PHARMACOLOGICAL AND TOXICOLOGICAL EFFECTS OF SELECTED MEDICINAL PLANTS TRADITIONALLY USED TO TREAT MALARIA IN MSAMBWENI SUB-COUNTY, KENYA.

By

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DECLARATION

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

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DEDICATION

This work is dedicated to my family. To my parents Mr. Bernard Ndii and Mrs. Medrine Ndii who took me to School and my wife Pauline and our children Melody, Mike and Marvin for paying my School fees. It is only through the sacrifice they all made that I was able to complete my studies at UON successfully. May God bless you all.

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LIST OF ACRONYMS

ANOVA	-	Analysis of variance
CAVS	-	College of Agriculture and Veterinary Sciences
CBPS	-	College of Biological and Physical Sciences
CHS	-	College of Health Sciences
CSD	-	Clinical Studies department
CTMDR	-	Centre for Traditional Medicine and Drug Research
CQ	-	Chloroquine
$CHCL_3$	-	Chloroform
DMSO	-	Dimethyl Sulphoxide
DW	-	Distilled Water
KEMRI	-	Kenya Medical Research Institute
LD	-	Lethal Dose
MeoH	-	Methanol
OECD	-	Organisation for Economic Cooperation and Development.
PHPT	-	Public Health, Pharmacology and Toxicology
PSG	-	Phosphate Saline Glucose
RBCs	-	Red Blood Cells
SBS	-	School of Biological Sciences
SPSS	-	Statistical Package for the Social Sciences
TLC	-	Thin Layer Chromatography
WMI	-	Wangari Maathai Institute
WHO	-	World Health Organization
UON	-	University of Nairobi

ABSTRACT

Malaria is a major public health problem and continues to kill a million people each year, with more than 90% of these cases found in sub-Saharan Africa. In Kenya, more than half of the population is exposed to malaria with minimal opportunities for treatment. The management of malaria is complicated because the parasites that cause the disease are resistant to most of the safest and cheapest first line treatments developed so far. There is therefore an urgent need for discovery of new antimalarial agents.

This study was conducted to investigate pharmacological and toxicological effects of some selected plants from Msambweni district that have been claimed to possess antimalarial properties. The antimalarial and hematological activities of aqueous and organic plant extracts were determined using a mouse model infected with *Plasmodium berghei* (ANKA). Cytotoxicity and oral acute toxicity was evaluated using brine shrimp and the mouse models respectively. The phytochemical compounds were screened using standard methodologies for possible active compounds.

Crude extracts were prepared from three plant species; *Zanthoxylum chalybeum* Engl.[(Rutaceae) (Root bark)], *Ocimum suave* willd [(Lamiaceae) (Leaves)] and *Plectranthus barbatus* Andr.[(Lamiaceae) (Root bark]. Plants depending on the part traditionally used to treat malaria were collected from Shimoni in Msambweni Sub-County, Kenya.

Adult healthy swiss albino mice (*Mus musculus* L.) were infected intraperitoneally with *Plasmodium berghei* (ANKA) to induce malaria and then treated orally with a dose of 100 mg/kg body weight of each crude extract according to standard procedures. The negative control was treated with distilled water and the positive with chloroquine (CQ).

Brine shrimp lethality assay was used to test for cytotoxicity while healthy female swiss albino mice were used for oral acute toxicity as per Organization for Economic Co-operation and Development (OECD 420-2001) guidelines. The chemosuppression means obtained from the 4 - days suppressive test were analyzed using one way ANOVA and Dunnett test for multiple comparisons. Aqueous extracts of Z. *chalybeum*, *O. suave* and *P. barbatus* had percentage chemosuppression of 81.45, 55.23 and 67.70% respectively. The organic extracts on the other hand exhibited percentage chemosuppression of 78.39, 54.78 and 78.69%

respectively. Chloroquine, which was the positive control, had a chemosuppression of 97.76%. There was no significant difference between the chemosuppression of the aqueous extracts of Z. *chalybeum* and the organic extract of *P. barbatus* and that of chloroquine ($p<0.05$).

Oral administration of *Z. chalybeum* extracts in mice caused an increase in the red blood cell count, packed cell volume (PCV), hemoglobin and the neutrophils while extracts from *O. suave* and *Plectranthus barbatus* only caused an increase on neutrophils and monocytes respectively. Organic extract of *Z. chalybeum* had an LD_{50} lower than 50 ug/ml (42.73) and hence considered highly toxic to brine shrimp while those of *O. suave, P. barbatus* and the aqueous extracts of *Z. chalybeum and P. barbatus* were moderately toxic to the brine shrimp (LD_{50} >100<500). Aqueous leaf extract of *O. suave* was weakly toxic to brine shrimp (LD_{50}>500<1000).

Acute oral toxicity studies showed that the aqueous and the organic extracts of the three plants investigated were not toxic to mice at a concentration of 2000 mg/kg body weight. Alkaloids, flavonoids, sesquiterpene lactones and tannins were found present in all extracts while glycosides were only present in *P. barbatus*. Saponins were found present in extracts of *O. suave* and *P. barbatus* but found absent in *Z. chalybeum*.

The findings suggest that folkloric medicinal application of the three plants by Msambweni Community has a pharmacological basis and to some extent safe. Alkaloids, flavonoids, saponins, sesquiterpenes and tannins found present in the three plant extracts upon phytochemical screening are perhaps compounds responsible for the antimalarial activity. It is also evident that the three screened plant extracts has ability to increase RBCs and WBCs. Bioactivity guided fractionation and isolation of bioactive molecules from the two most active species could lead to new hits against *P. falciparum* malaria.

CHAPTER ONE

1.0 INTRODUCTION

Malaria is a vector-borne disease caused by protozoan plasmodia parasites. There are four plasmodium species which are significant as far as malaria is concerned namely: *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae and Plasmodium ovale.* The most severe form of malaria is caused by *P. falciparum*. Malaria is a serious cause of mortality globally and about 3.3 billion people are at risk of Malaria (WHO, 2014). It is estimated that there are as many as 300 million acute cases resulting in one million deaths. Ninety percent of these deaths occur in Sub-Saharan Africa, and most of victims are children aged less than 5 years (WHO, 2014). Malaria, with AIDS and Tuberculosis, is one of the three major communicable diseases linked to poverty and a major obstruction to social and economic development (Gathirwa *et al*., 2011). The disease contributes significantly to poverty firstly, through lost productive days when sick or when tending to sick relatives. Secondly, the cost of acquiring treatment or barriers to mosquito nets is high for the average Kenyan. Malaria causes enormous misery and suffering through the pain of fevers and the anguish of bereavement (Gathirwa *et al*., 2011).

In Kenya, 22 million people are at risk of malaria, 70% of them living in the rural areas where conventional drugs are often unaffordable or inaccessible. Even where they are accessible, some health facilities are poorly equipped with personnel and medicine and so people turn to traditional medicine (Nguta *et al*., 2010a). About 34,000 Kenyan children die every year from malaria compared to a total estimate of 42,000 deaths (KMIS 2011). Each year, there are over 8.2 million malaria infections in Kenya (Jean Marie 2002) mostly due to inadequate medical care, unavailability of insecticide treated nets and increased resistance of the parasites to drugs.

There were 9.2 million clinically diagnosed malaria cases in 2007 in which 30% were outpatient cases, 15% hospital admissions and 3-5% inpatient deaths (Kenya Malaria Programme Performance Review, 2009). Children aged 5 years and below, and pregnant women are the most affected. In children, it does not only lead to illness and death but also long term consequences on child development such as low birth weight, chronic anaemia, reduced growth and in some cases, severe mental retardation. In pregnancy, malaria may

cause maternal anemia, premature births, low-weight babies (which is the principal contributor to infant mortality), still births, and miscarriages. The disease is endemic in the Lowlands, particularly the coastal strip where transmission is sufficiently intense (Muthaura *et al*., 2011).

Malaria is of global and national interest in Kenya because of development of resistant strains of *P. falciparum* to many existing drugs such as chloroquine which has made treatment of malaria increasingly problematic (Nguta *et al*., 2011). Four major problems are associated with the management of malaria. The most important problem is that parasites which cause malaria are resistant to or are developing resistance to the most widely available, affordable and safest first line treatments (Kilama 2005; Sendagire *et al.,* 2005). Secondly, the overall control of the mosquitoes which transmit malaria is made difficult by their resistance to a wide range of insecticides. The third, which is a new and developing problem, is the widespread production of fake antimalarial drugs. For example, in Mainland Southeast Asia 38% and 53% of "artesunate" blister packs sampled contained no active ingredient (Newton *et al*., 2006). Lastly, many countries in Africa lack the necessary infrastructure and resources to manage and control malaria (WHO, 2004). The appearance of drug resistance *P. falciparum* strains since 1960 in particular to chloroquine has made the treatment of malaria increasingly problematic in virtually all malarious regions of the world.

Historically, the vast majority of the existing antimalarial drugs have been derived from medicinal plants or from structures modeled on plant derived compounds (Phillipson and Wright, 1991; Nguta *et al*., 2010). These include drugs in use today such as quinine and artemisinin and its derivatives. The current drug of choice in malaria chemotherapy is artemisinin. Emerging resistance of *P. falciparum* to artemisinin has however been reported in the recent past (Rahmtullah *et al*., 2012). So in the absence of a functional safe and widely available malaria vaccine, efforts to develop new antimalarial drugs continue being urgently needed. Thus new drugs with unique structures and mechanism of action are required in order to circumvent or delay the development of drug resistance. (Nguta *et al*., 2011).

There is consensus among the scientific community that natural products have been playing a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases (Newman *et al*., 2003). Indeed, the vast majority of the existing antimalarial chemotherapeutic agents are based on natural products, and this fact anticipates that new leads

may certainly emerge from the tropical plant sources. Plants provide a rich source of antimalarial drugs (Julie *et al*., 2007), and there are many plants that can be used to control and treat malaria. However, very few plants have been analyzed chemically (Gravito *et al*., 2007). Due to the wide biodiversity of plants used by different communities to treat malaria and the development of resistance by plasmodium to currently available antimalarial drugs, the search for new and more effective antimalarial drugs becomes imperative, elusive and a continuous process (Nguta *et al*., 2010a).

One of the disadvantages of traditional medicine is the lack of precision in dosage (Kokwaro, 2009). A high concentration of the active principles of the plants can be lethal. Toxicity studies are thus necessary to determine lethality of the drugs and the appropriate dosage. Owing to the widespread suffering and death due to malaria and the failure of the safest and most affordable antimalarial drugs due to drug resistance, there is an urgent need to develop new drugs or vaccine for the treatment, management, prevention and control of malaria (Kilama *et al*., 2005, Nguta *et al*., 2010b).

Furthermore, with changing lifestyles and customs, lack of proper documentation can easily lead to erosion of the traditional medicinal knowledge and resources, which threatens the sustainability of rural healthcare systems (Srithi *et al*., 2009). Medicinal plants are commonly used in Kenyan traditional healthcare to treat a range of ailments, including malaria and its associated symptoms. Those plants currently used by indigenous people to treat malaria should be documented and investigated as potential sources of new antimalarial drugs.

1.1 Problem Statement

Malaria is a major public health problem that is presently complicated by the increasing resistance of *P. falciparum* against the mainstay drugs (Batista *et al*., 2009, Nguta *et al.,* 2011). Malaria is endemic in Msambweni and most of the residents have no access to modern health facilities. In addition, most of them live in poverty, cannot afford modern medicine and have traditionally treated themselves using medicinal plants (Nguta *et al*., 2010a). Despite the existence of effective interventions both preventive and curative, there is persistence in the use of traditional medicine by Msambweni community owing to the fact that herbal medicines are accessible, affordable, culturally accepted, socially sanctioned, and easy to prepare. Thus there is an urgent need for bioprospecting of new drugs, more affordable, accessible and with

unique structures/mechanism of action to treat drug-resistant strains of malaria and medicinal plants from Msambweni can serve as useful leads.

In view of the increasing consumption of medicinal plants as alternative therapy, it is necessary to carry out toxicity studies to determine lethality and the safest and effective dose that is appropriate for human consumption with a view to validate and document the safety of these medicinal plants with antimalarial properties to the society.

This study was carried out to determine the pharmacological and toxicological effects of three selected antimalarial medicinal plants commonly used in Msambweni Sub-county, South Coast of Kenya, namely : *Zanthoxylum chalybeum* (root bark), *Ocimum suave* (Leaves) and *Plectranthus barbatus* (root bark). The study was aimed at validating the medicinal use of these plants by the Community as well as screening them for possible candidature for further investigation as potential sources of new plant based alternative antimalarial drugs.

1.2 Justification

About 80% of the World's people rely on Traditional medicine (UNEP, 2010) and about 75% of Kenya's Population seeks health care among traditional healers, (Sandiga *et al*; 1995). Plants have always been considered to be an alternative source of new drugs and some of the antimalarial drugs in use today such as quinine and artemesinin were derived directly from medicinal plants or structures modeled on plant derived compounds (Nguta et al; 2010).The important advantages for therapeutic uses of medicinal plants in various ailments are their safety, economical, effective and easy availability.

Increased side effects of conventional drugs/development of resistance by parasites that causes malaria has become a global concern and thus highlights the need to develop novel antimalarial drugs that are not only active against drug resistant parasites, but more importantly, kill persistent parasites and shorten the length of treatment.

1.3 Hypothesis

H_0: Crude extracts of plants under investigation do not possess *in vivo* antimalarial activity and are not toxic to mice.

H_A: Crude extracts of plants under investigation possess *in vivo* antimalarial activity and are toxic to mice.

1.4 Objectives

1.4.1 General Objective

To determine pharmacological and toxicological effects of selected antimalarial plants traditionally used to treat malaria in Msambweni Sub- County.

1.4.2 Specific Objectives

1. To determine the antimalarial effects of crude extracts of *Zanthoxylum chalybeum* (root bark), *Ocimum suave* (leaves) and *Plectranthus barbatus* (root bark) in mice.
2. To assess hematological effects in infected mice treated with the crude extracts of *Zanthoxylum chalybeum* (root bark), *Ocimum suave* (leaves) and *Plectranthus barbatus* (root bark).
3. To establish the acute toxicity of aqueous and organic crude extracts of *Zanthoxylum chalybeum* (root bark), *Ocimum suave* (leaves) and *Plectranthus barbatus* (root bark) in brine shrimp larva and mice.
4. To screen for the major phytochemical compounds present in organic and aqueous crude extracts of *Zanthoxylum chalybeum* (root bark), *Ocimum suave* (leaves) and *Plectranthus barbatus* (root bark).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Malaria prevalence in Kenya and its Socio-economic impact

Malaria is still a major cause of morbidity and mortality in Kenya. There was 76% of an estimated 43 million people in 2012 at the risk of Malaria (Presidents' Malaria Initiative, 2012). Malaria accounts for 30-50% of all outpatient attendance and 20% of all admissions to the health facilities with the most vulnerable group to malaria infections being pregnant women and children under five years of age. Approximately 1.5 million women become pregnant each year and up to 70% cannot live in areas of moderate to intense transmission of malaria. Malaria contributes to about 2- 15% of severe anaemia and 8-14% of low birth weight in Kenya (Kenya Malaria Performance Review, 2009). Malaria transmission patterns in Kenya are influenced by factors such as rainfall, relative humidity, vector species and intensity of biting, altitude and presence of susceptible new human hosts (Muthaura *et al*., 2011).

Malaria is endemic in lowlands, particularly the coastal strip and Lake Victoria where transmission is sufficiently intense. According to the Kenya Malaria Fact Sheet (2012), Kenya has four malaria epidemiological zones: Endemic areas- altitude ranges from 0 – 1300 metres for example around Lake Victoria and Coastal regions. Seasonal malaria transmission area is an epidemiological zone in arid and semi-arid areas of northern and south eastern parts of the country. Malaria epidemic prone areas of Western Highlands of Kenya- transmission are seasonal, with considerable year-to-year variation. Low risk Malaria areas - the Central highlands of Kenya including Nairobi where the temperatures are usually too low to allow completion of the sporogonic cycle of the malaria parasite in the vector.

About 4 million Kenyans were living in areas of high malaria transmission in 1997 (Chuma *et al*., 2010). Most of the Kenyan population lives in areas with minimal malaria transmission of *P. falciparum.* In 2009, only 11% of the total population was exposed to highest transmission intensity of *P. falciparum* (Abdsalam *et al*., 2009). Figure 1 shows malaria endemicity in Kenya. Gucha and Makueni districts have high fevers due to Malaria in older children and adults. Total household cost of treatment of malaria is high (US$19.6 per month) and low in

Bondo (US$ 9.2 per month), these costs result to increased poverty and low economic growth in the country (Chuma *et al*., 2010).

Malaria is understood to be a disease of poverty and a cause of poverty resulting to reduced economic growth. Countries with intensive malaria have a low economic growth of about 1.3% per person compared to other neighbouring countries which don't have intensive malaria and a decrease in malaria by 10% results to a 0.3% increase in annual economic growth (Enato and Okhamafe, 2005). In areas where people are infected with malaria, most people are poor. Therefore, a healthy nation is important to a thriving economy (Sachs and Pia, 2002).

Figure 1: Map of Kenya showing the prevalence of malaria

(Source: Kenya Malaria Indicator Survey 2010**)**

2.2 Conventional drugs used to treat malaria and antimalarial drug resistance

Decades ago, malaria was treated using antimalarial drugs such as chloroquine, quinine, sulfadoxine- pyrimethamine, mefloquine and Malarone (Chenq *et al*., 2012). Resistance to the above antimalarial medicines has however made their continued use untenable (WHO, 2012). Despite development of resistance to the above drugs, their use still persists because new effective antimalarial therapies are also faced with parasite resistance.

Today, artemesinin based combination therapy (ACT) is used as the first line of treatment in uncomplicated *P. falciparum* malaria in over 100 countries (chenq *et al*., 2012). Quinine has been used for treatment for almost 400 years since it was first documented. It is mostly used for complicated malaria (Achan *et al*., 2011). However, in recent years, parasite resistance to artemesinin has been detected in four countries: Cambodia, Myanmar, Thailand and Vietnam (WHO, 2012). There being no other alternative antimalarial drug available, WHO 2012 warned that, if resistance to artemesinin spreads to other larger geographical areas, the health consequences could be dire.

2.3 Ethnopharmacology

Herbal medicine as a component of Traditional medicine is widely practiced in Kenya, where this has been documented by ethnobotanical surveys (Miaron *et al*., 2004, kareru *et al*., 2007, Njoroge and Bussmann 2007). Moreover, herbal medicines are an important part of the culture and traditions of the African people (Nanyingi *et al*., 2008). In addition, they are a preserve of the cultural heritage, ethnopharmacological base for drug discovery and biological diversity (Weldogerima, 2009). On the other hand, many rural areas have few health centers, and where they are, some are poorly equipped with personnel and medicine and so people turn to traditional medicine (Nguta *et al*., 2010a).

Herbal medicine tends to look primitive and unscientific when compared to synthetic (conventional) drugs which are thought to be more reliable than those made from plants. Herbal medicine is still the mainstay of about 75-80% of the World population mainly in the developing countries for primary health care. This is primarily because of the general belief that herbal drugs are without any side effects, cheap and locally available. The use of plants for healing purposes predates human history and forms the origin of much modern medicine.

Many synthetic drugs originated from plant sources: a century ago, most of the few effective drugs were plant based. Examples include: Aspirin (a chemical copy of the analgensic chemical in the bark of Willow trees), digoxin (from Fox glove), quinine (from the bark of various Cinchona tree species which was used in the treatment of Malaria) and Morphine (from the opium poppy).

2.4 Plants as a source of antimalarial drugs

Herbal medicine is the oldest form of healthcare known to mankind. Herbs have been used by all cultures throughout history to treat various human and livestock ailments. The whole plant or sometimes plant parts such as leaves, roots or bark were used to prepare the drugs. Different methods were used to prepare the drugs such as boiling, soaking, burning, and pounding, chewing, heating or roasting (Kokwaro, 2009).

