ANTIPLASMODIAL AND CYTOTOXIC ACTIVITIES OF SELECTED MEDICINAL PLANTS IN KAKAMEGA COUNTY, KENYA

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APRIL, 2024

DECLARATION

Declaration by the student

I declare that this thesis is my original work and has not been presented for master degree or any other award in any other university.

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DEDICATION

I dedicate this thesis to my family, for their endless love, support and encouragement in my education.

ACKNOWLEDGMENTS

I would like to express my sincere thanks to the study participants for consenting to the study. I could not have completed this work without the unwavering support of my supervisors Dr. Nathan Shaviya and Mr. Mambo, your patience and guidance made this work possible. In addition, I acknowledge the clinical, laboratory staffs and management of Masinde Muliro University for their support during laboratory work. To my friends, classmates and family. Finally, my appreciation goes to God for his guidance and provision during my study period.

ABSTRACT

Female Anopheles mosquitoes spread malaria. Pregnant women, young children, and older adults are particularly prone to this sickness due to immune system impairment. The licensed antimalarials cause intolerance and toxicity. The conventional malaria management technique is expensive, especially for low-income nations. Thus, alternative and additional treatments are needed. Due of their lower cost, adverse effects, and convenience, therapeutic plants may be available. The lack of study on antimalarial medicinal plants as effective and affordable pharmacotherapies reveals a research need. The current study studied Kakamega County medicinal plants' antiplasmodial and cytotoxic effects. Twenty adults, male and female, were asked about plant use. Statistics show 16 traditional medicine plant species. These plants were chosen for their uses and bibliographies. Biological investigations were performed on 16 plant leaves, barks, and roots. Materials were solvent-extracted and dried. Water provided the most plant extracts. Dichloromethane produced the least solvent. Water released the most plant extracts. Dichloromethane produced the least solvent. Leguminosae produced the most crops of the examined plant species. Senna didmobotrya and occidentalis yielded the most water, 12.6% and 11.6%. The highest methanol yield was 6.3% from Senna didmobotrya. Following closely was 6.2% Lantana trifolia L. The highest dichloromethane yields were 2.7% and 2.4% from Trichilia emetic and Spathodea campanulata. The aqueous extracts of three plant species shown significant antiplasmodial activity against Plasmodium falciparum strain 3D7, with an IC50 value of ≤10 μg/ml. Three plant extracts were also effective against P. falciparum W2 strains. This study analyses plant species' antiplasmodial activity against 3D7 Plasmodium falciparum strains. Ten aqueous plant extracts shown moderate antiplasmodial activity (IC50 values: 11-49.9 μg/ml) against P. falciparum strains 3D7 and W2. The 3D7 strain demonstrated moderate antiplasmodial activity in 10 of 16 plants. Nine of 16 W2 samples demonstrated moderate antiplasmodial effectiveness. Two plant specimens did not inhibit Plasmodium falciparum 3D7 strain growth in lab trials. The only plant extract with modest W2 strain antiplasmodial activity was investigated. This extract was inactive against 3D7 strains, with an IC50 of ≥100 μg/ml. Inactive three people were exposed to W2 strains. Six of sixteen plant methanol extracts efficiently inhibited 3D7 bacteria. Methanol extract of the plant demonstrated significant antiplasmodial action against W2 mutant strain. Methanol-extracted plants demonstrated moderate antiplasmodial efficacy against the 3D7 strain in 6 of 16. Most methanol-extracted herbs (11/16) have modest antiplasmodial activity against W2. Three methanol-extracted plants have modest 3D7 strain antiplasmodial activity. W2 P. falciparum strains performed similarly in two experiments. Methanol extractions of 1 and 2 did not kill 3D7 or W2 strains. Dichloromethane leaf extracts from 16 plants inhibited 3D7 and W2. Six compounds strongly inhibited 3D7 strains. Four of sixteen tests revealed high W2 antiplasmodial activity. Five plants had moderate 3D7 antiplasmodial activity. A lot of samples have modest W2 activity. Four plants showed limited antiplasmodial activity against the 3D7 strain, but only two against the W2 strain. The plant had no 3D7 antiplasmodial action. dichloromethane-extracted compounds demonstrated no antiplasmodial action on W2. The results of this study offer significant insights for stakeholders who are interested in

investigating the potential of herbal remedies as an alternate strategy for the treatment of malaria.

TABLE OF CONTENTS

PLAGIARISM STATEMENT ii

DECLARATION iii

COPY RIGHT iv

DEDICATION v

ACKNOWLEDGMENTS vi

ABSTRACT vii

LIST OF FIGURES xii

LIST OF ABBREVIATIONS AND ACRONYMS xiii

OPERATIONALIZATION OF TERMINOLOGIES xiv

CHAPTER ONE 1

1.0 INTRODUCTION 1

1.1 Background information of the study 1

1.2 Problem statement 3

1.3 Objectives 5

1.3.1 General objective 5

1.3.2 Specific objectives 5

1.4 Research questions 5

1.5 Justification of the study 5

1.6 Significance of the study 6

1.7 Scope of the study 6

1.7.1 Limitations of the study 7

1.7.2 Delimitations of the study 8

CHAPTER TWO 10

2.0 LITERATURE REVIEW 10

2.1 Burden of malaria and herbal treatment 10
2.2 Mechanisms of Actions of Novel Phytochemicals in Malaria Treatment 13
2.3 Herbs and Plant Parts Used to Manage and Treat Malaria across Communities in Kenya.....15
2.4 Mode of Preparation and Use of Herbs in Treatment of Malaria in Kenya 16
2.5 Antimalarial Activities.....18
2.6 Toxicity of Herbs Used in Kenya for Malaria Treatment.....20
2.7. Traditional Health Care Practice and Policy Framework in Kenya 21
2.6.3. Flavonoids.....24
3.1 Study area 25
3.2 Study design.....25
3.3 Collection of plants.....25
3.4 Extraction methods 26
3.4.1 Aqueous extraction 26
3.4.2 Organic extraction 27
3.5 Antiplasmodial assays 28
3.5.1 Preparation of stock crude drugs 28
3.5.2 Culture of malaria parasites 28
3.5.3 *In vitro* antiplasmodial assays.....28
3.5.4 *In vitro* cytotoxicity assays 30
3.6 Data Analysis and Management 31
3.7 Ethical Considerations 31
5.1 Medicinal plant use in Kakamega County.....44
5.2 Yield and *in vitro* antiplasmodial activities of selected plants 46
CHAPTER SIX.....56
6.0 CONCLUSION AND RECOMMENDATIONS 56
6.1. Conclusions.....56
6.2. Recommendation for action.....56

6.3. Recommendation for further study 57
REFERENCES 58
APPENDICES 70
Appendix I: Map of the Study Site 70
Appendix II: MMUST Ethical Clearance 71
Appendix III: NACOSTI Permit 72

LIST OF TABLES

Table 4. 1. A summary of botanical and local identification as well as percentage yields per solvent ……34

Table 4.2 Showing antiplasmodial activities of based on different solvents against 3D7…….38

Table 4.3 Showing antiplasmodial activities of based on different solvents against W2……..39

LIST OF FIGURES

Figure 4.1. Showing percentage (%) yields of the solvents. Water, H_2O; Methane, METH; Dichloromethane,
DCM……..……..…32

Figure 4.2 showing inhibitory concentrations (IC_{50}) of selected plant extracts by different solvents against
3D7……...……...…40

Figure 4.3 showing inhibitory concentrations (IC_{50}) of selected plant extracts by different solvents
against……..……..41

LIST OF ABBREVIATIONS AND ACRONYMS

CC50	Cytotoxic concentration 50%
CO2	Carbon dioxide
CPM	Count per minute
DCM	Dichloromethane
DMSO	Dimethyl Sulphoxide
G6PDH	Glucose 6 Phosphate Dehydrogenase
IC50	Inhibition concentration 50%
LD50	Lethal dose 50% (Median Lethal Dose)
MEM	Minimum Essential Media
MTT	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
Nacl	Sodium Chloride
PCR	Polymerase Chain Reaction
PET	Petroleum Ether
RPMI	1640 25mM HEPES (N-Hydroxyethylpiperazine-N‟-2-ethanol sulfonic acid)
WHO	World Health Organization

OPERATIONALIZATION OF TERMINOLOGIES

Herbal Remedy- Natural products extracted from medicinal plants use for therapy.

Traditional Healers- Practitioners of indigenous healing practices in promoting psychological, physical, and spiritual.

Herbalist- Practitioner of herbal medicine

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information of the study

Malaria is caused by five Plasmodium species: *P. falciparum*, *P. vivax, P. malariae, P. ovale*, and *P. knowlesi. P. falciparum* had the greatest negative impact among the species studied. In 2016, the World Health Organisation observed high malaria mortality and morbidity in sub-Saharan Africa, Asia, and Latin America (WHO, 2016).

Kamau *et al*. (2020) estimate that over 3 billion people are exposed to malaria annually, with 1.2 billion at high risk. Malaria remains the most common tropical disease, with over 219 million symptomatic cases and 435,000 deaths (Kamau *et al*., 2020; Kapesa, 2018). The highest rates of illnesses and deaths occur in Africa, especially among pregnant women and children under five, who are most vulnerable (Barber *et al*., 2017).

After being bitten by female anopheles' mosquitoes, Plasmodium parasites mature and proliferate in the liver. This causes malaria symptoms include headache, fever, weakness, pain, nausea, stomach discomfort, and profuse sweating (WHO, 2016). According to Chipwaza *et al*. (2014), untreated and prolonged malaria can cause severe anemia, brain damage, kidney failure, pulmonary edema, and skin yellowing.

Despite malaria treatment advances, drug-resistant forms of *P. falciparum* against artemisinin combination therapies (ACTs), the main antimalarials, are causing worry (Kamau *et al*., 2020).

Malaria treatment still relies on chemotherapy (Staines & Krishna, 2012). Single or combination antimalarial medicines are available. The single category includes antimalarial medicines from several classes. Quinine (Cinchona alkaloid), Amodiaquine (AQ), Piperaquine, and Chloroquine (CQ) are 4-aminoquinolines, Primaquine (8-aminoquinolines), Pyrimethamine (Diaminopyrimidines), Sulfadoxine (Sulfonamides), Artesunate (ART) and Artemeter (Sesquiterpine lactones), Mefloquine ART + Amodiaquine, ART + Meflquine, Artemether + Lumefantrine, and ART + Sulfadoxine/Pyrimethamine are combination therapies.

Antimalarials without artemisinin have several downsides. These limitations include reduced efficacy, as shown by Li *et al*. (2019). These medications can also cause toxicity and be expensive, reducing patient compliance. Due to the molecular similarities of many antimalarial drugs, resistance is the biggest challenge (Muregi *et al*., 2003). In 2017, Ajayi *et al*. reported cross resistance between 4-aminoquinolines chloroquine (CQ) and amodiaquine (AQ). Elfawal *et al*. (2012) and Krishna *et al*. (2004) found chloroquine (CQ) to be an effective and cost-effective malaria treatment before resistance. Tse *et al*. (2019) found artemisinin and its semi-synthetic derivatives more effective than quinine. Thus, artemisinin-based combination therapy (ACTs) is the recommended treatment for malaria caused by *P. falciparum* infection (Esu *et al*., 2019; Panda *et al*., 2018). Even though ACTs are the main antimalarial treatment in tropical regions, artemisinin-resistant Plasmodium parasites still exist. Thus, additional research is needed to find safer, more effective treatments.

Malaria care has relied on medicinal plants to prevent Plasmodium parasite resistance to traditional antimalarials (Batista *et al*., 2009; Okumu *et al*., 2017). Herbal therapy has been the main malaria treatment for thousands of years. Quinine, the first antimalarial drug, was extracted from the bark of the *Rubiaceae* Cinchona tree, a traditional herbal medicine plant. A Cinchona bark solution was used to treat malaria in humans in the early 17th century (World Health Organisation, 2018). Ancient medicinal plant *Artemisia annua* was rediscovered in China and used to isolate artemisinin (Mbengue *et al*., 2015). The scientific community is interested in using herbal medicine to develop natural antimalarial drugs. This method controls malaria well. Herbal medicine is becoming more popular in developed and developing countries due to its cost-effectiveness, availability, social acceptance, and accessibility (Muregi *et al*., 2003).

Between 2001 and 2017, 2000 plant compounds were shown to be antimalarial against *P. falciparum* (Lemma *et al*., 2017). Kenyan communities treat malaria with 150 plant species from 60 plant families. Of these 60 plant families, *Asteraceae* is the most widely used for malaria treatment, accounting for up to 15% of all plant species (Muthaura *et al*., 2011). Herbal plants have several biologically active compounds that fight malaria (Philip *et al*., 2017). According to Batista *et al*. (2009), these substances can be alkaloids, sesquiterpenes, quinones, triterpenoids, flavonoids, quassinoids, limonoids, terpenes, chalcones, coumarins, or other random forms. These compounds' concentrations vary by herbal plant locale. The antiplasmodial and cytotoxic characteristics of various medicinal plants must be studied according to their regional ranges. Thus, this study examines the antiplasmodial and cytotoxic characteristics of different Kakamega County medicinal herbs.

