, A.A., Mohanakrishnan, D., Bagavan, A., Elango, G., Zahir, A.A., Santhoshkumar, T., Marimuthu, S., Jayaseelan, C., Kirthi, A.V., Rajakumar, G., Velayutham, K., Sahal, D., 2012. Antimalarial activities of medicinal plants traditionally used in the villages of Dharmapuri regions of South India. J. Ethnopharmacol. 141 (3), 796–802.

Kaou, A.M., Mahiou-Leddet, V., Hutter, S., Aïnouddine, S., Hassani, S., Yahaya, I., Azas, N., Ollivier, E., 2008. Antimalarial activity of crude extracts from nine African medicinal plants. J. Ethnopharmacol. 116 (1), 74–83.

Karou, D., Dicko, M.H., Sanon, S., Simpore, J., Traore, A.S., 2003. Antimalarial activity of Sida acuta Burm. f. (Malvaceae) and Pterocarpus erinaceus Poir. (Fabaceae). J. Ethnopharmacol. 89 (2–3), 291–294.

Khaomek, P., Ichino, C., Ishiyama, A., Sekiguchi, H., Namatame, M., Ruangrungsi, N., Saifah, E., Kiyohara, H., Otoguro, K., Omura, S., Yamada, H., 2008. In vitro antimalarial activity of prenylated flavonoids from Erythrina fusca. J. Nat. Med. 62 (2), 217–220.

Klyushnichenko, V.E., Yakimov, S.A., Tuzova, T.P., Syagailo, Y.V., Kuzovkina, I.N., Wulfson, A.N., Miroshnikov, A.I., 1995. Determination of indole alkaloids from R. serpentina and R. vomitoria by high-performance liquid chromatography and high-performance thin-layer chromatography. J. Chromatogr. A 704 (2), 357–362.

Köhler, I., Jenett-Siems, K., Kraft, C., Siems, K., Abbiw, D., Bienzle, U., Eich, E., 2002. Herbal remedies traditionally used against malaria in Ghana: bioassay-guided fractionation of Microglossa pyrifolia (Asteraceae). Z. Naturforsch. C J. Biosci. 57 (11–12), 1022–1027.

Konaté, K., Bassolé, I.H.N., Hilou, A., Aworet-Samseny, R.R.R., Souza, A., Barro, N., Dicko, M.H., Datté, J.Y., M'Batchi, B., 2012. Toxicity assessment and analgesic activity investigation of aqueous acetone extracts of Sida acuta Burn f. and Sida cordifolia L. (Malvaceae), medicinal plants of Burkina Faso. BMC Complement. Altern. Med. 12 (1), 120 (11 pp.).

Koudouvo, K., Karou, D.S., Kokou, K., Essien, K., Aklikokou, K., Glitho, I.A., Simpore, J., Sanogo, R., De Souza, C., Gbeassor, M., 2011. An ethnobotanical study of antimalarial plants in Togo Maritime Region. J. Ethnopharmacol. 134 (1), 183–190.

Macfoy, D.C., 2013. Medicinal Plants and Traditional Medicine in Sierra Leone. iUniverse LCC, Bloomington. Retrieved from ⟨http://books.google.com/books?id¼tDGGAgAAQBAJ&pgis¼1⟩.

Madureira, M., do, C., de, Martins, A.P., Gomes, M., Paiva, J., Cunha, A.P., da, Rosário, V., do, R., 2002. Antimalarial activity of medicinal plants used in traditional medicine in S. Tome and Principe islands. J. Ethnopharmacol. 81, 23–29.

Makinde, J.M., Amusan, O.O., Adesogan, E.K., 1988. The antimalarial activity of Spathodea campanulata stem bark extract on Plasmodium berghei berghei in mice. Planta Medica 54 (2), 122–125.

Makinde, J.M., Obih, P.O., 1985. Screening of Morinda lucida leaf extract for antimalarial action on Plasmodium berghei berghei in mice. Afr. J. Med. Med. Sci. 14 (1–2), 59–63.

