INVESTIGATION OF SYNERGISTIC ANTIMALARIAL ACTIVITY OF *CORYMBIA CITRIODORA, MAYTENUS SENEGALENSIS* AND *WARBUGIA UGANDENSIS* USED IN TRADITIONAL MEDICINE IN KENYA

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Investigation of Synergistic Antimalarial Activity of *Corymbia citriodora, Maytenus senegalensis* and *Warbugia ugandensis* Used in Traditional Medicine in Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature……Date……

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This thesis has been submitted for examination with our approval as University Supervisors:

Signature……Date……

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DEDICATION

I dedicate this work to almighty God and my parents; Mr. Joseph Atambo Nyakundi and late mother, Mrs. Mary Nyaboke Atambo for sowing the seed of resilience within me. God's abundant grace and guidance have been sufficient for the completion of this work. All I can say is thank you God with everlasting praise.

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ACRONYMS AND ABBREVIATIONS

ACT	Artemisinin-Based Combination Therapy
ANOVA	Analysis of Variance
CC_{50}	The Concentration Inhibiting 50% of the Vero cells
CTMDR	Centre for Traditional Medicine and Drug Research
DDT	Dichlorodiphenyltrichloroethane
ERC	Ethical Review Committee
ED50	The Concentration Inhibiting 50% of *P. berghei*
KEMRI	Kenya Medical Research Institute
LD_{50}	Lethal Dose for 50% of the Experimental Animals
MTT	3-[4,5-Dimethylthiazol-2-yl]-2,5-Diphenyltetrazolium Bromide
OECD	Organization for Economic Cooperation and Development
PSG	Phosphate Saline Glucose Buffer
SFIC	Sum of Fractional Inhibitory Concentrations
SEM	Standard Error of Mean
WHO	World Health Organization

ABSTRACT

Malaria is majorly caused by *Plasmodium falciparum i*n Kenya where 30% of the population relies on traditional medicine for treatment due to cost and accessible medical facilities especially in the rural areas. *W. ugandensis* (W), *M. senegalensis* (M) and *C. citriodora* (C) are plants used in herbal medicine for treating malaria. However, their combined antimalarial efficacy and safety is yet to be determined. The negative control and solvent used was water and chloroquine as positive control. Cytotoxic properties (CC_{50}) of the plants were carried out against Vero cell-lines *in vitro* via MTT assay. Acute oral toxicity (LD50) was conducted according to OECD protocol. Antimalarial properties (ED_{50}) of aqueous plant extracts were analyzed against *Plasmodium berghei in vivo.* The combinations of C: M: W (1:1:1) used in the study exhibited no cytotoxicity, (CC_{50}) of 101.47±3.17 µg/ml. All the plant extracts demonstrated LD_{50} above 2000 mg/kg with no adverse effects hence recognized as safe. The result indicated that the combination of M:W (1:1) had the highest antimalarial activity, ED_{50} of 1.05mg/kg and C:M:W (1:1:1) (ED_{50} of 2.26 mg/kg). Phytochemical profile of plant extracts not conducted but activity attributed to individual plant tannins, flavonoids, sesquiterpenes, and saponins. The SFIC determines the interaction between two or more drug compounds. Two-plant extracts exhibited synergistic effect whereas C.M.W in the ratio 1:1:1 showed antagonism with SFIC 1.92. Non-linear analysis was used to determine CC_{50}, ED_{50} and LD_{50}, one- way ANOVA for means between treatment groups and Tukey's post hoc for pairwise control of means. From a molecular medicine perspective, this study elucidates the potential of combination therapy at the molecular level by evaluating the interactions between plant-derived compounds through the Synergistic Fractional Inhibitory Concentration (SFIC) index. The determination of cytotoxicity and effective concentrations provides critical insights into the molecular mechanisms underlying the safety and efficacy of these herbal extracts, paving the way for their optimization as targeted antimalarial therapies.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The species that infect humans are four and these include *P. vivax, P. ovale, P. malariae, and P.falciparum* and recently *P.knowlesi* (CDC, 2024). Mammalian hosts are the exclusive targets for *Plasmodium falciparum* and *Plasmodium vivax* with the former causing the most malaria burden globally (WHO, 2020).

Pregnant women and children under the age of five are the most vulnerable populations due to reduced immunity and the increased risk in severe malaria. Malaria transmission and infection rates are reduced via preventive strategies such as vector control and chemoprophylaxis (CDC, 2024).

A first-line treatment is Artemisinin-based combination therapies (ACTs) which treat uncomplicated malaria because of their high therapeutic efficacy (Diallo *et al*., 2020). Pregnant women likely to suffer maternal anemia have Sulfadoxine–pyrimethamine administered to reduce malaria infection (Mlugu *et al*., 2020). Doxycycline is another effective chemoprophylactic agent but it is not recommended for use in pregnant women and young children because of safety concern (CDC, 2024).

Malaria chemotherapy involves a combination drug regimen for both chemoprophylaxis and treatment after infection. Chemoprophylaxis doses provide temporal protection to individuals travelling to malaria endemic hotspots and areas at risk of endemic outbreaks (DeVos *et al*., 2020). The drugs that have been used for treatment of malaria include atovaquone–proguanil, efloquine, sulfadoxine, quinine, quinidine, halofantrine and artemisinin derivatives. Atovaquone disrupts mitochondrial function, artemisinin generates toxic radicals, proguanil and sulfadoxine inhibits folate metabolism and quinine and halofantrine blocks heme detoxification (Alghamdi *et al*., 2024). A first-generation vaccine against *P.falciparum* known as RTS,S/AS01 has been developed that reduces malaria cases in young children (Laurens, 2019) It stimulates the production of CD4+ T cell activation and

cytokine production against circumsporozoite protein on the sporozoite surface preventing Plasmodium from invading the liver cells interrupting their development (Quin *et al.,* 2025).

The emergence and spread of drug-resistant strains of *Plasmodium falciparum* threatens current malaria control programs. Resistance in malaria-endemic areas to antimalarial drugs such as chloroquine and Sulfadoxine-pyrimethamine has been reported (Habarugira et al., 2026). There are cases of reduced sensitivity to artemisinin-based combination therapies raising concerns about their long-term efficacy (Ocan *et al*., 2023). There is an urgent need for novel antimalarial agents due to the accessibility, cost and rising resistance of the malaria parasites to treatment drugs.

Medicinal plants have been used as sources of antimalarial drugs and are still important for drug discovery (Habibi *et al*., 2022). Antimalarial drugs such as artemisinin and quinine which are important in malaria chemotherapy originated from plants (Ceravolo *et al*., 2021). Approximately, 75% of the African population rely on herbal medicine to treat malaria. The clinical applicability such as dosing and cytotoxicity data has not been established limiting their use (Okaiyeto *et al*., 2021). The safety and efficacy of some of these herbal remedies are yet to be scientifically determined.

Isolation of antimalarial natural products obtained from plants used in traditional medicine, provides a novel source of antimalarial therapeutics. It has been suggested that the development of direct herbal formulations will provide dosage descriptions leading to cheaper and more affordable sources of drugs to the communities in which they belong. Due to the natural origin and prolonged traditional use of herbal medicines, they are considered safe (John *et al.,* 2023). Mortality and morbidity rates caused by malaria have reduced because of the use of local herbal therapeutics particularly in parts of developing countries where conventional antimalarial drugs are not readily accessible, affordable and available (Noronha *et al*., 2020).

It is essential to establish the efficacy and safety of herbal medicine so as to develop affordable therapeutic agents. The safety profile and selectivity of the herbal extracts are determined through cytotoxicity studies using mammalian Vero cell lines (Gavanji

et al., 2023). Potential adverse effects and safe doses are determined by acute toxicity studies in mice. *Plasmodium berghei* infected mice are used to test antimalarial activity *in vivo* through a four-day chemosuppressive test (Omagha *et al*., 2021).

Plant extracts are combined to increase therapeutic efficacy. The combinations may cause synergistic, additive or antagonistic interactions which is dependent on their fractional inhibitory concentration index (FIC) (Caesar *et al*., 2019).

In East Africa, medicinal plants including *Maytenus senegalensis*, *Warburgia ugandensis* and *Corymbia citriodora* are commonly used to treat malaria and have demonstrated antimalarial activity (Omara, 2020). *Corymbia citriodora* has traditionally been used in the past to treat; fever, respiratory infections, and inflammatory conditions (Valdiviezo-Campos *et al*., 2024), symptoms that are often associated with malaria. *Corymbia citriodora* is known for its natural mosquito repellent and antimalarial activity (Corzo-Gómez *et al*., 2024). Phytochemical studies showed flavonoids, tannins and terpenoids as the bioactive antimalarial compounds (Muthengi *et al.*, 2025).

In communities where access to conventional drugs is limited, *Maytenus senegalensis* is a plant that has been used to treat inflammatory conditions and infectious diseases. The stem bark, leaves, and roots are the commonly used plant parts. The antimalarial activity of *Maytenus senegalensis* is linked to active compounds such as sesquiterpenes, flavonoids and triterpenoids (Kassimu *et al*., 2024). The key bioactive antiplasmodial compounds are triterpenes, maytenoic acid and pristimerin with notable potential efficacy against drug-resistant malaria strains (Jigam *et al*., 2020).

Similarly, *Warburgia ugandensis* is extensively used across East Africa where decoctions of stem bark, roots and leaves are used to treat microbial infections and malaria with relatively low cytotoxicity (Opiyo., 2023). Phytochemical investigations of *Warbugia ugandensis* have demonstrated antiparasitic and antimicrobial activity attributed to the bioactive compounds namely; flavonoids, tannins, warburganal, saponins, and drimane sesquiterpenes (Okello *et al*., 2019). *Warbugia ugandensis* antiplasmodial activity has been demonstrated in both *in vivo* and *in vitro* models producing significant chemosuppression in mice models (Were *et al*., 2020).

These plants have shown promising antimalarial properties individually. There is limited information on the safety and efficacy of their combined use to treat malaria. This study aimed to evaluate the potential synergistic cytotoxicity, acute toxicity and *in vivo* antimalarial activity of *Maytenus senegalensis, Warburgia ugandensis* and *Corymbia citriodora* combined plant extracts.

1.2 Problem Statement

Malaria is a major public health burden due to the drawback that is associated with cost and accessibility to treatment drugs in developing countries (Mezieobi *et al*., 2025). Kenya records 3.4 million clinical malaria cases yearly particularly in the Western and Coastal regions. Transmission of the disease, hospital consultations and deaths is still high despite reduction in incidences. Approximately 12,000 deaths occur every year mostly among pregnant women and children under the age of five (CDC, 2024). Subsidies are given by donors and the government for malaria drugs, reducing the cost to under 1$ in turn causing over treatment (Institute of Medicine (US), 2024). In severe cases that require medical attention, costs go up to 50$ for both direct and indirect expenses (Watts et al., 2021). This is a high cost where a majority of the population live on less than 3$ a day (World Bank Group, 2022). This causes adults to purchase cheaper alternatives despite being less effective.

Additionally, the perpetually evolving drug-resistant strains of *Plasmodium* species compromises malaria control. Emerging resistance to the current regimen for malaria treatment artemisinin and its derivative such as artemether and artesunate have been reported (Rasmussen *et al.,* 2021).