1.2 Problem statement

Malaria remains a significant public health concern, mostly impacting children and pregnant women. Kakamega County is recognized as one of the regions where malaria is endemic, exhibiting a substantial prevalence rate. Nevertheless, there is a growing body of evidence indicating that malaria parasites, particularly *P. falciparum*, are displaying resistance to the now prescribed treatments, which primarily consist of artemisinin derivatives. Moreover, the expenses linked to the standard method of malaria management are potentially substantial, particularly for individuals residing in low-income nations. Consequently, there is a necessity for alternate and supplementary treatments. Kakamega County possesses a diverse array of medicinal plants, predominantly sourced from the tropical rainforest. Moreover, there exists documented information regarding the utilization of some medicinal herbs for the purpose of malaria management within this particular geographical area. Although the antiplasmodial and cytotoxic properties of certain medicinal herbs have been confirmed. Studies have demonstrated that plants originating from diverse geographical regions have variations in the levels of active metabolites. This suggests that the antiplasmodial and cytotoxic activities of plants can exhibit variations based on geographical location, as well as the techniques employed for extraction. Furthermore, it should be noted that various components of medicinal plants, such as roots, barks, and leaves, exhibit variations in the levels of active metabolites. These variations ultimately govern the antiplasmodial and cytotoxic activities of the plants. It is imperative to conduct investigations on the antiplasmodial and cytotoxic properties of medicinal plants across various geographical

regions, as this would facilitate the development of alternate therapeutic interventions for malaria.

1.3 Objectives

1.3.1 General objective

To determine antiplasmodial and cytotoxic activities of selected medicinal plants in Kakamega County.

1.3.2 Specific objectives

1. To determine percentage yields of selected medicinal plants in Kakamega County.
2. To determine antiplasmodial activities of selected medicinal plants in Kakamega County.
3. To determine cytotoxic activities of selected medicinal plants in Kakamega County.

1.4 Research questions

1. What are the percentage yields of selected medicinal plants in Kakamega County?
2. What are the *in vitro* antiplasmodial activities of selected medicinal plants in Kakamega County?
3. What are the cytotoxic activities of selected medicinal plants in Kakamega County?

1.5 Justification of the study

Malaria affects millions of children and pregnant women globally and is still is a major public health concern. Kakamega County has a malaria prevalence of 33% being one of the highest in the region. The development of resistance to antimalarial medications is the major problem in this field. As a result, it is essential to find and develop novel chemical compounds having anti-malarial action. Two primary medications, quinine and artemisinin, have been used to treat malaria for decades, and both were originally discovered as medicinal herbs. Therefore, there is enormous hope that medicinal plants will provide novel anti-malarial substances. Kakamega County has a tropical rain forest that is a source of medicinal plants some that have been used in the management of malaria. These plants are prescribed by herbal practitioners as well as within the community. There is need to identify and determine antiplasmodial and cytotoxic activities of some of the plants commonly used in the treatment of malaria.

1.6 Significance of the study

Malaria remains a significant public health issue, impacting a substantial number of children and pregnant women on a global scale. Kakamega County has a malaria prevalence rate of 33%, which is among the highest within the region. One of the most significant obstacles encountered by antimalarial medications is the formation of drug resistance. Hence, it is imperative to consistently discover novel chemical compounds exhibiting anti-malarial properties. Medicinal plants have long been recognised for their significant contribution to the treatment of malaria, as seen by the global utilisation of two prominent medications, quinine and artemisinin, which have been discovered over the course of several decades.

Therefore, medicinal plants possess significant potential in the discovery of novel anti-malarial compounds. Kakamega County is home to a tropical rainforest, which serves as a valuable reservoir of medicinal plants, including those that have been traditionally employed in the treatment and control of malaria. Herbal practitioners, as well as members of the community, recommend the use of these plants. It is imperative to ascertain and evaluate the antiplasmodial and cytotoxic properties of certain plant species that are frequently employed in the management of malaria.

1.7 Scope of the study

The primary objective of this study was to investigate the potential advantages and disadvantages linked to the utilisation of specific medicinal plants within Kakamega County for the purpose of treating malaria and other ailments. The primary objective of this study was to assess the antiplasmodial (anti-malaria) and cytotoxic (cell toxicity) properties exhibited by these botanical specimens. A comprehensive assortment of varied botanical specimens with therapeutic properties was procured from multiple sites situated within Kakamega County. The plant selection procedure considered not just the traditional utilisation of plants among local communities but also their cultural significance. Ensuring the dependability of results necessitates the meticulous identification and appropriate preparation of plant samples.

In order to isolate bioactive components from the plant materials that were gathered, suitable extraction techniques were utilised, which involved the use of solvents such as ethanol or methanol. Subsequently, the aforementioned extracts underwent thorough antiplasmodial

testing, which encompassed the evaluation of their efficacy against both wildtype and mutant strains of the P. falciparum parasite. The objective of this study is to assess the efficacy of plant extracts in combating malaria by conducting a series of experiments. These tests will enable the determination of the IC_{50} values associated with the plant extracts. Furthermore, the investigation involved the implementation of cytotoxicity assays on human cell lines in order to evaluate the potential harmlessness of these botanical extracts for human consumption. The assessment provided valuable perspectives on the possible medicinal uses of the botanical specimens and any accompanying hazards.

1.7.1 Limitations of the study

The observed diversity in the chemical composition of the chosen therapeutic plants. Inconsistencies in the composition of bioactive chemicals within samples may arise due to various factors, including ambient circumstances, plant growth phases, and collecting procedures. The observed variability has the potential to impact the dependability and replicability of the study's findings.

Furthermore, although the selected assays yielded significant findings regarding the antiplasmodial and cytotoxic properties of the plant extracts, they do not completely simulate the intricate dynamics of the human organism. Validation of the findings and evaluation of the efficacy and safety of the extracts in a comprehensive context would require the inclusion of in vivo research.

In addition, there exist significant ethical problems pertaining to the acquisition of plant samples and the utilisation of human cell lines. Although diligent attempts were undertaken

to adhere to ethical protocols, the presence of obstacles pertaining to the acquisition of informed permission, preservation of indigenous knowledge, and observance of cultural sensitivity posed significant problems.

1.7.2 Delimitations of the study

The research primarily concentrated on utilising *in vitro* assays to evaluate the antiplasmodial efficacy against the *P. falciparum* parasite and the cytotoxic effects on human cell lines. The aforementioned assays offer controlled conditions for testing purposes; but, they do not fully reproduce the intricate array of interactions that take place within a living organism. Therefore, it is possible that the results may not have direct applicability to real-life scenarios.

Furthermore, the choice of medicinal herbs was determined by their traditional usage and cultural importance. Although this methodology guarantees the inclusion of plant species that are relevant to local practises, it may inadvertently overlook other plant species that possess untapped qualities that could be of significant value. Furthermore, the study's temporal constraints and budget limitations may impose restrictions on the quantity of plant specimens that may be gathered and examined.

Moreover, the research investigation was centred on a particular geographical region, namely Kakamega County. The establishment of a focused focus is necessary in order to situate the findings within a distinct cultural and ecological framework. Nevertheless, this implies that the outcomes may not be readily transferable to other areas characterised by distinct plant species, temperatures, and cultural practises.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Burden of malaria and herbal treatment

Through the bite of an infected female Anopheles mosquito, people can contract malaria, a serious and potentially fatal disease caused by Plasmodium parasites (WHO, 2016). This disease is quite prevalent in contemporary times, not just inside the borders of Kenya but also across the entire African continent. Therefore, it is imperative to implement diligent surveillance measures for malaria, given its potential for severe manifestations and high mortality rates, particularly in light of the rising frequency of resistance to existing antimalarial medications. According to Pan *et al*. (2018), *P. falciparum* and *P. vivax* are considered the most lethal of the five-parasite species responsible for causing malaria in

humans. According to the World Health Organisation (2018), *P. falciparum* and *P. vivax* are the predominant malaria parasites in sub-Saharan Africa and parts of the Americas, respectively. In 2017, these two species were responsible for approximately 99.7% and 74.1% of reported malaria cases. *P. knowlesi* is the predominant etiological agent of malaria in Southeast Asia, including a substantial proportion of malaria cases, estimated to be as high as 70% (Barber *et al*., 2017). However, it is worth noting that this parasite has a higher affinity for infecting Old-World monkeys (Barber *et al*., 2017). *P. malariae* and *P. ovale*, two more Plasmodium species, typically induce moderate febrile symptoms. In 2016, the World Health Organisation (WHO) recorded around 216 million cases of malaria, resulting in a mortality rate of up to 445,000 individuals. According to the research conducted by Njoroge and Bussman (2006), it has been found that malaria is accountable for an estimated annual mortality rate of one to two million individuals in Africa. The characteristic manifestations of malaria encompass elevated body temperature, exhaustion, cephalalgia, myalgia, queasiness, abdominal unease, and excessive perspiration. Nevertheless, in instances of severe pathology and extended absence of medical intervention, it is possible to observe brain tissue damage, pulmonary edoema, renal dysfunction, profound anaemia, jaundice, and hypoglycemia (Pan *et al*., 2018; WHO, 2016). Malaria is a significant contributor to morbidity and mortality in Kenya (Njoroge & Bussmann, 2006). According to statistical data, it has been observed that this particular condition contributes to 46% of illnesses among children, nearly 40% of visits to outpatient facilities such as hospitals and clinics, 25% of admissions to hospitals, 14% of deaths that occur during inpatient care, and around 23% of baby mortalities. These findings have been reported by Kamau *et al*. (2020)

and Kapesa *et al*. (2018). The utilisation of herbs and herbal extracts for the management and treatment of malaria is widely practised throughout many regions globally. This prevalence can be attributed to the affordability, accessibility, and efficacy of herbs in combating the disease. Indeed, there has been a noticeable increase in the global utilisation of herbal medicine as a kind of therapeutic intervention. According to James *et al*. (2018), a significant majority of the Kenyan population, specifically over 80%, depends on herbal plants as their primary source of healthcare. The utilisation of traditional herbal medicines is prevalent among a significant proportion of the population due to the substantiated therapeutic efficacy associated with such remedies (James *et al*., 2018). The growing inclination towards herbal remedies, along with the emergence of drug-resistant pathogenic strains such as Plasmodium species, has sparked the interest of researchers in exploring herbal plants as potential alternatives to develop more efficacious antimalarial drugs (Okello & Kang, 2019; Tse *et al*., 2019). The leaves of plants are widely utilised for the treatment of malaria, followed by the roots and bark. While other plant parts and whole plants are less typically employed for this purpose. A specific botanical specimen is frequently employed individually, albeit occasionally in conjunction with other botanicals. The predominant method of utilisation involves the process of boiling the specific anatomical component of the medicinal plant in water, followed by consumption of the resulting decoction. Additionally, the intake of fresh extracts and powdered formulations of the herbal substances is also commonly observed.

Various herbal medicines are utilised throughout diverse populations across different regions of the country, with their selection being influenced by the geographical distribution of specific medicinal plant species. For instance, in Western Kenya, *Warburgia ugandensis* holds particular significance and is commonly employed for medical purposes. Nevertheless, it is worth noting that certain herbal plant species, such as *Bidens pilosa* L., are widely distributed across the nation and have gained recognition for their efficacy in treating malaria within the local population. The varied ethnic groups in Kenya, such as the Luhya, Luo, Kalenji, Kikuyu, Maasai, Turkana, and Kisii, have assigned various local names to these medicinal plants.

In Kenya, many strategies are used throughout different communities to mitigate the spread of malaria. These efforts encompass the elimination of stagnant water sources, the clearance and incineration of vegetation, the utilisation of insecticide-treated mosquito nets for sleeping, and the application of insecticides through home spraying.

