UNIVERSITY OF GHANA

COLLEGE OF BASIC AND APPLIED SCIENCES

SCHOOL OF BIOLOGICAL SCIENCES

IMMUNOMODULATORY, ANTIOXIDANT, AND PROPHYLACTIC EFFECTS OF THE COA PLUS MIXTURE IN *PLASMODIUM BERGHEI* INFECTED MICE

BY DOROTHY NAA LOMOLEY LOMO-MAINOO

(10938085)

THIS THESIS IS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES, UNIVERSITY OF GHANA – LEGON, IN

PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF

MPHIL IN APPLIED PARASITOLOGY DEGREE.

DEPARTMENT OF ANIMAL BIOLOGY AND CONSERVATION SCIENCE

SEPTEMBER, 2024

DEDICATION

I dedicate this thesis to my parents, Rev. and Rev. Mrs. Gifty Lomo-Mainoo, for their unwavering support and encouragement, and to my Pastor, Prophet Michael Divine for his prayers and spiritual guidance throughout this academic journey.

DECLARATION

I, Dorothy Lomo-Mainoo, hereby declare that this MPhil thesis titled '**Immunomodulatory, antioxidant and prophylactic effects of the COA Plus mixture in *Plasmodium berghei* infected mice'** is my original work carried out at the Noguchi Memorial Institute for Medical Research and the Department of Biochemistry, Cell and Molecular Biology, University of Ghana Legon under the supervision of Prof. Augustine Ocloo, Dr. Daniel Oduro and Dr. G. T Mensah. All sources of information and material used in this thesis has been duly acknowledged. Any assistance received from individuals or organizations in the preparation of this thesis has also been acknowledged in the acknowledgement section. I affirm that this thesis has not been submitted for any other degree or qualification, and all data and findings presented herein are genuine and have not been manipulated or falsified in any way.

DOROTHY LOMO-MAINOO (Student)

SIGNATURE. 

DATE June 2, 2025.

PROF. AUGUSTINE OCLOO (PRINCIPAL SUPERVISOR)

SIGNATURE……

DATE……

DR. DANIEL ODURO (CO-SUPERVISOR)

SIGNATURE 

DATE. June 2, 2025.

DR. GEORGE TETTEH MENSAH (CO-SUPERVISOR)

SIGNATURE 

DATE June 2, 2025.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Almighty God for granting me the strength throughout this research journey. My heartfelt appreciation goes to Prof. Augustine Ocloo for his unwavering commitment to academic excellence, which pushed me to be thorough and critical in my work. I am also immensely grateful to Dr. Daniel Oduro and Dr. G. T. Mensah for their regular guidance, insightful feedback, and support in navigating the challenges of my research. Their encouragement was vital in the completion of this thesis.

I would like to thank Prof. Mike Yaw Osei-Atweneboana, Director of the Water Research Institute, for giving me the opportunity to pursue my academic goals. Special appreciation goes to my seniors and colleagues in the Environmental Biology and Biotechnology and Health Division. Dr. Mark Akrong, Dr. Ayesha Adams, Borbor Selorm, and Getrude Nortey—for their constant support and motivation. My sincere thanks also extend to the staff at the Animal Experimentation Unit of the Noguchi Memorial Institute for Medical Research, particularly Mr. Bonni and Ms. Padikie, for their guidance and professionalism in the laboratory. I am also grateful to Mr. Jones and Mr. Andrew Obese of the Immunology Department for their expertise, which contributed significantly to the success of my experiments. Additionally, I would like to thank Mr. Donkor, technical officer in the Department of Biochemistry, Cell Biology, and Molecular Biology, for his assistance with sample preparation and data acquisition. I also acknowledge the West African Centre for Cell Biology of Infectious Pathogens (WACCBIP) and COA Manufacturing Limited Company for their financial support, which made this research possible.

TABLE OF CONTENTS

DEDICATION ..... **ii**

DECLARATION iii

ACKNOWLEDGEMENTS iii

LIST OF FIGURES vii

LIST OF TABLES viii

LIST OF ABBREVIATIONS x

ABSTRACT xii

CHAPTER ONE 1

1.0 INTRODUCTION 1

1.1 BACKGROUND 1

1.2 PROBLEM STATEMENT AND JUSTIFICATION 5

1.3 AIM AND SPECIFIC OBJECTIVES 6
1.3.1 Aim 6
1.3.2 Specific Objectives 6

CHAPTER TWO 7

2.0 LITERATURE REVIEW 7
2.1 Plant Medicine and Healthcare 7

2.1.1 Historical Background 7

2.1.2 Plant Medicine in Africa 8

2.1.3The Importance of Plant Medicine in Healthcare Delivery 10
2.2 The Immune System 13

2.2.1 Immunomodulation 15

2.2.2 Plants as Sources of Immunomodulators 16
2.3 Oxidative Stress 19

2.3.1 Antioxidants 22

2.3.2 Plants as Sources of Antioxidants 23
2.4 The Importance of Prophylaxis in healthcare 26

2.4.1 Plant Medicine as Prophylaxis *28*

2.4.2 Plant Medicine prophylaxis for malaria prevention *30*
2.5 The COA Plus Mixture and its Constituent Plants **31**

CHAPTER THREE **34**

3.0 MATERIALS AND METHODS **34**
3.1 Materials **34**

3.1.1 Reagents and Chemicals *34*

3.1.2 Experimental Animals *34*
3.2 Ethical Approval **35**
3.3 Methods **35**

3.3.1 Grouping and treatment regimen *35*

3.3.2 Selection and preparation of Plasmodium berghei infected blood. *36*

3.3.3 Collection of blood and tissue samples *37*

3.3.4 Determination of prophylactic properties. *37*

3.3.5 Determination of in vivo antioxidant activity *38*
Determination of Lipid Peroxidation **38**

3.3.6 Investigation of the immunomodulatory properties *38*

3.3.7 Determination of the Effect of COA Plus mixture on selected Haematological Parameters 41
3.4 Data analyses **42**

CHAPTER FOUR 4.0RESULTS **43**
4.1 Effect on Immunological Markers **43**
4.2 Effect on Parasitaemia **46**
4.3 Effect on Lipid Peroxidation **47**
Figure 8: Level of Malondialdehyde (MDA) in Liver **48**
4.4 Effect on Haematological Parameters **48**
4.5 Effect of COA on Mean Organ Weight **51**

CHAPTER FIVE **52**

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS **52**
5.1 Discussion **52**

5.1.1 The immunomodulatory effect of COA mixture *52*

5.1.2 The prophylactic properties of COA Plus mixture against P. berghei *54*

5.1.3 In vivo antioxidant properties of the COA Plus mixture *55*

5.1.4 Effect of the COA Plus mixture on haematological profile *57*

5.15. Effect of the COA Plus mixture on Organ weight *58*

5.2 Conclusion **58**

5.3 Strengths and Limitations **58**

5.4 Recommendations **59**

REFERENCES **60**

APPENDICES **90**

LIST OF FIGURES

Figure 1: Preparation of standard cocktail dilutions..... 54

Figure 2: Assay Plate 41
Figure 3: Levels of Interleukin-4 (IL-4) 44
Figure 4: Levels of Interleukin-10 (IL-10) in mice 45
Figure 5: Levels of Interferon-gamma (IFN-gamma) in mice 46
Figure 6: Time course analysis of parasitaemia 47
Figure 7: Parasitaemia at 72 hours after inoculation with *P. berghei* 48
Figure 8: Level of MDA in Liver 49

LIST OF TABLES

Table 1: Grouping of experimental animals 35

Table 2: Haematology Parameters..... 50
Table 3: Mean Organ Weights..... 51

LIST OF ABBREVIATIONS

BHT- Butylated hydroxytoluene

BSA-Bovine Serum Albumin Standards

CNS-Central Nervous System

DAMPs-Damage Associated Molecular Patterns

DNA-Deoxyribonucleic Acid

DOXY-Doxycycline

EDTA- Ethylenediaminetetraacetic acid

FDA-Food and Drugs Authority

GAFCO chow diet- Ghana Agro Food Company chow diet

HCL-Hydrochloric acid

HCT-Hematocrit

HGB-Haemoglobin

HIV/AIDS- Human Immunodeficiency Virus/Acquired Immune Deficiency Virus

IFN-γ - Interferon Gamma

IgE- Immunoglobin E

IL1-Interleukin 1

IL-2- Interleukin 2

IL4-Interleukin4

IL6-Interleukin6

IL10-Interleukin10

IL17-Interleukin17

IP-Intraperitoneal injection

LNCaP-Lymph Node Carcinoma of the Prostate

MCF-7- Michigan Cancer Foundation-7

MDA- Malondialdehyde

MPV-Mean Platelet Volume

MRSA-Methicillin Resistant *Staphylococcus aureus*

MSCs- Mesenchymal Stem Cells

NK cells- Natural Killer Cells

NO-Nitric Oxide

PARA-Parasite

PBS-Phosphate Buffered Saline

PO-Protein Carbonyl

RDW-Red Cell Distribution

RDWSD -Red Cell Distribution with Standard Deviation

ROS-Reactive Oxygen Species

TBA-Thiobarbituric acid

TCA- Trichloroacetic acid

TEP-Tetraloxypropane

Th1-T-Helper cells 1

Th2-T-Helper cells 2

TNF-α Tumor Necrosis factor alpha

TNF-β Tumor Necrosis factor beta

WHO- World Health Organization

ABSTRACT

The COA Plus mixture, an enhanced formulation of the original COA mixture developed by COA Research and Manufacturing Ltd in 2024, is registered by the Food and Drug Authority as an immune booster. While existing studies support the efficacy of the original COA mixture, data on COA Plus are lacking**.** This study assessed its immunological, antioxidant, and prophylactic potential in female ICR mice. Thirty-five mice were divided into five groups: Normal Control, Parasite Control (infected with *Plasmodium berghei*, Parasite+ Doxycycline, Parasite + COA, and COA only. The Normal Control and Parasite Control groups were administered distilled water for 8 weeks, with the Parasite Control group inoculated with *P. berghei* after this period. The Parasite + Doxycycline group received doxycycline (0.5 mg/kg) prior to parasite inoculation, while the COA groups were treated with COA Plus (0.30 ml/kg) for 8 weeks, with parasite inoculation after the 8th week for the COA + Parasite group. Blood samples were collected for cytokine profiling, parasitemia monitoring, and hematological analysis. Organs were harvested, and liver was used for lipid peroxidation assessment. The COA-only group showed reduced IL-4 levels suggesting positive immunomodulatory effects. Elevated IL-10 levels in the COA-only group indicated an enhanced inflammatory response, while high IFN- γ levels in the COA + Parasite group reflected a strengthened Th1 response, critical for malaria control. The COA + Parasite group also exhibited a reduction in parasitemia, reaching nearly 0% within 72 hours, demonstrating COA Plus Mixture's prophylactic potential. Both the COA and Doxycycline groups had elevated malonyl dialdehyde (MDA) levels, indicating oxidative stress, yet COA likely triggered both pro-oxidant and antioxidant responses, promoting immune resilience. COA treatment enhanced platelets count, confirming its immune-signaling (cytokines and chemokines) effects. In conclusion, the COA Plus Mixture exhibited significant immunomodulatory, antioxidant, and prophylactic effects against *P. berghei* infection, validating the purported claim of its use as a therapeutic agent for malaria prevention and immune support**.**

CHAPTER ONE
1.0 INTRODUCTION

1.1 BACKGROUND

The utilization of herbal and plant-based medicine dates to the earlier stages of human history, which can be traced to at least 5,000 years ago with the Sumerians, while archaeological evidence points to earlier practices (Matole et al., 2021). Humans have relied on plants for healing, utilizing plant – derived products as food components or in the form of botanical preparations for treatment and prevention from various diseases with varying degrees of efficacy (Raskin et al., 2002; Sun & Shahrajabian, 2023). Herbal medicines are the main healthcare foundation for approximately 75-80% of the global population, predominantly in developing countries (Ghosh et al., 2023; Hussain et al., 2009; Kamboj, 2000) . This widespread reliance is heavily due to the perception that herbal treatments are affordable, readily accessible, and free of significant side effects (Dean, 2024; Gupta et al., 2021). The World Health Organization (WHO) reports that the global use of herbal remedies exceeds that of conventional pharmaceuticals by two or three times (Pal & Shukla, 2003; Sagheer et al., 2023). Herbal medicines have recently become increasingly popular as dietary supplements and treatments for various diseases, with a wide range of these products now available in markets worldwide.

During the HIV/AIDS pandemic, many patients sought to immune boosters (Gqalenia et al., 2012), and since the COVID-19 pandemic, there has been an increased interest in plant-based foods, beverages, dietary supplements, and herbal extracts, among others that may have positive immunomodulatory effects (Arshad et al., 2020). Thus, for the past two to three decades, consumers' top health concerns have been associated with improved immunity given that immunocompromised individuals are more susceptible to complications from parasitic, viral and bacterial infections (Arshad et al., 2020). Hence, in recent years, there has been growing interest in the immunomodulatory properties of plants, driven by an increasing

awareness of the relevance of immune system regulation in disease prevention Many plant-based remedies show anti-infective properties not entirely through direct action on pathogens but also, by improving the host's natural and adaptive immune response. This binary mechanism highlights the therapeutic potential of plant-derived compounds in supporting immune health and preventing infections (Gond et al., 2022; Rios & Recio, 2005).

The immune system is a complex network of cells, tissues, organs, and the substances they make help the body fight both infectious and non-infectious diseases. Nutrition influences physiological processes in the body, and nutritional status can have a definite impact on immune functions, infection resistance, and autoimmunity (Chandra & Kumari, 1994; Hatch-McChesney & Smith, 2023; Lakra & Gahlawat, 2016). Certain nutrients are essential for maintaining optimal immune responses, and their deficiency or excess intake can have a negative impact on the number and activity of immune cells (Ghatak & Panchal, 2012). Nutrients that are rich in antioxidants play a crucial role in supporting the immune system. Immune cells, such as T cells, NK and T-Helper cells, produce reactive Oxygen species (ROS) as part of their defense mechanisms to eradicate pathogens (Yang et al., 2013). Natural herbs, containing diverse phenolic compounds, vitamins, carotenoids, flavonoids, show multiple pharmacological effects, including immunomodulatory, anti-oxidative, anti-allergic, and anticancer activities (Sun & Shahrajabian, 2023; Yu et al., 2006). This has encouraged increasing interest in herbal medicine, particularly in the identification of natural compounds with immunomodulatory potential (Shukla et al., 2014).

The two primary defense mechanisms of the immune system are natural and acquired immunity. Natural immunity involves the innate immune cells such as macrophages, neutrophils, eosinophils, natural killer cells, and basophils. Adaptive immunity, on the other hand, comprises the humoral response mediated by B cells and the cytotoxic response mediated by T cells (Latha,

2012). Additionally, there are specialized intercellular signaling molecules called (IL-6, IL-10, TNF-α, IL-2, IFN-γ, etc.), which are important mediators of immune response. These mediate the functions between immune cells and pathogens to elicit an immune response that can be referred to as inflammation (Abdulkhaleq et al., 2018). Immunomodulation is the modification process of immune reactions that function to regulate immune responses. The immune system maintains homeostasis within the body in a healthy organism. Exogenous and endogenous factors influence immune system function and efficiency, resulting in either immunosuppression or immune stimulation. Immunomodulators are agents that could normalize or modulate pathophysiological processes. Numerous studies have highlighted the immunomodulatory properties of various herbs, spices, and medicinal plants (Evanjalin Monica et al., 2022; Shukla et al., 2014). The very well-known plant of garlic or *Allium sativum* used in most of the Indian houses is found to lower IL-1 and IL-6 levels, acting as anti-inflammatory, and as an antioxidant (Singh et al., 2016). The Neem leaf extract for instance is also proven to reduce the circulating pro-inflammatory cytokines, IL-1, IL-6, TNF-α, and Interferon γ (IFN-γ) in *in-vivo* studies (Morris et al., 2019).

Recent advancements in traditional medicine have led to the development of improved herbal formulations, utilizing modern and technologically advanced production methods. These innovations aim to improve the efficacy, safety, and standardization of herbal products, bridging traditional knowledge with contemporary scientific approaches. By employing appropriate technology, the COA Research and Manufacturing Ltd Company has developed COA Plus Mixture, an antiviral herbal product for the human immunodeficiency (HIV) and other human viruses from fresh leaves of *Azadirachta indica, Carica papaya, Spondias mombin, Ocimum viride* and *Persea americana* using novel combination of maceration, maturation, and distillation process. COA Plus Mixture is registered (FDA/DRID/HMD/HMU/16/0981, 2016) under the guidelines of FDA, Ghana, for use for immune system support. A number of studies have

reported the biological activities of some of the constituent plants of the COA Plus Mixture including their antimicrobial and immunomodulatory properties. Polysaccharides derived from *Azadirachta indica* have been shown to inhibit poliovirus (PV- 1) by disrupting the early stages of viral replication (Faccin-Galhardi et al., 2012). Additionally, extracts from *Azadirachta indica* exhibit antiviral activity against the Newcastle disease virus (Mahmood et al., 2018) dengue fever virus and Herpes Simplex Virus (HSV-1) (Faccin-Galhardi et al., 2012). Neem infusion has been found to improve antibody titers and increase growth performance when administered at a concentration of 50 ml/L in drinking water (Rahmani et al., 2018). The therapeutic benefits of *Carica papaya* are attributed to its rich content of vitamins A, B, and C, along with proteolytic enzymes such as papain and chymopapain, which exhibit antiviral, antifungal, and antibacterial properties (Radhakrishnan et al., 2017; Vij & Prashar, 2015). Papaya extracts have demonstrated activity against the dengue virus (Sharma et al., 2019) and have shown anti- inflammatory and immunomodulatory effects(Pandey & Rizvi, 2009). Furthermore, *Spondias mombin* extracts have been found to be active against HSV (Siqueira et al., 2020) while Geraniin from the same plant has emerged as a promising inhibitor of the Ebola virus based on molecular docking studies (Boadu et al., 2023). *Spondias mombin* leaf extract also exhibits anti-inflammatory properties by inhibiting tumor necrosis factor-α and nitric oxide production (Nworu et al., 2011).

Ocimum basilicum, commonly known as sweet basil, has been extensively researched for its wide range of bioactive properties. These include antimicrobial, antifungal, insecticidal, antiparasitic, antioxidant, immunomodulatory, anti-inflammatory, hepatoprotective, anti- osteoporotic, cardio protective, neuroprotective, and anticancer activities, among other health-promoting effects (Dhama et al., 2023; Kamelnia et al., 2023). Extracts from *Persea Americana* commonly known as Avocado pear has been shown to have anti-viral activity against a wide range of virus including anti-HIV activity (Darshana et al., 2023; Wigg et al., 1996) , anti-HSV activity (Miranda et al., 1997; Yasir et al., 2010). The arabinogalactan-protein-rich fraction from an aqueous extract of

avocado leaves demonstrated an inhibitory effect on the classical pathway of the complement system (Yamassaki et al., 2018).

1.2 PROBLEM STATEMENT AND JUSTIFICATION

The COA Plus mixture an upgraded form of the COA mixture, which was produced and launched by the COA Research and Manufacturing Ltd Company in 2024 and registered by the Ghana Food and Drug Authority as an herbal medicine for immune system support. Although there are some published scientific data on the COA mixture, there are none on the COA Plus mixture. For instance, a computational analysis of the COA mixture revealed that some phytocompounds in the mixture have the potential to slow down the elimination of protease inhibitors (PIs), thereby maintaining optimal PIs concentrations. They are also able to serve as natural inhibitors of HIV pro and used as important standard in developing novel drugs to inhibit the activity of HIV pro (Kehinde et al., 2019). A retrospective analysis of clinical data in 74 HIV patients with ages ranging between 12 to 60 years, who had been placed on the COA Mixture revealed a decrease in viral load in the 59.5% of them (Ocloo et al., 2021).The product is also reported to possess good antioxidant property *in vitro* and antiproliferative assay conducted, which revealed that it was not toxic to Hep G2 cells and MCF-7 but potent against PC-3 cells, LNCaP and Jurkat cells (Languon et al., 2018). It is reported to be one of the herbal medicines that are widely patronised by HIV-infected individuals (Nlooto & Naidoo, 2016) and is widely marketed as an immune supplement. Indeed, the product gained prominence during the early stage of the COVID-19 pandemic. With the advent of HIV and COVID-19, most individuals' top health concern in recent times has been boosting their immune system. Hence, there has been an increase in the patronage of potential immune boosters, including vitamin C and medicinal plant extracts. The use of herbal medicines is popular in many countries, especially in developing countries, where about 80% of the population is estimated to use herbal medicine for their primary health care needs (Kala, 2017). Medicinal plants are to be good sources of antioxidants, which have been established to play a significant role in

maintaining the immune system (Hajian, 2014). Enhanced immunity is known to assist not only in fighting communicable diseases but also in non-communicable diseases such as cancers. Therefore, generating scientific data on the *in vivo* antioxidant properties, immunomodulatory and protective effects of COA Plus mixture, will not only provide scientific evidence in support of its claims but will provide baseline data for its development into an herbal immune supplement and a supportive treatment to other conventional antimalarial drugs and therapies. The outcome of the study will contribute to the realization of good health and wellbeing. It will contribute to scientific research of natural compounds to drastically reduce the effect of one of the most prevalent parasitic diseases in Africa -malaria (Simwela & Waters, 2022). It will also promote the use of murine models for exploring efficacy and mechanisms before human trials to ensure safety and mechanistic insight, which is ethically and scientifically necessary before clinical testing (Adebayo et al., 2025; Messerli et al., 2019).

1.3 AIM AND SPECIFIC OBJECTIVES

1.3.1 Aim

To investigate the *in vivo* antioxidant properties, the immunomodulatory effect and prophylactic activity against *Plasmodium berghei* infection of the COA Plus mixture.

1.3.2 Specific Objectives

- To assess the effect of the COA Plus mixture on IFN-γ, IL-4, IL-10 in mice.
- To ascertain the effect of the COA Plus mixture on lipid peroxidation in mice liver
- To determine the effect of the COA Plus mixture on the level of parasitaemia in *Plasmodium berghei* infected mice.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Plant Medicine and Healthcare

Plant medicine, also known as herbal medicine or phytotherapy, has a long and diversified history, which cuts across multiple cultures and civilizations. It has played an important role in human health for ages, from the use of medicinal plants in traditional Chinese medicine to Native American tribes' indigenous healing methods (Petrovska, 2012). Plants were once considered valuable resources for treating ailments and promoting overall well-being.

2.1.1 Historical Background

The Ebers Papyrus, an ancient Egyptian medical document dating back to 1550 BCE, contains references to over 700 plant-based remedies. Also, Ayurvedic texts in India, such as the Charaka Samhita and Sushruta Samhita, describe the use of many plants(herbs) for medicinal purposes (Mills et al., 2006; Petrovska, 2012).. Throughout civilizations, plants have played a role in sustaining life by providing resources such as shelter, nutrition, and medicine (Balick & Cox, 2020; Myer, 1997) . The historical record is full of evidence from various cultures acknowledging the therapeutic potential of herbs and their extracts (Alves & Rosa, 2007). Ayurvedic and Chinese medical manuscripts highlighted the use of plants for medicinal purposes, emphasizing their importance in early medical practices (Eisenberg et al., 1993). In the same way, civilizations like the ancient Egyptian made use of botanical remedies, found in documents such as the Ebers Papyrus, highlighted the reliance on plant-based healing (Singh, 2017; Srivastava & Vaidya, 1999). These practices and documented uses of plant-based remedies have not only endured the test of time but have also paved the way for the development of traditional medicine systems globally (Fabricant & Farnsworth, 2001). These systems are based on regional biodiversity and indigenous knowledge systems, where accumulated knowledge passed down through generations has contributed to the identification, use, and preservation of medicinal flora (Heinrich et al., 2021; Thomas et al., 2008).