Herbal medicines have been utilized since ancient times for the management of a multitude of human ailments and diseases. Plants provide a novel source for the development of human therapeutics (Chaachouay et al., 2024). In malaria treatment, plant combinations have been used. For instance, artemisinin which is the current drug regimen used for the treatment of malaria is isolated from *Artemisia annua* highlighting plants as novel sources of therapeutic agents (Barend *et al*., 2023).

Plants namely *Corymbia citriodora, Warbugia ugandensis* and *Maytenus sengalensis* have demonstrated antimalarial activity. Most studies have evaluated the plant extract antimalarial properties in isolation. In comparison to single plant extracts, studies have shown that plant extract combination in malaria treatment increases efficacy, synergistic effects and reduced toxicity (Angupale *et al.*, 2024). The determination of effectiveness of these combined plant extracts is crucial in developing complementary herbal formulations and isolation of pure antimalarial compounds.

1.3 Justification of the Study

Malaria remains a major public health issue in Sub- Saharan Africa. In Kenya, 30% of the population relies on traditional medicine for treatment. High rates of mortality are observed in children under the age of five as well as high infection rates in pregnant women. Management of malaria is becoming problematic due to the development of resistance to the drugs used for treatment.

Most of the antimalarial drugs have a mono-therapeutic mechanism by inhibiting a single pathway or target such as folate synthesis or heme detoxification. Plant extract combination gives a multi-target therapeutic approach that act on both the parasite biochemical pathways and modulate host-immune responses (Donkor *et al*., 2023). This shows a need for novel antimalarial therapeutics with multi-target mechanisms. This is a crucial approach in overcoming drug resistance.

Majority of the population heavily rely on traditional medicine to treat malaria. Historically, plants have provided novel compounds for drug discovery and development. Studies on medicinal plants used in traditional are a source of affordable, effective and culturally acceptable therapeutics (Tabuti *et al*., 2023). This justifies the study to address drug resistance while advancing the development of alternative therapies

1.4 Research Hypothesis

1.4.1 Null Hypothesis

The combined extracts of *Corymbria citriodora, Maytenus senegalensis and Warbugia ugandensis* are not safe and have no antimalarial activity.

1.5 Objectives

1.5.1 General Objective

To assess the synergistic antimalarial activity of *Corymbia citriodora, Maytenus senegalensis* and *Warbugia ugandensis* used in the management of malaria in Kenya

1.5.2 Specific Objectives

i. To evaluate the *in vitro* cytotoxicity of combined extracts of *Corymbia citriodora, Maytenus senegalensis* and *Warburgia ugandens*is in Vero cells.
ii. To evaluate the acute toxicity of combined extracts of *Corymbia citriodora, Maytenus senegalensis* and *Warburgia ugandens*is in mice.
iii. To evaluate the synergistic *in vivo* anti-malarial activity of extracts of *Corymbia citriodora, Maytenus senegalensis* and *Warburgia ugandens*is utilizing the *Plasmodium berghei.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria Etiological Agent and Lifecycle

Four species of the protozoal genus cause malaria in humans. *Plasmodium falciparum* is the most perilous followed by several other species. *P. falciparum* is the most severe cause of malaria infection and highly prevalent in Africa (Sato, 2021). *P. vivax* is more prevalent than *P. falciparum* because it has the ability to infect *anopheles'* vectors during lower temperatures, higher altitudes and in cooler weather conditions. *Plasmodium knowlesi* is more predominant in the Asian peninsula where it causes feeble illnesses (Price *et al*., 2020). *Plasmodium ovale* highly occurs in the islands of the Western Pacific and Sub-Saharan Africa where it causes infection that is characterized by low parasitemia levels (Okafor, 2019).

The vector for malaria is the female *anopheles* mosquito that is comprised of approximately 400 different species whereby only 30 species are involved in malaria transmission (CDC, 2024). During a blood meal, an infected female *anopheles* mosquito injects sporozoites into the human host. The sporozoites are lancet shaped, 7µm and uninucleate. They migrate to the hepatocytes parenchymal cells where they develop into spherical multinucleate schizonts. Upon maturity schizonts burst releasing uninucleate merozoites that migrate to erythrocytes. In the erythrocytes, merozoites may develop into gametocytes or schizonts carrying about 10 to 36 merozoites (Hofer *et al*., 2024). This stage causes severe damage of erythrocytes resulting in the clinical symptoms of the disease. Mosquito biting infected humans, at this stage will be infected with the gametocytes which are deposited at the gut where they mature into sporozoites hence completing the cyclic life of plasmodium (Alemayehu *et al.*, 2023).

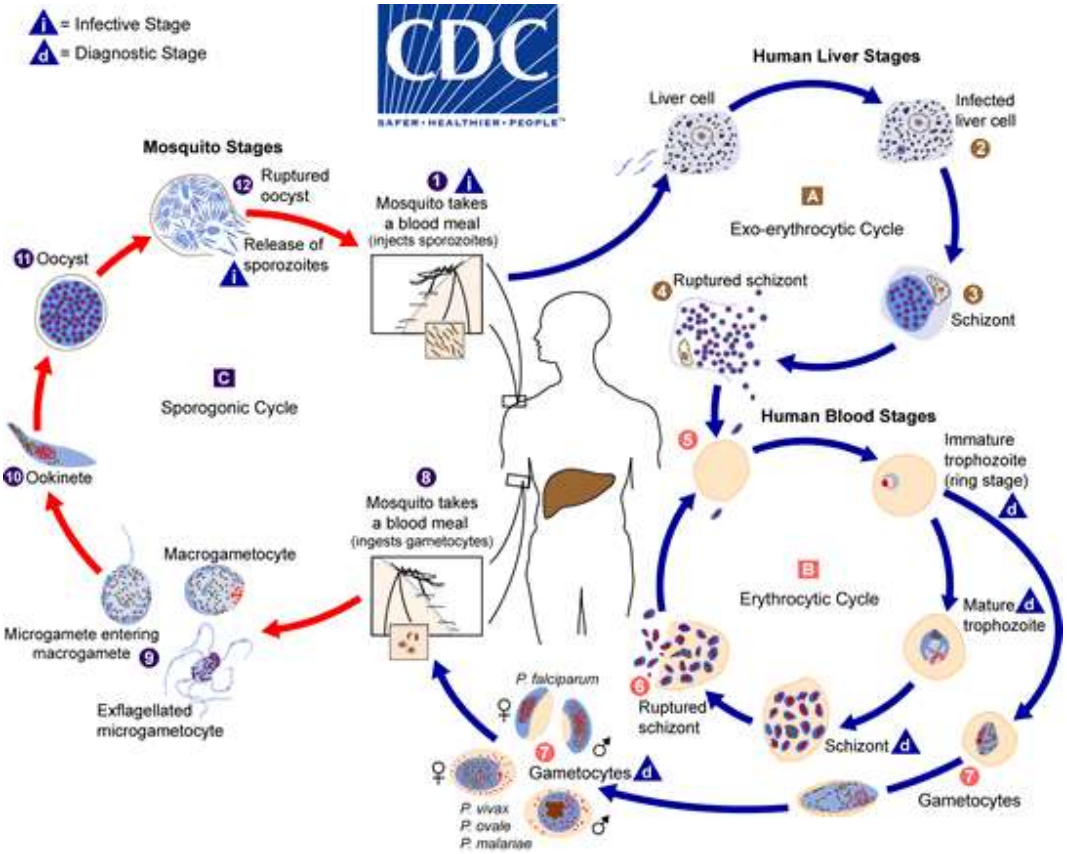

Figure 2.1: Illustration of the Life Cycles of Malaria Parasites, Plasmodium Species

Source: CDC (2019)

2.2 Transmission

Malaria is transmitted by the anopheles' mosquito whose species determines the virulence of the disease. In Turkana *Anopheles arabiensis* transmits *Plasmodium falciparum* and may reside indoors due to unfavorable site conditions and is spread throughout the country (Karungu *et al*., 2019). In the highlands, *Anopheles funestus* is the primary vector, *Anopheles gambiae sensulato* complex mostly occurs during the non-rainy season transmitting the parasites into the human host (Ogola *et al*., 2018). The life span of the above-mentioned species is long enough to encourage virulence, especially during the rainy seasons that increases breeding site.

2.3 Epidemiology of Malaria

Malaria caused close to half a million of deaths in 2019 and 94% of the deaths occurred in Sub Saharan Africa. Further, over 200 million clinical incidents of malaria occur every year. Among these deaths, 67% occurred in children under the age of five years (Li., 2024). It's most prevalent in Sub Saharan Africa and Latin America whose presence varies within these regions depending on climate change and immigration into an endemic area.

Elimination of malaria in the African continent is deeming difficult as it is wide spread. The numbers and infectivity of the anopheles' mosquito is dependent on temperatures between 25-30 ˚C and proximity of households to natural water sources and dams (Chapoterera et al., 2025). Approximately 26% of the global endemic regions are exposed to unstable malaria and the remaining percentage has hypoendemic prevalence to the disease. Resurgence of malaria in the past occurred due to resistant strains to drugs, poor funding and increase in disease transmissibility (Fola *et al*., 2023).

The highest victims of malaria infection are pregnant women and children under five years of age. Kenya is divided into zones depending on the degree of infections observed (prevalence) and weather patterns that promote the spread of disease but study in endemic areas showed a rise of infection amongst the young (Zablon et al., 2026).

2.4 Seasonality of Malaria Infection in Kenya

Temperature and rainfall are not uniform across the country and this affects the distribution of the malaria vector (Kioko et al., 2024). In the highland areas of Kenya, there has been a change in behavior of mosquitos as well as change in climate. Seasonal peaks of malaria infection have been observed in the months of April, July, November and December similar to Kwale (Cook *et al*., 2018).

2.5 Clinical Symptoms Associated with Malaria

Plasmodium falciparum malaria symptoms are observed in a period not exceeding two weeks after a mosquito bite (Markwalter *et al.*, 2024). Clinically, malaria may be uncomplicated where the individual experiences chills and muscle joints or in children as lethargy, pallor or loss of appetite. In severe malaria jaundice, abnormal bleeding and respiratory distress is present due to the high level of parasites in the blood.

Asymptomatic malaria may appear mostly in persons that reside in the high-risk areas. Symptoms are observed due to the growth of parasites in the erythrocytes (Prah *et al*., 2023). Longer periods of infection reduce hemoglobin levels and this is a cause of anemia. An individual becomes fatigued with the presence of sweating, renal failure, cerebral malaria, the latter occurring majorly in children, renal failure and respiratory problems resulting to ultimate death (White, 2018).

2.6 Laboratory Diagnosis

Early detection and intervention of malaria is important to avoid further complications, transmission of the disease and enable effective treatment of the same. This is through a number of methods such as the more commonly used light microscopy and dipsticks (Mbanefo *et al*., 2020). Microscopy uses Giemsa stain to observe both thick and thin blood smear though this technique is costly due to the equipment used and skilled individuals required for accurate diagnosis through microscopy. Fluorescence microscopy involves use of fluorochromes for staining and yields precise results (Poostchi *et al*., 2018).

Rapid diagnostic kits are used in the diagnosis of malaria especially caused by *Plasmodium falciparum* where a finger prick is placed on the kit that detects production of metabolic enzyme by the parasite. The principle is immunochromatography where the blood containing the antigen (HRP-2, pLDH and *Plasmodium aldolase*) from the parasite is captured (Kayode *et al.,* 2024).

Molecular techniques such as PCR are used to determine the level of infection in blood PCR is a sensitive technique that demonstrates the variations within the gene or strain

of the parasite, accurately identifies the species and determines past exposures present (Grabias *et al*., 2019).