2.2 Mechanisms of Actions of Novel Phytochemicals in Malaria Treatment

Herbal plants have several phytochemicals that treat and prevent malaria. Sesquiterpenes, lactones, fluoroquinolones, chalcones, flavanones, phenolics, quinones, coumarins, and alkaloids are covered in this study. These chemical components are also found in malaria-preventive herbs. Quinine and artemisinin synthesis has advanced antimalarial medications. These chemicals, from different chemical classes, have shown great efficacy in fighting malaria, leading in disease management successes. Artemisinins, derived from the Asteraceae species *Artemisia annua*, have helped prevent malaria. Artemisinin-based

combination therapy has advanced this disease's treatment (Tse *et al*., 2019). These medicines have worked against different strains of P. falciparum, including drug-resistant strains (Bekono *et al*., 2020; Krishna *et al*., 2004). Academic literature has extensively examined artemisinin's pharmacological mechanism. The general view is that artemisinin activates through heme interaction. Bekono *et al*. (2020) found that the activation mechanism produces free radicals that specifically interact with and damage parasite-essential proteins. It is hypothesized that the antimalarial properties of artemisinin, a sesquiterpene lactone, are due to the uncommon peroxide linkage bridge it contains. The peroxide linkage bridge cleaves with heme-produced iron (II) ions. The text describes how cleavage creates highly reactive free radicals that rapidly rearrange to generate stable carbon-centered radicals. According to Tse *et al*. (2019), carbon-centered radicals chemically alter the parasite, inhibiting parasite molecular functions. This kills the parasite. Artemisinin mainly inhibits parasite trophozoite formation, slowing illness progression. According to Krishna *et al*. (2004), this method eliminates ring-stage parasites, improving therapeutic outcomes. According to Mok *et al*. (2014), artemisinin induces unfolded protein response pathways, which reduces parasite proliferation and maturation. Based on their research, Shandilya *et al*. (2013) suggested that iron activates artemisinin. PfATP6, a calcium pump, inhibits phosphorylation, nucleotide binding, and actuator domains after activation. Finally, this perturbation causes PfATP6 to degrade, killing the Plasmodium parasite. Mbengue *et al*. (2015) discovered that artemisinin blocks the activity of phosphoinositide-3-kinase (PfPI3K), a biochemical regulator of Plasmodium falciparum development, proliferation, and survival.

Since 1632, quinine, a malaria treatment made from cinchona tree bark, has been in use (Staines & Krishna, 2012). In order to keep healthcare systems running smoothly, quinine is an essential drug, as stated by the WHO (2015). This medication is reserved for cases of malaria caused by a specific strain of Plasmodium falciparum that is resistant to chloroquine. This is important when artemisinins, an alternate treatment, are unavailable (Esu *et al*., 2019). It is well established that quinine works by studying chloroquine, a medicine closely related to quinoline. Quinine blocks the biocrystallization pathway of hemozoin, causing the parasite to accumulate cytotoxic heme and die, according to Foley and Tilley (1997). Kenyan malaria cure plants mostly contain alkaloids, which have been related to antiplasmodial properties. Numerous alkaloids have a unique affinity for the Plasmodium parasite's apicoplast. Additionally, benzylisoquinoline alkaloids from Cissampelos mucronata, a Menispermaceae plant, restricted parasite protein synthesis (Chinwuba *et al*., 2015).

Flavonoids, found in *Asteraceae* plants like *B. longipes*, *A. conyzoides*, and *A. africana*, are used to cure malaria in Kenya. However, other herbal plants, such as C. roseus in the *Apocynaceae* family and *A. zygia* and *A. nilotica* in the *Mimosaceae* family, also contain these flavonoids as active components with antiplasmodial properties. Chinwuba *et al*. (2015) discovered flavonoids antiplasmodial against several malaria strains. The mechanism behind their antimalarial action is unknown. According to Chinwuba *et al*. (2015), multiple studies demonstrate that flavonoids can prevent myoinositol and L-glutamine from entering

infected erythrocytes. Chinwuba *et al*. (2015) found that certain flavonoids increase erythrocyte oxidation and inhibit malaria parasite protein synthesis. According to Freundlich *et al*. (2005), flavonoids can inhibit Plasmodium's FAS II pathway. *P. falciparum* has artemisinin resistance in Vietnam, Cambodia, Muang Lao, and Thailand. Tse *et al*. (2019) found over 30 artemisinin resistance cases in Southeast Asia in a 2018 report. Resistance slows parasite elimination, increasing gametocyte concentration. Thus, this event raises selective pressure on alternative medicinal partners, promoting resistance. Therefore, this is a major health risk. Thus, extensive research on Africa's vast medicinal plant resources is needed to generate alternative medications with different mechanisms of action. Local populations have used these resources to treat malaria. However, its antimalarial potential has not been thoroughly studied. Amoa Onguéné *et al*. (Bekono *et al*., 2020) stressed Africa's role in developing a novel antimalarial medication.

2.3 Herbs and Plant Parts Used to Manage and Treat Malaria across Communities in Kenya

A comprehensive survey conducted in Kenya revealed that approximately 150 plant species, belonging to around 60 distinct plant families, are employed for the treatment of malaria within various communities. Notably, among these plant families, the Asteraceae family exhibits the highest prevalence in the country for malaria treatment, accounting for up to 15% of the total plant species employed (Muthaura *et al*., 2011). According to Omara (2020), the taxa listed below include species from the Fabaceae (9%), Lamiaceae (8%), Euphorbiaceae (6%), and Mimosaceae (4%) families. Additionally, the Myrtaceae,

Aloeaceae, and Rutaceae families each account for approximately 3% of the total number of species used for malaria therapy in Kenya. The remaining groups comprise just 49% of the total plant species used for malaria therapy.

2.4 Mode of Preparation and Use of Herbs in Treatment of Malaria in Kenya

The ways in which herbs were prepared and used differed among different communities, depending on the properties of the herb and the specific plant parts used for treating malaria (Adia et al., 2014). According to Philip et al. (2017) and Stangeland et al. (2011), herbal remedies are commonly prepared in the form of aqueous extracts, such as decoctions and infusions, or alternatively, as steam baths. As stated by Philip et al. (2017), the primary procedure for preparing the herbal plant water extract entails the boiling of a limited amount of medicinal plant constituents, such as leaves, in a volume of one liter of water. Following this, the aforementioned extract is supplied to the patient through the route of oral ingestion. The appropriate dosage of the provided extract is dependent on the patient's age and the effectiveness of the herbal cure, occasionally taking into account the patient's weight (Philip et al., 2017; Stangeland et al., 2011). The optimal dosage of the extract is contingent upon the specific age cohort. The ideal range for adults is 100 to 500 ml. The recommended range for older children is 100 to 250 ml. For children under the age of 5, the recommended dosage is 1 to 3 tea or tablespoons. The administration frequency ranges from 1 to 3 times per day. The prescribed treatment plan is commonly adhered to for a duration of around one week or until the patient has achieved complete recuperation (Anywar et al., 2016; Philip et al., 2017). The extractions are predominantly obtained from individual botanical specimens

or a blend of two botanical specimens. The combination of Tamarindus indica and Mangifera indica is frequently employed, as demonstrated by Anywar et al. (2016).

In certain instances, the botanical components with medicinal properties undergo a process of dehydration followed by pulverisation into a fine powder. Subsequently, a quantity of 2–5 tablespoons of this powdered substance is incorporated into water and subjected to boiling, thereby producing a therapeutic decoction. According to Anywar *et al*. (2016), the bark of *M. indica* stem and the roots of *V. lasiopus*, along with their respective powders, are subjected to a prolonged boiling process until the volume of water is reduced by half. According to Adia *et al*. (2014), the powdered form of the herbal plant can be incorporated into both cold and hot water, followed by stirring, and thereafter consumed as per the prescribed guidelines.

According to Stangeland *et al*. (2011), a medicinal remedy for malaria derived from the herb *B. pilosa* can be prepared by extracting the juice from a handful of newly harvested leaves and consuming 1-3 teaspoons of the extract on a daily basis. In certain instances, malaria herbal remedies can be acquired by the preparation of various plant components in conjunction. For instance, a solution can be created by utilising freshly harvested leaves and crushed roots of *V. amygdalina* (Anywar *et al*., 2016). Subsequently, the substance is administered via the oral route at a suitable dosage. The utilisation of certain botanical components, such as leaves, for medical purposes involves the extraction of their therapeutic properties by the process of squeezing. These extracted substances can then be combined with either cold or warm water to create a bath solution. For instance, the leaves of *B.*

adoensis have been identified as a potential candidate for this practise (Anywar *et al*., 2016). Certain plants that are often consumed as vegetables have been traditionally used as a preventive measure against malaria. Additionally, some of these herbs are cultivated in pots and strategically placed about households, or burned, with the intention of repelling mosquitoes.

2.5 Antimalarial Activities

Several researches have been conducted to investigate the antiplasmodial/antimalarial properties of some herbal plants commonly employed in Kenya for the treatment of malaria. These studies employed different strains of malarial parasites to ascertain the efficacy of these plants as potential treatments for malaria (Adia *et al*., 2014; Obbo *et al*., 2019). Moreover, a diverse array of phytochemicals, which are accountable for the biological functionalities exhibited by certain antimalarial plants, have been successfully extracted and identified in a study conducted by Philip *et al*. (2017). In Kenya, a comprehensive examination of plant species employed for malaria therapy has revealed that out of the 150 species studied, 112 have been subjected to scrutiny regarding their antimalarial properties. Encouragingly, 108 of these plants exhibited favourable outcomes, while a mere four species failed to provide favourable findings when assessed for their antimalarial potential. Approximately 39% of the plant species utilised by various groups in Kenya for malaria therapy lack documented research on their antimalarial properties.

The effectiveness of herbal plants in combating malaria can be ascribed to the existence of several bioactive constituents (Philip et al., 2017). Batista et al. (2009) have reported that these chemicals have the potential to express themselves in several chemical structures,

including alkaloids, sesquiterpenes, quinones, triterpenoids, flavonoids, quassinoids, limonoids, terpenes, chalcones, coumarins, and other miscellaneous forms. The extraction solvent employed has a substantial impact on the concentrations of the active metabolites present in the extract. One notable observation is that methanolic extracts derived from herbal plants demonstrate a higher level of in vitro activity in comparison to water extracts. The observed difference in activity could perhaps be ascribed to the heightened abundance of lipophilic compounds, which are recognized for their enhanced bioactivity (Muthaura et al., 2015).

The effectiveness of antimalarial plant extracts is dependent on the concentration of the active antimalarial secondary metabolites, as stated by Muthaura et al. (2015). To provide an example, it has been noted that gedunin, a chemical known for its strong efficacy against Plasmodium, has been detected in the foliage of A. indica. The measured IC50 against P. falciparum was 0.02 μg/ml. Nevertheless, the plant has a relatively low concentration of gedunin, leading to a moderate level of physiological activity in its extract (Muthaura et al., 2015; Philip et al., 2017).

The observed high levels of antiplasmodial activity in specific herbal plant extracts can be ascribed to the synergistic impact arising from the interaction of diverse active secondary metabolites. In the case of A. afra, the efficacy of individual flavonoids and sesquiterpenes extracted from the plant against P. falciparum was found to be negligible, as evidenced by

their low IC50 values. Nevertheless, the plant extract exhibited a significant IC50 value of 3.9 μg/ml, indicating that the collective activity of the constituents within the extract plays a role in its heightened effectiveness (Muthaura et al., 2015). The presence of specific active components considerably impacts the antimalarial effectiveness of herbal plant extracts. The antiplasmodial activity of M. pyrifolia has been attributed to the diterpene molecule 6E-geranylgeraniol-19-oic-acid, derived from its aqueous extract. Adia et al. (2014) reported that nitidine, derived from Z. chalybeum, demonstrated a very low IC50 value of 0.17 μg/ml against P. falciparum 3D7. Furthermore, Muthaura et al. (2015) have established that pristimerin, the primary active component found in the extract of M. senegalensis, exhibited a notably elevated antiplasmodial activity, as evidenced by an IC50 value of 0.5 mg/ml.

2.6 Toxicity of Herbs Used in Kenya for Malaria Treatment

Adia et al. (2014) shown that the efficacy of herbal extracts in the treatment of malaria may be ascribed to the existence of a minimum of two secondary metabolites inside the extract. The choice of pathogenic strains utilized in in vitro studies may exhibit variability, resulting in potential variations in the parasite's susceptibility to the active metabolites. As a result, this diversity may lead to differences in the observed efficacy of the extracts against malaria (Adia et al., 2016). According to Philip et al. (2017), the absence of antiplasmodial activity in herbal plants suggests that their extracts may not include metabolically active compounds capable of successfully combating Plasmodium parasites. According to Abdelgadir and Van Staden (2013), the level of toxicity can differ depending on factors such as the sensitivity of

animals, the type of tissue or cells used, the type of extract, the nature of the test chemical, the dosage, and the mode of administration. Muthaura et al. (2011) conducted a study which revealed that around one third of the herbal treatments investigated for the treatment of malaria in Kenya displayed significant antiplasmodial capabilities, while displaying low toxicity. Numerous botanical components have exhibited noteworthy antiplasmodial and antimalarial characteristics, while displaying little or negligible toxicity. The plant parts encompassed in this list consist of the leaves of Artemisia annua, Artemisia africana, Swietenia pinnata, Carica papaya, and Flueggea virosa, among other instances. Several plant extracts have demonstrated efficacy in combating Plasmodium, the causative agent of malaria. Nevertheless, it is important to acknowledge that certain extracts, such as the petroleum ether leaf extract of V. amygdalina and the dichloromethane leaf extract of M. pyrifolia, demonstrate significant toxicity (Lacroix et al., 2011; Muganga et al., 2010). The plant species Clerodendrum rotundifolium exhibits notable antimalarial and antiplasmodial activities. However, its potential toxicity has not been thoroughly examined in scientific research (Kaur et al., 2018).