Plants are a rich source of antimalarial drugs as evidenced by antimalarial properties of Quinine, an alkaloid isolated from the bark of Cinchona, which has been used for over 300 years to treat malaria. Development of Artemensinin derivatives, which is currently the first line of treatment of malaria from Artemesia annua plant has re-affirmed the potential of plant species to provide effective drugs for the treatment of malaria (Muthaura *et al*., 2011).

Kenya possesses rich floristic wealth and diversified genetic resources of medicinal plants with many of the plants being used by indigenous people to prepare drugs for treating various ailments including Malaria (Nguta *et al*., 2011). Moreover, indigenous local communities in Kenya use herbal preparations to control parasitic diseases like malaria (Kigondu *et al*., 2009).

Some ethnobotanical studies have been accomplished in Kenya targeting the different cultures and localities among others (Johns *et al*., 1990). Studies on specialized knowledge on antimalarial herbal remedies in Msambweni, one of the malaria endemic districts in South Coast Kenya have been accomplished (Nguta *et al*., 2010b). Sixty species were documented from this ethnobotanical survey where Rutaceae and Lamiaceae families had a higher number of species cited as sources of antimalarial remedies. However, few have been screened for antiplasmodial activity and phytochemical compounds. Because of the past successes in obtaining antimalarial drugs from plants such as quinine and artemensinin and the continued

use of plants as source of drugs by the indigenous people, there is a possibility that ethnopharmacological approaches could lead to discovery of more antimalarial drugs (Pillay *et al*., 2008).

2.5 Phytochemicals as potential chemotherapeutic agents

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive Properties. Plants produce these chemicals to protect themselves against microbial infections or infestations by pests (Doughari *et al*., 2009), but research demonstrate that they can also protect humans against diseases. Classes of phytochemicals include: Alkaloids, Flavonoids, Saponins, Terpenes, Tannins, Glycosides, Phenolics, Anthraquinones, Essential oils and Steroids.

2.6 Significance of hematological parameters

The use of blood examination as a way of assessing the health status of animals has been documented (Owoyele *et al*., 2003). This is because it plays a vital role in physiological, nutritional and pathological status of organisms (Muhammed *et al*., 2000). They range from giving the level of the blood to detecting ailments or disorders through them. Hematological parameters are those parameters that are related to the blood forming organs (Stenesh 1975).

Hematological parameters are blood characteristics which affect both health and nutritional state of an animal. The efficacy or toxicity of a drug could therefore be reflected through parameters such as white blood cells (WBCs), red blood cells (RBCs, packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), lymphocutes, basophils, eosinophils and neutrophils. The full blood count (FBC), sometimes referred to as full blood examination or complete blood count, is one of the most commonly performed blood tests, as it can tell us so much about the status of our health.

2.7 Significance of Toxicity studies

Many plants produce toxic secondary metabolites as natural defense from adverse conditions. These secondary metabolites have served as useful leads in developing drugs for human consumption. Some of the phytochemicals produced by plants against herbivorous insects also end up being harmful to humans, because highly conserved biological similarities are

shared between both taxa as seen in most pathways involving protein, nucleic acid, carbohydrate and lipid metabolism.

Another implication in the toxicity of certain herbs is the presence of toxic minerals and heavy metals like mercury, arsenic, lead and cadmium. Lead and mercury can cause serious neurological impairment when a herbal medicinal product contaminated with these metals is ingested. In view of the increasing consumption of medicinal plants as alternative therapy, it is necessary to carry out toxicity studies to ensure that the plants are safe for human consumption (Sim *et al*., 2010).

2.8 Description, traditional use and chemical constituents of the three selected plant species

2.8.1 *Zanthoxylum chalybeum* Engl.(Rutaceae)

Figure 2: *Zanthoxylum chalybeum* shrub

(Photo taken by Micheni N.K. at Shimoni)

2.8.1.1 Taxonomic description

Zanthoxylum chalybeum is a deciduous spiny shrub or tree that may stand at 1.5 – 10 m tall. It is commonly found in semi-evergreen or dry forests, wooded grasslands and in coastal thickets near the sea (Beentje, 1994). The trunk is furrowed with corky knobs or ridges crowned with spines. The bark is pale grey; smooth dark with scales and prickles. The branches also bear curved spines up to 2 cm with conspicuous dark scales. The leaves are compound, usually 3-5 pairs of shiny leaflets plus a terminal leaflet, margins entire or crenulate. The flowers are yellow green, sweet scented in racemes or little branched panicles to 10 cm, usually borne below the leaves at the base of the new branchlets. The fruit is pink, obliquely ellipsoid, about 5-8 mm long, splitting to allow the shiny black seeds to partly protrude.

2.8.1.2 Traditional medicinal use

Zanthoxylum chalybeum is widely used in traditional medicine. It is commonly referred as *Mjafari* by the inhabitants of Msambweni district. Among the Giriama, Duruma and the Digo of South Coast, the root is used to treat malaria, (Nguta *et al.,* 2010a). In a general study of East African medicinal plants, Kokwaro (1993) reported multiple uses for this plant as follows; Stem bark decoctions or root bark decoctions are widely taken to treat malaria, given to goats suffering from diarrhea., leaves used against snake bite, boiled leaf decoction drunk for the treatment of oedema in kwashiorkor, fruits used for chest pain, fever and sore throat. Similar observations were earlier recorded by Timberlake (1987) among the Pokot of northern Kenya. According to Simiyu *et al.*, (1996), the Luo of Siaya extensively used *Z. chalybeum* roots and seeds for the treatment of chest pains and stomach pains.

2.8.2 *Plectranthus barbatus* Andr. (Lamiaceae)

Figure 3: *Plectranthus barbatus* herb

(Photo taken by Micheni N.K. at Shimoni)

2.8.2.1 Taxonomic description

Plectranthus barbatus is a perennial branched, aromatic herb belonging to the botanical family of Lamiaceae (Labiatae). It is one of the 10 species of the genus Plectranthus having greatest number of synonyms and most number of uses. It is a herbaceous plant with a thick and perennial rootstalk. The stems grow up to 1-2 feet and become decumbent, when grown larger. The inflorescence and flowers are typical of the family Labiatae. The roots are fasciculate, thick, succulent, and contain the unique chemical forskolin.

2.8.2.2 Traditional medicinal use and chemical constituents

Plectranthus barbatus is locally known as *Mraga dare* by the people of Msambweni, where the root bark has been used in traditional ethnomedicine against malaria (Nguta *et al.*, 2010a). In Kenya, the plant is used in the treatment of wounds and ringworms (Githinji and Kokwaro 1993). Other benefits include help in losing weight by improving the breakdown of fats, improving digestion and nutrient absorption, lowering cholesterol, and immune system support. It is also used in the treatment of stomach ache and as a purgative, for nausea. In India the roots are used in the treatment of pickles.

In Brazil, it is used in the treatment of gastritis and intestinal spasms. In Kenya and the Democratic Republic of Congo, the plant is used in the treatment of wounds and ringworms. *P. barbatus* contains many chemical compounds that are diterpenes in nature. The roots contain an essential oil with an attractive and spicy note. It can find application in the food industry as a flavourant. The active phytochemical in *P.barbatus* is forskolin, has a vast array of effects on the body, working primarily on the enzymatic level, raising the level of cyclic AMP (adenosine 3, 5- monophosphate) a substance that activates all sorts of other cellular enzymes.

2.8.3 *Ocimum suave willd* (Lamiaceae)

Figure 4 : *Ocimum suave* herb
(Photo taken by Micheni N.K. at Shimoni)

2.8.3.1 Taxonomic description

Ocimum suave is an herb belonging to Lamiaceae family and ocimum genus. This herb mainly grows in India, Africa and parts of South- East Asia are commonly known by names such as African basil, clove basil and wild basil. The herb is woody at the base and grows somewhere around 1- 3 metres in height. It has an erect, round- quadrangular and much branched stem, which are smooth or hairy. The leaves are narrow and oval in shape growing 5-13 cm in length, 3-9 cm width and arranged opposite. The leaves are green in colour.

The stalk is 2-4.5 cm long, slender and hairy. The blade is elliptical to egg-shaped. The inflorescence is arranged in a terminal with simple or 30-50 cm long raceme. The flower stalk is acuminate, sessile egg shaped spreading or ascending and slightly curved. The flowers are pale yellow in color and give out a sweet scent of camphor. The petals are greenish white, bell shaped and hairy on the outside. Stamens are four, bent in two pairs and inserted in a petal tube. The filaments are distinctly protruding. Ovary is superior consisting of two fruits, each two celled.

2.8.3.2 Traditional medicinal use and chemical constituent

Ocimum suave, locally known as *Murihani* is traditionally used to treat malaria by the Msambweni community (Nguta *et al.,* 2010a). In folk medicine, *O. Suave* is extensively used as febrifuge, anti-malarial and anti-convulsant. The crushed leaf juice is used in the treatment of convulsion, stomach pain, cough and influenza. The leaves, which are strongly scented, are rubbed between palms and sniffed to treat blocked nostrils. An infusion of leaves acts as a disinfectant and insecticide (Kokwaro, 2009). The oil from the leaves has been found to be active against *E. coli, Shigella, Salmonella, Proteus* and fungi (Nakamura *et al.,* 1999).

In Tanzania, a mixture of *O. Suave* and *Zingiber officinalis* is used in the treatment of candida including oral candidiasis (Nguta *et al*., 2010). There are reports that *O. Suave* is used as anti – diarrhea agent and for the treatment of conjunctivitis by instilling directly into the eyes. The dried leaves are shuffed to alleviate headaches, and fever among other uses (Iwu, 1993). *O. suave* and their essential oils have been used as fragrances and as insect repellent particularly mosquitoes (Josiane *et al*., 2003). Oil from the leaves has been found to possess antiseptics, antibacterial and antifungal activities. The oil is also active against several species of bacteria (*E.coli*, shigella, Salmonella and Proteus) and fungi (Nakamura *et al*., 1999).

2.8.4 Significance of the three plants

Ethnobotanical studies done in Msambweni reported three plant families most commonly used traditionally by residents for malaria treatment namely: Rutaceae, Lamiaceae and Liliaceae (Nguta *et al.,* 2010a). In Rutaceae family, *Z. chalybeum* was reported as frequently utilized antimalarial plant species while in Lamiaceae, *Ocimum suave* and *Plectranthus barbatus* were among the species reported. This serves as an important lead to the species that can be targeted for further pharmacological analysis and it's against this background that this study was taken.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Study Area

Msambweni Sub-County is located in South Coast, in Kwale County of Kenya (Figure 5). The area is hot and humid all year round. The annual mean temperature ranges between 23^{o}C and 34^{o}C and the relative humidity ranges between 60% and 80%. The soils are made of sandstone and grit and are fairly fertile for cultivation. The area has monsoon climate, hot and dry from January to April while June to August is the coolest period. Rainfall comes in two seasons with short rains from October to December and long rains from March/April to July. The study area is mainly inhabited by the Digo community, a Bantu tribe with a population of 288,000 (2009 Kenya Population and Housing Census), 90% of whom are Muslims and are concentrated on the southern coastal strip of Kenya between Mombasa and the boarder of Tanzania. The community is rural and depends on crop agriculture as its major source of livelihood (Nguta *et al*., 2010a).

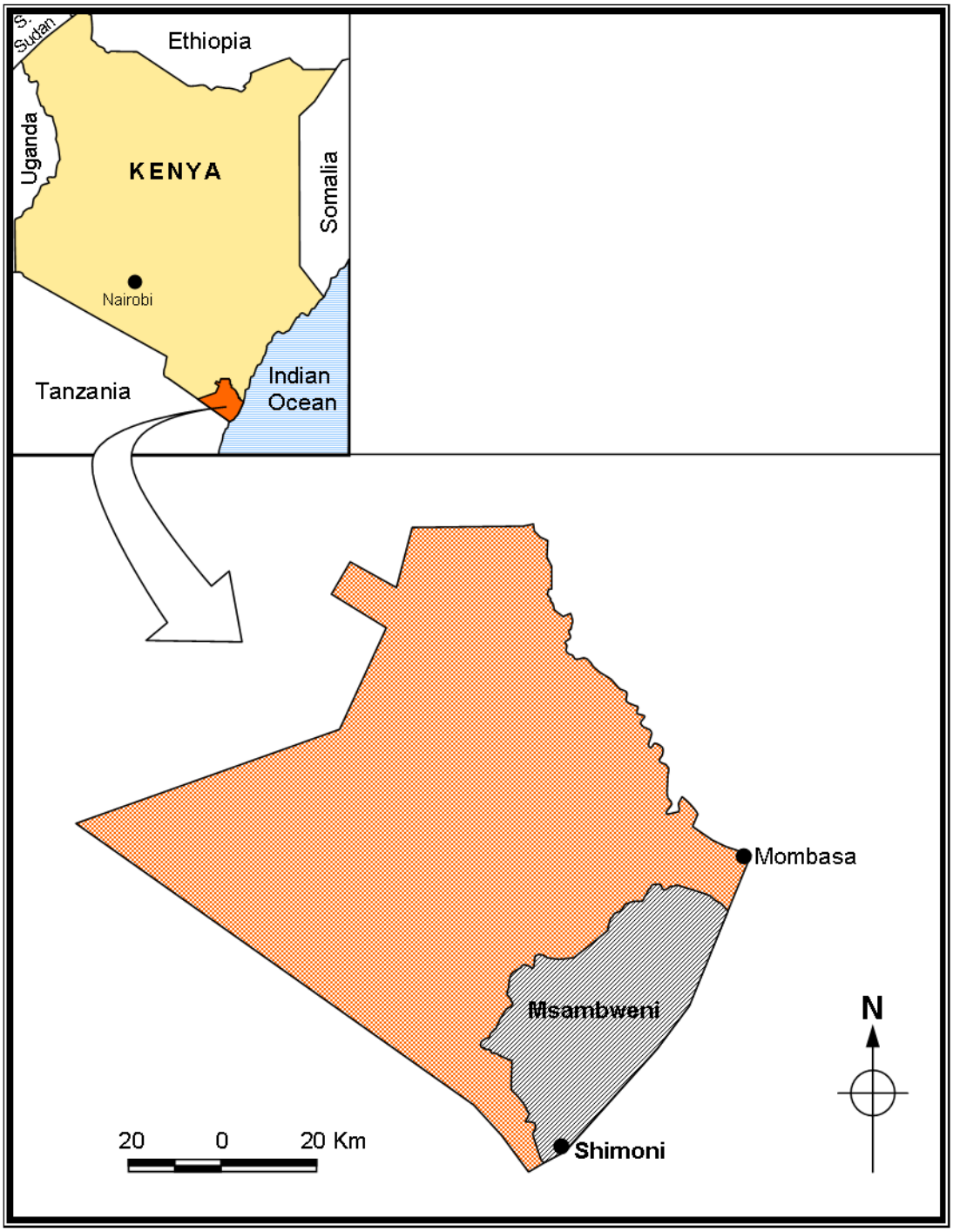

Figure 5: Study site: Map of Kenya showing location of Shimoni in Msambweni Sub-County in Kwale County

(Source: School of Biological Sciences – UON Scientific Illustrator, Lawrence Kigondu)

3.2 Collection of plant material

The plant samples used in the current study were collected from Shimoni in Msambweni district of Kwale County based on data collected during ethnopharmacological survey on plants used as antimalarial drugs (Nguta *et al*., 2010a) and with help from an identified traditional health practitioner. The information gathered included part of the plant used and the method of preparation of the herbal antimalarial remedies. A written informed consent was obtained from the community representatives. The plants were identified by Mr Kimeu Musembi and Mr Patrick Mutiso both taxonomists at the University of Nairobi herbarium, where voucher specimens were deposited. The plants voucher numbers are as follows: *Z. chalybeum* Engl. (MNK 01/2014), *O. suave* Willd. (MNK 02/2014) and *P. barbatus* Andrews (MNK 03/2014). Species nomenclature follows the flora for tropical East Africa.

The plant parts normally used in the preparation of traditional medicine were collected as follows: *Zanthoxylum chalybeum* (Rutaceae*)* root bark, *Ocimum suave* (Lamiaceae) leaves *and Plectranthus barbatus* (Lamiaceae), root bark. The roots of *Z. chalybeum* and *P. barbatus* were collected through digging while the leaves of *O. suave* were harvested from twigs. The plants were shade dried (room temperature at 25^{o} C) for one month, chopped into small pieces and pulverized using a laboratory mill.

3.3 Preparation of crude extracts

People of Msambweni usually use hot water to prepare their herbal remedies as decocortions and sometimes concortions. In view of this fact, an aqueous hot infusion of each plant part was prepared. Fifty grams of the ground plant material was extracted using 500 mls of distilled water and heated at 60^{o} C in a water bath for one hour. The extracts were then filtered using a muslin cloth (gauze) and the filtrate kept in a deep freezer for 24 hours and later dried into powder using a freeze drier. The dry extracts were stored in stoppered sample vials at 4^{o} c until the time that they were used.

The organic extracts were prepared by soaking 50g of powdered plant material in 500 mls of the organic solvent (Chloroform: methanol mixture (1:1) for 48 hours according to standard extraction methods (Harbourne, 2002). The extracts were later filtered through whatman filter paper No. 1. The filtrate was concentrated to dryness in vacuum by rotary evaporator and later

in oven at 40^{o} C. The dry solid extracts were weighed and stored in airtight containers until they were used.

3.4 Preparation of test extracts

Stock solutions of 10,000 ug/ml for the aqueous extracts were prepared by dissolving 0.1 g of each of the extracts in 10 ml of distilled water. The same concentration of organic extracts were prepared by dissolving 0.1 g of each of the organic samples in 0.1 ml (0.001%) of Dimethyl sulphoxide (DMSO) followed by dilution with water to make 10 ml of solution (Wanyoike *et al*., 2004).

3.5 Acquisition and maintenance of *plasmodium berghei* parasites in mice

The animal experiments were conducted in Kenya Medical Research Institute (KEMRI) Animal house facility. Mice were used as an animal model to carry out the antimalarial, acute toxicity and the hematological parts of the experiments. Cryopreserved chloroquine sensitive *Plasmodium berghei* (ANKA strain) parasites stored at -80^{o} C was obtained from KEMRI. Three naive mice were used to revive, stabilize the *plasmodium berghei* parasites in the host and maintained the parasite by continuous reinjection of the parasite to new mice according to Ravindran *et al*., (1982).

New mice were injected with the parasite inoculums to ensure that stabilized parasites were always available. Passages were done when parasitaemia levels reached about 20-30%. Mice were anaesthetized using chloroform in an anaesthetizing chamber and blood was collected by cardiac puncture into heparinised bottles to make inoculums for infecting new mice. Reinjection of the parasite inoculums to new mice ensured that stabilized parasites were available as infected mice die after some time due to increased parasitaemia levels (Jambou *et al*., 2011). Donor mice were sacrificed by cervical dislocation.

3.6 *In vivo* determination of antimalarial activity

In vivo anti-malarial activity of the aqueous and organic extracts of all three plants was determined using the 4-days suppressive anti-malarial assay (Peters *et al.,* 1975; Waako *et al*., 2005*; Madara et al.,* 2010). Forty Swiss albino mice (8 weeks old) of either sex were infected by administration of 0.2ml of blood suspension containing about 1×10^{7} parasitized red blood

cells (1% parasitaemia) intraperitoneally (*Waako et al.*, 2005). To obtain 1% parasitaemia for infection, parastaemia in the donor mouse was first determined then a dilution was done with Phosphate Saline Glucose (PSG) since blood obtained from donor mice was normally of high concentration with parasitized cells. Percentage parasitaemia in the donor mouse was calculated as follows:

$$\text{Percentage parasitaemia} = \frac{\text{Total number of parasitized cells}}{\text{Total number of cells}} \times 100$$

Table 3.1 summarizes the steps that were used to determine percentage parasitaemia levels of the three mice in order to come up with a donor mouse that was used to provide blood inoculum for the experimental animals. Mouse number three with parasitaemia level of 28.75% was selected as donor mouse because its parasitaemia level was most stabilized (20-30%) among the three mice (Table 1). 9.5 ml of PSG was used to dilute 0.5 ml of blood from the donor mouse with 28.75% parasitaemia to obtain 10 ml of blood with 1% parasitaemia. This blood was used to infect forty experimental animals where each was infected with 0.2 ml of the diluted blood through intraperitoneal injection.