2.7 Treatment

Artemisinin based Combination therapy in Africa is used as the first or second line of treatment since there is reduced cases of resistance. Children and pregnant women are considered to be the vulnerable population as far as malaria is concerned. Sulfadoxine-Pyrimethamine is administered to pregnant women that helps prevent both anemia and malaria (Sundararaman *et al.,* 2022). Doxycycline may be used for preventive measures despite the reactions from drug use and is thus not advisable for pregnant women or children to use (Fisher *et al*., 2024) Some of the antimalarial developed drugs have been rendered obsolete by *Plasmodium* species resistance. Malaria vaccines; RTS,S/AS01 and R21/Matrix-M are administered to children above the age of five months are used to reduce infection by 40-75% (Oduoye *et al*., 2024)). The vaccines inhibit Plasmodium falciparum infection in children aimed to increase survival rates from the illness. Despite this, funding is affecting the uptake of the vaccine at national level (Okanda et al.,2024).

2.8 Control Measures

To prevent the spread of malaria, several control measures have been put in place through funding that ensures the continuity of the set controls. In endemic countries one of the major strategies is to reduce contact of human with the mosquito. In Kenya long lasting insecticidal nets are given to those at high risk at health care facilities and also in endemic areas whereas market prices are fair to encourage their use (Haileselassie *et al*., 2022).

Indoor residual spraying can be carried out to reduce the numbers of vectors mostly in endemic areas using DDT and dieldrin. The substances sprayed can last up to one year and is more efficient when neighboring areas undergo the same process (Zhou *et al*., 2022).

Drugs and vaccines are used to prevent infection of erythrocytes by the parasite. Antimalarial are given for people travelling to a prone region, pregnant women and children vaccination. The vaccines Mosquirix/ RTS,S/AS01 and R21/Matrix-M are administered to children above the age of five months are used to reduce infection by 40-75%. Chemoprophylaxis is advocated for travelers to prevent infection that would be carried back to their native countries (CDC, 2025).

2.9 Challenges to Current Anti-Malarial Therapeutics

In Kenya, government health facilities offer free Artemether Lumefantrine to children. However, the cost of treatment for uncomplicated and severe malaria cases range between $5-$100 or higher in private facilities (Mori *et al*., 2024). In 2022, Kenya reported a poverty rate of 39.8% with a higher percentage within the rural areas with limited access to infrastructure such as health and schools (KNBS, 2022). The poverty line is based on the monthly expenditure per person which was $40 for those in the rural areas and $80 in the urban areas. This encourages individuals to seek alternative forms of treatment from traditional healers that are more cost effective or offer flexible payment options (Berhe *et al*., 2024).

Accessibility to effective malaria treatment is another challenge especially in the rural settings. The medical facilities may be too far or the area may have poor roads or dependable means of transportation. This causes delay to treatment which is crucial especially in severe cases (Obeagu *et al*., 2026). The health facilities may be poorly stocked, lack diagnostic tools or have counterfeit medicines further affecting treatment outcomes. These factors undermine malaria control and elimination as it increases the risk of mortality.

Malaria treatment is also worsened by the extensive resistance of the *Plasmodium* parasite to some available treatment drugs leading to a decline in the effectiveness of the drugs. This has led to a dire need to come up with newer antimalarial drugs (Duffey *et al*., 2021).

The resistance to sulfadoxine-pyrimethamine and chloroquine, which are the lowest costing antimalarial, have been prevalent. Other than resistance, there are safety issues

that have been associated with the antimalarial or properties that tend to decrease compliance. Mefloquine has continued to be effective in most parts across the globe but its cost and side effects, which are anxiety neurosis, acute psychosis and seizures, may make an individual reconsider taking it (Coban, 2020). There has been a decrease in sensitivity to emerging first line drugs such as artemisinin-based combination treatment (ACT) and Artemether-Lumefantrine (Nsanzabana, 2019). Mutations in the parasites *pfkelch13* gene is reducing the rate of parasite clearance by Artemisinin-based combination treatment (Habarugira *et al.,* 2026).

Cases of resistance to Artemisinin-based combination therapies have been reported (Conrad *et al*., 2023). Resistance observed is due to mutations in the parasite's Kelch 13 gene (K13 gene) that reduces activation of the artemisinin drug. *Plasmodium falciparum chloroquine* resistance transporter (PfCRT) gene mutation alters piperaquine transport within the parasite's digestive vacuole and *Plasmodium falciparum* multi- drug resistance 1 (PfMDR1) gene amplification causes parasite efflux and reduced intracellular concentration of mefloquine (Ward *et al*., 2022).

2.10 Plants as a Source of Antimalarial Agents

The challenges such as drug resistance and accessibility hinder the effective management of malaria. Natural products have historically been instrumental in drug discovery and development of modern pharmaceuticals (Nasim *et al*. 2022). Most antimalarial drugs that are currently being used were obtained from the advent of traditional medicine (artemisinin and quinine). Others have been developed by the synthesis of analogues (atovaquone, mefloquine, chloroquine and primaquine) (Moyo *et al*., 2023). The modification of chemicals of the active natural products has played vital roles in the drug development (artemether, artesunate and arteether) Quinine was the initial effective antimalarial drug isolated from plants; the cinchona tree. Its chemical structure of the quinine was used for synthesizing antimalarial like primaquine and chloroquine (Ceravolo *et al*., 2021).

Plant extraction is an important step to evaluating bioactivity of plant extracts. Different methods can be used to target the plant chemical properties using polar and non-polar solvents. Polar solvents include water, ethanol or methanol which extract

hydrophilic compounds such as flavonoids, sapponins, glycosides and tannins. Non-polar solvents include terpenoids and sterols which are lipophilic in nature (Lee *et al*., 2024). The plant extract is cleaned, dried and ground to powder to increase surface area for solvent to penetrate. The desired extraction such as decoction, maceration or Soxhlet extraction is applied to yield the crude product. Aqueous extraction is used to mimic traditional preparation in ethnopharmacological studies (Hlatshwayo *et al*., 2025).

Plant derivatives such as quinine from the Cinchona tree served as the first effective treatment of malaria (Lokole *et al.*, 2024). Artemisinin from the plant *Artemisia annua* replaced chloroquine and sulfadoxine pyrimethamine due to parasite resistance to these drugs. Artemisinin derivatives such as artesunate and dihydroartemisinin is combined with synthetic antimalarial drugs such as lumefantrine producing a synergistic effect (Czechowski *et al*., 2020).

The plants used in this study; *Corymbia citriodora, Maytenus senegalensis* and *Warbugia ugandensis* have shown individual antimalarial activity. Each plant has bioactive compounds that are attributed to their antimalarial and antiplasmodial activity. *Corymbia citriodora* has several bioactive compounds that give antimalarial, repellant and antiplasmodial activity (Ahmed *et al*., 2023). The repellant and antimalarial bioactive compounds of *Corymbia citriodora* include; Citronellol, Ellagic acid, Citronellal, Isopulegol, p-Mentane- 3,8- diol and Citriodiol with observed antiplasmodial activity. The antiprotozoal and antiplasmodial isolated compounds activity has been attributed to phytochemicals such as phenolic, flavonoids and glycosides (Perry *et al*., 2023).

Maytenus senegalensis has reported pristimerine, terpenoids, anthraquinones as bioactive compounds that contribute to pharmacological activity (Ramadwa *et al*., 2025). The root bark methanolic extract has strong antimalarial and antiplasmodial activity (Kassimu *et al*.,2024). *Warbugia ugandensis* is widely used and has bioactive antimalarial compounds like sesquiterpenoids, tannins, sterols and saponins that inhibit parasite and possess antimicrobial activities (Opiyo., 2023).

It is imperative that discovery of new antimalarial is necessary due to the limitations of drug availability, safety as well as the spread of the resistance. Attention has increasingly been directed to natural products to combat antimalarial drug resistance. In fact, commonly used antimalarial drugs which include artermisinin and quinine have been isolated from plants (Pan *et al*., 2018).

2.11 Plant Used in the Study

2.11.1 *Corymbia citriodora*

Species Identity

Taxonomy (Authority: Hook*) Corymbia citriodora* is classified as follows; Kingdom: Plantae Order: Myrtales Family*: Myrtaceae* Genus*: Corymbia* Species*: Corymbia citriodora* (POWO, 2025).

Common Names

Corymbia citriodora is commonly known as the Spotted gum, Lemon scented gum, Blue spotted gum, *Eucalyptus citriodora* and Lemon eucalyptus (Faria *et al*., 2025).

Geographical Distribution

It is natively found in Queensland, Australia. It is a plant exotic to the countries; Albania, Algeria, Brazil, Cyprus, Egypt, Ethiopia, Fiji, Ghana, Greece, India, Italy, Kenya, Libyan Arab Jamahiriya, Malaysia, Malta, Morocco, Nigeria, Portugal, Spain, Sri Lanka, Tanzania, Tunisia, Uganda, United States of America, Vietnam and Zimbabwe (Healey *et al*., 2021).

Description and Functional Uses of *Corymbia citriodora*

Corymbia citriodora is a medium tree that ranges from 24- 50 m in height with a smooth colored bark with slender twigs and lemon scented leaves once ground. Suitable soil is loam soil which is slightly acidic and upon aging, p-menthane 3, 8-diols (PMD) is formed from the citronellal present in the plant (Caetano *et al*., 2024).

It grows best in regions with uniformly distributed rainfall. The lemon scented essential oil produced from the plant after hydro distillation is used as an insect repellant in the developed countries and is availed commercially. *Corymbia citriodora* contains 85% of citronella in the essential oil and can repel parasites such as mosquitos and arthropods at concentrations of 5-10% for several hours (Salem *et al.*, 2018).

Corymbia citriodora antimalarial biomechanism involves generating reactive oxygen species that damage the parasite's lipids, DNA and proteins (Mayana *et al*., 2018). Citronellal disrupts the parasite cell membrane which increases permeability and parasite lysis. The monoterpenoids impair ATP production and mitochondrial function (Martinez-Tellez *et al*., 2026).

2.11.2 *Maytenus senegalensis*

Species Identity

Kingdom: Plantae, Order: Celestrales Family: Celestaceae Subfamily: *Celestroideae* Genus: *Maytenus* Species: *Maytenus senegalensis* (World Agroforestry., 2026).

Common Names

Maytenus senegalensis is commonly known as the red spike thorn or confetti tree in English, Mutsotsova or Musosaguva or Chizhuzhu in Shona, Isihlangu in Ndebele. In Bungoma, Kenya it is known as kumwayakhafu (PlantNet, 2018).

Geographical Distribution

It is native to Senegal with a worldwide distribution. Other locations include; Southern Spain, Aldabra, Madagascar, India, North Africa, Arabia, Afghanistan and the Sub-Saharan Africa (Kamaka Kassimu *et al*., 2022).

Description and Uses of Plant

Maytenus senegalensis is a shrub with spines reaching a maximum height of 15 m. It has reddish brown seeds and is a plant widely used across Africa. Maytenus senegalensis has reported antimalarial and antiplasmodial activity (Tajbakhsh *et al*.,

2021). It also has anti-inflammatory herbal remedy, an antimicrobial, treating respiratory illnesses through infusions as well as applied topically treating wounds. Maytenus senegalensis extract is currently at the second phase of a standardized clinical test of uncomplicated malaria (Kassim Kamaka *et al*., 2022).