Martey, O.N.K., Shittah-Bay, O., Owusu, J., Okine, L.K.N., 2013. The antiplasmodial activity of an herbal antimalarial, AM207, in Plasmodium berghei- infected Bald/ c mice: absence of organ specific toxicity. J. Med. Sci. 13 (7), 537–545.

Mbatchi, S.F., Mbatchi, B., Banzouzi, J.T., Bansimba, T., Nsonde Ntandou, G.F., Ouamba, J.-M., Berry, A., Benoit-Vical, F., 2006. In vitro antiplasmodial activity of 18 plants used in Congo Brazzaville traditional medicine. J. Ethnopharmacol. 104 (1–2), 168–174.

Ménan, H., Banzouzi, J.-T., Hocquette, A., Pélissier, Y., Blache, Y., Koné, M., Mallié, M., Assi, L.A., Valentin, A., 2006. Antiplasmodial activity and cytotoxicity of plants used in West African traditional medicine for the treatment of malaria. J. Ethnopharmacol. 105 (1–2), 131–136.

Mesfin, A., Giday, M., Animut, A., Teklehaymanot, T., 2012. Ethnobotanical study of antimalarial plants in Shinile District, Somali Region, Ethiopia, and In vivo evaluation of selected ones against Plasmodium berghei. J. Ethnopharmacol. 139 (1), 221–227.

Ministry of Health, Republic of Ghana, 2008. Recommended list of Herbal Medicines Essential for Primary Healthcare Services. Accra.

Moura, A.C., Silva, E.L., Fraga, M.C., Wanderley, A.G., Afiatpour, P., Maia, M.B., 2005. Antiinflammatory and chronic toxicity study of the leaves of Ageratum conyzoides L. in rats. Phytomedicine. Int. J. Phytother. Phytopharmacol. 12 (1–2), 138–142.

Mshana, R.N., Abbiw, D.K., Addae-Mensah, I., Adjanouhoun, E., Ahyi, M.R.A., Ekpere, J., Enow-Rock, E.G., Gbile, Z.O., Noamesi, G.K., Odei, M.A., Odunlami, H., OtengYeboah, A.A., Sarpong, K., Soforowa, A., Tackie, A., 2001. Traditional Medicine and Pharmacopoeia; Contribution to the Revision of Ethnobotanical and Floristic Studies in Ghana. Science and Technology Press, CSIR, Accra.

Muganza, M.D., Fruth, B.I., Nzunzu Lami, J., Mesia, G.K., Kambu, O.K., Tona, G.L., Cimanga Kanyanga, R., Cos, P., Maes, L., Apers, S., Pieters, L., 2012. In vitro antiprotozoal and cytotoxic activity of 33 ethonopharmacologically selected medicinal plants from Democratic Republic of Congo. J. Ethnopharmacol. 141 (1), 301–318.

Mutabingwa, T.K., 2005. Artemisinin-based combination therapies (ACTs): best hope for malaria treatment but inaccessible to the needy!. Acta Tropica 95 (3), 305–315.

Nagendrappa, P.B., Naik, M.P., Payyappallimana, U., 2013. Ethnobotanical survey of malaria prophylactic remedies in Odisha, India. J. Ethnopharmacol. 146 (3), 768–772.

Nguta, J.M., Mbaria, J.M., Gakuya, D.W., Gathumbi, P.K., Kiama, S.G., 2010. Antimalarial herbal remedies of Msambweni, Kenya. J. Ethnopharmacol. 128 (2), 424–432.

Njan, A.A., 2012. Herbal medicine in the treatment of malaria: Vernonia amygdalina: an overview of evidence and pharmacology. In: B. Acree (Ed.), Toxicity and Drug Testing (pp. 167–186). InTech.

Oduola, T., Bello, I., Adeosun, G., Ademosun, A.-W., Raheem, G., Avwioro, G., 2010. Hepatotoxicity and nephrotoxicity evaluation in Wistar albino rats exposed to Morinda lucida leaf extract. North Am. J. Med. Sci. 2 (5), 230–233.