In the Americas, indigenous communities have relied on their botanical knowledge for healing; (Alves & Rosa, 2007; Moerman et al., 1999). Native American healing traditions and Amazonian herbal practices make use of region-specific plants for various medicinal purposes (Alves & Rosa, 2007). These traditions are intertwined with cultural identities and have persisted despite evolving healthcare landscapes (Alves & Rosa, 2007). The contributions of scientists such as Hippocrates, known as the "father of Western medicine," highlighted the importance of plant medicine throughout history. Hippocrates said, "Let food be thy medicine and medicine be thy food," emphasizing the healing power of natural things, which include plants (Halberstein, 2005).

.

2.1.2 Plant Medicine in Africa

The use of medicinal plants have a place in regional contexts, often reflecting the rich biodiversity and indigenous knowledge systems within specific geographical areas (Alves & Rosa, 2007). Across various regions, worldwide, traditional medicine systems rely on the use of region-specific plants, indicating a deep-rooted association between culture, nature, and healthcare practices (Ahmad et al., 2023; Anyinam, 1999).

Plant medicine has a place in African cultural and spiritual traditions, and it plays an important part in their healing rituals. The usage of medicinal plants in Africa is thousands of years old and has been transferred through generations, defining our continent's perception and use of plant medicine (Bodeker & Ong, 2005; Cunningham et al., 2012). With a diverse flora, estimated to exceed 4,000 plant species used for medicinal purposes, Africa stands out as a reservoir of botanical remedies (Okaiyeto & Oguntibeju, 2021). This diverse plant wealth contributes to the local healthcare landscape, meeting an estimated annual consumption of 50,000 tons of medicinal plants (Cunningham et al., 2012; Kumar et al., 2021). The reliance on traditional medicine within African communities remains substantial, with about 75% of urban and rural

populations incorporating medicinal plants into their everyday healthcare practices (Ahmed, 2021). Traditional healers, custodians of this knowledge, play vital roles in providing remedies and guidance, holding influence within communities (Grace et al., 2017).

Africa's diverse habitats have led to the continent's abundance of medicinal plants. Each location has its own distinct flora, resulting in a various range of plant species used for various health purposes. For example, the San people of Southern Africa have a thorough awareness of the medicinal powers of native plants and use them to cure a variety of illness and injuries (Mahomoodally, 2013) . However, the introduction of Europeans in Africa caused considerable modifications in traditional medical practices. Colonialism had influence on African traditional medicine (Busia, 2005). Colonial rulers saw traditional African medicine as primitive and attempted to replace it with Western medical procedures. This resulted in a decline in the usage and appreciation of plant medicine in many African tribes. However, in recent years, there has been a renewed interest in traditional plant medicine throughout Africa (Sofowara, 1993). This comeback can be linked to a variety of causes, including a rising appreciation for the worth and efficacy of ancient therapeutic techniques, a desire for more natural and holistic approaches to healthcare, and a reclamation of cultural history (Abdullahi, 2011). For example, a report from *The Washington Post* in 2024 on King Charles's diagnosis of prostate cancer highlighted his role as a founder of a charity organization that advocates for alternative medicine. He is an advocate of homeopathy and views alternative medicine as integral to his fundamental beliefs. King Charles expressed his concern that humanity has deviated from a more traditional, natural, and Edenic state, becoming overly reliant on mechanistic, technological, and modernist ideologies. In contrast, he promotes "whole-ism" (his preferred spelling) as the more appropriate approach to health and well-being.

2.1.3The Importance of Plant Medicine in Healthcare Delivery

The dependence on traditional medicine is an accepted practice, in regions where access to conventional healthcare may be limited or where cultural and historical practices favour indigenous healing methods (WHO, 2002; Winiger, 2022). WHO's statistics shows the substantial global dependence on traditional medicine for primary healthcare, estimating that about 80% of the global population relies on traditional remedies to address various health concerns. This dependence emerged from a combination of factors such as cultural beliefs, accessibility, affordability, and the perceived efficacy of traditional healing practices (Dutta et al., 2021).

The rates of dependence on traditional medicine show variations across different regions worldwide, with high dependence observed in certain areas like Africa (WHO, 2002) In specific African regions, reliance rates soar high, ranging between 60% and 95% of the population turning to traditional medicine as their primary source of healthcare (Cunningham et al., 2012; WHO, 2002; Witol, 2010). Factors contributing to such high dependence include cultural heritage, limited access to modern medical facilities in rural areas, and the trust placed in traditional healers within communities (Fokunang et al., 2011). Continued dependence on traditional medicine in developing regions calls for the acknowledgment and integration of indigenous healing practices within broader healthcare frameworks, to ensure culturally sensitive and effective healthcare delivery (Absolon, 2010; Mills et al., 2006). Efforts aimed at integrating traditional medicine with modern healthcare systems, as seen in initiatives by various governments and health organizations, seek to capitalize on the strengths of both systems to provide comprehensive and accessible healthcare for diverse populations (Alleyne et al., 2006; Burton et al., 2015). This dependence on traditional medicine indicates not just a gap in conventional healthcare accessibility but also the preservation of cultural heritage and the resilience of age-old healing practices intertwined with the societal fabric (Petzer &

Mngqundaniso, 2008). As global healthcare strives for inclusivity and sustainability, recognizing the importance of traditional medicine in meeting healthcare needs becomes imperative for fostering equitable and competent cultural healthcare systems (Dutta et al., 2021; WHO, 2002).

Throughout the history of life on Earth, plants have been vital in providing shelter, oxygen, food, and medicine for higher life forms. As societies emerged, humans began to recognize and categorize plant materials suitable for fulfilling their basic needs. Among these needs, the use of herbs and their extracts; for healing purposes can be traced back to ancient myths, traditions, and written records that documented plants with pain-relieving and disease-treating properties (Fernando et al., 2015). Traditional medicine and the utilization of medicinal plants are vital components of healthcare systems, particularly in developing nations. Approximately 80% of the global population depends on traditional medicine for their primary healthcare requirements (Holley & Cherla, 1998; Mulliken, 2003). In developed countries, there is a growing acceptance and use of traditional, complementary, and alternative medicines. Significant utilization rates have been reported, including 48% in Australia, 31% in Belgium, 70% in Canada, 49% in France, and 42% in the United States (WHO, 2002).

Medicinal plants serve as a reflection of a country's medicinal flora and highlight the importance of traditional medicine in addressing health concerns. Tropical Africa stands out with its use of over 4,000 plant species for medicinal purposes and an annual consumption of 50,000 tons of medicinal plants (Ahenkan & Boon, 2008). Medicinal plants are gaining recognition in healthcare delivery, in developing countries, due to their affordability, consumer acceptance, and local availability (Burton et al., 2015; Lambert et al., 1997).

There is a global interest in medicinal plants, with about 50,000 known plant species used in traditional and modern medicine (Mulliken, 2003; Schippmann et al., 2002). The World Health

Organization (WHO, 2002) estimated that the annual demand for medicinal plants was around US $14 billion and is increasing at a rate of 15 to 25% per year. It was reported that the demand would increase to over US $5 trillion by 2050 (Kala et al., 2006). Concerns about the effects of synthetic medication, questioning of allopathic medicine, and increased access to health information have contributed to the demand of traditional medicine in developed countries (WHO, 2002). In Ghana, for instance, medicinal plants are vital, with about 75% of the population in urban and rural areas relying on them for everyday healthcare needs (Ahenkan & Boon, 2008) .

Governments around the world have appreciated the significance of traditional medicine and have introduced policies to incorporate it into formal healthcare systems. Countries such as China and Mongolia have established healthcare frameworks rooted in traditional medical practices. In Africa, there is increasing recognition of traditional healers, with ongoing efforts to harmonize and integrate indigenous and Western medical practices (Fokunang et al., 2011; Mutombo et al., 2023).

2.2 The Immune System

The immune system is a multi-scale system that includes genes, molecules, cells, and organs arranged in complex networks of synergistic connections to combat many forms of threats to the organism (Subramanian et al., 2015). The two ways of immune defense are the innate and the adaptive/ immunity.

The innate immunity is the initial line of protection and exists from birth. It consists of physical barriers like the skin and mucous membranes, as well as cellular and molecular components that react to invading pathogens in a nonspecific manner, providing a rapid but generalized defense against infection (Turvey & Broide, 2010). The innate immunity is non-specific and rapid response mediated by immune cells from hematopoietic and non-hematopoietic origin. The Hematopoietic cells involved include macrophages, dendritic cells, mast cell, neutrophils, eosinophils, natural killer (NK) cells. Natural Killer cells, a type of lymphocyte, are important in anti-viral immunity and can shape the nature of an adaptive immune response (Hamerman et al., 2005). These also have the ability to adapt and develop into long-lived memory cells, providing new insights into the roles of innate immune cells (Sharrock & Sun, 2020). NK cells are involved in the early defense against infectious agents and have a potential immunoregulatory role in modulating autoimmunity and infectious diseases (Aktas et al., 2009). Macrophages are innate immune system cells that can eliminate pathogens via phagocytosis and then recruit other immune cells to attack intruders (Linehan & Fitzgerald, 2015). They are part of the mononuclear phagocytic system (formerly known as the reticuloendothelial system), they serve as the first line of defense against infections. They are categorized into two types: "conventionally activated" (M1) and "alternatively activated" (M2) (Mantovani et al., 2004; Yao et al., 2019). M1 macrophages are activated by type 1/Th1 cytokines (e.g., IFN-gamma, TNF- alpha), pathogen-associated molecular patterns (e.g., LPS), and damage-associated molecular patterns (DAMPs) (Murray, 2017; Zhang & Mosser, 2008). M2 macrophages play

anti-inflammatory roles in wound healing, matrix deposition, tissue repair, fibrosis, remodelling, immunological modulation, immune suppression, allergies, and parasitic infections (Italiani & Boraschi, 2014; Song et al., 2023). Dendritic cells (DCs) are crucial in the innate immune response, serving as a link to the adaptive response (Clark et al., 2000). They are key regulators of immune reactions, controlling early innate responses and contributing to the maintenance of self-tolerance. DCs interact with microbes through pattern-recognition receptors, priming natural killer cells and specific T-cell responses (Granucci et al., 2004). Their ability to take up, process, and present antigen to T cells makes them potent antigen-presenting cells, bridging innate and adaptive immunity (Chu et al., 2011). Mast cells are associated with allergic responses, also playing a role in innate immunity. They are involved in the defense against bacterial, viral, and fungal infections, and are activated by various mechanisms, including complement components and pattern-recognition receptors (Malaviya & Abraham, 2001). In addition to their role in immediate allergic reactions, mast cells are important for both innate and adaptive immunity in tissues that interface with the environment (Tete et al., 2012). Their unique ability to interact with the vasculature and expedite cell recruitment further elaborates their importance in the immune response. In the skin, mast cells act as sentinels and effector cells in innate immune responses (Metz et al., 2008).

Adaptive immunity is a specialized defense mechanism that involves the activation of B and T lymphocytes in response to specific antigens. This immune response is highly specific and is developed after the body is exposed to a particular pathogen or antigen, enabling a targeted and efficient defense against subsequent exposures (Alberts et al., 2015; Chaplin, 2010). The adaptive immune response, though slower than the innate immune system, exhibits a higher specificity toward pathogens and utilizes immunological memory to enhance future responses upon re-exposure. In the presence of antigens from invading pathogens, B cells produce

specific antibodies that can neutralize the pathogen directly or facilitate its clearance by promoting phagocytosis by macrophages. Additionally, these antibodies contribute to the activation of the complement system, which targets the microbial membrane for destruction. (Iwasaki & Pillai, 2014). Cytotoxic T cells eliminate host cells that contain foreign molecules, while helper T (TH) cells enhance the immune response by coordinating the functions of other immune cells, including B cells and cytotoxic T cells. Upon exposure to antigens, naïve T cells differentiate into distinct subtypes of T cells, which play critical roles in the immune response (Alberts et al., 2015). In cancer, for example, there is the natural ability to improve the human T lymphocytes to identify malignant cells (Leko & Rosenberg, 2020). Paracrine anti-inflammatory capabilities of mesenchymal stem cells (MSCs) are used to treat some disorders. Another option is to use monoclonal antibodies to inactivate certain proinflammatory factors (e.g., TNF-alpha, IL-6) (Gilotra et al., 2021).

As agonists, monoclonal antibodies can mimic immunomodulatory signaling on antigen-presenting cells. There are diverse sets of medications that modulate inflammasome function. Furthermore, established immunomodulatory medications such as corticosteroids, nonsteroidal anti-inflammatory medicines, anti-histamine, and interferons have been reintroduced with radical innovations which had enhancement on the context of research aimed at improving their efficacy (Suresh & NP, 2020).

2.2.1 Immunomodulation

Immunomodulation involves the regulation and modulation of immune responses to maintain balance within the immune system, ensuring specific reactions to pathogens and preventing immune activation leading to inflammation or autoimmunity (Iwasaki & Pillai, 2014; Libbey et al., 2010). Various factors, including dietary components and natural compounds from plants, exhibit immunomodulatory effects, influencing immune cell function and cytokine production

(Pandey & Rizvi, 2009; Scalbert et al., 2005). In recent times, immunomodulation has gained attention because it has direct applications in both basic science and clinical medicine. Modulation of an insufficient, exaggerated immune response allows for a disease's clinical course to be slowed and equilibrium to be restored (Strzelec et al., 2023).

The targets for modulating immunity are as diverse as the immune system's components, creating many avenues for intervention. The immune system is relevant for resistance to harmful infections and maintaining homeostasis. An immunological response to a threatening situation is a viable and conscious balance-saving mechanism (Strzelec et al., 2023). An amplified and out-of-control immune response, on the other hand, can act as a self-driving positive feedback loop with consequences and is linked to the development of a wide spectrum of diseases (Heneka et al., 2015). The chronic inflammatory process causes some accumulation of an inappropriate immune reaction, against specific cells, leading to the development of autoimmune disorders. The growth of inflammation contributes to disease aggravation and patient deterioration. It is necessary to suppress the immune response from an external source. This indicates that chronic inflammation, which involves an increase in proinflammatory cytokine levels, is part of the pathogenesis of many diseases. This include asthma, rheumatoid arthritis, hepatitis, heart disease, and some of the common central nervous system (CNS) disorders such as Alzheimer's disease, epilepsy, depression, and schizophrenia (Strzelec et al., 2023). Therefore, immunomodulation is an advancing field in terms of pharmacokinetics and pharmacodynamics.

2.2.2 Plants as Sources of Immunomodulators

Plants have been recognized for their potential to enhance the immune system, offering many compounds that support immune function and overall health. Phytochemicals found in plants, including polyphenols, flavonoids, alkaloids, tanins and vitamins play roles in modulating

immune responses (Pandey & Rizvi, 2009; Scalbert et al., 2005). Plant-derived compounds like curcumin, ginsenosides, and quercetin exhibit immunomodulatory effects, influencing immune responses by regulating immune cell function and cytokine production. Perceiving the mechanisms of these immunomodulators, offers avenues for managing immune-related disorders and supporting overall immune health.

The ability of these plant-derived compounds to modulate immune responses has promised many health benefits. Immunomodulators, through their regulation of immune cell function and cytokine balance, contribute to managing inflammatory conditions, supporting immune health, and reducing the risk of autoimmune diseases (Scalbert et al., 2005; Sharma et al., 2017). While plant-derived immunomodulators offer potential benefits, challenges exist in standardization, bioavailability, and dosage optimization. Further research focusing on the mechanistic perception and clinical applications of these compounds is relevant to harness their full potential as immunomodulators (Pandey & Rizvi, 2009; Scalbert et al., 2005).

Studies highlight that curcumin, a bioactive compound in turmeric, modulates immune responses by regulating cytokine production, reducing inflammation, and enhancing immune cell activity (Gupta et al., 2021; Vikou et al., 2023). Curcumin's multifaceted actions make it a promising immunomodulatory agent. Ginsenosides found in Panax, ginseng exhibits immunomodulatory effects, influencing immune cell function and cytokine regulation. Research suggests their ability to enhance immune responses by stimulating immune cell activity, particularly natural killer (NK) cells, aiding in combating infections (Gupta et al., 2021; Hui et al., 2018). Quercetin, abundant in foods like onions and apples, produces immunomodulatory effects by regulating immune cell activity and cytokine production (D Archivio et al., 2007; Hui et al., 2018). Its ability to modulate immune responses contributes to its recognized role in immune health.

Various medicinal plants have garnered attention for their potential in enhancing the immune system and overall health. For instance, Echinacea, known for its immune-stimulating properties, contains compounds like polysaccharides and alkamides that activate macrophages and increase cytokine production (Hudson et al., 2005; Shahrajabian et al., 2019). Astragalus renowned for its immunomodulatory and antioxidant characteristics, stimulates T-cell function and promotes antibody production, augmenting the body's immune response (H. Bower & R. Cundell, 2014; Wang et al., 2021). In the same vein , garlic, rich in sulphur compounds like allicin, exhibits antimicrobial effects while stimulating immune cells and antibody production (Bayan et al., 2014; Nantz et al., 2012). Elderberry, abundant in flavonoids, modulates immune responses and demonstrates antiviral effects that could be beneficial in managing respiratory infections (Hawkins et al., 2019; Tiralongo et al., 2016). Elderberry is rich in flavonoids known for their immune-enhancing effects. Studies suggest that elderberry extracts modulate immune responses, aiding in the prevention and management of respiratory infections (Hawkins et al., 2019; Tiralongo et al., 2016). A study that was conducted on BALB/c mice indicated a definite increase in CD4+ and CD8+ cells when treated with the aqueous extract of neem leaves. Lymphocyte and monocyte count after the treatment showed a definite increase (Beuth et al., 2006).

The mechanism of action of plant-derived bioactive immunomodulators involves the activation and production of signal molecules, such as cytokines, which are water-soluble glycoproteins. These cytokines include colony-stimulating factors, interleukins, and interferons, produced by host cells and commonly used in vaccines and therapeutic agents (Beuth et al., 2006). These

signaling molecules mediate the activation of both the innate immune system, including natural killer cells and macrophages, and the adaptive immune system, involving humoral (T and B cells) and cellular (antibody production) responses. The immune response is initiated through the interaction between Toll-like receptor (TLR) proteins and antigens, leading to an appropriate immune reaction (Kennedy, 2010).

Immune system modulation with medicinal herbs and phytochemicals is a new treatment option for disorders involving immune response. Plants, fungi (mushrooms), and algae produce secondary metabolites that can modulate the immune response of the host, either by enhancing or inhibiting it. Various traditional medicinal plants, including *Andrographis paniculata, Curcuma longa, Echinacea purpurea, Withania somnifera, Tinospora cordifolia, Ocimum sanctum, Azadirachta indica, Boswellia serrata, Momordica charantia, and Panax ginseng,* have been utilized for their immunostimulatory and immunosuppressive properties, targeting multiple components and mediators of both the innate and adaptive immune responses. Alkaloids (berberine, chelerythrine, piperine), polyphenols (quercetin, rutin, apigenin, luteolin, epigallocatechin-3-gallate, daidzein, genistein, resveratrol), and isoprenoids (triptolide, 14-deoxyandrographolide, ginsan, oleanolic acid) are among the immunomodulatory phytochemicals.

2.3 Oxidative Stress

The interaction of oxidative stress, immunomodulation, and antioxidants is a triad in the maintenance of immune function and overall health (Meydani, 1995; Tavassolifar et al., 2020). Oxidative stress is defined as an imbalance between the production of Reactive Oxygen Species (ROS) and the body's ability to neutralize them using antioxidants (Haleng et al., 2007; Preiser, 2012).

Reactive Oxygen Species, also known as free radicals are formed in our body due to internal and external factors. Internal factors such as a by-product of energy metabolism by the mitochondria (Lobo et al., 2010). The external factors like excessive drinking, smoking, food additives, exposure to environmental pollution and heavy metals also contribute to free radicals in the body (Phaniendra et al., 2015; Smilin Bell Aseervatham et al., 2013). Damage to lipids, proteins, and DNA results from elevated ROS levels. The lipid membrane can be damaged by ROS, which can also increase membrane fluidity and permeability (Tai et al., 2010; Yadav et al., 2019). According to (Ayala et al., 2014) protein damage includes proteolysis susceptibility, peptide chain fragmentation, cross-linked reaction product aggregation, electric charge alteration, and enzymatic inactivation.

Reactive oxygen species can induce DNA damage, especially double strand breaks (DSBs), and influence the cellular response to genotoxic therapy for instance in cancer (Srinivas et al., 2019). DNA damage alters replication and transcription, resulting in cell death leading to mutations and neoplastic transformation in the human system.

Reactive Oxygen Species-mediated deleterious effects contribute to human degenerative conditions including neurological disorders; (Dröge, 2002; Singh et al., 2019), cardiac dysfunction (Alexander et al., 2000; Singh et al., 2019), and cancers (Dreher & Junod, 1996; (Dreher & Junod, 1996; Waris & Ahsan, 2006) as well as the process of aging (Harman, 1981; Waris & Ahsan, 2006). DNA damage, whether from external sources like UV radiation and ionizing radiation, or internal sources like reactive oxygen species, can have biological consequences. It can lead to mutations, genetic instability, and an increased risk of skin cancer (Marrot & Meunier, 2008). Inherited or sporadic defects in DNA repair mechanisms can result in cellular outcomes that underlie disease and aging, such as cancer, neurological disease, and premature aging (Tiwari & Wilson, 2019). Due to this, our bodies need to maintain a certain

balance of free radicals and antioxidants. Antioxidants are the molecules that prevent the harmful effects of ROS and free radicals by donating electrons or hydrogen atoms, thereby reducing oxidative stress and preventing cellular damage (Valko et al., 2007). It is known that oxidative stress affects immune cell functions such as antigen presentation, lymphocyte activation, and cytokine production (Sharma et al., 2012). ROS, a hallmark of oxidative stress, also function as signaling molecules in immune cells, regulating processes such as cytokine production, phagocytosis, and T cell activation (Sharma et al., 2012). This dual role of ROS highlights their relevance in immune responses and how oxidative stress, modulates immune activities. Oxidative stress has a complex link to inflammation, which is a fundamental immune response. It plays a dual role in inflammation in that, it is necessary for defense against pathogens, but excessive oxidative stress can lead to chronic inflammation and damage of the tissues. This implies that, while oxidative stress is necessary for initiating immune responses, too much can be harmful (Sharma et al., 2012).

The National Institutes of Health defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention (Zhang et al., 2004) . The biomarkers of oxidative stress are grouped as molecules that are changed by the interactions with ROS in the environment and molecules of the antioxidant system that are modified in response to redox stress. Molecules like DNA, lipids (including phospholipids), proteins and carbohydrates are examples of the molecules that can be changed by ROS *in vivo*.

