COMPARATIVE CLINICAL STUDY OF MIST AMEN FEVERMIX AND EDHEC MALACURE: TWO POLYHERBAL PRODUCTS USED FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN GHANA AGAINST ARTEMETHER/LUMEFANTRINE

By

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A thesis submitted to the Department of Pharmacognosy, Kwame Nkrumah University of Science and Technology, Kumasi in partial fulfilment of the requirements for the award degree of

DOCTOR OF PHILOSOPHY IN PHARMACOGNOSY

NOVEMBER, 2020

DECLARATION

I hereby declare that this submission is my own work towards the award of a Doctor of Philosophy (Pharmacognosy option) and that, to the best of my knowledge, it contains neither material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in this thesis.

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ETHICAL CERTIFICATE

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL
COMMITTEE ON HUMAN RESEARCH, PUBLICATION AND ETHICS

Our Ref: CHRPE/AP/424/19 — 9th July, 2019.

Mr. Turkson Bernard Kofi
Department of Pharmacognosy
Faculty of Pharmacy and
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Dear Sir,

LETTER OF APPROVAL

Protocol Title: ***"Comparative Clinical Studies of Artemether/Lumefantrine and Mist. Amen Fevermix and Edhec Malacure Mixture, Two Herbal Product Used for the Treatment of Uncomplicated Malaria in Ghana."***

Proposed Site: ***Herbal Medicine Unit, Tafo Government Hospital, Kumasi.***

Sponsor: ***Principal Investigator.***

Your submission to the Committee on Human Research, Publications and Ethics on the above-named protocol refers.

The Committee reviewed the following documents:

- A notification letter of 18th April, 2019 from the Tafo Hospital (study site) indicating approval for the conduct of the study at the Hospital.
- A Completed CHRPE Application Form.
- Participant Information Leaflet and Consent Form.
- Research Protocol.
- Questionnaire and Interview Guide.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, beginning 9th July, 2019 to 8th July, 2020 renewable thereafter. .The Committee may however, suspend or withdraw ethical approval at any time if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at the close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Thank you, Sir, for your application.

Yours faithfully,

Osomfo Prof. Sir J. W. Acheampong MD, FWACP
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DETAILS OF PUBLICATIONS FROM THE DISSERTATION

1. Turkson Bernard K, Merlin L.K. Mensah, George H. Sam, Abraham Y. Mensah, Isaac K. Amponsah, Edmund Ekuadzi, Gustav Komlaga and Emmanuel Achaab (2020). Evaluation of the Microbial Load and Heavy Metal Content of Two Polyherbal Antimalarial Products on the Ghanaian Market. Evidence-Based Complementary and Alternative Medicine. Volume 2020. DOI.org/10.1155/2020/1014273.

2. Turkson, B.K., Mensah, M.L.K., Amponsah, I.K., Mensah, A.Y., Achaab, E., Mensah, R.B., Atakorah, E., Attah, E.O., Zoiku, F (2020). *In vitro* and *in vivo* Activity of Mist Amen Fevermix and Edhec Malacure, Polyherbal Antimalarial Products on Field Isolates of *Plasmodium falciparum* and *Plasmodium berghei*. Discovery Phytomedicine. Vol. 7. No. 3. Pp. 97-102. DOI: 10.15562/ phytomedicine.2020.129.

DEDICATION

To my parents Mr Anthony Kwesi and Mrs Mary Abena Turkson, my wife Mrs Araba Turkson, my children: Nhyira Ekow Annan Turkson, Praise Abena Seiwaa Turkson and Ama Aseda Simmons Turkson, and my siblings Dorothy, Dora, Eric, Elizabeth, Emmanuel and the late Judith Turkson, and all those who inspired me to this status.

ACKNOWLEDGEMENT

My first and foremost gratitude extends to the all-knowing and mighty God, whose Divine inspiration, guidance and direction has sustained me up to this time.

I acknowledge the Ghana Education Trust Fund (GetFund) for funding this PhD and providing all the needed support throughout this study. I give special recognition, acknowledgement and most sincere thanks to my dynamic supervisors Professor M. L. K. Mensah, for his guidance and patience with me, Dr I. K Amponsah for his guidance and Professor A.Y. Mensah for his encouragement and corrections. I would never have finished the thesis without you. Sir, words cannot describe what you have done for me, may God bless you in all your endeavours.

My appreciation goes to Dr Emmanuel Achaab, a co-supervisor at the Tafo Government Hospital for his support and guidance throughout the study period.

My deepest appreciation also goes to all the lecturers at the Departments of Pharmacognosy and Herbal Medicine in the Kwame Nkrumah University of Science and Technology, Kumasi for their support and encouragement.

My deepest gratitude goes to my wife, Araba and my children Nhyira, Praise and Aseda for their love, support and patience during the time I spent away while pursuing my studies. I am forever thankful to my parents and family members for their love and support. I am grateful to Dorothy for the support she gave me. God bless you sister.

I would also like to thank the graduate students at the Department of Pharmacognosy, and Pharmaceutical Chemistry, for their support and encouragement during the whole period of my study.

Finally, I would also like to thank Mr Titi Accam and Dr Paul Osei Kofi for their support.

ABSTRACT

The use of herbal medicinal products for the treatment of malaria an infectious and a lifethreatening disease, has increased globally. However, inadequate scientific studies, questions about the quality, safety and efficacy of such herbal products have been raised. On the other hand, the reduced sensitivity of the malaria parasites to artemisinin-based combination therapies is also of concern. There is therefore the need for new antimalarial medications including those from alternative sources such as herbal medicinal products. In this study, methods for the quality control of *Mist Amen Fevermix* and *Edhec Malacure*, two polyherbal antimalarial products used in Ghana for the management of uncomplicated malaria was undertaken. The development of the quality parameters for the test samples was based on phytochemical, physicochemical, chromatographic and spectroscopic methods. The set parameters were found to be sufficient to evaluate *Mist Amen Fevermix* and *Edhec Malacure*, and can be used as reference standards for the quality control purposes. Qualitative phytochemical screening and fingerprinting were undertaken based on standard analytical methods. The antiplasmodial activity was assessed *in vitro* by using field isolates of *Plasmodium falciparum* with SYBR® Green assays to measure parasite growth inhibition. Thermo Elemental M5 Atomic Absorption Spectrophotometer (AAS) fitted with Graphite furnace and an auto sampler was used to determine the heavy metal contents of the herbal products. The herbal samples were evaluated for microbial load by using the appropriate culture media. *In vivo* antiparasitic activity in mice was assessed using the Rane's curative method using ANKA strain of *Plasmodium berghei* parasites. A comparative clinical study was done to assess the safety and effectiveness of the test samples at the Tafo Government Hospital, Kumasi after Committee on Human Research, Publication and Ethics approval. Male and female patients aged 15-45 years with clinically established malaria were treated with *Mist Amen Fevermix* and *Edhec Malacure*, at the specified doses of 45 mls (0.1063 g) and 30 mls (0.0521 g) three times daily after meals for three days. Basic phytochemical screening of the two products indicated the presence of the following phytochemicals: alkaloids, saponins, tannins, phytosterols and flavonoids. From the data, it was established that *Mist Amen Fevermix* and *Edhec Malacure* complied with the pharmacopoeial standards after testing for microbes. The following heavy metals were present in *Mist Amen Fevermix* and *Edhec Malacure*: Fe, Ni, K, Zn, Hg, Cu, Mn, Cr, Cd, Pb, Fe, Cu, K and Na. Ni was below detectable limit in *Edhec Malacure*. The phytochemical screening of the products revealed the presence of alkaloid flavonoid, tannin, steroid and saponin. The HPLC method was validated for linearity, limits of detection and quantification, precision and accuracy. The test products were found not to have been adulterated with lumefantrine, artemether and quinine. The test herbal products showed *in vitro* and *in vivo* antiplasmodial activities against *Plasmodium falciparum* and *Plasmodium berghei* parasites. Inhibitory concentration (IC_{50}) values for *Edhec Malacure* was 70.89 ng/ml and that of *Mist Amen Fevermix* was 112.5 ng/ml. *Edhec Malacure* suppressed 76.17% of parasitaemia while *Mist Amen Fevermix* suppressed 69.03% of parasitaemia. *Edhec Malacure* demonstrated curative chemo suppressive potentials of 80.93% at the dose of 2.234 $mgkg^{-1}$ and *Mist Amen Fevermix* % suppression was 69.03% at a dose of 4.56mg/kg^{-1}. Both products demonstrated antiplasmodial activity in human red blood cells. The clinical evaluation of the test samples showed that *Mist Amen Fevermix* exhibited a statistically significant difference between the mean malaria parasite load recorded at the first visit and those recorded at the second visit, $t(23) = 4.59$, $p = 0$ .000. Similarly, there was a significant difference between the mean parasite count recorded on the second visit and the third visit, $t(6) = 1.49$, $p = 0$ .187. No difference were recorded for the third and fourth visits $t(3) = 1.00$, $p = 0$ .391. *Edhec Malacure* also exhibited a significant difference in efficacy between the mean malaria parasite count recorded at the first visit and those recorded at the second visit, $t(26) = 3.77$, $p = 0$ .001. Similarly, there is a statistically significant difference between malaria parasite count at the second visits and third visits, $t(16) = 1.74$, $p = 0$ .100. This shows the significant effectiveness of the products. Kidney and liver panel as well as full blood count and vital signs were within normal

reference range at the end of the 28-day study and thus established the safety of *Mist Amen Fevermix* and *Edhec Malacure* in the treatment of uncomplicated malaria. The results support claims that *Mist Amen Fevermix* and *Edhec Malacure* may be useful antimalarial agents. This study has demonstrated the *in vitro* and *in vivo* antiplasmodial activities of *Mist Amen Fevermix* and *Edhec Malacure,* and suggests that, the products have promising antimalarial activity. The *in vivo* findings showed that *Mist Amen Fevermix* and *Edhec Malacure* are relatively safe for oral administration at doses tested. In addition, the study supports the use of *Mist Amen Fevermix* and *Edhec Malacure*, two polyherbal products for the treatment of uncomplicated malaria. Both products achieved a comparable clinical treatment outcome to the reference control medication artemether/lumefantrine.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ACD	Acid Citrate Dextrose
ACT	Artemisinin Combination Therapy
AIDS	Acquired Immunodeficiency Syndrome
A-L	Artemether Lumefantrine
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
APIs	Active Pharmaceutical Ingredient
AS-AQ	Artesunate Amodiaquine
ASHH	Amen Scientific Herbal Hospital
AST	Aspartate Transaminase
CMC	Chemical-Manufacturing-Control
CRF	Case Record Folder
CSP	Circumsporozoite Protein
CHRPE	Committee for Human Research, Publications and Ethics
CPM	Counts Per Minute
CG	Cycloguanil
DHAP	Dihydroartemisinin Piperaquine
DNA	Deoxyribonucleic Acid
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
EHC	Edu Herbal Clinic
EHML	Essential Herbal Medicines List
FBC	Full Blood Count
FDA	Food and Drug Authority
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
GGT	Gamma-glutamyl Transferase
GHP	Ghana Herbal Pharmacopoea
GHS	Ghana Health Service
GNDP	Ghana National Drugs Programme
GACP	Good Agricultural and Collection Practices
GLP	Good Laboratory Practices

GMP	Good Manufacturing Practices
GSP	Good Storage Practices
GSK	GlaxoSmithKline
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin-Layer Chromatography
HIV	Human Immunodeficiency Virus
IFA	Immunofluorescence Antibody
IRS	Indoor Residual Spraying
ITN	Insecticide Treated Net
IRB	Institutional Review Board
ITT	Intention To-Treat
IP	Investigational Product
IB	Investigator's Brochure
IR	Institutional Review
KFT	Kidney Function Test
KNUST	Kwame Nkrumah University of Science and Technology
LFT	Liver Function Test
LC	Liquid Chromatography
LLINs	Long-Lasting Insecticidal Nets
LOD	Limit Of Detection
LOQ	Limit Of Quantification
MVI	Malaria Vaccine Initiative
MIC	Minimum Inhibitory Concentration
MOH	Ministry of Health
NGOs	Non-Governmental Organizations
NMCP	National Malaria Control Programme
NOAEL	No Observed Adverse Effect Level
OECD	Organization for Economic Co-operation and Development
OPD	Out Patient Department
PA	Public Address
PCR	Polymerase Chain Reaction
PDA	Photodiode Array Detector
PG	Proguanil
PHA	Public Health Act
PLDH	Parasite Lactate Dehydrogenase

PYR	Pyrimethamine
RBCs	Red Blood Cells
RBM	Roll Back Malaria
RCT	Randomized Controlled Trial
RDT	Rapid Diagnostic Test
RPMI	Roswell Park Memorial Institute
RT-PCR	Real-Time Polymerase Chain Reaction
SAP	Statistical Analysis Plan
SDX	Sulfadoxine
SOPs	Standard Operating Procedures
SPSS	Statistical Package for the Social Sciences
TB	Tuberculosis
T3	Test, Treat and Track
TLC	Thin –Layer Chromatography
TM	Traditional Medicine
TCM	Traditional Chinese Medicine
TKM	Traditional Korean Medicine
UV	Ultraviolet
WAHP	West Africa Herbal Pharmacopoiea
WBC	White Blood Cells
WHO	World Health Organization
WHOQOL	World Health Organization Quality of Life

LIST OF APPENDICES

CHAPTER ONE

INTRODUCTION

1.1. General Introduction

Malaria is a life-threatening mosquito-borne infectious ailment which causes hundreds of thousands of deaths every year. It is one of the globally most important infectious ailments which leads to substantial morbidity, mortality with negative socioeconomic influence, and human suffering every year (WHO, 2020; WHO, 2018). Globally, the World Health Organization (WHO) states that approximately 228 million cases of malaria was estimated to have occurred in the year 2018 leading to about 435,000 deaths, the majority, 93 per cent, occurred in Africa and over 405,000 deaths have been recorded in children under age 5 years, which account for 67 per cent of all deaths (WHO, 2018). As in most sub-Saharan countries, malaria is prevalent in Ghana and happens to be a serious public health challenge accounting for 4 per cent of the global burden and 7 per cent of the malaria burden in West Africa (WHO, 2018). Malaria resulted in 38 per cent of all Out-Patient Department (OPD) attendances, 35 per cent of all admissions, and 34 per cent of under-five year's hospital admissions in the country. It has been noted to be responsible for the cause of poverty and low productivity (NMCP/ MOH, 2009; GHS, 2011). Malaria was responsible for 19 per cent of all deaths recorded in Ghana in the year 2018 (The Global Fund, 2019). Malaria admission increased from 280,000 to 340,000 persons between the years 2010 and 2017 (WHO, 2018).

According to the WHO, the total expenditure for malaria control and eradication globally reached an estimated US$ 3.1 billion in the year 2017 (WHO, 2018). Funding from governments of nations with widespread cases amounted to US$ 900 million, constituting 28 per cent of the bulk funding (WHO, 2018). Most of the above statistics do not reflect the reality since the most vulnerable population have little or no access to modern medical facilities and as such most expenditure are not registered. Such

population make up the bulk of the estimated 80 per cent of the world's population that rely on herbal medicinal and products for their primary healthcare needs (WHO, 2002).

Malaria infection is categorised as either complicated (severe) or uncomplicated. Complicated malaria is characterised by severe organ dysfunction or abnormality in the patient's blood or metabolism. The presentations of severe malaria, where more than 5 per cent of the red blood cells are infected by malaria parasites include cerebral malaria with abnormal behaviour, impairment of consciousness, seizures, coma, or other neurologic abnormalities, severe anaemia (due to haemolysis), haemoglobinuria, low blood pressure, acute renal failure and hyperparasitemia (WHO, 2018; www.cdc.gov/malaria). Uncomplicated malaria refers to the presence of fever with confirmed laboratory investigation in the absence of any signs of severe disease and lasts 6-10 hours. The symptoms consist of the following: a cold stage (sensation of cold, shivering), a hot stage (fever, headaches, vomiting, and seizures in young children) and lastly a sweating stage (sweats, return to normal temperature). Uncomplicated malaria is normally treated with oral medications and the current effective treatment is the use of artemisinin in combination with other antimalarials as firstline treatment (Kokwaro, 2009; WHO, 2018).

Treatment of malaria and strategies aimed at terminating the infection, preventing the spread of infection, treatment of clinical manifestation, eradication of the parasites from the liver and prevention of recurrence in the future, has been investigated for hundreds of years and continues up to the present day. However, *Plasmodium* parasites have become resistant to the previously known and therapeutically potent antimalarial agent and many of the existing antimalarial medicines including amodiaquine and sulphadoxine-pyrimethamine. The current gold standard treatment is the use of the fixed-dose artemisinin combination therapy consisting of derivatives of artemisinin and a longeracting antimalarial agent. However, there are emerging signs of resistance and treatment failure to artemisinins, with patients taking longer to clear their fever and parasite (WHO, 2018). In Ghana,

artesunate-amodiaquine is currently the first-line therapy of choice for uncomplicated malaria. Alternative first-line therapy involves the use of artemether-lumefantrine and dihydroartemisininpiperaquine for patients who cannot tolerate artesunate-amodiaquine (WHO, 2018). Oral quinine is recommended as the medicine of choice for the treatment of uncomplicated malaria in the case of treatment failure with artesunate-amodiaquine (WHO, 2018; www.ghanahealthservice.org). Before the advent of the use of synthetic compounds as medicines, herbal medicinal products were used as therapy for malaria for thousands of years and are the basis of the two principal groups of modern antimalarial drugs-quinine and artemisinin derivatives from *Cinchona* and *Artemisia* respectively (Nkunya, 2002; White, 2008; Achan *et al*, 2011).

According to the WHO, about 80 per cent of the citizens of most developing countries depend on traditional medicines for their primary healthcare needs (WHO, 2012). Considering this fact and also the inadequacy of modern healthcare delivery services to meet the needs of those in deprived communities and member states of WHO were urged to advance and institutionalize traditional medicine in their national healthcare systems (WHO, 2012). This initiative led to the institutionalization of traditional medicine as viable treatment option providing an opportunity to introduce polyherbal antimalarials as standardized products, as well as treatment alternatives (WHO, 2001; MOH, 2005). Despite the various claims for the benefits of herbal products in the treatment of various disease conditions including malaria, concerns have been raised regarding their quality, safety and efficacy. Quality related to the correct starting materials used and the absence of impurities is of paramount concern. Also, safety related to less side and adverse effects linked with the use of the herbal medicinal products is essential to minimize toxicity. Herbal therapies should be effective for the disease or condition indicated. Results of some clinical studies have suggested that some herbal products may be safe and effective in the treatment of diseases, many were not randomized nor were they placebo-controlled (Yuyan *et al*., 2019). There is therefore the need to clinically validate such

herbal products. There is little evidence to support the claim of safety and efficacy of herbal medicinal products. Such studies are essential today to ensure that polyherbal products are well researched into. Most herbal medicinal products, have a long history of traditional use justifying their safety. However, the efficacy of most of them are unproven by standard scientific methods (WHO, 2001). Safety and efficacy depend on the indications of the therapy. A therapy has no clinical value if it is safe but lacks efficacy or if it is active on a relevant therapeutic target but its use is unsafe (Moreira *et al*., 2014). It is therefore important to undertake a clinical study to validate the quality, safety and effectiveness of herbal medicinal products used in the treatment of diseases.

1.2. Problem Statement

In Ghana, about 75 per cent of the population relies on herbal medicines for their primary health care needs (WHO, 2001). However, there is paucity of data on the quality, safety and efficacy of herbal products in circulation. In addition, some herbal drugs have been adulterated with synthetic drugs (Patwardhan *et al*., 2008).

Since herbal products are natural, there is the belief that the use of such products for therapeutic purpose is safe and this has led to the widespread use of herbal products globally (Moreira *et al*., 2014). As the global use of herbal medicinal products continues to increase, public health issues and concerns encompassing their safety are also increasingly being recognized. Although some herbal medications have promising potentials and are broaldy utilized, large number of them remain untested. This makes knowledge of their potential adverse effects very limited and identification of the safest and most effective therapies as well as the promotion of their rational utilization more troublesome (WHO, 2002b). It is also common knowledge that the safety of most herbal products is further compromised by lack of suitable quality controls, inadequate labelling, and the absence of appropriate patient information (Raynor *et al*., 2011). It has become relevant, therefore, to provide the general public and healthcare professionals with enough information on the quality, safety and

efficacy of herbal products to ensure that all medicines are safe and does not cause harm to the body when used.

Even though information on the quality, safety and efficacy of *Mist Amen Fevermix* is available (Turkson *et al*., 2015), there is no comparative clinical study data with the standard treatment for malaria. Also, there is inadequate data on the quality, safety and efficacy of *Edhec Malacure*. In addition, there is high patronage and patient's acceptability of the selected herbal products. Therefore, there is the need to conduct a clinical study of these products, with the view to establishing the definitive safety profile and efficacy of these herbal antimalarials, for the benefit of Medical Herbalists, clients and the scientific community as a whole.

1.3. Hypothesis

Patients with uncomplicated malaria are more likely to be completely treated when administered with *Mist Amen Fevermix* or *Edhec Malacure* than Artemether/Lumefantrine.

1.4. Justification

The influx of substandard and falsified medicinal products coupled with non-adherence to therapy by patients has resulted in many disease-causing organisms especially, the malaria parasites becoming resistant to therapy. This phenomenon does not only threaten the safety of patients and the success of therapy but also undermines healthcare delivery which is crucial in reducing morbidity and restoring health. Malaria is responsible for employee absenteeism, increased health care spending, and decreased productivity, all of which can lead to negative socioeconomic impact and human suffering. Hence, there is the need to look for different therapies, with low toxicity and efficacy in the treatment of malaria, as more people are turning increasingly to herbal products usage. This calls for new quality medicines which are safe and with broad therapeutic activity in the treatment of malaria infection (Chinsembu *et al*., 2010). Herbal products may contain potentially toxic constituents which make

them unsafe and therefore there is the need to assess quality standards for herbal products. To control the quality of herbal products, some European countries like Germany, France, Sweden, Denmark and Switzerland have developed specific national parameters for the evaluation of the quality, safety and efficacy for herbal products (Busse, 2000; Ang-Lee *et al*., 2001).

In Ghana, the Ghana Standards Authority (GSA) has also developed some guidelines for the quality control of herbal medicines based on standards from some European contries; Germany, Netherlands among others (www.gsa.gov.gh). In order to reduce the risk of adverse events attributable to unsafe and poor-quality herbal medicines, the World Health Organization (WHO) has also developed some guidelines for assessing the quality of herbal medicines with reference to contaminants and residues (WHO, 2007). In Ghana, however, even though, herbal medicine service is wholly integrated into the public healthcare service (Appiah, 2012), the products on the recommended Essential Herbal Medicines List, used for the management and treatment of various diseases, lack data on quality, safety and efficacy (NMCP/ MOH, 2010). Therefore, clinical study to validation need to be undertaken to ensure the quality, safety and effectiveness of herbal products as enshrined in the Public Health Act 851, (PHA, 2012).

Comparative clinical study of herbal antimalarial products has become more imperative because of the gradual rise in the number of patients reporting at the herbal medicine units of the Ghana Health Service (GHS) seeking an alternative to the orthodox anti-malarial treatment. It is also important to explore the potentials of herbal medicinal products through rigorous scientific analysis in Ghana.

According to the Ghana National Drugs Programme (GNDP, 2004), there was no literature on the quality and randomized controlled trial of medicinal plants and products that were manufactured in the country implying that claims of safety and effectiveness are unsubstantiated (Turkson, 2006).

Edhec Malacure, though approved by the Food and Drugs Authority (FDA) since the year 2014, there is inadequate data to support claims by the manufacturer for its quality, safety and efficacy. However, preliminary clinical data indicates *Edhec Malacure* possesses antiplasmodial properties *in vitro* and *in vivo* (Turkson *et al*., 2020). Also, *Mist Amen Fevermix* which is on the recommended Essential Herbal Medicines List (EHML) of the Ministry of Health, Ghana (MOH, 2008), has data to support claims by the manufacturer for the quality, safety and effectiveness. However, there is no comparative clinical study with conventional medicine. The outcome of an observational study conducted using *Mist Amen Fevermix,* for the treatment of uncomplicated malaria in humans was very safe and effective (Turkson *et al.,* 2015). Thus, validating its clinical use in the management of uncomplicated malaria as an alternative antimalarial agent is deemded necessary. It is, therefore, essential to undertake a comparative clinical study on the two products compared with Artemether-Lumefantrine as a positive control to validate the claim or otherwise.

The assurance of the quality, safety and efficacy profiles of these herbal antimalarial products will help to standardize, validate and prioritize new antimalarial products from herbal medicines.

1.5. Aim

The aim of the study is to compare clinical safety and effectiveness and also to establish the quality of MAF and MEM, two polyherbal products used for the treatment of uncomplicated malaria against Artemether/Lumefantrine, one of the standards or recommended treatments of malaria (WHO, 2015), using data from preclinical and clinical studies of the two products.

1.6. Objectives of the Study

The primary objective of the study is to perform an open prospective clinical study on *Mist Amen Fevermix* and *Edhec Malacure,* two polyherbal antimalarial products.

The specific objectives of the study are as follows:

1. Establish the quality parameters of *Mist Amen Fevermix* and *Edhec Malacure*
    a. Organoleptic parameters of *Mist Amen Fevermix* and *Edhec Malacure*
    b. Phytochemical assessment of *Mist Amen Fevermix* and *Edhec Malacure*.
    c. Physicochemical parameters of *Mist Amen Fevermix* and *Edhec Malacure*.
    d. Assess microbial load and contaminants in *Mist Amen Fevermix* and *Edhec Malacure.*
    e. IR Spectroscopy
        i. IR Fingerprint of *Mist Amen Fevermix* and *Edhec Malacure* and pants component.
        ii. IR Chemometrics to establish the presence or otherwise of the component plants.
    f. HPLC
        i. HPLC chromatographic analysis of *Mist Amen Fevermix* and *Edhec Malacure*.
        ii. Evaluation for possible adulteration of *Mist Amen Fevermix* and *Edhec Malacure* with artemether, lumefantrine and quinine.
    g. Evaluate the *in vitro* and *in vivo* antiplasmodial activities of *Mist Amen Fevermix* and *Edhec Malacure.*
2. Establish the safety parameters *Mist Amen Fevermix* and *Edhec Malacure.*
    a. Assess laboratory outcome of *Mist Amen Fevermix* and *Edhec Malacure* on renal and hepatic function, haematological indices, effects on blood pressure, body weight, and body temperature.
    b. Determine any adverse effect of *Mist Amen Fevermix* and *Edhec Malacure* in study participants.
    c. To compare the safety of *Mist Amen Fevermix* and *Edhec Malacure* against artemether/lumefantrine.

d. To assess the safety of *Mist Amen Fevermix* and *Edhec Malacure* by assessing the quality of life using the Karnofsky's scale.

3. Effectiveness parameters.
    a. To assess the effectiveness of *Mist Amen Fevermix* and *Edhec Malacure* by evaluating clinical outcomes.
        i. Improved symptoms.
    b. To assess the effectiveness of *Mist Amen Fevermix* and *Edhec Malacure* by assessing laboratory outcomes.
        i. Clearance of malaria parasites.
4. To compare the clinical effectiveness of *Mist Amen Fevermix* and *Edhec Malacure* against artemether/lumefantrine.

CHAPTER TWO

LITERATURE REVIEW

2.1. Overview of Traditional Medicine

The World Health Organization defines traditional medicine as "the sum total of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the preservation of health as well as in the prevention, diagnosis, improvement of treatment of physical and mental disorders" (WHO/EDM/TRM, 2001; WHO, 2011a). Some of the most widely used traditional medicine practices today include Traditional Chinese medicine (TCM), Ayurveda, Kampo, traditional Korean medicine (TKM), Unani and African traditional medicine (Fabricant and Farnsworth, 2001). Traditional medicine is the oldest form of health care known to mankind in the world. Different societies and cultures historically developed various useful healing methods to treat various kinds of diseases (WHO, 2000; Cragg *et al.*, 2001; Abdullahi, 2011). According to the WHO, about 80% of the population of many countries in African, Asia and Latin America are known to use traditional medicine (TM) to meet their primary health care needs. Traditional medicine service has been successfully used in other countries where conventional medicines are predominant in the national healthcare system (WHO, 2002). The utilization of traditional medicines has expanded globally and has gained popularity in the last few decades. Traditional practitioners include bonesetters, traditional birth attendants, tooth extractors, circumcisers, herbalists and spiritual healers (Papadopoulos *et al.*, 2002).

Historically, the study and use of herbs dates back 5,000 years and it is attributed to the ancient Sumerians, who described well-established medicinal uses for plants (Phillipson, 2001). However, archaeological studies have shown that the practice of herbal medicine dates as far back as 60,000 years and 8,000 years ago in Iraq and China respectively (Gourhan, 1975). For thousands of years,

animal parts, minerals and medicinal plant and products have played significant roles in healthcare: in the diagnosis, treatment and prevention of diseases. Natural products are not only important sources of new medicines but also provide leads and templates suitable in drug development (Newman *et al*., 2000; Balunas *et al*., 2005). Some examples of natural products are *galegine* obtained from Galega officinalis L and *papaverine* from Papaver somniferum (Fabricant and Farnsworth., 2001).

The substantial use of traditional medicine in developing countries, made up of mainly herbal medicinal plants, is linked to cultural and economic reasons. Therefore, the WHO encouraged member states to promote and integrate traditional medical practices into the health care delivery system (WHO, 2002).

2.2. Malaria

Malaria is an endemic and potentially deadly infectious ailment caused by obligate, intracellular protozoan parasites of the genus *Plasmodium* which infect and divide in red blood cells (RBCs) of various kinds of vertebrates which include mammals, birds and reptiles. Four distinct species *P. falciparum, P. ovale, P. malariae* and *P. vivax* are known to cause infections in humans. *Plasmodium falciparum* is known to be the most prevalent and virulent malaria parasite in the WHO African Region and causes severe infections which result in about 99.7 per cent of estimated malaria cases, 90 per cent of deaths and other deformities in affected patients. Also, *P. falciparum* accounts for 62.8 per cent of estimated malaria cases in the WHO regions of South-East Asia, the Eastern Mediterranean 69 per cent and the Western Pacific 71.9 per cent. *P. vivax* is the main parasite in the WHO Region of the Americas, representing 74.1 per cent of malaria cases (WHO, 2018). In recent years, however, a fifth parasite, *P. knowlesi,* which causes malaria infection in monkeys and occurs in certain forest areas of South-East Asia has been found to cause malaria infections in humans too (Sabatini *et al*., 2010).

2.2.1. Epidemiology of Malaria

The majority of malaria infection cases representing 65 per cent occurred in children below age 5 years in developing countries. It has been estimated that at least about 125 million pregnant women are at risk of being infected each year in sub-Saharan Africa (Hartman *et al*., 2010; Murray *et al*., 2012; WHO, 2018). In Western Europe and the United States, there were estimated 10,000 and 1300–1500 malaria cases per year respectively (www.cdc.gov, 2018; WHO, 2018). The global distribution of malaria transmission is as shown in Figure 2.1.

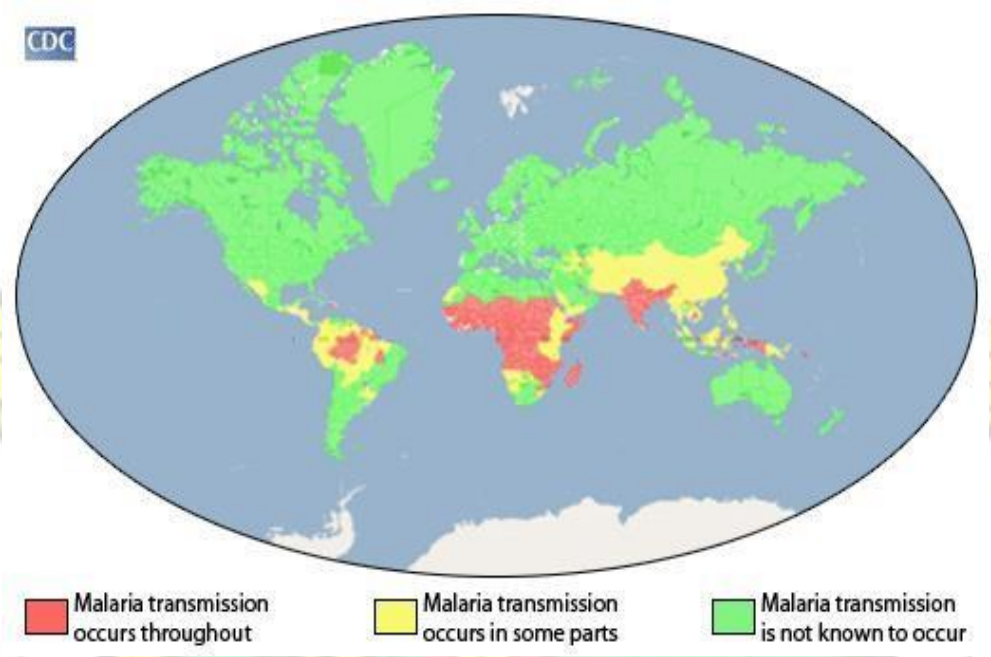


Figure 2.1: Malaria Distribution in the World (www.cdc.gov, 2018)

2.2.2. Life Cycle of the Malaria Parasite

The malaria parasite has a complex life cycle consisting of an insect vector, the female anopheline mosquito and a human host. Three stages are involved in the life cycle: the exo-erythrocytic cycle, the erythrocytic cycle, and the sporogonic cycle (Figure 2.2). The cycle starts when an infected female anopheles mosquito feeds on human blood and introduces the parasite in its saliva in the form of sporozoites into the bloodstream. From the bloodstream, the sporozoites invade hepatocytes where they undergo asexual reproduction and develop into schizonts from which merozoites are produced

(exo-erythrocytic schizogony). The erythrocytic cycle begins when, the merozoite undergoes asexual multiplication in the erythrocytes (erythrocytic schizogony) progressing into trophozoites, schizonts and infective merozoites with the ability to reinfecting other erythrocytes when freed again and replicating the erythrocytic cycle. Some merozoites from the blood upon entering a red blood cell change into gametocytes (sexual forms) which are taken up by a feeding anopheline mosquito. The parasites' multiplication in the mosquito is referred to as the sporogonic cycle. In the mosquito's gut, microgametes penetrate the macrogametes producing zygotes. The zygotes then become motile and elongated (ookinetes), which capture the midgut wall of the mosquito, where they progress into oocysts. The oocysts grow, rupture, and produce sporozoites, which are released to the mosquito's salivary glands. introduction of the sporozoites into a new human host continue the malaria life cycle (www.cdc.gov, 2018) Figure 2.2.

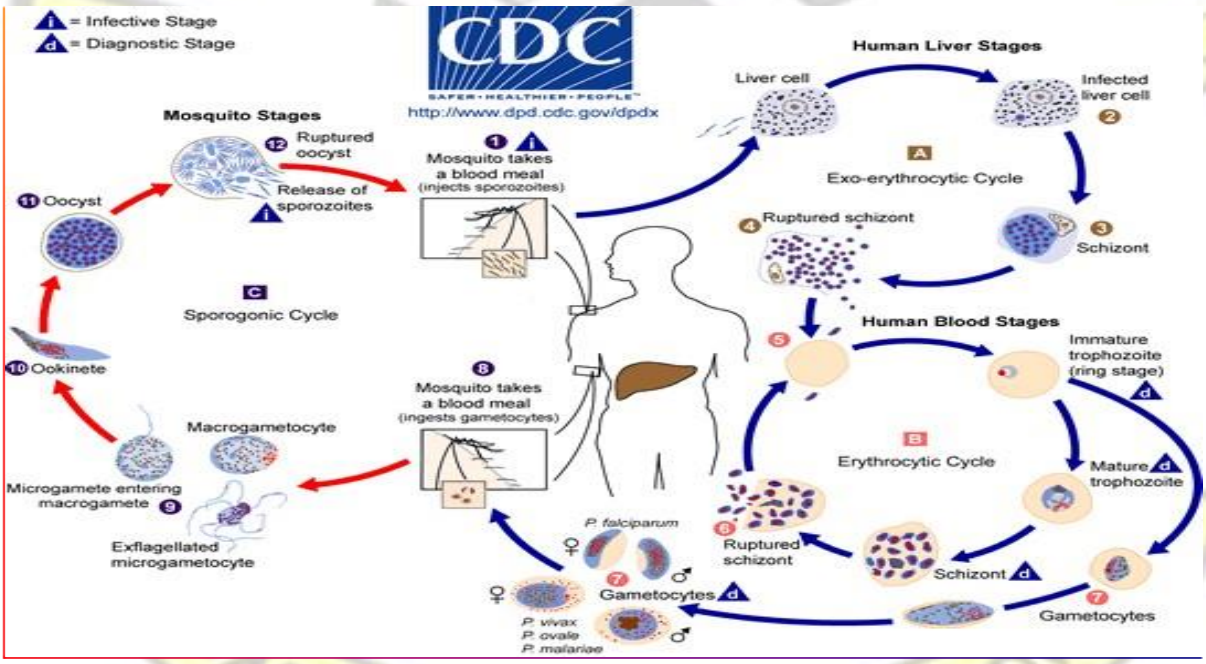


Figure 2.2: Overview of life cycle of malaria parasite (www.cdc.gov, 2018)

2.2.3. Signs and Symptoms of Uncomplicated Malaria

Malaria is a rapid onset febrile illness; symptoms appear seven days or more (usually 10–15 days) after pathogenic female mosquito bites. Symptoms of malaria infection are usually relatively mild and consist only of episodes of fever, malaise, rigours, anorexia, headache, chills, vomiting and sometimes diarrhoea, usually, there are no severe complications. However, if left untreated for twenty-four (24) hours, *Plasmodium falciparum* infection can progress to severe malaria often resulting in death (WHO, 2018).

2.2.4. Diagnosis of Malaria

Several approaches to the diagnosis of malaria can be employed; depending on clinical manifestations and also confirmed by examination and identifying malaria parasites in the patient's blood via microscopy. Rapid and precise diagnosis of malaria is vital for effective and successful management. High-quality diagnosis is essential in all settings as misdiagnosis can result in high morbidity and mortality. Clinical diagnosis can be achieved based on the patient's symptoms and on physical findings at examination. However, clinical doubt of malaria should be confirmed with a parasitological diagnosis (WHO, 2010). Also, another routine method employed is the rapid diagnostic tests (RDTs), which detect parasite-specific antigens (Bell *et al*., 2005; WHO, 2018). Molecular diagnosis invloving polymerase chain reaction (PCR) can also be used (WHO, 2018; WHO, 2010).

2.3. Treatment of Malaria

Malaria is an entirely preventable and treatable disease. The choice of treatment is dependent mainly on the infecting species, the severity of infection, age of the patient, and susceptibility of parasites to antimalarial medicines, the cost and availability of medicines. The goal of malaria treatment is to ensure rapid and complete elimination of the *Plasmodium* parasites from the patient's blood to help prevent progression of uncomplicated malaria to complicated illness that leads to malaria-related

anaemia and death. From a public health perspective, treatment is meant to reduce transmission of the infection to others, by reducing the infectious reservoir and to prevent the emergence and spread of resistance to antimalarial medicines (Ishengoma *et al.*, 2009; WHO, 2013).

Antimalarials used in the treatment of malaria infection come from the following five groups of chemical compounds: quinolines and arylaminoalcohols, antifolate, artemisinin derivatives, the hydroxynaphthaquinones and antibacterial agents (Salfi *et al*., 2013).

i. Quinolines 4-aminoquinolines (chloroquine, amodiaquine and piperaquine), 8-aminoquinolines (e.g. primaquine and pamaquine) belong to the quinolines. **Chloroquine [1]**, a 4-aminoquinoline exhibits its antimalarial activity largely on the large ring-form and mature trophozoites stage of the parasite. The side-effects of chloroquine include pruritus, skin-rashes, cephalgia, gastrointestinal disturbances and rarely bone marrow suppression, alopecia and convulsions (Tripathi, 2006; WHO, 2007). Chloroquine was withdrawn from use because of a decline in effectiveness resulting from resistance strains of the parasite and fatal side effects (Martin *et al*., 2009). Chloroquine is currently on the MLEM for the treatment of *P. vivax* infection in regions where resistance has not developed (WHO, 2019). **Amodiaquine [2]**, also a Mannich base 4-aminoquinoline and its mechanism of action involve the suppression of the breakdown of haemoglobin. The drug also suppresses the glutathionedependent destruction of ferriprotoporphyrin IX in the malaria parasite, leading to the accumul*ation* of this peptide, which is unsafe to the survival of the parasite. Amodiaquine is therapeutically potent as compared to chloroquine in treating chloroquine-resistant *Plasmodium falciparum* malaria infections. These two drugs were widely used in the past for both prophylaxis and treatment of malaria. However, amodiaquine has serious adverse effects of hepatitis and agranulocytosis associated with its long-term use and therefore not generally recommended in malaria treatment

(Parhizgar and Tahghighi, 2017).

Primaquine [3] is a member of the 8-aminoquinoline range of antimalarials that includes tafenoquine and pamaquine. Primaquine is primarily used in the treatment of *P. vivax* or *P. ovale* malaria, specifically to eliminate the inactive liver forms of these parasites (hypnozoites). To achieve this, a 14-day course of primaquine is required (Baird *et al*., 2003). Usual adverse effects associated with the administration of primaquine include nausea, vomiting, and stomach cramps. The most dangerous adverse effect of primaquine is haemolysis in patients who are deficient in G6PD enzyme, Africans or Caucasians of Mediterranean descent. Primaquine is the only antimalarial currently recommended as a therapy in *P vivax* malaria (Recht *et al*., 2018).

Piperaquine is a bisquinoline compound which was first synthesized in the 1960s and was widely used in China and Indochina as a preventive agent for treatment purposes for over 20 years. Due to resistant strains of *P. falciparum* and the introduction of artemisinin-based antimalarial products, the usage of piperaquine declined (Davis *et al*., 2005). Currently, piperaquine is used in combination with dihydroartemisinin to treat malaria (WHO, 2015).

Mefloquine [4] is a quinoline methanol compound which resembles quinine and it is active against the asexual stages of malaria; however, its precise mode of action is not known. Mefloquine is therapeutically potent as a preventive agent against malaria and is extensively used in therapy against chloroquine-resistant *P. falciparum* malaria infection. Mefloquine is effective against all five strains of malaria parasites known to affect humans (WHO, 2018). Frequent treatment using mefloquine is associated with asymptomatic, transient serum enzyme elevations in up to 18 per cent of patients. Adverse reactions such as skin-rash and autoantibody formation are also rare. Reported side effects of mefloquine include nausea, vomiting, abdominal pains, dizziness, neurotoxic effects and chronic neuropsychiatric adverse effects (Ritchie *et al.*, 2013; Nevin, 2014). Mefloquine is currently not widely used due to the perception of central nervous system toxicity (Nevin and Croft, 2016).

ii. **Arylaminoalcohols.** Quinine, quinidine, mefloquine, lumefantrine and halofantrine, belong to the arylaminoalcohols**. Quinine** is a drug obtained from the stem bark of the cinchona tree and was the first therapy used for malaria (Achan *et al*., 2011). The most common adverse effects of quinine involve a group of symptoms called cinchonism; headache, vasodilation and sweating, nausea, tinnitus, hearing impairment, vertigo or dizziness, blurred vision, and interference in colour perception. Quinine is a common cause of drug-induced disorders, including thrombocytopenia and thrombotic microangiopathy (Reese *et al*., 2015). Quinine can also have severe adverse effects involving multiple organ systems, among which are immune system effects and fever, hypotension, hemolytic anaemia, acute kidney injury, liver toxicity, and blindness. Quinine excites the secretion of insulin and may lead to hyperglycaemia which is a risk in pregnancy (Kremsner *et al*., 2012). The mode of action of quinine is not clear but it is believed to interfere with the parasite's ability to breakdown haemoglobin leading to the inhibition of self-generated formation of beta-haematin (haemozoin or malaria pigment) which is a poisonous product of the breakdown of haemoglobin by parasite (Salfi *et al*., 2013). Quinine is currently not used a front-line therapy for malaria due to the high-quality evidence of the efficacy superiority of artesunate over quinine in adults and children with severe malaria (WHO, 2015).

iii. **Antifolate**. The principal antifolates are pyrimethamine **[5]** (PYR), proguanil (PG; broken-down *in vivo* to the active form cycloguanil [CG]). The sulfa drugs, the most significant of the antifolate are the outstanding, sulfadoxine (SDX), and the sulfone, dapsone. Antifolates were initially made available in the late 1960s, and established to be of long-term use, particularly, as a low-cost substitute to combat the CQ-resistant parasites that were distributed across Africa from the late 1970s onwards (Hyde, 2007). Currently, antifolate are not widely used as a preventative therapy because of high levels of resistance (Lumb *et al*., 2011).

iv. **Hydroxy naphthoquinones** have been widely investigated over the past 50 years for their antimalarial effect (Srivastava, 1997). Atovaquone **[6]** is a hydroxyl naphthoquinone that is used in combination with proguanil for prophylaxis and therapy of uncomplicated malaria (Baggish and Hill, 2002). Atovaquone has outstanding anti-malarial property but demonstrates poor pharmaceutical activities, such as poor bioavailability and high plasma protein binding. The mechanism of action of atovaquone is through the prevention of the electron transport system at the level of cytochrome BC1 complex. Atovaquone also ensures the breakdown of the parasite mitochondrial membrane potential. Atovaquone is used as a fixed-dose combination with proguanil for the treatment of uncomplicated malaria. No serious or life-threatening adverse effects have been reported. Hydroxy naphthoquinones are taken one dose per day and for 7 consecutive days (Dressman and Reppas, 2000; www.cdc.gov).

v. **Artemisinin** and its derivatives (Artesunate, Artemether, and Dihydroartemisinin) represent a new category of antimalarials. Fixed-dose formulations (combining two different active ingredients coformulated in one tablet, Artesunate-Amodiaquine and Artemether-Lumefantrine are ideally favoured and recommended over co-blistered, co-packaged or loose tablet combinations, since it enhances adherence to treatment and cuts down the possible use of the individual components of co-blistered drugs as monotherapy (WHO 2014). The WHO advocates for the use of artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated malaria caused by the *P. falciparum* parasite. ACTs are the most therapeutically potent antimalarial medicines available today (WHO, 2014). The current trend in the treatment of uncomplicated malaria caused by *P. falciparum* is the use of ACTs with one of the following artemisinin-based combination therapies:

- Artesunate+Amodiaquine (AS-AQ)

- Artemether+Lumefantrine (A-L)
- Dihydroartemisinin+Piperaquine (DHAP).
- Artesunate+mefloquine
- Artesunate+ sulfadoxine+pyrimethamine (WHO, 2015).