Table 3.1: Percentage parasitaemia for the donor mice used at different passages

Donor Mouse 1 (1st passage)	**Magnification (Four fields were used)**				**Total**	**% Parasitaemia**
	1	2	3	4		
Parasitized cells	32	62	34	39	167	21.72
Total cells	165	236	194	174	769	
Donor Mouse 2 (2nd passage)	**Magnification (Four fields were used)**				**Total**	**% Parasitaemia**
	1	2	3	4		
Parasitized cells	42	51	32	39	164	22.19
Total cells	186	207	160	186	739	
Donor Mouse 3 (3rd passage)	**Magnification (Four fields were used)**				**Total**	**% Parasitaemia**
	1	2	3	4		
Parasitized cells	59	64	58	53	234	28.75
Total cells	192	247	176	199	814	

Aqueous and organic crude extracts of the selected plants were administered orally every day, and at the same time for four days at a dosage of 100 mg/kg from day one to day four. Two control experiments were conducted i.e. chloroquine (20 mg/kg/day) as a positive control drug and normal distilled water was used as a negative control in the experiment.

3.7 Experimental design

A completely randomized design was employed in carrying out the experiment. Forty mice were infected with *Plasmodium berghei* parasites and kept in the main chamber as shown in Table 3.2. Eight cages of mice were selected and in each 5 infected mice assigned randomly from the main chamber. Mice in each cage were numbered by marking their tails with a felt pen for easy identification and administration of the drug. The cages were given treatments randomly. Mice in 3 cages received aqueous extracts orally, 3 received organic extracts and 2 were controls: one received chloroquine (positive control) and the other distilled water (negative control). Drugs were administered orally on daily basis for 4 days. On the first day administration of the drug was done 2 hours after infecting the mice with *Plasmodium berghei* parasites.

Table 3.2: Distribution of the experimental mice in cages for drug administration

Treatments	**Aqueous**			**Organic**			**Controls**	
	A_a	B_a	C_a	A_o	B_o	C_o	-ve	+ve
Day 0	Cage 5	Cage 1	Cage 8	Cage 3	Cage 7	Cage 2	Cage 4	Cage 6
Day 1	Cage 5	Cage 1	Cage 8	Cage 3	Cage 7	Cage 2	Cage 4	Cage 6
Day 2	Cage 5	Cage 1	Cage 8	Cage 3	Cage 7	Cage 2	Cage 4	Cage 6
Day 3	Cage 5	Cage 1	Cage 8	Cage 3	Cage 7	Cage 2	Cage 4	Cage 6
Day 4								
Preparation of thin blood smears from each mouse								

Key:

+ve = Positive

-ve = Negative

A_a= *Z. chalybeum* - aqueous extract

B_a= *O. suave* – aqueous extract,

C_a= *P. barbatus* – aqueous extract

A_o= *Z. chalybeum* – organic extract

B_o= *O. suave* – organic extract

C_o= *P. barbatus* – organic extract

On the fourth day, blood was obtained from a tail cut of each of the mice and thin blood smears prepared and stained using 10% Giemsa stain in phosphate buffer, PH 7.2 and examined microscopically. A photographic compound microscope was used to observe the slides under oil immersion (×1000) to determine the number of parasitized cells per given magnification field (MF). Four magnification fields were observed for each blood smear of a given mouse. The number of parasitized cells (Schizonts) and the total number of cells in each magnification field was determined and recorded. The data obtained was used to determine percentage parasitaemia and chemosuppression (Appendix Ia), in each mouse and for each extract as follows:

$$\% \text{ Parasitaemia} = \frac{\text{Total Number of parasitized cells} \times 100}{\text{Total Number of cells}}$$

Inhibition of growth of parasites by the drugs was calculated according to Hilou *et al.,* 2006 and Gathirwa *et al*., 2011 as follows:

$$\text{Chemosuppression} = \frac{(\text{Parasitaemia in control animals}) - (\text{Parasitaemia with Drug}) \times 100}{(\text{parasitaemia in control animals})}$$

3.8 Evaluation of hematological activity

A 4-day suppressive test against *Plasmodium berghei* infection in mice was done. Three groups of mice consisting of the normal, negative and positive controls were used while two other groups of infected mice were treated with 100 and 200 mg/kg body weight plant extracts respectively. On the 5^{th} day, the mice were stunned and in this unconscious state, the thoracic and abdominal regions were opened to expose the heart and other organs and blood collected through heart puncture into EDTA vacuutainers. The whole blood samples were analyzed for packed cell volume (PCV) and haemoglobin (Hb) concentration. The total white blood cells (WBCs), white blood differentials, red blood cells (RBCs) and the platelet counts were counted using the Vet MS4 blood analyzer and recorded (Appendix IV).

3.9 Toxicity testing against the brine shrimp

Brine shrimp Leech (*Artemia salina L*.) larvae were used to determine the toxicity of the crude extracts as previously described by Meyer *et al*., (1982). Brine shrimp eggs were

hatched in a shallow rectangular dish (22 cm x 32 cm) filled with artificial sea water which was prepared with a commercial salt mixture and double distilled water. The hatching tank had two compartments; one small and one large compartment with a plastic divider with several 2 mm holes, which allowed hatched brine shrimp larvae to pass through.

Artificial sea water was prepared by dissolving 33 grams of commercial salt mixture in one litre of double distilled water at PH 7. About 200 mls of this saline solution was poured into the hatching tank and approximately 50 mg of brine shrimp eggs were sprinkled to the larger compartment which was darkened, because brine shrimp eggs hatch in dark while the smaller compartment was illuminated. A bulb was placed near the hatching tank to provide warmth and light. The shrimps were allowed to hatch for 48 hours after which nauplii were collected by pipette from the illuminated side, having been separated by the divider from the shells.

A quantity of 0.1 g of each aqueous plant extract was dissolved in 10 ml distilled water to make stock solutions (10,000 ug/ml). The same concentration of organic extracts was prepared by separately dissolving, 0.1 g of each of the samples in 0.1 ml (0.01%) of Dimethyl sulphoxide (DMSO) followed by dilution with water to make 10 ml (stock solution). Ten (10) larvae were transferred into each sample vial (three serial dilutions of different aqueous and organic plant extracts: 10, 100, 1000 ug/ml). A control group containing artificial sea water and *A. salina* only was included in the experiment. Each experiment was carried out in triplicate and the survivors were counted after 24 hours using a magnifying glass (Meyer et al., 1982), and the percentage of deaths at each dose was recorded (Appendix IIc). Lethal concetration (LC_{50}) was then calculated using Finney computer program.

3.10 Evaluation of acute oral toxicity

Female Swiss albino mice about weighing about 20 g (18-22g) were used to determine the acute toxicity of the aqueous and organic extracts of the three selected antimalarial plants. The mice were fasted for twelve hours prior to dosing. Five mice were used for each sample and were given a single dose as per the OECD (420-2001) guidelines. The mice were weighed before the plant extracts were given. Food was withheld for one hour. Three doses of 300, 500 and 2000 mg/kg body weight were administered to the mice. The mice were observed individually once for the first 30 minutes and periodically during the first twenty four hours with special attention during the first four hours and daily thereafter for at least 14 days

(Appendix IIb). Signs of toxicity such as rough fur, dullness, watery eyes, rapid and shallow respiratory, heart-rate, tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma was noted. Time of onset of signs of toxicity or death if any was recorded. Weights of the mice were recorded after every two days for 14 days.

3.11 Phytochemical screening for Secondary metabolites using Thin Layer Chromatography

The plant crude extracts were screened for secondary metabolites namely flavonoids, alkaloids, sesquiterpene lactones, tannins, glycosides and saponins according to Harbone (2002). The extracts were dissolved in chloroform: methanol (1:1). Aluminium TLC plates measuring 6.6 cm by 5 cm were used for phytochemical characterization. Two millimeter diameter spots of the dissolved extracts were made on the plates using micropippetes. The origin was 1 cm from the base of the plates and the solvent front was 5 cm from the base of the plates. The plates were placed in a chamber containing the appropriate chromatographic solvent system specific for the determination of the presence of a given class of phytochemical constituents (Table 3.3).

Table 3.3: Criteria for detection of various phytochemical compounds

Secondary Metabolite	Solvent System	Detection
Alkaloids	Dichloromethane: Methanol 9:1	Drangedroff's reagent was sprayed on the developed TLC to detect alkaloids. The plates were viualized under 254 nm and 266 nm. Brown or orange spots indicated prescence of alkaloids.
Tannins	Ethyl acetate: Methanol: Water (100: 135: 10)	The developed plates were sprayed with iron (III) chloride solution and visualized under UV light. Dark blue spots indicated prescence of tannins.
Saponins	Dichloromethane: Ethylacetate	The developed plates were sprayed with a mixture of ethanol and sulphuric acid (9:1) and heated for 10 minute. Saponins were detected as black spots.
Sesquiterpene Lactones	n- hexane: Ethylacetate (9:1)	The developed plates were placed in a chamber containing iodine crystals. They wer detected as brown, yellow, or red spots on the plate.
Flavonoids	n- hexane: ethylacetate (1:9)	At 254 nm, flavonoids were detected as dark blue zones on a yellow background on the developed TLC. At 365 nm, flavonoids fluorescent yellow, blue or green. The plates were sprayed with vanillin dissolved in 2 ml sulphuric acid and 8 ml of ethanol added. The plates were then heated for five minutes at 110^{o} C. Flavonoids were detected as purple, red or blue spots.

3.12 Data analysis

Ms Excel 2007 was used to determine the mean parasitaemia and the percentage chemosuppression and to draw charts and graphs. Chemosuppression was analyzed using SPSS Version 16. One way ANOVA was used to analyze chemosuppression means obtained from the four day suppressive assay to determine whether chemosuppression caused by one plant extract was different from chemosuppressions caused by the other plant extracts according to Morgan *et al*., (2004). Once the means were found to be different from each other, Dunnett test was then used for multiple comparisons of chemosuppressions to determine whether chemosuppressions arising from the various treatments were different from the chemosuppression induced by chloroquine (positive control). LSD (Least Significance Difference Test) was also used to compare chemosuppression of two treatments at ago. The significance level used in the analysis was 0.05 (Alpha Level $\leq$ 0.05).

Brine shrimp toxicity was determined by Finney probit analysis according to Meyer *et al.*, (1982). The average mortality in the three concentrations (1000 ug/ml, 100 ug/ml and 10 ug/ml) for each plant was fed into the program to estimate the LD_{50}.

CHAPTER FOUR

4.0 RESULTS

4.1 Yield of crude plant extracts

The plant materials were extracted using water (Aqueous) and Chloroform: Methanol [($CHcl_3$:MEOH (1:1)]. The aqueous extracts varied from 11% to 7% w/w while the organic extracts varied from 20% to 12% w/w. *Plectranthus barbatus* had the highest yield (20.1%) under organic extraction compared to *Ocimum suave* which had the lowest yield (12.06%). Under aqueous extraction, *Zanthoxylum chalybeum* had the highest yield (11.4%) compared to *Plectranthus barbatus* which had the lowest (6.78%) as shown in Table 4.1.

Table 4.1: Percentage yield of aqueous and organic crude plant extracts

Plant species and voucher specimen no.	Part used	Extraction type	Initial sample weight (g)	Sample weight after extraction (g)	Standard deviation	Standard error	% yield (w/w)
Zanthoxylum chalybeum MNK 01/2014	Root bark	Aqueous Organic	50 50	5.57 6.27	.12000 .01000	.06928 .00577	11.14 12.54
Ocimum suave MNK 02/2014	Leaves	Aqueous Oganic	40 50	5.22 6.03	.11000 .02000	.06351 .01155	10.44 12.06
Plectranthus barbatus MNK 03/2014	Root bark	Aqueous Organic	50 50	2.71 10.05	.05859 .05000	.03383 .02887	6.78 20.1

4.2 Parasitaemia and parasite growth inhibition

Peter's 4- day suppressive test assay was used in antimalarial activity evaluation. On the 4th day of the suppressive test, thin blood smears were prepared and observed under oil immersion (× 1000) of a Photographic Compound Microscope (LEICA ICC50). The estimate number of parasitized cells and the total number of cells visible in the field of view were counted (Appendix 1). The photomicrographs shown on plate 1 show non-parasitized cells, parasitized but no treatment and parasitized and treated cells from one magnification field.

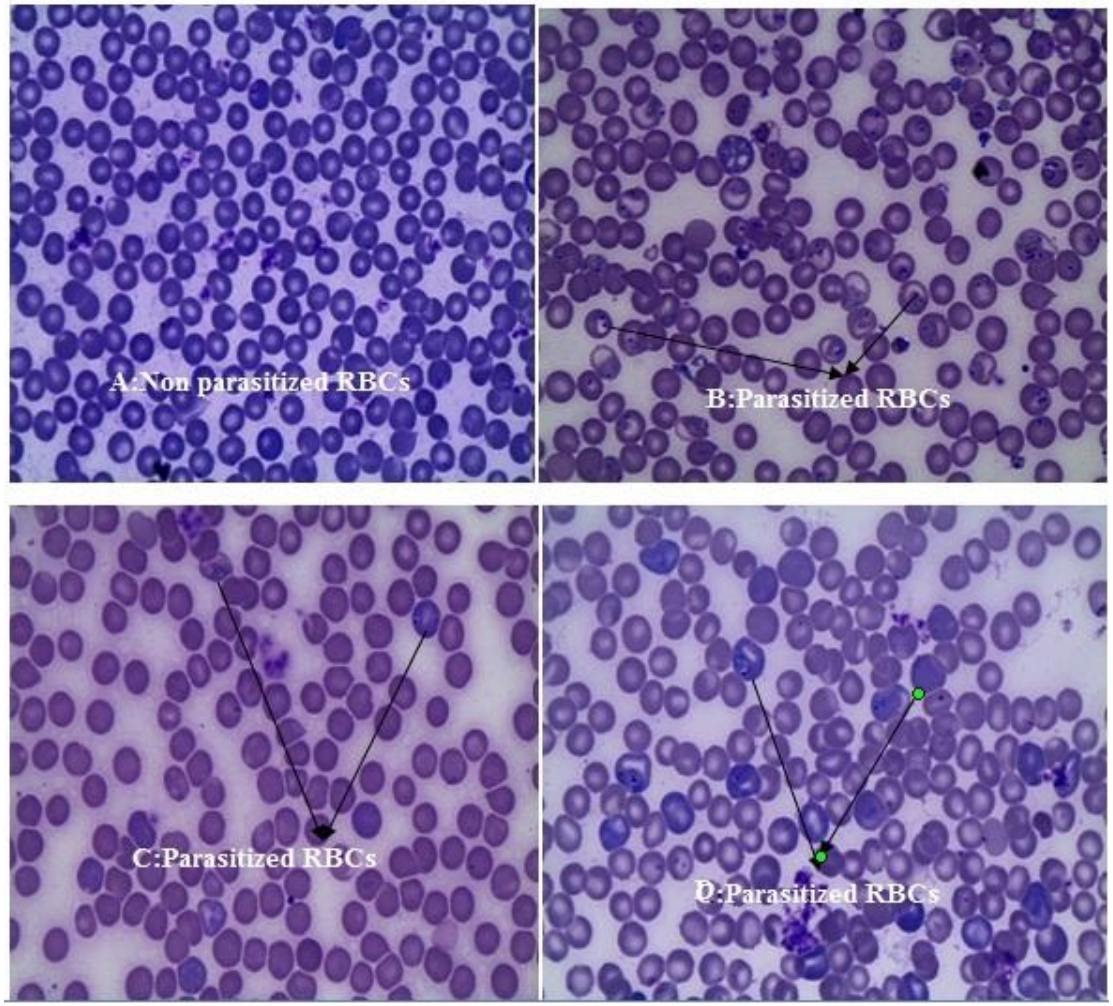

Plate 1: Blood smear microslides of Red blood cells

KEY:

A: Blood smear slide of non-parasitized red blood cells of a normal mouse

B: Blood smear slide showing some parasitized red blood cells from a mouse that was infected by *P. berghei* and no treatment (-ve control)

C: Blood smear slide of parasitized red blood cells of a mouse treated with chloroquine (+ve control)

D: Blood smear slide of parasitized red blood cell of a mouse treated with aqueous extract root extract of *Z. chalybeum.*

Magnification was × 1000

4.3: *In vivo* antimalarial activity

Table 4.2 summarizes the parasite densities after the 4-days suppressive antimalarial screening of the extracts at a dose of 100 mg/kg body weight/day and the positive (Chloroquine) and negative (Distilled water) controls in mice parasitized with *P. berghei*.

Both aqueous and organic extracts were subjected to ANOVA. Table 4.3 shows the results of preliminary ANOVA. Means of the various treatments were compared to determine whether there were significant differences between them. Probability level/significance level used in the analysis was $p \leq 0.05$.

NB: When the significance level obtained is $p < 0.05$: Reject the null hypothesis.

When the significance level obtained is $p > 0.05$: Accept the null hypothesis.

From the table, the significance level obtained is $p < 0.000$ which is $p < 0.05$. Hence reject the null hypothesis and conclude that the chemosuppressions of the various treatments were significantly different from each other. This called for further ANOVA (posthoc ANOVA) to compare two treatments at ago to determine whether they are significantly different or not. Posthoc ANOVA was performed using LSD and Dunnet test (Table 4.4).

From table 4.4, the significance levels obtained from the comparison of the chemosuppression of the *Z. chalybeum* and chloroquine from both LSD and Dunnet test are 0.195 and 0.413 respectively. These significance levels are $p > 0.05$. Hence the conclusion that the chemosuppression induced by aqueous extracts of *Z. chalybeum* is not statistically significantly different from the chemosuppression induced by chloroquine. Levels of significance obtained when both *O. suave* and *P. barbatus* were compared with chloroquine are 0.002 and 0.000 respectively which are $p<0.05$ (Table 4.5). Hence the chemosuppression of these aqueous extracts were different from that of chloroquine.

ANOVA was performed for chemosuppression of the organic extracts. The level of significance obtained was 0.000; hence all the treatments had chemosuppressions which were significantly different from each other (Table 4.6).

Table 4.2: Mean parasitaemia for the six treatments and the controls

Treatment	**Dose**	**Parasite density (P.d)**					
		Aqueous	Standard deviation	Standard error	Organic	Standard deviation	Standard error
Z.anthoxylum chalybeum	100 mg/kg/ b.w	4.14	0.1200	0.0692	4.83	0.3151	0.1819
Ocimum suave wild	100 mg/kg b.w	7.21	0.2100	0.1212	11.01	2.0100	1.1604
Plectranthus barbatus	100 mg/kg b.w	10.0	1.5000	0.8660	4.76	0.1400	0.0808
Chloroquine	20 mg/kg b.w	0.5	0.2516	0.1453	0.5	0.3000	0.1732
Distilled water	N/A	22.33	0.5150	0.2973	22.33	0.3300	0.1905

Data expressed as mean for five determinants per group

Table 4.3: ANOVA (Analysis of Variance) for the aqueous extracts

Chemosuppression					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3201.147	3	1067.049	18.228	0.000
Within Groups	936.625	16	58.539		
Total	4137.771	19			

Table 4.4: Least Significance Difference test and Dunnet test for the aqueous extracts

Dependent Variable Chemosuppression

				Sig.	95% Confidence Interval	
	(I) Treatments	(J) Treatments	Mean Difference (I-J)		Lower Bound	Upper Bound
LSD	Z.chalybeum	O.suave	13.75000*	0.012	3.4918	24.0082
		P.barbatus	26.22000*	0.000	15.9618	36.4782
		Chloroquine	-6.55000	0.195	-16.8082	3.7082
	O.suave	Z.chalybeum	-13.75000*	0.012	-24.0082	-3.4918
		P.barbatus	12.47000*	0.020	2.2118	22.7282
		Chloroquine	-20.30000*	0.001	-30.5582	-10.0418
	P.barbatus	Z.chalybeum	-26.22000*	0.000	-36.4782	-15.9618
		O.suave	-12.47000*	0.020	-22.7282	-2.2118
		Chloroquine	-32.77000*	0.000	-43.0282	-22.5118
	Chloroquine	Z.chalybeum	6.55000	0.195	-3.7082	16.8082
		O.suave	20.30000*	0.001	10.0418	30.5582
		P.barbatus	32.77000*	0.000	22.5118	43.0282
Dunnett t (2-sided)[a]	Z.chalybeum	Chloroquine	-6.55000	0.413	-19.0943	5.9943
	O.suave	Chloroquine	-20.30000*	0.002	-32.8443	-7.7557
	P.barbatus	Chloroquine	-32.77000*	0.000	-45.3143	-20.2257

*. The mean difference is significant at the 0.05 level.