The biochemical process that occurs in cell membranes and includes the oxidative degradation of polyunsaturated fatty acids is called lipid peroxidation. This occurrence is linked to many pathological conditions such as aging, neurodegenerative diseases, cardiovascular disorders,

and cancer. The abundant reactive double bonds in the molecular structure of lipids make them prone to oxidation (Nam, 2011; Porter et al., 1995; Zhang et al., 2004; Zhang & Mosser, 2008). Isoprostanes (IsoPs) and malondialdehyde (MDA) are two of the most well studied lipidperoxidation markers. Perceiving the mechanisms of lipid peroxidation and its consequences provides valuable insights for the development of therapeutic interventions targeting oxidative stress-related diseases.

2.3.1 Antioxidants

Antioxidants are molecules that inhibit the oxidation of other molecules, thereby preventing the formation of free radicals and the chain reactions that can damage cells (Aldred, 2020). They play a role in maintaining health and preventing diseases, including cancer, diabetes, heart disease, stroke, Alzheimer's disease, rheumatoid arthritis, and cataracts (Kaur et al., 2024). The criteria for evaluating antioxidant activity include the ability to destroy biological oxygen-derived species *in vitro* and evidence of functioning as an antioxidant *in vivo* (Baliyan et al., 2022; Halliwell et al., 1995). Antioxidants include vitamins E and C, carotenoids, and plant phenolics, which can prevent free radical-induced tissue damage (Kruk et al., 2022; Vaishali, 2014) .

The body's antioxidant defense system is a complex network of enzymatic and non-enzymatic components that work together to protect against oxidative damage. This system includes enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, which play a basic role in the defense strategy (Ighodaro, 2018). Glutathione, a component of this system, interacts with other antioxidants such as vitamin C to alleviate oxidant stresses (Jacob, 1995; Lee et al., 2023). The activity of the enzymatic antioxidant system is regulated by many factors, including age, physiological condition, and the presence of coenzymes and inhibitors

(Lavryshyn et al., 2020). The antioxidant defense system is relevant for protecting the body from oxidative damage and maintaining overall health.

2.3.2 Plants as Sources of Antioxidants

Antioxidants are compounds that play a role in combating oxidative stress by neutralizing free radicals in the body, thereby mitigating cellular damage and reducing the risk of various chronic diseases. Plants are sources of antioxidants, offering an array of phytochemicals (Cao & Prior, 2000) with antioxidant properties, including polyphenols, flavonoids, carotenoids, and vitamins (Pandey & Rizvi, 2009; Rice-Evans et al., 1997). The free radical scavenging antioxidants that are in plants include phenols, flavonoids, anthocyanins, carotenoids, dietary glutathione, and vitamins (Prakash & Gupta, 2009; Rahaman et al., 2023). When these free- radicals are neutralized and absorbed by antioxidants certain diseases like atherosclerosis, ageing, cancer, diabetes mellitus, inflammation, and sometimes AIDS can be managed by improving immune function leading to healthy living.

Phenols are a major group of antioxidant phytochemicals with properties that can play a relevant role in adsorbing and neutralizing free radicals. They possess antioxidant, anticancer, antibacterial, antiviral, and anti-inflammatory activities (Chang et al., 2002; Hechaichi et al., 2023). There are types of phenolic compounds, which are flavonoid and non-flavonoid. Phenolic and polyphenolic acts alone and/or together with vitamins, carotenoids, vitamin E, and vitamin C, which work as antioxidants to defend the body's tissues from the harmful effects of oxidative stress (Minatel et al., 2017). Polyphenolics are the common antioxidants found in vegetables and fruits. Common benzoic acids consumed by humans are gallic, ellagic, protocatechuic, and 4-hydroxybenzoic acids, while the common cinnamic acids are caffeic, ferulic, sinapic, and p-coumaric acids (Chandrasekara & Shahidi, 2010; M Dos Santos Lima et al., 2014).

Plant-based diets are high in polyphenols, which offer nutritional benefits and help prevent chronic diseases. For instance, flavonoids, which are phenols, play an essential role in treating skin inflammation in the case of an inflammatory skin condition (Maleki et al., 2022). The anaerobic bacterium *Propionibacterium acnes* carry out a vital role of the pathophysiology of skin inflammation. Retinoids are a comprehensive approach to treating skin rashes including antimicrobials and may offer an alternative to standard care (Bandyopadhyay, 2021; Leyden et al., 2017). Some phenolic compounds with antibacterial effects against *Propionibacterium acnes* include honokiol and magnolol (isolated from *Magnolia sp.*), as well as gallic, caffeic, chromogenic, ferulic, myricetin, and cinnamic acids, quercetin, apigenin, luteolin, and thymol, derived from wild watermelon leaves.

A member of the phenolic group, anthocyanins are coloured and water-soluble pigments. The pigments are in glycosylated forms. Fruits and vegetables containing anthocyanins occur in the colours red, purple, and blue. There are high levels of anthocyanins in berries, currants, grapes, and other tropical fruits. Leafy vegetables, grains, roots, and tubers with colours ranging from red to purplish-blue are edible vegetables with high anthocyanin content as well (Hock et al., 2021). Among the anthocyanin pigments, cyanidin-3-glucoside is the major anthocyanin found in most of the plants. The coloured anthocyanin pigments have been used as a natural food colorant. The colour and stability of these pigments are influenced by pH, light, temperature, and structure (Hock et al., 2021). Anthocyanin has been used as a phytopharmaceutical, choleretic agent and appetite stimulant as it is one of the potent nutraceutical or pharmaceutical ingredients found in medicinal plants, and hence it is used in treating several kinds of other diseases.

Antioxidants, which are relevant in neutralizing ROS, also play a role in maintaining immune function. They act as defense mechanisms against oxidative stress, preserving immune cell

function and overall immune response (Pisoschi & Pop, 2015) . This emphasizes the importance of antioxidants in preserving the balance between oxidative and immune activation. Some herbal plants have antioxidant properties that provide natural remedies for oxidative stress-related illnesses. Garlic (*Allium Sativum)* for instance is a culinary staple around the world, contains allicin, a sulphur-containing compound known for its antioxidant and anti-inflammatory properties (Banerjee & Sarkar, 2003). *Ginkgo biloba*, an ancient tree species, the extract from its leaves, which contains a high flavonoid content. These flavonoids, which include quercetin and kaempferol, have antioxidant properties. According to (Ahlemeyer & Krieglstein, 2003) and (Cheung & Yew, 2020) *biloba* extract protects against oxidative cell death by inhibiting the formation of ROS.

Polyphenols, distributed in plants, exhibit relevant antioxidant activity due to their ability to scavenge free radicals and modulate various cellular signaling pathways (Sandoval-Acuña et al., 2014; Scalbert et al., 2005) . Berries, such as blueberries and strawberries, are known for their high polyphenol content, in particular anthocyanin and flavonoids, showing robust antioxidant properties (Seeram et al., 2008; Wu et al., 2004). In addition, green tea, rich in like epigallocatechin gallate (EGCG), have antioxidant effects, contributing to its health-promoting properties (Higdon & Frei, 2003; Musial et al., 2020).

Flavonoids, a subclass of polyphenols, found in various fruits, vegetables, and herbs, offering notable antioxidant capabilities (Middleton Jr et al., 2000; Panche et al., 2016; Williams et al., 2004). Citrus fruits, rich in flavonoids like hesperidin and naringin, have potent antioxidant effects, contributing to their protective role against oxidative stress-related diseases (Hou et al., 2003; Kawai et al., 2000; Zaidun et al., 2018). Onions and broccoli, also contain quercetin and kaempferol, respectively, exhibiting antioxidant potential, offering protective benefits against oxidative damage (D Archivio et al., 2007; Zhang & Zhang, 2014).

Carotenoids, the pigments responsible for the vibrant colors in fruits and vegetables, serve as antioxidants (Krinsky & Johnson, 2005; Rao & Rao, 2007) . Beta-carotene, found in carrots and sweet potatoes, and lycopene, abundant in tomatoes, have antioxidant activity, contributing to cellular protection against oxidative stress (Rao & Rao, 2007; Stahl et al., 2000).

Vitamins C and E, present in many fruits, vegetables, and nuts, function as antioxidants, protecting cells from oxidative damage (Brigelius-Flohé & Traber, 1999; Pruteanu et al., 2023). Citrus fruits, including oranges and lemons, have vitamin C offering antioxidant benefits (Padayatty et al., 2003) . In the same vein , nuts, such as almonds and sunflower seeds, contain high levels of vitamin E, contributing to their antioxidant properties (Amarowicz & Pegg, 2020; Lopez-Bote et al., 2003).

2.4 The Importance of Prophylaxis in healthcare

Prophylaxis encompasses a range of measures aimed at preventing disease, including medical and sanitary interventions (Blancou, 2006). It is a relevant aspect of public health, with an impact on both individual (patients and healthcare workers) and community well-being (Alipour & Mofarrah, 2022). The range of measures include the use of antibiotics to prevent infection in patients (Hulscher et al., 2010) . For instance, in the case of severe haemophilia .A and B, prophylaxis is a standard of care, involving regular infusions of clotting factor concentrates to prevent bleeding complications and preserve joint health (Fischer et al., 2014). In specific medical conditions, such as venous thromboembolism and HIV infection, appropriate prophylaxis has been shown to be highly beneficial (Dobesh, 2010; Gallant et al., 1994).

Moghadas emphasizes the importance of protecting healthcare workers during an influenza pandemic, highlighting the use of antiviral drugs as a key strategy (Moghadas, 2010; Sinha et

al., 2014) that elaborates the role of systemic antimicrobial prophylaxis in preventing surgical site infections, with a focus on the selection and dosing of antimicrobial drugs (Sinha et al., 2014). Gallant also discusses the importance of prophylaxis in preventing opportunistic infections in patients with HIV, emphasizing the need for efficacy, safety, and cost-effectiveness (Gallant et al., 1994). Weber (2010) summarizes the key components of an effective infection control program, including pre-exposure immunization, adherence to standard precautions, and post exposure prophylaxis (Weber et al., 2010).

Prophylaxis also plays an important role in healthcare, particularly in preventing laboratory-acquired infections (Setiawan, 2011). While some parasitic infections are difficult to control, others can be managed through preventative chemotherapy and vector control (Molyneux, 2006). A holistic approach, including chemotherapeutic intervention, is recommended for addressing intestinal parasite infections (Alum et al., 2010). In the case of malaria, adherence to effective measures for preventing contact with mosquitoes and bites is emphasized, with personal protection measures shown to significantly reduce the risk of infection (Debboun & Strickman, 2013; Durrheim et al., 1999) .Infections, spanning bacterial, viral, and parasitic origins, often promote inflammation and oxidative stress within the body. Bacterial culprits such as *Staphylococcus aureus*, *Escherichia coli*, and *Mycobacterium tuberculosis*, alongside viruses like influenza, hepatitis strains, and HIV, trigger oxidative damage through immune responses and replication processes. Parasitic invaders such as Plasmodium (malaria), Trypanosoma, and Leishmania also contribute to oxidative stress during their life cycles (Iliev et al., 2017). These infections provoke the generation of free radicals and subsequent tissue harm, amplifying the progression of diseases. The interplay between immune reactions, oxidative stress, and these infections elaborates the importance of preventive measures (Roche & Romero-Alvira, 1995). The use of antioxidants to counteract this oxidative stress and prevent tissue damage is a potential strategy for disease prevention and management (Amaral et al.,

2020).

Vaccination, antimicrobial treatments, and lifestyle adjustments that bolster the immune system serve as pivotal defenses against these infections. Moreover, exploring natural compounds from diverse sources, including plants, rich in antioxidant and anti-inflammatory properties, holds promise in fortifying the body's resilience against a spectrum of infections. Perceiving these dynamics remains critical in developing effective preventive and therapeutic strategies. This method has enabled the determination of oxidative stress levels in plasma samples from malaria patients. High levels of oxidative stress have also been detected in mice infected with *P. berghei, P. yoelii, or P. chabaudi* as well as monkeys infected with *P. knowlesi* suggesting that oxidative stress is a widespread phenomenon in Plasmodium (Nneji et al., 2013). There are various sources of host-derived oxidative stress during the blood stage of malaria. These sources may arise either directly from the infection of erythrocytes by *Plasmodium*, such as heme, or from the host's response to the infection, which involves the systemic upregulation of oxidative enzymes and the oxidative burst associated with phagocytic activity (Splettstoesser & Schuff-Werner, 2002).

2.4.1 Plant Medicine as Prophylaxis

Herbal plants have been explored for their potential in offering preventive measures against various health concerns. While not specifically addressing malaria, these plants have been traditionally used and investigated for their prophylactic properties in supporting immune function and potentially preventing infections. Plants have been studied extensively for their potential prophylactic effects on many health issues, including supporting immune function and potentially preventing infections. Many herbs and plants have compounds known for their immunomodulatory properties that could contribute to boosting the body's defense mechanisms against infections (Alhazmi et al., 2021). For instance, plants like echinacea, astragalus, garlic,

and ginger have been traditionally used for their immune-boosting properties. These plants contain bioactive compounds that may help enhance the immune response, although their specific effects on preventing common tropical diseases like malaria have not been extensively studied.

Additionally, plants like *Artemisia annua* (sweet wormwood) have received attention for their anti-malarial properties due to the presence of artemisinin. While traditionally used in Chinese medicine, artemisinin and its derivatives have become a critical part of malaria treatment worldwide. Research on the prophylactic effects of various plants against malaria or other infections often involves investigating their bioactive compounds, understanding their mechanisms of action, and conducting clinical trials to validate their effectiveness and safety (E. Feng et al., 2020). The exploration of plants and their potential prophylactic effects is an ongoing area of research, holding promise for novel preventive measures and supporting overall health and immunity. Echinacea, known for its immune-stimulating effects, has shown promise in reducing the occurrence and severity of common colds and upper respiratory tract infections, possibly serving as a preventive measure against such ailments (Raus, 2013). Garlic, recognized for its antimicrobial properties, particularly its active compound allicin, has historical use suggesting potential as a preventive measure against infections (Bayan et al., 2014). Similarly, elderberry, rich in antioxidants and flavonoids, has demonstrated the ability to shorten the duration and severity of colds and flu-like symptoms, potentially acting as a prophylactic agent against respiratory infections (Tiralongo et al., 2016). Astragalus, valued for its immunomodulatory effects, is being explored for its potential in enhancing immune responses and potentially preventing respiratory infections (Shi et al., 2020). While these herbal plants exhibit promising properties, further research is needed to comprehensively understand their efficacy and safety as preventive agents before integrating them into healthcare practices.

2.4.2 Plant Medicine prophylaxis for malaria prevention

Malaria caused by *plasmodium* parasites and transmitted by *Anopheles* mosquitos continues to claim hundreds of thousands of lives each year, especially in sub-Saharan Africa. The WHO malaria report showed 263 million cases and 597, 000 deaths worldwide in 2023 as compared to 252 million and 600,000 more cases and deaths, in 2022 (WHO, 2023). Preventing the malaria infection before it occurs is a critical strategy in malaria control because up to 80% of people in Africa rely on traditional medicine for primary healthcare, making herbal prophylaxis a practical public health tool in malaria prevention (WHO, 2023).

Several medicinal plants have long been used to prevent malaria, either by repelling mosquitos, increasing immunity, or directly obstructing the growth of the *Plasmodium* parasite. *Cryptolepis sanguinolenta*, a popular West African plant, contains cryptolepine, which has been shown to have strong antiplasmodial activity (Parvatkar et al., 2024; Tona et al., 2004). *Azadirachta indica* (neem) has potential due to its immunostimulant and mosquito-repelling characteristics (Asghar et al., 2022; Udeinya et al., 2004). Another prominent herb is *Artemisia annua*, which has been used in Chinese medicine for centuries and has been shown to be efficient in inhibiting malaria parasites (X. Feng et al., 2020; Tu, 2011). Over the years *in vivo* studies using murine models are crucial for understanding pathogenesis testing the efficacy of antimalarial drugs, vaccines, and plant-based medicines (Chetia, 2019). Mice are infected with rodent-specific Plasmodium species, such as *Plasmodium berghei, P. yoelii, P. chabaudi, and P. vinckei*, which mimic various aspects of human malaria (Otun et al., 2024). These murine models enable researchers to evaluate parasitemia levels, survival rates, immunological responses, and the protective efficacy of preventive medicines. Numerous herbal extracts and drugs have undergone testing using murine models. For instance, a study was done in mice infected with *P. berghei* and treated with *Cryptolepis sanguinolenta* extract. The results showed significantly reduced parasitemia and improved survival, suggesting strong antiplasmodial activity (Eze et al., 2018). *Azadirachta indica*

(neem) and *Artemisia annua* have demonstrated same immunomodulatory and parasite-inhibiting properties in infected mice (Attemene et al., 2018; Willcox & Chamberlain, 2004).

2.5 The COA Plus Mixture and its Constituent Plants

The COA Plus Mixture just like the COA mixture is produced via the methodical distillation of fresh leaves of *Azadirachta indica, Carica papaya, Spondias mombin, Ocimum viride,* and *Persea Americana. Azadirachta indica,* often known as neem, is a plant that has found several uses in the ecological, medical, and agricultural sectors. A study found that extract of *A. indica* leaves at a dose of 200 mg/kg had significant anti-inflammatory activity in rats in a cotton pellet granuloma assay (Chattopadhyay, 1998). Other research has found that neem leaf extract has a significant anti-inflammatory effect, but it is less effective than dexamethasone (Mosaddek & Rashid, 2008). The extracts of the neem tree over the past years have shown to have immense immomodulation functions. A study was also done on 24 adult wistar rats that were given extract of *Spondias mombin* at 400 mg/kg via (Asuquo et al., 2013). The results revealed that *Spondias Mombin* administration improved glucose tolerance and lipid peroxidation, as well as the antioxidant capacity, superoxide dismutase, and glutathione peroxidase activities in the liver of the rats. Another study was conducted to determine if administering *Vernonia amygdalina* and *Carica papaya* plants had synergistic benefits in alleviating plasmodium infection in mice. The study found a substantial ($p<0.05$) reduction in parasite load between the infected treatment groups and the disease control group on day 3 after infection, which remained stable throughout the trial (Okpe et al., 2016).

Azadirachta indica, commonly referred to as neem, has been identified in numerous studies as a significant source of antioxidants. This species belongs to the Meliaceae family and is recognized for its therapeutic properties in health management, attributed to its diverse range of bioactive compounds. Key constituents include azadirachtin, along with other compounds

such as nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin (Singh, 2017). The leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol (Rahmani et al., 2018). Polyphenolic flavonoids isolated from fresh neem leaves include quercetin and ß-sitosterol, which are known to have antibacterial and antifungal activities. Parts of the neem (*Azadirachta indica*) plant have antibacterial properties through microbial growth inhibition and potential cell wall rupturing. Experiments conducted showed the activity of the leaves of neem against *Staphylococcus aureus and* MRSA with a large zone of inhibition when used at 100% concentration of leave extract. The active ingredient of neem, plays a role in preventing disease and infections by the enzymatic action of antioxidants rupturing the cell wall of bacteria and playing a role as chemo preventive, through the regulation of cellular pathways (Rahmani et al., 2018).

Carica Papaya is an herbaceous succulent known as pawpaw and belongs to the Caricaceae family of flowering plants. They are tropical plants found in Central America and Africa. According to recent research, the leaves, fruits, and seeds of the *Carica papaya* plant species contain levels of natural antioxidants (Da Silva et al., 2007) . Caffeic acid, myricetin, rutin, quercetin, -tocopherol, papain, benzyl isothiocyanate (BITC), and kaempferol are among the chemical components that are present in *Carica Papaya* . *Carica Papaya* can therefore inhibit pro-oxidants by activating many of signalling pathways that either increase the expression of antioxidant enzymes or lower ROS generation. These signalling pathways trigger the body's anti-oxidant defense systems, which guard it against both internal and external oxidative stress (Da Silva et al., 2007; Maity et al., 2022). S*pondias mombin*, belonging to the family Anacardiaceous, is a plant native to Brazil, where it is known as "cajá" (Hog plums). It has been observed that extracts isolated from the leaves of the plant contained vitamin E and vitamin C, phenolic acids, flavonoids, tannins and triterpenes which have proven to have anti-

oxidants, anti-inflammatory and antiviral potentials (Corthout et al., 1991; Rahmani et al., 2018). Therefore, the leaves and back of the *S. mombin* are added to drugs and supplements during oxidative stress conditions.

Vernonia amygdalina, also known as bitter leaf, is a perennial shrub in the Asteraceae family that thrives in tropical Africa. It is a medicinal plant in the genus Vernonia that has many phytochemicals (including anti-nutritional factors) is present in the leaves of this plant. The phytochemicals found include terpenes, coumarins, phenolic acids, lignans, xanthones and anthraquinones identified bioactive peptides known as edotides in the leaves *of V. amygdalin.* These phytochemicals are responsible for the plant's abundance of bioactivities. These bioactive components may work singularly or synergistically to achieve the outcomes for which *V. amygadlin* therapeutic values have been extensively researched (Farombi & Owoeye, 2011). In traditional medicine, practitioners use the herb as an anti-helminth, anti-malarial, and laxative. Others make use of it as a digestive tonic, appetizer, febrifuge, and wound treatment (Dalziel, 1937). It was among the first to note that the plant's root and twig are used by the Hausas of Northern Nigeria to treat stomach and gastrointestinal issues, while an extract of the leaves is used to treat malaria fever and cough in Ghana and Guinea.

Ocimum viride, commonly known as basil, is a tender aromatic annual herb indigenous to West Africa and cultivated in India. This herb, along with other aromatic spices, is utilized in culinary applications not only for its distinct flavour and strong aroma but also for its notable medicinal properties, which include antioxidant, anti-inflammatory, antiviral, and antimicrobial effects (Purushothaman et al., 2018). For these properties, extracts from Basil have been formulated into herbal medicines to help boost the immune system and combat diseases. Basil is used fresh in developing nations, particularly Ghana, in food preparations (such as flavouring in chicken soups, sauces, etc.).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

The materials used in this study are detailed in the sections below, with additional information provided in the appendix.

3.1.1 Reagents and Chemicals

Pierce BCA Protein Assay Kit was purchased from Thermo Scientific, USA. The Luminex Discover Assay (Mouse Premixed Multi-Analyte Kit) was purchased from R&D systems. Butylated hydroxytoluene (BHT), Methanol Sigma-Aldrich, N-butanol, Standard MDA solution, Thiobarbituric acid (TBA, Trichloroacetic acid (TCA) were all purchased from the Thermo Fisher Scientific, USA. Doxycycline was obtained from Ernest Chemist Ghana Limited, Giemsa stain was obtained from Sigma-Aldrich, Hydrochloric acid (HCl) and the Phosphate Buffer Saline was purchased from Caisson Laboratories Inc. Pierce. The COA Plus Mixture was obtained from the COA Research and Manufacturing Ltd. Company, Ghana.

3.1.2 Experimental Animals

Thirty-five (35) female pathogen free, five-weeks-old ICR strain mice, were obtained from the Animal Experimentation Unit of the Noguchi Memorial Institute for Medical Research (University of Ghana) and acclimatized to laboratory conditions for one week. The mice were kept at a relative humidity of 50–65% at 20ºC–24ºC and 12 h: 12 h light: darkness and fed on standard pellet diet (GAFCO Chow diet, Accra, Ghana), and sterilized drinking water *ad libitum*.