Okokon, J.E., Udokpoh, A.E., Antia, B.S., 2007. Antimalarial activity of ethanolic extract of Tetrapleura tetraptera fruit. J. Ethnopharmacol. 111 (3), 537–540.

Okpekon, T., Yolou, S., Gleye, C., Roblot, F., Loiseau, P., Bories, C., Grellier, P., Frappier, F., Laurens, A., Hocquemiller, R., 2004. Antiparasitic activities of medicinal plants used in Ivory Coast. J. Ethnopharmacol. 90 (1), 91–97.

Olorunnisola, O.S., Adetutu, A., Balogun, E.A., Afolayan, A.J., 2013. Ethnobotanical survey of medicinal plants used in the treatment of malaria in Ogbomoso, Southwest Nigeria. J. Ethnopharmacol. 150 (1), 71–78.

Owuor, B.O., Ochanda, J.O., Kokwaro, J.O., Cheruiyot, A.C., Yeda, R.A., Okudo, C.A., Akala, H.M., 2012. In vitro antiplasmodial activity of selected Luo and Kuria medicinal plants. J. Ethnopharmacol. 144 (3), 779–781.

Oyen, L.P.A., 2010. Cyclosorus interruptus (Willd.) H.Itô. In: Brink, M., Achigan-Dako, E.G. (Eds.), Prota 16: Fibres/Plantes à fibres. [CD-Rom]. PROTA, Wageningen, Netherlands, Available at: ⟨http://database.prota.org/PROTAhtml/Cyclosorus% 20interruptus_En.htm⟩..

Patel, J.P., Gami, B., Patel, K., 2010. Evaluation of in vitro schizonticidal properties of acetone extract of some Indian medicinal plants. Adv. Biol. Res. 4 (5), 253–258.

Public Health Act, 2012. The 851 Act of the Parliament of the Republic of Ghana, 2012. Ghana.

Raji, Y., Salman, T.M., Akinsomisoye, O.S., 2005. Reproductive functions in male rats treated with methanolic extract of Alstonia boonei stem bark. Afr. J. Biomed. Res. 8, 105–111.

Raman, A., Lau, C., 1996. Anti-diabetic properties and phytochemistry of Momordica charantia L. (Cucurbitaceae). Phytomedicine 2 (4), 349–362.

Randrianarivelojosia, M., Rasidimanana, V.T., Rabarison, H., Cheplogoi, P.K., Ratsimbason, M., Mulholland, D.A., Mauclère, P., 2003. Plants traditionally prescribed to treat tazo (malaria) in the eastern region of Madagascar. Malar. J. 2 (1), 25.

Sanon, S., Gansane, A., Ouattara, L.P., Traore, A., Ouedraogo, I.N., Tiono, A., Taramelli, D., Basilico, N., Sirima, S.B., 2013. In vitro antiplasmodial and cytotoxic properties of some medicinal plants from western Burkina Faso. Afr. J. Lab. Med. 2 (1), 1–7.

Sanon, S., Ollivier, E., Azas, N., Mahiou, V., Gasquet, M., Ouattara, C.T., Nebie, I., Traore, A.S., Esposito, F., Balansard, G., Timon-David, P., Fumoux, F., 2003. Ethnobotanical survey and in vitro antiplasmodial activity of plants used in traditional medicine in Burkina Faso. J. Ethnopharmacol. 86 (2–3), 143–147.

Saotoing, P., Toua, V., Tchobsala, Tchuenguem, F.-N.F., Alexandre-Michel, N.N., Jean, M., 2011. Medicinal plants used in traditional treatment of malaria in Cameroon. J. Ecol. Nat. Environ. 3, 104–117.

Schmelzer, G.H., Gurib-Fakim, A., 2013. Plant Resources of Tropical Africa. 11. PROTA, Wageningen, p. 221 Available at: ⟨books.google.com/books?id¼3clmAgAAQBAJ&pgis¼1⟩..