Table 4.5: Chemosuppressions of *P. berghei* by the treatments versus chloroquine

Treatment	Dose	Chemosuppression				
		Aqueous	Standard deviation	Standard error	Organic	Standard deviation	Standard error
Zanthoxylum chalybeum	100 mg/kg/ b.w	81.45	8.1183	3.6306	78.39	1.0845	0.4850
Ocimum suave wild	100 mg/kg b.w	67.70	10.4675	4.6812	54.78	4.7671	2.1319
Plectranthus barbatus	100 mg/kg b.w	55.23	7.3606	3.2917	78.69	2.0070	0.8975
Chloroquine	20 mg/kg b.w	97.76	2.1213	0.9486	97.76	2.1679	0.9695

Data expressed as mean for five determinants per group

Table 4.6: ANOVA for the organic extracts

Chemosuppression

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2438.339	3	812.780	99.636	0.000
Within Groups	130.519	16	8.157		
Total	2568.858	19			

Levels of significance obtained when both *Z. chalybeum* and *P. barbatus* was compared with chloroquine using Dunnet test were $p<0.067$ and $p<0.091$ respectively (Table 4.7). These levels are $P> 0.05$ and hence the conclusion that the chemosuppressions induced by the organic extracts of *Z. chalybeum* and *P. barbatus* were not significantly different from the chemosuppression induced by chloroquine. The level of significance obtained when *O. suave* was compared to chloroquine is 0.000 which is $p< 0.05$ hence the chemosuppression induced by the organic extract of *O.suave* is significantly different from that of chloroquine.

Table 4.7: LSD and Dunnet for the organic extracts

Dependent Variable: Chemosuppression

					95% Confidence Interval	
	(I) Treatments	(J) Treatments	Mean Difference (I-J)	Sig.	Lower Bound	Upper Bound
LSD	Z.chalybeum	O.suave	23.61000*	0.000	19.7807	27.4393
		P.barbatus	-.30000	0.870	-4.1293	3.5293
		Chloroquine	-4.41000*	0.027	-8.2393	-.5807
	O.suave	Z.chalybeum	-23.61000*	0.000	-27.4393	-19.7807
		P.barbatus	-23.91000*	0.000	-27.7393	-20.0807
		Chloroquine	-28.02000*	0.000	-31.8493	-24.1907
	P.barbatus	Z.chalybeum	.30000	0.870	-3.5293	4.1293
		O.suave	23.91000*	0.000	20.0807	27.7393
		Chloroquine	-4.11000*	0.037	-7.9393	-.2807
	Chloroquine	Z.chalybeum	4.41000*	0.027	.5807	8.2393
		O.suave	28.02000*	0.000	24.1907	31.8493
		P.barbatus	4.11000*	0.037	0.2807	7.9393
Dunnett t (2-sided)[a]	Z.chalybeum	Chloroquine	-4.41000	**0.067**	-9.0927	0.2727
	O.suave	Chloroquine	-28.02000*	0.000	-32.7027	-23.3373
	P.barbatus	Chloroquine	-4.11000	**0.091**	-8.7927	0.5727

*. The mean difference is significant at the 0.05 level.

Figure 6 summarizes Parasite density versus chemosuppression for the three plant extracts and the controls. Th**e** highest parasitaemia was exhibited in the group treated with distilled water (22.33%) while the highest chemosuppression was exhited in the group treated with chloroquine (97.76%). Both aqueous and organic root extract of *Z. chalybeum* and the organic root extract of *P. barbatus* exhibited the lowest parasitaemia levels of 4.14%, 4.83% and 4.76% respectively, and hence high chemosuppression of 81.45%, 78.39% and 78.69% respectively. These chemosuppressions were not significantly different from that of chloroquine ($p>0.05$). Both aqueous and organic leaf extracts of *O. suave* had chemosuppressions that were significantly different from that of chloroquine ($p<0.05$).

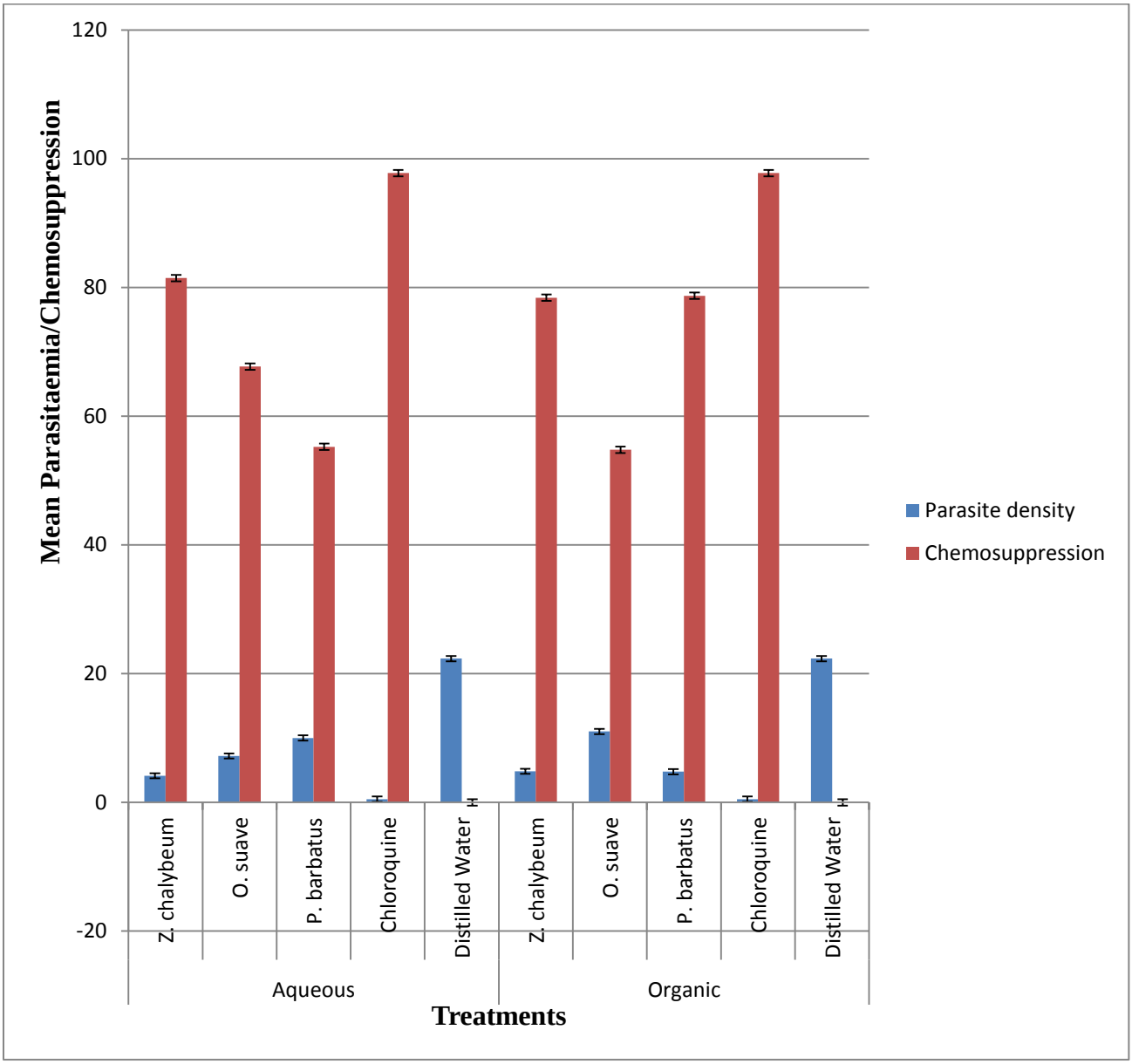

Figure 6: Mean plots for parasitaemia and chemosuppression for both aqueous and organic plant extracts compared to the controls

4.4 Mean survival time (days) of *P. berghei* infected mice

Figure 7 summarizes the survival time of different groups of mice treated with the plant crude extracts. Those mice treated with chloroquine, the test drug survived for the 14 days while those treated with distilled water survived for approximately 6 days. The mice treated with aqueous extracts of *Z. chalybeum* and *P. barbatus* survived longest (9.8 and 9 days) respectively. This was close to the survival time of mice treated with chloroquine. In organic treatments, those treated with *Z. chalybeum* and *P. barbatus* again survived longest (9.6 and 9.4 days) respectively. This is again close to those treated with chloroquine. There is a relationship between chemosuppression and the survival time. The group that was treated with plant extracts with high chemosuppression survived longest (close to the survival time of those treated with chloroquine). The group that was treated with *O. suave* which had a low chemosuppresssion showed a survival time that was slightly higher than those treated with distilled water (7.9 days).

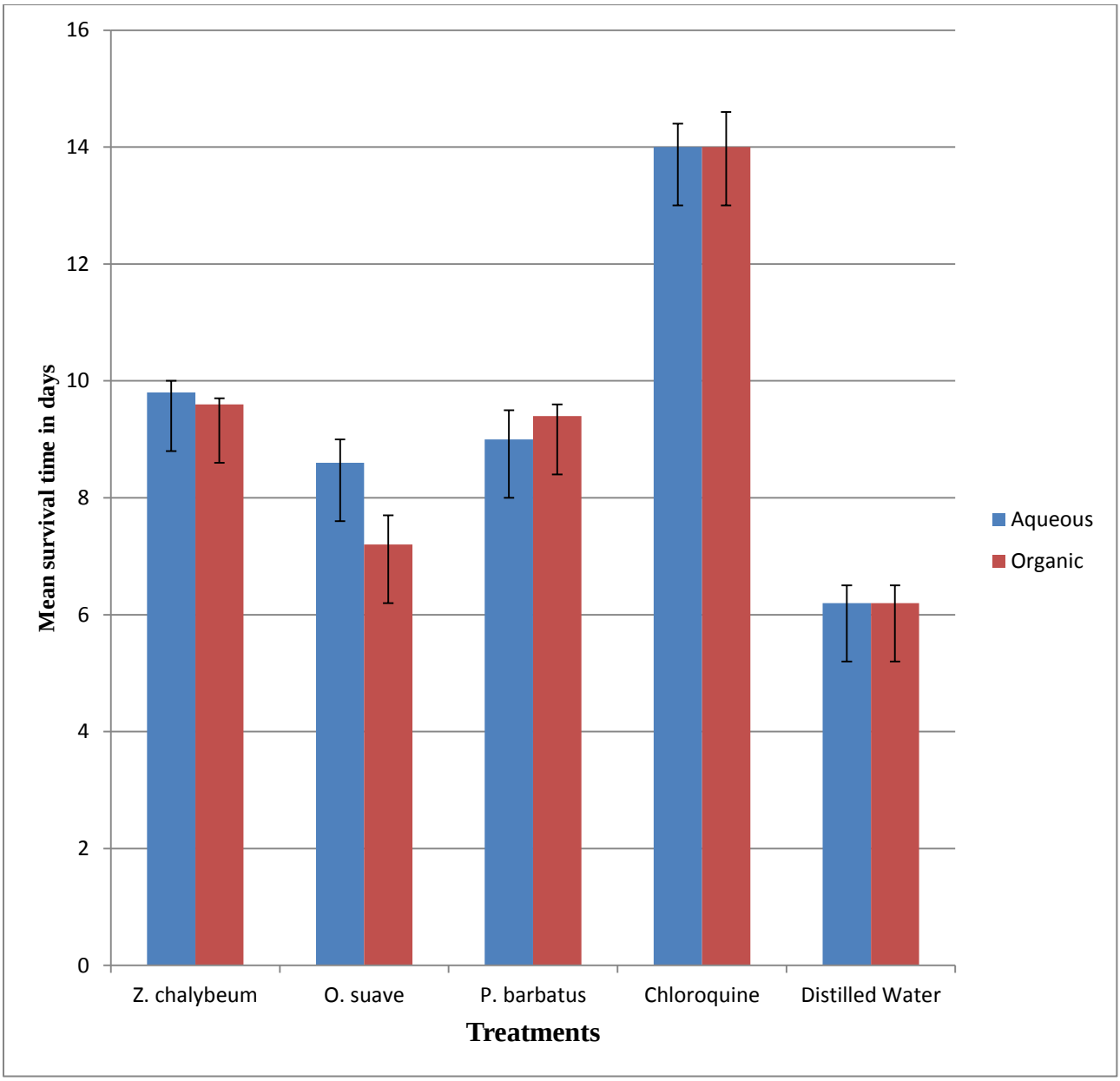

Figure 7: Mean survival time (days) of mice using the six plant extracts and controls

4.5 Hematological activity

Figure 8 summarizes the effects induced in the hematological parameters following treatment of *P. berghei* infected mice with the aqueous plant extracts (dose of 100 mg/kg body weight), and chloroquine. The highest PCV was observed in the positive control (41.4) while the lowest PCV was observed in the group treated with *P. barbatus*. Both PCV of the normal and the negative control group had PCV of 31. *Z. chalybeum* and *O. suave* had a slightly lower PCV of 28.9 and 22 respectively. Highest Hb content was observed in the positive control group while the lowest was observed in the group treated with *P. barbatus, Z. chalybeum* and *O.suave* had moderate Hb content. Platelet count was highest in the *P. barbatus* and positive control groups and was lowest in *Z. chalybeum* and *O. suave* group. On the contrary WBC were lowest in chloroquine (CQ) group and highest in the groups treated with Z. chalybeum, *O. suave* and *P. barbatus*

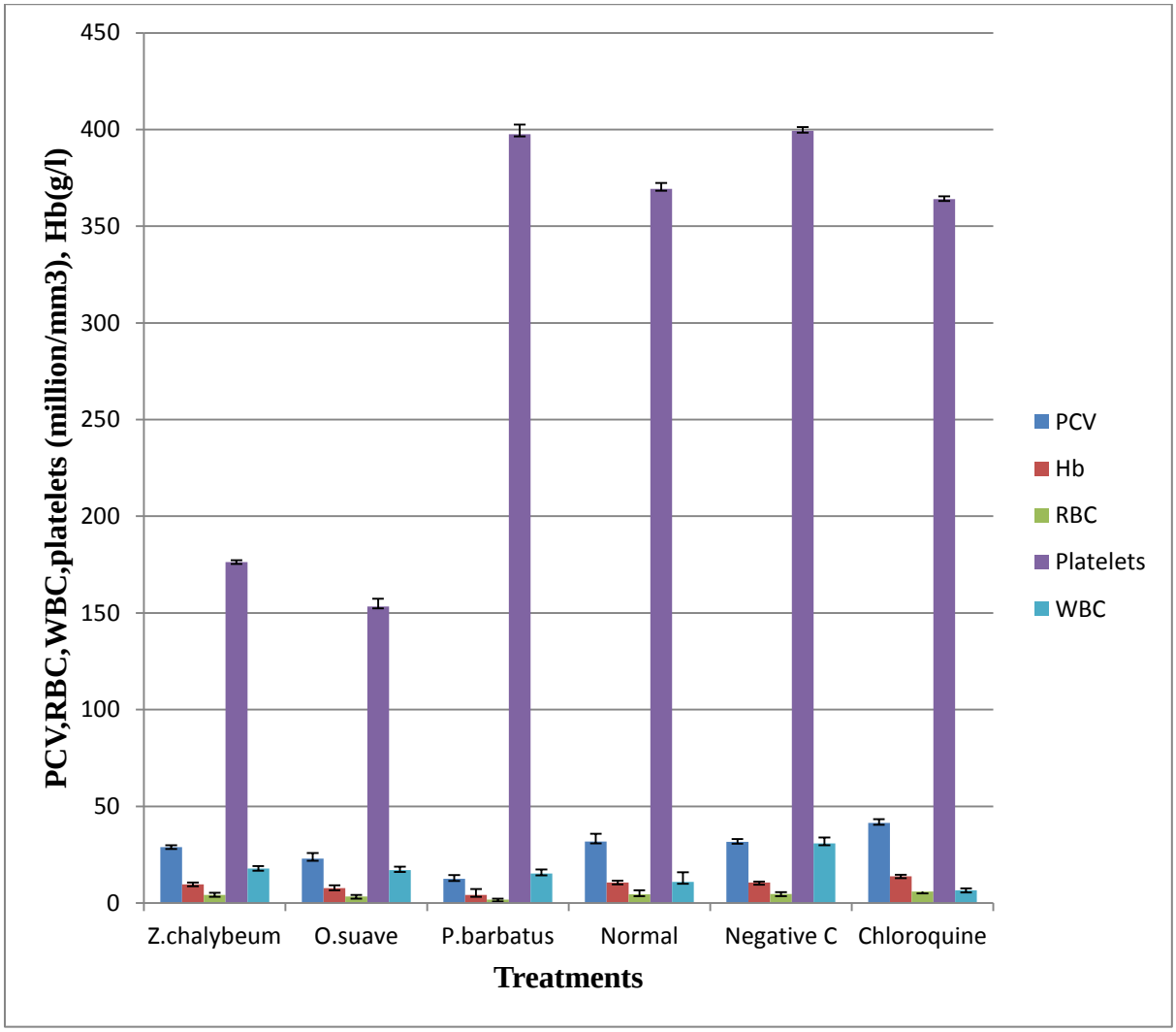

Figure 8: Effects of chloroquine (CQ) and aqueous plant extracts on hematological parameters of *P. berghei* infected mice (CQ= 20 mg/kg; Extracts= 100 mg/kg).

Figure 9 summarizes the effects induced in the hematological parameters following treatment of *P. berghei* infected mice with the aqueous plant extracts (dose of 200 mg/kg body weight), and chloroquine. High PCV, RBC, Hb and Platelets were observed in the group treated with CQ while *Z. chalybeum, O. suave* and *P. barbatus* groups has slightly lower content of these hematological parameters compared to CQ group, with *P. barbatus* having the least amount and *Z. chalybeum* having the highest content. However, WBC were least in CQ group, and among *Z. chalybeum*, *O. suave* and *P. barbatus*, it was highest in *Z. chalybeum* and lowest in *P. barbatus*.