3.2 Ethical Approval

This study was approved by the University of Ghana Institutional Animal Care and Use Committee (UG-IACUC 022/22-23).

3.3 Methods

The methodologies employed in this study are outlined in the subsequent sections below.

3.3.1 Grouping and treatment regimen

The Thirty-five (35) mice were divided into five groups of seven (7) mice per group (Table 1 below). Group 1 was the Negative Control group (CONTROL), which was only administered distilled water throughout the treatment period (8 weeks). Groups 2 and 3 were the Parasite group (PARA) and Doxycycline group (DOXY) respectively. The animals in these groups were administered distilled water throughout the treatment period and inoculated with 0.2 ml of the *P. berghei* infected blood after the eight weeks treatment. However, the animals in the DOXY group received doxycycline through oral gavage (malaria prophylaxis) at a dose of (0.5 mg/day/mouse) in water (Bayo College of Medicine) for three (3) days before inoculation. Groups 4 and 5 were designated COA+Parasite group (COA+PARA) and COA group (COA) respectively. The animals in these groups were administered COA Plus mixture (0.3 ml/kg body weight) daily for eight weeks after which the animals in group 4 (COA+Parasite) were challenged with *P. berghei* whilst those in the group 5 (COA) were not. Animals were assessed for signs of distress twice daily to ensure their well-being. To assess the effect of the treatments on body weight, the weights of the animals were measured and recorded at the beginning of every week throughout the period of experimentation (8 weeks).

Table 1: Experimental Groups

GROUPS	NUMBER OF ANIMALS	DESIGNATION
GP 1	7	CONTROL
GP 2	7	PARA
GP 3	7	DOXY
GP 4	7	COA+PARA
GP 5	7	COA

3.3.2 Selection and preparation of *Plasmodium berghei* infected blood.

The rodent malaria parasite, *Plasmodium berghei*, was originally obtained from the Animal Experimentation Unit of Noguchi Memorial Institute for Medical Research, Ghana. Per the standard protocol, the parasite had been maintained at the Animal Experimentation Unit by passing them through female ICR strain and preserved through cryoscopic storage. The serial passage was initiated after storage at - 70°C by intraperitoneal (IP) injection into non-experimental normal ICR strain laboratory mice with 0.2 ml blood containing parasites maintained in buffer solution (Basir et al., 2012). They were monitored for 4 days to confirm the growth of the parasites in the animals. The passage was repeated every four days to increase the levels of parasitaemia to at least 35% (Franco et al., 2023). On the day of passage to the experimental mice, parasitaemia was determined and 0.1 ml of blood was collected from the infected mice by cardiac puncture while the animal was anesthetized with chloroform. The blood was diluted with sterile, 0.85% saline to give 2×10^7 PRBC in an injection volume of 0.2 ml. The percentage parasitaemia determined the amount of saline used in the dilution of the infected blood (e.g., if 1 ml of blood was removed, the dilution was with (x-1) ml of saline, where x was the percentage parasitaemia). The experimental mice were then infected by interfemoral injecting of 0.2 ml of 2×10^7 PRBC (Franco et al., 2023; Orr et al., 2012).

3.3.3 Collection of blood and tissue samples

After the treatment period, tail bleeding was done to monitor parasitaemia. The mice were subjected to euthanasia in a chloroform chamber, blood was obtained by cardiac puncture and their organs (heart, spleen, liver, kidneys, and lungs) were harvested and placed in falcon tubes containing phosphate buffer saline on ice. The organs were stored at –4◦C until analysed. Blood from each animal was collected into EDTA tubes for immunology, lipid peroxidation, protein concentration and haematology analysis.

3.3.4 Determination of prophylactic properties.

The level of Parasitaemia was measured in GP 2, GP 3 and GP 4 animals, at 24, 48 and 72 hours after inoculation with the *P. berghei* infected blood using microscopy (Berhan et al. 2012). Thin blood smears were prepared from tail bleeding on the frosted microscope glass sides. The smears were allowed to dry completely on the staining racks and were fixed with absolute methanol for 5 min. The smears were stained with 10% Giemsa Stain solution and was washed with distilled water after 5 min. The stained blood smears were examined under the microscope to determine the level of parasitaemia. The number of parasitized red blood cells (RBCs) per 100 RBCs were counted and the percentage parasitaemia was calculated using the formula below:

$$\text{Parasitaemia (\%)} = \frac{\text{Number of Parasitized RBC}}{\textbf{Total Number of RBCs}} \times 100$$

The blood smear preparation was repeated three times, and a mean value was determined for each mouse to obtain representative data. The specialized counting chamber was used for higher accuracy as well as appropriate controls used to validate the experimental setup and staining procedure.

3.3.5 Determination of in vivo antioxidant activity

To assess the *in vivo* antioxidant properties of the COA Plus mixture, the levels of lipid peroxidation (Malonaldehyde) was measured in the liver of all the groups using the thiobarbituric acid reactive substances (TBARS) assay (De Leon & Borges, 2020; Esterbauer & Cheeseman, 1990).

Determination of Lipid Peroxidation

One (1) g of liver sample from each mouse was weighed, cut into pieces, and suspended in 10 ml of Phosphate Buffer Saline (PBS) containing 10 µL of 0.5 M Butylated hydroxytoluene (BHT) Solution. The mixture was homogenised on ice, to prevent further oxidation during the process. The samples were centrifuged at 15,000 g for 10 min to obtain a clear supernatant. An aliquot (20 µl) of each supernatant was then transferred into labelled Eppendorf tube in duplicates. To each aliquot was added 20 µl of 8.1% SDS. After which 150 µL of 20% acetic acid was added followed by 150 µL of 8% TBA. The reaction mix was topped up to 400 µl with 60 µl distilled water and incubated in a water bath at 95 ^{o}C for 60 min. It was then cooled to room temperature and topped up to 500 µl with 100 µl distilled water. Exactly 500 µl pyridine: butanol (1:15 v/v) was added. The mixture was centrifuged at 3000 rpm for 10 min. The upper layer (150 µL) was transferred to the wells of the microplate in triplicate, and the absorbance read at 532 nm with 1,1,3,3-tetraethoxypropane (TEP) (1.56 – 100 µM) as the standard against a solvent blank using a microplate reader (Varioskan™).

3.3.6 Investigation of the immunomodulatory properties

Blood samples (0.5ml) were collected through cardiac puncture individually from all seven (7) mice in each of the five (5) groups of mice and placed in labelled EDTA tubes. Samples were then

centrifuged at 2,000 g for 20 min and plasma was aliquoted into Eppendorf tubes and stored at -20 °C freezer until ready to use. Frozen plasma samples were centrifuged at 16,000 g for 4 minutes.

Assay Procedure

Per manufacturer's (Biotechne®, Rand D systems- Luminex ® Discovery Assay) protocol**,** all reagents were brought to room temperature before use. Twenty milliliters **(**20 mL) of Wash Buffer Concentrate was added to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer. Calibrator Diluent RD6-52 (275µL) was added to the mouse standard cocktail. The concentrate was labelled as Standard 1 and allowed to sit for 15 min. Serial dilutions of standard one was prepared as shown in Figure 1 below.

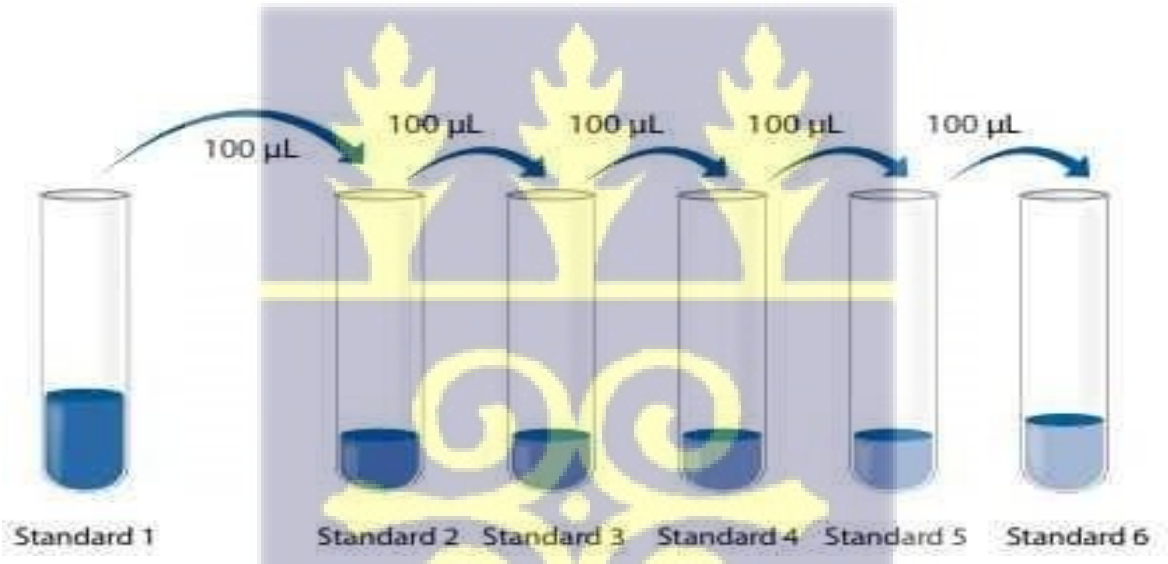


Figure 1: Preparation of standard cocktail dilutions.

Reagent Preparation

Diluted microparticle cocktail preparation

The microparticle cocktail vial was centrifuged for 30seconds at 1000xg prior to removing the cap. The vial was gently vortexed to suspend the microparticle taking precautions not to invertthe vial. For using 57 wells, 296 µl of microparticle cocktail was diluted in the mixing bottle by adding 2.96 ml of the Assay diluent RD1W.

Diluted Biotin-Antibody Cocktail Preparation

The Biotin-Antibody Cocktail vial was centrifuged for 30 seconds at 1000 x g prior to removing the cap. The vial was vortexed taking into precautions not to invert the vial. For using 57 wells, 296 µl of Biotin-Antibody Cocktail was diluted in the mixing bottle by adding 2.96 ml of Assay Diluent RD1W.

Streptavidin-PE Preparation

The Streptavidin-PE vial was centrifuged for 30 seconds at 1000 x g prior to removing the cap. It was gently vortexed-taking precautions not to invert the vial. For 57 wells, it was diluted by adding 130 µl of Streptavidin-PE concentrate in 3.15 ml Wash Buffer in a 15 ml falcon tube wrapped with aluminum foil away from light during handling and storage

Fifty microlitres (50 µl) each of standards and samples were aliquoted into respective wells in duplicates (Figure 2). The diluted Microparticle Cocktail was resuspended by vortexing and 50 µl was added to each well of the microplate. A foil plate sealer was used to cover the microplate securely. The plate was incubated for 2 h at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. After incubation, a magnetic device designed to accommodate a microplate, was used to wash by applying the magnet to the bottom of the microplate, allowing one (1) minute before removing the liquid, filling each well with Wash Buffer (100 µL) and allowing another minute before removing the liquid again. The wash procedure was performed three times. Fifty microlitres (50 µl) of diluted Biotin- Antibody Cocktail was added to each well, covered with a foil plate sealer and incubated for 1 h at room temperature on the shaker set at 500 ± 50 rpm. The wash procedure was repeated three times after incubation. Fifty microlitres (50 µl) of diluted Streptavidin-PE was added to each well and securely covered with a foil plate sealer and incubated for 30 min at room temperature on the shaker set at 500 ± 50 rpm. The wash procedure was again repeated three times and the

microparticles were suspended by adding 100 µl of Wash Buffer to each well. It was then incubated for 2 min on the shaker set at 500 ± 50 rpm. The concentration was read within 90 min using a Luminex® analyser with wavelength set at 570nm (Chitnis et al., 2011).

Figure 2: Assay Plate

3.3.7 Determination of the Effect of COA Plus mixture on selected Haematological Parameters

Whole blood from each animal was collected into EDTA tubes for haematological analysis. The whole blood samples of the mice were analyzed at the Noguchi Memorial Institute for Medical Research using an automated haematology analyzer (Sysmex KX-21 Haematology Analyzer) to determine the following haematological parameters: erythrocyte count (RBC), mean corpuscular volume (MCV), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), platelet count (PLT), haematocrit (HCT), mean platelet volume (MPV), red cell distribution width (RDW), and RDW expressed with standard deviation (RDW SD). Additionally, platelet distribution width (PDW), lymphocyte (LYM), neutrophils (NEU), and total white blood cells (WBCs) were analyzed.

3.4 Data analyses

Statistical analysis was performed using Excel and the GraphPad Prism software (version 3.3.1). All data was expressed as mean ± standard error of the mean (S.E.M.). Kruskal Wallis was used to estimate the significance among the five (5) study groups. Dunn's multiple comparison test was used for multiple pairwise comparisons of groups where applicable. Organ weights and haematology parameters were analysed using ANOVA test and Dunn's multiple comparison test was used for multiple pairwise comparisons of groups where applicable. This was done using the RM one-way Anova Multiple-Sample Comparison Analysis software to compare significant differences, correlations and homogeneity between the parasitaemia, optical densities of the immunological biomarkers and lipid peroxidation.

According to variance equality, one-way ANOVA test with Tukey's HSD or Games-Howell post hoc tests was applied for comparing between the mice groups. P value < 0.05 indicated statistical significance.

CHAPTER FOUR

4.0RESULTS

4.1 Effect on Immunological Markers

The levels of IL4, IL10 and INF-gamma in the study groups are shown in Figures 3, 4 and 5 below. As shown in Figure 3, the levels of IL-4 in the COA and the COA+PARA groups were significantly lower ($p = 0.0137$ and $p = 0.0319$ respectively) than the PARA group but only slightly lower than the CONTROL group ($p > 0.05$). DOXY group on the other hand had significantly lower IL-4 values than both the CONTROL ($p = 0.0434$) and the PARA groups ($p = 0.0144$). This indicates that all the groups that were treated (drug or herbal mixture) had lower IL-4 levels than those that were not treated.

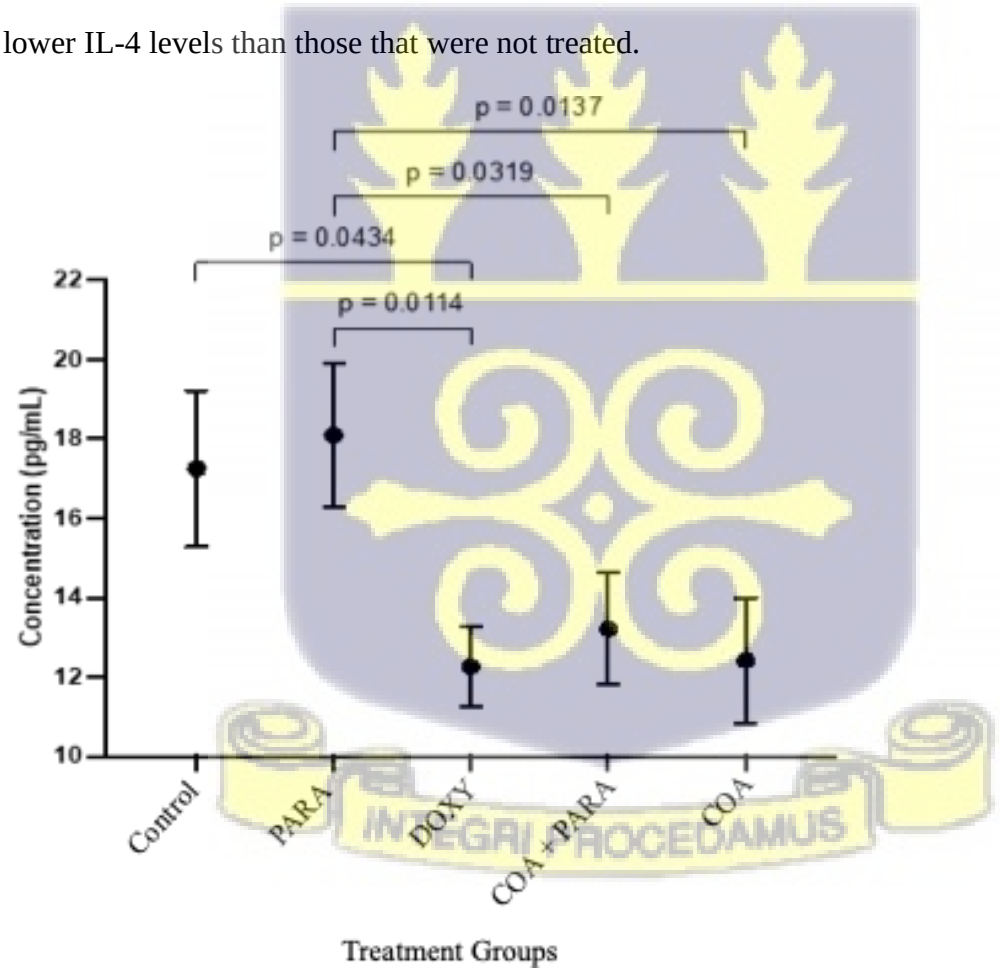


Figure 3: Levels of Interleukin-4 (IL-4)

In the case of IL-10, the level in the COA group was comparable to that of the CONTROL. Interestingly, the level of IL-10 was significantly higher in the COA+PARA compared to the CONTROL ($p < 0.0001$), the PARA ($p = 0.0009$) and the COA groups ($p < 0.0001$). Thus, treatment with the COA Plus mixture before inoculation with the parasite resulted in a significant increase in IL-10 levels. The DOXY group also had significantly higher IL-10 levels than the CONTROL ($p = 0.0005$), the PARA ($p = 0.0250$) and the COA ($p = 0.0001$) groups (Figure 4).

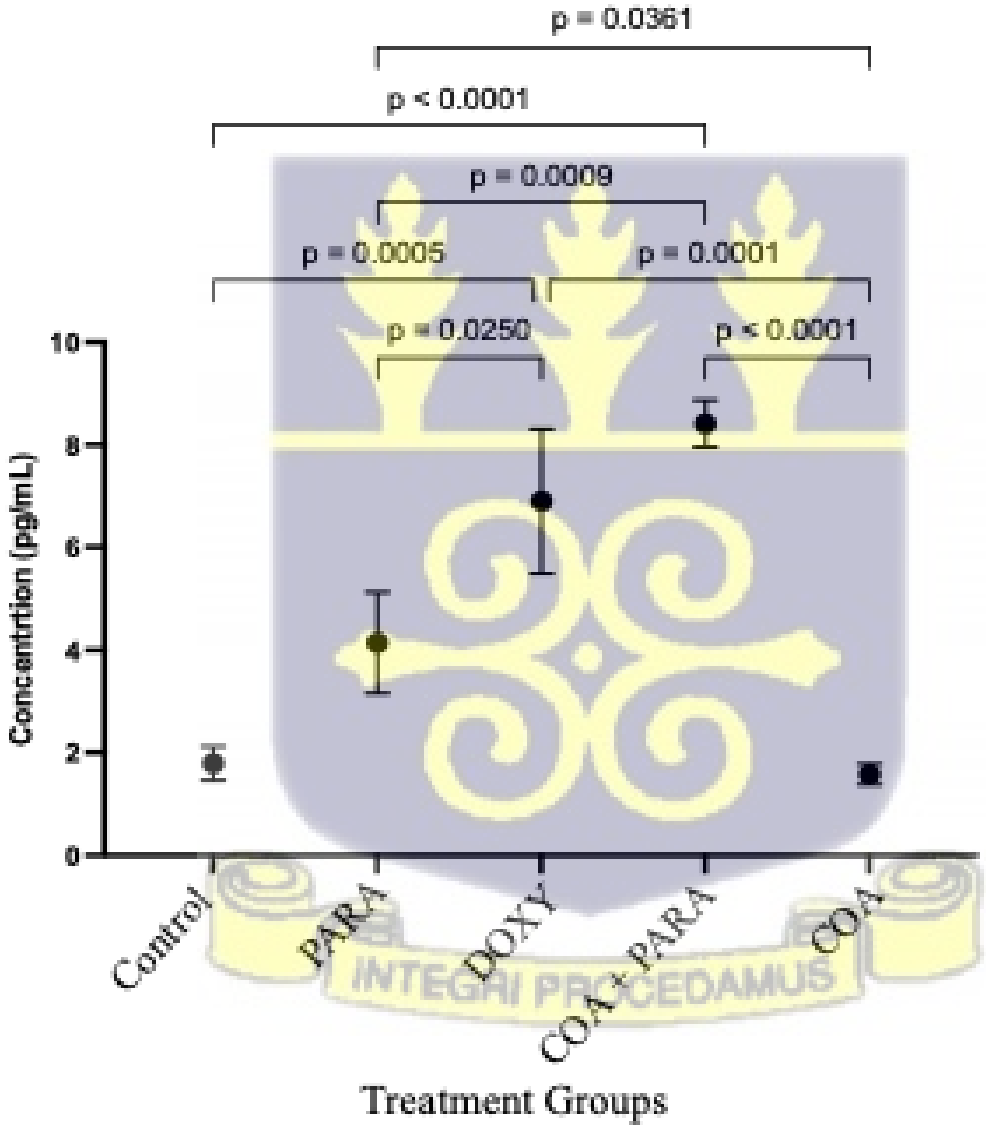


Figure 4: Levels of Interleukin-10 (IL-10) in mice

The level of IFN-γ in the COA group was comparable to the CONTROL group whilst the COA+PARA group had significantly higher IFN-γ than the CONTROL (p = 0.0188) and the COA (p = 0.0135) groups implying that treatment with the COA Plus mixture before inoculation with the parasite resulted in a significant increase in IFN-γ levels. The PARA group also had a significantly higher IFM-γ than CONTROL (p = 0.0079) and the COA (p = 0.0052) groups. Similarly, the DOXY group had a significantly higher IFN-γ than CONTROL (p = 0.0016) and the COA (p = 0.0011) groups. Thus, the levels of IFN-γ was significantly higher in all the groups inoculated with the parasite than the non-inoculated groups (Figure 5).