Artemisinin-based Combination Therapy (ACTs) has been used since 2004 in Ghana for the treatment of uncomplicated malaria. This initiative was important because the malaria parasite became resistant to Chloroquine and other monotherapies. Artemisinin is administered in combination with a second, long-acting antimalarial to enhance treatment and protect against the development of drug resistance (MOH, 2014).

vi. New Product under Development.

DDD107498 [7] (Figure 2.4) is a compound with the chemical name 6-Fluoro-2-[4-(4morpholinylmethyl) phenyl]-N-[2-(1-pyrrolidinyl) ethyl]-4-quinolinecarboxamide. It is a novel chemical compound developed based on a 2, 6-disubstituted quinoline-4-carboxamide scaffold against the blood stage of the multi-drug-sensitive *Plasmodium falciparum* 3D7 strain. The compound has a powerful and wide spectrum of antimalarial activity against varied life-cycle phases of the *Plasmodium* parasite, with better pharmacokinetic activities and a satisfactory safety profile. DDD107498 has sub-micromolar efficacy against the parasites. The compound has shown excellent activity against 3D7 strain parasites. It is also effective against several drug-resistant strains. It is more effective as compared to artesunate in (*ex vivo)* assays against a range of clinical isolates of both *P. falciparum* and *P. vivax* and is not toxic to human cells (Baragana *et al*., 2015, www.glixxlabs.com). DDD107498 which is now called M5717 entered the first stages of human clinical trials in 2017 (Baragana *et al*., 2015; MMV, 2018). Some examples of synthetic compounds used in the management of malaria are shown in Figure 2.3.

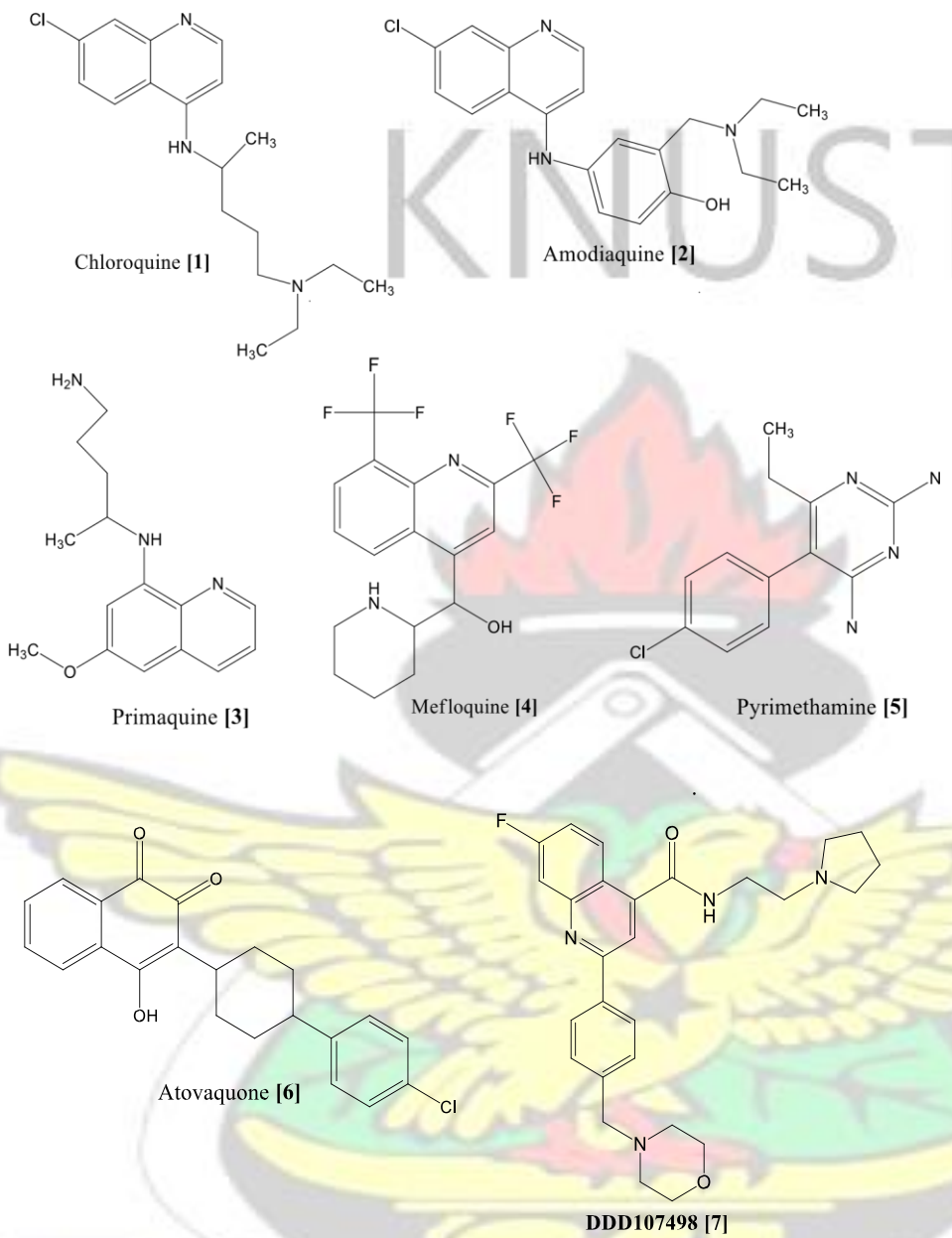


Figure 2.3: Chemical Structures of Some Synthetic Compounds used as Antimalarial

2.4. Vaccine for Malaria

The only approved vaccine as of 2015 is *RTS,S*, known by the trade name *Mosquirix*. *RTS,S/AS01* is the most recently developed recombinant protein-based malaria vaccine. *RTS,S/AS01* was engineered using genes from the outer protein of *P. falciparum* malaria parasite (circumsporozoite protein (CSP) from the pre-erythrocytic stage and a portion of a hepatitis B virus plus a chemical adjuvant (AS01) to boost the immune response. Infection is prevented by inducing humoral and cellular immunity, with high antibody titres that block the parasite from infecting the liver (www.malariavaccine.org, 2013; Foquet *et al*., 2014; Clinical Trials Partnership, 2015). RTS,S was developed by PATH Malaria Vaccine Initiative (MVI) and GlaxoSmithKline (GSK), and it is the world's first licensed malaria vaccine and also the first vaccine licensed for use against a human parasitic disease of any kind. It requires four injections (WHO, 2019). The RTS, S-based vaccine formulation had previously been demonstrated to be safe, well-tolerated, immunogenic, and to potentially confer partial efficacy in both children and adults in malaria-endemic areas (Regules *et al*., 2011).

Initial data from a phase III clinical trial indicated that *RTS,S/AS01* reduced the number of malaria cases among young children by almost 50 per cent and among infants by around 25 per cent. The administration of a booster dose showed a positive result. After four years of trial, there was a reduction of 36 per cent of infection for children who received three shots and a booster dose. The vaccine is shown to be less effective for infants. Three doses of vaccine plus a booster decreased the risk of clinical occurrence by 26 per cent over three years but offered no notable protection against severe malaria (Borghino, 2015).

A vaccination programme to pilot the vaccine in three high-malaria endemic countries in Africa (Ghana, Malawi and Kenya) began in April 2019. This is ongoing and it is being done to establish the feasibility, impact and safety of RTS,S, when used as part of a routine immunization programme

(www.who.int/immunization/diseases/malaria/). Therefore, *RTS,S/AS01* does not confer total immunity against malaria. It is also partially effective in children than in adult and not effective in infants and in severe malaria.

2.5. Natural Products used in the Treatment of Malaria

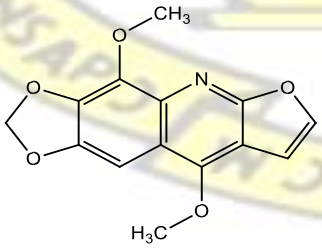
The use of natural products for the treatment of parasitic and infectious diseases is well known in history, for instance, the use of *Cinchona succirubra* (Rubiaceae) for the treatment of malaria has been known for centuries. Some medicinal plants which have been used in the treatment of malaria in West Africa are shown in Table 2.1 (Mshana *et al.,* 2000; GHP, 2007; WAHP, 2013). Several compounds used as antimalarial agents such as flindersiamine **[8]**, liriodenine **[9]**, skimmanine **[10]**, palmatine **[11]**, artemisinin **[12]**, alstonine **[13]**, quinine **[14]**, aborinine **[15]**, nitidine **[16]**, melicopicine **[17]** and evoxine **[18]** as shown in Figure 2.4 have been isolated from some medicinal plants such as *Alstonia boonei, Cinchona officinalis, Artemisia annua, Zanthoxylum nitidum,* among others (Kaur *et al*., 2009; Onguéné *et al.,* 2013).

Table 2.1: Some Medicinal Plants used for the Treatment of Malaria in West Africa.

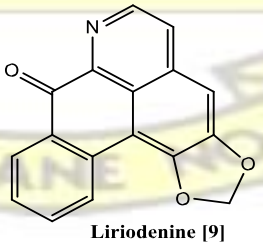
Botanical Name of plant	Common name/English	Plant part used	Preparation/dosage form
Adansonia digitata (Bombacaceae)	Baobab	Leaf	About 30g dried leaf is boiled in 1000 ml of water. Dosage: 200mL three times daily (www.henriettes-herb.com).
Alchornea cordifolia (Euphorbiaceae)	Christmas bush	Leaf	About 30g of dried leaf is boiled in one litre of water. Dosage: 3-4 teacups daily. (www.expressfsgroup.com).
Alstonia boonei (Apocynaceae)	Alstonia	Stem bark	Dried leaves or stem bark are boiled with ginger. Dosage: Drink decoction thrice daily (Mshana *et al.*, 2000).
Azadirachta indica (Meliaceae)	Neem	Leaf	About 30g of stem bark is boiled one-litre water and drank as a decoction (Mshana *et al.*, 2000).
Balanites aegyptiaca (balanitaceae)	Desert tree	Stem bark	About 30g of dried stem bark is boiled. Dosage: Drink as required (Mshana *et al.*, 2000).
Bidens pilosa (Asteraceae)	Bur marigold	Leaf	About 30g of dried leaf is boiled. Dosage: half glass full three times daily (www.rain-tree.com)
Carica papaya (Caricaceae)	Pawpaw	Leaves	The leaf of *Carica papaya* is pounded and boiled. Dosage: 160mL three times daily till cured (WAHP, 2013).
Citrus aurantifolia (Rutaceae)	Lime	Fruit and leaf	The leaf is boiled in water. Dosage: Drink as required (Mshana *et al.*, 2000).
Combretum micranthum (Combretaceae)	Combretum	Leaf	Hot water is poured on leaves and roots. Infusion is drunk as required (Mshana *et al.*, 2000).
Cryptolepis sanguinolenta (*Periplocaceae/Asclepiadaceae*)	Cryptolepis	Root	The root is boiled with water for 30minutes. Dosage: 40mL three times daily (Mshana *et al.*, 2000).
Hallea stipulosa (Rubiaceae)	African linden	Stem bark and leaf	About 30g of dried leaf is boiled in 900ml of water. Dosage: 30mL three times daily (WAHP, 2013).
Harrisonia abyssinica (Simaroubaceae)	Baingou	Leaf and stem bark	About 30g of dried leaf is boiled in 900ml of water. Dosage: 30ml three times daily (WAHP, 2013).
Khaya senegalensis (Meliaceae)	African mahogany	Stem bark	30g of dried leaf is boiled in 900mL of water. Dosage: 200mL three times daily (Mshana *et al.*, 2000).
Lippia multiflora (Verbenaceae)	Bush Tea	Root bark	Boil pulverized leaves with 200mL of water and drink 200mL thrice daily (Mshana *et al.*, 2000).
Momordica charantia (Cucurbitaceae)	African cucmber	Aerial part	About 30g of dried aerial part is boiled in 600mL of water. Dosage: one teacup full three times daily (Mshana *et al.*, 2000).

Moringa oleifera (Moringaceae)	*Moringa*	Leaf	About 30g of dried leaf is boiled in 600mL of water. Dosage: one teacup full three times daily (WAHP, 2013).

Botanical Name of plant	Common name/English	Plant part used	Preparation/dosage form
Phyllanthus niruri (Euphorbiaceae)	Stone breaker	Leaf	About 30g of dried leaf is boiled in 600mL of water. Dosage: 1-3 teacup full three times daily (WAHP, 2013)
Pterocarpus erinaceus (Papilionaceae)	African rosewood	Leaf and stem bark	About 30g of dried aerial part is boiled in 600mL of water. Dosage: two tablespoonfuls two times daily (WAHP, 2013).
Rauwolfia vomitoria (Apocynaceae)	Devil's-pepper	Root	About 30g of dried aerial part is boiled in 600ml of water. Dosage: 1-3 teacups daily (Mshana *et al.*, 2000).
Sarcocephalus latifolius (Rubiaceae)	Negro peach	Root	Macerate 250g in about 600mL of hot water. Dosage: 100mL thrice daily (WAHP, 2013).
Senna occidentalis (Leguminosae)	Coffee senna	Leaf	About 10g of dried leaf is boiled in 500mL of water. Take 1 teacup twice daily (WAHP, 2013).
Vernonia amygdalina (Asteraceae)	Bitter leaf	Leaf and root bark	About 30g of the dried leaf or root bark is boiled. Dosage: 30mL three times daily (WAHP, 2013).
Xylopia aethiopica (Annonaceae)	Ethiopian pepper	Fruit	Boil pounded fruit with water. Dosage: 15mL daily (Mshana *et al.*, 2000).



Flindersiamine [8]



Liriodenine [9]

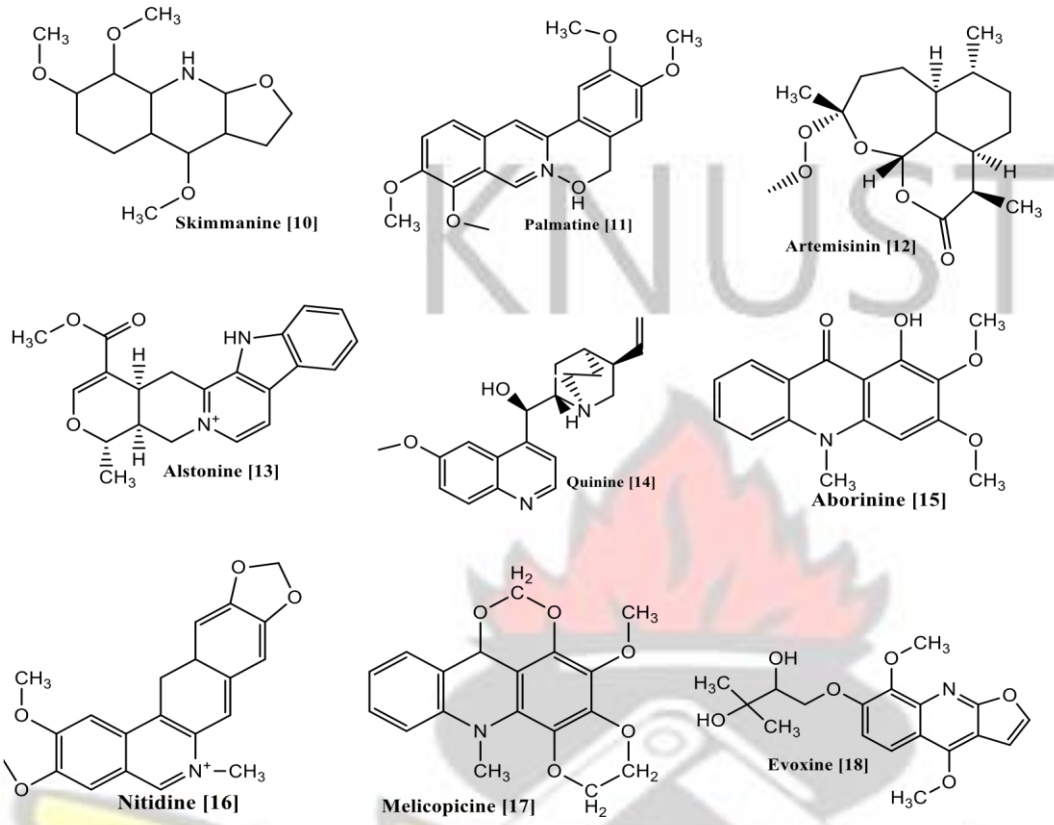


Figure 2.4: Chemical Structures of Some Antimalarial Compounds from Medicinal Plant Sources

2.6. Quality of Herbal Products

The quality of herbal products is defined as the status of the product, which is determined either by identity, purity, active content, and other chemical, physical or biological properties or by the manufacturing process. It includes the correct medicinal plant material used, or the absence of impurities above the maximum permitted level (Houghton, 2003). Quality is a key issue which affects the safety and efficacy of herbal products. Quality control and standardization of herbal products are fundamental aspects for pharmacological evaluation and therapeutic use (EMEA, 2005). Quality is

an important parameter which affects the safety and efficacy of herbal products. Quality control is, therefore, a basic part of the standardization of herbal products for biological assessment and therapeutic application.

Quality, safety and efficacy of herbal products are affected by many extrinsic and intrinsic factors. The extrinsic factors include; environmental factors, inclusive of altitude, soil, atmospheric humidity, shade, light, water, temperature and supplied nutrients, can influence their phytochemical composition (Chadwick and Fong, 2006). Occasionally, insects, animals and their excreta can also be introduced at any stage of the manufacturing process, leading to poor quality of herbal products which become unsafe. Unfavourable storage conditions during storage may enhance the levels of chemical and biological toxins. Accidental or intentional substitution with different plant species is also a very common phenomenon for lot of herbal products (Koh and Woo., 2000). Intrinsically, every medicinal plant or part used in the manufacture of herbal products contains various kinds of components, which may interact during the post-harvest processing including washing of starting materials and the manufacturing process (source of water, type of boiler used). This may affect the quality and purity of some herbal products. The quality and composition of many herbal products are not always assured unlike synthetic pharmaceutical drugs which makes the standardization of herbal products extraordinarily demanding and very essential (Zhang *et al*., 2012; Wani, 2007).

It is vital to assure product safety and efficacy in humans (Heinrich, 2015). Therefore, it is very essential to adhere to analytical standards in manufacturing herbal products.

Many standard operating procedures (SOPs) can be employed to control the purity of herbal preparations, including Good Agricultural and Collection Practices (GACP), Good Laboratory Practices (GLP), Good Storage Practices (GSP) and Good Manufacturing Practices (GMP) for producing herbal products (WHO, 2014; WHO, 2003).

With the increased utilization of herbal products issues of quality, safety and efficacy is of uppermost importance. It is therefore important that standards and acceptable quality control requirements suitable for herbal products be established using standard analytical methods to ensure the manufacture of quality, safe and efficacious herbal products on the market to prevent serious adverse effects or death. Without quality herbal preparations, the outcome of any clinical research on herbal products will be compromised.

2.6.1. Quality Assessment with Standardization of Herbal Products

The assessment of the quality of herbal products may involve the determination of organoleptic properties (taste, odour and colour), chromatographic investigation, the physicochemical characteristics such as the pH and the extractives (dry weight per millilitre), microbial contaminants and heavy metals since they can be very toxic to humans (Evans, 2009).

Phytochemical assessment to identify secondary metabolites such as alkaloid, tannin, saponin and steroid is conducted to determine compounds which tend to possess physiological effects on humans. Thin-layer chromatography (TLC), a physicochemical method may be used for determining the variety and quantity of the secondary metabolites. Extracts of the product are made and compared chromatographically with standard reference solutions of the known constituents (Evans, 2009).

2.7. Safety Assessment of Herbal Products

While herbal products use is on the increase, issues relating to safety and the monitoring of adverse effects is of paramount importance. This is because some herbs can pose serious health complications when used. Herbal products are generally considered safe because it is natural in origin and based on their long-standing usage (www.pac.iupac.org).

According to the WHO, a product is defined as being safe, if it causes no known or potentially harmful effects to consumers. The safety of any product is dependent on the substances which it contains. Toxicity and possible adverse effects from the use of herbal medicines may arise from source of

starting raw materials, source of water among others. A single herb always contains many kinds of active comnstituents, each of which may contribute to the herb's pharmacological effects and toxicities (Muller *et al*., 2001). The causes of adverse reactions of herbal products include, allergic reactions, toxic effects from contaminants, adulterations of other herb or synthetic substances and interactions with drugs or other herbs (WHO, 2002).

Herbal products contain many active constituents and some of them may well be toxic (WHO, 2002; Pal and Shukla, 2003).

The safety assessment of herbal products deserves paramount consideration, and should be an important consideration for approval. More research to develop new and cheap analytical methods for safety profiling and identification of herbal products are needed.

2.7.1. Guidelines to Safety Assessment of Herbal Products

Assessment of the safety of herbal products is paramount in herbal medicine usage because of the potential of toxicity or other adverse effects and, therefore, is a vital principle in the provision of herbal products for health care. Safety is a measure of the risk: benefit ratio. Any assessment of the safety of herbal medicines must be based on identification and characterization of the constituents where possible (WHO, 2004).

Detailed phytochemical and pharmacological studies are required for the evaluation of the safety of medicinal plant products. The safety parameters normally studied are full blood count, liver and kidney functions among others (WHO, 2004).

The safety of the herbal medicinal therapies should not be compromised. The fundamentals underlying the provision of high-quality herbal products are a basic tenet of society. Therefore, standards should not be compromised.

2.8. Effectiveness Assessment of Herbal Products

The extent to which a therapy achieves its intended effect coupled with its capacity to enhance health and well-being is referred to as effectiveness. Herbal medicinal plants and products are believed to be very effective and mostly justified based on their long history of usage (Mosihuzzaman *et al.,* 2008).

2.8.1. Guidelines for the Assessment of the Effectiveness of Herbal Products

Presently assessment of the effectiveness of herbal medicines uses methods currently used in conventional clinical trials (Mosihuzzaman *et al.,* 2008). The effectiveness is determined by a clinical, laboratory, or diagnostic outcomes (Mosihuzzaman *et al.,* 2008). Clinical outcomes are varied and include parameters such as improved morbidity outcomes: low death rates, reduced pain or discomfort, improved desire to eat, improved gain in weight, reduction of blood pressure, and enhanced quality of life generally. Laboratory and other diagnostic outcomes which are essential indicators for good health include; decrease of blood glucose, enhancement of haemoglobin status, and improvement in electrocardiogram (ECG) findings (Mosihuzzaman *et al.,* 2008).

The Karnofsky's scale is also used as a means to measure quality of life. Quality of life measurement evaluate how comfortable people are faring in relation to the impact of disease. It is a wide-ranging principle in a complex way assessed by the person's physical and mental status, level of independence, social relationships, personal believes and their relationship to important characteristics of their surroundings (Burckhardt and Anderson, 2003).

The tools that are usually applied for evaluating the effectiveness of herbal medicines are:

i. **Case reports**, these are the starting point for assessing the efficacy of many herbal medicines scientifically, as evident from the contents of many reputable clinical journals. This can lead to the identification of efficacious and new interventions which were previously unknown. Case reports can be retrospective or prospective (Mosihuzzaman *et al.,* 2008).

Meta-analysis, Meta-analysis is defined as the statistical analysis that combines the results of multiple scientific studies for the purpose of integrating the findings. Meta-analysis can be performed when multiple scientific study area addressing the same question, with each individual study reporting measurements that are expected to have some degree of error (Greenland *et al*., 2008). Meta-analysis is also the putting together of individual case reports and organized to establish a particular pattern. Case series may be retrospective or prospective (observational or interventional) in nature (Mosihuzzaman *et al*., 2008).

Meta-analysis aims to use approaches from statistics to derive a pooled estimate closest to the unknown common truth based on how this error is perceived. In addition to providing an estimate of the unknown common truth, meta-analysis has the capacity to contrast results from different studies and identify patterns among study results, sources of disagreement among those results, or other interesting relationships that may come to light in the context of multiple studies (Greenland *et al*., 2008; Gravetter *et al*., 2008).

Meta-analysis is useful for resolving unexpected differences in clinical research and includes only published studies. As such, a meta-analysis is an objective, quantitative synthesis of research findings (Walker *et al*., 2008). Well and properly conducted meta-analysis of medical studies is considered decisive evidence, as it occupies a top-level in the hierarchy of evidence (Guyatt *et al*., 1995). This justifies a meta-analysis to be a more efficient and effective standard procedure for putting together the results of many studies than is subjective judgment.

There are two statistical models for a meta-analysis: the fixed effect and random effect models. The fixed-effect model assumes that all of the studies in the meta-analysis have one true effect size, and the observed variation among studies is caused by sampling errors or chance (Smith and Egger, 1997).

The random effect model assumes that different studies exhibit substantial diversity, and the true effect size may vary from study to study (DerSimonian and Kacker, 2007).

A major advantage of a meta-analysis is that it produces a precise estimate of the effect size with considerably increased statistical power, which is especially important when the power of the primary study is limited because of the small sample size. A meta-analysis also analyses the variation in the results of different studies and quantifies result inconsistency (heterogeneity) across studies. It is also an objective and quantitative procedure that provides a less biased estimate on a specific topic. A meta-analysis can also resolve conflicts between studies, and yield conclusive results when individual studies are inconclusive. Meta-analysis is an invaluable bridge between past and future studies (Walker *et al*., 2008).

The main criticism of a meta-analysis is that it combines different types of studies (Walker *et al*., 2008).

The use of meta-analysis in medicine has increased in recent years due to a growing interest from both physicians and statisticians. This is because; it helps in understanding the results of intervention in medicine and contributes to many aspects of clinical research, such as;

- increases the statistical power of a comparison
- improves the estimation of the effect of a treatment
- combines the results of studies that are contrasting
- answers new questions
- Analyses sub-groups of subjects selected from different studies
- analyses trends within a time-frame, in a sub-group of patients with the same characteristics

- Defines areas in which further studies are needed

It is always possible to update a meta-analysis if it is not conclusive when new studies are published (Gioacchino, 2005).

In herbal medical practice currently, medical herbalists need to be updated with the results of the most important clinical studies on herbal products. Also, they are to be part of clinical trials and to evaluate the results of new herbal products.

iii. **Randomized clinical trials.** A randomized controlled trial (RCT) is a prospective, comparative, quantitative study undertaken under controlled conditions with random allocation of interventions to comparison groups. Randomized controlled trials assess the safety and effectiveness of a new intervention or treatment (Hariton and Locascio, 2018). A randomized controlled clinical trial with double-blind studies is the gold standard in clinical trial study and the ultimate measure of safety and effectiveness in clinical research (Mosihuzzaman *et al*., 2008). A randomized controlled clinical trial is a comparative study design with a treatment group and a control group. The assignment of participants to a group is determined by the formal procedure of randomization. Randomization, in the simplest case, is a process by which all participants are equally likely to be assigned to either the treatment group or the control group (Friedman *et al*., 2010). Randomization reduces bias and provides a rigorous strategy to examine cause-effect relationships between an intervention and outcome (Hariton and Locascio, 2018). This is because the act of randomization balances participant characteristics (both observed and unobserved) between the groups allowing attribution of any differences in relation to the outcome of the study intervention or treatment. This is not possible with any other study design (Hariton and Locascio, 2018).

Randomized controlled trials can be assessed by intention-to-treat (ITT) analysis; subjects analysed in the groups to which they were randomized, per protocol; only participants who completed the

intervention originally allocated are analysed or other variations. Intention to-treat is often regarded as the least biased. Randomized controlled trials should have pre-specified primary outcomes, be registered with a clinical trials database and should have appropriate ethical approvals (Hariton and Locascio, 2018).

The treatment or intervention being tested is allocated to various study groups (two or more groups) that are followed prospectively. Outcomes of interest are recorded, and comparisons are made between treatment or intervention and control groups. The control group may receive no intervention, a standard treatment, or a placebo. The intervention can be treatment or preventive. Randomized controlled trials are suitable for both preclinical and clinical trial study. For clinical trials, the proposed treatment or intervention is sometimes based on logic, but mostly on data obtained from invitro laboratory studies, animal experiments or preliminary serendipitous observation in an uncontrolled setting. Observational (case-control or cohort) studies may suggest the benefit of an intervention, but they are prone to bias (Bhide *et al*., 2018).

Randomized controlled trials are becoming steadily popular in herbal medicine. However, randomized controlled trials can have drawbacks; designing and conducting a trial, analysing data, interpreting findings and disseminating results, high cost in terms of time and money, problems with generalizability (participants that volunteer to participate might not be representative of the population being studied) and loss to follow up (Bhide *et al*., 2018; Hariton and Locascio, 2018).

2.9. The Herbal Product, Mist Amen Fevermix

Mist Amen Fevermix is a finished herbal product, a decoction, prepared from the stem bark of *Morinda lucida* Benth (Family: Rubiaceae) and the stem bark of *Parinari robusta* Oliv. (Family: Chrysobalanaceae). The product has been registered with the FDA, Ghana, since the year 2008 and

is on the 'Recommended Essential Herbal Medicines List (EHML)' for primary healthcare services of the Ministry of Health and used in the Herbal Medicine Units of Ghana Health Service (MOH, 2008). *Mist Amen Fevermix* is produced by Amen Scientific Herbal Hospital (ASHH) in Kumasi.

2.9.1. Component Plants of *Mist Amen Fevermix*

2.9.1.1. *Morinda lucida* Benth. Taxonomy and Description

Morinda lucida Benth (Figure 2.5) belongs to the family *Rubiaceae*. It is a tropical Africa rainforest tree also called Brimstone tree. The plant is distributed from Senegal to Sudan and southwards to Angola and Zambia. It grows in grassland, exposed hillsides, thickets, forest, often on termite mounds, from sea level up to 1300 meters altitude (Burkill, 1997). The genus *Morinda* comprises about 90 species (Mabberley, 2008) and occurs throughout the tropics. In Africa, 5 species are known, namely: *Morinda morindiodes* (Baker) Milne-Redh., *M. asteroscepa* K. Schum., *M. longiflora* G. Don., *M. lucida* Benth.and *M. indet* (Linn) (Sambamurty, 2005). It is an evergreen shrub or small to medium-sized tree up to 18-25 meters high, the branches are often gnarled, projecting from a stem covered with both smooth and rough-forming irregular shaped grey-brown patches, often showing purple colouration (Abbiw, 1990). Also, the tree has slender branchlets and a dense crown. The leaves are simple, broad, ovate and tapering end, with sizes ranging from 7-15 cm long and 3.5-7.5 cm wide. The plant has a characteristic yellow wood from which it derives its name ''brimstone tree''. Its bole and branches are often crooked or gnarled, slender branchlets and a dense crown. The bark is smooth to roughly scaly, grey to brown, often with some distinct purple layers (Abbiw, 1990).

Morinda lucida is a flowering plant with aromatic leaves and produces fragrant white flowers from January to July and September to October and also bears fruits from March to April (Irvine, 1961). The flowers are bisexual and have narrow glabrous corolla tube of about 2.5 cm. The fruits produced are classified as drupe, arranged together into an almost globose succulent syncarp 1-2.5 cm in diameter, which is soft and black when matured. The fruit has a size measuring up to 6.5 mm x 4 mm

in diameter, dark red brown and one seeded (Irvine, 1961). In Ghana, *Morinda lucida* is known as *Konkroma* (Twi), *Onkroma* (Fante) and *Amake* (Ewe).

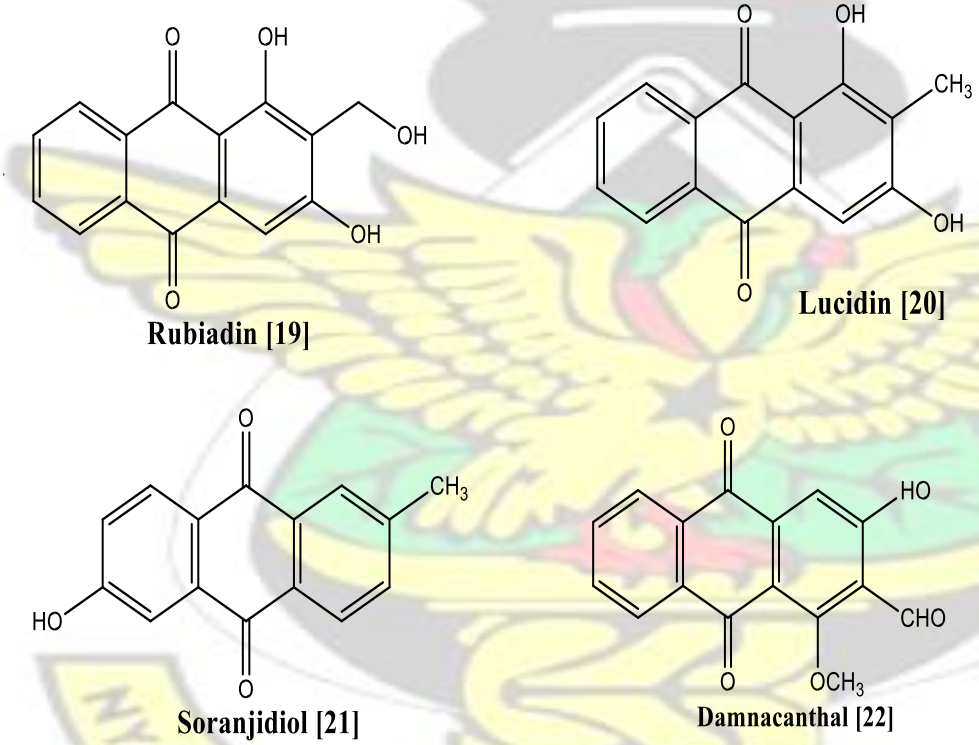
Figure 2.5: A photograph showing *Morinda lucida* plant taken at the Tafo Government Hospital, Kumasi, Ghana, Table 3.1.

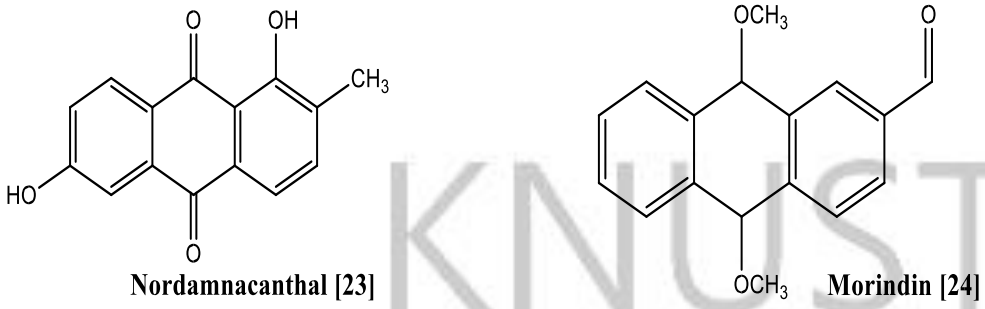
2.9.1.2. Traditional Medicinal Uses *of Morinda lucida* Benth.

Morinda lucida has several uses in traditional medicine, the leaf is used as a tea to treat malarial fevers and other infections (Koumaglo, 1992). Decoctions and infusions of various parts of the plant are employed in the treatment of diabetes, hypertension, dysentery, stomach-ache, ulcers, severe jaundice, leprosy and gonorrhoea (Adesida and Adesogan, 1972; Oliver-Bever, 1986; Kemabonta and Okogbue, 2000). In DR Congo, the leaves and stem bark decoction is used for treating ringworm infections and itches (Abbiw, 1990). Aqueous extract of the leaf is applied to the breast of women during the weaning of their infants due to its bitterness, and also to prevent infections. It is reported that *M lucida* is used as laxative, analgesic and febrifuge (Burkill *et al*., 1997; Raji *et al*., 2005). In Ghana, the decoction of the stem bark or leaf is used to treat typhoid, gonorrhoea, bone fracture, high blood pressure, rheumatism and candidiasis (Ofori *et al*., 2012).

2.9.1.1.3. Chemical Constituents of Morinda lucida

Anthraquinones like rubiadin **[19]**, lucidin **[20]**, soranjidiol **[21]**, damnacanthal **[22]** nordamnacanthal **[23]**, morindin **[24]** oruwacin and oruwal, have been isolated from *Morinda lucida* as also tannin, flavonoid, and saponosides (Fain, 2006; Suzuki *et al*., 2015). In Ghana recently, a tetracyclic iridoid known as molucidin and its derivatives have been isolated from the leaves of *Morinda lucida* and characterized through a bioassay-guided fractionation (Suzuki *et al*., 2015; Kwofie *et al*., 2016). Figure 2.6 shows the chemical structures and compounds of some anthraquinones found in *Morinda lucida.*





Nordamnacanthal [23] Morindin [24]

Figure 2.6: Chemical Structures of Some Anthraquinones isolated from *Morinda lucida*

2.9.2. Parinari robusta Oliv.

2.9.2.1. Taxonomy and Description

Parinari robusta Oliv. belongs to the family Chrysobalanaceae. It is a tropical West African rain forest tree (www.theplantlist.org). The plant occurs in West Africa, from Ghana, La Côte d'Ivoire to Nigeria (Turkson *et al*., 2015; Aubréville, 1959). The genus Parinari comprises 12 species, some of which occur in tropical Africa, Asia and tropical America. The species are *P.capensis* Harv., *P.excelsa* Sabine., *P.curatellifolia* Planch., *P.oblongifolia* Hook.F., *P. occidentalis* Prance., *P.nonda* F.Muell., *P. papuana* C.T.White., *P. anamensis* Hance., *P. macrophylla* Sabine., *P. polyandra* Benth., *P.glaberrimum* Hassk., and *P.robusta* Oliv. In Ghana, it is known as kukuodua (Twi) (www.plants.jstor.org). It is small to a medium-sized deciduous tree with a characteristic habitat of swamp-forest. It grows up to 13 meters high and low-branching in coastal areas, or up to 35 meters or more inland with a cylindrical bole up to 1.70 meters girth (Taylor, 1960; Keay *et al*., 1989).

P. robusta regenerates well in shade and in Ghana, flowering usually occurs seasonally in January-

July and September. It is widespread but there is no current data on its abundance (www.prota4u.org).

Figure 2.7: A photograph showing leaves, fruits and stem of *Parinari robusta* plant taken at Nokwareasa village, Ejura, Ghana, Table 3.1

2.9.2.2. Traditional Medicinal Uses of Parinari robusta

In La Côte d'Ivoire, bark decoctions and pounded leaves of *Parinari robusta* are applied as an analgesic. Pregnant women take a decoction of the bark as a tonic (www.prota4u.org; www.plants.jstor.org). In Ghana, it is a component plant of a finished bi-herbal product, used in the treatment of uncomplicated malaria (Turkson *et al*., 2015).

2.9.2.3. Chemical Constituents of Parinari robusta

Parinari robusta is known to contain saponin (Turkson *et al*., 2015).

2.10. The Herbal Product's, *Edhec Malacure*

Edhec Malacure is a finished herbal product and a decoction prepared from the stem bark of *Morinda lucida* Benth (Family: Rubiaceae), leaves of *Cleistopholis patens* Benth. Engl. and Diels (Family: Annonaceae), and stem bark of *Mangifera indica* Linn. (Family: Anacardiaceae). *Edhec Malacure* is manufactured and distributed by Edu Herbal Clinic (EHC), located in Baafikrom, near Mankessim in

the Central Region, Ghana. *Edhec Malacure* is not on the EHML, however, it has been approved by the FDA since the year 2014 and available on the market.

2.10.1. Plant Components of *Edhec Malacure* Herbal Product

2.10.1.1. Taxonomy and Description of Cleistopholis patens (Benth.) Engl. and Diels.

Cleistopholis patens (Benth.) Engl. and Diels belong to the family *Annonaceae*. It is small to a medium-sized tree which can grow up to 20-30 meters tall. It is usually straight, cylindrical and slender, up to 0.8-0.9 meters in diameter. It is sometimes slightly fluted at the base; bark surface smooth, shallowly fissured, greyish white to grey, inner bark strongly fibrous, peel-able in long strips, white to pale orange-brown, scented; crown with horizontal branches drooping at tips; twigs often with small ridges, glabrous. The leaves are alternate, simple and entire but stipules are absent. *C patens* is distributed in various parts of tropical Africa, in the rain forest region from Burkina Faso, La Cote d' Ivoire, Ghana, Liberia, Sierra Leone and Togo. *Cleistopholis* comprises 3 species, *C. patens, C. glauca* Pierre ex Engl. and Diels and *C. staudtii* Engl and Diels all in tropical Africa (Adonu *et al*., 2013).

In La Cote d' Ivoire and Ghana ripe fruits occur in August–November. *C. patens* is most commonly found in riverine and swamp forest, and in secondary forest. It prefers flat, disturbed and wet sites, but can also be found in evergreen forest on slopes, up to 1100 meters altitude (Adonu *et al*., 2013

Figure 2.8: A photograph showing the leaves of *Cleistopholis patens* plant taken in the KNUST Botanical Gardens, Kumasi, Ghana, Table 3.1.

2.10.1.2. Traditional Medicinal uses of *Cleistopholis patens* (Benth.) Engl. and Diels.

Cleistopholis patens (Benth.) Engl. and Diels. has been used as antimicrobial, anthelmintic and antimalarial agents. Bark decoctions are taken to treat stomach ache, diarrhoea, tuberculosis and bronchitis. Bark pulp is applied against swellings, oedema and whitlow, and bark sap is instilled into the nose to treat headache and rubbed in to treat rickets in children. In Uganda, crushed bark is used in preparations to treat malaria and measles. In Nigeria, the bark is used to treat typhoid fever and also in the treatment of menstrual irregularities. The root bark is used as an emetic. Leaf infusions are administered against infective hepatitis, fever, trypanosomiasis and rheumatic arthritis, and as a vermifuge. The leaf and stem bark also have anti-plasmodial activity, treatment of jaundice and stomach disorders (Mshana *et al.*,2000; Addo-Fordjour *et al*., 2008; Boyoma *et al*., 2011).

2.10.1.3. *Chemical Constituents of Cleistopholis patens (Benth.) Engl. and Diels.*

Cleistopholis patens is rich in monoterpenes, sesquiterpenes (Hufford *et al*., 1987), azaoxoaporphinoid and aporphinoid alkaloids. Aporphinoid alkaloids like *cleistopholine* **[25]**, *onychine* **[26]**, *eupolauridine* **[27]**, eupolauridine *N*-oxide and eupolauridine di-*N*-oxide) have been

isolated from the root bark of *C patens* as also 3-methoxysampangine **[29]** (Liu *et al*., 1990), and 8hydroxysampangine, an azaoxoaporphinoid (Akendengué *et al.*, 1999). Also, cleistriosides and cleistetrosides acetylated tri- and tetrarhamnoside dodecanyl ether derivatives have been obtained as well as *liriodenine* **[28]**, a copyrine alkaloid from the plant (Hufford *et al*., 1987; Waterman 1999). Structures of some compounds found in *C. patens* are shown in Figure 2.9.