2.7. Traditional Health Care Practice and Policy Framework in Kenya

In Kenya, the healthcare system consists of three primary sectors: the public sector, the private for-profit sector, and the private non-profit sector. A substantial proportion of health care providers in the informal health care market encompass traditional medicine practitioners, drug shops, prescription dealers, and complementary and alternative practitioners. Okumu et al. (2017) have emphasized that the acknowledgment and

admiration of the contribution made by traditional health practitioners in Kenya's healthcare system has occurred very recently. The negative perspective can be traced back to the colonial period, wherein cultural customs, such as the use of traditional medicine like herbal treatments for healing, were considered early and aggressively disapproved of (Okumu et al., 2017). The government is currently making efforts to promote the use of traditional medicine, recognizing the important role that traditional health practitioners play in the primary healthcare system (Okumu et al., 2017). The Ministry of Health implemented a public-private partnership with the aim of integrating traditional health practitioners into the conventional health system, as proposed by Kigen et al. (2019) and Okumu et al. (2017). A policy was implemented to govern the practice of Traditional and Complementary Medicine, with a particular emphasis on research and development. The aforementioned policy prioritizes the advancement, protection, and sustainable utilization of medicinal plant resources (Kigen et al., 2019; Okumu et al., 2017). A legislative proposal has been put out by the Ministry of Health with the objective of establishing the Centre for Traditional Medicine and Research. This initiative seeks to foster cooperation between the conventional healthcare industry and traditional health practitioners. The aforementioned semiautonomous entity also aims to protect the intellectual property rights of cultural health professionals (Kigen et al., 2019; Okumu et al., 2017).

The regulatory bodies responsible for regulating the quality control of medicinal commodities, including herbal medicines, in Kenya are the Kenya Bureau of Standards (KEBS) and the Kenya Pharmacy and Poisons Board. According to Gakuya et al. (2020),

these entities function within the legal framework that has been established by the government. The regulatory system pertaining to herbal medicines in Kenya lacks significant elements that differentiate it from the regulations governing conventional pharmaceuticals. Herbal medical items are subject to the same regulations and procedures that control conventional pharmaceutical medicines. Gakuya et al. (2020) reported the implementation of a strategy in 2002 aimed at the registration of herbal medicines. At present, there has been no registration of herbal medication in accordance with this policy.

Despite the widespread utilization of herbal treatments in Kenya, there exists a dearth of sufficient regulation pertaining to their usage. Currently, the nation does not possess a comprehensive structure for the authorization and supervision of conventional healthcare professionals and their products. In addition, the incorporation of traditional and complementary medicine into the conventional healthcare system continues to be an elusive objective, with substantial advancements still to be achieved.

2.6 Phytochemical Compounds

Compounds having anti-malarial action and other benefits have been discovered through phytochemical studies of a variety of trees, shrubs, and lianas. Examples of such chemicals are:

2.6.1. Phenols

Numerous phenols that exist naturally have exhibited unique inhibitory properties against the proliferation of the malaria parasite. A prenylated phloroglucinol derivative produced from Hypericum calycinum (Hypericaceae) shown inhibitory effects on the growth of Plasmodium falciparum, a parasite responsible for malaria, in an in vitro setting. The EC50 value, which represents the specific concentration at which 50% inhibition of growth was observed, was determined to be 0.88[1]g/ml. The anti-plasmodial action of 2'-epicycloisobrachycoumarinone epoxide and its 29 stereoisomers, derived from Vernonia brachycalyx (Asteraceae), was shown by Silva et al. (2013). The compounds under investigation have exhibited similar effectiveness in laboratory settings against both chloroquine-sensitive and chloroquine-resistant strains of P. falciparum, as evidenced by their respective EC50 values of 0.11 and 0.15 µg/ml.

2.6.2. Chalcones

Phlorizidin, derived from *Micromelum tephrocarpum* (Rutaceae) plant species, was among the initial chalcone glycosides documented for its demonstrated anti-parasitic properties. Licochalcone A emerges as the most auspicious chemical within this particular category of natural compounds. The first isolation of the compound in question was conducted from Glycyrrhiza glabra, a plant belonging to the Fabaceae family. Subsequently, extensive preclinical investigations were undertaken, with licochalcone A serving as the primary compound of interest. As a result, numerous chalcones were synthesized and their structure-activity connections were thoroughly characterized (Silva *et al*., 2013).

2.6.3. Flavonoids

Flavonoids exhibit a vast distribution throughout the plant kingdom. Since the identification of anti-plasmodial flavonoids derived from *Artemisia annua* (Asteraceae), there has been a resurgence of interest in this particular group of compounds. In vitro, the combined action of methoxylated flavonones artemetin and casticin demonstrates a synergistic effect with artemisinin against Plasmodium falciparum. Previous investigations on Artemisia species have identified the isolation of exigua flavanone A and B from *Artemisia indica* (Asteraceae). These compounds have demonstrated in vitro activity against P. falciparum, with EC50 values of 4.6 and 7.1 µg/ml, respectively (Silva *et al*., 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

This study was conducted in Kakamega County. This county is characterized by high temperatures and long rainy seasons ideal for mosquito breeding. The Kakamega tropical rain forest traverses the Kakamega County. The County has 12 sub-counties with Shinyalu and Malava sub-counties having the bulk of the forest and by extension most of the traditional healers and medicinal plants practitioners reside in these sub-counties. Kakamega County has a malaria prevalence of 33%. There is documented evidence of herbal use by residents of Kakamega County in the treatment of malaria (Mukungu *et al*., 2016).

3.2 Study design

This study adopted an experimental laboratory study design. Sixteen selected medicinal plants were harvested, identified and classified taxonomically. Various parts (leaves, root and bark) of the plant were then extracted and their antiplasmodial and cytotoxic activities tested.

3.3 Collection of plants

The collection of medicinal plants was conducted in Kakamega County, facilitated by traditional herbalists who possess knowledge of their ethnomedical applications. To ensure accuracy, a taxonomist at the East African Herbarium, located at the National Museum of Kenya in Nairobi, authenticated the obtained plants. Voucher specimens were then placed at this institution. The plant materials that were gathered were subjected to a process of air-

drying for a duration of one week in a chamber with sufficient air circulation and maintained at the ambient temperature. Following this, the materials were further processed by being mechanically ground into a granular form with a somewhat rough texture. Subsequently, the individual granules were carefully placed into appropriately marked plastic containers and securely stored on arid laboratory shelves, in anticipation of the extraction process.

3.4 Extraction methods

The plant material, which had been dried and turned into powder, underwent maceration for a duration of three days at room temperature. This process was carried out separately and simultaneously in 10 L of four different solvents: a mixture of methylene chloride and methanol in a 1:1 ratio, methylene chloride alone, hexane, and methanol. The mixture underwent filtration using Whatman paper and was subsequently concentrated to dryness, resulting in the formation of viscous residues. This process was carried out utilizing a Rotavapor system manufactured by BÜCHI Labortechnik AG in Switzerland. The crude extracts were kept at a temperature of 4°C for subsequent use.

3.4.1 Aqueous extraction

The Bibi et al. (2012) study's methodology was followed to in the manufacture of the aqueous extracts. The powdered plant materials, weighing 100 grams, were meticulously transferred into sterile beakers with a volumetric capacity of 1000 ml. Following that, a volume of 600 ml of desalinated water was introduced into each vial. The beakers containing the different mixtures were sealed with aluminum foil and immersed in a water bath maintained at a temperature of 80°C for a period of one and a half hours to facilitate the

extraction process. Following this, the mixtures were subjected to filtering using Whatman's prime filter papers before being subjected to freeze drying for a period of 48 hours. The desiccated and freeze-dried samples were transferred into standard containers that were sterile, desiccated, and pre-weighed. The percentage yields of these samples were then computed and stored at a temperature of -20°C in a freezer until they were prepared for use.

3.4.2 Organic extraction

The approach outlined by Bibi et al. (2012) was followed for implementing the sequential extraction method that uses dichloromethane and methanol. The plant components were macerated in 600 ml of liquid over 1000 ml beakers at a temperature of 25°C for 48 hours. The mixtures were subsequently subjected to filtering using double-layer Whatman's number one filter sheets. Subsequently, the filtrates obtained were underwent vacuum reduction at a temperature of 40°C using a rotating evaporator. Following the same technique as the dichloromethane extraction, the relevant samples were subsequently exposed to maceration in 600 ml quantities of methanol in beakers with a capacity of 1000 ml. The solution obtained was further filtered and subjected to vacuum concentration using a rotary evaporator operating at a temperature of 56°C. Subsequently, the obtained extracts were transferred into pre-weighed sterile glass bottles. The extracts' yields were subsequently quantified, and the bottles were stored in a freezer at a temperature of -20°C until they were prepared for utilization.

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3.5 Antiplasmodial assays

3.5.1 Preparation of stock crude drugs

The stock solutions were produced using sterile deionized water and then filtered using 0.22 μm membrane filters under aseptic conditions in a laminar flow hood. The extracts that do not dissolve in water were initially dissolved in a solution of dimethyl sulphoxide (DMSO) with a concentration of 0.02%. They were then diluted to the correct ratios using sterile deionized water, following the method described by Muregi et al. (2003). A temperature of -20°C was maintained for all of the therapeutic substances.

3.5.2 Culture of malaria parasites

The malaria-resistant genotypes of Plasmodium falciparum utilized in this study were obtained from the Malaria Laboratories of the Kenya Medical Research Institute (KEMRI), situated in Nairobi. The culture medium was created by mixing RPMI 1640 with 10% human serum, 25 mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES), 25 mM NaHCO3, and 50 mg/ml gentamycin (0.5 ml). The study utilized human type O+ erythrocytes that were under 28 days old as the host cells. The samples were subjected to incubation at a temperature of 37°C within an atmosphere comprising 3% carbon dioxide, 5% oxygen, and 92% nitrogen.

3.5.3 *In vitro* antiplasmodial assays

The potential of the extracts to inhibit the absorption of [G-3H]-hypoxanthine by the malaria parasite was assessed using an in vitro semiautomated microdilution test method (Dame et al., 2013). A volume of 25 µl of the culture media was applied to each well, excluding row B, in a 96-well flat-bottomed microculture plate. Three replicates of the 50 µl test solutions were placed in row B. A titertek motorized hand diluter was employed to serially dilute each sample, resulting in a concentration range of 64 times. The dilutions ranged from 200 µg/ml (100%) to 3.125 µg/ml (1.56%). In the culture media, rows R9–R12 contained erythrocytes that were not parasitized, while the other rows were supplemented with suspensions of parasitized erythrocytes (200 µl, 1.5% v/v). The suspensions exhibited a parasitemia rate of 0.4%. The plates were incubated at a temperature of 37°C in an environment consisting of ninety-two percent nitrogen, five percent oxygen, and three percent carbon dioxide. Following a 48-hour incubation period, each well was subjected to a 25 µl pulse of culture medium containing 0.5 µCi of [G-3H]-hypoxanthine. Next, the plates were subjected to an additional incubation period of eighteen hours. The specimens obtained from each plate were collected onto glass fiber mats, subjected to a meticulous cleansing process using distilled water, and subsequently dried. Liquid scintillation was employed for the quantification of radioactivity.

The data from the beta counter was imported into a spreadsheet using Microsoft Excel 2016. Subsequently, the data was inputted into an Oracle database program to determine the IC50 values. To estimate the drug concentration that leads to a 50% blockage of [G-3H]-

hypoxanthine absorption (also known as IC50), the logarithmic conversion of concentration and counts per minute (CPM) data was interpolated using the following formula:

$$\mathrm{IC}_{50} = \text{antilog}\left(\text{Log}\, X_1 + \frac{(\text{Log}\, Y_{50} - \text{Log}\, Y_1)(\text{Log}\, X_2 - \text{Log}\, X_1)}{(\text{Log}\, Y_2 - \text{Log}\, Y_1)}\right)$$

In this context, the CPM value Y50 represents the midpoint between control cultures that have been parasitized and those that have not; the concentrations and CPM values for the data points above and below the CPM midpoints are denoted as X1, Y1, X2, and Y2, respectively.