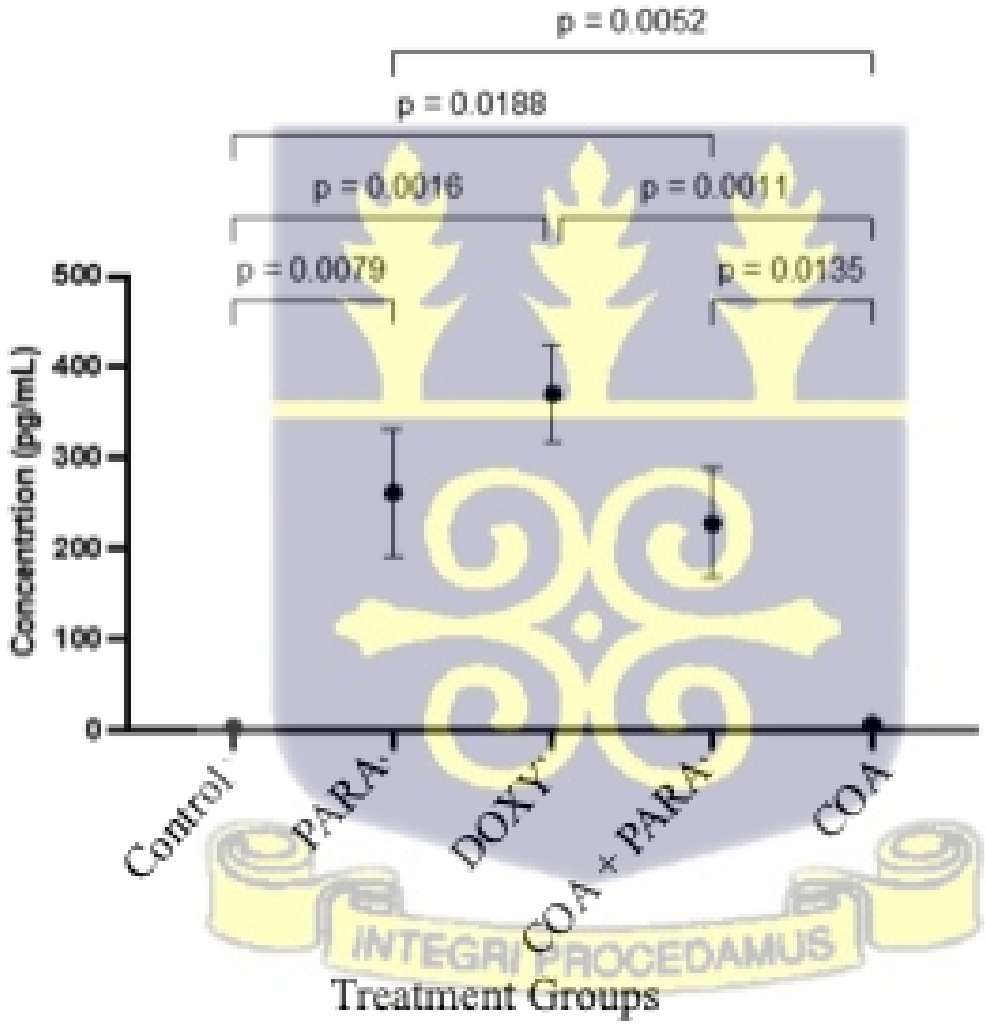


Figure 5: Levels of Interferon-gamma (IFN-γ) in mice.

4.2 Effect on Parasitaemia

The effects of treatment with the COA Plus mixture and Doxycycline on the level of parasitaemia in the *P. berghei* mice were as shown in Figures 6 and 7. Figure 6 shows the results of the time course (24, 48 and 72 h) analysis of parasitaemia among the study groups while figure 7 represents the parasitaemia after 72 h of inoculation. The parasitaemia increased consistently in the PARA group over the time course reaching 3% after 72 h. The parasitaemia in the COA+PARA group was highest (1%) after 24 h but reduced to almost 0% after 72 h. In the case of the DOXY group, the parasitaemia increased from almost 0% after 48 h to 1.5% after 72 h of inoculation. The level of parasitaemia was significantly lower in the COA+PARA group compared to the PARA group ($p = 0.0006$) but not the DOXY group. Thus, treatment with the COA Plus mixture before inoculation resulted in an increase in the rate of clearance of the parasites after 72 h compared to the doxycycline, which rather protected the animals initially and the effect reduced with time leading to increased parasitaemia from about 0% on day 1 to about 0.5% after 72 h.

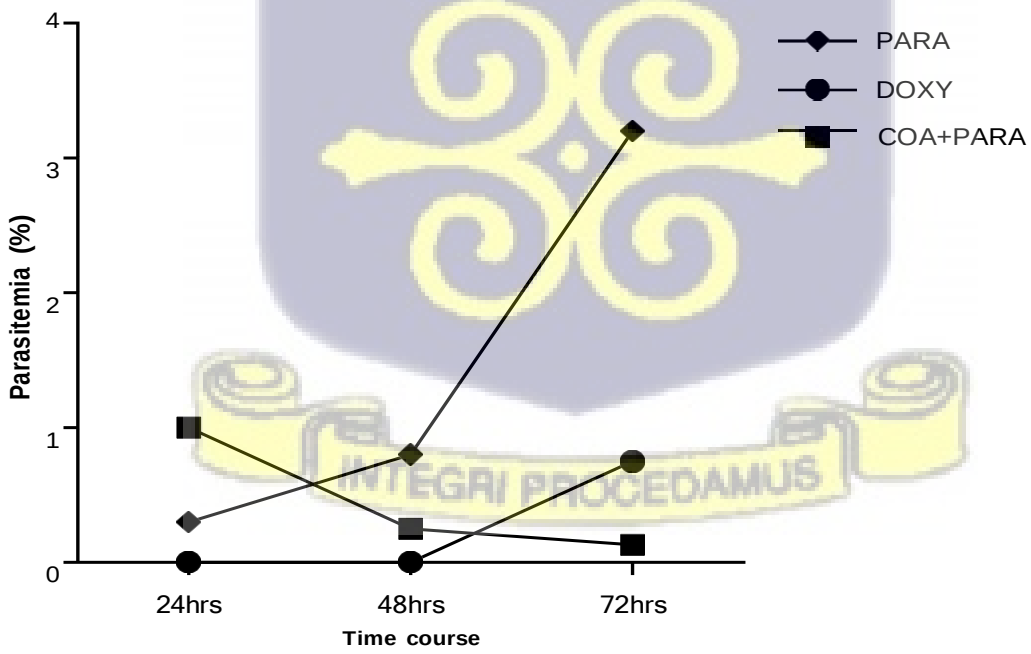


Figure 6: Time course analysis of parasitaemia

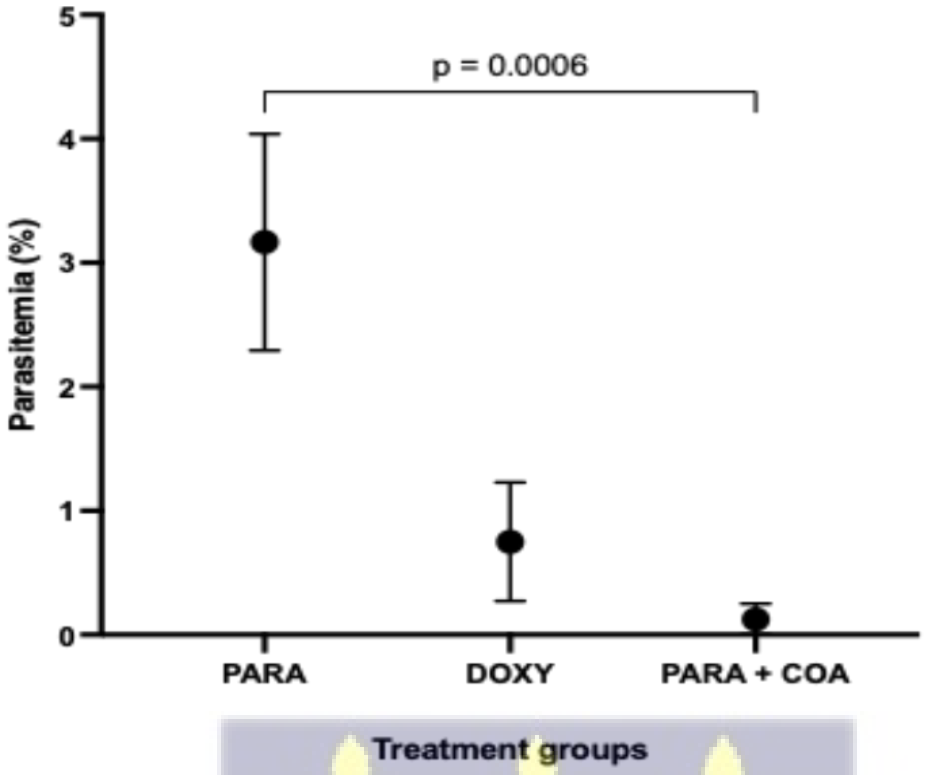


Figure 7: Parasitaemia at 72 hours after inoculation with *P. berghei*

4.3 Effect on Lipid Peroxidation

Figure 8 shows the level of lipid peroxidation in the liver of the experimental animals at the end of the treatment period. Treatment with the doxycycline or the COA Plus mixture resulted in significant increase in malonyl dialdehyde (MDA) levels compared to the CONTROL and the PARA group. That is, the DOXY group had significantly higher MDA level compared to the CONTROL ($p = 0.0004$) and the PARA ($p = 0.0152$) groups, the COA group had significantly higher MDA level compared to the CONTROL ($p < 0.0001$) and the PARA ($p = 0.0044$) groups and the COA+PARA group had significantly higher MDA level compared to the CONTROL ($p < 0.0001$) and the PARA ($p = 0.0028$) groups. The MDA levels among the groups that were treated with doxycycline or COA Plus mixture were not significantly different from each other.

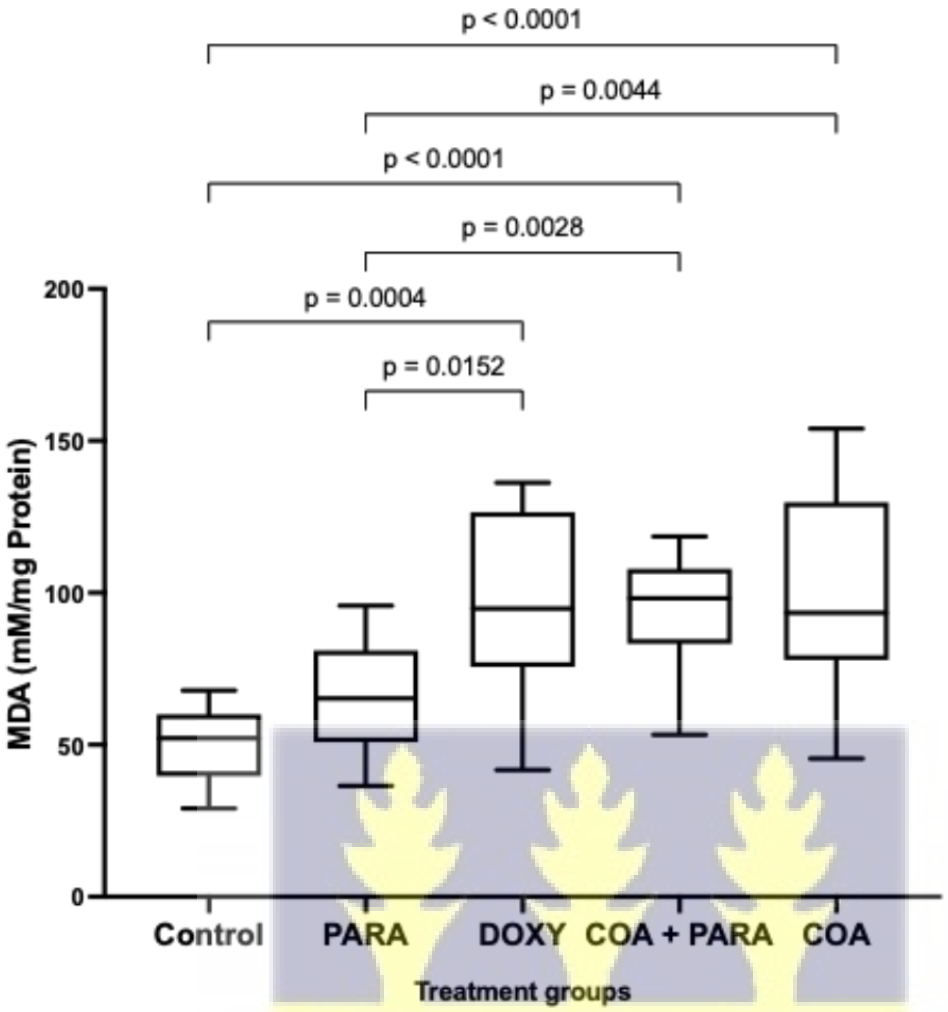


Figure 8: Level of Malondialdehyde (MDA) in Liver

4.4 Effect on Haematological Parameters

Table 2 below shows the values of selected haematological parameters in the test groups compared to controls. Overall, apart from the platelets, which were significantly higher in the COA group than CONTROL, treatment with COA did not result in significant changes in any of the other haematological parameters. However, the levels of NEUT were significantly higher in the COA+PARA group than the CONTROL. The DOXY group had significantly higher RBCs, LYMP and MXD compared to the CONTROL and the PARA group had significantly higher levels of NEUT than the CONTROL.

Table 2: Haematology Parameters

HAEMATOLOGICAL PARAMETERS	STUDY GROUPS					
	Control	**PAR**	**DOX**	**COA-Parasite**	**COA**	**p-value**
WBC (10^3µL)	8.16±0.65	6.71±1.3	6.35±0.53	15.39±8.46	11.66±2.33	0.23
RBC(x10^6/µL)	6.29 ±1.4[a]	7.4 ±053[a]	8.82 ± 0.11[b]	8.30±0.19[a,b]	7.79±0.34[a]	<0.05
NEUT# (x 10^3/µL)	1.26 ± 0.17[a]	3.33 ± 0.99[b]	2.32 ± 0.32[a,b]	3.34±0.42[b]	2.08±0.66[a,b]	<0.05
LYMP# (x 10^3/µL)	6.66 ± 0.64[a]	4.62 ± 0.94[a,b]	3.68 ± 0.26[b]	4.76±1.23[a,b]	5.93±1.47[a,b]	<0.05
MXD#(x 10^3/µL)	0.78 ± 0.55[a]	0.82 ± 0.45[a]	3.68 ± 0.26[b]	1.22±0.70[a,b]	1.20±0.96[a]	<0.05
HGB (g/dL)	12.06 ± 0.80	10.93 ± 0.75	12.85 ± 0.75	9.06±4.3	12.20±0.68	0.53
PLT (10^3µL)	1174± 229.7[a,b]	838.2 ± 287.0[a]	681.3 ± 322.0[a]	971.3±207.1[a,b]	1763±115.8[b]	<0.05

The data in the table above are represented as mean and standard error of mean. P value was obtained by Kruskal-Wallis and ANOVA. A post hoc test was done by Dunn's Multiple Test to detect significant differences between paired groups. In each case, there is no significant difference for values with the same alphabet ([a, b]). Significant values are all $p<0.05$.

4.5 Effect of COA on Mean Organ Weight

The mean organ weights at the end of the study period are detailed in Table 3. There were no significant differences in the mean weight of all the organs (heart, spleen, lung, liver, right kidney and left kidney) among the groups (p>0.05). The mean weight of all the organs in all the treatment groups were comparable to the CONTROL.

Table 3: Mean Organ Weights

	MEAN WEIGHTS OF ORGANS (KG)					
ORGANS	**Control**	**PARA**	**DOXY**	**COA+PARA**	**COA**	**p-value**
HEART	0.10 ± 0.00	0.12 ± 0.02	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.53
SPLEEN	0.12 ± 0.02	0.22 ± 0.06	0.20 ± 0.00	0.32 ± 0.15	0.20 ± 0.03	0.36
LUNG	0.26 ± 0.02	0.27 ± 0.04	0.25 ± 0.03	0.28 ± 0.08	0.18 ± 0.02	0.30
LIVER	1.22 ± 0.06	1.38 ± 0.16	1.25 ± 0.05	1.34 ± 0.10	1.36 ± 0.09	0.76
RIGHT KIDNEY	0.10 ± 0.00	0.13 ± 0.02	0.13 ± 0.03	0.14 ± 0.03	0.12 ± 0.02	0.64
LEFT KIDNEY	0.10 ± 0.00	0.13 ± 0.05	0.13 ± 0.05	0.12 ± 0.02	0.12 ± 0.04	0.75

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

This section discusses the effect of the COA Plus mixture on immunomodulatory, antioxidant, and prophylactic effects of *plasmodium berghei*. Emphasis is placed on immune response, modulation of oxidative stress as indicated by malondialdehyde (MDA) levels, and its potential in preventing malaria infection.

5.1.1 The immunomodulatory effect of COA mixture

The study assessed the effect of the COA Plus mixture on a spectrum of markers in immune responses. The observed effects were diverse across the selected immunological markers (IFN γ, IL-4 and IL-10).

IL-4 levels are usually elevated during acute malaria but decrease during convalescence (Kotepui et al., 2022). This study showed significantly lower levels of IL-4 in study groups treated with the COA Plus mixture indicating an immunomodulatory effect of the herbal mixture. This immunomodulatory effect of COA is similar to what was observed in the standard drug, doxycycline. Other studies have shown some traditional herbal treatments ability to reduce inflammation, balance cytokine levels and subsequently resulting in lower Il-4 production (Li et al., 2020; Spelman et al., 2006). This is consistent with COA used in this study. In the group challenged with only malaria parasite (PARA), elevated IL-4 levels were observed during acute malaria infection, which is part of the immune system's Th2 response to the malaria parasite. IL-4 is also an important cytokine that plays a role in promoting the activation and differentiation of naïve T cells into Th2 cells resulting in the stimulation of B cell activity (Okada et al., 2003). The reduction of IL-4 levels in the COA treated groups

indicates the influence of the herbal mixture on cytokine production. Studies have shown that herbal natural extracts which reduce cytokine profiles is beneficial in balancing Th1/Th2 responses (Ahui et al., 2008). When the Th1/Th2 responses are balanced, the immune system can respond effectively to a wide array of pathogens while mitigating harmful effects such as excessive inflammation, autoimmunity and allergies (Berger, 2000).

Th2 cytokines including IL-4 is known to defend against extracellular pathogens such as parasites and promote antibody production (Zhu, 2015). Elevated levels of IL-4 are usually associated with allergic diseases thereby contributing to IgE antibody production which plays a crucial role in allergic reaction (Philips et al., 2018). The ability of COA herbal mixture to reduce IL4 levels, not only shows promise in helping the immune system against malaria, but also beneficial in managing allergies, airway hyper responsiveness and inflammation in asthma patients.

IL-10 is an inflammatory cytokine that mitigates excessive immune responses and prevents tissue damage during malaria (Ouyang et al., 2011). When infected with malaria parasites, the immune system defends the body by elevating IL-10 levels. This is consistent with the observation in this study where the PARA group had elevated level of IL-10. Treatment with the COA Plus mixture prior to inoculation with the parasite (COA+PARA), caused a significant increase in the elevated level of IL-10 levels indicating the benefits of the herbal mixture. Doxycycline, the standard drug, also showed similar trends as the COA Plus mixture. This suggest that both the herbal mixture and the doxycycline have immunomodulatory effects that enhances the immune system to produce more IL-10 upon encountering of the parasite in the body. The potential of the COA Plus mixture to elevate IL-10 levels when challenged with malaria parasites could mitigate the inflammatory damage that accompanies the malaria disease, thus promoting recovery and reducing the risk of severe complication (Niikura et al., 2011).

IFN-γ levels were significantly higher in study groups treated and challenged with malaria parasites (COA+PARA and DOXY). IFN-γ plays an important role in controlling malaria parasite by activating macrophages and natural killer (NK) cells which are responsible for clearing plasmodium-infected red blood cells during malaria infection (Pombo et al., 2002). During the early stages of malaria infection, the Th1 response, dominated by IFN-γ is activated to prevent the parasite from multiplying within host cells. Some herbal mixtures have been demonstrated to regulate the immune system by promoting Th1 cytokines, including IFN-γ, thereby strengthening the body's ability to fight infections (Spelman et al., 2006) . The COA Plus mixture maintained the levels of IFN-γ after mice were treated and challenged with parasites while the standard drug showed a slight increase in the levels of the cytokine. This is probably to manage inflammation whiles promoting other cytokines such as IL-10, which is an anti-inflammation. As observed in the CONTROL and the COA groups, it is expected that IFN-γ levels would remain at normal levels when not challenged with parasitic infection, as no Th1 activation

5.1.2 The prophylactic properties of COA Plus mixture against P. berghei

The study also evaluated the preventive effects of COA Plus mixture against *P. berghei* infection. In the COA+PARA group, Parasitaemia count was initially high (1% at 24 hours) but dropped to nearly 0% after 72 h. This reduction in parasite burden can be attributed to the COA Plus mixture's ability to boost the immune system. The elevated levels of IL-10 and reduced level of IL-4 in COA + PARA group could be associated with the reduction in parasite burden. IL-10 is regarded as necessary for suppressing severe pathology during *Plasmodium* infection by reducing the parasite burden (Niikura et al., 2011). Some herbal compounds possess antimalarial effects, which aid in eradicating malaria parasites. For instance artemisinin derivatives from *Artemisia annua* are well-known for their antimalarial properties (Li et al., 2020) .The constituents of the COA Plus mixture like neem tree contain

comparable bioactive compounds that target plasmodium parasites directly. A study was conducted on the antiplasmodial activity of neem, finding significant effectiveness in clearing *Plasmodium berghei* in infected mice at certain doses (Biswas et al., 2002). Some herbal mixtures are also known to improve innate and adaptive immunity, delivering a faster approach and organised response to infections (Spelman et al., 2006).

In the DOXY group, parasitaemia levels at the onset remained low (approximately 0%) but increased to 1.5% after 72 h, which suggests that doxycycline provided an initial protection, but the efficacy reduced over time. Studies have shown that Doxycycline is known to suppress protein synthesis which is often used as a prophylaxis drug against malaria (Wasko, 2016). However, the drug works at a much slower pace and does not rapidly eradicate the parasites. It is known to interfere with parasite growth, which explains why levels where low from the on-set and increased after the drug concentration decreased within the three-day therapy period. The reduced efficacy of Doxycycline over the 72 hours results from its dependency on immune clearance mechanisms to fully eliminate parasites, which could be less efficient as compared to the immune response seen in the COA+PARA group. Another reason can stem from Doxycycline's primary effect which is antibiotic rather than immunomodulatory (Ruh et al., 2017).

5.1.3 In vivo antioxidant properties of the COA Plus mixture

Treatment with the standard drug, doxycycline and the COA Plus mixture resulted in significant increase in malondialdehyde (MDA) levels as compared to the CONTROL and the PARA group. This development can be attributed to the oxidative stress induced by the treatments and the immune response to malaria infection in the COA+PARA group. Lipid peroxidation is the oxidative degradation of lipids, which leads to formation of by-products like malondialdehyde (MDA) (Ayala et al., 2014). There are herbal products that contain

antioxidants, which aid in neutralization of free radicals and reduction of oxidative stress. On the other hand, some herbal remedies from the on-set act as pro-oxidants by increasing reactive oxygen species (ROS) and oxidative stress markers like MDA, activating an immune response.

If the herbal products like COA, eventually leading to an antioxidant response after the initial period of oxidative stress, the holistic effect will be protective for the body (Poljšak & Milisav, 2012) . Artemisinin, a traditional Chinese treatment for malaria, also promotes the production of ROS, which in turn raises MDA levels. Acting as a prooxidant in certain situations, it generates ROS that can induce apoptosis in some medical conditions, similar to the impact of the COA Plus mixture on MDA levels (Efferth, 2009).

The increase in MDA in the COA group will also act as a form of defense for the immune system by training it to assist the body prepare for more serious infections (Ristow & Zarse, 2010) . Doxycycline is an antibiotic drug that is also known to produce reactive oxygen (ROS) as part of the mechanism for clearing parasites and bacteria (Clemens et al., 2018) .The significantly higher MDA level in the DOXY group demonstrates an increase in oxidative stress induced by the therapeutic effects of the drug, which is consistent with studies that demonstrate that doxycycline increases oxidative stress, including MDA, as part of its mode of action (Shan et al., 2022)(Rahman et al., 2020).