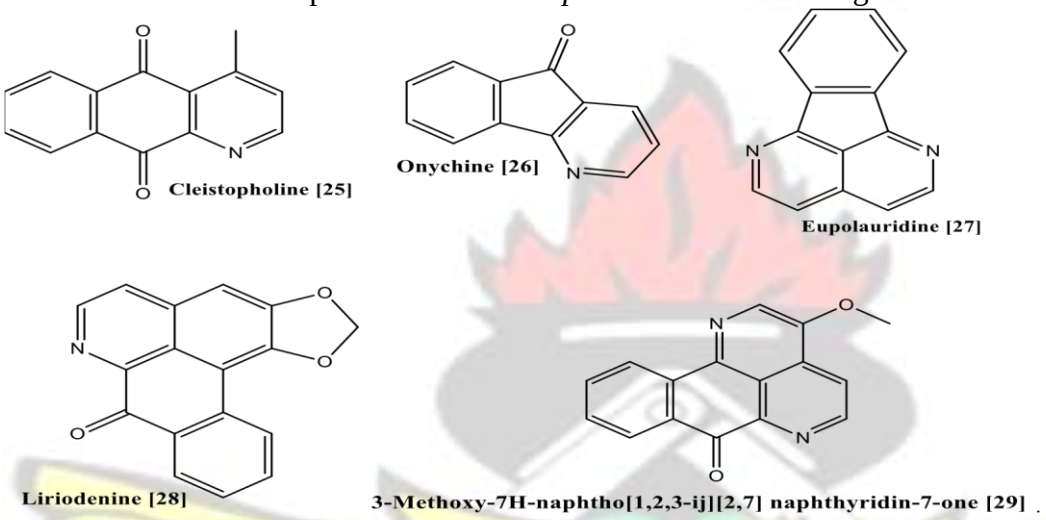


Figure 2.9: Chemical Structures of Some Compounds found in Cleistopholis patens

2.10.1.4. Mangifera indica *L.*

2.10.1.4.1. Taxonomy and Description of *Mangifera indica* L.

Mangifera indica commonly called mango belongs to the genus *Mangifera* which consists of about 30 species of tropical fruiting trees in the flowering plant family *Anacardiaceae*. It is a large evergreen tree which can grow to a height of about 20-25 meters tall with a dark green, umbrella-shaped crown. *Mangifera indica* has a trunk of about 90 centimetres in diameter. It has a bark which is brownish, smoothish, with many thin fissures; thick, becoming darker, rough and scaly or furrowed; branchlets rather stout, pale green and hairless. Inner bark light brown and bitter (Litz., 2009). The leaves of mango are alternate, simple, leathery and oblong-lanceolate measuring 16-30 x 3-7 centimetres.

The mango fruit has a varied irregularly egg-shaped and slightly compressed fleshy drupe, with a maximum size of 8-30 centimetres attached at the broadest end on a pendulous stalk. *M. indica* is native to tropical Asia, and has been cultivated in the Indian subcontinent for over 4000 years and is now found in most tropical countries (Litz, 2009; www.worldagroforestry.org).

Figure 2.10: A Photograph showing *Mangifera indica* plant taken at the Tafo Government Hospital, Kumasi, Ghana, Table 3.1.

2.10.1.4.2. Traditional Medicinal uses of Mangifera indica L.

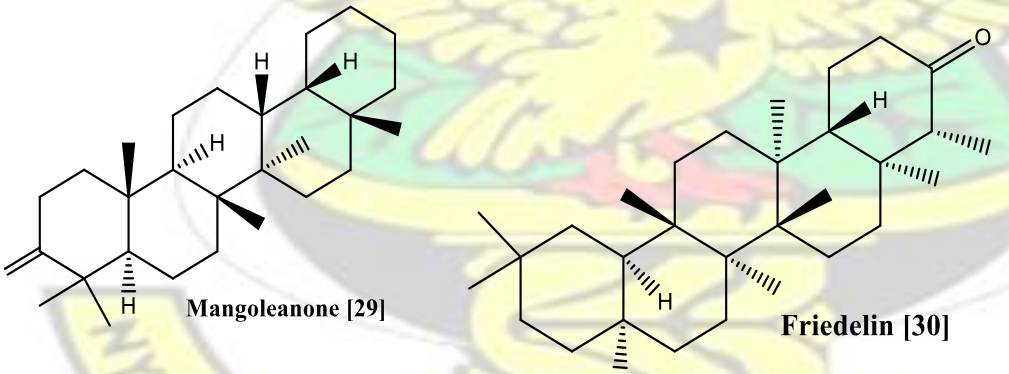
Mangifera indica has been used for traditional medicinal purposes. The unripe pulp has been used therapeutically as an antibacterial agent against foodborne bacteria (Gupta *et al*., 2008). The leaf possesses antibacterial activity, antiulcerogenic action, hypoglycemic activity and atherogenic activity (Aderibigbe *et al*., 2001; Muruganandan *et al*., 2005; Doughari and Manzara, 2008; Severi *et al*., 2009). The seed kernel possesses anti-diarrhoeal activity and antidyslipidemic. The stem bark possesses immunomodulatory activity, anti-inflammatory and neuroprotective activity (Sairam *et al*., 2003; Lemus-Molina *et al*., 2009).

Plaster is made from the charred and pulverised leaves to remove warts and also act as a styptic. The seeds are used to treat chronic colds and coughs, obstinate diarrhoea and bleeding piles. The bark is

astringent, homeostatic and anti-rheumatic. All parts of mango are used to treat abscesses, rabid dog, tumour, snakebite, stings, datura poisoning, heatstroke, miscarriage, anthrax, blisters, mouth ulcers, tympanitis, colic, diarrhoea, glossitis, indigestion, bacillosis, bloody dysentery, liver disorders, excessive urination, tetanus and bronchial asthma (Shah *et al*., 2010).

2.10.1.4.3. Chemical Constituents of *Mangifera indica* L.

Some of the chemical constituents present in *Mangifera indica* include: polyphenolics, flavonoids, and triterpenoids. Mangiferin a xanthone glycoside is a major bioactive constituent, isomangiferin, tannins and gallic acid derivatives. The bark is reported to contain protocatechuic acid, catechin, mangiferin, alanine, glycine, γ-aminobutyric acid, kinic acid, shikimic acid and the tetracyclic triterpenoids cycloart-24-en-3β,26diol, 3-ketodammar-24 (*E*)-en-20S,26-diol, C-24 epimers of cycloart-25 en 3β,24,27-triol and cycloartan-3β,24,27-triol (Scartezzini and Speroni., 2007; Gupta *et al*., 2008). Structures of some compounds Mangoleanone (**29**), Friedlin (**30**), Mangiferin (**31**) and Myricetin (**32**) present in *Mangifera indica* are as shown in below (Figure 2.11).



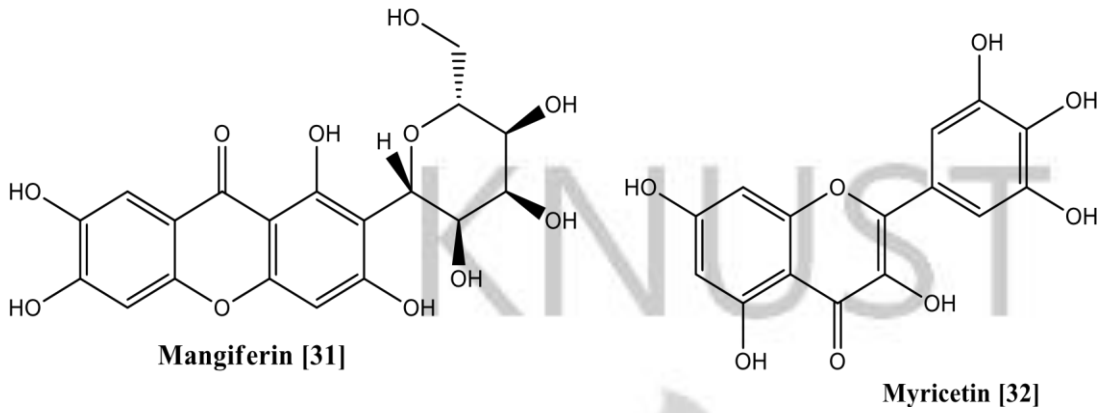


Figure 2.11: Chemical Structures of Some Compounds found in *Mangifera indica*

2.11. Overview of Clinical Studies of Herbal Products

Herbal therapies are in widespread use throughout the world because of their perceived safety and effectiveness. However, such widespread use does not assure that herbal therapies have a favourable risk-benefit ratio. The actual benefits and risks need to be evaluated by clinical studies (WHO, 2005). The purpose of clinical study is to find ways to effectively prevent, diagnose, or treat disease. The WHO posits that succinct data is needed which will lead to well supported clinical studies of herbal products, approvable by national regulatory authorities (WHO, 2005).

Clinical studies are carried out on herbal products after standardization to ensure that the substances being evaluated are always the same. Herbal remedies should be prepared incorporating GMP guidelines. A number of preclinical tests regarding safety are required for a therapy in animals before human use. Once the therapy has proven to be effective in the preclinical trials, a clinical study is undertaken (WHO, 2005).

2.11.1. Requirements for Conducting Clinical Studies of Herbal Products

There are four phases involved in clinical trials. Phases 1 and 2 studies are performed on a few participants under strict medical supervision. The requirements for this phase include details on the standardization of the product (WHO, 2005). For the trial herbal product, the amount of active ingredient, list of excipients, type of product (tablet, capsule, decoction, etc.) and its method of manufacture, analysis of the supposed active ingredient(s) using chemical or biological parameters, analysis of chemical constituent (analytical marker compound), analysis using chemical fingerprint (analytical markers), analysis for lack of contamination by pesticides, herbicides, heavy metals, synthetic drug adulterants, microbial load and toxins, storage conditions and stability over the length of the trial, specification against which a certificate of analysis can be assessed before the clinical trial material is released (WHO, 2005). Phase 1 and 2 are to establish the safety and efficacy of products.

For Phase 3 trials, a large number of participants are used. GMP standards are employed before phase 3 trials. Generally, Phase 3 trials more extensively done and with more stringent oversight (WHO, 2005).

CHAPTER THREE QUALITY ASSESSMENT OF THE HERBAL PRODUCTS TOWARDS THE

DEVELOPMENT OF QUALITY STANDARDS

3.1. INTRODUCTION

The quality of an herbal product has a direct impact on its safety and efficacy. Research has shown that there are many contaminants and impurities that may cause harm to the end-users of herbal products. Many of such contaminants and residues may include naturally occurring radionuclides, toxic metals and bacteria (WHO, 2007). Inadvertent contamination, like heavy metals and microbial

contamination during the production stage, can also lead to deterioration in quality. This is because production of mycotoxins such as aflatoxin, have shown mutagenic, carcinogenic, teratogenic, neurotoxic, nephrotoxic, and immunosuppressive activities (Ashiq *et al.*, 2014).

The establishment of quality standards for the herbal products in this study was undertaken to provide some relevant globally acceptable information on these herbal medications.

The standardization process of *Mist Amen Fevermix*, *Edhec Malcure* and their component plants is represented schematically (Figure 3.1)

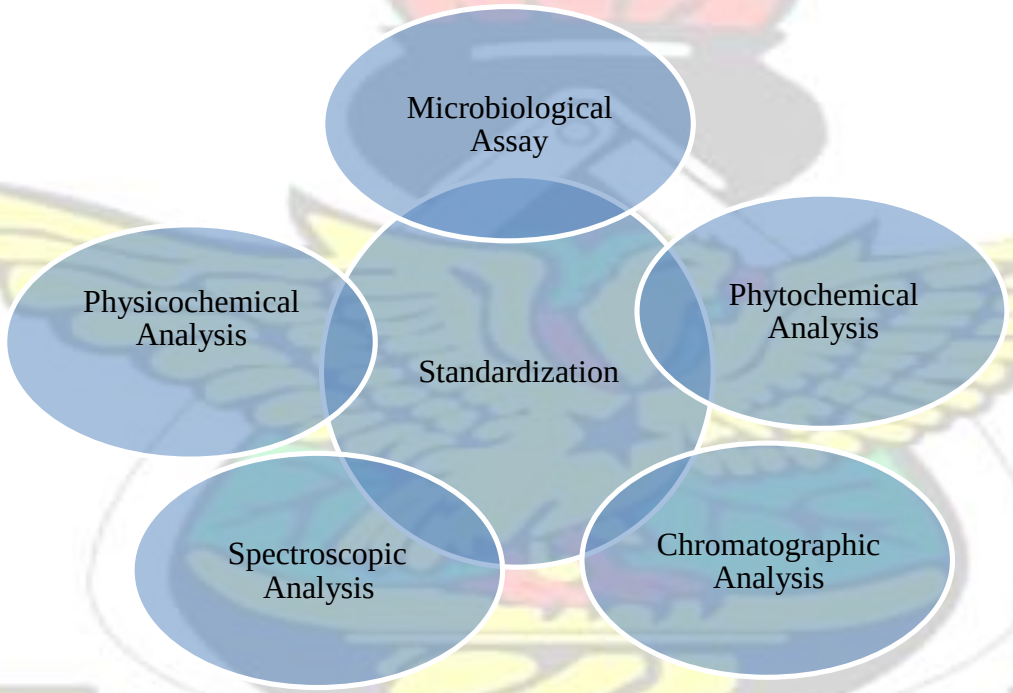


Figure 3.1: Schematic Representation of the Standardization Process

3.2. Materials and Methods

3.2.1. Herbal Products

Four bottles each containing 330 mL of *Mist Amen Fevermix* in amber plastic bottles and *Edhec Malacure* in 500 mL amber bottle (test samples) were bought from Danny Herbal Shop, an herbal medicine distributor in Kumasi.

3.2.2. Collection and Authentication of Components Plants of *Mist Amen Fevermix* and *Edhec Malacure* used in the Study

Mist Amen Fevermix contains the stem bark of *Morinda lucida* Benth. and *Parinari robusta* Oliv. *Edhec Malacure* contains three plant materials; leaves of *Cleistopholis patens* (Benth.) Engl. and Diels., stem bark of *Morinda lucida* Benth. and *Mangifera indica* L. (Table 3.1). The stem bark of *Morinda lucida* and *Mangifera indica* were harvested from the premises of the Tafo Government Hospital on October 19, 2019. The leaves of *Cleistopholis patens* (Benth.) Engl. and Diels. was also collected from the KNUST Botanic Garden on October 18, 2019. *Parinari robusta* Oliv. was collected from Nokwareasa village, East of Ejura in the Ashanti Region of Ghana and about 100 km from Kumasi on October 20, 2019. Geographical location coordinates of the plants were documented (Table 3.1). The plants were authenticated by Mr Clifford Osafo Asare of the Department of Herbal Medicine, KNUST where Voucher Specimen with numbers were allocated and specimen deposited in the herbarium of the Department (Table 3.1).

Table 3.1: Voucher Specimen Numbers of Plant Materials Used

Test Samples	Batch No.	Plant Material	Voucher Specimen Number	GPS Coordinates
Mist Amem Fevermix	044	*Morinda lucida*	KNUST/HM1/2019/SB023	latitude 1º 36' 47.214"N and longitude 6º 43' 28.272"W
		Parinari robusta	KNUST/HM1/2019/SB022	latitude 7º 23' 8.088"N and longitude 1º 21' 22.212"W.
Edhec Malacure	EHC 003	*Cleistopholis patens*	KNUST/HM1/2019/L016	latitude 1º 33' 54.972"N and longitude 6º 40' 48.838"W
		Morinda lucida	KNUST/HM1/2019/SB023	latitude 1º 36' 47.214"N and longitude 6º 43' 28.272"W
		Mangifera indica	KNUST/HM1/2019/SB021	latitude 1º 36' 45.468"N and longitude 6º 43' 31.962"W

3.2.3. Plant materials and test samples processing

About 330 mL of *Mist Amen Fevermix* and 500 mL of *Edhec Malacure* were separately lyophilised. The stem barks of *Morinda lucida, Parinari robusta, Mangifera indica and* fresh leaves of *Cleistopholis patens*, were thoroughly washed under running water to rid it of dirt and other foreign materials. They were then cut into smaller pieces and separately sun-dried for two days. The dried samples were communited to coarse powders using a mechanical grinding machine (YF-150, USA) and stored in airtight amber glass containers until required for use.

3.2.4. Quality Establishment of *Mist Amen Fevermix* and *Edhec Malacure* and Component Plants

Quality evaluation and the standardization of *Mist Amen Fevermix* and *Edhec Malacure* were performed in the laboratory of the Department of Pharmacognosy. Microbial load analysis was done at the Department of Pharmaceutical Microbiology, Faculty of Pharmacy and Pharmaceutical Sciences, IR and HPLC were performed at the Central laboratory, Kwame Nkrumah University of Science and Technology, Kumasi. Parameters assessed included the: organoleptic characterization (colour, odour and taste), basic phytochemical screening, physicochemical analysis (pH, relative density and elemental contents analysis), microbial load determination, chromatographic profiles (HPLC) and IR spectroscopy.

3.2.4.1. Organoleptic Tests.

About 200 mL each of *Mist Amen Fevermix* and *Edhec Malacure* were used for the evaluation of sensory characteristics such as colour, odour and taste.

3.2.4.2. Phytochemical Screening of *Mist Amen Fevermix* and *Edhec Malacure*

3.2.4.2.1. Reagents and Chemicals

The following analytical grade solvents and reagents; Methanol, 1% lead acetate, ammoniacal alcohol, 1% H_2SO_4, 20% NaOH, dilute NH_3, HCl (Aldrich Sigma, USA), chloroform, ethanol, Fehling's solution A and B, and Dragendorff's reagents, purchased from Lab Chem, Kumasi.

3.2.4.2.2. Methods

Mist Amen Fevermix and *Edhec Malacure* were each screened for alkaloids, saponins, phenols, flavonoids, sterols and triterpenes, anthracene glycosides and cyanogenic glycosides.

3.2.4.2.3 Tannin Test

About 0.5 g of lyophilised *Mist Amen Fevermix* and *Edhec Malacure* were each separately added to 25 mL of water respectively and boiled for 5 minutes at a temperature of about 100^0C. It was then allowed to cool filtered and the volume adjusted to 25 mL. To 1mL aliquot of the aqueous extracts was added 10 mL of water and 2 to 10 drops of 1% $FeCl_3$, and observed for any colour formed (Evans, 2009).

3.2.4.2.4. Alkaloids Test

About 30 mL of ammoniacal alcohol (ammonia: alcohol, 1:9) was separately added to 0.5 g of *Mist Amen Fevermix* and *Edhec Malacure* and filtered. The filtrate was then evaporated to dryness and the residue extracted with 1% H_2SO_4. The acidic extract was then filtered and the filtrate rendered alkaline with dilute ammonia solution. The alkaline solution of the extract was then transferred into a separation funnel and extracted with chloroform. The chloroformic layer was then separated and evaporated to dryness. The residue was again dissolved in 1% H_2SO_4 and few drops of Dragendorff's reagent added in a test tube. A yellowish to orange colouration was recorded as a positive test. (Evans, 2009).

3.2.4.2.5. Saponin Test

About 5 mL of water was separately added to about 0.2 g each of the dried powdered *Mist Amen Fevermix* and *Edhec Malacure* and each shaken in a test tube and the mixture observed for the presence of a froth which does not break readily upon standing for about ten minutes (Evans, 2009).

3.2.4.2.6. Phytosterol Test

About 30 mL of chloroform was separately added to about one gram of *Mist Amen Fevermix* and *Edhec Malacure* shaken and filtered. About 3 mL of acetic anhydride was added followed by few drops of concentrated sulphuric acid. Appearance of bluish-green colour should show the presence of sterols (Tiwari *et al*., 2011).

3.2.4.2.7. Glycoside Test

About 200 mg of the dried powdered *Mist Amen Fevermix* and *Edhec Malacure* samples were each separately warmed in a test tube with 5mL dilute H_2SO_4 on a water bath for 2 minutes. The acidic extract was then filtered and the filtrate made distinctly alkaline with 2 to 5 drops of 20% NaOH. 1mL each of Fehling's solution A and B was then added to the filtrate and heated on the water bath for 2 minutes. A brick-red precipitate indicates the presence of glycosides (Evans, 2009).

3.2.4.2.8. Flavonoid Test

About 0.5g of *Mist Amen Fevermix* and *Edhec Malacure* were each extracted with 15 mL of ethanol (98%). To the ethanolic extract was added a small piece of zinc metal, this was followed by dropwise addition of concentrated hydrochloric acid. Colours ranging from orange to red would indicate flavones, red to crimson indicated flavonols, crimson to magenta indicate flavanones (Evans, 2009).

3.2.5. Physicochemical Test

3.2.5.1. pH Determination

The pH of *Mist Amen Fevermix* and *Edhec Malacure* were determined separately using a pH meter (Schott Instrument Lab 860, Germany) on a 20 mL sample at room temperature of 29.6 ^{O}C.

3.2.5.2 Residue on Drying

The weight of 330 mL of *Mist Amen Fevermix* and 500 mL of *Edhec Malacure* were determined on a balance and was placed on a water bath to evaporate until a constant mass was obtained. This was done in triplicate and the average calculated to establish the weight per millilitre of each product on drying.

3.2.5.3. Heavy and Non-Heavy Metal contents of *Mist Amen Fevermix* and *Edhec Malacure*

3.2.5.3.1. Equipment, Chemicals and Reagents

Analytical grade concentrated nitric acid and perchloric acid (Sigma Aldrich, USA) were purchased from Lab Chem, Kumasi. Thermo Elemental M5 Atomic Absorption Spectrophotometer (AAS), Model ICE3000; Thermo Scientific, USA, fitted with Graphite furnace and an autosampler. The analysis was performed at the Faculty of Agriculture, Department of Soil Science laboratory, Kwame Nkrumah University of Science and Technology (KNUST), Ghana.

3.2.5.3.2. Preparation of Mist Amen Fevermix and Edhec Malacure

Nine heavy metals (Arsenic, iron, nickle, copper, lead, mercury, magnesium, cadmium and zinc) and two non-heavy metals (sodium and potassium) were analysed in each product. An aliquot of 1 mL each of *Mist Amen Fevermix* and *Edhec Malacure* were separately placed in a 250 mL beaker and 5mL each of freshly prepared mixture of concentrated HNO_3, concentrated HCl and distilled H_2O in the ratio 1.5:0.5:0.5 were added. The mixture was gently heated on a hot plate at a temperature of 150°C until the sample had completely dissolved to give a clear solution. During the digestion process, the inner walls of the beaker were washed with deionized water to prevent sample loss. After digestion, *Mist Amen Fevermix* and *Edhec Malacure* were made up to 50 mL with deionized water and analysed. Multi-element standard solutions of all the elements involved were prepared by dilution of 1000 mg/L stock solutions with 5 per cent nitric acid solution (WHO 2007).

3.2.6. Microbial Load Analysis

3.2.6.1. Materials and Methods

Potato dextrose agar, Nutrient Agar, MacConkey agar, Salmonella Shigella and Pseudomonas Cetrimide agar (Sigma Aldrich), were obtained from the stores of the Department of Microbiology, KNUST, Ghana. Laboratory Incubator (Gallenkamp, Germany), oven (Gallenkamp), electrical balance (Mettler Toledo, Switzerland) and general laboratory glasswares.

3.2.6.2 Preparation of Media

3.2.6.2.1. Nutrient Agar

About 8.75 gm of Nutrient Agar was weighed and dissolved in 500 mL distilled water in an infusion bottle and stirred using s stirring rod. The bottle was appropriately closed and the mixture heated to boil to dissolve the agar completely. It was sterilized by autoclaving at 121^0C for 15 minutes. About 1 mL was poured into two separate Petri dishes, exposed under aseptic conditions, the surface was allowed to dry and 0.5 mL of samples each of *Mist Amen Fevermix* and *Edhec Malacure* were plated and incubated (Downes and Ito, 2001). This was to establish the presence of non-fastidious organisms.

3.2.6.2.2. MacConkey Agar

About 26.5 gm *of MacConkey Agar* was weighed and mixed with 500 ml distilled water in an infusion bottle and stirred. The bottle was appropriately corked and the mixture heated to boil to dissolve the agar completely. It was sterilized by autoclaving at 121^0C for 15 minutes. About 10 mL was poured into two separate Petri dishes, exposed under aseptic conditions, the surface was allowed to dry and 0.5 mL samples each of *Mist Amen Fevermix* and *Edhec Malacure* were plated and incubated (Cheesbrough, 2006). This was to determine the presence of bacteria.

3.2.6.2.3. Salmonella, Shigella Agar

About 31.5 gm of *Salmonella, Shigella agar* was weighed and dissolved in 500 mL distilled water in an infusion bottle and stirred. The bottle was appropriately corked and the mixture heated to boil to dissolve the agar completely. It was cooled to about 51^{O}C, and well mixed. About 10 mL was poured into two separate Petri dishes, exposed under aseptic conditions, the surface was allowed to dry and 0.5 mL of samples each of *Mist Amen Fevermix* and *Edhec Malacure* were inoculated and incubated (BP, 2018). This was to determine the presence of salmonella and shigella.

3.2.6.2.4. Potato Dextrose Agar

About 19.5 gm of *Potato Dextrose Agar* was weighed and mixed with 500 mL distilled water in an infusion bottle and stirred. The bottle was appropriately corked and the mixture heated to boil to dissolve the agar completely. It was sterilized by autoclaving at 121^{o}C for 15 minutes. About 10 mL was poured into two separate Petri dishes, exposed under aseptic conditions, the surface was allowed to dry and 0.5 mL of samples each of *Mist Amen Fevermix* and *Edhec Malacure* were inoculated and incubated (BP, 2018). This was to determine the presence of yeast and mold.

3.2.6.5. Pseudomonas Cetrimide Agar

About 22.65 gm of *Pseudomonas Cetrimide Agar* was weighed and mixed with 500 mL distilled water in an infusion bottle and stirred. The bottle was appropriately corked and the mixture heated to boil to dissolve the agar completely. It was sterilized by autoclaving at 121^{o}C for 15 minutes. About 10 mL was poured into two separate Petri dishes, exposed under aseptic conditions, the surface was allowed to dry and 0.5 mL of samples each of *Mist Amen Fevermix* and *Edhec Malacure* were inoculated and incubated (BP, 2018). This was to determine the presence of *Pseudomonas aeruginosa.*

3.2.7. Development of FT-IR Fingerprint of *Mist Amen Fevermix, Edhec Malacure* and Component Plants

Fourier transform Infrared (FT-IR) fingerprint was developed for the test samples and their component plants according to the method described by Wulandari *et al.*, (2016). About 50 mL each of *Mist Amen Fevermix* and *Edhec Malacure* were evaporated on a water bath at a temperature of about 40 ^{o}C until a dry residue of constant weight was obtained. For the component plants, about 0.5 gm of the respective plant parts were air-dried for two weeks and ground into fine powder using a mortar and pestle. The products were scanned between spectra range of 400-4000 cm^{-1} using Perkin Elmer Spectrum Version 10.03.09 model, USA. This was used to obtain a fingerprint for the test samples and component plants for the quality control.

3.2.8. High-Performance Liquid Chromatographic (HPLC) Profile of *Mist Amen Fevermix* and *Edhec Malacure* and Plants Component

HPLC chromatogram was developed for the test samples and component plants as a quality control parameter.

3.2.8.1. Chemicals, Reagents and Instrumentation Conditions

A Liquid Chromatographic system was used Perkin Elmer Flexar and comprised of a binary pump, autosampler, degasser and PDA detector. Separation was achieved on Zorbax 300SB C18 (250×6mm, 5µm) column from Agilent. All reagents and chemicals used were of analytical grade (Sigma Aldrich, USA), purchased from Lab Chem, Kumasi. Mobile phase consisted of 0.05% Trifluoroacetic acid (TFA) (A) and Acetonitrile (B). Gradient elution was used. The gradient program was 0 min A (90%), 0 – 4 min A (90%) 4 – 14 min A (20%), 14 – 18 min A (20%) 18 – 18.1 min A (90%), 18.1 – 23.1 min A (90%). The detection of the wavelength was by scanning the test samples and component plants over a wide range of wavelength from 200nm to 400nm. A fixed concentration of analyte (10µg/mL) was analyzed at different wavelength. As per the response of analyte, the λ max value was found to be 210 nm. Injection volume was 20µL

and flow rate of 1ml/min was also set. The analysis was done at ambient temperature. The system was controlled and data acquired and processed using Chromera software version 3.4.

3.2.8.2. Preparation of *Mist Amen Fevermix* and *Edhec Malacure*

Three batches each of *Mist Amen Fevermix* and *Edhec Malacure* were sampled. They were thoroughly shaken to ensure complete mixing of the components. About 50 mL each of the products were taken and sonicated for 10 minutes. The samples were then filtered using a 0.45µm membrane filter into 2.5 mL vials and placed in the HPLC autosampler for injection.

3.2.8.3. Preparation of Component Plants

About 50 mL aqueous extract each of the stem bark of *Morinda lucida, Parinari robusta, Mangifera indica* and the leaves of *Cleistopholis patens* were lyophilised. Each sample was reconstituted in methanol to achieve a concentration of 100 mg/mL. It was then sonicated for about 10 minutes. The samples were then filtered using 0.45µm membrane filter (Thermo Fischer Scientific, USA) into 2.5 mL vials and set in the HPLC autosampler for injection; each injection was done in triplicate.

3.2.9. HPLC Analysis and FT-IR fingerprint of *Mist Amen Fevermix* and *Edhec Malacure* to Identify their Component Medicinal Plants

Anecdotal evidence claims some manufacturers of herbal products do not completely declare entirely the plant materials used in the formulation. This could endanger the health of consumers in case of sensitivity to the undisclosed plant material. Hence, in the present study, an FT-IR fingerprint and HPLC were developed for the test samples and their component plants. This was to establsih whether the plant components listed on the lables are present in the test samples.

3.2.9.1. Chemicals, Reagents and Instrumentation Conditions

The chromatographic conditions developed in section 3.2.8.1 was applied for the identification assessment of the presence of the plant component in *Mist Amen Fevermix* and *Edhec Malacure*.

3.2.9.2. Medicinal Plants Component Preparation

In order to identify the presence of the plant component of the test samples, about 0.5 g of dried extract of *Morinda lucida*, *Parinari robusta*, *Mangifera indica* and *Cleistopholis patens* were accurately weighed and transferred into a 20 mL test tube. It was then sonicated for about 10 minutes to completely dissolve in a solvent which is a mixture of methanol and water in a ratio of 1:1 to make a total volume of 10 mL. The solution was filtered through a 0.45µm membrane filter into 2.5ml vial and set in the HPLC autosampler for injection.

3.2.9.3. Sample Preparations

The test sample preparations described in section 3.2.8.3 was applied for the identification assessment of the presence of the component plants in *Mist Amen Fevermix* and *Edhec Malacure*.

3.2.10. Chemometric Analysis to Identify Component Plants of *Mist Amen Fevermix and Edhec Malacure* using FT-IR Fingerprint

FT-IR fingerprint was developed as described in section 3.2.7 and was subjected to chemometric analysis. In this analysis, hierarchical cluster analysis using Ward method with squared cuclidean (Strauss and Maltitz., 2017; Ward, 1963) statistical method was used for further classification of the resultant data by means of Euclidean distance as a measure of similarity. A plot of distances versus samples was used to represent the data based on their similarities (Li *et al*., 2009). Also, principal component analysis (PCA) was used to cluster the samples. PCA was used as an unsupervised clustering analysis technique. All the principal components (PCs) were extracted from the resultant matrix of data using singular value decomposition algorithm. PCA theory is based on ranking the PCs according to their eigenvalues in such a way that the first PC contains the most variation in the data set. Accordingly, the second PC is calculated to be orthogonal with respect to the first one. The plot of the first two PCs represent data scattering in a two-dimensional space (Sundaram *et al.*, 2012).

3.2.11. HPLC Analysis to Check Possible Adulteration of *Mist Amen Fevermix* and *Edhec Malacure* with Conventional Antimalarials

3.2.11.1. Equipment, Chemicals and Reagents

Analytical grade Acetronile, methanol, acetic acid (Sigma Aldrich, USA) was used. Stationary phase was C 18. The reference antimalarial drugs (Artemether, Lumefantrine and Quinine, Sigma Aldrich, USA) were obtained from the Department of Pharmaceutical Chemistry, KNUST.

3.2.11.2. Preparation of the Mobile Phase

The mobile phase was composed of methanol and 0.05%TFA. About 500µL of the Trifluoroacetic acid was pipetted and transferred into a 1L volumetric flask. It was then topped with deionized water to yield 1000ml of 0.05%TFA.

3.2.11.3. Chromatographic Method Development and Conditions for Eluting Artemether, Lumefantrine and Quinine

The mobile phase selected for the chromatographic separation was; Acetronile (ACN), methanol (MeOH) and acetic acid (CH_3COOH). These reagents were selected based on the separation, retention time, peak heights and the area obtained. Detection wavelength was selected by scanning standard drug over a wide range of wavelength from 200nm to 400nm. A fixed concentration of analyte (10µg/mL) was analyzed at different wavelength. As per the response of analyte, the λ max value was found to be 210 nm, 250 nm and 345 nm for Quinine, Artemether and Lumefantrine, respectively.

The flow rate was between 1.0 ml/min to 1.54 ml/min and an injection volume of 20µl was used. Column ambient temperature of 26ºC was used. Isocratic elution mode was used in the HPLC method development. The chromatographic conditions developed were applied for the establishment of adulteration**.** This was developed for the detection or otherwise of the presence of the reference antimalarial drugs in the test samples. The three reference antimalarial drugs were run simultaneously. Chromatogram elution of the three reference antimalarial drugs (Appendix 17).

3.2.11.4. Preparation of Reference Antimalarials

A quantity of 100 mg of Artemether was accurately weighed and transferred into a 100 mL volumetric flask. About 50 mL of diluent which consisted of the mobile phase in the ratio of 0.05%TFA: CAN, 20:80, was added and sonicated for about 10 minutes to completely dissolve the Artemether. The volume was made up to the mark with the diluent to a final concentration of 1000 mg/L. The solution was filtered through a 0.45µm membrane filter. Similarly, 50 mg each of lumefantrine and quinine, were prepared as described for artemether but achieving a final concentration of 500 mg/mL each.

3.2.11.5. Validation of the Methods

3.2.11.5.1. Calibration and Linearity

Standard stock solution was prepared for Artemether, Lumefantrine and Quinine the standard solutions were in the range of 100µg/mL to 500µg/mL for Artemether, 2.5µg/mL to 40µg/mL for Lumefantrine and 10µg/mL to 160µg/mL for quinine. These linear solutions were injected in triplicates. Calibration graphs were plotted for the three active pharmaceutical ingredients (APIs) and were found to be linear. The correlation coefficient was found to be 0.993, 0.999 and 0.999 for Artemether, Lumefantrine and Quinine respectively (ICH, 1997).

3.2.11.5.2. Precision

In the precision studies, 150µg/mL, 30µg/mL and 50µg/mL of Artemether, Lumefantrine and Quinine respectively were prepared. The solution was analysed six times on day one. The solution was also analysed six times on day 2 and the data analysed.

3.2.11.5.3. Accuracy/Recovery

The accuracy of the method was determined by a recovery test. A control blank sample was analysed at the start of the analytical block (Appendix 15). The recovery test was conducted by spiking three different known concentrations of standard compounds (APIs) to test samples; 250 mg/L, 350 mg/L and 450 mg/L for artemether, 15 mg/L, 25 mg/L and 35 mg/L for lumefantrine and 30 mg/L, 50 mg/L

and 70 mg/L for quinine respectively (Appendix 16). This was analysed to determine the amount that will be recovered.

3.2.11.5.4. Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by the use of the equations:

LOD = 3 σ/s.

LOQ = 10 σ/s

Where:

σ is the standard deviation of intercept of calibration plot and s is the average of the slope of the corresponding calibration plot.

3.2.11.5.5. Robustness

In the robustness test, chromatographic conditions were kept constant; however, few parameters were deliberately altered. These include flow rate, wavelength and pH. The retention times for the control samples (injected two times for each pH reading) were recorded. The corresponding concentrations were then calculated. The relationship between pH and retention time as well as pH and concentration were compared.

3.2.11.6. Flow Rate versus Retention time

The flow rate was 1 mL per minute and the retention time noted. The effect of the flow rate on the retention time was noted.

3.3. ESTABLISHING THE EFFICACY AND ACUTE TOXICITY of *MIST AMEN FEVERMIX* AND *EDHEC MALACURE*

3.3.1. *In Vitro* Antiplasmodial Activity of *Mist Amen Fevermix* and *Edhec Malacure*

3.3.1.1. Equipment, Chemicals and Reagents

SYBR Green, artesunate, 5% O_2, 5% CO_2 and 90% Nitrogen were obtained from the Department of Pharmacology, KNUST, and Kumasi. RBC (O^-, Rhesus positive), field isolate strains of *P. falciparum*, Falcon and ACD tubes were obtained from the Tafo Government Hospital, Kumasi, Ghana. Incubation was done using incubator (RS Biotech, USA) at the Department of Pharmacology, KNUST.

3.3.1.2. Preparation of *Mist Amen Fevermix* and *Edhec Malacure*

About 1320 mL of *Mist Amen Fevermix* and 1500 mL of *Edhec Malacure* were lyophilised to obtain 3.1175 g and 2.6067 g of powders respectively. About 25 mg of weighed powders (*Mist Amen Fevermix* and *Edhec Malacure*) were transferred into 15 mL Falcon tubes containing 5 mL of 70% ethanol to obtain a stock concentration of 3000 µg/mL. About 1.7 mL each of the stock solution was transferred into a 15 mL Falcon tubes and serially diluted 9-fold to obtain 1000, 500, 250, 125, 62.5, 31.3, 15.6, 7.8 and 3.9 µg/mL.

3.3.1.3. Parasite Collection and Culturing

In vitro susceptibility assays of *Mist Amen Fevermix* and *Edhec Malacure Mixture* were performed on *P. falciparum* field isolate obtained from the Tafo Government Hospital after ethical approval was granted (CHRPE/AP/424/19). About 2.5 mL of blood samples containing *P. falciparum* field isolates were separately collected aseptically from a venous puncture using the vacutainer system from six patients into acid citrate dextrose (ACD) tubes and then stored by placing it in liquid nitrogen. The parasites were then transferred into parasite vials and cultured as described by Hout *et al*., (2006). The parasite vials were appropriately thawed in a water bath at a temperature of 37ºC. The vials (cell culture) were centrifuged at 2000 rpm for 10 minutes and the resultant supernatant was discarded. A mixture of 3.5% NaCl in distilled water was added to each of the pellet, which was centrifuged at 2000 rpm for 10minutes. The pellets were gently disengaged and 1 mL aliquot of complete parasite

medium (5 mL of L-glutamine, 2.5 mL of 10 mg/mL and 50 mL Albumax in 500 mL of Roswell Park Memorial Institute (RPMI 1640) was added and centrifuged again at 2000 rpm for 10 minutes (Jensen and Trager, 1980). This procedure was duplicated, and the parasites were then suspended in 25 mL BD Falcon tubes (culture flask) containing 200 µL freshly prepared pack of RBC (O^-, Rhesus positive) and 5 mL of complete parasite medium to have a haematocrit of 4%. A 2% Oxygen, 5.5% Carbon dioxide and 92.5% Nitrogen was used to gas the culture for 30 seconds in a 25 mL culture flask. The flasks were quickly closed and put into an incubator (RS Biotech Laboratory Equipment Ltd., UK) at a temperature of 37ºC in 5 per cent O_2, 5 per cent CO_2 and 90 per cent Nitrogen. Parasites were allowed to grow for 3 days before use in the assay.

3.3.1.4. Parasite preparation and in vitro antiplasmodial Assay

After three weeks of adaptation and growth of the parasites in the culture, they were harvested at the ring stage (trophozoites) and initial parasitaemia estimated for each sample concentration using Giemsa stained slides and light microscope at 100X magnification. Samples were then processed and 2% haematocrit with 1% parasitaemia prepared using uninfected blood to make a total of 14mL parasite mixture in a complete culture medium. One hundred microliters (100 µL) of each of the nine dilutions (1000 µg/mL, 500 µg/mL, 250 ug/mL, 125 µg/mL, 62.5 µg/mL, 31.3 µg/mL, 15.6, 7.8 µg/mL and 3.9 µg/mL) were plated in duplicate 96 well coastal plate. Test control drug, 200 ng/mL artesunate was plated alongside the *Mist Amen Fevermix* and *Edhec Malacure* herbal mixtures. One hundred microliters of the parasite were mixed with 2 per cent haematocrit and 1 per cent parasitaemia was added to each treated well starting from the 2nd well to the tenth well. One hundred microliters of parasite mixture were added to the 11th wells as a negative control. The procedure was repeated for the other five samples and the plates were arranged in a modular Chamber under an atmosphere of 5% Oxygen, 5% Carbon dioxide and 90% Nitrogen and kept at 37°C for 72 hours. The assay was paused by adding 100 uL lysing buffer containing SYBR Green to each 96-well micro-titre plate and

was thoroughly and gently spun to avoid the production of bubbles. The *in vitro* activities on strains of *P. falciparum* were then determined (Izumiyama *et al*., 2009). A thin blood smear was prepared on microscope slides, fixed in absolute methanol, stained with 10% Giemsa in phosphate buffer under sterile conditions in a laminar flow safety cabinet (Hitachi Clean Bench, Japan) for 10 minutes. The slides were dried and observed under a compound light microscope using 100X oil immersion objective lens and also using FLUOstar OPTIMA Fluorometer plate reader with control software version 2.20 at 470 nm and 520 nm wavelengths (Hout *et al*., 2006; Lambros and Vanderberg, 1979). Various IC_{50} values were then determined.

The level of parasitaemia was estimated by measuring lactate dehydrogenase activity (Kenmogne *et al*., 2006). The in vitro antiplasmodial results were expressed as the mean IC_{50} (the concentration of a drug that reduced the level of parasitaemia to 50%).

3.3.2. Acute Toxicity Testing (Single Dose Toxicity Testing)

The acute oral toxicity of *Mist Amen Fevermix* and *Edhec Malacure* was evaluated in Swiss albino mice according to the protocol from the Organization for Economic Co-operation and Development (OECD, 2001). Ten animals (male n = 5) and (female n = 5), nulliparous and non-pregnant), weighing 18-23g, obtained from the Noguchi Memorial Institute for Medical Research. The animals were maintained under ambient environmental conditions (22–25 °C, 12 hours/12 hours light/dark cycle) and had free access to a standard pellet diet, water *ad libitum* prior to the start of the study in the animal house of the Department of Pharmacology, KNUST. The mice were fasted for 16 hours before the test commenced. Each animal was subjected to treatment with a single dose of 5,000 mg/kg of the study product *per os* by gavage. Animals were observed individually for the first 30 minutes after dosing and then periodically during the first 24 hours with special attention during the first 4 hours, and daily thereafter for 3 days. The animals were observed for altered autonomic effects such as:

lacrimation, salivation, and piloerection, and central nervous system effect such as; tremors, convulsion, drowsiness, skin piloerection, body weight, food consumption, water consumption and mortality (Balogun and Ashafa 2016).

3.3.3. *In Vivo* Antiplasmodial Activity of Mist Amen Fevermix and Edhec Malacure

3.3.3.1. Experimental Animals

About eighty Swiss albino mice were bought from the Noguchi Memorial Institute for Medical Research (NMIMR), were housed in standard cages at a room temperature of 26°C, a constant lightdark schedule (12 hours light and 12-hour dark cycle). They were maintained on a standard feed (pellets) and water was given *ad libitum*. The chloroquine-sensitive strain of *P. berghei* was donated by the Department of Pharmacology, KNUST.

3.3.3.2. Ethical Approval

Ethical approval for the use of experimental animals was obtained from the Ethics Committee on Animal Studies, Department of Pharmacology, KNUST. The care and use of experimental animals described in the rationale and methodology of this research are in accordance with the goals, outcomes and considerations defined in the guide for care and use of laboratory animals, by the Committee for the update of this guide, National Research Council of the National Academies (2010).

3.3.3.3. Inoculation of Experimental Animals with Parasite

Cryo-frozen stock of parasitized red blood cells (PRBCs) was diluted with phosphate-buffered saline (PBS) based on parasitaemia level of each donor and the RBC count of normal mice, such that 1 mL blood contained 5×10^7 *P. berghei* strain parasites. The study animals were each inoculated intraperitoneally with 1×10^7 RBCs (Basir *et al.*, 2012).

3.3.3.5. Evaluation of the Suppressive Activity (Peter's 4-Day Test)

Suppressive activity of *Mist Amen Fevermix* and *Edhec Malacure* were evaluated in *P. berghei* infected Swiss albino mice using the method described by Knight and Peters (1980). Twenty mice

were randomly divided into four groups of five each. Group one was positive control, group two negative control, groups three and four for *Mist Amen Fevermix* and *Edhec Malacure* (test products) respectively. On the first day (D_0), the mice in all the groups were each infected with 1×10^7 *P. berghei* infected RBCs. Three hours later, the study animals in Group one (positive control group) were administered artesunate (5 $mgkg^{-1}$) intraperitoneally while groups three and four received *Mist Amen Fevermix* and *Edhec Malacure* orally at the stated dose of 4.56 mgkg-^{1} and 2.234 $mgkg^{-1}$ bodyweights respectively for four consecutive days ($D_0 - D_3$). Group 1 (negative control) received normal saline. The body weight of each mouse was measured before infection (D_0) and on the fifth day (D_4) using a sensitive digital analytical weighing balance. On the fifth day (D_4), a thin blood film was made from the tail blood of each study animal, fixed in methanol and stained with Giemsa to reveal parasitized erythrocytes out of 500 in a random field of the microscope. Parasitaemia was determined by light microscopy using a 100X objective lens and the following equation:

$$\%\ \text{Parasitaemia} = \frac{\text{Total number of parasitized erythrocytes}}{\text{Total number of erythrocytes counted}} \times 100$$

The average percentage of chemo suppression was calculated from the formula:

$$\%\ \text{Suppression} = \frac{\text{Parasitemia in Negative Control} - \text{Parasitemia in Test Group}}{\text{Parasitemia in Negative Control}} \times 100$$

Average percentage chemo-suppression was calculated as:

$$100〔(\mathrm{A} - \mathrm{B})/A]$$

Where **A** is the average percentage parasitaemia in the negative control group and **B** is the average percentage parasitaemia in the test group.