3.5.4 *In vitro* cytotoxicity assays

Per the protocol laid out by Bibi et al. (2012), the cytotoxicity of the extracts was assessed using quick calorimetric tests utilizing 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT). To catalyze the breakdown of the tetrazolium ring of the light yellow MTT molecule, the experimental approach makes use of a mitochondrial dehydrogenase enzyme that is produced from live cells. As a byproduct of this procedure, dark-blue formazan crystals are produced, which are unable to cross the cell membrane. According to Bibi et al. (2012), there is a substantial correlation between cell number and formazan production, since the presence of formazan crystals was found exclusively in live cells. The Vero (E196) kidney cell line, which is derived from African green monkeys, was

cultured in Eagle's Minimum Essential Media (MEM) with 10% fetal bovine serum added. A 20,000 cell solution was added to 100 µl of 96-well plates, and then left to incubate for 24 hours at 37°C with a 5% CO2 concentration. A confluence level greater than 90% was the target of this incubation procedure. At the beginning, after a 24-hour incubation period, the concentration of the test extracts and control was 1000 µg/ml. A chloroquine concentration of 100 µg/ml served as the positive control that was administered to the experimental group. For 48 hours, the cells were left to their own devices with the test extracts. Afterwards, 10 microliters of MTT test reagent (10 mg/ml) was added to every well and left to incubate for another 4 hours under the same conditions. To help dissolve the formazan crystals, 100 µl of dimethyl sulfoxide (DMSO) was added after the medium was removed from the plate. Spectrophotometers were then used to examine the plates. The optical density (OD) measurements for each medicine concentration in each well were included in the dataset.

3.6 Data Analysis and Management

The quantitative data was organized and calculated using Microsoft Excel 2016, and afterwards transferred to IBM SPSS software version 25 for further analysis. The data underwent descriptive statistical analysis and was presented as the mean plus or minus the standard error of the mean (SEM). The in vitro antiplasmodial activities were assessed through the determination of IC50 values, while the evaluation of acute toxicity and cytotoxicity was conducted by obtaining CC50 values. The calculation of toxicity and cytotoxicity involved the use of logarithmic transformation.

3.7 Ethical Considerations

The MMUST Institutional Ethics Review Committee granted ethical approval. The study received institutional permission from the medical authorities of the health facility. Approval to carry out the study was obtained by the National Council for Science and Technology and Innovations (NACOSTI). The data was safeguarded with password protection, limiting access solely to the chief investigator in order to maintain secrecy.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage plant yields

Three solvents water, methanol and dichloromethane were utilized in the extraction of the selected plants. Overall, water gave the highest percentage yield of the plant extracts. Dichloromethane gave the lowest percentage yield of the three solvents. In the case of the selected plants, from plants of the *Leguminosae* family produced the highest yield *Senna didmobotrya* (12.6%) and *Senna occidentalis* (11.6%) in water. Additionally, *Senna didmobotrya* gave the highest percentage yield in methanol solvent of 6.3% followed by *Lantana trifolia L* 6.2%. *Trichilia emetic* and *Spathodea campanulata* produced the highest percentage yield when dichloromethane was the solvent of 2.7% and 2.4% respectively. The summary of the overall percentage yields from the three solvent is shown in Figure 4.1.

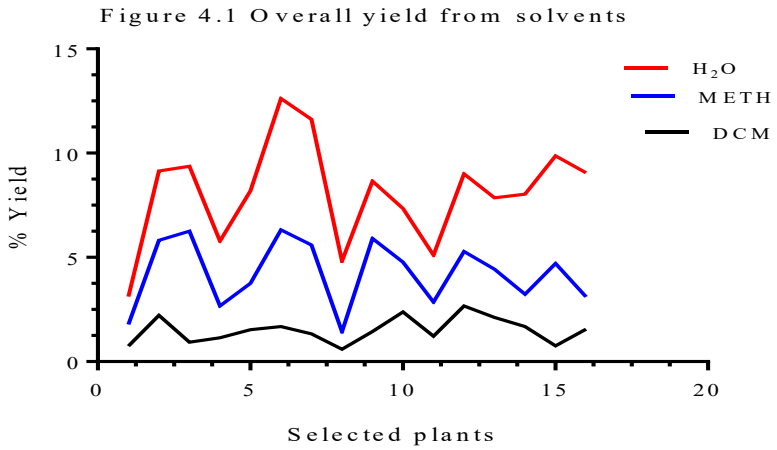

Figure 4.1. Showing percentage (%) yields of the solvents. Water, H_2O; Methane, METH; Dichloromethane, DCM.

A total of 16 plants were selected for *in vitro* antiplasmodial and cytotoxic analyses. The summary of botanical and local identification as well as the percentage yields per solvent is summarized in Table 4.1. Majority of the plants utilized in Kakamega County for malaria treatment are from the family *Lamiaceae*. These included *Ajuga integrifolia*, *Fuerstia africana*, *Ocimum kilimandscharicum* and *Rotheca myricoides*. Plants in this family gave fairly high percentage yields across all the solvents with water being the better solvent in terms of yield. Conversely, plants in the families *Compositae* and *Canellaceae Acmella caulirhiza* and *Warbugia ugandensis* respectively gave the least percentage yields across the three solvents.

Table 4.1: Showing a summary of botanical and local identification as well as percentage yields per solvent.

Table 4. 1. A summary of botanical and local identification as well as percentage yields per solvent.

Voucher No	Family	Plant name	Local name	Yield (%)		
				H_2O	METH	DCM
KKA001	Compositae	*Acmella caulirhiza*	Shituti	3.12	1.78	0.74
KKA002	Apocynaceae	*Carissa edulis*	Shikata	9.14	5.82	2.22
KKA003	Verbenaceae	*Lantana trifolia*	Shimenenwa	9.37	6.25	0.93
KKA004	Solanaceae	*Solanum incanum*	Indalandalu	5.78	2.67	1.15
KKA005	Rutaceae	*Zanthoxylum gilletii*	Shikuma	8.19	3.76	1.53
KKA006	Leguminosae	*Senna didmobotrya*	Lubinu	12.62	6.32	1.68
KKA007	Leguminosae	*Senna occidentalis*	Imbindi	11.62	5.6	1.33
KKA008	Canellaceae	*Warbugia ugandensis*	Apachi	4.82	1.43	0.59
KKA009	Lamiaceae	*Ajuga integrifolia*	Imbuli yu mtakha	8.66	5.91	1.44
KKA010	Bignoniaceae	*Spathodea campanulata*	Mutsulio	7.35	4.77	2.38
KKA011	Rutaceae	*Clausena anisata*	Shihunya bukundu	5.11	2.86	1.22
KKA012	Meliaceae	*Trichilia emetica*	Munyama	9.01	5.28	2.67
KKA013	Lamiaceae	*Rotheca myricoides*	Shisilangokho	7.86	4.43	2.12
KKA014	Lamiaceae	*Fuerstia africana*	Muvesemu	8.03	3.23	1.68
KKA015	Lamiaceae	*Ocimum kilimandscharicum*	M'monyi	9.87	4.71	0.76
KKA016	Moraceae	*Ficus thonningii*	Mutoto	9.06	3.11	1.56

Data presented as percentages (%). Water, H_2O; Methane, METH; Dichloromethane, DCM.

4.2 *In vitro* antiplasmodial activities of selected plants

Results showed that the chosen plants had antiplasmodial activity in vitro. According to earlier research (Gathirwa et al., 2011; Waiganjo et al., 2020b), this study followed the classification of antiplasmodial activities as follows: high activity (IC50 of ≤10 µg/ml), moderate activity (IC50 of 11-49.9 µg/ml), low activity (IC50 of 50-100 µg/ml), and inactive (IC50 of ≯100 µg/ml). The antiplasmodial activities were evaluated in vitro using three different solvents: methanol, organic dichloromethane, and water. The plant extracts were evaluated using two different P. falciparum strains: 3D7, which is sensitive to chloroquine in its native form, and W2, which is mutant and resistant to chloroquine. The 3D7 P. falciparum strains were effectively inhibited by the aqueous plant extracts of Rotheca myricoides, Ficus thonningii, and Acmella caulirhiza, with an IC50 value of less than or equal to 10 µg/ml. On the other hand, the W2 P. falciparum strains were effectively countered by the aqueous plant extracts of Ficus thonningii, Trichilia emetic, and Fuerstia africana. Both Table 4.2 and Table 4.3 describe the antiplasmodial activity of chosen plants against 3D7 P. falciparum strains, and W2 strains, respectively. Most of the aqueous plant extracts showed moderate antiplasmodial activity (IC_{50} of 11-49.9 µg/ml) against both 3D7 and W2 *P. falciparum* strains. Ten of the sixteen selected plants exhibited moderate antiplasmodial activity against 3D7. These include *Lantana trifolia*, *Solanum incanum*, *Zanthoxylum gilletii*, *Senna didmobotrya*, *Ajuga integrifolia*, *Spathodea campanulata*, *Clausena anisata*, *Trichilia emetic*, *Fuerstia africana*, and *Ocimum kilimandscharicum*. Nine out of 16 showed moderate antiplasmodial activity against W2. They include: *Acmella caulirhiza*, *Carissa edulis*, *Lantana trifolia*, *Solanum incanum*, *Senna didmobotrya*, *Senna occidentalis*, *Clausena anisata*, *Rotheca myricoides*, and *Ocimum kilimandscharicum*.

Two plants exhibited low antiplasmodial *in vitro* activity against 3D7 strains. These plants are: *Carissa edulis* and *Warbugia ugandensis*. Low antiplasmodial activity against W2 was only exhibited by *Spathodea campanulata*. *Senna occidentalis* was the only plant among the selected that was inactive (IC_{50} of ≥100 µg/ml) against 3D7 strains. On the other hand, *Zanthoxylum gilletii*, *Warbugia ugandensis* and *Ajuga integrifolia* were inactive against W2 strains.

Organic extracts were done using methanol and dichloromethane. Methanol extracts of 6 out of the 16 selected plants *Acmella caulirhiza*, *Lantana trifolia*, *Ajuga integrifolia*, *Spathodea campanulata*, *Clausena anisata* and *Fuerstia Africana* produced high antiplasmodial activity against 3D7 strains. *Lantana trifolia* methanol was the only based plant extracts that exhibited high antiplasmodial activity against the mutant strain W2. Similarly, 6 of the 16 selected methanol extracted plants showed moderate antiplasmodial activity against 3D7. These plants include: *Solanum incanum*, *Zanthoxylum gilletii*, *Rotheca myricoides*, *Senna didmobotrya Ocimum kilimandscharicum*, and *Ficus thonningii*. Additionally, majority of the methanol extracted plants 11 out of 16 produced moderate antiplasmodial activity against W2. These plants are: *Acmella caulirhiza*, *Carissa edulis*, *Solanum incanum*, *Senna didmobotrya*, *Senna occidentalis*, *Clausena anisata*, *Trichilia emetic*, *Rotheca myricoides*, *Fuerstia africana*, *Ocimum kilimandscharicum* and *Ficus thonningii*.

Low antiplasmodial activity against 3D7 was exhibited by the following three methanol extracted plants; *Carissa edulis*, *Senna didmobotrya* and *Warbugia ugandensis*. Also, the same was shown in *Ajuga integrifolia* and *Spathodea campanulata* against W2 *P.*

falciparum strains. Absence of antiplasmodial activity was exhibited by *Senna occidentalis* methanol extracted against 3D7, while methanol extracted *Zanthoxylum gilletii* and *Warbugia ugandensis* showed no antiplasmodial activity against W2 strains.

The other organic solvent dichloromethane was used as a solvent. Antiplasmodial activities of dichloromethane-based plant extracts were determined against both 3D7 and W2. Of the 16 plants, 6 exhibited high antiplasmodial activities against 3D7 strains. These plants are: *Solanum incanum*, *Spathodea campanulata*, *Clausena anisata*, *Ocimum kilimandscharicum*, *Ficus thonningii* and *Lantana trifolia*. Four of the sixteen plants including *Carissa edulis*, *Senna occidentalis*, *Rotheca myricoides* and *Ficus thonningii* showed high antiplasmodial activity against W2. Moderate antiplasmodial activity was shown by *Carissa edulis*, *Senna didmobotrya*, *Ajuga integrifolia*, *Trichilia emetic* and *Zanthoxylum gilletii* against 3D7. Moreover, a majority *Lantana trifolia*, *Solanum incanum*, *Senna didmobotrya*, *Ajuga integrifolia*, *Spathodea campanulata*, *Trichilia emetic*, *Rotheca myricoides*, *Fuerstia africana* and *Ocimum kilimandscharicum* produced moderate activity against W2.

Dichloromethane plant extracts that exhibited low antiplasmodial activities against 3D7 included *Acmella caulirhiza*, *Senna occidentalis*, *Clausena anisata* and *Fuerstia africana*. Low antiplasmodial activity against W2 was shown by *Acmella caulirhiza* and *Warbugia ugandensis*. Lack of antiplasmodial activity was shown by *Warbugia ugandensis* when tested against 3D7. Similarly, no antiplasmodial activity was detected when Zanthoxylum gilletii extracted in dichloromethane was tested against W2. Figures 4.2 and 4.3 are showing the actual inhibitory concentrations of the selected plants.