In the COA+PARA group, MDA levels were higher as compared to the control and the PARA group. This increase can be attributed to the combined effect of the herbal treatment (immunomodulatory) and the malaria parasites causing oxidative stress, as malaria itself causes oxidative damage through the formation of free radicals and the body's immune response (Becker et al., 2004). The PARA group had lower MDA levels as compared to the DOXY and COA-treated groups. Some studies have demonstrated the mechanism of the plasmodium parasite to change the host's system and mitigate oxidative stress in order to extend their

survival rate which can account for the lower MDA levels (Kurtzhals et al., 1998). The effects of COA and DOXY treatments may have enhanced immune responses, leading to higher levels of MDA. The PARA group's lower MDA levels in comparison demonstrated an absence of the oxidative effect, emphasizing the importance of therapeutic interventions in modulating oxidative stress.

5.1.4 Effect of the COA Plus mixture on haematological profile

Overall, haematological parameters did not show significant differences in study groups. However, platelets counts were significantly elevated in the COA groups compared to the other groups. This affirms the immune signalling (release of chemokines and cytokines) properties of the COA Plus mixture. Even though the standard drug showed similar effects on the haematological parameters, the COA Plus mixture was more effective in most cases. Some herbal compounds are known to influence haematopoiesis, for instance, studies have demonstrated that herbal mixtures can improve platelet counts, usually in conditions like thrombocytopenia (Tan et al., 2022). Neutrophils play an important role in the initial stages of an immune response, especially when a pathogen has been introduced into the body (Malech et al., 2014) . The higher levels of NEUT in both the COA+PARA and PARA compared to the control demonstrates an active immune response to the malaria parasite evidenced by the release of neutrophils to the site of infection, which includes the blood stream in the case of malaria. Some herbal remedies have been demonstrated to stimulate neutrophil activity, increasing the body's defense against pathogens (Spelman et al., 2006).

The malaria parasite causes a destruction of the red blood cells resulting in anaemia in infected individuals (Paul & Brey, 2003).

5.15. Effect of the COA Plus mixture on Organ weight

The organs that were harvested maintained standard weights across all groups. There was no significant difference in the treatment groups. The elevated liver weight in the PARA group can be as a result of parasite sequestration and clearance of harmful substances during malaria infection, (Sowunmi, 1996) which is termed as hepatomegaly (an enlargement of the liver). This is usually characterized by immune cell infiltration by the Kupffer cells and lymphocytes (Wynn et al., 2013).

5.2 Conclusion

The findings from this study demonstrate the immunomodulatory properties of the COA Plus mixture, particularly its ability to modulate cytokine profiles by reducing IL-4 levels and enhancing IL-10 and IFN-γ production. These effects suggest that the COA Plus mixture supports immunological function. Additionally, the COA Plus mixture showed efficacy in enhancing the rate of clearance of *P. berghei* parasite. The observed increase in malonyl dialdehyde (MDA) levels indicates its potential to balance oxidative stress. Collectively, these results position COA Plus mixture as promising natural alternative to conventional treatments like doxycycline, offering therapeutic benefits as both an immune enhancer and a prophylactic agent.

5.3 Strengths and Limitations

The study was meticulously designed to investigate the role of specific cytokines, namely interleukin-4 (IL-4), interferon-gamma (INF-γ), and interleukin-10 (IL-10), given their critical involvement in immune responses and potential impact on disease progression. These cytokines were carefully selected due to their significant influence on immune mechanisms. The research focused exclusively on malaria as the disease condition of interest, enabling a

comprehensive and in-depth analysis of the interactions between these cytokines within the context of malaria infection. This targeted approach provided valuable insights into the complex immune responses associated with malaria, thereby contributing to a deeper understanding of the disease's pathogenesis and offering potential directions for the development of future preventive strategies.

Incorporating additional immune markers and considering a broader range of disease conditions could have significantly expanded the scope of the study. This approach would allow for a more comprehensive analysis of the immune system's response across different disease conditions and enhance the generalizability of the findings, making the study's conclusions more robust and applicable to a wider range of medical conditions.

5.4 Recommendations

The COA Plus mixture shows promise as an immune booster, other immune markers are recommended for further studies. A comprehensive analysis of cytokines including TNF-α, IL-6, and IL-12 can shed light on how the COA Plus mixture affects the immunological response to malaria and the general immune system. Further studies could be done to identify the active compounds in the COA Plus mixture that causes immunomodulatory effects. The protective effect of the COA Plus mixture can be explored against other infections. The COA Plus mixture can be considered as a supportive treatment for malaria infections during combination therapy with other proven traditional antimalarial drugs. Further clinical trials can be done in immunocompromised individuals to identify the possible beneficial benefits that it may have for individuals with co infections and weakened immune systems.

REFERENCES

Abdulkhaleq, L., Assi, M., Abdullah, R., Zamri-Saad, M., Taufiq-Yap, Y., & Hezmee, M. (2018). The crucial roles of inflammatory mediators in inflammation: A review. *Veterinary world*, *11*(5), 627.

Abdullahi, A. A. (2011). Trends and challenges of traditional medicine in Africa. *African journal of traditional, complementary and alternative medicines*, *8*(5S).

Absolon, K. (2010). Indigenous wholistic theory: A knowledge set for practice. *First peoples child & family review*, *5*(2), 74-87.

Adebayo, G., Ayanda, O. I., Rottmann, M., Ajibaye, O. S., Oduselu, G., Mulindwa, J., Ajani, O. O., Aina, O., Mäser, P., & Adebiyi, E. (2025). The Importance of Murine Models in Determining In Vivo Pharmacokinetics, Safety, and Efficacy in Antimalarial Drug Discovery. *Pharmaceuticals*, *18*(3), 424.

Ahenkan, A., & Boon, E. (2008). Impact of deforestation on medicinal plants in Ghana. In: GRIN Verlag.

Ahlemeyer, B., & Krieglstein, J. (2003). Neuroprotective effects of Ginkgo biloba extract. *Cellular and Molecular Life Sciences CMLS*, *60*, 1779-1792.

Ahmad, S., Akram, M., Riaz, M., Munir, N., Mahmood Tahir, I., Anwar, H., Zahid, R., Daniyal, M., Jabeen, F., & Ashraf, E. (2023). Zootherapy as traditional therapeutic strategy in the Cholistan desert of Bahawalpur-Pakistan. *Veterinary Medicine and Science*, *9*(4), 1861-1868.

Ahmed, T. (2021). *Contributions of traditional medicine in health care delivery system in the west mamprusi municipality*

Ahui, M. L. B., Champy, P., Ramadan, A., Van, L. P., Araujo, L., André, K. B., Diem, S.,

Damotte, D., Kati-Coulibaly, S., & Offoumou, M. A. (2008). Ginger prevents Th2-mediated immune responses in a mouse model of airway inflammation. *International immunopharmacology*, *8*(12), 1626-1632.

Aktas, E., Ciftci, F., Bilgic, S., Sezer, O., Bozkanat, E., Deniz, O., Citici, U., & Deniz, G. (2009). Peripheral immune response in pulmonary tuberculosis. *Scandinavian Journal of Immunology*, *70*(3), 300-308.

Alberts, B., Bray, D., Hopkin, K., Johnson, A. D., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2015). *Essential cell biology*. Garland Science.

Aldred, S. (2020). Nitric Oxide Synthase (NOS). *Encyclopedia of Behavioral Medicine*, 1506-1507.

Alexander, K. P., Anstrom, K. J., Muhlbaier, L. H., Grosswald, R. D., Smith, P. K., Jones, R. H., & Peterson, E. D. (2000). Outcomes of cardiac surgery in patients age≥ 80 years: results from the National Cardiovascular Network. *Journal of the American College of Cardiology*, *35*(3), 731-738.

Alhazmi, H. A., Najmi, A., Javed, S. A., Sultana, S., Al Bratty, M., Makeen, H. A., Meraya, A. M., Ahsan, W., Mohan, S., & Taha, M. M. (2021). Medicinal plants and isolated molecules demonstrating immunomodulation activity as potential alternative therapies for viral diseases including COVID-19. *Frontiers in immunology*, *12*, 637553.

Alipour, M., & Mofarrah, R. (2022). Probiotics for treatment of helicobacter pylori infections and gastric cancer prevention. *Journal of Genetic Resources*, *8*(2), 244-254.

Alleyne, G., Breman, J., Claeson, M., Evans, D., Jamison, D., Jha, P., Measham, A., Mills, A., & Musgrove, P. (2006). Disease control priorities in developing countries. In: World Bank/OUP.

Alum, A., Rubino, J. R., & Ijaz, M. K. (2010). The global war against intestinal parasites—should we use a holistic approach? *International journal of infectious diseases*, *14*(9), e732-e738.

Alves, R. R., & Rosa, I. M. (2007). Biodiversity, traditional medicine and public health: where do they meet? *Journal of ethnobiology and ethnomedicine*, *3*, 1-9.

Amaral, A. L., Mendonça, A. M., Giolo, J. S., Costa, J. G., Mariano, I. M., de Souza, T. C., Batista, J. P., Rodrigues, M. L., de Souza, A. V., & Caixeta, D. C. (2020). The effects of isoflavone supplementation plus combined exercise on salivary markers of oxidative stress in postmenopausal women. *Journal of Clinical Biochemistry and Nutrition*, *66*(1), 43-48.

Amarowicz, R., & Pegg, R. B. (2020). Tree nuts and peanuts as a source of natural antioxidants in our daily diet. *Current pharmaceutical design*, *26*(16), 1898-1916.

Anyinam, A. C. I. (1999). ETHNOMEDICINE, SACRED SPACES. *Sacred Spaces and Public Quarrels: African Cultural and Economic Landscapes*, 127.

Arshad, M. S., Khan, U., Sadiq, A., Khalid, W., Hussain, M., Yasmeen, A., Asghar, Z., & Rehana, H. (2020). RETRACTED: Coronavirus disease (COVID-19) and immunity booster green foods: A mini review. *Food Science & Nutrition*, *8*(8), 3971-3976.

Asghar, H. A., Abbas, S. Q., Arshad, M. K., Jabin, A., Usman, B., Aslam, M., & Asghar, A. (2022). Therapeutic potential of Azadirachta indica (Neem)-a comprehensive review. *Sch Int J Tradit Complement Med*, *5*(3), 47-64.

Asuquo, O., Udonwa, U., Eluwa, M., & Ekanem, T. (2013). Effects of Spondias mombin leaf extract on the cytoarchitecture of the cerebal cortex and on learning and memory in Wistar Rats. *International Journal of Science and Research*, *2*(9), 5-8.

Attemene, S. D. D., Beourou, S., Tuo, K., Gnondjui, A. A., Konate, A., Toure, A. O., Kati-Coulibaly, S., & Djaman, J. A. (2018). Antiplasmodial activity of two medicinal plants against clinical isolates of Plasmodium falciparum and Plasmodium berghei infected mice. *Journal of parasitic diseases*, *42*, 68-76.

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative*

medicine and cellular longevity, *2014*(1), 360438.

Balick, M. J., & Cox, P. A. (2020). *Plants, people, and culture: the science of ethnobotany*. Garland Science 1003049079.

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C.-M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. *Molecules*, *27*(4), 1326.

Bandyopadhyay, D. (2021). Topical antibacterials in dermatology. *Indian journal of dermatology*, *66*(2), 117-125.

Banerjee, M., & Sarkar, P. K. (2003). Inhibitory effect of garlic on bacterial pathogens from spices. *World Journal of Microbiology and Biotechnology*, *19*, 565-569.

Basir, R., Rahiman, S. F., Hasballah, K., Chong, W., Talib, H., Yam, M., Jabbarzare, M., Tie, T., Othman, F., & Moklas, M. (2012). Plasmodium berghei ANKA infection in ICR mice as a model of cerebral malaria. *Iranian journal of parasitology*, *7*(4), 62.

Bayan, L., Koulivand, P. H., & Gorji, A. (2014). Garlic: a review of potential therapeutic effects. *Avicenna journal of phytomedicine*, *4*(1), 1.

Becker, K., Tilley, L., Vennerstrom, J. L., Roberts, D., Rogerson, S., & Ginsburg, H. (2004). Oxidative stress in malaria parasite-infected erythrocytes: host–parasite interactions. *International journal for parasitology*, *34*(2), 163-189.

Berger, A. (2000). Th1 and Th2 responses: what are they? *Bmj*, *321*(7258), 424.

Beuth, J., Schneider, H., & Ko, H. (2006). Enhancement of immune responses to neem leaf extract (Azadirachta indica) correlates with antineoplastic activity in BALB/c-mice. *in vivo*, *20*(2), 247-251.

Biswas, K., Chattopadhyay, I., Banerjee, R. K., & Bandyopadhyay, U. (2002). Biological activities and medicinal properties of neem (Azadirachta indica). *Current science*, 1336-1345.

Blancou, J. (2006). Old prophylactic methods. In *Rinderpest and Peste des Petits Ruminants* (pp.

185-X). Elsevier.

Boadu, A., Karpoormath, R., & Nlooto, M. (2023). Exploration of alternate therapeutic remedies in Ebola virus disease: the case of reported antiviral phytochemical derived from the leaves Spondias Mombin linn. *Advances in Traditional Medicine*, *23*(1), 157-168.

Bodeker, G., & Ong, C.-K. (2005). *WHO global atlas of traditional, complementary and alternative medicine* (Vol. 1). World Health Organization.

Brigelius-Flohé, R., & Traber, M. G. (1999). Vitamin E: function and metabolism. *The FASEB journal*, *13*(10), 1145-1155.

Burton, A., Smith, M., & Falkenberg, T. (2015). Building WHO's global strategy for traditional medicine. *European Journal of Integrative Medicine*, *7*(1), 13-15.

Busia, K. (2005). Medical provision in Africa–Past and present. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *19*(11), 919-923.

Cao, G., & Prior, R. L. (2000). Postprandial increases in serum antioxidant capacity in older women. *Journal of Applied Physiology*, *89*(3), 877-883.

Chandra, R. K., & Kumari, S. (1994). Nutrition and immunity: an overview. *The Journal of nutrition*, *124*, 1433S-1435S.

Chandrasekara, A., & Shahidi, F. (2010). Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. *Journal of agricultural and food chemistry*, *58*(11), 6706-6714.

Chang, L.-W., Yen, W.-J., Huang, S. C., & Duh, P.-D. (2002). Antioxidant activity of sesame coat. *Food chemistry*, *78*(3), 347-354.

Chaplin, D. D. (2010). Overview of the immune response. *Journal of Allergy and Clinical Immunology*, *125*(2), S3-S23.

Chattopadhyay, R. (1998). Possible biochemical mode of anti-inflammatory action of Azadirachta indica A. Juss. in rats. *Indian journal of experimental biology*, *36*(4), 418-

420.

Chetia, M. R. D. (2019). In vitro in vivo and models used for antimalarial activity: A brief review. *Asian Journal of Pharmacy and Pharmacology*, *5*(6), 1251-1255.

Cheung, H. M., & Yew, D. T. W. (2020). Neuroprotective mechanisms of Ginkgo biloba against oxidative stress. In *Oxidative Stress and Dietary Antioxidants in Neurological Diseases* (pp. 271-290). Elsevier.

Chitnis, V., Strange, C., Gu, J., Curtis, D., Spencer, J., Huuskonen, J., & Blankfard, M. (2011). Magnetic bead-based immunoassays for the quantification of human and mouse cytokines. JOURNAL OF IMMUNOLOGY,

Chu, C.-C., Di Meglio, P., & Nestle, F. O. (2011). Harnessing dendritic cells in inflammatory skin diseases. Seminars in immunology,

Clark, G. J., Angel, N., Kato, M., López, J. A., MacDonald, K., Vuckovic, S., & Hart, D. N. (2000). The role of dendritic cells in the innate immune system. *Microbes and infection*, *2*(3), 257-272.

Clemens, D. L., Duryee, M. J., Sarmiento, C., Chiou, A., McGowan, J. D., Hunter, C. D., Schlichte, S. L., Tian, J., Klassen, L. W., & O'Dell, J. R. (2018). Novel antioxidant properties of doxycycline. *International journal of molecular sciences*, *19*(12), 4078.

Corthout, J., Pieters, L., Claeys, M., Vanden Berghe, D., & Vlietinck, A. (1991). Antiviral ellagitannins from Spondias mombin. *Phytochemistry*, *30*(4), 1129-1130.

Cunningham, A. B., Shanley, P., & Laird, S. (2012). Health, habitats and medicinal plant use. In *Human Health and Forests* (pp. 35-62). Routledge.

D Archivio, M., Filesi, C., Di Benedetto, R., Gargiulo, R., Giovannini, C., & Masella, R. (2007). Polyphenols, dietary sources and bioavailability. *Annali-Istituto Superiore di Sanita*, *43*(4), 348.

Da Silva, J. T., Rashid, Z., Nhut, D. T., Sivakumar, D., Gera, A., Souza, M. T., & Tennant, P. (2007). Papaya (Carica papaya L.) biology and biotechnology. *Tree and forestry science*

and biotechnology, *1*(1), 47-73.

Dalziel, J. M. (1937). The useful plants of west tropical Africa. 612-pp.

Darshana, D., Chutiwitoonchai, N., Paemanee, A., Sureram, S., Mahidol, C., Ruchirawat, S., & Kittakoop, P. (2023). Virucidal and antiviral polyhydroxylated fatty alcohols in avocado fruit pulp (Persea americana) of booth 7 Cultivar¶. *ACS Food Science & Technology*, *3*(10), 1759-1767.

De Leon, J. A. D., & Borges, C. R. (2020). Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. *Journal of visualized experiments: JoVE*(159), 10.3791/61122.

Dean, M. (2024). Green Medicine: Exploring the Role of Herbal Treatments in Disease Therapy. *Golden Ratio of Data in Summary*, *4*(1), 01-10.

Debboun, M., & Strickman, D. (2013). Insect repellents and associated personal protection for a reduction in human disease. *Medical and Veterinary Entomology*, *27*(1), 1-9.

Dhama, K., Sharun, K., Gugjoo, M. B., Tiwari, R., Alagawany, M., Iqbal Yatoo, M., Thakur, P., Iqbal, H. M., Chaicumpa, W., & Michalak, I. (2023). A comprehensive review on chemical profile and pharmacological activities of Ocimum basilicum. *Food Reviews International*, *39*(1), 119-147.

Dobesh, P. (2010). The importance of appropriate prophylaxis for the prevention of venous thromboembolism in at-risk medical patients. *International journal of clinical practice*, *64*(11), 1554-1562.

Dreher, D., & Junod, A. F. (1996). Role of oxygen free radicals in cancer development. *European Journal of cancer*, *32*(1), 30-38.

Dröge, W. (2002). Free radicals in the physiological control of cell function. *Physiological reviews*.

Durrheim, D. N., Ogunbanjo, G. A., & Blumberg, L. (1999). Managing re-emergent malaria in South Africa. *South African Family Practice*, *21*(1).

Dutta, T., Anand, U., Saha, S. C., Mane, A. B., Prasanth, D. A., Kandimalla, R., Proćków, J., & Dey, A. (2021). Advancing urban ethnopharmacology: A modern concept of sustainability, conservation and cross-cultural adaptations of medicinal plant lore in the urban environment. *Conservation physiology*, *9*(1), coab073.

Efferth, T. (2009). Artemisinin: a versatile weapon from traditional Chinese medicine. *Herbal drugs: ethnomedicine to modern medicine*, 173-194.

Eisenberg, D. M., Kessler, R. C., Foster, C., Norlock, F. E., Calkins, D. R., & Delbanco, T. L. (1993). Unconventional medicine in the United States--prevalence, costs, and patterns of use. *New England journal of medicine*, *328*(4), 246-252.

Esterbauer, H., & Cheeseman, K. H. (1990). [42] Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. In *Methods in enzymology* (Vol. 186, pp. 407-421). Elsevier.

Evanjalin Monica, V., Nimbkar, S., Elumalai, A., Moses, J., & Anandharamakrishnan, C. (2022). Potential Role of Herbs and Spices on the Immune System. In *Immunomodulators and Human Health* (pp. 39-70). Springer.

Eze, C. C., Attama, A. A., Ibezim, E. C., Berebon, D. P., & Agbo, M. C. (2018). Malaria parasite clearance rate of crude methanol extract of Cryptolepis sanguinolenta in mice infected with chloroquine sensitive strain of Plasmodium berghei. *Journal of Medicinal Plants Research*, *12*(25), 396-403.

Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental health perspectives*, *109*(suppl 1), 69-75.

Faccin-Galhardi, L. C., Yamamoto, K. A., Ray, S., Ray, B., Linhares, R. E. C., & Nozawa, C. (2012). The in vitro antiviral property of Azadirachta indica polysaccharides for poliovirus. *Journal of ethnopharmacology*, *142*(1), 86-90.

Farombi, E. O., & Owoeye, O. (2011). Antioxidative and chemopreventive properties of Vernonia amygdalina and Garcinia biflavonoid. *International journal of environmental*

research and public health, *8*(6), 2533-2555.

Feng, E., Shen, K., Lin, F., Lin, W., Zhang, T., Zhang, Y., Lin, F., Yang, Y., & Lin, C. (2020). Improved osteogenic activity and inhibited bacterial biofilm formation on andrographolide-loaded titania nanotubes. *Annals of Translational Medicine*, *8*(16), 987.

Feng, X., Cao, S., Qiu, F., & Zhang, B. (2020). Traditional application and modern pharmacological research of Artemisia annua L. *Pharmacology & therapeutics*, *216*, 107650.

Fernando, D. M., Wijesundera, R. L., Soysa, P., de Silva, D., & Nanayakkara, C. M. (2015). Antioxidant potential, in vitro cytotoxicity and apoptotic effect induced by crude organic extract of Anthracophyllum lateritium against RD sarcoma cells. *BMC complementary and alternative medicine*, *15*, 1-9.

Fischer, K., Konkle, B., Broderick, C., & Kessler, C. (2014). Prophylaxis in real life scenarios. *Haemophilia*, *20*, 106-113.

Fokunang, C., Ndikum, V., Tabi, O., Jiofack, R., Ngameni, B., Guedje, N., Tembe-Fokunang, E., Tomkins, P., Barkwan, S., & Kechia, F. (2011). Traditional medicine: past, present and future research and development prospects and integration in the National Health System of Cameroon. *African journal of traditional, complementary and alternative medicines*, *8*(3).

Franco, A., Flores-Garcia, Y., Zavala, F., & Sullivan, D. J. (2023). Plasmodium berghei purified hemozoin associated with DNA strongly inhibits P. berghei liver-stage development in BALB/c mice after intravenous inoculation. *Infection and immunity*, *91*(1), e00304-00322.

Gallant, J. E., Moore, R. D., & Chaisson, R. E. (1994). Prophylaxis for opportunistic infections in patients with HIV infection. *Annals of internal medicine*, *120*(11), 932-944.

Ghatak, S. B., & Panchal, S. J. (2012). Investigation of the immunomodulatory potential of oryzanol isolated from crude rice bran oil in experimental animal models. *Phytotherapy*

Research, *26*(11), 1701-1708.

Ghosh, S., Bishal, A., Ghosh, S. K., Jana, K., Gayen, B., Sahu, S., & Debnath, B. (2023). Herbal medicines: A potent approach to human diseases, their chief compounds, formulations, present status, and future aspects. *Int. J. Membr. Sci. Technol*, *10*, 442-464.