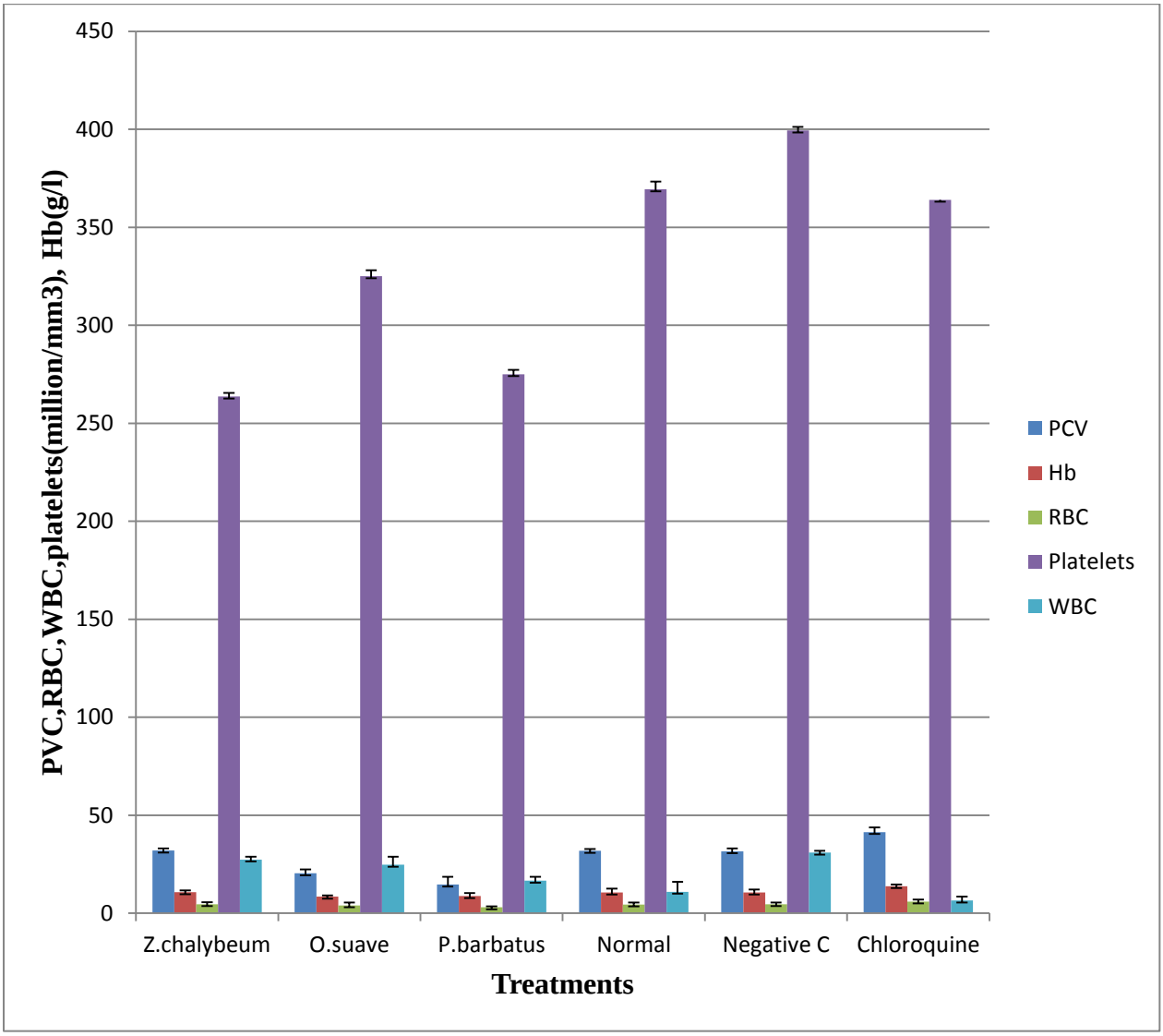

Figure 9: Effects of Chloroquine (CQ) and aqueous plant extracts on hematological parameters of *P. berghei* infected mice (CQ = 20 mg/kg, Extracts = 200 mg/kg

Figure 10 summarizes the effects induced in the hematological parameters following treatment of *P. berghei* infected mice with the organic plant extracts (dose of 100 mg/kg body weight), and chloroquine. Among Chloroquine (CQ), *Z. chalybeum, O. suave* and *P. barbatus* treatment groups, CQ group had high content of PCV, RBC, Hb and Platelets, followed by *Z. chalybeum* while *O. suave* had the least content of these hematological parameters. Still among these four groups, CQ group had the least amount of WBCs, while *Z. chalybeum* and *P. barbatus* groups had the highest number of WBCs and *O. suave* had slightly lower number of WBCs compared to *Z. chalybeum* and *P. barbatus* groups.

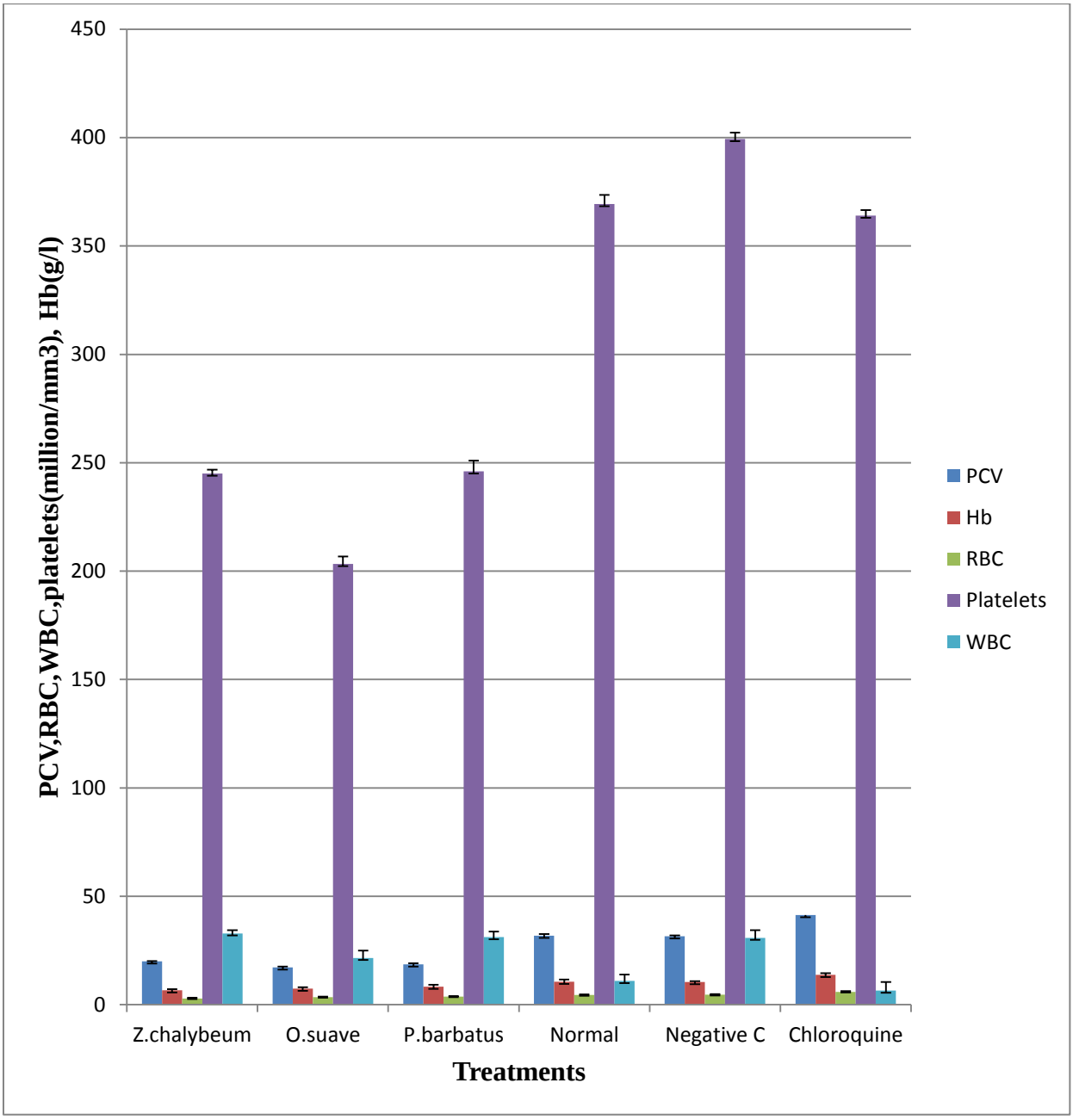

Figure 10: Effects of chloroquine (CQ) and organic plant extracts on hematological parameters of *P. berghei* infected mice (CQ= 20 mg/kg; Extracts= 100 mg/kg).

Figure 11 summarizes the effects induced in the hematological parameters following treatment of *P. berghei* infected mice with the organic plant extracts (dose of 200 mg/kg body weight), and chloroquine. Among Chloroquine (CQ), *Z. chalybeum, O. suave* and *P. barbatus* treatment groups, CQ group had High content of PCV, RBC and Hb, followed by *Z. chalybeum* while *P. barbatus* had the least content of these hematological parameters. Platelets were highest in the CQ group, followed by *Z. chalybeum* group; *P. barbatus* group had lower platelet number compared to *Z. chalybeum* group but higher number of platelets compared to *O. suave* group. On the other hand CQ group had the least amount of WBCs, while *Z. chalybeum* followed by *O. suave* group, *P. barbatus* group had lower number of WBCs compared to *O. suave* group.

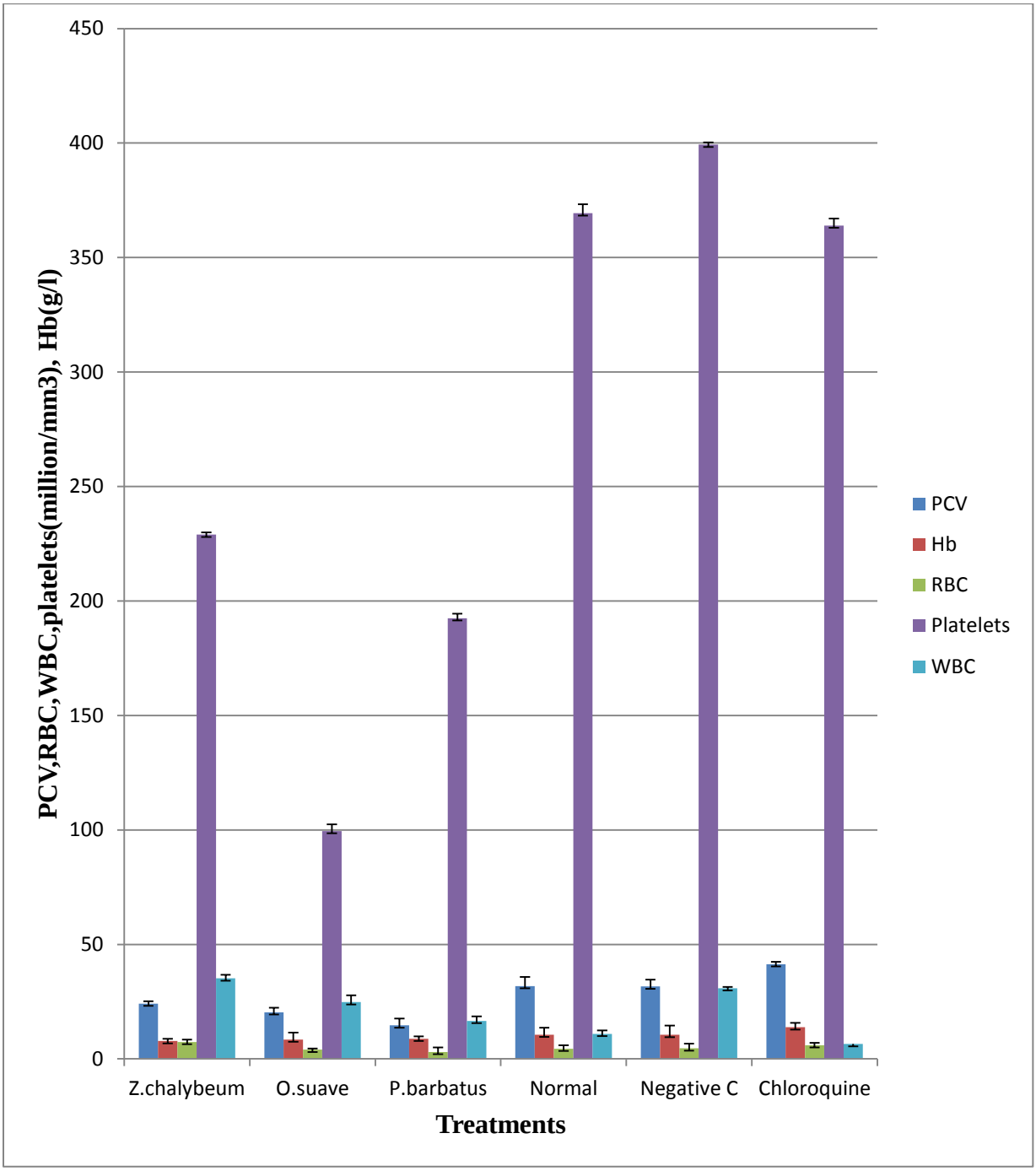

Figure 11: Effects of chloroquine (CQ) and organic plant extracts on hematological parameters of *P. berghei* infected mice (CQ= 20 mg/kg; Extracts= 200 mg/kg).

Figure 12 summarizes the effects on WBC differential count following treatment of *P. berghei* infected mice with the aqueous plant extracts (dose of 100 mg/kg body weight), and chloroquine. In all the treatments, no eosinophils and basophils were detected. CQ group had the highest number of neutrophils and lymphocytes compared to *Z. chalybeum, O. suave* and *P. barbatus* groups which had almost the same number of neutrophils and lymphocytes. Monocytes were lowest in the CQ group and highest in the *O. suave* group with *Z. chalybeum* and *P. barbatus* groups having lower monocytes number than *O. suave* group.

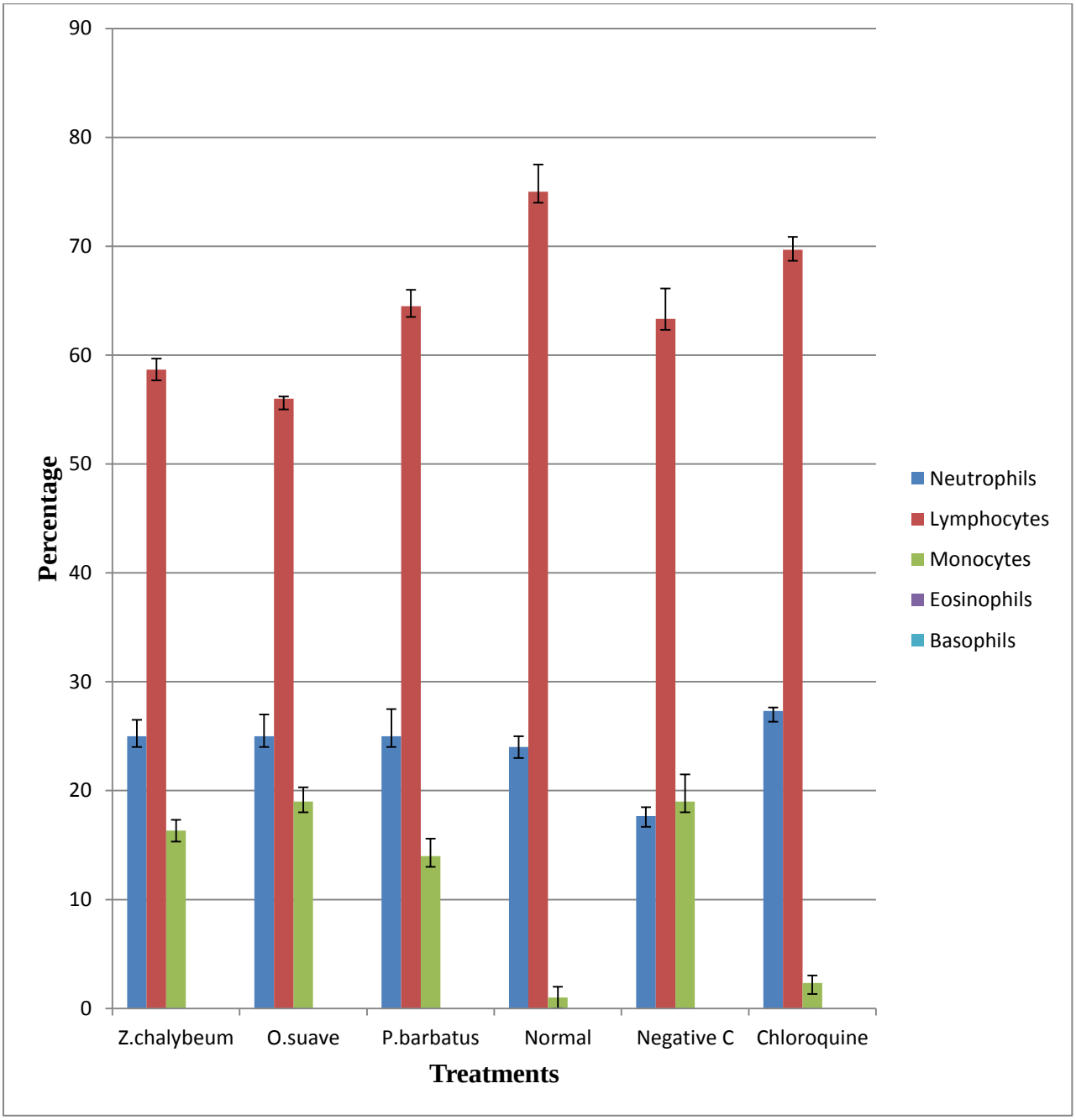

Figure 12: Effects of chloroquine (CQ) and aqueous plant extracts on WBC differential count on *P. berghei* infected mice (CQ= 20 mg/kg; Extracts= 100 mg/kg).

Figure 13 summarizes the effects on WBC differential count following treatment of infected *P. berghei* mice with the aqueous plant extracts (dose of 200 mg/kg body weight) and chloroquine. The number of neutrophils and lymphocytes were almost the same in the CQ, *Z. chalybeum* group, *O. suave* and *P. barbatus* group. No eosinophils and basophils could be detected in all the mice treated in all the groups. Monocytes were lowest in the CQ group and almost the same in number in *Z. chalybeum, O. suave* and *P. barbatus* groups.

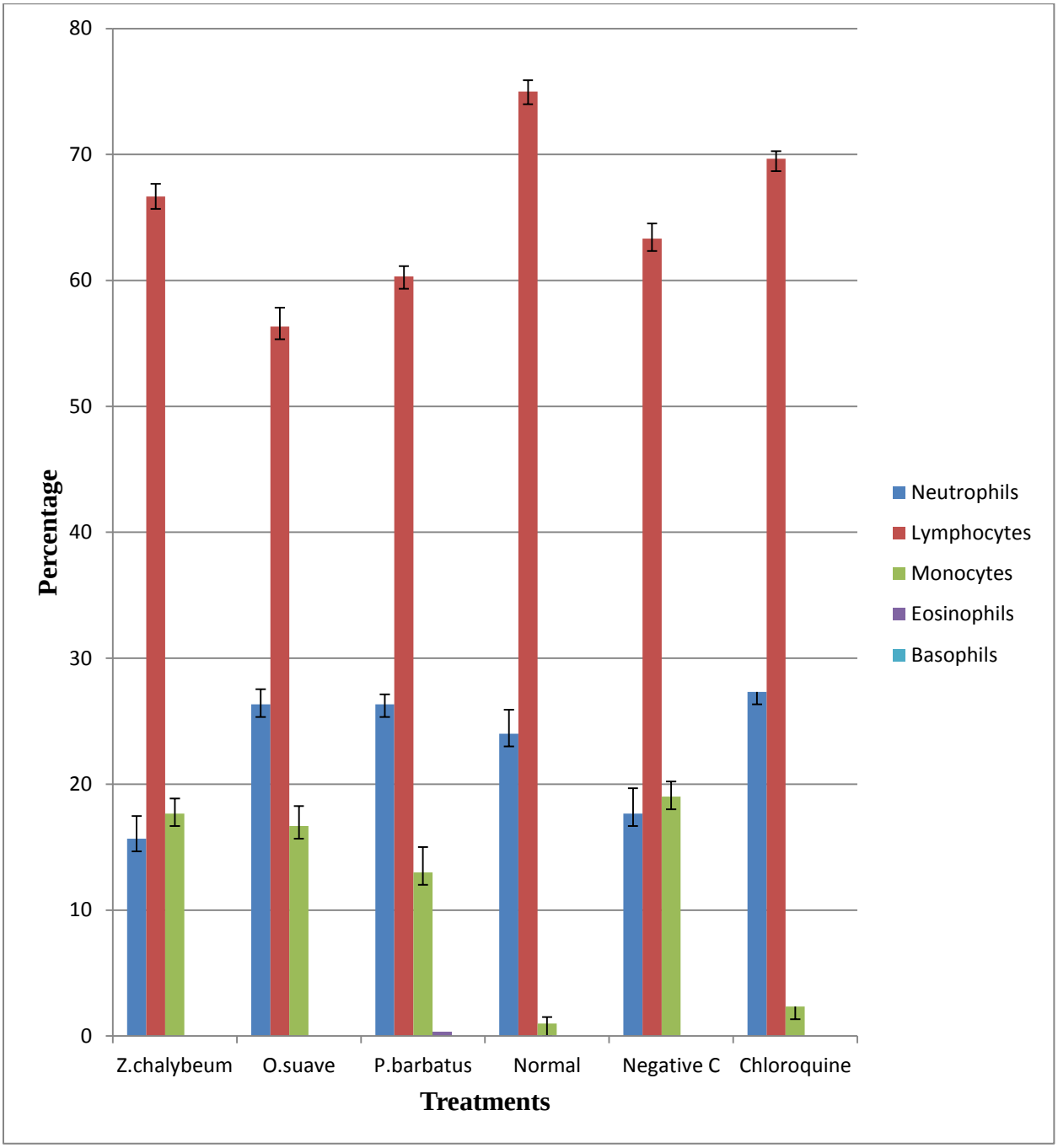

Figure 13: Effects of chloroquine (CQ) and aqueous plant extracts on WBC differential count on *P. berghei* infected mice (CQ= 20 mg/kg; Extracts= 200 mg/kg).