Sharma, O.P., Sharma, S., Pattabhi, V., Mahato, S.B., Sharma, P.D., 2007. A review of the hepatotoxic plant Lantana camara. Crit. Rev. Toxicol. 37 (4), 313–352.

Sittie, A.A., Lemmich, E., Olsen, C.E., Hviid, L., Brøgger Christensen, S., 1998. Alkamides from Phyllanthus fraternus. Planta Medica 64 (2), 192–193.

Snow, R.W., Guerra, C.A., Noor, A.M., Myint, H.Y., Hay, S.I., 2005. The global distribution of clinical episodes of Plasmodium falciparum malaria. Nature 434 (7030), 214–217.

Stangeland, T., Alele, P.E., Katuura, E., Lye, K.A., 2011. Plants used to treat malaria in Nyakayojo sub-county, western Uganda. J. Ethnopharmacol. 137 (1), 154–166.

Tabuti, J.R.S., 2008. Herbal medicines used in the treatment of malaria in Budiope County, Uganda. J. Ethnopharmacol. 116 (1), 33–42.

Tchinda, A.T., Fuendjiep, V., Sajjad, A., Matchawe, C., Pascal, Wafo, Khan, S., Tane, P., Choudhary, M.I., 2009. Bioactive compounds from the fruits of Zanthoxylum leprieurii. Pharmacologyonline 1, 406–415.

Tchoumbougnang, F., Zollo, P.H., Dagne, E., Mekonnen, Y., 2005. In vivo antimalarial activity of essential oils from Cymbopogon citratus and Ocimum gratissimum on mice infected with Plasmodium berghei. Planta Medica 71 (1), 20–23.

Tepongning, R.N., Lucantoni, L., Nasuti, C.C., Dori, G.U., Yerbanga, S.R., Lupidi, G., Marini, C., Rossi, G., Esposito, F., Habluetzel, A., 2011. Potential of a Khaya ivorensis–Alstonia boonei extract combination as antimalarial prophylactic remedy. J. Ethnopharmacol. 137 (1), 743–751.

Tona, L., Cimanga, R.K., Mesia, K., Musuamba, C.T., De Bruyne, T., Apers, S., Hernans, N., Van Miert, S., Pieters, L., Totté, J., Vlietinck, A.J., 2004. In vitro antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. J. Ethnopharmacol. 93 (1), 27–32.

Tona, L., Ngimbi, N.P., Tsakala, M., Mesia, K., Cimanga, K., Apers, S., De Bruyne, T., Pieters, L., Totté, J., Vlietinck, A.J., 1999. Antimalarial activity of 20 crude extracts from nine African medicinal plants used in Kinshasa, Congo. J. Ethnopharmacol. 68 (1–3), 193–203.

Traore, M.S., Baldé, M.A., Diallo, M.S.T., Baldé, E.S., Diané, S., Camara, A., Diallo, A., Balde, A., Keïta, A., Keita, S.M., Oularé, K., Magassouba, F.B., Diakité, I., Pieters, L., Baldé, A.M., 2013. Ethnobotanical survey on medicinal plants used by Guinean traditional healers in the treatment of malaria. J. Ethnopharmacol. 150 (3), 1145–1153.

Ueno, H.M., Doyama, J.T., Padovani, C.R., Salata, E., 1996. Effect of Momordica charantia L. in mice infected with Plasmodium berghei. J. Braz. Soc. Trop. Med. 29 (5), 455–460.

Ukwe, V.C., Epueke, E.A., Ekwunife, O.I., Okoye, C., Akudor, G.C., Ubaka, C.M., 2010. Antimalarial activity of aqueous extract and fractions of leaves of Ageratum conyzoides in mice infected with Plasmodium berghei. Int. J. Pharm. Sci. 2 (1), 33–38.

Valentin, A., Benoit-Vical, F., Pélissier, Y., Koné-Bamba, D., Mallié, M., 2000. Antiplasmodial activity of plant extracts used in West African traditional medicine. J. Ethnopharmacol. 73 (1–2), 145–151.