3.3.3.6. Evaluation of the Prophylactic Activity

The prophylactic antiplasmodial activity of *Mist Amen Fevermix* and *Edhec Malacure* were evaluated using the method described by Peters (1965). The twenty mice were randomly divided into four

groups of five albino mice each. Group 1 (negative control) was treated with normal saline, Group 2 (positive control) 1.2 mgkg-^{1} of pyrimethamine, group 3 and 4 (test groups) were treated with *Mist Amen Fevermix* 4.56 mgkg-^{1} and *Edhec Malacure* at dose of 2.234 mgkg^{-1} (The differences in doses is based on the dose stated on the labels). The administration of the test samples and pyrimethamine continued for three consecutive days ($D_0 – D_2$). On the fourth day (D_3), the mice were inoculated with 10^7 *P. berghei* and parasitemia level was assessed by blood smear 72 hours later.

3.3.3.7. Evaluation of the Curative Activity (Rane's Test)

The curative antiplasmodial activity of *Mist Amen Fevermix* and *Edhec Malacure* were evaluated using the method described by Peters (1970). This was used to evaluate the schizontocidal activity of the products. About 1×10^7 *P. berghei* parasitized RBCs were injected intraperitoneally into each of thirty mice on the first day (D_0). Seventy-two hours later (D_3), the mice were randomly divided into five groups of five mice each. The study samples were administered orally at 2 dose levels; *Mist Amen Fevermix* (9.12 and 18.24) mg kg-^{1}, and *Edhec Malacure* (4.468 and 8.936) mg kg-^{1} respectively for three consecutive days ($D0 – D_2$). Two control groups (n = 5) were used namely; normal (infected and untreated), positive (infected and treated with 8 mg/kg artemether/lumefantrine). Blood samples were collected from the tip of the tails of the animals on day 4 and day 7 post-treatment. Giemsa stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor parasitaemia level. The body weight was measured before infection (D_0) and from the fourth day (D_3) to the eighth day (D_7) while the mean survival time (MST) of the mice in each treatment group was determined over 28 days ($D_0 – D_{28}$) as follows:

$$\text{MST} = \frac{\text{Sum of survival time of all mice in a group (days)}}{\text{Total number of mice in that group}}$$

The *in vitro, in vivo* studies and acute toxicity testing process of *Mist Amen Fevermix* and *Edhec Malacure* is represented schematically (Figure 3.2)

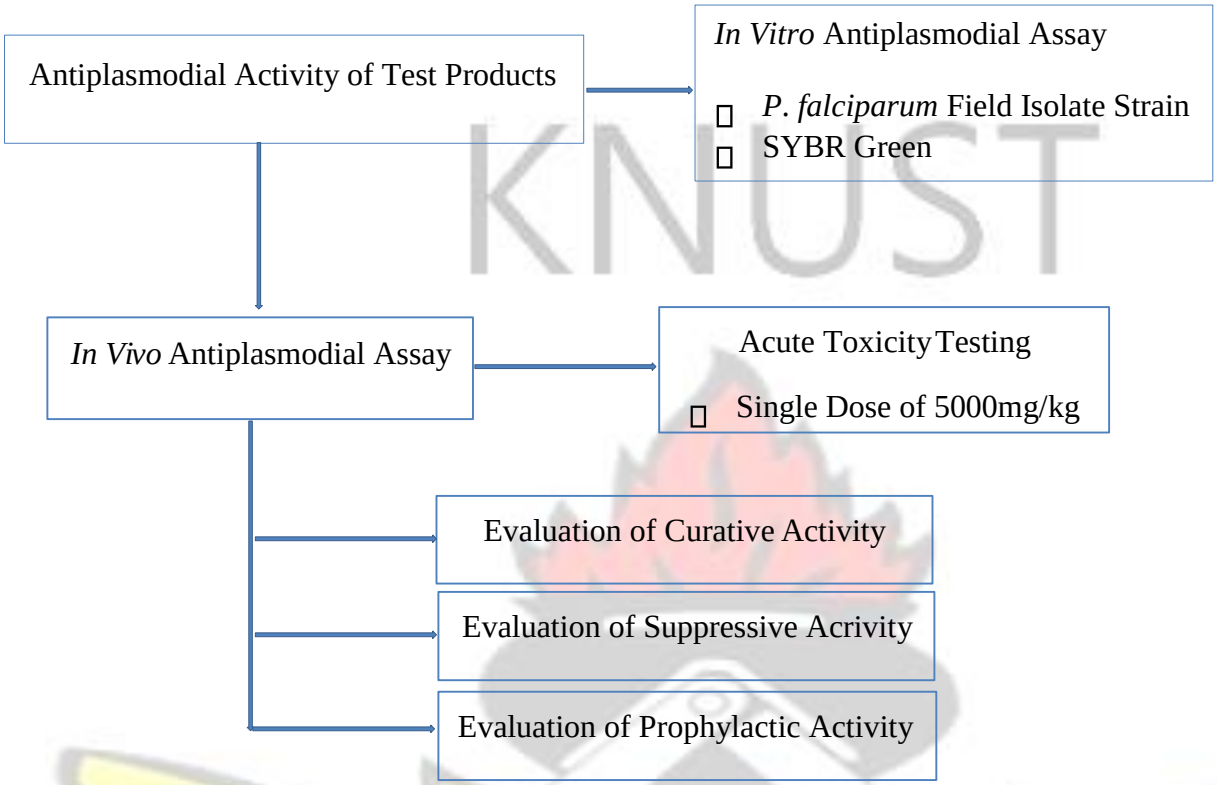


Figure 3.2: Schematic Representation of Efficacy Studies of *Mist Amen Fevermix* and *Edhec Malacure*.

3.4. CLINICAL ASSESSMENT OF THE SAFETY AND EFFECTIVENESS OF *MIST AMEN FEVERMIX* AND *EDHEC MALACURE*

3.4.1. Introduction

Clinical study is the most recognised and accepted form of evidence required for the safety and efficacy for any therapeutic agent. Currently, there is inadequate clinical data to support the continual usage of most herbal products. However, even when such evidence is available, questions have always been raised about the quality of the procedural process used in these evaluations. It is therefore recommended that, clinical evaluation, involving randomozed control trial, which is the gold standards for assessing medicines, is used to evaluate herbal products to provide the needed evidence that will safeguard the safety of the consumer (WHO, 2004).

The clinical study of *Mist Amen Fevermix* and *Edhec Malacure* was carried out to evaluate their safety and effectiveness to satisfy the criteria set forth by the WHO for medicinal products. The absence of any adverse reactions from preliminary studies (Turkson *et al*., 2015; Turkson *et al*., 2020) and acute toxicity study (section 3.3.2) provided a basis for the clinical studies to be undertaken. The procedures for the evaluation used in this study were in conformity to the recommendations of the Consolidated Standards for Reporting Trials (CONSORT) (Gagniera *et al*., 2006).

3.5. Methodology

3.5.1. Study Site

The study was conducted at the Herbal Medicine Unit of the Tafo Government Hospital, Kumasi, between July and November 2019. The Hospital serves about 261,584 people in Manhyia North submetro which constitutes 16 per cent of the population of the Kumasi Metropolis (Tafo Government Hospital, Annual Performance Review Report, 2018). The Hospital was established in 1976, as the Tafo Urban Health Centre and upgraded to hospital status in the year 2000. The Hospital lies on land extending from latitude 6^{o} $44^{'}$ $9^{''}$N and longitude 1^{o} $36^{'}$ $29^{''}$W in Manhyia North Sub-metro within Kumasi Metropolis, Ashanti region (www.gps-coorndinates.net) (Appendix 1).

The Hospital has a total of 42 beds for, male, female and children. The average daily attendance of patients is about 400. The Hospital provides a 24-hour service including nine specialist clinics: Herbal Medicine, Ear Nose and Throat, Eye Clinic, Dermatology, Urology, Paediatric, Diet Therapy, Physiotherapy, Obstetrics and Gynaecology.

The Herbal Medicine Unit started operation on 23rd January 2012 with one Medical Herbalist but currently has two other Medical Herbalists posted to the Herbal Unit on 22nd March 2012 and June, 2018 respectively.

3.5.2. Health Team of Tafo Government Hospital

The Tafo Government Hospital has a staff strength of 219 with 202 being permanent staff and 17 casual workers (Table 3.2).

Table 3.2: Health Team Members

Health Care Members	Number
General Practitioners	7
Gynaecologist	2
Surgeon	1
Dermatologist	1
Urologist	1
Physician Assistant (Medical)	5
Medical Herbalists	3
Nurse Practitioners	2
Pharmacists	6
Nurses	113

(Annual performance Review Report, Tafo Government Hospital, 2018)

3.5.3. Study Design

The research design employed is a prospective, open-proof, comparative clinical trial and data was collected using a structured questionnaire (Appendix 2). All data were collected and written in a case record folder (CRF) of the Herbal Unit of the Tafo Government Hospital between August to November 2019.

3.5.4. Patients Selection Criteria and Monitoring for Malaria

3.5.4.1. Inclusion Criteria

Patients were recruited and managed as outpatients in a normal clinical setting. The selection criteria included the following:

- Gender: Male and female
- Age: 18 to 45 years

- Disease state: Uncomplicated malaria ○ Absence of severe anaemia ○ Presence of axillary temperature ~37.5 and < 39.5°C at visit Informed consent of participants (Appendix 3). Patient able and willing to return for follow up.

3.5.4.2. Exclusion Criteria

- Participants with anaemia (haemoglobin <8g/dl)
- Patients on treatment with orthodox antimalarial
- Any disease condition which might compromise the renal, hepatic or any other body system
- Intake of any medication within 14 days before the start of the study
- Presence of clinically significant abnormal laboratory results during screening
- Pregnant women
- Use of any recreational drugs or a history of drug addiction
- Any chronic and communicable disease condition (WHO, 2004).

The clinical study process for the safety and effectiveness of *Mist Amen Fevermix* and *Edhec Malacue* is represented schematically (Figure 3.3).

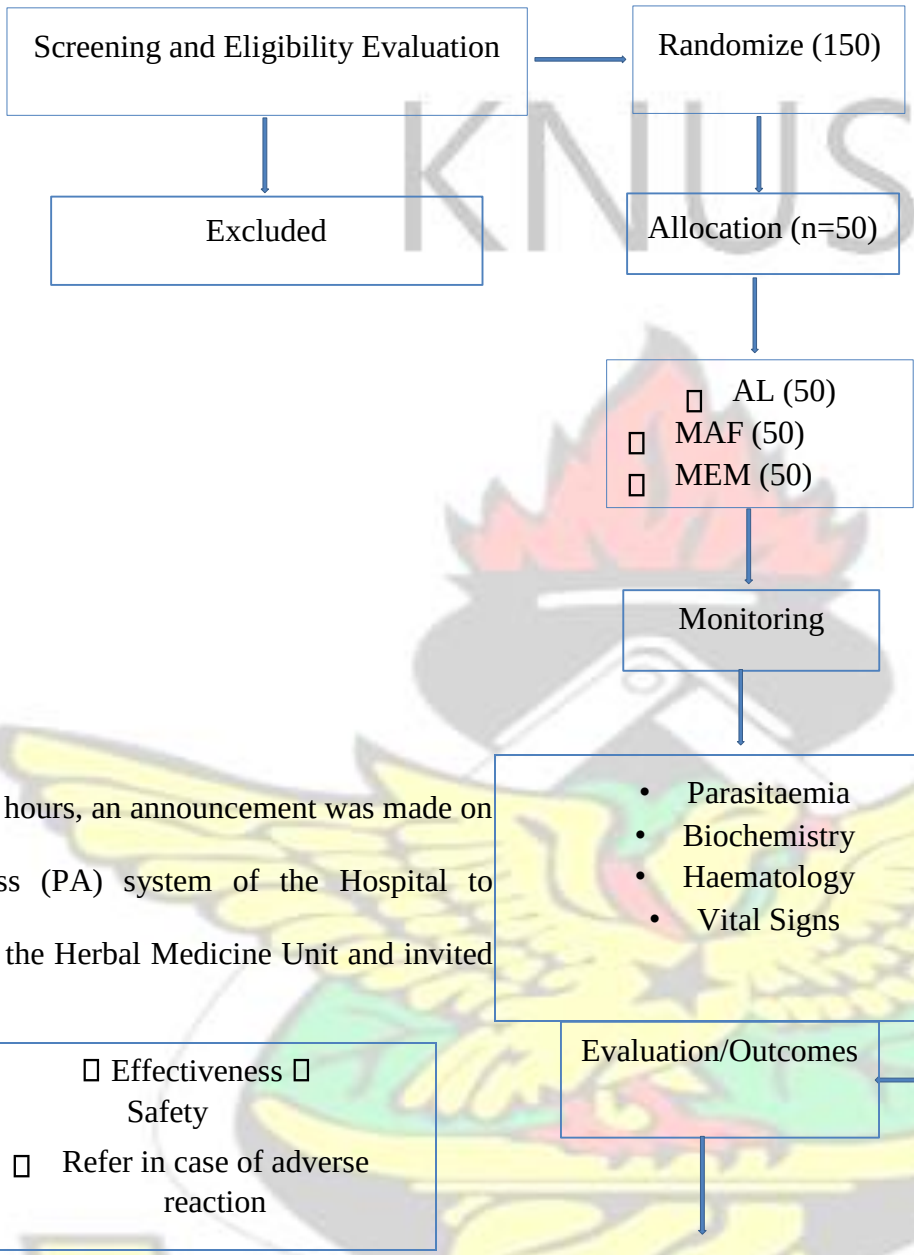


Figure 3.3: Schematic Representation of Clinical Study

3.5.5. Recruitment of Participants

During out-patient department's (OPD) herbal medicine clinic hours, an announcement was made on the public address (PA) system of the Hospital to inform patients about the Herbal Medicine Unit and invited clients who were willing to use the services of the unit. The participants who volunteered and presented with malaria signs and symptoms were informed about the study. They were examined and made to undergo laboratory tests to confirm the presence of malaria parasites or otherwise. Those with uncomplicated malaria were made to do the following laboratory investigations; renal and hepatic panel tests, and full blood count (FBC).

A total of 150 participants were recruited with 50 in each arm of the test products and 50 in the control group of study. The participants were briefed and enrolled with their consent. The participants were randomly selected.

3.5.6. Withdrawal from study

The withdrawal criteria for participants involved in the study were recorded as persons who were unable to comply with the protocol and those who developed any reaction to the test samples were withdrawn from the study and referred to the OPD to be attended to.

3.5.7. Sample Size Calculation

The population size of 50 participants (males and females) on each arm of the study was used. This was based on total attendance for 2017 and 2018. The sample size was determined according to Pocock's formula for the sample size for a dichotomous or continuous response (Pocock, 1983).

$$n = \frac{[P1\,(1-P1) + P2\,(1-P2)]}{(P1-P2)^2} \times (Z\alpha/2 + Z\beta)^2$$

Where:

n required sample size

P₁ estimated proportion of study outcome in the exposed group ***P₂***

estimated proportion of study outcome in the unexposed group **α**

is the level of statistical significance

$\mathbf{Z_{\alpha/2}}$ represents the desired level of statistical significance (typically 1.96 for 95% for α=0.05)

$\mathbf{Z_{\beta}}$ represents the desired power (typically 0.84 for 80% power)

n for each group *2=total sample (i.e. for the two groups)

3.5.8. Ethical Consideration

Recruitment of participants was done after approval for the study was obtained from the Committee for Human Research, Publications and Ethics (CHRPE), Kwame Nkrumah University of Science and Technology, School of Medical Sciences and Komfo Anokye Teaching Hospital (CHRPE/AP/424/19). The study was conducted in accordance with the protocol and Good Clinical and Laboratory Practice (GCLP) to ensure the protection of all aspects of the ethical rights and welfare of study participants (WHO, 2009). An emergency team headed by a medical officer with a public health background was constituted as required for ethical clearance during the study period. This was to ensure that participants who may experience any andverse reactions would be attended to.

3.5.9. Informed Consent Forms

Participants were asked to complete an informed consent form. The details of the clinical study were explained to participants in the local dialect or the language of choice by the principal investigator before forms were signed or thumb printed.

3.6. Artemether/Lumefantrine, *Mist Amen Fevermix and Edhec Malacure* Administration

Mist Amen Fevermix and *Edhec Malacure* were dispensed according to recommended dosing for seven days. Each participant was given three bottles of the product, making a total of one hundred and fifty (150) bottles for participants on *Mist Amen Fevermix* and (150) bottles for participants on *Edhec Malacure*. Also, tablet Artemether/Lumefantrine (80/480mg) was dispensed according to recommended dosing for three days. Each participant was given one pack containing six tablets of the product, making a total of fifty (50) packs.

3.6.1. Dosing

Mist Amen Fevermix was dispensed at the recommended dose of 45 mL thrice daily after meals and *Edhec Malacure* at 30 mL thrice daily after meals for seven days. Artemether/Lumefantrine was dispensed at the recommended dose of (80/480mg) twice daily after meals for three days.

3.6.2. Monitoring Participants for Malaria

Patients were monitored and reviewed on days; 3, 7, 14, 21 and 28. During the review period, the history was retaken and assessment was made to establish treatment outcomes and any side effect noted. Examination of blood films for malaria parasites was also done at the review.

On the 7^{th}, 14^{th} and 28^{th}-day visits, clinical evaluation of the patients, remission of signs and symptoms; using a checklist for signs and symptoms (Appendix 4) or otherwise were noted: full blood count to check for malarial parasites, liver and kidney panel tests were conducted and any side effects recorded using a checklist (Appendix 5 WHO, 2004).

3.6.3. Data Collection

Demographic data (age, gender, marital status, and education) of participants were captured and entered the moment they were enrolled in the study. Codes were given to participants to ensure their identity was anonymous. Adverse reaction, recurrence of signs and symptoms, and quality of life were also recorded accordingly.

3.7. Clinical Assessment of the Effectiveness of *Mist Amen Fevermix* and *Edhec Malacure*

The efficacy of *Mist Amen Fevermix* and *Edhec Malacure* were assessed based on the clinical outcomes after the duration of treatment (laboratory outcome). Treatment was measured by the clearance of parasite at the end of the study.

3.8. Clinical Assessment of the Safety of *Mist Amen Fevermix* and *Edhec Malacure*

The reagents (Tridem Eng., Italy) for the tests (LFT, KFT, and FBC) were all purchased from Tridem Chemicals, Kumasi, Ghana.

The following vital signs, parameters (Blood pressure, temperature, body weight) of all participants enrolled in the study were taken on days (0, 3, 7, 21 and 28). Haematological tests were done by using Abacus 5 Differential Haematology Analyzer (Diatron MI Zrt, Hungary) and the hepatic function and renal function tests were done by using Faith Mindray BS-230 Auto Clinical Chemistry Analyzer (BS-120/BS-200/BS-240, China).

Hepatic and renal panel test and FBC baseline parameters were compared at the end of the study. This was done in relation to the reference range and, any significant change in a parameter, whether below or above the accepted reference range was considered to have compromised the integrity of the said parameter.

3.9. Assessment of Quality of Life and Adverse Reaction

This was done by using Karnofsky's performance status scale (Appendix 4). A high score is an indication that there was an improvement in the condition and therefore the quality of life improved in the course of the study and indication of the effectiveness of the study products.

3.10. Data Analysis

Data on the safety and effectiveness studies of *Mist Amen Fevermix* and *Edhec Malacure* were statistically analysed using IBM Statistical Package for the Social Sciences (SPSS), version 19. Exploratory statistics were computed to measure the frequency distribution, central tendencies and dispersions of the data. Graph pad prism version 8 was used for the animal data analysis. The mean variables in both liver and kidney panel were calculated and statistically tested against the control range; a hypothesis was postulated. A paired sample t-test of the mean variables over the three subsequent visits to test the difference between the first visit and the second visit and then that of the second and the third. To this, a hypothesis was postulated. The null hypothesis was that the mean

variables at various visits was no different from each other or that the alternate hypotheses for the variables tested over the visits are not equal. The null hypothesis for the pairing of the first visit and second visit test is:

i. The mean levels of malaria parasite load are equal. The alternate hypothesis states that the first visit's level of malaria parasite load is not the same as the second visit.

ii. Similarly, the null hypothesis for the pairing of the second visit and third visit states that there is an equal level of malaria parasite loads and the alternate states there is a difference.

iii. Finally, the null hypothesis for the pairing of the third and fourth visit states that there is an equal level of malaria at both visits while the alternates state otherwise.

CHAPTER FOUR

RESULTS AND DISCUSSION 4.1. Quality Control Assessment of *Mist Amen Fevermix*, *Edhec Malacure* and Component

Plants

Mist Amen Fevermix, is a decoction with insipid taste, aromatic in odour and brown in colour. Also, *Edhec Malacure*, is a decoction, bitter in taste, aromatic in odour and brown in colour. Stem bark of *Morinda lucida*, was brown in colour, woody in odour and bitter in taste. Also, stem bark of *Parinari robusta*, was aromatic in odour, bitter in taste and brown in colour. Stem bark of *Mangifera indica* was sour in taste, brown in colour and aromatic in odour. Leaves of *Cleistopholis patens* was green in colour, leafy in odour and had a bitter in taste (Table 4.1). One of the quality parameters used in

the evaluation of finished herbal products is organoleptic evaluation. The present study established the organoleptic properties of *Mist Amen Fevermix* and *Edhec Malacure* with the help of the sensory organs such as colour, odour and taste (Table 4.2). Changes in these parameters may signal adulteration or detrerioration.

Mist Amen Fevermix and *Edhec Malacure*, contained all the phytochemical constituents analysed (Table 4.2). Also, *Morinda lucida* and *Mangifera indica* contained all the phytochemical constituents. *Parinari robusta* contained the phytoconstituents except alkaloids and phytosterols whereas tannins were the only constituents not detected in *Cleistopholis patens* (Table 4.2). Basic phytochemical screening revealed the presence of some secondary plant metabolites. These secondary metabolites included alkaloids, saponins, tannins, glycosides, flavonoids and steroidal compounds (Table 4.2). The secondary plant metabolites detected in the test samples have also been reported to be present in *Morinda lucida, Cleistopholis patens, and Mangifera indica*. These constituents have also been reported to exhibit antimalarial activities (Adeyemi *et al*., 2014; Oludare, 2018; Okwu and Ezenagu, 2008), the medicinal plants contained in *Mist Amen Fevermix* and *Edhec Malacure*. The activity of the test samples is due to the presence of the secondary metabolites they contain.

Table 4.1: Organoleptic Characteristics of *Mist Amen Fevermix, Edhec Malacure* and Component Plants

Characteristics	MAF	*MEM*	*Morinda lucida*	*Parinari robusta*	*Mangifera indica*	*Cleistopholis patens*
Dosage (Form)	Decoction	Decoction	Stem bark	Stem bark	Stem bark	Leaf
Taste	Insipid	Bitter	Bitter	Aromatic	Sour	Bitter
Odour	Aromatic	Aromatic	Woody	Woody	Aromatic	Leafy
Colour	Brown	Brown	Brown	Brown	Brown	Green

Key: MAF- *Mist Amen Fevermix, MEM- Edhec Malacure*

Table 4.2: Phytochemical Constituents of *Mist Amen Fevermix* and *Edhec Malacure* and their Plant Components.

Phytoconstituents	MAF	MEM	*Morinda lucida*	*Parinari robusta*	*Mangifera indica*	Cleistopholis patens
Alkaloids	+	+	+	-	+	+
Glycosides	+	+	+	+	+	+
Tannins	+	+	+	+	+	-
Saponins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Phytosterols	+	+	+	-	+	+

Key: MAF- *Mist Amen Fevermix, MEM- Edhec Malacure*

Four samples each of test products were used for the physichochemical analysis. *Mist Amen Fevermix* was found to be slightly acidic than *Edhec Malacure* (Table 3.4). The pH of the products was within the normal pH of the stomach (4-6.5) and also enhances the stability and absorption of medicines (www.alleganynutrition.com; Allen *et al*., 2011). The heavy metals: arsenic, cadmium, iron, mercury, manganese, nickel, lead, zinc, and copper were detected in *Mist Amen Fevermix* and *Edhec Malacure*. The levels of the heavy and non-heavy metals present were within the permissible limits (Table 4.3). This implies that, the two polyherbal products comply with safety regulations related to toxic metals (Gajalakshmi *et al*., 2012).

Table 4.3: Physicochemical Properties of *Mist Amen Fevermix* and *Edhec Malacure*

Physicochemical parameters	**Samples**		**Permissible limits (mg/kg)/reference**
	Mist Amen Fevermix	**Edhec Malacure**	
As	0.074±0.012	0.005±0.002	5.0(FAO/WHO., 1984).
Cu	0.013±0.003	4.384±0.852	(Ulla *et al*., 2012).
Cd	0.007±0.002	0.050±0.030	0.3 (FAO/WHO., 1984).
Fe	0.078±0.012	25.140±1.581	(Ulla *et al*., 2012).

Hg	0.011±0.002	0.00103±0.00019	0.5 (FAO/WHO., 1984).
Mn	0.285±0.065	2.309±0.087	(Ulla *et al*., 2012).
Ni	0.005±0.003	BDL	1.683 (FAO/WHO., 1984).
Pb	0.009±0.008	0.00147±0.00122	10 (FAO/WHO., 1984).
Zn	0.089±0.013	0.430±0.008	27.4
K	3.830±0.140	355.747±50.575	(Ulla *et al*., 2012).
Na	0.625±0.255	40.1053±1.1097	(Ulla *et al*., 2012).
pH	4.93±0.05	5.47±0.13	
Weight per mL g/mL	0.002362±0.022	0.001738±0.1.13	
Volume Per Bottle (mL)	330	500	

Key: BDL-Below detectable limit. Results are Mean ± S.E.M

4.1.2. Microbial Load Analysis

Four samples each of the test products were used for the analysis. Total bacterial and fungal counts detected in *Mist Amen Fevermix* (Table 4.4) and *Edhec Malacure* were within the specified set limit (Table 4.5) *Salmonella, Shigella* and *Pseudomonas* were absent. These microbial counts were below the maximum permissible limit of 1.0×10^5 cfu/mL. In addition, the amount of yeast and moulds in *Mist Amen Fevermix* was 1.09×10^3 cfu/mL and *Edhec Malacure* had 1.83×10^3 cfu/mL counts. The microbes present in Mist *Amen Fevermix* and *Edhec Malacure Mixture* were below the acceptable limit of 1.0×10^7 cfu/mL (BP, 2007). This implies that, *Mist Amen Fevermix* and *Edhec Malacure* were produced based on good manufacturing practices observed. This may have resulted from the pH of the products which was within suitable range (pH 5–8.5) to promote bacterial growth (Zamir *et al*., 2015). Also, contamination may result from unhygienic conditions in the manufacutirng unit coupled with improper handling of the starting materials, source of water and the manufacturing process. Plants materials used in the manufacture of herbal products may be contaminated by absorbing toxic metals from soil, water and air. In addition, some aspect of the manufacturing process; bottling,

capping and labelling can introduce microbes into the finished products (Gajalakshmi *et al*., 2012). Therefore, extreme care should be taken to minimize the introduction of these microbes into herbal drugs. Also, the sourcing of the starting materials should be from a reliable source and away from human settlement to reduce contamination.

Table 4.4: Microbial Load of *Mist Amen Fevermix*

Test	Results	Acceptable Limits (BP, 2018)
Total aerobic viable count NA; 37^{O}C; 24hrs) ≤1×10^{5} cfu/mL	1.27×10^{3}±0.06	Not more than 1.0×10^{7} cfu/mL
Test for Salmonella Shigella. (BSA/37^{O}C/48hrs. Nil/L)	0	Absent
Test for *Escherichia coli* (MAC/37^{O}C/48hrs. Nil/L)	0	Not more than 1.0×10^{2} cfu/mL
Test for *Pseudomonas* (PCA/37^{O}C/48hrs. Nil/L)	0	Absent
Test for yeast and moulds (PDC/SAB/25^{O}C/5 days)	1.09×10^{3}±0.08	Not more than 1.0×10^{5} cfu/mL

Results are Mean ± S.E.M

Table 4.5: Microbial Load of *Edhec Malacure*

Test	Results				Acceptable Limits
	A	B	AVE	SD	
					(BP, 2018)
Total aerobic viable count NA; 37^{O}C; 24hrs) ≤1×10^{5} cfu/mL			2.17×10^{3}±0.06		Not more than 1.0×10^{7} cfu/mL

Test for Salmonella Shigella. (BSA/37^{O}C/48hrs. Nil/L)	0	Absent
Test for *Escherichia coli* (MAC/37^{O}C/48hrs. Nil/L)	0	Not more than $1.0\times10_2$ cfu/mL
Test for *Pseudomonas* (PCA/37^{O}C/48hrs. Nil/L)	0	Absent
Test for yeast and moulds (PDC/SAB/25^{O}C/5 days)	$1.83\times10_3\pm0.6$	Not more than $1.0\times10_5$ cfu/mL

Results are Mean ± S.E.M

4.2. FT-IR Spectroscopic Analysis

The FT-IR fingerprint of *Mist Amen Fevermix* showed two characteristic peaks at 3332.22 cm^{-1} (broad) and 1636.99 cm^{-1} (weak) (Figure 4.1). Similarly, two characteristic peaks were recorded by *Edhec Malacure* at 3316.94(broad) cm^{-1} and 1636.76 cm^{-1} (weak) (Figure 4.2). the FT-IR fingerprint of the plant components with their respective characterisitc peaks were documented (Figures 4.3-4.6). In order to establish the identity of component plants as well as adulteration and the purity of *Mist Amen Fevermix* and *Edhech Malacure*, chemical fingerprinting and profiling of the test samples and their plant components was done. This assessment involved FT-IR spectroscopy analysis. The respective wave numbers produced (Figures 4.1 and 4.2) were indicative of the type of chemical bonds and functional groups that may be present in *Mist Amen Fevermix* and *Edhec Malacure*. The FT-IR spectra can be used as characteristic fingerprint for the quality evaluation of *Mist Amen Fevermix* and *Edhec Malacure.* In addition, it can be used to assess the possibility of adulteration in

Mist Amen Fevermix and *Edhec Malacure.* FT-IR spectroscopy helps authenticate herbal products. Similarly, FT-IR spectroscopy has also been used to identify adulterants in finished herbal products (Black *et al*., 2016).

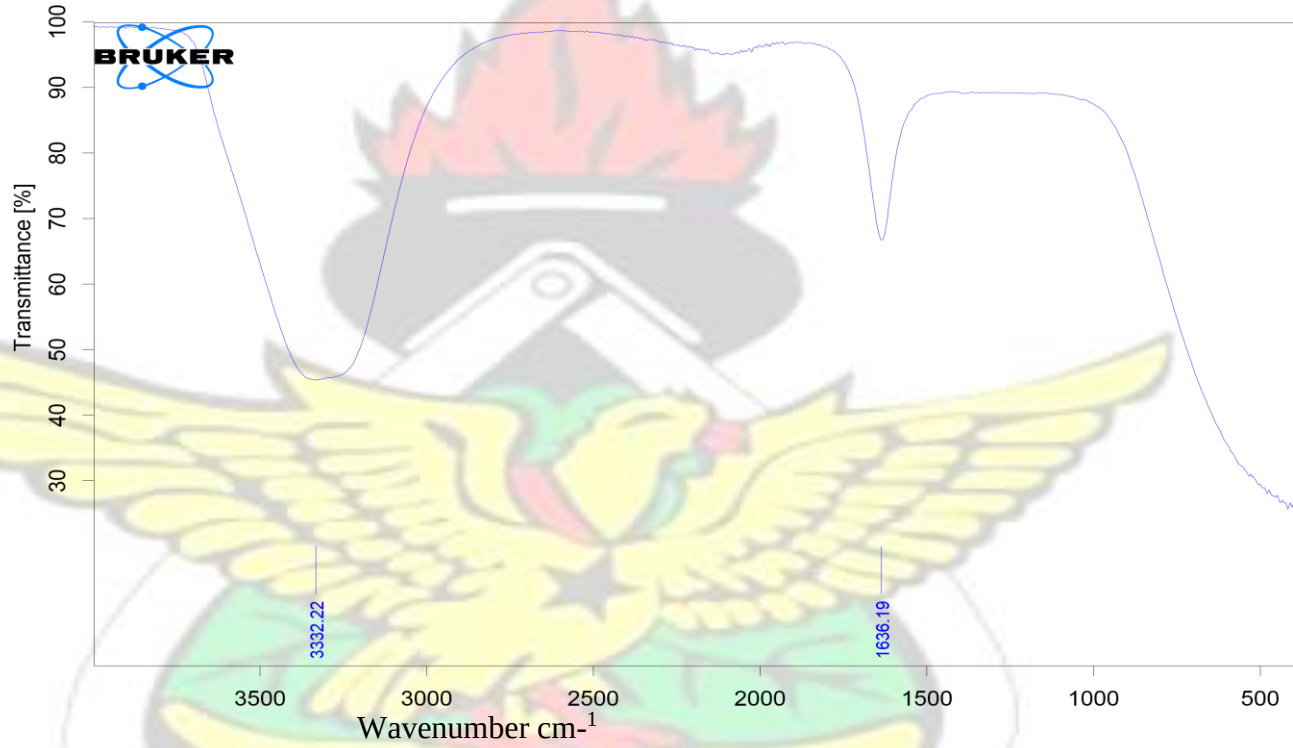


Figure 4.1: IR Characteristic Fingerprint of Mist Amen Fevermix

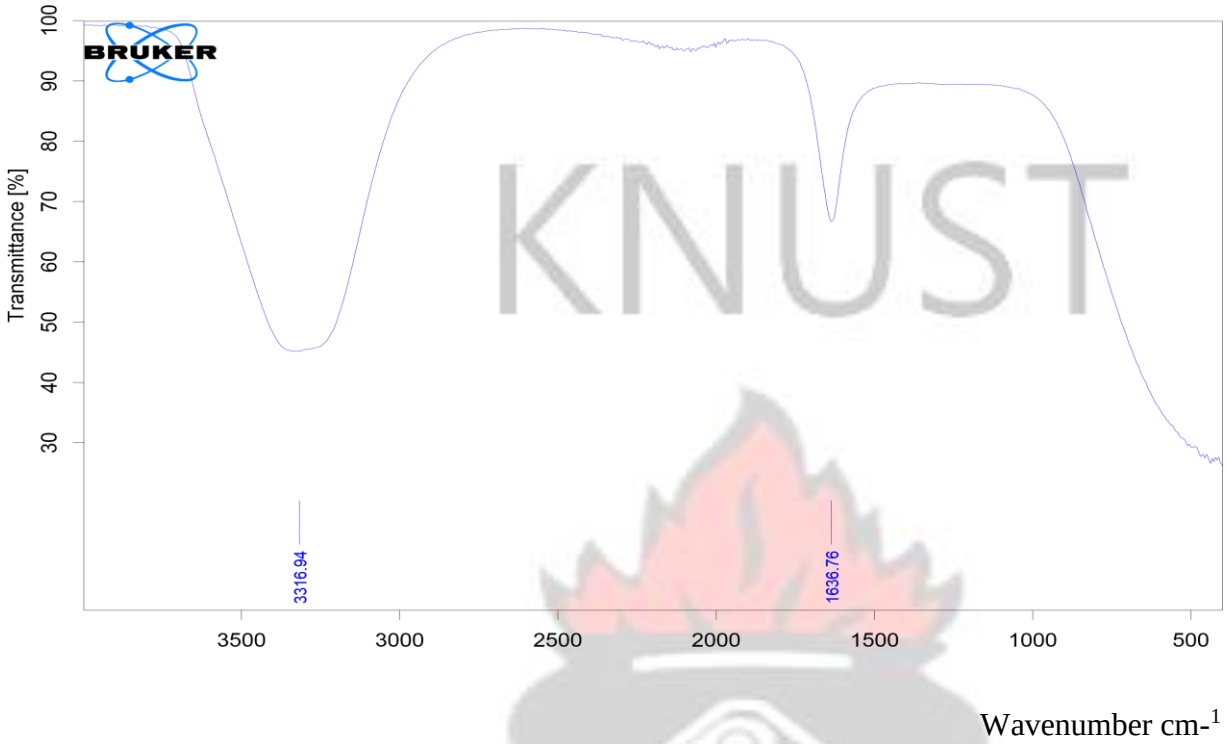


Figure 4.2: IR Characteristic Fingerprint of *Edhec Malacure*

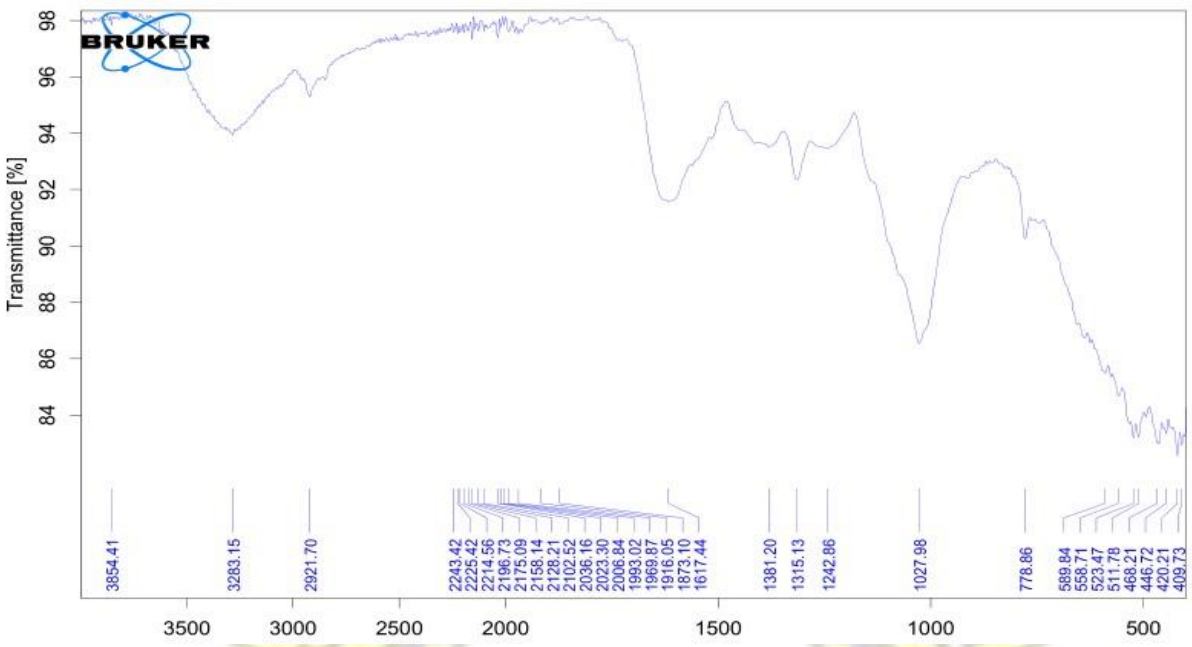


Figure 4.3: IR Characteristic Fingerprint of *Morinda lucida*

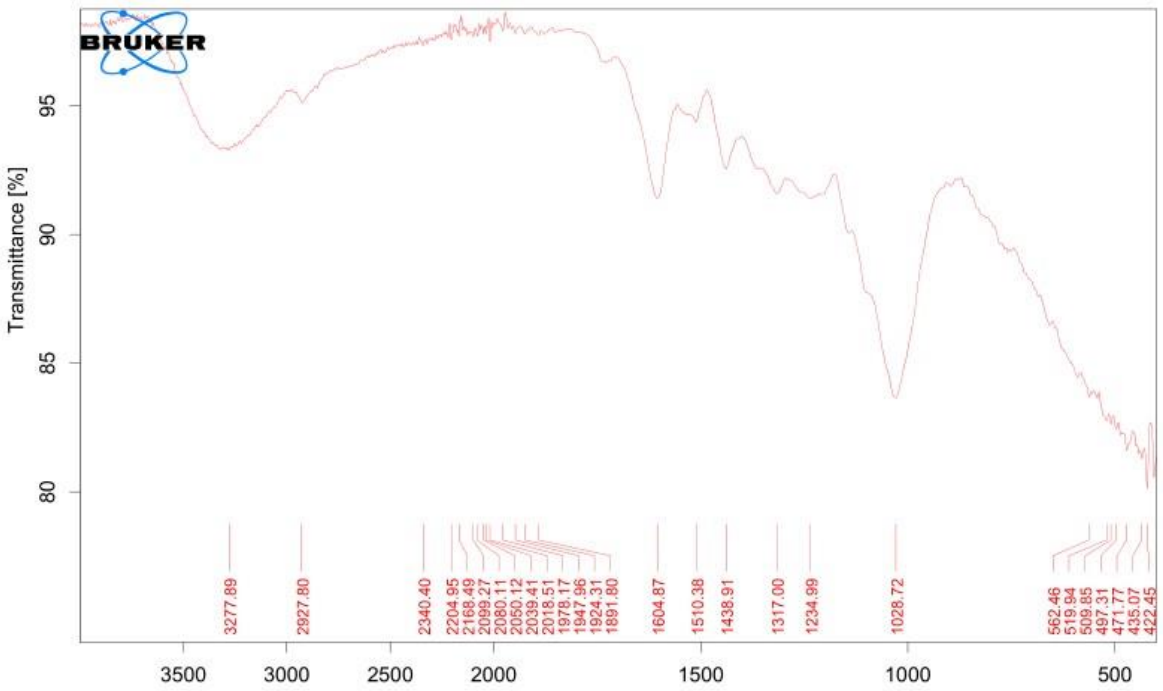


Figure 4.4: IR Characteristic Fingerprint of *Parinari robusta*

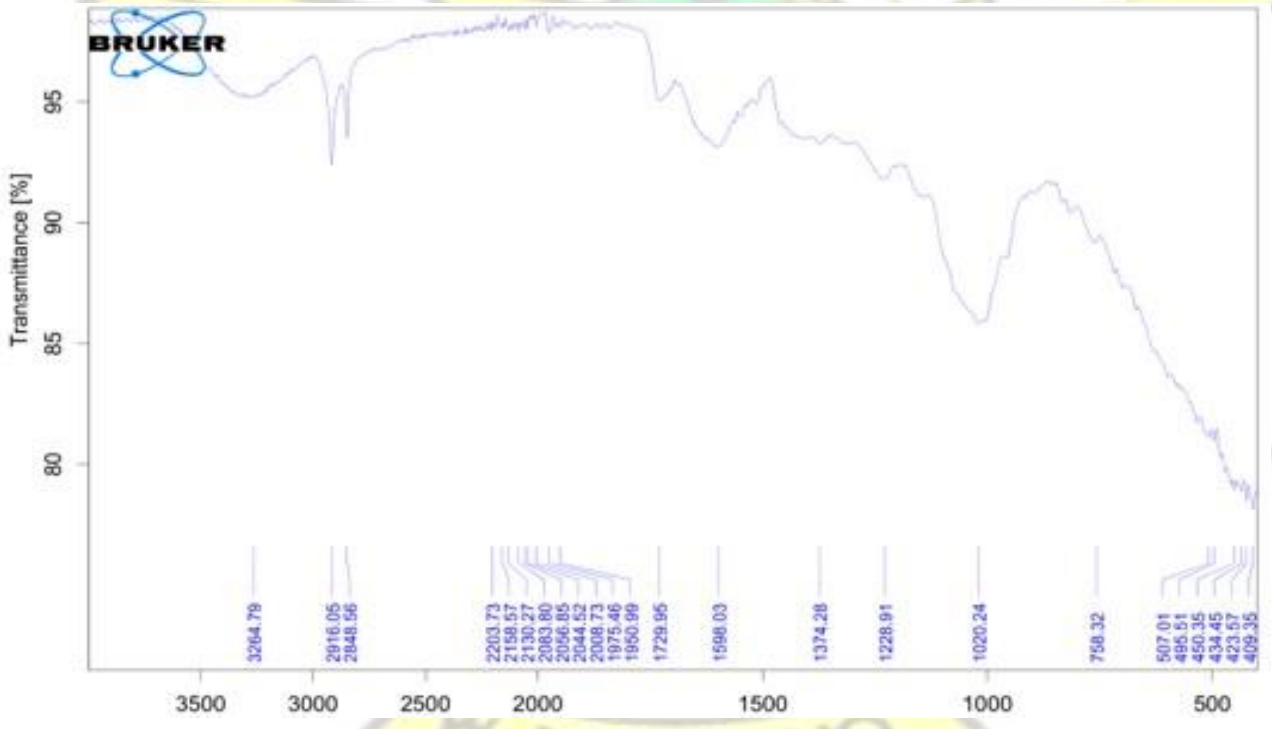
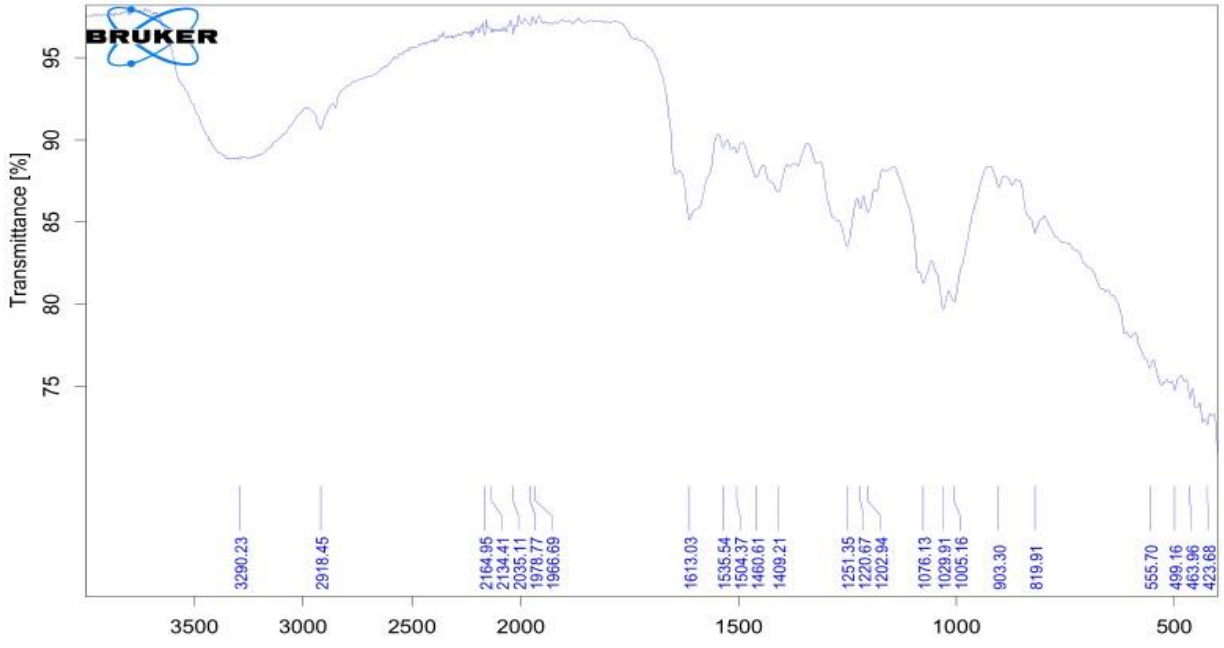


Figure 4.5 IR Characteristic Fingerprint of *Cleistopholis patens*



Wavenumber cm-[1] **Figure 4.6: IR Characteristic Fingerprint of *Mangifera indica***

4.3. Chromatographic Characterization

The HPLC chromatogram of *Mist Amen Fevermix* produced 7 prominent peaks at a wavelength of 210 nm, all eluting within 17.70 minutes (Appendix 9). Similarly, *Edhec malacure* produced 13 prominent peaks eluting within 17.79 minutes (Appendix 10). Also, the component plants, *Morinda lucida* produced 11 prominent peaks eluting within 21.82 minutes (Appendix 11), *Parinari robusta* produced 7 prominent peaks eluting within 21.88 minutes (Appendix 12), *Cleistopholis patens* on the other hand produced 21 prominent peaks eluting within 22.29 minutes (Appendix 13) and *Mangifera indica* produced 6 prominent peaks eluting within 17.83 minutes (Appendix 14). The HPLC chromatograms can be used as characteristic fingerprint for *Mist Amen Fevermix* and *Edhec Malacure.* In addition, it can be used to assess the possibility of adulteration in *Mist Amen Fevermix* and *Edhec Malacure.* HPLC has been successfully used for characterization of herbal products (Boligon *et al*., 2014).