Figure 14 summarizes the effects on WBC differential count following treatment of infected *P. berghei* mice with the organic plant extracts (dose os 100 mg/kg body weight) and chloroquine. Neutrophils were highest in the CQ group, *Z. chalybeum*, *O. suave* group and were lowest in *P. barbatus* group. Lympocytes were highest in the *P. barbatus* group followed by the CQ group, followed by *O. suave* group. *Z. chalybeum* had the lowest lymphocytes content. Monocytes were lowest in CQ group and highest in the *Z. chalybeum* and *O. suave* groups while *P. barbatus* group had slightly lowest monocytes content compared to the *Z. chalybeum* and *O. suave* groups. No Eosinophils and Basophils were detected in the entire treatment groups.

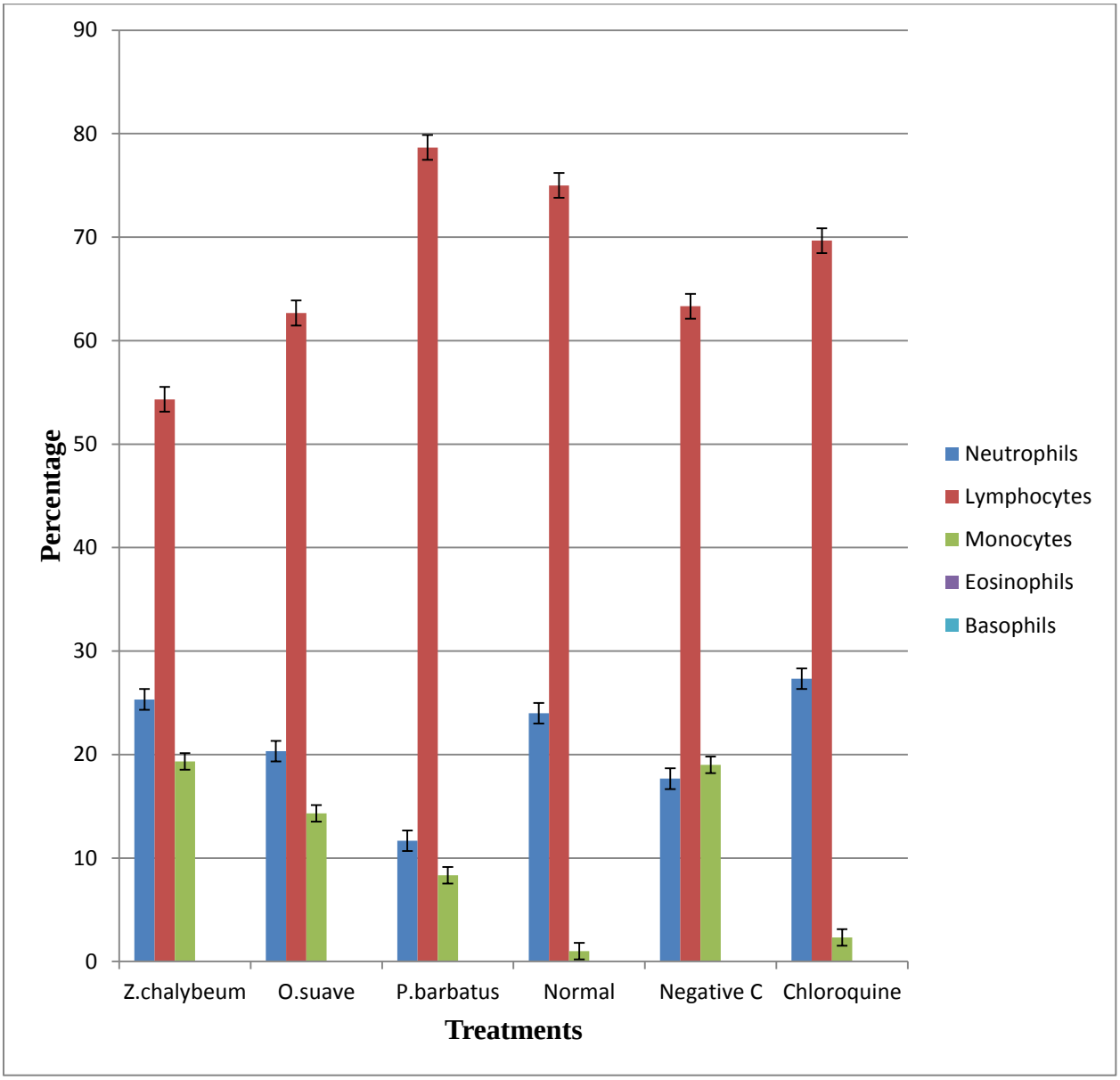

Figure 14: Effects of chloroquine (CQ) and organic plant extracts on WBC differential count on *P. berghei* infected mice (CQ= 20 mg/kg; Extracts= 100 mg/kg).

Figure 15 summarizes the effects on WBC differential count following treatment of infected *P. berghei* mice with the organic plant extracts (dose of 200 mg/kg body weight) and chloroquine. *Z. chalybeum* and CQ group had the highest neutrophil content, while *O. suave* had the least neutrophil content; *P. barbatus* group has slightly higher neutrophil content compared to *O. suave* group. Lypmhocytes were highest in *O. suave* and *P. barbatus* groups and were least in *Z. chalybeum* group. Monocytes as usual were least in the CQ group and highest in the *Z. chalybeum*, *O. suave* and *P. barbatus* groups, with *P. barbatus* having the highest number of monocytes. Eosinophils and Basophils could not be detected in all the treatment groups.

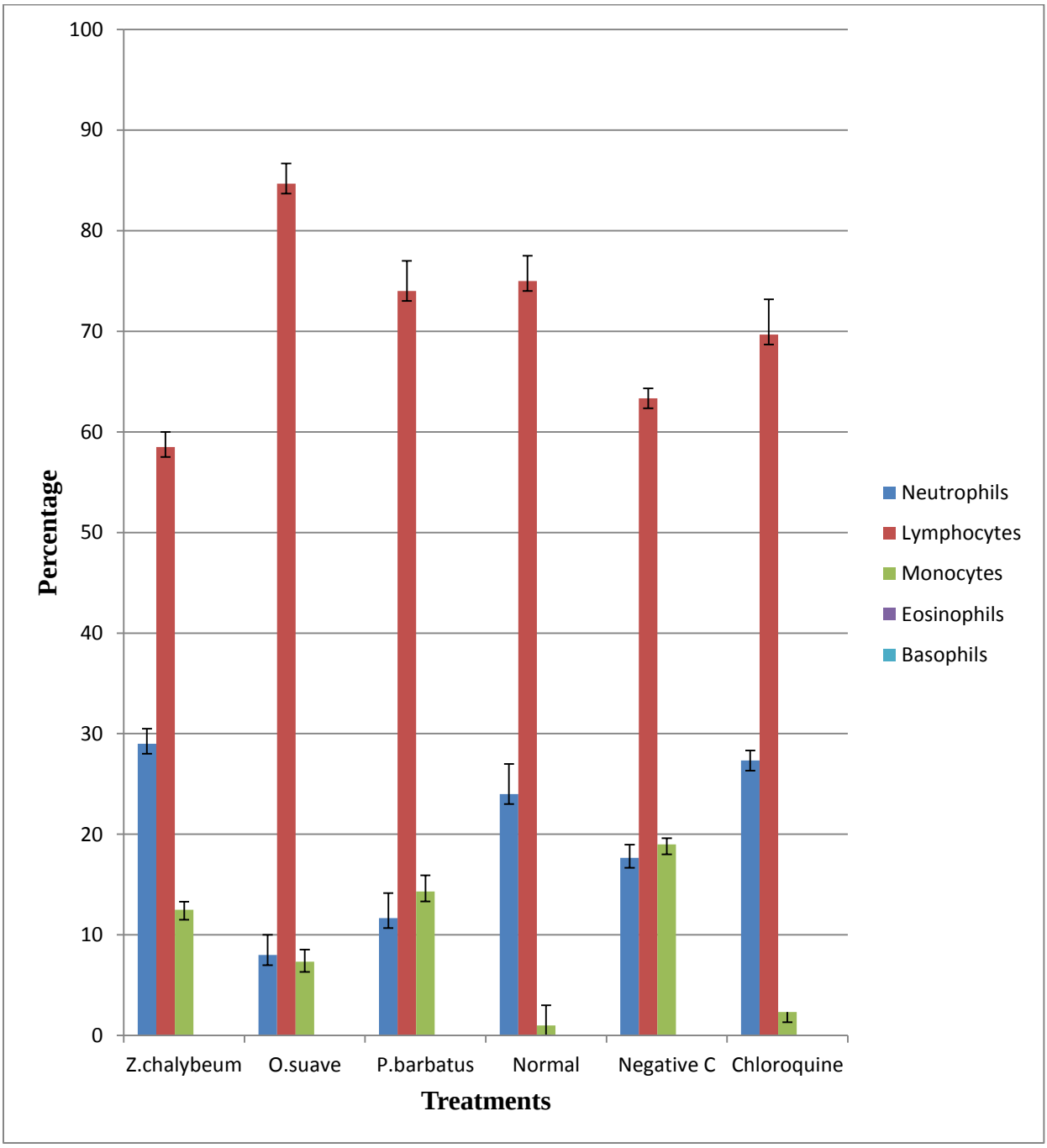

Figure 15: Effects of chloroquine (CQ) and organic plant extracts on WBC differential count on *P. berghei* infected mice (CQ= 20 mg/kg; Extracts= 200 mg/kg).

4.6: Acute oral toxicity

None of the three plant extracts produced mortality after giving a dose of 2000 mg/kg/ body weight. All the mice given the extracts survived the 14 days period of observation and were sacrificed on the 14th day. All the three plant extracts did not show signs of toxicity. In terms of weight, the aqueous treatments showed a steady increase in the average weight up to day 5 and a decrease by day 7 followed by an increase by day 9 which was afterwards followed by a fluctuated pattern up to day 14 when the mice were sacrificed. *Z. chalybeum* and *P. barbatus* showed the highest increase while *O. suave* had the least (Figure 16).

For the organic extracts treatments, none of the extracts produced mortality after giving a dose of 2000 mg/kg/body weight. All the mice given the extracts survived the 14 days period of observation and were sacrificed on the 14th day. In terms of weight, the organic treatments showed a steady increase in the average weight up to day 5 and a decrease by day 7 followed by an increase by day 9 which was afterwards followed by a fluctuated pattern up to day 14 when the mice were sacrificed. Organic extracts of *O. suave* showed the highest increase followed by *P. barbatus* and *Z. chalybeum* with the average weights of 25.2, 22.8 and 20.2 grams respectively (Figure 17).

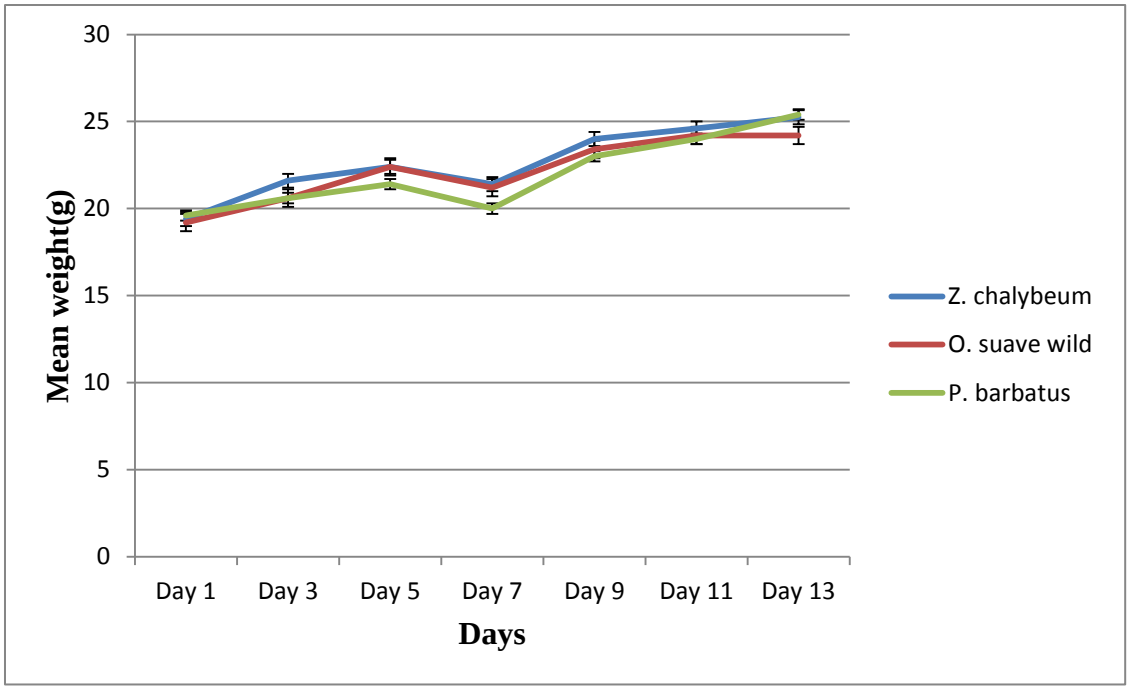

Figure 16: Variation of mean weights of the mice treated with aqueous extracts with time.

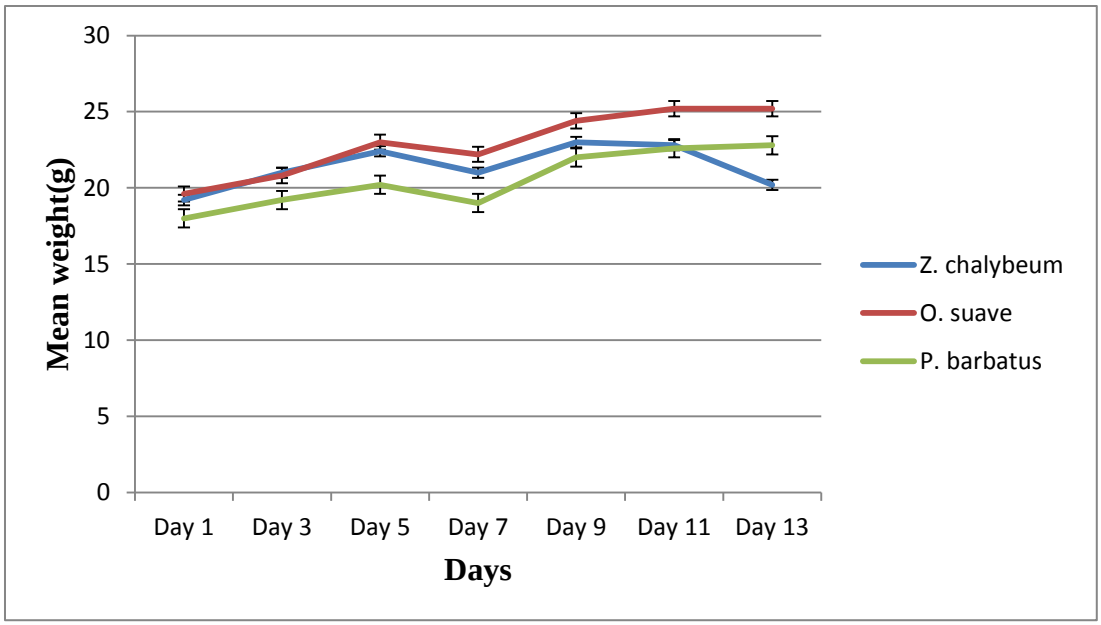

Figure 17: Variation of mean weights of the mice treated with organic extracts with time.

4.7 Brine shrimp lethality assay

The aqueous root extracts of *Z. chalybeum* and *P. barbatus* had a LD_{50} of 576.97 and 566.45 respectively meaning that they were weakly toxic to brine shrimp while the aqueous leaf extract of *O. suave* had a LD_{50} of 324.44 meaning that it was moderately toxic to brine shrimp (Table 4.8).

The organic leaf extract of *O. suave* and the root extract of *P. barbatus* had a LD_{50} of 150.14 and 418.76 respectively meaning that they were moderately toxic to the brine shrimp while *Z. chalybeum* had a LD_{50} of 42.73 meaning that it was highly toxic to the brine shrimp (Table 4.9).

Table 4.8: LD_{50} values for brine shrimp treated with aqueous extracts

Extract	Concetration (ug/ml)	Total number of shrimps	% Mortality	Average Mortality	LD_{50}
Zanthoxylum chalybeum	0	30	0	0	576.97
	10	30	0	0	
	100	30	0	0	
	1000	30	100	10	
Ocimum suave	0	30	0	0	324.44
	10	30	0	0	
	100	30	46.7	4.67	
	1000	30	100	10	
Plectranthus barbatus	0	30	0	0	566.45
	10	30	0	0	
	100	30	30	3	
	1000	30	86.7	8.67	

Table 4.9: LD_{50} values for brine shrimp treated with organic extracts

Extract	Concetration (ug/ml)	Total number of shrimps	% Mortality	Average Mortality	LD_{50}
Zanthoxylum chalybeum	0	30	0	0	
	10	30	56.7	5.67	
	100	30	100	10	42.73
	1000	30	100	10	
Ocimum suave	0	30	0	0	
	10	30	0	0	
	100	30	86.7	8.67	150.14
	1000	30	100	10	
Plectranthus barbatus	0	30	0	0	
	10	30	0	0	
	100	30	46.7	4.67	418.76
	1000	30	90	9	

KEY:
LD_{50} < 100 = Strongly/highly toxic
LD_{50} > 100 < 500 = moderately toxic
LD_{50} > 500 < 1000 = weakly toxic
LD_{50} > 1000 = Non toxic (Nguta *et al.*, 2011)

4.8 Phytochemicals in crude extracts

Thin layer chromatography (TLC) was carried out to determine the various major compounds that were present in the aqueous and the organic crude extracts of the plants. Alkaloids, Flavonoids and Tannins were found to be present in all both aqueous and organic extracts of the three plants. Saponins were found present in all extracts except for *Zanthoxylum chalybeum*. Glycosides were only found present in both extracts of *Plectranthus barbatus* while sesquiterpene lactones were only found present in the organic ($CHCL_3$: MEOH) extracts of the three plants (Table 4.10).