Van Andel, T., Myren, B., Van Onselen, S., 2012. Ghana's herbal market. J. Ethnopharmacol. 140 (2), 368–378.

White, N.J., Pukrittayakamee, S., Hien, T.T., Faiz, M.A., Mokuolu, O.A., Dondorp, A.M., 2014. Malar. Lancet 383 (9918), 723–735.

WHO, 2007. WHO Monographs on Selected Medicinal Plants. vol. 3. WHO Press, World Health Organization, Geneva, Switzerland, pp. 88–101.

WHO, 2010a. Guidelines for Registration of Traditional Medicines in the WHO African Region. Publication and Language Services Unit, WHO Africa Regional Office, Brazzaville, Republic of Gongo.

WHO, 2010b. Guidelines for the Treatment of Malaria, WHO Press, Second edition. World Health Organization, Geneva, Switzerland.

WHO, 2011. World Malaria Report 2011, WHO Press. World Health Organization, Geneva, Switzerland.

WHO, 2013a. WHO Traditional Medicine Strategy 2014–2023. WHO Press, World Health Organization, Geneva, Switzerland, pp. 1–78.

WHO, 2013b. World Malaria Report 2013. WHO Press, World Health Organization, Geneva, Switzerland.

WHO, 2014. WHO Malaria Report 2014. WHO Press, World Health Organization, Geneva, Switzerland.

Willcox, M., 2011. Improved traditional phytomedicines in current use for the clinical treatment of malaria. Planta Medica 77 (6), 662–671.

Willcox, M., Benoit-Vical, F., Fowler, D., Bourdy, G., Burford, G., Giani, S., Graziose, R., Houghton, P., Randrianarivelojosia, M., Rasoanaivo, P., 2011. Do ethnobotanical and laboratory data predict clinical safety and efficacy of anti-malarial plants? Malar. J. 10 (Suppl. 1), S7 (9 pp.).

Yenesew, A., Induli, M., Derese, S., Midiwo, J.O., Heydenreich, M., Peter, M.G., Waters, N.C., 2004. Anti-plasmodial flavonoids from the stem bark of Erythrina abyssinica. Phytochemistry 65 (22), 3029–3032.

Yetein, M.H., Houessou, L.G., Lougbégnon, T.O., Teka, O., Tente, B., 2013. Ethnobotanical study of medicinal plants used for the treatment of malaria in plateau of Allada, Benin (West Africa). J. Ethnopharmacol. 146 (1), 154–163.

Zirihi, G.N., Mambu, L., Guédé-Guina, F., Bodo, B., Grellier, P., 2005. In vitro antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. J. Ethnopharmacol. 98 (3), 281–285.

Appendix 15a. *Sample* questionnaire

Ethnobotanical survey of medicinal plants used to treat malaria in the Bosomtwi /Sekyere East Districts of the Ashanti Region, Ghana.

Date: ___/___/12

Practitioner's Demographics

1. Name……
   ID:…….
2. Gender: Male [ ] Female [ ]
3. Age: 20- 30 [ ] 31 -40 [ ] 41-50 [ ] 51-60 [ ] 60+ [ ]
4. Level of Education: Primary [ ] JHS/Middle sch. [ ] Secondary [ ] Tertiary [ ] None [ ]
5. Profession: Farmer [ ] Teacher [ ] Health worker [ ] Lawyer [ ] Banker [ ] Others .....
6. How long have you been practicing: ≤10 [] 11 -20 [] 21-30 [] 31-40 [] >40 []
7. How did you acquire this skill? Understudied an experienced herbalist(s) [ ] Divine gift [ ] Learnt it in my family [] Taught by spirits [] through dreams [] others.....