Maytenus senegalensis possesses antimalarial activity through a combination of bioactive compounds. Maytenin which is a quinone methide generates reactive oxygen species that damage the parasite's lipids, DNA and proteins (Ramadwa *et al.*, 2025). Flavonoid, another bioactive compound, interferes with heme detoxification in the parasite's digestive vacuole during the breakdown of host red blood cell causing oxidative stress and eventual parasite death. Triterpenoids disrupt the metabolic pathways and parasite membrane integrity (Noushahi *et al*., 2022).

2.11.3 Warbugia ugandensis

Species Identity

Taxonomy of the plant as per the Sprague Authority scientifically classifies the plant as Kingdom: Plantae, Division: Angiospermae, Order: Canellales, Family: Magnoliidae, Genus: Warburgia, Species: *Warbugia ugandensis* (Useful Tropical Plants, 2026).

Common Names

It is a plant commonly known as the East African green wood green heart, Kenya green heart and pepper bark tree in English. It is also known as Muthiga in Kikuyu; zogdom in Amharic and muwiya and mukazanume in Luganda (Wambua *et al.*, 2021).

Geographical Distribution

Warbugia ugandensis is found naturally occurring in Democratic Republic of Congo, Ethiopia, Kenya, Malawi, South Africa, Swaziland, Tanzania, Uganda and is exotic to India (Giday *et al*., 2020).

Description and Uses of Plant

Warbugia ugandensis is an evergreen plant that grows from seeds directly sown or seedlings in natural environs where it germinates within a period slightly exceeding two weeks. It's a hermaphroditic plant that grows in altitudes between 100-2200 having a mean annual rainfall of 1000-1500 mm. In Africa, several locally available plant stems, leaves, bark roots or at times the whole plant is used in treatment of the diseases. *Warbugia ugandensis* is a member of the Canellaceae family and is known to be an effective antimalarial with its use evident in parts of Kenya (Schultz *et al.,* 2020). The tree is used to treat malaria, bacterial infections, trypanocidal activity, stomach ache and constipation (Denis *et al*., 2018).

Warbugia ugandensis is rich in drimane-type sesquiterpenoids namely; warbuganal, muzigadial, polygodial, mukaadial, and ugandensidial. The main mechanism is in disruption of the parasite's proteins, enzyme inhibition and membrane integrity leading to parasite death (Okello *et al*., 2023). They also cause oxidative damage by depleting intracellular antioxidants such as glutathione in *Plasmodium falciparum* (Zhuang *et al*., 2021).

Plants have been an excellent source of antimalarial drugs since time immemorial. Despite the massive efforts to produce new synthetic antimalarial drugs medicinal plants have been the highest contributors of new antimalarial drugs. (Sharma *et al*., 2023). Plants have been significant sources of the current antimalarial treatments including artemisinin. The combination of plant extracts is essential in forming novel therapeutics and overcoming drug resistance because of their mechanism of action and multi- target nature (Noriman *et al*., 2026).

Plant extracts exhibit a polypharmacological nature from the complex mixture of the bioactive compounds which increase efficacy and reduction in dosage as compared to individual plant extracts. This approach is important in overcoming drug resistance and multiple biological pathways such as disrupting cell division and increasing permeability of the cell membrane (Tarkang *et al*., 2016).

The standard drug used in this study is chloroquine which is a synthetic derivative of Quinine despite the presence of resistant *Plasmodium falciparum* strains. It is a standardized control for resistance mapping by highlighting the re-emergence or disappearance of resistant strains. It is used in pre-clinical studies since its pharmacological profile and mechanism of action is known and reproducible (de Villiers *et al*., 2021). This is key in comparing the efficacy of new antimalarial compounds. Once in the host's red blood cells, the parasite digests hemoglobin for food releasing heme which is toxic but the parasite is able to neutralize it. Chloroquine then blocks the detoxification of heme leading to the buildup of heme and death of *Plasmodium falciparum* (Coban, 2020).

Malaria pathology is highly dependent on parasite proliferation and inflammatory response in mammalian host (Mavondo *et al*., 2019). The individual antimalarial activity of *C. citriodora, M. senegalensis* and *W. ugandensis* has been attributed to specific bioactive compounds with identified mechanisms. The observed antimalarial mechanism of the individual plant extract includes; oxidative damage, mitochondrial disruption, inhibition of antioxidant defenses, membrane and macromolecule damage, and heme- mediated oxidative reactions.

These plants are often used in combination in traditional medicine suggesting interactions that may enhance efficacy or reduce toxicity. However, there is limited scientific evidence evaluating the synergistic combined effects of *Corymbia citriodora*, *Maytenus senegalensis*, and *Warburgia ugandensis*. This study aims to address this gap by investigating the *in vitro* cytotoxicity, acute toxicity, and *in vivo* antimalarial activity of these plant extracts in combination.

2.12 *In Vitro* Cytotoxicity, Acute Toxicity and *In Vivo* Antimalarial Activity of Herbal Remedies

Herbal remedies are used in Kenya to treat different ailments based on culture as well as knowledge of the plant extracts in most communities. The accessibility and affordability encourage the use of herbal extracts though its safety and drug efficacy raises concerns (Chebii *et al*., 2020).

In vitro cytotoxicity of plant extracts is carried out to determine the concentration of the extract that may kill 50% the cells. Vero cells are used to identify cytotoxicity as they mimic human cells (Abd'quadri-Abojukoro *et al*., 2022).

Acute toxicity in mice is important to observe any toxicity that may be induced by the plant extract. Plant extract is given in increasing dose concentration and toxicity observed (Chandrashekar *et al*., 2022). Changes in food intake, occurrence of tremor and/or convulsions among others are monitored to determine acute toxicity (Daskum *et al.*, 2022).

In vivo antimalarial activity is carried out in mice using herbal extracts to ascertain the safety of the herbal extracts to treat malaria using *Plasmodium berghei*. *P. berghei* is a *Plasmodium* vector rodent species that is used to study the relationship between the vector and the host (Girmaw *et al*., 2022). This is a 4-day chemo suppressive test that is used to investigate the chemotherapeutic activity of plant extracts (Habte *et al*., 2023).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study area was Kinango in Kwale County (approximately −4.3550° S, 39.4089° E). It is situated in the coastal region and is characterized by semi-arid to dry sub-humid weather conditions. The area has average temperatures of 22-30℃ with an annual rainfall of 700-1000mm ideal for growth of indigenous plants. Kinango was selected due to plant availability and its ethnopharmacological application of the plant materials. The three plant extracts *Corymbia citriodora, Maytenus senegalensis* and *Warbugia ugandensis* are used in treating malaria. They were collected from community farmland and forest patches. A qualified botanist authenticated the plant material before processing. The extraction, cytotoxicity and antimalarial analysis was carried out at the Kenya Medical Research Institute (KEMRI), Centre of Traditional Medicine and Drug Research (CTMDR).

3.2 Study Design

This was a laboratory based experimental study to evaluate the synergistic antimalarial activity of *Corymbia citriodora, Maytenus senegalensis* and *Warbugia ugandensis* Used in traditional medicine in Kenya. The plant materials were cleaned, ground and filtered extract turned into powder and extraction was conducted using water to mimic ethnopharmacological preparation. The extracts were tested *in vitro* using Vero cells, *in vivo* antimalarial tests utilizing Plasmodium berghei. The antimalarial experimental groups included negative control distilled water, positive control chloroquine group and the plant extract in different combination ratios and concentration. The antimalarial study on infected mice was conducted over a 4-day period and parasitemia levels checked. This study design aimed to align with traditional medicine practices whilst providing a controlled and reproducible assessment of the synergistic activity of the plant extracts.

3.3 Plant Materials

Plant material (roots, stem bark and leaves) were collected from Kinango at optimal maturity. 5kg each of the healthy plant parts were collected in gunny bags to avoid moisture accumulation and growth of fungi. Authentication was done at the East African Herbarium; National Museum of Kenya and the plant material was subsequently deposited at the herbarium and voucher number collected. A taxonomist was involved during the collection and authentication process. The plant parts were then delivered to CTMDR, KEMRI cleaned and left to dry for 2 weeks at room temperature.

Table 3.1: Collection and Identification of the Plants

Botanical Name	Family	Voucher Number	Parts used
Corymbia citriodora	**Myrtle**	**PK03**	**Leaves**
Warbugia Ugandensis	**Magnoliidae**	**TFm11**	**Stem bark**
Maytenus senegalensis	**Celestaceae**	**TFm10**	**Stem bark**

3.3.1 Preparation of the Plant Extracts

Clean plant parts were air- dried at room temperature (25^{o} C) for a fortnight. Once completely dried, the plant parts were ground using an electric mill (Christy & Norris Ltd., Chelmsford, England) into powder form then separately stored in sealed containers at room temperature. Aqueous plant extraction was used to replicate traditional preparation where *Corymbia citriodora*, *Maytenus senegalensis*, and *Warbugia ugandensis* are administered as decoctions. A portion (100g) of each of the powdered plant part was put in 1 liter of distilled water and heated in a water bath at 70°C for 90 minutes. The extract was decanted into a clean dry 3-liter conical flask followed by filtering through a cotton gauze. *Corymbia citriodora* yielded 15g, *Maytenus senegalensis* yielded 18g and *Warbugia ugandensis* yielded 12g which was adequate for the study. The filtered extract was then lyophilized using an Edwards freeze dryer Modulyo into powder form and then pooled into an air tight bottle, weighed, labeled and stored at 4^{0} C awaiting use.

3.3.2 Handling of the Experimental Animals

90 female Swiss mice 6-8 weeks old weighing 18 to 25g were obtained from the KEMRI animal house where they are bred and under standard laboratory conditions before any experiment. Female mice were used to maintain statistical validity and reduce variability in results (Smarr *et al.,* 2022). Male Swiss mice have been reported to experience territorial aggression especially in groupings and this may affect observations such as behavioral or weight changes (Weber *et al*., 2022). They are acclimatized in the experimental room for a week before commencement of the experiment. The mice were housed in a 15x 21 x29 cm standard steel cage containing wood shavings at the base which was replaced after every two days, a nipple watering device and mouse pellets (Mice pellets UNGA® food) given as a source of food. The cages were clearly labeled, covered with metallic mesh and placed in a room that was locked during the entire period of the experiment. To avoid infection, a laboratory coat and a pair of gloves was worn at all times while handling the animals. The animals were humanely handled with no physical harassment, at all times and fed as they were at the beginning of the experiment. At completion of the study, the mice were euthanized in a CO_2 chamber then incinerated (Hickman, 2021).

3.3.3 Determination of Cytotoxicity

3.3.3.1 Cell Cultures

Vero E6 cell line, 1ml, from the American Type Culture Collection containing 1500-2000 cells was retrieved from liquid nitrogen storage. The cells were thawed and cultured in T-75 flasks with Earl's Minimum Essential Media (EMEM), supplemented with penicillin & streptomycin (1%) and 10% Fetal Bovine Serum maintained at 37˚C in a humidified atmosphere of 5% CO_2 to achieve a monolayer.

Cell monolayer was broken into single cells using trypsin after achieving 70-100% confluence. Cell density count of viable cells was determined and maintained at 37˚C in a humidified atmosphere of 5% CO_2 awaiting use.

3.3.3.2 MTT Assay

In vitro cytotoxicity assay was carried out following a modified rapid calorimetric assay Mosmann (Ziemba, 2025). The plant extracts of *Corymbia citriodora, Maytenus Senegalensis* and *Warbugia Ugandensis* that was stored at 4º C were retrieved and combined in the ratio of 1:1:1, 1:0.5:1 and 0.5:1:0.5.