4.4. Chemical Profiling to Identify the Presence of Component Plants in Test Products

4.4.1. *Mist Amen Fevermix* and Component Plant Materials

There were 8 peaks identified in the *Mist Amen Fevermix* fingerprint (Appendix 9), 11 peaks in *Morinda lucida* (Appendix 11) and 7 peaks in *Parinari robusta* (Appendix 12). Some of these peaks were observed to be similar to two or more of the test samples. A similarity analysis then carried out to identify the common peaks, especially peaks present in both *Mist Amen Fevermix* and either of the plant materials or both. Due to potential peak shifting, which could arise from variations in the chromatographic conditions, the retention times were converted to relative retention times for direct comparison. One prominent peak was selected as the reference peak to calculate the relative retention times of the other peaks in each of the chromatograms (Figure 4.7). The results showed that there were common peaks (peaks 2, 8 and 13) (Table 4.6) to *Mist Amen Fevermix* and the constituent's plants, *Morinda lucida* and *Parinari robusta*. These peaks were identical, and their similarity was further confirmed with their percentage deviations which were not more than 5% (Table 4.6). Some of the peaks (3) also showed up in *Mist Amen Fevermix* and the two plants; for example, peaks 3 and 9 were present in *Mist Amen Fevermix* and *Parinari robusta* while peak 11 was present in *Mist Amen Fevermix* and *Morinda lucida*. Figure 4.7 is a fingerprint of the plants and the product in a comparative mode. In addition to the above peaks (2, 8 and 13), there were also peaks 12 and 15. This depicted that the two plants shared some similar chemical constituents between them.

Table 4.6: Relative retention times for identified peaks in the chromatographic fingerprints of Amen Fevermix and constituents' plant materials

	Amen Fevermix		*Morinda lucida*			*Parinari robusta*			Comment (if any)
	Retention time (mins)	Relative retention time	Retention time (mins)	Relative retention time	% deviation from Fevermix	Retention time (mins)	Relative retention time	% deviation from Fevermix	
Peak 1	-	-	2.44	0.17	-	-	-	-	
Peak 2	2.68	0.19	2.64	0.19	0.00	2.83	0.20	5.00	
Peak 3	4.01	0.28	-	-	-	4.00	0.28	0.00	
Peak 4	9.92	0.70	-	-	-	-	-	-	
Peak 5	-	-	11.70	0.82	-	-	-	-	
Peak 6	-	-	12.76	0.90	-	-	-	-	
Peak 7	-	-	13.33	0.94	-	-	-	-	
Peak 8	14.21	1.00	14.22	1.00	0.00	14.29	1.00	0.00	Reference peak
Peak 9	16.05	1.13	-	-	-	16.18	1.13	0.00	
Peak 10	16.25	1.14	-	-	-	-	-	-	
Peak 11	17.40	1.22	17.40	1.22	0.00	-	-	-	
Peak 12	-	-	17.82	1.25	-	17.89	1.25	-	
Peak 13	18.87	1.33	19.26	1.35	1.50	19.21	1.34	0.75	
Peak 14	-	-	20.65	1.45	-	-	-	-	
Peak 15	-	-	21.82	1.53	-	21.88	1.53	-	

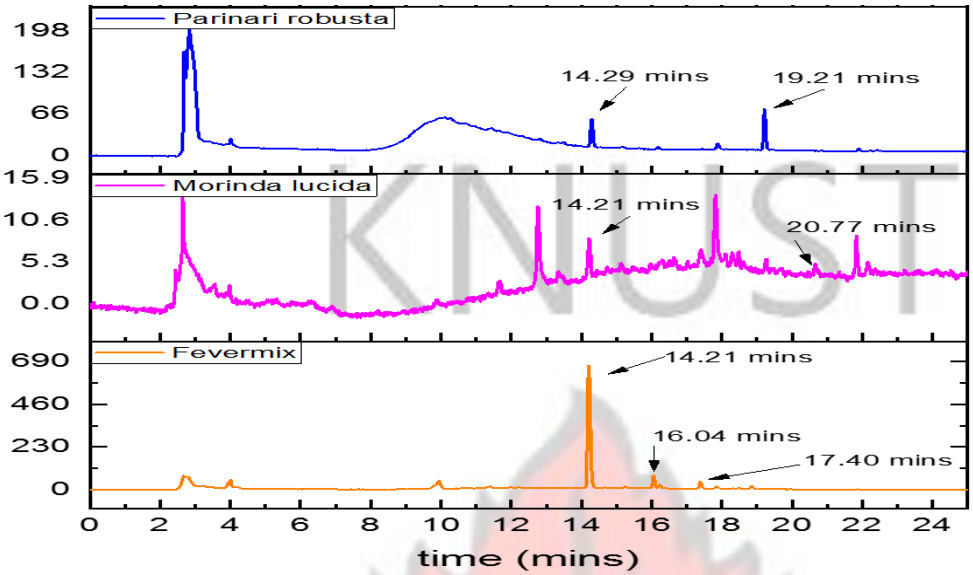


Figure 4.7: HPLC Spectra of Mist Amen Fevermix, Morinda lucida and Parinari robusta

4.4.2. *Edhec Malacure* and its Component Plants

Similarity analysis was carried out on *Edhec Malacure* (Appendix 10) and its component plant materials. Chromatographic fingerprints of the aqueous extract of the stem bark of *Morinda lucida* (Appendix 11), aqueous extract of the stem bark of *Mangifera indica* (Appendix 14) and aqueous extract of the leaf of *Cleistopholis patens* (Appendix 13) were obtained and compared by determining the relative retention times using a common peak which appeared in all the samples as the reference peak (peak 20). It was observed from the analysis that 32 peaks in all were identified. Out of this number, 13 of them were identified in the fingerprint of *Edhec Malacure* (Appendix 11), while 10, 6 and 21 were respectively identified in the fingerprints of *Morinda lucida* (Appendix 10) *Mangifera indica* (Appendix 14) and *Cleistopholis patens* (Appendix 13). Three peaks (peaks 16, 20 and 25) were found to occur in all the plant materials and the herbal product as well. These peaks were also thought to be similar as their percentage deviations were not more than ± 5% (Table 4.7). Figure 4.8 shows the fingerprints of the samples in a comparative mode.

Table 4.7: Relative retention times for peaks in the chromatographic fingerprints of Edhec Malacure and components plant

	Edhec Malacure		*Morinda lucida*			*Mangifera indica*			*Cleistopholis patens*			Comment (if any)
	Retention time (mins)	Relative retention time	Retention time (mins)	Relative retention time	% deviation from Fevermix	Retention time (mins)	Relative retention time	% deviation from Fevermix	Retention time (mins)	Relative retention time	% deviation from Fevermix	
Peak 1	-	-	2.44	0.17	-	-	-	-	-	-	-	
Peak 2	2.67	0.19	2.64	0.19	-1.08	-	-	-	-	-	-	
Peak 3	-	-	-	-	-	-	-	-	2.74	0.19	-	
Peak 4	-	-	-	-	-	-	-	-	3.09	0.22	-	
Peak 5	-	-	-	-	-	-	-	-	3.99	0.28	-	
Peak 6	-	-	-	-	-	-	-	-	6.62	0.47	-	
Peak 7	-	-	8.39	0.59	-	-	-	-	-	-	-	
Peak 8	9.17	0.65	-	-	-	-	-	-	-	-	-	
Peak 9	9.95	0.70	-	-	-	-	-	-	-	-	-	
Peak 10	10.23	0.72	-	-	-	-	-	-	-	-	-	
Peak 11	10.9	0.77	-	-	-	-	-	-	10.88	0.76	-0.39	
Peak 12	11.4	0.80	-	-	-	-	-	-	11.37	0.80	-0.47	
Peak 13	11.69	0.82	-	-	-	11.69	0.82	0.14	-	-	-	
Peak 14	12.47	0.88	-	-	-	-	-	-	-	-	-	
Peak 15	-	-	-	-	-	12.63	0.89	-	12.64	0.89		
Peak 16	12.76	0.90	12.76	0.90	-0.11	12.76	0.90	0.14	12.78	0.90	-0.05	
Peak 17	13.34	0.94	-	-	-	13.34	0.94	0.14	13.29	0.93	-0.58	
Peak 18	13.87	0.98	-	-	-	-	-	-	13.91	0.98	0.08	
Peak 19	-	-	14.14	0.99	-	-	-	-	-	-	-	
Peak 20	14.2	1.00	14.22	1.00	0	14.18	1	0	14.23	1.00	0	Reference peak
Peak 21	-	-	-	-	-	-	-	-	15.01	1.05	-	
Peak 22	-	-	-	-	-	-	-	-	15.67	1.10	-	
Peak 23	-	-	-	-	-	-	-	-	16.45	1.16	-	
Peak 24	-	-	17.38	1.22	-	-	-	-	-	-	-	

Peak 25	17.79	1.25	17.82	1.25	0.04	17.83	1.257405	0.366206273	17.83	1.25	0.01	
Peak 26	-	-	18.28	1.29	-	-	-	-	18.33	1.29	-	
Peak 27	-	-	-	-	-	-	-	-	19.39	1.36	-	
Peak 28	-	-	-	-	-	-	-	-	19.61	1.38	-	
Peak 29	-	-	20.65	1.45	-	-	-	-	-	-	-	
Peak 30	-	-	21.83	1.54	-	-	-	-	21.84	1.53	-	
Peak 31	-	-	-	-	-	-	-	-	22.01	1.55	-	
Peak 32	-	-	-	-	-	-	-	-	22.29	1.57	-	

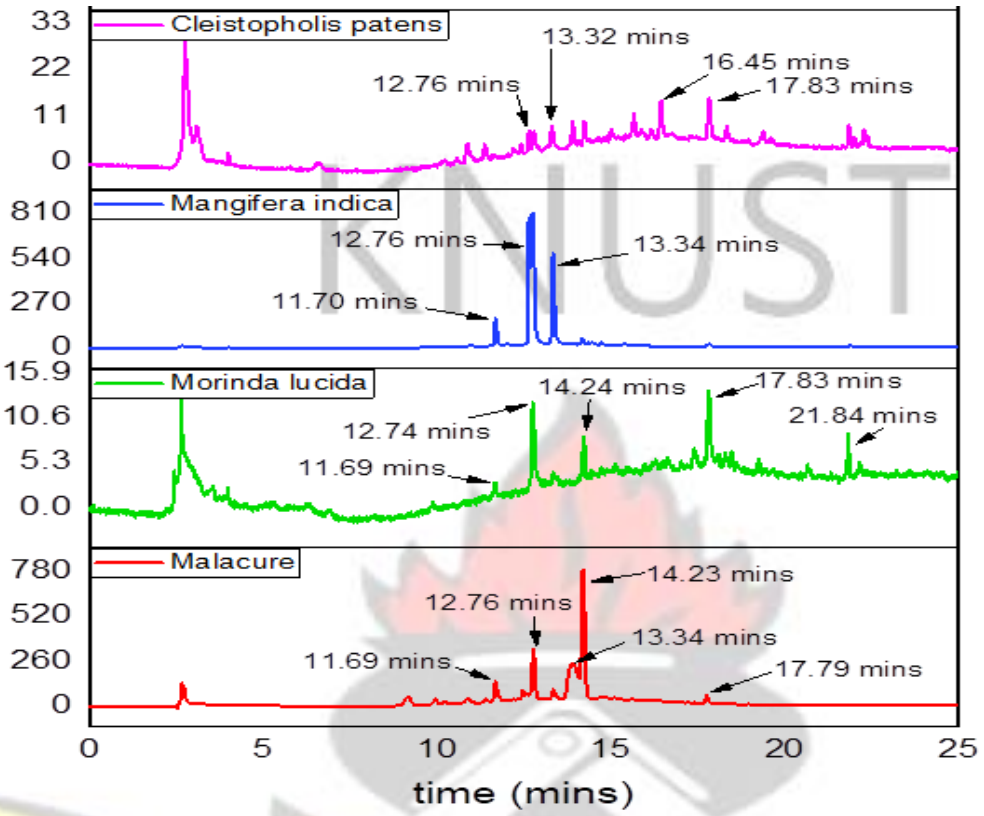


Figure 4.8: HPLC Chromatogram of Edhec Malacure, Morinda lucida, Cleistopholis patens and Mangifera indica

4.4.3. Result of HPLC Comparative Chromatographic Analysis of *Mist Amen Fevermix* and *Edhec Malacure*

The constituents of *Mist Amen Fevermix* and *Edhec Malacure* are different except for one prominent peak eluting at 11.48 minutes (Figure 4.9).

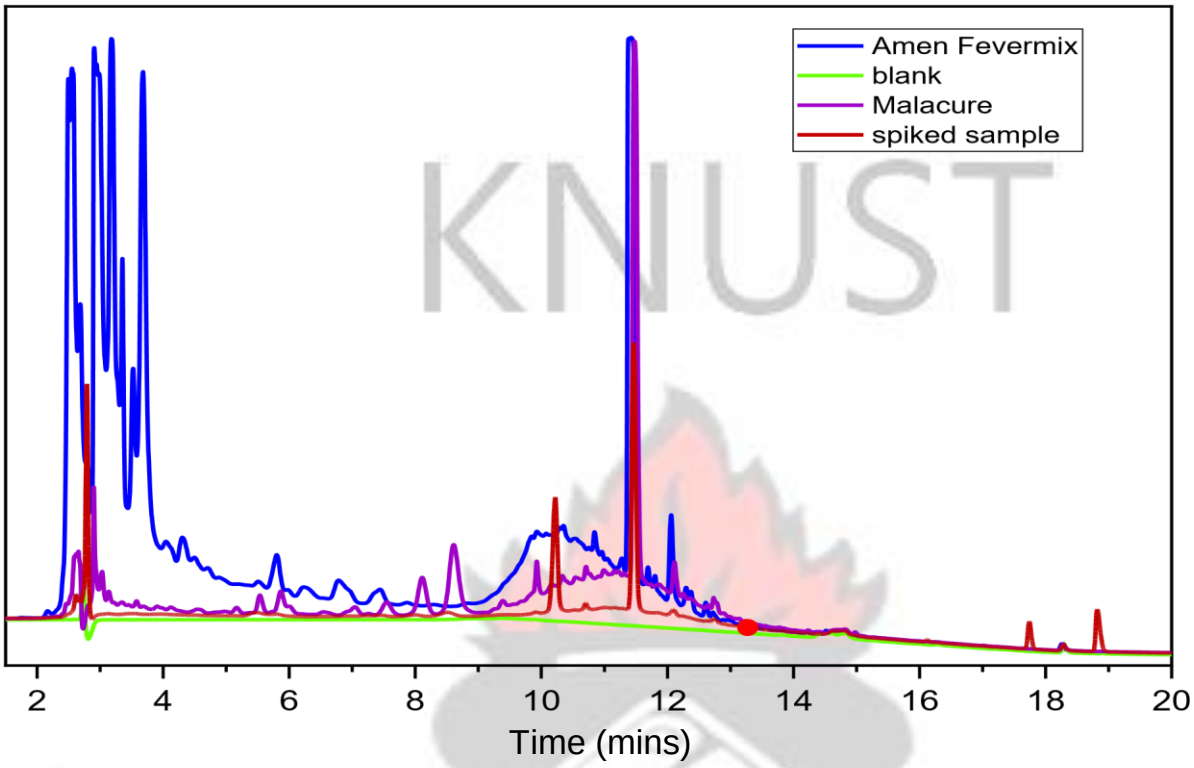


Figure 4.9: HPLC Chromatogram for *Mist Amen Fevermix* and *Edhec Malacure*

4.5. Results of Chemometric Profile

4.5.1. Chemometric Profile of *Mist Amen Fevermix*

The IR spectrum of *Mist Amen Fevermix* (Figure 4.1 and Figure 4.10) and component plants; *Morinda lucida* (Figure 4.3) and *Parinari robusta* (Figure 4.4) as well as that of *Edhec Malacur*e (Figure 4.2 and Figure 4.12) and its component plants, *Morinda lucida* (Figure 3. 5), *Cleistopholis patens* (Figure 4.5) and *Mangifera indica* (Figure 4.6), were recorded within the spectral range, 400 cm^{-1} to 4000 cm^{-1}. From the IR spectra, some key functional groups were evident; the following key bonds N-H, O-H, C-H, C=C and C-O stretches as well as aromatic overtones were evident. The presence of these bonds partially confirms the presence of the phytochemicals identified in the products and plant materials. For example, the presence of the N-H bond may be indicative of the presence of alkaloids,

which were shown to be present in the test sample (Table 4.2). Most of the peaks below 1500 cm^{-1} may be attributable to a number of functional groups (fingerprint region). Further exploration of the

IR data using Hierarchical Cluster Analysis was performed using Ward Method with Squared Euclidean (Randriamihamison *et al*., 2020; Ward, 1963) distance type for *Mist Amen Fevermix* and its component plants (Figure 4.10), while Ward Method with Pearson correlation distance type was adopted for *Edhec Malacure* with its plant constituents (Figure 4.12). Similarities were observed in one instance, between *Morinda lucida* and *Parinari robusta* at a similarity level of 52.41% (Figure 4.11) and then between *Morinda lucida* and *Edhec Malacure* at a similarity level of 52.81% and between *Mangifera indica* and *Cleistopholis patens* at a similarity level of 91.58% (Figure 4.13). The similarities in the chromatograms and spectra of the various samples were analysed. Upon aligning all the peaks, the reference chromatograms and dendrograms were generated. IR spectral analysis of *Mist Amen Fevermix, Morinda lucida* and *Parinari robusta* (Figure 4.10) using chemometrics, it was realized that there were some similarities. This implies that *Morinda lucida* and *Parinari robusta* were contained in *Mist Amen Fevermix.* This observation supports the use of chemometric approaches to identify the presence of a plant material in an herbal product (Sima *et al*., 2018). Chemometric analysis and the resultant dendrogram, showed similarity between *Morinda lucida* and *Edhec Malacure* at a similarity level of 52.81% and that between *Magnifera indica* and *Cleistopholis patens* at a similarity level of 91.58% (Figure 4.13). This is an indication that the plant materials may be present in *Edhec Malacure.* This may serve as a quality control indicator for the authentication of the finished product.

The outcomes from FTIR fingerprinting (dendrograms) (Figures 4.11 and 4.13) confirms the results of the chromatographic fingerprints, such that the plant materials in each of the products contained plant constituents which have been demonstrated from the spectral and chromatographic

fingerprinting analysis to be present in the products. The results and observations made from this study confirm a study which established the authentication and identification of the components of dietary supplements which were achieved with HPLC and IR combined with chemometric evaluation of data (Sima *et al*., 2018).

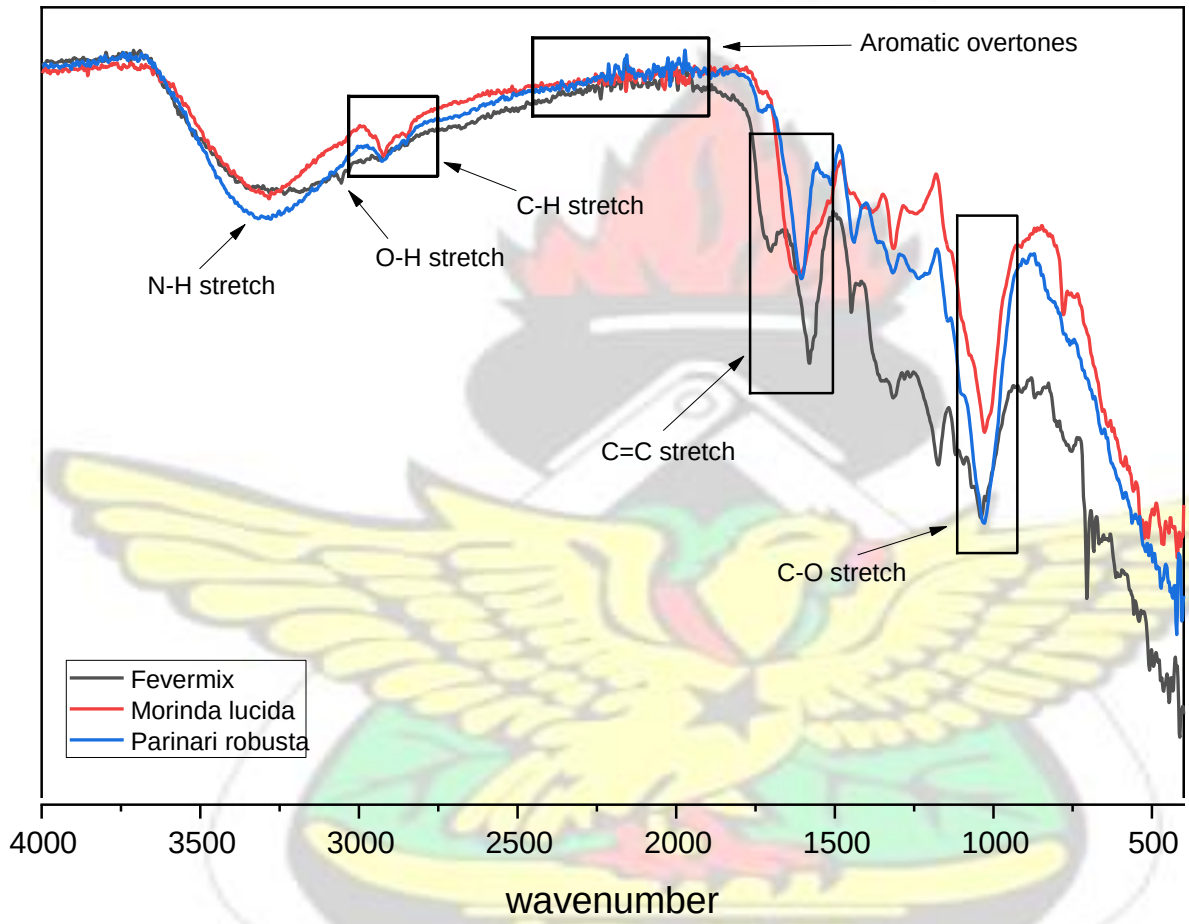


Figure 4.10: IR Spectra of Mist Amen Fevermix, Morinda lucida and Parinari robusta

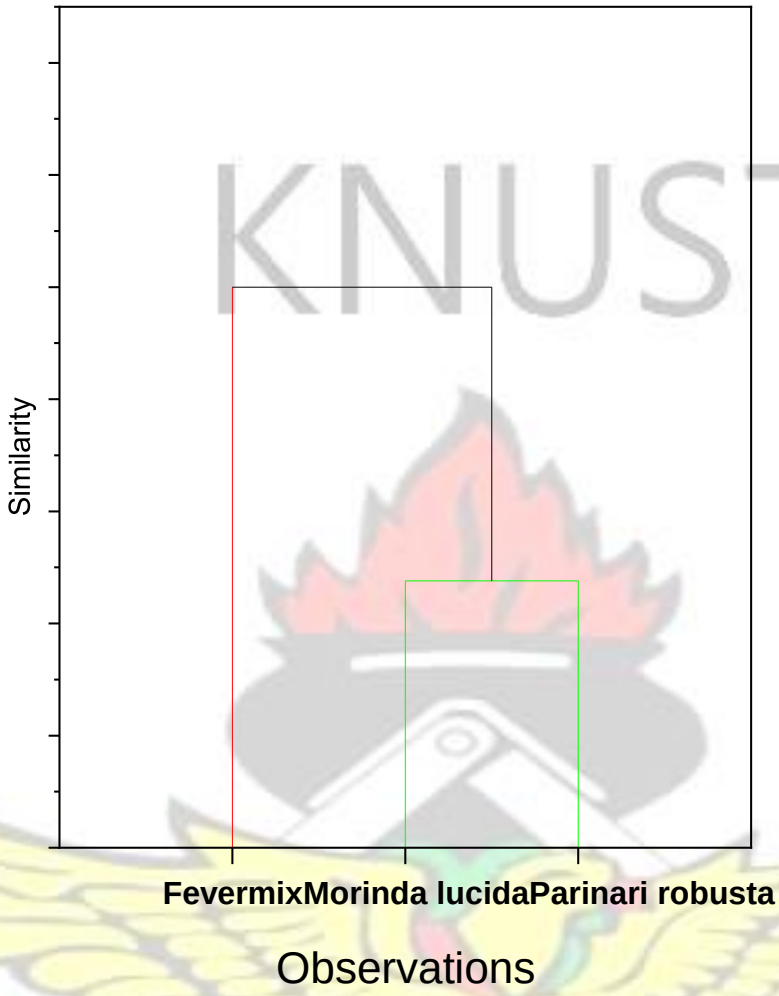


Figure 4.11: Dendrogram obtained for Mist Amen Fevermix, Morinda lucida and Parinari robusta

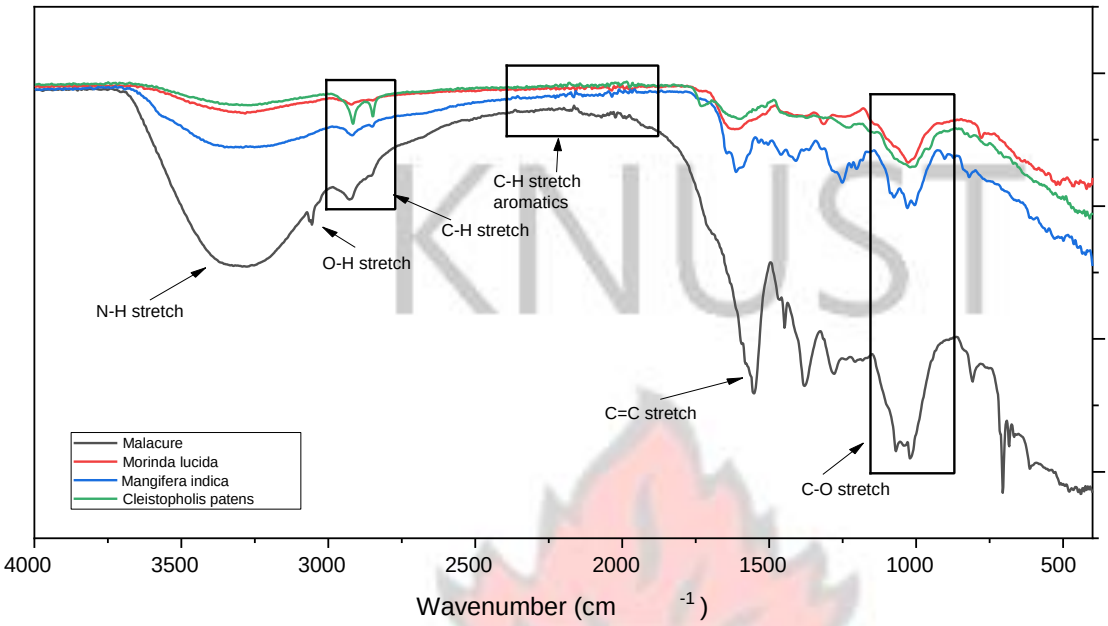


Figure 4.12: IR Spectra of Edhec Malacure, Morinda lucida, Mangifera indica and Cleistopholis patens

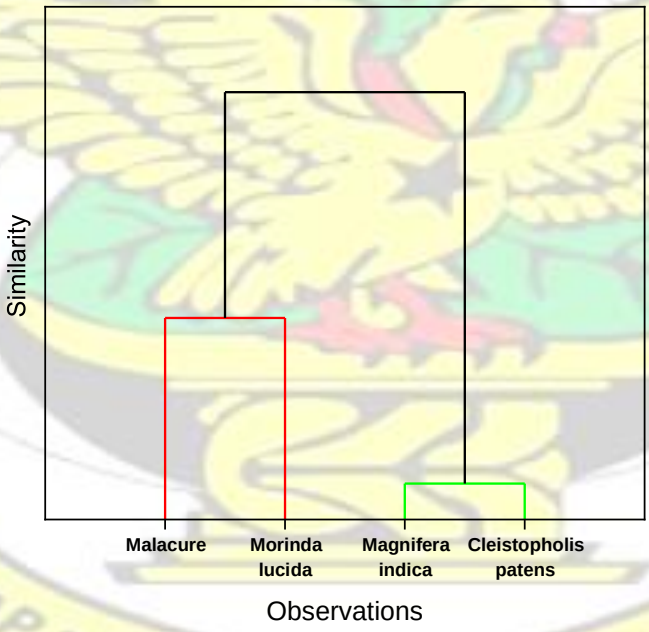


Figure 4.13: Dendrogram obtained for Edhec Malacure, Morinda lucida, Mangifera indica and Cleistopholis patens

4.6. Results of Chromatographic Analysis for Adulteration

The purity and the possibility of adulteration of *Mist Amen Fevermix* and *Edhech Malacure* with artemether, lumefantrine and quinine was assessed using HPLC (Tables 4.8, 4,9, Figures 4.17, 4.19A and 4.19B) and *Edhech Malacure* (Figures 4.18, 4.20A, 4.20B). It was established that the test products were not adulterated.

4.6.1. Validation of Chromatographic Method

The chromatographic method developed was validated for linearity and range, precision, recovery and system suitability according to guidelines by the International Conference on Harmonisation (ICH) (ICH, 1997).

4.6.2. Linearity and Range

An assessment of the peak area (y-axis) versus concentration (x-axis) revealed that, Artemether had a regression equation of y = 1070.4x + 7934.9, Lumefantrine's regression equation was y = 29326x + 36079 and that of quinine was y = 23781x - 3270.3 (Table 4.8). The correlation coefficients (R^2) of Artemether was 0.993 and that of Lumefantrine and Quinine was 0.999 (Table 4.8). The retention times for Artemether, Lumefantrine and Quinine were 17.71, 18.76 and 10.18 minutes respectively (Table 4.9). The total run time was 23.1minutes. Concentration range of 100-500mg/l (100, 200, 300, 400, and 500mg/L) for Artemether, 2.5-40mg/L (2.5, 5, 10, 20, 40mg/L) for Lumefantrine and 10160mg/L (10, 20, 40, 80 and 160mg/L) for Quinine were used (Table 4.9). All these three analytes gave linear curve plots. In addition, they gave a very good correlation coefficient for the selected concentration range for the individual analytes. Calibration curve plots obtained for concentrations injected (Figures 4.14-4.16).

Table 4.8: Validation Data from the Calibration Curves of the Standard Antimalarial Drugs

Antimalarial Drugs	Regression Equation	Correlation Coefficient (R^2)	Linearity Range (mg/ml)
Artemether	y = 1070.4x + 7934.9	0.993	100-500
Lumefantrine	y = 29326x + 36079	0.999	2.5-40
Quinine	y = 23781x - 3270.3	0.999	10-160

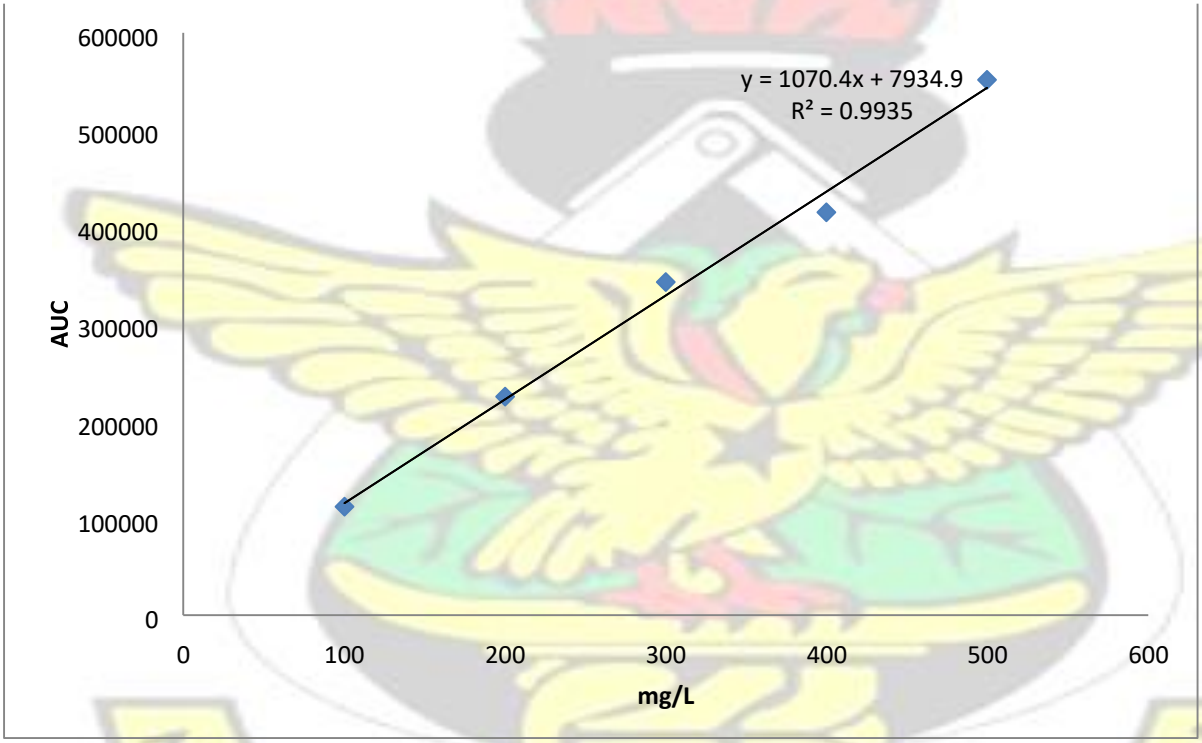


Figure 4.14: Calibration curve of Artemether

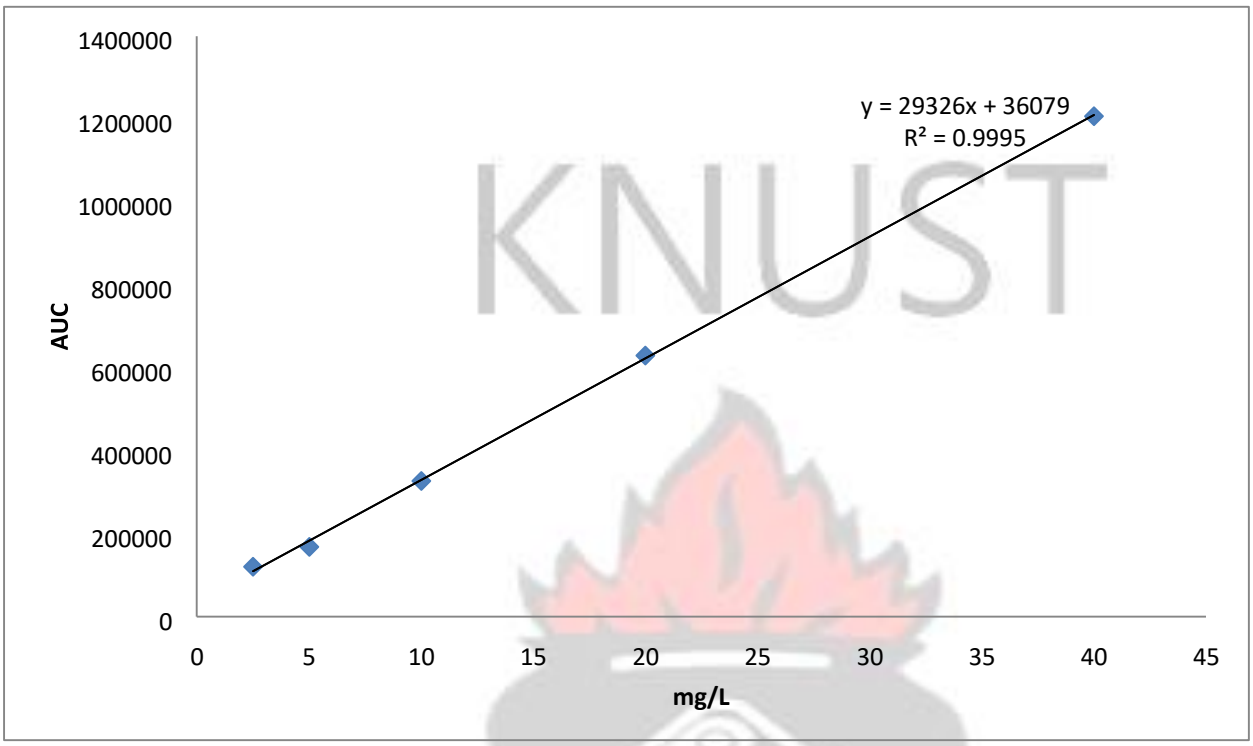


Figure 4.15: Calibration curve of Lumefantrine

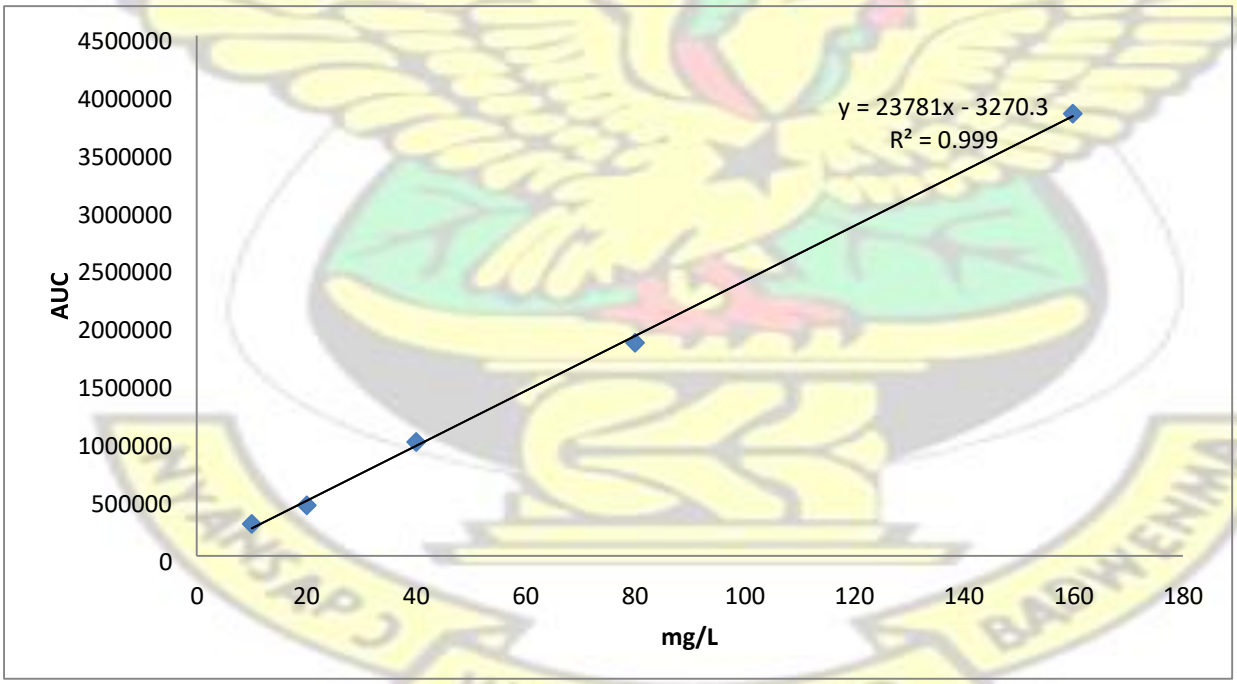


Figure 4.16: Calibration curve of Quinine

4.6.3. Accuracy and Recovery

The peak area produced indicated percentage recovery for artemether as 94.214±2.292%, lumefantrine 92.696±2.172% and quinine as 99.226±5.022% (Table 4.9). The calibration curves were performed in triplicate.