Information on malaria

8. Do you know what malaria is? Yes [ ] No [ ] **9.** How do you tell if someone has malaria?
   High temperature [ ] Bitter mouth [ ] Nausea and/or vomiting [ ] Chills [ ] Loss of appetite [ ] Insomnia [ ] Stomach ache [ ] Other symptoms.....
10. Do people come to you for help when they have malaria? Yes [ ] No [ ]
11. If yes, how often do you encounter malaria cases? Everyday [ ] One/more cases per week [ ] Once in a while [ ]
12. How do you tell if your patient is successfully healed of the malaria disease she/he had……..……

Plant Information

13. What do you use to treat malaria? Herbs [ ] Prayer [ ] Medicines bought from drug stores [ ]
    Others…….
14. If herbs, which plant(s)? Vernacular names, or/and botanical name if known, please
    1. .....
    2. .....
    3. .....
15. How do you obtain the plant? Self-harvesting/collection 1[] 2 [] 3 [] 4 [] 5 []
    Buy from market 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ] Supply by customers 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ]

16. In what condition is the plant used? Fresh 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ]
Dried 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ]

Medicinal/pharmaceutical information

17. Plant part(s) used: Leaf 1 [] 2 [] 3 [] 4 [] 5 [] Stem bark 1 [] 2 [] 3 [] 4 [] 5 []
Root/root bark 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ] Whole plant 1 [] 2 [ ] 3 [ ] 4 [ ] 5 []
Flower 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ] Fruit 1 [ ] 2 [ ] 3 [] 4 [] 5 []
Seed 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ] Others…….....

18. How do you prepare them for use? (mode of preparation and usage form)
1.....
2.....
3.....

19. Side effects: Diarrhoea: 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ] Sedation: 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 []
Vomiting: 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ] None: 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ]
Others……

20. Contraindications: Pregnancy :1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ]
Breastfeeding: 1 [] 2 [ ] 3 [ ] 4 [ ] 5 [ ] Hypertension: 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ]
Stomach ulcer: 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ] None 1 [] 2 [] 3 [] 4 [] 5 []
Others……

21. Any special warning or caution?
1……
2……
3…….

22. Any other uses of plant
1……
2.....
3.....
4.....
5.....……..

Geographical/geological information

23. . Village name…….....
24. . Vegetation type……..
25. Category of respondent……..

Appendix 15b. Survey of finished herbal antimalarial products in the Kumasi Metropolis

Commercially available herbal antimalarials in the Kumasi Metropolis
Facility information

1. Name of facility……
2. Type of facility:
1. Herbal medicine wholesale shop 2. Herbal medicine retail shop
3. Community pharmacy 4. Herbal clinic 5. Other……..
3. Profession of supervisor of facility
1. Traditional Herbalist 2. Medical herbalist.
3. Pharmacist 4. Other…….

Label information

4. Name of product …….
5. FDA number……....
6. Indications: 1……
2……
3……..…… 4……
7. Dosage form: Liquid [ ] Tablet [ ] Powder [ ] others……
8. Name and part of the plant ingredients / preparation / quantity
1……
2……
3……
9. Stated route of administration if any: Oral [ ] Rectal [ ] Topical [ ]
Nostril [ ] Others……
10. Presence of Dosage information: Adult Yes [ ] No [ ]
Children Yes [ ] No [ ]
Elderly Yes [ ] No [ ]
11. Stated side effects:
1……
2……
3……..……..……
12. Stated Contraindications:
1……
2…….
3…….
13. Stated warnings and precautions:
1…….
2……..
3……..……..……

14. Manufacturing date indicated:	Yes [ ]	No [ ]
15. Expiry date indicated:	Yes [ ]	No [ ]
16. Batch number present:	Yes [ ]	No [ ]
17. Manufacturer's address present:	Yes [ ]	No [ ]
18. Informative leaflet Present:	Yes [ ]	No [ ]

Appendix 15c. Respondent's information and informed consent

RESPONDENT'S INFORMATION AND INFORMED CONSENT

Study Title

Ethnobotanical survey of medicine plants use to treat malaria in the Bosomtwe District of the Ashanti Region, Ghana.