Table 4.2 Showing antiplasmodial activities of based on different solvents against 3D7

Antiplasmodial activity ~ 3D7	**Solvent/Plant**		
	H_2O	**METH**	**DCM**
High activity (IC_{50} of ≤10 µg/ml)	*Acmella caulirhiza, Rotheca myricoides Ficus thonningii*	*Acmella caulirhiza, Lantana trifolia, Ajuga integrifolia, Spathodea campanulata, Clausena anisata, Fuerstia Africana*	*Solanum incanum, Spathodea campanulata, Clausena anisata, Ocimum kilimandscharicum, Ficus thonningii, Lantana trifolia*
Moderate activity (IC_{50} of 11-49.9 µg/ml)	*Lantana trifolia, Solanum incanum, Zanthoxylum gilletii, Senna didmobotrya, Ajuga integrifolia, Spathodea campanulata, Clausena anisata, Trichilia emetic, Fuerstia africana, Ocimum*	*Solanum incanum, Zanthoxylum gilletii, Rotheca myricoides, Senna didmobotrya Ocimum kilimandscharicum, Ficus thonningii*	*Carissa edulis, Senna didmobotrya, Ajuga integrifolia, Trichilia emetic, Zanthoxylum gilletii*

	kilimandscharicum		
Low activity (IC_{50} of 50-100 µg/ml)	*Carissa edulis Warbugia ugandensis*	*Carissa edulis, Senna didmobotrya, Warbugia ugandensis*	*Acmella caulirhiza, Senna occidentalis, Clausena anisata, Fuerstia Africana*
Inactive (IC_{50} of ≥100 µg/ml)	*Senna occidentalis*	*Senna occidentalis*	*Warbugia ugandensis*

IC_{50}, Inhibitory concentration; Water, H_2O; Methane, METH; Dichloromethane, DCM

Table 4.3 Showing antiplasmodial activities of based on different solvents against W2

Antiplasmodial activity ~ W2	**Solvent/Plant**		
	H_2O	**METH**	**DCM**
High activity (IC_{50} of ≤10 µg/ml)	*Trichilia emetic, Fuerstia Africana, Ficus thonningii*	*Lantana trifolia*	*Carissa edulis, Senna occidentalis, Rotheca myricoides, Ficus thonningii*
Moderate activity (IC_{50} of 11-49.9 µg/ml)	*Acmella caulirhiza, Carissa edulis, Lantana trifolia, Solanum incanum, Senna didmobotrya, Senna occidentalis, Clausena anisata, Rotheca myricoides, Ocimum kilimandscharicum*	*Acmella caulirhiza, Carissa edulis, Solanum incanum, Senna didmobotrya, Senna occidentalis, Clausena anisata, Trichilia emetic, Rotheca myricoides, Fuerstia africana, Ocimum kilimandscharicum, Ficus thonningii.*	*Lantana trifolia, Solanum incanum, Senna didmobotrya, Ajuga integrifolia, Spathodea campanulata, Trichilia emetic, Rotheca myricoides, Fuerstia Africana, Ocimum kilimandscharicum*

Low activity (IC_{50} of 50-100 µg/ml)	*Spathodea campanulata*	*Ajuga integrifolia, Spathodea campanulata*	*Acmella caulirhiza, Warbugia ugandensis*
Inactive (IC_{50} of ≥100 µg/ml)	*Zanthoxylum gilletii, Warbugia ugandensis Ajuga integrifolia*	*Zanthoxylum gilletii Warbugia ugandensis*	*Zanthoxylum gilletii*

IC_{50}, Inhibitory concentration; Water, H_2O; Methane, METH; Dichloromethane, DCM

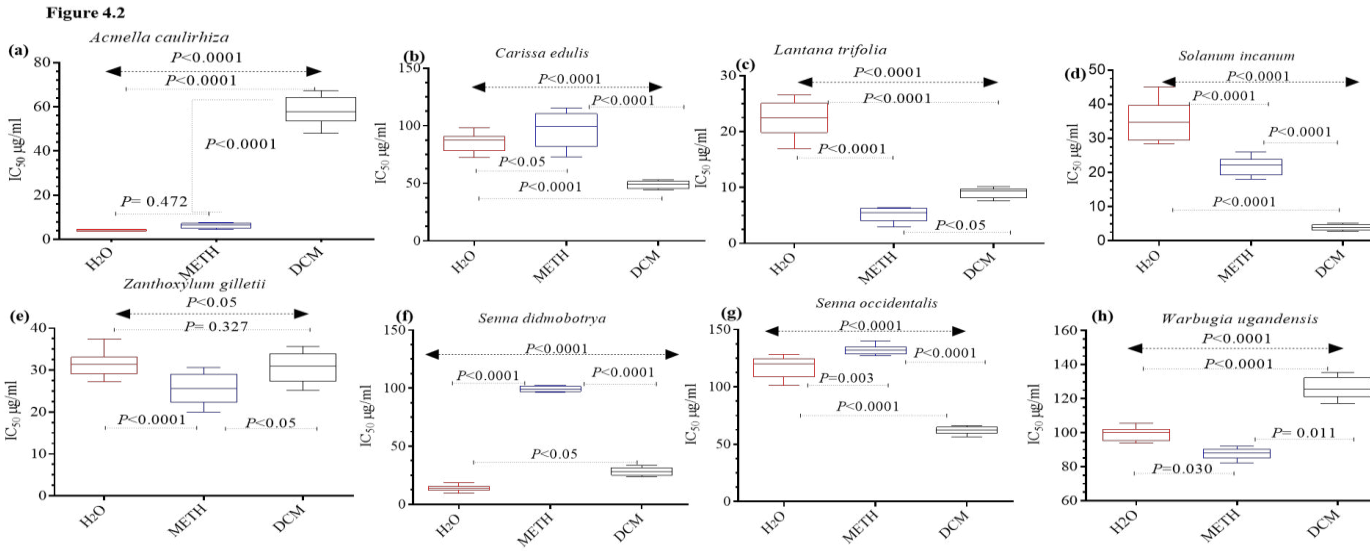

Figure 4.2 showing inhibitory concentrations (IC_{50}) of selected plant extracts by different solvents against 3D7. Water (H_2O), Methanol (METH) and Dichloromethane (DCM). Data compared across the groups (solvents) by ANOVA. *Post-hoc* analyses were done using Barnett's test. (a) *Acmella caulirhiza*; (b) *Carissa edulis*; (c) *Lantana trifolia*; (d) *Solanum incanum*; (e) *Zanthoxylum gilletii*; (f) *Senna didmobotrya*; (g) *Senna occidentalis*; (h) *Warbugia ugandensis*.

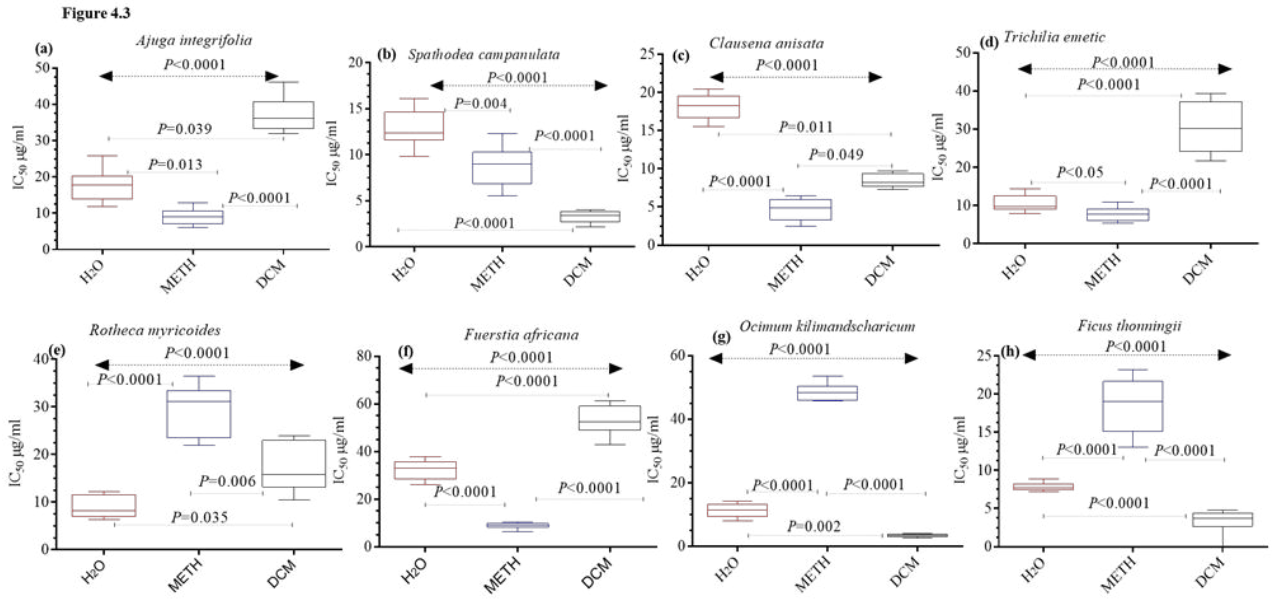

Figure 4.3 showing inhibitory concentrations (IC_{50}) of selected plant extracts by different solvents against 3D7. Water (H_2O), Methanol (METH) and Dichloromethane (DCM). Data compared across the groups (solvents) by ANOVA. *Post-hoc* analyses were done using Barnett's test. (a) *Ajuga integrifolia*; (b) *Spathodea campanulata*; (c) *Clausena anisata*; (d) *Trichilia emetic*; (e) *Rotheca myricoides*; (f) *Fuerstia Africana*; (g) *Ocimum kilimandscharicum*; (h) *Ficus thonningii*.

4.3 *In vitro* cytotoxic activities of selected plants

Previous studies have described biological efficacies of plant extracts as not being as a result of *in vitro* cytotoxicity if the selectivity index is ≥ 10 (Muthaura *et al*., 2015; Waiganjo *et al*., 2020b). Therefore, this study set low selectivity index at < 10 while high selectivity index was considered at ≥ 10. The selectivity indices were obtained by dividing IC_{50} of Vero cell lines with those of 3D7 and W2 *P. falciparum* strains. The analysis was done per

solvent. Of the 16 selected aqueous plant extracts, 7 had a high selectivity index ≥ 10 when tested against 3D7. The plants included: *Acmella caulirhiza*, *Carissa edulis*, *Lantana trifolia*, *Solanum incanum*, *Senna occidentalis*, *Warbugia ugandensis* and *Spathodea campanulata*. The rest of the plants had a low selectivity index against 3D7 that is; *Zanthoxylum gilletii*, *Senna didmobotrya*, *Ajuga integrifolia*, *Clausena anisata*, *Trichilia emetic*, *Rotheca myricoides*, *Fuerstia africana*, *Ocimum kilimandscharicum* and *Ficus thonningii*. Aqueous selectivity indices against W2 *P. falciparum* strains were high for *Lantana trifolia, Solanum incanum, Warbugia ugandensis, Spathodea campanulata,* and *Rotheca myricoides*. The indices were low for *Acmella caulirhiza, Carissa edulis, Zanthoxylum gilletii, Senna didmobotrya, Senna occidentalis, Ajuga integrifolia, Clausena anisata, Trichilia emetic, Fuerstia africana, Ocimum kilimandscharicum* and *Ficus thonningii*.