An amount of 50 mg each of the different ratios of the combined extract was dissolved in 5ml double distilled water to give stock solutions of 10mg/ml each. This stock solution was then dissolved in 0.5 ml dimethyl sulfoxide (DMSO). Double distilled water was then added to give a stock solution of 10mg/ml ensuring that the final concentration of DMSO is 1%. The use of 1% DMSO concentration ensures minimal toxicity to *Vero* cells and improves solubility of aqueous plant extracts. This one-part stock solution was diluted to 99 parts of Earl's Minimum Essential Medium (MEM) (ratio of 1:99), containing 2% Fetal Bovine Serum (FBS, maintenance medium), which was 10µl of the extract in 990 µl of media giving a start concentration of 1000 µg/ ml in 1% DMSO used in the MTT ((3- [4, 5- dimethylthiazol -2- yl) -2,5- diphenyltetrazolium bromide) assay.

DMSO 100% concentration was the solvent used for this procedure whereas 5mg/ml MTT in PBS served as the solution after filter sterilization using a 0.22 µm pore size Millex syringe driven filter after adding MTT.

A 96- well micro titer plate was used. A Vero cell suspension (10 µl) containing 2 x 10^5 viable cells was then seeded onto columns 1,2,4,5,7,8,10,11 whereas 10 µl of media without Vero cells was added onto rows 3,6,9 and 12. The plate was then incubated at 37ºC in 5 % CO_2 for 24 hours to allow cells to attach. The next day, media from row H was removed. Different concentration of the drugs was serially added to the cells *Corymbia citriodora, Maytenus senegalensis* and *Warbugia ugandensis* combined in the ratio of 1:1:1, 1:0.5:1 and 0.5:1:0.5.

The plates were then incubated for 48 hours at 37ºC in 5 % CO_2 to allow the drug to take effect on the growing cells. Upon incubation and ensuring normal cell growth using an inverted microscope, 10 µL of MTT dye was added to all the wells in the

plates. The plates were incubated for another 4 hours then entire media plus MTT dye was aspirated off followed by addition of 100 µl of DMSO to dissolve the formazin.

The plates were read on a scanning multi well spectrophotometer (Multiskan Ex Labssystems) at 562 nm and 690 nm as reference to determine $CC_{50.}$ The percentage viability was calculated using the formula;

$$\%\ \text{Cell viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \text{ x } 100$$

Cytotoxicity is then obtained by calculating; 100 - %Cell viability. These values are then determined across different concentrations to give a dose-response curve.

3.3.4 Acute Oral Toxicity

The acute oral toxicity test was run according to OECD 425 protocol (OECD, 2022). The mice were monitored closely from onset to 48 hours in intervals of 0, 5, 10, 15, 30, 45min, 1hour, 3hr, 6hr, 9hr, 21hr, 36hr, 48hr. These intervals ensured systematic observation of any behavioral or physiological changes such as shivering, change in feeding and responsiveness which could be attributed to the treatment. Fifteen nulliparous and non-pregnant female (8-12 weeks old, 18 – 25g) Swiss mice were acclimatized to laboratory conditions for five days prior to dosing. Five groups (comprised of three mice) were randomly picked and placed in a marked cage. The mice were fasted for 3 hours prior dosing and their weight determined. A single dose of 0.2 ml (1ml/ 100g of mouse weight) containing combined plant extract at 5, 50 ,300 and 2000 mg/kg body weight doses was orally administered per group of mice using a 20-gauge oral gavage stainless steel cannula. Food was withheld for 2 hours after treatment with the plant extract.

A single group of mice were first administered with 5mg/kg initial dose and monitored for 48 hours. In case of death within this period then a lower dose would be administered. If none or one mouse dies, then a higher dose would be administered but less than 2000mg/kg to another group of three mice. The first 48 hours for observation is crucial to ensure survival of the group of mice before proceeding to a higher dose. Toxicity was monitored and the vehicle/ negative control (distilled water) was given

to the normal group. The mice were weighed at the start and end of the experiment. Weight loss that was significant in comparison to control group would indicate systemic toxicity. Signs of toxicity such as changes in breathing patterns, weight loss, reduced activeness and eventual death were checked within the first 24 hours after treatment. Severe toxicity, presence and absence of death was noted to determine cause of death in 50% of the animals. At the end of 14 days, weight of the surviving animals was recorded and humanely sacrificed.

3.3.5 Determination of Antimalarial Activity *In Vivo*

The rodent parasite *Plasmodium berghei* ANKA was used for investigation of the antimalarial properties of the plant extracts in a four-day chemosuppressive assay (Gizachew *et al.*, 2023). An inoculum vial containing the cryopreserved Plasmodium stored at -80° C was retrieved and used to infect three Swiss mice. A volume of 0.2ml containing 1×10^7 parasitized erythrocytes (1% concentration), was infected into each mouse intraperitoneally. The mice were placed in a cage where they were fed and given water. On the fourth day, a syringe laced with heparin was used to draw blood (0.6ml) from the heart. The Swiss mouse containing *P. berghei* ANKA at a parasitemia level of 20-30% was used as the donor. Parasitized erythrocytes were harvested from the donor mouse using a sterile needle and syringe. The blood was collected in containers coated with heparin. A portion of the collected blood (0.4ml) was diluted using 9.6ml phosphate saline glucose buffer (PSG) to obtain 10 ml blood containing 1×10^7 parasitized erythrocytes (1% concentration). A concentration above 1% may lead to death or anemia whereas a concentration lower than 1% will lead to low parasitemia levels in the mice (Azizah *et al*., 2023). A volume of 0.2ml containing 1x 10^7 parasitized erythrocytes was then intraperitoneally injected into 30 mice using a 27-gauge needle. The animals were then randomly divided into six groups each comprising of five animals. After 2 hours, a randomized group of animals was orally treated with the vehicle (distilled water), reference drug (Chloroquine) and aqueous (water) plant extracts as shown in table 3.2 using a 26-gauge stainless steel cannula. The group treated with the vehicle served as the negative control while the chloroquine treated group served as the positive control. The mice were administered the same dose

as per treatment group for four consecutive days at the same time (Gebrehiwot *et al*., 2019).

On the 4th day, the mice were pricked at the tail tip and drops of blood collected on a labelled microscope slide. A drop of blood was used to prepare a thin smear on a microscope slide. The blood cells were fixed onto the microscope slides by submerging them into a staining tank containing methanol for four minutes. The methanol was then poured off and the microscope slides set to air dry. Giemsa dye was then added onto the slides and incubated for 40minutes at 37ºC for staining purposes. The slides were then washed using the overflow method and dried in a mechanical incubator. The glass slides were viewed under a microscope at x100 magnification to observe and count the number of infected red blood cells. Parasitemia was determined microscopically by counting four fields on the microscope slide having approximately 100 erythrocytes per field. The number of infected erythrocytes against the number of total erythrocytes in the field was recorded. The percentage growth inhibition of the plant extracts was determined using the formula (Gedefaw et al., 2021);

$$\%\text{Parasitemia} = \frac{\text{Number of Parasitized RBCs}}{\text{Total number of RBCs}} x100$$

$$\text{Chemosuppression} = \frac{\text{Parasitemia in Negative Control} - \text{Parasitemia in treatment group}}{\text{Parasitemia in Negative Control}} \; x100$$

3.4 Data Management and Analysis

The data from the antimalarial analysis, MTT assay and acute toxicity test were keyed in excel spreadsheets. The data was exported to MinTab version 18.0 for descriptive analysis and express as mean±SEM. Normality was assessed using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. Once assumptions of normality and equal variance were met, parametric tests were conducted.

The ED_{50}, CC_{50} and LD_{50} were determined by nonlinear analysis using Graph Pad prism version 8.0. A four-parameter logistic (4PL) model with variable slope, was used

to fit dose response curves from which the half-maximal effective (ED_{50}), cytotoxic (CC_{50}), and lethal (LD_{50}) concentrations were derived.

One-way ANOVA was used to compare the means between treatment groups, negative and positive controls. This was followed by Tukey's post hoc for pairwise comparison of means. The means with $p \leq 0.05$ was considered significant. Non parametric tests were considered for data that violated parametric assumptions. The interquartile range was used to identify any outliers. Listwise deletion was used to address missing data from animal loss. A p-value of ≤ 0.05 was considered statistically significant.

3.5 Ethical Considerations

Appraisal of the proposal were carried out by; KEMRI Scientific Steering Committee, KEMRI Ethical Review Committee (ERC) approval number KEMRI/ RES/ 7/3/1 and Animal Care and Use Committee (ACUC) approval number KEMRI/ ACUC/ 01.05.14 who provided approval for the study. Mice handling and maintenance before, during and after the study were humanely conducted and at the end of the study the mice were euthanized in a CO_2 chamber then incinerated.

The plants were obtained from their natural habitats and the communities were engaged about the purpose of the study, the procedures and the outcomes. After the findings, the communities were encouraged to conserve the medicinal plants for sustainability and apply safe traditional practices with evidence-based recommendations.

CHAPTER FOUR

RESULTS

4.1 Cytotoxicity of *C. citriodora, M. senegalensis* and *W. ugandensis*

In vitro safety profile of the plant extract was established by determining the lethal dose that reduced Vero cells by 50% (CC_{50}). Chloroquine had the highest cytotoxic effect with a CC_{50} value of 71.13±1.63 µg/ml followed by *C. citriodora: M. senegalensis: W. ugandensis* (1:1:1) and *C. citriodora: M. senegalensis: W. ugandensis* (1:0.5:1) with CC_{50} value of 101.47±3.17 µg/ml and 190.37±3.47 µg/ml respectively (table 4.6). The least cytotoxicity was shown by *C. citriodora: M. senegalensis: W. ugandensis* (0.5:1:0.5) at of 268.70±31.40 µg/ml (Table 4.1).

Table 4.1: Cytotoxic Effects of Various Extract Combination on Vero Cells

Treatments	**CC_{50} Values (µg/ml)**
Chloroquine	71.13±1.63
C. citriodora: M. senegalensis: W. ugandensis (1:1:1)	101.47±3.17
C. citriodora: M. senegalensis: W. ugandensis (1:0.5:1)	213.55±3.47
C. citriodora: M. senegalensis: W. ugandensis (0.5:1:0.5)	575.80±31.40

All values are expressed as Mean±SEM,

4.2 Acute Toxicity of *C. citriodora, M. senegalensis* and *W. ugandensis*

Acute toxicity of the plant extracts was carried out in Swiss mice and body weights determined. Generally, there was an increment of the mice body weight after administration of the extract (Table 4.2). The normal control group had the highest percentage weight gain of 27.67±3.17% followed by the group treated with the 2000mg/kg at 26.59±2.05% (Table 4.2). No animals died at the highest concentration of the treatment of 2000mg/kg.

Table 4.2: Effects of C. citriodora: M. senegalensis: W. ugandensis (1:1:1) on Mice Body Weight

Concentrations	Weight before extract administration (g)	Weight after extract administration (g)	Percentage Weight change (g)
Distilled water	19.80±0.37	27.00±0.71	27.67±3.17
5mg/kg	20.20±0.66	27.60±0.51	24.60±1.08
50mg/kg	20.20±0.66	27.00±0.45	25.13±2.64
300mg/kg	19.60±0.51	26.40±0.81	24.98±3.35
2000mg/kg	19.40±0.51	26.00±0.32	24.54±2.46
Chloroquine	19.00±0.32	26.00±1.00	26.50±2.05

LD_{50} >2000mg/kg

All values are expressed as Mean±SEM,

The behavioral changes assessed in the study included loose stool, activeness, convulsion and mortality. No mortality nor adverse behavioral changes were observed across all the treatment concentrations (Table 4.3).