Principal Investigator

Mr Gustav Komlaga, University of Science and Technology, Kumasi, Ghana. Tel. 0244632880

Purpose

You are being asked to take part in a research study to document medicinal plants use to treat malaria by herbalists in the Bosomtwe District. You have been chosen because of your reputation as an herbalist in the district. The purpose of this research study is to learn how herbalists treat malaria and which medicinal plants they employ to treat the disease.

Procedures

If you agree to take part in this research study, you will be requested to help fill the questionnaire concerning your knowledge about malaria and the medicinal plants you use to treat it. You may also be required to show us these medicinal plants on the field.

Risks/Discomforts

There is no anticipated discomfort for those contributing to this study, so risk to participants is minimal.

Benefits

Your help with this study may help document the medicinal plants use to treat malaria in the Bosomtwe District and would prevent the loss of such important knowledge from the community.

Confidentiality

Your records will be kept confidential and will not be released without your consent except as required by law. In addition, if the results of this study are written in a scientific journal or presented at a scientific meeting, your name will not be used

Voluntary Participation/Withdrawal

Your decision to take part in this research study is entirely voluntary. You may refuse to take part in or you may withdraw from the study at any time without penalty or loss of benefits to which you are normally entitled.

Questions

You may wish to discuss this with others before you agree to take part in this study. If you have any questions about the research now or during the study contact Gustav Komlaga (0244632880)

Statement of Consent

I have read the above description of this research study. I have been informed of the risks and benefits involved, and all my questions have been answered to my satisfaction. Furthermore, I have been assured that any future questions I may have will also be answered by a member of the research team. I voluntarily agree to take part in this study. I understand I will receive a copy of this consent form.

Name of respondent

_____ _____

Respondent's Signature Date

Appendix 15d. Medicinal plant species cited for the first time in Ghana

Plants not yet recorded in Ghana but recorded elsewhere with references
Afrostyrax lepidophyllus Mildbr. (Muganza et al. 2012)
Albizia ferruginea (Guill. and Perr.) Benth. (Zirih et al., 2005)
Allium cepa L. (Idowu et al. 2010; Olorunnisola et al. 2013; Saotoing et al. 2011)
Allium sativum L. (Adebayo & Krettli, 2011; Saotoing et al., 2011)
Aloe vera (L.) Burm. f. (Nguta et al. 2010; Saotoing et al. 2011)
Argemone mexicana L. (Idowu et al. 2010; Kamaraj et al. 2012; Yetein et al. 2013)
Citrus × *limon* (L.) Burm. f. (Betti et al. 2013; Saotoing et al. 2011; Yetein et al. 2013)
Cyathula prostrata (L.) Blume (Mbatchi et al. 2006)
Dioscorea dumetorum (Kunth) Pax (Dike et al, 2012)
Ficus exasperata Vahl. (Koudouvo et al. 2011)
Ficus sycomorus L. (Sanon et al. 2003)
Microdesmis puberula Hook.f. ex Planch. (Betti et al. 2013)
Mimosa pudica L. (Aarthi and Murugan, 2011)
Ocimum canum L. (Koudouvo et al. 2011)
Parinari excelsa Sabine (Attioua et al. 2012)
Phragmanthera capitata (Spreng.) Balle (Jiofack et al. 2009)
Solanum tuberosum L. (Chen et al. 2010)
Trema orientalis (L.) Blume (Betti et al. 2013)
Uapaca guineensis Müll. Arg. (Macfoy, 2013)
Sida cordifolia (Konaté et al. 2012)

Appendix 15e. Medicinal plant species cited for the first time

Plants cited for the first time for treating malaria

Amphimas pterocarpoides Harms

Cola nitida (Vent.) Schott and Endl.

Cyclosorus afer Ching

Erythrina mildbraedii Harms

Grossera vignei Hoyle

Justicia carnea Lindl.

Launaea taraxacifolia (Willd.) Amin ex C. Jeffrey

Periploca nigrescens Afzel.

Pterygota macrocarpa K. Schum.

Sida cordata L.

Zanthoxylum leprieurii Guill. & Perr.