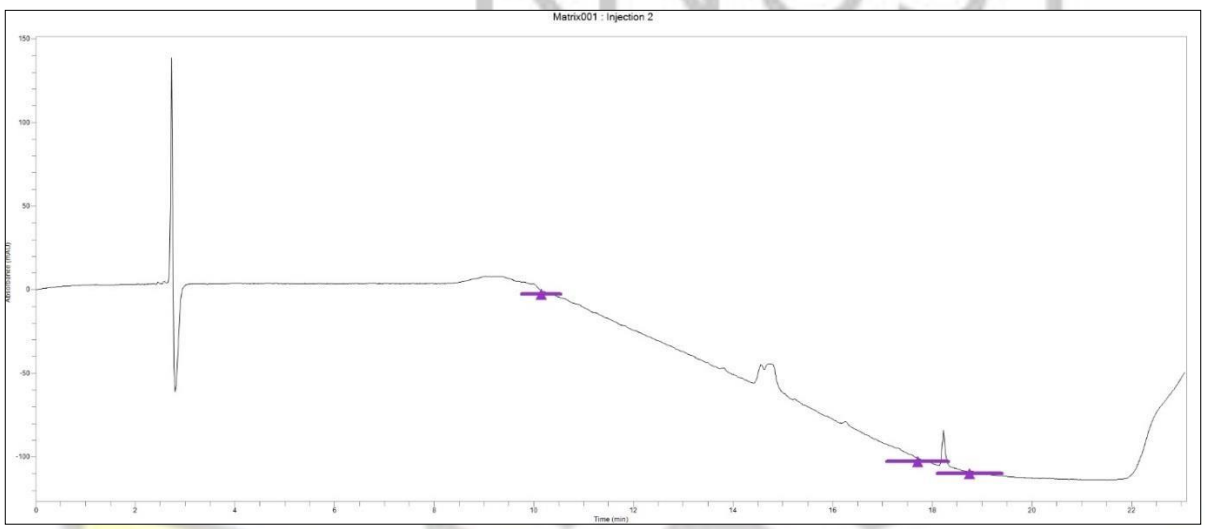


Figure 4.17: HPLC Linearity, Precision and Recovery test (Blank)

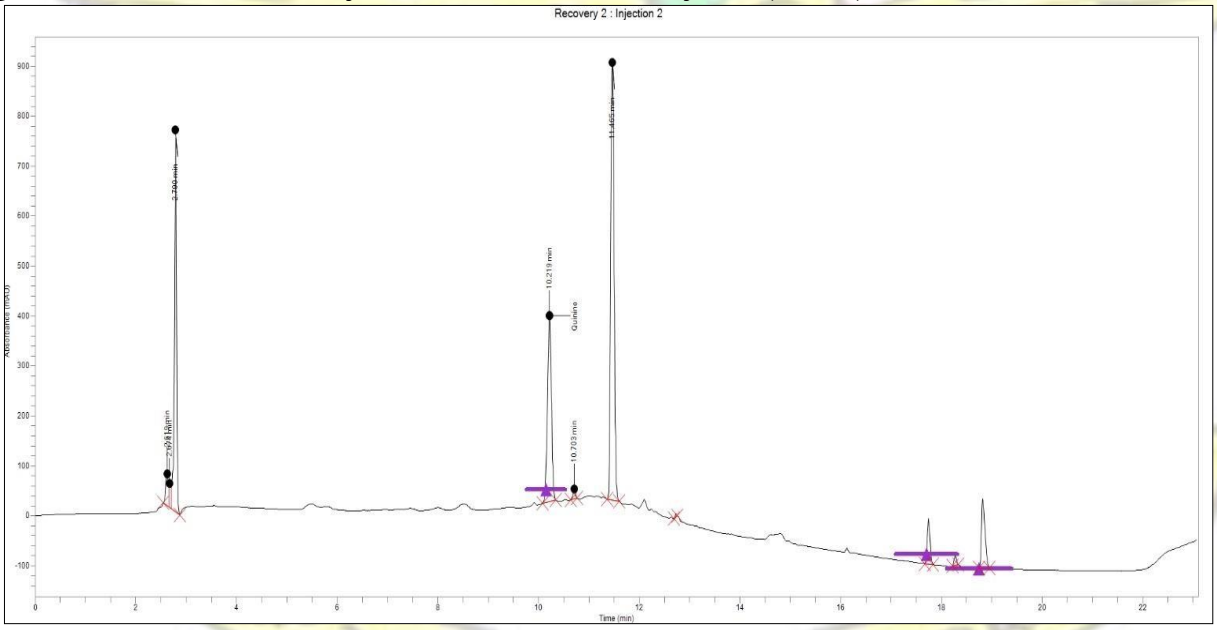


Figure 4.18: HPLC Linearity, Precision and Recovery test (Spiked)

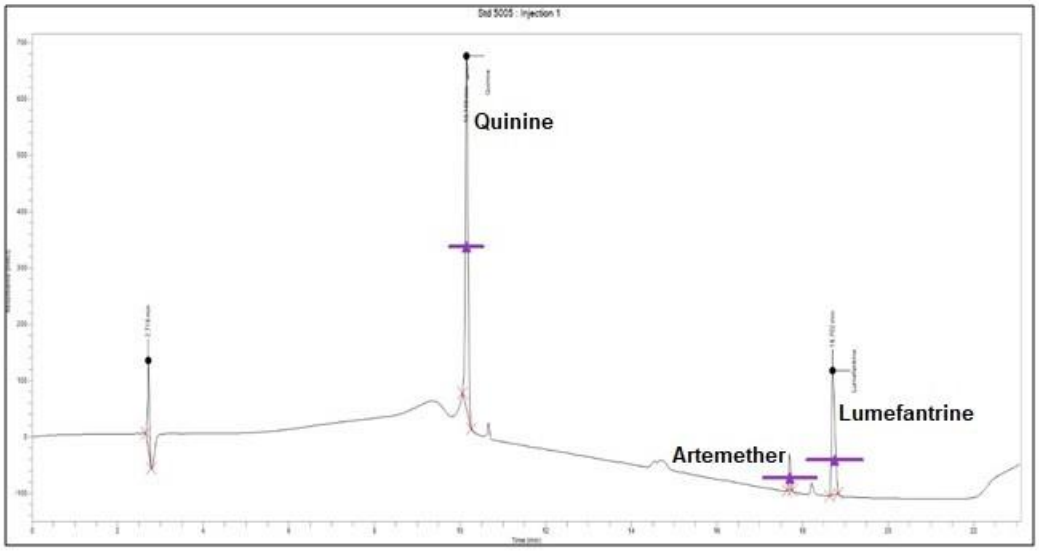


Figure 4.19A Amen fevermix

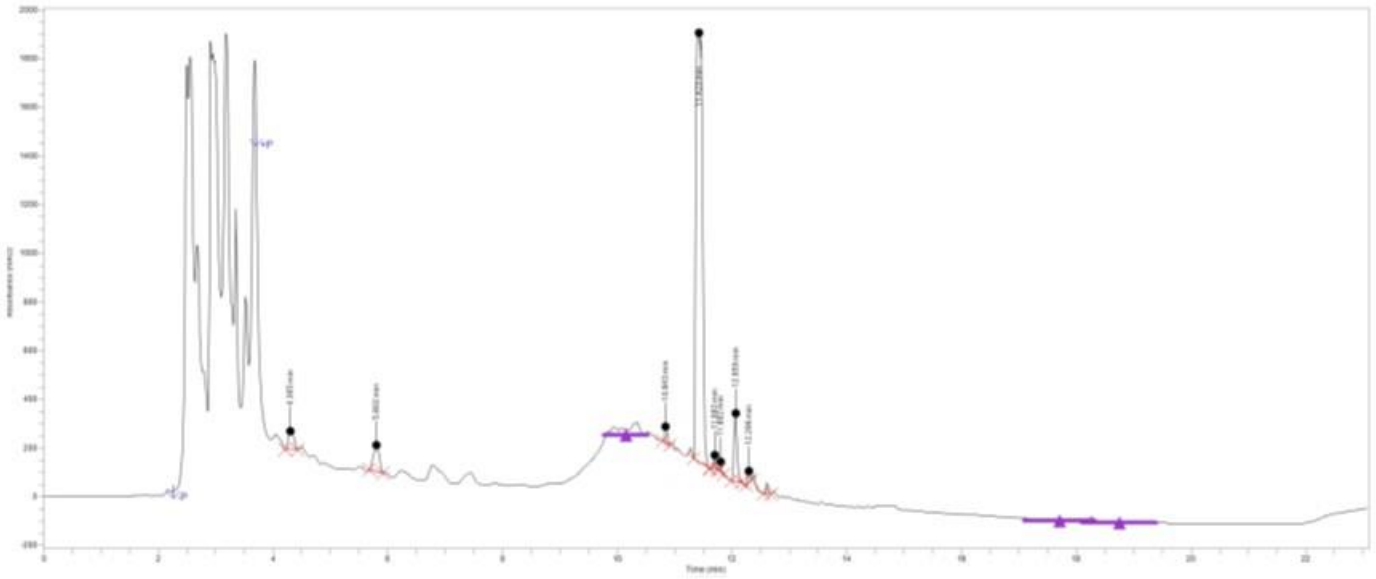


Figure 4.19B

Figure 19A: chromatogram of Quinine, Artemether and Lumefantrine

Figure 19B: chromatogram of *Mist Amen Fevermix*, Quinine, Artemether and Lumefantrine

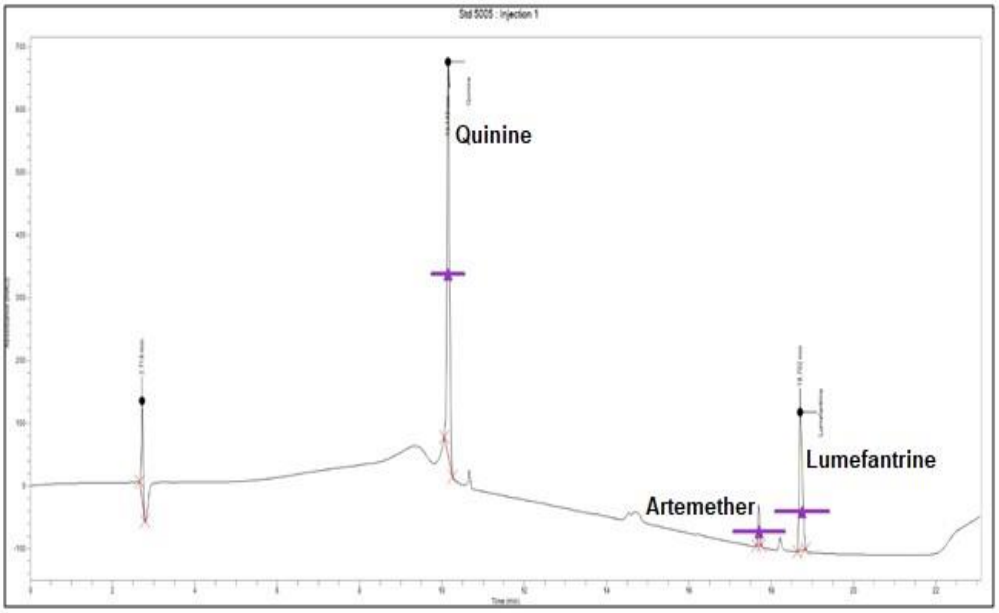


Figure 4.20A

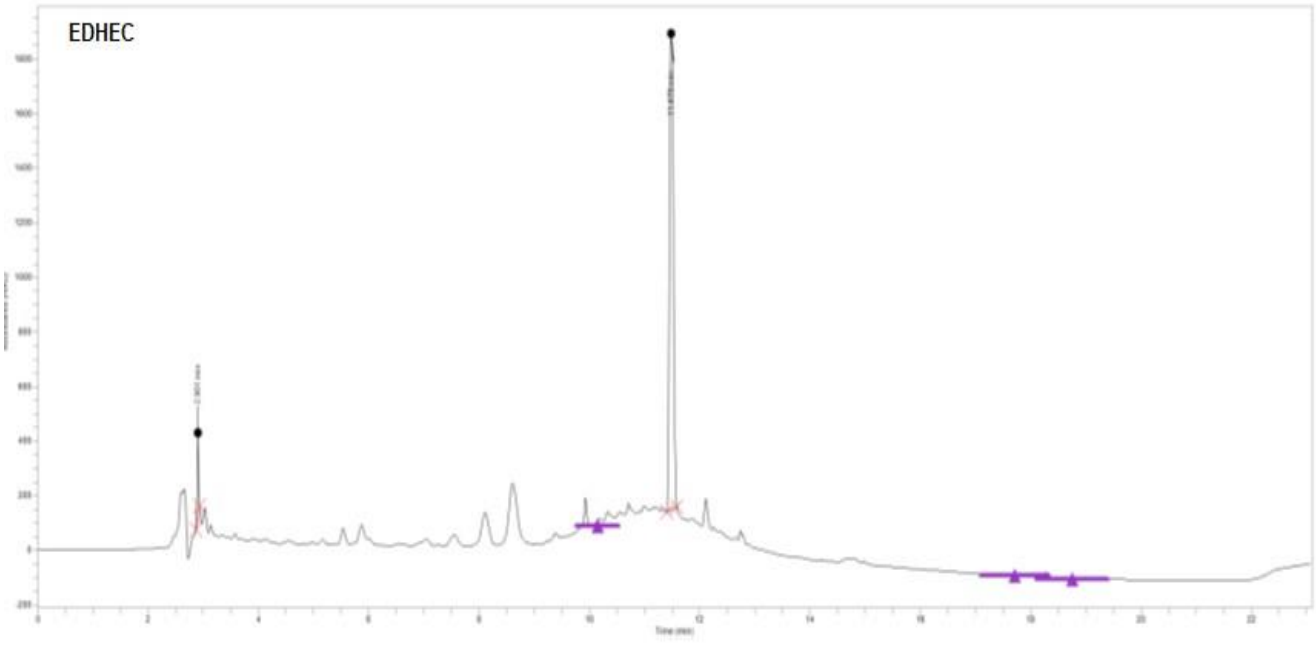


Figure 4.20B

Figure 20A: Chromatogram of Quinine, Artemether and Lumefantrine Figure 20B: Chromatogram of *Edhec Malacure*, Quinine, Artemether and Lumefantrine

4.6.4. Limits of Detection and Limits of Quantitation

Limits of detection (LOD) and limits of quantitation (LOQ) for the HPLC method were assessed using the signal to noise ratio. LOD was determined as 3.3 times the signal to noise ratio and the LOQ was also determined as 10 times the signal to noise ratio using the calibration curve method. LOD and LOQ for artemether were 114.494 mg/mL and 346.951 mg/mL respectively, lumefantrine (1.943 mg/mL and 5.889 mg/mL) and quinine (11.053 mg/mL and 33.492 mg/mL) respectively (Table 4.9).

Table 4.9: Limits of Detection, Quantitation and Recovery Data for the Determination of the Standard Antimalarial Drugs in Test Samples.

Control Drugs	Retention Time (min)	Spiked Conc. (mg/L)	LOD (mg/L)	LOQ (mg/L)	Recovery (%)
Artemether	17.71	500	114.494	346.951	94.214±2.292
Lumefantrine	18.76	40	1.943	5.889	92.696±2.172
Quinine	10.18	160	11.053	33.492	99.226±5.022

Values are Mean ± S.E.M

4.7. EFFICACY AND ACUTE TOXICITY

4.7.1. *In Vitro* Antiplasmodial Activity

Edhec Malacure exhibited antiplasmodial activity with an IC_{50} value of 70.89 µg/ mL, and *Mist Amen Fevermix* exhibited antiplasmodial activity with an IC_{50} value of 112.5µg/mL) (Tables 4.10). However, artesunate, a known anti-malarial used as a reference control in this study, exhibited a much higher activity IC_{50} value of 1.571 ng/mL than the study samples. This indicated very low sensitivity of the test samples on parasite growth *in vitro.*

Table 4.10: IC_{50} Values of *Mist Amen Fevermix* and *Edhec Malacure* against Reference Drug (Artesunate)

Antimalarial Products	Geometric Mean
AS	1.571 ng/ml

MEM	70.89 µg /ml
MAF	112.5 µg /ml

Key: **AS**-artesunate, **MEM**-*Edhec Malacure*, **MAF**-*Mist Amen Fevermix*

4.8. Results of *In Vivo* Toxicological and Antiplasmodial Activities of *Mist Amen Fevermix* and *Edhec Malacure* in Mice

4.8.1. Acute (Single Dose) Oral Toxicity Testing of *Mist Amen Fevermix and Edhec Malacure*

Mist Amen Fevermix had no-adverse-effect following oral administration at a dose of 5000mg/kg per body weight. All the mice survived and physical observation did not reveal any signs of toxicity such as changes on the eyes and mucus secretion, behaviour patterns, trembling, diarrhoea, falling of the fur, sleep or coma. Similarly, *Edhec Malacure* showed no-adverse-effect following oral administration of a dose of 5000 mg/kg with no signs of acute toxicity. There were no changes in their body weights. This implies that, both test samples may be safe using the dose (45 mL thrice daily for *Mist Amen Fevermix* and 30 mL thrice daily for *Edhec Malacure)* as listed on the labels.

4.8.2. Evaluation of the Suppressive Activity of *Mist Amen Fevermix and Edhec Malacure* (Peter's 4-Day Test)

Evaluation of the suppressive activity of *Mist Amen Fevermix* and *Edhec Malacure* in *P. berghei* infected mice revealed both study products to show chemo suppressive activity on parasitaemia. *Mist Amen Fevermix* showed a chemo suppression of 78.95 per cent at a dose of 4.56 $mgkg^{-1}$. This was statistically significant ($p<0.0001$) relative to the positive control at 71.50 per cent. *Edhec Malacure* also showed 70.73 per cent chemo suppression and was statistically significant ($p<0.0001$) at a dose of 2.234 $mgkg^{-1}$ as compared to the positive control (Table 4.12). No significant increases in weight were observed in mice treated with *Edhec Malacure*, however, there was a reduction in weight in the animals treated with *Mist Amen Fevermix* (23.96± 3.62 to 18.88± 9.72) (Table 4.11). This implies that the test products possess good *in vivo* suppressive activity and schizonticidal in action.

Table 4.11: Bodyweight (Day 0 and Day 4) of *Plasmodium*-infected Animals treated with *Mist Amen Fevermix* and *Edhec Malacure* in the 4-day Suppressive Test

Sample	Initial Weight /g	Final Weight /g
Negative control	22.44± 1.11	22.53± 2.35
MAF	23.96± 3.62	18.88± 9.72
MEM	21.92± 1.8	20.85± 1.83
Pyrimethamine	22.38± 2.27	22.72± 1.59

Key: MAF-Mist *Amen Fevermix,* **MEM-***Edhec Malacure,* **Values are Mean ± S.E.M**

Table 4.12: Antiplasmodial effect of *Mist Amen Fevermix* and *Edhec Malacure* in *P. bergheiinfected* mice on day 4

Sample	Dose ($mgkg^{-1}$)	% Parasitaemia (mean ± SEM)	% Suppression
Negative control		7.92 ± 1.24	N/A
MAF	4.56	1.64 ± 0.82****	78.95%
MEM	2.234	2.28 ± 0.33****	70.73%
Artesunate	5	2.22 ± 0.23****	71.50%

Key: MAF-*Mist Amen Fevermix*, **MEM-***Edhec Malacure.* N/A-Not Applicable, Values are Mean ± S.E.M

4.8.3. Antiplasmodial Prophylactic Activity of *Mist Amen Fevermix and Edhec Malacure*

At a dose of 4.56 $mgkg^{-1}$, *Mist Amen Fevermix* demonstrated statistically significant (p<0.001) antiplasmodial activity of 60.52%, which was higher when compared to the reference control pyrimethamine tested at 1.2 $mgkg^{-1}$. Also, *Edhec Malacure* demonstrated statistically significant (p<0.001) antiplasmodial activity of 63.77% at a dose of 2.234 $mgkg^{-1}$, when compared to the control pyrimethamine (55.75%) (p<0.01) (Table 4.14).

There was no marked change in the body weight of the animals in the test group. This confirms a previous study where some herbal plants are used as prophylactic measures to prevent malaria

infection (Okello and Kang., 2019). However, there was a significant reduction in weight of the animals in the negative control (Table 4.13).

Table 4.13: Body weight (Day 0 and Day 4) of *Plasmodium*-infected animals treated with *Mist Amen Fevermix and Edhec Malacure* in the 4-day Prophylactic Test

Sample	Initial Weight (g)	Difference in Weight (g)
NC	23.70± 2.37	21.35± 1.62
MAF	22.90± 2.69	21.73± 2.54
MEM	23.90± 2.11	22.53± 2.29
Pyrimethamine	23.20± 2.36	23.13± 2.51

Key: NC-negative control, **MAA**-Mist Amen Fevermix, **MEM**-Edhec Malacure, Values are Mean ± S.E.M

Table 4.14: Antiplasmodial Prophylactic Effect of *Mist Amen Fevermix* and *Edhec Malacure* against *P. berghei* Infection in Mice in a 4-day Test.

Sample	Dose ($mgkg^{-1}$)	% Parasitaemia (mean ± SEM)	% Suppression
Negative control		4.61 ± 0.64	N/A
MAF	4.56	1.82 ± 0.14***	60.52%
MEM	2.234	1.67 ± 0.33****	63.77%
Pyrimethamine	1.2	2.04 ± 0.25**	55.75%

**p<0.01, ***p<0.001 and ****p<0.0001 as compared to the negative control group.
Key: MAF-*Mist Amen Fevermix*, MEM-*Edhec Malacure*, N/A-Not Applicable.Values are Mean ± S.E.M

4.8.4. Evaluation of the Curative Activity of *Mist Amen Fevermix and Edhec Malacure* (Rane's Test)

In the Rane's curative test, both *Mist Amen Fevermix* and *Edhec Malacure* caused a statistically significant ($p<0.0001$) reduction of parasitaemia compared to the control. This implies the test samples were more effective than the control. The chemo suppression exhibited by *Mist Amen Fevermix* at a dose level of 4.56 $mgkg^{-1}$ on day three (3) was (69.03%) and *Edhec Malacure* at a dose

level of 2.234 $mgkg^{-1}$ was (80.93%). This result was significant compared to that of artesunate (98.01%), the reference drug used. Since the duration for the treatment indicated (test samples) was seven days, treatment continued for seven days. After the seventh day treatment period, chemo suppression exhibited by *Mist Amen Fevermix* was 74.48% respectively and for *Edhec Malacure* 80.93% while that of AL was 98.45% (Table 4.16). Chemo suppression exhibited by *Mist Amen Fevermix* using two dose levels (9.12 and 18.24) $mgkg^{-1}$ was 97.80% and 98.12% and *Edhec Malacure* 4.468 and 8.936 $mgkg^{-1}$ was 97.67% and 97.81% while that of AL was 97.84% (Table 4.17). This is an indication that *Mist Amen Fevermix* and *Edhec Malacure* could be potential curative agents for malaria. There was a reduction in the weight of animals treated with *Mist Amen Fevermix* from 14.033±4.69 to 13.767±4.71 g. Also, there was a non-significant increase in the weight of the animals treated with Edhec Malacure (Table 4.15).

Table 4.15: Effect of Test Samples on Weight of Mice

SAMPLE	Initial bodyweight of mice (g)	Difference in bodyweight (g)
Negative control	22.40 ± 1.38	21.55 ± 1.75
MAF	14.033 ± 4.69	13.767 ± 4.71
MEM	12.32 ± 3.93	12.47 ± 3.97
AL	16.98 ± 1.44	18.98 ± 1.37

Initial weight was taken on day 3 and final weight was taken on day 7.
Key: MAF-*Mist Amen Fevermix*, **MEM**-*Edhec Malacure*, AL-Artemether/Lumefantrine Values are presented as mean ± SEM

Table 4.16: Antiplasmodial Curative Effect of Test Samples Using a Single Dose on day 7

Dose	%Parasitaemia	%Suppression

Drugs		Day 3	Day 7	Day 10	Day 7	Day 10
NC		63.90 ± 7.09	75.90 ± 4.93	79.85 ± 3.25	N/A	N/A
MAF	9.12	74.74 ± 4.75	23.51 ± 13.23	20.38 ± 0.89	69.03	74.48
MEM	4.468	72.95 ± 10.23	18.09 ± 6.21	15.23 ± 8.20	76.17	80.93
AL	20/120mg	69.30 ± 1.72	1.51 ± 0.21	1.24 ± 0.17	98.01	98.45

Key: **NC**-negative control, **MAF**-*Mist Amen Fevermix*, **MEM**-*Edhec Malacure*, AL-Artemether/Lumefantrine, **N/A**-Not Applicable, Values are Mean ± S.E.M

Table 4.17: Antiplasmodial Curative Effect using two Dose levels of Test Products on day 4

Drugs	Dose mg/kg	% Parasitaemia Day 3	% Parasitaemia Day 7	% Suppression Day 7
NC		63.90 ± 7.09	74.55 ± 1.35	N/A
MAF	9.12	72.545 ± 1.215	1.64 ± 0.14	97.80
	18.24	70.05 ± 3.03	1.40 ± 0.1	98.12
MEM	4.468	69.75 ± 0.75	1.74 ± 0.04	97.67
	8.936	68.50 ± 0.50	1.635 ± 0.05	97.81
AL	20/120mg	71.10 ± 0.10	1.61 ± 0.01	97.84

Key: **NC**-negative control, **MAF**-Mist Amen, **MEM**-*Edhec Malacure*, Fevermix, AL-Artemether/Lumefantrine, **N/A**-Not Applicable, Values are Mean ± S.E.M

In this study, the *in vitro* and *in vivo* antiplasmodial activity of *Mist Amen Fevermix* and *Edhec Malacure* were demonstrated.

The *in vitro* and *in vivo* outcomes confirm the antiplasmodial therapeutic potential of *Mist Amen Fevermix* and *Edhec Malacure*.

4.9. CLINICAL SAFETY AND EFFECTIVENESS

4.9.1. Sample Characteristics

The study participants comprised both male and female patients. Most of the patients (62.2%) were aged between 18 and 33 years with an average age being 31.1 (SD = 8.23) years. All drug arms had an equal number of participants taking part in the study Table 4.18. Out of the total sample population of 150 patients, 90 (60%) are female, whereas 60 (40%) are males Table 4.18. There were 64% females in both the control group (AL), *Mist Amen Fevermix* and 52% in *Edhec Malacure*, and 36% males in the control group (AL), *Mist Amen Fevermix* and 48% in *Edhec Malacure* (Figure 4.19).

Table 4.18: Sample Characteristics of Participants

	Drug Type			
Variable	Control Artemether/Lumefantrine n (%)	MEM n(%)	MAF n (%)	Total n (%)
sex				
Male	18 (36)	24(48)	18 (36)	60 (40.0)
Female	32 (64)	26(52)	32 (64)	90 (60.0)
Age Groups				
18-21	5 (10)	9(18)	6 (12)	20 (13.3)
22-25	9 (18)	7(14)	10 (20)	26 (17.3)
26-29	9 (18)	11(22)	9 (18)	29 (19.3)
30-33	7 (14)	6(12)	6 (12)	19 (12.7)
34-37	2 (4)	4(8)	6 (12)	12 (8.0)
38-41	9 (18)	6(12)	9 (18)	24 (16.0)
42-45	9 (18)	7(14)	4 (8)	20 (13.3)
Total	100	100	100	

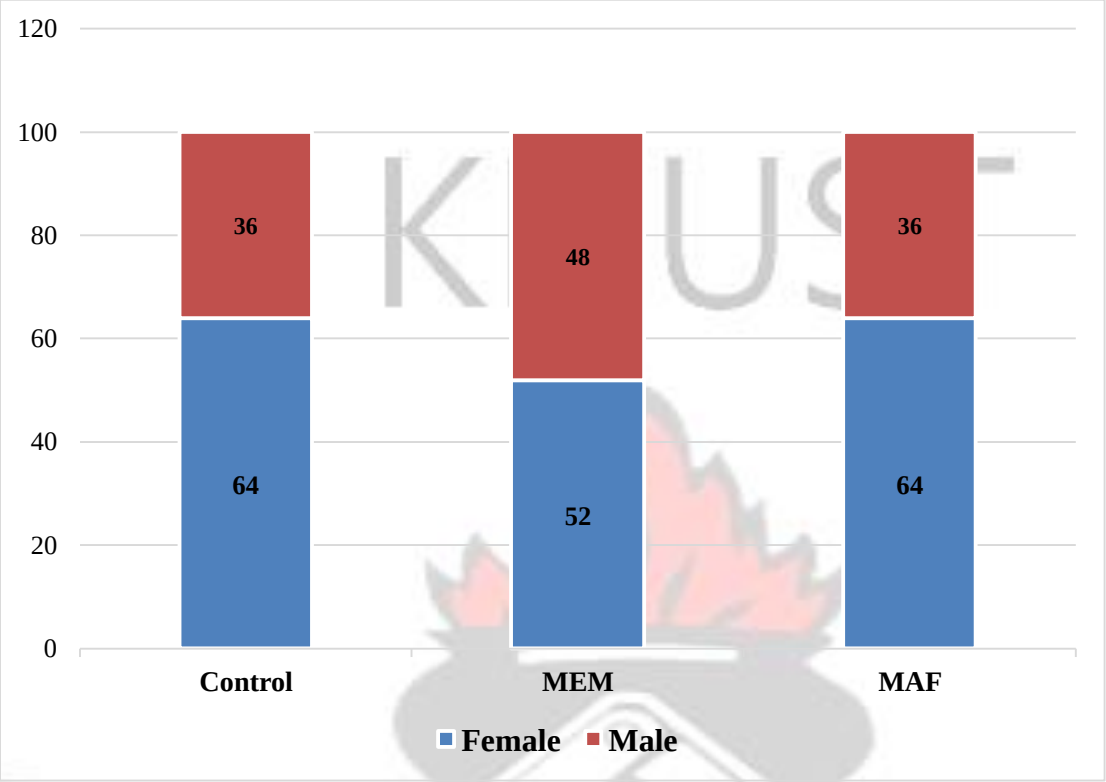


Figure 4.21: Gender Distribution for Test Drugs (n=50)

4.9.2. Age Distribution of Participants

The age distribution of the participants shows that the ages of 19.3% participants fall within the 26 to 29 years age group; 17.3% of participants fall within the 22 to 25 years age group, and 16% were within the age group of 38 to 41 years. Some 13.3% each were in the age group of 18 to 21 years and 42 to 45 years. Also, 12.7% of participants were within the 30 to 33 years age group while 8% fell within ages 34 to 37 years. Cumulatively the majority of participants belong to the age bracket of 18 – 33 years, which constitutes a very youthful age (Figure 4.22). This is because younger people appear to prefer herbal products due to their safety and effectiveness (Rashrash *et al*., 2017).

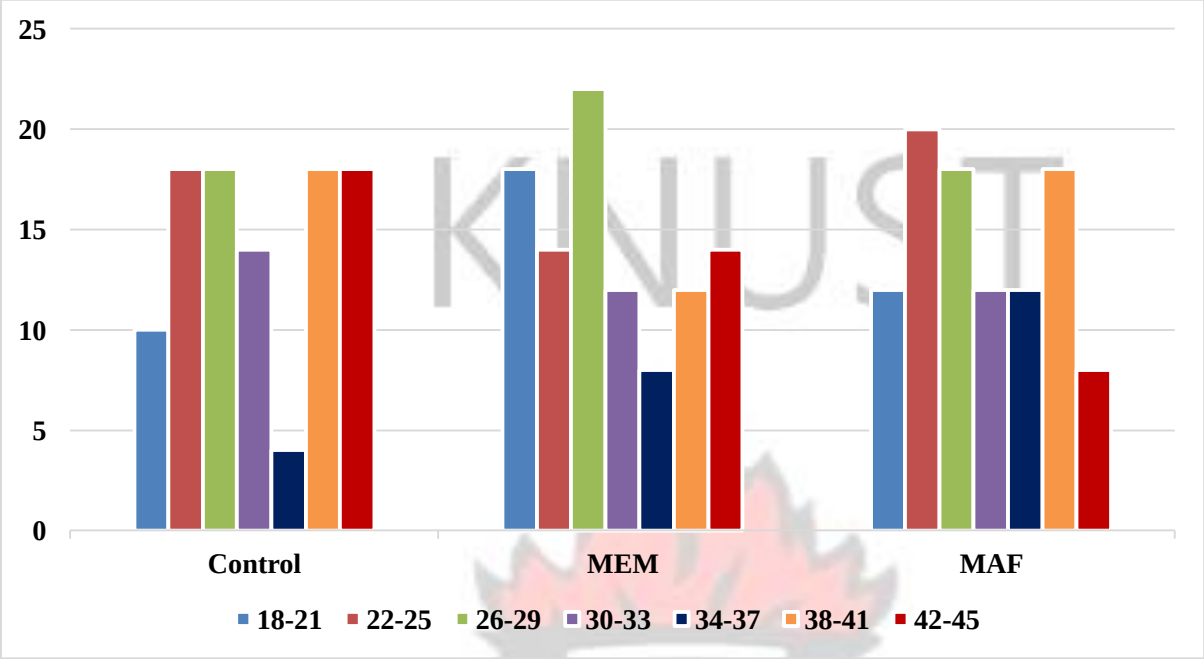


Figure 4.22: Age Distribution of Patients

4.9.3. Assessment of the Effectiveness of Test Samples

4.9.3.1. Control Drug (Artemether/Lumefantrine)

A paired-sample t-test to evaluate the statistical difference between the parasite load at first visit (day 0) against the second visit, the second against third, and third against fourth visits (Figure 4.23) was done. For the Control AL, the null hypothesis for the pairing of the *t*-test is that the mean levels of malaria parasite load are the same/equal (i.e. there is no difference between the parasite counts for the first visit and the second visit). The alternate hypothesis tested here is that the malaria parasite load at the first visit is not the same as the second visit (i.e. there is a statistical difference between the first visit and the second visit counts). Similarly, the null hypothesis for the comparison between the second and third visit; the third and fourth visits; and the fourth and fifth visits, stated that there are equal levels of malaria parasite loads and the alternate states otherwise.

The test results indicate statistically significant differences between the mean malaria parasite counts recorded at the first visit and those recorded at the second visit, t (18) = 3.42, p=0.003.

Correspondingly, there was a statistically significant difference between the malaria parasite load at the second visit and a third visit, t (4) = 2.12, p =0.101. Finally, no significant differences [t (3) = 1.00, p =0.391] were reached at the third and final visits for counts of the malaria parasite. This shows the significant effectiveness of Control AL used by the patients. The fourth and fifth visits difference test was not possible as a result of the incalculability of the value of t and its correlates; all the parasites were completely cleared on those visits.

4.9.3.2. Assessment of the Effectiveness of *Mist Amen Fevermix*

The results (Figure 4.24) is a paired-sample t-test performed to test the difference between mean parasite counts at first visit against the second visits, the second against third visits and third against fourth, and fourth and fifth visits. The test indicates a statistically significant difference between the mean malaria parasite load recorded at the first visit and those recorded at the second visit, t (23) = 4.59, p =0.000. Similarly, there was a significant difference between the mean parasite count recorded on the second visit and that of the third visit, t (6) = 1.49, p =0.187. No difference was achieved for the third and fourth visits t (3) = 1.00, p =0.391. This shows the significant effectiveness of *Mist Amen Fevermix* used by the patients. The fourth and final pairing difference test was not possible due to the apparent lack of patient visits for the fifth test.

4.9.3.3. Assessment of the Effectiveness of *Edhec Malacure*

Figure 4.24 shows the results of paired-sample t-tests performed to test the difference between the mean parasite counts between consecutive visits. Statistically, there was a significant difference between the mean malaria parasite count recorded at the first visit and those recorded at the second visit, t (26) =3.77, p =0.001. Similarly, there is a statistically significant difference between malaria parasite count at the second visits and third visits, t (16) = 1.74, p =0.100. Comparison of the third and fourth visits and the fourth and fifth visits were not possible due to the incalculability of the value

of t and its correlate. This shows a significant effectiveness of *Edhec Malacure* after the first and second visits.

4.9.3.4. Comparative Effectiveness of Test Products

Control (*AL*) was most effective in reducing the parasite counts as the mean reduced parasite count [mean = 14268.68, SD = 18167.06] was the highest reduction of all the drugs on the second visit. Likewise, effectiveness at the third visit was highest for Control AL [mean = 392.20, SD = 413.37]. Results of the reduced parasite counts for first, second, third and fourth days (Table 4.19).

Table 4.19: Parasite Counts During Visits and Treatment

Comparison	Drug	Mean	Std. Deviation	Min.	Max.
First	Control (AL)	14268.68	18167.06	-250	53320
	MEM	3069.81	4233.36	120	21374
	MAF	2072.38	2212.71	320	9374
Second	Control	392.20	413.37	0	1080
	MEM	249.71	590.53	-434	2090
	MAF	85.14	151.23	0	370
Third	Control	0	0	0	0
	MEM	0	0	0	0
	MAF	0	0	0	0

Key: MAF-Mist Amen Fevermix, **MEM**-Edhec Malacure

In general, whereas Control drug (AL), *Edhec Malacure* and *Mist Amen Fevermix* recorded a relatively minor reduction in parasite counts reduced after the second and third visits, no reduction of parasite counts was recorded at the fourth visits (Figure 4.23).

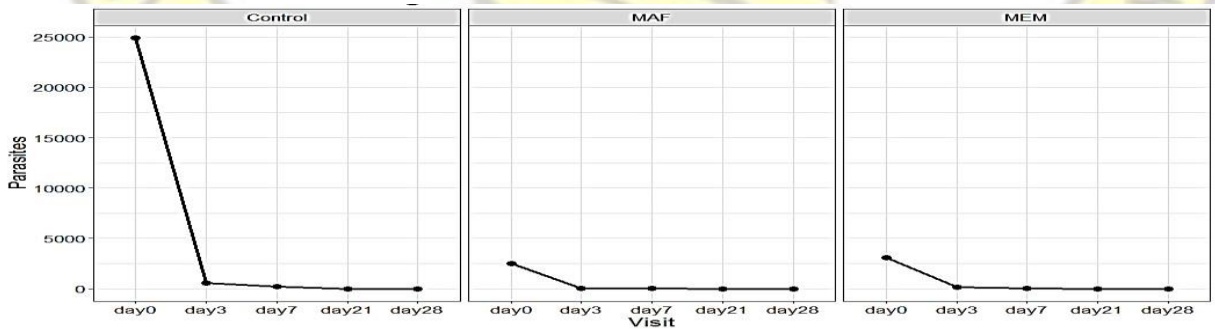


Figure 4.23: Parasite Counts for Drugs At Visiting Days

Using a one-way ANOVA test, results of comparison of *Control (AL), Edhec Malacure* and *Mist Amen Fevermix* showed significant differences in effectiveness (number of resolved parasites) of the three drugs at second visits [F (2, 67) = 9.75, $p < 0.001$] (Figure 4.24). No difference in effectiveness was shown for the three drugs at the third visit [F (2, 26) = 0.58, p =0.568]. At the fourth visit, there were no recorded parasite counts in the participants.

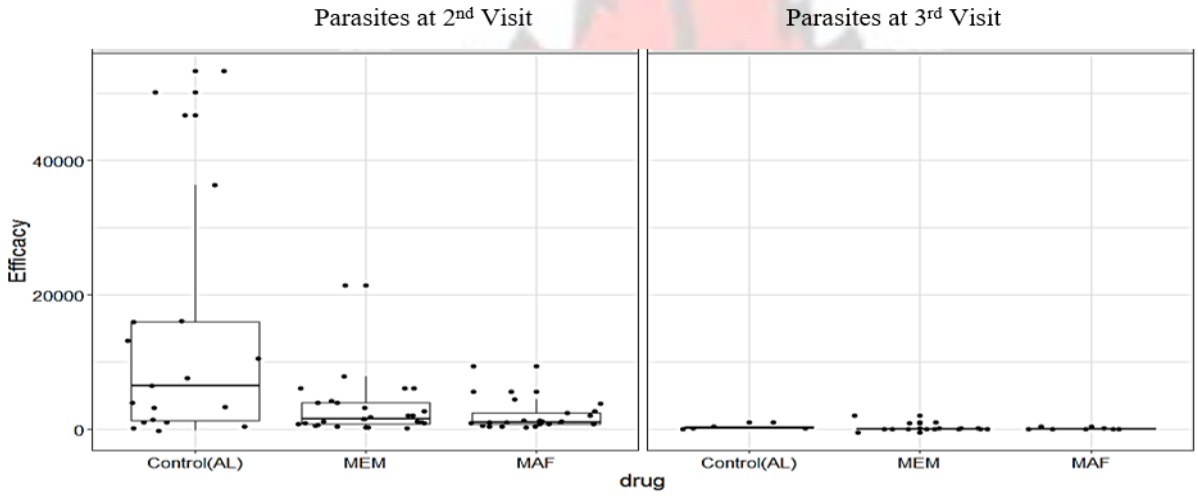


Figure 4.24: Reduction in Parasite Counts

Post-hoc analysis for reduced parasite count at second and third visits, using Dunnett's *t* test (a 2sided *t*-test), revealed higher effectiveness of the Control drug (AL) when compared to *Edhec Malacure* (p =0.001), and to *Mist Amen Fevermix* ($p < 0.001$) Figure 4.24.

4.9.4 Assessment of the Safety of Test Products on Renal Panel

The effects of AL, *Mist Amen Fevermix* and *Edhec Malacure* were assessed using the paired sample *t*-test to evaluate their significant effects on the levels of Potassium, Sodium, Chlorine, Urea and

Creatine in participants (Tables 4.10 and 4.21). There was no significant difference between baseline parameters and subsequent visit parameters. This implies the test products did not cause any adverse injury to the renal system and therefore safe.

4.9.4.1. Assessment of the Safety of Artemether/Lumefantrine on Renal Panel

Results of comparative statistical analyses indicates that for patients who used the AL drug, the levels of all kidney function variables at first visit is statistically not different from the levels recorded at second visit. Figure 4.23 shows that there were no changes in the levels of Potassium [$t(44) = 0.325$, $p = 0.747$]; Sodium [$t(44) = 0.363$, $p = 0.719$]; Chlorine [$t(44) = 0.173$, $p = 0.864$]; Urea [$t(44) = -.682$, $p = 0.499$] and Creatine [$t(44) = 0.865$, $p = 0.391$] before and after use of control drug *AL*. The results of analysis of difference between tested substances before and after use of AL Figure 4.23.

4.9.4.2. Assessment of the Safety of *Mist Amen Fevermix* on Renal Panel

Similarly, no significant differences between levels of Potassium [$t(24) = -.110$, $p = 0.913$]; Sodium [$t(24) = -.116$, $p = 0.909$]; Chlorine [$t(24) = -.249$, $p = 0.805$]; Urea [$t(24) = -.232$, $p = 0.817$]; Creatinine [$t(24) = .108$, $p = 0.915$]; and eGFR levels[$t(41) = .142$, $p = 0.888$] before and after use of the *Mist Amen Fevermix* were revealed in the analyses (Table 4.20).

Table 4.20: Effect of *Mist Amen Fevermix* on Kidney

Parameter	Range	1st Visit	2nd Visit	*p*-value
		$\chi^{\pm}s$	$\chi^{\pm}s$	
Potassium (K)	3.5 – 5.5	4.17 ±0.51	4.18 ±0.5	.913
Sodium (Na)	135 – 155	140.14 ±2.82	140.21 ±2.65	.909
Chloride (Cl)	96 – 110	100.18 ±2.92	100.27 ±2.67	.805
Urea	2.1 – 7.1	4.67 ±1.42	4.74 ±1.28	.817
Creatinine	M = 61.88 –123.8 F = 61.88 – 106.1	87.66 ±15.59	87.32 ±16.96	.915
eGFR	>60mL/min/1.73m^2	95.47 ±2.92	95.37 ±2.65	.888

Results are Mean ± S.E.M

4.9.4.3. Assessment of the Safety of *Edhec Malacure* on Renal Panel

Also, no significant differences were recorded between levels of Potassium [$t(45) = -.357$, $p = 0.723$]; Sodium [$t(45) = 1.207$, $p = 0.234$]; Chlorine [$t(45) = 1.019$, $p = 0.314$]; Urea [$t(45) = -1.319$, $p = 0.194$]; Creatinine [$t(45) = 0.609$, $p = 0.546$] and eGFR [$t(45) = .518$, $p = 0.607$] before and after use of *Edhec Malacure* (Table 4.21)

Table 4.21: Effect of *Edhec Malacure* on Kidney

Parameter	Range	1st Visit	2nd Visit	*p*-value
		$\chi \pm s$	$\chi \pm s$	
Potassium (K)	3.5 – 5.5	4.14 ±0.54	4.18 ±0.52	.723
Sodium (Na)	135 – 155	139.64 ±2.53	136.9 ±15.26	.234
Chloride (Cl)	96 – 110	119.8 ±1.73	100.2 ±2.45	.314
Urea	2.1 – 7.1	4.87 ±1.39	6.85 ±10.08	.194
Creatinine	M = 61.88 –123.8 F = 61.88 – 106.1	96.95 ±17.5	95.41 ±15.42	.546
eGFR	7 – 32	95.53±2.42	95.27±2.71	.607

Values are Mean ± S.E.M

4.9.4.4. Comparative Effect of Test Products on Renal Panel

The results of a one-way ANOVA test, comparing the effect of *AL, Mist Amen Fevermix* and *Edhec Malacure* on patient's kidney, showed no significant differences in levels of Potassium [$F(2, 130) = .124$, $p = 0.884$], Sodium [$F(2, 130) = 1.195$, $p = 0.306$], Chlorine [$F(2, 130) = 0.98$, $p = 0.378$], Urea [$F(2, 130) = 1.361$, $p = 0.26$]; Creatinine [$F(2, 130) = 0.648$, $p = 0.525$] and eGFR [$F(2, 130) = 0.834$, $p = 0.437$] after first visits. Post hoc analysis was not needed as there were no significant differences warranting the test. Results of comparative analysis of drugs on the test variables of kidney panel Figure 4.25.

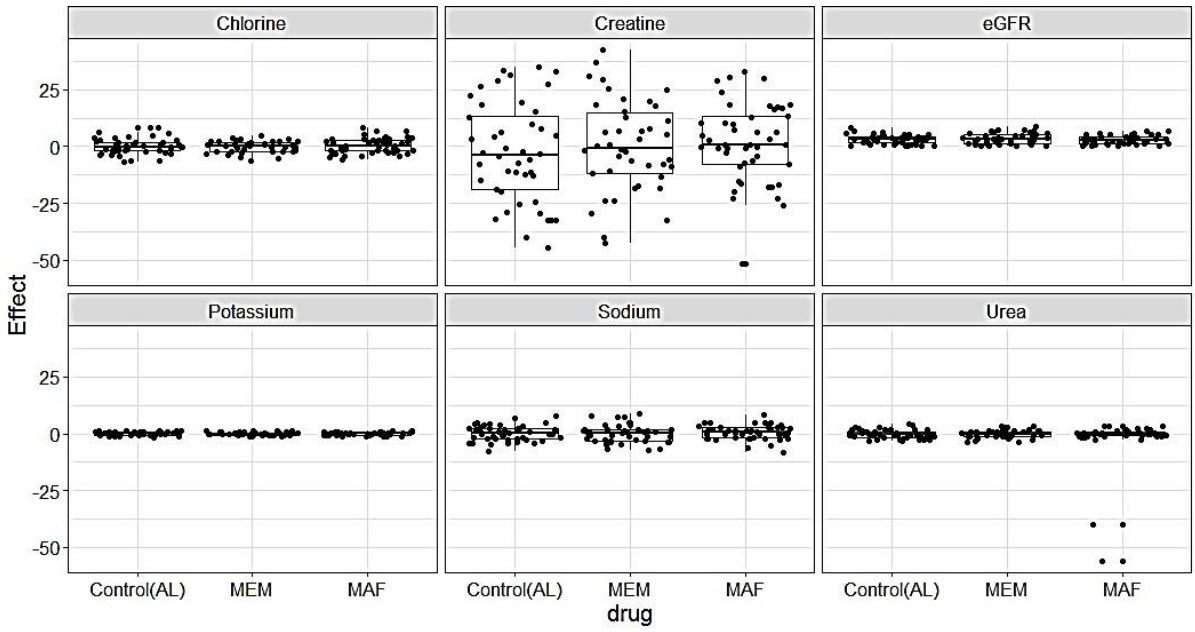


Figure 4.25: Levels of Kidney Variables for Drugs

4.9.5. Assessment of the Effect of Test Products on Liver Panel

Test drugs *Edhec Malacure* and *Mist Amen Fevermix* were tested using the paired sample *t*-test to evaluate their significant effects on the levels of health indicators of the liver in participants. Tables 5.6 and 5.7 below show the results of the difference in levels of test indicators between visits.