Table 4.3: Behavioral Indicator of Toxicity in Swiss Mice

Observations	5mg/kg	50mg/kg	300mg/kg	2000mg/kg
Salivation	-	-	-	-
Reduced activity	-	-	-	-
Paw licking	-	-	-	-
Frequent urination	-	-	-	-
Vomiting	-	-	-	-
Diarrhea	-	-	-	-
Convolution	-	-	-	-
Hyper-activity	-	-	-	-

4.3 *In Vivo* Antimalarial Activity of Extracts of *C. citriodora, M. senegalensis* and *W. ugandens*is

The synergistic antimalarial activity of aqueous plant extracts of *Corymbia citriodora, Maytenus senegalensis* and *Warbugia ugandensis* was evaluated using the 4- day suppressive test in mice. The chemosupression was noted with *Maytenus senegalensis* and *Corymbia citriodora* having dose dependent activity where the level of chemosppression increased with increase in dose (Figure 4.1). *Corymbia citriodora* had a chemosupression of 70.65±0.36 and *Maytenus sengalensis* had 71.40±0.48 at

the highest dose concentration of 600mg/kg. *Warbugia ugandensis* exhibited the highest chemosuppression of 84.36±0.39 at the lowest dose of 75mg/kg. Chloroquine exhibited a standard concentration of 99.35±0.19 whereas negative control (water) did not show any chemosuppression.

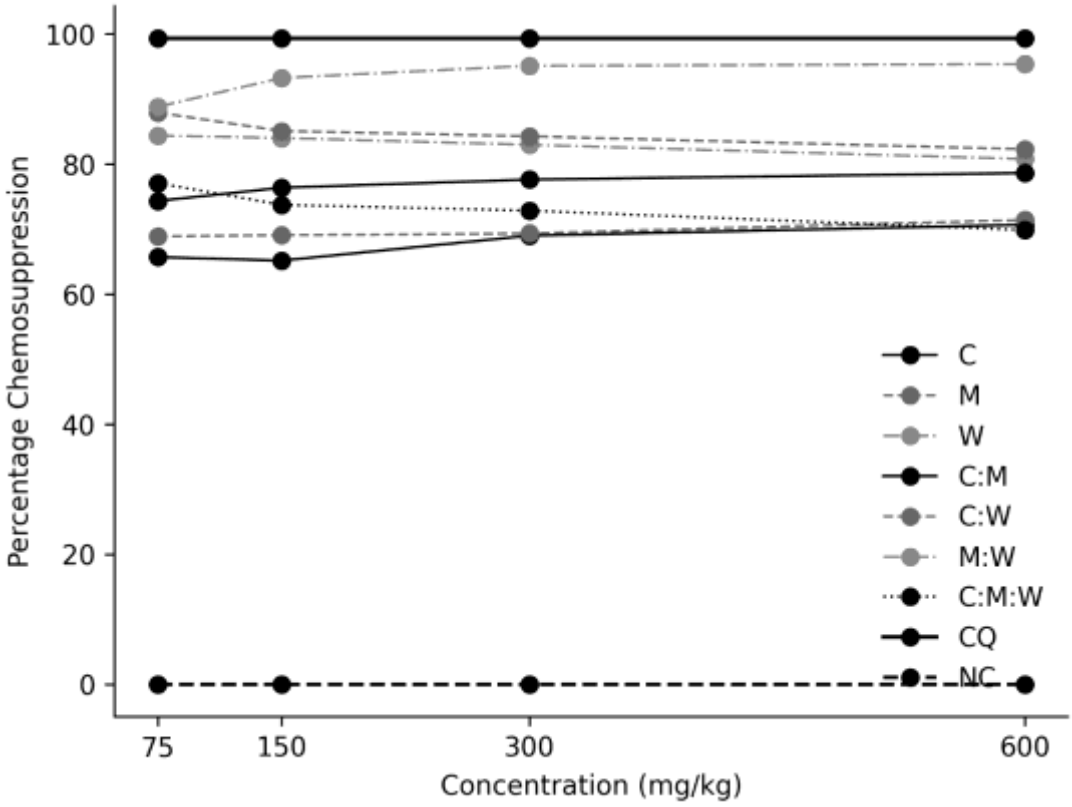

Figure 4.1: In Vivo Antimalarial Effect of Single Plant, Two- Plant and Three-Plant Combination of *C. citriodora*, *M.* senegalensis and *W. ugandensis* on *P. berghei* Inhibition

4.3.1 *In Vivo* Antimalarial Effect of Combined Two- Plant Extracts

Combinations of two plants extracts were tested for antimalarial activity. The combinations tested included *C. citriodora: M. senegalensis* (C: M), *C. citriodora: W. ugandensis* (C: W) *and M. senegalensis: W. ugandensis* (M: W) at the ratio of 1:1.

M. senegalensis: W. ugandensis (M: W) had the highest chemosuppression activity of 95.35±0.17 at 600mg/kg. *C. citriodora: W. ugandensis* (C: W) had a chemosuppression activity of 87.89±0.56 at 75mg/kg while the lowest was *C. citriodora: M. senegalensis* (C: M) with chemosuprression of 78.61±0.26.

4.3.2 *In Vivo* Antimalarial Effect of Combined Three- Plant Extracts

Further, the three plants (*C. citriodora: W. ugandensis: M. senegalensis*) were combined and their antimalarial activities determined. Consequently, the highest percent chemosuppression (77.06±0.41%) was observed at the lowest concentration (75mg/kg) of the plant extracts (Figure 4.1).

Warbugia ugandensis exhibited the highest activity (ED_{50}) value of 3.3mg/kg followed by *M. senegalensis* (ED_{50} value of 3.44mg/kg) and the lowest was *C. citriodora* (ED_{50} value of 3.92mg/kg). The combination of *Maytenus senegalensis* and *Warbugia ugandensis* had the highest activity of 1.05mg/kg followed by the combination of *Corymbia citriodora* and *Warbugia ugandensis* (ED_{50} value of 1.45mg/ kg) then *Corymbia citriodora* and *Maytenus senegalensis* (ED_{50} value of 1.53mg/kg). The combined three-plant extracts showed an ED_{50} value of 2.26mg/kg (Figure 4.2).

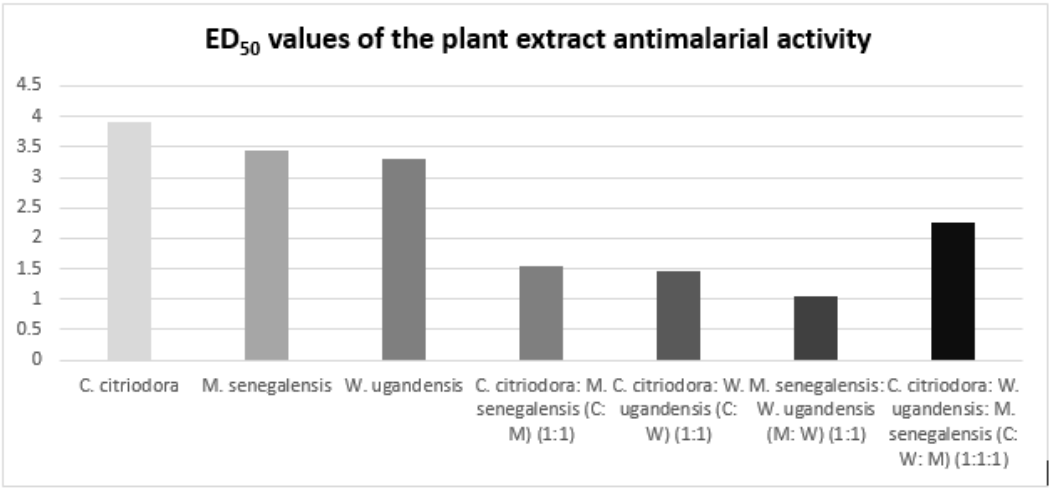

Figure 4.2: ED_{50} Values of the Plant Extract Antimalarial Activity

An independent t-test was conducted to determine potentiation of the combined plant extracts (Figure 4.3)

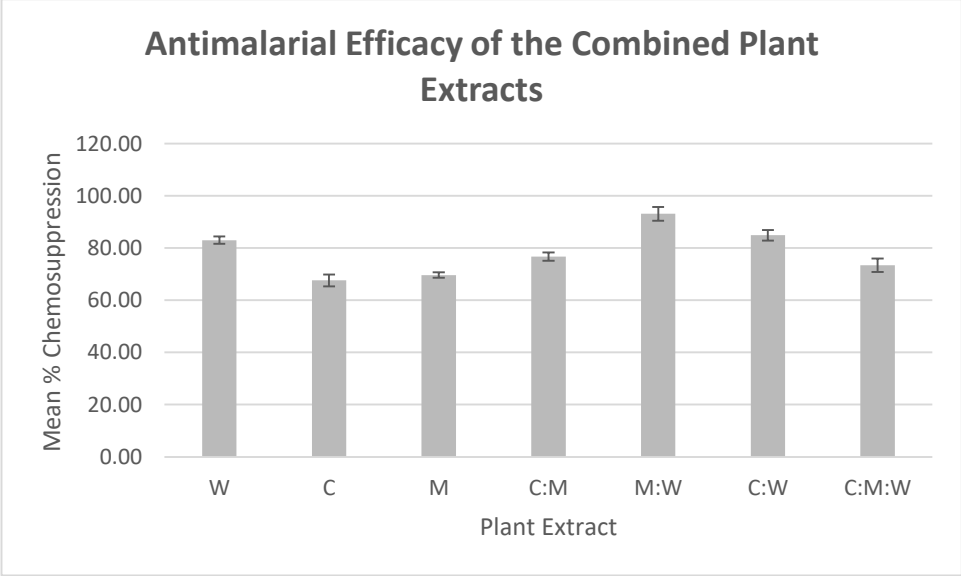

Figure 4.3: Antimalarial Efficacy of the Combined Plant Extracts

The p values were determined and recorded as follows; C:M:W p< 0.0006, C:W p< 0.1814, M:W p< 0.0005, C:M p<0.0010.

The interactive potential of the combined extracts was analyzed by determining the sum of Fraction Inhibition Concentration (SFIC) (*K*). The SFIC indexes for *C. citriodora*: *M. senegalensis* (C: M), *C. citriodora*: *W. ugandensis* (C: W) and *M. senegalensis*: *W. ugandensis* (M: W) were 0.67, 0.83 and 0.28 respectively (Table 4.4).