Gilotra, N. A., Wand, A. L., Pillarisetty, A., Devraj, M., Pavlovic, N., Ahmed, S., Saad, E., Solnes, L., Garcia, C., & Okada, D. R. (2021). Clinical and imaging response to tumor necrosis factor alpha inhibitors in treatment of cardiac sarcoidosis: a multicenter experience. *Journal of cardiac failure*, *27*(1), 83-91.

Gond, S. P., Sahu, S., Rawat, S., Rajendiran, A., & Singh, A. (2022). Immunomodulatory natural product. *Asian J Pharm Clin Res*, *15*(5), 5-9.

Gqalenia, N., Ngcoboa, M., Parboosing, R., & Naidoo, A. (2012). In vitro testing of African traditional medicines for cytotoxic, immune modulatory and anti-HIV activities. *African journal of traditional, complementary and alternative medicines*, *9*(3S), 2-11.

Grace, M., Smith, P., Raskin, I., & Lila, M. (2017). Africa: Achillea millefolium: Re-Exploring Herbal Remedies for Combating Malaria. In *Ethnobotany: A Phytochemical Perspective* (pp. 160-166). wiley.

Granucci, F., Zanoni, I., Pavelka, N., Van Dommelen, S. L., Andoniou, C. E., Belardelli, F., Degli Esposti, M. A., & Ricciardi-Castagnoli, P. (2004). A contribution of mouse dendritic cell–derived IL-2 for NK cell activation. *Journal of Experimental Medicine*, *200*(3), 287-295.

Gupta, A., Gupta, S. K., Priyam, M., Siddik, M. A., Kumar, N., Mishra, P. K., Gupta, K. K., Sarkar, B., Sharma, T. R., & Pattanayak, A. (2021). Immunomodulation by dietary supplements: A preventive health strategy for sustainable aquaculture of tropical freshwater fish, Labeo rohita (Hamilton, 1822). *Reviews in Aquaculture*, *13*(4), 2364-2394.

H. Bower, A., & R. Cundell, D. (2014). Immunomodulatory Activity of Astragalus, Ginseng and

Echinacea: From Keith Block and Mark Mead to Today, Have We Moved On? *Current Immunology Reviews*, *10*(2), 99-112.

Hajian, S. (2014). Positive effect of antioxidants on immune system. *Immunopathologia Persa*, *1*(1), e02.

Halberstein, R. A. (2005). Medicinal plants: historical and cross-cultural usage patterns. *Annals of epidemiology*, *15*(9), 686-699.

Haleng, J., Pincemail, J., Defraigne, J.-O., Charlier, C., & Chapelle, J.-P. (2007). Le stress oxydant. *Revue médicale de Liège*, *62*(10).

Halliwell, B., Aeschbach, R., Löliger, J., & Aruoma, O. (1995). The characterization of antioxidants. *Food and Chemical Toxicology*, *33*(7), 601-617.

Hamerman, J. A., Ogasawara, K., & Lanier, L. L. (2005). NK cells in innate immunity. *Current opinion in immunology*, *17*(1), 29-35.

Harman, D. (1981). The aging process. *Proceedings of the National Academy of Sciences*, *78*(11), 7124-7128.

Hatch-McChesney, A., & Smith, T. J. (2023). Nutrition, immune function, and infectious disease in military personnel: a narrative review. *Nutrients*, *15*(23), 4999.

Hawkins, J., Baker, C., Cherry, L., & Dunne, E. (2019). Black elderberry (Sambucus nigra) supplementation effectively treats upper respiratory symptoms: A meta-analysis of randomized, controlled clinical trials. *Complementary therapies in medicine*, *42*, 361-365.

Hechaichi, F. Z., Bendif, H., Bensouici, C., Alsalamah, S. A., Zaidi, B., Bouhenna, M. M., Souilah, N., Alghonaim, M. I., Benslama, A., & Medjekal, S. (2023). Phytochemicals, antioxidant and antimicrobial potentials and LC-MS analysis of Centaurea parviflora Desf. extracts. *Molecules*, *28*(5), 2263.

Heinrich, M., Jiang, H., Scotti, F., Booker, A., Walt, H., Weckerle, C., & Maake, C. (2021). Medicinal plants from the Himalayan region for potential novel antimicrobial and anti-

inflammatory skin treatments. *Journal of Pharmacy and Pharmacology*, *73*(7), 956-967.

Heneka, M. T., Golenbock, D. T., & Latz, E. (2015). Innate immunity in Alzheimer's disease. *Nature immunology*, *16*(3), 229-236.

Higdon, J. V., & Frei, B. (2003). Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions.

Hock, K., Acton, R. B., Jáuregui, A., Vanderlee, L., White, C. M., & Hammond, D. (2021). Experimental study of front-of-package nutrition labels' efficacy on perceived healthfulness of sugar-sweetened beverages among youth in six countries. *Preventive medicine reports*, *24*, 101577.

Holley, J., & Cherla, K. (1998). Medicinal plants sector in India: a review.

Hou, W.-C., Lin, R.-D., Cheng, K.-T., Hung, Y.-T., Cho, C.-H., Chen, C.-H., Hwang, S.-Y., & Lee, M.-H. (2003). Free radical-scavenging activity of Taiwanese native plants. *Phytomedicine*, *10*(2-3), 170-175.

Hudson, J., Vimalanathan, S., Kang, L., Amiguet, V. T., Livesey, J., & Arnason, J. T. (2005). Characterization of antiviral activities in Echinacea. Root preparations. *Pharmaceutical biology*, *43*(9), 790-796.

Hui, D. S., Lee, N., Chan, P. K., & Beigel, J. H. (2018). The role of adjuvant immunomodulatory agents for treatment of severe influenza. *Antiviral research*, *150*, 202-216.

Hulscher, M. E., Grol, R. P., & Van Der Meer, J. W. (2010). Antibiotic prescribing in hospitals: a social and behavioural scientific approach. *The Lancet infectious diseases*, *10*(3), 167-175.

Hussain, K., Majeed, M. T., Ismail, Z., Sadikun, A., & Ibrahim, P. (2009). Traditional and complementary medicines: quality assessment strategies and safe usage. *Southern med review*, *2*(1), 19.

Ighodaro, O. M. (2018). Molecular pathways associated with oxidative stress in diabetes mellitus. *Biomedicine & pharmacotherapy*, *108*, 656-662.

Iliev, P., Kirkova, Z., Ivanov, A., Prelezov, P., Tonev, A., & Kalkanov, I. (2017). Retrospective analysis on helminthic and protozoan infections in dogs and cats in Bulgaria. *Bulg. J. Vet. Med*, *20*(Suppl 1), 389-393.

Italiani, P., & Boraschi, D. (2014). From monocytes to M1/M2 macrophages: phenotypical vs. functional differentiation. *Frontiers in immunology*, *5*, 514.

Iwasaki, A., & Pillai, P. S. (2014). Innate immunity to influenza virus infection. *Nature Reviews Immunology*, *14*(5), 315-328.

Jacob, R. A. (1995). The integrated antioxidant system. *Nutrition research*, *15*(5), 755-766.

Kala, C. P. (2017). Traditional health care systems and herbal medicines. *European Journal of Environment and Public Health*, *1*(1), 03.

Kala, C. P., Dhyani, P. P., & Sajwan, B. S. (2006). Developing the medicinal plants sector in northern India: challenges and opportunities. *Journal of ethnobiology and ethnomedicine*, *2*, 1-15.

Kamboj, V. P. (2000). Herbal medicine. *Current science*, *78*(1), 35-39.

Kamelnia, E., Mohebbati, R., Kamelnia, R., El-Seedi, H. R., & Boskabady, M. H. (2023). Anti-inflammatory, immunomodulatory and anti-oxidant effects of Ocimum basilicum L. and its main constituents: A review. *Iranian Journal of Basic Medical Sciences*, *26*(6), 617.

Kaur, S., Kumari, A., Seem, K., Kaur, G., Kumar, D., Verma, S., Singh, N., Kumar, A., Kumar, M., & Jaiswal, S. (2024). Finger millet (Eleusine coracana L.): from staple to superfood—a comprehensive review on nutritional, bioactive, industrial, and climate resilience potential. *Planta*, *260*(3), 75.

Kawai, K., Liu, S.-X., Tyurin, V. A., Tyurina, Y. Y., Borisenko, G. G., Jiang, J. F., St. Croix, C. M., Fabisiak, J. P., Pitt, B. R., & Kagan, V. E. (2000). Antioxidant and antiapoptotic function of metallothioneins in HL-60 cells challenged with copper nitrilotriacetate. *Chemical Research in Toxicology*, *13*(12), 1275-1286.

Kehinde, I., Ramharack, P., Nlooto, M., & Gordon, M. (2019). The pharmacokinetic properties

of HIV-1 protease inhibitors: A computational perspective on herbal phytochemicals. *Heliyon*, *5*(10).

Kennedy, M. A. (2010). A brief review of the basics of immunology: the innate and adaptive response. *Veterinary Clinics: Small Animal Practice*, *40*(3), 369-379.

Kotepui, K. U., Thirarattanasunthon, P., Rattaprasert, P., & Kotepui, M. (2022). A systematic review and meta-analysis of blood interleukin-4 levels concerning malaria infection and severity. *Malaria journal*, *21*(1), 217.

Krinsky, N. I., & Johnson, E. J. (2005). Carotenoid actions and their relation to health and disease. *Molecular aspects of medicine*, *26*(6), 459-516.

Kruk, J., Aboul-Enein, B. H., Duchnik, E., & Marchlewicz, M. (2022). Antioxidative properties of phenolic compounds and their effect on oxidative stress induced by severe physical exercise. *The Journal of Physiological Sciences*, *72*(1), 19.

Kumar, M., Rawat, S., Nagar, B., Kumar, A., Pala, N. A., Bhat, J. A., Bussmann, R. W., Cabral-Pinto, M., & Kunwar, R. (2021). Implementation of the use of ethnomedicinal plants for curing diseases in the Indian Himalayas and its role in sustainability of livelihoods and socioeconomic development. *International journal of environmental research and public health*, *18*(4), 1509.

Kurtzhals, J. A., Adabayeri, V., Goka, B. Q., Akanmori, B. D., Oliver-Commey, J. O., Nkrumah, F. K., Behr, C., & Hviid, L. (1998). Low plasma concentrations of interleukin 10 in severe malarial anaemia compared with cerebral and uncomplicated malaria. *The Lancet*, *351*(9118), 1768-1772.

Lakra, P., & Gahlawat, I. N. (2016). The role of Nutrition in the Immune system functions. *Integrated Journal of Social Sciences*, *3*(1), 30-33.

Lambert, J., Srivastava, J., & Vietmeyer, N. (1997). *Medicinal plants: rescuing a global heritage* (Vol. 355). World Bank Publications.

Languon, S., Tuffour, I., Quayson, E. E., Appiah-Opong, R., & Quaye, O. (2018). In vitro

evaluation of cytotoxic activities of marketed herbal products in Ghana. *Journal of Evidence-Based Integrative Medicine*, *23*, 2515690X18790723.

Latha, M. P. (2012). *A Textbook of Immunology*. S. Chand Publishing.

Lavryshyn, Y., Gutyj, B., Kalyn, B., & Romanovych, M. (2020). The effect of Cadmium on the level of vitamins A and E in the blood of young cattle. Colloquium-journal,

Lee, Y.-R., Moon, G.-H., Shim, D., Kim, J. C., Lee, K.-J., Chung, K.-H., & An, J. H. (2023). Neuroprotective effects of fermented tea in MPTP-induced Parkinson's disease mouse model via MAPK signaling-mediated regulation of inflammation and antioxidant activity. *Food Research International*, *164*, 112133.

Leko, V., & Rosenberg, S. A. (2020). Identifying and targeting human tumor antigens for T cell-based immunotherapy of solid tumors. *Cancer cell*, *38*(4), 454-472.

Leyden, J., Stein-Gold, L., & Weiss, J. (2017). Why topical retinoids are mainstay of therapy for acne. *Dermatology and therapy*, *7*, 293-304.

Li, Y., Zhou, X., Liu, J., Yuan, X., & He, Q. (2020). Therapeutic potentials and mechanisms of artemisinin and its derivatives for tumorigenesis and metastasis. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, *20*(5), 520-535.

Libbey, J. E., Tsunoda, I., & Fujinami, R. S. (2010). Studies in the modulation of experimental autoimmune encephalomyelitis. *Journal of Neuroimmune Pharmacology*, *5*, 168-175.

Linehan, E., & Fitzgerald, D. (2015). Ageing and the immune system: focus on macrophages. *European Journal of Microbiology and Immunology*, *5*(1), 14-24.

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews*, *4*(8), 118.

Lopez-Bote, C., Isabel, B., Ruiz, J., & Daza, A. (2003). Effect of vitamin E supplementation and partial substitution of poly-with mono-unsaturated fatty acids in pig diets on muscle, and microsome extract a-tocopherol concentration and lipid oxidation. *Archives of Animal*

Nutrition, *57*(1), 11-12.

M Dos Santos Lima, M., Silani, I. d. S. V., Toaldo, I. M., Corrêa, L. C., Biasoto, A. C. T., Pereira, G. E., Bordignon-Luiz, M. T., & Ninow, J. L. (2014). Phenolic compounds, organic acids and antioxidant activity of grape juices produced from new Brazilian varieties planted in the Northeast Region of Brazil. *Food chemistry*, *161*, 94-103.

Mahmood, M. S., Amir, H. W., Abbas, R. Z., Bilal Aslam, B. A., & Azhar Rafique, A. R. (2018). Evaluation of antiviral activity of Azadirachta indica (Neem) bark extract against Newcastle disease virus.

Mahomoodally, M. F. (2013). Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. *Evidence-Based Complementary and Alternative Medicine*, *2013*(1), 617459.

Maity, S., Kinra, M., Nampoothiri, M., Arora, D., Pai, K. S. R., & Mudgal, J. (2022). Caffeic acid, a dietary polyphenol, as a promising candidate for combination therapy. *Chemical Papers*, 1-13.

Malaviya, R., & Abraham, S. N. (2001). Mast cell modulation of immune responses to bacteria. *Immunological reviews*, *179*(1), 16-24.

Malech, H. L., DeLeo, F. R., & Quinn, M. T. (2014). The role of neutrophils in the immune system: an overview. *Neutrophil methods and protocols*, 3-10.

Maleki, M. H., Daneshniya, M., Latifi, Z., Zarrin, Y. P., Behzadinia, M., & Morakabati, N. (2022). Evaluating the Potential of Phytochemicals as Natural Substitute for Synthetic Antioxidant: A. *Asian J. Res. Biochem*, *10*, 36-62.

Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., & Locati, M. (2004). The chemokine system in diverse forms of macrophage activation and polarization. *Trends in immunology*, *25*(12), 677-686.

Marrot, L., & Meunier, J.-R. (2008). Skin DNA photodamage and its biological consequences. *Journal of the American Academy of Dermatology*, *58*(5), S139-S148.

Matole, V., Thorat, Y., Ghurghure, S., Ingle, S., Birajdar, A., Nangare, G., Safwan, M., Madur, S., Patil, S., & Bagalkote, Z. (2021). A brief review on herbal medicines. *Research Journal of Pharmacognosy and Phytochemistry*, *13*(2), 101-102.

Messerli, P., Murniningtyas, E., Eloundou-Enyegue, P., Foli, E. G., Furman, E., Glassman, A., Hernández Licona, G., Kim, E. M., Lutz, W., & Moatti, J.-P. (2019). Global sustainable development report 2019: the future is now–science for achieving sustainable development.

Metz, M., Siebenhaar, F., & Maurer, M. (2008). Mast cell functions in the innate skin immune system. *Immunobiology*, *213*(3-4), 251-260.

Meydani, S. N. (1995). Vitamin E enhancement of T cell-mediated function in healthy elderly: mechanisms of action. *Nutrition reviews*, *53*(4), S52-S58.

Middleton Jr, E., Kandaswami, C., & Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological reviews*, *52*(4), 673-751.

Mills, E., Dugoua, J.-J., Perri, D., & Koren, G. (2006). *Herbal medicines in pregnancy and lactation: an evidence-based approach*. Taylor & Francis London; New York.

Minatel, I. O., Borges, C. V., Ferreira, M. I., Gomez, H. A. G., Chen, C.-Y. O., & Lima, G. P. P. (2017). Phenolic compounds: Functional properties, impact of processing and bioavailability. *Phenolic Compd. Biol. Act*, *8*, 1-24.

Miranda, M., Almeida, A., Costa, S., Santos, M., Lagrota, M., & Wigg, M. (1997). In vitro activity of extracts of Persea americana leaves on acyclovir-resistant and phosphonoacetic resistant Herpes simplex virus. *Phytomedicine*, *4*(4), 347-352.

Moerman, D. E., Pemberton, R. W., Kiefer, D., & Berlin, B. (1999). A comparative analysis of five medicinal floras. *Journal of ethnobiology*, *19*(1), 49-70.

Moghadas, S. (2010). Prophylaxis of healthcare workers in an influenza pandemic. *Handbook of disease burdens and quality of life measures*, 1419.

Molyneux, D. H. (2006). Control of human parasitic diseases: Context and overview. *Advances in Parasitology*, *61*, 1-45.

Morris, J., Gonzales, C. B., De La Chapa, J. J., Cabang, A. B., Fountzilas, C., Patel, M., Orozco, S., & Wargovich, M. J. (2019). The highly pure neem leaf extract, SCNE, inhibits tumorigenesis in oral squamous cell carcinoma via disruption of pro-tumor inflammatory cytokines and cell signaling. *Frontiers in oncology*, *9*, 890.

Mosaddek, A. S. M., & Rashid, M. M. U. (2008). A comparative study of the anti-inflammatory effect of aqueous extract of neem leaf and dexamethasone. ||| *Bangladesh Journal of Pharmacology*, *3*(1), 44-47.

Mulliken, T. (2003). Sustainable use of medicinal plants–a multi-sectoral challenge and opportunity. Seminar proceedings-Harvesting of Non-Wood Forest Products, Food and Agriculture Organisation (FAO), Rome. URL: http://www. fao. org/DOCREP/005/Y4496E/Y4496E42. htm,

Murray, P. J. (2017). Macrophage polarization. *Annual review of physiology*, *79*(1), 541-566.

Musial, J., Krakowiak, R., Mlynarczyk, D. T., Goslinski, T., & Stanisz, B. J. (2020). Titanium dioxide nanoparticles in food and personal care products—What do we know about their safety? *Nanomaterials*, *10*(6), 1110.

Mutombo, P. N., Kasilo, O. M. J., James, P. B., Wardle, J., Kunle, O., Katerere, D., Wambebe, C., Matsabisa, M. G., Rahmatullah, M., & Nikiema, J.-B. (2023). Experiences and challenges of African traditional medicine: lessons from COVID-19 pandemic. *BMJ global health*, *8*(8), e010813.

Myer, N. (1997). Plants, People and Culture: The Science of Ethnobotany. BY MICHAEL J. BALICK AND PAUL A. COX. *Environmental Conservation*, *24*(1), 90-96.

Nam, T.-g. (2011). Lipid peroxidation and its toxicological implications. *Toxicological research*, *27*(1), 1-6.

Nantz, M. P., Rowe, C. A., Muller, C. E., Creasy, R. A., Stanilka, J. M., & Percival, S. S. (2012).

Supplementation with aged garlic extract improves both NK and γδ-T cell function and reduces the severity of cold and flu symptoms: a randomized, double-blind, placebo-controlled nutrition intervention. *Clinical Nutrition*, *31*(3), 337-344.

Niikura, M., Inoue, S.-I., & Kobayashi, F. (2011). Role of interleukin-10 in malaria: focusing on coinfection with lethal and nonlethal murine malaria parasites. *BioMed Research International*, *2011*(1), 383962.

Nlooto, M., & Naidoo, P. (2016). Traditional, complementary and alternative medicine use by HIV patients a decade after public sector antiretroviral therapy roll out in South Africa: a cross sectional study. *BMC complementary and alternative medicine*, *16*, 1-12.

Nneji, C. M., Adaramoye, O. A., Falade, C. O., & Ademowo, O. G. (2013). Effect of chloroquine, methylene blue and artemether on red cell and hepatic antioxidant defence system in mice infected with Plasmodium yoelii nigeriensis. *Parasitology research*, *112*, 2619-2625.

Nworu, C. S., Akah, P. A., Okoye, F. B., Toukam, D. K., Udeh, J., & Esimone, C. O. (2011). The leaf extract of Spondias mombin L. displays an anti-inflammatory effect and suppresses inducible formation of tumor necrosis factor-α and nitric oxide (NO). *Journal of immunotoxicology*, *8*(1), 10-16.

Ocloo, A., Quasie, O., & Antwi, S. (2021). A retrospective study on the effect of COA Mixture® on viral load in HIV and Hep B Patients. *Int J Clin Stud Med Case Reports*, *14*(3).

Okada, H., Banchereau, J., & Lotze, M. T. (2003). Interleukin-4. *The cytokine handbook*, *1*, 227-262.

Okaiyeto, K., & Oguntibeju, O. O. (2021). African herbal medicines: Adverse effects and cytotoxic potentials with different therapeutic applications. *International journal of environmental research and public health*, *18*(11), 5988.

Okpe, O., Habila, N., Ikwebe, J., Upev, V. A., Okoduwa, S. I., & Isaac, O. T. (2016). Antimalarial potential of Carica papaya and Vernonia amygdalina in mice infected with

Plasmodium berghei. *Journal of tropical medicine*, *2016*(1), 8738972.

Orr, R. Y., Philip, N., & Waters, A. P. (2012). Improved negative selection protocol for Plasmodium berghei in the rodent malarial model. *Malaria journal*, *11*, 1-6.

Otun, S. O., Graca, R., Onisuru, O., & Achilonu, I. (2024). Evaluation of Plasmodium berghei models in malaria research. *Journal of Cellular Signaling*, *5*(3), 96-113.

Ouyang, W., Rutz, S., Crellin, N. K., Valdez, P. A., & Hymowitz, S. G. (2011). Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annual review of immunology*, *29*(1), 71-109.

Padayatty, S. J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J.-H., Chen, S., Corpe, C., Dutta, A., & Dutta, S. K. (2003). Vitamin C as an antioxidant: evaluation of its role in disease prevention. *Journal of the American college of Nutrition*, *22*(1), 18-35.

Pal, S. K., & Shukla, Y. (2003). Herbal medicine: current status and the future. *Asian pacific journal of cancer prevention*, *4*(4), 281-288.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of nutritional science*, *5*, e47.

Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative medicine and cellular longevity*, *2*(5), 270-278.

Parvatkar, P. T., Diagne, K., Zhao, Y., & Manetsch, R. (2024). Indoloquinoline Alkaloids as Antimalarials: Advances, Challenges, and Opportunities. *ChemMedChem*, *19*(18), e202400254.

Paul, R. E., & Brey, P. T. (2003). Malaria parasites and red blood cells: from anaemia to transmission. *Molecules and cells*, *15*(2), 139-149.

Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy reviews*, *6*(11), 1.

Petzer, K., & Mngqundaniso, N. (2008). Patients consulting traditional health practioners in the context of HIV/AIDS in urban areas in KwaZulu-Natal, South Africa. *African journal of*

traditional, complementary and alternative medicines, *5*(4), 370-379.