4.9.5.1. Assessment of the Effect of *Mist Amen Fevermix* on Liver Panel

Statistically, no significant differences in levels of Albumin, ALP, ALT, AST, GGT, Indirect Bilirubin, Protein and Total Bilirubin between the three test days for *Mist Amen Fevermix* were revealed. However, Globulin [t (41) = -39.12, $p < 0.001$] and Direct Bilirubin [t (41) = -2.75, $p < 0.01$] were shown to have been reduced after use of *Mist Amen Fevermix* on the second and third tests respectively. This implies that the test product may have hepatorestorative activities since they exerted alterations in protein profile in liver Table 4.22.

Table 4.22: Effect of *Mist Amen Fevermix* on Participants' Liver (n=46)

Parameter	Normal range	1st Visit	2nd Visit	3rd visit
		$\chi \pm s$	$\chi \pm s$	$\chi \pm s$
AST	30 –51	17.03 ±8.94	17.21 ±8.56	16.9 ±9.31
ALP	0 – 240	120.5 ±68.09	122.09 ±69.02	120.07 ±64.54
ALT	0 – 40	22.9 ±12.03	23.05 ±10.28	20.97 ±12.65
GGT	7-32	20.88 ±7.41	21.73 ±7.19	20.89 ±7.4
Bilirubin Total	0 – 26	12.16 ±8.01	13.52 ±6.84	11.51 ±7.71
Bilirubin Direct	0-8.67	5.05 ±2.65	5.72 ±2.66	5.20±2.38
Bilirubin Indirect	0 – 17.33	7.11 ±6.01	7.8 ±3.41	6.31 ±6.45
Total Protein	66 – 87	69.4 ±12.33	70.1 ±11.9	68.7 ±12.67
Albumin	18 – 51	37.0 ±7.71	34.8 ±4.54	35.2 ±4.19
Globulin	25–40	32.4 ±4.55	35.3 ±6.14	33.5 ±4.93

Values are Mean ± S.E.M

4.9.5.2. Assessment of the Effect of *Edhec Malacure* on Liver Panel

The effect of *Edhec Malacure* on patient's liver, showed significant differences between the levels of Albumin, ALP, ALT, AST, Direct Bilirubin, GGT, Globulin, Indirect Bilirubin, Protein, Total Bilirubin on the second visits. This implies that the test product may have hepatoprotective activities since they exerted alterations in protein profile in liver. This is an indication that the product may not have any harmful effect on the liver and therefore safe Table 4.23.

Table 4.23: Effect of *Edhec Malacure* on Participants' Liver

Parameter	Normal range	1st Visit	2nd Visit	3rd visit
		$\chi \pm s$	$\chi \pm s$	$\chi \pm s$
AST	30 –51	18.1 ±9.19	17.8 ±8.6	18.3 ±9.75

ALP	0 – 240	104.21 ±65.44	108.09 ±63.64	105.33 ±69.85
ALT	0 – 40	22.1 ±12.34	21.02 ±12.65	21.9 ±11.26
GGT	7-32	20.10 ±7.92	18.90 ±7.26	19.3 ±6.68
Bilirubin Total	0 – 26	12.26 ±7.67	14.45 ±8.38	14.13 ±8.41
Bilirubin Direct	0-8.67	4.21 ±2.69	4.2 ±2.78	5.03 ±2.47
Bilirubin Indirect	0 – 17.33	8.05 ±4.32	9.21 ±5.42	9.10 ±5.16
Total Protein	66 – 87	74.43 ±3.91	74.13 ±3.94	74.31±4.22
Albumin	18 – 51	40.1 ±8.57	39.10 ±2.11	37.3 ±0.20
Globulin	25–40	34.33 ±5.34	35.03 ±5.83	37.01 ±6.02

Values are Mean ± S.E.M

4.9.5.3. Comparative Effect Assessment of Test Products on Liver Panel

The results of the comparison of the effect of *Mist Amen Fevermix* and *Edhec Malacure* on liver health indicator variables showed no significant differences with the control.

4.9.6. Assessment of Vital Signs After the use of Test Products

Control drug *AL* and test drugs *Mist Amen Fevermix* and *Edhec Malacure* were tested using the paired sample *t*-test to evaluate their significant effects on the levels of body weight, systolic and diastolic blood pressure and body temperature in participants before and after uptake. Tables 4.24 and 4.25 shows the results of the difference in levels of test substances between visits.

4.9.6.1. Assessment of Vital Signs After the use of Artemether/Lumefantrine

Results of statistical analysis indicates that the bodyweight of patients before and after use of AL was not different [t (46) = -.754, p =0.455]. Similarly, no significant differences were recorded for diastolic blood pressure [t (45) = 1.751, p =0.087] before and after use of AL. Meanwhile, differences in systolic blood pressure [t (46) = 3.704, p =0.001] and body temperature [t (39) = 4.51, p < 0.001] of patients before and

after use of the AL were shown. Figure 4.26 shows the results of the analysis of the difference between tested variables before and after the use of Control AL and the herbal remedies.

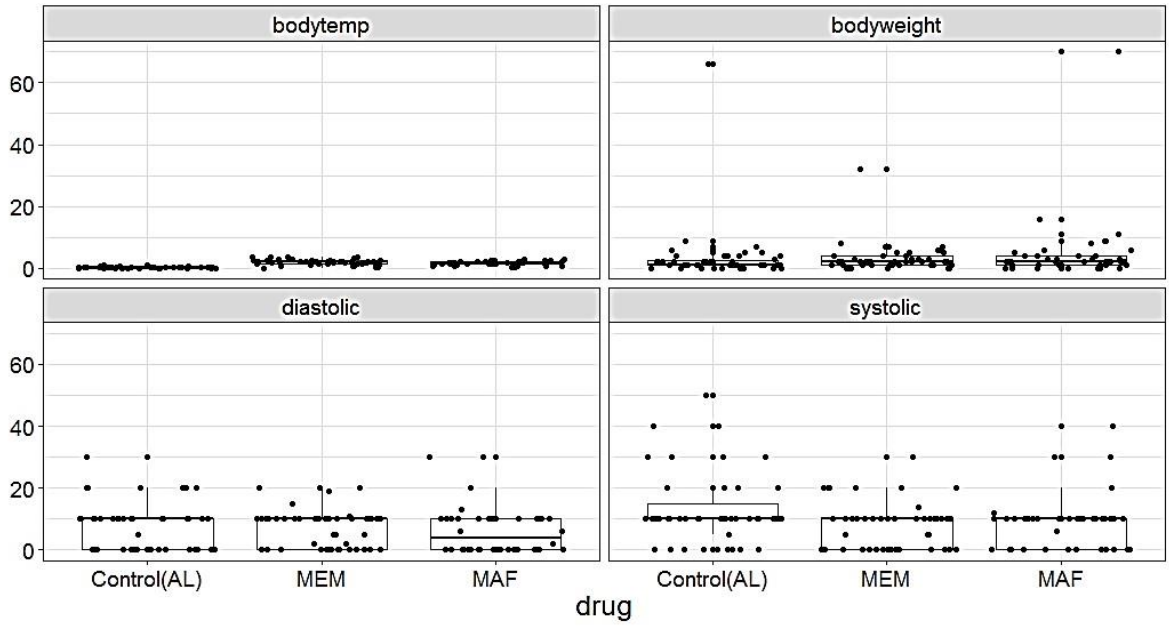


Figure 4.26: Vital Signs Comparison for Drugs

4.9.6.2. Assessment of Vital Signs after the use of *Mist Amen Fevermix*

Statistically, no significant differences between levels of bodyweight [t (41) = 0.352, p =0.726]; systolic blood pressure [t (41) = -.300, p =0.766]; and diastolic blood pressure [t (41) = 1.234, p =0.224] before and after use of the *Mist Amen Fevermix* were revealed. However, body temperature [t (41) = 2.50, p <0 .001] was shown to have been reduced after use of *Mist Amen Fevermix*. The result of this analysis is as shown below in Table 4.24

Table 4.24: Effect of *Mist Amen Fevermix* on Vital Signs

Parameter	**1st Visit**	**2nd Visit**	***p*-value**
	$\chi^{\pm}s$	$\chi^{\pm}s$	
Bodyweight	53.19 ±9.91	52.55 ±12.72	.726

Systolic	118.33 ±10.1	118.9 ±11.49	.766
Diastolic	79.43 ±8.33	77.6 ±10.8	.224
Body Temperature	38.79 ±0.55	37.1 ±0.48	.000

Values are Mean ± S.E.M

4.9.6.3. Assessment of Vital Signs After the use of *Edhec Malacure*

For *Edhec Malacure* on patients, there was no significant differences between the levels of bodyweight [$t(41) = -.63$, $p = 0.531$] before and after test, whereas systolic [$t(41) = 2.11$, $p = 0.041$]; diastolic [$t(41) = 2.25$, $p = 0.03$]; and body temperature [$t(41) = 15.02$, $p < 0.001$] before and after test. Table 4.25 depicts the differences between tested substances before and after use of *Edhec Malacure*.

Table 4.25: Effect of *Edhec Malacure* on Vital Signs

Parameter	1st Visit	2nd Visit	*p*-value
	$\chi^{\pm}s$	$\chi^{\pm}s$	
Bodyweight	56.11 ±8.89	56.65 ±10.58	.531
Systolic	119.67 ±10.13	116.43 ±8.77	.041
Diastolic	79.04 ±8.77	76.09 ±8.82	.030
Body Temperature	38.95 ±0.66	36.95 ±0.62	.000

Values are Mean ± S.E.M

4.9.6.4. Comparative Assessment of Vital Signs After the use of Test Products

Using the one-way ANOVA test, the results of comparison of effectiveness of *AL, Mist Amen Fevermix* and *Edhec Malacure* on health indicator variables showed no significant differences for bodyweight [$F(2, 132) = 0.351$, $p = 0.704$] and diastolic blood pressure [$F(2, 131) = .553$, $p = 0.576$] after the test. Meanwhile, significant differences were evident for systolic blood pressure [$F(2, 132) = 3.422$, $p = 0.036$] and body temperature [$F(2, 125) = 74.13$, $p < 0.001$] after test (Figure 4.26).

Post-hoc analysis using Dunnett's *t*-test showed higher effectiveness of *AL* on systolic when compared individually to *Edhec Malacure* (p =0.028) whereas AL and *Mist Amen Fevermix* (p =0.099) were not statistically different. For body temperature AL was found to have higher effect than both *Mist Amen Fevermix* (p <0.001) and *Edhec Malacure* (p < 0.001) (Figure 4.26).

4.9.7. Assessment of Full Blood Count after use of Test Products

4.9.7.1. Assessment of Full Blood Count after use of Artemether/Lumefantrine

Results of statistical analysis indicate that the levels of full blood count variables at first visit were different from those at second visit for HB [t (27) = -3.106, p =0 .004] and RBC [t (27) = 3.042, p =0 .005]. Meanwhile, WBC [t(27) = -1.454, p =0 .158]; Neutro [t(27) = 1.446, p =0 .160]; Lympho [t(27) = -.592, p =0.559]; Monocytes [t(27) = -.868, p =0 .393]; eosinophils [t(27) = -.868, p =0 .393]; and Basophils [t(27) = -.402, p =0 .691] showed no differences before and after use of the AL.

At the second test of effectiveness of AL, no statistical differences were recorded for HB [t(27) = .866, p =0 .394]; WBC [t(27) = -1.658, p =0 .109]; RBC [t(27) = -.644, p =0 .525]; Neutrophils [t(27) = 1.114, p =0 .275]; Lymphocytes [t(27) = -1.997, p =0 .056]; Monocytes [t(27) = .734, p =0 .469]; Eosinophils [t(27) = 0.734, p =0.469]; and Basophils [t(27) = 0.356, p =0.724]. Figure 4.27 below shows the results of analysis of differences between tested levels of Hb before and after use of Control AL at second and third visits.

4.9.7.2. Assessment of Full Blood Count after use of *Mist Amen Fevermix*

Also, no significant differences were shown between levels of HB [t(43) = -1.052, p =0 .299]; WBC [t(43) = -1.125, p =0 .267]; Neutrophils [t(43) = .485, p =0 .63]; Monocytes [t(43) = .350, p =0 .728]; Eosinophils [t(43) = 1.051, p =0 .299]; and Basophils [t(43) = 1.014, p =0 .316] before and after use of *Mist Amen Fevermix* at first test. Two variables RBC [t (43) = 2.381, p =0 .022]; and Lymphocytes [t (43) = 2.678, p =0 .01] were shown to have significant differences in levels before and after use of *Mist Amen Fevermix*. Table 4.26 depicts the differences between tested substances before and after use of *Mist Amen Fevermix*.

Table 4.26: Effect of *Mist Amen Fevermix* on FBC

Parameter	Reference Range	1st Visit	2nd Visit	3rd Visit
		$\chi \pm s$	$\chi \pm s$	$\chi \pm s$
HB	12.0-18.0 g/dL	12.15 ±2.11	12.6 ±1.68	12.75 ±1.56
WBC	4.5-11.0 × 10^9/L	7.2 ±2.87	7.55 ±3.22	7.56 ±3.25
RBC	4.3-5.9 x10^{12}/L	5.02 ±0.36	4.92 ±0.4	6.73 ±8.32
Neutro	40.0-75.0%	59.06 ±17.33	58.51 ±15.32	50.79 ±20.96
Lympho	21.0-40.0%	33.73 ±16.69	27.37 ±18.06	24.35 ±16.1
Monocy	3.0-7.0%	3.44 ±2.14	3.33 ±2.14	3.47 ±2.26
Eosi	0.0-5.0%	0.48 ±1.24	0.3 ±0.33	0.35 ±0.42
Baso	0.0-1.5%	12.15 ±2.11	12.6 ±1.68	12.75 ±1.56

Key: Hb-Haemoglobin, WBC-White Blood Cells, RBC-Red Blood Cells, Neutro-Neutrophils, Lympho-Lymphocytes, Monocy-Monocytes, Eosi-Eosinophils, Baso-Basophils. Values are Mean ± S.E.M

At the second test of effectiveness of *Mist Amen Fevermix*, Hb [$t(43) = -1.306$, $p = 0$ .199]; WBC [$t(43) = -052$, $p = 0$ .959]; RBC [$t(43) = -1.454$, $p = 0$ .153]; Lymphocytes [$t(43) = 1.518$, $p = 0$ .136]; Monocytes [$t(43) = -.514$, $p = 0$ .610]; and Basophils [$t(43) = -.740$, $p = 0$ .463] showed no statistical differences between the two visits. Neutrophils [t (43) = 2.681, $p = 0$ .01]; and Eosinophils [t (43) = 3.098, $p = 0$ .003] reported statistically significant differences between the second and third visits after use of *Mist Amen Fevermix*.

4.9.7.3. Assessment of Full Blood Count after use of *Edhec Malacure*

There were no statistical significant differences between levels of WBC [$t(55) = 1.351$, $p = 0$ .182]; Lymphocytes [$t(55) = .125$, $p = 0$ .901]; Monocytes [$t(55) = -1.136$, $p = 0$ .261]; Eosinophils [$t(55) = -.244$, $p = 0$ .81]; and Basophils [$t(55) = .702$, p =0 .485] before and after use of the *Mist Amen Fevermix*. On the other hand, Hb [t (55) = -3.651, $p = 0$ .001], RBC [t (55) = 3.132, $p = 0$ .003]; and Neutrophils [t (55) = 4.208, $p < .001$] showed differences at the first and second visits Table 4.27.

Table 4.27: Effect of *Edhec Malacure* on FBC

Parameter	Reference Range	1st Visit	2nd Visit	3rd Visit
		$\chi \pm s$	$\chi \pm s$	$\chi \pm s$
HB	12.0-18.0 g/dL	12.89 ±2.07	13.07 ±1.84	13.19 ±1.75
WBC	4.5-11.0 × 10^9/L	10.84 ±13.37	9.4 ±10.85	10.37 ±12.44
RBC	4.3-5.9 x10^{12}/L	5.05 ±0.39	4.88 ±0.45	5.69 ±5.77
Neutro	40.0-75.0%	59.94 ±14.47	56.8 ±13.33	57.82 ±13.74*
Lympho	21.0-40.0%	34.84 ±15.26	34.76 ±14.47	35.17 ±14.59
Monocy	3.0-7.0%	3.83 ±6.48	4.35 ±7.23	3.65 ±5.73
Eosi	0.0-5.0%	3.15 ±3.36	3.21 ±3.66	3.13 ±3.65*
Baso	0.0-1.5%	0.34 ±0.87	0.26 ±0.21	0.26 ±0.16

Key: Hb-Haemoglobin, WBC-White Blood Cells, RBC-Red Blood Cells, Neutro-Neutrophils, Lympho-Lymphocytes, Monocy-Monocytes, Eosi-Eosinophils, Baso-Basophils. Values are Mean ± S.E.M

At the second test of effectiveness of AL, no statistical differences were recorded for HB [$t(55) = 1.552$, $p = 0$ .126]; WBC [$t(55) = -.955$, $p = 0$ .344]; RBC [$t(55) = -1.047$, $p = 0$ .30]; Neutrophils [$t(55) = -1.148$, $p = 0$ .256]; Lymphocytes [$t(55) = -1.402$, $p = 0$ .166]; Monocytes [$t(55) = 1.503$, $p = 0$ .139]; eosinophils [$t(55) = 1.221$, $p = 0$ .227]; and Basophils [$t(55) = -.157$, $p = 0$ .876]. The result of this analysis is shown in Figure 4.27.

4.9.7.4. Comparative Assessment of the Effect of Test Samples on Full Blood Count

Analysis of variance of effectiveness of *AL*, *Mist Amen Fevermix* and *Edhec Malacure* on each of the indicators showed no significant differences in their effect on Hb ($p = .737$), WBC ($p = .15$), RBC ($p = .529$), Neutrophils ($p = .098$), Monocyte ($p = .518$), Eosinophils ($p = .328$) and Basophils ($p = .645$) after first visits. However, differences in effect of the three drugs on Lymphocytes ($p = .003$) were

recorded after the first visit (Figure 4.27). Post-hoc analysis showed effects of *Edhec Malacure* to be lower than the effects of Control (AL) on levels of Lymphocytes in the patients (Figure 4.28).

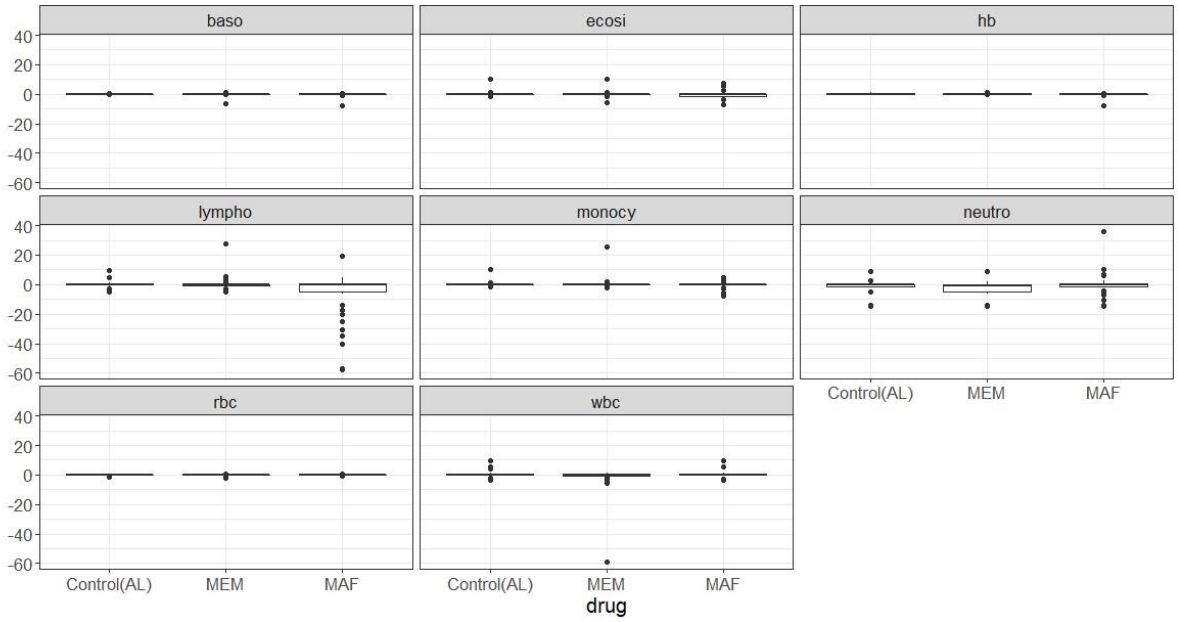


Figure 4.27: Effects of control against Test Samples at 2nd visit

4.9.7.5. Third Visit (second test) Assessment of the Effect of Test Samples

In the third visit, comparison of effectiveness of *AL*, *Mist Amen Fevermix* and *Edhec Malacure* on blood counts of patients showed no significant differences for Hb (p =.946), WBC (p =.091), RBC (p =.476), Monocytes (p =.238) and Basophils (p =.585).

Differences in effect of drugs on counts of Neutrophils (p =.004), Lymphocytes (p =.035) and Eosinophils (p =.001) were recorded after the second visit (Figure 4.28). Subsequent analysis showed higher effectiveness of Control (AL) when compared individually to *Mist Amen Fevermix* for Neutrophils (p =.116), Lymphocytes (p =.034) and Eosinophils (p =.008).

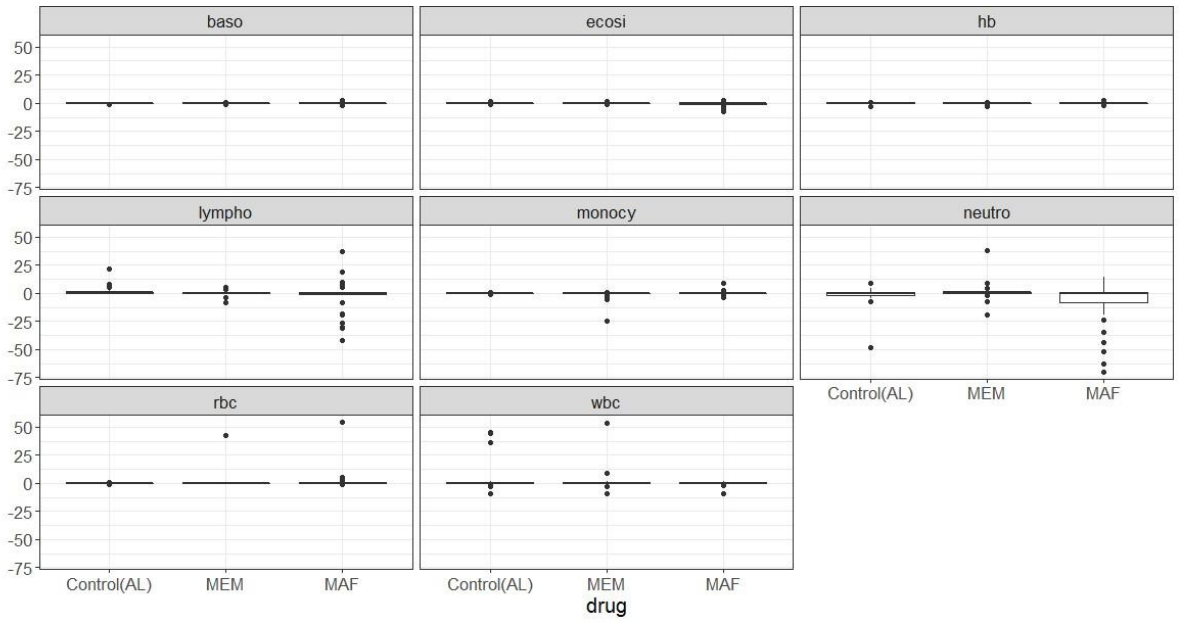


Figure 4.28: Effects of control against Test Samples at 3rd visit

4.9.8. Assessment of The Effect of Test Products on Malaria Symptoms

4.9.8.1. Symptoms

All drugs, after being used by participants showed a remarkable effect in alleviating the symptoms recorded on the first visits of patients (Figure 4.29).

4.9.8.2. Comparative Analysis the Effect of Test Products on Malaria Symptoms

Comparative analysis of drugs in terms of reducing the number of symptoms showed that there were no significant differences in the number of reduced cases of symptoms recorded for each drug [F (2, 33) = .071, p =.931]. Figure 4.29 depicts the mean number of resolved cases among participants for each drug.

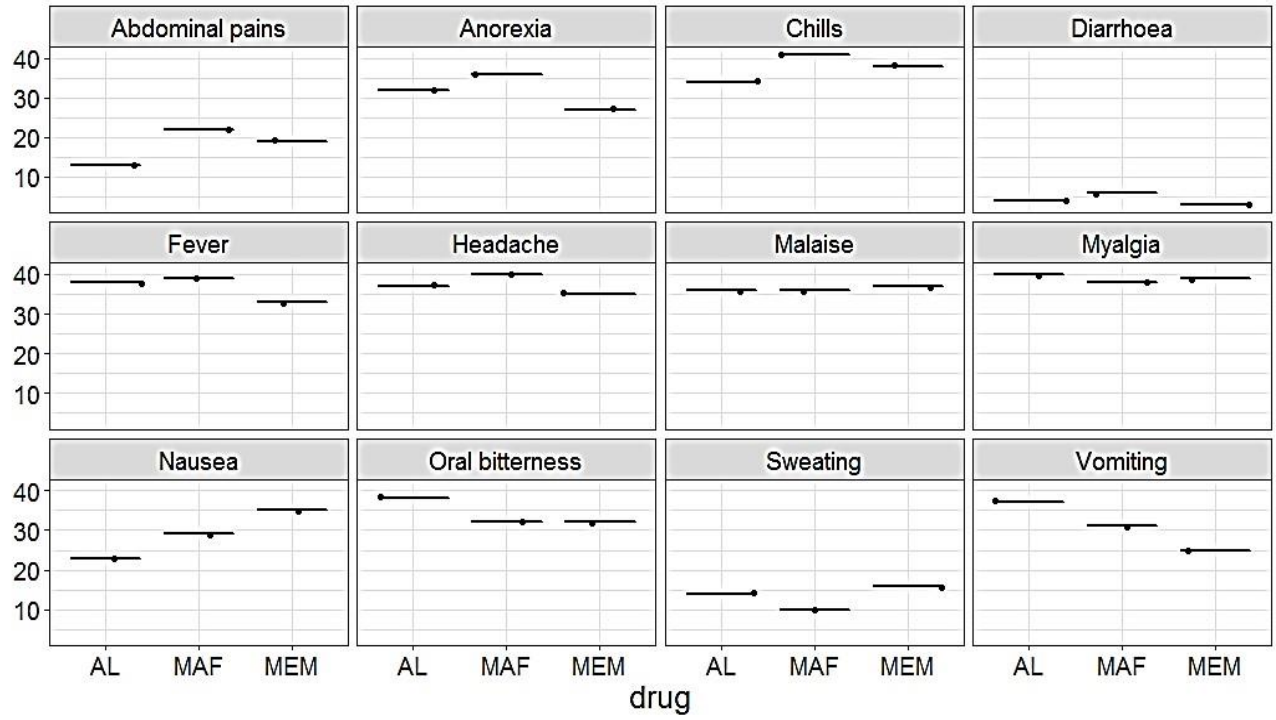


Figure 4.29: Malaria Symptoms after the use of test drugs

4.9.9. Assessment of Quality of Life using the Karnofsky's Scale of Performance

On day zero (0) before the administration of *Mist Amen Fevermix* the mean quality of life was 08.00±5.0. This improved to 95±5.0 with a *p* value of *>.0001* at the end of the study on day seven (7). Also, after the administration of *Edhec Malacure* on day zero (0), 85.0±5.0 was the mean quality of life, this also improved significantly on day seven (7) to 92±2.5 with a *p* value of *>.0001*. Details of the results (Tables 4.28 and 4.29) for *Mist Amen Fevermix* and *Edhec Malacure* respectively.

Table 4.28: Results of Quality of Life using Karnofsky's Scale between Baseline Day 0 and Day 7 for *Mist Amen Fevermix (n=46)*

Days	Karnofsky's Scale	Level of Significance
0	80.0±5.0	

7	95±5.0	*p*>0.0001

Values are Mean ± S.E.M

Table 4.29: Results of Quality of Life using Karnofsky's Assessment between Baseline Day 0 and Day 7 for *Edhec Malacure (n=42)*

Days	Karnofsky's Scale	Level of Significance
0	85.0±5.0	
7	92.5±2.5	*p*>0.0001

Values are Mean ± S.E.M

4.9.10. Referrals

There were no referrals as all the participants responded favourably to *Mist Amen Fevermix* and *Edhec Malacure.*

CHAPTER FIVE

GENERAL DISCUSSION

5.1. Introduction

The increasing usage of finished herbal products for the management and treatment of different kinds of ailments found in developing and developed countries poses a public health challenge due to the number of clinically untested herbal preparations. Herbal drugs and products have long been used to promote optimal health and well-being. They contain various phytochemicals which possess pharmacological activities (Pribitkin, 2005). They are also a source of important therapeutic remedies for alleviating human ailments. There is, therefore, the need to, harness the potential clinical use of herbal products as alternative therapies or options to conventional drugs. This has many benefits to the population who rely on herbal products for their primary health care needs as it improves the quality of life of consumers. *Mist Amen Fevermix* and *Edhec Malacure* have been used in clinical practice in Ghana since the year 2011 to date for the treatment of uncomplicated malaria. There is paucity of data from clinical studies that compare the safety and efficacy of herbal products with standard conventional medicines to justify their utilization. Thus, it is desirable to undertake a comparative clinical study of the two polyherbal products against artemether/lumefantrine using standard scientific methods to clinically evaluate the antimalarial activity for their benefits in humans. Quality control of the two herbal products was also undertaken.

The selection of two FDA registered polyherbal antimalarial remedies was based on acceptance, patronage and their subsequent utilization at the HMU of the Tafo Government Hospital. This was followed by the preclinical evaluation of the products to obtain data on the safety and efficacy. Therefore, acute toxicity testing coupled with *in vitro* and *in vivo* assay for efficacy were undertaken. This enabled the establishment of IC_{50} values, prophylactic and curative potentials of the test samples.

Evidence gathered in the pre-clinical study revealed that *Mist Amen Fevermix* and *Edhech Malacure* are safe and effective at the dosages tested. The dosage for *Mist Amen Fevermix* was 4.56 $mgkg^{-1}$and that of *Edhech Malacure* was 2.234 $mgkg^{-1}$. Therefore, clinical study was undertaken to ascertain the safety profile and effectiveness of the products in humans with uncomplicated malaria.

Mist Amen Fevermix and *Edhech Malacure,* polyherbal antimalarial products were found to be safe and effective. Some polyherbal products have been confirmed to be very effective in the treatment of wide variety of diseases (Parasuraman *et al*., 2014; Krettli *et al*., 2001).

Herbal medications have been widely utilized globally with the erroneous perception that they are natural and therefore quite safe compared to conventional medicines (Gurib-Fakim, 2006). In spite of the fact that the general occurrence of adverse effects from herbal medications appears to be low compared to those connected with allopathic drugs, injury from some herbal preparations can still happen due to plant misidentification, adulteration, contamination. Though not much can be said about the toxic effects of herbal medications in Ghana, which could be attributed to under-reporting and poor documentation, no one can rule out the fact that there are a number of herbal medicines whose toxicity assessment have not been well established and documented. This implies that they may have the potential to cause serious adverse effects on the health of consumers. Not only has this study evaluated the safety profile and effectiveness of the two polyherbal antimalarial agents, but also provided a scientific guideline (IR spectroscopy, IR chemometric and HPLC analysis) for the identification of adulterants and plant components not disclosed by manufacturers. This could be used for other herbal preparations by the FDA in Ghana prior to approval and registration.

Efforts to eliminate malaria calls for improvement in existing therapies and the development of new medicines (Burrows *et al.,* 2013; Diagana, 2015). This is because, there is global widespread of malaria parasite resistance against antimalarial medications in use now. This highlight the need for the use of polyherbal antimalarial medicines among others to propel the elimination of malaria (Willcox and Bodeker., 2004).

There are many herbal therapies available on the market without any evidence of their safety and efficacy. In order to broaden their acceptance especially in the scientific community and for policy direction, clinical evaluation of these phytotherapies should be carried out.

5.2. Acute Toxicity, *In Vitro* and *In Vivo* Efficacy

5.2.1. *In Vitro* Efficacy

Edhec Malacure had an IC_{50} value of 70.89 ng/mL whereas *Mist Amen Fevermix* had an IC_{50} value of 112.5 ng/mL as compared to the reference control artesunate which had an IC_{50} value of 0.001571 ng/mL. The differences in the IC_{50} values could be due to differences in the strains' sensitivities to *Mist Amen Fevermix* and *Edhec Malacure.* The outcome of the assay supports a previous study which found that some herbal antimalarials have antiplasmodial activity and their IC_{50} values recorded were 81.59±1.48 ng/mL and 82.25±1.91 ng/mL (Amoah *et al.,* 2015).

5.2.2. *In Vivo* Toxicity and Efficacy Assay

5.2.2.1. Acute Toxicity

Observations made after acute toxicity testing of the two herbal products showed that the test products were not lethal up to the dosage below 5000 mg/kg body weight. Thus, the test products would not cause any acute toxicity in consumers. This observation is in line with a study which established an herbal drug may not produce severe toxicological risk to consumers (Iwuanyanwu *et al*., 2012).

5.2.2.2. *In Vivo* Efficacy

In vivo efficacy assessment revealed that the test samples possess suppressive, prophylactic and curative antiplasmodial activities. The outcome of the study is in line with a study which showed that a herbal therapy exhibited effective chemo suppression activity against malaria (Tarkang *et al*., 2014).

Finally, both products exhibited much higher prophylactic antiplasmodial activity when compared to pyrimethamine (Table 4.14). The IC_{50} values indicated very low sensitivity of the test products on parasite growth *in vitro* but a much more potent antiplasmodial activity *in vivo*. This is the first record of a Ghanaian polyherbal product to the best of my knowledge, with the potential to be used for malaria prophylaxis. Hence, *Mist Amen Fevermix* and *Edhec Malacure* represents promising source of new prophylactic remedies against malaria. These observations support a study which proved the prophylactic prospects of a malaria polyherbal therapy (Nagendrappa *et al*., 2015). The significant anti-plasmodial activity of *Mist Amen Fevermix* and *Edhec Malacure* in established infection give prominence to these products for malaria treatment in Ghana. *Mist Amen Fevermix* and *Edhec Malacure* possess antiplasmodial properties *in vitro* and *in vivo*, thus validating their clinical use in the management of uncomplicated malaria and as alternative antima-larial agents.

5.3. Clinical Safety and Effectiveness

Comparative clinical study to evaluate and validate the safety and effectiveness of *Mist Amen Fevermix* and *Edhec Malacure* was undertaken. This was based on the data obtained from the preclinical studies' (acute toxicity testing and efficacy assay). The results and data obtained could serve as a potential guide for prescribers, consumers and to inform and improve policy direction on the utilization of herbal products. The study design used; open-proof, prospective clinical study involving the use of a well-established comparator therapy, in this case, Artemether/Lumefantrine, a known first-line conventional antimalarial medication agent. This makes the evidence gathered for the test products very reliable.

To validate the clinical safety and effectiveness of the test products, a total of 150 participants were recruited for the study. The study had three arms of two test groups and a control group. Each of the groups were assigned 50 participants after assessing for eligibility and consenting to be part of the

study. *Mist Amen Fevermix* was administered orally at a dose of 45 mL thrice daily after meals whereas *Edhec Malacure* was given at a dose of 30 mL thrice daily for seven days. The control group received artemether/lumefantrine at a dose of 80/480 mg twice daily after meals for three days.

5.3.1. Clinical Effectiveness

The comparative effectiveness of the test products proved that the control artemether/lumefantrine was most effective in the treatment of uncomplicated malaria. It was most effective in reducing the parasite counts. The effectiveness of AL visit was highest on the second visit (Table 4.19). The control drug and the test product recorded a relatively major reduction in parasite counts after the second and third (Figure 4.21 and 4.22). This implies the two herbal drugs may be useful alternative therapy in malaria endemic areas. The result obtained is similar to a study which showed there was a complete treatment of malaria infection in patients treated with an antimalarial phytomedicine against artemether/lumefantrine (Noudjiegbe *et al.*, 2020; Mesia *et al*., 2012).

The mechanism of action of *Mist Amen Fevermix* and *Edhec Malacure* is not known. However, the possible mechanism of action could be that they act on biochemical targets unique to protozoa, block oxidative metabolism or exhibit schizonticidal action and reduce gametocyte in *plasmodi*a transmission. This is because, the mechanism of action of many herbal medicinal products with antiprotozoal activities is presently not known (Wright, 2009).

5.3.2. Clinical Safety

Results from clinical analysis of renal panel variables revealed that the test samples did not exert any untoward effect based on the doses administered as compared to the control. This may mean that since the test samples were only used for a short period, the possibility of any untoward injury during therapy was minimized.

Hepatic panel assessment showed that direct bilirubin and globulin levels decreased during the second and the third visits when *Mist Amen Fevermix* was used. Also, there was an increase and followed by a decrease in the levels of globulin during the second and third visits when *Edhec Malacure* was used. The decrease in globulin serve as a measure for hepatic injury. This could be attributed to the reduced ability of the liver to synthesize protein and also to peroxidative injury (Kaneko *et al.*, 1997). Also, the increase in globulin levels is an indication that the test products may have hepatoprotective properties. This confirm a clinical study which found some plant products to possess hepatoprotective properties and does not interfere with hepatic function (Ekam and Udosen, 2012; Ganesh *et al*., 2009). Statistically, comparison of the effect of *Mist Amen Fevermix* and *Edhec Malacure* with AL on liver panel showed no significant differences. Also, decrease in globulin is an indication of hepatic injury (Kaneko *et al.*, 1997).

Analysis of variance of safety of *AL*, *Mist Amen Fevermix* and *Edhec Malacure* on each of the FBC variables showed no significant differences. However, there were differences in the effect of the three drugs on lymphocytes after the first visit (Figure 4.25). It has been found that some herbal drugs when administered can lead to enormous haemolysis resulting in a low FBC counts. The test products did not exert any untoward effect on haematological, renal and hepatic variables based on the doses administered as well as the control drug. This implies the two polyherbal drugs are relatively safe and could be used as alternative antimalarial agents with confidence.

It was hypothesized that *Mist Amen Fevermix* and *Edhec Malacure*, two incompletely evaluated polyherbal products claimed to have anti-malarial properties. Therefore, the two polyherbal products have been well-evaluated and found to be safe and effective to be used in humans with uncomplicated malaria.

5.4. Quality Control and Standardization

5.4.1. Introduction

The promising clinical safety profile, efficacy and *in vivo* assay outcomes called for the establishment of quality standards for the purpose of assuring of quality, identification and detection of adulteration. Therefore, quality control parameters were developed for *Mist Amen Fevermix* and *Edhec Malacure*. To achieve this, organoleptic characters, chemical fingerprinting and profiling utilizing basic phytochemical screening, HPLC and IR spectroscopic analysis. Also, HPLC analysis was done to exclude adulteration of the products with artemether, lumefantrine and quinine. In addition, HPLC profiling and IR chemometrics were done to determine the presence or otherwise of the component plants in the two polyherbal drugs as listed on their labels.

5.4.2. Quality Control Parameters

The quality control of *Mist Amen Fevermix* and *Edhec Malacure* started with the authentication of the plant materials listed as used in the manufacture of the test samples. This is very vital to avoid misidentification, detect adulteration and deterioration (Ang-Lee *et al.*, 2001).

Phytochemical screening revealed secondary plant metabolites in the products and have also been reported to be present in the plant component contained in the test samples. The presence of these phytochemicals may also serve as a means to establish the identity of subsequent manufactured products.

The pH of the test samples was consistent with the normal pH of the stomach at between 4-6.5. The stability and absorption of products is dependent on a pH within a specified acceptable range. This is an indication that the test samples may not be affected by the pH of the stomach (Mitra and Kesisoglou., 2013).

Heavy and non-heavy metals analysis revealed the presence of both macro and micro/trace elements in the test samples. However, all of them were within permissible set limits. This implies that *Mist Amen Fevermix* and *Edhec Malacure*, may be safe when used in humans. Consumption of heavy metals above permissible limits can steadily lead to muscular, physical and neurological degenerative processes (Jarup, 2003).

Salmonella Shigella, *Pseudomonas* and *E. coli* were not detected in the test samples; however, a total aerobic viable count of up to 2.17×10^3 cfu/mL was detected in both products. These microbial counts are below the maximum permissible limit of 1.0×10^5 cfu/mL. Also, the quantity of yeast and moulds was up to a maximum of 1.83×10^3 cfu/mL. These microbes even though present were below the acceptable maximum limit of 1.0×10 7 cfu/mL. This means that good harvesting and hygienic conditions were maintained during the manufacturing process. This implies the test samples are relatively free from microbial contaminants.

HPLC and IR spectroscopic fingerprint for *Mist Amen Fevermix*, *Edhec Malacure* and component plants were developed. This could be used as a characteristic fingerprint for *Mist Amen Fevermix* and *Edhec Malacure* for the purposes of identification and also to assess the possibility of adulteration.

HPLC analysis to determine the presence of artemether, lumefantrine and quinine as adulterants in the two herbal drugs was done using calibration curve plots and equation, it was revealed that the test samples were not adulterated. This was also confirmed by comparing the chromatograms produced by the test samples with that of the suspected adulterants. HPLC has been applied severally to determine adulteration in the herbal drug industry (Venhuis *et al.*, 2008).

Chromatographic profiling of *Mist Amen Fevermix* and *Edhec Malacure* to identify their component plants revealed that (Figure 4.9) there could be a plant component in *Mist Amen Fevermix* and *Edhec Malacure* which were not disclosed, however, these could also be attributed to breakdown of the

products or excipients or preservatives. This finding in the test samples is an indication that some medicinal plant components of finished herbal products are not listed on labels. This was evident from the comparative analysis (Figure 4.9). At a similarity level of 52.41%, *Morinda lucida* and *Parinari robusta* showed similarity to *Mist Amen Fevermix* (Figure 4.11). Also, the dendrogram for *Edhec Malacure* and plant components showed a similarity level of 91.58% (Figure 4.13). This implies the presence of *Morinda lucida, Magnifera indica* and *Cleistopholis patens* in *Edhec Malacure.* Another interesting revelation was that, there were some plant materials as components/ingredients of the test samples there were not listed on the labels. The results show the potentials of IR chemometrics for the identification and quality control of herbal products.

The set parameters for the quality control were found to be sufficient to evaluate the herbal drugs and can be used as reference standards for quality assurance and for routine analysis. The outcomes of the study; the quality control, validation and the provision of clinical evidence, indicate that *Mist Amen Fevermix* and *Edhec Malacure* possess potential prophylactic and curative antimalarial properties. The products were also found to be safe and did not cause any haematological and biochemical damage. Therefore, considering the high cost of orthodox medications (Mefloquine 250 mg weekly and Pyrimethamine 25 mg weekly) used as prophylactics with an average cost of about GH₵ 44.00 for mefloquine and Pyrimethamine is GH₵ 11.00 per tablet in Ghana (Lansah Pharmacy, 2020), it is recommended that, the test products be considered for use in malaria prophylaxis. This is because, the cost of the allopathic medicine is beyond the reach of the average Ghanaian. However, the cost of the test samples is GH₵ 8.00 per bottle each. Therefore, they could be used as alternatives.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

This study has established the quality parameters and validated the safety profile and effectiveness of *Mist Amen Fevermix* and *Edhec Malacure.*The study has also proven and confirm the potent antiplasmodial activity *in vivo* of *Mist Amen Fevermix* and *Edhec Malacure.* The acute-toxicity of *Mist Amen Fevermix* and *Edhec Malacure* in mice revealed that they are non-toxic and therefore safe. However, the *in vitro* antiplasmodial activity exhibited was weakly active.

The study has provided scientific evidence on the safety and effectiveness of the antimalarial properties of *Mist Amen Fevermix* and *Edhec Malacure,* which justified their use as herbal antimalarial products. The polyherbal products; *Mist Amen Fevermix* and *Edhec Malacure* achieved a comparable treatment outcome to the reference control medication artemether/lumefantrine. The two herbal products could therefore be considered as viable alternatives to the allopathic treatment with artemether/lumefantrine. The two polyherbal products were equally safe and effective.