Table 4.4: Sum of Fraction Inhibition Concentration (SFIC) (K) Ratio

Treatment	**(SFIC) (*K*) Ratio**	**Effect**
***C. citriodora*: *M. senegalensis* (C: M) (1:1)**	0.67	Synergistic
***C. citriodora*: *W. ugandensis* (C: W) (1:1)**	0.83	Synergistic
***M. senegalensis*: *W. ugandensis* (M: W) (1:1)**	0.28	Synergistic
***C. citriodora*:*M. senegalensis*:*W. ugandensis* (1:1:1)**	1.92	Antagonistic

CHAPTER FIVE

DISCUSSION

5.1 Discussion

5.1.1 *In Vitro* Cytotoxicity of the Plant Extracts

C. citriodora: M. senegalensis: W. ugandensis (1:1:1), C. citriodora: M. senegalensis: W. ugandensis (1:0.5:1) C. citriodora: M. senegalensis: W. ugandensis (0.5:1:0.5) showed no cytotoxic effects with CC_{50} values of 101.47±3.17 µg/ml, 213.55±3.47 µg/ml and 575.80±31.40 µg/ml respectively. The standard classification of cytotoxicity denotes that crude extracts exhibiting CC_{50} values less than 30 µg/ml is considered toxic whereas pure compound extracts exhibiting CC_{50} values less than 100 µg/ml is considered toxic (Konyanee et al., 2024). The different three- plant crude extract combinations exhibited no cytotoxicity.

The MTT ([3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyl tetrazolium bromide]) assay is used to test for cell viability in which living cells reduce tetrazolium salt in a reaction catalyzed by the mitochondrial dehydrogenase enzyme (Karatop *et al*., 2022). Vero cells are recommended for cytotoxicity assays of plant extracts and nutraceuticals in biomaterial research since they mimic normal body cells (M.A Mohd *et al*., 2021). The cell line was subjected to extract treatment upon which the CC_{50} values were determined.

Elsewhere, the cytotoxic properties of *M. senegalensis*, *C. citriodora* and *W. ugandensis* have been investigated. Nabende (2015) observed that the aqueous leaf extracts of *M. senegalensis* had no cytotoxic effects against the Vero cell lines having CC50 values > 1000 µg/ml. Similar to the findings in the present study, *W. ugandensis* has been reported as nontoxic and selective with methanolic stem bark extract showed a CC_{50} of 682.5 µg/ml (Nanyingi *et al*., 2010). Additional *in vitro* findings indicate that Warburgia ugandensis demonstrated no significant cytotoxicity in BALB/c macrophages even at concentrations as high as 1000 µg/mL, further supporting its safety profile in mammalian cell models (Githinji et al., 2010). Hence, previous

findings corroborate the safety properties of the aqueous extract of *W. ugandensis* observed in the current study.

Similarly, recent evaluations have indicated that *Corymbia citriodora* and *Corymbia torelliana* did not exhibit significant cytotoxic effects on Vero cell lines at concentrations up to 1000 µg/mL, suggesting a favorable *in vitro* safety profile at high exposure levels (Nobakht *et al*., 2017). The single plant extracts CC_{50} values observed in the above studies showed no cytotoxicity in *vero* cells for both crude and pure plant extract.

The application of natural products in healthcare requires prior evaluation of their cytotoxic profiles to ensure safety, biocompatibility, and an acceptable therapeutic index before clinical consideration (Atanasov *et al*., 2021). Modern therapeutic development emphasizes demonstrating selectivity toward target pathogens while sparing host cells, as this directly improves safety margins and clinical applicability of candidate compounds (Zhang, 2025). Therapeutic selectivity and selectivity index determination assist in advancing compounds from preclinical screening into nobel drug treatments (Nair *et al*., 2024). The combined extract of *C. citriodora, M. senegalemnsis and W. ugandensis* demonstrated selectivity pronouncing them as safe candidate plants for the development of antimalarial drugs.

5.1.2 Acute Oral Toxicity of the Plant Extracts In Swiss Mice

In the current investigation, no adverse effects were observed after administration of 2000mg/kg of the combined plant extracts. Additionally, no mortality was observed on combined plant extract administration at 2000mg/kg, hence the LD_{50} was above 2000mg/kg.

The three plant extract combination of *C. citriodora, M. senegalensis and W. ugandensis* in the ratio 1:1:1 was administered to a group of mice in different concentration increment. The mice were closely monitored for adverse reactions within 0-48 hours. The observations were done in intervals of 0, 5, 10, 15, 30, 45 minutes, 1hour, 3rd hour, 6th hour, 9th hour, 21st hour, 36th hour and 48th hour. The mice were closely monitored and no adverse reactions were noted past drug administration.

Some of the reactions observed include; writhing, fur erection, hyperactivity and vomiting.

Herbal medical practitioners are combining *C. citriodora, M. senegalensis and W. ugandensis* extracts to treat various ailments however, little information exists about their toxicity profiles. The assessment of medicinal plant toxicity properties will lead to the development of safe and affordable medical options for people in the third world (Patwardhan *et al.,* 2025). The acute oral toxicity *C. citriodora, M. senegalensis* and *W. ugandensis* was determined in the current study. Acute oral toxicity measures the adverse effects of oral administration of one or several doses of a drug within an initial duration of 24 hours (Erhirhie et al., 2018).

The findings of the current inquiry are supplemented by Dada and Muhammed (2018) who reported that mice treated with 2000 mg/kg body weight of ethanolic leaf extract of *C. citriodora* showed no signs of toxicities. *M. senegalensis* ethanolic root bark extract has also been found non-toxic at 1,600 mg/kg body weight (Malebo *et al.,* 2015). These observations, hereby confirm the safety properties of combined extract of *C. citriodora, M. senegalensis* and *W. ugandensis* (1:1:1) as they showed no adverse toxicity to test animals.

5.1.3 *In Vivo* Antimalarial Activity of Extracts of *C. citriodora, M. senegalensis* and *W. ugandensis*

The current study evaluated the synergistic antimalarial activity of the extracts of *Corymbia citriodora*, *Maytenus senegalensis* and *Warbugia ugandensis*. The test plant extracts showed chemosuppressive effects against *P. berghei* parasites in mice. All the extracts showed statistically different percent chemosuppression of *P. berghei* between the negative control and the standard drug chloroquine ($p \leq 0.05$).

The single plant *Warbugia Ugandensis* had the highest chemosuppression value at 84.36±0.39% at the lowest concentration of 75mg/kg. This is evident in documented studies where both antimalarial and antiplasmodial effects are evident at approximately 68-72% (Wekesa *et al*., 2025). Both *Corymbia citriodora* and *Maytenus senegalensis* exhibited higher chemosuppression values of 70.65±0.36% and

71.40±0.48% respectively at the highest dose concentration administered. Plants in this Myrtaceae genus have been reported to have antimalarial activities (Ramadhan *et al.,* 2015). The findings of Muhammed *et al.* (2018) reported that 800 mg/kg mice body weight of ethanol *C. citriodora* root extract was able to clear parasitemia levels in Swiss mice. Previous studies of ethanolic plant extracts of *Maytenus senegalensis* demonstrated a chemosuppression of 88-98.1% at a concentration of 25-100mg/kg (Malebo *et al*., 2015).

Combination therapy has been shown to enhance therapeutic efficacy of antimalarial drugs and reduce the risk of emerging drug resistance. Drug combination has been a novel approach to counter parasite resistance in anti-malarial chemotherapy as it applies a multi- target action (Tagbor *et al*., 2018). This was evident in this study as higher chemosupression was observed in the combined two- plant extract than that exhibited by the single plant extract.

The antimalarial activity in *Corymbia citriodora* is attributed to secondary metabolites namely; citronellal, flavonoids and phenolics. Flavonoid glycosides are among myrtaceae metabolites that is attributed to mechanistic bases for antimalarial activity (Perry *et al*., 2023). Various phytochemical alkaloids, saponins, tannins, anthraquinones, flavonoids and cardiac glycosides have been identified in *C. citriodora*. These compounds such as anthraquinones (Dada and Muhammed, 2018) have implicated antimalarial activities.

Maytenus senegalensis has reported secondary metabolites such as flavonoids, tannins, saponins, terpenes, phenols and alkaloids that cause the antimalarial activity (Huang *et al.,* 2021). Other phytochemicals include dihydroagarofuran sesquiterpene alkaloids mayteine, putterine A and putterine B, Quinonemethide triterpenoids, maytansinoids and flavonoids (Clarise *et al.,* 2017).

Warbugia ugandensis both stem and root extract have showed antimalarial and antiplasmodial activity. *Warbugia ugandensis* has over 60 phytochemicals of which drimane sesquiterpenes, flavonoids and macrocyclic glycosides are attributed to antimicrobial, antiplasmodial and antimalarial activity (Opiyo., 2023).

A low ED_{50} value suggests strong potency. The highest antimalarial efficacy was observed in the combination of *M. senegalensis: W. ugandensis* (M: W) (1:1) (ED_{50} =1.05mg/kg) which was higher than *C. citriodora: W. ugandensis: M. senegalensis* (C: W: M) (1:1:1) ED_{50} 2.26mg/kg. High potency was observed in the two plant extract combinations (1:1) in comparison to the three-plant extract combination (1:1:1) ($p<0.05$). The aqueous ED_{50} value of 3.44 of *M. senegalensis* observed in this study compares to previously reported *M. senegalensis* ethanolic root extract having an ED_{50} value of 3.3 mg/kg mouse body weight against P. berghei (Malebo *et al*., 2015). This shows consistent antimalarial activity across extraction methods. The current study reported that *C. citriodora* had ED_{50} values of 3.92 mg/kg. The current study reported antimalarial potency in the combination of the plant combination C:M:W $p< 0.0006$, C:M $p<0.0010$, M:W $p< 0.0005$. The plant extract combination. C:W $p< 0.1814$ was deemed not statistically significant.

The two-plant extract combination showed higher potency than the individual and the three- plant extracts. This can be attributed to the complementary and synergistic mechanisms from their unique and some similar phytochemical profiles that inhibit the parasite activity. *Maytenus senegalensis* rich in flavonoids and alkaloids (Jain *et al*., 2024) cause enzyme inhibition whereas Terpenoids and sesquiterpenes in *Corymbia citriodora* cause membrane disruption (Santos *et al.*, 2020). Tannin, terpenoids and alkaloids in Warbugia ugandensis causes oxidative damage by generating reactive oxygen species or disrupting the antioxidant defenses of the parasite (Okello *et al*., 2019).

Host related complementary and synergistic mechanism involves immunomodulation and anti-inflammatory effects. Immunomodulation occurs by interfering with signaling pathways and regulating pro-inflammatory TNF-α, IL-1β, and IL-6 cytokines due to malaria infection by increasing the production of IL-10 which is anti-inflammatory (Obeagu, 2024). Immunomodulation can be attributed to flavonoids, tannins, alkaloids, and phenolic compounds majorly from *Maytenus sengealensis* as well as the two plant extracts. This multi- target action explains the increase in potency in the combined two and three- plant extracts.

In combination therapy research, drugs are classified as synergistic, additive or antagonistic based on their *S*um of Fraction Inhibition Concentration (SFIC) index. Fraction Inhibition Concentration (SFIC) indexes of <1, 1 and >1 indicates synergism, additivity and antagonism respectively (Zahari *et al*., 2023). Synergism can be described as an increase in activity of two or more combined agents that produces an effect which is greater than the sum of the activity of the individual agents (Kamble *et al*., 2019). Antagonism is indicated by weaker effect of the combined agents than the sum of the activity of the individual agents whereas an additive interaction is indicated by equal effect of drug combinations to the sum of the effects of the two separate drugs (Chaachouay *et al*., 2025).