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian journal of clinical biochemistry*, *30*, 11-26.

Philips, N., Samuel, P., Samuel, M., Perez, G., Khundoker, R., & Alahmade, G. (2018). Interleukin-4 signaling pathway and effects in allergic diseases. *Current Signal Transduction Therapy*, *13*(1), 76-80.

Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European journal of medicinal chemistry*, *97*, 55-74.

Poljšak, B., & Milisav, I. (2012). Clinical implications of cellular stress responses. *Bosnian journal of basic medical sciences*, *12*(2), 122.

Pombo, D. J., Lawrence, G., Hirunpetcharat, C., Rzepczyk, C., Bryden, M., Cloonan, N., Anderson, K., Mahakunkijcharoen, Y., Martin, L. B., & Wilson, D. (2002). Immunity to malaria after administration of ultra-low doses of red cells infected with Plasmodium falciparum. *The Lancet*, *360*(9333), 610-617.

Porter, N. A., Caldwell, S. E., & Mills, K. A. (1995). Mechanisms of free radical oxidation of unsaturated lipids. *Lipids*, *30*(4), 277-290.

Prakash, D., & Gupta, K. (2009). The antioxidant phytochemicals of nutraceutical importance. *The Open Nutraceuticals Journal*, *2*(1), 20-35.

Preiser, J. C. (2012). Oxidative stress. *Journal of Parenteral and Enteral Nutrition*, *36*(2), 147-154.

Pruteanu, L. L., Bailey, D. S., Grădinaru, A. C., & Jäntschi, L. (2023). The biochemistry and effectiveness of antioxidants in food, fruits, and marine algae. *Antioxidants*, *12*(4), 860.

Purushothaman, B., PrasannaSrinivasan, R., Suganthi, P., Ranganathan, B., Gimbun, J., & Shanmugam, K. (2018). A comprehensive review on Ocimum basilicum. *Journal of Natural Remedies*, 71-85.

Radhakrishnan, N., Lam, K., & Norhaizan, M. (2017). Molecular docking analysis of Carica papaya Linn constituents as antiviral agent. *International Food Research Journal*, *24*(4).

Rahaman, M. M., Hossain, R., Herrera-Bravo, J., Islam, M. T., Atolani, O., Adeyemi, O. S., Owolodun, O. A., Kambizi, L., Daştan, S. D., & Calina, D. (2023). Natural antioxidants from some fruits, seeds, foods, natural products, and associated health benefits: An update. *Food Science & Nutrition*, *11*(4), 1657-1670.

Rahmani, A., Almatroudi, A., Alrumaihi, F., & Khan, A. (2018). Pharmacological and therapeutic potential of neem (Azadirachta indica). *Pharmacognosy reviews*, *12*(24), 250-255.

Rao, A. V., & Rao, L. G. (2007). Carotenoids and human health. *Pharmacological research*, *55*(3), 207-216.

Raskin, I., Ribnicky, D. M., Komarnytsky, S., Ilic, N., Poulev, A., Borisjuk, N., Brinker, A., Moreno, D. A., Ripoll, C., & Yakoby, N. (2002). Plants and human health in the twenty-first century. *TRENDS in Biotechnology*, *20*(12), 522-531.

Raus, K. (2013). *Continuous sedation at the end of life: practice, perspectives and ethical analyses* Ghent University].

Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in plant science*, *2*(4), 152-159.

Rios, J.-L., & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. *Journal of ethnopharmacology*, *100*(1-2), 80-84.

Ristow, M., & Zarse, K. (2010). How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). *Experimental gerontology*, *45*(6), 410-418.

Roche, E., & Romero-Alvira, D. (1995). Role of oxidative stress in gene expression: myocardial and cerebral ischemia, cancer and other diseases. *Medicina Clinica*, *104*(12), 468-476.

Ruh, C., Banjade, R., Mandadi, S., Marr, C., Sumon, Z., & Crane, J. K. (2017).

Immunomodulatory effects of antimicrobial drugs. *Immunological investigations*, *46*(8), 847-863.

Sagheer, M. S., Aslam, M. R., Jabbar, S., Ahmad, K., Aslam, N., Aslam, N., Ahmad, H. S., & Saeed, M. M. (2023). Current Updates on Pharmacological Profile, Chemical Constituents and Traditional Uses of Gymnema Sylvestre (Gurmar). *RADS Journal of Pharmacy and Allied Health Sciences*, *1*(2), 68-79.

Sandoval-Acuña, C., Ferreira, J., & Speisky, H. (2014). Polyphenols and mitochondria: An update on their increasingly emerging ROS-scavenging independent actions. *Archives of biochemistry and biophysics*, *559*, 75-90.

Scalbert, A., Johnson, I. T., & Saltmarsh, M. (2005). Polyphenols: antioxidants and beyond. *The American journal of clinical nutrition*, *81*(1), 215S-217S.

Schippmann, U., Leaman, D. J., & Cunningham, A. B. (2002). Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. *Biodiversity and the ecosystem approach in agriculture, forestry and fisheries*.

Seeram, N. P., Aviram, M., Zhang, Y., Henning, S. M., Feng, L., Dreher, M., & Heber, D. (2008). Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. *Journal of agricultural and food chemistry*, *56*(4), 1415-1422.

Setiawan, B. (2011). The role of prophylactic antibiotics in preventing perioperative infection. *Surgery*, *15*, 30.

Shahrajabian, M. H., Sun, W., & Cheng, Q. (2019). Clinical aspects and health benefits of ginger (Zingiber officinale) in both traditional Chinese medicine and modern industry. *Acta agriculturae scandinavica, section b—Soil & Plant Science*, *69*(6), 546-556.

Shan, J., Xiaoqian, D., Xia, L., Yu, W., Zhilong, Z., Zhihui, S., & Yanjie, Q. (2022). Oxidative stress, autophagy, and apoptosis induced by doxycycline in loach fin cells in vitro. *Science of the Total Environment*, *839*, 156379.

Sharma, N., Mishra, K. P., Chanda, S., Bhardwaj, V., Tanwar, H., Ganju, L., Kumar, B., & Singh, S. B. (2019). Evaluation of anti-dengue activity of Carica papaya aqueous leaf extract and its role in platelet augmentation. *Archives of virology*, *164*, 1095-1110.

Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of botany*, *2012*(1), 217037.

Sharma, P., Kumar, P., Sharma, R., Gupta, G., & Chaudhary, A. (2017). Immunomodulators: Role of medicinal plants in immune system. *National Journal of Physiology, Pharmacy and Pharmacology*, *7*(6), 552.

Sharrock, J., & Sun, J. C. (2020). Innate immunological memory: from plants to animals. *Current opinion in immunology*, *62*, 69-78.

Shi, H.-T., Wang, B.-Y., Bian, C.-Z., Han, Y.-Q., & Qiao, H.-X. (2020). Fermented Astragalus in diet improved laying performance, egg quality, antioxidant and immunological status and intestinal microbiota in laying hens. *Amb Express*, *10*, 1-11.

Shukla, S., Bajpai, V. K., & Kim, M. (2014). Plants as potential sources of natural immunomodulators. *Reviews in Environmental Science and Bio/Technology*, *13*, 17-33.

Simwela, N. V., & Waters, A. P. (2022). Current status of experimental models for the study of malaria. *Parasitology*, *149*(6), 729-750.

Singh, A., Kukreti, R., Saso, L., & Kukreti, S. (2019). Oxidative stress: a key modulator in neurodegenerative diseases. *Molecules*, *24*(8), 1583.

Singh, N. (2017). Pulses: an overview. *Journal of food science and technology*, *54*, 853-857.

Singh, N., Tailang, M., & Mehta, S. (2016). A review on herbal plants as immunomodulators. *International Journal of Pharmaceutical Sciences and Research*, *7*(9), 3602.

Sinha, A. C., Singh, P. M., Williams, N. W., Ochroch, E. A., & Goudra, B. G. (2014). Aprepitant's prophylactic efficacy in decreasing postoperative nausea and vomiting in morbidly obese patients undergoing bariatric surgery. *Obesity surgery*, *24*, 225-231.

Siqueira, E. M. d. S., Lima, T. L., Boff, L., Lima, S. G., Lourenço, E. M., Ferreira, É. G., Barbosa, E. G., Machado, P. R., Farias, K. J., & Ferreira, L. d. S. (2020). Antiviral potential of Spondias mombin L. leaves extract against herpes simplex virus type-1 replication using in vitro and in silico approaches. *Planta medica*, *86*(07), 505-515.

Smilin Bell Aseervatham, G., Sivasudha, T., Suganya, M., Rameshkumar, A., & Jeyadevi, R. (2013). Trichosanthes tricuspidata modulates oxidative toxicity in brain hippocampus against pilocarpine induced status epilepticus in mice. *Neurochemical research*, *38*, 1715-1725.

Sofowara, A. (1993). Medicinal plants and traditional medicine in Africa Spectrum books LTD. *Ibadan, Nigeria*, *289*.

Song, Z., Cheng, Y., Chen, M., & Xie, X. (2023). Macrophage polarization in bone implant repair: A review. *Tissue and Cell*, *82*, 102112.

Sowunmi, A. (1996). Hepatomegaly in acute falciparum malaria in children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *90*(5), 540-542.

Spelman, K., Burns, J., Nichols, D., Winters, N., Ottersberg, S., & Tenborg, M. (2006). Modulation of cytokine expression by traditional medicines: a review of herbal immunomodulators. *Alternative medicine review*, *11*(2), 128.

Splettstoesser, W. D., & Schuff-Werner, P. (2002). Oxidative stress in phagocytes—"the enemy within". *Microscopy research and technique*, *57*(6), 441-455.

Srinivas, N., Rachakonda, S., Hielscher, T., Calderazzo, S., Rudnai, P., Gurzau, E., Koppova, K., Fletcher, T., & Kumar, R. (2019). Telomere length, arsenic exposure and risk of basal cell carcinoma of skin. *Carcinogenesis*, *40*(6), 715-723.

Srivastava, I. K., & Vaidya, A. B. (1999). A mechanism for the synergistic antimalarial action of atovaquone and proguanil. *Antimicrobial agents and chemotherapy*, *43*(6), 1334-1339.

Stahl, W., Heinrich, U., Jungmann, H., Sies, H., & Tronnier, H. (2000). Carotenoids and carotenoids plus vitamin E protect against ultraviolet light–induced erythema in humans.

The American journal of clinical nutrition, *71*(3), 795-798.

Strzelec, M., Detka, J., Mieszczak, P., Sobocińska, M. K., & Majka, M. (2023). Immunomodulation—a general review of the current state-of-the-art and new therapeutic strategies for targeting the immune system. *Frontiers in immunology*, *14*, 1127704.

Subramanian, N., Torabi-Parizi, P., Gottschalk, R. A., Germain, R. N., & Dutta, B. (2015). Network representations of immune system complexity. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, *7*(1), 13-38.

Sun, W., & Shahrajabian, M. H. (2023). Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human health. *Molecules*, *28*(4), 1845.

Suresh, C., & NP, M. (2020). Immunomodulators. *International Journal of Pharmaceutical Research (09752366)*.

Tai, W.-Y., Yang, Y.-C., Lin, H.-J., Huang, C.-P., Cheng, Y.-L., Chen, M.-F., Yen, H.-L., & Liau, I. (2010). Interplay between structure and fluidity of model lipid membranes under oxidative attack. *The Journal of Physical Chemistry B*, *114*(47), 15642-15649.

Tan, S.-A., Bendran, A. P., Hamdan, M. R., Kamaruzaman, N. A., Rajoo, S., Agarwal, T., Ramanathan, S., & Murugaiyah, V. (2022). Platelet boosting effect of commercial herbal supplements in chemically-induced thrombocytopenic rats. *Annals of Phytomedicine*, *11*(1), 276-282.

Tavassolifar, M. j., Vodjgani, M., Salehi, Z., & Izad, M. (2020). The influence of reactive oxygen species in the immune system and pathogenesis of multiple sclerosis. *Autoimmune diseases*, *2020*(1), 5793817.

Tete, S., Tripodi, D., Rosati, M., Conti, F., Maccauro, G., Saggini, A., Salini, V., Cianchetti, E., Caraffa, A., & Antinolfi, P. (2012). Role of mast cells in innate and adaptive immunity. *Journal of biological regulators and homeostatic agents*, *26*(2), 193-201.

Thomas, E., Vandebroek, I., Goetghebeur, P., Sanca, S., Arrázola, S., & Van Damme, P. (2008). The relationship between plant use and plant diversity in the Bolivian Andes, with special

reference to medicinal plant use. *Human Ecology*, *36*, 861-879.

Tiralongo, E., Wee, S. S., & Lea, R. A. (2016). Elderberry supplementation reduces cold duration and symptoms in air-travellers: A randomized, double-blind placebo-controlled clinical trial. *Nutrients*, *8*(4), 182.

Tiwari, V., & Wilson, D. M. (2019). DNA damage and associated DNA repair defects in disease and premature aging. *The American Journal of Human Genetics*, *105*(2), 237-257.

Tona, L., Cimanga, R., Mesia, K., Musuamba, C., De Bruyne, T., Apers, S., Hernans, N., Van Miert, S., Pieters, L., & Totté, J. (2004). In vitro antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. *Journal of ethnopharmacology*, *93*(1), 27-32.

Tu, Y. (2011). The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. *Nature medicine*, *17*(10), 1217-1220.

Turvey, S. E., & Broide, D. H. (2010). Innate immunity. *Journal of Allergy and Clinical Immunology*, *125*(2), S24-S32.

Udeinya, I., Mbah, A., Chijioke, C., & Shu, E. (2004). An antimalarial extract from neem leaves is antiretroviral. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *98*(7), 435-437.

Vaishali, M. (2014). Antioxidants in health and diseases. *Research Journal of Pharmacy and Technology*, *7*(4), 489-493.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology*, *39*(1), 44-84.

Vij, T., & Prashar, Y. (2015). A review on medicinal properties of Carica papaya Linn. *Asian Pacific Journal of Tropical Disease*, *5*(1), 1-6.

Vikou, C., Semassa, J., Diaconeasa, Z., Roko, G., Tohoyessou, M., Dah-Nouvlessounon, D., Sina, H., Stanilă, A., & Baba-Moussa, L. (2023). Diversity, chemical compositions and

beneficial effects of some spices and aromatic leaves consumed in Benin and in the world: Critical review. *American Journal of Plant Sciences*, *14*(5), 569-598.

Wang, X., Hu, C., Ding, L., Tang, Y., Wei, H., Jiang, C., Yan, Q., Dong, Q., & Degen, A. A. (2021). Astragalus membranaceus alters rumen bacteria to enhance fiber digestion, improves antioxidant capacity and immunity indices of small intestinal mucosa, and enhances liver metabolites for energy synthesis in tibetan sheep. *Animals*, *11*(11), 3236.

Waris, G., & Ahsan, H. (2006). Reactive oxygen species: role in the development of cancer and various chronic conditions. *Journal of carcinogenesis*, *5*, 14.

Wasko, A. (2016). Doxycycline Resistance in Plasmodium falciparum Linked to Single Nucleotide Polymorphisms in the Plasmodium falciparum apicoplast Small Subunit Ribosomal RNA (pfSSrRNA) gene.

Weber, J., Tatoud, R., & Fidler, S. (2010). Postexposure prophylaxis, preexposure prophylaxis or universal test and treat: the strategic use of antiretroviral drugs to prevent HIV acquisition and transmission. In (Vol. 24, pp. S27-S39): LWW.

WHO. (2002). *The world health report 2002: reducing risks, promoting healthy life*. World Health Organization.

WHO. (2023). *World malaria report 2023*. World Health Organization.

Wigg, M., Al-Jabri, A., Costa, S., Race, E., Bodo, B., & Oxford, J. (1996). In-Vitro virucidal and virustatic anti HIV-1 effects of extracts from Persea americana Mill,(Avocado) leaves. *Antiviral Chemistry and Chemotherapy*, *7*(4), 179-183.

Willcox, M., & Chamberlain, J. (2004). Neem (Azadirachta indica). *Traditional medicinal plants and malaria*, 91-115.

Williams, R. J., Spencer, J. P., & Rice-Evans, C. (2004). Flavonoids: antioxidants or signalling molecules? *Free radical biology and medicine*, *36*(7), 838-849.

Winiger, F. (2022). The Spirituality of Others and the WHO. *The Spirit of Global Health: The World Health Organization and the'spiritual Dimension'of Health, 1946-2021*, 83.

Witol, D. (2010). *Medicinal plants: Uses and preservation to ensure their sustainability in the nadowli district of the upper west region, Ghana*

Wu, G., Lupton, J. R., Turner, N. D., Fang, Y.-Z., & Yang, S. (2004). Glutathione metabolism and its implications for health. *The Journal of nutrition*, *134*(3), 489-492.

Wynn, T. A., Chawla, A., & Pollard, J. W. (2013). Macrophage biology in development, homeostasis and disease. *Nature*, *496*(7446), 445-455.

Yadav, D. K., Kumar, S., Choi, E.-H., Chaudhary, S., & Kim, M.-H. (2019). Molecular dynamic simulations of oxidized skin lipid bilayer and permeability of reactive oxygen species. *Scientific Reports*, *9*(1), 4496.

Yamassaki, F., Campestrini, L., Zawadzki-Baggio, S., & Maurer, J. (2018). Chemical characterization and complement modulating activities of an arabinogalactan-protein-rich fraction from an aqueous extract of avocado leaves. *International Journal of Biological Macromolecules*, *120*, 513-521.

Yang, Y., Bazhin, A. V., Werner, J., & Karakhanova, S. (2013). Reactive oxygen species in the immune system. *International reviews of immunology*, *32*(3), 249-270.

Yao, Y., Xu, X.-H., & Jin, L. (2019). Macrophage polarization in physiological and pathological pregnancy. *Frontiers in immunology*, *10*, 792.

Yasir, M., Das, S., & Kharya, M. (2010). The phytochemical and pharmacological profile of Persea americana Mill. *Pharmacognosy reviews*, *4*(7), 77.

Yu, M.-H., Im, H.-G., Lee, H.-J., Ji, Y.-J., & Lee, I.-S. (2006). Components and their antioxidative activities of methanol extracts from sarcocarp and seed of Zizyphus jujuba var. inermis Rehder. *Korean Journal of Food Science and Technology*, *38*(1), 128-134.

Zaidun, N. H., Thent, Z. C., & Abd Latiff, A. (2018). Combating oxidative stress disorders with citrus flavonoid: Naringenin. *Life sciences*, *208*, 111-122.

Zhang, H. M., & Zhang, Y. (2014). Melatonin: a well-documented antioxidant with conditional pro-oxidant actions. *Journal of pineal research*, *57*(2), 131-146.

Zhang, R., Barker, L., Pinchev, D., Marshall, J., Rasamoelisolo, M., Smith, C., Kupchak, P., Kireeva, I., Ingratta, L., & Jackowski, G. (2004). Mining biomarkers in human sera using proteomic tools. *Proteomics*, *4*(1), 244-256.

Zhang, X., & Mosser, D. M. (2008). Macrophage activation by endogenous danger signals. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, *214*(2), 161-178.

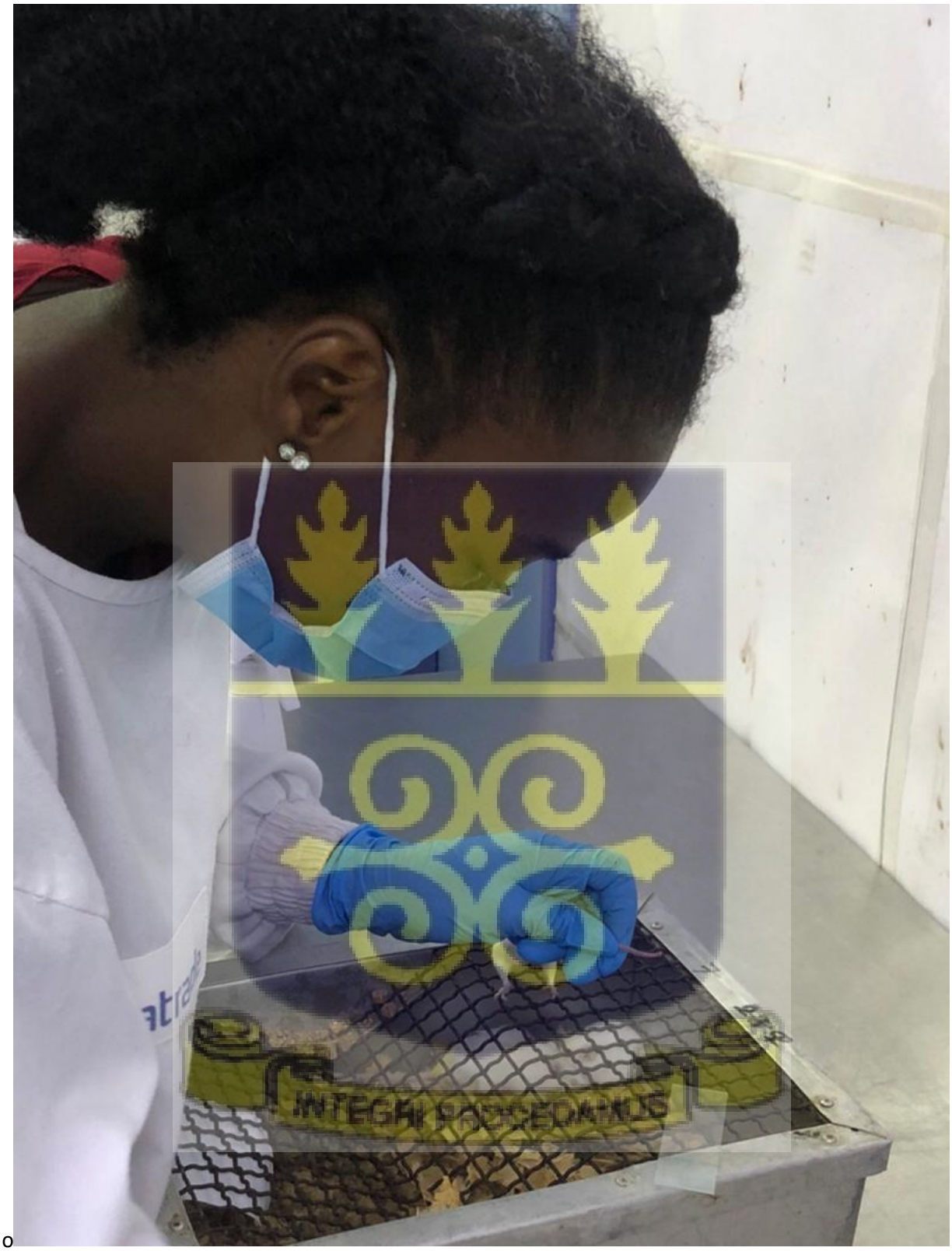
Zhu, J. (2015). T helper 2 (Th2) cell differentiation, type 2 innate lymphoid cell (ILC2) development and regulation of interleukin-4 (IL-4) and IL-13 production. *Cytokine*, *75*(1), 14-24.

APPENDICES

Appendix 1: Organ harvesting

Appendix 2: Administeration of COA Plus Mixture

Appendix3: Tail Bleeding for Parasitaemia Count



io