Organic extracts also generated diverse selectivity indices. For methanol extracts 3 out of 16 plants had high selectivity against 3D7 namely; *Carissa edulis, Warbugia ugandensis* and *Solanum incanum*. The remaining 13 plants had low selectivity indices against 3D7. These are; *Acmella caulirhiza, Lantana trifolia, Zanthoxylum gilletii, Senna didmobotrya, Senna occidentalis, Ajuga integrifolia, Spathodea campanulata, Clausena anisata, Trichilia emetic, Rotheca myricoides, Fuerstia africana, Ocimum kilimandscharicum* and *Ficus thonningii.* In the case of W2 strains, only 1 plant had a high index that is *Warbugia ugandensis* after methanol extraction. The rest, 15 selected plants had low indices against W2 namely; *Acmella caulirhiza, Carissa edulis, Lantana trifolia, Solanum incanum, Zanthoxylum gilletii, Senna didmobotrya, Senna occidentalis, Ajuga integrifolia, Spathodea*

campanulata, Clausena anisata, Trichilia emetic, Rotheca myricoides, Fuerstia africana, Ocimum kilimandscharicum and *Ficus thonningii*. Selectivity indices for dichloromethane extracts for both 3D7 and W2 *P. falciparum* strains were high in *Warbugia ugandensis* only. The rest of the plants namely *Acmella caulirhiza, Carissa edulis, Lantana trifolia, Solanum incanum, Zanthoxylum gilletii, Senna didmobotrya, Senna occidentalis, Ajuga integrifolia, Spathodea campanulata, Clausena anisata, Trichilia emetic, Rotheca myricoides, Fuerstia africana, Ocimum kilimandscharicum* and *Ficus thonningii* had low selectivity indices.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Medicinal plant use in Kakamega County

The utilization of traditional therapy methods has proven to be beneficial in the management of several disorders and in the identification of pharmacologically active agents. Medicinal herbs, possessing a diverse range of therapeutic characteristics, have demonstrated considerable potential in the treatment of malaria. For example, the compounds artemisinin and quinine are derived from botanical sources, specifically *Artemisia annua* and cinchona, respectively. The resistance of malaria to conventional chemotherapeutic, such as chloroquine, has arisen due to its improper utilisation. Therefore, Artemisinin-based combination therapy (ACT) has been universally implemented as the exclusive form of treatment. The utilisation of a combination therapy may be attributed to the presence of diverse pharmacologically active substances. Therefore, it has been demonstrated that ethnomedicinal plants, which contain a variety of phytochemical compounds, can be utilised in the production of chemotherapeutic agents that effectively combat the plasmodium parasite. Hence, the present investigation assessed the efficacy of 16 frequently employed botanical species in Kakamega County against plasmodium strains resistant to chloroquine, with the aim of offering a cost-effective and alternative therapeutic approach. The plant species present in the given list are *Acmella caulirhiza, Carissa edulis, Lantana trifolia, Solanum incanum, Zanthoxylum gilletii, Senna didmobotrya, Senna occidentalis, Warbugia*

ugandensis, Ajuga integrifolia, Spathodea campanulata, Clausena anisata, Trichilia emetic, Rotheca myricoides, *Fuerstia africana*, and *Ocimum kilimandscharicum*. Among these plants, the majority (40%) are classified under the family Lamiaceae. The present study aligns with a thorough investigation undertaken using multidisciplinary databases, which documented a total of 286 plant species belonging to 75 families. Among these families, the Lamiaceae family accounted for around 22% of the species exhibiting antiplasmodial activity (Omara, 2020). The family is widely acknowledged for possessing anti-malarial properties on a global scale. Nevertheless, this work represents the initial investigation into the possible utilisation of this treatment against plasmodium strains that have developed resistance to chloroquine. The plant extracts were only derived from leaves, in contrast to previous research that primarily focused on diverse plant parts such as roots bark, stem barks, whole plant, roots, flowers, and other components. Prior research has indicated a major utilisation of leaves for the purpose of extraction, as evidenced by studies conducted by Gontijo *et al*. (2020) and Tajuddeen *et al*. (2021). The potential cause of this phenomenon may be attributed to the provision of little nutrients by leaves, which contribute to the plant's survival. Consequently, this promotes the regular and secure utilisation of plant materials for the purpose of herbal preparation, in contrast to alternative plant components such as roots and barks. Furthermore, it has been observed that leaves have a greater propensity for regeneration in comparison to roots (Alebie *et al*., 2017). Similarly, leaves play a crucial role in the synthesis of the bulk of plant secondary metabolites. This characteristic renders them a plentiful reservoir of dynamic chemical compounds, which may be readily isolated (Katemo *et al*., 2012). In contrast, it is worth noting that plant root

structures have the potential to serve as abundant reservoirs of highly potent bioactive chemical compounds, in contrast to leaves, particularly in some plant species such as rhizomes. Nevertheless, the regular utilisation of plant roots for the purpose of creating herbal remedies may pose a potential threat to the long-term viability of plant species (Abdel-Azim, 2020).

5.2 Yield and *in vitro* antiplasmodial activities of selected plants

Aqueous Acmella caulirhiza leaf extract outperformed methanol and dichloromethane solvents in terms of percentage yield, according to the comparative study. The 3D7 strain of parasites was effectively inhibited by both the water and ethanol extracts. Still, the antiplasmodial activity of the aforementioned extract against the W2 strain was moderate. Results showed that the dichloromethane extract had weak antiplasmodial action against 3D7 and W2 strains. These findings provide more evidence that Acmella caulirhiza leaf aqueous extracts may be useful in the fight against plasmodia strains 3D7 and W2. In addition, the solvent characteristics of these extracts are excellent for extraction. This confirms the findings of an earlier research that looked at the Kuria and Luo people's medicinal plant usage in Luo Nyanza, Kenya. This study found that the Acmella caulirhiza DCM extract was highly effective against the W2 and D6 strains of parasitoides. Note that DCM extracts were the only ones used in the study (Owuor et al., 2012). Among the three extracts tested, the water-based Carissa edulis solution yielded the most, followed by the methanol and DCM solutions. This study's results are in agreement with those of Waiganjo et al. (2020), who found that methanol and dichloromethane (DCM) had the next best extraction yields, followed by the plant's aqueous leaf extract. While the

aqueous and methanol extracts of Carissa edulis showed low antiplasmodial properties against 3D6, the DCM leaf extract showed modest effectiveness. The aqueous and methanol extracts of DCM showed only moderate antiplasmodial activity against W2, but the leaf extract showed a considerable amount of activity. However, contrary to what was previously shown, the aqueous extract showed strong antiplasmodial effects, according to research by Waiganjo et al. (2020). The antiplasmodial activity of the methanol and DCM extracts were somewhat low, on the other hand. Research among Kenya's Maasai people has shown that Carissa edulis has antiplasmodial characteristics, lending credence to the plant's medicinal importance (Koch et al., 2005).

When compared to methanol and DCM, the extract yield from Lantana trifolia in water is significantly higher. The 3D7 and W2 strains of parasitoides were both significantly inhibited by the methanol extract of Lantana trifolia leaves. In terms of antiplasmodial activity, the DCM extracts were quite effective against the 3D7 strain and only moderately effective against the W2 strain. The 3D7 strain was not effectively targeted by the plant's aqueous extracts, however they showed significant antiplasmodial action against the W2 strain. The results show that polar solvents are less successful than non-polar ones when it comes to extracting active phytochemical components. Consistent with earlier studies, this one found that the ethanolic extract had little antiplasmodial action, whereas the petroleum ether and chloroform extracts showed moderate activity (Stangeland et al., 2011). Aqueous and methanol extracts of Solanum incanum leaves exhibited moderate antimalarial efficacy against the 3D7 strain of Plasmodium falciparum. On the other hand, the dichloromethane (DCM) extract showed strong antiplasmodial effects. The antiplasmodial

effectiveness of the aqueous, methanol, and DCM extracts of Solanum incanum leaves was moderate, but. The most favorable option for achieving the needed extract was DCM, even though it was not the ideal solvent for extraction. Anwar (2018) investigated the antiplasmodial activity of a DCM extract from Solanum incanum leaves, and his findings are in agreement with ours. A moderate antiplasmodial activity against P. falciparum was observed in the plant, according to the study (IC50 > 64). It should be mentioned that the study assessed both the water and methanol plant extracts.

The antiplasmodial activity of the aqueous, methanol, and DCM extracts of Zanthoxylum gilletii was moderate against the 3D7 strain, while it was not significant against the W2 strain. Omosa and Okemwa (2017) found that an aqueous extract of Zanthoxylum gilletii was very effective against protozoa. However, the current investigation's results disprove this claim.

After the methanol and DCM extracts, the aqueous leaf extract of Senna didmobotrya produced the highest yield of extract. All of the extracts showed some antimalarial action against the 3D7 and W2 strains, according to the data. This study's findings go counter to those of an earlier one out of Embu, Kenya, that found only weak antiplasmodial efficacy in aqueous and methanol leaf extracts of Senna didmobotrya against the 3D7 and W2 malaria parasite strains. But there was a lot of antiplasmodial action in the dichloromethane extract (Waiganjo et al., 2020). It is possible that the different geographical locations of the plants are to blame for the observed difference in findings. This suggests that a plant's phytochemical composition, even within the same species, can be affected by its geographical location.

After the methanol and DCM extracts, the aqueous leaf extract of Senna occidentalis had the highest yield. Results from leaf extractions using water, methanol, and hexane as solvents varied, as demonstrated in a study by Daskum et al. (2019). With a yield of 31.32%, hexane was the most productive, followed by methanol with 12.29%, and water with 8.9%. Beyond the percentage yield, the antiplasmodial effect is most strongly demonstrated by the hexane extract (3.47 μg/mL), then by the methanol extract (3.79 μg/mL), and finally by the aqueous extract (4.03 μg/mL). Research by Daskum et al. (2019) indicates that

The antiplasmodial efficacy of the aqueous and methanol extracts of Warbugia ugandensis was found to be low and negligible against the 3D7 and W2 strains, respectively. The antiplasmodial activity of the DCM leaf extract was found to be minimal against the 3D7 strain, whereas its antiplasmodial activity against the W2 strain was very moderate. The current findings are consistent with prior studies that have demonstrated the modest levels of antiplasmodial activity exhibited by the leaf extract of Warbugia ugandensis against P. falciparum (Lacroix et al., 2011). In an independent investigation carried out by Were et al. (2010), it was noted that the chloroform leaf extract derived from Warbugia ugandensis shown noteworthy antiplasmodial properties against P. knowlesi and P. falciparum. The IC50 values for these antiplasmodial agents were determined to be 3.14 μg/ml and 6.04 μg/ml, respectively.

Several extracts obtained from the leaves of Ajuga integrifolia have demonstrated different levels of antiplasmodial action. The antiplasmodial activity of the methanol extracts against

the 3D7 strain has been found to be substantial, whilst the aqueous and DCM leaf extracts have shown a moderate level of antiplasmodial activity against the same strain. However, the extracts derived from aqueous, methanol, and DCM solvents demonstrated ineffective, minimal, and significant antiplasmodial effects against W2, respectively. The current investigation supports prior scholarly conclusions, as demonstrated by the modest antiplasmodial properties (IC50 = 29.04 μg/ml) detected in the methanol leaf extracts of Ajuga integrifolia (Daskum et al., 2019). The findings of our investigation suggest that the extraction yields of Spathodea campanulata leaf extracts, namely aqueous, methanol, and DCM, displayed variability, with the aqueous extract demonstrating the highest quantity. In addition, the extracts demonstrated different degrees of effectiveness in combating plasmodial infections caused by the 3D7 and W2 strains. The antiplasmodial activity of the aqueous extracts against the 3D7 strain was found to be modest. In contrast, both the Methanol and DCM extracts shown a high level of antiplasmodial activity against the same strain. Although the aqueous and methanol leaf extracts of Spathodea campanulata shown modest effectiveness against the W2 strain in terms of antiplasmodial activity, the dichloromethane (DCM) extract exhibited a significant degree of activity against the W2 strain. The results suggest that the DCM extract possesses enhanced characteristics as an extract and exhibits effectiveness against both the 3D7 and W2 strains in terms of antiplasmodial activity. The current finding is consistent with previous studies that have shown the effectiveness of Spathodea campanulata in combating plasmodium infections. Makinde et al. (1988) conducted a study which demonstrated that the hexane and chloroform extracts obtained from the stem bark displayed significant inhibitory effects on

parasitaemia. The observed impact was notably significant when the administration occurred within the dosage range of 100 to 400 mg/kg/day. In a similar vein, the ethanol fraction has exhibited noteworthy antiplasmodial efficacy, as evidenced by an IC50 value of 18.7±2.23µg/ml, against both chloroquine-sensitive and chloroquine-resistant Plasmodium falciparum isolates (Dhanabalan, 2008).