The two plant combination (1:1) of *M. senegalensis*: *W. ugandensis, C. citriodora*: *M. senegalensis* and *C. citriodora*: *W. ugandensis* recorded synergistic activities with SFIC indexes of 0.28, 0.67 and 0.83 respectively. However, the three plant combination (1:1:1) of *C. citriodora: W. ugandensis: M. senegalensis* demonstrated antagonistic effects with SFIC index of 1.92. The antagonism from the three-plant extract combination means that a weaker effect was induced in comparison to the sum of the activity of the single plant extracts. The bioactive compounds from the three plant extracts may have cancelled each other or competed for the same biological target reducing potency (Caesar., 2019). The findings of this study demonstrate the candidacy of these plants and the importance of proper combination of plant extracts for the management of malaria.

The combined extract of *Corymbia citriodora, Maytenus senegalensis* and *Warbugia ugandensis* serve as a novel antimalarial therapy in comparison to commonly used remedies such as Chloroquine and Artemisinin-based treatments. Conventional antimalarial drugs tend to have one dominant mechanism of action. For instance, chloroquine inhibits heme detoxification (de Villiers *et al*., 2021) whereas artemisinin generates free radicals within the parasite causing parasite death (Ribbiso *et al*., 2021). This is an effective approach but has led to the rapid emergence of drug-resistant strains of *Plasmodium*.

The current vaccines RTS,S/ and R21/M-Matrix vaccine induce antibodies such as IgG1 and IgG3 which target NANP repeat region of the parasites' circumsporozoite as well as epitopes within the C-terminal region (Kurtovic 2021). These antibodies bind to the circumsporozoite protein impairing parasite motility while inducing opsonization. This mechanism inhibits liver-stage development and subsequent infection of the red blood cells (Pendyala et al., 2023).

Conversely, the combined plant extracts present a multi-target mechanism from the varied phytochemicals, including alkaloids, flavonoids, terpenoids, sesquiterpenes, and tannins. The synergistic approach affects both the host and parasite mechanism likely reducing the development of resistance. A multi- target action requires the parasite to adapt to different biochemical disruptions.

Moreover, the observed synergistic interactions and improved potency suggest that the combined extract demonstrates a natural combination therapy, similar to artemisinin-based combination therapies (ACTs). The rise in resistance to existing drugs present this combined extract as a candidate for novel drug development. The plant-based origin is beneficial in terms of accessibility and affordability. This is a viable antimalarial therapy once safety, standardization, and clinical efficacy studies are conducted.

5.2 Conclusion

i. The combined aqueous extracts of *Corymbia citriodora, Maytenus senegalensis* and *Warbugia ugandensis* exhibited synergism and low cytotoxicity among different combination ratios against Vero cells. Synergism and low cytotoxicity allow for the development of antimalarial therapies that overcome drug resistance and are safe. This supports further investigations of the combined two-plant extracts as potential antimalarial agents.
ii. The combined aqueous extracts of *Corymbia citriodora, Maytenus senegalensis* and *Warbugia ugandensis* did not cause mortality. The mice gained weight and did not exhibit any adverse effects from onset to the end of the 14 days suggesting safety of plant extracts in the tested concentrations. This

indicates absence of acute toxicity and positive drug efficacy as normal physiological functions such as feeding was not affected.

iii. The combined aqueous extracts of *Corymbia citriodora, Maytenus sengalensis* and *Warbugia ugandensis* individual extracts exhibited lower potency than two and three-plant extract combinations. The two-plant extract combination showed higher potency and synergistic than the three-plant extract combination which was antagonistic. The synergistic activity suggests enhanced therapeutic efficacy and the antagonism highlights compound interference hence the need for optimizing extract combinations.

5.3 Recommendations from the Study

Investigate different combination ratios of the three plant extracts to attain additivity or synergism. The three-plant extract exhibited antagonism. The determination of suboptimal ratios will enhance the therapeutic activity while maintaining low toxicity to develop a safe and effective formulation.

5.3.1 Future Recommendations

i. Sub-acute and chronic toxicity studies to validate long-term safety profiles of the extracts in malaria-endemic regions. This is critical to test prolonged or delayed effects from long-term use of the combined plant extract on organs. It also helps identify safe dose ranges that is necessary in developing reliable and effective long-term antimalarial therapies.

ii. Conduct IC_{50} (the half- maximal inhibitory concentration) to measure the antiplasmodial potency of the combined plant extracts. This is essential for drug development and dose optimization. The half-maximal inhibitory concentration identifies the optimal concentration with minimal cell toxicity while inhibiting parasite growth.

iii. Conduct histopathological studies on the combined plant extracts to confirm safety profile of the combined plant extracts at cellular and tissue level after long-term exposure. Histopathology is the microscopic examination of mice tissue samples to distinguish between healthy and infected cells or reveal early

signs of organ damage. It is crucial in risk assessment for therapeutic application.

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APPENDICES

Appendix I: Clearance Letter from KEMRI Scientific Steering Committee

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003. Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

ESACIPAC/SSC/102300 19th November, 2013

Sarah Bosibori

Thro'
Director, CTMDR
NAIROBI

Forwarded 20/11/2013

REF: SSC No. 2704 (Revised) – Investigation of synergistic antimalarial activity of herbal plants used in traditional medicine in Kenya

I am pleased to inform you that the above mentioned proposal, in which you are the PI, was discussed by the KEMRI Scientific Steering Committee (SSC), during its 208th meeting held on 5th November, 2013 and has since been approved for implementation by the SSC.

Kindly submit 4 copies of the revised protocol to SSC within 2 weeks from the date of this letter, i.e, 3rd December, 2013.

We advise that work on this project can only start when ERC approval is received.

for: Sammy Njenga, PhD
SECRETARY, SSC

19/11/13

In Search of Better Health

Appendix II: Clearance Letter from Animal Care and Use Committee

KENYA MEDICAL RESEARCH INSTITUTE

Centre for Virus Research, P.O. Box 54628 - 00200 NAIROBI - Kenya
Tel: (254) (020) 2722541, 254 02 2713349, 0722-205901, 0733-400003 Fax (254) (020) 2726115
Email: cvr@kemri.org

KEMRI/ACUC/ 01.05.14 **12th May, 2014**

Atambo Sarah Bosibori
C/O CTMDR
Nairobi

Atambo,

RE: Animal use approval for SSC 2704 - "Investigation of synergistic antimalarial activity of *Corymbia citriodora*, *Maytenus senegalensis* and *Warbugia ugandensis* used in traditional medicine in Kenya" protocol

The KEMRI ACUC committee acknowledges the resubmission of the above mentioned protocol. It has been confirmed that all the issues raised earlier have been addressed appropriately.

The committee grants you the approval to use laboratory mice in your study but recommends that you proceed after obtaining all the other necessary approvals that may be required.

Approval is granted for a period of two years starting from when the final ethical approval will be obtained. The committee expects you to adhere to all the animal handling procedures as described in the protocol.

The committee wishes you all the best in your work.

Yours sincerely,

Dr. Konongoi Limbaso
Chairperson KEMRI ACUC

Appendix III: Clearance Letter from KEMRI Ethics Review Committee

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003, Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **June 04, 2014**

TO: **SARAH ATAMBO,**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. JENNIFER ORWA,**
ACTING DIRECTOR, CTMDR,
NAIROBI

Dear Madam,

RE: **SSC PROTOCOL NO. 2704 – (*RESUBMISSION*). AN INVESTIGATION OF SYNERGISTIC ANTIMALARIAL ACTIVITY OF *CORYMBIA CITRIODORA*, *MAYTENUS SENEGALENSIS* AND *WARBUGIA UGANDENSIS* USED IN TRADITIONAL MEDICINE IN KENYA. (VERSION 1.1 DATED 22ND MAY 2014)**

Reference is made to your letter dated 22nd May 2014 and received at the KEMRI ERC on 23rd May 2014.

This is to inform you that the Committee notes that the following issues raised at the 224th meeting of the KEMRI Ethics Review Committee held on 18th February 2014 have been adequately addressed. Consequently, the study is granted approval for implementation effective this **4th June 2014** for a period of one year. Please note that authorization to conduct this study will automatically expire on **June 03, 2015.**

If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to the ERC Secretariat by **April 21, 2015**. The regulations require continuing review even though the research activity may not have begun until sometime after the ERC approval.

You are required to submit any proposed changes to this study to the SSC and ERC for review and the changes should not be initiated until written approval from the ERC is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of the ERC and you should advise the ERC when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

DR. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

Appendix IV: Publication

ANTI-MALARIAL ACTIVITY AND TOXICOLOGICAL EFFECT OF COMBINED CORYMBIA CITRIODORA, MAYTENUS SENEGALENSIS AND WARBUGIA UGANDENSIS AS USED IN TRADITIONAL MEDICINE IN KENYA

https://doi.org/10.7176/JNSR

Sarah Atambo [1,] Peter K. Njenga[1], [2*] Festus Tolo[2].

1. Jomo Kenyatta University of Agriculture and Technology, Nairobi CBD Campus
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Abstract

Malaria is majorly caused by *Plasmodium falciparum* resulting in thousands of deaths every year. In Africa, it is a key contributor to the disease burden notable in the disability adjusted life years (DALYs). About 243 million individuals are at a risk of contracting the disease and a higher rate of deaths are observed in children under the age of five. Conventional drugs are available at a subsidized rate but the rising problem is the resistance of the plasmodium parasite to these drugs. Hence, there is an urgent need for the development of new and alternative therapeutics for treatment of malaria. In some regions in Kenya, parts of locally available plants are harvested and used for treating malaria. It is estimated that locally, 30% of the population relies on traditional medicine for treating various ailments due to the lack of infrastructure and accessible medical facilities especially in the rural areas. *Warbugia ugandensis*, *Maytenus senegalensis* and *Corymbia citriodora* are amongst the plants used in herbal medicine for the treatment of malaria. However, their combinatorial antimalarial efficacy and safety is yet to be determined hence the aim of this study. The plants were harvested from their natural habitats and transported to the Centre of Traditional Medicine and Drug Research (CTMDR) at the Kenya Medical Research Institute (KEMRI), Nairobi. Antimalarial properties of single and combined extracts were analyzed against *Plasmodium berghei* in vivo. Cytotoxic properties of the plants were carried out against the Vero cell-lines in vitro by the MTT assay. Acute oral toxicity was conducted according to the OECD protocol. Effective concentration (ED_{50}), cytotoxicity concentration (CC_{50}) and median lethal dose (LD50) were derived. The result indicated that the combination of *M. senegalensis: W. ugandensis* (1:1) had the most antimalarial activity at ED_{50} of 1.05mg/kg whereas among the single plants *W. ugandensis* had the highest antimalarial activity (ED_{50} of 3.3mg/kg). The combinations of *C. citriodora*: *M. senegalensis*: *W. ugandensis* (1:1:1), *C. citriodora*: *M. senegalensis*: *W. ugandensis* (1:0.5:1) and *C. citriodora*: *M. senegalensis*: *W. ugandensis* (0.5:1:0.5) showed cytotoxicity concentration (CC_{50}) of 101.47±3.17 µg/ml, 213.55±3.47 µg/ml and 575.80±31.40 µg/ml respectively. All the plants combinations showed no cytotoxic effects. The synergistic antimalarial properties of combined *C. citriodora*: *M. senegalensis*, *C. citriodora*: *W. ugandensis* and *M. senegalensis*: *W. ugandensis* were confirmed as the extracts showed SFIC indexes of 0.67, 0.83 and 0.28 respectively. All the plant extracts demonstrated LD_{50} above 2000 mg/kg with no adverse effects hence recognized as safe. This study confirms the safety and antimalarial activities of these plants and justify their use in herbal medicine practices. The results of this study sets the precedence for the development of an antimalarial herbal formulation that is less toxic and more affordable.