Table 4.10: Phytochemical composition of crude extracts

Plant Extract	Extraction Type	Alkaloids	Flavonoids	Sesquiterpene Lactones	Saponins	Glycosides	Tannins
Z. chalybeum	Aqueous	+	+	-	-	-	+
	Organic	+	+	+	-	-	+
O. suave	Aqueous	+	+	-	+	-	+
	Organic	+	+	+	+	-	+
P. barbatus	Aqueous	+	+	-	+	+	+
	Organic	+	+	+	+	+	+

KEY

+: Present

-: Absent

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The current study was designed to evaluate the pharmacological and toxicological effects of some selected antimalarial plants in Msambweni folklore medicine. In the study, an animal model *Plasmodium berghei* parasite that infects rodents was used to imitate *P. falciparum*, an animal model swiss albino mouse to imitate human presentation of malaria infection and the crude extracts from these plants to represent potential malaria drugs. The antimalarial plants naturalized in Msambweni of Kwale County were: *Zanthoxylum chalybeum* (root bark), *Ocimum suave* (Leaves) and *Plectranthus barbatus* (root bark). These plants were screened for their pharmacological and toxicological effects.

Antimalarial activity of all extracts was tested using *P.berghei* (ANKA) in a mouse model. Phytochemical screening was done to establish the biological compounds (secondary metabolites) that could be responsible for the observed effects, while hematological parameters were assessed to investigate the mechanisms of action through which antimalarial plant extracts probably works.

Chemosuppression by both aqueous and organic root extracts of *Z. chalybeum* (81.45% and 78.38% respectively indicated antimalarial pharmacological activity since it was not significantly different from that of chloroquine ($p>0.05$). The decrease in parasitaemia density in the aqueous treatment (4.14%) and organic (4.83%) respectively suggests possible antimalarial activity in the extracts as compared to the controls (22.33%) for distilled water and 0.5% for chloroquine respectively. Chloroquine is a drug used in the treatment of malaria.

Methanolic extracts of the root bark of *Z. chalybeum* have been reported to have significant antimalarial activity *on P. falciparum* (Muganga *et al*., 2010). This is in agreement with the observations in the current study. Other studies have shown that aqueous extract of a related species *Z. usambarense* (Engl.) also exhibited significant antiplasmodial activity against *P. falciparum* (Kirira *et al*., 2006). Further, previous studies have shown that water extracts of *Z. chalybeum* have significant in vitro antimalarial activity against chloroquine sensitive and

chloroquine resistant strains of *P. falciparum* (Rukunga *et al.,* 2009) which is in line with the results on *Z. chalybeum* obtained in the current study. In addition, water and methanol stem extracts of *Z. chalybeum* has been reported to have *in vivo* antimalarial activity (Musila *et al*., 2013).

Out of the three plant extracts tested, the aqueous extract of *Z. chalybeum* showed the lowest parasitaemia (4.14%) compared to distilled water (22.33%) and the highest chemosuppression of 81.45% compared to that of chloroquine of 97.76%. Indeed, the survival time of mice treated with the aqueous extract of *Z. chalybeum* survived the longest duration compared to those treated with chloroquine indicating that there were bioactive compounds *against P. berghei* in the aqueous extract.

The effect of both aqueous and organic root extracts of *Z. chalybeum* on hematological parameters in *P. berghei* infected mice was investigated. The extract at high dose (200 mg/kg body weight caused an increase in the erythrocyte count. This was confirmed by the increased hematocrit (PCV) and percentage Hb in the high dose recipient group. In normal circumstances, local tissue anoxia apparently leads to the formation of a glycoprotein called erythropoietin, which stimulates increased production of erythrocytes. It is very likely that *Z. chalybeum* root extract contains erythropoietin-like agent(s) which is/are responsible for the increased production of erythrocytes. Administration of the extract caused a decrease in platelets.

The total WBC (Leucocyte) counts were also significantly increased following extract administration. Examination of the differential counts revealed that the neutrophil and monocyte counts increased. It is also possible that the extract contains agents that stimulate the bone marrow to produce neutrophils and release them into the blood. Neutrophils are the major granulocytes to be activated when the body is invaded by bacteria and they provide the first line of defense against invading microorganisms. The granules of the neutrophil contain many enzymes which makes it a powerful and effective killer machine. This effect on neutrophil count may be partly responsible for the claim that *Z. chalybeum* has antibacterial actions.

There was also some significant rise in lymphocytes. The results from the current study are in agreement with those of Kinney *et al.,* (1999), where he associated the significant rise in

lymphocytes to the potential usefulness of the plant as immune system stimulant, a factor that may justify the use of the plant decocortion in treatment of Measles in Uganda.

The root bark of *Z. chalybeum* was also screened for the presence or absence of the various classes of secondary metabolites using Thin Layer Chromatography. In both aqueous and organic root extracts, alkaloids, flavonoids and tannins were present. Sesquiterpene lactones were present in the organic extract but absent in the aqueous extracts. Alkaloids, Flavonoids and Sesquiterpenes have been reported to be potent plant secondary metabolites with broad spectrum of bioactivities (Mazid *et al*., 2011). Moreover, alkaloids are major classes of compounds possessing antimalarial activity, quinine is one of the most important and oldest antimalarial drugs which belong to this class of compounds (Saxena *et al*., 2003). In view of the above factors, it can be speculated that alkaloids and sesquiterpene lactones present in the root bark of *Z. chalybeum* may have been responsible for the observed *in vivo* antimalarial activity.

Upon testing for cytotoxicity on brine shrimp, the aqueous and the organic root bark extract of *Z. chalybeum* had LD_{50} of 576.97 and 42.73 respectively, meaning that the extracts were toxic to the shrimps. These results are in agreement with those of Nguta *et al*., (2011) while investigating toxicity of aqueous extracts of the leaves, stem bark and root bark of *Z. chalybeum* on brine shrimp larvae obtained LD_{50}< 500 ug/ml which supports the results obtained in the current study. Similarly, Musila *et al*., (2013), while investigating toxicity of aqueous and organic stem bark of *Z. chalybeum* on brine shrimp larvae obtained LD_{50}<500 ug/ml which was considered toxic.

In addition, significant cytotoxicity of *Z. chalybeum* methanolic root extracts on human foetal lung fibrolast cells has also been reported by Kamuhabwa *et al.,* (2000). This is again in agreement with the results obtained in this study. The results from the current study indicate that root bark extracts from *Z. chalybeum* could not make safe antimalarial remedies. However, in herbal practice, root extract from *Z. chalybeum* is usually prepared as a concortion with other antimalarial plants (Nguta *et al*., 2010) and this could explain why no adverse effects have been reported by those communities commonly using the plant as an antimalarial phytotherapeutic remedy.

At a dose of 2000 mg/kg body weight via the oral route, both aqueous and organic root extracts of *Z. chalybeum* did not exhibit any behavioural signs of toxicity to mice. There was also zero mortality in 24 hours and during the 14 days observation period. In addition, a general increase in weight was observed in the experimental animals up to day 14 at the time of sacrifice. The absence of significant weight loss in the study animals even at a high dose of 2000 mg/kg could suggest short term safety of the extracts on mice. Moreover, the significant weight gain observed indicates that the root bark extract may contain appetite stimulants.

The decrease in the weight could be an indicator of long term effect of the extract and further studies on the chronic toxicity of the aqueous and chloroform: methanol root extracts of Z. *chalybeum* should be carried out. In addition, it may be argued that though the root extract was toxic to brine shrimp and non-toxic to mice, there was a possibility that some of the toxic compounds were rendered harmless as the drug inform of the extract underwent the various stages of metabolism and this could have led to the witnessed non- toxicity scenario of the extracts within the 14 days. Otherwise chronic toxicity may set in over time.

The aqueous leaves extract of *Ocimum suave* had a parasitaemia of 7.21% compared to that of distilled water (negative control) of 22.33%. The organic leaves extract had a parasitaemia of 11.01% compared to that of distilled water (22.33%). Both aqueous and organic extracts had a chemosuppression of 67.7 and 54.78% respectively compared to chloroquine (positive control) which had a parasitaemia of 0.5% and a chemosuppression of 97.76% respectively. The chemosuppression induced by both aqueous and organic leaf extracts of *O. suave* was significantly different from that of chloroquine ($p<0.05$). However, since the parasitaemia levels in both cases were lower than that of distilled water, the plants extracts showed possible antimalarial activity evidenced by the chemosuppressions of 67.70% and 54.78% respectively for aqueous and organic extracts. It may be argued that mice do not metabolize extracts in the same way as humans and this could have led to the witnessed low chemosuppression of both aqueous and organic extracts of *O. suave*. In addition, anecdotal efficacy reported by the study community could be related to synergism of phytoconstituents since the assayed plant extracts are used in combination with others to treat malaria.

Moreover, it should be noticed that pure compounds isolated from *O. suave* may exhibit more activity than the crude extract. Indeed, crude extracts contain many ingredients that can interact with each other. Leaves of *O. suave* have been used in traditional medicine for

treatment of malaria in Msambweni Sub-County, Kenya (Nguta *et al*., 2010b). This study therefore provides insights in the use of the plant for the treatment of malaria in folk medicine. Other species of the genus Ocimum have been reported to have antimalarial activity.

Earlier studies on *in vitro* antiplamodial activity of ethanol extracts of some members of the genus Ocimum revealed that the leaf extracts of *O. canum*, root extracts of *O. sanctum* and *O. basilicum* and the flower and stem extracts of all the three species of Ocimum tested had LC_{50} values of between 50 and 100 ug/ml (Inbaneson *et al*., 2011). In addition, Murithi *et al*., (2014), while studying *O. gratissimum* reveals that the organic leaf extracts induced 88.07% chemosuppression on *P. berghei* infected mice. The chemosuppression by the organic extract was not significantly different from that of chloroquine ($p>0.05$). *O. suave* and *O. gratissimum* are closely related species of the genus Ocimum and therefore the results obtained in the current study are in line with those of the previous study (Murithi *et al*., 2014).

The effect of both aqueous and organic leaf extract of *O. suave* on hematological parameters in *P. berghei* infected mice was investigated. The leaf extracts had no effect on the packed cell volume (PCV) and Red blood cells (RBCs) counts. The total WBC (Leucocyte) counts were also significantly increased following extract administration. On examination of the differential counts, neutrophils, lymphocytes and monocytes increased following administration of the leaf extract.

Cytotoxicity test revealed that both aqueous and organic leaf extracts of *O. suave* were moderately toxic to the brine shrimp. These results are in agreement with those reported by Murithi *et al*., (2014), on *O. gratissimum* which is a close relative of *O. suave*. Acute oral toxicity revealed that a dose of 2000 mg/kg body weight aqueous and organic leaf extracts of *O.suave* were safe to mice. During the observation period, no significant signs of toxicity such as changes in breathing, skin effects, defecation, yellowing or loss of hair, postural abnormalities or impaired food intake were observed (Mequanint *et al*., 2011). However, the fluctuations in weight over the 14 days of survival observation may be suggestive of chronic toxicity. The other possibility would also be that any toxins present in the extracts were neutralized during the metabolism by the mice over the 4- days treatment period.

Upon screening the leaf extracts of *O. suave* for the phyochemicals, for both aqueous and organic leaf extracts, alkaloids, flavonoids, saponins and tannins were found present. Sesquiterpene lactones were found present in organic leaf extract but absent in aqueous extract while glycosides were absent in both extracts. Earlier studies on phytochemical screening of the ethanol and aqueous extracts of *O. gratissimum* a close relative of *O. suave*, revealed the presence of phenolic acids, flavonoids, tannins and triterpenes which were perhaps responsible for the antiplasmodial activity (Birhanesey *et al.,* 2012). Similarly, the presence of the same phytochemicals in the present study may have been responsible for the in vivo antimalarial chemosuppression observed against the *P. berghei* (ANKA).

Both aqueous and organic extracts of *Plectranthus barbatus* had a parasitaemia level of 10 % and 4.76% respectively compared to that of distilled water (22.33%) and chloroquine (0.5%), and a chemosuppression of 55.23% and 78.69% respectively compared to chloroquine (97.76%). The chemosuppression from the organic root extract of *P. barbatus* was not significantly different from that of chloroquine. These results suggest possible antimalarial activity in the extract compared to the controls. These results are in agreement with those of Al- Muayeib *et al*., (2012), where results from the in vitro antiplasmodial studies using several Plectranthus species showed antiplasmodial activity against *P. falciparum* 3D7 strain. In addition, the results obtained in the present screen are in agreement with the literature data and ethnobotanical survey done by Nguta *et al*., (2010) in Msambweni and hence justifies the folkloric use.

On hematological activity, the extract did not exhibit a significant effect on RBCs hematocrit, hemoglobin at all treatment doses relative to the control. There was an increase in lymphocytes and neutrophils in the *P. barbatus* root extract treatments compared to the negative controls. Asiimwe *et al.,* (2014) while working with *P. amboinicus* a close relative of *P. barbatus* observed that the plant caused a significant increase in lymphocytes, platelets, basophils and neutrophils in the test animals. These results are in line with results in the current study. The significant increase in lymphocytes in the test animals suggests that the extract may have immunostimulating properties. This is due to the presence of saponins, flavonoids and alkaloids which enhance immune functions by stimulating cell division and transformation in lymphocytes (Bruneton, 1995; Govid *et al*., 2012; Hoffmann., 2003).

Upon testing for cytotoxicity, the aqueous root extract of *P. barbatus* was found to be weakly toxic while organic root extract was found to be moderately toxic. When tested for cytotoxicity using the brine shrimp. *P. barbatus* has been reported to generally have low toxicity (Figueiredo *et al.,* 2010) and this is in agreement with results obtained in the current study.

Acute oral toxicity revealed that a dose of 2000 mg/kg body weight aqueous and organic root extracts of *P. barbatus* were safe to mice. All experimental mice were alive and normal for all 14 days experimentation period. No mortality or signs of toxicity was observed. However, the fluctuations in weight over the 14 days of survival observation period may be suggestive of chronic toxicity. The other possibility would also be that any toxins present in the extracts were neutralized during the metabolism by the mice over the 4- days treatment period. The root bark may as well be containing some appetite stimulants which might have made the mice to feed more on the pellets thereby causing an increase in their weights over the experimental period.

Phytochemical screening of *P. barbatus* revealed the presence of alkaloids, flavonoids, saponins, glycosides and tannins in both aqueous and organic root extracts of *P. barbatus*. Sesquiterpene lactones were found present in organic extract and absent in aqueous extract. Van Zyl *et al*., (2008) attributes the antiplasmodial activity of the Plectranthus species he worked on to the presence of abietane diterpenes. This is in agreement with results obtained in the current study where sesquiterpene lactones were found present during the phytochemical characterization of *P. barbatus* root bark.

In summary, good antimalarial activity was observed in the aqueous extracts of *Z. chalybeum* and the organic extracts of *P. barbatus* and *Z. chalybeum* with a chemosuppression of 81.45%, 78.68% and 78.38% respectively. These chemosuppressions were not significantly different from that of chloroquine ($p>0.05$). These extracts though elicited signs of cytotoxicity in brine shrimp did not elicit signs of toxicity in the mice at a dose of 2000 mg/kg body weight. They can therefore be considered safe for use. Even though chemosuppressions induced by the aqueous crude extracts of *O. suave* and *P. barbatus* and the organic crude extract of *O. suave* were significantly different from that of chloroquine ($p<0.05$), their parasitaemia was lower than that of the negative control (distilled water). This suggests that they have some antimalarial activity. Even though there was some mild cytotoxicity detected

in brine shrimp lethality assay, the extracts were safe to mice at a dose of 2000 mg/kg body weight since no mortality or signs of toxicity were observed in acute toxicity tests.

Alkaloids, flavonoids, saponins, sesquiterpenes tannins and glycosides were found to be present in the crude extracts of the three plants. According to literature, alkaloids, flavonoids and sesquterpenes are plant phytochemicals with broad spectrum of bioactivities and these may have been responsible for the observed plants antimalarial and toxicity activities (Mazid *et al.*, 2011). An aminoquinoline alkaloid (quinine) isolated from the bark of Cinchona species (Rubiaceae), has been widely used for treatment of malaria (Ronnan *et al.,* 2009).

5.2 Conclusions

Results show that scientific studies carried out on medicinal application of the three plants having antimalarial traditional claims of effectiveness yielded fruitful results. Results of the pharmacological studies show that they indeed have some antimalarial activity and no serious toxicological effects. This to some extent validates the use of these plants by the people of Msambweni in the treatment of malaria related signs and symptoms. In addition, the findings from this study have identified two out of the three plants studied, as promising candidates for further investigation as potential sources of a new plant based class of antimalarial drugs. The current study to the best of our knowledge reports for the first time *in vivo* antimalarial activity of *P. barbatus*.

5.3 Recommendations

The following recommendations were made:

i. In vitro studies of antimalarial activity of the three plants should be carried out to investigate the effects of the extracts on *P. falciparum* which is the major cause of malaria in humans.

ii. Further toxicity studies should be done on the plants to promote knowledge on their safety.

iii. Further studies aimed at the isolation and structural elucidation of antimalarial active constituents from the investigated plants should be done.

iv. Combined therapy studies should be done to establish whether there is synergism among plant extracts.

v. The Communities using roots as sources of drugs need to be educated on sustainable harvesting/propagation techniques to mitigate the challenge of uprooting the whole plant which poses danger to the conservation status of some of these rare indigenous plant species.