The findings of the study suggest that the leaf extracts of Clausena anisata, specifically those obtained in aqueous, methanol, and DCM forms, demonstrated antiplasmodial properties against both the 3D7 and W2 strains. Although the percentage yield was rather low, it is noteworthy that DCM had the highest antiplasmodial activity, suggesting that the extraction of phytochemical active components is more efficient when employing DCM. The findings align with prior studies that have demonstrated the limited antimalarial effects of the unrefined leaf extract of Clausena anisata in an in vitro study (Okokon et al., 2012). The Trichilia emetic leaves' aqueous, methanol, and DCM extracts have shown a notable antiplasmodial effectiveness against both 3D7 and W2 strains, on average. The results of this inquiry are consistent with a prior investigation carried out in Côte d'Ivoire by Obbo et al. (2019), which shown that the root bark extract of Trichilia emetic exhibits significant efficacy against Plasmodium falciparum, as evidenced by an IC50 value of 8.36 g/ml. Additionally, it has been observed that the leaf extracts derived from Trichilia emetic aldehyde demonstrate inhibitory properties against the growth of Plasmodium falciparum (with an IC50 value of 76 µM) and slow-proliferating breast cancer cells MCF7 (with an IC50 value of 78 µM). Furthermore, Traore et al. (2007) have reported that it exhibits significant inhibitory effects on the proliferation of S180 cancer cells, as evidenced by its

IC50 value of 7.4 μM. The antiplasmodial efficacy of Rotheca myricoides leaf extracts, including aqueous, methanol, and DCM extracts, has been seen against both 3D7 and W2 strains. The current investigation is consistent with a prior study carried out in Kakamega, East sub-county, which highlights Rotheca myricoides as a commonly utilized medicinal plant for the treatment of several illnesses, including malaria (Mukungu et al., 2016). The antiplasmodial efficacy of the aqueous, methanol, and DCM leaf extracts of Fuerstia africana was shown against the 3D7 and W2 strains. The findings align with a prior investigation carried out by Kigondu et al. (2011), which shown that both petroleum ether and methanol displayed noteworthy antiplasmodial properties against D6 and W2 strains, as indicated by IC50 values of 1.56 ± 0.00 μg/ml and 2.5 ± 0.4 μg/ml, respectively. The percentage yield of Ocimum kilimandscharicum leaf extracts, including aqueous, methanol, and DCM extracts, exhibited a greater magnitude in comparison to a previous investigation that assessed the yield of extracts derived from the leaves and twigs of the identical plant species, which yielded 3.8%. All of the plant extracts demonstrated a moderate amount of antiplasmodial action. This observation is consistent with a prior investigation carried out in Kenya, which specifically examined a range of therapeutic herbs frequently employed by the Luo and Kuria populations. The research conducted by Owuor et al. (2012) shown that the unrefined extracts derived from the leaves and twigs of Ocimum kilimandscharicum had noteworthy antiplasmodial properties. The IC50 values for the 3D7 strain and the W2 strain were determined to be 1.5477±0.226 μg/ml and 0.8437±0.123 μg/ml, respectively.

The aqueous leaf extracts of Ficus thonningii exhibited substantial antiplasmodial activity

against both the 3D7 and W2 strains of P. falciparum, as evidenced by an IC50 value of ≤10 µg/ml. However, it is important to acknowledge that both methanol and DCM extracts exhibited a moderate degree of antiplasmodial impact on both 3D7 and W2 strains of P. falciparum, as indicated by an IC50 range of 11-49.9 µg/ml. Falade et al. (2014) conducted a study which revealed that methanol and hexane shown notable antiplasmodial efficacy against both the K1 (multi-resistant) and NF54 (sensitive) strains of P. falciparum. The K1 and NF54 strains exhibited IC50 values of 5.3 ± 2.3 µg/mL and 21.1 ± 2.3 µg/mL, respectively, for methanol. The K1 and NF54 strains exhibited IC50 values of 2.7 ± 1.6 µg/mL and 10.4 ± 1.6 µg/mL, respectively, for hexane.

5.3 *In vitro* cytotoxic activities of selected plants

Prior research has examined the biological effectiveness of plant extracts and their selectivity indices. According to Muthaura *et al*. (2015) and Waiganjo *et al*. (2020), the effectiveness of plant extracts in biological contexts cannot be attributed exclusively to their in vitro cytotoxicity. Instead, it is also influenced by the selectivity index, as discussed by Ghosh *et al*. (2020) and Adamu *et al*. (2013). In the conducted investigations, a selectivity index equal to or greater than 10 was classified as high, whilst a selectivity index less than 10 was classified as low.

The present investigation involved the determination of selectivity indices for 16 specifically chosen aqueous plant extracts against the 3D7 and W2 strains of *P. falciparum*. Out of the several extracts examined, it was observed that 7 of them exhibited a significant selectivity index (≥ 10) against the 3D7 strain. The plant species seen in this study encompassed *Acmella caulirhiza, Carissa edulis, Lantana trifolia, Solanum incanum, Senna occidentalis, Warbugia ugandensis*, and *Spathodea campanulata*. The selectivity index against the 3D7 strain was found to be poor for the remaining plants. Comparable findings were noted with the selectivity indices against the W2 strain, wherein *Lantana trifolia, Solanum incanum, Warbugia ugandensis, Spathodea campanulata*, and *Rotheca myricoides* exhibited elevated selectivity indices, whereas the remaining plants had diminished selectivity indices.

The investigation also examined the selectivity indices of organic extracts, namely methanol and dichloromethane extracts. The methanol extracts of Carissa edulis, *Warbugia ugandensis*, and *Solanum incanum* exhibited significant selectivity indices against the 3D7 strain, whereas the other plant extracts demonstrated comparatively lower selectivity indices. Among the tested plant species, it was observed that only *Warbugia ugandensis* exhibited a notable selectivity index against the W2 strain following the process of methanol extraction. Conversely, the remaining plant species demonstrated comparatively poor selectivity indices. Among the dichloromethane extracts examined, it was observed that only *Warbugia ugandensis* exhibited a notable selectivity index against both the 3D7 and W2 strains. Conversely, the remaining plant extracts demonstrated relatively low selectivity indices.

Upon comparing these data with prior research, it becomes apparent that the selectivity indices of the plant extracts exhibit variability contingent upon the solvent employed for extraction and the particular strains of *P. falciparum* that were subjected to testing. The present investigation has identified a number of plant species exhibiting notable selectivity indices, including *Carissa edulis, Lantana trifolia, Solanum incanum,* and *Warbugia ugandensis*. These plants have also been previously documented to possess significant biological efficacies in studies conducted by Ghosh *et al*. (2020), Adamu *et al*. (2013), and Jiménez-Medina *et al*. (2006). Nevertheless, it is crucial to acknowledge that the present investigation did not identify the precise chemicals accountable for the reported biological activities and selectivity indices. Additional investigation is required to separate and delineate these substances in order to ascertain their potential medicinal uses.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1. Conclusions

i. The maximum yield was obtained from the aqueous leaves extract of the studied plants, followed by methanol and DCM. This indicates that aqueous solvents may be the preferred choice.

ii. The plant extracts exhibiting low IC_{50} values (≤10 μg/ml) following evaluation have the potential to serve as promising candidates for the discovery of novel antiplasmodial drugs, subject to additional research and development.

iii. The cytotoxic activities of the methanol and DCM leaf extracts from certain plants were found to be significantly high.

6.2. Recommendation for action

i. In addition to aqueous, methanol, and DCM solvents, solvents such as petroleum ether, hexane, and chloroform should be employed for extracting compounds from additional plant components, such as root and stem bark.

ii. The identification and comparison of the phytochemical active component of the selective plant should be conducted, taking into consideration the specific location from which they are extracted and the various solvents employed in the extraction process.

iii. Detailed investigations involving bioassay-guided fractionation and isolation of active compounds from these plants have the potential to facilitate the identification of molecules that could serve as valuable starting points for the discovery and development of antimalarial drugs.

6.3. Recommendation for further study

i. An experimental study design should incorporate the utilization of supplementary solvents such as chloroform, hexane, and petroleum ether to facilitate the extraction process of certain plant specimens.

ii. Conduct tests on various plant components, including flowers, fruits, seeds, root, and stem bark, in order to ascertain the extent of diversity in the antiplasmodial activity exhibited by the chosen medicinal plants against both the 3D7 and W2 strains of P. falciparum.

iii. An *in-vitro* study should be conducted to elucidate the mechanism by which the detected phytochemical component induces cytotoxic effects against both the 3D7 and W2 strains of Plasmodium falciparum.

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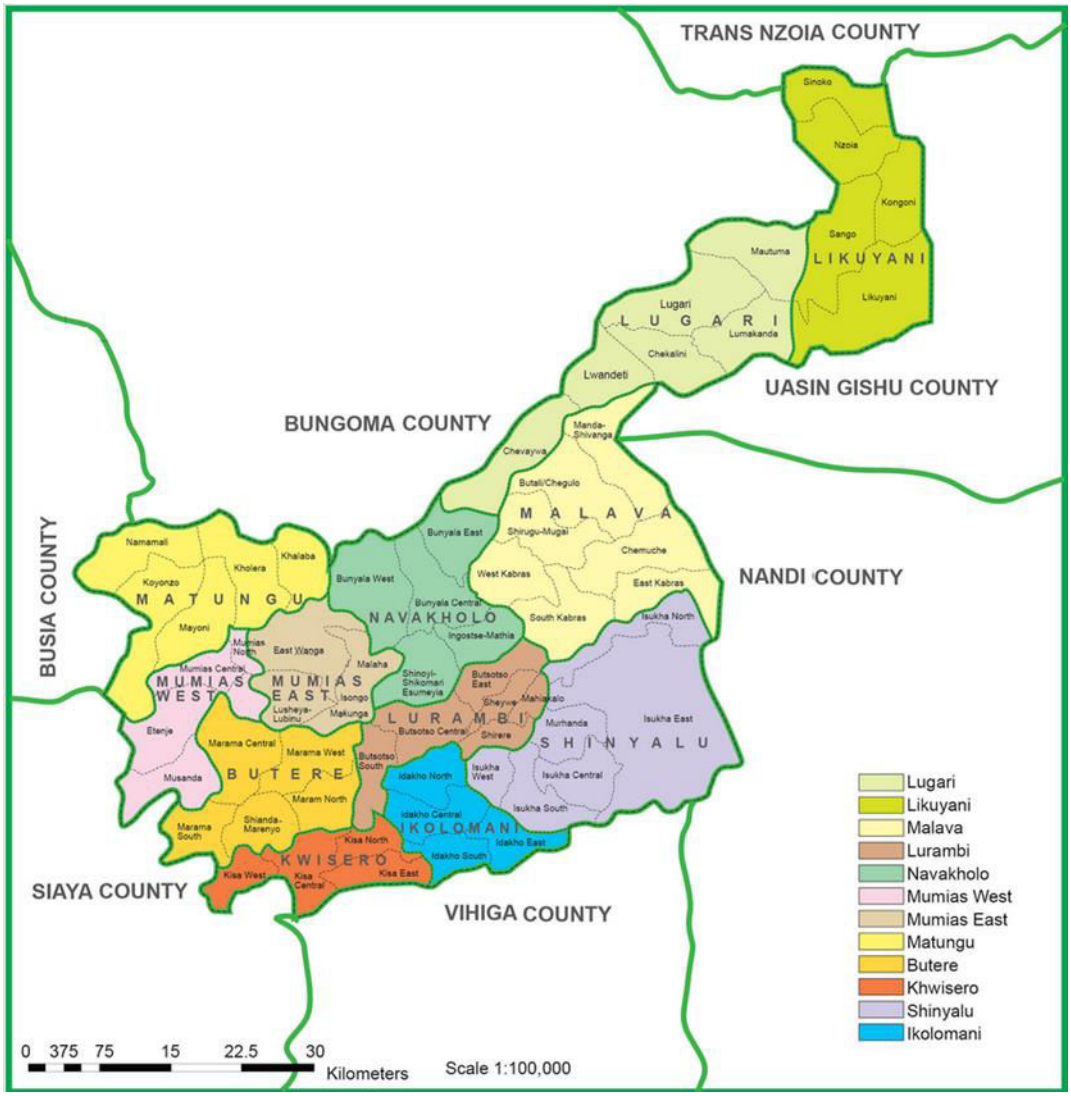
APPENDICES

Appendix I: Map of the Study Site



Appendix II: MMUST Ethical Clearance

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY
Tel: 056-31375
Fax: 056-30153
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Website: www.mmust.ac.ke
P. O. Box 190,
50100,
Kakamega,
KENYA

Institutional Ethics and Review Committee (IERC)

REF: MMU/COR:403012 Vol 5 (01)

Date: October 08th, 2021

To: Daniel Wekesa

Dear Sir/Madam,

RE: ANTIPLASMODIAL AND CYTOTOXIC ACTIVITIES OF SELECTED MEDICINAL PLANTS IN KAKAMEGA COUNTY, KENYA.

This is to inform you that *Masinde Muliro University of Science and Technology Institutional Ethics and Review Committee (MMUST-IERC)* has reviewed and approved your above research proposal. Your application approval number is **MMUST/IERC/015/2021.** The approval period is *08th October, 2021 - 08th October, 2022.*

This approval is subject to compliance with the following requirements:

i. Only approved documents including informed consents, study instruments, MTA will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by *MMUST-IERC.*
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to *MMUST-IERC* within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to *MMUST-IERC* within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to *MMUST-IERC.*

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours Sincerely,

Dr. Gordon Nguka (PhD)
Chairman, Institutional Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)

Appendix III: NACOSTI Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **697182**

Date of Issue: **22/November/2021**

RESEARCH LICENSE

This is to Certify that Mr.. Daniel Wamalwa Wekesa of Masinde Muliro University of Science and Technology, has been licensed to conduct research in Kakamega on the topic: Antiplasmodial and cytotoxic activities of selected medicinal plants in Kakamega County, Kenya for the period ending : 22/November/2022.

License No: **NACOSTI/P/21/14450**

697182

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