The antimalarial polyherbal products, *Mist Amen Fevermix* and *Edhec Malacure* may be characterized qualitatively by their content of alkaloid, tannin, steroid, saponin and flavonoid. The following elements; copper, chromium, iron, zinc, potassium, sodium and manganese were found to be present in the test products. Heavy metals such as aluminium, arsenic, cadmium, mercury, lead, and nickel were also present. All the elemental contents were within the permissible limits. The results of the qualitative chemical fingerprinting and profiling provide adequate standards by which *Mist Amen Fevermix* and *Edhec Malacure* can be assessed. The combination of these characteristics can significantly contribute to the quality control, identification and detection of adulteration.

6.2 RECOMMENDATIONS

It is recommended that;

i. Reformulation of the dosage form to an improved pharmaceutical dosage form (tablet). This will, enhance the adjustment in the frequency of administration which is necessary to promote adherence.

ii. Bioavailability studies to establish essential pharmacokinetic parameters including absorption, distribution, metabolism and elimination to validate the dosage regimen and the optimization of the effectiveness of *Mist Amen Fevermix* and *Edhec Malacure.*

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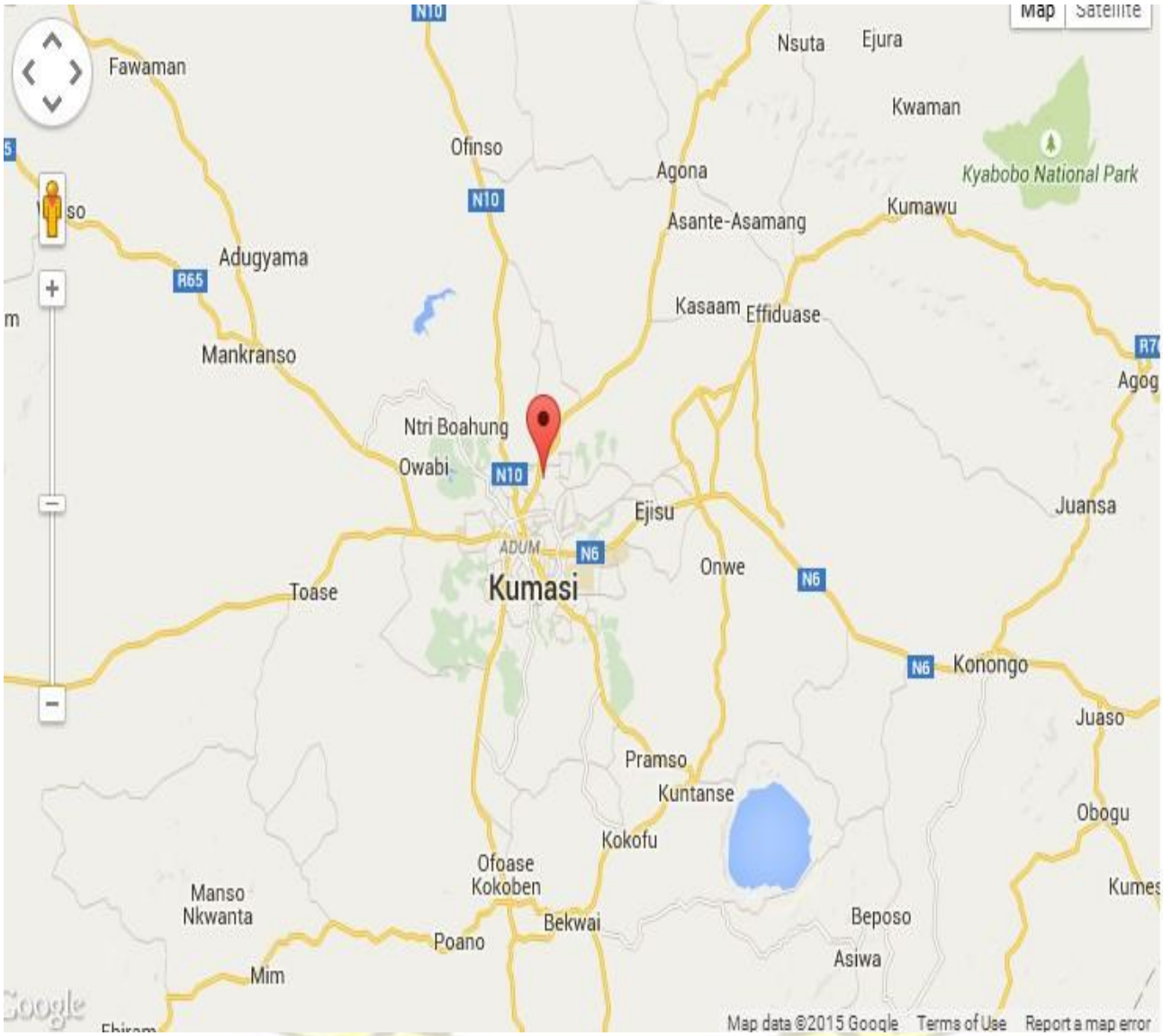
APPENDICIES

APPENDIX 1

Map Showing Tafo Government Hospital Site



APPENDIX 2

Patients Questionnaire

This questionnaire is designed to gather information on the safety and effectiveness of *Mist Amen Fevermix* and *Edhec Malacure*, two Ghanaian polyherbal products, used in the management of uncomplicated malaria.

Date

1. Card No……
2. Age…….
3. Sex: M ( )/F ( )
4. Body weight.....

1. Symptoms and Signs:

( ) General malaise
( ) Temperature> 37^0 C
( ) Body aches
( ) Joint weakness
( ) Loss of appetite
( ) Chills
( ) Rigours
( ) Fever
( ) Headaches
( ) General weakness
( ) Drenching sweat
( ) Dizziness
( ) Insomnia
( ) Easy fatiguability
( ) Shortness of breath
( ) Palpitations

Other(s)……

2. Laboratory findings:

Malaria parasites present: Yes ( ), Parasitaemia……..

Haemoglobin…….g/dl Range (11-18)

Urea……..mmol/L Range (3.6-9.3)

Creatinine……mmol/L Range (53-124)

Liver function tests: Normal ( ), Abnormal ( )

3. Diagnosis:
    a. Malaria ( )Malaria with aneamia
4. Treatment Regimen:
    a. Dosage(s)…….
    b. Duration of treatment ……
5. Treatment Outcome:
    a. Excellent ( )
    b. Very good ( )
    c. Good ( )
    d. Poor ( )
    e. Very poor ( )

    Patient feeling of well- being (absence of signs and symptoms as above)

    Please state……
6. Post-treatment Laboratory Findings:
    a. Malaria parasites present: Yes ( ), Magnitude (++++), None seen ( )
    b. Haemoglobin ……g/dl
    c. Urea……mmol/L
    d. Creatine…….mmol/L
    e. Liver function test : Normal ( ), Abnormal ( )

APPENDIX 3 Informed

Consent Form

1. Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

Date: _____ Name: _____ Statement

of person giving consent:

I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).
I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.
I understand that I may freely stop being part of this study at any time without having to explain myself.
I have received a copy of this information leaflet and consent form to keep for myself.
Name:_____

Date: _____ Signature/Thumb Print: _____

2. Statement of person witnessing consent (Process for Non-Literate Participants):

I _____ (Name of Witness) certify that information given to

_____ (Name of Participant), in the local language, is a true reflection of what l have read from the study Participant Information Leaflet, attached.

Witness' Signature (maintain if participant is non-literate): _____

Mother's Signature (maintain if participant is under 18 years): _____

Mother's Name: _____

Father's Signature (maintain if participant is under 18 years): _____

Father's Name: _____

APPENDIX 4

Karnofsky Scale for Quality of Life Assessment

Karnofsky Performance Status Scale Definitions Rating (%) Criteria

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of the disease.
	80	Normal activity with effort; some signs or symptoms of the disease.

Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

APPENDIX 5

Reference Range for Safety Parameters (RFT)

The reference ranges used in the study during the safety assessments (RFT) are listed in Tables 4.23 and 4.24.

Parameter	Reference ranges
Renal Function	
Potassium (K^+)	3.5 – 5.5(mmol/L)
Sodium(Na^+)	135 – 155(mmol/L)
Chloride(Cl^-)	96 – 110(mmol/L)
Urea	2.1 – 7.1(mmol/L)

Creatinine	M = 61.88 –123.8(µmol/L) F = 61.88 – 106.1(µmol/L)
eGFR	7 – 32 (>90ml/min/1.73m2)

APPENDIX 6

Reference Range for Safety Parameters (LFT)

The reference ranges used in the study during the safety assessments (LFT) are listed in Tables 4.25 and 4.26.

Parameter	Reference Ranges
Liver Function	
Alkaline Phosphatase (ALP)	98-279 U/L
Alanine Aminotransferase (ALT)	Males Up to 40 U/L Females Up to 32 U/L
Aspartate Transaminase (AST)	Male Up to 38 U/L Females Up to 31 U/L
Albumin (ALB)	34-48 g/dl
Gamma Glutamyl Transferase (GGT)	Male 11 to 51 U/L Females 7 to 33 U/L
Direct Bilirubin	0 – 8.67(µmol/L)
Globulin	25 – 40(g/dL)
Indirect Bilirubin	0 – 17.33(µmol/L)
Protein	66 – 87 l (g/L)
Total Bilirubin	0 – 26 (µmol/L)

APPENDIX 7

Reference Range for Safety Parameters (FBC)

The reference ranges used in the study during the safety assessments (FBC) are listed in Tables 4.29 and 4.30.

Parameter	Reference ranges
Hb	12.0-18.0 g/dL
WBC	4.5-11.0 × 10^9/L
RBC	4.3-5.9 x10^{12}/L

Neutrophils Count	2-7.5 x10^9/L
Lymphocytes Count	1.5-4.5 x 10^9/L
Monocytes Count	0.2-0.8 x 10^9/L
Eosinophil Count	0-0.4 x 10^9/L
Basophil Count	0-0.1 x 10^9/L

APPENDIX 8

Checklist for Possible Side Effect

DAY	0	3	7	14	28
Nervous system					
Drowsiness					
Nervousness					
Insomnia					
Nightmares					
Shakiness					
Numbness					
Tinnitus					
Blurred vision					
Unpleasant taste					
Thirst					
Cardiovascular					
Fast heartbeat					

Irregular heartbeat					
Respiratory					
Cough					
Chest pain					
Stuffy nose					
Gastrointestinal					
Heartburn					
Abdominal pain					
Diarrhoea					
Constipation					
Intestinal wind					
Black stools					
Genito-urinary					
Dysuria					
Nocturia					
Dark urine					
Change in sexual ability/desire					
Muco-cutaneous					
Skin rash					
Pruritus					
Easy brushing					
Dry mouth					
Jaundice					
Other(s) (specify)					

(WHO, 2004)

APPENDIX 9

HPLC Characteristic Fingerprint for Mist Amen Fevermix

Chromatogram Report

Sample Name

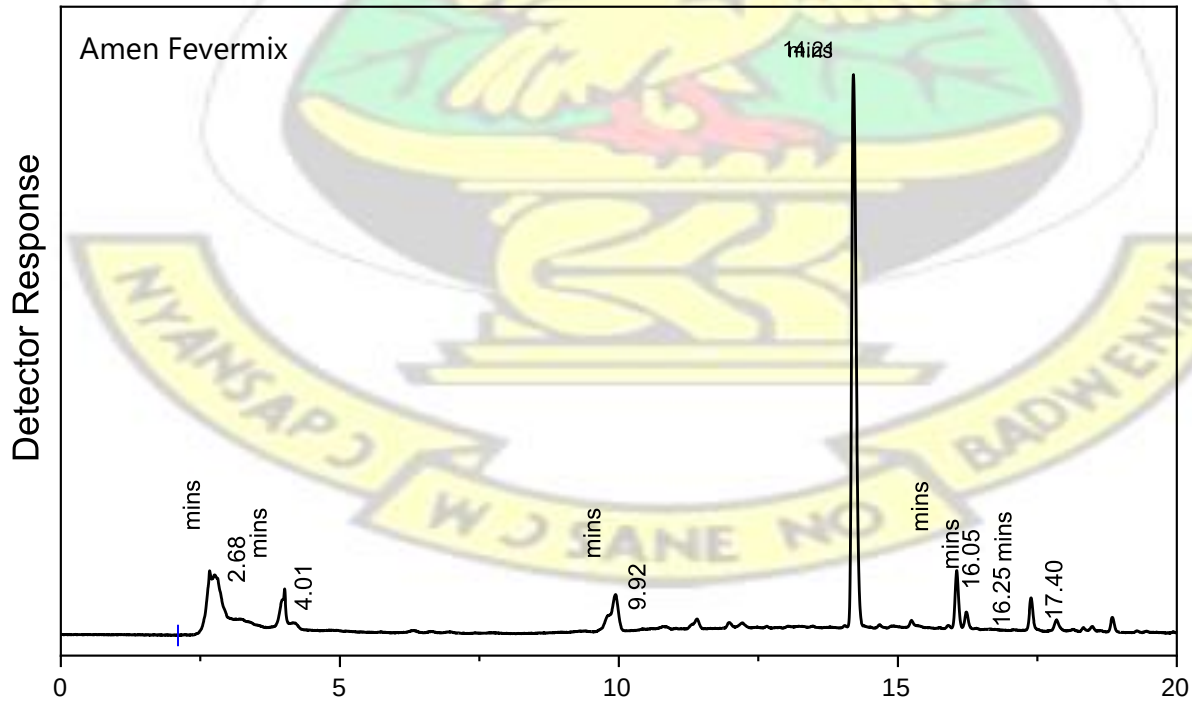
MIST AMEN FEVERMIX

Batch Group/Name DNA 2020/DNA 2

Acquisition Method

Processing Method DNA 2

Instrument Name	HPLC	Channel Name	270:10:400:10
Vial Number	3	Injection Number	1
Operator	CENTRAL LAB	Chromera Version	3.4.0.5712
Acquisition Date/Time	6/18/2020 10:34:26 AM		



time (mins)

HPLC Retention Time for Mist Amen Fevermix

Peak #	RT (min)	Component Name	Area	Height	BL	Final Amount	Units
1	2.668		77,707.7	23,573.3	BB		
2	4.010		198,668.2	40,042.6	BB		
3	9.941		158,622.6	28,826.8	BB		
4	14.205		3,649,458.8	659,322.6	BB		
5	16.056		285,839.5	67,943.0	BB		
6	16.230		66,401.6	17,449.8	BB		
7	17.388		154,744.7	37,232.9	BB		
8	18.846		59,863.5	15,518.4	BB		
Total			4,651,306.6				

Sample Name

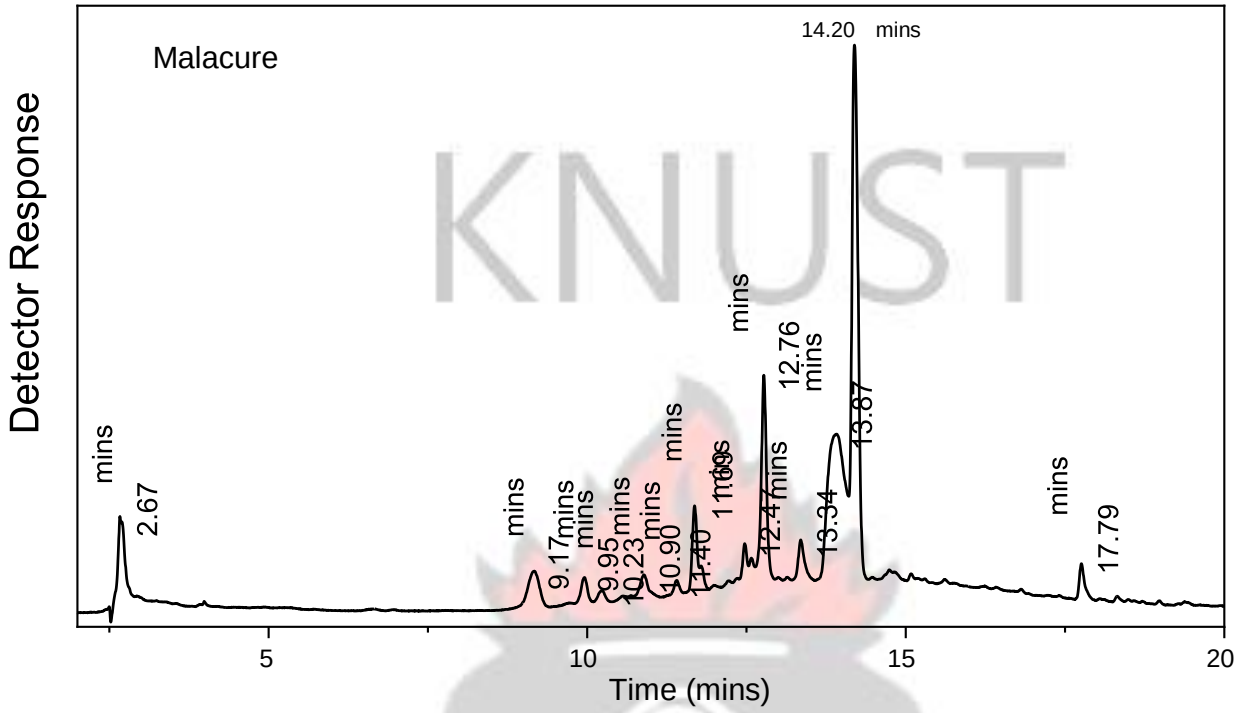
APPENDIX 10

HPLC Characteristic Fingerprint of Edhec Malacure

Chromatogram Report

EDHEC MALACURE
Batch Group/Name
DNA 2020/DNA 2
Acquisition Method
Processing Method
DNA 2

Instrument Name	HPLC	Channel Name	270:10:400:10
Vial Number	6	Injection Number	1
Operator	CENTRAL LAB	Chromera Version	3.4.0.5712
Acquisition Date/Time	6/18/2020 12:20:12 PM		



HPLC Retention Time for Edhec Malacure

Peak #	RT (min)	Component Name	Area	Height	BL	Final Amount	Units
1	2.667		73,248.8	35,125.0	BB		
2	9.169		50,185.9	3,918.0	BB		
3	9.958		184,004.1	31,167.0	BB		
4	10.895		143,584.1	23,212.9	BB		
5	11.406		73,324.3	15,798.5	BB		
6	11.687		454,457.3	101,453.0	BB		
7	12.474		146,145.8	39,255.8	BB		
8	12.577		7,324.0	3,675.7	BB		
9	12.774		1,488,606.9	275,501.9	BB		

Sample Name

10	13.353		317,421.7	52,760.2	BB		
11	13.915		3,742,706.2	202,145.0	BV		
12	14.196		5,077,084.4	747,072.9	VB		
13	17.757		234,880.0	46,947.6	BB		
Total			11,992,973.5				

APPENDIX 11

HPLC Characteristic Fingerprint of Morinda lucida

Chromatogram Report

MORINDA

Batch Group/Name DNA 2020/DNA 2

Acquisition Method

Processing Method

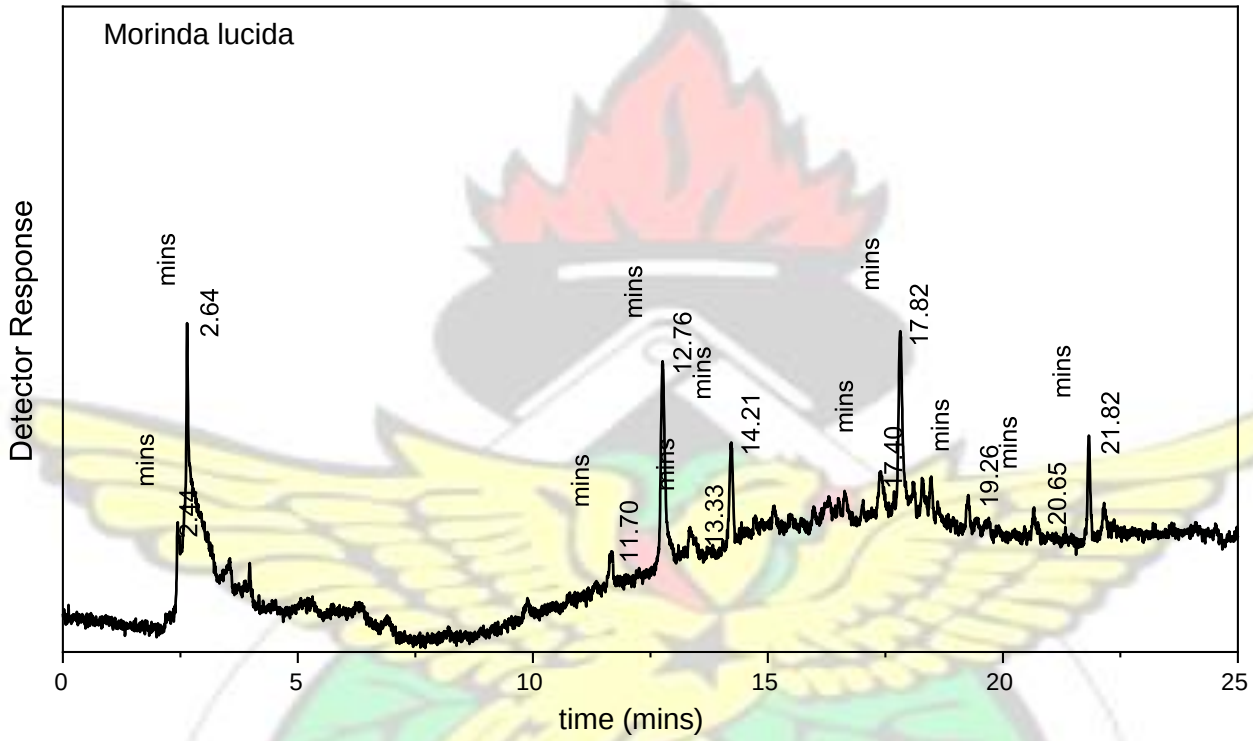
DNA 2

Instrument Name HPLC Channel Name 270:10:400:10

Vial Number 8 Injection Number 1

Operator CENTRAL LAB Chromera Version 3.4.0.5712

Acquisition Date/Time 6/18/2020 1:20:10 PM



HPLC Retention Time for Morinda lucida

Peak #	RT (min)	Component Name	Area	Height	BL	Final Amount	Units
1	2.443		3,950.5	2,066.6	BB		
2	2.644		21,321.9	8,542.8	BB		
3	8.388		614.4	556.2	BB		

Sample Name

4	12.760		50,681.9	9,263.2	BB		
5	14.135		1,097.3	940.4	BV		
6	14.215		26,144.8	5,035.9	VB		
7	17.383		2,737.6	927.9	BB		
8	17.816		43,264.2	8,257.1	BB		
9	18.281		9,101.9	1,768.9	BB		
10	20.650		3,464.5	1,124.0	BB		
11	21.831		19,053.1	5,031.1	BB		
Total			181,432.0				

APPENDIX 12

HPLC Characteristic Fingerprint of Parinari robusta

Chromatogram Report

PARINARI ROBUSTA

Batch Group/Name DNA 2020/DNA 2

Acquisition Method

Processing Method

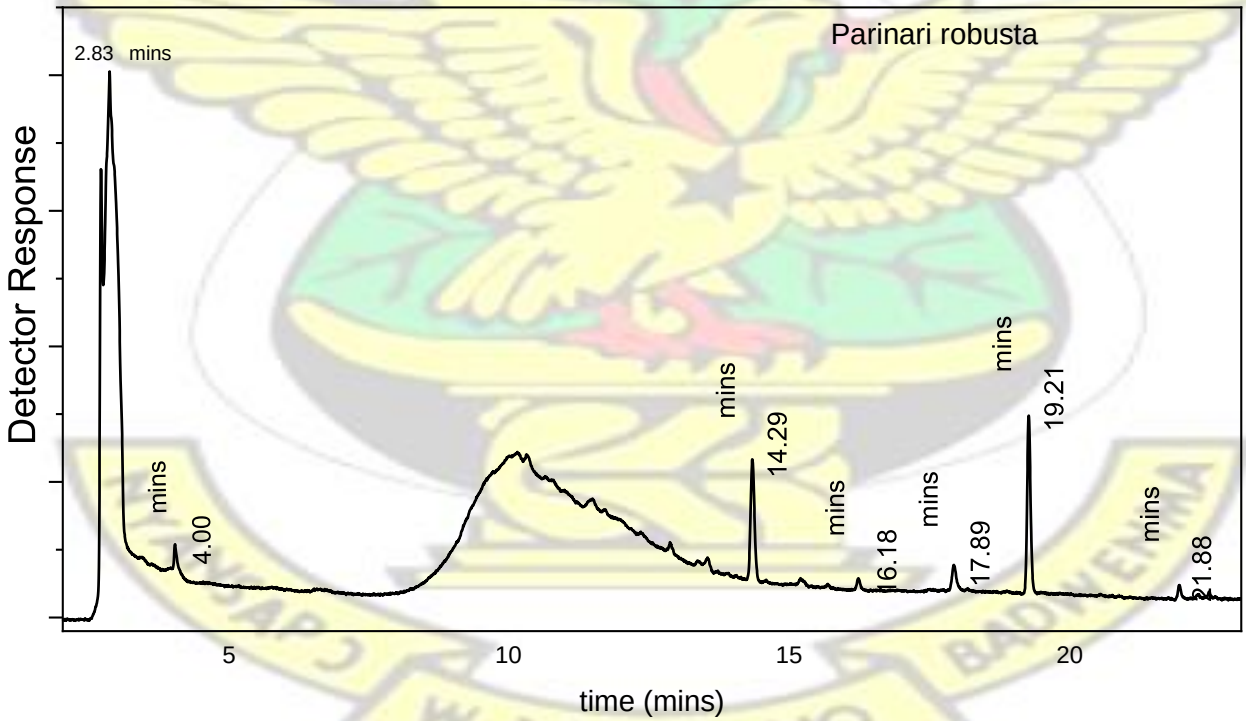
DNA 2

Instrument Name HPLC Channel Name 270:10:400:10

Vial Number 2 Injection Number 1

Operator CENTRAL LAB Chromera Version 3.4.0.5712

Acquisition Date/Time 6/18/2020 10:01:45 AM



Sample Name

HPLC Retention Time for Parinari robusta

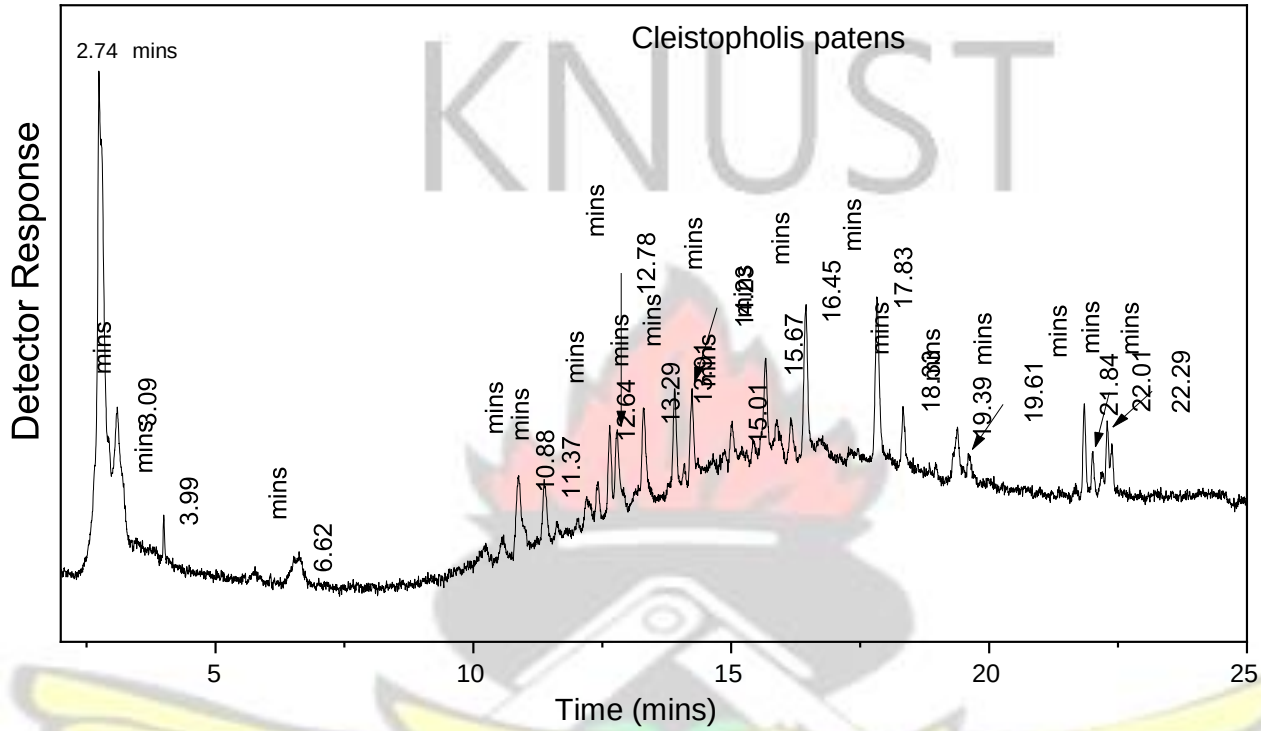
Peak #	RT (min)	Component Name	Area	Height	BL	Final Amount	Units
1	4.000		39,337.0	9,884.3	BB		
2	9.677		111,114.8	1,891.1	BB		
3	9.951		3,960.7	802.3	BB		
4	10.265		22,038.0	3,489.2	BB		
5	14.288		221,984.9	45,022.8	BB		
6	16.178		16,874.6	4,141.1	BB		
7	17.877		45,728.1	9,095.0	BB		
8	19.213		279,797.3	65,528.8	BB		
9	21.895		19,633.8	5,126.4	BB		
Total			760,469.1				

APPENDIX 13

HPLC Characteristic Fingerprint of Cleistopholis patens

Batch Group/Name	DNA 2020/DNA 2		
Acquisition Method			
Processing Method	DNA 2		
Instrument Name	HPLC	Channel Name	270:10:400:10
Vial Number	7	Injection Number	1
Operator	CENTRAL LAB	Chromera Version	3.4.0.5712
Acquisition Date/Time	6/18/2020 12:50:35 PM		

Sample Name



HPLC Retention Time for Cleistopholis patens

Peak #	RT (min)	Component Name	Area	Height	BL	Final Amount	Units
1	2.741		36,727.0	13,169.2	BB		
2	3.097		12,127.8	3,149.2	BB		
3	3.998		4,167.8	2,350.1	BB		
4	10.875		5,064.6	1,301.0	BB		
5	11.373		1,775.4	965.8	BB		

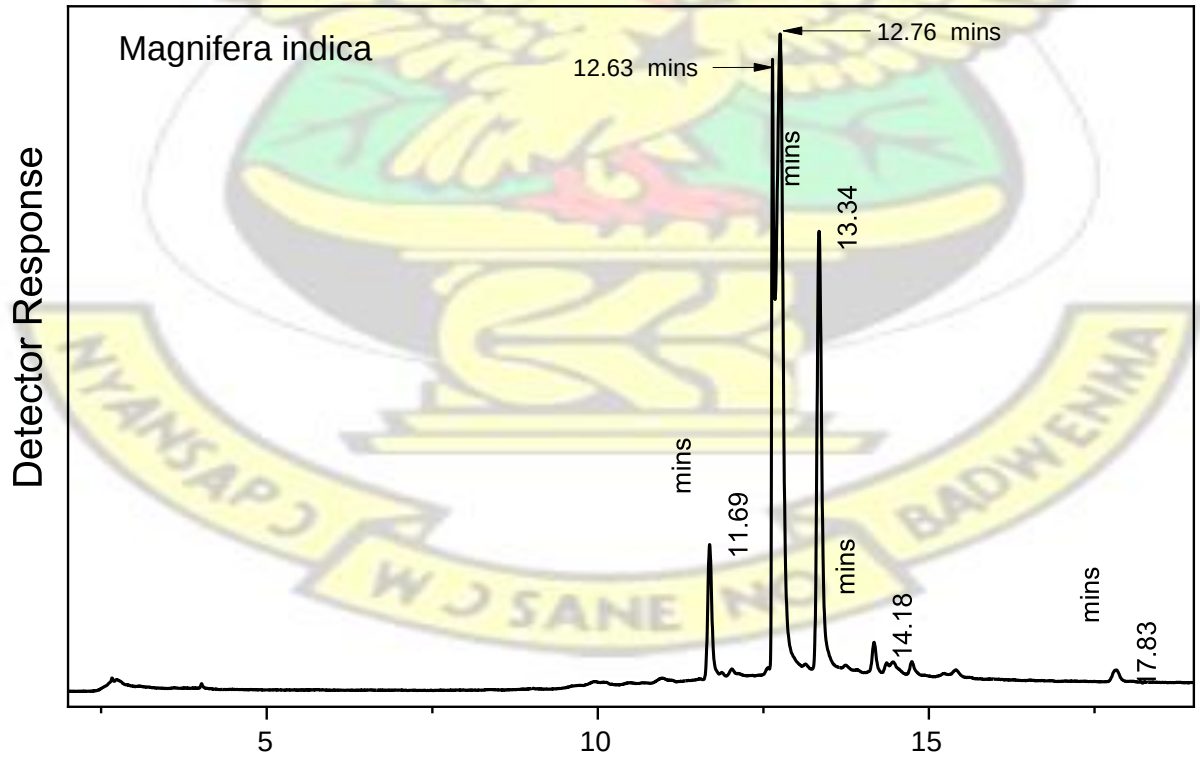
6	12.644		2,757.4	977.2	BB		
7	12.789		9,993.1	2,854.0	BB		
8	13.305		17,393.7	4,001.2	BB		
9	13.903		21,660.4	5,387.8	BB		
10	14.240		21,390.4	5,111.3	BB		
11	15.667		13,086.3	3,860.3	BB		
12	16.449		34,316.2	7,801.1	BB		
13	17.827		40,008.3	8,585.5	BB		
14	21.846		16,235.2	4,886.8	BB		
15	22.289		7,370.8	2,885.5	BB		
Total			244,074.5				

APPENDIX 14

HPLC Characteristic Fingerprint of Mangifera indica

CHROMATOGRAM for MANGIFERA INDICA

Sample Name	MANGIFER INDICA		
Batch Group/Name	DNA 2020/DNA 2		
Acquisition Method			
Processing Method	DNA 2		
Instrument Name	HPLC	Channel Name	270:10:400:10
Vial Number	5	Injection Number	1
Operator	CENTRAL LAB	Chromera Version	3.4.0.5712
Acquisition Date/Time	6/18/2020 11:48:56 AM		



Time (mins)

HPLC Retention Time for Mangifera indica

Peak #	RT (min)	Component Name	Area	Height	BL	Final Amount	Units
1	11.688		677,046.7	162,823.7	BB		
2	12.638		1,874,435.6	757,567.6	BV		
3	12.753		5,381,057.0	782,672.7	VB		
4	13.341		2,716,618.0	542,159.5	BB		
5	14.171		135,396.2	35,207.3	BB		
6	14.742		43,652.8	12,617.8	BB		
Total			10,828,206.3				

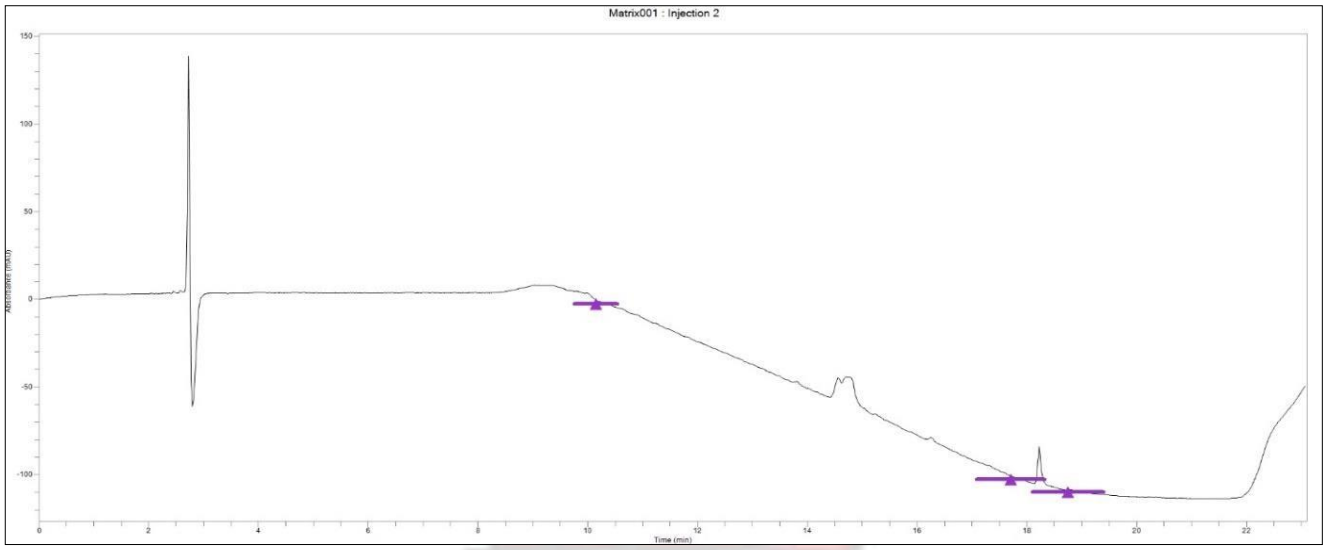
APPENDIX 15

HPLC Profiling for Adulteration

Blank

Sample Name	Matrix001		
Batch Group/Name	DNA 2020/Turkson Linearity, Precision and Recovery test		
Acquisition Method	DNATurkson Final		
Processing Method	DNATurkson Final		
Instrument Name	HPLC	Channel Name	210:10:400:10
Vial Number	1	Injection Number	2
Operator	CENTRAL LAB	Chromera Version	3.4.0.5712

3/2/2020 1:32:16 PM



Peak #	RT (min)	Component Name	Area	Height	BL	Final Amount	Units
Total							

APPENDIX 16

HPLC Profiling for Adulteration

Spiked Sample

Sample Name Recovery 2

Batch Group/Name DNA 2020/Turkson Linearity, Precision and recovery test

Acquisition Method DNATurkson Final

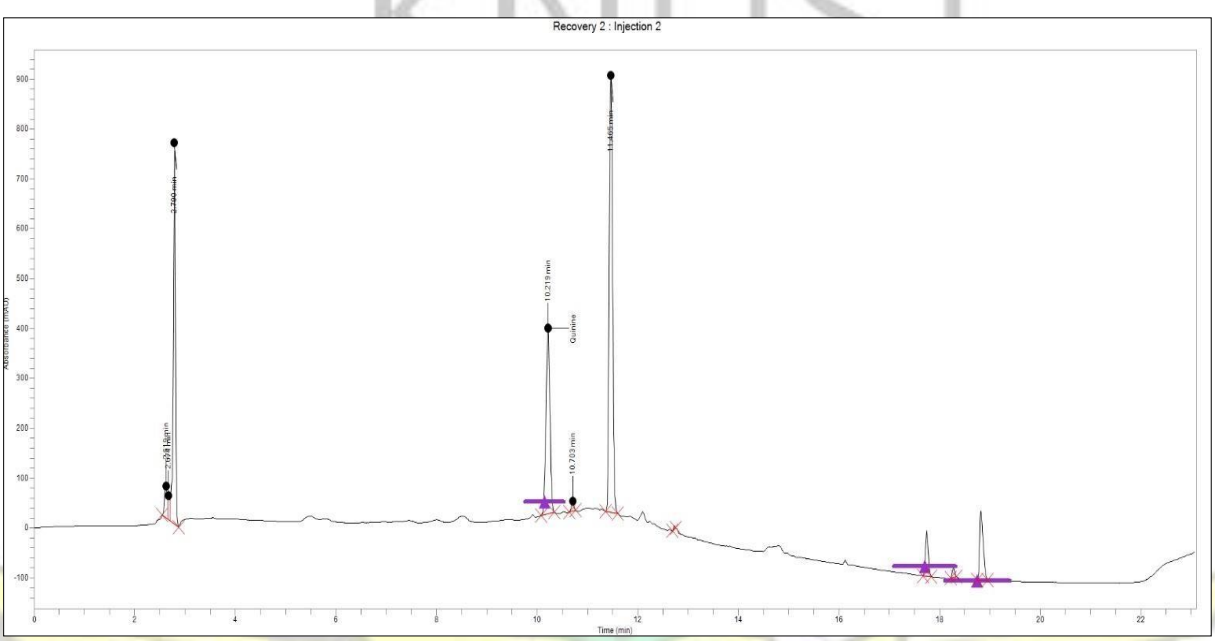
Processing Method DNATurkson Final

Instrument Name HPLC Channel Name 210:10:400:10

Vial Number 9 Injection Number 2

Operator CENTRAL LAB Chromera Version 3.4.0.5712

Acquisition Date/Time 3/2/2020 10:06:19 PM



HPLC Retention Time for Spiked Sample for Detection of Adulteration

Peak #	RT (min)	Component Name	Area	Height	BL	Final Amount	Units
1	2.619		215,827.5	63,384.2	BV		
2	2.674		126,484.0	48,009.7	VV		
3	2.790		2,249,044.5	764,849.1	VB		
4	10.219	Quinine	1,980,564.0	373,397.5	BB	83.4194	ppm
5	10.703		64,749.6	20,429.6	BB		
6	11.465		3,927,336.0	877,162.2	BB		

7	12.730		14,908.4	7,174.7	BB		
8	17.742	Artemether	348,894.2	90,636.8	BB	370.9677	ppm
9	18.277		67,099.9	19,191.5	BB		
10	18.819	Lumefantrine	664,136.1	138,929.5	BB	42.8328	ppm
Total			9,659,044.1			497.2200	

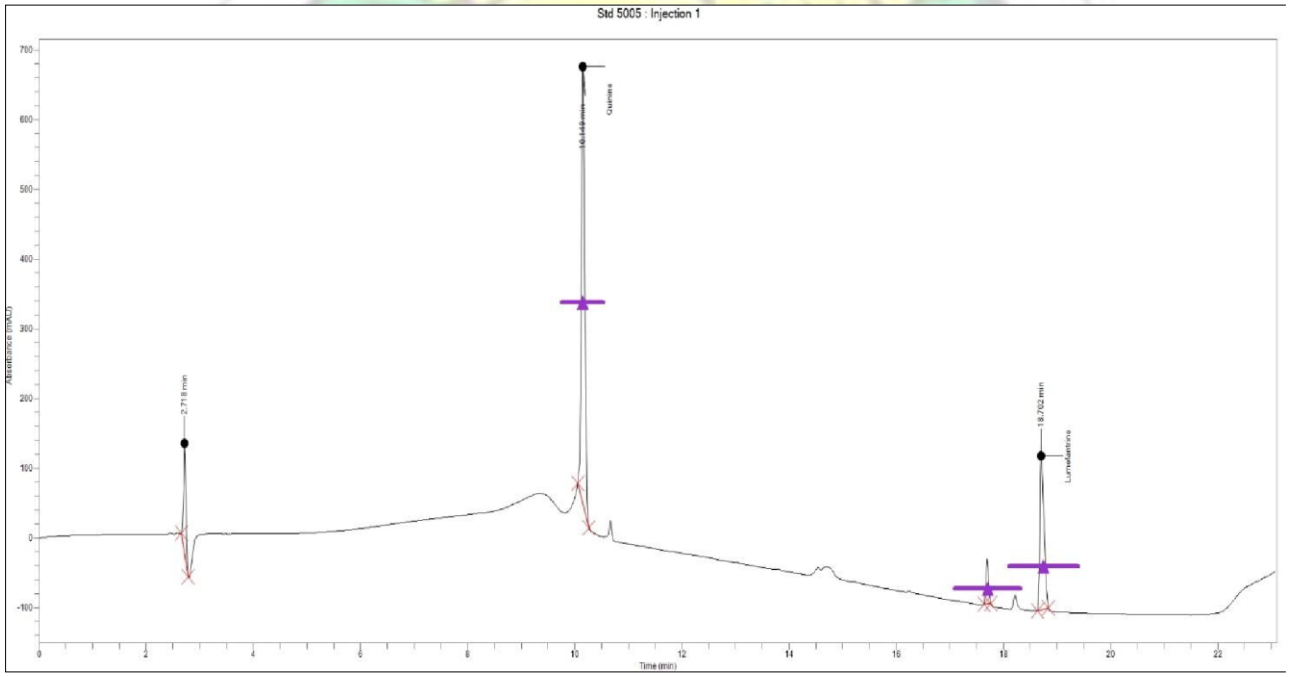
APPENDIX 17

Simultaneous HPLC Chromatogram Elution of the Reference Antimalarial Drugs

Sample Name Std 5005

DNA 2020/Turkson Linearity, Precision and recovery test

Batch Group/Name	DNATurkson Final		
Acquisition Method	DNATurkson Final		
	HPLC	Channel Name	210:10:400:10
Processing Method	6	Injection Number	1
	CENTRAL LAB	Chromera Version	3.4.0.5712
Instrument Name	3/2/2020 5:13:11 PM		
Vial Number			
Operator			
Acquisition Date/Time			



HPLC Retention Time for Simultaneous Elution of the Reference Antimalarial Drugs

Peak #	RT (min)	Component Name	Area	Height	BL	Final Amount	Units
1	2.718		452,199.7	159,786.2	BB		
2	10.149	Quinine	2,715,500.8	627,014.3	BB		
3	17.694	Artemether	228,569.9	64,474.4	BB		
4	18.702	Lumefantrine	1,174,444.6	221,148.5	BB		
Total			4,570,715.0				