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APPENDICES

Appendix 1: Antimalarial Activity

Percentage parasitaemia and chemosuppression of mice treated with aqueous crude extracts of Zanthoxylum *chalybeum* at a dosage of 100 mg /kg/ body weight per mouse for 4 days

Mouse		**MF1**	**MF2**	**MF3**	**MF4**	**TOTAL**	**% Parasitaemia**	**% Chemosuppression**
1	Parasitized cells (PCs)	10	14	9	9	**42**	5.11	77.12
	Total Cells (TCs)	224	237	146	215	**822**		
2	Parasitized cells (PCs)	1	5	4	3	**13**	1.34	94.0
	Total Cells (TCs)	190	245	267	270	**972**		
3	Parasitized cells (PCs)	2	0	1	0	**3**	0.33	98.52
	Total Cells (TCs)	252	287	170	202	**911**		
4	Parasitized cells (PCs)	17	28	28	26	**99**	9.79	56.16
	Total Cells (TCs)	242	269	216	284	**1011**		
5	Parasitized cells (PCs)	N/A	N/A	N/A	N/A	N/A		
	Total Cells (TCs)	N/A	N/A	N/A	N/A	N/A		
Average							**4.14**	**81.45**

KEY:

MF1- Magnification Field 1

MF2- Magnification Field 2

MF3- Magnification Field 3

MF4- Magnification Field 4

Percentage parasitaemia and chemosuppression of mice treated with aqueous crude extracts of *Ocimum suave* at a dosage of 100 mg /kg/body weight per mouse for 4 days

Mouse		**MF1**	**MF2**	**MF3**	**MF4**	**TOTAL**	**% Parasitaemia**	**% Chemosuppression**
1	Parasitized cells (PCs)	19	19	26	23	**87**	8.02	64.08
	Total Cells (TCs)	213	310	305	257	**1085**		
2	Parasitized cells (PCs)	5	6	5	7	**23**	2.22	90.06
	Total Cells (TCs)	268	244	216	310	**1038**		
3	Parasitized cells (PCs)	16	16	15	35	**82**	11.70	47.60
	Total Cells (TCs)	174	168	160	199	**701**		
4	Parasitized cells (PCs)	16	15	15	23	**69**	7.45	66.64
	Total Cells (TCs)	243	242	222	219	**926**		
5	Parasitized cells (PCs)	15	20	15	19	**69**	6.67	70.13
	Total Cells (TCs)	248	280	249	257	**1034**		
Average							**7.21**	**67.70**

KEY:

MF1- Magnification Field 1

MF2- Magnification Field 2

MF3- Magnification Field 3

MF4- Magnification Field 4

Percentage parasitaemia and chemosuppression of mice treated with aqueous crude extracts of *Plectranthus barbatus* at a dosage of 100 mg /kg/ body weight per mouse for 4 days

Mouse		**MF1**	**MF2**	**MF3**	**MF4**	**TOTAL**	**% Parasitaemia**	**% Chemosuppression**
1	Parasitized cells (PCs)	10	8	6	5	**29**	2.52	88.71
	Total Cells (TCs)	329	305	290	229	**1153**		
2	Parasitized cells (PCs)	21	42	38	44	**148**	20.11	9.94
	Total Cells (TCs)	158	176	196	206	**736**		
3	Parasitized cells (PCs)	2	1	1	3	**7**	0.73	96.73
	Total Cells (TCs)	224	198	230	301	**953**		
4	Parasitized cells (PCs)	34	41	33	41	**149**	14.77	33.86
	Total Cells (TCs)	238	268	236		**267**	1009	
5	Parasitized cells (PCs)	2	4	3	3	**12**	11.86	46.89
	Total Cells (TCs)	266	262	246	238	**1012**		
Average							**10.0**	**55.23**

KEY:

MF1- Magnification Field 1

MF2- Magnification Field 2

MF3- Magnification Field 3

MF4- Magnification Field 4

Percentage parasitaemia and chemosuppression of mice treated with organic crude extracts of *Zanthoxylum chalybeum* at a dosage of 100 mg /kg/ body weight per mouse for 4 days

Mouse		**MF1**	**MF2**	**MF3**	**MF4**	**TOTAL**	**% Parasitaemia**	**% Chemosuppression**
1	Parasitized cells (PCs)	22	14	13	6	**55**	7.12	68.11
	Total Cells (TCs)	267	173	184	149	**773**		
2	Parasitized cells (PCs)	13	18	22	19	**72**	7.55	66.19
	Total Cells (TCs)	199	236	265	254	**954**		
3	Parasitized cells (PCs)	3	4	1	1	**9**	0.92	95.88
	Total Cells (TCs)	287	258	273	165	**983**		
4	Parasitized cells (PCs)	2	2	3	2	**9**	0.85	96.19
	Total Cells (TCs)	305	247	286	221	**1059**		
5	Parasitized cells (PCs)	24	24	25	27	**100**	7.69	65.56
	Total Cells (TCs)	319	325	331	325	**1300**		
Average							**4.83**	**78.39**

KEY:

MF1- Magnification Field 1

MF2- Magnification Field 2

MF3- Magnification Field 3

MF4- Magnification Field 4

Percentage parasitaemia and chemosuppression of mice treated with organic crude extracts of *Ocimum suave* at a dosage of 100 mg /kg/ body weight per mouse for 4 days

Mouse		MF1	MF2	MF3	MF4	TOTAL	% Parasitaemia	% Chemosuppression
1	Parasitized cells (PCs)	46	39	60	43	**188**	24.61	- 10.21
	Total Cells (TCs)	178	165	235	186	**764**		
2	Parasitized cells (PCs)	22	21	20	21	**84**	8.20	63.28
	Total Cells (TCs)	263	271	256	235	**1025**		
3	Parasitized cells (PCs)	29	17	22	27	**95**	12.18	45.45
	Total Cells (TCs)	180	172	184	244	**780**		
4	Parasitized cells (PCs)	7	6	5	6	**24**	2.60	88.36
	Total Cells (TCs)	178	296	245	203	**922**		
5	Parasitized cells (PCs)	19	19	16	15	**69**	7.46	66.59
	Total Cells (TCs)	207	249	219	250	**925**		
Average							**11.01**	**54.78**

KEY:

MF1- Magnification Field 1

MF2- Magnification Field 2

MF3- Magnification Field 3

MF4- Magnification Field 4

Percentage parasitaemia and chemosuppression of mice treated with organic crude extracts of Plectranthus *barbatus* at a dosage of 100 mg /kg/ body weight per mouse for 4 days

Mouse		**MF1**	**MF2**	**MF3**	**MF4**	**TOTAL**	**% Parasitaemia**	**% Chemosuppression**
1	Parasitized cells (PCs)	10	11	10	10	**41**	4.63	79.27
	Total Cells (TCs)	174	235	254	223	**886**		
2	Parasitized cells (PCs)	3	4	3	3	**13**	1.67	92.52
	Total Cells (TCs)	239	173	186	179	**777**		
3	Parasitized cells (PCs)	10	10	7	6	**33**	4.05	81.86
	Total Cells (TCs)	190	222	172	231	**815**		
4	Parasitized cells (PCs)	18	25	19	13	**75**	7.88	64.71
	Total Cells (TCs)	237	242	330	143	**952**		
5	Parasitized cells (PCs)	16	11	9	15	**51**	5.56	75.10
	Total Cells (TCs)	213	237	221	246	**917**		
Average							**4.76**	**78.69**

KEY:

MF1- Magnification Field 1

MF2- Magnification Field 2

MF3- Magnification Field 3

MF4- Magnification Field 4

Controls

Percentage parasitaemia and chemosuppression of mice treated with Chloroquine at a dosage of 20 mg /kg/ body weight per mouse for 4 days

Mouse		**MF1**	**MF2**	**MF3**	**MF4**	**TOTAL**	**% Parasitaemia**	**% Chemosuppression**
1	Parasitized cells (PCs)	1	1	1	0	**3**	0.31	98.61
	Total Cells (TCs)	251	250	230	240	**971**		
2	Parasitized cells (PCs)	0	2	0	1	**3**	0.36	98.39
	Total Cells (TCs)	212	242	223	159	**836**		
3	Parasitized cells (PCs)	0	1	0	1	**2**	0.23	98.97
	Total Cells (TCs)	213	261	199	214	**887**		
4	Parasitized cells (PCs)	3	3	1	2	**9**	1.13	94.94
	Total Cells (TCs)	201	226	209	161	**797**		
5	Parasitized cells (PCs)	0	2	1	1	**4**	0.47	97.90
	Total Cells (TCs)	208	232	245	167	**852**		
Average							**0.5**	**97.76**

KEY:

MF1- Magnification Field 1

MF2- Magnification Field 2

MF3- Magnification Field 3

MF4- Magnification Field 4

Percentage parasitaemia and percentage chemosuppression of mice treated with distilled water at a dosage of 0.2 ml /kg/ body weight per mouse for 4 days

Mouse		**MF1**	**MF2**	**MF3**	**MF4**	**TOTAL**	**% Parasitaemia**	**% Chemosuppression**
1	Parasitized cells (PCs)	28	43	50	44	**165**	17.04	N/A
	Total Cells (TCs)	180	231	222	259	**892**		
2	Parasitized cells (PCs)	32	62	34	39	**167**	21.72	N/A
	Total Cells (TCs)	165	236	194	174	**769**		
3	Parasitized cells (PCs)	42	51	32	39	**164**	22.19	N/A
	Total Cells (TCs)	186	207	160	186	**739**		
4	Parasitized cells (PCs)	33	39	55	43	**170**	21.96	N/A
	Total Cells (TCs)	153	184	207	230	**774**		
5	Parasitized cells (PCs)	59	64	58	53	**234**	28.75	N/A
	Total Cells (TCs)	192	247	176	199	**814**		
Average							**22.33**	**N/A**

KEY:

MF1- Magnification Field 1

MF2- Magnification Field 2

MF3- Magnification Field 3

MF4- Magnification Field 4

Appendix II: Mortality

a, Antimalarial tests

Mice survival time after treatment with crude plant extracts at a dosage of 100 mg/kg/body weight per mouse for 14 days

Treatment	**M1**	**M2**	**M3**	**M4**	**M5**	**Mean survival time**
Zanthoxylum chalybeum (Aqueous)	6	7	7	13	16	9.8
Zanthoxylum chalybeum (Organic)	5	5	12	13	13	9.6
Ocimum suave (Aqueous)	6	9	9	9	10	8.6
Ocimum suave (Organic)	5	6	6	8	11	7.2
Plectranthus barbatus (Aqueous)	6	7	9	10	13	9
Plectranthus barbatus (Organic)	6	7	10	11	13	9.4
Distilled Water	5	6	6	7	7	6.2
Chloroquine	14	14	14	14	14	14

KEY:

M1- Mouse 1

M2- Mouse 2

M3- Mouse 3

M4- Mouse 4

M5- Mouse 5

b, Acute oral toxicity

Number of mice that were alive per cage after treatment at a dosage of 300 mg/kg body weight per mouse for 14 days

Days / Extract	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Zanthoxylum chalybeum A	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Zanthoxylum chalybeum O	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Ocimum suave A	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Ocimum suave O	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Plectranthus barbatus A	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Plectranthus barbatus O	5	5	5	5	5	5	5	5	5	5	5	5	5	5

KEY: A= Aqueous, O= Organic

Number of mice that were alive per cage after treatment at a dosage of 2, 000 mg/kg body weight per mouse for 14 days

Days / Extract	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Zanthoxylum chalybeum A	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Zanthoxylum chalybeum O	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Ocimum suave A	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Ocimum suave O	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Plectranthus barbatus A	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Plectranthus barbatus O	5	5	5	5	5	5	5	5	5	5	5	5	5	5

KEY: A= Aqueous, O= Organic

c, Brine shrimp lethality Assay results

Brine shrimp results for aqueous extracts

Plant extract	Concentration	1000ug/ml		100ug/ml		10ug/ml		0ug/ml	
	No. of shrimps	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Z. chalybeum	10	0	10	10	0	10	0	10	0
	10	0	10	10	0	10	0	10	0
	10	0	10	10	0	10	0	10	0
O. suave	10	0	10	5	5	10	0	10	0
	10	0	10	5	5	10	0	10	0
	10	0	10	6	4	10	0	10	0
P. barbatus	10	2	8	10	0	10	0	10	0
	10	2	8	0	10	10	0	10	0
	10	0	10	10	0	10	0	10	0

Brine shrimp results for organic extracts

Plant extract	Concentration	1000ug/ml		100ug/ml		10ug/ml		0ug/ml	
	No. of shrimps	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Z. chalybeum	10	0	10	0	10	2	8	10	0
	10	0	10	0	10	1	9	10	0
	10	0	10	0	10	0	10	10	0
O. suave	10	0	10	1	9	10	0	10	0
	10	0	10	1	9	10	0	10	0
	10	0	10	2	8	10	0	10	0
P. barbatus	10	1	9	6	4	10	0	10	0
	10	1	9	5	5	10	0	10	0
	10	0	10	5	5	10	0	10	0

Appendix III:

Weight changes (g) of mice treated at a dosage of 2000 mg/kg body weight

Average Weight changes of mice treated with aqueous extracts for 14 days

Treatment	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
Z. chalybeum A	19.4	21.6	22.4	21.4	24	24.6	25.25
O. suave A	19.2	20.6	22.4	21.2	23.4	24.2	24.2
P. barbatus A	19.6	20.6	21.4	20	23	24	25.4

Data expressed as average weight of five determinants per group

KEY: A= Aqueous

Weight changes of mice treated with organic extracts for 14 days

Treatment	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
Z. chalybeum O	19.2	21	22.4	21	23	22.8	20.2
O. suave O	19.6	20.8	23	22.2	24.4	25.2	25.2
P. barbatus O	18	19.2	20.2	19	22	22.6	22.8

Data expressed as average weight of five determinants per group

KEY: O= Organic

Appendix IV:

Hematological activity

Effect of Chloroquine and Aqueous plant extracts on hematological parameters of *P. berghei* infected mice (Dosage: CQ= 20 mg/kg b.w; Extracts = 100 mg/kgb.w)

	Packed cell volume (PCV)	Hemoglobin (Hb) concentration (g/l)	Red blood cell (RBC) count (million/mm^3)	Platelet count/ (mm^3)	White blood cell (WBC) count (/mm^3)
Normal control	31.8	10.6	4.46	369.33	9.29
Negative control (untreated *P. berghei* infected mice.	31.63	10.53	4.61	399.33	30.88
Positive control (*P. berghei* infected mice treated with 20mg/kg b.w chloroquine)	41.4	13.8	5.97	364.0	6.51
P. berghei infected mice treated with100mg/kg b.w *Z. Chalybeum* A	28.9	9.63	4.16	176.33	17.76
P. berghei infected mice treated with100mg/kg b.w *O. suave* A	22.9	7.63	3.3	153.33	17.06
P. berghei infected mice treated with100mg/kg b.w *P. barbatus* A	12.45	4.15	1.79	397.5	15.26

KEY: CQ – Chloroquine, O= Organic

Effect of Chloroquine and Aqueous plant extracts on hematological parameters of *P. berghei* infected mice (Dosage: CQ= 20 mg/kg b.w; Extracts = 200mg/kgb.w)

	Packed cell volume (PCV)	Hemoglobin (Hb) concentration (g/l)	Red blood cell (RBC) count (million/mm^3)	Platelet count/mm^3	White blood cell (WBC) count (/mm^3)
Normal control	31.8	10.6	4.46	369.33	9.29
Negative control (untreated *P. berghei* infected mice)	31.63	10.53	4.61	399.33	30.88
Positive control (*P. berghei* infected mice treated with 20mg/kg b.w chloroquine)	41.4	13.8	5.97	364	6.51
P. berghei infected mice treated with200mg/kg b.w *Z. Chalybeum* A	32	10.67	4.61	263.67	27.39
P. berghei infected mice treated with200mg/kg b.w *O. suave* A	20.4	8.45	4.03	325.0	24.77
P. berghei infected mice treated with200mg/kg b.w *P. barbatus* A	14.65	8.8	3.04	275.0	16.58

KEY: CQ – Chloroquine

A= Aqueous

Effect of Chloroquine and organic plant extracts on hematological parameters of *P. berghei* infected mice (Dosage: CQ= 20 mg/kg b.w; Extracts = 100mg/kgb.w)

	Packed cell volume (PCV)	Hemoglobin (Hb) concentration (g/l)	Red blood cell (RBC) count (million/mm^3)	Platelet count/mm^3	White blood cell (WBC) count (/mm^3)
Normal control	31.8	10.6	4.46	369.33	9.29
Negative control (untreated *P. berghei* infected mice.	31.63	10.53	4.61	399.33	30.88
Positive control (*P. berghei* infected mice treated with 20mg/kg b.w chloroquine)	41.4	13.8	5.97	364.0	6.51
P. berghei infected mice treated with100mg/kg b.w *Z. Chalybeum* O	20	6.67	2.88	245.0	32.92
P. berghei infected mice treated with100mg/kg b.w *O. suave* O	17.23	7.43	3.51	203.33	21.60
P. berghei infected mice treated with100mg/kg b.*w P. barbatus* O	18.57	8.27	3.75	246.0	31.19

KEY: CQ – Chloroquine

O- Organic

Effect of Chloroquine and organic plant extracts on hematological parameters of *P. berghei* infected mice (Dosage: CQ= 20 mg/kg b.w; Extracts = 200mg/kgb.w)

	Packed cell volume (PCV)	Hemoglobin (Hb) concentration (g/l)	Red blood cell (RBC) count (million/mm^3)	Platelet count/mm^3	White blood cell (WBC) count (/mm^3)
Normal control	31.8	10.6	4.46	369.33	9.29
Negative control (untreated *P. berghei* infected mice.	31.63	10.53	4.61	399.33	30.88
Positive control (*P. berghei* infected mice treated with 20mg/kg b.w chloroquine)	41.4	13.8	5.97	364.0	6.51
P. berghei infected mice treated with200mg/kg b.w *Z. Chalybeum* O	24.15	7.8	7.41	229.0	35.20
P. berghei infected mice treated with200mg/kg b.w *O. suave* O	20.4	8.45	4.03	99.5	24.77
P. berghei infected mice treated with200mg/kg b.w *P. barbatus* O	14.65	8.8	3.04	192.5	16.58

KEY: CQ – Chloroquine

O= Organic

Effect of chloroquine and aqueous plant extracts on WBC differential count on *P. berghei* infected mice (Dosage: CQ = 20 mg/kg/b.w; Extracts = 100 mg/kg b.w)

	Neutrophil count (% WBC)	Lymphocyte Count (% WBC)	Monocyte count (% WBC)	Eosinophil count (% WBC)	Basophil count (% WBC)
Normal control	24	75	1	0	0
Negative control (Untreated *P. berghei* infected mice)	17.67	63.33	19	0	0
Positive control (*P. berghei* infected mice treated with 20 mg/kg b.w chloroquine)	27.33	69.67	2.33	0	0
P. berghei infected mice treated with 100 mg/ kg b.w *Z. Chalybeum* A	25	58.67	16.33	0	0
P. berghei infected mice treated with 100 mg/ kg b.w *O. suave* A	25	56	19	0	0
P. berghei infected mice treated with 100 mg/ kg b.w *P. barbatus* A	25	64.5	14	0	0

KEY:

CQ= Chloroquine, A= Aqueous

Effect of chloroquine and aqueous plant extracts on WBC differential count on *P. berghei* infected mice (Dosage: CQ = 20 mg/kg/b.w; Extracts = 200 mg/kg b.w)

	Neutrophil count (% WBC)	Lymphocyte Count (% WBC)	Monocyte count (% WBC)	Eosinophil count (% WBC)	Basophil count (% WBC)
Normal control	24	75	1	0	0
Negative control (Untreated *P. berghei* infected mice)	17.67	63.33	19	0	0
Positive control (*P. berghei* infected mice treated with 20 mg/kg b.w chloroquine)	27.33	69.67	2.33	0	0
P. berghei infected mice treated with 200 mg/ kg b.w *Z. Chalybeum* A	15.67	66.67	17.67	0	0
P. berghei infected mice treated with 200 mg/ kg b.w *O. suave* A	26.33	56.33	16.67	0	0
P. berghei infected mice treated with 200 mg/ kg b.w *P. barbatus* A	26.33	60.33	13	0.33	0

KEY:

CQ= Chloroquine, A= Aqueous

Effect of chloroquine and organic plant extracts on WBC differential count on *P. berghei* infected mice (Dosage: CQ = 20 mg/kg/b.w; Extracts = 100 mg/kg b.w)

	Neutrophil count (% WBC)	Lymphocyte Count (% WBC)	Monocyte count (% WBC)	Eosinophil count (% WBC)	Basophil count (% WBC)
Normal control	24	75	1	0	0
Negative control (Untreated *P. berghei* infected mice)	17.67	63.33	19	0	0
Positive control (*P. berghei* infected mice treated with 20 mg/kg b.w chloroquine)	27.33	69.67	2.33	0	0
P. berghei infected mice treated with 100 mg/ kg b.w *Z. Chalybeum* O	25.33	54.33	19.33	0	0
P. berghei infected mice treated with 100 mg/ kg b.w *O. suave* O	20.33	62.67	14.33	0	0
P. berghei infected mice treated with 100 mg/ kg b.w *P. barbatus* O	11.67	78.67	8.33	0	0

KEY:

CQ= Chloroquine, O= Organic

Effect of chloroquine and organic plant extracts on WBC differential count on *P. berghei* infected mice (Dosage: CQ = 20 mg/kg/b.w; Extracts = 200 mg/kg b.w)

	Neutrophil count (% WBC)	Lymphocyte Count (% WBC)	Monocyte count (% WBC)	Eosinophil count (% WBC)	Basophil count (% WBC)
Normal control	24	75	1	0	0
Negative control (Untreated *P. berghei* infected mice)	17.67	63.33	19	0	0
Positive control (*P. berghei* infected mice treated with 20 mg/kg b.w chloroquine)	27.33	69.67	2.33	0	0
P. berghei infected mice treated with 200 mg/ kg b.w *Z. Chalybeum* O	29	58.5	12.5	0	0
P. berghei infected mice treated with 200 mg/ kg b.w *O. suave* O	8	84.67	7.33	0	0
P. berghei infected mice treated with 200 mg/ kg b.w *P. barbatus* O	11.67	74	14.33	0	0

KEY:

CQ= Chloroquine, O= Organic